METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES FROM HAND-DUG WELLS IN THE CUVELAI-ETOSHA BASIN, NAMIBIA

BY

BILLY McBENEDICT

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LUSAKA

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DECLARATION

I, Billy McBenedict, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

……………………………………………… Date…………………………………………

Billy McBenedict

APPROVAL

This thesis of Billy McBenedict has been approved as fulfilling the requirements for the award of Doctor of Philosophy in Microbiology by The University of Zambia.

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Internal Examiner Signature Date

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External Examiner Signature Date

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Chairperson Signature Date

(Board of Examiner)

Prof Bernard M. Hang’ombe ……………………………………... ………………………………

Supervisor Signature Date

ABSTRACT

The total surface area of planet earth is about 510 million Km^2 and about 71% of this area is water and the remaining 29% is formed by land mass. The proportion of water is much higher, as it is a major component of physiological existence and includes all types of natural water resources; oceans, seas, rivers, lakes and groundwater. Africa has about 64% water coverage, which is the lowest among the continents in the world. This situation retards the development of most African countries because water is crucial for social-economic development and this situation demands that African countries explore the use of groundwater and rainfall in addition to surface water. Water scarcity is commonly experienced in southern Africa due to the increased arid conditions and unpredictable rainfall patterns. The region has countries such as Botswana, Angola and Zambia with unpredictable rainfall patterns while Namibia is a desert country with short rain and long dry seasons. The Cuvelai Etosha Basin of Namibia is a rural setting in which most people depend on groundwater to circumvent water scarcity by the construction of hand-dug wells. However, groundwater presents another problem because it is saline in most parts of the basin and the situation is worsened by lack of perennial rivers within the regions. A Metagenomics and culturing study was conducted to explore the bacterial communities in hand-dug well water of the Cuvelai Etosha Basin and its safety for human and livestock consumption. The influence of hand-dug well type, region and season on bacterial; colony forming units, coliforms and particular genera, phyla, species richness, diversity and evenness, human and livestock pathogens, zoonotic pathogens, and grey bacteria was revealed. The dominant bacterial phyla and major water physicochemical parameters influencing phyla abundance were determined leading to conclusions; hand-dug well type and region does not influence the subjects investigated except for colony forming unitss that are influenced by hand-dug well type. Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Cyanobacteria are dominant and main physicochemical factors influencing their abundance were phosphate, manganese, potential of hydrogen, and temperature. Seasonality did not affect coliforms and *Proteus* species presence, bacterial species diversity and evenness except richness and abundance. The wet season had pronounced abundances of human, livestock and zoonotic pathogens and grey bacteria. Overall, Cuvelai Etosha Basin hand-dug well water is not safe for human and livestock consumption unless sanitized.

Keywords: Bacteria, Cuvelai Etosha Basin, hand-dug wells, metagenomics, water

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DEDICATION

I dedicate this work to my family, Beauty, Benedict, Lydia, Ben, Bertha, Britney, Constance and Eveline I am very grateful for the unlimited love, support, encouragement and prayers that you continuously give to me. You have always given me the drive to pull through hardships and challenges and to you I owe the hard work implemented in this research. Furthermore, I would also love to thank my best friend Wilhelmina Hauwanga who gave me unlimited encouragement, love and support during this study. Above all, I thank the almighty God for the strength and elevation for me to complete a Doctoral study. I am greatly humbled and honoured for such blessings.

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ABBREVIATIONS AND ACRONYMS

°C	Degree Celsius
µl	Microliter
µM	Micromole
Al	Aluminium
AMR	Antimicrobial resistance
ANOSIM	Analysis of similarities
ANOVA	Analysis of Variance
ARISA	Automated ribosomal intergenic spacer analysis
As	Arsenic
ATP	Adenosine triphosphate
Ba	Barium
Be	Beryllium
Bo_2	Oxido borane
bp	Base pair
Br	Bromine
C	Cytosine
Ca	Calcium
$CaCO_3$	Calcium carbonate
CARD-FISH	Catalysed reporter deposition FISH
Cd	Cadmium
cDNA	Complementary DNA
CEB	Cuvelai Etosha Basin
CFU	Colony Forming Unit's
Cl	Chloride
Co	Cobalt
Cr	Chromium
Cu	Copper
D	Simpson index
d.f	Degree of freedom

DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
E	Evenness
Ec	Electrical conductivity
EH	Shannon’s equitability
F	Fluorine
FISH	Fluorescence in situ hybridization
gm	Grams
G	Guanine
H’	Shannon-Wiener index
H_2S	Hydrogen sulphide
Hco_3^-	Bicarbonate
HPC	Heterotrophic plate count
ITS	Internal transcribed spacer
K	Potassium
Kb	Kilobytes
Km^2	Squared kilometres
Li	Lithium
Mbp	Mega base pairs
MCL	Maximum Composite Likelihood
MDS	Multidimensional scaling
MEGA	Molecular Evolutionary Genetics Analysis
Mg	Magnesium
Mg^{2+}	Magnesium ion
mL	Millilitres
Mn^{2+}	Manganese
MPN	Most probable number
mRNA	Messenger RNA
MR–VP	Methyl Red and Voges-Prosakuer
ms^{-1}	Metre per second
Na	Sodium

NCBI	National Centre for Biotechnology Information
Ng	Nanogram
NH_4	Ammonium
Ni	Nickel
Nm	Nanometres
NMS	Nonmetric multidimensional scaling
No_2^-	Nitrite
No_3^-	Nitrate
NPoc	Non-purgeable organic carbon
O_2	Oxygen
OTUs	Operational taxonomic units
Pb	Lead
PCA	Principle component analysis
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain reaction
pH	Potential of hydrogen
Po_4^{3-}	Phosphate
R	Richness
RDP	Ribosomal database project
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
$S0_4^{2-}$	Sulphate
Sc	Scandium
Sio_2	Silica
Sr	Strontium
Ti	Titanium
Tic	Total inorganic carbon
TN_b	Total nitrogen bound
TRFLP	Terminal restriction fragment length polymorphism
UPGMA	Unweighted pair group method using arithmetic averages
UV	Ultra-violet

V	Vanadium
WHO	World Health Organisation
X^2	Chi-Square
Xg	Times gravity
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Orientation of the study

The total surface area of planet earth is about 510 million Km^2 and about 71% of this area is water and the remaining 29% is formed by land mass (Reynolds, 2014). The proportion of water is much higher, as it is a major component of physiological existence. The total amount of water on the earth is termed the hydrosphere. The hydrosphere includes all types of natural water resources; oceans, seas, rivers, lakes and groundwater (Shiklomanov and Rodda, 2004). Rivers are complex systems of uni-directional flowing waters at an average velocity ranging from 0.1 to 1 ms^{-1} as they drain particular land surfaces called river basins or watersheds (Chapman, 1996). A lake is a body of water (usually fresh water) that is enclosed with a low average current velocity of 0.001 to 0.01 ms^{-1} and entirely surrounded by land which prevents its direct access to the sea (Chapman, 1996). In some cases, lakes are saline due to evaporation or as a result of input from groundwater (Thomas *et al.*, 1996). Groundwater is the water found beneath the surface of the earth characterized by a flow pattern that is steady in direction and velocity ranging from 10^{-10} to 10^{-3} ms^{-1} governed mainly by the porosity and permeability of the geological material (Chapman, 1996). Reservoirs are another source of water that are human made and are reliable due to easy control.

Africa has about 64% water coverage which is the lowest among the continents in the world (Allan, 2012). This situation retards the development of most African countries because water is crucial for social-economic development and water shortages negatively affect food production, health and industrial development. African countries should explore the use of groundwater and rainfall in addition to surface water especially that water is an important factor in land investments (Pereira *et al.*, 2009; Allan, 2012). Groundwater constitutes two thirds of the world's fresh water resources and is mostly available close to where water is needed (Chapman, 1996). Water scarcity is commonly experienced in southern Africa due to the increased variable arid conditions and unpredictable rainfall patterns (Msangi, 2014). The region has countries such as Botswana, Angola and Zambia with unpredictable rainfall patterns, while Namibia is a desert country with high temperatures and this results in increased evaporation of rain water. Namibia experiences short rain seasons and long dry seasons which cause water scarcity especially in rural areas that lack developed water pipelines and rely on rain water harvesting or groundwater sources such as boreholes, open deep wells and shallow wells (Msangi, 2014).

The Cuvelai Etosha Basin is located in central northern Namibia, and part of it is shared between Angola and Namibia. In Angola, the Basin covers 36% with Cunene province having a larger portion of the northern Cuvelai while Cuando Cubango and Huila provinces share a minor piece (DRFN and HIWAC, 2013). As for Namibia, Oshikoto, Omusati, Ohangwena and Oshana regions contribute 64% (Figure 1.1), while Kunene and Otjozondjupa regions have an intersection with minor areas in the southern part of the Basin (DRFN and HIWAC, 2013). This Basin harbours about half of the Namibian population amounting to one million people (Zimmermann, 2010). The Cuvelai system originates from Angola spreading into Namibia's flat plains leading to shallow ephemeral water resources locally termed as "*oshanas"* in Namibia. At times, floods form a wide network of water in Namibia as a result of overflow from Angola or a combination of local rainfall and floods.

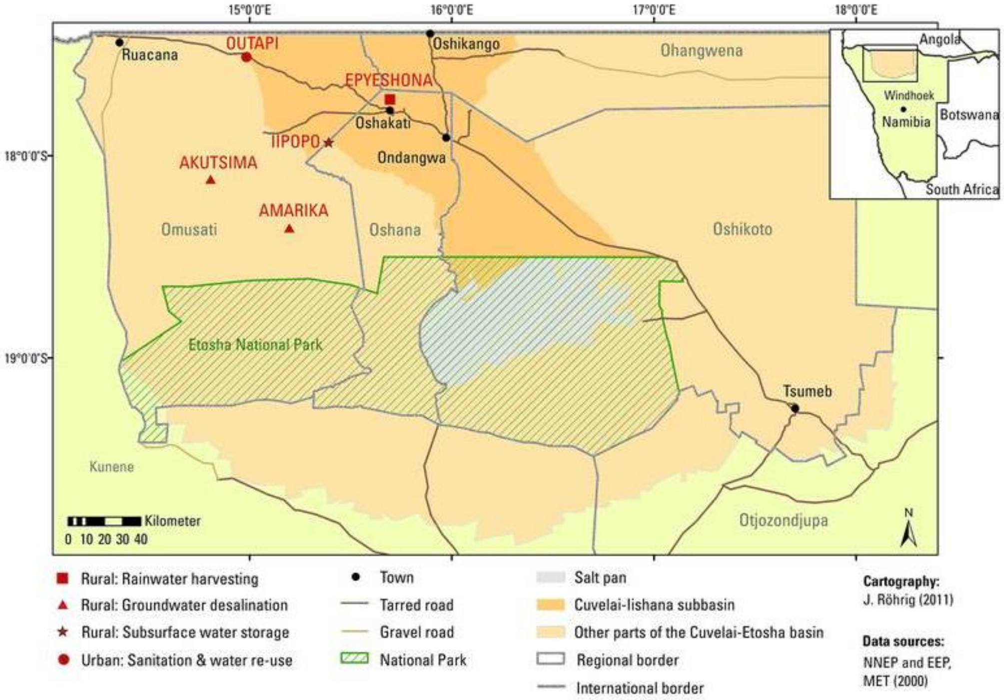

Figure 1.1 Location of the four regions sharing the Cuvelai-Etosha basin in Namibia (retrieved from https://www.google.com.na/Cuvelai-Etosha+Basin+Atlas&biw).

The Cuvelai system serves as a water resource for the communities in Oshikoto, Omusati, Ohangwena and Oshana regions. This water resource is widely utilized since these regions lack a developed water pipeline system that can provide water to sustain agriculture and house hold consumption (Christelis and Struckmeier, 2011). Most people rely on domestic water supply by constructing private hand-dug wells which are near their houses for convenience and preference because deeper groundwater may be saline in large parts of the Basin. Lack of a developed water supply system in some parts of the region increases the risk of water borne infections in these areas because people utilize water from hand-dug wells for house hold use regardless of its quality and safety (McBenedict *et al.,* 2017). Since hand-dug wells are a part of freshwater environments which are known to possess variable resources and conditions that promote microbial growth, it is suggestive that; hand-dug wells in the Cuvelai Etosha Basin of Namibia are a habitat for microorganisms that may pose a health threat to humans and livestock, and there is a change in microbial communities in these wells as a result of seasonal changes driving turnover. Water body turnover is the natural mixing of the top and bottom layer in water bodies due to variations in surface temperature that occurs as a result of seasonal changes (Posch *et al.*, 2012).

The Cuvelai Etosha Basin has three distinct hand-dug well forms (Figures; 3.1, 3.2 and 3.3) that differ according to structure. These structural differences determine whether or not animals have access. Hand-dug well water may harbour microorganisms such as viruses, bacteria, fungi and protozoa which may be pathogenic and induce diseases to both humans and livestock leading to death in severe cases (Abinah, 2013; Yakubu, 2013). Most hand-dug wells in the Cuvelai Etosha Basin of Namibia are not covered and lack a protection zone which allows animals to access the water troughs which are often placed besides the hand-dug wells. In the case of shallow hand-dug wells, animals may walk in and defecate in the vicinity increasing the risk of contamination. Furthermore, surface run off, the construction of pit latrines, and dumping of wastes near wells are also ways that get hand-dug well water contaminated. In addition, studies have shown that shallow perched aquifers are not appropriate water resources for human consumption due to high vulnerability to contamination (Christelis and Struckmeier, 2011).

1.2 Statement of the problem and justification of the study

The problem under investigation is that the microbial water quality and safety of hand-dug wells being utilized for house hold consumption in the Cuvelai-Etosha Basin is unknown and the only research found was by Wanke *et al.* (2014) and Li *et al.* (2017). However, Wanke *et al.* (2014) and Li *et al.* (2017) focused on isotope analysis and hydro-geochemical processes respectively, and did not pay particular attention to the microbiological water quality. Based on this, there is a knowledge gap in relation to the microbial water quality in the Cuvelai Etosha Basin. This is undesirable since water is a habitat for some pathogenic microorganisms. Moreover, disease outbreaks transmitted through contaminated drinking water are of grave concern worldwide especially in underdeveloped countries which experience 99.8% deaths of the cases annually (WHO, 2006).

In addition, lack of knowledge on the microbial water quality and safety of these hand-dug wells is a barrier towards relating the cause of water borne disease outbreaks to the water quality of the hand-dug wells and the development of appropriate remediation strategies. Furthermore, there is a poor understanding of the ecology of microbial communities in hand-dug wells since most aquatic microbial studies focus on lakes, rivers and oceans (Cottrell *et al.*, 2005; Debroas *et al.*, 2009; Konstantinidis *et al.*, 2009; Kristiansson *et al.*, 2011). Hence, this study employed culture-dependent (enrichment and culturing) and culture-independent (Metagenomics) bacterial analyses of hand-dug wells to reveal the entire microbial communities, and the identity of the species present.

1.3 General objective

The main aim of this study was to conduct Metagenomics analysis of bacterial communities in hand-dug wells in the Ohangwena and Omusati regions of the Cuvelai Etosha Basin of Namibia.

1.4 Specific objectives

Culture dependent objectives are the specific objectives that derive the results using culturing techniques. Metagenomics specific objectives yielded results through molecular techniques.by isolating, amplifying and sequencing bacteria DNA directly from the sampled environment.

Water chemical specific objectives focussed on the analysis of the physical and chemical factors in hand-dug well water. The following are the specific objectives of the research:

A. Culture-dependent specific objectives

1. To investigate the influence of hand-dug well type, region, and season (wet or dry) on the abundance of bacterial Colony Forming Units's (cfu) and bacteria genus.
2. To investigate the presence of *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species using culturing techniques.

B. Metagenomics specific objectives

1. To determine the absolute abundance of the different bacterial phyla.
2. To explore the influence of hand-dug well type, region and season on the abundance of bacterial phyla and species richness, diversity and evenness.
3. To examine the effect of hand-dug well type, region and season on the abundance of human, livestock, zoonotic and grey bacterial pathogens.

C. Water chemical/mineral composition specific objectives

1. To investigate the major water physicochemical parameters that influence the abundance of bacterial phyla.

1.5 Null hypothesis

A. Culture-dependent null hypothesis

1. There is no significant difference in the abundance of bacterial CFUs based on; hand-dug well type, region and season.
2. There is no *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella* and *Pseudomonas* species in hand-dug wells.
3. There is no significant difference in the presence of *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species based on; hand-dug well type, region, and season.

B. Metagenomics null hypothesis

1. There is no significant difference in absolute abundance of the different bacterial Phyla.
2. Hand-dug well type, region, and season have no influence on bacterial phyla richness, diversity and evenness.
3. There is no significant difference in the abundance of bacterial Phyla based on; hand-dug well type, region, and season.
4. There is no significant difference in the abundance of human and livestock bacterial pathogens based on; hand-dug well type, region, and season.
5. There is no significant difference in the abundance of zoonotic bacterial pathogens based on; hand-dug well type, region, and season.
6. There is no significant difference in the abundance of grey bacteria based on; hand-dug well type, region, and season.

C. Water chemical/mineral composition null hypothesis

1. There is no relationship between water physicochemical parameters and the abundance of bacterial phyla.

1.6 Expected benefits

The dependence on hand-dug wells as the source of good drinking water in most communities in the Cuvelai Etosha Basin makes the assessment of the water quality from hand-dug wells imperative. This study therefore provided information regarding the bacteriological quality and safety of water from hand-dug wells in the Cuvelai Etosha Basin. This is important in order to understand the possible cause of the water borne diseases such as cholera, and typhoid that are prominent in this area (Shivute, 2008) and appropriate measures can be taken to improve the safety of the water and safe guard the health of the communities. In addition, it enhanced the knowledge and understanding of the bacterial communities in the hand-dug wells in the Cuvelai Etosha Basin of Namibia and their possible interactions. The results of this study may serve as a foundation for a water safety campaign to educate the communities on the risks involved in the consumption of contaminated water and how to treat the water before consumption.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bacterial species associated with water

Water is a natural resource that is essential for sustaining life on earth. It is critical to the survival of living organisms as they may survive without food for a number of weeks. This is not possible in the case of water because it is needed to replace fluids lost due to physiological activities not limited to urination, evaporation through skin, sweating and respiration (Murray *et al.*, 2003). Chinedu *et al.* (2011) revealed that about 1.36 billion Km^3 of water is contained in the hydrosphere of which only 0.3% of it is present as fresh water in rivers, streams, springs and aquifers for human consumption, while 99.7% exists in seas and oceans. Of the fresh water resources, groundwater serves as the largest source of domestic, public and agricultural fresh water for the majority of the population (Assaf and Saadeh 2009; Carreira *et al.,* 2010).

Freshwater environments possess variable resources and conditions that promote microbial growth. These environments are water bodies that are characterized by a dissolved salt concentration of less than one percent. They cover only one percent of the earth's surface and exist as; lakes, ponds, inland wetlands, streams and rivers (Brient *et al.*, 2008). These environments support both oxygen-producing and oxygen-consuming microorganisms that are relevant in regulating the balance between respiration and photosynthetic processes responsible for maintaining the natural cycles of oxygen, carbon and other nutrients such as nitrogen, phosphorus and metals (Madigan *et al.*, 2015).

The safety and quality of water for human consumption is important because drinking contaminated water may lead to waterborne diseases (WHO, 2008). Most water microbial studies focus on the use of microbial indicator organisms to investigate water safety which often has a limitation as described by Payment and Franko (1993). The limitation is that no single microorganism meets the requirements of a good indicator as defined by Grabow (1986), WHO (1993) and NRC (2004). These authors define a microbial indicator as a microorganism that serves as a representative of a particular group or type of microorganisms which when present in a sample indicates the potential presence of the microorganisms it represents. *Citrobacter*, *Escherichia*, *Klebsiella* and *Enterobacter* are collectively referred to as coliform bacteria and serve as indicator organisms for faecal contamination of which *E. coli* is the gold standard (Ashbolt *et al.*, 2001).

Coliforms are gram-negative, none spore forming, oxidase negative, rod shaped aerobic and facultative anaerobic bacteria that ferment lactose (with β-galactosidase) to acid and produce gas within 48 hours at 35 ± 2 °C (WHO, 2004). According to WHO (2004) guidelines on drinking water quality testing, total coliforms should not be detected per 100 ml. Ashbolt *et al.* (2001) revealed that indicator organisms are divided into three categories namely; general (process) microbial indicators, faecal indicators such as *E. coli*, and index organisms and model organisms. Ashbolt *et al.* (2001) defined; general indicators as an assemblage of organisms that validates the effectiveness of a process such as total coliforms for chlorine disinfection, faecal indicators as an assemblage of organisms that indicate the presence of faecal contamination, and index and model organisms as an assemblage of organisms that suggest the presence of a pathogen based on similar behaviour such as *E. coli* is an index for *Salmonella* and F-RNA coliphages are models of human enteric viruses.

Other indicator organisms for water contamination that are gram-positive, and/or anaerobic in nature include faecal streptococci, faecal enterococci, sulphite-reducing clostridia and *Clostridium perfringens*. Faecal streptococci are gram-positive facultative anaerobes and obligate anaerobes, and catalase-negative cocci from selective media (e.g. azide dextrose broth or m Enterococcus agar) that grow on Bile esculin agar at 45° C (Ashbolt *et al.*, 2001). Faecal streptococci belongs to the genera *Streptococcus* and contain the Lancefield group D antigen while faecal enterococci are gram-positive anaerobic cocci that grow at pH 9.6, between 10° C and 45° C, and in 6.5% NaCl (Ashbolt *et al.*, 2001). Sulphite-reducing clostridia are gram-positive obligate anaerobic rods that are spore-forming, non-motile, and reduce sulphite to hydrogen sulphide (Ashbolt *et al.*, 2001).

Bacteria inhabiting water usually exists in groups as they benefit from their interaction for survival especially in freshwater environments due to highly variable available resources and conditions. Hussain (2010) successfully isolated bacteria from drinking water in three cities (Khairpur, Sukkur and Rohri) in Pakistan. In all three cities, it was observed that the number of *E. coli* in summer months was more than in winter months in at least 70% of the water samples, because there is an increased amount of *E. coli* in ruminants in the summer months and decreased to low or undetectable levels in the winter months (Edrington *et al.*,2006). This is because most countries have summer rainfall in which water and conducive temperature (35° C – 47° C) are available to activate metabolic activities enhancing *E. coli* growth at sites where livestock feed. Water activity and appropriate temperature is needed for bacterial growth and survival and low water levels terminate metabolism (Holt *et al.*, 1994; Potts, 1996; Stevenson and Hallsworth, 2014). Using membrane filtration techniques and analytic profile index system for Enterobacteriaceae (API 20E), Hussain (2010) isolated and identified species from families Enterobacteriaceae, Vibrionaceae, Aeromonadaceae and Pseudomonadaceae. In addition, bacteria such as *Chryseobacterium meningosepticum, Providencia rettgeri, Providencia stuarti* and *Citrobacter youngae* were isolated from water even though they are not widely documented as water residents.

Zvidzai *et al.* (2006) conducted a study on the microbial community analysis of drinking water sources (boreholes, open deep wells, shallow wells and rivers). Their findings showed that open deep wells, shallow wells and rivers were more contaminated than boreholes and that protected water sources were less contaminated as compared to unprotected ones. The bacteria species identified were gram-negative *Escherichia coli, Shigella, Salmonella* and *Enterobacter aerogenes*. Igwe *et al.* (2015) investigated the microbiological water quality of 40 samples of which 13 were from rivers, nine from boreholes and 18 from hand-dug wells. The bacterial isolation and identification was based on morphological tests that included; gram stain, spore stain and biochemical reactions based on motility, catalase, coagulase, indole, MR–VP, urease, citrate, oxidase and sugar fermentation. In this investigation, the results indicated that hand-dug wells contained; *Bacillus subtilis, Klebsiella spp., Escherichia coli, Staphylococcus spp, Shigella spp.* and *Pseudomonas aeruginosa,* rivers had a low pH (<5.99) and contained; *Klebsiella spp., Shigella spp., Bacillus subtilis, Escherichia coli, Staphylococcus spp., Salmonella spp.,* and *Yersinia enterocolitica,* while in the boreholes; *Klebsiella spp., Shigella spp., Bacillus subtilis, Staphylococcus spp.* and *Salmonella spp* were detected. These water resources disclosed the presence of pathogenic bacteria at unacceptable levels according to the WHO drinking water guidelines of zero CFU/ml. Hand-dug wells revealed a total bacterial count ranging from 2.00 x 10^3 to 7.50 x 10^3 CFU/ml, boreholes had from zero to 4.92 x 10^2 CFU/ml, and rivers ranged from

1.25×10^4 to 5.83×10^4 CFU/ml. The presence of increased microbial loads in hand-dug wells compared to rivers translates into poor hygiene, safety measures and poor construction of hand-dug wells.

In a study by Boamah *et al.* (2011) on microbial quality of household water sources and incidences of diarrhoea in three peri-urban communities in Kumasi (Ghana), hand-dug wells were found to be susceptible to high levels of contamination than boreholes which is in agreement with Zvidzai *et al.* (2006). Hand-dug wells experience easy contamination mostly influenced by activities that occur on the surface and these include; animals such as rodents and livestock that can drink from water troughs usually placed near the wells especially when the well lacks a protection zone, contaminated water can enter the wells through floods that overtops the well cover. In the study by Boamah *et al.* (2011), faecal *Streptococci* were the most isolated compared to *Escherichia coli*, *Salmonella*, *Enterobacter sakazakii*, *Enterobacter cloacae*, and *Serratia marcescens* due to the high persistence and resistance to natural pressures and treatment of faecal *Streptococci* in water environments. Faecal *Streptococci* have been reported to possess a survival rate similar to enteric viruses compared to other coliforms (Cohen *et al.*, 1973; Fulazzaky *et al.*, 2010). The study showed a link between the number of diarrhoeal cases reported to the level of water contamination and that the major source of contamination was by livestock and pit latrines constructed in close proximity to the hand-dug wells. The isolated organisms indicated faecal contamination by humans or animals or both and could also signal the possible presence of protozoa and helminths (Boamah *et al.*, 2011).

In a microbial water quality study involving the evaluation of hand-dug wells in Ibadan, Oyo state, Nigeria in 2013, Ayantobo *et al.* (2013) observed a high amount of total coliforms in the hand-dug wells that were constructed close to possible sources of contamination (domestic refuse waste, abattoir, pit latrine, stagnant water and drainages) and a low contamination in hand-dug wells far from these sources. This study also indicated the importance of protecting the wells because protected hand-dug wells showed an improved water quality as compared to partially protected and non-protected ones. Odeyemi *et al.* (2012) isolated bacteria from hand-dug well water and a flowing stream in which they analysed the microbiological and physicochemical quality of 10 water samples from hand-dug wells near Omisanjana stream comparable to the quality of the stream in Nigeria. Their results indicated that the total bacteria and coliform counts of the water samples from hand-dug wells ranged between 2.80 x 10^3 – 6.56 x 10^4 CFU/ml and 0.3 x 10^3 – 5.9 x 10^4 CFU/ml and water samples from the stream showed comparable values of 3.0 x 10^4 CFU/ml and 2.45 x 10^4 CFU/ml for total bacteria and coliform counts respectively. A total of 10^6 bacteria were isolated from the hand-dug wells while only 40 were isolated from the stream. These bacteria were characterized and grouped into eleven different genera as follows; *Acinetobacter* spp., *Flavobacterium* spp., *Bacillus* spp., *Proteus* spp., *Klebsiella* spp. and *Shigella* spp. In addition, they further screened for susceptibility of bacteria to various antibiotics commonly used in the community and found that most of the gram-negative bacteria isolated exhibited resistance in the range of three to eight antibiotics. This suggests the need to treat water that is obtained from hand-dug wells in order to ensure its safety.

Abinah (2013) assessed the water quality of a river (Asuotia) and six hand-dug wells and the findings showed the occurrence of high microbial indicator counts which is unacceptable in drinking water (WHO, 2011) as the recommended guideline limit is zero count of coliform bacteria per 100 ml sample of drinking water. Mean total coliform values of between 2107.00 ± 241.70 CFU to 26184.00 ± 447.06 CFU per 100 ml and 158.30 ± 10.83 CFU to 1689.00 ± 151.10 CFU were obtained for river and hand-dug well samples respectively. From which faecal coliform counts of 217.00 ± 23.76 and *E. coli* counts of 32.88 ± 3.89 CFU per 100 ml sample of hand-dug well water were obtained. Most microbial infections are associated with the ingestion of contaminated water especially that with faecal matter from either human or animal. Faeces are a potential source of pathogenic bacteria, viruses, protozoa and helminths (WHO, 2008). However, bacterial infections can also be transmitted through contact with water (bathing) and inhalation (aerosols), and this presents a public health concern depending on the disease severity associated with the particular pathogen, their infectivity and the population at risk. In addition, there is diversity in the bacteria transmitted through drinking water due to factors such as; animal and human population density, water treatment strategies and medical intervention, waste water management, and the emergence of new pathogens and mutants as a result of selective pressures (WHO, 2008).

Immunity plays a role in the infection of an individual and varies considerably. Infection is acquired by direct contact with a pathogen or transmission from person to person and vector to person in the case of communicable diseases and can be influenced by factors such as age, sex, state of health and living conditions. Water plays a role in the transmission of pathogens by faecal–oral route in addition to contaminated food, hands, utensils and clothing, and poor domestic sanitation and hygiene (WHO, 2011). Hence it is necessary to monitor and improve the quality and availability of water in general hygiene and excreta disposal (WHO, 2011).

WHO (2011) argued that several pathogens progressively lose viability and pathogenicity at an exponential rate when they leave their host's body making them become undetectable after a certain period. The lack of detection is attributable to the fact that culturing techniques are the routine way of testing for the presence of pathogens in water. Pathogens with minimal persistence are the most vulnerable and their survival depends on infecting new hosts, and are least potently transmitted through drinking water but rather through other means such as person-to-person contact. Several factors, among which temperature is the most important influence the persistence of bacteria in water environments. Higher temperatures acting on the water accompanied by ultra-violet radiation in sunlight have been implicated to be the reason for rapid decay of bacteria found in water (WHO, 2011). Water may harbour conditions that promote the growth of bacteria. Water that contains high amounts of decomposed organic carbon, warm temperature (on the surfaces) and low concentrations of chlorine supports most bacterial species growth not limited to *Legionella*, *Vibrio cholerae*, *Naegleria fowleri*, and *Acanthamoeba*. However, bacteria such as human normal flora that rely on particular hosts to complete their life cycles are deprived of proliferation. Pathogens commonly known to be transmitted through water mostly infect the gastrointestinal tract and are excreted in the faeces of infected humans and animals (WHO, 2011).

Acinetobacter species are gram-negative, oxidase negative, none motile coccobacilli. These species are also referred to as *Acinetobacter calcoaceticus baumannii* complex in some classification schemes to cover all subgroups of this species, such as *A. baumannii*, *A. iwoffii* and *A. junii* (WHO, 2011). The *Acinetobacter* species are known to be commensal organisms, but may be opportunistic pathogens in immunocompromised individuals in which they predominantly cause pneumonia, secondary meningitis, urinary tract infections, bacteraemia, and wound infections. *Acinetobacter* infections mostly occur in people experiencing burns, surgery, infants and old individuals. These species are ubiquitously found in soil, water and sewage environments (Bartram *et al.,* 2003). WHO (2011) argued that *Acinetobacter* has been isolated from 97% of natural surface water samples in numbers of up to 100 CFU/ml. The high numbers support the evidence that these bacteria are abundantly distributed. Furthermore, a study of untreated groundwater supplies in the USA revealed the presence of *Acinetobacter* species in 38% of the groundwater supplies at an arithmetic mean density of 8/100 ml (Bartram *et al.,* 2003). Despite the detection of *Acinetobacter* species in drinking water, there is a lack of evidence linking their detection to clinical disease. Thermotolerant coliforms such as *E. coli* cannot be used as an index for the presence/absence of *Acinetobacter* species due to their ubiquitous distribution.

Aeromonas species are gram-negative, none spore forming, facultative anaerobic bacilli belonging to the family Vibrionaceae. The Vibrionaceae family is similar to the Enterobacteriaceae and is composed of two groups namely the psychrophilic none motile and the mesophilic motile. Psychrophilic none motile aeromonads comprise of only one species, *A. salmonicida* (an obligate fish pathogen) while mesophilic motile aeromonads are potential human pathogens and are composed of *A. hydrophila*, *A. caviae, A. veronii* subsp. *sobria*, *A. jandaei*, *A. veronii* and *A. schubertii* (Bartram *et al.,* 2003). *Aeromonas* species are extensively distributed in fresh water, soil, and food, not limited to meat and milk. These species have been reported to infect humans resulting in septicaemia which may develop from aeromonads present in the gastrointestinal tract, and respiratory tract infections especially in immunocompromised patients (Bartram *et al.,* 2003). *Aeromonas* species growth in water is associated with organic content, temperature, and the presence of residual chlorine. These species are usually detected in fresh waters but are also found in the soil. However, the species found in water have been described to possess different DNA homology groups compared to those associated with cases of gastroenteritis. In addition, thermotolerant coliforms cannot be used as an index for the presence/absence of *Aeromonas* species because they are ubiquitous and autochthonous in aquatic environments (Igbinosa *et al.*, 2012).

Bacillus species are gram-positive, encapsulated bacilli that are strictly aerobic or facultative anaerobic. These species have the ability to produce spores that are highly resistant to unfavourable conditions. *Bacillus* species are categorized into the subgroups *B. polymyxa*, *B. subtilis* (which includes *B. cereus* and *B. licheniformis*), *B. brevis* and *B. anthracis* (WHO, 2008). Most *Bacillus* species are not harmful leaving a few pathogenic to both humans and animals such as *Bacillus cereus* which causes food poisoning that's mostly accompanied by vomiting within one to five hours of ingestion or diarrhoea within 10 – 15 hours, and bacteraemia in immunocompromised patients. These species have been isolated from soil and water, and are readily detected in most drinking water supplies owing to the formation of spores and the resistance of spores to disinfection processes. *Bacillus cereus* is known to cause disease through ingestion of the organisms or toxins produced by the organisms. However, drinking water is not known to be a source of infection of pathogenic *Bacillus* species such as *Bacillus cereus*, and transmission of *Bacillus* gastroenteritis via water is yet to be established (WHO, 2008). Thermotolerant coliforms or *E. coli* cannot be used as an index for the presence/absence of *Bacillus* species because they form spores which tend to be resistant to detection and disinfection processes (WHO, 2008).

Burkholderia pseudomallei is a gram-negative bacillus found in natural environments such as soil and muddy water. These species are prevalent in tropical regions such as northern Australia and southeast Asia (Currie, 2000; Currie *et al.*, 2001). *Burkholderia pseudomallei* have the ability to endure a none nutrient water environment for lengthy periods, and are acid tolerant. These species are known to cause melioidosis, and a fatal form of pneumonia. Melioidosis is prevalent in northern Australia and various tropical regions and is capable of developing into community acquired pneumonia or severe septicaemic pneumonia. Further complications from *Burkholderia pseudomallei* infections include skin abscesses and ulcers, abscesses in internal organs and unusual neurological illnesses not limited to brainstem encephalitis and acute paraplegia (WHO, 2011). Various groups of people ranging from healthy children, adults, and immunocompromised people are susceptible to melioidosis (Inglis *et al.,* 2000). *Burkholderia pseudomallei* infections are transmitted through drinking water although the concentrations needed for infection are unknown, inhalation, and skin contact with cuts or bruises. Thermotolerant coliforms or *E. coli* are not appropriate for use as index for the presence/absence of *Burkholderia pseudomallei* owing to its ubiquitous existence in the environment (WHO, 2008).

Campylobacter species are gram-negative curved spiral rod, microaerophilic and capnophilic bacteria. These species contain a single unsheathed polar flagellum and are one of the main causes of acute gastroenteritis globally. Acute diarrhoeal disease is reported to be mainly caused by *Campylobacter jejuni* as evidenced by isolated species from patients with acute diarrhoeal disease, while *Campylobacter coli*, *Campylobacter laridis* and *Campylobacter fetus* are reported in a small number of cases (Frost, 2001). Unlike other bacteria, *C. jejuni* is highly pathogenic with an increased infectivity even at low bacterial counts such as 1000 organisms (WHO, 2011).

Infection by this organism leads to abdominal pain, diarrhoea, reactive arthritis, meningitis, vomiting, chills and fever. Furthermore, WHO (2011) also indicated that *C. jejuni* has been implicated to be an associated pathogen of acute demyelinating disease of the peripheral nerves called Guillain-Barré syndrome. *Campylobacter* species have been detected in different environments including water, and inhabiting; wild and domestic animals, poultry, wild birds and cattle (WHO, 2011). This organism is transmitted through ingestion of animal and poultry products, and unclean drinking water. Since *Campylobacter* species are faecally borne pathogens and are susceptible to decontamination, *E. coli* is an appropriate indicator for the presence/absence of *Campylobacter* species in sources of drinking water (WHO, 2008).

Enterobacter sakazakii is a gram-negative motile rod shaped bacterium. These species are none spore forming bacterium described to be contaminants of infant formulas. *Enterobacter* species are distinguished from *Klebsiella* on the basis of ornithine positivity, but share similar biochemical characteristics (WHO, 2011). In comparison to the Enterobacteriaceae family, *Enterobacter sakazakii* are reported to be more resistant to osmotic and dry stress. Infection by *Enterobacter sakazakii* results in sepsis, enterocolitis, meningitis, cerebritis and necrotizing, and is mostly detected in low birth weight infants and prematurely born babies (WHO, 2011). There is lack of evidence that *E. sakazakii* are transmitted through drinking water since it has not been detected in most water, soil, mud, and bird faeces, but it’s presence in contaminated water cannot be ruled out (WHO, 2011). However, this bacterium is mostly detected in infant formula milk, probably due to contamination during the production process. Since the detection of *E. sakazakii* is linked to products made from cow milk (WHO, 2008; Casalinuovo *et al.*, 2014), perhaps cows are the source of *E. sakazakii* which tends to be persistent and undetected throughout the production process.

Escherichia coli is an intestinal normal flora of humans and animals, and exists in vast numbers and is ubiquitous in nature. *E. coli* has the ability to cause severe infections in other parts of the body including urinary tract infections, bacteraemia and meningitis (O'Connor, 2002). Some enteropathogenic strains have been implicated to cause acute diarrhoea and have been identified on the basis of different virulence factors, including enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) as described by Nataro (1998) and WHO (2011). *E. coli* O157:H7 and *E. coli* O111 are serotypes of EHEC that are known to cause various types of diarhoea such as mild, none bloody or highly bloody diarhoea that cannot be differentiated from haemorrhagic colitis. About two percent to seven percent of diseased individuals can progress into the potentially lethal haemolytic uraemic syndrome (HUS) leading to acute renal failure and haemolytic anaemia which mostly affects children under five years old (WHO, 2008).

EAEC and DAEC strains are less documented and the pathogenicity and prevalence are relatively unknown. The strains of EHEC are the most pathogenic and have the ability to induce disease even at low numbers such as 100 organisms. ETEC is known to produce heat labile or heat stable *E. coli* enterotoxin, or both toxins at the same time making it one of the main causes of diarrhoea affecting infants in developing countries (O'Connor, 2002). Infection with ETEC mainly presents with mild watery diarrhoea, abdominal cramps, nausea and headache while EPEC infections mainly presents with fatal chronic, none bloody diarrhoea, vomiting and fever in infants and are most commonly in developing countries (O'Connor, 2002). EIEC infections present with watery and rarely bloody diarrhoea and these bacterial strains have a similar pathogenic mechanism to that of *Shigella* in their attack on colon cells. Humans serve as a reservoir of EPEC, ETEC and EIEC strains, while livestock, such as cattle, sheep, and in reduced amounts goats, pigs and chickens are the main source of EHEC strains (O'Connor, 2002). However, these enteropathogenic strains of *E. coli* have also been detected in different water environments and documented to be water borne transmissible. Routine *E. coli* testing is a suitable index for the enteropathogenic strains of *E. coli* since there is no evidence suggestive that water treatment, response to treatment and decontamination of enteropathogenic strains of *E. coli* or other *E. coli* vary (O'Connor, 2002).

Helicobacter pylori was previously known as *Campylobacter pylori*. This is a gram-negative microaerophilic spirally shaped motile bacterium (WHO, 2008). *H. pylori* is a distinguished human pathogen among the 14 known species of *Helicobacter*. *Helicobacter pylori* is a resident of the stomach and is linked to chronic gastritis. Chronic gastritis is implicated in the development of complex conditions like peptic and duodenal ulcer disease and gastric cancer. Chronic gastritis resulting from *H. pylori* infections mostly occur in childhood due to lack of treatment and this is mostly problematic in developing countries. *H. pylori* has been detected in water, and domestic cats although humans seem to be the primary host (Mazari-Hiriart *et al.*, 2001). WHO (2008) disclosed that the evidence that *H. pylori* is sensitive to bile salts suggest that it should not be present in faecal excretion although it has been detected in faeces of young children, and surface water and shallow groundwater samples. Furthermore, it was revealed that *H. pylori* is unlikely to grow in water, but has the ability to survive for three weeks in biofilms and up to 20 – 30 days in surface waters (Mazari-Hiriart *et al.*, 2001; WHO, 2008). For these reasons, *E. coli* or thermotolerant coliforms do not serve as suitable index for the presence/absence of *H. pylori* although water has been described to be a potential source of *H. pylori* infection.

Klebsiella species are gram-negative none motile bacilli belonging to the Enterobacteriaceae family (WHO, 2008). *K. pneumoniae*, *K. oxytoca*, *K. planticola* and *K. terrigena* are all members of the *Klebsiella* genus. *Klebsiella* species are distinguished in morphology from other family members by possession of an outermost layer comprised of a large polysaccharide capsule. *K. pneumonia* is the most isolated *Klebsiella* species from about 60% - 80% faecal and clinical specimens and indicate a positive thermotolerant coliform test (Ainsworth, 2004). *Klebsiella oxytoca* and *Klebsiella pneumonia* have been identified as pathogens capable of inducing destructive pneumonia and are common in colonizing hospital patients, patients with impaired immune systems (old aged or very young), patients with burns or excessive wounds, and those undergoing immunosuppressive therapy or those with HIV/AIDS infection (Ainsworth, 2004). *Klebsiella* species are usual residents of various water environments and have the capacity to proliferate in nutrient rich waters (WHO, 2008). These species serve as indicators of faecal contamination because they are excreted in the faeces of many healthy humans and animals making them easily detected in sewage polluted water (Ainsworth, 2004). Hence, routine total coliform tests can be used to detect *Klebsiella* since it is a coliform organism.

Legionellae are gram-negative rod shaped non spore forming bacteria that require L-cysteine for growth and primary isolation (WHO, 2008). These species are at least 42 in number and are under the *Legionella* genus and Legionellaceae family. *Legionella* species inhabit a variety of water environments such as rivers in fairly low numbers and have the ability to proliferate at temperatures exceeding 25° C (WHO, 2008). These bacteria tend to make use of the warm temperatures and nutrients found in water for their growth and multiplication. *Legionella* genus grow in both piped and un-piped water distribution systems. All *Legionella* species are described as a potential threat to human health and among them, *L. pneumophila* is the main pathogen responsible for waterborne infections termed legionellosis and exists in two clinical forms known are Legionnaire's disease and Pontiac fever (WHO, 2008). Pontiac fever is a pneumonic illness in which males are more susceptible than females and the most affected age range is 40 – 70 years' age group (WHO, 2008).

WHO (2011) argued that Legionellae can be ingested by trophozoites of certain amoebae such as *Acanthamoeba*, *Hartmanella* and *Naegleria*, and this is implicated in their extensive survival periods in water environments. These bacteria can also be acquired through the inhalation of water droplets (aerosols). The old, infants, patients with burns or wounds, and those subjected to immunosuppressive therapy or those with acquired immunodeficiency syndrome (AIDS) are more susceptible to such infections. Some bacteria species such as *Pseudomonas aeruginosa,* and members of *Flavobacterium, Acinetobacter, Klebsiella, Serratia*, *Aeromonas* and *Mycobacteria* (none tuberculous) have the potential to infect the skin and the mucous membranes of the eye, ear, nose and throat even when consumed at low but adequate amounts in the water (WHO, 2008). Since *Legionella* species display extensive survival periods in water and cannot be detected by HPC techniques*, E. coli* or thermotolerant coliforms are not an appropriate index for the presence/absence of *Legionella* species (WHO, 2008).

Leptospires are aerobic spirochetes composed of two genera namely *Leptospira*, which includes the pathogenic *L. interrogans*, and *Leptonoma* (Bharti *et al.,* 2003). These species are mostly housed in host animals but have the ability to survive several days in water. *Leptospira interrogans* is known to be a zoonotic pathogen that causes the disease leptospirosis. WHO (2008) revealed that about 200 pathogenic serovars have been identified and categorized into 25 serogroups according to their serologic relatedness. Leptospirosis is widespread worldwide in temperate and tropical climates found in both rural and urban areas. Leptospirosis clinical complications include fever, headache, muscle pain, chills, redness in the eyes, abdominal pain, jaundice, haemorrhages in skin and mucous membranes (including pulmonary bleeding), vomiting, diarrhoea and rash (WHO, 2003; Pond, 2005).

WHO (2008) further revealed that Weil's disease, which manifests with conditions of jaundice, renal failure, haemorrhage and myocarditis is another term for leptospirosis, although it represents a subset of the manifestations. Various *Leptospira* serovars have been described to inhabit different hosts for example; rats are a reservoir for *Leptospira interrogans* serovars icterohaemorrhagiae and copenhageni, cattle are the main reservoir for serovar hardjo, and field mice (*Microtus arvalis*) and muskrats (*Ondatra zibethicus*) are the main reservoirs for serovar grippotyphosa, house mice (*Crocidura russula*) is a suggested reservoir for serovar mozdok type three (WHO, 2011). Water that is polluted with urine and tissues of diseased animals is a well-known mode of infection by pathogenic leptospires. These species have a high susceptibility to adverse environmental conditions such as low pH, desiccation, and direct sunlight. Since leptospires inhabit water for lengthy periods due to their persistence in favourable conditions, *E. coli* or thermotolerant coliforms are not an appropriate index for the presence/absence of leptospires (WHO, 2008).

Mycobacterium are divided into two groups namely; typical *Mycobacterium* tuberculous species and atypical *Mycobacterium* species (WHO, 2008). Typical *Mycobacterium* tuberculous species include *M. tuberculosis, M. bovis, M. africanum* and *M. leprae* (WHO, 2008). These species are not transmitted through water and solely inhabit humans or animals (WHO, 2008). However, atypical *Mycobacterium* species have the ability to occupy water environments and the species include *M. gordonae*, *M. kansasii*, *M. marinum*, *M. scrofulaceum*, *M. xenopi*, *M. intracellulare*, *M. avium*, *M. chelonae* and *M. fortuitum* (WHO, 2008), all of which have not been documented to inhabit any water resource in Namibia. Atypical *Mycobacterium* species are rod shaped aerobic acid fast bacteria with the ability to proliferate at a relatively slow rate in optimum water environments (WHO, 2008). *Mycobacterium* species are distinguished from other bacteria in that they have a cell wall with high lipid content making them easily identified using acid fast staining. Atypical *Mycobacterium* species are known to cause disease not limited to pulmonary disease, Buruli ulcers, osteomyelitis and septic arthritis, and these conditions are exacerbated in immunocompromised patients and mostly cause death in HIV positive persons. Atypical *Mycobacteria* are relatively resistant to disinfection and are not detected by HPC techniques (WHO, 2008). Hence *E. coli* or thermotolerant coliforms do not serve as an appropriate index for the presence/absence of *Mycobacterium* species.

Pseudomonas aeruginosa are aerobic gram-negative rod shaped polar flagellated bacteria. These bacteria are members of the family Pseudomonadaceae. *Pseudomonas aeruginosa* is widely distributed in a variety of environments such as faeces, soil, water and sewage. *Pseudomonas aeruginosa* produces pyocyanin when cultivated on appropriate media which is a none fluorescent bluish pigment. *Pseudomonas aeruginosa* is known to cause infections mostly in immunocompromised individuals, and cystic fibrosis patients leading to pulmonary complications (de Victorica and Galván, 2001). This bacterium thrives on ulcerations, burns and surgical wounds, as well as the respiratory tract of individuals with underlying disease and physically damaged eyes (WHO, 2008). *P. aeruginosa* causes various diseases including septicaemia, meningitis, water related folliculitis and ear infections. In addition, this organism is linked to a change in odour, turbidity and taste of water, and there is lack of evidence that normal uses of drinking water supplies are a source of infection in the general population (Bartram, 2003). Since *Pseudomonas aeruginosa* is ubiquitously distributed in the environment, *E. coli* or thermotolerant coliforms are not suitable indicator organisms for the presence/absence of *Pseudomonas aeruginosa* (WHO, 2008).

Salmonella species are motile gram-negative bacilli belonging to the Enterobacteriaceae family and are widely distributed in the environment. These species are unable to ferment lactose but are capable of producing hydrogen sulphide or gas from carbohydrate fermentation. *Salmonella* species are classified into; *Salmonella enterica* or *Salmonella choleraesuis, Salmonella bongori* and *Salmonella typhi* (WHO, 2008). *S. enterica* consists of the entire of enteric pathogens except *S. typhi.* Clinical presentations of *Salmonella* infections include gastroenteritis, bacteraemia or septicaemia, typhoid fever / enteric fever and a carrier state in persons with previous infections (Escartin, 2002). On the basis of enteric illness, *Salmonella* species are categorised into typhoidal species/serovars which are *Salmonella typhi* and *S. paratyphi*, and the rest are none typhoidal species/serovars (WHO, 2011). Some *Salmonella* species demonstrate host specificity such as *S. typhi* and *S. paratyphi* which are restricted to humans and in some cases *S. paratyphi* is present in livestock (WHO, 2008). Humans and various animals such as poultry, cows, pigs, sheep, birds and reptiles are susceptible to infection by *S. typhimurium* and *S. enteritidis. Salmonella* species are transmitted through the faecal oral route especially typhoid species through the consumption of unclean water or food while non-typhoid species are mostly spread by direct person to person contact (WHO, 2008). *S. typhimurium* has been linked to the consumption of contaminated groundwater and surface water supplies. Although *Salmonella* species are widely distributed, they are sensitive to disinfection which makes thermotolerant coliforms or *E. coli* a suitable index for the presence/absence of *Salmonella* species in drinking water supplies (WHO, 2008).

Shigella species are gram-negative rod shaped bacteria that are unable to form spores, and belong to the Enterobacteriaceae family (Alamanos *et al.,* 2000). These bacteria are none motile and able to grow in the presence or absence of oxygen and are classified based on their somatic O antigens which are also found in other enteric bacilli such as *E. coli*. *Shigella* are divided into four species namely *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* (Alamanos *et al.,* 2000). *Shigella* species are known to cause severe intestinal diseases, such as bacillary dysentery which mostly occurs in children under 10 years of age. *Shigella* species have a high infectivity, and thus consumption of a few organisms ranging from zero to 100 can induce disease (Alamanos *et al.,* 2000). Of all the species, *S. sonnei* has been reported to cause less severe disease and is self-limiting while *S. dysenteriae* causes severe disease and is known to produce Shiga toxin that induces ulcerations (WHO, 2011). *Shigella* species are mostly transmitted by the faecal oral route, person to person contact, and contaminated food and water. These species cannot survive for long periods in water and their detection in water indicates recent human faecal pollution since *Shigella* species seem to only inhabit humans and other higher primates (WHO, 2008). For this reason, *E. coli* or thermotolerant coliforms is an appropriate index for the presence/absence of *Shigella* species in drinking water supplies (Alamanos *et al.*, 2000).

There are 15 species contained in the *Staphylococcus* genus, humans are susceptible to infection by *S. aureus*, *S. epidermidis* and *S. saprophyticus* (Antai, 1987; WHO, 2008). *S. aureus* is among the human microflora but has the ability to induce disease and illness such as gastrointestinal disease due to heat-stable *Staphylococcal* enterotoxin, boils, skin sepsis, post-operative wound infections, enteric infections, septicaemia, endocarditis, osteomyelitis and pneumonia (WHO, 2008). *Staphylococcus aureus* is a gram-positive aerobic or anaerobic none spore forming coccus bacterium (WHO, 2008). These species are none motile and display a catalase and coagulase positive test. *Staphylococcus aureus* is widely distributed in the environment but mostly inhabits the skin and mucous membranes of animals (WHO, 2008). In addition, it is also transmitted from person to person through hand contact, and to the water through human contact with various water bodies. Since *S. aureus* is widely distributed and its period of survival in water is unknown, *E. coli* or thermotolerant coliforms is not an appropriate index for the presence/absence of *S. aureus* in drinking water supplies (WHO, 2008).

Tsukamurella species are gram-positive rod shaped obligate aerobic bacterium. These species belong to the Nocardiaceae family and are none motile acid fast positive, and are *Actinomycetes* related to *Rhodococcus*, *Nocardia* and *Mycobacterium* (WHO, 2008). *Tsukamurella* species are widely spread in the environment especially in soil and water. *Tsukamurella* species cause disease such as necrotizing tenosynovitis, bone infections, meningitis, peritonitis, bacteraemia, chronic lung diseases, immune suppression (leukaemia, tumours, HIV/AIDS infection) and post-operative wound infections mostly in immunocompromised people (Kattar *et al.*, 2001). However, it is not yet established that the organisms in water causes illness. Since *Tsukamurella* is ubiquitously distributed in the environmental, *E. coli* or thermotolerant coliforms are not an appropriate index for *Tsukamurella* species.

Vibrio species are gram-negative bacteria with a single polar flagellum. These species are characterized according to the O antigens they contain and include pathogenic species such as *V. cholerae, V. parahaemolyticus* and *V. vulnificus*. However, of the three species, *Vibrio cholerae* is the only one that is associated with contaminated freshwater environments and is known to cause diarrhoea. Serovars O1 and O139 mostly possess the virulence factors causing cholera and produce an enterotoxin (cholera toxin) which disturbs ionic balance across the intestinal mucosa resulting in severe loss of water, electrolytes and dehydration (Kaper *et al.*, 1995; WHO, 2008). However, some strains of *V. cholerae* that are none toxigenic can result in self-limiting gastroenteritis, wound infections and bacteraemia (Kaper *et al.*, 1995). According to WHO (2011), none toxigenic *V. cholerae* is widely distributed in water environments compared to toxigenic *V. cholerae.* Humans are reported to be a source of toxigenic *V. cholerae* and the incidences are described to decrease with decreasing water temperatures especially below 20° C (WHO, 2002). Polluted water is a common cause of cholera and is typically transmitted by the faecal oral route and infection mainly occurs by ingestion of polluted water and food but high numbers of the organism are required for infection to occur. *E. coli* or thermotolerant coliforms are not an appropriate index for the presence or absence of *V. cholerae* in drinking water because *Vibrio cholerae* O1 and none O1 are detectable in the absence of *E. coli* (WHO, 2008).

Yersinia species are gram-negative rods that are motile at 25° C but not at 37° C (WHO, 2008). These species belong to the Enterobacteriaceae family and consists of seven species some of which are *Y. pestis*, *Y. pseudotuberculosis* and certain serotypes of *Y. enterocolitica* which are human pathogens. *Yersinia pestis* is known to cause bubonic plague which results from contact with rodents and their fleas while *Yersinia enterocolitica* causes ulcerations of the intestinal mucosa after invading the cells of the ilium and presents with acute gastroenteritis, diarrhoea, fever, enlarged painful lymph nodes and abdominal pain (Waage *et al.*, 1999). *Yersinia* species have been described to inhabit water, domestic and wild animals. It is established that *Y. enterocolitica* and *Y. pseudotuberculosis* mostly inhabits water and is transmitted to humans through faecal oral route mainly by consumption of contaminated drinking water. Humans and animals are a source of pathogenic *Yersinia* species with pigs being the major reservoir of pathogenic *Y. enterocolitica* while rodents and small animals are the major reservoir of *Y. pseudotuberculosis* (WHO, 2008). WHO (2008) argued that most *Y. enterocolitica* strains found in water do not cause disease and are widely distributed in the environment. Since some *Yersinia* species have the capacity to proliferate in water and are able to survive long periods especially in the presence of nitrogen, *E.*

coli or thermotolerant coliforms are not a suitable index for the presence/absence of *Yersinia* species in water sources (WHO, 2008).

Most of the bacterial species associated with water described above have not been documented to contaminate drinking water in Namibia. The only study documented on microbiological water quality assessment is by McBenedict *et al.* (2017), who found *Bacillus aerophilus*, *Bacillus amyloliquefaciens*, *Bacillus aquimaris*, *Bacillus aryabhattai*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus safensis*, *Bacillus samanii*, *Bacillus stratophericus*, *Bacillus subtilis*, *Pseudomonas mendocina*, *Staphylococcus haemolyticus* and *Streptomyces celuloflavus* in hand-dug wells of the Cuvelai Etosha Basin. *Bacillus* species were the most isolated in these hand-dug wells, while none of the indicator organisms were detected confirming that the absence of indicator organisms does not assert that water is safe for drinking.

It is sufficing to state that the WHO documented list of water borne pathogens is not comprehensive due to lack of widespread research on water pathogens using highly specific and effective techniques such as Metagenomics. Hence, there is need to conduct more microbial research in water in order to reveal the vast microbial life forms and their interactions. This study comprehensively disclosed the diversity of bacteria inhabiting the hand-dug wells in the Cuvelai Etosha Basin and adds on to the known pathogens for which water is a mode of transmission.

2.2 Methods used to investigate bacteria in water

A. Culture-dependent analyses of microbial communities

Bacterial culturing enables the characterization of properties and the prediction of contributions of individual populations to the environment. However, molecular studies have revealed that 99% of the bacteria in nature have under no circumstances been cultured (Madigan *et al.*, 2015). Culturing methods that aim at isolating pure cultures of particular microbial species have since been established.

A1. Enrichment techniques

Enrichment cultures are cultures that are prepared using a medium and appropriate incubation conditions that favour the organism of interest and inhibit the undesired organisms (Madigan *et al.,* 2015). The success of a bacterial enrichment culture is dependent on its ability to provide a favourable environment that supports the growth of desired bacterium that is similar to the natural environment in which these bacteria are found. A sample having the bacteria of interest is obtained from the environment, placed on the selective media and incubated at particular conditions that support growth. Resources (nutrients) and conditions are a key component to the successful growth of the bacteria of interest because inaccurate resources or conditions yield no growth (Madigan *et al.,* 2015). The use of bacterial enrichment cultures has a limitation because absence of growth does not mean the bacteria of interest is absent but a positive growth confirms the presence (a firm positive is possible but not a firm negative) (Madigan *et al.,* 2015). In addition, the ecological function and abundance of the bacteria in its habitat cannot be determined using the enrichment culture technique because more than 99% of bacteria observed through microscopy in any environment are not cultivable (Rastogi and Sani, 2011).

A2. Bacterial culturing and isolation

Bacteria are grown in laboratory nutrient solutions called culture mediums (Madigan *et al.,* 2015). There are two broad types of media; defined media and complex media. Culture media enables the isolation and identification of bacteria, long term storage of pure cultures, and the analysis of microbial metabolic pathways. In addition, bacterial culturing has the advantage of diagnosing infectious diseases, studying bacterial morphology and properties, used in genetic studies and development of serological assays or vaccines, estimating bacterial numbers that are viable, and isolating bacteria in mixtures (Vos *et al.*, 2011).

Bacteria cultures are prepared using media designed for various purposes extending from; general growth media which is non-selective and grows different culturable bacteria, enriched media intended to isolate fastidious bacteria, selective media designed to allow the growth of a single type of bacteria while inhibiting the growth of other types, and differential media used for the visual discrimination between two or more species (Vos *et al.*, 2011). Bacteria can either be isolated by streaking or pour plate method. The initial step involves the preparation of the desired media on petri dishes using manufacturer's protocol. Thereafter, the bacteria are successively serially diluted until the cell density is decreased enough to provide the visualization of single colonies (Madigan *et al.*, 2015). The bacteria are then streaked on the agar and incubated at the appropriate growth conditions, while with pour plate method, the bacterial dilution is added to molten agar and incubated at appropriate growth conditions. Once the colonies have grown, they are isolated to form pure cultures which are then identified in various biochemical tests using the Bergeys manual for bacterial identification (Holt *et al.*, 1994; Vos *et al.*, 2011).

A3. The Winogradsky Column

The Winogradsky column developed by Sergei Winogradsky creates a virtual microbial ecosystem and a long-term source of various bacteria for enrichment cultures (Madigan *et al.*, 2015). Some anaerobes, phototrophic purple and green bacteria, and sulphate-reducing bacteria have been isolated using Winogradsky columns (Madigan *et al.,* 2015). This technique involves placing half volume of mud that is organically rich comprising carbon substrates into a glass cylinder (Madigan *et al.*, 2015). Sulphide-containing mud is rather used for this purpose and the substrates selects for the desired bacterial species (Madigan *et al.*, 2015). Substrates (glucose) that yield acidic conditions are avoided due to potential gas pocket formations that interrupt the enrichment process. Calcium carbonate ($CaCO_3$) is added as a buffer while gypsum ($CaSO_4$) acts as a source of sulphate (Yasa *et al.*, 2006). The mud is compacted in the cylinder to avoid formation of air compartments and then covered with freshwater or marine water. Evaporation is avoided by covering the top of the cylinder upon which the container is positioned close to a window transmitting diffuse sunlight for a period of months (Madigan *et al.,* 2015). A diverse community of microorganisms develops of which Algae and cyanobacteria grow rapidly and occupy the upper section of the water column. Algae and cyanobacteria yield oxygen (O_2) that helps maintain the upper zone with oxygen. Organic acids, alcohols, and hydrogen serve as substrates for sulphate reducing microorganisms and are formed as a result of decomposition (Madigan *et al.*, 2015).

Purple and green sulphur bacteria (anoxygenic phototrophs) that depend on sulphide as a photosynthetic electron donor emerge from the production of hydrogen sulphide (H_2S) from the sulphate reducers (Rogan *et al.*, 2005). The microorganisms mostly grow in biofilms in the mud on the sides of the column and can possibly grow in the water itself if oxygenic phototrophs are scarce. The Winogradsky columns have the ability to successfully isolate both aerobic and anaerobic prokaryotes (Madigan *et al.,* 2015). However, culture techniques suffer the limitation of most rapidly growing organisms dominating for the chosen set of conditions (Madigan *et al.,* 2015). This limitation is circumvented by molecular techniques that have revealed that most cultured fast growing organisms mostly form a minor fraction of the microbial community as opposed to the most abundant and ecologically significant organisms (Madigan *et al.,* 2015). Once an enrichment culture has been developed, a pure culture is then obtained by ways of streak plate, the agar dilution, and liquid dilution (Madigan *et al.,* 2015).

The agar dilution method involves the dilution of a mixed culture into tubes of molten agar medium thereby promoting growth of colonies inserted in the agar (Madigan *et al.,* 2015). Anaerobic organisms (sulphur bacteria and sulphate-reducing bacteria) are successfully isolated using this method. Pure cultures are obtained by repeating the procedure using colonies from the most dilute tube (Madigan *et al.,* 2015). Liquid dilution makes use of serial dilutions of an inoculum until the final tube in the series of dilution reveals no growth. The liquid dilution is also used to estimate the number of viable cells using the most-probable-number (MPN) technique (Sutton, 2010). Selective media and specific conditions can be used in an MPN count to target a particular organism or group of organisms or an MPN can be performed on a general purpose media to get an overview estimate of viable cells (Madigan *et al.,* 2015).

A4. The Laser Tweezers and Flow Cytometry (selective single cell isolation)

Laser tweezers and flow cytometry techniques are also used for obtaining pure cultures. They are essential and effective in isolating slow growing microorganisms that are mostly undetected and dominated by fast growing micro organisms. Laser tweezers contain an inverted light microscope furnished with an infrared laser and a micromanipulation device. Laser tweezers are able to isolate a microorganism because the laser beam exerts a force on the microbial cell and pushes it down (Wang *et al.,* 2005). The force traps the cell making it move whenever the laser beam moves; this enables optical trapping and separation of a single cell especially if a mixed sample is in a capillary tube. The trapped cell is then obtained by breaking the tube at a point between the cell and the contaminants and transferring the cell into sterile medium. Specific microorganisms can be isolated from a culture mixture using laser tweezers when it is combined with staining techniques

(Madigan *et al.,* 2015). Similarly, the flow cytometry technique is used to selectively isolate single cells. It enables counting and exploration of microscopic particles suspended in liquid when they are passed through an electronic detector. Flow cytometers are able to discriminate between cells based on size, shape, or fluorescent properties (Lau *et al.*, 2008).

B. Culture independent microscopic analyses of bacterial Communities

B1. General staining methods

Cell staining is important because it enables evaluation of relative abundances of different species in a habitat. However, staining methods do not reveal the physiology or phylogeny of the cells. General staining methods include; fluorescent staining with dyes that have an affinity for and bind to nucleic acids, viability staining, and fluorescent proteins as cell tags and reporter genes (Madigan *et al.,* 2015).

B1.1 Fluorescent dyes

Fluorescent dyes such as DAPI (4′, 6-diamidino-2-phenylindole), Acridine orange and SYBR Green I can be used to generally stain microorganisms from various microbial habitats. DAPI is widely used in general staining, while SYBR Green I is mostly used for the advantage that it is able to stain viruses inducing fluorescence (Yin *et al.*, 2008). The stains bind to DNA and induce fluorescence when ultraviolet (UV) radiation is introduced. Different stains have different specific ultraviolet (UV) radiation requirements (DAPI absorption maximum is 400 nm; acridine orange's absorption maximum is 500 nm; SYBR Green I's absorption maximum I's 497 nm). The stains make isolation easier due to enhanced visibility. The stains have different colour fluorescence of; blue for DAPI, orange for acridine orange, and green for SYBR Green I (Johnson *et al.*, 2007). However, DNA staining is a nonspecific process and hence is unable to discriminate between different species of microorganisms, and between viable and non-viable cells. In addition, these stains are unable to track species of microorganisms in an environment (Madigan *et al.,* 2015).

B1.2 Viability Staining

Viability staining is able to discriminate between live and dead cells (Madigan *et al.,* 2015). The abundance of microorganisms and their viability can be simultaneously assessed using viability stains. These stains rely on the integrity of the cytoplasmic membrane (Madigan *et al.,* 2015). A pair of dyes are added to the sample to asses both abundance and viability, one dye fluoresces green and the other red. The green fluorescing dye penetrates all cells regardless of the viability state whereas the red dye only penetrates dead cells (Auty *et al.*, 2001). The red dye contains the chemical propidium iodide which enables penetration only in dead cells because their cytoplasmic membrane is no longer intact. The stained cells are then viewed under the microscope to differentiate the live ones stained green and the dead ones stained red (Comer *et al.*, 2013). In order to avoid nonspecific staining of background materials in the case of water samples, filtration is employed and the filters are stained, and examined using a microscope (Madigan *et al.,* 2015).

B2. Fluorescence in Situ Hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a technique in which Microorganisms can be identified and quantified using nucleic acid probes. A nucleic acid probe is a piece of DNA or RNA sequence that is complementary to a sequence in a target gene or RNA and induces hybridization when in contact with the target sequence (Martinez *et al.*, 2013). Fluorescent dyes are added to the nucleic acid probes in order to introduce fluorescence. FISH is also used in a method termed FISH phylogenetic staining in which a fluorescing probe complementary in base sequence to a conserved region sequence such as 16S rRNA and 23S rRNA in prokaryotes or 18S rRNA and 28S rRNA in eukaryotes is employed. Phylogenetic stains are non-destructive as they penetrate cells and hybridize with the target sequence in the ribosomes. The number of ribosomes is then determined by the number of fluorescent probes bound to a cell. FISH can be used in microbial tracking, in combination with DAPI in determination of microbial populations and percentages of each species in a community, in clinical diagnostics and food industry for microscopic detection (Perez *et al*., 2013).

FISH can also be used to investigate gene expression of microorganisms in an environment using a method called CARD-FISH (Madigan *et al.,* 2015). This method is specific to mRNA and differs from the standard FISH techniques because it employs amplification of the signal (fluorescence) owing to its name, catalysed reporter deposition FISH (CARD-FISH). CARD-FISH employs specific nucleic acid probes that possess a molecule of the peroxidase enzyme attached to it instead of a fluorescent dye (Kubota, 2013). Once hybridization has occurred, a fluorescently labelled soluble compound called tyramide is added to serve as a substrate for peroxidase. The cells having the nucleic acid probe convert tyramide with the aid of peroxidase into a very reactive intermediate which covalently binds to adjacent proteins leading to amplification of the signal suitable for microscopy detection (Lefort and Gasol, 2013). A single peroxidase molecule is capable of activating multiple tyramide molecules enabling the detection of low abundance mRNAs. CARD-FISH has proved effective in phylogenetic studies of slow growing prokaryotes due to limited habitat resources and unfavourable conditions thereby bypassing the weak signal limitations of standard FISH (Fakruddin and Mannan, 2013).

B3. ATP Assay

Adenosine triphosphate (ATP) measurements can be used to determine cell viability, cell proliferation and cytotoxicity of various compounds and biological response modifiers (Madigan *et al.*, 2015). The method involves a lysis step in which the ATP degrading enzymes (ATPases) are irreversibly inactivated producing a luminescent signal that corresponds to the endogenous levels of ATP (Vang *et al.*, 2014). Hammes *et al.* (2010) and Vang *et al.* (2014) revealed that ATP is found in drinking water as a microbial ATP fraction from active and viable cells, and a free ATP fraction (Total ATP = microbial ATP + free ATP). Dying cells are thought to be the source of free ATP. The total ATP is determined by adding a lysing agent followed by a luciferin/luciferase reagent while Free ATP is measured by adding only the luciferin/luciferase reagent to the sample without cell lysis (Hammes *et al.*, 2010). The microbial ATP concentration is then determined by the difference between the value of total ATP and free ATP (microbial ATP = total ATP - free ATP). The ATP assay is based on the production of light due to the interaction between ATP and the added luciferase and D-luciferin. The intensity of the light produced is proportional to the amount of ATP inside the cell (Vang *et al.*, 2014).

ATP measuring kits or methods are a potentially effective way of measuring microbial quality of drinking water and have the advantages of providing quick results (van der Wielen and van der Kooij, 2010). It also has a possibility of continuous monitoring of ATP enabling early detection of contamination in drinking water supplies especially in those that distribute non disinfected drinking water (Smeets *et al.*, 2009). In addition, it enables a more accurate measure of the total active biomass in a drinking water sample compared to heterotrophic plate counts with a threshold of less than one percent (Vang *et al.*, 2014) and can be used to assess the efficiency of treatment procedures at waterworks and assessing regrowth of bacteria in treated water (Hammes *et al.*, 2008; Vital *et al.*, 2012; Liu *et al.*, 2013). According to Vang *et al.* (2014), ATP assays present challenges in determination of the extent to which the technique can detect contaminations arising from microbial ingression from wastewater or surface water and the amount of contaminated water capable of raising ATP concentrations. In addition, variations in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can compromise the interpretation of the results (Vang *et al.*, 2014).

C. Culture-independent genetic analyses of bacterial communities

Culture-independent genetic analyses of bacterial communities are increasingly proving effective in ecological studies (Madigan *et al.*, 2015). Genetic based analyses utilize specific genes to investigate metabolic capacity and biodiversity of microorganism (Madigan *et al.*, 2015). The most commonly used techniques of microbial community analysis employ polymerase chain reaction (PCR), DNA fragment analysis by gel electrophoresis (DGGE, T-RFLP, ARISA) or molecular cloning, and DNA sequencing and analysis (Cocolin *et al.*, 2013). Furthermore, genomic techniques are employed to assess the whole genomes and activities of organisms present in an environmental sample (Madigan *et al.*, 2015).

C1. PCR based methods of bacterial community analysis

The PCR technique involves three major steps: (i) A primer pair hybridizes to a complementary sequence of the gene of interest, (ii) DNA polymerase replicates the gene of interest and (3) synthesis of multiple copies of the gene of interest then occurs by repetitive melting of complementary strands and hybridization of primers (Madigan *et al.*, 2015). Bacterial community analysis employs the use of phylogenetically informative genes which are highly conserved because it is possible to amplify these genes using different primers in all organisms regardless of the phylogenetic distance involved (Cocolin *et al.*, 2013). Alternatively, some studies may focus on analysing the ecological function of different species in a community in which case the genes amplified are those that encode enzymes for metabolic functions exclusive to a particular organism or class of species. A bacterial community analysis study can be achieved by the isolation of total DNA/RNA from a microbial habitat using commercially available extraction kits or traditional extraction methods. The kits isolate the entire DNA/RNA from the different microorganisms that were present in the environmental sample and a PCR reaction is performed. Upon successful PCR, the PCR products of different phylotypes are then sorted using one of the three methods: (1) physical separation by gel electrophoresis, (2) clone library construction, and (3) next-generation sequencing technology (Madigan *et al.*, 2015).

C1.1 Denaturing gradient gel electrophoresis (DGGE)

Denaturing gradient gel electrophoresis (DGGE) discriminates between genes of the same size due to their different melting (denaturing) profile based on different base sequences (Madigan *et al.*, 2015). A DNA denaturant (a mixture of urea and formamide) gradient is used in this method. The DNA denaturant melts and stops the migration of a double-stranded DNA fragment through the gel once it reaches a point having sufficient denaturant (Strathdee and Free, 2013). The bands separate based on different denaturing temperatures that are a consequence of different base sequences and this reveals the different phylotypes (of the target gene) present in the sample. The bands are then excised and sequenced to identify the species and infer phylogenetic relationships (Strathdee and Free, 2013).

C1.2 T-RFLP and ARISA

Terminal restriction fragment length polymorphism (T-RFLP) is a method used in microbial community analysis that employs PCR in which one of the primers is end-labelled with a fluorescent dye (Madigan *et al.*, 2015). Restriction enzymes are then used to digest the DNA at specific sequences. The cut pieces of DNA are usually short due to the use of restriction enzymes with recognition sites of only four base pairs (Van Dorst *et al.*, 2014). Multiple DNA fragments with different sizes are consequently generated in which the number of fragments is determined by the amount of restriction sites in the DNA (Madigan *et al.*, 2015). After restriction, gel electrophoresis is performed to separate the fluorescently labelled terminal fragments which are analysed on an automated DNA sequencing machine that identifies fluorescing fragments (terminal dye-labelled) (Madigan *et al.*, 2015). The fluorescing reveals the rRNA gene variation in sequence in the sample from a microbial habitat. Although DGGE and T-RFLP are similar in that they target a single gene, they vary because a DGGE gel reveals the number of same-length sequence variants of a single gene while a T-RFLP gel reveals variants differing in DNA sequence of a single gene due to different fragment sizes arising from restriction enzyme digestion. T-RFLP analysis is advantageous for giving information regarding the diversity and population abundances of a microbial community, the existence or non-existence of a restriction site in a target sequence, the fragment size and the exact sequences flanking the restriction enzyme cut site (Fakruddin and Mannan, 2013). However, this technique is unable to discriminate closely related sequences and consequently generally underestimates the analysis of variations in a microbial habitat (Madigan *et al.*, 2015).

Automated ribosomal intergenic spacer analysis (ARISA) is a technique similar to T-RFLP, but is rather more informative regarding analysis of microbial communities in that it scrutinizes the vicinity of the 16S rRNA and 23S prokaryotic rRNA genes. The 16S rRNA and 23S prokaryotic rRNA genes are separated by a fragment termed the internal transcribed spacer (ITS) region (Madigan *et al.*, 2015). The ITS region size (length) varies among and across species and often varies in length among the multiple rRNA gene operons of a single species (Fakruddin and Mannan, 2013). ARISA employs PCR primers with complementary sequences to conserved sequences that flank the 16S and 23S rRNA genes spacer region. After amplification, ARISA displays banding patterns that are informative in community analysis. Unlike T-RFLP, ARISA does not make use of restriction enzyme digestion. In addition, ARISA is used to investigate microbial community dynamics especially the abundance of particular species in a community and their change over time and space (Ghosh *et al.*, 2015).

C1.3 Clone libraries and Next-Generation Sequencing (NGS)

Clone libraries have been used to separate DNA molecules after amplification on the basis of using each clone with a unique sequence as the template strand in sequence identification. When a target gene such as the 16S rRNA gene is amplified from an environmental sample, the PCR product none denaturing gel electrophoresis shows a single band (Mardis, 2013). This single band contains DNA from multiples different cells and needs to be sorted out prior to sequencing. Sorting can be performed by molecular cloning, DGGE, or by sequencing methods. The introduction of next-generation sequencers has eliminated the need for the cloning step because the DNA fragments are isolated and amplified by the sequencer (Mardis, 2013). Next-generation sequencers simultaneously amplify multiple (thousands) template DNA strands and yields massive sequence reads as compared to sequencing individual clones from a library. The assembly of clone libraries and sequencing are standard useful tools in investigating the phylogenetic diversity of microbial communities and evaluating the species ecological functional. Next-generation sequencing is able to detect both the low abundance and high abundance phylotypes in a sample, thereby eliminating the limitation of missing out minor phylotypes presented by clone libraries (Koboldt *et al.*, 2013). In addition, sequencing technologies have revealed that phylogenetically distinct microorganisms are abundant in nature whose rRNA gene sequences are different from the known laboratory cultures, and current laboratory cultures are unable to grow most of the dominating phylotypes in natural microbial communities (Madigan *et al.,* 2015).

C1.3.1 Microarrays

Microarrays are employed in the analysis of phylogenetic and functional diversity of microbial communities (Madigan *et al.,* 2015). Microarrays specifically created to measure overall gene expression in microorganisms are called DNA chips, whereas those constructed for biodiversity studies are called phylochips (Paul, 2014). Phylochips are able to discriminate between specific groups of microorganisms by targeting genes that encode metabolic processes specific to the respective groups (Madigan *et al.,* 2015). Microarrays are equipped with multiple probes in order to detect a wide coverage of natural diversity and genes encoding functionally comparable enzymes. Phylochips are created by attaching rRNA gene probes or rRNA gene–targeted oligonucleotide probes to the chip surface in a known pattern (Chan *et al.*, 2013) such as construction of the probe to possess oligonucleotides complementary to specific sequences in the 16S rRNA genes of the nitrogen fixing bacteria (Madigan *et al.*, 2015). Phylochips can be general or specific depending on the probes attached to the chips, multiples probes can also be affixed to the chip enabling the detection of thousands of species (Madigan *et al.*, 2015).

After successful total DNA extraction, PCR, and fluorescence labelling of the 16S rRNA genes, the fluorescently labelled PCR products are then hybridized with the probes on the phylochip. Confirmation of the presence of any species is performed by investigating probes that hybridize with sample DNA. In the case of rRNA gene, there is no need to amplify. The rRNA is extracted from the sample, labelled with a fluorescent dye, and hybridized directly to the phylochip (Paul, 2014). The techniques that involve PCR, DGGE, cloning, and sequencing are time consuming.

However, Phylochips and functional gene microarrays such as GeoChip avoid this. In addition, Phylochips are reproducible methods especially when dealing with low-abundance taxa as opposed to the sequencing methods. Nevertheless, gene microarray methods suffer the possibility of nonspecific hybridization due to the high level of sequence similarities between closely related species (Tu *et al.*, 2014). Furthermore, false positives occur when distinct genes possess sequences that are able to hybridize to the probe due to complementarity (Madigan *et al.*, 2015).

C1.3.2 Metagenomics

Metagenomics is a technique that employs the sequencing and analysis of the entire microbial community genomes in order to define and understand the genetic content of the environment in question (Madigan *et al.*, 2015). Metagenomics is also termed environmental genomics owing to its ability to capture and analyse the total DNA of an environment (Sharon and Banfield, 2013). Metagenomics currently utilizes high-through put sequencing of the entire DNA directly from the environment and has eliminated the DNA cloning step involving the inserting of environmental DNA fragments into plasmids to generate clone libraries for sequencing or screening for novel genes (Schloss and Handelsman, 2003). Metagenomics is a sequence-based and functional analysis of the entire microbial genomes from a microbial habitat (Zeyaullah *et al.*, 2009). Metagenomics has the ability to reveal an inclusive measure of genetic diversity, species composition, evolution, and ecological functions of respective species in microbial communities (Simon and Daniel, 2011). Hence, current metagenomics studies screen for entire genes present in a microbial community of interest and this provides information enhancing the understanding of the structure and function of species of the community as opposed to earlier studies that analysed a single-gene. The results of a metagenomics study also show the phylogeny of the organisms corresponding to the genes detected. Algorithms have been developed for the use of metagenomics sequence data assembly: these algorithms have improved the frequent construction and growth of metagenomics databases (Ercolini, 2013).

Genomes assembled from entire environmental DNA sequence reads present challenges of being potentially unlikely to be clonal, but contain DNA sequences of closely related species (Lasken and McLean, 2014). It is also imperative to determine if the genes relevant for the survival of any living organism are present (stable RNAs—tRNAs and rRNAs). This indicates an inclusive assessment of a complete genome. In addition, this investigates the interactions between species in a microbial habitat and how their relative gene abundance changes as they respond to their interactions and environmental changes (Madigan *et al.*, 2015). Madigan *et al.* (2015) argued that an environment that is limited in NH_4^+, NO_3^-, and alternative nitrogen forms will select for nitrogen-fixing bacteria and this can be supported by the detection of multiple genes responsible for nitrogen fixation. Whole genome metagenomics is more informative compared to rRNA gene based community analyses due to detection failures owing the low-sensitivity detection of single gene Metagenomics (Tringe *et al.*, 2005). This is because some sequences present in a sample are possibly not amplified by the PCR primers and minor species are frequently omitted during clone library sequencing. Analysis of microbial communities suggests that more research ought to be performed in order to fully understand the structure and function of microbial communities. Madigan *et al.* (2015) also argued that current technologies are efficient for a thorough analysis of microbial communities yet not a single environment has been sequenced completely.

C1.3.3 Metatranscriptomics and Metaproteomics

Genomics has given rise to two fields termed metatranscriptomics and metaproteomics (Madigan *et al.*, 2015). Metatranscriptomics is also called functional genomics and is similar to metagenomics, but only that it analyses RNA and not DNA (Ishii *et al.*, 2013). Upon successful RNA extraction, the RNA is converted to cDNA by reverse transcription prior to sequencing. Metagenomics and Metatranscriptomics analysis differ in that the functional capacities of the community and the relative abundance of specific genes are revealed by Metagenomics while metatranscriptomics provides information regarding the entire expressed genes in the community and their relative level of expression at a specific time and place (Fakruddin and Mannan, 2013). In addition, metatranscriptomics is employed to investigate metabolic processes occurring in the microbial community at the time of sampling by analysing gene transcript abundance (mRNA) owing to regulation of gene expression in prokaryotes mostly occurring at the transcription stage (Lim *et al.*, 2013).

Metaproteomics is a technique that investigates the immediate catalytic potential of a microbial community or rather the measure of the diversity and abundance of different proteins in a community (Schloss and Handelsman, 2003). Metaproteomics is a more specific measure of functionality because it analyses the proteins as opposed to metatranscriptomics that focuses on mRNAs that have varying half-lives and efficiencies of translation which leads to the production of different protein copy numbers. Metaproteomics is more demanding compared to both Metagenomics and metatranscriptomics because the PCR amplification step and sequencing of protein nucleic acid sequences is not possible thereby making protein identification challenging (Madigan *et al.,* 2015). In addition, protein identification requires the availability of natural material since it is usually performed by mass spectrometric classification of peptides produced by digestion of the entire protein content by a protease that cleaves at arginine or lysine residues (Madigan *et al.*, 2015). Furthermore, Metaproteomics analysis faces problems of uneven species distribution. Microorganisms exhibit a wide range of protein expression levels, and microbial communities possess large genetic heterogeneity (Simon and Daniel, 2011). Metaproteomics analysis experiences retrieval of membrane-bound and cytoplasmic proteins that are inconsistent and this confines the technique to be frequently used in qualitative characterization of rather simple microbial communities and analysis of complex communities but with the focus on very abundant proteins (Madigan *et al.*, 2015). However, metaproteomics can be effectively used to study an ecosystem and assess the contribution of the species in a microbial community (Madigan *et al.*, 2015).

2.3 Applications of Metagenomics in bacteriology

Metagenomics has been applied in soil, digestive tract, marine and lake habitats (Madigan *et al.*, 2015). This technique has successfully enhanced the efficiency and turnaround time for the detection of bacteria in various studies. In addition, the interaction of various bacteria and their role in the various ecosystems has been improved even though there is still lack of knowledge regarding most uncultured species. Furthermore, some bacterial niches are yet to be explored and fully understood. Described below are studies in which Metagenomics has been performed in soil, digestive tract, marine and lake habitats.

2.3.1 Soil habitat Metagenomics

Soil habitat possesses the largest diversity of bacterial communities compared to other habitats. Rosello-mora and Amman (2001) revealed that a gram of soil contains approximately 10 billion microorganisms and thousands of different species. This abundant diversity results from the complexity and spatial heterogeneity of soil habitations compared to other environments owing to soil particles possessing minerals of different shapes, sizes and chemical compositions, mixed with biotic and organic compounds in different phases of decomposition (Daniel, 2005). In addition, the water content and availability of nutrients plays a role in the survival and growth of microorganisms, and the differential distribution of these factors lead to entirely different microbial habitats which are subject to change over time. These different microbial habitats display distinguished phylogenetic, genomic and metabolic diversity (De Bruijn, 2011). In addition, De Bruijn, (2011) revealed that soil microorganisms are in close association with soil particles such as clay- organic matter and sand grains rendering them immobile.

Kirby *et al.* (2011) explored the Actinobacteria diversity associated with Antarctic dry valley mineral soils. The phylogenetic analysis results revealed the identification of clones that are closely related to culturable species such as *Modestobacter multiseptatus, Kineococcus radiotolerans.* In addition, a monophyletic group was created by four clones with members of the *Nocardioidacea* family. However, the sequence similarity of one of the four clones with members of the *Nocardioidacea* was less than 90% suggesting a novel genus of this family. Furthermore, six clones were reported to be distantly related from all the known Actinobacteria genera and formed a distinct clade. This study also highlights the importance of Metagenomics in the discovery of new species. Smith, J. *et al.* (2006) investigated the bacterial diversity in three different Antarctic cold desert mineral soils namely underneath a crabeater seal carcass on Bratina Island (BIS), the midslopes of Miers Valley (MVG), and fine gravels from Penance Pass, a high-altitude site between the Miers and Shangri La Valleys (PENP). The results indicated the presence of different phylotypes namely, Cyanobacteria, Actinobacteria, Acidobacteria, Unclassified Bacteroidetes, Verrucomicrobia, Chloroflexi, Alphaproteobacteria, and Betaproteobacteria. The mineral sites also indicated diversity of MVG 64%, PENP 73%, BIS 56% by calculation of the coverage index. These findings show that a different prokaryote phylotypes are present in Antarctic dry valley cold desert mineral soils which is also in agreement with Lipson and Schmidt (2004).

Kutovaya *et al.* (2015) studied the metagenomic characterization of biodiversity in the extremely arid desert soils of Kazakhstan in which two sites were sampled. It was revealed that the dominant bacteria identified were from the phyla Proteobacteria (43.9% and 50.8%), Actinobacteria (9.5 and 10%), Firmicutes (2.4 and 0.8%), Verrucomicrobia (1.1 and 3%), Acidobacteria (1.1 and 2%), and Bacteroidetes (1.4 and 1.2%) and the less dominant were represented insignificantly (< 1%). These phyla are mostly the abundant in microbial soil studies and this is indicative that they are highly versatile and have the potential to survive adverse condition (Kutovaya *et al.*, 2015).

Arjun and Harikrishnan (2011) conducted a metagenomics bacterial diversity study in the rice rhizosphere soil microbiome. The results disclosed the presence of four phyla namely; Proteobacteria, Firmicutes, Bacteroidetes and Acidobacteria. Most clones were closely related to Proteobacteria (7/12) followed by Firmicutes (2/12), Bacteroidetes (2/12) and Acidobacteria (1/12). Their findings are in agreement with Chowdhury *et al.* (2009), indicating that Proteobacteria, Firmicutes, Bacteroidetes and Acidobacteria are the major phylotypes found in the soil of which Proteobacteria is the most abundant and metabolically diverse (Liles *et al.,* 2003; Tringe *et al.,* 2005; Yergeau *et al.,* 2009; Zarda *et al.,* 1997). In addition, Arjun and Harikrishnan (2011) argued that the soil ecosystem is complex and metagenomics has revealed several uncultured bacterial species and more species are yet to be revealed alongside their role in the ecosystem.

Metagenomics has the potential to reveal undiscovered species and has been used to investigate soil bacteria in various studies. It has been be used for: (i) taxonomic profiling and metagenome analysis of a microbial community from a habitat contaminated with industrial discharges (Shah *et al.,* 2013), (ii) tackling soil diversity with the assembly of large, complex metagenomes (Howe *et al.,* 2014), (iii) soil bacterial Metagenomics analysis from uranium ore deposit of Domiasiat in Northeast India (Kumar, R. *et al.,* 2013), (iv) environmental microbial sequencing and identification methods for ecologists (Zimmerman, 2014), (v) bacterial community structures of Antarctic soils (Bottos *et al.,* 2014). Other studies that have employed metagenomics include Silveira *et al.* (2006), Oliveira *et al.* (2017), Priyanka and Koel (2015), Fierer *et al.* (2012), Meier (2014), Castañeda and Barbosa (2017), and Riesenfeld *et al.* (2004).

2.3.2 Digestive tract habitat metagenomics

Studies involving microbial communities inhabiting the gastrointestinal tract of livestock indicate a transition from the conventional culturing techniques to metagenomics approaches (Deusch *et al.,* 2015). However, culturing still retains its usefulness in the characterization of microbial physiological properties. Ojima *et al.* (2016) investigated the dynamic changes of whole gut microbiota in the acute phase of intensive care unit patients (ICU) by metagenomics analysis. The patients were admitted on the basis of diagnosis of trauma in four patients, cardiac arrest in four patients, sepsis in three patients, and acute respiratory distress syndrome in one patient. Their results indicated that bacteria belonging to the phyla Firmicutes and Bacteroidetes were largely represented in each sample. Ojima *et al.* (2016) argued that ICU patients had dynamic fluctuations in the microbiota of the gut and these changes were possibly associated with patient prognosis. These changes were undoubtedly due to the consumption of antibiotics, vasoactive agents, agents to neutralize gastric secretions, sedatives or analgesics, agents that impair intestinal motility, and diet as indicated in previous studies (Iapichino *et al.,* 2008; Rhee *et al.,* 2009).

Monira *et al.* (2013) studied the metagenomics profile of gut microbiota in children during cholera and recovery. Nine children aged between two to three years who were suffering from acute dehydrating diarrhoea primarily confirmed as cholera were studied. The study was carried out throughout the illness until recovery and indicated variations among individuals in the abundance and dominance of bacterial families which accounted for more than 90% of the bacterial flora in nearly all of the children. The family Vibrionaceae was commonly found in all nine children and displayed the highest relative abundance in six children, while Enterobacteriaceae, and Prevotellaceae were predominant in rest of the children. Preantibiotic patterns disclosed that the family's Enterobacteriaceae, Prevotellaceae, Actinomycetaceae, Mycoplasmataceae, Streptococcaceae, and Veillonellaceae seemed to be the second most abundant in all nine children (Monira *et al.,* 2013).

Monira *et al.* (2013) also disclosed that antibiotic therapy resulted in dynamic change in microbial populations and relative abundances as the children progressed towards recovery. For example, certain bacteria belonging to the family Bacteroidaceae, Bifidobacteriaceae, and Ruminococcaceae that were initially low at day zero became abundant at day 28. Furthermore, similar trends were observed at phyla level for Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria which are the main bacterial phyla in the human gut. Initially, the relative abundance (mean ± sem %) of Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria were 55 ± 7, 18 ± 4, 13 ± 4, and 8 ± 4, respectively, in the total faecal microbiota of all the nine children with cholera. However, the relative abundance of the phyla Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria changed (mean ± sem %) to 12 ± 4, 43 ± 4, 33 ± 3, and 12 ± 2 percent respectively at day 28. The changes in microbial communities was attributed to the changes in bowel movements and excessive loss of stomach contents that occur in acute watery diarrhoea due to cholera. Monira *et al.* (2013) further argued that the changes were due to washing out of the gut commensal bacteria due to extensive diarhoea thereby enabling perhaps harmful Proteobacteria to colonize it.

Dinan *et al.* (2015), argued that the gut of infants comprises of low diversity and a relative dominance of the phyla Proteobacteria and Actinobacteria soon after birth. It was further revealed that with time, the gut becomes increasingly diverse allowing Firmicutes and Bacteroidetes to dominate. Infant gut microbiome is extremely dynamic, low in diversity and considered unstable compared to that of an adult which has been described to be more complex with increasing stability over time (Hamady and Knight, 2009; Dinan *et al.,* 2015). In addition, it was argued that regardless of the differences in gut microbiota, there is a shared core gut microbiome that is necessary for the functionality of the host and this is evidenced by the dominance of members of the Bacteroidetes and Firmicutes phyla in an adult gut. The disturbances in the core gut microbiome is indicative of illness and this has been observed in studies that have assessed microbiomes of individuals transitioning from health to illness (Zhang, Y. *et al.,* 2015; Dubourg, 2016; Ehrlich, 2016; Lagier, 2016; Ross *et al.,* 2016; Boulygina *et al.,* 2017; Hosny *et al.,* 2017).

Other studies that have reported variations in the gut microbiome include Revised computational Metagenomics processing uncovers hidden and biologically meaningful functional variation in the human microbiome (Manor and Borenstein, 2017), faecal Metagenomics profiles in subgroups of patients with myalgic encephalomyelitis/chronic fatigue syndrome (Nagy-Szakal *et al.,* 2017), Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct Metagenomics functions (Estaki *et al.,* 2016), The gut microbiota and host health: a new clinical frontier (Marchesi *et al.,* 2015), Phylogeny, culturing, and Metagenomics of the human gut microbiota

(Walker *et al.,* 2014), Development of the gut microbiota in infancy and its impact on health in later life (Tanaka and Nakayama, 2017), Metagenomics surveys of gut microbiota (Mandal *et al.*, 2015).

2.3.3 Marine and freshwater habitat Metagenomics

There have been studies conducted on marine and freshwater environments in an attempt to understand their relationship. Some studies have indicated that marine and freshwater microbes are usually not closely related and form different groups in phylogenetic trees (Logares *et al.*, 2009). This is in agreement with Marshall *et al.* (2008)'s study on metagenomics profiles of aquatic microbial communities for environmental assessments. However, Tamames *et al.* (2010) and Wang *et al.* (2012) disputed suggesting that freshwater bacterial populations are relatively equally complex and rich compared to marine environments highlighting the need for further extensive research to characterize both environments.

Mohiuddin *et al.,* (2017) argued that recreational waters and adjacent beach sands possess various microbial communities with the potential to cause human disease and these pathogens cannot be detected through culturing techniques. Metagenomics analysis results showed a significantly high ($P < 0.001$) alpha diversity and average taxonomic richness in beach sands than associated water (Mohiuddin *et al.,* 2017). Furthermore, novel unclassified phylotypes were recognized from the sand beach than the associated water including species from Aquificae, Candidatus Microgenomates, Latescibacteria, and Candidatus Aminicenantes (Mohiuddin *et al.*, 2017). The phyla Proteobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia were detected in water as the most abundant. Betaproteobacteria, Alphaproteobacteria, and Gammaproteobacteria were the more abundant assemblages within Proteobacteria in both environments. Pathogens and faecal indicator bacteria were identified in both water and beach sands (Mohiuddin *et al.,* 2017). Furthermore, pathogen and indicator bacteria were explored based on their relevance and public health impact and found that *E. coli* was the most abundant and revealed no significant difference in abundance between the beach environments. Mohiuddin *et al.,* (2017) also detected vibrio species in water but not in sand. These findings are in agreement with findings of Brown *et al.* (2015) and Jung *et al.* (2010) who reported that half of the bacteria species identified were Proteobacteria.

Fahrenfeld *et al.* (2017) investigated shifts in microbial community structure and function in surface waters impacted by unconventional oil and gas wastewater using metagenomics. The results indicated that communities at all sampled sites mostly contained Proteobacteria mainly represented by the classes, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria. Impacted sites were dominated mostly by Deltaproteobacteria (9.8 ± 0.1%) compared to none impacted sites (6.2 ± 1.2%), followed by unclassified bacteria (21.9 – 27.1%). These findings revealed a change in geochemical properties of the stream due to activities at a UOG waste water disposal facility and this influenced the community composition and potential metabolic activity of the microbes. Fahrenfeld *et al.* (2017) further suggested that there were potential shifts in nutrient cycling and redox conditions as evidenced by the loss of nitrate-oxidizing Nitrospira, reductions in ammonia oxidizing Thaumarchaeota, and elevation in anaerobic Methanomicrobia.

Das *et al.* (2017)'s study used the metagenomics approach to decipher the indigenous microbial communities of arsenic contaminated groundwater of Assam. Their results disclosed that the phyla Proteobacteria was the most abundant (62.6%), followed by Bacteroidetes (11.7%), Planctomycetes (7.7%), Verrucomicrobia (5.6%), Actinobacteria (3.7%) and Firmicutes (1.9%) while other phyla such as Acidobacteria, Ascomycota, Chlamydia, Chlorobi, Chloroflexi and Chordata were represented in 0.5–3% of the entire metagenome. These results are in agreement with the findings of Fahrenfeld *et al.* (2017) and Uyaguari-Diaz *et al.* (2016), who also reported Proteobacteria to be abundant among the phyla detected. This can be explained based on Proteobacteria's ability to survive in harsh and metal contaminated stressed environments as evidenced by Sheik *et al.* (2012) and Lu *et al.* (2017). In addition, Uyaguari-Diaz *et al.* (2016) further disclosed that Betaproteobacteria was the most abundant within the phyla Proteobacteria and consisted of about 17%, 35%, and 11 % of the bacterial community in amplicon and metagenome libraries from urban, agricultural impacted, and protected watersheds respectively.

Huang *et al.* (2016) used Metagenomics to study the distinct bacterial communities in biofilters among different marine recirculating aquaculture systems. This study revealed that Proteobacteria were the most abundant taxa with approximately 36% – 50% of the metagenome of a certain filter.

Other filters revealed abundances of Bacteroidetes with 13 – 34%, Chloroflexi with two to 23%, Nitrospirae with one to seven percent, Planctomycetes with one to four percent, and Actinobacteria with two to five percent, while fluidized sand filters had abundances of 19% for Bacteroidetes, 17% for Nitrospirae, and 11% for Planctomycetes. The results are in agreement with findings of Martins *et al.* (2013)'s study in which they noted that Proteobacteria were the most abundant taxa alongside Bacteroidetes. They also argued that there were changes in bacterial community structure and composition between recirculating aquaculture systems producing turbot and sole, signifying a strong influence of species cultured on associated microbial communities.

Matishov *et al.* (2015) used metagenomics analysis to investigate bacterial communities of the sea of Azov. Water samples (10) were collected from surface and bottom regions of the sea from five stations from the Deneb research station. The results showed the following proportions; Proteobacteria (23.9 – 66.4%), Bacteroidetes (7.37 – 32.2%), Cyanobacteria (1.62 – 33.6%), Actinobacteria (up – 18.5%), Firmicutes (up – 8.18%), Planctomycetes (0.89 –8.41%), and Verrucomicrobia (up – 15.9%). Among the Proteobacteria, Gammaproteobacteria (10.7 – 55.5%) and Alphaproteobacteria (4.40 – 16.0%) were predominant on the surface layer, probably due to their uniform distribution and autotrophic properties of this group. Matishov *et al.* (2015) also argued that Bacteroidetes combine heterotrophic bacteria with the ability to adapt to multiple physiological parameters, and this permits them to colonize different ecological niches especially that their multienzyme systems have the ability to utilize various substrates to derive carbon and energy. Mamaeva *et al.* (2016) also used Metagenomics analysis to investigate microbial communities of the sediments of the Kara Sea shelf and the Yenisei Bay and found that the predominant phyla were Cyanobacteria (29.3%), Verrucomicrobia (26.9%), Actinobacteria (16.0%), and Proteobacteria (13.7%). In addition, an increase in salinity was positively correlated with an increase in abundance of Gammaproteobacteria and decreased abundance of Alphaproteobacteria and Betaproteobacteria, as well as of the phyla Verrucomicrobia, Chloroflexi, Chlorobi, and Acidobacteria. Other metagenomics studies performed on water include Guo *et al.* (2017), Crovadore *et al.* (2017), Jünemann *et al.* (2017), Nakayama *et al.* (2017), Mineta and Gojobori, (2016), Klippel *et al.* (2014), Nakai (2011), Li et al. (2016), Edwards (2007), Carrino-Kyker *et al.* (2013), (Bik, 2014), Kaluzhnaya *et al.* (2012), Thomas, *et al.* (2007), and Somboonna *et al.* (2012).

Ferrer *et al.* (2011) studied the taxonomic and functional metagenomics profiling of the microbial community in the anoxic sediment of a sub-saline shallow lake; Laguna de Carrizo in Central Spain. The findings revealed that the lake had an abundance of Ca^{2+} and $S0_4^{2-}$ being dominant. Two thirds of the bacteria identified belonged to the phylum Proteobacteria and the classes Betaproteobacteria and Deltaproteobacteria were the most abundant from which some sequences were found to cluster with branches represented only by uncultured microorganisms (Ferrer *et al.*, 2011). The genus *Burkholderia* was the most prominent genus among the Betaproteobacteria and the less prominent were *Gallionella* species, *Thiobacillus* species, and *Rhodocyclus* species. The Deltaproteobacteria is comprised of the major groups of sulphate-reducing bacteria. Most of the bacteria (75%) within the Deltaproteobacteria belonged to the families; Desulfobacteraceae, Desulfobulbaceae, Syntrophaceae, and Syntrophobacteraceae (Ferrer *et al.*, 2011).

2.4 Significance of Metagenomics

Metagenomics is an important tool that targets to comprehensively describe the diversity and function of microorganisms in an ecosystem. It also links functional and phylogenetic information to the biological and physicochemical parameters that characterise an environment (Sun *et al.*, 2017). This tool is significant in the discovery of new species and genes encoding functional proteins, and has gained its widespread use in the investigation of microbial diversity and ecology of environments (Thomas *et al.*, 2015). Furthermore, metagenomics allows the rapid and cost effective characterisation of whole genome information and bypasses the limitations of culture dependent techniques that are less informative and laborious. With the rapid development of next-generation sequencing techniques (Thomas *et al.*, 2015), metagenomics can be used in combination with other techniques such as metatranscriptomics and metaproteomics to provide a more comprehensive understanding of the central dogma of biology (Thomas *et al.*, 2015).

2.5 Metagenomics data analysis tools

2.5.1 Sample processing

The processing of samples is important because it determines the amount and quality of information a metagenomics project reveals. It is necessary that high quality DNA that is representative of the whole environment under investigation is extracted and this determines the amount and quality of nucleic acids that would be available for subsequent library production and sequencing (Thomas *et al.,* 2012). In a bid to improve extraction efficiency, sample specific extraction methods have been documented (Venter *et al.,* 2004; Burke *et al.,* 2009; Thomas *et al.,* 2010; Delmont *et al.,* 2011; Delmont *et al.,* 2012). DNA processing is vital in exploring microbial communities through metagenomics since it is widely accepted that DNA extraction methods affect the outcome of community profile analysis (Delmont *et al.,* 2012; Thomas *et al.,* 2012). Thomas *et al.* (2015) argued that various protocols are tailored for particular sample types and no protocol is best suited for all sample types, and DNA enrichment techniques are applied prior to extraction such as selective filtration or centrifugation (Venter *et al.,* 2004; Palenik *et al.,* 2009; Delmont *et al.,* 2011). Some sample types such as groundwater yield lesser amounts of DNA and need an amplification step in which random hexamers and phage phi29 polymerase may be used (Ishoey *et al.,* 2008; Lasken, 2009; Abbai *et al.,* 2012; Thomas *et al.,* 2015).

2.5.2 Sequencing technologies and quality control

The choice of sequencing technology affects the perceived characterization and composition of microbial communities. The main sequencing technologies used in Metagenomics studies are Sanger sequencing, 454/Roche and the Illumina/Solexa systems. Thomas *et al.* (2012) stated that Sanger sequencing is widely known and is considered the gold standard for sequencing due to its low error rate, long read length (> 700 bp) and large insert sizes (e.g. > 30 Kb for fosmids or bacterial artificial chromosomes). Sanger sequencing is most appropriate when investigating and constructing large or genomes in low diversity environments (Goltsman *et al.,* 2009; Thomas *et al.,* 2015). However, Sanger sequencing is expensive (~ USD 40,000 per gigabase pair), labour intensive and is associated with bias against genes toxic for the cloning host (Thomas *et al.,* 2012).

In comparison to Sanger, 454/Roche and the Illumina/Solexa systems are widely used in metagenomics studies. The 454/Roche system amplifies random DNA fragments embedded on microscopic beads using emulsion polymerase chain reaction (ePCR). Pyrosequencing is performed on these beads after they are deposited into the wells of a picotitre plate, Pyrosequencing is done on individual beads in a parallel pattern (Thomas *et al.,* 2012). The pyrosequencing progresses by the successively adding complementary deoxynucleoside triphosphates bases to the template strand and a pyrophosphate coupled with light is released in the process. After approximately 1.2 million reactions, the light can be detected using a charge-coupled device (CCD) camera and translated into actual complementary sequences of the template. The key steps that affect Metagenomics studies are: ePCR which can produce excess replicates thereby influencing estimates of gene abundance that need knowledge of bioinformatics when analysing (Niu *et al.,* 2010; Teal and Schmidt, 2010), and the interpretation of light intensity that occurs when the polymerase runs through a homopolymer making it decipher the actual number of nucleotide positions which may lead to insertions or deletion errors in homopolymers and reading frameshifts (Thomas *et al.,* 2015). However, Thomas *et al.* (2012) stated that 454/Roche pyrosequencing offers multiplexing with a maximum of 12 samples in a single run of ~500 Mbp, has an informative average read length of 600 - 800 bp (Wommack *et al.,* 2008), and is cheaper (~ USD 20,000 per gigabase pair) than Sanger sequencing making it a widespread choice for shotgun sequencing Metagenomics. A detailed description of 454/Roche system is provided by Mardis (2008) and Metzker (2010).

PCR amplification using Illumina/Solexa technology is performed after embedding random DNA fragments on a surface leading to clusters of identical DNA fragments (Thomas *et al.,* 2012). Sequencing is then done using reversible terminators in a sequencing-by-synthesis process (Bentley *et al.,* 2008). Thomas *et al.* (2012) disclosed that there is a massive cluster density containing hundreds of millions of reads per surface channel and 16 channels per run on the HiSeq2000 instrument making it possible to generate ~60 Gbp in a single channel. Illumina/Solexa sequencing is not documented to have multiple limitations except that of having a limited read length and high error rates at the tail ends of reads (Nakamura *et al.,* 2011), but advantages include that it is cheap (~ USD 50 per Gbp), can be applied in metagenomics, can be used to construct draft genomes from complex dataset, multiplexing of samples and faster runtime using the new

Illumina MiSeq instrument (Thomas *et al.,* 2015). A comprehensive description of Illumina MiSeq system is outlined by Mardis (2008) and Metzker (2010).

2.5.3 Assembly

Assembly methods used in metagenomics are reference-based assembly (co-assembly) and de novo assembly. Thomas *et al.* (2012) argued that metagenomics studies aimed at investigating the genome of uncultured organisms and not functional description of the community can construct longer genomic contigs from the assembly of short read fragments. Most assembly software's were engineered for assembly of single, clonal genomes, and caution should be taken when using them on complex pan-genomic mixtures. Software packages such as Newbler (Roche), AMOS http://sourceforge. net/projects/amos/, or MIRA can perform reference-based assembly (Chevreux *et al.,* 1999; Thomas *et al.,* 2012). Reference based assembly is optimised when metagenomic dataset contains sequences with available closely related reference genomes (Thomas *et al.,* 2012). Thomas *et al.* (2012) also revealed that variations between the true genome of the sample and the reference such as large insertions, deletions, or polymorphisms can be interpreted that the assembly is fragmented or that divergent regions are not covered.

De novo assembly makes use of tools based on the de Bruijn graphs that can handle very large amounts of data since it typically requires larger computational resources (Miller *et al.,* 2010). Zerbino and Birney (2008) and Li *et al.* (2008) argued that Bruijn assemblers Velvet or SOAP have considerably high machine requirements compared to reference-based assembly (co-assembly) and longer run times which normally are days (Thomas *et al.,* 2012). Thomas *et al.* (2015) further argued that since most microbial communities differ significantly on a strain and species level, assembly algorithms that assume clonal genomes are inappropriate for metagenomics due to suppression of information, and this assumption is engineered into most assemblers. This limitation is however circumvented by Bruijn-type assemblers MetaVelvet and Meta-IDBA (Peng *et al.,* 2011). Downstream pipelines determine which kind of assembly is to be performed for example MG-RAST threshold is 75 bp or longer for analysis (Glass *et al.,* 2010). Li and Godzik (2006) and Edgar (2010) argued that clustering near identical reads with cd-hit or uclust is a suitable option opposed to assembling reads into contigs since there is a reduction in data. However, Thomas *et al.* (2012) argued that the quality is enhanced by merging reads which allows the analysis of complex genetic elements. There is need to develop metagenomics assemblers since their use is still in its infancy, and comparisons or assertion of accuracy cannot be made due to lack of reference studies (Thomas *et al.,* 2015).

2.5.4 Binning

Binning is the process of arranging DNA sequences into clusters based on similarity to represent an individual genome or genomes of related organisms (Thomas *et al.,* 2012). Binning and classification of DNA fragments is enhanced by long, contiguous sequences and the use of appropriate tools (Thomas *et al.,* 2012). There are two types of binning; compositional binning and similarity based binning. Phylopythia, S-GSOM, PCAHIER and TACAO are the commonly used compositional based binning algorithms, IMG/M, MG-RAST, MEGAN, CARMA, SOrt-ITEMS and MetaPhyler are similarity-based binning algorithms and PhymmBL and MetaCluster use both composition and similarity based binning (Diaz *et al.,* 2009; Glass *et al.,* 2010; Thomas *et al.,* 2012). Furthermore, Thomas *et al.* (2012) revealed that composition based binning is not reliable for short reads due to insufficient information. A detailed discussion on binning is provided by Thomas *et al.* (2012).

2.5.5 Annotation

Annotation of metagenomes can either be performed by either using existing pipelines for genome annotations or entire community and relies on unassembled reads or short contigs (Thomas *et al.,* 2012). The use of existing pipelines such RAST or IMG requires minimal contigs length of 30,000 bp or longer (Aziz *et al.,* 2008; Markowitz *et al.,* 2009). Thomas *et al.* (2012) revealed that annotation of metagenomics sequence data has two steps; feature prediction and functional annotation. Feature prediction is the process of identifying genes from sequences using algorithms such as FragGeneScan, MetaGeneMark, MetaGeneAnnotator (MGA)/ Metagene and Orphelia that detect coding sequences using internal information (McHardy *et al.,* 2007; Noguchi *et al.,* 2008; Rho *et al.,* 2010; Yok and Rosen, 2011; Thomas *et al.,* 2012). BLAST search is then used to identify none labelled sequences due to missing information in these programs. Annotation can be achieved through a variety of pipelines such as MG-RAST, CAMERA’s RAMCAPP, SILVA, Greengenes and RDP databases (Thomas *et al.,* 2012; Thomas *et al.,* 2015).

Functional annotation involves the labelling of sequences that code for known proteins. Thomas *et al.* (2012) argued that only about 20 - 50% of a metagenomics sequences can be annotated presently and this presents a challenge since annotation is done by mapping sequences to known genes or protein libraries. Since annotation largely depends on available known genes, some sequences termed ORFans cannot be mapped due to lack of their representation in the available databases. Due to the large sizes of metagenomics datasets, it is impossible to manually annotate making the use of automated annotation viable and computationally inexpensive. KEGG, eggNOG, COG/KOG, PFAM, TIGRFAM and recent versions of MG-RAST and IMG/M are among the reference databases that are available for functional annotation. A broad account of these tools is provided by Thomas *et al.* (2012) and Thomas *et al.* (2015).

2.5.6 Experimental Design and Statistical Analysis

The interpretation of metagenomics studies is also influenced by experimental designs and statistical analysis (Thomas *et al.*, 2015). Good experimental designs are aimed at avoiding or reducing potential biases that get introduced into a metagenomics study from sampling to interpretation of data such as sampling methods and sample size. Inappropriate use of statistics may give inaccurate interpretations while proper use can reduce the vast data to succinct conclusions (Thomas *et al.*, 2015). The Primer-E package and Functionalize R package are the reliably widely used tools since the can perform robust multivariate statistical analyses such as principal coordinates analysis (PCoA), principle component analysis (PCA), multidimensional scaling (MDS) and analysis of similarities (ANOSIM) (Thomas *et al.*, 2012).

2.5.7 Metadata sharing and storage

Sharing and storage of data is important for the advancement of Omics studies since it allows the identification and mapping of sequences under investigation to reference sequences in shared databases (Thomas *et al.*, 2015). The National Centre for Biotechnology Information (NCBI) is responsible for the storage and dissemination of all metagenomics data. This database has played a significant role in the characterisation of sequences in molecular studies including metagenomics. Metagenomics heavily relies on the existence of databases such as IMG/M, CAMERA and MG-RAST, and the addition of new sequences to these databases as research continues. Thomas *et al.* (2012) and Yilmaz *et al.* (2011) revealed that a standard for the representation of sequences is provided such as the Minimum Information about any (x) Sequence checklists (MIxS).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites

The population of interest were hand-dug wells (shallow, shallow-na and deep) found in the Cuvelai Etosha Basin which is located in north central Namibia (see Figure 1.1). The Oshikoto, Omusati, Ohangwena and Oshana regions of Namibia form the Cuvelai Etosha Basin in Namibia. However, Omusati and Ohangwena regions were the source of the water samples because they have the three types of hand-dug wells targeted and were the primary focus area of water chemical analysis funded by Southern African Science Service Centre for Climate Change and Adaptive Land Management (SASSCAL) task 007: *Improving knowledge and understanding of groundwater flow, water quality and quantity variations, improve methodology of groundwater availability study in Cuvelai* since 2014.

The Omusati, Oshana, Ohangwena and Oshikoto communities were the populations under study. These regions have a projected total population of 918 010 for the year 2018 (Namibia Statistics Agency report, 2014). These regions practice livestock farming of cattle, sheep, goats, horses, donkeys, and pigs. According to the 2013 consus records (Ministry of Agriculture Water and Forestry Directorate of Veterinary Services, 2013), the livestock populations are as follows; Oshana region recorded; cattle (149 585), sheep (3 564), goats (71 003), horses (113), donkeys (11 891) and pigs (6 800), Omusati region; cattle (294 206) sheep (11 315) goats (187 246) horses (701) donkeys (31 620) and pigs (14 354), Ohangwena region; cattle (199 392), sheep (291), goats (125 944), horses (466), donkeys (11 512) and pigs (5 683), Oshikoto region; cattle (291 994), sheep (3 405), goats (199 153), horses (870), donkeys (28 356) and pigs (130 922). The livestock in these regions access water through the water troughs that are placed close to the hand-dug wells, and through walking in particularly in the shallow hand-dug wells thereby potentially contaminating the water (McBenedict *et al.*, 2017). Other activities that may interfere with hand-dug well water quality is open defecation and the use of manure and fertilizers during crop farming that takes place within the vicinity (McBenedict *et al.*, 2017; Thomas, 2016).

3.2 Research design

The study employed a repeated cross sectional design that enabled once off sample collections in the dry and wet seasons in order to study the seasonal influence on bacterial populations in the defined area, and to reveal the particular seasonal parameters associated with any observable changes. The study targeted hand-dug wells which for the purpose of this study are defined as a hand man-made hole, shaft, or excavation created to extract ground water for domestic use (Harter, 2003). The hand-dug wells were categorized into three groups namely; shallow, shallow-na and deep. Shallow indicated the shallow hand-dug wells that are accessed by livestock (Figure 3.1) while shallow-na represented those that are not accessed by livestock (Figure 3.2). The deep hand-dug wells represented the conventional hand-dug well architecture with a depth of at least 18 m (Figure 3.3). Both qualitative and quantitative data collection and analysis was employed in the present study.

Figure 3.1: An illustrative diagram of the shallow hand-dug wells found in the Ohangwena region of the Cuvelai Etosha Basin in which animals have access to the water.

Figure 3.2: An illustrative diagram of the shallow-na hand-dug wells found in the Omusati region of the Cuvelai Etosha Basin in which animals do not have access to the water.

Figure 3.3: An illustrative diagram of the deep hand-dug wells found in the Omusati region of the Cuvelai Etosha Basin in which animals do not have access to the water.

3.3 Sampling strategy

Purposive sampling was employed in this study by targeting hand-dug wells that were also being investigated for water chemical composition. The intention was to use the water chemical composition data to better understand bacteria community structure and composition. The water samples were collected from 40 hand-dug wells targeted by SASSCAL to generate information on their microbiological water quality in the dry and wet seasons. Half the total number were collected from Ohangwena and 20 from Omusati regions of Namibia. Water samples were collected from the same hand-dug wells in the wet and dry seasons. The water samples were collected in the following proportions; three from shallow hand-dug wells, three from the shallow-na hand-dug wells and four from the deep hand-dug wells in each season.

3.4 Sampling procedure

Two water samples were collected in sterile 200 ml falcon bottles from each hand-dug well. The bottles were lowered into the hand-dug wells for water collection using a rope which was tied to the sterile bottles (Figure 3.3). The bottles were then placed on ice during transportation to the University of Namibia for analysis. One of the duplicate water samples was sent to Bundesanstalt für Geowissenschaften und Rohstoffe (BGR) in Hannover (Germany) for analysis of potassium (K), sodium (Na), chloride (Cl), magnesium (Mg), calcium (Ca), sulphate (So4), bicarbonate

(Hco3), Iron (II) oxide [Fe (II)], manganese (Mn), nitrate (No3), bromine (Br), ammonium (NH4), nitrite (No2), fluorine (F), phosphate (Po4), aluminium (Al), arsenic (As), oxido (oxo) borane (Bo2), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lithium (Li), nickel (Ni), lead (Pb), scandium (Sc), silica (Sio2), strontium (Sr), titanium (Ti), vanadium (V), zinc (Zn), total inorganic carbon (Tic), non-purgeable organic carbon (NPoc), and total nitrogen bound (TN_b), while the other was used for microbial water quality investigation. Temperature, electrical conductivity (Ec), redox potential and potential of hydrogen (pH) of the water were measured at the sampling site as these parameters change in response to environmental changes occurring during transportation of samples (Li *et al.*, 2017; Wanke *et al.*, 2014).

3.5 Culture of bacteria from water

The collected water samples were processed for bacterial culture. In order to widen the scope of bacterial isolation, the water in each 200 ml falcon tube was centrifuged at a speed of 7 000 *xg* for one hour to concentrate the bacteria. After centrifugation, each volume was then reduced to 10 ml by discarding the supernatant thereby leaving the pellet suspended in 10 ml and then 0.1 ml was streaked on the selective and differential MacConkey agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA). This media was used to isolate and detect gram-negative bacteria according to manufacturer’s guidelines. Briefly, MacConkey agar was prepared by suspending 52 g of the medium in 1000 ml of distilled water and boiled to dissolve completely. The media was sterilized using an autoclave at 121° C for 15 minutes. The agar was then left to cool at room temperature (26° C) in a fume hood, upon which about 30 ml of agar was poured per petri dish left to solidify. After solidifying, the sterility test was performed at key points where contamination can potentially be introduced such as the incubator where microbial growth is enhanced, and the fume hood where inoculation was done. The sterility test was performed by placing uncapped petri dishes containing Tryptone soya agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for seven days at the work stations to detect contamination by fungi and bacteria before inoculation. After confirmation of sterility, inoculation was then performed under the fume hood and the plates were inverted and incubated at 35° C for 48 hours for total coliform counts. With regards to thermotolerant coliform counts, the plates were incubated at 45° C for 48 hours. This media provided a basis for total coliform counts and thermotolerant coliform counts, and also distinguished between lactose-fermenting and none lactose fermenting gram-negative enteric bacilli.

Following the coliform counts, single colonies were isolated and grown as pure cultures on MacConkey agar. MacConkey agar enabled the discrimination of lactose fermenting and none lactose fermenting gram-negative bacteria. The two groups namely; (A) lactose fermenting and (B) none lactose fermenting were then subjected to a different flow of biochemical tests as described below and outlined in Figure 3.4.

A. Lactose positive isolates which appeared pink in colour on the MacConkey agar were tested for indole production using the differential SIM media (Thermo Fisher Scientific, Waltham, Massachusetts, USA). This medium tests the bacteria's ability in; sulphur reduction, indole production, and mobility/motility. The SIM media was prepared by suspending 30 g of medium in 1000 ml of demineralized water and heating it to boil with agitation to aid complete dissolution. The SIM media was then dispensed into tubes and sterilized by autoclaving at 121° C for 15 minutes. The bacteria were then inoculated into the tubes by stubbing down the centre of the medium using an inoculating loop to within the bottom ⅓ of the tube. The tubes were incubated with loosened caps at 35° C for 18 - 24 hours and observed for H_2S production and motility. To detect indole production, three to four drops of Kovac's reagent were added to the tubes and observed for a red colour development. The indole positive strains were then tested for citrate utilization using Simmons citrate agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA) slants to separate those capable of using citrate as a sole carbon source from those that could not. Simmons citrate agar slants were prepared by suspending 24.2 g of the medium in 1000 ml of distilled water, heating with frequent agitation and boiling for one minute to completely dissolve the medium. The medium was then dispensed into tubes and autoclaved at 121° C for 15 minutes upon which it was placed to solidify in a slanted position.

   Pure colonies of respective bacteria were then streaked on Simmons citrate agar slants with a light inoculum and tubes incubated at 35° C for 48 hours with loosened caps. The tubes were then observed for a positive reaction indicated by growth on the slant with a colour change of green to blue (alkaline reaction) while a negative reaction was indicated by lack of growth or poor growth without change in colour (medium remained green). The indole negative strains were then tested using Urea agar base (Thermo Fisher Scientific, Waltham, Massachusetts, USA) medium slants to isolate strains that could hydrolyse urea using the enzyme urease. Urea agar base medium slants were prepared by suspending; 29 g of the medium and 15 g of Bacteriological agar in 100 ml and 900 ml purified water respectively. Each bottle was then heated with frequent agitation for one minute to completely dissolve the medium and sterilized by autoclaving at 121° C for 15 minutes. The sterilized agar was then cooled to a range of 45 - 50° C and aseptically mixed thoroughly with the sterile Urea agar base. The mixture was then dispensed into sterile tubes placed in a slanted position. The respective bacteria were inoculated by streaking back and forth over the entire slant surface, and the tubes were incubated at 35° C with loosened caps. The tubes were then left for observation for about 6 days with daily inspections. This provided the identity of the lactose fermenting, indole negative bacterial species.

B. None lactose fermenting gram-negative strains were tested for the ability to metabolize glucose to form acid using Dextrose casein-peptone agar (Merck, Kenilworth, USA) plates. Dextrose casein-peptone agar plates were prepared by suspending 27 g of the medium in 1000 ml of distilled water, boiled to dissolve completely, and sterilized using an autoclave at 121° C for 15 minutes. A positive test showed bacterial colonies that metabolize dextrose to form acid by causing the indicator Bromocresol purple in the medium to change its colour from purple to yellow. The glucose positives were subsequently tested for motility using SIM media as described in the section above, while the glucose negative species were identified at this point. The glucose positive motile strains were then tested for the ability to hydrolyse urea using the enzyme urease on Urea agar base medium slants as described in the section above. At this point, the identity of the none lactose fermenting, glucose negative, gram-negative bacterial species was revealed.

The outline above and flow chart below were modified from Bergey's Manual (Holt *et al.*, 1994; Garrity *et al.,* 2004) for the identification of unknown bacteria. The scheme below shows the steps that were employed to identify the isolated genus; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species.

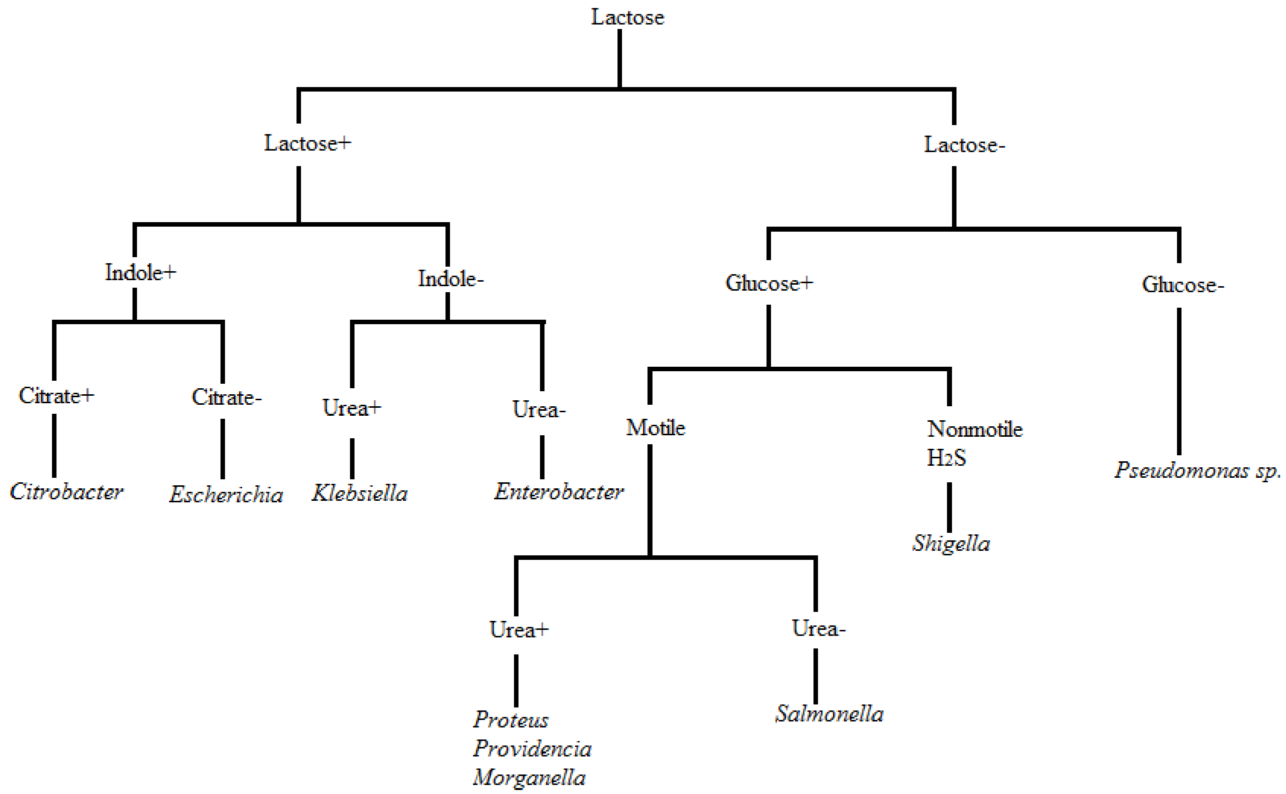

Figure 3.4: An illustration of the steps used to isolate and identify gram-negative bacterial species, modified from Bergey's Manual (Holt *et al.*, 1994; Garrity *et al.*, 2004).

3.6 Metagenomics analysis of water bacteria

3.6.1 DNA extraction and 16S rRNA gene amplification.

Each water sample containing a volume of 200 ml was centrifuged at a speed of 7 000 *xg* for one hour in order to concentrate the bacteria. Each volume was then reduced to 10 ml of water after centrifugation by discarding the supernatant. DNA was extracted from a two microliter volume using SEEPREP 12 ^{TM} kit (Seegene, Rockville, USA), and its concentration and quality determined by the NanoDrop-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). PCR was then used to amplify the 16S rRNA gene of each variant (phylotype) using universal primer sets 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5'

TACGGYTACCTTGTTACGACTT 3'). The thermo-cycler (Bio-Rad, Hercules, CA) was used with reaction conditions of; one cycle of pre-denaturation at 94° C for four minutes, 35 cycles of denaturation at 94° C for one minute, annealing at 55° C for 30 seconds, and extension at 72° C for two minutes, and a final extension at 72° C for 10 minutes. The amplicons were then sent for next generation sequencing diversity assay using Illumina 16S sequencing at Mr. DNA Next Generation Sequencing provider in Texas, United States of America.

3.6.2 PCR product preparation and sequencing

The PCR amplicons from above were prepared for sequencing. Initially, the 16S rRNA gene V4 variable region with PCR primers 515/806 with barcodes on the forward primer were used in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). The following reaction conditions were used: 94° C for three minutes, followed by 28 cycles of 94° C for 30 seconds, 53° C for 40 seconds and 72° C for one minute, after which a final elongation step at 72° C for five minutes was performed. After amplification, PCR products were checked on a two percent agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were then purified using calibrated Ampure XP beads. The pooled and purified PCR products were subsequently used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing (20k 2x300bp illumina 16s) was performed at MR. DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer’s guidelines. Sequence data were processed using a proprietary analysis pipeline (MR. DNA, Shallowater, TX, USA).

3.7 Data analyses

3.7.1 Bacterial culturing data collection and analysis

The results from culturing were recorded as binary data. The bacterial cultures from each water sample were subjected to genus identification as; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species. The data was then manually scored into a binary matrix for subsequent analysis. Each water sample was obtained from a single hand-dug well and was scored for the presence (1) of any of the aforementioned species or absence (0) of the specie(s). The binary data was then entered into IBM SPSS statistics for windows version 24.0 software for Frequency and Crosstab calculations (Corp, I.B.M., 2016). The Frequency variables entered were; hand-dug well type, region, season, *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas*. The Crosstab tables were generated by entering hand-dug well type, season, and region and respectively relating (crossing) it to; *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species. The Chi-Square statistical method was used in the Crosstab analysis to investigate the influence of hand-dug well type, region and season on the presence of *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* in hand-dug wells.

3.7.2 16S Metagenomics data collection and analysis

The Metagenomics sequence data obtained from Mr. DNA Next Generation Sequencing provider (Texas, United States of America) were processed and edited using a proprietary analysis pipeline (www.mrdnalab.com, MR. DNA, Shallowater, TX). The Q25 sequence data derived from the sequencing process were depleted of barcodes and primers, and short sequences less than 150 bp were removed. In addition, sequences with ambiguous base calls, and homopolymer runs exceeding six bp were removed. The sequences were then denoised and chimeras also removed. Operational taxonomic units (OTUs) were defined after removal of singleton sequences, clustering at three percent divergence (97% similarity) according to other workers (Dowd, Callaway *et al.*, 2008; Dowd, Sun *et al.*, 2008; Edgar, 2010; Capone *et al.,* 2011; Eren *et al.*, 2011; Swanson *et al.*, 2011). OTUs were then taxonomically classified by performing a BLASTn against a curated GreenGenes, RDPII (http://rdp.cme.msu.edu) and NCBI (www.ncbi.nlm.nih.gov) databases and compiled into each taxonomic level (DeSantis *et al.*, 2006). The files were compiled based on counts and percentages with counts revealing the actual number of sequences while the percentages displayed the relative proportion (in percentage) of sequences within each sample that map to the designated taxonomic classification. Hence, the bacterial communities and the percentage of each species in the community were explored.

3.7.2.1 Determination of the relative abundance of bacterial phyla

The taxonomically classified phyla from section 3.7.2 were then entered into Microsoft Excel. The relative abundance of each phylum was determined by dividing the sum of counts of each phylum from all samples by the total counts of all the phyla from all the samples and multiplying the product by 100 to get a percentage. The percentages were rounded to two decimals places and subsequently used to generate a Sunburst chart. This chart displayed the relative abundance of each bacterial phylum detected. Hence, the representation of each phylum was explored and only the phyla with a significant (> 1%) representation was shown on a Sunburst chart while the rest were combined to form the "Others" group on the Sunburst chart.

3.7.2.2 Influence of hand-dug well type, region and season on the abundance of bacterial phyla

The counts from each of the bacterial phyla were used to determine the influence of hand-dug well type, region and season on the abundance of bacterial phyla. Each phylum was assessed for normal distribution across the three types of hand-dug wells, the Ohangwena and Omusati regions, and the wet and dry seasons using SPSS version 24. The Shapiro-Wilk test values were used to determine the distribution of phyla based on hand-dug well type, region and season. The Independent Samples Kruskal-Wallis test was then performed on all the phyla to examine the influence of hand-dug well type on the abundance of bacterial phyla. The Independent Samples Mann-Whitney U test was employed to examine the influence of geographic location (Ohangwena and Omusati regions) on the abundance of bacterial phyla. The Wilcoxon test was used to examine the influence of season (wet and dry) on the abundance of bacterial phyla.

3.7.2.3 Influence of hand-dug well type, region and season on bacterial species diversity, evenness and richness

Species diversity is the number of species and abundance of each species present in a particular area, while species richness is the number of species present in a particular area (Pielou, 1975). Species evenness is the measure of the relative abundance of different species in a particular area (Pielou, 1975). Species diversity, evenness and richness are fundamental in determining ecosystem health, and in the present study it gave an indication of contamination levels. In each hand-dug well, bacterial species richness was counted, and Shannon-Wiener diversity indices, Simpson's diversity indices and species evenness were calculated to determine the species diversity, richness and evenness using the formulas described by Uthappa *et al.* (2016) as outlined below;

Shannon-Wiener index (H'):

$$H' = -\sum(pi * \ln pi)$$

Where, H'= Shannon index of diversity, and Pi= the proportion of individuals found in the ith species.

Simpson index (D):

$$D = 1 - \left(\frac{\sum \mathrm{n}(\mathrm{n}-1)}{N(N-1)}\right)$$

Where, n = the number of individuals of each different species, and N = the total number of individuals of all the species.

Shannon's equitability (EH) can be calculated by dividing H' by $H_{\max}$ (here $H_{\max}$ = ln$S$). Equitability assumes a value between zero and one with one being complete evenness.

Shannon's equitability (EH):

$$E_H = \frac{H'}{H_{max}}$$

The Shapiro-Wilk test values were used to determine the distribution of bacterial species based on hand-dug well type, region and season. Possible differences in Shannon-Wiener diversity indices and species evenness of bacterial species between the wet and dry seasons were tested using a Paired sample t-test while differences in Simpson diversity indices and species richness of bacteria between the wet and dry seasons were tested using the Wilcoxon test. Assessments of significant differences in Shannon-Wiener diversity indices and species evenness of bacterial species among the shallow, shallow-na and deep hand-dug wells were tested using a One-Way ANOVA while a Kruskal-Wallis test was used to evaluate differences in Simpson diversity indices and species richness of bacterial species among the shallow, shallow-na and deep hand-dug wells.

3.7.2.4 Metagenomics bacterial species grouping's analysis

The taxonomically classified sequences with bacterial identity were separated into four categories namely; zoonotic, humans, livestock, and grey with various emphases as described below;

1. Zoonotic category focused on bacteria which cause diseases in both humans and livestock.
2. Human's category focused on bacterial species that cause diseases in humans only.
3. Livestock category focused on bacterial species that cause diseases in livestock only, and
4. Grey category focused on bacterial species not known to cause diseases in both humans and livestock.

The counts of each sequence gave an indication of the abundance of that particular bacterial species. These groups allowed the selection of smaller number of species for analysis, and a comprehensive analysis of all the species without loss of information that arises in handling huge data sets. During the analysis, bacterial species that were present only in one season were given a value of zero (0) for the season in which they were absent. The counts of each group were then entered into SPSS version 24 for analysis as described in the sections below.

3.7.2.4.1 Human, livestock and zoonotic phylogenetic trees

A total of three phylogenetic trees were constructed from sequences of the categories; human, livestock, and zoonotic using the Maximum Likelihood Method based on the Tamura-Nei model (1993). Only the trees with the highest log likelihood were chosen and percentages of trees in which the associated taxa clustered together were shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Bootstrap was performed and the consensus trees inferred from 1000 replicates were taken to represent the evolutionary history of the taxa analysed (Kumar *et al.,* 2016). Branches corresponding to partitions reproduced in less than 70% bootstrap replicates were collapsed and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were also indicated next to the branches. The evolutionary analyses were conducted in MEGA 7 (Kumar *et al.,* 2016). The *V. cholerae* strain with accession number *KJ725364.1* was retrieved from the NCBI website and used as the outgroup to root the human pathogen's phylogenetic tree while a *B. anthracis* strain with accession number *AJ516943.1* was retrieved from the NCBI website and used as the outgroup to root the livestock and zoonotic phylogenetic trees.

3.7.2.4.2 Influence of hand-dug well type, region and season on the abundance of human bacterial pathogens

The counts of the human bacterial pathogens were subjected to analysis. The distribution of these pathogens across different hand-dug well types, region and season was evaluated by entering the data into SPSS version 24 to; generate the Shapiro-Wilk test values. An Independent Samples Kruskal-Wallis test was employed to study the influence of hand-dug well type on the abundance of the identified human bacterial pathogens. An Independent Samples Mann-Whitney U test was then used to investigate the influence of region on the abundance of the identified human bacterial pathogens while a Wilcoxon test was employed to investigate the influence of season on the abundance of the identified human bacterial pathogens.

3.7.2.4.3 Influence of hand-dug well type, region and season on the abundance of livestock bacterial pathogens

The distribution of the livestock bacterial pathogens across different hand-dug well types, region and season was evaluated by entering the data into SPSS version 24 in order to; generate the Shapiro-Wilk test values. An Independent Samples Kruskal-Wallis test was employed to study the influence of hand-dug well type on the abundance of the identified livestock bacterial pathogens. An Independent Samples Mann-Whitney U test was then used to investigate the influence of region on the abundance of the identified livestock bacterial pathogens, and a Wilcoxon test was then performed to investigate the influence of season on the abundance of the identified livestock bacterial pathogens.

3.7.2.4.4 Influence of hand-dug well type, region and season on the abundance of zoonotic bacterial pathogens

The distribution of the zoonotic bacterial pathogens across different hand-dug well types, regions (Ohangwena and Omusati) and seasons (wet and dry) was evaluated by entering the data into SPSS version 24 and subsequently generating the Shapiro-Wilk test values. An Independent Samples Kruskal-Wallis test was employed to study the influence of hand-dug well type on the abundance of the identified zoonotic bacterial pathogens. An Independent Samples Mann-Whitney U test was then used to determine the influence of region on the abundance of the identified zoonotic bacterial pathogens, and a Wilcoxon test was then performed to investigate the influence of season on the abundance of the identified zoonotic bacterial pathogens.

3.7.2.4.5 Effect of hand-dug well type, region and season on the abundance of grey bacteria

The grey bacteria counts were assessed for their distribution across different hand-dug well types, regions (Ohangwena and Omusati) and seasons (wet and dry) by entering the data into SPSS version 24, generating the Shapiro-Wilk test values. An Independent Samples Kruskal-Wallis test was employed to study the influence of hand-dug well type on the abundance of the identified grey bacterial category. An Independent Samples Mann-Whitney U test was then used to investigate the effect of region on the abundance of the identified grey bacterial species, and a Wilcoxon test was then performed to investigate the influence of season on the abundance of the identified grey bacterial species.

3.7.3 Water physicochemical data collection and analysis

The physicochemical analysis of the water samples assessed the physical parameters: temperature, potential of hydrogen (pH) and electrical conductivity (Ec). The chemical parameters assessed were: potassium (K), sodium (Na), chloride (Cl), magnesium (Mg), calcium (Ca), sulphate (So4), bicarbonate (Hco3), Iron (II) oxide [Fe (II)], manganese (Mn), nitrate (No3), bromine (Br), ammonium (NH4), nitrite (No2), fluorine (F), phosphate (Po4), aluminum (Al), arsenic (As), oxido (oxo) borane (Bo2), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lithium (Li), nickel (Ni), lead (Pb), scandium (Sc), silica (Sio2), strontium (Sr), titanium (Ti), vanadium (V), zinc (Zn), total inorganic carbon (Tic), non-purgeable organic carbon (NPoc), and total nitrogen bound (TN_b). The measurements were then entered into PC-ORD version 7 in order to determine the particular parameters responsible for the bacterial phyla abundance based on hand-dug well type, region, and season. The Nonmetric multidimensional scaling (NMS) multivariate analysis was used for this purpose (Holland, 2008).

3.8 Research ethics

The water samples obtained from the Cuvelai Etosha Basin were used solely for this study. Prior to sampling, permission was obtained from the owners of the hand-dug wells through the councillor. The constituency councillor and regional council provided permission since they are responsible for undertakings that involve the community, and communicated to the communities on the importance of their participation in this study. In addition, all contributions (intellectual or physical) made to the success of this study were acknowledged.

CHAPTER FOUR

RESULTS

A total of 40 water samples were collected in this study in the wet and dry seasons. Half the number was collected in the wet season, with the rest being the dry season. A general assessment of bacterial loads in the water samples indicated that total coliform counts ranged from 160 CFU/ml to 297 CFU/ml in the wet season, and 110 CFU/ml to 243 CFU/ml in the dry season.

4.1 Colony forming Units (CFU) in sampled hand-dug wells

Of the six shallow hand-dug wells investigated per season (wet and dry), it was revealed that three shallow hand-dug wells from Ohangwena region had counts of 297, 273 and 286 CFU/ml in the wet season. The remaining three shallow hand-dug wells from Omusati region had counts of 266, 283 and 289 CFU/ml in the wet season. In the dry season, the three shallow hand-dug wells from Ohangwena region had counts of 241, 233 and 230 CFU/ml, and three shallow hand-dug wells from Omusati region had counts of 225, 236 and 243 CFU/ml. A total of six shallow-na hand-dug wells were investigated for each season (wet and dry). Half the number was collected from Ohangwena region and the other half from Omusati region. The three shallow-na hand-dug wells from Ohangwena region had counts of 173, 165 and 171 CFU/ml, and three shallow-na hand-dug wells from Omusati region had counts of 160, 166 and 169 CFU/ml in the wet season. The dry season revealed that three shallow-na hand-dug wells from Ohangwena region had counts of 120, 110 and 115 CFU/ml, and three shallow-na hand-dug wells from Omusati region had counts of 118, 126 and 129 CFU/ml.

A total of eight deep hand-dug wells were analysed for each season (wet and dry). Half the number was collected from Ohangwena region and the other half from Omusati region. The four deep hand-dug wells from Ohangwena region had counts of 199, 205, 193 and 178 CFU/ml, and those from Omusati region had counts of 203, 189, 186 and 175 CFU/ml in the wet season. In the dry season, the four deep hand-dug wells from Ohangwena region had counts of 145, 140, 135 and 133 CFU/ml, and those from Omusati region had counts of 143, 130, 125 and 123 CFU/ml. The colony forming unit counts from the shallow, shallow-na and deep hand-dug wells were used to generate a bar graph showing an overview of the contamination levels in the different hand-dug well types. The bar charts (Figures 4.1 and 4.2) depicted visible differences in the abundance of bacterial CFU's in shallow, shallow-na and deep hand-dug wells.

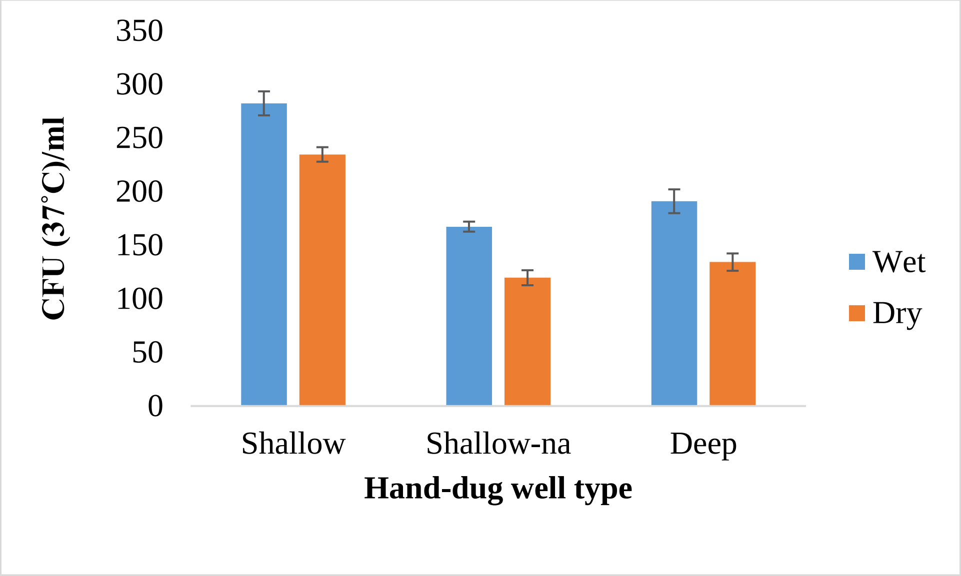

Figure 4.1: An illustration of the abundance (CFU) of bacteria cultured at 37˚C from shallow, shallow-na and deep hand-dug wells of the Cuvelai Etosha Basin in the wet and dry seasons.

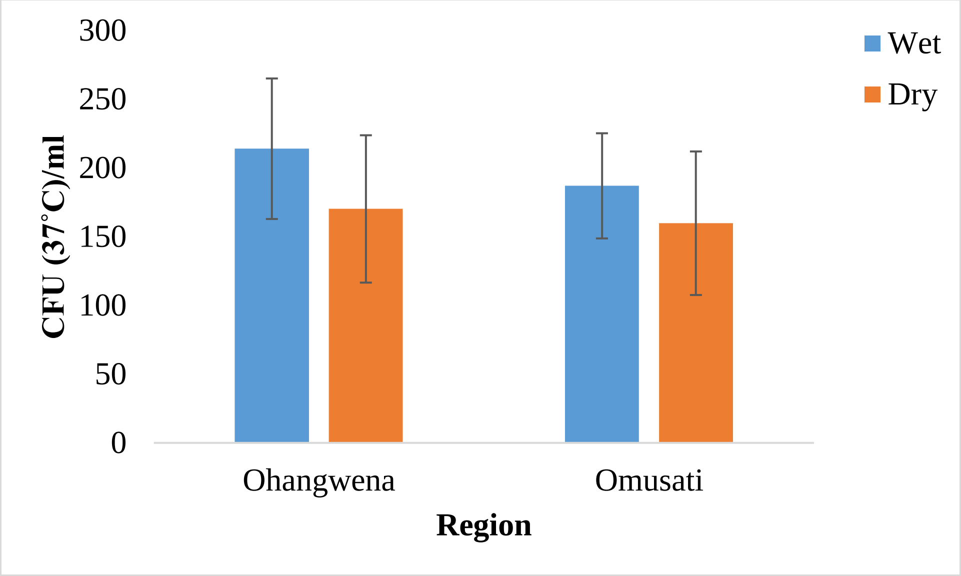

Figure 4.2: An illustration of the abundance (CFU) of bacteria cultured at 37˚C from Ohangwena and Omusati hand-dug wells of the Cuvelai-Etosha Basin in the wet and dry seasons.

4.2 Identified bacteria by culture from water

Culturing revealed the presence of *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species in the hand-dug wells (Figures 4.3 – 4.6). *Citrobacter* species were detected in 11 hand-dug wells, *Escherichia* species were detected in nine hand-dug wells, *Klebsiella* species were detected in 36 hand-dug wells, *Enterobacter* species were detected in 33 hand-dug wells, *Proteus* species were detected in two hand-dug wells, *Salmonella* species were detected in four hand-dug wells, *Shigella* species were detected in 19 hand-dug wells, and *Pseudomonas* species were detected in nine hand-dug wells.

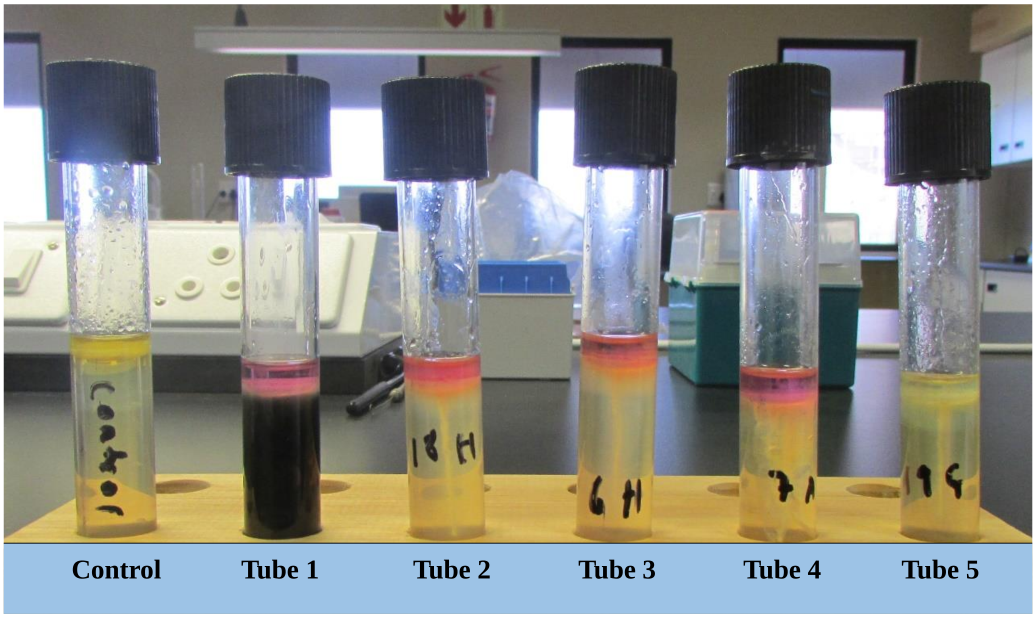

Figure 4.3: A depiction of gram-negative bacteria tested for hydrogen sulphide production, indole formation and motility on SIM agar. Test tube one indicated the presence of *Proteus* species, tubes two, three and four had *Enterobacter* species, while tube five indicated the presence of *Salmonella* species.

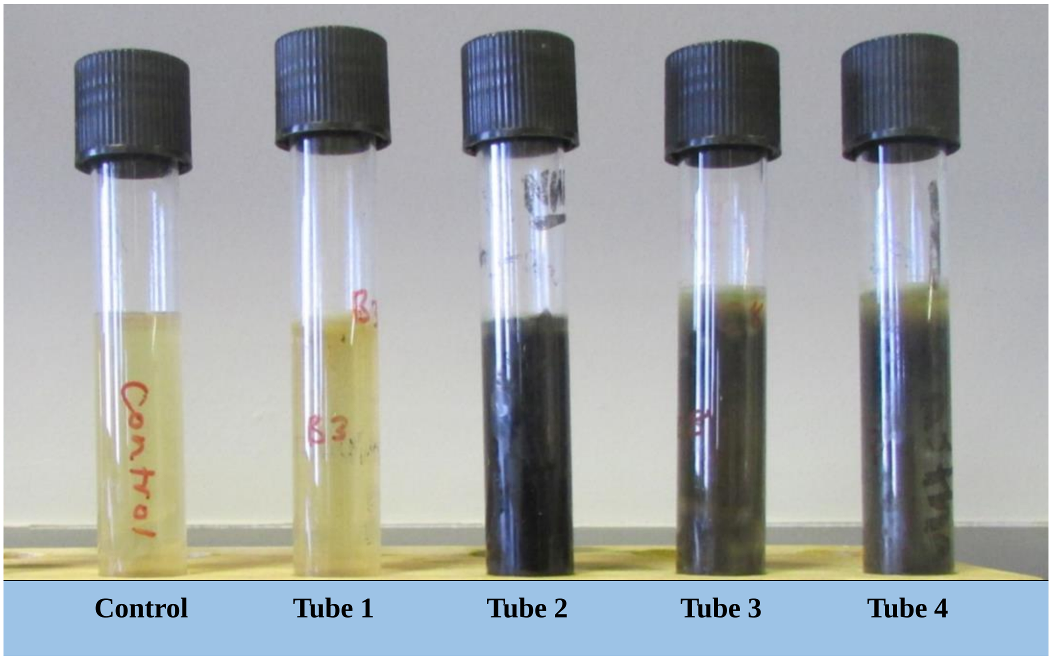

Figure 4.4: A depiction of gram-negative bacteria tested for hydrogen sulphide production and motility on SIM agar. Test tube one showed no growth while tubes two, three and four showed the presence of *Shigella* species (H_2S +).

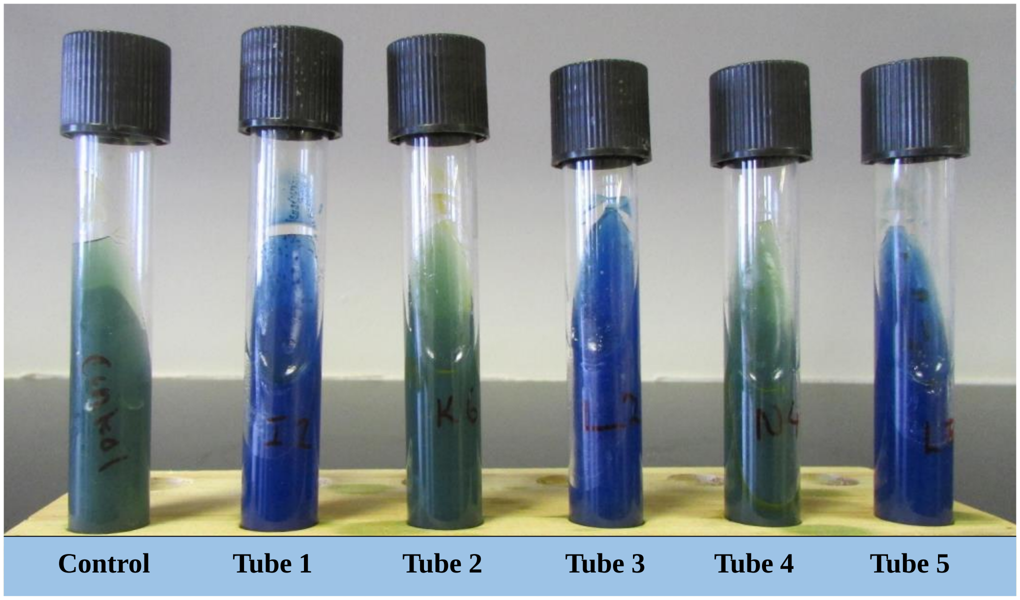

Figure 4.5: A depiction of gram-negative bacteria tested for citrate utilization by means of Simmons citrate agar slants. Test tubes one, three and five showed the presence of *Citrobacter* species (citrate +) while tubes two and four indicated the presence of *Escherichia* species (Citrate -).

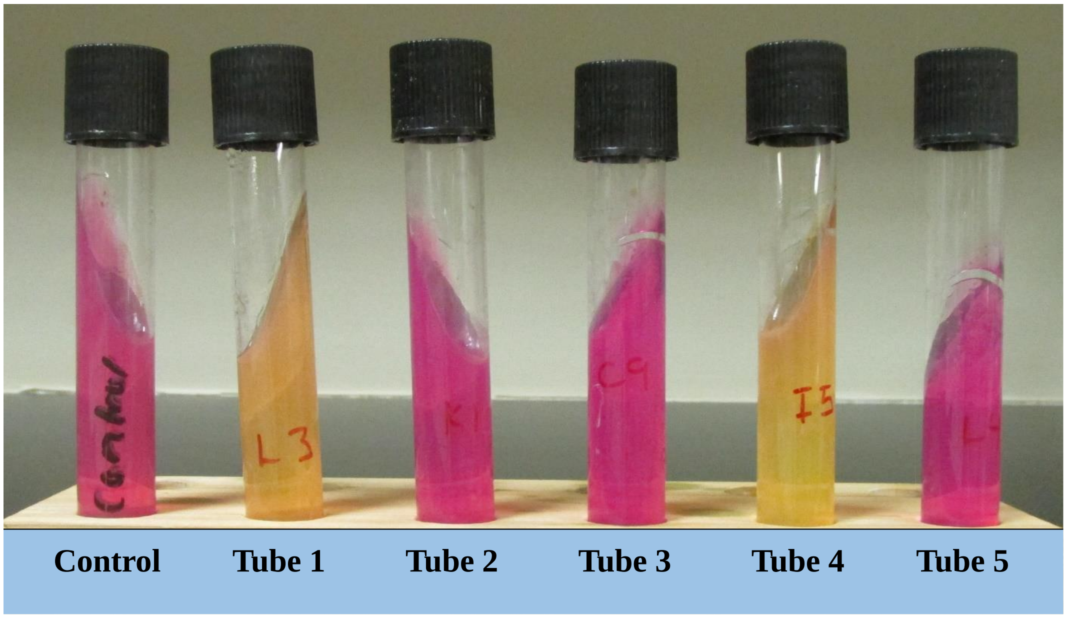

Figure 2.6: An illustration of gram-negative bacteria tested for urease production using Urea agar base slants. Test tubes two, three and five showed the presence of *Klebsiella* species (Urease +), while tube one indicated the presence of *Enterobacter* species, (Urease -), and tube four the presence of *Salmonella* species.

4.2.1 Shallow hand-dug wells

A total of six water samples were collected from wells per season (wet and dry). Half the number was from Ohangwena region and the others from Omusati region. *Escherichia* species were detected in two wells from Ohangwena region and two wells from Omusati region in the wet season. The dry season indicated the absence of *Escherichia* species in all (six) the wells. *Citrobacter* species were detected in one well from Ohangwena region and two wells from Omusati region in the wet season, and in only one well from Ohangwena region and one well in Omusati region in the dry season. *Klebsiella* species were detected in three wells from Ohangwena region and two wells from Omusati region in the wet season, and in three wells from Ohangwena region and three wells from Omusati region in the dry season. *Enterobacter* species were detected in three wells from Ohangwena region and three wells from Omusati region in the wet season, and in three wells from Ohangwena region and three wells from Omusati region in the dry season.

Proteus species were detected in only one well from Ohangwena region and none from Omusati region in the wet season, and was not detected in any of these wells from both regions in the dry season. *Salmonella* species were detected in only one well from Omusati region and none from

Ohangwena region in the wet season, and was not detected in all wells from Ohangwena and Omusati regions in the dry season. *Shigella* species were detected in one well from Ohangwena region and two wells from Omusati region in the wet season, and in three wells from Ohangwena region and three wells from Omusati region in the dry season. *Pseudomonas* species were not detected in all wells from Ohangwena region and Omusati region in the wet season but were detected in only one well from Omusati region and none from Ohangwena region in the dry season.

4.2.2 Shallow-na hand-dug wells

A total of six water samples were also collected from wells per season (wet and dry). Half the number was from Ohangwena region and three from Omusati region. *Escherichia* species were not detected in all the wells from Ohangwena and Omusati regions in the wet season but were detected in two wells from Ohangwena region and none from Omusati region in the dry season. *Citrobacter* species were detected only in one well from Ohangwena region and none from Omusati region in the wet season, and in only one well from Omusati region and one well from Ohangwena region in the dry season. *Klebsiella* species were detected in two wells from Ohangwena region and three wells from Omusati region in the wet season and in three wells from Ohangwena region and three wells from Omusati region in the dry season. *Enterobacter* species were detected in three wells from Ohangwena region and three wells from Omusati region in the wet season, and in three wells from Ohangwena region and one well from Omusati region in the dry season.

Proteus species were not detected in all wells from Ohangwena region and Omusati region in the wet and dry seasons. *Salmonella* species were detected in only one well from Omusati region and none from Ohangwena region in the wet season, and was not detected in all wells from Ohangwena and Omusati regions in the dry season. *Shigella* species were detected in only one well from Ohangwena region and none from Omusati region in the wet season, and in two wells from Ohangwena region and two wells from Omusati region in the dry season. *Pseudomonas* species were detected in two wells from Omusati region and none from Ohangwena region in the wet season, and in only one well from Omusati region and none from Ohangwena region in the dry season.

4.2.3 Deep hand-dug wells

A total of eight water samples were collected from wells per season (wet and dry). Half the number were from Ohangwena region and four from Omusati region. *Escherichia* species were only detected in one well from Omusati region and none from Ohangwena region in the wet season, and in one well from Ohangwena region and one well from Omusati region in the dry season. *Citrobacter* species were detected in only one well from Ohangwena region and one well from Omusati region in the wet season, and in only one well from Ohangwena region and none from Omusati region in the dry season. *Klebsiella* species were detected in four wells from Ohangwena region and four wells from Omusati region in the wet season, and in four wells from Ohangwena region and two wells from Omusati region in the dry season. *Enterobacter* species were detected in four wells from Ohangwena region and two wells from Omusati region in the wet season, and in three wells from Ohangwena region and two wells from Omusati region in the dry season.

Proteus species were only detected in one well from Ohangwena region and none from Omusati region in the wet season, and none from Ohangwena and Omusati regions in the dry seasons. *Salmonella* species were detected in two wells from Omusati region and none from Ohangwena region in the wet season, and none from Ohangwena and Omusati regions in the dry season. *Shigella* species were not detected in all such wells from Ohangwena and Omusati regions in the wet season but was detected in three wells in Ohangwena region and two wells in Omusati region in the dry season. *Pseudomonas* species were not detected in all the wells from Omusati and Ohangwena regions in the wet season but were detected in four wells from Ohangwena region and only one well from Omusati region in the dry season.

4.3 Metagenomics of water analysed

Metagenomics was used to detect the entire operational taxonomic units (OTU's) in the shallow, shallow-na and deep hand-dug wells of the Cuvelai Etosha Basin in the wet and dry seasons. The overall OTU's for shallow, shallow-na and deep hand-dug wells in the wet season were 807 634, 678 101 and 990 129 respectively. The overall OTU's for shallow, shallow-na and deep hand-dug wells in the dry season were 201 011, 174 558 and 251 870 respectively. Hence, the wet season had a total of 2 475 864 OTU's while the dry season had 627 439 OTU's.

4.3.1 Shallow hand-dug wells

Of the six shallow hand-dug wells investigated per season (wet and dry), it was revealed that three shallow hand-dug wells from Ohangwena region had overall abundances of 137 972, 68 978 and 94 105 operational taxonomic units (OTU's) in the wet season. The remaining three hand-dug wells from Omusati region had overall abundances of 133 697, 138 830 and 234 052 OTU's in the wet season. In the dry season, the three shallow hand-dug wells from Ohangwena region had overall abundances of 31 385, 40 260 and 37 720 OTU's, and three shallow hand-dug wells from Omusati region had overall abundances of 36 333, 31 630 and 23 683 OTU's.

4.3.2 Shallow-na hand-dug wells

A total of six shallow-na hand-dug wells were investigated for each season (wet and dry). The three shallow-na hand-dug wells from Ohangwena region had overall abundances of 61 730, 61 250 and 87 958 OTU's, and three shallow-na hand-dug wells from Omusati region had overall abundances of 196 443, 143 032 and 127 688 OTU's in the wet season. The dry season revealed that three shallow-na hand-dug wells from Ohangwena region had overall abundances of 21 674, 25 905 and 24 518 OTU's, and three shallow-na hand-dug wells from Omusati region had overall abundances of 23 109, 48 223 and 31 129 OTU's.

4.3.3 Deep hand-dug wells

A total of eight deep hand-dug wells were analysed for each season (wet and dry). The four deep hand-dug wells from Ohangwena region had overall abundances of 182 405, 74 383, 190 195 and 96 074 OTU's, and those from Omusati region had overall abundances of 175 430, 88 541, 83 357 and 99 744 OTU's in the wet season. In the dry season, the wells from Ohangwena region had overall abundances of 38 612, 45 323, 25 983 and 28 901 OTU's, and while those from Omusati region had overall abundances of 29 846, 33 142, 24 858 and 25 205 OTU's.

4.4 Relative abundance and seasonal variations of bacterial phyla

Across all hand-dug wells, 30 bacterial phyla were identified as indicated in Table 4.1. The relative abundance calculations of all these phyla showed that the predominant phyla were Proteobacteria (65.6%), Firmicutes (12.8%), Actinobacteria (7.94%), Bacteroidetes (7.48%), Cyanobacteria

(2.95%) and the rest had each a relative abundance less than one percent. Hence they were combined and represented as the “others” (3.17%) category (Figure 4.7).

Table 4.1: Shows the overall abundance based on counts of bacteria species belonging to particular phyla in the wet and dry seasons.

Phyla	**Abundance in wet season**	**Abundance in dry season**
Acetothermia	Not detected	28
Acidobacteria	8 770	708
Actinobacteria	210 728	54 451
Aquificae	Not detected	3
Bacteroidetes	164 459	80 504
Caldiserica	Not detected	3
Candidatus saccharibacteria	137	Not detected
Chlamydiae	19 947	1 325
Chlorobi	7	22
Chloroflexi	21 567	1 243
Cloacimonetes	Not detected	23
Cyanobacteria	12 782	1 437
Deferribacteres	Not detected	9
Deinococcus thermus	340	1 034
Elusimicrobia	Not detected	30
Fibrobacteres	Not detected	57
Firmicutes	341 110	70 345
Fusobacteria	6 419	113
Gemmatimonadetes	377	327
Ignavibacteriae	218	22
Lentisphaerae	524	8
Nitrospinae	Not detected	22
Nitrospirae	259	253
Planctomycetes	8 178	648
Proteobacteria	1 644 409	411 453
Spirochaetes	9 735	421
Synergistetes	Not detected	27
Tenericutes	4 255	293
Thermodesulfobacteria	703	19
Verrucomicrobia	20 940	2 551

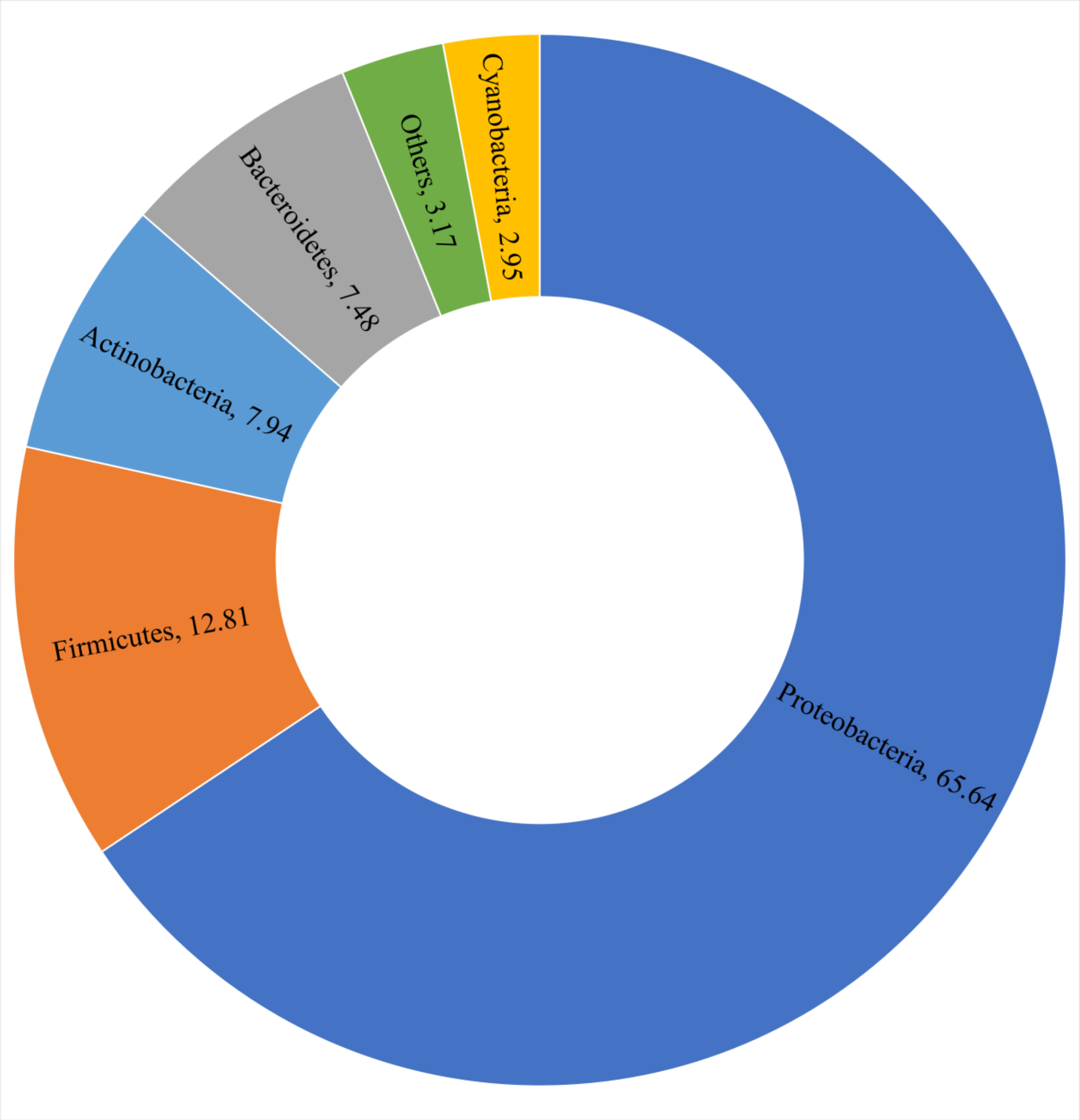

Figure 4.7: A depiction of the relative abundance (%) of all the dominant detected phyla in the Cuvelai Etosha Basin hand-dug wells of Namibia.

It was noted that water samples from the hand-dug wells in the wet season had 21 phyla while those from the dry season revealed 29 phyla (Table 4.1). The relative abundance calculations of phyla from the wet season showed that the predominant phyla were Proteobacteria (65.7%), Firmicutes (13.1%), Actinobacteria (7.80%), Bacteroidetes (6.50%) and Cyanobacteria (3.45%), and the rest had each a relative abundance less than one percent and were combined as "others"

(3.49%.) as shown in Figure 4.8. The relative abundance of the dominant (> 1%) phyla from the dry season were Proteobacteria (65.6%), Bacteroidetes (12.8%), Firmicutes (11.2%), Actinobacteria (8.68%), and "Others" (1.70%) as shown in Figure 4.9.

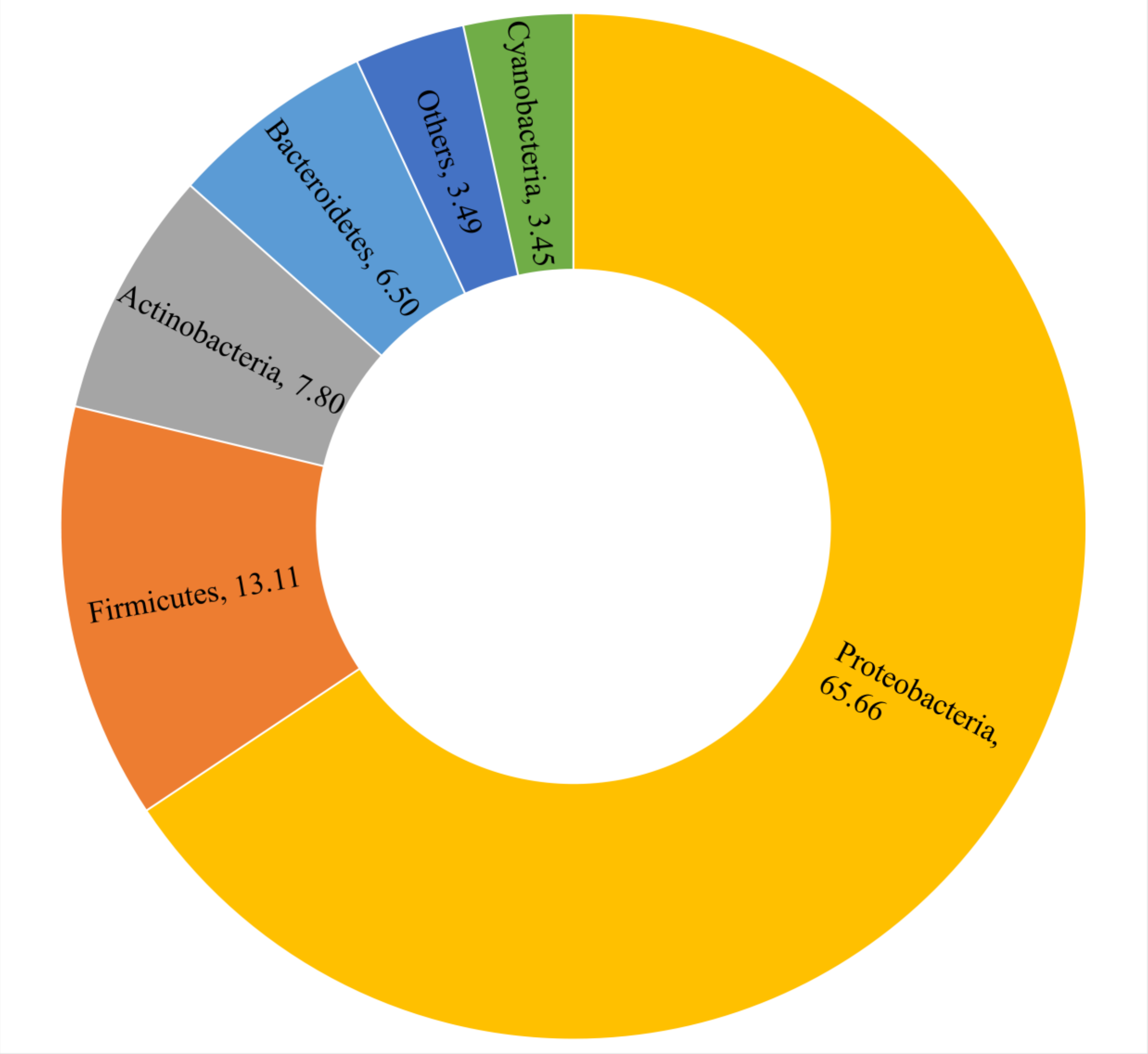

Figure 4.8: A depiction of the relative abundance (%) of the dominant detected phyla in the wet season from hand-dug wells in the Cuvelai Etosha Basin of Namibia.

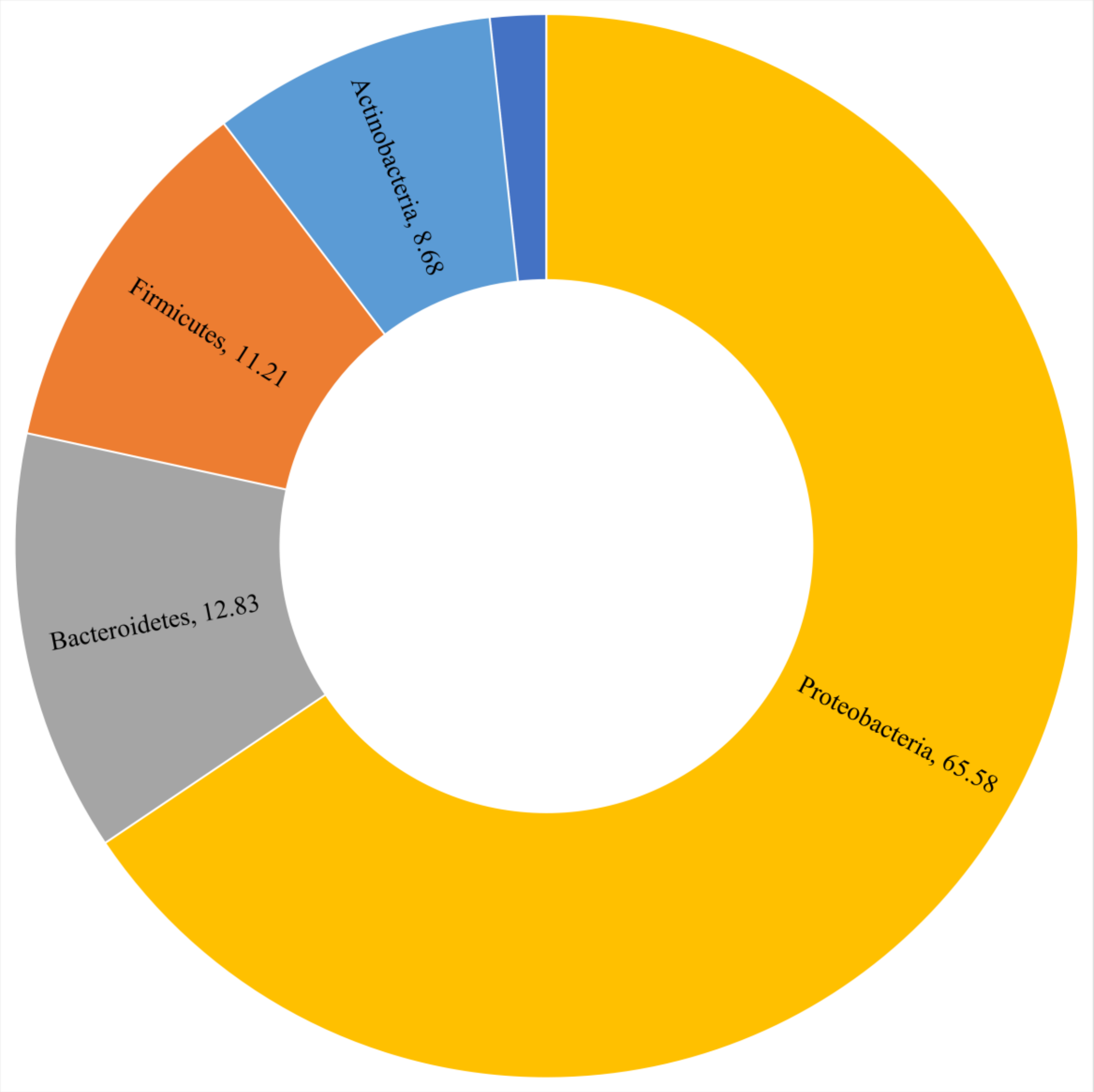

Figure 4.9: A depiction of the relative abundance (%) of the dominant detected phyla in the dry season from hand-dug wells in the Cuvelai Etosha Basin of Namibia.

4.5 Bacterial species diversity, richness and evenness

The bacterial species diversity, richness and evenness in the shallow, shallow-na and deep hand-dug wells of the Cuvelai Etosha Basin was explored using Metagenomics data. The averages of Shannon species diversity for all shallow, shallow-na and deep hand-dug wells in the wet season were 3.37, 2.89 and 3.10 respectively, while the averages of Simpson species diversity for all shallow, shallow-na and deep hand-dug wells in the wet season were 0.907, 0.916 and 0.919 respectively. The averages of species richness for all shallow, shallow-na and deep hand-dug wells in the wet season were 336, 301 and 315 respectively. The averages of species evenness for all shallow, shallow-na and deep hand-dug wells in the wet season were 0.576, 0.507 and 0.539 respectively.

In the dry season, the average Shannon species diversity for all shallow, shallow-na and deep hand-dug wells were 3.69, 3.39 and 3.16, respectively, while the average Simpson species diversity for all shallow, shallow-na and deep hand-dug wells were 0.931, 0.904 and 0.891, respectively. The averages of species richness for all shallow, shallow-na and deep hand-dug wells in the dry season were 554, 392 and 410 respectively. The average species evenness for all shallow, shallow-na and deep hand-dug wells in the dry season were 0.584, 0.570 and 0.527, respectively.

4.5.1 Shallow hand-dug wells

The six shallow hand-dug wells examined per season (wet and dry) revealed that three shallow hand-dug wells from Ohangwena region had Shannon diversity values ranging from 1.94 – 3.77, Simpson diversity values ranging from 0.755 – 0.969, richness values ranging from 276 – 334 and evenness values ranging from 0.345 – 0.648 in the wet season. The three shallow hand-dug wells from Omusati region had Shannon diversity values ranging from 3.81 – 4.41, Simpson diversity values ranging from 0.964 – 0.978, richness values ranging from 347 – 397 and evenness values ranging from 0.651 – 0.736 in the wet season. In the dry season, three shallow hand-dug wells from Ohangwena region had Shannon diversity values ranging from 2.65 – 4.46, Simpson diversity values ranging from 0.863 – 0.969, richness values ranging from 429 – 665 and evenness values ranging from 0.437 – 0.686, and three shallow hand-dug wells from Omusati region had Shannon diversity values ranging from 3.49 – 4.21, Simpson diversity values ranging from 0.913 – 0.963, richness values ranging from 504 – 654 and evenness values ranging from 0.560 – 0.650.

4.5.2 Shallow-na hand-dug wells

The three shallow-na hand-dug wells from Ohangwena region had Shannon diversity values ranging from 2.62 – 2.88, Simpson diversity values ranging from 0.898 – 0.928, richness values ranging from 246 – 302 and evenness values ranging from 0.475 – 0.507 in the wet season. The three shallow-na hand-dug wells from Omusati region had Shannon diversity values ranging from 2.79 – 3.23, Simpson diversity values ranging from 0.887 – 0.939, richness values ranging from 319 – 355 and evenness values ranging from 0.483 – 0.550 in the wet season. In the dry season, three shallow-na hand-dug wells from Ohangwena region had Shannon diversity values ranging from 2.61 – 3.29, Simpson diversity values ranging from 0.835 – 0.927, richness values ranging from 245 – 402 and evenness values ranging from 0.435 – 0.575, and three shallow-na hand-dug wells from Omusati region had Shannon diversity values ranging from 3.48 – 4.17, Simpson diversity values ranging from 0.912 – 0.955, richness values ranging from 372 – 563 and evenness values ranging from 0.586 – 0.658.

4.5.3 Deep hand-dug wells

A total of eight deep hand-dug wells were studied per season (wet and dry) in Ohangwena and Omusati regions. The four deep hand-dug wells from Ohangwena region had Shannon diversity values ranging from 2.48 – 3.38, Simpson diversity values ranging from 0.828 – 0.945, richness values ranging from 266 – 388 and evenness values ranging from 0.423 – 0.586 in the wet season. The four deep hand-dug wells from Omusati region had Shannon diversity values ranging from 2.86 – 3.71, Simpson diversity values ranging from 0.909 – 0.956, richness values ranging from 283 – 351 and evenness values ranging from 0.507 – 0.633 in the wet season. In the dry season, the four deep hand-dug wells from Ohangwena region had Shannon diversity values ranging from 2.84 – 3.86, Simpson diversity values ranging from 0.821 – 0.946, richness values ranging from 400 – 517 and evenness values ranging from 0.454 – 0.645, and four deep hand-dug wells from Omusati region had Shannon diversity values ranging from 2.86 – 3.38, Simpson diversity values ranging from 0.859 – 0.932, richness values ranging from 295 – 479 and evenness values ranging from 0.464 – 0.582.

4.6 The effect of hand-dug well type, region and season on the abundance or presence of bacteria

The influence of hand-dug well type, region and season on the abundance of bacteria was determined using colony forming units and relative abundances of bacterial phyla data. The relationship between hand-dug well type, region and season, and particular genera (*Escherichia*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species) was determined. Since season produces notable changes in physicochemical parameters, the effect of seasonality in physicochemical parameters on the abundance of the detected bacterial phyla was determined. In addition, the influence of hand-dug well type, region and season on bacterial species diversity, richness and evenness was also determined.

4.6.1 The effect of hand-dug well type, region and season on the abundance of bacterial colony forming units (CFU)

This study recorded total coliform counts ranging from 160 CFU/ml to 297 CFU/ml in the wet season, and 110 CFU/ml to 243 CFU/ml in the dry season. The CFU data was not normally distributed ($P < 0.05$) and analysis indicated a statistically significant difference ($P < 0.05$) in the abundance of bacterial CFU's in the shallow, shallow-na and deep hand-dug wells of the Cuvelai Etosha Basin of Namibia (test statistic; 18.8, d.f; 2, $P < 0.01$). Although the bar chart in Figure 4.2 illustrated visible differences in the abundance of bacterial CFU's between Ohangwena and Omusati regions, there was no significant difference in the abundance of bacterial CFU's between Ohangwena and Omusati regions ($P > 0.05$). The wet season had a higher abundance of bacterial CFU's compared to the dry season (Figures 4.1 and 4.2). However, there was a significant difference in the abundance of bacterial CFU's between the wet and dry seasons ($P < 0.05$).

4.6.2 The effect of hand-dug well type, region and season on the presence of coliforms, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species

On the basis of hand-dug well type, region and season, there was no significant difference in the presence of *Escherichia* and *Klebsiella* species ($P > 0.05$, Tables; 4.2, 4.3 and 4.4). There was no significant difference in the presence of *Salmonella* species with regards to hand-dug well type and region ($P > 0.05$, Tables; 4.2 and 4.3), but a significant difference was observed based on season ($P < 0.05$, Table 4.4). There was no significant difference in the presence of *Shigella* species based on hand-dug well type and region ($P > 0.05$, Tables; 4.2 and 4.3), but a significant difference was observed based on season ($P < 0.05$, Table 4.4). Based on hand-dug well type, region and season, there was no significant difference in the presence of *Enterobacter* species ($P > 0.05$, Tables; 4.2, 4.3 and 4.4). There was no significant difference in the presence of *Citrobacter* species on the basis of hand-dug well type, region and season ($P > 0.05$, Tables; 4.2, 4.3 and 4.4). It was shown that there was no significant difference in the presence of *Pseudomonas* species based on region and season ($P > 0.05$, Tables; 4.3 and 4.4), but a significant difference existed in terms of hand-dug well type ($P < 0.05$, Table 4.2). There was no significant difference in the presence of

Proteus species on the basis of hand-dug well type, region and season (P > 0.05, Tables; 4.2, 4.3 and 4.4). In addition, *Proteus* species were only detected in two hand-dug wells in the wet season.

Table 4.2: Influence of hand-dug well type on the presence of *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas* and coliform species.

Bacterial species	X^2- value	Deg. of freedom	P-value
Citrobacter	6.29	2	0.430
Escherichia	2.13	2	0.346
Klebsiella	0.346	2	0.841
Enterobacter	4.61	2	0.100
Proteus	2.69	2	0.261
Salmonella	5.30	2	0.071
Shigella	5.86	2	0.053
Pseudomonas	6.76	2	0.034

Table 4.3: Influence of region on the presence of *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas* and coliforms species.

Bacterial species	X^2- value	Deg. of freedom	P-value
Citrobacter	2.46	1	0.117
Escherichia	0.957	1	0.332
Klebsiella	0.341	1	0.560
Enterobacter	0.296	1	0.587
Proteus	2.68	1	0.167
Salmonella	3.77	1	0.060
Shigella	3.50	1	0.062
Pseudomonas	0.783	1	0.377

Table 4.4: Influence of season on the presence of *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas* and coliforms species.

Bacterial species	X^2- value	Deg. of freedom	P-value
Citrobacter	0.000	1	1.000
Escherichia	0.005	1	0.946
Klebsiella	0.068	1	0.795
Enterobacter	2.30	1	0.132
Proteus	2.50	1	0.186
Salmonella	8.06	1	0.016
Shigella	15.1	1	<0.001
Pseudomonas	3.18	1	0.076

4.6.3 Effect of hand-dug well type, region and season on the relative abundance of bacterial phyla

The bacterial phyla data was not normally distributed ($P < 0.05$). The assessment of the effect of hand-dug well type and region on the relative abundance of bacterial phyla did not yield any significant results. However, the results revealed that there was a highly significant difference in Actinobacteria abundance between the wet and dry season ($P < 0.01$, Table 4.5). It was noted that Actinobacteria were the only class detected in the Actinobacteria phylum, and Actinomycetales were the only order detected in the Actinobacteria class in both the dry and wet seasons. Relative abundances of families from the order Actinomycetales for the wet season indicated that; Actinomycetaceae (6.67%), Corynebacteriaceae (62.6%), Dietziaceae (18.1%), Mycobacteriaceae (1.05%), Micromonosporaceae (1.34%), Nocardioidaceae (6.54%) and Streptomycetaceae (2.74%) were dominant. In the dry season, it was found that Actinomycetaceae (8.49%), Corynebacteriaceae (5.38%), Dietziaceae (2.45%), Mycobacteriaceae (2.12%), Micromonosporaceae (8.73%), Nocardioidaceae (7.58%), Pseudonocardiaceae (2.00%) and Streptomycetaceae (61.3%) were dominant.

Bacteroidetes were among the dominant phyla in hand-dug wells but no significant seasonal difference was observed ($P > 0.05$, Table 4.5). At class level, there were also no significant differences in the abundance within classes of Bacteroidetes although a slight increase in relative abundance of Bacteroidia and Sphingobacteria in the wet season was observed as follows; Bacteroidia (27.4%), Cytophagia (22.2%), Flavobacteria (13.3%), and Sphingobacteria (37.1%), while the dry season had Bacteroidia (6.25%), Cytophagia (32.2%), Flavobacteria (40.9%), and Sphingobacteria (20.7%). Within the order Bacteroidales, the families Bacteroidaceae (24.6%), Marinilabiliaceae (2.87%), Porphyromonadaceae (52.9%), Prolixibacteraceae (3.78%) and Rikenellaceae (15.6%) had significant relative abundances in the wet season, and families Bacteroidaceae (17.1%), Marinilabiliaceae (12.8%), Porphyromonadaceae (45.2%), Prevotellaceae (7.64%), Prolixibacteraceae (3.36%) and Rikenellaceae (13.9%) had significant relative abundances in the dry season.

Cyanobacteria were also among the dominant phyla and had a significant seasonal difference based on sequence counts ($P < 0.05$, Table 4.5). Cyanobacteria had a higher relative abundance in the wet season (3.45%, Figure 4.8) compared to the dry season (< 1%, Figure 4.9). Since the Cyanobacteria class was unassigned, the analysis proceeded to order level. At order level, it was observed that Oscillatoriales (90.4%) had the highest relative abundance followed by Chroococcales (6.43%) and Prochlorales (3.13%). Oscillatoriales and Chroococcales could not be identified to family level. Within the order Prochlorales, only the family Prochlorococcaceae was present.

Relative abundance results of hand-dug wells indicated that Firmicutes were part of the dominant phyla in both the wet (13.1%, Figure 4.8) and dry (11.2%, Figure 4.9) seasons. This study found a highly significant difference ($P < 0.01$, Table 4.5) in the abundance of Firmicutes between the wet and dry seasons. The wet season recorded a higher relative abundance (13.1%) than the dry (11.2%) season. Relative abundances of Firmicutes classes revealed that Bacilli (84.7%), Clostridia (15.5%) and Erysipelotrichia (2.55%) were dominant (>1%) in the dry season, and Bacilli (39.9%), Clostridia (56.5%), Erysipelotrichia (1.91%), and Negativicutes (1.67%) were dominant (>1%) in the wet season. Relative abundances at the order level for the dry season showed that within the class Bacilli, Bacillales (77.6%) and Lactobacillales (22.4%) were dominant and in the class Clostridia, Clostridiales (97.9%) and Thermoanaerobacterales (2.14%) were dominant. The class Erysipelotrichia only had Erysipelotrichales. However, relative abundances at order level for the wet season indicated that within the class Bacilli, Bacillales (69.0%) and Lactobacillales (31.1%) were dominant. Within the class Clostridia, only Clostridiales (99.4%) were dominant. The class Erysipelotrichia only had Erysipelotrichales, and the class Negativicutes only had the order Selenomonadales.

Relative abundances of families within respective orders for the dry season indicated that within the Bacillale, Bacillaceae (43.1%), Paenibacillaceae (1.13%), Planococcaceae (53.4%) and Staphylococcaceae (2.14%) were dominant (>1%). Within the Lactobacillales, Carnobacteriaceae (7.28%), Enterococcaceae (2.76%), Lactobacillaceae (56.0%), Leuconostocaceae (28.5%) and Streptococcaceae (5.42%) were dominant. Within the Clostridiales, Clostridiaceae (61.5%), Eubacteriaceae (4.52%), Lachnospiraceae (1.95%), Peptococcaceae (5.48%), Peptostreptococcaceae (14.7%) and Ruminococcaceae (10.8%) were dominant, while Erysipelotrichaceae is the only family in the Erysipelotrichales order that was dominant.

Relative abundances of families within individual orders of the wet season indicated that; within the Bacillale, Bacillaceae (16.0%), Planococcaceae (9.34%) and Staphylococcaceae (74.5%) were dominant. Within the Lactobacillales, Carnobacteriaceae (8.47%), Enterococcaceae (7.74%), Lactobacillaceae (16.0%), Leuconostocaceae (32.8%) and Streptococcaceae (34.0%) were dominant. Within the Clostridiales, Clostridiaceae (89.3%), Eubacteriaceae (2.26%), Lachnospiraceae (2.62%), Peptococcaceae (1.26%), Ruminococcaceae (3.06%) and Syntrophomonadaceae (1.19%) were dominant, and Erysipelotrichaceae was the only family in the Erysipelotrichales order that was dominant. The Selenomonadales data did not give resolution at family level. It was disclosed that Proteobacteria was the most abundant phylum inhabiting hand-dug wells in both the wet (65.7%) and dry (65.6%) seasons. In addition, there was highly significant ($P < 0.010$, Table 4.5) difference in the abundance of Proteobacteria between the wet and dry seasons.

Relative abundance of Proteobacteria classes revealed that Gammaproteobacteria (45.1%), Betaproteobacteria (36.4%), Alphaproteobacteria (11.4%), Epsilonproteobacteria (5.14%), and Deltaproteobacteria (1.88%) were dominant (>1%) in the wet season, and Betaproteobacteria (44.7%), Alphaproteobacteria (28.1%), Gammaproteobacteria (17.8%), Epsilonproteobacteria (8.24%) and Deltaproteobacteria (1.19%) were dominant (>1%) in the dry season. The relative abundance of orders within respective classes for the wet season showed that within the Alphaproteobacteria, Caulobacterales (3.23%), Rhizobiales (28.0%), Rhodobacterales (37.9%), Rhodospirillales (2.93%) and Sphingomonadales (27.3%) were dominant. Within the Betaproteobacteria, Burkholderiales (85.2%), Methylophilales (2.64%), Neisseriales (1.96%), Nitrosomonadales (1.46%), and Rhodocyclales (8.46%) were dominant. Within the Deltaproteobacteria, Bdellovibrionales (19.7%), Desulfobacterales (17.6%), Desulfovibrionales (2.45%), Desulfuromonadales (15.4%), Myxococcales (17.7%) and Syntrophobacterales (27.1%) were dominant. Within the Epsilonproteobacteria, Campylobacterales were the only detected order. Within the Gammaproteobacteria, Alteromonadales (2.18%), Chromatiales (2.88%), Enterobacteriales (14.4%), Methylococcales (3.26%), and Pseudomonadales (76.3%) were dominant.

The dry season revealed that within the Alphaproteobacteria, Rhizobiales (8.11%), Rhodobacterales (68.2%), Rhodospirillales (2.22%) and Sphingomonadales (19.8%) were dominant. Within the Betaproteobacteria, Burkholderiales (86.4%), Methylophilales (3.20%), Neisseriales (1.76%), and Rhodocyclales (8.23%) were dominant. Within the Deltaproteobacteria, Bdellovibrionales (10.7%), Desulfobacterales (10.7%), Desulfovibrionales (2.95%), Desulfuromonadales (58.3%), Myxococcales (9.90%) and Syntrophobacterales (7.43%) were dominant. Within Epsilonproteobacteria, Campylobacterales were the only detected order. Within Gammaproteobacteria, Alteromonadales (1.93%), Chromatiales (14.3%), Enterobacteriales (2.85%), Legionellales (2.82%), Methylococcales (2.66%), Oceanospirillales (11.5%), Pseudomonadales (60.8%), Thiotrichales (1.30%) and Vibrionales (1.19%) were dominant.

Relative abundances of families within respective orders for the wet season indicated that within the Rhizobiales, Beijerinckiaceae (3.19%), Bradyrhizobiaceae (37.0%), Brucellaceae (5.81%), Hyphomicrobiaceae (22.8%), Methylobacteriaceae (15.4%) and Rhizobiaceae (15.8%) were dominant. The Caulobacterales, Enterobacteriales, Methylophilales, Nitrosomonadales, Rhodobacterales, Rhodocyclales and Syntrophobacterales had one family each; Caulobacteraceae, Enterobacteriaceae, Methylophilaceae, Nitrosomonadaceae, Rhodobacteraceae, Rhodocyclaceae and Syntrophobacteraceae respectively. In the order Rhodospirillales, Acetobacteraceae (54.0%) and Rhodospirillaceae (46.0%) were dominant. Within the Sphingomonadales, Erythrobacteraceae (17.0%) and Sphingomonadaceae (83.0%) were dominant. In the order Burkholderiales, Alcaligenaceae (3.78%), Burkholderiaceae (21.8%), Comamonadaceae (61.8%) and Oxalobacteraceae (12.6%) were dominant. Within the Neisseriales, Chromobacteriaceae (58.3%) and Neisseriaceae (41.7%) were dominant.

In the order Bdellovibrionales, Bacteriovoracaceae (14.6%) and Bdellovibrionaceae (85.4%) were dominant. Within the Desulfobacterales, Desulfobacteraceae (39.7%) and Desulfobulbaceae (60.3%) were dominant. Within the Desulfovibrionales, Desulfonatronumaceae (26.6%) and Desulfovibrionaceae (73.4%) were dominant. Within the Desulfuromonadales, Desulfuromonadaceae (1.28%) and Geobacteraceae (98.7%) were dominant. Within the Myxococcales, Nannocystaceae (12.2%), Polyangiaceae (74.3%) and Sandaracinaceae (13.6%) were dominant. Within the Campylobacterales, Campylobacteraceae (89.5%) and Helicobacteraceae (10.5%) were dominant. Within the Alteromonadales, Alteromonadaceae (18.3%) and Shewanellaceae (81.7%) were dominant. Within the Chromatiales, Chromatiaceae (19.4%), Ectothiorhodospiraceae (14.1%) and Halothiobacillaceae (66.5%) were dominant. Within the Methylococcales, Methylococcaceae (100%) was the only dominant. Within the Pseudomonadales, Moraxellaceae (30.4%) and Pseudomonadaceae (69.6%) were dominant.

The dry season indicated that within the Rhizobiales, Bradyrhizobiaceae (1.99%), Brucellaceae (4.97%), Hyphomicrobiaceae (49.0%), Methylobacteriaceae (11.0%), Methylocystaceae (10.6%), Phyllobacteriaceae (3.87%), Rhizobiaceae (15.9%) and Xanthobacteraceae (2.39%) were dominant. The Enterobacteriales, Rhodobacterales, Methylophilales, Rhodocyclales and Vibrionales had one family each; Enterobacteriaceae, Rhodobacteraceae, Methylophilaceae, Rhodocyclaceae and Vibrionaceae respectively. Within the Rhodospirillales, Acetobacteraceae (21.1%) and Rhodospirillaceae (78.9%) were dominant. Within the Sphingomonadales, Erythrobacteraceae (9.83%) and Sphingomonadaceae (90.2%) were dominant. Within the Burkholderiales, Alcaligenaceae (5.66%), Burkholderiaceae (7.51%), Comamonadaceae (84.8%) and Oxalobacteraceae (2.03%) were dominant. Within the Neisseriales, Chromobacteriaceae (32.0%) and Neisseriaceae (68.0%) were dominant. Within the Bdellovibrionales, Bacteriovoracaceae (14.5%) and Bdellovibrionaceae (85.5%) were dominant. Within the Desulfobacterales, Desulfobacteraceae (89.0%), Desulfobulbaceae (6.74%) and Nitrospinaceae (4.24%) were dominant. Within the Desulfovibrionales, Desulfomicrobiaceae (24.1%), Desulfonatronumaceae (17.5%) and Desulfovibrionaceae (58.4%) were dominant.

Within the Desulfuromonadales, Geobacteraceae (99.5%) was the only dominant family. Within the Myxococcales, Cystobacteraceae (12.0%), Myxococcaceae (2.60%), Kofleriaceae (26.0%), Nannocystaceae (27.0%), Phaselicystidaceae (5.52%), Polyangiaceae (25.7%) and Sandaracinaceae (1.30%) were dominant. Within the Syntrophobacterales, Syntrophaceae (59.1%) and Syntrophobacteraceae (40.9%) were dominant. Within the Campylobacterales, Campylobacteraceae (65.5%) and Helicobacteraceae (34.5%) were dominant. Within the

Alteromonadales, Alteromonadaceae (87.4%), Idiomarinaceae (9.72%) and Pseudoalteromonadaceae (2.12%) were dominant. Within the Chromatiales, Chromatiaceae (10.5%), Ectothiorhodospiraceae (8.38%) and Halothiobacillaceae (80.7%) were dominant. Within the Legionellales, Coxiellaceae (65.2%) and Legionellaceae (34.8%) were dominant. Within the Methylococcales, Methylococcaceae (100%) was the only dominant. Within the Oceanospirillales, Halomonadaceae (3.01%) and Oceanospirillaceae (96.0%) were dominant. Within the Pseudomonadales, Moraxellaceae (93.0%) and Pseudomonadaceae (7.03%) were dominant. Within the Thiotrichales, Francisellaceae (2.11%), Piscirickettsiaceae (72.6%) and Thiotrichaceae (25.3%) were dominant.

Table 4.5: Influence of season on the abundance of all detected bacterial phyla.

Phyla	**Z value**	**P – Value**
Acetothermia	-1.00	0.317
Acidobacteria	-280	0.779
Actinobacteria	-3.55	0.000
Aquificae	-1.00	0.317
Bacteroidetes	-896	0.370
Caldiserica	-1.00	0.317
Candidatus saccharibacteria	-2.20	0.028
Chlamydiae	-0.443	0.658
Chlorobi	-0.355	0.723
Chloroflexi	-1.03	0.305
Cloacimonetes	-1.34	0.180
Cyanobacteria	-2.28	0.023
Deferribacteres	-1.84	0.066
Deinococcus thermus	-3.10	0.002
Elusimicrobia	-2.26	0.024
Fibrobacteres	-1.84	0.066
Firmicutes	-3.02	0.002
Fusobacteria	-0.328	0.743
Gemmatimonadetes	-2.83	0.005
Ignavibacteriae	-0.339	0.735
Lentisphaerae	-0.265	0.791
Nitrospinae	-1.34	0.180
Nitrospirae	-1.93	0.053
Planctomycetes	-0.322	0.748
Proteobacteria	-3.92	0.000
Spirochaetes	-1.27	0.204
Synergistetes	-1.60	0.109
Tenericutes	-1.20	0.232

Thermodesulfobacteria	-0.948	0.343
Verrucomicrobia	-1.06	0.287

4.6.4 Seasonal changes in physicochemical parameters on the abundance of the detected bacterial phyla

The NMS analysis revealed that phosphate (r = 0.658, tau = 0.518), manganese (r = 0.468, tau = 0.290), potential of hydrogen (r = 0.835, tau = 0.631), and temperature (r = -0.855, tau = -0.631) were the main physicochemical factors responsible for the abundance of bacterial phyla in the hand-dug wells in the wet and dry seasons (Figures 4.10 and 4.11).

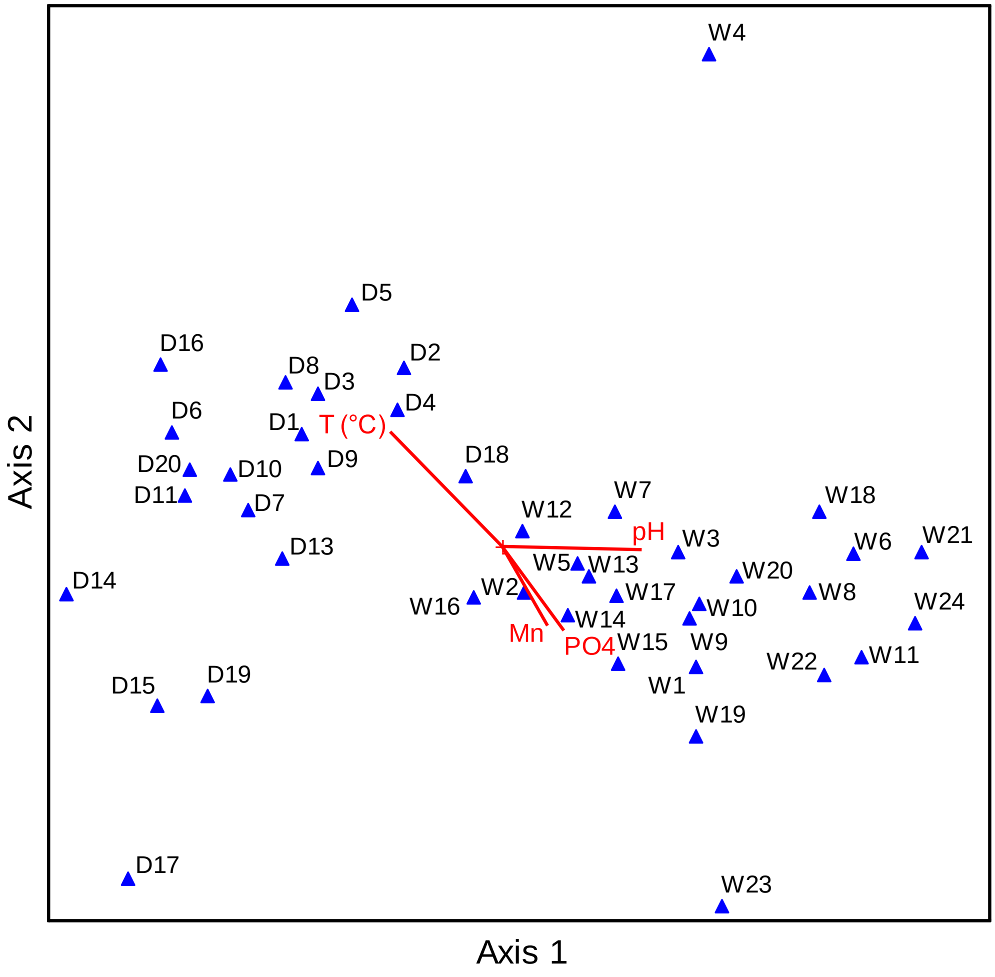

Figure 4.10: Depicts the correlation between the hand-dug well samples in the wet and dry seasons, and the major physicochemical parameters influencing bacterial phyla abundance (D = dry season samples, W = wet season samples).

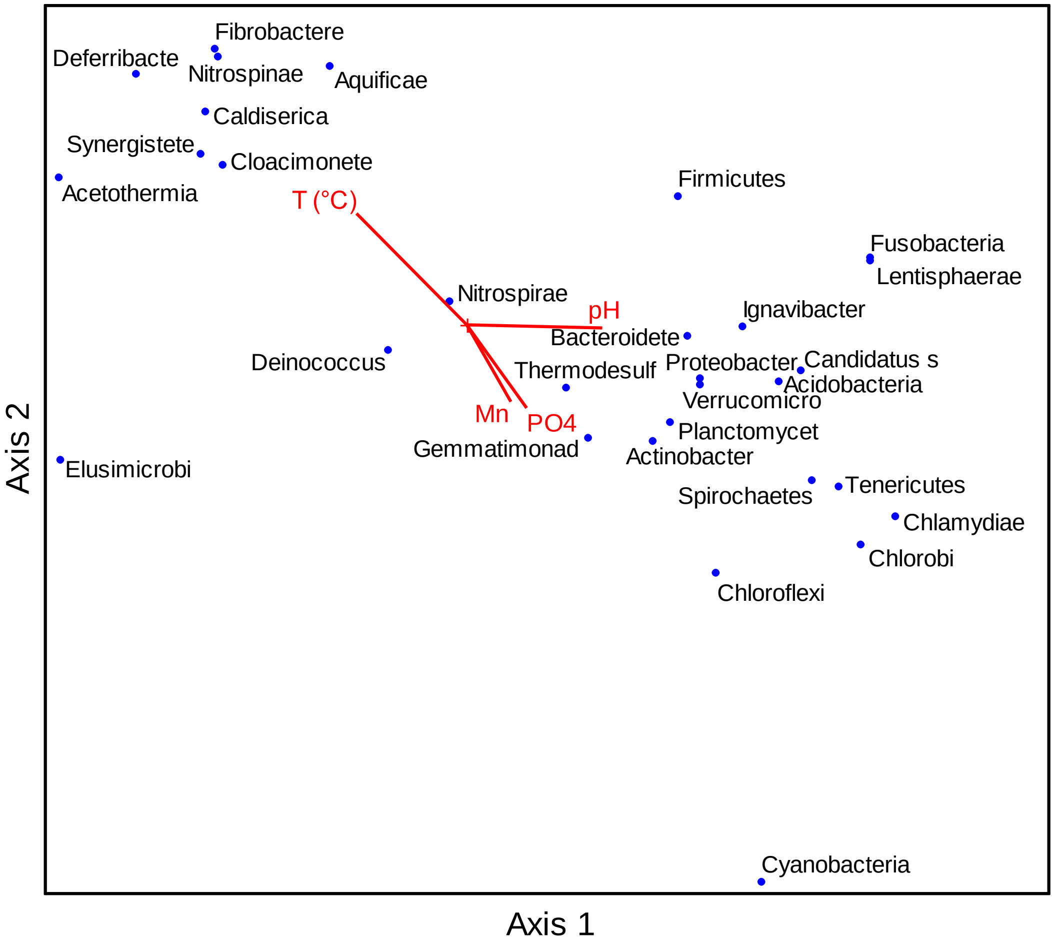

Figure 4.11: Depicts the major physicochemical parameters contributing to the abundance of the detected bacterial phyla in hand-dug well samples from the wet and dry seasons.

The abundance of bacteria in the dry season was associated with temperature while in the wet season it was associated with pH, PO_4^{3-} and Mn^{2+} as indicated by Figures 4.10 and 4.11. The pH values ranged from 7.18 to 8.31 in the wet season and 5.68 to 8.34 in the dry season, and temperature values ranged from 13.2° C to 26.3° C in the wet season and 20.5° C to 34.6° C in the dry season. The PO_4^{3-} values ranged from 0.03 mg/l to 6.05 mg/l in the wet season and 0.00 mg/l to 3.34 mg/ in the dry season, and Mn^{2+} values ranged from 0.00 mg/l to 0.93 mg/l in the wet season and 0.00 mg/l to 0.39 mg/l in the dry season.

The phyla Acetothermia, Aquificae, Caldiserica, Cloacimonetes, Deferribacteres, Deinococcus-Thermus, Elusimicrobia, Fibrobacteres, Nitrospinae, Nitrospirae and Synergistetes henceforth referred to as the cluster of phyla from the dry season, were associated with hand-dug well samples from the dry season. The phyla Acidobacteria, Actinobacteria, Bacteroidetes, Candidatus saccharibacteria, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria, Firmicutes, Fusobacteria, Gemmatimonadetes, Ignavibacteriae, Lentisphaerae, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes, Thermodesulfobacteria and Verrucomicrobia henceforth referred to as the cluster of phyla from the wet season, were associated with hand-dug well samples from the wet season (Figure 4.11).

4.6.5 Effect of hand-dug well type, region and season on bacterial species diversity, richness and evenness

The species diversity and species evenness data were normally distributed ($P > 0.05$), and analysis showed that there was no significant difference between species diversity, and species evenness of bacterial species in the wet and dry seasons (Table 4.6). The species richness data were not normally distributed ($P < 0.05$) and analysis showed that bacterial species richness differed significantly between the wet and dry seasons ($P < 0.05$, Table 4.6). There was no significant difference in bacterial species evenness between the Ohangwena and Omusati regions ($P > 0.05$, Table 4.7). There was no significant difference in species richness between the Ohangwena and Omusati regions ($P > 0.05$, Table 4.7). There was no significant difference in bacterial species diversity and species richness among the shallow, shallow-na and deep hand-dug wells ($P > 0.05$, Tables 4.8 and 4.10 respectively). There was no significant difference in bacterial species diversity and species evenness among the shallow, shallow-na and deep hand-dug wells ($P > 0.05$, Table 4.9 and 4.11 respectively).

Table 4.6: Influence of season on bacterial species richness (R), evenness (E) and diversity [Shannon (H') and Simpson (D)].

		Mean	**St.Dev**	**Test statistics value**	**df**	**P-value**
Wet season	**H'**	3.12	0.652	T=-1.45	19	$P > 0.05$
	D	0.914	0.058	Z=-0.373	19	$P > 0.05$
	R	317	44.8	Z=-3.36	19	$P < 0.05$
	E	0.541	0.103	T=-0.599	19	$P > 0.05$
Dry season	**H'**	3.33	0.536	T=-1.45	19	$P > 0.05$
	D	0.904	0.046	Z=-0.373	19	$P > 0.05$

	R	436	110	Z=-3.36	19	P < 0.05
	E	0.557	0.077	T=-0.599	19	P > 0.05

Table 4.7: Influence of region on bacterial species richness (R), evenness (E) and diversity [Shannon (H') and Simpson (D)].

	Parameter	**Mean**	**St.Dev**	**Test statistics value**	**df**	**P-value**
Ohangwena region	**H'**	3.21	0.683	T = -0.472	9.03	P > 0.05
	D	0.9	0.063	U = 221	19	P > 0.05
	R	392	98.7	U = 159	19	P > 0.05
	E	0.539	0.108	T = -0.871	8.8	P > 0.05
Omusati region	**H'**	3.33	0.536	T = -0472	9.03	P > 0.05
	D	0.904	0.046	U = 221	19	P > 0.05
	R	436	110	U = 160	19	P > 0.05
	E	0.557	0.077	T = -0.871	8.8	P > 0.05

Table 4.8: Influence of hand-dug well type on bacterial species diversity.

	Shannon Diversity				
One-way ANOVA	**mean**	**st-dev**	**F-value**	**df**	**P-value**
Shallow	3.12	0.924	0.230	2.00	P > 0.05
Shallow-na	3.29	0.520	0.122	2.00	P > 0.05
Deep	3.29	0.521	0.122	2.00	P > 0.05

Table 4.9: Influence of hand-dug well type on bacterial species diversity.

	Simpson diversity index				
Kruskal-Wallis test	**mean**	**st-dev**	**H-value**	**df**	**P-value**
Shallow	0.891	0.079	0.328	2.00	P > 0.05
Shallow-na	0.922	0.035	0.328	2.00	P > 0.05
Deep	0.907	0.050	0.328	2.00	P > 0.05

Table 4.10: Influence of hand-dug well type on bacterial species richness.

	Species richness				
One-way ANOVA	**mean**	**st-dev**	**F-value**	**df**	**P-value**
Shallow	422	145	1.89	2.00	P > 0.05
Shallow-na	365	111	1.89	2.00	P > 0.05
Deep	382	70.1	1.89	2.00	P > 0.05

Table 4.11: Influence of hand-dug well type on bacterial species evenness.

	Species evenness				
Kruskal-Wallis test	**mean**	**st-dev**	**H-value**	**df**	**P-value**
Shallow	0.516	0.132	0.662	2.00	P > 0.05
Shallow-na	0.559	0.070	0.662	2.00	P > 0.05
Deep	0.555	0.090	0.662	2.00	P > 0.05

4.7 Metagenomics of pathogenic bacteria detected in hand-dug wells

Of the entire bacterial communities detected in the hand-dug wells, 181 species are known human pathogens, five species are known livestock pathogens and 66 species were known zoonotic pathogens. The relationship of bacterial species within; the human pathogens group, livestock pathogens group and zoonotic pathogens group was explored using phylogenetic trees. The effect of hand-dug well type and region on the abundance of human, livestock and zoonotic pathogens did not reveal notable trends (see Appendices). However, the effect of season on the abundance of human, livestock and zoonotic pathogens was determined and is outlined below.

4.7.1 Human pathogens detected

The human pathogen's phylogenetic tree revealed the relationship between the detected human pathogens (Figure 4.12). It indicated that *Lactococcus garvieae, Lactococcus lactis, Lactococcus sp., Streptococcus lutetiensis, Streptococcus gordonii, Streptococcus sanguinis, Leuconostoc pseudomesenteroides, Lactobacillus iners, Lactobacillus paraplantarum, Lactobacillus fermentum, Lactobacillus plantarum, Enterococcus faecalis, Bacillus parabrevis, Brevibacillus sp., Bacillus coagulans, Sporosarcina spp., Exiguobacterium aurantiacum, Exiguobacterium sp., Libanicoccus massiliensis* and *Globicatella sanguinis* formed a cluster at 77% bootstrap. *L. garvieae, L. lactis, Lactococcus sp., S. lutetiensis, S. gordonii* and *S. sanguinis* formed a sub-cluster at 84% bootstrap within which *S. lutetiensis, S. gordonii* and *S. sanguinis* formed a smaller sub-cluster at 71% bootstrap, and *L. paraplantarum, L. fermentum* and *L. plantarum* formed a sub-cluster at 77% bootstrap. *B. parabrevis* and *Brevibacillus sp.* formed a sub-cluster at 89% bootstrap, and *E. aurantiacum* and *Exiguobacterium sp.* formed a sub-cluster at 79% bootstrap. *Anaerococcus sp.* and *F. magna* formed a cluster at 77% bootstrap.

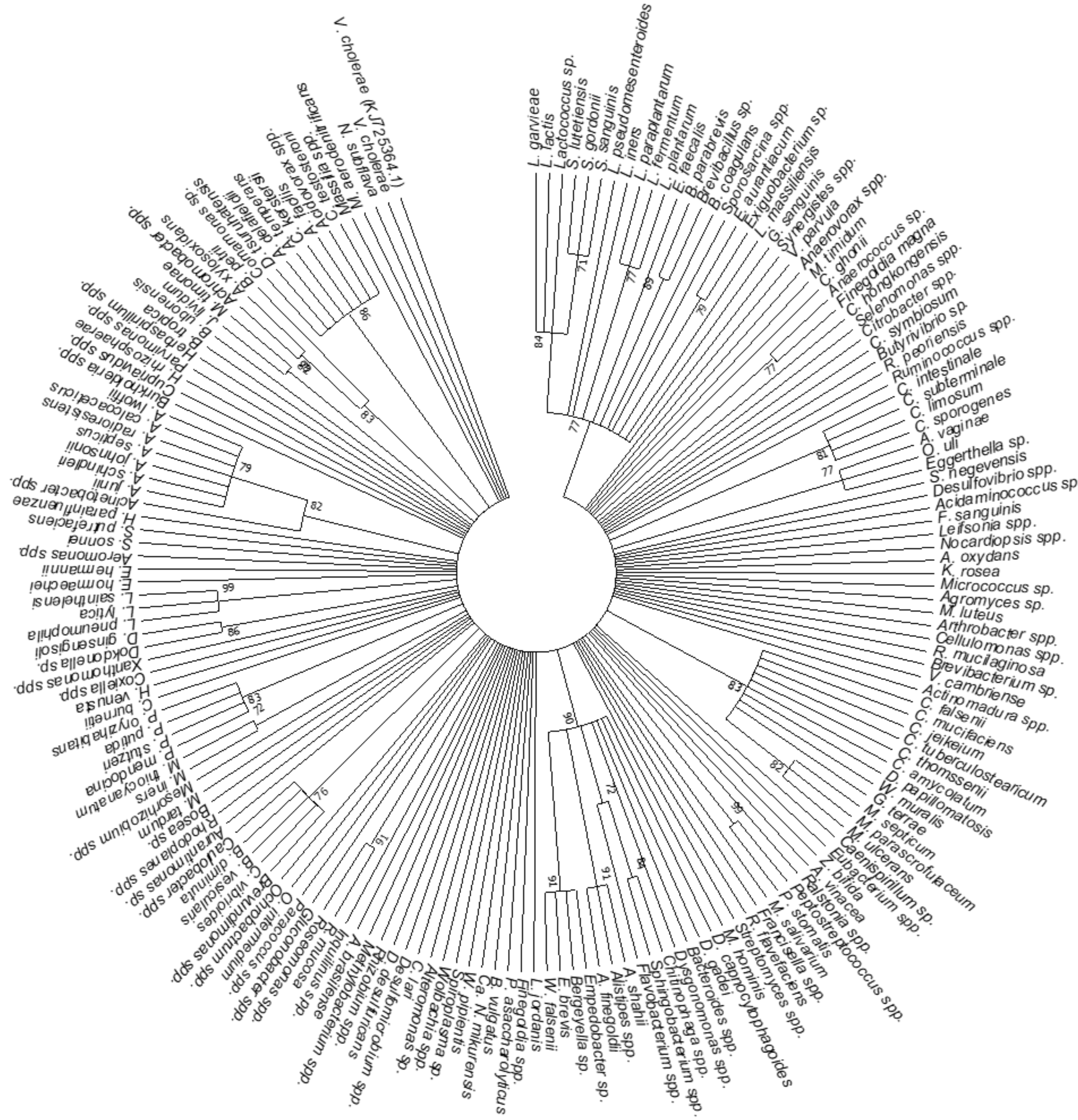

Figure 4.12: Phylogenetic tree depicting the evolutionary history of the detected human bacterial pathogens. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates were collapsed.

Clostridium intestinale, Clostridium subterminale, Clostridium limosum and *Clostridium sporogenes* formed a cluster at 81% bootstrap while *Atopobium vaginae, Olsenella uli* and *Eggerthella sp.* formed a cluster at 77% bootstrap. *Corynebacterium falsenii, Corynebacterium mucifaciens, Corynebacterium jeikeium, Corynebacterium tuberculostearicum, Corynebacterium thomssenii, Corynebacterium amycolatum, Dietzia papillomatosis, Williamsia muralis, Glyphidocera terrae, Mycobacterium septicum, Mycobacterium parascrofulaceum* and *Mycobacterium ulcerans* formed a cluster at 83% bootstrap within which *M. septicum, M. parascrofulaceum* and *M. ulcerans* formed a sub-cluster at 82% bootstrap, and *Peptostreptococcus spp.* and *Peptostreptococcus stomatis* formed a cluster at 99% bootstrap. *Dysgonomonas capnocytophagoides, Dysgonomonas gadei, Bacteroides spp., Dysgonomonas spp., Chitinophaga spp., Sphingobacterium spp., Flavobacterium spp., Alistipes shahii, Alistipes spp., Alistipes finegoldii, Empedobacter sp., Bergeyella sp., Empedobacter brevis* and *Wautersiella falsenii* formed a cluster at 90% bootstrap within which; *Chitinophaga spp., Sphingobacterium spp.* and *Flavobacterium spp.* formed a sub-cluster at 72% bootstrap and another sub-cluster was formed by *Chitinophaga spp.* and *Sphingobacterium spp.* at 84% bootstrap.

A. shahii, Alistipes spp. and *A. finegoldii* formed a sub-cluster at 91% bootstrap, and *Empedobacter sp., Bergeyella sp., E. brevis* and *W. falsenii* formed a sub-cluster at 91% bootstrap. *Roseomonas mucosa* and *Roseomonas spp.* formed a cluster at 91% bootstrap while *Brevundimonas spp., Caulobacter vibrioides, Brevundimonas vesicularis, Brevundimonas diminuta* and *Caulobacter spp.* formed a cluster at 76% bootstrap, and *Methylobacterium iners* and *Methylobacterium thiocyanatum* formed a cluster at 72% bootstrap. *Pseudomonas mendocina, Pseudomonas stutzeri, Pseudomonas putida* and *Pseudomonas oryzihabitans* formed a cluster at 83% bootstrap while *Dokdonella sp.* and *Dyella ginsengisoli* formed a cluster at 86% bootstrap, and Legionella *pneumophila,* Legionella *lytica* and Legionella *sainthelensi* formed a cluster at 99% bootstrap. *Acinetobacter spp., Acinetobacter junii, Acinetobacter schindleri, Acinetobacter johnsonii, Acinetobacter septicus, Acinetobacter radioresistens, Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* formed a cluster at 82% bootstrap within which *A. junii, A. schindleri, A. johnsonii, A. septicus, A. radioresistens, A. calcoaceticus* and *A. lwoffii* formed a sub-cluster at 79% bootstrap, and *Janthinobacterium lividum* and Massilia timonae formed a cluster at 82% bootstrap. *Achromobacter spp., Achromobacter xylosoxidans* and *Bordetella petrii* formed a cluster at 83% bootstrap within which *Achromobacter spp.* and *A. xylosoxidans* formed a sub-cluster at 78% bootstrap, and *Comamonas sp., Delftia tsuruhatensis, Acidovorax delafieldii, Acidovorax temperans, Comamonas kerstersii, Acidovorax facilis, Acidovorax spp.* and *Comamonas testosteroni* formed a cluster at 86% bootstrap.

However, the species; *Acidaminococcus spp., Actinomadura spp., Actinomadura vinacea, Aeromonas spp., Agromyces sp., Alteromonas sp., Anaerovorax spp., Arthrobacter oxydans, Arthrobacter spp., Aurantimonas sp., Azospirillum brasilense, Bacteroides vulgatus, Bosea sp., Brevibacterium sp., Burkholderia spp., Burkholderia tropica, Burkholderia ubonensis, Butyrivibrio sp., Caenispirillum sp., Campylobacter lari, Candidatus neoehrlichia mikurensis, Catabacter hongkongensis, Cellulomonas spp., Citrobacter spp., Clostridium ghonii, Coxiella burnetii, Coxiella spp., Cupriavidus spp., Desulfomicrobium spp., Desulfovibrio desulfuricans, Desulfovibrio spp., Enterobacter hormaechei, Escherichia hermannii, Eubacterium spp., Fastidiosipila sanguinis, Finegoldia spp., Francisella spp., Gluconobacter spp., Haemophilus parainfluenzae, Halomonas venusta, Herbaspirillum rhizosphaerae, Herbaspirillum spp., Inquilinus spp., Kocuria rosea, Lachnoclostridium clostridium symbiosum, Legionella jordanis, Leifsonia spp., Massilia spp., Mesorhizobium spp., Methylobacterium spp., Methylobacterium tardum, Micrococcus luteus, Micrococcus sp., Microvirgula aerodenitrificans, Mogibacterium timidum, Mycoplasma hominis, Mycoplasma salivarium, Neisseria subflava, Nocardiopsis spp., Ochrobactrum intermedium, Ochrobactrum spp., Paracoccus spp., Parvimonas spp., Peptoniphilus asaccharolyticus, Pseudoclavibacter zimmermannella bifida, Ralstonia spp., Rhizobium spp., Rhodoplanes spp., Robinsoniella peoriensis, Rothia mucilaginosa, Ruminococcus flavefaciens, Ruminococcus spp., Selenomonas spp., Shewanella putrefaciens, Shigella sonnei, Simkania negevensis, Spiroplasma sp., Streptomyces spp., Synergistes spp., Varibaculum cambriense, Veillonella parvula, Vibrio cholerae, Wolbachia pipientis, Wolbachia spp.* and *Xanthomonas spp.* did not form any clusters. The *Vibrio cholerae* strain with accession number *KJ725364.1* was retrieved from the NCBI website and used as the outgroup to root the human pathogen’s tree.

The results showed that human pathogens data was not normally distributed (P < 0.05). Analysis was performed to determine the influence of season on the abundance of the detected human bacterial pathogens (Table 4.12). *H. parainfluenzae* (P < 0.05), *L. lytica* (P < 0.05), *L. sainthelensi* (P < 0.05), *P. mendocina* (P < 0.05), *P. oryzihabitans* (P < 0.05), *P. putida* (P < 0.05), *P. stutzeri* (P < 0.05) and *S. sonnei* (P < 0.05) showed a significant difference in abundance between the wet and dry seasons (Table 4.12). *L. sainthelensi* (P < 0.05), *P. oryzihabitans* (P < 0.05), *P. putida* (P < 0.05), *P. stutzeri* (P < 0.05) and *S. sonnei* (P < 0.05) were significantly abundant in wet season compared to the dry season. *H. parainfluenzae* (P < 0.05), *L. lytica* (P < 0.05), and *P. mendocina* (P < 0.05) were significantly abundant in the dry season compared to the wet season. However, there was no significant difference in the abundance of *Citrobacter spp.* (P > 0.05), *L. pneumophila* (P > 0.05), *L. jordanis* (P > 0.05) and *V. cholera* (P > 0.05) between the wet and dry seasons (Table 4.12).

Table 4.12: Influence of season on the abundance of the detected human bacterial pathogens.

Bacterial species	**Z value**	**P – Value**
Achromobacter spp.	-1.94	0.052
Achromobacter xylosoxidans	-3.92	P < 0.001
Acidaminococcus spp.	-1.60	0.109
Acidovorax delafieldii	-1.01	0.313
Acidovorax facilis	-3.83	P < 0.001
Acidovorax spp.	-0.112	0.911
Acidovorax temperans	-3.30	0.001
Acinetobacter calcoaceticus	-0.019	0.985
Acinetobacter johnsonii	-3.62	P < 0.001
Acinetobacter junii	-3.92	P < 0.001
Acinetobacter lwoffii	-3.88	P < 0.001
Acinetobacter radioresistens	-3.92	P < 0.001
Acinetobacter schindleri	-3.85	P < 0.001
Acinetobacter septicus	-3.73	P < 0.001
Acinetobacter spp.	-1.05	0.296
Actinomadura spp.	-2.21	0.027
Actinomadura vinacea	-2.68	0.007
Aeromonas spp.	-3.07	0.002
Agromyces sp.	-3.52	P < 0.001
Alistipes finegoldii	-3.74	P < 0.001

Alistipes shahii	-1.00	0.317
Alistipes spp.	-2.11	0.035
Alteromonas sp.	-1.00	0.317
Anaerococcus sp.	-2.00	0.046
Anaerovorax spp.	-3.54	P < 0.001
Arthrobacter oxydans	-2.02	0.043
Arthrobacter spp.	-0.65	0.514
Atopobium vaginae	-1.34	0.180
Aurantimonas sp.	-2.03	0.042
Azospirillum brasilense	-1.00	0.317
Bacillus coagulans	-3.37	0.001
Bacteroides spp.	-0.69	0.490
Bacteroides vulgatus	-1.00	0.317
Bergeyella sp.	-2.54	0.011
Bordetella petrii	-2.82	0.005
Bosea sp.	-2.83	0.005
Brevibacillus parabrevis	-1.00	0.317
Brevibacillus sp.	-1.89	0.059
Brevibacterium sp.	-2.99	0.003
Brevundimonas diminuta	-3.74	P < 0.001
Brevundimonas spp.	-2.99	0.003
Brevundimonas vesicularis	-1.34	0.180
Burkholderia spp.	-3.00	0.003
Burkholderia tropica	-1.34	0.180
Burkholderia ubonensis	-3.92	P < 0.001
Butyrivibrio sp.	-1.00	0.317
Caenispirillum sp.	-1.00	0.317
Campylobacter lari	-1.00	0.317
Candidatus neoehrlichia mikurensis	-0.365	0.715
Catabacter hongkongensis	-1.00	0.317
Caulobacter spp.	-2.67	0.008
Caulobacter vibrioides	-2.53	0.011
Cellulomonas spp.	-3.42	0.001
Chitinophaga spp.	-1.16	0.245
Citrobacter spp.	-1.00	0.317
Clostridium ghonii	-3.54	P < 0.001
Clostridium intestinale	-1.63	0.102
Clostridium limosum	-1.00	0.317

Clostridium sporogenes	-1.84	0.066
Clostridium subterminale	-1.60	0.109
Comamonas kerstersii	-3.06	0.002
Comamonas sp.	-3.62	P < 0.001
Comamonas testosterone	-0.242	0.809
Corynebacterium amycolatum	-1.84	0.066
Corynebacterium falsenii	-3.83	P < 0.001
Corynebacterium jeikeium	-3.74	P < 0.001
Corynebacterium mucifaciens	-3.44	0.001
Corynebacterium thomssenii	-3.92	P < 0.001
Corynebacterium tuberculostearicum	-3.92	P < 0.001
Coxiella burnetii	-1.34	0.180
Coxiella spp.	-3.07	0.002
Cupriavidus spp.	-1.71	0.087
Delftia tsuruhatensis	-3.92	P < 0.001
Desulfomicrobium spp.	-2.03	0.042
Desulfovibrio desulfuricans	-1.34	0.180
Desulfovibrio spp.	0.00	1.000
Dietzia papillomatosis	-2.21	0.027
Dokdonella spp.	-0.464	0.642
Dyella ginsengisoli	-1.83	0.068
Dysgonomonas capnocytophagoides	-3.22	0.001
Dysgonomonas gadei	-3.92	P < 0.001
Dysgonomonas spp.	-3.09	0.002
Eggerthella sp.	-1.00	0.317
Empedobacter brevis	-2.23	0.026
Empedobacter sp.	-1.84	0.066
Enterobacter hormaechei	-3.25	0.001
Enterococcus faecalis	-3.63	P < 0.001
Escherichia hermannii	-2.39	0.017
Eubacterium spp.	-1.25	0.212
Exiguobacterium aurantiacum	-2.94	0.003
Exiguobacterium sp.	-3.62	P < 0.001
Fastidiosipila sanguinis	-1.00	0.317
Finegoldia magna	-3.83	P < 0.001
Finegoldia spp.	-1.00	0.317
Flavobacterium spp.	-3.29	0.001
Francisella spp.	-0.530	0.596

Gemella sanguinis	-3.76	P < 0.001
Gluconobacter spp.	-2.96	0.003
Gordonia terrae	-2.12	0.034
Haemophilus parainfluenzae	-2.12	0.034
Halomonas venusta	-2.55	0.011
Herbaspirillum rhizosphaerae	-0.46	0.647
Herbaspirillum spp.	-0.040	0.968
Inquilinus spp.	-2.06	0.039
Janthinobacterium lividum	-3.92	P < 0.001
Kocuria rosea	-3.92	P < 0.001
Lachnoclostridium clostridium symbiosum	-1.34	0.180
Lactobacillus fermentum	-1.60	0.109
Lactobacillus iners	-3.83	P < 0.001
Lactobacillus paraplantarum	-1.00	0.317
Lactobacillus plantarum	-2.26	0.024
Lactococcus garvieae	-2.54	0.011
Lactococcus lactis	-3.55	P < 0.001
Lactococcus sp.	-1.00	0.317
Legionella jordanis	-1.00	0.317
Legionella lytica	-2.81	0.005
Legionella pneumophila	-1.84	0.066
Legionella sainthelensi	-3.93	P < 0.001
Leifsonia spp.	-.100	0.317
Leuconostoc pseudomesenteroides	-2.23	0.026
Lysinibacillus massiliensis	-3.83	P < 0.001
Massilia spp.	-1.34	0.180
Massilia timonae	-2.19	0.029
Mesorhizobium spp.	-3.31	0.001
Methylobacterium iners	-2.23	0.026
Methylobacterium spp.	-3.22	0.001
Methylobacterium tardum	-3.02	0.003
Methylobacterium thiocyanatum	-0.55	0.583
Micrococcus luteus	-0.626	0.538
Micrococcus sp.	-686	0.493
Microvirgula aerodenitrificans	-1.63	0.102
Mogibacterium timidum	-1.84	0.066
Mycobacterium parascrofulaceum	-2.33	0.020
Mycobacterium septicum	-3.52	P < 0.001

Mycobacterium ulcerans	-2.97	0.003
Mycoplasma hominis	-1.00	0.317
Mycoplasma salivarium	-1.00	0.317
Neisseria subflava	-2.68	0.007
Nocardiopsis spp.	-1.17	0.242
Ochrobactrum intermedium	-1.84	0.066
Ochrobactrum spp.	-2.02	0.043
Olsenella uli	-2.97	0.003
Paracoccus spp.	-3.36	0.001
Parvimonas spp.	-2.06	0.039
Peptoniphilus asaccharolyticus	-1.00	0.317
Peptostreptococcus spp.	-1.00	0.317
Peptostreptococcus stomatis	-1.34	0.180
Pseudoclavibacter zimmermannella bifida	-1.00	0.317
Pseudomonas mendocina	-3.54	$P < 0.001$
Pseudomonas oryzihabitans	-3.84	$P < 0.001$
Pseudomonas putida	-3.92	$P < 0.001$
Pseudomonas stutzeri	-3.92	$P < 0.001$
Ralstonia spp.	-3.31	0.001
Rhizobium spp.	-3.92	$P < 0.001$
Rhodoplanes spp.	-1.91	0.056
Robinsoniella peoriensis	-2.02	0.043
Roseomonas mucosa	-1.34	0.180
Roseomonas spp.	-0.150	0.881
Rothia mucilaginosa	-1.34	0.180
Ruminococcus flavefaciens	-0.518	0.605
Ruminococcus spp.	-3.13	0.002
Selenomonas spp.	-1.84	0.066
Shewanella putrefaciens	-3.92	$P < 0.001$
Shigella sonnei	-3.92	$P < 0.001$
Simkania negevensis	-1.34	0.180
Sphingobacterium spp.	-0.967	0.334
Spiroplasma sp.	-1.60	0.109
Sporosarcina spp.	-1.92	0.054
Streptococcus gordonii	-3.92	$P < 0.001$
Streptococcus lutetiensis	-1.84	0.066
Streptococcus sanguinis	-2.53	0.011
Streptomyces spp.	-0.944	0.345

Synergistes spp.	-1.60	0.109
Varibaculum cambriense	-3.93	$P < 0.001$
Veillonella parvula	-3.74	$P < 0.001$
Vibrio cholera	-1.23	0.219
Wautersiella falsenii	-1.34	0.180
Williamsia muralis	-2.46	0.014
Wolbachia pipientis	-1.34	0.180
Wolbachia spp.	-3.10	0.002
Xanthomonas spp.	-1.04	0.299

4.7.2 Livestock pathogens detected

A total of five livestock pathogens were identified and a phylogenetic tree was generated (Figure 4.13). The *Bacillus anthracis* strain with accession number *AJ516943.1* was retrieved from the NCBI website and used as the outgroup to root the livestock pathogen's phylogenetic tree. *Acholeplasma morum, Psychrobacter pulmonis, Acetivibrio spp.* and *Acholeplasma laidlawii* formed a cluster at 99% bootstrap while *Acholeplasma spp.* did not form any cluster The results revealed that livestock pathogens data was not normally distributed ($P < 0.05$). Analysis was performed to determine the influence of season on the abundance of the detected livestock bacterial pathogens (Table 4.13). *A. laidlawii* ($P > 0.05$), *A. morum* ($P > 0.05$), *Acetivibrio spp.* ($P > 0.05$) and *P. pulmonis* ($P > 0.05$) showed no significant difference in abundance between the two seasons (Table 4.13). It was also noted that there was a significant difference in the abundance of *Acholeplasma spp.* ($P < 0.05$) between the two seasons.

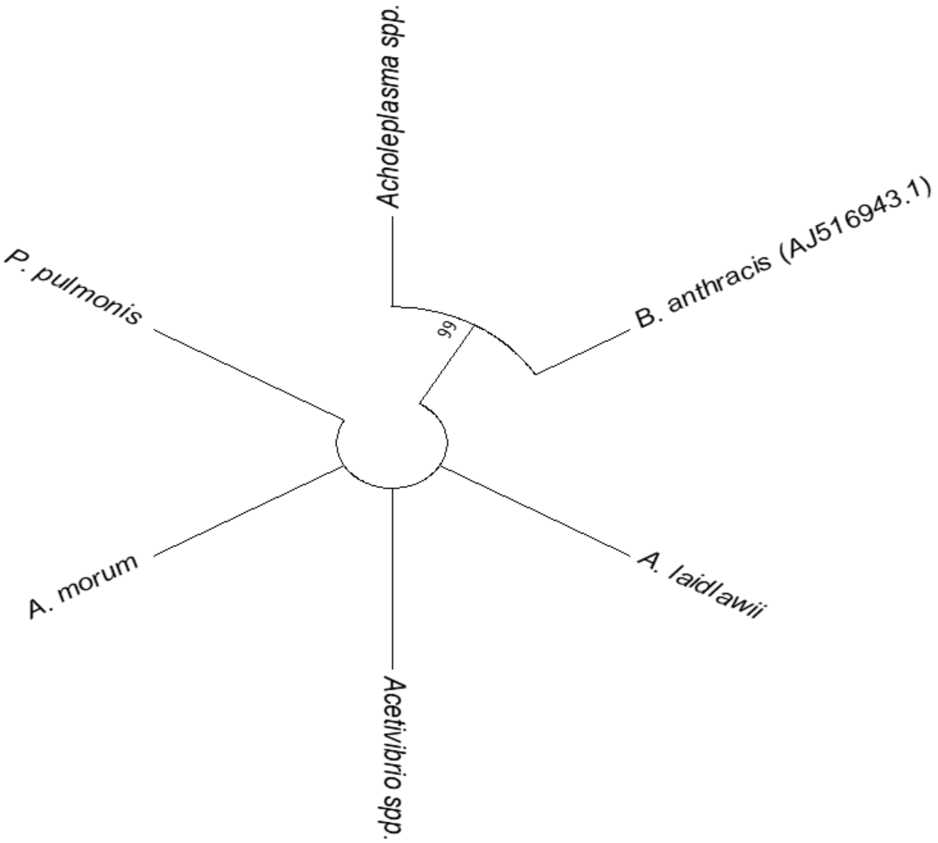

Figure 4.13: Phylogenetic tree depicting the evolutionary history of the detected livestock bacterial pathogens. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates were collapsed.

Table 4.13: Influence of season on the abundance of livestock bacterial pathogens.

Bacterial species	**Z value**	**P – Value**
Acetivibrio spp.	-1.26	0.206
Acholeplasma laidlawii	-1.84	0.066
Acholeplasma morum	-1.83	0.068
Acholeplasma spp.	-2.23	0.026
Psychrobacter pulmonis	-1.60	0.109

4.7.3 Zoonotic pathogens detected

A total of 66 zoonotic pathogens (Table 4.14) and 1224 grey bacteria (see Appendix) were detected.

Table 4.14: Influence of season on the abundance of the detected zoonotic bacterial pathogens.

Bacterial species	**Z value**	**P – Value**
Actinomyces spp.	-0.938	0.348
Actinomyces viscosus	-1.34	0.180
Aerococcus viridans	-2.65	0.008
Afipia sp.	-2.92	0.004
Alcaligenes faecalis	-3.92	P < 0.001
Alcaligenes sp.	-1.84	0.066
Anabaena spp.	-1.00	0.317
Anaerorhabdus spp.	-1.00	0.317
Anaplasma phagocytophilum	-1.34	0.180
Anaplasma spp.	-1.00	0.317
Arcobacter butzlerii	-2.03	0.042
Arcobacter cryaerophilus	-3.93	P < 0.001
Arcobacter spp.	-1.76	0.079
Bacillus cereus	-2.26	0.024
Bacillus pumilus	-3.93	P < 0.001
Bacillus spp.	-3.81	P < 0.001
Bacillus subtilis	-3.20	0.001
Bordetella sp.	-3.92	P < 0.001
Brucella spp.	-2.81	0.005
Chlamydia spp.	-2.21	0.027
Clostridium perfringens	-2.06	0.039
Clostridium spp.	-2.30	0.022
Corynebacterium spp.	-3.93	P < 0.001
Corynebacterium urealyticum	-3.44	0.001
Cyanobacterium spp.	-2.04	0.041
Dietzia maris	-1.69	0.092
Dietzia spp.	-3.47	0.001
Ehrlichia spp.	-1.00	0.317
Enterobacter cloacae	-2.12	0.034
Enterococcus sp.	-3.84	P < 0.001

Erysipelothrix spp.	-0.35	0.726
Escherichia coli	-1.34	0.180
Fusobacterium nucleatum	-1.60	0.109
Fusobacterium spp.	-3.22	0.001
Hafnia sp.	-3.92	P < 0.001
Helicobacter heilmannii	-1.00	0.317
Helicobacter spp.	-0.923	0.356
Klebsiella sp.	-1.34	0.180
Legionella spp.	-2.69	0.007
Leptospira interrogans	0.000	1.000
Leptospira spp.	-3.21	0.001
Microcystis spp.	-2.94	0.003
Morganella morganii	-1.00	0.317
Mycobacterium spp.	-3.37	0.001
Mycoplasma sp.	-3.23	0.001
Nocardia nova	-1.34	0.180
Paenibacillus polymyxa	-1.73	0.083
Paenibacillus spp.	-3.31	0.001
Porphyromonas spp.	-2.24	0.025
Propionibacterium acnes	-2.56	0.011
Pseudomonas aeruginosa	-1.00	0.317
Pseudomonas spp.	-3.21	0.001
Rhodococcus spp.	-1.63	0.102
Rickettsia spp.	-1.72	0.086
Salmonella enterica	-3.43	0.001
Sphingobium paucimobilis	-1.60	0.109
Sphingobium spp.	-3.08	0.002
Sphingomonas spp.	-0.728	0.467
Staphylococcus epidermidis	-3.58	P < 0.001
Staphylococcus spp.	-3.83	P < 0.001
Stenotrophomonas maltophilia	-1.00	0.317
Stenotrophomonas spp.	-3.20	0.001
Treponema spp.	-0.445	0.656
Vibrio spp.	-1.99	0.046
Waddlia sp.	-1.34	0.180
Wohlfahrtiimonas sp.	-2.12	0.034

The relationship between the detected zoonotic pathogens was investigated by generating a phylogenetic tree (Figure 4.14). It was shown that *Corynebacterium spp., Corynebacterium urealyticum, Dietzia maris, Dietzia spp., Mycobacterium spp., Nocardia nova, Actinomyces viscosus, Actinomyces spp.* and *Propionibacterium acnes* formed a cluster at 95% bootstrap within which *Corynebacterium spp., C. urealyticum, D. maris, Dietzia spp., Mycobacterium spp., N. nova* and *A. viscosus* formed a sub-cluster at 77% bootstrap. In addition, *Corynebacterium spp., C. urealyticum, D. maris, Dietzia spp., Mycobacterium spp.* and *N. nova* formed a small sub-cluster at 85% bootstrap within which a smaller sub-cluster consisting *Corynebacterium spp.* and *C. urealyticum* was formed at 70% bootstrap. *Cyanobacterium spp.* and *Microcystis spp.* formed a cluster at 99% bootstrap while *Anaerorhabdus spp.* and *Erysipelothrix spp.* formed a cluster at 99% bootstrap. *Paenibacillus polymyxa* and *Paenibacillus spp.* formed a cluster at 79% bootstrap while *Bacillus pumilus* and *Bacillus subtilis* formed a cluster at 79% bootstrap, and *Chlamydia spp.* and *Waddlia sp.* formed a cluster at 86% bootstrap.

Arcobacter butzlerii, Arcobacter cryaerophilus and *Arcobacter spp.* formed a cluster at 99% bootstrap within which *A. butzleri* and *A. cryaerophilus* formed a sub-cluster at 96% bootstrap. *Pseudomonas aeruginosa* and *Pseudomonas spp.* formed a cluster at 94% bootstrap while *Alcaligenes sp., Bordetella sp.* and *Alcaligenes faecalis* formed a cluster at 100% bootstrap. *Vibrio spp., Enterobacter cloacae, Klebsiella sp., Hafnia sp., Morganella morganii, Escherichia coli* and *Salmonella enterica* formed a cluster at 79% bootstrap within which *E. cloacae, Klebsiella sp., Hafnia sp., M. morganii, E. coli* and *S. enterica* formed a sub-cluster at 78% bootstrap. In addition, *E. cloacae* and *Klebsiella sp.* formed a small sub-cluster at 99% bootstrap, and *E. coli* and *S. enterica* formed a small sub-cluster at 97% bootstrap.

Anaplasma spp. and *Ehrlichia spp.* formed a cluster at 90% bootstrap while *Aerococcus viridans, Afipia sp., Anabaena spp., Anaplasma phagocytophilum, Bacillus cereus, Bacillus spp., Brucella spp., Clostridium perfringens, Clostridium spp., Enterococcus sp., Fusobacterium nucleatum, Fusobacterium spp., Helicobacter heilmannii, Helicobacter spp., Legionella spp., Leptospira interrogans, Leptospira spp., Mycoplasma sp., Porphyromonas spp., Rhodococcus spp., Rickettsia spp., Sphingobium paucimobilis, Sphingobium spp., Sphingomonas spp., Staphylococcus epidermidis, Staphylococcus spp., Stenotrophomonas maltophilia, Stenotrophomonas spp.,* *Treponema spp.* and *Wohlfahrtiimonas sp.* did not form any clusters. The *Bacillus anthracis* sequence with accession number *AJ516943.1* was retrieved from the NCBI website and used as the outgroup to root the zoonotic pathogen's phylogenetic tree.

Results showed that zoonotic pathogens data was not normally distributed ($P < 0.05$) and analysis was performed to determine the influence of season on the abundance of the detected zoonotic bacterial pathogens (Table 4.14). *Brucella spp.*, *Bacillus spp.*, *Chlamydia spp.*, *Cyanobacterium spp.*, *Enterococcus sp.*, *Legionella spp.*, *Leptospira spp.*, *Microcystis spp.*, *Mycobacterium spp.*, *Salmonella enterica* and *Staphylococcus spp.* showed a significant difference in abundance between the wet and dry season ($P < 0.05$, Table 4.14). *Brucella spp.*, *Bacillus spp.*, *Chlamydia spp.*, *Cyanobacterium spp.*, *Enterococcus sp.*, *Legionella spp.*, *Microcystis spp.* and *Salmonella enterica* had higher abundances in the dry season compared to the wet season. *Leptospira spp.*, *Mycobacterium spp.* and *Staphylococcus spp.* had significantly higher abundances in the wet season compared to the dry season. However, there was no significant difference in the abundance of *Ehrlichia spp.*, *Escherichia coli*, *Helicobacter spp.*, *Treponema spp.* and *Klebsiella sp.* between the wet and dry seasons.

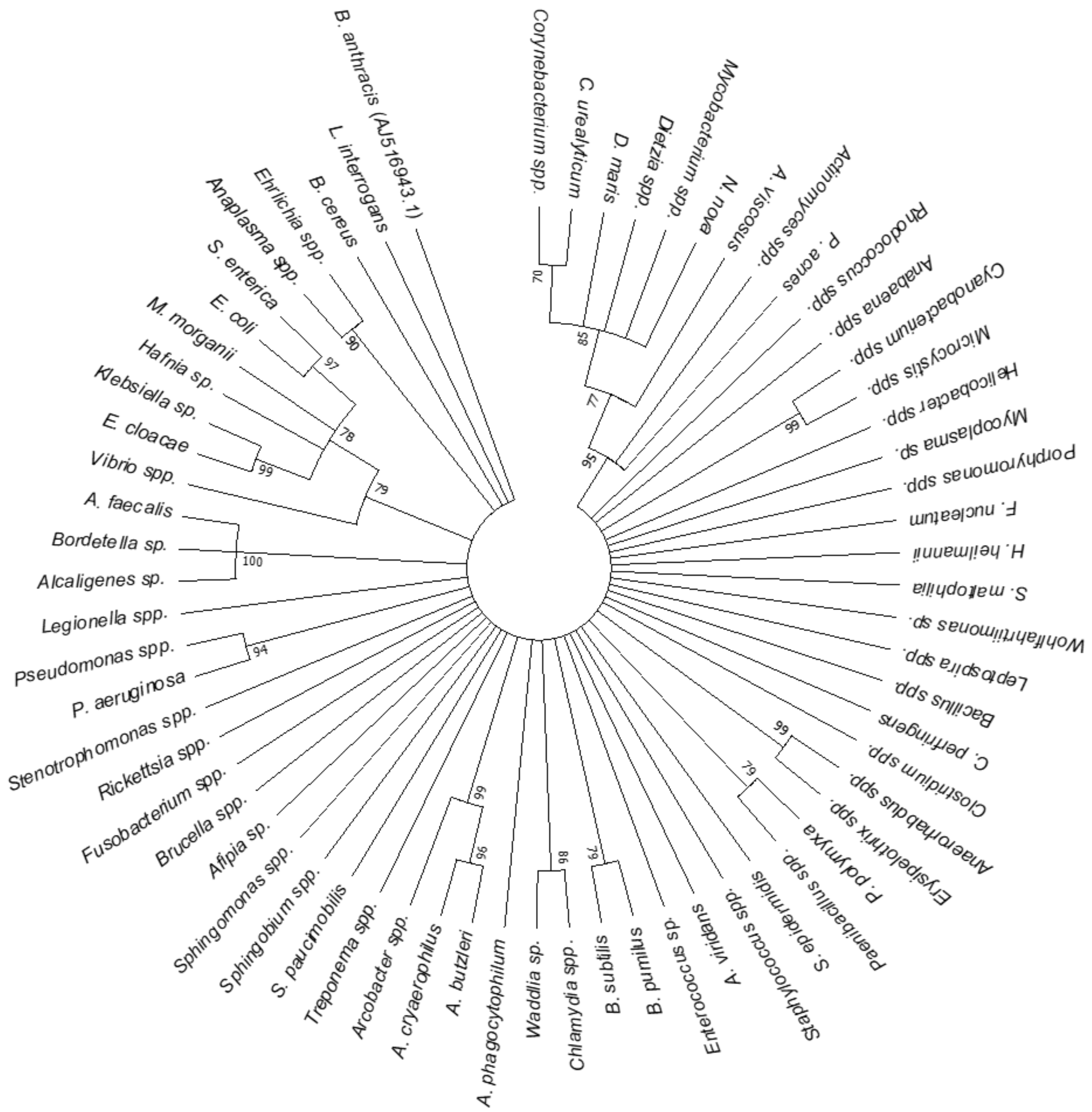

Figure 4.14: Phylogenetic tree depicting the evolutionary history of the detected zoonotic bacterial pathogens. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates were collapsed.

CHAPTER FIVE

DISCUSSION

5.1 Bacterial culturing based water quality assessment

Water is a universal need for survival across all life forms and serves as a habitat for some creatures. Although important, water can be a source and driver of diseases to humans and livestock if contaminated (WHO, 2011) and this highlights the need to ensure the safety of drinking water supplies. Since groundwater microbial water quality assessment is not prioritised in most developing countries, water related diseases account for 10% of the disease burden in these countries (Park, 2002). However, approximately one billion people in developing countries do not have access to safe drinking water in which Namibia is not an exception (WHO, 2004). The safety and quality of drinking water sources are a global concern especially in rural areas where water scarcity and contamination can be alarming. Odonkor and Addo (2013) argued that rural areas of developing countries experience high rates of waterborne diseases compared to other ailments due to bacteriological contamination. The water shortages in developing countries have led to communities depending on the use of groundwater to supplement the surface water supplies. This is the first study aimed at comprehensively investigating the microbial water quality in Namibia except for a pilot study that was conducted and documented (McBenedict *et al.,* 2017) in the early phases of this study.

This study successfully isolated cultures of *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species from the hand-dug well water samples. *Klebsiella* and *Enterobacter* species were the most isolated in the wet and dry seasons. *Escherichia* species were the least isolated in the dry season, while *Proteus* and *Pseudomonas* species were the least isolated in the wet season. *Proteus* and *Salmonella* species were not isolated in the dry season. This study's low detection levels of *Escherichia* species in the dry season is striking, since *Escherichia* species are known to be abundant in the dry season (Edrington *et al.,* 2006; Hussain, 2010). *Escherichia* species were more readily detected in the wet season probably due to rainfall in which water and conducive temperature (35° C – 47° C) was available to activate metabolic activities enhancing *E. coli* growth.

Olowe *et al.* (2015), isolated 382 *E. coli* strains from different drinking water sources; hand-dug wells, water pipes, boreholes, streams and packaged water in Ado-Ekiti in Nigeria. The proportions of isolation were 267 *E. coli* strains (69.9%) from hand-dug well water samples, 33 *E. coli* strains (8.64%) from pipe-borne water samples, 23 *E. coli* strains (6.02%) from borehole water samples, 56 *E. coli* strains (14.7%) from stream water samples and three *E. coli* strains (0.79%) from packaged water. Their findings revealed high *E. coli* counts from hand-dug well water samples compared to other water sources indicating the increased vulnerability of hand-dug wells to contamination probably due to nearby faecal sources, surface runoff or animal faeces. Vagarali *et al.* (2011) analysed water samples from Jawaharlal Nehru Medical College hostel overhead tanks in India. Of the 30 drinking water samples analysed, *Pseudomonas* species were the most detected in the water samples (20%) followed by *Escherichia coli* (10%) and *Klebsiella pneumoniae* (10%), and lastly *Proteus vulgaris* (three percent). Olowe *et al.* (2015)'s results were contrary to the present study that recorded *Pseudomonas* species to be the least detected, but agrees with Hussain (2010) and Akrong *et al.* (2012)'s findings.

Hussain (2010) investigated the microbial quality of drinking water samples (municipal water) from Khairpur city water works, Sukkur city water works, and Rohri city water works in Pakistan. They found that; *P. aeruginosa* (75%) was the most isolated bacteria followed by *E. coli* (70%), *P. mirabilis* (67%), *P. rettgeri* (67%), *C. youngae* (65%), *P. stuartii* (63%), *Non-fermenter* spp. (59%) from various genera such as *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavobacter*, *Oligella, Flavimonas, Agrobacter* and *Weeksiella*, C. meningosepticum (55%), *K. oxytoca* (49%), and *Salmonella species* were not detected in all samples. The isolation rate of all species was significantly higher in summer months than in winter months in drinking water of Khairpur, Sukkur and Rohri city, except for *P. aeruginosa* which was significantly higher in both seasons in drinking water of Khairpur, Sukkur and Rohri city. The steady high abundance of *P. aeruginosa* was due to its mesophilic nature (Havelaar *et al.*, 1992; Hussain, 2010).

Adakole *et al.* (2010) assessed the water quality of hand-dug wells in Samaru – Zaria in Nigeria and detected *E. coli*, *Enterobacter sp*. and *Klebsiella sp.* As opposed to the present study, Adakole *et al.* (2010), found more *Enterobacter sp*. and *Klebsiella sp.* in the dry season than the wet season, while *E. coli* was less in the dry season than the wet season. In addition, Adakole *et al.* (2010), predominantly isolated *E. coli* in most samples. This agrees with Humayun *et al.* (2015)'s study in which *E. coli* (26.7%) was the most isolated species followed by *P. aeruginosa* (12.2%), *H. pylori* (8.88%) and *Salmonella* (6.66%) species. However, the present study found *Enterobacter sp*. and *Klebsiella sp.* in most samples, indicating that disparities in results can exist due to variations in bacterial survival that is greatly influenced by available nutrients and incubation temperature (Sautour *et al.*, 2003).

5.1.1 The effect of hand-dug well type, region and season on the abundance of bacterial colony forming units

There were differences in the abundance of bacterial CFU's in shallow, shallow-na and deep hand-dug wells. Higher total coliform counts were recorded in the wet season compared to the dry season in all hand-dug well types. The shallow hand-dug wells had the highest amount of CFU's followed by the deep hand-dug wells and lastly the shallow-na hand-dug wells (refer to Figures 4.1). The high CFU's from shallow wells were mainly because these wells are not protected, making them most vulnerable to contamination and this agrees with Ayantobo *et al.* (2012)'s study on water quality evaluation of hand-dug wells in Ibadan, Nigeria. Ayantobo *et al.* (2012) categorized hand-dug wells into three kinds namely; "protected wells", "semi-protected wells" and "unprotected wells" and found that unprotected hand-dug wells had the highest *E. coli* and total coliform counts (74.09 CFU/100ml and 685.00 CFU/100ml respectively) followed by semi-protected hand-dug wells (58.37 CFU/100ml and 424.86 CFU/100ml respectively) and protected hand-dug wells (23.5 CFU/100ml and 348.19 CFU/100ml respectively). This agrees with Amenu *et al.* (2014) who recorded similar trends of protected water sources containing less CFU's than the unprotected ones. The shallow hand-dug wells had a poor structure in that; they appear as though they are water logged land depressions with irregular outlines, and are shallow in nature which allows livestock to walk in since they are generally not well protected with a fence. In addition, the irregular outline of shallow hand-dug wells could not allow the construction of covers to appropriately protect the water surface leading to easy access of livestock, and other domestic and wild animals or birds to the hand-dug well water thereby increasing the level of contamination and thus the high CFU. The animals could walk into the water in the shallow hand-dug wells and defecate thereby increasing the level of enteric microorganisms.

This study revealed a significant difference in the abundance of bacterial CFU's between the wet and dry seasons in shallow hand-dug wells ($P < 0.05$). This showed that rainfall increased the levels of contamination and CFU's in the shallow hand-dug wells through surface water runoff. Moreover, the Ohangwena and Omusati regions commonly experience floods during the rainy seasons that are prolonged by poor drainage systems (Thomas, 2016). These flood water can transport bacteria from soil, human and animal wastes, and organic debris such as dead plants into these hand-dug wells, thereby increasing bacterial numbers and simultaneously providing a carbon source for bacterial growth. The results of this study agree with Cronin *et al.* (2006), who investigated the water quality of hand-dug wells in Niassa province of northern Mozambique and found higher average coliform counts in hand-dug wells in the wet season (121.2 CFU/100ml), compared to the dry season (39.1 CFU/100ml).

The shallow-na hand-dug wells revealed the lowest bacterial CFU abundance compared to the shallow and deep hand-dug wells, probably due to restricted animal access. Shallow-na hand-dug wells were built with some form of staircases in addition to fences that were mostly placed around them. The staircases and fencing make it difficult for livestock or animals to access the water within these hand-dug wells and increase the distance between the site at which livestock droppings are found and the hand-dug wells. The restricted animal access in the shallow-na hand-dug wells led to reduced CFU's. These findings agree with those of Ayantobo *et al.* (2012) that restricted animal access lowers levels of water contamination in hand-dug wells. Higher CFU values were recorded in the shallow-na hand-dug wells in the wet season compared to the dry season in this study as a result of surface runoff. This agrees with Isikwue *et al.* (2011) but is contrary to Kang (2013), who found lower total coliform counts ranging between 12 MPN/100ml – 26 MPN/100ml in hand-dug wells found in Accra region of Ghana in the wet season and 17 MPN/100ml – 79 MPN/100ml in the dry season probably owing to reduced hand-dug well water volume because of evaporation in the dry season leading to increased bacterial concentrations.

The deep hand-dug wells had the second highest abundance in bacterial CFU's following the shallow hand-dug wells. All the deep hand-dug wells surveyed in this study lacked fences around them and were thus not protected from contamination. However, due to their structure, livestock could not have contact with the water although they had access to the vicinity of the wells. The livestock could drink water from the troughs that are placed besides the deep hand-dug wells. Hence, livestock faeces close to the wells could be transported into these wells by wind or water running from the troughs since they lacked a top covering. A trend of increased well depth with a reduction in CFU's was noticed confirming that soil texture and profiles naturally filter out contaminants during indirect flow of water into the hand-dug wells via porous layers of the soil (Isikwue *et al.*, 2011). Bolaji and Martins (2008) reported similar trends confirming that the level of contamination is influenced by well depth.

This study recorded higher CFU values in deep hand-dug wells in the wet season compared to the dry season which are ostensibly propelled by precipitation. These findings corroborate with those of Isikwue *et al.* (2011), who investigated the effect of depth on microbial pollution of shallow wells in Makurdi Metropoilis in Nigeria and found high CFU's in the wet season ranging from 48 CFU/ml - 155 CFU/ml compared to the dry season ranges of 26 CFU/ml - 102 CFU/ml. Hence, the microbiological water quality of the present study's hand-dug wells could have been influenced by season. Surface runoff transports various bacteria into the hand-dug wells thereby elevating the levels of contamination and CFU's especially that Omusati and Ohangwena regions are known to experience floods during the rainy season (Thomas, 2016). In addition, Thomas (2016) disclosed that Namibia recorded the highest rate of open defecation in southern Africa. This makes it easy for the hand-dug wells to be polluted since water serves as a transporter of these contaminants especially in the rainy season when the water penetrates the permeable soil layers reaching the aquifers below that are shared by the hand-dug wells within the same vicinity (Van der Wal, 2008).

Colony forming unit (CFU) estimates are useful as they give an idea of the bacteriological water quality in most cases with a focus on faecal contamination (Cho *et al*., 2010; Herschy, 2012). Based on CFU, it is not possible to describe the identity of the bacteria unless further tests were performed. However, the presence of high microbial loads showed that the water was not definitely safe for human consumption and undoubtedly harmful to livestock as well. Conversely, the use of CFU's to compare levels of water contamination in different samples could be deceiving because some bacteria display fastidious growth patterns leading to an interpretation that a particular water sample was less contaminated. Thus, it is possible that a particular water sample could have a high diversity and amount of pathogenic bacteria that are fastidious in nature and be asserted less contaminated than a water sample that has a low diversity and copy number of un-fastidious pathogenic bacteria.

5.1.2 The effect of hand-dug well type, region and season on the presence of coliforms, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species

Citrobacter, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* species were detected in the hand-dug wells. On the basis of hand-dug well type, region and season, there was no significant difference in the presence of *Escherichia* species. Since *Escherichia* species exist as normal flora in the intestine of humans and animals, their presence translates into faecal contamination that occurs regardless of season or region. The uncontrolled animal access to the shallow hand-dug wells coupled with the surface runoff during the wet season explains the contamination of these hand-dug wells by *Escherichia* species (McBenedict *et al*., 2017). The shallow-na and deep hand-dug wells are also vulnerable to contamination although animals could not walk in the water due to poor architecture such as lack of an elevated design and covers on the top. McBenedict *et al.* (2017) also stated that the Cuvelai Etosha Basin hand-dug wells were vulnerable to bacterial contamination because they share the same aquifers. Anderson *et al.* (2003) defined an aquifer as a geological formation consisting saturated permeable rocks or sands or gravels that transmits groundwater to wells or springs. The aquifer systems are recharged by precipitation and this allows the transportation of contaminants from the surface through the porous layers into the aquifers that recharge hand-dug wells thereby spreading contaminants.

Van Elsas *et al.* (2011) revealed that *Escherichia* species could grow and survive outside their primary hosts (humans and animals) in open environments with appropriate resources. Appropriate resources include carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur availability coupled with a suitable pH and temperature. Since these hand-dug wells are in contact with the soil, have animal droppings and visible floating debris, it can be hypothesized that they are propitious for microbial growth. Furthermore, this soil-water environment can enhance the ability of the microorganisms to cope with various or fluctuating environmental conditions by the transfer and exchange of genes owed to microbial interactions. This is evident because *Escherichia* species were once described to be unable to survive lengthy periods outside the intestines of warm blooded animals and it’s on this basis that *Escherichia coli* is used as a water quality indicator for faecal contamination and a predictor of the potential presence of other contaminant species (WHO, 2008). Recent studies have indicated that *E. coli* strains survive in soil and water that’s not known to be faecally contaminated (Ishii *et al.*, 2007; NandaKafle *et al.*, 2017).

The current status quo demands the development of a more suitable indicator of recent faecal contamination and further research to explore these emerging patterns. Although not harmful under normal physiological conditions, *Escherichia* species have the ability to cause urinary tract infections, bacteraemia, acute renal failure and meningitis when there is a breach in the immune system (O’ Connor, 2002; Durso *et al.*, 2005). In addition, *Escherichia* species are zoonotic pathogens and can therefore infect and be transmitted among livestock and humans. *Escherichia* species that have been implicated in disease include enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (O’ Connor, 2002; Herschy, 2012).

There was no significant difference in the presence of *Klebsiella* species based on hand-dug well type, region and season. The presence of *Klebsiella* species in these hand-dug wells is plausible given their ubiquitous distribution in nature in addition to the gut microbiota in humans and livestock. These species inhabit diverse environments ranging from surface waters, gastrointestinal tract of mammals, soil, and plants (Podschun *et al.*, 2001; Vos *et al.*, 2011). Disease conditions due to infection by these species include community acquired pneumonia, urinary tract infections, chronic genital ulcerative disease and bacteraemia (Madigan *et al.*, 2015). *Klebsiella* species are part of the coliform indicator organism list and their detection is described to indicate faecal contamination (Herschy, 2012).

Due to the wide distribution of *Klebsiella* species, it is arguable that these species are not suitable indicators of faecal contamination. *K. pneumonia* is recorded to be the most isolated species especially in the clinical setting from about 60 - 80% of human clinical specimens (Umeh *et al.*, 2002; Herschy, 2012). *Klebsiella* species can present a problem especially that they are zoonotic pathogens and can therefore play a role in the development and transfer of antimicrobial resistance, and this agrees with the studies of Sikarwar and Batra (2011), Wand *et al.* (2013), and Vuotto *et al.* (2014) who isolated multidrug resistant *Klebsiella* species. Furthermore, Sikarwar and Batra (2011) revealed that *Klebsiella* has recorded an alarming emergence of multi-drug resistant strains especially those involved in nosocomial diseases. Their results indicated that from 50 samples collected, 10 of the *K. pneumoniae* isolates were found to be multidrug resistant. Hence, the presence of *Klebsiella* species in this study confirmed that the water from these hand-dug wells was not safe for human and livestock consumption.

Salmonella species showed no significant difference in their detection based on hand-dug well type and region but a significant difference was observed in terms of season. *Salmonella* species occur in humans, warm and cold blooded animals, foods and the environment (Holt *et al.,* 1994; Vos *et al.,* 2011). However, these species are considered foreign and pathogenic to humans and livestock (Vos *et al.,* 2011). *Salmonella* species are widely distributed in the environment and can easily gain entry into the hand-dug wells. *Salmonella* species were not detected in the dry season in the present study and this finding agrees with results of Polo *et al.* (1999) and Adingra *et al.* (2012) who indicated that the presence of *Salmonella* species increased with high levels of rainfall and its prevalence was significantly increased in higher rainfall seasons. Hence, rainfall is among the major factors determining the presence and distribution of *Salmonella* species in the hand-dug wells especially those lacking proper architecture with increased vulnerability to surface runoff or animal entry.

There are several disease conditions caused by *Salmonella* species (Holt *et al.,* 1994; Vos *et al.,* 2011) and their presence in water indicates that it is not safe for human and livestock consumption. *Salmonella* species cause gastroenteritis, bacteraemia, typhoid fever and a carrier state in persons with previous infections (Escartin, 2002). According to Ahmer and Gunn (2011), and Herschy (2012), the presence of *Salmonella* species in drinking water is of high concern since they are a global major cause of human morbidity and mortality. Ahmer and Gunn (2011) argued that *Salmonella* species were equipped with the ability to overcome the opposition mediated by the gut microbiota and the innate immune system during colonization.

Shigella species revealed no significant difference in their detection based on hand-dug well type and region but a significant difference was noted based on season. *Shigella* species are intestinal pathogens of humans and other primates in which they cause dysentery (Vos *et al.,* 2011). Although the present study indicated that *Shigella* species were readily detected in the dry season compared to the wet season, Phung *et al.* (2017) revealed that these species show a high peak in the rainy season. Hence, the findings of this study hypothesise that nutrients and climatic conditions favouring *Shigella* species growth and survival were present in these hand-dug wells especially during the dry season. Moreover, *Shigella* species are chemoorganotrophic having both a respiratory and fermentative type of metabolism making them versatile. Phung *et al.* (2017) also found that temperature, humidity, and precipitation were positively associated with the incidences of *Shigella* species in the rainy season. Although Phung *et al.* (2017) argued that *Shigella* species incidence rates are higher in the rainy season, it cannot be ruled out that suitable conditions for their growth exist in the dry season as well as evidenced in the present study.

The presence of *Shigella* species in these hand-dug wells is of grave concern because it is highly infectious. Bacterial counts between zero to 100 are adequate to induce shigellosis in humans (Alamanos *et al.,* 2000). This bacterium is not stable and can be used as an indicator for recent human faecal pollution (WHO, 2008). *Shigella* species are reported to cause illness and disease such as bacillary dysentery and stomach ulcerations and are of global health concern. Puzari *et al.* (2017) argued that there is an increase in multidrug resistant (MDR) *Shigella* species leading to acute gastroenteritic diarrhoeal infections responsible for about 700,000 deaths per year globally. Puzari *et al.* (2017) further revealed that *S. dysenteriae* manifests as an epidemic, *S. flexneri* and *S. sonnei* display endemicity to developing and developed nations, and *S. boydii* is prominently reported in India and nearby countries. Since *Shigella* species are known to inhabit humans and primates as hosts, it is logical to state that the detected *Shigella* species was a result of human or livestock faecal contamination. Therefore, the detection of *Shigella* species in the hand-dug wells clearly was an indication that the water was not safe for consumption by humans and requires treatment prior to use.

For the *Enterobacter* species, there was no significant difference in their presence based on hand-dug well type, region and season. These results confirmed earlier reports by Iversen *et al.* (2004)

and Vos *et al.* (2011) that *Enterobacter* species are widely distributed in nature, and occur in fresh water, soil, sewage, plants, and animal and human faeces. However, Hussain *et al.* (2013) indicated that *Enterobacter* strains can be detected in soil and vegetation which are sources of pathogenic bacteria that are found in water. *Enterobacter* strains share similar biochemical characteristics with *Klebsiella* species and have been reported to cause urinary tract infections, enterocolitis, septicaemia, meningitis, and cerebritis in humans (Vos *et al.* 2011; Herschy, 2012). They are part of the normal flora of human and animal gastrointestinal tracts, but can cause disease in animals leading to death in some cases. However, in immunocompetent livestock and humans, *Enterobacter* species are unlikely to cause serious harm. Nonetheless, diseased animals quickly recuperate after administration of antibiotics (Sserunkuma *et al.,* 2017).

Hussain *et al.* (2013) argued that consumption of water containing *Enterobacter* strains regardless of their source is a major risk to the health of human beings. Hussain (2010) and Boamah *et al.* (2011) also isolated *Enterobacter* species from water especially *Enterobacter sakazakii* which are now known as *Cronobacter* species (Vojkovska *et al.*, 2016) and *Enterobacter cloacae* in hand-dug wells both of which are pathogenic to humans (Drudy *et al.,* 2006; Petrosillo *et al.,* 2016; Fei *et al.*, 2017) and animals (Wilberger *et al.,* 2012; Sharif *et al.,* 2017). The isolation of *Enterobacter* species from most hand-dug wells regardless of season, region and hand-dug well type is due to the versatility of these species allowing them to grow and survive over a wide range of temperatures, pH values and nutrient compositions (Vos *et al.*, 2011).

There was no significant difference in their presence of *Citrobacter* species according to hand-dug well type, region and season. *Citrobacter* species are widely distributed in the soil, food, sewage, water and intestinal tracts of humans and animals (Lai *et al.*, 2010; Vos *et al.*, 2011). These species were isolated from all hand-dug well types in both dry and wet seasons and this is in agreement with findings of Liu *et al.* (2017) that *Citrobacter* species are commonly found in water, soil, food, and the intestines of animals and humans. Since water in these hand-dug wells is in contact with the soil, and based on *Citrobacter* species ubiquitous distribution, it is no surprise that these species were detected. In addition, animal and human defecation can gain entry into these hand-dug wells due to poor architectural design especially in the wet season.

The presence of *Citrobacter* species in drinking water signifies a public health threat and emphasizes the need to treat the water prior to consumption. These species have been reported to cause a broad range of infections affecting the urinary tract, liver, biliary tract, peritoneum, intestines, bone, respiratory tract, endocardium, wounds, soft tissue, meninges, and the bloodstream (Kumar *et al.*, 2013; Hirai *et al.*, 2016; Kesler *et al.*, 2016; Oyeka and Antony, 2017; Stewart *et al.,* 2017). These species are opportunistic especially in the immunocompromised individuals and infants (Dervisoglu *et al.*, 2008; Adesoji *et al.*, 2016). Although *Citrobacter* species are classified into 11 species namely; *Citrobacter freundii*, *Citrobacter koseri*, *Citrobacter amalonaticus*, *Citrobacter farmeri*, *Citrobacter youngae*, *Citrobacter braakii*, *Citrobacter werkmanii*, *Citrobacter sedlakii*, *Citrobacter rodentium*, *Citrobacter gillenii,* and *Citrobacter murliniae* (Liu *et al.*, 2017), Hirai *et al.* (2016) argued that *Citrobacter freundii* and *Citrobacter koseri* are the commonly isolated human pathogens while *Citrobacter braakii* is rarely reported.

There was no significant difference in their presence of *Pseudomonas* species based on region and season but a significant difference existed in terms of hand-dug well type. It can be postulated that the absence of *Pseudomonas* species in the shallow hand-dug wells could have been due to increased competition for space and resources within the diverse collection of microbial species. In addition, floating debris and livestock droppings were observed in and near shallow hand-dug wells, indicating a plausible high level of contamination compared to shallow-na and deep hand-dug wells. These high levels of contamination could also be viewed as environments with the potential to reveal the interactions within and between bacterial species in ecological studies. The understanding of bacterial interactomics can allow the prediction of which bacteria are most likely to co-exist with the detected bacterial species, and serve as a more accurate molecular marker facilitating the prevention of water related diseases. It is widely accepted that some bacterial species inhibit or restrict the growth and survival of others and this has been shown by various studies (Deines and Bosch, 2016; Schiessl *et al.,* 2016; Zilelidou *et al.,* 2016; Hachicho *et al.*, 2017).

The presence of *Pseudomonas* species in the rest of the hand-dug wells (deep and shallow-na) confirmed their extensive distribution in soil, faeces, water and sewage. Although these bacterial species are ubiquitous, the commonly isolated clinical species is *Pseudomonas aeruginosa*

(Herschy, 2012). *Pseudomonas* species were reported to cause septicaemia, meningitis, ear infections and water related folliculitis. *Pseudomonas* species are also found on the surfaces of plants and animals, and are opportunistic and nosocomial pathogens of the gastrointestinal tract, heart, blood, respiratory system, central nervous system, ear, eye, bone and joints, skin, and soft tissues (Amin, 2011).

Vaz-Moreira *et al.* (2012) argued that *Pseudomonas* species were residents of various aquatic environments and this explains their occurrence in the hand-dug wells studied. Vaz-Moreira *et al.* (2012) isolated a total of 14 *Pseudomonas* species from 32 water sampled sites in which all the isolates had a distinct genotype based on the type of water from which they were obtained (water treatment plant/distribution system, tap water, cup fillers, biofilm, and mineral water). Moreover, *Pseudomonas* species are persistent and prevalent in water as demonstrated by Vagarali *et al.* (2011) who found that *Pseudomonas aeruginosa* grew at the same site in Jawaharlal Nehru Medical College hostel overhead tanks in India after cleaning and treatment, while *E. coli, K. pneumoniae* and *P. vulgaris* were not detected any longer after cleaning and treatment.

There was no significant difference in their presence of *Proteus* species on the basis of hand-dug well type, region and season. In addition, these species were only detected in two hand-dug wells in the wet season which was probably a result of faecal contamination (WHO, 2011). Although it is established that *Proteus* species are found in water environments (Vos *et al.*, 2011; Drzewiecka, 2016), the findings of this study propose that these species have short periods of survival in water unless optimal conditions are available in which case it seldom occurs due to bacterial competition or interaction. *Proteus* species are members of the human and livestock gastrointestinal tract (Fernández-Delgado *et al.*, 2007; Ahmed, 2015). These species are also widely distributed in environments such as water, faeces, and soil, and are known to contribute to the decomposition of organic matter of animal origin (Fernández-Delgado *et al.*, 2007). *Proteus* species have mostly been documented as opportunistic pathogens responsible for infection of the urinary tract, respiratory tract, wounds, burns, skin, eyes, ears, nose, throat, and responsible for kidney stone formation (Amin, 2011; Ahmed, 2015; Norsworthy *et al.*, 2017). *Proteus* species can also cause gastroenteritis and infective endocarditis which presents clinical complications (Liu *et al.*, 2015).

Hence, the presence of *Proteus* species in the hand-dug wells highlights that this water is not safe for both human and livestock consumption.

In this study, water quality assessment by culturing found no resemblance in the presence of *Escherichia coli*, which is currently recognized as the gold standard for microbial water quality assessment and *Salmonella*, *Shigella*, *Pseudomonas*, *Citrobacter*, *Klebsiella*, *Enterobacter* and *Proteus* species. This is due to the different rates of survival of these species in water and this highlights the inappropriateness of asserting water to be safe based on the presence or absence of *Escherichia coli*. Similar results have been reported by other studies, although they focused on comparing *Salmonella* to *Escherichia coli* (Winfield and Groisman, 2003; Dechesne and Soyeux, 2007; Tracogna *et al.,* 2013). The emergency of multi-drug resistant *Shigella* (Baker *et al.*, 2016; Poramathikul *et al.,* 2016), *Escherichia* (Brennan *et al.*, 2016; Chen *et al.*, 2017), *Klebsiella* (Carasso *et al.*, 2016; Moradigaravand *et al.,* 2017), *Enterobacter* (Janecko *et al.,* 2016), *Proteus* (Jain *et al.,* 2016; Korytny *et al.,* 2016), *Salmonella* (Begum *et al.,* 2017; Ferstl *et al.,* 2017), *Citrobacter* (Liu *et al.,* 2016; Reinheimer *et al.,* 2016), and *Pseudomonas* (Li *et al.,* 2016; Magalhaes *et al.,* 2016) species is problematic. This extremely highlights the importance of implementing preventive measures against infection to prevent outbreaks and deaths.

Overall, various factors influence the level of bacterial contamination in the Cuvelai Etosha Basin and include open defecation, the flat terrain, increasing population coupled with inappropriate waste disposal and poor sewage systems (McBenedict *et al.*, 2017). Galadima, *et al.* (2011) argued that heavy rainfall, flat terrain and poor drainage systems can cause severe floods even with minimal precipitation. Furthermore, the site of hand-dug well construction is vital since hand-dug wells near refuse or waste dumps and pit latrines experience high levels of contamination (Kiptum and Ndambuki, 2012; Ochuko and Thaddeus, 2013). Yakubu (2013) assessed the water quality of hand-dug wells in Zaria Local Government Area of Kaduna State, Nigeria and found that hand-dug wells were primarily contaminated due to their close proximity to refuse dumpsites. The increased detection of coliforms in the wet season in this study could have been due to deposition and permeation of coliform-rich surface water across spongy soil profiles into the aquifers of the hand dug wells, and possibly the construction of hand-dug wells close to toilet facilities.

The existence of a viable but none-culturable state in bacteria presents problems of incorrectly asserting the absence of a particular pathogen. It is widely known that when exposed to harsh conditions such as low nutrients, prolonged exposure to water, and inappropriate pH and salinity, bacteria can respond by entering a phase whereby they can metabolize, survive and retain their infective potential but cannot produce colonies on artificial media on which they are usually grown. Fricker (2003) and Cenciarini-Borde *et al.* (2009) revealed that members of the genera *Vibrio*, *Campylobacter*, *Aeromonas*, *Legionella* and members of the Enterobacteriaceae family such as *E. coli, Klebsiella*, *Citrobacter* and *Enterobacter* species can exist in the viable but none-culturable state. It is therefore worth noting that it cannot be ruled out that some of the targeted bacteria in this study may have not grown on culture media because they were in the viable but none-culturable state.

5.2 Metagenomics based water quality assessment

Metagenomics provided vast information regarding the microbial communities and safety of the water from the hand-dug wells in the Cuvelai Etosha Basin for household consumption compared to the culture based approach by bypassing limitations of culturing based methods that lead to the inability to quantify the total natural diversity within a given habitat. Metagenomics was able to disclose counts (abundance) at different taxonomic level of bacteria namely; phylum, class, order, family, genus and species and enabled the investigation of trends occurring at each taxonomic level due to the influence of hand-dug well type, region and season. In addition, it gave a detailed account of bacterial communities found in the hand-dug wells and also added to the list of known water resident pathogens documented by WHO (2008).

Due to Metagenomics robustness, 1332 bacterial species (species richness) belonging to 29 phyla in the dry season and 518 bacteria species (species richness) belonging to 21 phyla in the wet season were identified. The reason for a high number of phyla in the dry season is most likely due to increased evaporation of hand-dug well water leading to a reduced volume of water with concentrated bacteria. Odonkor and Addo (2013) argued that reduced water volumes coupled with increased water-animal contact leads to high bacterial abundance and richness. The wet season showed that the predominant phyla were Proteobacteria followed by Firmicutes, Actinobacteria, Bacteroidetes and Cyanobacteria while the dominant phyla from the dry season were

Proteobacteria followed by Bacteroidetes, Firmicutes and Actinobacteria. These findings agree with Sun *et al.* (2017), who also detected Proteobacteria, Actinobacteria, and Bacteroidetes as the dominant phyla of bacterioplankton communities in the Dongjiang River in Hong Kong.

Since metagenomics is a PCR-based analyses of microbial diversity, it is entrenched with some biases that are inherent to PCR applications and are worth noting (Filippidou *et al.,* 2015. Factors such as extraction efficiency and hybridization specificity are problematic in asserting the accuracy of microbial abundance, composition and diversity of indigenous microbial communities in a metagenomics study. Biases can occur at every step of the study including the type of environment being assessed (Delmont *et al.,* 2011; Lombard *et al.*, 2011), DNA extraction methods leading to different yields (Pinard *et al.,* 2006; Wunderlin *et al.*, 2013; Filippidou *et al.,* 2015), formation of PCR chimeric structures (Ashelford *et al.,* 2006; De Bruijn, 2011), and analysis of 16S rRNA gene sequence data that largely depends on the available datasets in public databases which contain considerable errors (De Bruijn, 2011).

De Bruijn, (2011) argued that sequence diversity analysis is a glance of a fraction of the actual diversity in nature, and metagenomics is significantly affected by the number of rrn operons, preferential amplification, misprimed elongation, suppression of minority populations, short sequences, sequence alignment, and the quality and selection of reference sequences. Martin-Laurent *et al.* (2001) revealed that DNA extraction protocols or kits display preferential disruption of cells and this affects the phylotype abundance, composition and interpretation of microbial diversity of indigenous bacterial communities. DNA extraction at times produces fragmented nucleic acids which are sources of artefacts during PCR amplification and possibly leads to the creation of chimeric PCR products that falsely suggest new species discovery (De Bruijn, 2011).

The challenges of Metagenomics based studies are not limited to the above mentioned. Most importantly, this study detected a vast amount of bacteria in the hand-dug wells of the Cuvelai Etosha Basin which included human, livestock and zoonotic pathogens of public health significance by using the 16S Metagenomics approach. However, it should be noted that since Metagenomics as opposed to Metatranscriptomics is a DNA based technique, the microbial communities detected potentially included DNA from dead bacteria thereby displaying an over representation of bacterial communities or omitted some bacteria due to DNA extraction difficulties, especially in spore forming Firmicutes as described by Filippidou *et al.* (2015). This might have led to a low coverage of less abundant taxa known as "depth bias" and underrepresentation of certain taxa.

5.2.1 Determination of relative abundance and seasonal variations of bacterial phyla using Metagenomics analysis

Across all hand-dug wells, 30 bacterial phyla were identified from which relative abundance calculations showed that the predominant phyla were Proteobacteria followed by Firmicutes, Actinobacteria, Bacteroidetes and Cyanobacteria. The analysis of phyla abundance based on hand-dug well type and region did not yield noticeable trends (see Appendix). This established that hand-dug well type and region do not have an obvious influence on the abundance of bacteria at phyla level. This is because phyla classification includes several genera with different abilities to withstand environmental pressures (Madigan *et al.*, 2015; Sun *et al.*, 2017). Hence, only drastic environmental changes such as climatic shifts in different seasons or variations in water nutrients and chemical composition can yield evident patterns.

In addition, these hand-dug wells and regions had some certain similarities in physicochemical conditions that influence community structures, and were all generally inappropriately constructed making them vulnerable to contamination in ways not limited to surface runoff especially in the rainy season. The minor insignificant differences in composition of a bacterial community were due to local environmental selection as stated by Ragon *et al.* (2012). This agrees with Mohiuddin *et al.* (2017)'s disclosure that geographic location does not seem to have major impacts on bacterial abundance and diversity. Thus this section only discusses the abundance and seasonal variation of bacterial phyla following metagenomics analysis.

Actinobacteria are widely distributed gram-positive bacteria which consists of features of both fungi and bacteria. This phylum can exist in water, animal gut and soil were they are responsible for recycling refractory biomaterials by decaying polymers in dead plants and animals (Anandan *et al.*, 2016). Hence, they replenish carbon stores and this is a key aspect of humus formation and nutrient recycling necessary for bacterial growth and survival. Actinobacteria have a high guanine and cytosine content in their DNA, lack distinct cell walls, and produce a non-septate mycelium, hyphae and conidia/sporangia like fungi in culture media (Anandan *et al.,* 2016). Actinobacteria are a diverse phylum that can inhabit various environments due to their adaptability and can be classified based on this as thermophilic, acidophilic, halophilic, endophytic, symbiotic, endosymbiotic, and gut Actinobacteria (Madigan *et al.,* 2015; Sun *et al.,* 2017). The existence of these classes of Actinobacteria explains their detection in the hand-dug wells since they are able to easily adapt and survive.

In addition, it is known that water availability and nutrients are among the main limiting factors influencing bacterial growth and survival (Stevenson and Hallsworth, 2014). Stevenson and Hallsworth (2014) reported that species of Actinobacteria can germinate and grow at 0.5 water activity (a_w) but non-halophilic species are probably not metabolically active below 0.80 water activity (a_w). Connon *et al.* (2007) revealed that a drop in water activity below 0.88 a_w causes termination of metabolism in bacteria although viability is maintained. The contact between soil and water in the hand-dug wells is a potential source of Actinobacteria. Since this phylum has mostly been reported to be found in the soil (Goodfellow and Williams, 1983; Mohammadipanah and Wink, 2016), it is plausible that a faction of the Actinobacteria detected originated from the soil and were either active or dormant. With growing evidence that Actinobacteria are dominant commonly isolated freshwater bacteria (Crump and Hobbie, 2005; Allgaier *et al.,* 2007; Wilhelm *et al.,* 2014; Sun *et al.,* 2017), the traditionally accepted idea that they are soil based organisms is evolving.

Mohammadipanah and Wink (2016) revealed that bacteria can regulate their water requirements in order to maintain physiological processes as evidenced by bacteria found in arid habitats. Actinobacteria cell dormancy also contributes to the perceived structure of microbial communities making it difficult to distinguish the active and dormant species in metagenomics studies, leading to partial understanding of their role in these hand-dug wells. Bull (2011) reported that extremophiles can grow and survive at extreme ranges of physicochemical parameters, and extremotrophs although not well optimized can also grow and survive in extreme conditions, but at a slow rate. The existence of acid-tolerant, alkaliphilic, psychrotolerant, thermotolerant, halotolerant, alkalitolerant, haloalkalitolerant, and xerophilous Actinobacteria culminates into a conclusion that they can survive in water and effectively reproduce. Moreover, Actinobacteria has been isolated from a broad range of extreme ecosystems in which water is not ruled out (Lubsanova *et al.*, 2014).

Mohammadipanah and Wink (2016) stated that extremotolerants may have larger genetic and metabolic plasticity. It can be argued that such bacteria can adapt to changes in physicochemical parameters in the hand-dug wells. Nonetheless, this study showed that there was a highly significant difference in Actinobacteria abundance between the wet and dry season in which the wet season had a higher abundance. Relative abundance calculations between the wet and dry season showed a slight decrease in relative representation of Actinobacteria in the wet season, revealing that the seasonal changes in physicochemical parameters have an influence. Pearce *et al.* (2013) studied the bacterial diversity of Lake Hodgson and detected Actinobacteria (23%), Proteobacteria (21%), Planctomycetes (20.2%) and Chloroflexi (11.6%) as the dominant phyla while the present study found Proteobacteria to be the most dominant in the wet and dry season, and Planctomycetes and Chloroflexi were insignificantly represented.

Sun *et al.* (2017) studied the effect of season on the diversity and composition of bacterioplankton communities in Dongjiang River, a drinking water source of Hong Kong. Relative abundances indicated that the dominant phyla were Proteobacteria (45.7%), Actinobacteria (24.6%), and Bacteroidetes (14.6%). Sun *et al.* (2017)'s relative abundance calculations for both seasons revealed that the dry season had about 25.2% Actinobacteria and 17.8% Bacteroidetes, while the wet season had 22.2% Actinobacteria and 13% Bacteroidetes indicating a significantly high relative abundance in the dry than wet season ($P < 0.01$), and Proteobacteria showed a minor reduction in the dry season than wet season respectively (45.8% - 46.5%). Relating to the present study, this showed that although hand-dug wells and rivers are both freshwater environments, the patterns of relative abundances of dominant phyla varies probably because river water flows thereby inherently inducing variations in physicochemical parameters while hand-dug well water is stationary.

The present study established that Actinobacteria were the third relatively abundant phylum in the wet season and overall across all hand-dug wells from both seasons but was the fourth relatively abundant in the dry season probably due to an increase in abundance of Bacteroidetes. Seasonal patterns showing a high relative abundance of Actinobacteria in the dry season than the wet have been reported in other freshwater studies by Allgaier *et al.* (2007) and Wilhelm *et al.* (2014), emphasizing the correlation with the seasonal shifts in physicochemical parameters. Furthermore, their dominance in both seasons confirmed their pronounced ecophysiological plasticity that permits them to adapt to various freshwater ecosystems and dynamic seasonal changes (Allgaier *et al.,* 2007; Sun *et al.,* 2017).

The present study revealed that Bacteroidetes were among the dominant phyla in hand-dug wells but had no significant seasonal difference. Bacteroidetes also known as *Cytophaga–Flexibacter–Bacteroides* (CFB) group are a diverse gram-negative bacterial phylum with about 7000 different species (Thomas *et al.,* 2011). This phylum is described to occupy various ecosystems such as freshwater, soil, ocean, humans, animals, and plants (Newton *et al.,* 2011). Thomas *et al.* (2011) and Vos *et al.* (2011) revealed that the Bacteroidetes phylum consists of four classes namely Bacteroidia, Flavobacteria, Sphingobacteria, and Cytophagia. Flavobacteria is the largest and diverse class consisting of about four times the quantity represented in the rest (Thomas *et al.,* 2011). Bacteroidetes consist of physiological types covering the spectrum from strictly anaerobic Bacteroides to strictly aerobic Flavobacteria, and can degrade complex organic matter which makes them easily inhabit various ecological niches (Thomas *et al.,* 2011).

Bacteroidetes and Firmicutes are responsible for about 98% of the mammal gut microbiota. Zhang *et al.* (2015) argued that the main gut bacterial phyla in healthy humans in the order of abundance are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria. Various studies have also indicated that Bacteroidetes and Firmicutes are dominant in the gut of humans and livestock Keijser *et al.,* 2008; Scupham *et al.,* 2008; Lu *et al.,* 2009; Matsui *et al.,* 2010; Middelbos *et al.,* 2010; Leng *et al.,* 2011; Parfrey *et al.,* 2011), and a change in their relative abundance is associated with disease state. Bacteroidetes have been detected in various environments indicating their adaptability. Reichenbach (2006) and Thomas *et al.* (2011) disclosed that Bacteroidetes have been detected in ecosystems not limited to soil, activated sludge, plants, dung, freshwater, oceans, algae, dairy products, and diseased animals. Flavobacteria, Cytophagia, and Sphingobacteria are predominantly found in the environment whereas Bacteroidia are the main gut Bacteroidetes.

Bacteroidetes have been described to be among the abundant phylum detected in marine environments following Proteobacteria and cyanobacteria (Fernández-Gomez *et al.*, 2013). Alonso *et al.* (2007) and Pommier *et al.* (2007) revealed that Bacteroidetes are generally distributed in diverse marine ecosystems such as coastal, offshore, sediments and hydrothermal vents. It is widely accepted that the Bacteroidetes mainly survive through adhesion to particles and degradation of polymers. Fernández-Gomez *et al.* (2013) argued that Bacteroidetes relative abundance increases after algal blooms indicating a preference for consuming polymers rather than monomers, and have adhesion ability and gliding motility, abundant glycosyl transferases, and numerous polymer degrading enzymes. This confirms the role of this abundant group of marine bacteria as degraders of particulate matter and is indicative of a high genetic plasticity.

Although most aquatic studies focused and detected Bacteroidetes to be dominant in marine ecosystems (Pommier *et al.*, 2007; Staufenberger *et al.*, 2008; Edwards *et al.*, 2010; Julies *et al.*, 2010; Salaun *et al.*, 2010), they can also dominate freshwater environments as indicated in this study. This agrees with Zhang *et al.* (2015), Staley *et al.* (2013) and Sun *et al.* (2017)'s studies in which Bacteroidetes were among the main phyla in freshwater lakes and rivers respectively. However, the present study disclosed that there was no significant difference in the abundance of the Bacteroidetes phylum between the dry and wet seasons. This could be because Bacteroidetes colonize various environments and can generally adapt to diverse conditions. Furthermore, Bacteroidetes can metabolise complex polymers which makes them easily survive in low nutrient environments that would otherwise limit the growth of other bacterial phyla. This corroborates with Lauber *et al.* (2009)'s study that revealed that Bacteroidetes can survive and grow at various soil pH values ranging from acidic (<4) to basic (pH > 8).

It can be hypothesised that the slight increases in relative abundances of Bacteroidia and Sphingobacteria in the wet season probably due to surface runoff from rain. This is evident because Bacteroidia are the main gut Bacteroidetes, hence their four fold increased abundance in hand-dug wells in the wet season reflected the transportation and contamination of these hand-dug wells by faecal matter mainly by overland flow. Herschy (2012) disclosed that Namibia recorded the highest rate of open defecation in southern Africa. In addition, Bacteroidia are the most versatile class in the Bacteroidetes phylum and this is evident in their relatively stable abundance at different pH values that exist in different compartments of the gastrointestinal tract (Bik *et al.*, 2006). Bik *et al.* (2006) reported that Bacteroidetes are abundant in the gastrointestinal tract despite the changes in conditions such as pH, nutrients, and oxygen availability, and aid in bile acid metabolism, and transformation of toxic and mutagenic compounds (Smith *et al.*, 2006). However, Sun *et al.* (2017) examined water from rivers and found that Bacteroidetes relative abundances were significantly higher in the dry season compared to the wet season. This could have been due to the continuous flow of rivers as opposed to hand-dug wells which retain contaminants for lengthy periods.

The present study indicated that Cyanobacteria were among the dominant phyla and had a highly significant seasonal difference in which the wet season had a higher abundance than the dry season. Cyanobacteria also known as blue-green algae are a diverse phylum consisting gram-negative bacteria with ecological importance. Cyanobacteria are photosynthetic prokaryotes that lack internal organelles, histone proteins associated with eukaryotic chromosomes, and a distinct nucleus. The Cyanobacteria phylum is composed of unicellular (coccoid) and filamentous classes involved in the cycling of nitrogen (diazotrophic) through a nitrogenase complex and can split water yielding oxygen and electrons during photosynthesis unlike other bacteria that split H_2S. Cyanobacteria are supposed to have brought about the Earth's early oxygenic atmosphere due to their photosynthetic abilities (Schopf and Walter, 1982).

Cyanobacteria colonize diverse environments such as soil, lakes, oceans, acidic bogs, deserts and volcanoes (Falkowski and Raven (2013). Havens (2008), Azúa-Bustos *et al.*, (2011), and Falkowski and Raven (2013) argued that Cyanobacteria preferably inhabit alkaline aquatic environments in comparison to soil, rocks, atmosphere, rain and fog. This phylum is characterised by both bacteria and algae (Fay, 1983), and can withstand harsh conditions such as desiccation, and nutrient deprivation. Potts (1996) argued that some species of Cyanobacteria can retain their metabolic activity upon rehydration after being desiccated for as long as ten years. Cyanobacteria can form microbial mats which are microbial communities with a multi-layered structure that grow in various habitats including freshwater environments, hypersaline ponds, and hot springs. Stal (2012) revealed that microbial mats are commonly made by filamentous and entwined organisms that can make macroscopic mat resembling structures. Other organisms such as benthic microbial communities have no coherent mats (Stal, 2012). These mats show pronounced diversity in appearance and composition (Allen *et al.,* 2009), and may include diatoms and various immobilized microorganisms (Skyring and Bauld, 1990).

Unicellular forms of Cyanobacteria are known to fix nitrogen in micro-aerobic ecosystems only while filamentous forms can fix nitrogen aerobically by forming specialized cells called heterocysts responsible for nitrogen fixation (Stal, 2012). The nitrogenase is sensitive to oxygen and hence Cyanobacteria have developed ways of protecting this enzyme such as loss of the oxygen evolving photosystem II apparatus, the loss of the reductive pentose phosphate pathway (Calvin cycle), and a relatively thick cell wall, decreasing the effective diffusion of gases into the cell, and temporal separation of photosynthetic respiration and nitrogen fixation activities (Stal, 2012). Cyanobacteria contain a blue-green pigment, phycocyanin which together with chlorophyll are responsible for its blue-green appearance.

This study found that Oscillatoriales had the highest relative abundance followed by Chroococcales and Prochlorales. The increased abundance of Oscillatoriacean Cyanobacteria led to a theory that since they are mobile and can glide, they maintained photosynthesis because they could secure and establish a fundamental niche (Azúa-Bustos *et al.,* 2011; Madigan et al., 2015). The observed increased relative abundance was due to surface runoff which carried Cyanobacteria species from soil into hand-dug wells and activated their metabolic activities. Some Cyanobacterial species were probably inactive in soil due to desiccation. This agrees with Potts (1996)'s findings that dehydrated Cyanobacteria can retain their metabolic activity upon rehydration. This led to a theory that water availability coupled with high temperatures were instrumental in the increased abundance. Namibia is a dry country that has a wet season characterised by high temperatures (Midgley *et al.,* 2005), favouring the growth of Cyanobacteria since they preferably inhabit aquatic environments with high temperatures (Havens, 2008; Azúa-Bustos *et al.,* 2011; Falkowski and Raven, 2013). Stevenson and Hallsworth (2014) argued that water activity is needed for bacterial growth and survival and low water levels terminate metabolism. Sun *et al.* (2017) reported similar results with a 10-fold increase in relative abundance of Cyanobacteria in the wet season compared to the dry season. Wilhelm *et al.* (2014) disclosed that Cyanobacteria dominated in summer at all the studied stations of Lake Erie, confirming that temperature influences Cyanobacteria distribution.

This study found a highly significant difference in the abundance of Firmicutes between two seasons in which the wet season had a higher abundance than the dry season. The Firmicutes phylum is composed of both gram-negative and gram-positive bacteria that inhabit various ecosystems. This phylum currently consists of seven classes namely Bacilli, Clostridia, Erysipelotrichia, Limnochordia, Negativicutes (gram-negative), Thermolithobacteria and Tissierellia (Marchandin *et al.*, 2010; Watanabe *et al.*, 2015). However, Zhang *et al.* (2015) described Firmicutes as gram-positive bacteria with a low G + C content including the large class of *Clostridia* and the lactic acid bacteria, indicating changes in bacterial taxonomy with new knowledge. Firmicutes together with Bacteroidetes form the majority (> 98%) of the microbes inhabiting the gastrointestinal tract of humans and mammals (Zhang *et al.*, 2015). Zhang *et al.* (2015) revealed that the gastrointestinal tract's dominant bacterial phyla in order of importance are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria.

Firmicutes are among the dominant phyla in freshwater environments, and were dominant in both the dry and wet seasons of the present study. It is generally accepted that this phylum is widely distributed across various habitats especially in the soil (Zhang *et al.*, 2015). Poor hand-dug well construction leading to contact between soil and water contributed to the high Firmicutes abundance. Hence, the abundant detection of this phylum in both seasons confirmed that they are versatile, dominant in freshwater and can adapt to various environmental changes. The increased Firmicutes abundance in the wet season could have been due to surface runoff that transported faecal matter into the hand-dug wells. Zhang *et al.* (2015) investigated 13 freshwater lakes in the Yunnan–Guizhou Plateau in southwest China and found that Bacteroidetes and Firmicutes dominated in two lakes, and there was no discernible factor attributable to their dominance.

Bai *et al.* (2012) investigated bacterial communities in the sediments of Dianchi Lake in southern China in different seasons in which samples were obtained from two basins within Dianchi Lake, Caohai with higher organic carbon levels and Waihai with lower organic carbon levels. Bai *et al.* (2012) found that Firmicutes were among the dominant phyla in all samples from different seasons regardless of organic carbon levels, highlighting their high genetic plasticity and ability to attain nutrients from degrading complex compounds in freshwater ecosystems. It has been reported that Firmicutes can degrade various organic compounds which makes them survive in nutrient deprived environments (Thomas *et al.,* 2011; Cupples, 2013; Fuentes *et al.*, 2014; Gomes *et al.,* 2014). Some Firmicutes can form spores and this enables them to inhabit diverse ecosystems with various stresses such as desiccation, organic solvents and oxidizing agents, Ultra-Violet irradiation, and predation by protozoa (Schleifer, 2009; Horneck *et al.*, 2010). Galperin (2013) disclosed that Firmicutes that can form spores that inhabit most aquatic and terrestrial ecosystems.

The increased relative abundance of families Staphylococcaceae, Streptococcaceae and Clostridiaceae in the wet season suggested that hand-dug wells were faecally contaminated mainly by surface runoff. In addition, some members of Clostridiaceae, Staphylococcaceae and Streptococcaceae are ubiquitously distributed in the soil (Badhai *et al.,* 2015), hence contact between hand-dug well water and soil in the hand-dug well walls could have contributed to their high detection levels. Lagier *et al.* (2012) revealed that Clostridiaceae species are abundant in the gut of mammals with values of about 10^{11} species per gram of faeces. This agrees with Girija *et al.* (2013) and Kim *et al.* (2017) who found Firmicutes in particular Bacillales and Clostridiales to be highly abundant in cattle microbiota respectively.

Besides, Firmicutes are the most abundant in the gastrointestinal tract of mammals followed by Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria (Zhang *et al.* 2015). Badhai *et al.* (2015) argued that orders Bacillales, Clostridiales, and Thermoanaerobacterales are decomposers of organic matter and involved in carbon cycling. These orders contain species that can form spores enabling them to inhabit both terrestrial and aquatic ecosystems in which they survive harsh conditions and resume metabolism and growth when conditions are favourable. The wet season is characterized with increased organic deposits and moderate to high temperatures in hand-dug wells, necessitating the increased abundance of

Firmicutes. In addition, increased water availability can trigger metabolism in dormant spore forming bacteria.

There was a highly significant difference in the abundance of Proteobacteria between two seasons, the wet season recorded a higher abundance than the dry season. Proteobacteria are among the main division within the prokaryotes and consists of gram-negative bacteria. Proteobacteria also known as purple bacteria consist of most known gram-negative pathogens which display various phenotypic and physiological characteristics (Gupta, 2000). This phylum consists of many phototrophs which produce purple features, heterotrophs and chemolithotrophs (Hedrich *et al.*, 2011). Proteobacteria consists of the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria. Dworkin (2006), argued that Proteobacteria are the largest and most phenotypically diverse phylogenetic lineage. This phylum is widely documented among others and demonstrates pronounced metabolic diversity.

Dworkin (2006) revealed that this phylum is of great biological importance since it contains most bacteria of medical, veterinary, industrial and agricultural importance. Holt *et al.* (1994) and Garrity (2001) disclosed that members of the Proteobacteria phylum have diverse characteristics not limited to aerobic or microaerophilic metabolism, motility, facultative anaerobic metabolism, obligate anaerobic metabolism, anoxygenic phototrophic metabolism, aerobic chemolithotrophic metabolism and sulphate or sulphur-reducing. Proteobacteria inhabit diverse environments including soil, plants, animals, and different kinds of water bodies (Holt *et al.*, 1994). The present study found that Proteobacteria was the most abundant phylum inhabiting hand-dug wells in both the wet and dry seasons. This phylum is the most abundant in the bacterial domain and has a wide distribution across different ecosystems.

It was also evident that the Bradyrhizobiacea relative abundance drastically increased in the wet season compared to the dry. Bradyrhizobiacea is predominantly a soil inhabiting family although it is also found in plants, freshwater and animals (Garrity, 2001). The high relative abundance of Bradyrhizobiacea in the wet season indicated that surface runoff and soil-water contact in the hand-dug wells were the main determinants. Additionally, species in this family display metabolic diversity are photosynthetic and play a role in biogeochemical cycles. de Souza *et al.* (2014)

revealed that Bradyrhizobium and other diazotrophic members can fix nitrogen and this property can be exploited in agriculture. These species can employ both aerobic and/or anaerobic respiration (Garrity, 2001), and this allows them to occupy and survive in diverse environments. The findings of this study agree with Oh *et al.* (2011)'s investigation of Metagenomics microbial communities of Lake Lanier in which Proteobacteria was the most dominant (37%) followed by Actinobacteria (32%) and Verrucomicrobia (14%).

Sun *et al.* (2017) did not detect significant differences in the abundance of Proteobacteria between the wet and dry seasons, confirming the versatility of this phylum and leading to the conclusion that the significant differences recorded in the present study are linked to livestock and human faecal matter, and soil transportation into the hand-dug wells due to surface runoff. Most families that have been described to be part of the human and livestock gastrointestinal tract were detected in the present study and this correlates with the high levels of open defecation. It is widely documented that the mammalian intestinal tract is characterized by the dominance of Bacteroidetes (23%) mostly from the genus Bacteroides, Firmicutes (64%) consisting of *Bacilli*, *Clostridia* and Mollicutes mostly from the genus *Streptococcus* and *Clostridium*, Proteobacteria (eight percent) mostly from the Desulfobulbaceae, Lactobacillaceae and Enterobacteriaceae while Fusobacteria, Verrucomicrobia and Actinobacteria constitute about three percent (Andersson *et al.,* 2008; Qin *et al.,* 2010; Clemente *et al.,* 2012; Lisko *et al.,* 2017).

5.2.2 Effect of seasonal changes in physicochemical parameters on the abundance of the detected bacterial phyla

The main factors that influenced bacterial phyla abundance in the hand-dug wells were phosphate (PO_4^{3-}), manganese (Mn^{2+}), potential of hydrogen (pH), and temperature. It was noted that temperature was positively correlated with the dry season and negatively correlated with the wet season, while pH, PO_4^{3-}, and Mn^{2+} were positively correlated with the wet season and negatively correlated with the dry season. Phosphate is a key macronutrient needed for the growth of bacteria, and manganese aids various enzymes in catalysing the transfer of phosphate groups (Madigan *et al.,* 2015). Manganese is a key component of water-splitting enzymes in oxygenic phototrophs (photosystem II) and certain superoxide dismutase's. Madigan *et al.* (2015) revealed that the main nutrients needed for bacterial growth and survival are carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulphur (CHONPS). Phosphorous is necessary for nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell components, and is obtained from inorganic phosphates and integrated directly.

Hence, manganese and phosphorous were the main chemical factors responsible for the pronounced bacterial growth in hand-dug wells of the wet season and this agrees with Miettinen *et al.* (1997)'s findings on the effect of phosphorus on bacterial growth in drinking water in which the addition of phosphorus (PO_4-P) of up to 10 micrograms per litre increased microbial growth in freshwater from the surface, groundwater and water in distribution networks. However, Miettinen *et al.* (1997) also reported that sodium, potassium, magnesium and calcium did not significantly affect microbial growth although low amounts (one microgram) of phosphorus had noticeable effects.

It is well known that pH and temperature have an influence on the growth and survival of bacteria. Haley *et al.* (2009) and Parker *et al.* (2010), argued that temperature is an important factor influencing both the die-off and growth of bacteria in water aquatic ecosystems. This agrees with the findings of Bull (2011), and Mohammadipanah and Wink (2016) who reported that pH, salinity, water content, temperature, pressure and radiation are the major physicochemical parameters that regulate bacterial growth and survival. Optimal bacterial growth occurs at various levels of pH ranging from low, moderate, and high in different species. Organisms that grow best; at low pH (< 5.5) are classified as acidophiles, at moderate pH (5.5 - 7.9) are classified as neutrophils and at high pH (≥ 8) are called alkaliphiles (Madigan *et al.*, 2015). The present study's pH values ranged from 7.18 to 8.31 in the wet season and 5.68 to 8.34 in the dry season promoting the growth of mainly neutrophils and minor alkaliphiles in the wet and dry seasons.

Similarly, various bacterial species have different temperature ranges for optimal growth (Madigan *et al.*, 2015). Northern Namibia's (study site) hand-dug well water temperature values ranged from 13.2° C to 26.3° C in the wet season and 20.5° C to 34.6° C in the dry season which supports the growth of mostly mesophilic bacteria. The bacterial species are categorised into psychrophiles, mesophiles, thermophiles and hyperthermophiles based on their cardinal temperatures. Temperature can affect microorganisms in dual contrasting ways; increasing temperatures favour the rate of enzymatic reactions promoting growth while beyond a particular temperature, denaturation of proteins, enzymes and cell components occurs. A psychrophile is an organism with an optimal growth temperature of 15° C or lower, and a maximum growth temperature below 20° C and a minimal growth temperature at 0° C or lower (Madigan *et al.*, 2015). A mesophile is an organism with an optimum temperature typically between 20° C and 45 °C. Organisms whose growth temperature optimum exceeds 45° C are called thermophiles and those whose optimum exceeds 80° C are called hyperthermophiles (Madigan *et al.*, 2015). Organisms that grow at 0° C but have optimal growth at 20° C – 40° C are called psychrotolerant.

In this study, hand-dug well samples from the wet season were positively correlated with pH, Mn^{2+} and PO_4^{3-}, and negatively correlated with temperature while hand-dug well samples from the dry season displayed a positive association with temperature and a negative association with pH, Mn^{2+} and PO_4^{3-}. The separate clustering of the wet season hand-dug well samples from the dry season hand-dug well samples confirmed that hand-dug wells from each respective season had similar physicochemical parameters, and established that there is a significant shift in these parameters between seasons. Although carbon, hydrogen, nitrogen and sulphur are major nutrients for microbial growth, they were not responsible for the variation in abundance of bacterial growth between seasons because the Cuvelai Etosha basin hand-dug wells normally contain large amounts of organic matter and humus substances which is exacerbated by inappropriate hand-dug well construction.

This study found that the potential sources of organic matter found in hand-dug wells were mainly; dead plant material from plants that grew inside on the walls of hand-dug wells, birds and small mammals that died in the hand-dug wells, and faecal matter that gained entry into these hand-dug wells. Carbon has been documented to be the principle determinant of microbial growth (LeChevallier *et al.*, 1991; van der Kooij, 1992; Proctor *et al.*, 2017) but the present study confirmed the findings of Miettinen *et al.* (1997) in which assimilated organic carbon had a poor correlation with microbial growth in drinking water, highlighting that other macronutrients can influence microbial growth in freshwater. Miettinen *et al.* (1997) also emphasized that nitrogen had insignificant effects on microbial growth.

The present study indicated that the cluster of phyla from the dry season were positively correlated with temperature and negatively correlated with PO_4^{3-}, Mn^{2+} and pH. While the cluster of phyla from the wet season were positively correlated with PO_4^{3-}, Mn^{2+} and pH, and negatively correlated with temperature. The detected abundant phyla; Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria and Cyanobacteria were all part of the cluster of phyla from the wet season. Although none of the phyla from the cluster of phyla from the dry season recorded a significant relative abundance in both the wet and dry season, it was established that the growth of bacteria belonging to this cluster of phyla was mainly influenced by temperature, and was largely composed of autotrophic thermophilic bacteria. In addition, the growth of bacteria belonging to the cluster of phyla from the wet season was largely influenced by the hand-dug well concentrations of PO_4^{3-} and Mn^{2+}, and pH. This agrees with Lauber *et al.* (2009), who studied soils from 88 different places and observed a positive correlation between the pH of the substrate and the relative abundance of major phyla such as Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria, and these relative abundances ranged from 1.7% at low pH (<4) to 17% in basic soils (pH > 8).

The cluster of phyla from the wet season was largely composed of autotrophic chemolithotrophic bacteria and this agrees with Madigan *et al.* (2015)'s report on the growth requirements and preferred habitats of bacterial species under these clusters. In general, since carbon is abundant and widely distributed across habitats compared to PO_4^{3-} and Mn^{2+}, it is logical that the growth of bacteria in freshwater with lack/extremely low concentrations of PO_4^{3-} and Mn^{2+} is disadvantaged thereby highlighting PO_4^{3-} and Mn^{2+} as the limiting factors. Carbon was abundant in the hand-dug wells and thus could not limit bacterial growth due to the presence of faecal matter and soil organic matter, plant and animal residues, cells and tissues of soil organisms, and materials produced by soil organisms. It was observed that faecal matter was largely found on the soil within the vicinity of hand-dug wells and this was probably the reason for increased hand-dug well macronutrients levels including carbon in the wet season.

This study found that potential sources of PO_4^{3-} and Mn^{2+} were mainly agriculture through the use of fertilizers, pesticides, salts, animal manure, and high level of human and livestock open defecation. Chemicals from agricultural activities are deposited into hand-dug wells through direct surface runoff, leaching from natural vegetation, and through seepage to ground water that discharges to a surface water outlet (Galadima, *et al.*, 2011). Galadima, *et al.* (2011) argued that surface runoff affects hand-dug well water physicochemical parameters which may lead to an increased temperature and decreased oxygen water environment. Furthermore, deposits of animal manure in hand-dug wells can elevate bacterial populations and increase diversity by several magnitudes since animal manure is about one hundred times more concentrated with bacteria than domestic sewage (Galadima, *et al.*, 2011).

5.2.3 Effect of hand-dug well type, region and season on bacterial species diversity, richness and evenness

There was no significant difference in species diversity, richness and evenness based on hand-dug well type and region, confirming that geographic location and hand-dug well type does not seem to have major impacts on bacterial abundance and diversity (Mohiuddin *et al.*, 2017). Therefore, this section discusses the relationship between season and species diversity, richness and evenness. There was no significant difference in species diversity and evenness based on season. However, there was a significant difference in species richness based on season, with the dry season having a higher species richness compared to the wet season. Bacterial species diversity and evenness did not show a significant difference between the wet and dry season indicating that hand-dug well bacterial diversity and evenness is independent of season. The sustained species diversity and evenness could have been caused by the poor structure, lack of protection from animal access and lack of a covered top throughout the year thereby allowing bacteria to be deposited into these wells. The easy access of livestock, other domestic and wild animals or birds to the water exposes these hand-dug wells to diverse forms of bacteria regardless of season.

The diversity and evenness of the bacteria in the hand-dug wells was similar in both season due to continuous contact between soil and water in the hand-dug wells. Since various bacteria belonging to different phyla have mostly been reported to be found in the soil (Mohammadipanah and Wink, 2016), it is plausible that most of the bacteria detected originated from the soil, and were either active or dormant which is an inherent limitation of Metagenomics. The water-soil bacteria interface allows these bacteria to survive thereby maintaining the diversity and evenness within the hand-dug wells and this agrees with Bull (2011) who reported that bacteria can grow and survive at various ranges of physicochemical parameters, and growth occurs at a slow rate because the environment is not well optimized. The findings of this study confirmed Sun *et al.* (2017)'s results which showed a high diversity of bacteria in a river in both the wet and dry seasons with no significant difference between the two seasons ($P > 0.05$). Furthermore, the sustained diversity of bacteria in both seasons confirmed that bacteria have pronounced ecophysiological plasticity that permits them to adapt to various freshwater ecosystems and dynamic seasonal changes (Allgaier *et al.,* 2007; Sun *et al.,* 2017).

The present study indicated that there was a significant difference in abundance and richness of bacterial species between the dry and wet seasons. The wet season had higher abundances of bacterial species than the dry season, and bacterial species richness was higher in the dry season compared to the wet season. The variation in abundance was due to surface runoff and the downward transportation of bacteria by water through the permeable soil layers in the wet season. Surface runoff transports various bacteria into the hand-dug wells thereby elevating their abundance in the wet season. The reason for an elevated bacterial species richness in the dry season is most likely due to increased evaporation of hand-dug well water leading to a reduced volume of water with concentrated bacteria (Odonkor and Addo, 2013). However, it's worth noting that surface runoff increases the abundance of bacterial species in the wet season while increased evaporation that occurs in the dry season increases bacterial species richness but not species diversity and evenness especially that the water is mostly in contact with soil.

5.2.4 Human, livestock and zoonotic bacterial pathogens detected in hand-dug wells

Human, livestock and zoonotic pathogens, and grey bacteria (see Appendices 3, 6, 9 and 12) were identified. The analysis of the effect of hand-dug well type and region on the abundance of human, livestock and zoonotic pathogens did not show significant trends (see Appendix), highlighting that these factors did not influence the abundance of pathogens in hand-dug wells. Hence, the discussion below focuses on the relationship between season, and human, livestock and zoonotic pathogens. The high numbers of detected human pathogens highlighted that humans are more at risk of getting bacterial infections from drinking hand-dug well water compared to livestock. The high numbers of shared (zoonotic) pathogens is alarming due to possibilities of transferring untreatable bacterial infections between humans and livestock that arise as a result of the inappropriate use of antibiotics in food animals. Detected genera from the Enterobacteriaceae family that are known human pathogens and can express multidrug resistance genes include *Shigella* (Baker *et al.*, 2016), *Escherichia* (Chen *et al.*, 2017), *Klebsiella* (Moradigaravand *et al.*, 2017), *Enterobacter* (Janecko *et al.*, 2016), *Proteus* (Jain *et al.*, 2016), *Salmonella* (Begum *et al.*, 2017), *Citrobacter* (Liu *et al.*, 2016) and *Pseudomonas* (Magalhaes *et al.*, 2016).

5.2.4.1 Human bacterial pathogens detected in hand-dug wells

Most clusters of the human pathogens in the phylogenetic tree were formed by species belonging to the same genus indicating their close relation. The detection of multiple species in each genus and their close relation confirmed intra-genus versatility. The notable human pathogens of public health concern were; *Citrobacter spp.* known to cause urinary tract infections (UTI), meningitis, bacteraemia and haemolytic–uraemic syndrome (eMedMD.com) *H. parainfluenzae* is known to cause sinusitis, otitis media, pneumonia, abscesses, endocarditis, and biliary tract infections (eMedMD.com; Frankard *et al.*, 2004). *Legionella* species (*L. jordanis, L. lytica, L. pneumophila* and *L. sainthelensi*) cause Legionnaires' disease, respiratory tract infections and Pontiac fever (eMedMD.com). *Pseudomonas* species (*P. mendocina*, *P. oryzihabitans*, *P. putida, P. stutzeri*) cause bacteraemia, UTI, wound infection, abscesses, septic arthritis, conjunctivitis, endocarditis, meningitis, otitis, sepsis, peritonitis, pneumonia and urinary tract infections (Ragone *et al.*, 1992; Lalucat *et al.*, 2006; Yoshino *et al.*, 2011; Tena and Fernández, 2015). *S. sonnei* causes enteric infections (Bowen *et al.*, 2015; Thompson *et al.*, 2015), and *V. cholerae* causes cholera (Robins and Mekalanos, 2014; Bhuiyan *et al.*, 2016).

H. parainfluenzae, L. lytica, L. sainthelensi, P. mendocina, P. oryzihabitans, P. putida, P. stutzeri and *S. sonnei* showed a significant difference in abundance between the wet and dry seasons. *L. sainthelensi*, *P. oryzihabitans*, *P. putida*, *P. stutzeri* and *S. sonnei* were more abundant in wet season compared to the dry season, demonstrating that the bacterial communities of the Cuvelai Etosha Basin are exposed to these pathogens to a higher extent in the wet season than the dry season. *H. parainfluenzae, L. lytica* and *P. mendocina* were more abundant in the dry season compared to the wet season, indicating that diseases caused by these species are expected to surge in the dry season. However, there was no significant difference in the abundance of *Citrobacter spp.*, *L. jordanis, L. pneumophila* and *V. cholerae* between the wet and dry seasons, indicating that the Cuvelai Etosha Basin communities are exposed to these pathogens continuously. This explains the none seasonal sporadic cholera outbreaks that occur in these communities and highlights the necessity of adhering to hygiene practices and implementing routine hand-dug well water bacteriological analysis. The rest of the detected human pathogens are reported to mostly cause endocarditis, meningitis and bacteraemia (see Appendix 5**)**.

5.2.4.2 Livestock bacterial pathogens detected in hand-dug wells

A phylogenetic tree was also generated for the detected livestock pathogens which excluded zoonotic bacteria. However, the livestock category had only five bacterial species making it inapplicable to infer phylogenetic relationships. Livestock pathogens detected were *Acetivibrio spp.* known to cause diarrhoea and is associated with dysentery in pigs as reported by Robinson and Ritchie (1981). Robinson and Ritchie (1981) found that *Acetivibrio ethanolgignens* constituted 20% or more of the bacterial population from the colon of pigs infected with dysentery and was not found in healthy pigs. *A. laidlawii*, *A. morum* and *Acholeplasma spp.* cause mystery swine disease and cattle dermatitis (Wensvoort *et al.*, 1991; Yano *et al.*, 2010), and *P. pulmonis* causes lung infections in sheep (Vela *et al.*, 2003).

Acholeplasma spp. showed a significant difference in abundance between the wet and dry seasons with higher abundances in the wet than dry season, while *Acetivibrio spp.* and *P. pulmonis*, *A. laidlawii* and *A. morum* showed no significant difference in abundance between the wet and dry seasons. The occurrence of these pathogens in the hand-dug well water taken by livestock indicated that the water was not fit for livestock consumption and was potentially the source of diarrhoea, eye and mouth infections, and cough that were observed in livestock in this study. There is a lack of research and documentation on livestock diseases in the Cuvelai Etosha Basin communities except for foot and mouth disease which seems to be prioritised by the government. However, these results demonstrated that livestock had a higher exposure to *Acholeplasma spp.* in the wet season compared to the dry season, and a continuous exposure to *Acetivibrio spp., A. laidlawii*, *A. morum*, and *P. pulmonis* in both seasons.

5.2.4.3 Zoonotic bacterial pathogens detected in hand-dug wells

Most clusters of the zoonotic pathogens in the phylogenetic tree were formed by species belonging to the same family indicating their close relation. The detection of multiple species in each family and their close relation confirmed the complexity and versatility of families. Among others, the notable zoonotic pathogens of public health concern were *Brucella spp.* known to cause Brucellosis (Assenga *et al.,* 2015; eMedMD.com). Some members of the genus *Bacillus* may cause pneumonia (*B. cereus*), and anthrax (*Bacillus anthracis*) as reported by Logan (1988). Some species of the genus *Chlamydia* may cause abortion (*Chlamydia abortus*) and psittacosis (*Chlamydia psittaci*) in animals, birds and humans (Ni *et al.,* 2015). Some members of the genus *Ehrlichia* (*Ehrlichia equi* and *E. phagocytophila*) can cause Ehrlichiosis (Ehrlichiosis *et al.,* 2013). Some members of the genus *Enterococcus* (*Enterococcus faecalis*, *Enterococcus faecium*) can cause mastitis and bacteraemia (Devriese *et al.,* 1999).

Escherichia coli is known to cause diarrhoea, haemorrhagic colitis, haemolytic uremic syndrome, thrombotic thrombocytopenic Purpura, urinary tract infections, bacteraemia, wound infections, meningitis, enteric infection, uraemic syndrome (Durso *et al.,* 2005; eMedmD.com). Some members of the genus *Helicobacter* (*H. pylori, H. heilmannii*) are known to cause chronic gastritis (Meining *et al.,* 1998; Morgner *et al.,* 2000; Bento-Miranda and Figueiredo, 2014). Some members of the genus *Klebsiella* (*K. pneumoniae*) are known to cause intra-mammary infections, and Donovanosis (*K. granulomatis*) as reported by Umeh and Berkowitz (2002) and Bannerman *et al.* (2004). Some members of the genus *Legionella* (*L. jordanis*, *L. lytica*, *L. pneumophila* and *L. sainthelensi*) are known to cause pneumonia, Legionnaire’s disease and Pontiac fever (Fabbi *et al.,* 1998). Some *Leptospira* species such as *Leptospira interrogans* is known to cause leptospirosis (Bolin and Koellner, 1988; Bharti *et al.,* 2003; Fabijanski, 2008).

Some members of the genus *Microcystis* (*M. aeruginosa*) are known to cause poisoning (*Oehrle et al., 2017*). Some members of the genus *Mycobacterium* (*M. bovis, M. tuberculosis, M. leprae*) are known to cause tuberculosis and leprosy (Palmer *et al.,* 2011; Amato *et al.,* 2017). *Salmonella enterica* is known to cause gastroenteritis, enteric fever, osteomyelitis and diarrhoea (Zhang *et al.,* 2002; Harvey *et al.,* 2017). Some members of the genus *Staphylococcus* (*S. aureus*, *S. epidermidis*, *S. saprophyticus*) are known to cause skin disease, bacteraemia, wound infections, endocarditis, catheter-related sepsis, UTI, toxic shock syndrome, food poisoning, eye infection and osteomyelitis (Vuong and Otto, 2002; WHO, 2008; Manji *et al.*, 2012; Foster, 2012). Some members of the genus *Treponema* (*Treponema pedis*, *Treponema pallidum*) are known to cause dermatitis and syphilis (Evans *et al.*, 2009; Correa *et al.*, 2017).

Brucella spp., *Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp.*, *Legionella spp.*, *Leptospira spp.*, *Microcystis spp.*, *Mycobacterium spp.*, *Salmonella enterica* and *Staphylococcus spp.* showed a significant difference in abundance between the wet and dry seasons. *Brucella spp.*, *Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp.*, *Legionella spp.*, *Microcystis spp.* and *Salmonella enterica* had higher abundances in the dry season compared to the wet season, indicating that the exposure of the populace and livestock in the Cuvelai Etosha Basin to these species is pronounced in the dry season although disease cases are not documented. However, culturing results indicated that *Salmonella* species were more in the wet season which could have been due to the existence of a viable but none culturable state in such species in the dry season or due to Metagenomics detecting none viable cells as discussed earlier. It can be argued that since pathogens show seasonality in aquatic environments and correlate with temperature, it cannot be ruled out that the warmer temperatures in the dry season can also support the growth of *Brucella spp.*, *Bacillus spp.*, *Chlamydia spp.*, *Enterococcus sp.*, *Legionella spp.*, *Microcystis spp.* and *Salmonella enterica* since bacterial species respond quickly to higher temperatures when appropriate resources are available (Kirchman and Rich, 1997).

Leptospira spp., *Mycobacterium spp.* and *Staphylococcus spp.* had higher abundances in the wet season compared to the dry season, demonstrating that exposure to these species is pronounced in the wet season compared to the dry season. Moreover, these species are mesophilic in nature and so the water temperatures were within their optimal growth or survival range since hand-dug well water temperature values ranged from 13.2° C to 26.3° C in the wet season and 20.5° C to 34.6° C in the dry season which supports the growth of mostly mesophilic bacteria. However, *Ehrlichia spp.*, *Escherichia coli*, *Helicobacter spp.*, *Treponema spp.* and *Klebsiella sp.* did not show a significant difference in abundance between the wet and dry seasons demonstrating that the Cuvelai Etosha Basin populace and livestock experience a continuous exposure to these pathogens. This leads to the conclusion that some water related diseases that occur in these communities can be predicted and appropriate prevention measures ascertained based on pathogen’s seasonal variations in abundance.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The aim of this study was to conduct Metagenomics analysis of bacterial communities in hand-dug wells in the Ohangwena and Omusati regions of the Cuvelai Etosha Basin of Namibia. The findings of this study gave rise to the following conclusions and recommendaions:

6.1 Conclusions

1. The microbial communities of the Cuvelai Etosha Basin hand-dug wells and the safety of the hand-dug well water for human and livestock consumption was determined using Metagenomics and culturing. It was found that the hand-dug well water is not safe for human and livestock consumption.
2. Hand-dug well type and region did not influence the abundance of bacterial CFU's while season (wet and dry) had an influence on the abundance of bacterial CFU's. The wet season exhibited higher CFU abundances than the dry season.
3. Bacterial species of the genera *Citrobacter*, *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, and *Pseudomonas* were found in hand-dug wells in both the wet and dry seasons.
4. Hand-dug well type, region and season did not influence the presence of *Citrobacter Escherichia*, *Enterobacter* and *Klebsiella* species, which are the known indicator bacteria and confirms their ubiquitous distribution in nature with the ability to inhabit various environments including water.
5. Furthermore, this study confirmed that nutrients and climatic conditions favouring bacterial growth and survival are not exclusive to the wet season, and hand-dug well type can have an effect on the abundance of bacterial species due to increased competition for space and resources within the diverse collection of microbial species existing in the shallow hand-dug wells.
6. Overall, Proteobacteria followed by Firmicutes, Actinobacteria, Bacteroidetes and Cyanobacteria are the predominant phyla in hand-dug wells of the Cuvelai Etosha Basin. In the wet season, Proteobacteria followed by Firmicutes, Actinobacteria, Bacteroidetes

and Cyanobacteria predominated, while Proteobacteria followed by Bacteroidetes, Firmicutes and Actinobacteria predominate in the dry season.

7. Season has no influence on the abundance of versatile bacterial species in hand-dug wells because they can degrade complex organic matter making them occupy various ecosystems such as freshwater, soil, ocean, humans, animals, and plants. However, season has an effect on the abundance of indigenous faecal matter and soil bacteria because their abundance is pronounced in hand-dug wells in the wet season compared to the dry season due to transportation of faecal matter and soil into the hand-dug wells by surface runoff.
8. Surface runoff increases the abundance of bacterial species in the wet season while increased evaporation in the dry season increases bacterial species richness but not species diversity and evenness especially that the water in hand-dug wells is mostly in contact with soil.
9. The main factors that influenced bacterial phyla abundance in hand-dug wells were phosphate (PO_4^{3-}), manganese (Mn^{2+}), potential of hydrogen (pH) and temperature. With manganese and phosphorous being the main chemical factors responsible for the pronounced bacterial growth in hand-dug wells in the wet season.
10. Hand-dug well type and region do not influence the abundance of human, livestock and zoonotic pathogens and grey bacteria in hand-dug wells.
11. The wet season has a pronounced abundance of human, livestock and zoonotic pathogens and grey bacterial species in hand-dug wells with only a few exceptional species that thrive in higher temperatures.

6.2 Recommendations

The hand-dug wells in the Cuvelai Etosha Basin displayed high levels of contamination with pathogens of public and veterinary importance, signifying that the water is not fit for human and livestock consumption unless appropriate measures are implemented that establish safety. The hand-dug wells in the study area contained high coliform counts and various pathogens that are unacceptable with respect to WHO (2011). It is therefore recommended that;

1. The site of hand-dug well construction should be appropriate with consideration of the probabilities of hand-dug well contamination since hydro-geologists and geophysicists

mainly focus on the site that has more groundwater and neglect environmental interactions such as the proximity of pit latrines that give rise to water pollution.

2. The government should implement guidelines that regulate the construction of hand-dug wells and should frequently inspect the adherence.
3. It is necessary for the government to host cycles of health campaigns that serve to educate these communities since they are unaware of the consequences of constructing hand-dug wells close to sources of contamination.
4. The government should include focus areas such as; water as a reservoir for deadly pathogens into the school curriculum to edify the public.
5. The communities should employ simple water treatment and improvement methods such as sieving, boiling water, disinfection with chlorine, and lining the walls of the hand-dug wells with concrete.
6. The government should implement recurring assessment of hand-dug well water quality through council of elders especially that significant populations rely on hand-dug wells as a water resource.
7. To the science community, it is recommended that extensive research must be performed to develop better and more accurate ways of accessing the safety of drinking water due to the evident limitations of the current indicators as established in this study. In addition, research that involves the die off times of various pathogens in water is necessary. Water based research is of vital importance and should be among the top priorities of research at every institution, this will allow not only ensuring the safety of water but also broaden the knowledge on the microbial interactions that occur in various water bodies and possibly the discovery of new indigenous water microbes.

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APPENDICES

Appendix 1: Kruskal-Wallis test performed to determine the influence of hand-dug well type on the abundance of the detected bacterial phyla.

Phyla	X^2- value	Deg. of freedom	P-value
Acetothermia	3.000	2	0.223
Acidobacteria	2.178	2	0.337
Actinobacteria	3.603	2	0.165
Aquificae	2.667	2	0.264
Bacteroidetes	2.571	2	0.277
Caldiserica	3.000	2	0.223
Candidatus saccharibacteria	1.711	2	0.425
Chlamydiae	0.711	2	0.701
Chlorobi	1.365	2	0.505
Chloroflexi	0.151	2	0.927
Cloacimonetes	1.870	2	0.393
Cyanobacteria	1.393	2	0.498
Deferribacteres	1.789	2	0.409
Deinococcus thermus	0.013	2	0.994
Elusimicrobia	0.947	2	0.623
Fibrobacteres	5.568	2	0.062
Firmicutes	3.038	2	0.219
Fusobacteria	1.144	2	0.564
Gemmatimonadetes	0.131	2	0.937

Ignavibacteriae	3.640	2	0.162
Lentisphaerae	0.156	2	0.925
Nitrospinae	1.886	2	0.390
Nitrospirae	5.345	2	0.069
Planctomycetes	2.052	2	0.358
Proteobacteria	1.032	2	0.597
Spirochaetes	0.581	2	0.748
Synergistetes	2.710	2	0.258
Tenericutes	1.104	2	0.576
Thermo desulfobacteria	0.273	2	0.872
Verrucomicrobia	2.493	2	0.287

Appendix 2: Mann-Whitney U test performed to determine the influence of region on the abundance of the detected bacterial phyla.

Phyla	**Mann-Whitney U Test value**	**Mean Rank (Ohangwena)**	**Mean Rank (Omusati)**	**P – Value**
Acetothermia	231	22.96	22	0.339
Acidobacteria	196	24.48	20.33	0.285
Actinobacteria	287	20.52	24.67	0.285
Aquificae	231	22.96	22	0.339
Bacteroidetes	176	25.35	19.38	0.124
Caldiserica	231	22.96	22	0.339
Candidatus saccharibacteria	211.5	23.80	21.07	0.268
Chlamydiae	207.5	23.98	20.88	0.424
Chlorobi	260	21.70	23.38	0.589
Chloroflexi	234.5	22.80	22.17	0.869
Cloacimonetes	220.5	23.41	21.50	0.172
Cyanobacteria	288	20.48	24.71	0.275
Deferribacteres	221	23.39	21.52	0.334
Deinococcus thermus	244.5	22.37	22.64	0.942
Elusimicrobia	266	21.43	23.67	0.334
Fibrobacteres	223	23.30	21.62	0.383
Firmicutes	311	19.48	25.81	0.102
Fusobacteria	265	21.48	23.62	0.576
Gemmatimonadetes	235.5	22.76	22.21	0.887

Ignavibacteriae	201.5	24.24	20.60	0.162
Lentisphaerae	235	22.78	22.19	0.840
Nitrospinae	220.5	23.41	21.50	0.172
Nitrospirae	192	24.65	20.14	0.203
Planctomycetes	293.5	20.24	24.98	0.221
Proteobacteria	241	22.52	22.48	0.991
Spirochaetes	225.5	23.20	21.74	0.706
Synergistetes	232	22.91	22.05	0.609
Tenericutes	214	23.70	21.19	0.517
Thermo desulfobacteria	249	22.17	22.86	0.768
Verrucomicrobia	229.5	23.02	21.93	0.778

Appendix 3: Kruskal-Wallis test performed to determine the influence of hand-dug well type on the abundance of human bacterial pathogens.

Bacterial species	**X^2- value**	**Deg. Of Freedom**	**P-Value**
Achromobacter spp.	0.794	2	0.672
Achromobacter xylosoxidans	1.068	2	0.586
Acidaminococcus spp.	2.880	2	0.237
Acidovorax delafieldii	2.565	2	0.277
Acidovorax facilis	0.036	2	0.982
Acidovorax spp.	3.939	2	0.140
Acidovorax temperans	1.148	2	0.563
Acinetobacter calcoaceticus	1.470	2	0.480
Acinetobacter johnsonii	0.555	2	0.758
Acinetobacter junii	2.083	2	0.353
Acinetobacter lwoffii	0.391	2	0.823
Acinetobacter radioresistens	0.646	2	0.724
Acinetobacter schindleri	2.973	2	0.226
Acinetobacter septicus	0.567	2	0.753
Acinetobacter spp.	1.776	2	0.412
Actinomadura spp.	0.973	2	0.615
Actinomadura vinacea	1.619	2	0.445
Aeromonas spp.	2.039	2	0.361
Agromyces sp.	0.932	2	0.627
Alistipes finegoldii	0.744	2	0.689
Alistipes shahii	1.095	2	0.578
Alistipes spp.	1.798	2	0.407
Alteromonas sp.	3.000	2	0.223
Anaerococcus sp.	1.472	2	0.479
Anaerovorax spp.	2.941	2	0.230
Arthrobacter oxydans	0.240	2	0.887
Arthrobacter spp.	0.590	2	0.745
Atopobium vaginae	1.118	2	0.572
Aurantimonas sp.	2.182	2	0.336
Azospirillum brasilense	3.000	2	0.223
Bacillus coagulans	0.268	2	0.875
Bacteroides spp.	0.166	2	0.920
Bacteroides vulgatus	3.000	2	0.223

Bergeyella sp.	0.338	2	0.844
Bordetella petrii	0.702	2	0.704
Bosea sp.	1.215	2	0.545
Brevibacillus parabrevis	3.000	2	0.223
Brevibacillus sp.	0.002	2	0.999
Brevibacterium sp.	2.358	2	0.308
Brevundimonas diminuta	0.009	2	0.996
Brevundimonas spp.	0.615	2	0.735
Brevundimonas vesicularis	1.051	2	0.591
Burkholderia spp.	0.255	2	0.880
Burkholderia tropica	1.886	2	0.390
Burkholderia ubonensis	1.797	2	0.407
Butyrivibrio sp.	3.000	2	0.223
Caenispirillum sp.	3.000	2	0.223
Campylobacter lari	1.095	2	0.578
Candidatus neoehrlichia mikurensis	1.531	2	0.465
Catabacter hongkongensis	3.000	2	0.223
Caulobacter spp.	0.716	2	0.699
Caulobacter vibrioides	0.148	2	0.929
Cellulomonas spp.	0.412	2	0.814
Chitinophaga spp.	1.980	2	0.317
Citrobacter spp.	2.667	2	0.264
Clostridium ghonii	0.047	2	0.977
Clostridium intestinale	0.214	2	0.899
Clostridium limosum	1.095	2	0.578
Clostridium sporogenes	1.638	2	0.441
Clostridium subterminale	3.175	2	0.204
Comamonas kerstersii	0.234	2	0.890
Comamonas sp.	0.477	2	0.788
Comamonas testosteroni	1.026	2	0.599
Corynebacterium amycolatum	1.328	2	0.515
Corynebacterium falsenii	1.962	2	0.375
Corynebacterium jeikeium	1.401	2	0.496
Corynebacterium mucifaciens	0.958	2	0.619
Corynebacterium thomssenii	1.207	2	0.547

Corynebacterium tuberculostearicum	1.097	2	0.578
Coxiella burnetii	0.866	2	0.648
Coxiella spp.	2.874	2	0.238
Cupriavidus spp.	3.797	2	0.150
Delftia tsuruhatensis	2.932	2	0.231
Desulfomicrobium spp.	0.708	2	0.702
Desulfovibrio desulfuricans	1.118	2	0.572
Desulfovibrio spp.	2.511	2	0.285
Dietzia papillomatosis	1.050	2	0.592
Dokdonella spp.	1.476	2	0.478
Dyella ginsengisoli	1.104	2	0.576
Dysgonomonas capnocytophagoides	3.016	2	0.221
Dysgonomonas gadei	0.904	2	0.636
Dysgonomonas spp.	2.106	2	0.349
Eggerthella sp.	1.095	2	0.578
Empedobacter brevis	1.944	2	0.378
Empedobacter sp.	0.587	2	0.746
Enterobacter hormaechei	0.478	2	0.787
Enterococcus faecalis	0.906	2	0.636
Escherichia hermannii	1.921	2	0.383
Eubacterium spp.	0.509	2	0.775
Exiguobacterium aurantiacum	3.227	2	0.199
Exiguobacterium sp.	2.534	2	0.282
Fastidiosipila sanguinis	2.667	2	0.264
Finegoldia magna	0.313	2	0.855
Finegoldia spp.	2.667	2	0.264
Flavobacterium spp.	1.715	2	0.424
Francisella spp.	0.292	2	0.864
Gemella sanguinis	0.587	2	0.746
Gluconobacter spp.	1.882	2	0.390
Gordonia terrae	0.552	2	0.759
Haemophilus parainfluenzae	0.552	2	0.759
Halomonas venusta	5.377	2	0.68
Herbaspirillum rhizosphaerae	1.180	2	0.554
Herbaspirillum spp.	0.241	2	0.886

Inquilinus spp.	3.465	2	0.177
Janthinobacterium lividum	0.281	2	0.869
Kocuria rosea	0.578	2	0.749
Lachnoclostridium clostridium symbiosum	0.866	2	0.648
Lactobacillus fermentum	1.180	2	0.554
Lactobacillus iners	0.199	2	0.905
Lactobacillus paraplantarum	1.095	2	0.578
Lactobacillus plantarum	0.947	2	0.623
Lactococcus garvieae	0.353	2	0.838
Lactococcus lactis	1.856	2	0.395
Lactococcus sp.	2.667	2	0.264
Legionella jordanis	3.000	2	0.223
Legionella lytica	2.189	2	0.335
Legionella pneumophila	2.055	2	0.358
Legionella sainthelensi	1.100	2	0.577
Leifsonia spp.	1.095	2	0.578
Leuconostoc pseudomesenteroides	0.988	2	0.610
Lysinibacillus massiliensis	0.194	2	0.908
Massilia spp.	2.386	2	0.303
Massilia timonae	0.654	2	0.721
Mesorhizobium spp.	0.941	2	0.625
Methylobacterium iners	5.643	2	0.060
Methylobacterium spp.	3.066	2	0.216
Methylobacterium tardum	1.453	2	0.484
Methylobacterium thiocyanatum	0.943	2	0.624
Micrococcus luteus	3.929	2	0.140
Micrococcus sp.	4.348	2	0.114
Microvirgula aerodenitrificans	2.993	2	0.224
Mogibacterium timidum	1.889	2	0.389
Mycobacterium parascrofulaceum	1.598	2	0.450
Mycobacterium septicum	2.980	2	0.225
Mycobacterium ulcerans	0.325	2	0.850
Mycoplasma hominis	3.000	2	0.223
Mycoplasma salivarium	3.000	2	0.223

Neisseria subflava	2.081	2	0.353
Nocardiopsis spp.	1.906	2	0.386
Ochrobactrum intermedium	0.014	2	0.993
Ochrobactrum spp.	2.958	2	0.228
Olsenella uli	0.119	2	0.942
Paracoccus spp.	2.927	2	0.231
Parvimonas spp.	0.209	2	0.901
Peptoniphilus asaccharolyticus	1.118	2	0.572
Peptostreptococcus spp.	3.000	2	0.223
Peptostreptococcus stomatis	1.118	2	0.572
Pseudoclavibacter zimmermannella bifida	3.000	2	0.223
Pseudomonas mendocina	0.277	2	0.871
Pseudomonas oryzihabitans	1.203	2	0.548
Pseudomonas putida	1.376	2	0.503
Pseudomonas stutzeri	0.022	2	0.989
Ralstonia spp.	1.830	2	0.400
Rhizobium spp.	1.686	2	0.430
Rhodoplanes spp.	3.254	2	0.197
Robinsoniella peoriensis	0.530	2	0.767
Roseomonas mucosa	1.118	2	0.572
Roseomonas spp.	4.500	2	0.105
Rothia mucilaginosa	1.870	2	0.393
Ruminococcus flavefaciens	0.395	2	0.821
Ruminococcus spp.	0.417	2	0.812
Selenomonas spp.	0.026	2	0.987
Shewanella putrefaciens	1.457	2	0.483
Shigella sonnei	2.868	2	0.238
Simkania negevensis	1.118	2	0.572
Sphingobacterium spp.	0.139	2	0.933
Spiroplasma sp.	2.710	2	0.258
Sporosarcina spp.	0.890	2	0.641
Streptococcus gordonii	0.373	2	0.830
Streptococcus lutetiensis	1.852	2	0.396
Streptococcus sanguinis	2.072	2	0.355
Streptomyces spp.	1.436	2	0.488
Synergistes spp.	2.710	2	0.258

Varibaculum cambriense	0.677	2	0.713
Veillonella parvula	2.246	2	0.325
Vibrio cholera	1.315	2	0.518
Wautersiella falsenii	0.220	2	0.896
Williamsia muralis	0.935	2	0.627
Wolbachia pipientis	0.940	2	0.625
Wolbachia spp.	0.554	2	0.758
Xanthomonas spp.	7.581	2	0.023

Appendix 4: Mann-Whitney U test performed to determine the influence of region on the abundance of the detected human bacterial pathogens.

Bacterial species	U Test Value	Mean Rank Ohangwena)	Mean Rank (Omusati)	P-Value
Achromobacter spp.	204	24.13	20.71	0.376
Achromobacter xylosoxidans	258.5	21.76	23.31	0.683
Acidaminococcus spp.	231	22.96	22	0.572
Acidovorax delafieldii	181.5	25.11	19.64	0.158
Acidovorax facilis	241	22.52	22.48	0.990
Acidovorax spp.	176.5	25.33	19.40	0.126
Acidovorax temperans	261	21.65	23.43	0.579
Acinetobacter calcoaceticus	230.5	22.98	21.98	0.795
Acinetobacter johnsonii	250.5	22.11	22.93	0.831
Acinetobacter junii	226.5	23.15	21.79	0.700
Acinetobacter lwoffii	223.5	23.28	21.64	0.672
Acinetobacter radioresistens	234	22.83	22.14	0.853
Acinetobacter schindleri	247.5	22.24	22.79	0.876
Acinetobacter septicus	238.5	22.63	22.36	0.940
Acinetobacter spp.	187.5	24.85	19.93	0.204
Actinomadura spp.	266.5	21.41	23.69	0.325
Actinomadura vinacea	278	20.91	24.24	0.224
Aeromonas spp.	289	20.43	24.76	0.155
Agromyces sp.	243.5	22.41	22.60	0.956
Alistipes finegoldii	267	21.39	23.71	0.528
Alistipes shahii	253	22	23.05	0.295
Alistipes spp.	285.5	20.59	24.60	0.273
Alteromonas sp.	231	22.96	22	0.339
Anaerococcus sp.	236	22.74	22.24	0.839
Anaerovorax spp.	210	23.87	21	0.446
Arthrobacter oxydans	253	22	23.05	0.624
Arthrobacter spp.	273.5	21.11	24.02	0.452
Atopobium vaginae	242	22.48	22.58	0.974
Aurantimonas sp.	254.5	21.93	23.12	0.579
Azospirillum brasilense	231	22.96	22	0.339
Bacillus coagulans	224	23.26	21.67	0.637
Bacteroides spp.	236	22.74	22.24	0.897

Bacteroides vulgatus	231	22.96	22	0.339
Bergeyella sp.	254	21.96	23.10	0.689
Bordetella petrii	267	21.39	23.71	0.414
Bosea sp.	227	23.13	21.81	0.642
Brevibacillus parabrevis	231	22.96	22	0.339
Brevibacillus sp.	242.5	22.46	22.55	0.962
Brevibacterium sp.	295	20.17	25.05	0.127
Brevundimonas diminuta	238.5	22.63	22.36	0.940
Brevundimonas spp.	209	23.91	20.95	0.435
Brevundimonas vesicularis	253	22	23.05	0.536
Burkholderia spp.	221.5	23.37	21.55	0.636
Burkholderia tropica	220.5	23.41	21.50	0.172
Burkholderia ubonensis	279.5	20.85	24.31	0.371
Butyrivibrio sp.	231	22.96	22	0.339
Caenispirillum sp.	231	22.96	22	0.339
Campylobacter lari	253	22	23.05	0.295
Candidatus neoehrlichia mikurensis	233	22.87	22.10	0.754
Catabacter hongkongensis	231	22.96	22	0.339
Caulobacter spp.	217	23.57	21.33	0.414
Caulobacter vibrioides	252.5	22.02	23.02	0.701
Cellulomonas spp.	224	23.26	21.67	0.626
Chitinophaga spp.	188	24.83	19.95	0.204
Citrobacter spp.	231	22.96	22	0.339
Clostridium ghonii	234	22.83	22.14	0.838
Clostridium intestinale	233	22.87	22.10	0.648
Clostridium limosum	253	22	23.05	0.295
Clostridium sporogenes	451.5	23.41	21.50	0.322
Clostridium subterminale	232	22.91	22.05	0.609
Comamonas kerstersii	226.5	23.15	21.79	0.653
Comamonas sp.	216	23.61	21.29	0.494
Comamonas testosterone	209	23.91	20.95	0.445
Corynebacterium amycolatum	222	23.35	21.57	0.358
Corynebacterium falsenii	286	20.57	24.62	0.267
Corynebacterium jeikeium	244.5	22.37	22.64	0.940
Corynebacterium mucifaciens	223.5	23.28	21.64	0.670
Corynebacterium thomssenii	275.5	21.02	24.12	0.401

Corynebacterium tuberculostearicum	274.5	21.07	24.07	0.415
Coxiella burnetii	243	22.43	22.57	0.922
Coxiella spp.	192.5	24.63	20.17	0.142
Cupriavidus spp.	230.5	22.98	21.98	0.733
Delftia tsuruhatensis	269.5	21.28	23.83	0.490
Desulfomicrobium spp.	232.5	22.89	22.07	0.701
Desulfovibrio desulfuricans	242	22.48	22.52	0.974
Desulfovibrio spp.	241.5	22.50	22.50	1.000
Dietzia papillomatosis	267.5	21.37	23.74	0.306
Dokdonella spp.	206.5	24.02	20.83	0.338
Dyella ginsengisoli	222	23.35	21.57	0.358
Dysgonomonas capnocytophagoides	304	19.78	25.48	0.086
Dysgonomonas gadei	254.5	21.93	23.12	0.748
Dysgonomonas spp.	225	23.22	21.71	0.621
Eggerthella sp.	253	22	23.05	0.295
Empedobacter brevis	222.5	23.33	21.60	0.454
Empedobacter sp.	236	22.74	22.24	0.815
Enterobacter hormaechei	241	22.52	22.48	0.991
Enterococcus faecalis	268	21.35	23.76	0.501
Escherichia hermannii	209.5	23.89	20.98	0.237
Eubacterium spp.	220	23.43	21.48	0.610
Exiguobacterium aurantiacum	242.5	22.46	22.55	0.975
Exiguobacterium sp.	248.5	22.20	22.83	0.851
Fastidiosipila sanguinis	231	22.96	22	0.339
Finegoldia magna	252	22.04	23	0.795
Finegoldia spp.	231	22.96	22	0.339
Flavobacterium spp.	206	24.04	20.81	0.403
Francisella spp.	247.5	22.24	22.79	0.847
Gemella sanguinis	268.5	21.33	23.79	0.496
Gluconobacter spp.	285	20.61	24.57	0.179
Gordonia terrae	232	22.91	22.05	0.685
Haemophilus parainfluenzae	232	22.91	22.05	0.685
Halomonas venusta	206.5	24.02	20.83	0.221
Herbaspirillum rhizosphaerae	287.5	20.50	24.69	0.279

Herbaspirillum spp.	259.5	21.72	23.36	0.672
Inquilinus spp.	234	22.83	22.14	0.749
Janthinobacterium lividum	243.5	22.41	22.60	0.961
Kocuria rosea	246.5	22.28	22.74	0.898
Lachnoclostridium clostridium symbiosum	243	22.43	22.57	0.922
Lactobacillus fermentum	254	21.96	23.10	0.502
Lactobacillus iners	254.5	21.93	23.12	0.750
Lactobacillus paraplantarum	253	22	23.05	0.295
Lactobacillus plantarum	266	21.43	23.67	0.334
Lactococcus garvieae	227	23.13	21.81	0.612
Lactococcus lactis	237.5	22.67	22.31	0.913
Lactococcus sp.	231	22.96	22	0.339
Legionella jordanis	231	22.96	22	0.339
Legionella lytica	211	23.83	21.05	0.329
Legionella pneumophila	241.5	22.50	22.50	1.000
Legionella sainthelensi	247.5	22.24	22.79	0.882
Leifsonia spp.	253	22	23.05	0.295
Leuconostoc pseudomesenteroides	266.5	21.41	23.69	0.325
Lysinibacillus massiliensis	241	22.52	22.48	0.990
Massilia spp.	220	23.43	21.48	0.397
Massilia timonae	233.5	22.85	22.12	0.851
Mesorhizobium spp.	209	23.91	20.95	0.414
Methylobacterium iners	223.5	23.28	21.64	0.478
Methylobacterium spp.	250	22.13	22.90	0.804
Methylobacterium tardum	268	21.35	23.76	0.528
Methylobacterium thiocyanatum	212	23.78	21.10	0.360
Micrococcus luteus	324.5	18.89	26.45	0.051
Micrococcus sp.	310	19.52	25.76	0.038
Microvirgula aerodenitrificans	233	22.87	22.10	0.648
Mogibacterium timidum	243.5	22.41	22.60	0.925
Mycobacterium parascrofulaceum	224.5	23.24	21.69	0.502
Mycobacterium septicum	194	24.57	20.24	0.195
Mycobacterium ulcerans	239	22.61	22.38	0.938

Mycoplasma hominis	231	22.96	22	0.339
Mycoplasma salivarium	231	22.96	22	0.339
Neisseria subflava	210.5	23.85	21.02	0.456
Nocardiopsis spp.	291	20.35	24.86	0.194
Ochrobactrum intermedium	244	22.39	22.62	0.906
Ochrobactrum spp.	211	23.83	21.05	0.193
Olsenella uli	231	22.96	22	0.744
Paracoccus spp.	230	23.0	21.95	0.775
Parvimonas spp.	252	22.04	23	0.654
Peptoniphilus asaccharolyticus	242	22.48	22.52	0.974
Peptostreptococcus spp.	231	22.96	22	0.339
Peptostreptococcus stomatis	242	22.48	22.52	0.974
Pseudoclavibacter zimmermannella bifida	231	22.96	22	0.339
Pseudomonas mendocina	228	23.09	21.86	0.712
Pseudomonas oryzihabitans	276.5	20.98	24.17	0.383
Pseudomonas putida	264.5	21.5	23.6	0.589
Pseudomonas stutzeri	247	22.26	22.76	0.897
Ralstonia spp.	230	23	21.95	0.786
Rhizobium spp.	236.5	22.72	22.26	0.898
Rhodoplanes spp.	209.5	23.89	20.98	0.443
Robinsoniella peoriensis	234	22.83	22.14	0.749
Roseomonas mucosa	242	22.48	22.52	0.974
Roseomonas spp.	254.5	22.33	22.69	0.924
Rothia mucilaginosa	220.5	23.41	21.50	0.172
Ruminococcus flavefaciens	226.5	23.15	21.79	0.689
Ruminococcus spp.	246.5	22.28	22.74	0.903
Selenomonas spp.	243	22.43	22.57	0.944
Shewanella putrefaciens	239	22.61	22.38	0.951
Shigella sonnei	289.5	20.41	24.79	0.236
Simkania negevensis	242	22.48	22.52	0.974
Sphingobacterium spp.	256	21.87	23.19	0.733
Spiroplasma sp.	232	22.91	22.05	0.609
Sporosarcina spp.	229	23.04	21.90	0.768
Streptococcus gordonii	254.5	21.93	23.12	0.748
Streptococcus lutetiensis	244	22.39	22.62	0.906

Streptococcus sanguinis	200.5	24.28	20.55	0.152
Streptomyces spp.	217.5	23.54	21.36	0.563
Synergistes spp.	232	22.91	22.05	0.609
Varibaculum cambriense	245.5	22.33	22.69	0.921
Veillonella parvula	301	19.91	25.33	0.134
Vibrio cholera	269	21.3	23.81	0.498
Wautersiella falsenii	233	22.87	22.10	0.648
Williamsia muralis	262.5	21.59	23.50	0.462
Wolbachia pipientis	242	22.48	22.52	0.974
Wolbachia spp.	255	21.91	23.14	0.685
Xanthomonas spp.	139	26.96	17.62	0.012

Appendix 5: Human diseases/clinical conditions caused by human bacterial pathogens detected in the present study.

Bacterial species	**Diseases/clinical conditions**	**Citations**
Achromobacter spp.	RTI, septicaemia, CAPD peritonitis, pneumonia, ear infection, pulmonary infection in cystic fibrosis, keratitis, vascular line sepsis	Spilker *et al.*, 2012; eMedMD.com
Achromobacter xylosoxidans	RTI, septicaemia, CAPD peritonitis, pneumonia, ear infection, pulmonary infection in cystic fibrosis, keratitis, vascular line sepsis	Reverdy *et al.*, 1984; eMedMD.com
Acidaminococcus spp.	Abscesses, postsurgical infections, Malnutrition	Gough *et al.*, 2016; eMedMD.com
Acidovorax delafieldii	Wound infection, UTI, bacteraemia, meningitis, septic arthritis	eMedMD.com
Acidovorax facilis	Wound infection, UTI, bacteraemia, meningitis, septic arthritis	eMedMD.com
Acidovorax spp.	Wound infection, UTI, bacteraemia, meningitis, septic arthritis	Shetty *et al.*, 2005; eMedMD.com
Acidovorax temperans	Wound infection, UTI, bacteraemia, meningitis, septic arthritis	eMedMD.com
Acinetobacter calcoaceticus	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	Li *et al.*, 2015; eMedMD.com

Acinetobacter johnsonii	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	eMedMD.com
Acinetobacter junii	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	Cayo *et al.,* 2011; eMedMD.com
Acinetobacter lwoffii	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	eMedMD.com
Acinetobacter radioresistens	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	Visca *et al.,* 2001; eMedMD.com
Acinetobacter schindleri	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	eMedMD.com
Acinetobacter septicus	Bacteraemia	Kilic *et al.,* 2008
Acinetobacter spp.	Septicaemia, UTI, wound infections, abscesses, endocarditis, meningitis, osteomyelitis	eMedMD.com
Actinomadura spp.	Actinomycetoma, Madura foot	eMedMD.com
Actinomadura vinacea	Actinomycetoma, Madura foot	eMedMD.com

Aeromonas spp.	Wound infection, abscesses, septicaemia, meningitis, leech-bite infection, alligator-bite infection, acute diarrhoea	Parker *et al.*, 2011; eMedMD.com
Agromyces sp.	Bacteraemia	Sridhar *et al.*, 2015
Alistipes finegoldii	Appendicitis, peritonitis, abdominal abscess	eMedMD.com
Alistipes shahii	Appendicitis, peritonitis, abdominal abscess	eMedMD.com
Alistipes spp.	Appendicitis, peritonitis, abdominal abscess	eMedMD.com
Alteromonas sp.	Bacteraemia	Vignier *et al.*, 2013
Anaerococcus sp.	Mixed anaerobic infections, abscesses	Song *et al.*, 2007; eMedMD.com
Anaerovorax spp.	Associated with genital ulcer disease	Mehta *et al.*, 2012
Arthrobacter oxydans	UTI, bacteraemia, skin infection	eMedMD.com
Arthrobacter spp.	UTI, bacteraemia, skin infection	eMedMD.com
Atopobium vaginae	Bacterial vaginosis	Ferris *et al.*, 2004; eMedMD.com
Aurantimonas sp.	Bacteraemia	Mendes *et al.*, 2009
Azospirillum brasilense	CAPD peritonitis, line sepsis	eMedMD.com
Bacillus coagulans	Pneumonia, septicaemia, corneal infections, meningitis, food	eMedMD.com

	poisoning, eye infection, lung infection	
Bacteroides spp.	Abscesses, bacteraemia, bite infections, wound infections, chronic otitis media, pelvic inflammatory disease, neonatal sepsis	eMedMD.com
Bacteroides vulgatus	Abscesses, bacteraemia, bite infections, wound infections, chronic otitis media, pelvic inflammatory disease, neonatal sepsis	Wexler, 2007; eMedMD.com
Bergeyella sp.	Wound infection, septicaemia, meningitis	eMedMD.com
Bordetella petrii	Associated with chronic pulmonary obstructive disease	Le Coustumier *et al.*, 2011
Bosea sp.	Linked with ventilator-associated pneumonia	eMedMD.com
Brevibacillus parabrevis	Bacteraemia, abscess	eMedMD.com
Brevibacillus sp.	Bacteraemia, abscess	eMedMD.com
Brevibacterium sp.	Bacteraemia, endocarditis, meningitis, chest infection, pericarditis, vascular catheter sepsis	Bal *et al.*, 2015; eMedMD.com
Brevundimonas diminuta	Septicaemia, endocarditis	Han and Andrade, 2005; eMedMD.com

Brevundimonas spp.	Septicaemia, endocarditis	Han and Andrade, 2005; eMedMD.com
Brevundimonas vesicularis	Septicaemia, endocarditis	Yang *et al.*, 2006; eMedMD.com
Burkholderia spp.	Lung infection in cystic fibrosis, septic arthritis, bacteraemia, meningitis, glanders, melioidosis	Baldwin *et al.*, 2007; eMedMD.com
Burkholderia tropica	Septicaemia	Deris *et al.*, 2010
Burkholderia ubonensis	Septicaemia	Price *et al.*, 2013
Butyrivibrio sp.	Endophthalmitis	eMedMD.com
Caenispirillum sp.	Bacteraemia	Romano-Bertrand, 2015
Campylobacter lari	Diarrhoea, bacteraemia, abscess	eMedMD.com
Candidatus neoehrlichia mikurensis	Bacteraemia	Welinder-Olsson *et al.*, 2010
Catabacter hongkongensis	Bacteraemia	Lau *et al.*, 2012
Caulobacter spp.	Bacteraemia, peritonitis	Justesen *et al.*, 2007
Caulobacter vibrioides	Bacteraemia, peritonitis	Justesen *et al.*, 2007
Cellulomonas spp.	Bacteraemia, meningitis, pilonidal abscess, wound infection, homograft valve infection, Infective endocarditis, osteomyelitis	Lai *et al.*, 2009; eMedMD.com
Chitinophaga spp.	Bacteraemia	Cremet *et al.*, 2009

Citrobacter spp.	UTI, meningitis, bacteraemia, haemolytic–uraemic syndrome	eMedMD.com
Clostridium ghonii	Wound infection, bacteraemia, abscesses	eMedMD.com
Clostridium intestinale	Bacteraemia	Elsayed and Zhang, 2005
Clostridium limosum	Wound infection, bacteraemia, abscesses	eMedMD.com
Clostridium sporogenes	Wound infection, bacteraemia, abscesses	eMedMD.com
Clostridium subterminale	Wound infection, bacteraemia, abscesses	eMedMD.com
Comamonas kerstersii	Intra-abdominal infections	Almuzara *et al.*, 2013
Comamonas sp.	Bacteraemia, UTI, conjunctivitis, endocarditis, wound infection, abdominal abscess, peritonitis, meningitis	eMedMD.com
Comamonas testosterone	Bacteraemia, UTI, conjunctivitis, endocarditis, wound infection, abdominal abscess, peritonitis, meningitis	eMedMD.com
Corynebacterium amycolatum	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis,	Berner *et al.*, 1997; eMedMD.com

	septic arthritis, meningitis, abscesses	
Corynebacterium falsenii	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses, Bacteraemia	Tam *et al.,* 2010; eMedMD.com
Corynebacterium jeikeium	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses, Bacteraemia	Ifantidou *et al.,* 2010; eMedMD.com
Corynebacterium mucifaciens	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses, Bacteraemia	eMedMD.com
Corynebacterium thomssenii	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses, Bacteraemia	eMedMD.com
Corynebacterium tuberculostearicum	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses, Bacteraemia	Abreu *et al.,* 2012; eMedMD.com

Coxiella burnetii	Bacteraemia, Q fever, endocarditis	Botelho-Nevers *et al.*, 2007; eMedMD.com
Coxiella spp.	Bacteraemia, Q fever, endocarditis	Botelho-Nevers *et al.*, 2007; eMedMD.com
Cupriavidus spp.	Meningitis, pulmonary infection in cystic fibrosis, line sepsis	Langevin *et al.,* 2011; eMedMd.com
Delftia tsuruhatensis	Bacteraemia	Tabak *et al.*, 2013
Desulfomicrobium spp.	Periodontitis	Langendijk *et al.*, 2001; eMedMD.com
Desulfovibrio desulfuricans	Bacteraemia, liver abscess	Goldstein *et al.*, 2003; eMedMD.com
Desulfovibrio spp.	Bacteraemia, liver abscess	Goldstein *et al.*, 2003; eMedMD.com
Dietzia papillomatosis	Bacteraemia	Rammer *et al.*, 2013
Dokdonella spp.	Bacteraemia	Lee and Weinstein, 2014
Dyella ginsengisoli	Bacteraemia, RTI	Duus *et al.*, 2013; Hakima *et al.*, 2017
Dysgonomonas capnocytophagoides	Diarrhoea, bacteraemia, abscess	Hironaga *et al.*, 2008; eMedMD.com
Dysgonomonas gadei	Diarrhoea, bacteraemia, abscess	eMedMD.com
Dysgonomonas spp.	Diarrhoea, bacteraemia, abscess	Almuzara *et al.*, 2009; eMedMD.com

Eggerthella sp.	Rectal abscess, bacteraemia	Gardiner *et al.*, 2015; eMedMD.com
Empedobacter brevis	Endophthalmitis, bacteraemia, UTI	Bokhari *et al.*, 2015; Sharma *et al.*, 2016; eMedMD.com
Empedobacter sp.	Endophthalmitis, bacteraemia, UTI, Meningitis,	Sharma *et al.*, 2016; eMedMD.com
Wautersiella falsenii	UTI, RTI	van der Velden *et al.*, 2012; Giordano *et al.*, 2016
Enterobacter hormaechei	Bacteraemia, respiratory tract infections, UTI	Wenger *et al.*, 1997; eMedMD.com
Enterococcus faecalis	Bacteraemia, abscesses, endocarditis, meningitis, UTI, peritonitis, osteomyelitis, wound infection	eMedMD.com
Escherichia hermannii	Wound infection, Bacteraemia	Kaewpoowat *et al.*, 2013; eMedMD.com
Eubacterium spp.	Wound infection, abscesses, septicaemia, periodontitis	Hill *et al.*, 1987; eMedMD.com
Exiguobacterium aurantiacum	Wound infection, bacteraemia	Pitt *et al.*, 2007; eMedMD.com
Exiguobacterium sp.	Wound infection, bacteraemia	Pitt *et al.*, 2007; eMedMD.com

Fastidiosipila sanguinis	Osteitis	Beauruelle *et al.*, 2014
Finegoldia magna	Prosthetic joint infections, Gingivitis, periodontitis	Levy *et al.*, 2009; eMedMD.com
Finegoldia spp.	Prosthetic joint infections, Gingivitis, periodontitis	Levy *et al.*, 2009; eMedMD.com
Flavobacterium spp.	Bacteraemia	Hsueh *et al.*, 1996
Francisella spp.	Septicaemia, invasive systemic infection, Tularaemia	eMedMD.com
Gemella sanguinis	Bacteraemia, endocarditis, prosthetic joint infection	Collins *et al.*, 1998; Leung *et al.*, 2011; eMedMD.com
Gluconobacter spp.	Bacteraemia, endocarditis, RTI	Alauzet *et al.*, 2010; Bassetti *et al.*, 2013
Gordonia terrae	Pulmonary infection, cholecystitis, breast abscess, sternal wound sepsis, brain abscess, bacteraemia, otitis	Blanc *et al.*, 2007; eMedMD.com
Haemophilus parainfluenzae	Sinusitis, otitis media, pneumonia, abscesses, endocarditis, biliary tract infections	Frankard *et al.*, 2004; eMedMD.com
Halomonas venusta	Wound infection	von Graevenitz *et al.*, 2000
Herbaspirillum rhizosphaerae	Associated with aortic aneurism, RTI	Spilker *et al.*, 2008; eMedMD.com

Herbaspirillum spp.	RTI, associated with aortic aneurism	Spilker *et al.*, 2008; eMedMD.com
Inquilinus spp.	Pulmonary infection in cystic fibrosis, endocarditis	Spilker *et al.*, 2008; eMedMD.com
Janthinobacterium lividum	Septicaemia	Patjanasoontorn,1992
Kocuria rosea	Bacteraemia, Cholecystitis, line-related sepsis	Altuntas *et al.*, 2004; eMedMD.com
Lachnoclostridium clostridium symbiosum	Bacteremia	Elsayed and Zhang, 2004
Lactobacillus fermentum	Abscesses, bacteraemia, endometritis, endocarditis, lung infection, UTI	eMedMD.com
Lactobacillus iners	Abscesses, bacteraemia, endometritis, endocarditis, lung infection, UTI	eMedMD.com
Lactobacillus paraplantarum	Abscesses, bacteraemia, endometritis, endocarditis, lung infection, UTI	eMedMD.com
Lactobacillus plantarum	Abscesses, bacteraemia, endometritis, endocarditis, lung infection, UTI	eMedMD.com
Lactococcus garvieae	Bacteraemia, endocarditis, UTI, associated with gastrointestinal disorders	Wang *et al.*, 2007; eMedMD.com
Lactococcus lactis	Bacteraemia, endocarditis, UTI	eMedMD.com
Lactococcus sp.	Bacteraemia, endocarditis, UTI	eMedMD.com

Legionella jordanis	Legionnaires' disease, Pontiac fever	eMedMD.com
Legionella lytica	Legionnaires' disease, Pontiac fever	eMedMD.com
Legionella pneumophila	Legionnaires' disease, Pontiac fever	eMedMD.com
Legionella sainthelensi	Legionnaires' disease, Pontiac fever, RTI	Loeb *et al.*, 1999; eMedMD.com
Leifsonia spp.	Peritonitis, UTI, endocarditis, meningitis, CAPD peritonitis	Gardenier *et al.*, 2012; eMedMD.com
Leuconostoc pseudomesenteroides	Meningitis, bacteraemia, pulmonary infection	eMedMD.com
Lysinibacillus massiliensis	Sepsis	Jin *et al.*, 2017
Massilia spp.	Bacteraemia, wound infection, otitis media	Lindquist *et al.*, 2003; Park *et al.*, 2013; eMedMD.com
Massilia timonae	Bacteraemia, wound infection	Lindquist *et al.*, 2003; eMedMD.com
Mesorhizobium spp.	Pneumonia	eMedMD.com
Methylobacterium iners	Bacteraemia	UY *et al.*, 2013.
Methylobacterium spp.	Bacteraemia, CAPD peritonitis, UTI, septic arthritis	Lai *et al.*, 2011; eMedMD.com
Methylobacterium tardum	Bacteraemia	Szwetkowski, 2017
Methylobacterium thiocyanatum	Bacteraemia	Szwetkowski, 2017
Micrococcus luteus	Bacteraemia, endocarditis, septic arthritis	eMedMD.com

Micrococcus sp.	Bacteraemia, endocarditis, septic arthritis	eMedMD.com
Microvirgula aerodenitrificans	Bacteraemia	Murphy *et al.*, 2012
Mogibacterium timidum	Periodontitis	Casarin *et al.*, 2012
Mycobacterium parascrofulaceum	Pulmonary infection, cervical adenitis	Teruya *et al.*, 2010; eMedMD.com
Mycobacterium septicum	Bacteraemia	Schinsky *et al.*, 2000
Mycobacterium ulcerans	Buruli ulcer	Sizaire *et al.*, 2006; eMedMD.com
Mycoplasma hominis	Respiratory infection, postpartum fever, pyelonephritis, pelvic inflammatory disease, myocarditis, pericarditis, meningitis	eMedMD.com
Mycoplasma salivarium	Respiratory infection, postpartum fever, pyelonephritis, pelvic inflammatory disease, myocarditis, pericarditis, meningitis	Grisold *et al.*, 2008; eMedMD.com
Neisseria subflava	Meningitis, bacteraemia, endocarditis, osteomyelitis	Marri *et al.*, 2010; eMedMD.com
Nocardiopsis spp.	Mycetoma, cutaneous infection, pulmonary infection, conjunctivitis	Bennur *et al.*, 2015; eMedMD.com
Ochrobactrum intermedium	Bacteraemia, endophthalmitis, liver abscess	Teyssier *et al.*, 2005; eMedMD.com

Ochrobactrum spp.	Bacteraemia, endophthalmitis, liver abscess	Teyssier *et al.*, 2005; eMedMD.com
Olsenella uli	Periodontitis, UTI, septicaemia	Göker *et al.*, 2010; eMedMD.com
Paracoccus spp.	Bacteraemia	Funke *et al.*, 2004; eMedMD.com
Parvimonas spp.	Infectious endocarditis	Gomez *et al.*, 2015
Peptoniphilus asaccharolyticus	Mixed anaerobic infections, abscesses	eMedMD.com
Peptostreptococcus spp.	Mixed anaerobic infections, abscesses	eMedMD.com
Peptostreptococcus stomatis	Mixed anaerobic infections, abscesses, endocarditis	eMedMD.com
Pseudoclavibacter zimmermannella bifida	Bacteremia	Oyaert *et al.*, 2013.
Pseudomonas mendocina	Bacteraemia, UTI, wound infection, abscesses, septic arthritis, conjunctivitis, endocarditis, meningitis, otitis	Ragone *et al.*, 1992; eMedMD.com
Pseudomonas oryzihabitans	Bacteraemia, sepsis, prosthetic valve endocarditis, peritonitis, meningitis, abscesses, pneumonia and urinary tract infections	Tena and Fernández, 2015
Pseudomonas putida	Bacteraemia, UTI, wound infection, abscesses, septic arthritis,	Yoshino *et al.*, 2011; eMedMD.com

	conjunctivitis, endocarditis, meningitis, otitis	
Pseudomonas stutzeri	Bacteraemia, UTI, wound infection, abscesses, septic arthritis, conjunctivitis, endocarditis, meningitis, otitis	Lalucat *et al.*, 2006; eMedMD.com
Ralstonia spp.	Meningitis, peritonitis, bacteraemia, UTI, pulmonary infection	Coenye *et al.*, 2002; eMedMD.com
Rhizobium spp.	Bacteraemia	Lai *et al.*, 2004
Rhodoplanes spp.	Bacteraemia	Zhang *et al.*, 2011
Robinsoniella peoriensis	Bacteremia	Cassir et a., 2012
Roseomonas mucosa	Bacteraemia, wound infection, peritonitis, septic arthritis	Sipsas *et al.*, 2006; Bard *et al.*, 2010; eMedMD.com
Roseomonas spp.	Bacteraemia, wound infection, peritonitis, septic arthritis	Sipsas *et al.*, 2006; Bard *et al.*, 2010; eMedMD.com
Rothia mucilaginosa	Endocarditis, meningitis, neutropenic sepsis, necrotizing fasciitis, septic arthritis	Kaasch *et al.*, 2011; eMedMD.com
Ruminococcus flavefaciens	Abdominal sepsis, abscesses	eMedMD.com
Ruminococcus spp.	Abdominal sepsis, abscesses	eMedMD.com
Selenomonas spp.	Bacteraemia, lung abscess	eMedMd.com
Shewanella putrefaciens	Abdominal sepsis, meningitis, bacteraemia, ear infection,	Vignier *et al.*, 2013; eMedMD.com

	abdominal, biliary tract infections	
Shigella sonnei	Enteric infection	Bowen *et al.*, 2015; Thompson *et al.*, 2015; eMedMD.com
Simkania negevensis	Bronchiolitis, pneumonia, bacteraemia	Friedman *et al.*, 2003; Kumar *et al.*, 2005; eMedMD.com
Sphingobacterium spp.	Bacteraemia, pulmonary infection	Gupta *et al.*, 2016; eMedMD.com
Spiroplasma sp.	Eye infection (cataract)	Lorenz *et al.*, 2002
Sporosarcina spp.	RTI	Chomarat *et al.*, 1990
Streptococcus gordonii	Bacteraemia, endocarditis, wound infection	Bosch *et al.*, 1996; eMedMD.com
Streptococcus lutetiensis	Bacteremia, endocarditis, CAPD peritonitis	Almuzara *et al.*, 2013; eMedMD.com
Streptococcus sanguinis	Bacteraemia, endocarditis, wound infection, mycotic popliteal aneurysm	Jolly *et al.*, 2014; eMedMD.com
Streptomyces spp.	Actinomycetoma, bacteraemia, abscess, pericarditis, endocarditis, pneumonia	Dunne *et al.*, 1998; Rose *et al.*, 2008; eMedMD.com
Synergistes spp.	Endodontic infections	Horz *et al.*, 2006.
Varibaculum cambriense	UTI, abscess, skin and soft tissue infections	Chu *et al.*, 2009; eMedMD.com

Veillonella parvula	Abscesses, bacteraemia, meningitis	Bhatti *et al.*, 2000; eMedMD.com
Vibrio cholera	Cholera	Robins, and Mekalanos, 2014; Bhuiyan *et al.*, 2016; eMedMD.com
Williamsia muralis	Pulmonary infection	Del Mar Tomas *et al.*, 2005; eMedMD.com
Wolbachia pipientis	Filariasis	eMedMD.com
Wolbachia spp.	Filariasis	eMedMD.com
Xanthomonas spp.	Bacteraemia	eMedMD.com

Appendix 6: Kruskal-Wallis test performed to determine the influence of hand-dug well type on the abundance of livestock bacteria.

Bacterial species	**X^2 value**	**Deg. of freedom**	**P-value**
Acetivibrio spp.	3.148	2	0.207
Acholeplasma laidlawii	2.055	2	0.358
Acholeplasma morum	1.214	2	0.545
Acholeplasma spp.	3.187	2	0.203
Psychrobacter pulmonis	1.179	2	0.555

Appendix 7: Mann-Whitney U test performed to determine the influence of region on the abundance of livestock bacterial pathogens.

Bacterial species	**U Test Value**	**Mean Rank (Ohangwena)**	**Mean Rank (Omusati)**	**P-Value**
Acetivibrio spp.	175.5	25.37	19.36	0.093
Acholeplasma laidlawii	241.500	22.500	22.500	1.000
Acholeplasma morum	223.000	23.300	21.620	0.383
Acholeplasma spp.	242.000	22.480	22.520	0.984
Psychrobacter pulmonis	255.000	21.910	23.140	0.468

Appendix 8: Livestock diseases/clinical conditions caused by livestock bacterial pathogens detected in the present study.

Bacterial species	**Diseases/clinical conditions**	**Citations**
Acetivibrio spp.	Associated with dysentery, diarrhoea	Robinson and Ritchie, 1981; Allison, 1989
Acholeplasma laidlawii	Mystery swine disease, Cattle dermatitis	Wensvoort *et al.*, 1991; Yano *et al.*, 2010
Acholeplasma morum	Cattle dermatitis	Wensvoort *et al.*, 1991; Yano *et al.*, 2010
Acholeplasma spp.	Mystery swine disease, Cattle dermatitis	Wensvoort *et al.*, 1991; Yano *et al.*, 2010
Psychrobacter pulmonis	lung infections	Vela *et al.*, 2003

Appendix 9: Kruskal-Wallis test performed to determine the influence of hand-dug well type on the abundance of zoonotic bacteria.

Bacterial species	**X^2 value**	**Deg. of freedom**	**P-value**
Actinomyces spp.	1.627	2.000	0.443
Actinomyces viscosus	1.886	2.000	0.390
Aerococcus viridans	0.685	2.000	0.710
Afipia sp.	0.870	2.000	0.647
Alcaligenes faecalis	0.656	2.000	0.720
Alcaligenes sp.	1.307	2.000	0.520
Anabaena spp.	1.095	2.000	0.578
Anaerorhabdus spp.	2.667	2.000	0.264
Anaplasma phagocytophilum	1.029	2.000	0.598
Anaplasma spp.	3.000	2.000	0.223
Arcobacter butzlerii	0.517	2.000	0.772
Arcobacter cryaerophilus	0.583	2.000	0.747
Arcobacter spp.	2.065	2.000	0.356
Bacillus cereus	0.393	2.000	0.822
Bacillus pumilus	0.763	2.000	0.683
Bacillus spp.	0.909	2.000	0.635
Bacillus subtilis	0.453	2.000	0.797
Bordetella sp.	1.677	2.000	0.432
Brucella spp.	1.504	2.000	0.471
Chlamydia spp.	2.913	2.000	0.233

Clostridium perfringens	1.874	2.000	0.392
Clostridium spp.	0.511	2.000	0.774
Corynebacterium spp.	0.738	2.000	0.692
Corynebacterium urealyticum	2.510	2.000	0.285
Cyanobacterium spp.	0.669	2.000	0.716
Dietzia maris	1.256	2.000	0.534
Dietzia spp.	1.843	2.000	0.398
Ehrlichia spp.	3.000	2.000	0.223
Enterococcus sp.	0.738	2.000	0.692
Erysipelothrix spp.	4.087	2.000	0.130
Escherichia coli	1.886	2.000	0.390
Fusobacterium nucleatum	3.175	2.000	0.204
Fusobacterium spp.	0.391	2.000	0.822
Hafnia sp.	0.461	2.000	0.794
Helicobacter heilmannii	1.095	2.000	0.578
Helicobacter spp.	0.027	2.000	0.987
Klebsiella sp.	1.870	2.000	0.393
Legionella spp.	1.036	2.000	0.596
Leptospira interrogans	2.292	2.000	0.318
Leptospira spp.	0.001	2.000	0.999
Microcystis spp.	0.037	2.000	0.982
Morganella morganii	3.000	2.000	0.223
Mycobacterium spp.	1.848	2.000	0.397

Mycoplasma sp.	1.539	2.000	0.463
Nocardia nova	1.118	2.000	0.572
Paenibacillus polymyxa	1.065	2.000	0.587
Paenibacillus spp.	0.396	2.000	0.820
Porphyromonas spp.	2.077	2.000	0.354
Propionibacterium acnes	1.227	2.000	0.542
Pseudomonas aeruginosa	3.442	2.000	0.179
Pseudomonas spp.	0.823	2.000	0.663
Rhodococcus spp.	3.275	2.000	0.194
Rickettsia spp.	2.123	2.000	0.346
Salmonella enterica	0.579	2.000	0.749
Sphingobium paucimobilis	0.261	2.000	0.878
Sphingomonas spp.	0.025	2	0.988
Staphylococcus epidermidis	1.116	2.000	0.572
Staphylococcus spp.	1.481	2.000	0.477
Stenotrophomonas maltophilia	1.095	2.000	0.578
Stenotrophomonas spp.	0.226	2.000	0.893
Treponema spp.	2.241	2.000	0.326
Vibrio spp.	1.671	2.000	0.434
Waddlia sp.	1.029	2.000	0.598
Wohlfahrtiimonas sp.	0.658	2.000	0.720

Appendix 10: Mann-Whitney U test performed to determine the influence of region on the abundance of zoonotic bacterial pathogens.

Bacterial species	**U Test Value**	**Mean Rank (Ohangwena)**	**Mean Rank (Omusati)**	**P-Value**
Actinomyces spp.	210.000	23.870	21.000	0.454
Actinomyces viscosus	220.500	23.410	21.500	0.172
Aerococcus viridans	230.500	22.980	21.980	0.767
Afipia sp.	280.500	20.800	24.360	0.357
Alcaligenes faecalis	249.000	22.170	22.860	0.853
Alcaligenes sp.	222.5	23.33	21.60	0.371
Anabaena spp.	253.000	22.000	23.050	0.295
Anaerorhabdus spp.	231.000	22.960	22.000	0.339
Anaplasma phagocytophilum	243.000	22.430	22.570	0.922
Anaplasma spp.	231.000	22.960	22.000	0.339
Arcobacter butzlerii	234.000	22.830	22.140	0.749
Arcobacter cryaerophilus	224.000	23.260	21.670	0.653
Arcobacter sp.	227.000	23.130	21.810	0.709
Arcobacter spp.	293.500	20.240	24.980	0.222
Bacillus cereus	241.500	22.500	22.500	1.000
Bacillus pumilus	261.500	21.630	23.450	0.621
Bacillus sp.	261.000	21.650	23.430	0.640
Bacillus spp.	214.500	23.670	21.210	0.523

Bacillus subtilis	224.000	23.260	21.670	0.610
Bordetella sp.	227.000	23.130	21.810	0.710
Brucella spp.	206.500	24.020	20.830	0.262
Chlamydia spp.	178.000	25.260	19.480	0.132
Clostridium perfringens	210.000	23.870	21.000	0.179
Clostridium sp.	225.500	23.200	21.740	0.707
Clostridium spp.	252.500	22.020	23.020	0.796
Corynebacterium sp.	245.500	22.350	22.670	0.919
Corynebacterium spp.	226.000	23.170	21.760	0.678
Corynebacterium urealyticum	239.000	22.610	22.380	0.947
Cyanobacterium spp.	233.000	22.870	22.100	0.717
Dietzia maris	231.500	22.930	22.020	0.801
Dietzia spp.	211.000	23.830	21.050	0.473
Ehrlichia spp.	231.000	22.960	22.000	0.339
Enterococcus sp.	218.000	23.520	21.380	0.541
Erysipelothrix spp.	324.000	18.910	26.430	0.051
Escherichia coli	220.500	23.410	21.500	0.172
Fusobacterium nucleatum	232.000	22.910	22.050	0.609
Fusobacterium spp.	261.500	21.630	23.450	0.559
Hafnia sp.	215.000	23.650	21.240	0.513
Helicobacter heilmannii	253.000	22.000	23.050	0.295
Helicobacter spp.	238.000	22.650	22.330	0.919

Klebsiella sp.	220.500	23.410	21.500	0.172
Legionella sp.	210.000	23.870	21.000	0.090
Legionella spp.	219.000	23.480	21.430	0.546
Leptospira interrogans	252.000	22.040	23.000	0.654
Leptospira sp.	248.000	22.220	22.810	0.845
Leptospira spp.	241.500	22.500	22.500	1.000
Microcystis spp.	244.500	22.370	22.640	0.926
Morganella morganii	231.000	22.960	22.000	0.339
Mycobacterium spp.	272.000	21.170	23.950	0.417
Mycoplasma sp.	231.500	22.930	22.020	0.806
Nocardia nova	242.000	22.480	22.520	0.974
Paenibacillus polymyxa	254.000	21.960	23.100	0.501
Paenibacillus sp.	227.000	23.130	21.810	0.680
Paenibacillus spp.	221.000	23.390	21.520	0.334
Porphyromonas spp.	224.500	23.240	21.690	0.502
Propionibacterium acnes	236.500	22.720	22.260	0.877
Pseudomonas aeruginosa	276.000	21.000	24.140	0.064
Pseudomonas sp.	268.5	21.33	23.79	0.525
Pseudomonas spp.	285	20.61	24.57	0.307
Rhodococcus spp.	210.000	23.870	21.000	0.090
Rickettsia spp.	231.000	22.960	22.000	0.798
Salmonella enterica	229.500	23.020	21.930	0.738
Sphingobium paucimobilis	232.000	22.910	22.050	0.609

Sphingomonas sp.	210	23.87	21.00	0.359
Sphingomonas spp.	245.5	22.33	22.69	0.925
Staphylococcus epidermidis	265.500	21.460	23.640	0.573
Staphylococcus spp.	267.500	21.370	23.740	0.517
Stenotrophomonas maltophilia	253.000	22.000	23.050	0.295
Stenotrophomonas sp.	237.500	22.670	22.310	0.907
Stenotrophomonas spp.	278.5	20.89	24.26	0.361
Treponema spp.	233.500	22.850	22.120	0.816
Vibrio sp.	238.500	22.630	22.360	0.920
Vibrio spp.	231.000	22.960	22.000	0.339
Waddlia sp.	243.000	22.430	22.570	0.922
Wohlfahrtiimonas sp.	221.500	23.370	21.550	0.430

Appendix 11: Zoonotic diseases/clinical conditions caused by zoonotic bacterial pathogens

Bacterial species	Diseases/clinical conditions	Citations
Actinomyces spp.	Sepsis, RTI	Holt *et al.*, 1994
Actinomyces viscosus	Infection of the lungs, Actinomycosis	Eng *et al.*, 1981
Aerococcus viridans	Endocarditis, UTI, wounds, meningitis, abscesses, CAPD peritonitis, lymphadenitis, spondodactylitis, mastitis	Saishu *et al.*, 2015; eMedMD.com
Afipia sp.	Cat-scratch disease, Bone marrow infection, septic arthritis, Bone infection	eMedMD.com
Alcaligenes faecalis	Pneumonia, otitis, UTI, osteomyelitis, bacteraemia, skin and soft tissue infection, peritonitis	Montgomery *et al.*, 1983; Kahveci *et al.*, 2011; Tena *et al.*, 2015; eMedMD.com
Alcaligenes sp.	Pneumonia, otitis, UTI, osteomyelitis, bacteraemia, skin and soft tissue infection, peritonitis	Montgomery *et al.*, 1983; Kahveci *et al.*, 2011; Tena *et al.*, 2015; eMedMD.com
Anabaena spp.	respiratory illness, poisoning, weakness, diarrhoea, vomiting	Hunter, 1992
Anaerorhabdus spp.	Lung abscess, appendix and abdominal abscesses	Holt *et al.*, 1994; eMedMD.com

Anaplasma phagocytophilum	Anaplasmosis, pasture fever, leucopenia, thrombocytopenia	Amusategui *et al.*, 2006; Jilintai *et al.*, 2009; eMedMD.com
Anaplasma spp.	Anaplasmosis, pasture fever, leucopenia, thrombocytopenia	Amusategui *et al.*, 2006; Jilintai *et al.*, 2009; eMedMD.com
Arcobacter butzlerii	Abdominal cramps, diarrhoea, mastitis	Vandenberg *et al.*, 2004; Giacometti *et al.*, 2015; eMedMD.com
Arcobacter cryaerophilus	Abdominal cramps, diarrhoea, mastitis	Vandenberg *et al.*, 2004; Giacometti *et al.*, 2015; eMedMD.com
Arcobacter spp.	Abdominal cramps, diarrhoea, mastitis	Vandenberg *et al.*, 2004; Giacometti *et al.*, 2015; eMedMD.com
Bacillus cereus	Food poisoning, wound infection, cutaneous lesions, bacteraemia, endocarditis, eye infection	Logan, 1988; eMedMD.com
Bacillus spp.	Pneumonia, septicaemia, corneal infections, meningitis, food poisoning, eye infection, lung	Logan, 1988; eMedMD.com

	infection, wound infection, cutaneous lesions, bacteraemia, endocarditis, eye infection, anthrax	
Bacillus subtilis	Food poisoning, wound infection, cutaneous lesions, bacteraemia, endocarditis, eye infection	Logan, 1988; eMedMD.com
Bordetella sp.	RTI, Bacteraemia, otitis, wound infection, Whooping cough, respiratory tract infection	Holt *et al.*, 1994; eMedMD.com
Brucella spp.	Brucellosis	Assenga *et al.*, 2015; eMedMD.com
Chlamydia spp.	Chlamydioses, Trachoma, genital infection, neonatal infection, lymphogranuloma venereum	Longbottom and Coulter, 2003; eMedMD.com
Clostridium perfringens	Enteritis, clostridial myonecrosis, gas gangrene, food poisoning, wound infection, bacteraemia, abscesses	Uzal *et al.*, 2015; Uzal *et al.*, 2016; eMedMD.com
Clostridium spp.	Enteritis, clostridial myonecrosis, gas gangrene, food poisoning, wound infection, bacteraemia, abscesses	Uzal *et al.*, 2015; Uzal *et al.*, 2016; eMedMD.com
Cyanobacterium spp.	Pneumonia, adult respiratory distress syndrome, liver and kidney damage, gastroenteritis, muscle pain, dermatitis, poisoning, hypersalivation, agitation, anorexia, pale mucus membranes, weakness, dyspnea, recumbancy, depression, ataxia, diarrhea, muscle tremors and fasciculations,	Chorus and Bartram, 1999; Hilborn and Beasley, 2015; Salmaso *et al.*, 2016

	convulsions, apparent blindness and sudden death	
Dietzia maris	Mastitis, prosthetic hip infection, bacteraemia	Hamid, 2013; eMedMD.com
Dietzia spp.	Mastitis, prosthetic hip infection, bacteraemia	Hamid, 2013; eMedMD.com
Ehrlichia spp.	Ehrlichiosis	Ehrlichiosis *et al.*, 2013; eMedMD.com
Enterococcus sp.	Intramammary infections, bacteraemia, abscesses, endocarditis, meningitis, UTI, peritonitis, osteomyelitis, wound infection	Devriese *et al.*, 1999; eMedMD.com Ooi *et al.*, 2006; eMedMD.com
Erysipelothrix spp.	Erysipelas, erysipeloid, skin lesions, acute septicaemia, chronic arthritis, polyarthritis, bacteraemia with endocarditis	Wang *et al.*, 2002; eMedMD.com
Escherichia coli	Diarrhoea, hemorrhagic colitis, HUS, TTP, UTI, bacteraemia, wound infection, meningitis, enteric infection, haemolytic fluoroquinolones, uraemic syndrome	Durso *et al.*, 2005; eMedMD.com
Fusobacterium nucleatum	Dermatitis in cattle, abscesses, bacteraemia, periodontitis, endocarditis, necrobacillosis	Castellarin *et al.*, 2012; Wilson-Welder *et al.*, 2015; eMedMD.com

Fusobacterium spp.	Dermatitis in cattle, abscesses, bacteraemia, periodontitis, endocarditis, necrobacillosis	Castellarin *et al.*, 2012; Wilson-Welder *et al.*, 2015; eMedMD.com
Helicobacter heilmannii	Chronic gastritis, ulcerations	Meining *et al.*, 1998; Morgner *et al.*, 2000; Bento-Miranda, and Figueiredo, 2014; eMedMD.com
Helicobacter spp.	Chronic gastritis, ulcerations	Meining *et al.*, 1998; Morgner *et al.*, 2000; Bento-Miranda, and Figueiredo, 2014; eMedMD.com
Klebsiella sp.	Intramammary infection, liver abscess, UTI, bacteraemia, wound infection, respiratory tract infection, Rhinoscleroma, Donovanosis	Umeh and Berkowitz, 2002; Bannerman *et al.*, 2004; eMedMD.com
Legionella spp.	Pneumonia, Legionnaires' disease, Pontiac fever	Fabbi *et al.*, 1998
Leptospira interrogans	Leptospirosis	Bolin and Koellner, 1988; Fabijanski,

		2008; eMedMD.com
Leptospira spp.	Leptospirosis	Bolin and Koellner, 1988; Fabijanski, 2008; eMedMD.com
Microcystis spp.	Poisoning	Ramos *et al.*, 2015; Harke *et al.*, 2016
Morganella morganii	Bacteraemia, RTI, UTI, wound infections	Holt *et al.*, 1994 Falagas *et al.*, 2006; Zhao *et al.*, 2012; eMedMD.com
Mycoplasma sp.	Chronic Pneumonia, Polyarthritis Syndrome, respiratory infection, postpartum fever, pyelonephritis, pelvic inflammatory disease, myocarditis, pericarditis, meningitis	Waites and Talkington, 2004; Perez-Casal and Prysliak, 2007; Suleman *et al.*, 2016; eMedMD.com
Nocardia nova	Nocardiosis, bacteraemia, pulmonary, soft tissue infections	Condas *et al.*, 2013; Condas, L.A.Z., 2015; eMedMD.com

Paenibacillus polymyxa	Bacteraemia, Toxic induced apoptosis, Septicaemia, meningitis, pneumonia	Nasu *et al.*, 2003; Mikkola *et al.*, 2017; eMedMD.com
Paenibacillus spp.	Bacteraemia, Toxic induced apoptosis, Septicaemia, meningitis, pneumonia	Nasu *et al.*, 2003; Mikkola *et al.*, 2017; eMedMD.com
Rhodococcus spp.	lymph node granulomas, lymphadenitis, pyogranulomatous bronchopneumonia, Bacteraemia, osteomyelitis, lung abscesses	Flynn *et al.*, 2001; Shitaye *et al.*, 2006; Macken *et al.*, 2015; Witkowski *et al.*, 2016; eMedMD.com
Rickettsia spp.	Rickettsial spotted fever, tick typhus, tick-bite fever, rickettsialpox	Ahmed *et al.*, 2016; Szekeres *et al.*, 2016; Cisak *et al.*, 2017; eMedMD.com
Sphingomonas paucimobilis	respiratory diseases, septicaemia, foot infections, bacteraemia, UTI, wound infections, CAPD peritonitis	Maragakis *et al.*, 2009; Lin *et al.*, 2010; Cengiz, *et al.*, 2015; eMedMD.com
Sphingomonas spp.	respiratory diseases, septicaemia, foot infections, bacteraemia, UTI, wound infections, CAPD peritonitis	Maragakis *et al.*, 2009; Lin *et al.*, 2010; Cengiz, *et al.*,

		2015; eMedMD.com
Staphylococcus epidermidis	skin disease, bacteraemia, wound infection, endocarditis, catheter-related sepsis, UTI, toxic shock syndrome, food poisoning, eye infection, osteomyelitis	Vuong and Otto, 2002; Foster, 2012
Staphylococcus spp.	skin disease, bacteraemia, wound infection, endocarditis, catheter-related sepsis, UTI, toxic shock syndrome, food poisoning, eye infection, osteomyelitis	Vuong and Otto, 2002; Foster, 2012
Stenotrophomonas maltophilia	Respiratory diseases, bacteraemia, meningitis, wound infection, UTI, pneumonia	Albini *et al.*, 2009; Brooke, 2012; eMedMD.com
Stenotrophomonas spp.	Respiratory diseases, bacteraemia, meningitis, wound infection, UTI, pneumonia, meningitis	Albini *et al.*, 2009; Brooke, 2012; eMedMD.com
Treponema spp.	Syphilis, Pinta, Yaws, dermatitis, ulcers	Evans *et al.*, 2009; Svartström, 2014; eMedMD.com
Vibrio spp.	Abortion in livestock, cholera	Laing, 1960; Robins and Mekalanos, 2014; Bhuiyan *et al.*, 2016; eMedMD.com

Waddlia sp.	Abortion in livestock and humans	Baud *et al.*, 2007; Wheelhouse *et al.*, 2016
Salmonella enterica	Gastroenteritis, enteric fever, osteomyelitis, diarrhoea	Zhang *et al.*, 2002; Harvey *et al.*, 2017; eMedMD.com
Mycobacterium spp.	Bacteraemia, tuberculosis, cervical adenitis, fish-tank granuloma, pulmonary infection	Palmer *et al.*, 2011; Amato *et al.*, 2017; eMedMD.com
Porphyromonas spp.	Mixed anaerobic infections at various sites, periodontitis, human and animal bites	Borsanelli *et al.*, 2015; eMedMD.com
Propionibacterium acnes	Placentitis and Abortion in livestock, bacteraemia, abscesses, endocarditis, septic arthritis, endophthalmitis	Lyons *et al.*, 2009; Saper *et al.*, 2015; eMedMD.com
Pseudomonas aeruginosa	Mastitis, bacteraemia, UTI, wound infection, abscesses, septic arthritis, conjunctivitis, endocarditis, meningitis, otitis	Mushin and Ziv, 1973; Turner *et al.*, 2014; eMedMD.com
Pseudomonas spp.	Bacteraemia, UTI, wound infection, abscesses, septic arthritis, conjunctivitis, endocarditis, meningitis, otitis	Mushin and Ziv, 1973; Yoshino *et al.*, 2011; Turner *et al.*, 2014; eMedMD.com

Wohlfahrtiimonas sp.	Bacteraemia, septicemia	Rebaudet *et al.*, 2009; Thaiwong *et al.*, 2014
Bacillus pumilus	Bovine mastitis, rectal fistula infection, food poisoning, wound infection, cutaneous lesions, bacteraemia, endocarditis, eye infection	Logan, 1988; eMedMD.com
Corynebacterium urealyticum	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses	Shallali *et al.*, 2001; Bailiff *et al.*, 2005; Soriano and Tauch, 2008; eMedMD.com
Corynebacterium spp.	Septicaemia, peritonitis, UTI, eye infection, wound infection, endocarditis, osteomyelitis, septic arthritis, meningitis, abscesses, bacteraemia	Shallali *et al.*, 2001; Bailiff *et al.*, 2005; Soriano and Tauch, 2008; eMedMD.com
Hafnia sp.	Septicaemia, endocarditis, meningitis, pneumonia, abscesses, urinary infections, peritonitis, endophthalmitis, cholecystitis, intestinal disorders, postenteritic arthritis	Albert *et al.*, 1991; Padilla *et al.*, 2015; Stanic *et al.*, 2015; eMedMD.com

Appendix 12: Kruskal-Wallis test performed to determine the influence of hand-dug well type on the abundance of Gray bacteria.

Bacterial species	**X^2 value**	**Deg. of freedom**	**P-value**
Acetanaerobacterium spp.	3.000	2	0.223
Acetobacterium wieringae	1.870	2	0.393
Achromatium oxaliferum	1.029	2	0.598
Acidaminobacter sp.	0.046	2	0.977
Acidimicrobium spp.	3.146	2	0.206
Acidisphaera sp.	0.840	2	0.657
Acidisphaera spp.	0.252	2	0.881
Aciditerrimonas sp.	1.037	2	0.595
Aciditerrimonas spp.	0.319	2	0.853
Acidithiobacillus spp.	9.439	2	0.009
Acidobacterium sp.	1.118	2	0.572
Acidobacterium spp.	1.337	2	0.513
Acidocella spp.	3.000	2	0.223
Acidothermus cellulolyticus	2.813	2	0.245
Acidovorax caeni	0.040	2	0.980
Acidovorax citrulli	0.730	2	0.694
Acidovorax konjaci	0.308	2	0.857
Acinetobacter brisouii	0.199	2	0.905
Acinetobacter genomosp. 3	2.660	2	0.264

Acinetobacter guillouiae	0.210	2	0.900
Acinetobacter marinus	1.095	2	0.578
Acinetobacter venetianus	1.196	2	0.550
Actinoallomurus iriomotensis	0.018	2	0.991
Actinocatenispora spp.	2.667	2	0.264
Actinophytocola sp.	1.180	2	0.554
Actinoplanes philippinensis	7.783	2	0.020
Actinoplanes spp.	1.212	2	0.545
Actinopolymorpha pittospori	1.029	2	0.598
Actinotalea fermentans	1.044	2	0.593
Adhaeribacter sp.	0.866	2	0.648
Adhaeribacter spp.	0.465	2	0.793
Advenella tetrathiobacter kashmirensis	0.027	2	0.987
Aeromicrobium sp.	2.295	2	0.317
Agrobacterium vitis	1.140	2	0.565
Akkermansia spp.	1.095	2	0.578
Alcanivorax spp.	9.748	2	0.008
Algidimarina propionica	1.870	2	0.393
Algorimarina spp.	0.046	2	0.977
Algoriphagus dokdonensis	3.441	2	0.179
Algoriphagus faecimaris	1.095	2	0.578

Algoriphagus hongiella halophile	2.284	2	0.319
Algoriphagus sp.	0.534	2	0.766
Algoriphagus spp.	5.457	2	0.065
Alicyclobacillus spp.	1.118	2	0.572
Alishewanella sp.	0.477	2	0.788
Alistipes indistinctus	3.000	2	0.223
Alistipes massiliensis	3.000	2	0.223
Alkalibacter saccharofermentans	1.185	2	0.553
Alkalibacter spp.	0.058	2	0.971
Alkalibacterium iburiense	0.355	2	0.837
Alkalibacterium kapii	0.026	2	0.987
Alkalibacterium spp.	1.029	2	0.598
Alkaliflexus spp.	3.867	2	0.145
Alkalilimnicola spp.	0.321	2	0.852
Alkaliphilus metalliredigens	1.095	2	0.578
Alkaliphilus sp.	1.562	2	0.458
Alkanibacter spp.	5.457	2	0.065
Alkanindiges hongkongensis	3.955	2	0.138
Alkanindiges illinoisensis	1.231	2	0.540
Alkanindiges sp.	5.125	2	0.077
Alkanindiges spp.	4.681	2	0.096

Allochromatium vinosum	0.621	2	0.733
Allokutzneria spp.	1.815	2	0.403
Alsobacter metallidurans	0.105	2	0.949
Altererythrobacter aestuarii	1.021	2	0.600
Altererythrobacter dongtanensis	0.700	2	0.705
Altererythrobacter sp.	4.616	2	0.099
Altererythrobacter spp.	1.478	2	0.478
Amaricoccus spp.	0.026	2	0.987
Ammonifex thiophilus	3.000	2	0.223
Ammoniphilus oxalivorans	5.142	2	0.076
Ammoniphilus sp.	0.776	2	0.678
Ammoniphilus spp.	2.132	2	0.344
Anaerobacterium chartisolvens	1.288	2	0.525
Anaerofilum spp.	2.667	2	0.264
Anaerolinea spp.	2.533	2	0.282
Anaeromusa sp.	0.013	2	0.994
Anaeromyxobacter dehalogenans	0.085	2	0.958
Anaeromyxobacter spp.	0.512	2	0.774
Anaerophaga spp.	0.426	2	0.808
Anaerosinus selenomonadaceae	0.234	2	0.889
Ancalomicrobium spp.	3.442	2	0.179
Angustibacter aerolatus	2.055	2	0.358

Anoxybacillus spp.	1.886	2	0.390
Aquabacterium sp.	2.667	2	0.264
Aquabacterium spp.	1.141	2	0.565
Aquaspirillum putridiconchylium	6.140	2	0.046
Aquaspirillum sp.	1.811	2	0.404
Aquicella siphonis	2.543	2	0.280
Aquicella spp.	0.842	2	0.656
Aquimonas sp.	1.625	2	0.444
Aquimonas spp.	0.683	2	0.711
Aquitalea magnusonii	0.011	2	0.995
Arcicella sp.	0.506	2	0.776
Arcicella spp.	2.176	2	0.337
Arenimonas daechungensis	1.675	2	0.433
Arenimonas sp.	0.379	2	0.828
Arenimonas spp.	1.092	2	0.579
Arhodomonas sp.	3.000	2	0.223
Aridibacter acidobacteria bacterium	2.111	2	0.348
Aromatoleum aromaticum	6.140	2	0.046
Arsenicicoccus sp.	0.214	2	0.899
Arsenophonus spp.	3.867	2	0.145
Arthrobacter agilis	1.120	2	0.571

Arthrobacter chlorophenolicus	1.466	2	0.480
Arthrobacter globiformis	0.234	2	0.889
Arthrobacter monumenti	2.036	2	0.361
Arthrobacter nicotianae	1.041	2	0.594
Arthrobacter protophormiae	0.241	2	0.887
Arthrobacter ramosus	0.396	2	0.820
Arthrospira platensis	1.095	2	0.578
Asticcacaulis biprosthecium	0.282	2	0.868
Asticcacaulis excentricus	5.011	2	0.082
Atopostipes sp.	1.029	2	0.598
Atopostipes spp.	1.074	2	0.585
Aureimonas ferruginea	10.612	2	0.005
Austwickia chelonae	3.000	2	0.223
Azoarcus sp.	0.760	2	0.684
Azoarcus spp.	5.717	2	0.057
Azonexus sp.	0.247	2	0.884
Azospira dechlorosoma sp.	0.431	2	0.806
Azospira oryzae	0.168	2	0.920
Azospirillum lipoferum	5.151	2	0.076
Azospirillum oryzae	1.127	2	0.569
Azospirillum picis	2.667	2	0.264
Azospirillum spp.	3.367	2	0.186
Azovibrio spp.	0.473	2	0.789

Bacillus alcalophilus	1.494	2	0.474
Bacillus andreesenii	2.187	2	0.335
Bacillus badius	0.409	2	0.815
Bacillus cellulosilyticus	0.409	2	0.815
Bacillus chandigarhensis	1.655	2	0.437
Bacillus clausii	0.306	2	0.858
Bacillus flexus	2.520	2	0.284
Bacillus horikoshii	0.991	2	0.609
Bacillus longiquaesitum	0.681	2	0.711
Bacillus nealsonii	1.135	2	0.567
Bacillus pocheonensis	1.563	2	0.458
Bacillus simplex	0.001	2	1.000
Bacillus vireti	0.936	2	0.626
Bacillus weihenstephanensis	0.169	2	0.919
Bacteriovorax marinus	3.000	2	0.223
Bacteriovorax sp.	4.178	2	0.124
Bacteriovorax spp.	2.565	2	0.277
Bacteroides coprocola	3.000	2	0.223
Bacteroides intestinalis	3.000	2	0.223
Bacteroides luti	2.446	2	0.294
Barnesiella viscericola	6.043	2	0.049
Bauldia consociate	0.324	2	0.850
Bdellovibrio bacteriovorus	7.947	2	0.019

Bdellovibrio exovorus	0.214	2	0.899
Bdellovibrio sp.	2.955	2	0.228
Bdellovibrio spp.	0.863	2	0.650
Beggiatoa sp.	0.345	2	0.842
Beggiatoa spp.	1.004	2	0.605
Beijerinckia spp.	3.449	2	0.178
Bellilinea spp.	0.289	2	0.866
Belnapia spp.	0.266	2	0.875
Blastococcus aggregatus	0.972	2	0.615
Blastococcus sp.	3.938	2	0.140
Blastococcus spp.	0.351	2	0.839
Blastomonas spp.	1.667	2	0.435
Blastopirellula marina	0.742	2	0.690
Blastopirellula spp.	0.971	2	0.615
Blautia product	0.477	2	0.788
Borrelia carolinensis	2.679	2	0.262
Bosea thiooxidans	2.667	2	0.264
Brachybacterium paraconglomeratum	0.313	2	0.855
Brachybacterium zhongshanense	3.000	2	0.223
Brachymonas denitrificans	5.027	2	0.081
Bradyrhizobium sp.	0.116	2	0.944

Bradyrhizobium spp.	1.057	2	0.589
Brevibacillus thermoruber	3.000	2	0.223
Brevibacterium daeguense	2.241	2	0.326
Brevundimonas abyssalis	0.010	2	0.995
Brevundimonas bacteroides	2.241	2	0.326
Buchnera aphidicola	0.604	2	0.739
Burkholderia xenovorans	2.267	2	0.322
Butyricimonas synergistica	3.000	2	0.223
Butyrivibrio clostridium proteoclasticum	0.885	2	0.643
Byssovorax spp.	2.667	2	0.264
Caedibacter spp.	0.728	2	0.695
Caenispirillum bisanense	2.667	2	0.264
Caldilinea spp.	1.512	2	0.470
Caldisericum spp.	3.000	2.000	0.223
Calditerricola sp.	1.029	2	0.598
Caloramator rice paddy	1.179	2	0.555
Caloramator spp.	1.265	2	0.531
Camelimonas alpha proteobacterium	1.870	2	0.393
Campylobacter Canadensis	2.667	2	0.264
Candidatus accumulibacter sp.	3.923	2	0.141

Candidatus acetothermum candidatus acetothermus autotrophicum	3.000	2	0.223
Candidatus alysiosphaera europeae	1.947	2	0.378
Candidatus aquiluna rubra	0.307	2	0.858
Candidatus arcobacter sulfidicus	0.386	2.000	0.824
Candidatus babela delta proteobacterium	2.241	2	0.326
Candidatus carsonella ruddii	1.896	2	0.388
Candidatus chloroploca chloroflexi bacterium	1.180	2	0.326
Candidatus cloacimonas acidaminovorans	3.000	2	0.223
Candidatus cloacimonas uncultured candidatus cloacamonas sp.	1.870	2	0.393
Candidatus clostridium anorexicamassiliense	2.345	2.000	0.310
Candidatus desulforudis audaxviator	2.667	2	0.264
Candidatus endobugula endosymbiont of bugula pacifica	1.254	2.000	0.534

Candidatus halomonas phosphatis	0.320	2	0.852
Candidatus lumbricincola sp.	1.118	2	0.572
Candidatus macropleicola muticae	5.457	2	0.065
Candidatus magnetobacterium uncultured magnetobacterium sp.	4.747	2	0.093
Candidatus magnetoovum mohavensis	2.667	2	0.264
Candidatus metachlamydia lacustris	1.767	2	0.413
Candidatus mycoplasma ravipulmonis	2.667	2	0.264
Candidatus nardonella endosymbiont of scyphophorus yuccae	3.000	2	0.223
Candidatus nardonella endosymbiont of sphenophorus levis	1.104	2	0.576
Candidatus nasuia deltocephalinicola	0.940	2	0.625
Candidatus nitrotoga arctica	1.095	2	0.578
Candidatus nucleicultrix amoebiphila	1.095	2	0.578

Candidatus odyssella thessalonicensis	1.870	2	0.393
Candidatus paenicardinium endonii	1.118	2	0.572
Candidatus paraholospora nucleivisitans	2.667	2	0.264
Candidatus pelagibacter uncultured pelagibacter sp.	2.667	2	0.264
Candidatus phytoplasma & apos	1.008	2.000	0.572
Candidatus phytoplasma mexican potato purple top phytoplasma	0.331	2	0.848
Candidatus planktoluna difficilis	0.222	2	0.895
Candidatus planktophila limnetica	0.536	2	0.765
Candidatus planktothricoides rosea	3.000	2	0.223
Candidatus protochlamydia amoebophila	1.029	2	0.598
Candidatus protochlamydia protochlamydia naegleriophila	0.331	2	0.848
Candidatus protochlamydia sp.	0.398	2	0.820
Candidatus rhabdochlamydia porcellionis	1.410	2	0.494

Candidatus rhabdochlamydia rhabdochlamydia crassificans	3.000	2	0.223
Candidatus rhabdochlamydia sp. cve88	1.179	2	0.555
Candidatus rhodoluna lacicola	0.259	2	0.879
Candidatus rhodoluna planktonica	1.684	2	0.431
Candidatus rhodoluna rhodoluna sp. kas9	1.170	2	0.557
Candidatus saccharimonas aalborgensis	1.711	2	0.425
Candidatus soleaferrea massiliensis	3.275	2	0.195
Candidatus thioglobus singularis	0.289	2	0.865
Candidatus trichorickettsia mobilis	3.941	2	0.139
Candidatus zinderia insecticola	2.501	2	0.286
Carboxydocella sp.	3.000	2	0.223
Carboxydothermus islandicus	1.095	2	0.578
Catalinimonas alkaloidigena	1.853	2	0.396
Catellatospora yuxiensis	3.262	2	0.196
Catenibacterium mitsuokai	3.000	2	0.223
Cellulomonas chitinilytica	0.310	2	0.856

Cellulomonas terrae	0.468	2	0.792
Cellulosilyticum ruminicola	1.029	2	0.598
Cellulosilyticum spp.	2.445	2	0.294
Cellvibrio gandavensis	1.118	2	0.572
Cellvibrio ostraviensis	2.072	2	0.355
Chitinibacter tainanensis	1.886	2	0.390
Chitinimonas koreensis	3.017	2	0.221
Chitinimonas taiwanensis	1.180	2	0.554
Chitinophaga flexibacter sancti	1.886	2	0.390
Chitinophaga pinensis	1.073	2	0.585
Chitinophaga spp.	1.871	2	0.392
Chlamydia ibidis	3.000	2	0.223
Chlorobium sp.	5.457	2	0.065
Chlorobium spp.	1.116	2	0.572
Chloroflexus spp.	1.053	2	0.591
Chloronema giganteum	1.095	2	0.578
Chondromyces crocatus	1.095	2	0.578
Chondromyces pediculatus	2.424	2	0.298
Chondromyces spp.	4.036	2	0.133
Chromatium okenii	1.920	2	0.383
Chromohalobacter spp.	0.067	2	0.967
Chryseobacterium anthropic	0.437	2	0.804
Chryseobacterium bovis	0.535	2	0.765

Chryseobacterium kwangyangense	0.866	2	0.648
Chryseobacterium soldanellicola	0.811	2	0.667
Chryseobacterium sp.	1.134	2	0.567
Chryseobacterium taiwanensis	0.940	2	0.625
Chryseomicrobium sp.	1.085	2	0.581
Chthoniobacter flavus	1.118	2	0.572
Cloacibacterium sp.	2.547	2	0.280
Cloacibacterium spp.	0.194	2	0.907
Clostridium aminobutyricum	0.930	2	0.628
Clostridium bovipellis	2.241	2	0.326
Clostridium bowmanii	3.626	2	0.163
Clostridium cavendishii	0.282	2	0.869
Clostridium cellulovorans	1.179	2	0.555
Clostridium disporicum	0.733	2	0.693
Clostridium enrichment	0.345	2	0.842
Clostridium frigidicarnis	1.838	2	0.399
Clostridium magnum	5.219	2	0.074
Clostridium quinii	0.866	2	0.648
Clostridium ruminantium	0.400	2	0.819
Clostridium scatologenes	3.867	2	0.145
Clostridium tunisiense	0.175	2	0.916

Cobetia marina	1.065	2	0.587
Cohnella sp.	0.663	2	0.718
Comamonas guangdongensis	0.390	2	0.823
Comamonas koreensis	0.664	2	0.717
Compostimonas spp.	10.014	2	0.007
Conexibacter sp.	1.118	2	0.572
Conexibacter spp.	1.327	2	0.515
Congregibacter litoralis	0.306	2	0.858
Coprococcus catus	0.866	2	0.648
Coprococcus eutactus	1.095	2	0.578
Corynebacterium appendicis	0.359	2	0.836
Corynebacterium lipophiloflavum	0.331	2	0.848
Corynebacterium maris	2.819	2	0.244
Corynebacterium matruchotii	0.806	2	0.668
Cosenzaea proteus myxofaciens	0.940	2	0.625
Couchioplanes caeruleus	0.550	2	0.760
Coxiella cheraxi	2.667	2	0.264
Craurococcus spp.	1.095	2	0.578
Crenothrix polyspora	0.282	2	0.868
Criblamydia sequanensis	1.385	2	0.500
Crocinitomix spp.	0.940	2	0.625
Cryobacterium spp.	1.087	2	0.581

Cryocola spp.	2.377	2	0.305
Cryptosporangium japonicum	1.185	2	0.553
Curvibacter sp.	0.225	2	0.894
Curvibacter spp.	0.657	2	0.720
Cyanothece spp.	6.049	2	0.049
Cycloclasticus spp.	1.870	2	0.393
Cystobacter spp.	0.388	2	0.824
Cystobacter violaceus	3.214	2	0.200
Cytophaga aurantiaca	0.289	2	0.865
Cytophaga sp.	0.039	2	0.981
Cytophaga spp.	1.511	2	0.470
Dactylosporangium spp.	0.220	2	0.896
Daeguia caeni	1.585	2	0.453
Dechloromonas denitrificans	2.064	2	0.356
Dechloromonas spp.	3.158	2	0.206
Dehalobacterium spp.	0.286	2	0.867
Dehalococcoides spp.	4.308	2	0.116
Dehalogenimonas spp.	1.409	2	0.494
Deinococcus alpinitundrae	0.320	2	0.852
Deinococcus deserti	0.028	2	0.986
Deinococcus geothermalis	6.140	2	0.046
Deinococcus hohokamensis	3.981	2	0.137
Deinococcus navajonensis	1.118	2	0.572

Deinococcus radiodurans	1.254	2	0.534
Deinococcus radiophilus	2.667	2	0.264
Deinococcus sp.	1.305	2	0.521
Deinococcus spp.	0.159	2	0.924
Deinococcus xinjiangensis	1.889	2	0.389
Delftia spp.	2.524	2	0.283
Demequina aestuarii	0.139	2	0.933
Demequina lutea	2.667	2	0.264
Denitratisoma sp.	0.074	2	0.964
Denitratisoma spp.	1.118	2	0.572
Denitrobacterium detoxificans	6.448	2	0.040
Derxia sp.	1.604	2	0.449
Desemzia incerta	3.735	2	0.155
Desertibacter roseus	1.172	2	0.557
Desulfatibacillum alkenivorans	3.000	2	0.223
Desulfatiglans desulfobacterium aniline	3.000	2	0.223
Desulfatitalea tepidiphila	3.000	2	0.223
Desulfitobacterium hafniense	1.792	2	0.408
Desulfitobacterium sp.	1.870	2	0.393
Desulfitobacterium spp.	0.004	2	0.998
Desulfobacter spp.	2.667	2	0.264
Desulfobacterium sp.	1.674	2	0.433

Desulfobacterium spp.	1.590	2	0.452
Desulfobulbus spp.	1.164	2	0.559
Desulfocapsa spp.	0.508	2	0.776
Desulfococcus biacutus	1.095	2	0.578
Desulfococcus spp.	3.374	2	0.185
Desulfofaba fastidiosa	1.886	2	0.390
Desulfofaba spp.	0.333	2	0.847
Desulfofrigus oceanense	3.271	2	0.195
Desulfomonile spp.	1.179	2	0.555
Desulfomonile tiedjei	0.064	2	0.968
Desulfonatronum thiosulfatophilum	3.781	2	0.151
Desulfonema limicola	1.095	2	0.578
Desulforegula spp.	1.815	2	0.403
Desulforhopalus spp.	3.655	2	0.161
Desulfosarcina spp.	1.029	2	0.598
Desulfosporomusa spp.	1.118	2	0.572
Desulfosporosinus meridiei	0.195	2	0.907
Desulfosporosinus spp.	0.481	2	0.786
Desulfotignum sp.	1.095	2	0.578
Desulfotomaculum acetoxidans	0.940	2	0.625
Desulfotomaculum solfataricum	2.667	2	0.264
Desulfotomaculum sp.	5.248	2	0.073

Desulfotomaculum spp.	0.261	2	0.878
Desulfovibrio mexicanus	1.029	2	0.598
Desulfovibrio oxyvorans	1.886	2	0.390
Desulfovibrio putealis	1.185	2	0.553
Desulfurobacterium spp.	2.667	2	0.264
Desulfuromonas spp.	2.667	2	0.264
Desulfuromusa spp.	1.328	2	0.515
Dethiosulfatibacter spp.	3.000	2	0.223
Devosia insulae	1.431	2	0.489
Devosia soli	0.003	2	0.999
Devosia sp.	3.268	2	0.195
Devosia spp.	0.296	2	0.862
Devosia subaequoris	0.261	2	0.878
Dissulfuribacter thermophiles	2.880	2	0.237
Dokdonella spp.	0.827	2	0.661
Dongia spp.	3.723	2	0.155
Dorea spp.	1.118	2	0.572
Draconibacterium orientale	3.925	2	0.141
Duganella sp.	0.143	2	0.931
Duganella zoogloeoides	0.634	2	0.728
Dyadobacter beijingensis	2.532	2	0.282
Dyadobacter psychrophilus	3.000	2	0.223
Dyadobacter sp.	1.928	2	0.381

Dyadobacter spp.	0.018	2	0.991
Ectothiorhodospira imhoffii	0.866	2	0.648
Ectothiorhodospira magna	6.149	2	0.046
Ectothiorhodospira sp.	1.029	2	0.598
Edaphobacter spp.	3.000	2	0.223
Elusimicrobium spp.	0.947	2	0.623
Emticicia oligotrophica	0.288	2	0.866
Emticicia spp.	3.000	2	0.223
Enhydrobacter aerosaccus	4.718	2	0.095
Ensifer adhaerens	1.732	2	0.421
Enteractinococcus sp.	3.442	2	0.179
Enterococcus columbae	3.419	2.000	0.181
Epulopiscium sp.	1.029	2	0.598
Erythrobacter gaetbuli	3.557	2	0.169
Erythrobacter litoralis	2.241	2	0.326
Erythrobacter piscidermidis	7.580	2	0.023
Erythrobacter sp.	2.612	2	0.271
Erythrobacter spp.	3.490	2	0.175
Ethanoligenens cellulosi	1.095	2	0.578
Ethanoligenens spp.	2.667	2	0.264
Eubacterium coprostanoligenes	3.000	2	0.223
Eubacterium oxidoreducens	0.063	2	0.969
Exiguobacterium indicum	0.581	2	0.748

Exiguobacterium lactigenes	3.146	2	0.207
Exiguobacterium panipatensis	1.732	2	0.421
Exiguobacterium profundum	1.074	2	0.585
Faecalibacterium prausnitzii	3.000	2	0.223
Ferrimicrobium spp.	1.388	2	0.500
Ferrithrix spp.	3.000	2	0.223
Ferrovum spp.	3.000	2	0.223
Ferruginibacter sp.	3.000	2	0.223
Fibrobacter spp.	5.568	2	0.062
Filibacter spp.	1.092	2	0.579
Filomicrobium sp.	3.000	2	0.223
Flavihumibacter sp.	2.065	2	0.356
Flavisolibacter flavosolibacter sp.	2.566	2	0.277
Flavisolibacter ginsengisoli	3.333	2	0.189
Flavisolibacter sp.	4.837	2	0.089
Flavisolibacter spp.	1.005	2	0.605
Flavobacterium aciduliphilum	0.839	2	0.657
Flavobacterium columnare	0.535	2	0.765
Flavobacterium indicum	1.660	2	0.436
Flavonifractor clostridium orbiscindens	0.866	2	0.648
Flectobacillus spp.	2.040	2	0.631

Flexibacter flexilis	0.214	2	0.899
Flexibacter spp.	1.304	2	0.521
Flexithrix dorotheae	1.095	2	0.578
Flexivirga spp.	0.668	2	0.711
Fluviicola spp.	3.410	2	0.182
Fluviicola taffensis	0.019	2	0.991
Fluviimonas pallidilutea	1.901	2	0.387
Fluviimonas sp.	0.349	2	0.840
Fonticella clostridiaceae bacterium	0.214	2	0.899
Formivibrio citricus	0.261	2	0.879
Frankia sp.	0.378	2	0.828
Frankia spp.	1.585	2	0.453
Frateuria aurantia	2.667	2	0.264
Frigoribacterium sp.	2.241	2	0.326
Fusibacter spp.	0.972	2	0.615
Gaiella occulta	3.000	2	0.223
Gaiella spp.	0.127	2	0.938
Gallaecimonas sp.	4.577	2	0.101
Gallionella spp.	0.584	2	0.747
Gelria spp.	1.118	2	0.572
Geminicoccus roseus	1.085	2	0.581
Gemmata sp.	1.118	2	0.572

Gemmata spp.	0.442	2	0.802
Gemmatimonas spp.	0.131	2.000	0.937
Gemmobacter catellibacterium sp.	4.276	2	0.118
Gemmobacter rhodobacter changlaii	1.712	2	0.425
Gemmobacter sp.	1.913	2	0.384
Geoalkalibacter spp.	1.097	2	0.578
Geobacter spp.	0.452	2	0.798
Geobacter thiogenes	3.374	2	0.185
Geodermatophilus obscurus	0.938	2	0.626
Geodermatophilus spp.	0.007	2	0.996
Geopsychrobacter electrodiphilus	6.140	2	0.046
Georgenia muralis	3.837	2	0.147
Georgenia sp.	0.866	2	0.648
Georgenia spp.	2.150	2	0.341
Geothermobacter spp.	0.922	2	0.631
Geothrix spp.	1.716	2	0.424
Geovibrio ferrireducens	1.789	2	0.409
Gloeobacter spp.	1.180	2	0.554
Gluconacetobacter spp.	1.886	2	0.390
Gordonibacter spp.	1.724	2	0.422

Gottschalkia eubacterium angustum	2.667	2	0.264
Gracilibacillus halotolerans	1.118	2	0.572
Gracilibacillus sp.	1.074	2	0.585
Gracilibacter spp.	0.940	2	0.625
Gracilimonas sp.	1.327	2	0.515
Granulicella spp.	3.000	2	0.223
Gulosibacter sp.	0.184	2	0.912
Haematobacter missouriensis	1.886	2	0.390
Halalkalibacillus halophilus	2.247	2	0.325
Haliangium spp.	0.064	2	0.968
Haliea mediterranea	0.752	2	0.687
Haliea sp.	2.880	2	0.237
Haliscomenobacter hydrossis	0.214	2	0.899
Haliscomenobacter spp.	0.882	2	0.643
Haloanella sp.	0.940	2	0.625
Halobacillus hunanensis	0.940	2	0.625
Halochromatium spp.	2.667	2	0.264
Halospirulina sp.	1.411	2	0.494
Halothiobacillus kellyi	1.029	2	0.598
Halothiobacillus sp.	2.048	2	0.359
Herbaspirillum rubrisubalbicans	1.487	2	0.476

Herbiconiux spp.	1.095	2	0.578
Hirschia sp.	0.701	2	0.704
Hoeflea sp.	3.194	2	0.202
Holdemania spp.	2.667	2	0.264
Holophaga foetida	3.274	2	0.195
Holophaga sp.	6.140	2	0.046
Holophaga spp.	0.573	2	0.751
Hydrogenophaga palleronii	2.304	2	0.316
Hydrogenophaga sp.	0.122	2	0.941
Hydrogenophaga spp.	0.547	2	0.761
Hydrogenophilus thermoluteolus	3.000	2	0.223
Hymenobacter gelipurpurascens	1.095	2	0.578
Hymenobacter sp.	0.197	2	0.906
Hymenobacter xinjiangensis	0.866	2	0.648
Hyphomicrobium spp.	0.900	2	0.638
Hyphomonas neptunium	5.339	2	0.069
Hyphomonas oceanitis	2.667	2	0.264
Hyphomonas spp.	0.013	2	0.994
Iamia majanohamensis	1.328	2	0.515
Iamia spp.	0.899	2	0.638
Ideonella sp.	0.864	2	0.649
Ideonella spp.	0.270	2	0.874
Idiomarina loihiensis	0.866	2	0.648

Idiomarina sp.	5.489	2	0.064
Idiomarina spp.	0.386	2	0.824
Ignavibacterium sp.	3.000	2	0.223
Ignavibacterium spp.	1.725	2	0.422
Ilumatobacter fluminis	3.271	2	0.195
Ilumatobacter spp.	1.592	2	0.451
Inhella inkyongensis	0.941	2	0.625
Insolitispirillum insolitospirillum peregrinum	0.497	2	0.780
Intestinimonas butyriciproducens	2.667	2	0.264
Isoptericola spp.	2.148	2	0.342
Jannaschia sp.	0.387	2	0.824
Jatrophihabitans endophyticus	6.140	2	0.046
Jeotgalicoccus psychrophilus	0.386	2	0.824
Jonesia sp.	0.308	2	0.857
Kaistia hirudinis	0.034	2	0.983
Kaistia sp.	1.074	2	0.585
Kaistobacter spp.	3.261	2	0.196
Kallotenue chloroflexi bacterium	0.940	2	0.625
Kineococcus radiotolerans	1.781	2	0.410
Kineococcus sp.	1.613	2	0.447
Kineosporia aurantiaca	5.220	2	0.074

Kitasatospora cystarginea	2.809	2	0.245
Kitasatospora spp.	1.185	2	0.553
Klugiella spp.	0.370	2	0.831
Knoellia sinensis	1.573	2	0.455
Knoellia subterranean	1.900	2	0.387
Kocuria carniphila	0.657	2	0.720
Kopriimonas spp.	1.029	2	0.598
Kouleothrix aurantiaca	2.710	2	0.258
Kouleothrix spp.	0.220	2	0.896
Ktedonobacter spp.	6.140	2	0.046
Labilithrix luteola	0.402	2	0.818
Labrenzia aggregate	3.552	2	0.169
Lachnoclostridium clostridium phytofermentans	1.095	2	0.578
Lachnoclostridium clostridium xylanolyticum	1.073	2	0.585
Lacibacter cauensis	0.751	2	0.687
Lacibacter sp.	1.187	2	0.552
Lacibacter spp.	1.095	2	0.578
Lacibacterium rhodospirillaceae bacterium	0.013	2	0.994
Lactobacillus farciminis	0.797	2	0.671
Lactobacillus gallinarum	0.024	2	0.988
Lactobacillus graminis	1.095	2	0.578

Lactobacillus helveticus	0.940	2	0.625
Lactobacillus kunkeei	1.095	2	0.578
Lactobacillus mali	1.095	2	0.578
Lactobacillus pentosus	1.029	2	0.598
Lactobacillus reuteri	1.095	2	0.578
Lactobacillus rossiae	3.000	2	0.223
Lactococcus plantarum	0.828	2	0.661
Larkinella sp.	1.877	2	0.391
Leadbetterella sp.	5.414	2	0.067
Leeia oryzae	0.010	2	0.995
Legionella dresdeniensis	0.210	2	0.900
Legionella geestiana	2.241	2	0.326
Legionella santicrucis	0.006	2	0.997
Lentzea spp.	1.896	2	0.387
Leptolinea sp.	1.366	2	0.505
Leptolinea spp.	5.457	2	0.065
Leptolyngbya frigida	1.095	2	0.578
Leptolyngbya saxicola	1.728	2	0.421
Leptolyngbya sp.	0.443	2	0.801
Leptolyngbya spp.	3.047	2	0.218
Leptospirillum ferrodiazotrophum	3.000	2	0.223
Leptospirillum spp.	1.118	2	0.572

Leptothrix sp.	0.573	2	0.751
Leptothrix spp.	9.573	2	0.008
Leucobacter sp.	0.693	2	0.707
Leuconostoc palmae	1.357	2	0.507
Levilinea spp.	2.552	2	0.279
Lewinella sp.	5.457	2	0.065
Lewinella spp.	0.866	2	0.648
Limnobacter litoralis	1.762	2	0.414
Limnobacter spp.	1.532	2	0.465
Limnohabitans curvus	2.929	2	0.231
Limnohabitans spp.	0.109	2	0.947
Loktanella salsilacus	5.339	2	0.069
Longilinea spp.	0.539	2	0.764
Luteimonas composti	4.821	2	0.090
Luteimonas sp.	2.502	2	0.286
Luteimonas spp.	0.921	2	0.631
Luteolibacter algae	1.095	2	0.578
Luteolibacter pohnpeiensis	0.214	2	0.899
Luteolibacter sp.	0.419	2	0.811
Luteolibacter spp.	0.336	2	0.845
Luteolibacter yonseiensis	3.377	2	0.185
Lutibaculum baratangense	1.095	2	0.578
Lutispora spp.	1.095	2	0.578

Lutispora thermophile	1.118	2	0.572
Lysinibacillus sphaericus	0.925	2	0.630
Lysobacter deserti	0.391	2	0.822
Lysobacter enzymogenes	0.762	2	0.683
Lysobacter sp.	4.117	2	0.128
Lysobacter spp.	0.392	2	0.822
Lyticum sinuosum	0.866	2	0.648
Magnetococcus spp.	2.247	2	0.325
Magnetospirillum sp.	1.250	2	0.535
Magnetospirillum spp.	2.243	2	0.326
Magnetovibrio blakemorei	2.548	2	0.280
Malikia spp.	0.072	2	0.964
Maribacter sp.	2.667	2	0.264
Marinilactibacillus sp.	2.710	2	0.258
Marinimicrobium koreense	1.095	2	0.578
Marininema halotolerans	2.775	2	0.250
Mariniphaga bacteroidales bacterium	5.457	2	0.065
Marinithermus spp.	1.832	2	0.400
Marinobacter sp.	0.866	2	0.648
Marinobacter spp.	0.965	2	0.617
Marinobacter zhanjiangensis	1.095	2	0.578
Marinobacterium spp.	1.029	2	0.598

Marinomonas arenicola	0.894	2	0.639
Marinomonas mediterranea	2.667	2.000	0.264
Marinomonas pontica	2.667	2.000	0.264
Marinomonas spp.	2.795	2.000	0.247
Marinomonas vaga	2.178	2.000	0.337
Marisediminicola spp.	0.636	2	0.727
Marispirillum spp.	2.667	2	0.264
Marmoricola sp.	0.368	2	0.832
Meniscus spp.	2.667	2	0.264
Merismopedia spp.	1.870	2	0.393
Methylibium petroleiphilum	0.930	2	0.628
Methylobacillus flagellates	1.674	2	0.433
Methylobacillus spp.	1.442	2	0.486
Methylobacter sp.	3.000	2	0.223
Methylobacter spp.	1.267	2	0.531
Methylobacter whittenburyi	0.866	2.000	0.648
Methylocaldum sp.	1.160	2	0.560
Methylocaldum spp.	1.129	2	0.569
Methylocella sp.	4.186	2	0.123
Methylococcus mobilis	0.220	2	0.896
Methylococcus spp.	0.700	2	0.705
Methylocystis parvus	0.328	2	0.849
Methylocystis spp.	3.271	2	0.195

Methylomicrobium spp.	3.271	2	0.195
Methylomonas fodinarum	3.000	2	0.223
Methylomonas methanica	0.582	2	0.747
Methylomonas sp.	1.591	2	0.451
Methylomonas spp.	1.002	2	0.606
Methylophaga sp.	3.175	2	0.204
Methylophaga spp.	2.136	2	0.344
Methylophilus spp.	0.348	2	0.840
Methylopila capsulate	1.118	2	0.572
Methylopila sp.	2.267	2	0.322
Methylosinus sp.	0.013	2	0.994
Methylosinus sporium	1.179	2	0.555
Methylosinus spp.	1.925	2	0.382
Methylosinus trichosporium	1.683	2	0.431
Methylosoma sp.	1.095	2	0.578
Methylotenera mobilis	1.327	2	0.515
Methylotenera spp.	0.024	2	0.988
Methylotenera versatilis	0.217	2	0.897
Methylothermus spp.	3.274	2	0.195
Methyloversatilis spp.	1.118	2	0.572
Methylovulum miyakonense	1.783	2	0.410
Microbacterium sediminicola	3.441	2	0.179
Microbispora rosea	1.510	2	0.470

Microcella putealis	0.964	2	0.618
Microcella spp.	3.938	2	0.140
Microcoleus spp.	0.659	2	0.719
Microcystis sp.	0.661	2	0.719
Micromonospora sp.	0.036	2	0.982
Micromonospora spp.	1.155	2	0.561
Microvirga spp.	2.956	2	0.228
Miniimonas arenae	0.358	2	0.836
Mitsuaria spp.	3.627	2	0.163
Modestobacter spp.	0.411	2	0.814
Mogibacterium pumilum	2.891	2	0.236
Moorella humiferrea	5.457	2	0.065
Moorella spp.	1.672	2	0.434
Moorella thermoacetica	6.140	2	0.046
Mucilaginibacter sp.	1.331	2	0.514
Mucilaginibacter spp.	0.708	2	0.702
Mucilaginibacter ximonensis	0.026	2	0.987
Mycoplana sp.	3.240	2	0.198
Mycoplasma alligatoris	1.825	2	0.402
Mycoplasma crocodyli	3.554	2	0.169
Mycoplasma phocidae	3.000	2	0.223
Mycoplasma zalophi	0.498	2	0.779
Myxococcus spp.	1.095	2	0.578

Nafulsella turpanensis	0.866	2	0.648
Nannocystis spp.	1.278	2	0.528
Natranaerovirga hydrolytica	1.065	2	0.587
Natranaerovirga pectinivora	3.274	2	0.195
Natronoanaerobium salstagnum	0.166	2	0.920
Neochlamydia hartmannellae	1.095	2	0.578
Neochlamydia sp.	2.667	2	0.264
Neochlamydia spp.	2.241	2	0.326
Neptunomonas spp.	3.441	2	0.179
Nevskia soli	2.667	2	0.264
Niabella sp.	1.886	2	0.390
Niastella sp.	0.127	2	0.939
Niastella spp.	0.210	2	0.900
Nitratireductor spp.	0.214	2	0.899
Nitrobacter spp.	1.095	2	0.578
Nitrosococcus spp.	1.095	2	0.578
Nitrosomonas spp.	3.000	2	0.223
Nitrosospira spp.	4.335	2	0.114
Nitrosovibrio spp.	2.763	2	0.251
Nitrospina spp.	1.886	2	0.390
Nitrospira sp.	1.886	2	0.390
Nitrospira spp.	4.024	2	0.134

Nitrospirillum azospirillum amazonense	1.095	2	0.578
Nocardioides furvisabuli	0.036	2	0.982
Nocardioides hankookensis	0.400	2	0.819
Nocardioides iriomotensis	0.082	2	0.960
Nocardioides maritimus	1.952	2	0.377
Nocardioides sp.	0.693	2	0.707
Nocardioides spp.	1.981	2	0.371
Nonomuraea sp.	0.866	2	0.648
Nonomuraea turkmeniaca	2.567	2	0.277
Nordella spp.	9.429	2	0.009
Nosocomiicoccus ampullae	2.795	2	0.247
Noviherbaspirillum malthae	0.318	2	0.853
Novosphingobium capsulatum	0.169	2	0.919
Novosphingobium mathurensis	0.227	2	0.893
Novosphingobium sp.	3.815	2	0.148
Novosphingobium spp.	3.521	2	0.172
Novosphingobium stygium	0.407	2	0.816
Novosphingobium subarcticum	1.986	2	0.370
Novosphingobium subterraneum	1.130	2	0.568
Nubsella sp.	3.000	2	0.223
Nubsella zeaxanthinifaciens	0.010	2	0.995
Oceanibaculum pacificum	5.457	2	0.065

Oceanibaculum spp.	3.000	2	0.223
Oceanimonas smirnovii	3.465	2	0.177
Oceanobacillus luteolus	0.561	2	0.756
Oceanobacillus sp.	0.076	2	0.963
Oculatella coburnii	1.095	2	0.578
Ohtaekwangia koreensis	1.085	2	0.581
Ohtaekwangia spp.	0.383	2	0.826
Oleiphilus messinensis	3.000	2	0.223
Oleiphilus spp.	10.646	2	0.005
Oleispira spp.	8.381	2	0.015
Oleomonas sp.	1.095	2	0.578
Opitutus sp.	9.672	2	0.008
Opitutus spp.	3.149	2	0.207
Opitutus terrae	3.245	2	0.197
Oribacterium sinus	9.425	2	0.009
Oribacterium sp.	1.095	2	0.578
Orientia tsutsugamushi	1.118	2.000	0.572
Ornatilinea apprima	1.185	2	0.553
Ornithinibacillus sp.	1.191	2	0.551
Ornithinicoccus hortensis	1.857	2	0.395
Ornithinimicrobium sp.	0.096	2	0.953
Oscillatoria sp.	1.095	2	0.578
Oscillatoria spp.	0.177	2	0.915

Oscillospira spp.	0.866	2	0.648
Owenweeksia spp.	8.110	2	0.017
Oxalicibacterium faecigallinarum	0.807	2	0.668
Oxobacter pfennigii	5.457	2	0.065
Paenibacillus cellulosilyticus	0.848	2	0.654
Paenibacillus chitinolyticus	1.328	2	0.515
Paenibacillus contaminans	1.052	2	0.591
Paenibacillus favisporus	0.331	2	0.848
Paenibacillus graminis	0.655	2	0.721
Paenibacillus konsidanse	1.886	2	0.390
Paenibacillus nanensis	2.241	2	0.326
Paenibacillus stellifer	3.000	2	0.223
Paenibacillus wynnii	0.024	2	0.988
Palleronia sp.	0.940	2	0.625
Paludibacter propionicigenes	1.970	2	0.373
Paludibacter sp.	3.072	2	0.215
Paludibacter spp.	1.180	2	0.554
Paludibacterium sp.	3.000	2	0.223
Pannonibacter sp.	3.000	2	0.223
Papillibacter cinnamivorans	5.457	2	0.65
Parabacteroides distasonis	9.429	2	0.009
Parabacteroides gordonii	3.000	2	0.223

Parabacteroides spp.	1.040	2	0.595
Parachlamydia acanthamoebae	0.449	2	0.799
Paracoccus denitrificans	0.576	2.000	0.750
Paracoccus marcusii	1.177	2	0.555
Paracoccus pantotrophus	2.471	2	0.291
Paracoccus spp.	1.052	2	0.591
Paracraurococcus spp.	1.191	2	0.551
Parasegetibacter luojiensis	2.622	2	0.270
Parvibaculum spp.	3.000	2	0.223
Parvimonas micra	1.095	2	0.578
Pediococcus lactobacillus plantarum	0.760	2	0.684
Pedobacter cryoconitis	0.006	2	0.997
Pedobacter glucosidilyticus	1.674	2	0.433
Pedobacter heparinus	0.498	2	0.780
Pedobacter lentus	1.118	2	0.572
Pedobacter metabolipauper	1.095	2	0.578
Pedobacter sp.	0.302	2	0.860
Pedobacter spp.	0.535	2	0.765
Pedobacter steynii	2.537	2	0.281
Pedobacter wanjuense	2.016	2	0.365
Pedomicrobium australicum	2.667	2	0.264
Pedomicrobium spp.	0.190	2	0.909

Pedosphaera parvula	4.233	2	0.120
Pedosphaera spp.	1.080	2	0.583
Pelagibacterium halotolerans	1.816	2	0.403
Pelagibius litoralis	1.095	2	0.578
Pelagibius spp.	3.000	2	0.223
Pelagicoccus mobilis	1.095	2	0.578
Pelobacter carbinolicus	1.266	2	0.531
Pelobacter spp.	4.257	2	0.119
Pelomonas sp.	0.928	2	0.629
Pelomonas spp.	0.843	2	0.656
Pelosinus sp.	1.118	2	0.572
Pelotomaculum spp.	3.652	2	0.161
Peptoclostridium clostridium bifermentans	0.088	2	0.957
Peptoclostridium clostridium difficile	2.441	2	0.295
Peptoclostridium clostridium sticklandii	3.000	2	0.223
Peptococcus sp.	3.000	2	0.223
Peredibacter spp.	1.345	2	0.510
Peredibacter starrii	3.307	2	0.191
Perlucidibaca piscinae	3.271	2	0.195
Perlucidibaca spp.	3.540	2	0.170
Persicirhabdus sediminis	1.268	2	0.530

Petrimonas spp.	1.870	2	0.393
Phaeospirillum fulvum	1.074	2	0.585
Phascolarctobacterium sp.	2.216	2	0.330
Phaselicystis spp.	0.866	2	0.648
Phenylobacterium sp.	0.532	2	0.766
Phenylobacterium spp.	2.465	2	0.292
Phycicoccus sp.	2.428	2	0.297
Phycisphaera spp.	1.095	2	0.578
Phyllobacterium sp.	2.616	2	0.270
Pirellula sp.	0.908	2	0.635
Pirellula spp.	0.965	2	0.617
Planctomyces maris	0.005	2	0.997
Planctomyces spp.	0.571	2	0.752
Planktothricoides spp.	1.816	2	0.403
Planococcus maitriensis	0.192	2	0.908
Planococcus sp.	4.088	2	0.130
Planomicrobium chinense	0.866	2	0.648
Planomicrobium koreense	1.671	2	0.434
Planomicrobium mcmeekinii	0.553	2	0.758
Plantactinospora sp.	5.808	2	0.055
Plasticicumulans lactativorans	5.457	2	0.065
Pleomorphomonas spp.	0.940	2	0.625
Polaribacter gangjinensis	0.602	2	0.740

Polyangium sp.	1.886	2	0.390
Polymorphospora rubra	2.547	2	0.280
Polynucleobacter cosmopolitanus	0.877	2	0.645
Polynucleobacter necessaries	0.685	2	0.710
Polynucleobacter rarus	0.277	2	0.871
Polynucleobacter spp.	1.749	2	0.417
Pontibacter korlensis	3.463	2	0.177
Pontibacter populi	1.095	2	0.578
Pontibacter sp.	0.807	2	0.668
Ponticoccus sp.	3.332	2	0.189
Porphyrobacter sp.	1.230	2	0.541
Porphyrobacter spp.	0.086	2	0.958
Porphyrobacter tepidarius	0.632	2	0.729
Porticoccus spp.	3.337	2	0.189
Prevotella amnii	1.297	2	0.523
Prevotella spp.	0.752	2	0.687
Prochlorococcus spp.	1.520	2	0.468
Prolixibacter spp.	1.204	2	0.548
Propionigenium spp.	0.473	2	0.789
Propionivibrio spp.	6.199	2	0.045
Prosthecobacter spp.	2.141	2	0.343
Prosthecobacter vanneervenii	1.095	2	0.578

Prosthecomicrobium spp.	1.564	2	0.457
Proteiniphilum acetatigenes	9.429	2	0.009
Proteiniphilum spp.	2.064	2	0.356
Proteinivorax tanatarense	2.294	2	0.318
Pseudoalteromonas sp.	2.548	2	0.280
Pseudoalteromonas spp.	5.457	2	0.065
Pseudoalteromonas tetraodonis	6.140	2	0.046
Pseudoclavibacter spp.	0.021	2	0.989
Pseudohongiella sp.	4.622	2	0.099
Pseudolabrys sp.	0.609	2	0.738
Pseudolabrys spp.	1.788	2	0.409
Pseudomonas luteola	0.470	2	0.791
Pseudomonas pachastrellae	0.754	2	0.686
Pseudomonas savastanoi	0.536	2	0.765
Pseudomonas straminea	1.597	2	0.450
Pseudomonas taiwanensis	1.884	2	0.390
Pseudomonas tuomuerense	3.486	2	0.175
Pseudomonas umsongensis	2.667	2	0.264
Pseudomonas veronii	1.309	2	0.520
Pseudonocardia spp.	1.623	2	0.444
Pseudorhodobacter sp.	1.837	2	0.399
Pseudospirillum spp.	2.667	2	0.264
Pseudoxanthomonas koreensis	1.495	2	0.473

Pseudoxanthomonas mexicana	0.266	2	0.875
Pseudoxanthomonas sp.	1.073	2	0.585
Pseudoxanthomonas taiwanensis	1.736	2	0.420
Psychrobacillus bacillus psychrodurans	1.095	2	0.578
Psychrobacter aquaticus	0.866	2	0.648
Psychrobacter sanguinis	1.095	2	0.578
Pullulanibacillus sp.	3.849	2	0.146
Puniceicoccus vermicola	6.140	2	0.046
Pusillimonas sp.	2.196	2	0.334
Pusillimonas spp.	1.095	2	0.578
Quadrisphaera sp.	2.817	2	0.244
Ramlibacter spp.	0.014	2	0.993
Ramlibacter tataouinensis	2.735	2	0.255
Rathayibacter tritici	1.185	2	0.553
Reyranella massiliensis	1.984	2	0.371
Reyranella soli	1.029	2	0.598
Reyranella sp.	3.000	2	0.223
Rheinheimera aquimaris	4.650	2	0.098
Rheinheimera chironomi	1.786	2	0.409
Rheinheimera sp.	1.596	2	0.450
Rheinheimera texana	4.096	2	0.129

Rhizobium leguminosarum	0.002	2	0.999
Rhizobium mongolense	2.150	2	0.341
Rhizobium tropici	1.095	2	0.578
Rhizomicrobium electricum	3.000	2	0.223
Rhodanobacter fulvus	2.714	2	0.257
Rhodanobacter sp.	1.016	2	0.602
Rhodobacter capsulatus	0.013	2	0.994
Rhodobacter gluconicum	0.306	2	0.858
Rhodobacter sphaeroides	1.172	2	0.556
Rhodobacter sp.	0.523	2	0.770
Rhodobacter spp.	0.232	2	0.891
Rhodobacter vinaykumarii	2.205	2	0.332
Rhodobium spp.	0.663	2	0.718
Rhodococcus kroppenstedtii	2.667	2	0.264
Rhodococcus rhodochrous	0.274	2	0.872
Rhodococcus yunnanensis	2.241	2	0.326
Rhodocyclus tenuis	0.948	2	0.623
Rhodocytophaga aerolata	1.506	2	0.471
Rhodocytophaga spp.	0.866	2	0.648
Rhodoferax albidiferax sp.	4.965	2	0.084
Rhodoferax antarcticus	0.187	2	0.911
Rhodomicrobium sp.	1.440	2	0.487
Rhodomicrobium spp.	0.830	2	0.660

Rhodomicrobium vannielii	5.457	2	0.605
Rhodopila globiformis	3.795	2	0.150
Rhodopirellula baltica	3.000	2	0.223
Rhodopirellula spp.	0.222	2	0.895
Rhodopseudomonas spp.	1.648	2	0.439
Rhodothermus spp.	3.000	2	0.223
Rhodovastum spp.	6.140	2	0.046
Rhodovibrio spp.	2.667	2	0.264
Rhodovulum marinum	2.667	2	0.264
Rhodovulum sulfidophilum	2.667	2	0.264
Rickettsia Canadensis	2.667	2	0.264
Rickettsiella grylli	0.561	2	0.755
Rikenella sp.	0.537	2	0.765
Rikenella spp.	1.130	2	0.568
Rivibacter sp.	2.226	2	0.329
Robiginitomaculum antarcticum	4.702	2	0.095
Roseburia faecis	1.118	2	0.572
Roseburia spp.	0.289	2	0.865
Roseibaca ekhonensis	5.457	2	0.065
Roseibacillus spp.	1.960	2	0.375
Roseicyclus spp.	3.806	2	0.149
Roseiflexus spp.	0.010	2	0.995
Roseinatronobacter sp.	2.844	2	0.241

Roseobacter sp.	2.241	2	0.326
Roseococcus sp.	4.129	2	0.127
Roseomonas lacus	3.083	2	0.214
Roseomonas ruber	1.385	2	0.500
Roseomonas stagni	1.202	2	0.548
Roseovarius sp.	1.104	2	0.576
Rothia sp.	0.731	2	0.694
Rubellimicrobium mesophilum	0.709	2	0.702
Rubellimicrobium spp.	3.258	2	0.196
Rubrimonas sp.	1.095	2	0.578
Rubrivivax gelatinosus	3.077	2	0.215
Rubrobacter spp.	2.994	2	0.224
Rudaea cellulosilytica	1.886	2	0.390
Rudanella sp.	2.445	2	0.294
Rufibacter sp.	1.411	2	0.494
Ruminiclostridium clostridium aldrichii	1.519	2	0.468
Ruminiclostridium clostridium cellobioparum	0.400	2	0.819
Ruminiclostridium clostridium josui	0.334	2	0.846
Ruminiclostridium clostridium papyrosolvens	1.877	2	0.391
Ruminococcus callidus	0.588	2	0.745

Rummeliibacillus pycnus	0.172	2	0.918
Runella slithyformis	0.594	2	0.743
Runella spp.	1.597	2	0.450
Saccharibacter spp.	0.361	2	0.835
Saccharofermentans acetigenes	3.795	2	0.150
Saccharomonospora azurea	1.095	2	0.578
Saccharophagus spp.	0.333	2	0.847
Saccharospirillum sp.	2.667	2	0.264
Saccharospirillum spp.	2.547	2	0.280
Saccharothrix xinjiangensis	1.095	2	0.578
Salinicoccus roseus	1.030	2	0.598
Salinicoccus sp.	0.917	2	0.632
Salinimicrobium sp.	1.095	2	0.578
Sandaracinus amylolyticus	1.095	2	0.578
Sandaracinus spp.	0.525	2	0.769
Sandarakinorhabdus sp.	1.029	2	0.598
Sandarakinorhabdus spp.	5.013	2	0.082
Sanguibacter antarcticus	1.300	2	0.522
Schlegelella spp.	1.118	2	0.572
Sedimentibacter spp.	2.667	2	0.264
Sediminibacterium salmoneum	0.001	2	0.999
Sediminibacterium sp.	0.020	2	0.990
Sediminibacterium spp.	0.501	2	0.779

Segetibacter spp.	0.234	2	0.890
Sejongia spp.	0.795	2	0.672
Seohaeicola saemankumensis	2.892	2	0.236
Serinicoccus sp.	0.433	2	0.805
Shimazuella kribbensis	3.000	2	0.223
Shimazuella sp.	1.870	2	0.393
Shinella spp.	1.040	2	0.595
Shinella zoogloeoides	3.000	2	0.223
Sideroxydans spp.	3.135	2	0.209
Silanimonas sp.	1.074	2	0.585
Simplicispira sp.	1.812	2	0.404
Singulisphaera sp.	0.304	2	0.859
Singulisphaera spp.	0.518	2	0.772
Sinorhizobium ensifer fredii	0.940	2	0.625
Sinorhizobium sp.	2.880	2	0.237
Skermanella sp.	2.967	2	0.227
Skermanella spp.	1.064	2	0.587
Smaragdicoccus niigatensis	0.412	2	0.814
Sneathiella sp.	5.457	2	0.065
Solimonas soli	4.813	2	0.090
Solirubrobacter spp.	2.732	2	0.255
Solitalea Canadensis	4.029	2	0.133
Solitalea spp.	2.548	2	0.280

Sorangium cellulosum	0.456	2	0.796
Sphaerobacter spp.	0.316	2	0.854
Sphaerobacter thermophiles	1.320	2	0.517
Sphaerotilus natans	1.012	2	0.603
Sphaerotilus spp.	0.780	2	0.677
Sphingobacterium faecium	0.138	2	0.934
Sphingobacterium siyangensis	0.930	2	0.628
Sphingobium chlorophenolicum	0.306	2	0.858
Sphingobium chungbukensis	0.940	2	0.625
Sphingobium faniae	2.667	2	0.264
Sphingobium xenophagum	4.875	2	0.087
Sphingobium yanoikuyae	0.601	2	0.741
Sphingomonas faeni	0.234	2	0.889
Sphingomonas melonis	2.854	2	0.240
Sphingomonas wittichii	0.196	2	0.907
Sphingomonas yunnanensis	1.095	2	0.578
Sphingopyxis chilensis	3.806	2	0.149
Sphingopyxis sp.	3.285	2	0.193
Sphingopyxis spp.	6.140	2	0.046
Sphingosinicella spp.	1.971	2	0.373
Spiribacter sp.	2.241	2	0.326
Spirobacillus cienkowskii	0.282	2	0.868
Spirochaeta aurantia	1.182	2	0.554

Spirochaeta bajacaliforniensis	0.866	2	0.648
Spirochaeta sp.	1.180	2	0.554
Spirochaeta spp.	1.262	2	0.532
Spirosoma linguale	2.667	2	0.264
Spongiibacter sp.	3.592	2	0.166
Sporichthya sp.	0.991	2	0.609
Sporichthya spp.	0.057	2	0.972
Sporobacter termitidis	0.167	2	0.920
Sporomusa spp.	2.319	2	0.314
Stappia spp.	1.497	2	0.473
Stella spp.	0.035	2	0.983
Stenotrophomonas acidaminiphila	1.278	2	0.528
Steroidobacter spp.	2.710	2	0.258
Sterolibacterium sp.	0.531	2	0.767
Sterolibacterium spp.	2.446	2	0.294
Streptomyces glaucescens	0.461	2	0.794
Streptomyces macrosporus	1.095	2	0.578
Streptomyces phaeopurpureus	1.029	2	0.598
Streptomyces scabrisporus	1.074	2	0.585
Streptomyces werraensis	4.975	2	0.083
Streptomyces yokosukanensis	0.047	2	0.977
Streptosporangium vulgare	1.118	2	0.572

Subdoligranulum spp.	2.842	2	0.242
Sulfuricurvum kujiense	0.889	2	0.641
Sulfuricurvum spp.	2.611	2	0.271
Sulfurimonas autotrophica	0.636	2	0.728
Sulfurimonas paralvinellae	1.852	2	0.396
Sulfurimonas spp.	2.015	2	0.365
Sulfurisoma sediminicola	3.104	2	0.212
Sulfurospirillum deleyianum	0.326	2	0.850
Sulfurospirillum spp.	0.470	2	0.791
Sulfurovum spp.	1.792	2	0.408
Sunxiuqinia faeciviva	5.457	2	0.065
Sunxiuqinia sp.	1.886	2	0.390
Symbiobacterium spp.	1.763	2	0.414
Synechococcus sp.	2.446	2	0.294
Synechococcus spp.	1.330	2	0.514
Synechocystis sp.	0.940	2	0.625
Syntrophobacter sp.	1.783	2	0.410
Syntrophobacter spp.	2.707	2	0.258
Syntrophomonas sp.	1.510	2	0.470
Syntrophomonas spp.	4.053	2	0.132
Syntrophus sp.	2.478	2	0.290
Syntrophus spp.	0.918	2	0.632
Tannerella spp.	1.052	2	0.591

Telmatobacter spp.	0.991	2	0.609
Tepidimonas spp.	2.282	2	0.320
Tepidiphilus petrobacter sp.	1.003	2	0.606
Teredinibacter sp.	1.095	2	0.578
Terrabacter sp.	0.061	2	0.970
Terribacillus halophilus	1.886	2	0.390
Terribacillus saccharophilus	2.321	2	0.313
Terriglobus roseus	2.667	2	0.264
Terrimonas sp.	7.969	2	0.019
Terrimonas spp.	1.627	2	0.443
Tetrasphaera spp.	0.125	2	0.939
Thalassobacillus devorans	0.894	2	0.639
Thalassobaculum sp.	1.035	2	0.596
Thalassolituus sp.	8.306	2	0.016
Thalassolituus spp.	0.769	2	0.681
Thauera mechernichensis	0.606	2	0.738
Thauera phenylacetica	1.483	2	0.477
Thauera selenatis	0.144	2	0.930
Thauera spp.	0.066	2	0.967
Thermacetogenium spp.	2.667	2	0.264
Thermaerobacter spp.	0.242	2	0.886
Thermincola spp.	1.118	2	0.572
Thermoanaerobacter uzonensis	2.667	2	0.264

Thermobacillus sp.	1.118	2	0.572
Thermodesulfobacterium spp.	0.273	2	0.872
Thermodesulfobium spp.	1.095	2	0.578
Thermodesulfovibrio spp.	4.746	2	0.093
Thermoleophilum album	2.667	2	0.264
Thermoleophilum spp.	2.124	2	0.346
Thermomicrobium spp.	0.243	2	0.886
Thermomonas brevis	1.342	2	0.511
Thermomonas fusca	1.882	2	0.390
Thermomonas haemolytica	1.876	2	0.391
Thermomonas sp.	0.289	2	0.865
Thermomonas spp.	1.083	2	0.582
Thermosporothrix spp.	1.886	2	0.390
Thermovum composti	1.095	2	0.578
Thermus sp.	2.203	2	0.332
Thermus spp.	1.815	2	0.404
Thermus thiopara	1.783	2	0.410
Thioalkalibacter halophilus	0.640	2	0.726
Thioalkalivibrio nitratireducens	2.737	2	0.254
Thioalkalivibrio spp.	2.289	2	0.318
Thiobaca spp.	1.095	2	0.578
Thiobacillus sp.	5.804	2	0.055
Thiobacillus spp.	0.071	2	0.965

Thiobacter spp.	3.000	2	0.223
Thiocystis violacea	1.886	2	0.390
Thiodictyon bacillosum	0.525	2	0.769
Thiohalophilus spp.	0.940	2	0.625
Thiomicrospira halophilus	2.775	2	0.250
Thiomicrospira sp.	1.319	2	0.517
Thioprofundum hispidum	6.140	2	0.046
Thioprofundum spp.	1.095	2	0.578
Thiorhodococcus bheemlicus	2.729	2	0.255
Thiorhodospira spp.	0.509	2	0.775
Thiorhodovibrio winogradskyi	0.456	2	0.796
Thiothrix caldifontis	0.006	2	0.997
Thiothrix disciformis	0.320	2	0.852
Thiothrix spp.	1.118	2	0.572
Thiovirga spp.	1.492	2	0.474
Thorsellia spp.	5.460	2	0.065
Tissierella spp.	3.665	2	0.160
Tistrella spp.	2.969	2	0.227
Tolumonas auensis	1.335	2	0.513
Tolumonas spp.	1.826	2	0.401
Treponema primitia	2.667	2	0.264
Treponema zuelzerae	0.940	2	0.625
Trichococcus pasteurii	1.783	2	0.410

Truepera spp.	1.886	2	0.390
Tumebacillus ginsengisoli	1.440	2	0.487
Tumebacillus permanentifrigoris	3.324	2	0.190
Tumebacillus sp.	2.964	2	0.227
Tumebacillus spp.	1.782	2	0.410
Turicibacter spp.	0.495	2	0.781
Turneriella parva	0.010	2.000	0.995
Uliginosibacterium gangwonense	0.693	2	0.707
Uliginosibacterium sp.	0.758	2	0.685
Uncultured candidatus brocadia sp.	0.282	2	0.868
Uncultured candidatus competibacter sp.	2.241	2	0.326
Uncultured candidatus microthrix sp.	0.482	2	0.786
Uncultured candidatus odyssella sp.	0.638	2	0.727
Uncultured candidatus pelagibacter sp.	3.000	2	0.223
Uncultured candidatus planktophila sp.	0.982	2	0.612
Uncultured candidatus protochlamydia sp.	2.667	2	0.264

Uncultured candidatus rhabdochlamydia sp.	7.018	2	0.030
Uncultured candidatus solibacter sp.	0.452	2	0.798
Undibacterium sp.	4.262	2	0.119
Undibacterium spp.	0.274	2	0.872
Vallitalea guaymasensis	1.095	2	0.578
Verrucomicrobium sp.	2.795	2	0.247
Verrucomicrobium spp.	0.409	2	0.815
Vibrio aestuarianus	1.947	2	0.378
Vibrio orientalis	0.389	2	0.823
Victivallis spp.	1.874	2	0.392
Victivallis vadensis	1.055	2	0.590
Virgibacillus halodenitrificans	0.445	2	0.800
Virgisporangium ochraceum	1.064	2	0.587
Vitreoscilla filiformis	0.561	2	0.755
Vogesella indigofera	0.523	2	0.770
Vogesella sp.	3.029	2	0.220
Vogesella spp.	0.834	2	0.659
Weissella cibaria	0.208	2	0.901
Weissella fabalis	1.312	2	0.519
Woodsholea maritima	3.982	2	0.137
Xanthobacillum maris	4.202	2	0.122

Xanthobacter spp.	0.692	2	0.708
Xenorhabdus nematophila	1.095	2	0.578
Xenorhabdus vietnamensis	1.278	2	0.528
Xylanimonas cellulosilytica	1.135	2	0.567
Zavarzinella spp.	3.000	2	0.223
Zoogloea oryzae	0.400	2	0.819
Zoogloea ramigera	0.949	2	0.622
Zoogloea resiniphila	2.191	2	0.334
Zoogloea spp.	1.411	2	0.494
Zymophilus spp.	1.118	2	0.572

Appendix 13: Mann-Whitney U test performed to determine the influence of region on the abundance of gray bacteria.

Bacterial species	**U Test Value**	**Mean Rank (Ohangwena)**	**Mean Rank (Omusati)**	**P-Value**
Acetanaerobacterium spp.	231.000	23	22.96	0.339
Acetobacterium wieringae	220.500	23.41	21.50	0.172
Achromatium oxaliferum	243.000	22.43	22.57	0.922
Acidaminobacter sp.	240.500	22.52	22.45	0.981
Acidimicrobium spp.	267.00	21.39	23.71	0.372
Acidisphaera sp.	234.000	22.83	22.14	0.782
Acidisphaera spp.	253.000	22.00	23.05	0.701
Aciditerrimonas sp.	278.000	20.91	24.24	0.308
Aciditerrimonas spp.	253.500	21.98	23.07	0.751
Acidithiobacillus spp.	210.000	23.87	21.00	0.090
Acidobacterium sp.	242.000	22.48	22.52	0.974
Acidobacterium spp.	22.500	23.02	21.93	0.777
Acidocella spp.	231.000	22.96	22.00	0.339
Acidothermus cellulolyticus	274.000	21.09	24.05	0.230
Acidovorax caeni	234.500	22.80	22.17	0.856
Acidovorax citrulli	255.000	21.91	23.14	0.707
Acidovorax konjaci	230.000	22.98	21.98	0.742
Acinetobacter brisouii	226.500	23.15	21.79	0.670
Acinetobacter genomosp. 3	205.000	24.09	20.76	0.385

Acinetobacter guillouiae	233.000	22.87	22.10	0.648
Acinetobacter marinus	253.00	22.00	23.05	0.295
Acinetobacter venetianus	241.500	22.50	22.50	1.000
Actinoallomurus iriomotensis	245.500	21.41	22.60	0.925
Actinocatenispora spp.	231.000	22.96	22.00	0.339
Actinophytocola sp.	254.00	21.96	23.10	0.502
Actinoplanes philippinensis	215	23.65	21.24	0.328
Actinoplanes spp.	208.500	23.93	20.93	0.271
Actinopolymorpha pittospori	243.000	22.43	22.57	0.922
Actinotalea fermentans	219.500	23.46	21.45	0.555
Adhaeribacter sp.	243.000	22.43	22.57	0.922
Adhaeribacter spp.	224.500	22.24	21.69	0.503
Advenella tetrathiobacter kashmirensis	239.500	22.59	22.40	0.944
Aeromicrobium sp.	267.000	21.39	23.71	0.315
Agrobacterium vitis	265.000	21.48	23.62	0.562
Akkermansia spp.	253.000	22.00	23.05	0.295
Alcanivorax spp.	174.000	25.43	19.29	0.069
Algidimarina propionica	220.500	23.41	21.50	0.172
Algorimarina spp.	238.500	22.63	22.36	0.942
Algoriphagus dokdonensis	276.000	21.00	24.14	0.064
Algoriphagus faecimaris	253.000	22.00	23.05	0.295

Algoriphagus hongiella halophile	266.500	21.41	23.69	0.325
Algoriphagus sp.	231.000	22.96	22.00	0.782
Algoriphagus spp.	220.200	23.41	21.50	0.172
Alicyclobacillus spp.	242.000	22.48	22.52	0.974
Alishewanella sp.	268	21.35	23.76	0.526
Alistipes indistinctus	231	22.96	22	0.339
Alistipes massiliensis	231.500	22.96	22.00	0.339
Alkalibacter saccharofermentans	254.500	21.93	23.12	0.484
Alkalibacter spp.	235.000	22.78	22.19	0.810
Alkalibacterium iburiense	219.000	23.48	21.43	0.552
Alkalibacterium kapii	244.000	22.39	22.62	0.902
Alkalibacterium spp.	243.000	22.43	22.57	0.922
Alkaliflexus spp.	210.000	23.87	21.00	0.090
Alkalilimnicola spp.	243.500	22.41	22.60	0.953
Alkaliphilus metalliredigens	253.000	22.00	23.05	0.295
Alkaliphilus sp.	211.500	23.8	21.07	0.268
Alkanibacter spp.	220.500	23.41	21.50	0.172
Alkanindiges hongkongensis	199.500	24.33	20.50	0.048
Alkanindiges illinoisensis	212.000	23.78	21.10	0.276
Alkanindiges sp.	189.000	24.78	20.00	0.025
Alkanindiges spp.	172.00	25.52	19.19	0.099

Allochromatium vinosum	223.500	23.28	21.54	0.627
Allokutzneria spp.	244.500	22.37	22.54	0.888
Alsobacter metallidurans	232.500	22.89	22.07	0.787
Altererythrobacter aestuarii	280.500	20.80	24.36	0.345
Altererythrobacter dongtanensis	240.000	22.57	22.43	0.970
Altererythrobacter sp.	210.000	23.87	21.00	0.370
Altererythrobacter spp.	219.000	23.48	21.43	0.522
Amaricoccus spp.	243.000	22.43	22.57	0.944
Ammonifex thiophilus	231.000	22.96	22.00	0.339
Ammoniphilus oxalivorans	177.500	25.28	19.45	0.025
Ammoniphilus sp.	235.000	22.78	22.19	0.810
Ammoniphilus spp.	198.500	24.37	20.43	0.197
Anaerobacterium chartisolvens	223.000	23.30	21.6	0.383
Anaerofilum spp.	231.000	22.96	22.00	0.339
Anaerolinea spp.	220.500	23.41	21.50	0.408
Anaeromusa sp.	244.500	22.41	22.60	0.925
Anaeromyxobacter dehalogenans	252.500	22.04	23.00	0.792
Anaeromyxobacter spp.	224.000	23.26	21.67	0.659
Anaerophaga spp.	221.500	23.37	21.55	0.634

Anaerosinus selenomonadaceae sb90	232.500	22.89	22.09	0.628
Ancalomicrobium spp.	276.000	21.00	24.14	0.064
Angustibacter aerolatus	241.500	22.50	22.50	1.000
Anoxybacillus spp.	220.500	23.41	21.50	0.172
Aquabacterium sp.	231.000	22.96	22.00	0.339
Aquabacterium spp.	209.000	23.91	20.95	0.396
Aquaspirillum putridiconchylium	220.500	23.41	21.50	0.172
Aquaspirillum sp.	265.000	21.48	23.61	0.268
Aquicella siphonis	275.500	21.02	24.12	0.147
Aquicella spp.	230.500	22.98	21.98	0.714
Aquimonas sp.	276.000	21.000	21.14	0.203
Aquimonas spp.	234.000	22.83	21.14	0.749
Aquitalea magnusonii	243.000	22.41	22.60	0.958
Arcicella sp.	229.500	23.02	21.93	0.765
Arcicella spp.	219.500	23.46	21.43	0.605
Arenimonas daechungensis	205.500	34.07	20.79	0.208
Arenimonas sp.	241.000	22.52	22.48	0.989
Arenimonas spp.	211.500	23.80	21.07	0.439
Arhodomonas sp.	231.000	22.96	22.00	0.339
Aridibacter acidobacteria bacterium	200.500	24.28	20.58	0.152

Aromatoleum aromaticum	220.500	23.41	21.50	0.172
Arsenicicoccus sp.	233.000	22.87	22.10	0.648
Arsenophonus spp.	210.000	23.87	21.00	0.090
Arthrobacter agilis	263.000	21.57	23.52	0.595
Arthrobacter chlorophenolicus	221.000	23.39	21.52	0.339
Arthrobacter globiformis	232.5	22.89	22.07	0.628
Arthrobacter monumenti	277.500	20.93	24.21	0.230
Arthrobacter nicotianae	238.000	22.65	23.33	0.897
Arthrobacter protophormiae	246.000	22.28	22.78	0.884
Arthrobacter ramosus	253.500	21.98	23.07	0.658
Arthrospira platensis	253.000	22.00	23.05	0.295
Asticcacaulis biprosthecium	232.000	22.91	22.05	0.609
Asticcacaulis excentricus	222.000	23.35	21.57	0.358
Atopostipes sp.	243.000	22.43	22.57	0.922
Atopostipes spp.	254.500	21.93	23.12	0.484
Aureimonas ferruginea	183.000	25.05	19.71	0.041
Austwickia chelonae	231.000	22.96	22.00	0.339
Azoarcus sp.	233.000	22.87	22.10	0.717
Azoarcus spp.	217.000	23.57	21.33	0.483
Azonexus sp.	588.5	21.76	23.31	0.635
Azospira dechlorosoma sp.	257.000	21.83	23.24	0.693
Azospira oryzae	234.000	22.83	22.14	0.782

Azospirillum lipoferum	301.000	19.91	25.53	0.028
Azospirillum oryzae	234	22.83	22.14	0.782
Azospirillum picis	231.000	22.96	22.00	0.339
Azospirillum spp.	231.000	22.96	22.00	0.572
Azovibrio spp.	261.000	21.65	23.43	0.515
Bacillus alcalophilus	260.000	21.70	23.38	0.643
Bacillus andreesenii	229000	23.04	21.90	0.722
Bacillus badius	229.000	21.67	23.40	0.526
Bacillus cellulosilyticus	256.500	21.85	23.21	0.522
Bacillus chandigarhensis	239.000	22.61	22.38	0.949
Bacillus clausii	231.500	22.93	22.02	0.591
Bacillus flexus	253.500	21.98	23. 07	0.755
Bacillus horikoshii	244.000	22.39	22.62	0.947
Bacillus longiquaesitum	225.500	23.20	21.74	0.681
Bacillus nealsonii	241.500	22.50	22.50	1.000
Bacillus pocheonensis	232.000	22.91	232.000	0.807
Bacillus simplex	242.000	22.48	22.52	0.981
Bacillus vireti	239.500	22.59	22.40	0.958
Bacillus weihenstephanensis	248.500	22.20	22.83	0.849
Bacteriovorax marinus	231.000	22.96	22.00	0.339
Bacteriovorax sp.	233.500	22.85	22.12	0.733
Bacteriovorax spp.	250.000	22.13	22.90	0.785
Bacteroides coprocola	231.000	22.96	22.00	0.339

Bacteroides intestinalis	231	22.96	22	0.339
Bacteroides luti	241.500	22.50	22.50	1.000
Barnesiella viscericola	221.000	23.39	21.51	0.334
Bauldia consociate	231	22.96	22.00	0.572
Bdellovibrio bacteriovorus	210.500	23.85	21.05	0.434
Bdellovibrio exovorus	233.000	22.87	22.08	0.648
Bdellovibrio sp.	223.500	23.28	21.64	0.658
Bdellovibrio spp.	225.000	23.22	21.71	0.680
Beggiatoa sp.	255.000	21.91	23.14	0.565
Beggiatoa spp.	211.500	23.80	21.07	0.317
Beijerinckia spp.	256	21.87	23.19	0.700
Bellilinea spp.	243.500	22.41	22.60	0.949
Belnapia spp.	232.000	22.91	22.05	0.609
Blastococcus aggregatus	205.5	24.07	20.79	0.395
Blastococcus sp.	289.5	20.41	24.79	0.058
Blastococcus spp.	260.5	21.67	23.40	0.651
Blastomonas spp.	232.000	24.39	20.41	0.268
Blastopirellula marina	263.500	21.54	23.55	0.441
Blastopirellula spp.	225.500	23.20	21.74	0.648
Blautia product	224.000	23.26	21.67	0.490
Borrelia carolinensis	207.000	24.00	20.86	0.286
Bosea thiooxidans	231.000	22.96	22.00	0.339

Brachybacterium paraconglomeratum	256.000	21.87	23.19	0.679
Brachybacterium zhongshanense	231.000	22.96	22.00	0.339
Brachymonas denitrificans	300.000	19.96	25.29	0.145
Bradyrhizobium sp.	236.5	22.72	22.26	0.893
Bradyrhizobium spp.	263	21.57	23.52	0.601
Brevibacillus thermoruber	231.000	22.96	22.00	0.339
Brevibacterium daeguense	264.500	21.50	23.60	0.134
Brevundimonas abyssalis	243.500	22.41	22.60	0.925
Brevundimonas bacteroides	264.500	21.50	23.60	0.134
Buchnera aphidicola	234.500	22.80	22.17	0.863
Burkholderia xenovorans	203.500	24.15	20.69	0.254
Butyricimonas synergistica	231.000	22.96	22.00	0.339
Butyrivibrio clostridium proteoclasticum	274.500	21.07	24.07	0.411
Byssovorax spp.	231.000	22.96	22.00	0.339
Caedibacter spp.	234.000	22.83	22.14	0.749
Caenispirillum bisanense	231.000	22.96	22.00	0.339
Caldilinea spp.	237	22.70	22.29	0.868
Caldisericum spp.	231	22.96	22	0.339
Calditerricola sp.	243	22.43	22.57	0.922
Caloramator rice paddy	253	22	23.05	0.536
Caloramator spp.	243.000	22.43	22.57	0.958

Camelimonas alpha proteobacterium	220.5	23.41	21.50	0.172
Campylobacter Canadensis	231	22.96	22	0.339
Candidatus accumulibacter sp.	303.000	19.83	25.43	0.147
Candidatus acetothermum candidatus acetothermus autotrophicum	231.000	22.96	22.00	0.339
Candidatus alysiosphaera europeae	223.000	23.30	21.62	0.466
Candidatus aquiluna rubra	232.000	22.91	22.05	0.823
Candidatus arcobacter sulfidicus	256	21.87	23.19	0.536
Candidatus babela delta proteobacterium babl1	191.000	24.70	20.10	0.160
Candidatus carsonella ruddii	258.500	21.78	23.31	0.627
Candidatus chloroploca chloroflexi bacterium um_3	264.500	21.50	23.60	0.134
Candidatus cloacimonas acidaminovorans	231.000	22.96	22.00	0.339
Candidatus cloacimonas uncultured	220.500	23.41	21.50	0.172

candidatus cloacamonas sp.				
Candidatus clostridium anorexicamassiliense	242.5	22.46	22.55	0.962
Candidatus desulforudis audaxviator	231.000	22.96	22.00	0.339
Candidatus endobugula endosymbiont of bugula pacifica	208.5	23.93	20.93	0.270
Candidatus halomonas phosphatis	231.000	22.96	22.00	0.572
Candidatus lumbricincola sp. lt_g1	242.000	22.48	22.52	0.974
Candidatus macropleicola muticae	220.500	23.41	21.50	0.172
Candidatus magnetobacterium uncultured magnetobacterium sp.	230.000	23.000	21.95	0.624
Candidatus magnetoovum mohavensis	231.000	22.96	22.00	0.339
Candidatus metachlamydia lacustris	278.500	20.89	24.26	0.217
Candidatus mycoplasma ravipulmonis	231.000	22.96	22.00	0.339

Candidatus nardonella endosymbiont of scyphophorus yuccae	231.000	22.96	22	0.339
Candidatus nardonella endosymbiont of sphenophorus levis	222.000	23.35	21.57	0.358
Candidatus nasuia deltocephalinicola	242.000	22.48	22.52	0.196
Candidatus nitrotoga arctica	253.000	22.00	23.05	0.295
Candidatus nucleicultrix amoebiphila	253.000	22.00	23.05	0.295
Candidatus odyssella thessalonicensis	220.500	23.41	21.50	0.172
Candidatus paenicardinium endonii	242	22.48	22.52	0.974
Candidatus paraholospora nucleivisitans	231.000	22.96	22.00	0.339
Candidatus pelagibacter uncultured pelagibacter sp.	231.000	22.96	22.00	0.339
Candidatus phytoplasma & apos	242	22.48	22.52	0.974
Candidatus phytoplasma mexican potato purple top phytoplasma	231.000	22.96	22.00	0.527

Candidatus planktoluna difficilis	225.500	23.20	21.74	0.677
Candidatus planktophila limnetica	251.500	22.07	22.98	0.814
Candidatus planktothricoides rosea	231.000	22.96	22.00	0.339
Candidatus protochlamydia amoebophila	243.000	22.43	22.57	0.922
Candidatus protochlamydia protochlamydia naegleriophila	231.000	22.96	22.00	0.572
Candidatus protochlamydia sp.	262.500	21.59	23.50	0.593
Candidatus rhabdochlamydia porcellionis	276.000	21.00	24.14	0.416
Candidatus rhabdochlamydia rhabdochlamydia crassificans	231.000	22.96	22.00	0.339
Candidatus rhabdochlamydia sp. cve88	253.000	22.00	23.05	0.536
Candidatus rhodoluna lacicola	220.43	23.43	21.48	0.613

Candidatus rhodoluna planktonica	187.500	24.85	19.93	0.200
Candidatus rhodoluna rhodoluna sp. kas9	199.500	24.33	20.50	0.318
Candidatus saccharimonas aalborgensis	211.500	23.80	21.07	0.268
Candidatus soleaferrea massiliensis	210.000	23.87	21.00	0.090
Candidatus thioglobus singularis	231.500	22.93	22.02	0.591
Candidatus trichorickettsia mobilis	199.500	24.33	20.50	0.048
Candidatus zinderia insecticola	302.500	19.85	25.40	0.119
Carboxydocella sp.	231.00	22.96	22.00	0.339
Carboxydothermus islandicus	253	22.00	23.05	0.295
Catalinimonas alkaloidigena	256.000	22.89	23.19	0.536
Catellatospora yuxiensis	210.500	23.85	21.02	0.186
Catenibacterium mitsuokai	231.000	22.96	22.00	0.339
Cellulomonas chitinilytica	258.00	21.78	23.29	0.629
Cellulomonas terrae	221.000	23.39	21.53	0.511
Cellulosilyticum ruminicola	243.000	22.43	22.57	0.922
Cellulosilyticum spp.	241.500	22.50	22.50	1.000
Cellvibrio gandavensis	242.000	22.48	22.53	0.974

Cellvibrio ostraviensis	198.500	24.37	20.45	0.184
Chitinibacter tainanensis	220.500	23.41	21.50	0.172
Chitinimonas koreensis	244.000	22.39	22.62	0.921
Chitinimonas taiwanensis	215.500	23.63	21.26	0.337
Chitinophaga flexibacter sancti	220.500	23.41	21.50	0.172
Chitinophaga pinensis	242.500	22.46	22.55	0.948
Chitinophaga spp.	202.000	24.22	20.62	0.342
Chlamydia ibidis	231.500	22.96	22	0.339
Chlorobium sp.	220.500	23.41	21.50	0.172
Chlorobium spp.	271.000	21.22	23.90	0.376
Chloroflexus spp.	218.500	23.50	21.40	0.580
Chloronema giganteum	253.000	22.00	23. 05	0.295
Chondromyces crocatus	253.000	22.00	23.05	0.295
Chondromyces pediculatus	221.000	23.39	21.52	0.582
Chondromyces spp.	234.500	22.80	22.17	0.765
Chromatium okenii	209.000	23.91	20.91	0.166
Chromohalobacter spp.	235.000	22.78	22.19	0.810
Chryseobacterium anthropic	257.000	21.83	23.24	0.509
Chryseobacterium bovis	265.500	21.54	23.55	0.509
Chryseobacterium kwangyangense	243.000	22.43	22.57	0.922
Chryseobacterium soldanellicola	214.500	23.67	21.21	0.368

Chryseobacterium sp.	244.500	22.37	22.64	0.917
Chryseobacterium taiwanensis	242.000	22.48	22.52	0.974
Chryseomicrobium sp.	255.000	21.91	23.14	0.468
Chthoniobacter flavus	242.000	22.48	22.52	0.974
Cloacibacterium sp.	233.000	22.87	22.10	0.648
Cloacibacterium spp.	238.000	22.65	22.33	0.934
Clostridium aminobutyricum	231.000	22.96	22.00	0.654
Clostridium bovipellis	264.500	21.50	23.60	0.134
Clostridium bowmanii	258.000	22.04	23.00	0.698
Clostridium cavendishii	241.000	22.52	22.48	0.989
Clostridium cellulovorans	253.000	22.00	23.05	0.536
Clostridium disporicum	262.000	21.61	23.48	0.550
Clostridium enrichment	255.000	21.91	23.14	0.565
Clostridium frigidicarnis	271.000	20.91	24.24	0.178
Clostridium magnum	211.000	23.83	21.05	0.260
Clostridium quinii	243.000	22.43	22.57	0.922
Clostridium ruminantium	245.000	22.35	22.67	0.934
Clostridium scatologenes	210.000	23.87	21.00	0.090
Clostridium tunisiense	243.500	22.41	22.60	0.937
Cobetia marina	254.000	21.96	23.10	0.501
Cohnella sp.	252.000	22.04	23.00	0.698

Comamonas guangdongensis	220.000	23.43	21.48	0.558
Comamonas koreensis	226.000	23.17	21.76	0.678
Compostimonas spp.	172.000	25.52	19.19	0.057
Conexibacter sp.	242.000	22.48	22.52	0.974
Conexibacter spp.	193.500	24.59	20.21	0.251
Congregibacter litoralis	231.500	22.93	22.02	0.591
Coprococcus catus	243.000	22.43	22.57	0.922
Coprococcus eutactus	253.000	22.00	23.05	0.295
Corynebacterium appendicis	254.500	21.93	23.12	0.741
Corynebacterium lipophiloflavum	231.000	22.96	22.00	0.572
Corynebacterium maris	246.500	22.28	22.74	0.844
Corynebacterium matruchotii	21.65	21.65	23.43	0.626
Cosenzaea proteus myxofaciens	242.000	22.48	22.52	0.974
Couchioplanes caeruleus	241.500	22.50	22.50	1.000
Coxiella cheraxi	231.000	22.96	22.00	0.339
Craurococcus spp.	253.000	22	23.05	0.295
Crenothrix polyspora	232.000	22.91	22.05	0.289
Criblamydia sequanensis	221.000	23.39	21.52	0.334
Crocinitomix spp.	242.000	22.48	22.52	0.974
Cryobacterium spp.	248.000	22.22	22.81	0.872

Cryocola spp.	251.500	22.07	22.98	0.812
Cryptosporangium japonicum	254.500	21.93	23.12	0.484
Curvibacter sp.	222.500	23.33	21.60	0.655
Curvibacter spp.	218.000	23.52	21.38	0.581
Cyanothece spp.	199.500	24.33	20.50	0.048
Cycloclasticus spp.	220.500	23.41	21.50	0.172
Cystobacter spp.	255.500	21.89	23.17	0.605
Cystobacter violaceus	236.000	22.74	22.24	0.815
Cytophaga aurantiaca	231.500	22.93	22.02	0.591
Cytophaga sp.	245.500	22.33	22.69	0.851
Cytophaga spp.	203.000	24.17	20.64	0.293
Dactylosporangium spp.	233.000	22.87	22.10	0.648
Daeguia caeni	238.000	22.65	22.33	0.928
Dechloromonas denitrificans	201.000	24.24	20.60	0.162
Dechloromonas spp.	259.500	21.72	23.36	0.672
Dehalobacterium spp.	244.500	22.37	22.64	0.906
Dehalococcoides spp.	184.500	24.98	19.79	0.172
Dehalogenimonas spp.	217.000	23.57	21.33	0.484
Deinococcus alpinitundrae	231.000	22.96	22.00	0.572
Deinococcus deserti	245.500	22.33	22.69	0.889
Deinococcus geothermalis	220.500	23.41	21.50	0.172

Deinococcus hohokamensis	199.500	24.33	20.50	0.048
Deinococcus navajonensis	242	22.48	22.52	0.974
Deinococcus radiodurans	222.000	23.35	21.57	0.358
Deinococcus radiophilus	231.000	22.96	22.00	0.339
Deinococcus sp.	235.500	22.76	22.21	0.878
Deinococcus spp.	254.000	29.96	23.10	0.715
Deinococcus xinjiangensis	243.500	22.41	22.60	0.925
Delftia spp.	259.500	21.72	23.36	0.656
Demequina aestuarii	242.500	22.46	22.55	0.973
Demequina lutea	231.000	22.96	22.00	0.339
Denitratisoma sp.	234.500	22.80	22.17	0.796
Denitratisoma spp.	242.000	22.48	22.52	0.974
Denitrobacterium detoxificans	257.000	21.83	23.34	0.629
Derxia sp.	275.500	21.02	24.12	0.208
Desemzia incerta	232.000	22.91	22.05	0.685
Desertibacter roseus	223.30	23. 30	21.62	0.383
Desulfatibacillum alkenivorans	231.000	22.96	22.00	0.339
Desulfatiglans desulfobacterium aniline	231.000	22.96	22.00	0.339
Desulfatitalea tepidiphila	231.000	22.96	22.00	0.339

Desulfitobacterium hafniense	284.000	20.64	24.52	0.188
Desulfitobacterium sp.	220.500	23.41	21.50	0.172
Desulfitobacterium spp.	241.500	22.50	22.50	1.000
Desulfobacter spp.	231.000	22.96	22.00	0.339
Desulfobacterium sp.	221.000	23.39	21.52	0.334
Desulfobacterium spp.	221.500	23. 37	21.55	0.431
Desulfobulbus spp.	219.500	23.46	21.46	0.339
Desulfocapsa spp.	257.500	21.80	23.26	0.690
Desulfococcus biacutus	253.000	22.00	23. 05	0.295
Desulfococcus spp.	210.000	23.87	21.00	0.090
Desulfofaba fastidiosa	220.500	23.41	21.50	0.172
Desulfofaba spp.	254.500	21.93	21. 12	0.579
Desulfofrigus oceanense	231.500	22.93	22.02	0.591
Desulfomonile spp.	253.000	22.00	23.05	0.536
Desulfomonile tiedjei	246.500	22.28	22.74	0.901
Desulfonatronum thiosulfatophilum	189.000	24.78	20.00	0.052
Desulfonema limicola	253.000	22.00	23.05	0.295
Desulforegula spp.	277.000	20.96	24.19	0.311
Desulforhopalus spp.	283.000	20.78	24.48	0.224
Desulfosarcina spp.	243.000	22.43	22.57	0.922
Desulfosporomusa spp.	242.000	22.48	22.52	0.974

Desulfosporosinus meridiei	232.500	22.89	22.07	0.740
Desulfosporosinus spp.	231.500	22.93	22.02	0.670
Desulfotignum sp.	253.000	22.00	23.05	0.295
Desulfotomaculum acetoxidans	242.000	22.48	22.52	0.974
Desulfotomaculum solfataricum	231.000	22.96	22.00	0.339
Desulfotomaculum sp.	323.000	18.96	20.38	0.028
Desulfotomaculum spp.	232.000	22.91	22.05	0.609
Desulfovibrio mexicanus	243.000	22.43	22.57	0.922
Desulfovibrio oxyvorans	220.500	23.41	21.50	0.172
Desulfovibrio putealis	254.500	21.93	23.12	0.484
Desulfurobacterium spp.	231.000	22.96	22.00	0.339
Desulfuromonas spp.	231.000	22.96	22.00	0.339
Desulfuromusa spp.	222.000	23.35	21.57	0.358
Dethiosulfatibacter spp.	231.000	22.96	22.00	0.339
Devosia insulae	217.500	23.54	21.36	0.495
Devosia soli	242.500	22.46	22.55	0.976
Devosia sp.	200.500	24.28	20.55	0.232
Devosia spp.	248.500	22.20	22.83	0.828
Devosia subaequoris	232.000	22.96	22.05	0.609
Dissulfuribacter thermophiles	231.000	22.96	22.00	0.572
Dokdonella spp.	244.500	22.37	22.64	0.927

Dongia spp.	210.000	23.87	21.00	0.090
Dorea spp.	242.000	22.48	22.52	0.974
Draconibacterium orientale	199.500	24.33	20.50	0.048
Duganella sp.	245.000	22.35	22.87	0.928
Duganella zoogloeoides	240.000	22.57	22.43	0.970
Dyadobacter beijingensis	205.000	24.09	20.78	0.356
Dyadobacter psychrophilus	231.000	22.96	22.00	0.339
Dyadobacter sp.	243.000	22.43	22.57	0.944
Dyadobacter spp.	243.000	22.43	22.57	0.944
Ectothiorhodospira imhoffii	243.000	22.43	22.57	0.922
Ectothiorhodospira magna	331.500	18.59	26.79	0.013
Ectothiorhodospira sp.	243.000	22.43	22.57	0.922
Edaphobacter spp.	231.000	22.96	22.00	0.339
Elusimicrobium spp.	266	21.46	23.67	0.334
Emticicia oligotrophica	233.500	22.85	22.12	0.824
Emticicia spp.	231.000	22.96	22.00	0.339
Enhydrobacter aerosaccus	228.500	23.50	21.40	0.588
Ensifer adhaerens	233.000	22.87	22.10	0.825
Enteractinococcus sp.	276.000	21.00	24.14	0.064
Enterococcus columbae	202	24.22	20.62	0.205
Epulopiscium sp.	243.000	22.43	22. 57	0.922
Erythrobacter gaetbuli	210.000	23.89	21.00	0.179
Erythrobacter litoralis	264.500	21.50	23.60	0.134

Erythrobacter piscidermidis	216.000	23.61	21.29	0.347
Erythrobacter sp.	198.500	24.37	20.43	0.257
Erythrobacter spp.	212.500	23.76	21.12	0.385
Ethanoligenens cellulosi	253.000	22.00	23.05	0.295
Ethanoligenens spp.	231.000	22.96	22.00	0.339
Eubacterium coprostanoligenes	231.000	22.96	22.00	0.339
Eubacterium oxidoreducens	236.000	22.74	22.24	0.839
Exiguobacterium indicum	267.500	21.37	23.74	0.540
Exiguobacterium lactigenes	270.500	21.24	23.88	0.311
Exiguobacterium panipatensis	243.500	22.41	22.60	0.959
Exiguobacterium profundum	254.500	21.93	23.12	0.484
Faecalibacterium prausnitzii	231.000	22.96	22.00	0.339
Ferrimicrobium spp.	223.000	23.30	21.62	0.383
Ferrithrix spp.	231.000	22.96	22.00	0.339
Ferrovum spp.	231.000	22.96	22.00	0.339
Ferruginibacter sp.	231.000	22.96	22.00	0.339
Fibrobacter spp.	223.000	23.30	21.60	0.383
Filibacter spp.	245.000	22.35	22.67	0. 928
Filomicrobium sp.	231.000	22.96	22.00	0.339
Flavihumibacter sp.	200.500	24.28	20.58	0.152
Flavisolibacter flavosolibacter sp.	233.500	22.85	22.12	0.789

Flavisolibacter ginsengisoli	188.000	24.83	19.95	0.109
Flavisolibacter sp.	169.000	25.65	19.05	0.044
Flavisolibacter spp.	218.000	23.52	21.38	0.513
Flavobacterium aciduliphilum	264.500	21.50	23.60	0.365
Flavobacterium columnare	245.000	22.35	22.67	0.890
Flavobacterium indicum	264.000	21.52	23.57	0.289
Flavonifractor clostridium orbiscindens	243.000	22.43	22.57	0.922
Flectobacillus spp.	287.000	20.52	24.67	0.279
Flexibacter flexilis	233.000	22.87	22.10	0.648
Flexibacter spp.	227.500	23.11	21.83	0.733
Flexithrix dorotheae	253.000	22.00	23.05	0.295
Flexivirga spp.	2 42.500	22.46	22.55	0.969
Fluviicola spp.	169.000	25.65	19.05	0.077
Fluviicola taffensis	242.5	22.46	22.55	0.962
Fluviimonas pallidilutea	247.000	22.26	22.76	0.860
Fluviimonas sp.	238.500	22.63	22.36	0.930
Fonticella clostridiaceae bacterium	233.500	22.89	22.1	0.648
Formivibrio citricus	232.000	22.91	22.05	0.609
Frankia sp.	236.000	22.74	22.24	0.015
Frankia spp.	212.000	23.78	21.10	0.208

Frateuria aurantia	231.000	22.96	22.00	0.339
Frigoribacterium sp.	254.500	21.50	23.60	0.134
Fusibacter spp.	264.500	21.50	23.80	0.535
Gaiella occulta	231.000	22.96	22.00	0.339
Gaiella spp.	251.000	22.09	22.95	0.751
Gallaecimonas sp.	231 .000	22.96	22.00	0.654
Gallionella spp.	246.000	22.30	22.71	0.859
Gelria spp.	242.000	22.48	22.52	0.974
Geminicoccus roseus	255.000	21.91	23.14	0.468
Gemmata sp.	242.000	22.48	22.52	0.974
Gemmata spp.	241.500	22.50	22.50	1.000
Gemmatimonas spp.	235.5	22.76	22.21	0.887
Gemmobacter catellibacterium sp.	204.500	24.11	20.74	0.342
Gemmobacter rhodobacter changlaii	224.000	22.26	21.64	0.599
Gemmobacter sp.	208.500	23.93	20.93	0.397
Geoalkalibacter spp.	210.500	23.83	21.01	0.301
Geobacter spp.	213.500	23.72	21.17	0.51
Geobacter thiogenes	210.00	23.87	21.00	0.090
Geodermatophilus obscurus	228.500	23.07	21.99	0.735
Geodermatophilus spp.	238.000	22.65	22.33	0.933

Geopsychrobacter electrodiphilus	220.500	23.41	21.50	0.172
Georgenia muralis	211.500	23.80	21.07	0.201
Georgenia sp.	243.000	22.43	22.57	0.922
Georgenia spp.	224.000	23.26	21.67	0.588
Geothermobacter spp.	230.000	23.00	21.95	0.624
Geothrix spp.	227.5	23.11	21.83	0.735
Geovibrio ferrireducens	210.000	23.39	21.52	0.334
Gloeobacter spp.	254	21.96	23.10	0.502
Gluconacetobacter spp.	220.5	23.41	21.50	0.172
Gordonibacter spp.	286.000	20.57	24.62	0.194
Gottschalkia eubacterium angustum	231	22.96	22.00	0.339
Gracilibacillus halotolerans	242.000	22.48	22.52	0.974
Gracilibacillus sp.	254.500	21.93	23.12	0.484
Gracilibacter spp.	242.000	22.48	22.52	0.974
Gracilimonas sp.	253.000	22.00	23.03	0.701
Granulicella spp.	231	22.96	22.00	0.339
Gulosibacter sp.	243.000	22.43	22.57	0.953
Haematobacter missouriensis	220.500	23.41	21.50	0.172
Halalkalibacillus halophilus	243.500	22.41	22.60	0.925
Haliangium spp.	249.000	22.17	22.86	0.802
Haliea mediterranea	238.500	22.63	22.36	0.926

Haliea sp.	231.000	22.96	22.00	0.572
Haliscomenobacter hydrossis	233.000	22.87	22.10	0.648
Haliscomenobacter spp.	207.000	24.00	20.86	0.394
Haloanella sp.	242.000	22.48	22.52	0.974
Halobacillus hunanensis	242.000	22.48	22.52	0.974
Halochromatium spp.	231.000	22.96	22.00	0.339
Halospirulina sp.	287.500	20.50	24.69	0.279
Halothiobacillus kellyi	243.000	22.43	22.57	0.922
Halothiobacillus sp.	245.000	22.35	22.67	0.925
Herbaspirillum rubrisubalbicans	270	21.26	23.86	0.481
Herbiconiux spp.	253.000	22.00	23.05	0.295
Hirschia sp.	231.500	22.93	22.02	0.670
Hoeflea sp.	264.500	21.50	23.60	0.421
Holdemania spp.	231.000	22.96	22.00	0.339
Holophaga foetida	210.000	23.87	21.00	0.090
Holophaga sp.	220.500	23.41	21.50	0.172
Holophaga spp.	222.000	23.35	21.57	0.532
Hydrogenophaga palleronii	241.500	22.50	22.50	1.000
Hydrogenophaga sp.	250.500	22.11	22.93	0.815
Hydrogenophaga spp.	238.000	22.65	22.33	0.928
Hydrogenophilus thermoluteolus	231.000	22.96	22.00	0.339

Hymenobacter gelipurpurascens	253.000	22.00	23.05	0.295
Hymenobacter sp.	256.000	21.87	23.19	0.663
Hymenobacter xinjiangensis	243.000	22.43	22.57	0.922
Hyphomicrobium spp.	202.500	24.20	20.64	0.359
Hyphomonas neptunium	220.500	23.41	21.50	0.322
Hyphomonas oceanitis	231.000	22.96	22.00	0.339
Hyphomonas spp.	243.500	22.41	22.60	0.925
Iamia majanohamensis	222.000	23.35	21.57	0.358
Iamia spp.	243.500	22.41	22.60	0.944
Ideonella sp.	207.500	23.98	20.88	0.354
Ideonella spp.	235.500	22.76	22.21	0.861
Idiomarina loihiensis	243.000	22.43	22.57	0.922
Idiomarina sp.	224.500	23.24	21.69	0.502
Idiomarina spp.	256.000	21.87	23.19	0.536
Ignavibacterium sp.	231.000	22.96	22.00	0.339
Ignavibacterium spp.	212.000	23.78	21.10	0.276
Ilumatobacter fluminis	231.500	22.93	22.02	0.591
Ilumatobacter spp.	212.500	23.76	21.12	0.397
Inhella inkyongensis	250.000	22.13	22.90	0.824
Insolitispirillum insolitospirillum peregrinum	260.000	21.70	23.38	0.618

Intestinimonas butyriciproducens	231.000	22.96	22.00	0.339
Isoptericola spp.	255.500	21.89	23.17	0.551
Jannaschia sp.	233.500	22.85	22.12	0.733
Jatrophihabitans endophyticus	220.500	23.41	21.50	0.172
Jeotgalicoccus psychrophilus	256.000	21.87	23.19	0.536
Jonesia sp.	254.500	21.93	23.12	0.579
Kaistia hirudinis	243.500	22.41	22.60	0.925
Kaistia sp.	254.500	21.93	23.12	0.484
Kaistobacter spp.	171.000	25.57	19.14	0.096
Kallotenue chloroflexi bacterium	242.000	22.48	22.52	0.974
Kineococcus radiotolerans	266.000	21.43	23.67	0.248
Kineococcus sp.	271.500	21.20	23.93	0.391
Kineosporia aurantiaca	226.000	23.17	21.76	0.541
Kitasatospora cystarginea	203.000	24.17	20.67	0.129
Kitasatospora spp.	254.500	21.93	23.12	0.484
Klugiella spp.	227.500	23.11	21.83	0.742
Knoellia sinensis	206.500	24.02	20.83	0.221
Knoellia subterranean	238.000	22.65	22.33	0.934
Kocuria carniphila	261.000	21.65	23.43	0.627
Kopriimonas spp.	243.000	22.43	22.57	0.922

Kouleothrix aurantiaca	232.000	22.91	22.05	0.609
Kouleothrix spp.	233.000	22.87	22.10	0.648
Ktedonobacter spp.	220.500	23.41	21.50	0.172
Labilithrix luteola	232.500	22.89	22.07	0.786
Labrenzia aggregate	276.000	21.00	24.14	0.389
Lachnoclostridium clostridium phytofermentans	253.000	22.00	23.05	0.295
Lachnoclostridium clostridium xylanolyticum	242.500	22.46	22.55	0.948
Lacibacter cauensis	265.500	21.46	23.64	0.533
Lacibacter sp.	197.500	24.41	20.40	0.276
Lacibacter spp.	253.000	22.00	23.05	0.295
Lacibacterium rhodospirillaceae bacterium	238.000	22.65	22.33	0.927
Lactobacillus farciminis	257.000	21.83	23.24	0.567
Lactobacillus gallinarum	244.500	22.37	22.64	0.888
Lactobacillus graminis	253.000	22.00	23.05	0.295
Lactobacillus helveticus	242.000	22.48	22.52	0.974
Lactobacillus kunkeei	253.000	22.00	23.05	0.295
Lactobacillus mali	253.000	22.00	23.05	0.295
Lactobacillus pentosus	243.000	22.43	22.57	0.922

Lactobacillus reuteri	253.000	22.00	23.05	0.295
Lactobacillus rossiae	231.000	22.96	22.00	0.339
Lactococcus plantarum	234	22.83	22.14	0.849
Larkinella sp.	220.500	23.41	21.50	0.172
Leadbetterella sp.	169.500	25.63	19.07	0.073
Leeia oryzae	241.500	22.50	22.50	1.000
Legionella dresdeniensis	233.000	22.87	22.10	0.648
Legionella geestiana	264.500	21.50	23.60	0.134
Legionella santicrucis	243	22.43	22.57	0.958
Lentzea spp.	254.000	21.96	23.10	0.594
Leptolinea sp.	220.500	23.41	21.50	0.322
Leptolinea spp.	220.500	23.41	21.50	0.322
Leptolyngbya frigida	253.000	22.00	23.05	0.295
Leptolyngbya saxicola	204.000	24.13	20.17	0.189
Leptolyngbya sp.	222.500	23.33	21.60	0.602
Leptolyngbya spp.	252.500	22.02	23.02	0.685
Leptospirillum ferrodiazotrophum	231.000	22.96	22.00	0.339
Leptospirillum spp.	242.000	22.48	22.52	0.974
Leptothrix sp.	255.500	21.89	23.17	0.605
Leptothrix spp.	217.500	23.54	21.36	0.572
Leucobacter sp.	223.000	23.30	21.62	0.518
Leuconostoc palmae	253.5	21.98	23.07	0.778

Levilinea spp.	196.500	24.46	20.36	0.251
Lewinella sp.	220.500	23.41	21.50	0.172
Lewinella spp.	243.000	22.43	22.57	0.922
Limnobacter litoralis	264.000	21.52	23.57	0.289
Limnobacter spp.	244.000	22.39	22.62	0.949
Limnohabitans curvus	197.500	24.41	20.40	0.301
Limnohabitans spp.	239.500	22.59	22.40	0.963
Loktanella salsilacus	220.500	23.41	21.50	0.322
Longilinea spp.	223.000	23.30	21.62	0.466
Luteimonas composti	233.000	22.87	22.10	0.754
Luteimonas sp.	185.500	24.93	19.83	0.166
Luteimonas spp.	261.000	21.65	23.43	0.630
Luteolibacter algae	253.000	22.00	23.05	0.295
Luteolibacter pohnpeiensis	233.000	22.87	22.10	0.648
Luteolibacter sp.	256.500	21.85	23.21	0.522
Luteolibacter spp.	245.000	22.35	22.67	0.890
Luteolibacter yonseiensis	261.000	21.65	23.43	0.557
Lutibaculum baratangense	253.000	22.00	23.05	0.295
Lutispora spp.	253.000	22.00	23.05	0.295
Lutispora thermophile	242.000	22.48	22.52	0.974
Lysinibacillus sphaericus	255.000	21.91	23.14	0.718
Lysobacter deserti	240.000	22.57	22.43	0.968
Lysobacter enzymogenes	261.500	21.63	23.45	0.559

Lysobacter sp.	194.000	24.57	20.24	0.259
Lysobacter spp.	224.500	23.24	21.69	0.643
Lyticum sinuosum	243.000	22.43	22.57	0.922
Magnetococcus spp.	243.500	22.41	22.60	0.925
Magnetospirillum sp.	220.500	23.41	21.50	0.463
Magnetospirillum spp.	264.500	21.50	23.60	0.134
Magnetovibrio blakemorei	233.000	22.87	22.10	0.648
Malikia spp.	231.500	22.93	22.02	0.797
Maribacter sp.	231.000	22.96	22.00	0.339
Marinilactibacillus sp.	232.000	22.91	22.05	0.609
Marinimicrobium koreense	253.000	22.00	23.05	0.295
Marininema halotolerans	278.000	20.91	24.24	0.119
Mariniphaga bacteroidales bacterium	220.5	23.41	21.50	0.172
Marinithermus spp.	258.000	21.78	23.29	0.482
Marinobacter sp.	243.000	22.43	22.57	0.922
Marinobacter spp.	214.000	23.70	21.19	0.470
Marinobacter zhanjiangensis	253.000	22.00	23.05	0.295
Marinobacterium spp.	243.000	22.43	22.57	0.922
Marinomonas arenicola	231	22.96	22	0.787
Marinomonas mediterranea	231	22.96	22	0.339
Marinomonas pontica	231	22.96	22	0.339

Marinomonas spp.	231.5	22.93	22.02	0.591
Marinomonas vaga	199.5	24.33	20.5	0.141
Marisediminicola spp.	259.000	21.74	23.33	0.518
Marispirillum spp.	231.000	22.96	22.00	0.339
Marmoricola sp.	261.500	21.63	23.45	0.591
Meniscus spp.	231.000	22.96	22.00	0.339
Merismopedia spp.	220.500	23.41	21.50	0.172
Methylibium petroleiphilum	247.500	22.24	22.79	0.857
Methylobacillus flagellates	221.000	23.39	21.52	0.334
Methylobacillus spp.	218.000	23.52	21.38	0.580
Methylobacter sp.	231.000	22.96	22.00	0.339
Methylobacter spp.	273.500	21.11	24.02	0.433
Methylobacter whittenburyi	243	22.43	22.57	0.922
Methylocaldum sp.	250.000	22.13	22.90	0.826
Methylocaldum spp.	231.000	22.96	22.00	0.770
Methylocella sp.	228.000	23.09	21.86	0.706
Methylococcus mobilis	233.000	22.87	22.10	0.648
Methylococcus spp.	231.000	22.96	22.00	0.654
Methylocystis parvus	258.000	21.78	23.29	0.646
Methylocystis spp.	231.500	22.93	22.02	0.591
Methylomicrobium spp.	231.500	22.93	22.02	0.591
Methylomonas fodinarum	231.000	22.96	22.00	0.339
Methylomonas methanica	223.000	23.30	21.62	0.466

Methylomonas sp.	222.500	23.33	21.60	0.543
Methylomonas spp.	262.500	21.59	23.50	0.614
Methylophaga sp.	232.000	22.91	22.05	0.609
Methylophaga spp.	201.500	24.24	20.60	0.200
Methylophilus spp.	221.000	23.39	21.52	0.575
Methylopila capsulate	242.000	22.48	22.52	0.974
Methylopila sp.	252.500	22.02	23.02	0.639
Methylosinus sp.	243.500	22.41	22.60	0.925
Methylosinus sporium	253.000	22.00	23.05	0.536
Methylosinus spp.	242.000	22.48	22.52	0.989
Methylosinus trichosporium	239.500	22.59	22.40	0.955
Methylosoma sp.	253.000	22.00	23.05	0.295
Methylotenera mobilis	223.000	23.30	21.62	0.579
Methylotenera spp.	242.000	22.48	22.52	0.989
Methylotenera versatilis	233.000	22.87	22.10	0.842
Methylothermus spp.	210.000	23.87	21.00	0.090
Methyloversatilis spp.	242.000	22.48	22.52	0.974
Methylovulum miyakonense	214.500	23.67	21.21	0.521
Microbacterium sediminicola	276.000	21.00	24.14	0.064
Microbispora rosea	222.500	23.33	21.60	0.371
Microcella putealis	249.500	22.15	22.88	0.835
Microcella spp.	289.500	20.41	24.79	0.058

Microcoleus spp.	231.000	22.96	22.00	0.654
Microcystis sp.	245.000	22.35	22.67	0.931
Micromonospora sp.	245.500	22.33	22.69	0.851
Micromonospora spp.	281.000	20.78	24.38	0.318
Microvirga spp.	186.000	24.91	19.86	0.190
Miniimonas arenae	245.000	22.35	22.67	0.890
Mitsuaria spp.	229.500	23.02	21.93	0.777
Modestobacter spp.	219.000	23.48	21.43	0.522
Mogibacterium pumilum	276.000	21.00	24.14	0.202
Moorella humiferrea	220.500	23.41	21.50	0.172
Moorella spp.	244.000	22.39	22.62	0.930
Moorella thermoacetica	220.500	23.41	21.50	0.172
Mucilaginibacter sp.	211.500	23.80	21.07	0.268
Mucilaginibacter spp.	232.500	22.89	22.07	0.701
Mucilaginibacter ximonensis	243.000	22.43	22.57	0.944
Mycoplana sp.	278.000	20.91	24.24	0.378
Mycoplasma alligatoris	220	23.43	21.48	0.311
Mycoplasma crocodyli	262.000	21.61	23.48	0.601
Mycoplasma phocidae	231.000	22.96	22.00	0.339
Mycoplasma zalophi	247.000	22.26	22.76	0.886
Myxococcus spp.	253.000	22.00	23.05	0.295
Nafulsella turpanensis	243.000	22.43	22.57	0.922
Nannocystis spp.	197.000	24.43	20.38	0.263

Natranaerovirga hydrolytica	254.000	21.96	23.10	0.501
Natranaerovirga pectinivora	210.000	23.87	21.00	0.090
Natronoanaerobium salstagnum	254.000	21.96	23.10	0.715
Neochlamydia hartmannellae	253	22	23.05	0.295
Neochlamydia sp.	231	22.96	22	0.339
Neochlamydia spp.	264.5	21.50	23.60	0.134
Neptunomonas spp.	276.000	21.00	24.14	0.064
Nevskia soli	231.000	22.96	22.00	0.339
Niabella sp.	220.500	23.41	21.50	0.172
Niastella sp.	235.500	22.76	22.21	0.841
Niastella spp.	226.500	23.15	21.79	0.653
Nitratireductor spp.	233.000	22.87	22.10	0.648
Nitrobacter spp.	253.000	22.00	23.05	0.295
Nitrosococcus spp.	253.000	22.00	23.05	0.295
Nitrosomonas spp.	231.000	22.96	22.00	0.339
Nitrosospira spp.	161.000	26.00	18.67	0.054
Nitrosovibrio spp.	250.000	22.13	22.90	0.841
Nitrospina spp.	220.500	23.41	21.50	0.172
Nitrospira sp.	220.500	23.41	21.50	0.172
Nitrospira spp.	191.000	24.70	20.10	0.175
Nitrospirillum azospirillum amazonense	253.000	22.00	23.05	0.295

Nocardioides furvisabuli	245.500	22.33	22.69	0.851
Nocardioides hankookensis	245.000	22.35	22.67	0.890
Nocardioides iriomotensis	253.500	21.98	23.07	0.777
Nocardioides maritimus	208.000	23.96	20.90	0.351
Nocardioides sp.	212.500	23.76	21.12	0.476
Nocardioides spp.	184.500	24.98	19.79	0.165
Nonomuraea sp.	243.000	22.43	22.57	0.922
Nonomuraea turkmeniaca	203.000	24.17	20.67	0.129
Nordella spp.	210.000	23.87	21.00	0.090
Nosocomiicoccus ampullae	231.500	22.93	22.02	0.591
Noviherbaspirillum malthae	244.000	22.39	22.62	0.922
Novosphingobium capsulatum	230.500	22.98	21.98	0.685
Novosphingobium mathurensis	229.500	23.02	21.93	0.758
Novosphingobium sp.	164.000	25.87	18.81	0.068
Novosphingobium spp.	178.500	25.24	19.50	0.133
Novosphingobium stygium	261.500	21.63	23.45	0.636
Novosphingobium subarcticum	194.500	24.54	20.26	0.221
Novosphingobium subterraneum	213.500	23.72	21.17	0.505
Nubsella sp.	231.000	22.96	22.00	0.339
Nubsella zeaxanthinifaciens	243.500	22.41	22.60	0.925

Oceanibaculum pacificum	220.500	23.41	21.50	0.172
Oceanibaculum spp.	231.000	22.96	22.00	0.339
Oceanimonas smirnovii	234.000	22.83	22.14	0.749
Oceanobacillus luteolus	221.500	23.37	21.55	0.485
Oceanobacillus sp.	250.500	22.11	22.93	0.787
Oculatella coburnii	253.000	22.00	23.05	0.295
Ohtaekwangia koreensis	255.000	21.91	23.14	0.468
Ohtaekwangia spp.	256.000	21.87	23.19	0.536
Oleiphilus messinensis	231.000	22.96	22.00	0.339
Oleiphilus spp.	156.000	26.20	18.45	0.006
Oleispira spp.	210.000	23.87	21.00	0.090
Oleomonas sp.	253.000	22.00	23.05	0.295
Opitutus sp.	147.000	26.61	18.00	0.008
Opitutus spp.	239.000	22.61	22.38	0.951
Opitutus terrae	192.500	24.63	20.17	0.128
Oribacterium sinus	210.000	23.87	21.00	0.090
Oribacterium sp.	253.000	22.00	23.05	0.295
Orientia tsutsugamushi	242	22.48	22.52	0.974
Ornatilinea apprima	254.500	21.93	23.12	0.484
Ornithinibacillus sp.	255	21.91	23.14	0.468
Ornithinicoccus hortensis	256.000	21.87	23.19	0.536
Ornithinimicrobium sp.	247.500	22.24	22.79	0.872
Oscillatoria sp.	253.000	22.00	23.05	0.295

Oscillatoria spp.	234.500	22.80	22.17	0.851
Oscillospira spp.	243.000	22.43	22.57	0.922
Owenweeksia spp.	210.000	23.87	21.00	0.179
Oxalicibacterium faecigallinarum	234.500	22.80	22.17	0.796
Oxobacter pfennigii	220.500	23.41	21.50	0.172
Paenibacillus cellulosilyticus	232.500	22.89	22.07	0.701
Paenibacillus chitinolyticus	222.000	23.35	21.57	0.358
Paenibacillus contaminans	253.000	22.00	23.05	0.536
Paenibacillus favisporus	231.000	22.96	22.00	0.572
Paenibacillus graminis	235.000	22.78	22.19	0.782
Paenibacillus konsidanse	220.500	23.41	21.50	0.172
Paenibacillus nanensis	264.500	21.50	23.60	0.134
Paenibacillus stellifer	231.000	22.96	22.00	0.339
Paenibacillus wynnii	244.500	22.37	22.64	0.888
Palleronia sp.	242.000	22.48	22.52	0.974
Paludibacter propionicigenes	208.500	23.93	20.93	0.270
Paludibacter sp.	251.500	22.07	22.98	0.785
Paludibacter spp.	204.500	24.11	20.74	0.377
Paludibacterium sp.	231.000	22.96	22.00	0.339
Pannonibacter sp.	231.000	22.96	22.00	0.339
Papillibacter cinnamivorans	220.5	23.41	21.50	0.172

Parabacteroides distasonis	210.000	23.87	21.00	0.090
Parabacteroides gordonii	231	22.96	22	0.339
Parabacteroides spp.	261.5	21.63	23.45	0.610
Parachlamydia acanthamoebae	224.5	23.24	21.69	0.503
Paracoccus denitrificans	260	21.7	23.38	0.660
Paracoccus marcusii	221.500	23.37	21.55	0.558
Paracoccus pantotrophus	216.000	23.61	21.29	0.494
Paracoccus spp.	253.000	22.00	23.05	0.536
Paracraurococcus spp.	255	21.91	23.14	0.468
Parasegetibacter luojiensis	213.000	23.74	21.14	0.224
Parvibaculum spp.	231.000	22.96	22.00	0.339
Parvimonas micra	253.000	22.00	23.05	0.295
Pediococcus lactobacillus plantarum	213.500	23.72	21.17	0.472
Pedobacter cryoconitis	241.500	22.50	22.50	1.000
Pedobacter glucosidilyticus	221.000	23.39	21.52	0.334
Pedobacter heparinus	258.000	21.78	23.29	0.482
Pedobacter lentus	242.000	22.48	22.52	0.974
Pedobacter metabolipauper	253.000	22.00	23.05	0.295
Pedobacter sp.	259.500	21.72	23.36	0.590
Pedobacter spp.	258.000	21.78	23.29	0.630
Pedobacter steynii	267.000	21.39	23.71	0.315

Pedobacter wanjuense	282.000	20.74	24.43	0.175
Pedomicrobium australicum	231.000	22.96	22.00	0.339
Pedomicrobium spp.	253.000	22.00	23.05	0.701
Pedosphaera parvula	210.000	23.87	21.00	0.179
Pedosphaera spp.	243.500	22.41	22.60	0.944
Pelagibacterium halotolerans	266.500	21.41	23.69	0.239
Pelagibius litoralis	253.000	22.00	23.05	0.295
Pelagibius spp.	231.000	22.96	22.00	0.339
Pelagicoccus mobilis	253.000	22.00	23.05	0.295
Pelobacter carbinolicus	282.000	20.74	24.43	0.268
Pelobacter spp.	186.500	24.89	19.88	0.147
Pelomonas sp.	227.500	23.11	21.83	0.729
Pelomonas spp.	243.000	22.43	22.57	0.972
Pelosinus sp.	242.000	22.48	22.52	0.974
Pelotomaculum spp.	161.000	26.00	18.67	0.056
Peptoclostridium clostridium bifermentans	243.500	22.41	22.60	0.944
Peptoclostridium clostridium difficile	229.500	23.02	21.93	0.733
Peptoclostridium clostridium sticklandii	231.000	22.96	22.00	0.339

Peptococcus sp.	231.000	22.96	22.00	0.339
Peredibacter spp.	215.5	23.63	21.26	0.434
Peredibacter starrii	193.000	24.61	20.19	0.073
Perlucidibaca piscinae	231.500	22.93	22.02	0.591
Perlucidibaca spp.	226.500	23.15	21.79	0.617
Persicirhabdus sediminis	267.000	21.39	23.71	0.372
Petrimonas spp.	220.500	23.41	21.50	0.172
Phaeospirillum fulvum	254.500	21.93	23.12	0.484
Phascolarctobacterium sp.	215.000	23.65	21.24	0.375
Phaselicystis spp.	243.000	22.43	22.57	0.922
Phenylobacterium sp.	272.000	21.17	23.95	0.467
Phenylobacterium spp.	200.000	24.30	20.52	0.274
Phycicoccus sp.	290.000	20.39	24.81	0.225
Phycisphaera spp.	253.000	22.00	23.05	0.295
Phyllobacterium sp.	237.000	22.70	22.29	0.889
Pirellula sp.	252.000	22.04	23.00	0.774
Pirellula spp.	257.000	21.83	23.24	0.682
Planctomyces maris	243.000	22.43	22.57	0.944
Planctomyces spp.	239.000	22.61	22.38	0.950
Planktothricoides spp.	266.500	21.41	23.69	0.239
Planococcus maitriensis	235.000	22.78	22.19	0.853
Planococcus sp.	256.000	21.87	23.19	0.629
Planomicrobium chinense	243.000	22.43	22.57	0.922

Planomicrobium koreense	235.000	22.78	22.19	0.867
Planomicrobium mcmeekinii	250.000	22.13	22.90	0.823
Plantactinospora sp.	201.000	24.26	20.57	0.157
Plasticicumulans lactativorans	220.500	23.41	21.50	0.172
Pleomorphomonas spp.	242.000	22.48	22.52	0.974
Polaribacter gangjinensis	223.000	23.30	21.62	0.517
Polyangium sp.	220.500	23.41	21.50	0.172
Polymorphospora rubra	233.000	22.87	22.10	0.648
Polynucleobacter cosmopolitanus	207.500	23.98	20.88	0.422
Polynucleobacter necessarius	208.500	23.93	20.93	0.434
Polynucleobacter rarus	245.000	22.35	22.67	0.890
Polynucleobacter spp.	265.500	21.46	23.64	0.258
Pontibacter korlensis	234.000	22.83	22.14	0.749
Pontibacter populi	253.000	22.00	23.05	0.295
Pontibacter sp.	253.000	22.00	23.05	0.671
Ponticoccus sp.	188.000	24.83	19.95	0.980
Porphyrobacter sp.	219.500	23.46	21.45	0.463
Porphyrobacter spp.	248.000	22.22	22.81	0.877
Porphyrobacter tepidarius	228.500	23.07	21.88	0.697
Porticoccus spp.	189.500	24.76	20.02	0.083
Prevotella amnii	222.000	23.35	21.57	0.358

Prevotella spp.	236.500	22.72	22.26	0.881
Prochlorococcus spp.	199	24.35	20.48	0.314
Prolixibacter spp.	221.000	23.39	21.52	0.605
Propionigenium spp.	232.000	22.91	22.05	0.819
Propionivibrio spp.	276.000	21.00	24.14	0.397
Prosthecobacter spp.	302.500	19.85	25.40	0.149
Prosthecobacter vanneervenii	253.000	22.00	23.05	0.295
Prosthecomicrobium spp.	221.000	23.39	21.52	0.334
Proteiniphilum acetatigenes	210.000	23.87	21.00	0.090
Proteiniphilum spp.	212.000	23.78	21.10	0.324
Proteinivorax tanatarense	243.000	22.43	22.57	0.944
Pseudoalteromonas sp.	233.000	22.87	22.10	0.648
Pseudoalteromonas spp.	220.500	23.41	21.50	0.172
Pseudoalteromonas tetraodonis	220.500	23.41	21.50	0.172
Pseudoclavibacter spp.	237.500	22.67	22.31	0.920
Pseudohongiella sp.	240.500	22.54	22.45	0.972
Pseudolabrys sp.	232.000	22.91	22.05	0.751
Pseudolabrys spp.	221.000	23.39	21.52	0.334
Pseudomonas luteola	250.000	22.11	22.93	0.830
Pseudomonas pachastrellae	269.5	21.28	23.83	0.413
Pseudomonas savastanoi	248.500	22.20	22.83	0.863

Pseudomonas straminea	237.500	22.67	22.31	0.883
Pseudomonas taiwanensis	258.000	21.78	23.29	0.684
Pseudomonas tuomuerense	210.000	23.87	21.00	0.090
Pseudomonas umsongensis	231.000	22.96	22.00	0.339
Pseudomonas veronii	219.000	23.48	21.43	0.568
Pseudonocardia spp.	222.000	23.35	21.57	0.559
Pseudorhodobacter sp.	213.000	23.74	21.14	0.464
Pseudospirillum spp.	231.000	22.96	22.00	0.339
Pseudoxanthomonas koreensis	279.000	20.87	24.29	0.305
Pseudoxanthomonas Mexicana	232	22.91	22.05	0.609
Pseudoxanthomonas sp.	242.500	22.46	22.55	0.948
Pseudoxanthomonas taiwanensis	270.000	21.26	23.86	0.482
Psychrobacillus bacillus psychrodurans	253.000	22.00	23.05	0.295
Psychrobacter aquaticus	243.000	22.43	22.57	0.922
Psychrobacter sanguinis	253.000	22.00	23.05	0.295
Pullulanibacillus sp.	300.000	19.96	25.29	0.051
Puniceicoccus vermicola	220.500	23.41	21.50	0.172
Pusillimonas sp.	236.500	22.72	22.26	0.867
Pusillimonas spp.	253.000	22.00	23.05	0.295
Quadrisphaera sp.	203.500	24.15	20.69	0.184

Ramlibacter spp.	239.500	22.59	22.40	0.958
Ramlibacter tataouinensis	261.500	21.63	23.45	0.627
Rathayibacter tritici	254.500	21.93	23.12	0.484
Reyranella massiliensis	222.000	23.35	21.57	0.442
Reyranella soli	243.000	22.43	22.57	0.922
Reyranella sp.	231.000	22.96	22.00	0.339
Rheinheimera aquimaris	200.500	24.28	20.55	0.262
Rheinheimera chironomi	264.500	21.50	23.60	0.278
Rheinheimera sp.	257.000	21.83	23.24	0.677
Rheinheimera texana	239.500	22.59	22.40	0.962
Rhizobium leguminosarum	242.500	22.46	22.55	0.962
Rhizobium mongolense	280.500	20.80	24.36	0.308
Rhizobium tropici	253.000	22.00	23.05	0.295
Rhizomicrobium electricum	231.000	22.96	22.00	0.339
Rhodanobacter fulvus	232.000	22.91	22.05	0.609
Rhodanobacter sp.	221.500	23.37	21.55	0.522
Rhodobacter capsulatus	243.500	22.41	22.60	0.925
Rhodobacter gluconicum	232.000	22.91	22.05	0.769
Rhodobacter sp.	241.000	22.52	22.48	0.991
Rhodobacter sphaeroides	226.000	23.17	21.76	0.687
Rhodobacter spp.	232.500	22.89	22.07	0.832
Rhodobacter vinaykumarii	196.500	24.46	20.36	0.290
Rhodobium spp.	231.000	22.96	22.00	0.654

Rhodococcus kroppenstedtii	231.000	22.96	22.00	0.339
Rhodococcus rhodochrous	239	22.61	22.38	0.934
Rhodococcus yunnanensis	264.500	21.50	23.60	0.134
Rhodocyclus tenuis	282.000	20.74	24.43	0.341
Rhodocytophaga aerolata	213.000	23.74	21.14	0.224
Rhodocytophaga spp.	243.000	22.43	22.57	0.922
Rhodoferax albidiferax sp.	151.500	26.41	18.21	0.034
Rhodoferax antarcticus	231.000	22.96	22.00	0.794
Rhodomicrobium sp.	223.000	23.30	21.62	0.383
Rhodomicrobium spp.	240.000	22.57	22.43	0.963
Rhodomicrobium vannielii	220.500	23.41	21.50	0.172
Rhodopila globiformis	210.000	23.87	21.00	0.090
Rhodopirellula baltica	231.000	22.96	22.00	0.339
Rhodopirellula spp.	260.500	21.67	23.40	0.639
Rhodopseudomonas spp.	250.000	22.13	22.90	0.822
Rhodothermus spp.	231.000	22.96	22.00	0.339
Rhodovastum spp.	220.500	23.41	21.50	0.172
Rhodovibrio spp.	231.000	22.96	22.00	0.339
Rhodovulum marinum	231.000	22.96	22.00	0.339
Rhodovulum sulfidophilum	231.000	22.96	22.00	0.339
Rickettsia Canadensis	231.000	22.96	22.00	0.339
Rickettsiella grylli	267.000	21.39	23.71	0.468
Rikenella sp.	265.500	21.46	23.64	0.563

Rikenella spp.	274.500	21.07	24.07	0.289
Rivibacter sp.	253.500	21.98	23.07	0.609
Robiginitomaculum antarcticum	287.500	20.50	24.69	0.030
Roseburia faecis	242.000	22.48	22.52	0.974
Roseburia spp.	231.500	22.93	22.02	0.591
Roseibaca ekhonensis	220.500	23.41	21.50	0.172
Roseibacillus spp.	235.000	22.78	22.19	0.841
Roseicyclus spp.	231.500	22.93	22.02	0.670
Roseiflexus spp.	244.500	22.37	22.64	0.934
Roseinatronobacter sp.	220.500	23.41	21.50	0.550
Roseobacter sp.	264.500	21.50	23.60	0.134
Roseococcus sp.	272.000	21.17	23.95	0.309
Roseomonas lacus	232.500	22.89	22.07	0.628
Roseomonas ruber	221.000	23.39	21.52	0.334
Roseomonas stagni	238.000	22.65	22.33	0.926
Roseovarius sp.	210.000	23.87	21.00	0.293
Rothia sp.	272.000	21.17	23.95	0.433
Rubellimicrobium mesophilum	262.500	21.59	23.50	0.588
Rubellimicrobium spp.	201.000	24.26	20.57	0.277
Rubrimonas sp.	253.000	22.00	23.05	0.295
Rubrivivax gelatinosus	217.000	23.57	21.33	0.563

Rubrobacter spp.	309.500	19.54	25.74	0.109
Rudaea cellulosilytica	220.500	23.41	21.50	0.172
Rudanella sp.	241.500	22.50	22.50	1.000
Rufibacter sp.	287.500	20.50	24.69	0.262
Ruminiclostridium clostridium aldrichii	220.000	23.43	21.48	0.311
Ruminiclostridium clostridium cellobioparum	249.000	22.17	22.86	0.809
Ruminiclostridium clostridium josui	229.500	23.02	21.93	0.675
Ruminiclostridium clostridium papyrosolvens	221.000	23.39	21.52	0.419
Ruminococcus callidus	252.000	22.02	23.02	0.685
Rummeliibacillus pycnus	254.000	21.96	23.10	0.699
Runella slithyformis	222.000	23.35	21.57	0.442
Runella spp.	248.000	22.22	22.81	0.872
Saccharibacter spp.	233.500	22.85	22.12	0.790
Saccharofermentans acetigenes	209.000	23.91	20.95	0.230
Saccharomonospora azurea	253.000	22.00	23.05	0.295
Saccharophagus spp.	237.500	22.67	22.31	0.923
Saccharospirillum sp.	231.000	22.96	22.00	0.339
Saccharospirillum spp.	233.000	22.87	22.10	0.648

Saccharothrix xinjiangensis	253.000	22.00	23.05	0.295
Salinicoccus roseus	226.000	23.17	21.76	0.672
Salinicoccus sp.	240.000	22.57	22.43	0.956
Salinimicrobium sp.	253.000	22.00	23.05	0.295
Sandaracinus amylolyticus	253.000	22.00	23.05	0.295
Sandaracinus spp.	248.000	22.22	22.81	0.835
Sandarakinorhabdus sp.	243.000	22.43	22.57	0.922
Sandarakinorhabdus spp.	193.000	24.61	20.19	0.073
Sanguibacter antarcticus	221.000	23.39	21.52	0.334
Schlegelella spp.	242.000	22.48	22.52	0.974
Sedimentibacter spp.	231.000	22.96	22.00	0.339
Sediminibacterium salmoneum	241.500	22.50	22.50	1.000
Sediminibacterium sp.	236.500	22.72	22.26	0.904
Sediminibacterium spp.	213.000	23.74	21.14	0.502
Segetibacter spp.	234.000	22.83	22.14	0.817
Sejongia spp.	238.500	22.63	22.36	0.936
Seohaeicola saemankumensis	210.500	23.85	21.02	0.426
Serinicoccus sp.	226.500	23.15	21.79	0.682
Shimazuella kribbensis	231.000	22.96	22.00	0.339
Shimazuella sp.	220.500	23.41	21.50	0.172
Shinella spp.	278.000	20.91	24.24	0.308

Shinella zoogloeoides	231.000	22.96	22.00	0.339
Sideroxydans spp.	237.000	22.70	22.29	0.885
Silanimonas sp.	254.000	21.93	23.12	0.484
Simplicispira sp.	210.000	23.87	21.00	0.179
Singulisphaera sp.	247.500	22.24	22.79	0.813
Singulisphaera spp.	230.500	22.98	21.98	0.639
Sinorhizobium ensifer fredii	242.000	22.48	22.52	0.974
Sinorhizobium sp.	231.000	22.96	22.00	0.572
Skermanella sp.	254.000	21.96	23.10	0.645
Skermanella spp.	254.000	21.96	23.10	0.502
Smaragdicoccus niigatensis	245.000	22.33	22.69	0.857
Sneathiella sp.	220.500	23.41	21.50	0.172
Solimonas soli	210.500	23.85	21.02	0.253
Solirubrobacter spp.	175.000	25.39	19.33	0.112
Solitalea Canadensis	279.500	20.85	24.31	0.306
Solitalea spp.	233.000	22.87	22.10	0.648
Sorangium cellulosum	234.000	22.83	22.14	0.749
Sphaerobacter spp.	225.000	23.22	21.71	0.697
Sphaerobacter thermophilus	225.000	23.22	21.71	0.516
Sphaerotilus natans	270.000	21.26	23.86	0.445
Sphaerotilus spp.	239.000	22.61	22.38	0.951
Sphingobacterium faecium	234	22.83	22.14	0.853

Sphingobacterium siyangensis	220.500	23.41	21.50	0.500
Sphingobium chlorophenolicum	231.500	22.93	22.02	0.591
Sphingobium chungbukensis	242.000	22.48	22.52	0.974
Sphingobium faniae	231.000	22.96	22.00	0.339
Sphingobium xenophagum	221.500	23.37	21.55	0.536
Sphingobium yanoikuyae	252.500	22.02	23.02	0.786
Sphingomonas faeni	232.500	22.89	22.07	0.628
Sphingomonas melonis	185.500	24.93	19.83	0.093
Sphingomonas wittichii	231.000	22.96	22.00	0.753
Sphingomonas yunnanensis	253.000	22.00	23.05	0.295
Sphingopyxis chilensis	231.500	22.93	22.09	0.670
Sphingopyxis sp.	184.500	24.98	19.79	0.113
Sphingopyxis spp.	220.500	23.41	21.50	0.172
Sphingosinicella spp.	242.500	22.46	22.55	0.962
Spiribacter sp.	264.500	21.50	23.60	0.134
Spirobacillus cienkowskii	232.000	22.91	22.05	0.609
Spirochaeta aurantia	254.000	21.96	23.10	0.501
Spirochaeta bajacaliforniensis	243.000	22.43	22.57	0.922
Spirochaeta sp.	254.000	21.96	23.10	0.502
Spirochaeta spp.	213.000	23.74	21.14	0.499
Spirosoma linguale	231.000	22.96	22.00	0.339

Spongiibacter sp.	210.000	23.87	21.00	0.090
Sporichthya sp.	204.500	24.11	20.74	0.344
Sporichthya spp.	251.500	22.07	22.98	0.813
Sporobacter termitidis	231.000	22.96	22.00	0.698
Sporomusa spp.	220.000	23.43	21.48	0.557
Stappia spp.	220.500	23.41	21.50	0.322
Stella spp.	243.500	22.41	22.60	0.925
Stenotrophomonas acidaminiphila	201.500	24.24	20.60	0.290
Steroidobacter spp.	223.000	23.30	21.62	0.620
Sterolibacterium sp.	253.000	22.00	23.05	0.749
Sterolibacterium spp.	241.500	22.50	22.50	1.000
Streptomyces glaucescens	218.000	23.52	21.38	0.571
Streptomyces macrosporus	253.000	22.00	23.05	0.295
Streptomyces phaeopurpureus	243.000	22.43	22.57	0.922
Streptomyces scabrisporus	254.500	21.93	23.12	0.484
Streptomyces werraensis	200.500	24.28	20.55	0.263
Streptomyces yokosukanensis	242.000	22.48	22.52	0.988
Streptosporangium vulgare	242.000	22.48	22.52	0.974
Subdoligranulum spp.	278.000	20.91	24.24	0.119
Sulfuricurvum kujiense	239.500	22.59	22.40	0.944
Sulfuricurvum spp.	307.000	19.65	25.62	0.107

Sulfurimonas autotrophica	272.000	21.17	23.95	0.467
Sulfurimonas paralvinellae	244.000	22.39	22.62	0.906
Sulfurimonas spp.	265.500	21.46	23.64	0.572
Sulfurisoma sediminicola	226.500	23.15	21.79	0.710
Sulfurospirillum deleyianum	263.500	21.54	23.55	0.580
Sulfurospirillum spp.	270.500	21.24	23.88	0.495
Sulfurovum spp.	195.500	24.50	20.31	0.274
Sunxiuqinia faeciviva	220.500	23.41	21.50	0.172
Sunxiuqinia sp.	220.500	23.41	21.50	0.172
Symbiobacterium spp.	217.500	23.54	21.30	0.490
Synechococcus sp.	241.500	22.50	22.50	1.000
Synechococcus spp.	238.500	22.63	22.36	0.928
Synechocystis sp.	242.000	22.48	22.52	0.974
Syntrophobacter sp.	266.000	21.43	23.67	0.248
Syntrophobacter spp.	276.500	20.98	24.17	0.136
Syntrophomonas sp.	222.500	23.33	21.60	0.371
Syntrophomonas spp.	208.000	23.96	20.90	0.417
Syntrophus sp.	222.500	23.33	21.60	0.454
Syntrophus spp.	223.000	23.30	21.62	0.590
Tannerella spp.	253.000	22.00	23.05	0.536
Telmatobacter spp.	214.000	23.70	21.19	0.359
Tepidimonas spp.	279.500	20.85	24.31	0.369
Tepidiphilus petrobacter sp.	241.500	22.50	22.50	1.000

Teredinibacter sp.	253.000	22.00	23.05	0.295
Terrabacter sp.	245.500	22.33	22.69	0.909
Terribacillus halophilus	220.500	23.41	21.50	0.172
Terribacillus saccharophilus	220.500	23.41	21.50	0.408
Terriglobus roseus	231.000	22.96	22.00	0.339
Terrimonas sp.	227.500	23.11	21.83	0.732
Terrimonas spp.	231.500	22.93	22.02	0.813
Tetrasphaera spp.	252.500	22.02	23.02	0.768
Thalassobacillus devorans	255.000	21.91	23.14	0.707
Thalassobaculum sp.	266.000	21.43	23.67	0.391
Thalassolituus sp.	189.000	24.78	20.00	0.025
Thalassolituus spp.	274.000	21.09	24.05	0.382
Thauera mechernichensis	256.000	21.87	23.19	0.592
Thauera phenylacetica	246.500	22.28	22.74	0.904
Thauera selenatis	235.500	22.76	22.21	0.861
Thauera spp.	232.000	22.91	22.05	0.799
Thermacetogenium spp.	231.000	22.96	22.00	0.339
Thermaerobacter spp.	224.500	23.24	21.69	0.643
Thermincola spp.	242.000	22.48	22.52	0.974
Thermoanaerobacter uzonensis	231.000	22.96	22.00	0.339
Thermobacillus sp.	242.000	22.48	22.52	0.974

Thermodesulfobacterium spp.	249.000	22.17	22.86	0.768
Thermodesulfobium spp.	253.000	22.00	23.05	0.295
Thermodesulfovibrio spp.	230.000	23.00	21.95	0.624
Thermoleophilum album	231.000	22.96	22.00	0.339
Thermoleophilum spp.	224.000	23.26	21.67	0.541
Thermomicrobium spp.	242.500	22.46	22.55	0.969
Thermomonas brevis	214.500	23.67	21.21	0.469
Thermomonas fusca	254.500	21.93	23.12	0.579
Thermomonas haemolytica	202.500	24.20	20.64	0.173
Thermomonas sp.	231.500	22.93	22.02	0.591
Thermomonas spp.	268.000	21.35	23.76	0.426
Thermosporothrix spp.	220.500	23.41	21.50	0.172
Thermovum composti	253.000	22.00	23.05	0.295
Thermus sp.	254.000	21.96	23.10	0.594
Thermus spp.	221.500	23.37	21.55	0.431
Thermus thiopara	266.000	21.43	23.67	0.248
Thioalkalibacter halophilus	211.000	23.83	21.05	0.443
Thioalkalivibrio nitratireducens	218.500	23.50	21.40	0.562
Thioalkalivibrio spp.	268.000	21.35	23.76	0.488
Thiobaca spp.	253.000	22.00	23.05	0.295
Thiobacillus sp.	199.500	24.33	20.50	0.048

Thiobacillus spp.	252.000	22.04	23.00	0.791
Thiobacter spp.	231.000	22.96	22.00	0.339
Thiocystis violacea	220.500	23.41	21.50	0.172
Thiodictyon bacillosum	248.000	22.22	22.81	0.835
Thiohalophilus spp.	242.000	22.48	22.52	0.974
Thiomicrospira halophilus	278.000	20.91	24.24	0.119
Thiomicrospira sp.	206.000	24.04	20.81	0.255
Thioprofundum hispidum	220.500	23.41	21.50	0.172
Thioprofundum spp.	253.000	22.00	23.05	0.295
Thiorhodococcus bheemlicus	230.000	23.00	21.95	0.748
Thiorhodospira spp.	225.500	23.20	21.74	0.576
Thiorhodovibrio winogradskyi	234.000	22.83	22.14	0.749
Thiothrix caldifontis	240.500	22.54	22.45	0.972
Thiothrix disciformis	231.000	22.96	22.00	0.572
Thiothrix spp.	242.000	22.48	22.52	0.974
Thiovirga spp.	258.000	21.78	23.29	0.698
Thorsellia spp.	220.500	23.41	21.50	0.172
Tissierella spp.	232.500	22.89	22.07	0.701
Tistrella spp.	270.000	21.26	23.86	0.480
Tolumonas auensis	248.500	22.20	22.83	0.842
Tolumonas spp.	212.500	23.76	21.12	0.284

Treponema primitia	231.000	22.96	22.00	0.339
Treponema zuelzerae	242.000	22.48	22.52	0.974
Trichococcus pasteurii	233.000	22.87	22.10	0.827
Truepera spp.	220.500	23.41	21.50	0.172
Tumebacillus ginsengisoli	223.000	23.30	21.62	0.383
Tumebacillus permanentifrigoris	210.000	23.87	21.00	0.090
Tumebacillus sp.	199.000	24.35	20.48	0.094
Tumebacillus spp.	245.000	22.35	22.67	0.869
Turicibacter spp.	271.000	21.22	23.90	0.485
Turneriella parva	243.5	22.41	22.6	0.925
Uliginosibacterium gangwonense	221.500	23.37	21.55	0.431
Uliginosibacterium sp.	255.000	21.91	23.14	0.618
Uncultured candidatus brocadia sp.	232.000	22.91	22.05	0.609
Uncultured candidatus competibacter sp.	264.500	21.50	23.60	0.134
Uncultured candidatus microthrix sp.	224.500	23.24	21.69	0.503
Uncultured candidatus odyssella sp.	257.000	21.83	23.24	0.567
Uncultured candidatus pelagibacter sp.	231.000	22.96	22.00	0.339

Uncultured candidatus planktophila sp.	199.500	24.33	20.50	0.322
Uncultured candidatus protochlamydia sp.	231.000	22.96	22.00	0.339
Uncultured candidatus rhabdochlamydia sp.	199.000	24.35	20.48	0.094
Uncultured candidatus solibacter sp.	233.500	22.85	22.12	0.733
Undibacterium sp.	293.000	20.26	24.95	0.221
Undibacterium spp.	258.500	21.76	23.31	0.677
Vallitalea guaymasensis	253.000	22.00	23.05	0.295
Verrucomicrobium sp.	231.500	22.93	22.02	0.591
Verrucomicrobium spp.	268.500	21.33	23.79	0.523
Vibrio aestuarianus	211.500	23.80	21.07	0.314
Vibrio orientalis	244.000	22.39	22.62	0.922
Victivallis spp.	210.000	23.87	21.00	0.179
Victivallis vadensis	267.500	21.37	23.74	0.305
Virgibacillus halodenitrificans	234.500	22.80	22.17	0.856
Virgisporangium ochraceum	254.000	21.96	23.10	0.502
Vitreoscilla filiformis	216.000	23.61	21.29	0.507
Vogesella indigofera	256.500	21.85	23.21	0.711
Vogesella sp.	229.000	23.04	21.9	0.768
Vogesella spp.	254.500	21.93	23.12	0.712

Weissella cibaria	239.000	22.61	22.38	0.944
Weissella fabalis	217.500	23.54	21.36	0.519
Woodsholea maritima	199.500	24.33	20.50	0.048
Xanthobacillum maris	184.500	24.98	19.79	0.104
Xanthobacter spp.	220.500	23.41	21.50	0.408
Xenorhabdus nematophila	253.000	22.00	23.05	0.295
Xenorhabdus vietnamensis	254.500	21.93	23.12	0.748
Xylanimonas cellulosilytica	268.500	21.33	23.79	0.287
Zavarzinella spp.	231.000	22.96	22.00	0.339
Zoogloea oryzae	261.000	21.65	23.43	0.557
Zoogloea ramigera	229.500	23.02	21.93	0.689
Zoogloea resiniphila	200.500	24.28	20.55	0.189
Zoogloea spp.	239.500	22.59	22.40	0.944
Zymophilus spp.	242.000	22.48	22.52	0.974

Appendix 14: Wilcoxon rank test performed to determine the influence of season on the abundance of gray bacteria.

Bacterial species	**Z Value**	**P - Value**
Acetanaerobacterium spp.	-1.000	0.317
Acetobacterium wieringae	-1.342	0.180
Achromatium oxaliferum	-1.342	0.180
Acidaminobacter sp.	-0.431	0.667
Acidimicrobium spp.	-2.585	0.010
Acidisphaera sp.	-2.456	0.014
Acidisphaera spp.	-1.377	0.168
Aciditerrimonas sp.	-2.969	0.003
Aciditerrimonas spp.	-1.433	0.152
Acidithiobacillus spp.	-1.732	0.083
Acidobacterium sp.	-1.732	0.083
Acidobacterium spp.	-0.521	0.602
Acidocella spp.	-1.000	0.317
Acidothermus cellulolyticus	-2.384	0.017
Acidovorax caeni	-3.826	0.000
Acidovorax citrulli	-3.408	0.001
Acidovorax konjaci	-3.086	0.002
Acinetobacter brisouii	-3.306	0.001
Acinetobacter genomosp.	-3.157	0.002
Acinetobacter guillouiae	-1.604	0.109
Acinetobacter marinus	-1.000	0.317

Acinetobacter venetianus	-3.928	0.000
Actinoallomurus iriomotensis	-1.826	0.068
Actinocatenispora spp.	-1.000	0.317
Actinophytocola sp.	-1.604	0.109
Actinoplanes philippinensis	-2.388	0.017
Actinoplanes spp.	-1.997	0.046
Actinopolymorpha pittospori	-1.342	0.180
Actinotalea fermentans	-3.624	0.000
Adhaeribacter sp.	-1.000	0.317
Adhaeribacter spp.	-2.207	0.027
Advenella tetrathiobacter kashmirensis	-2.585	0.010
Aeromicrobium sp.	-2.232	0.026
Agrobacterium vitis	-3.924	0.000
Akkermansia spp.	-1.000	0.317
Alcanivorax spp.	-0.760	0.448
Algidimarina propionica	-1.342	0.180
Algorimarina spp.	-0.260	0.795
Algoriphagus dokdonensis	-1.604	0.109
Algoriphagus faecimaris	-1.000	0.317
Algoriphagus hongiella halophile	-2.207	0.027
Algoriphagus sp.	-3.729	0.000
Algoriphagus spp.	-1.342	0.180
Alicyclobacillus spp.	-1.342	0.180

Alistipes massiliensis	-1.000	0.317
Alkalibacter saccharofermentans	-1.633	0.102
Alkalibacter spp.	-2.384	0.017
Alkalibacterium iburiense	-3.434	0.001
Alkalibacterium kapii	-1.841	0.066
Alkalibacterium spp.	-1.342	0.180
Alkaliflexus spp.	-1.604	0.109
Alkalilimnicola spp.	-3.213	0.001
Alkaliphilus metalliredigens	-1.000	0.317
Alkaliphilus sp.	-2.388	0.017
Alkanibacter spp.	-1.342	0.180
Alkanindiges hongkongensis	-1.890	0.059
Alkanindiges illinoisensis	-2.414	0.016
Alkanindiges sp.	-2.070	0.038
Alkanindiges spp.	-2.538	0.011
Allochromatium vinosum	-1.041	0.298
Allokutzneria spp.	-1.826	0.068
Alsobacter metallidurans	-3.076	0.002
Altererythrobacter aestuarii	-2.040	0.041
Altererythrobacter dongtanensis	-3.634	0.000
Altererythrobacter sp.	-3.325	0.001
Altererythrobacter spp.	-3.314	0.001
Amaricoccus spp.	-1.841	0.066

Ammonifex thiophilus	-1.000	0.317
Ammoniphilus oxalivorans	-2.552	0.011
Ammoniphilus sp.	-2.375	0.018
Ammoniphilus spp.	-3.074	0.002
Anaerobacterium chartisolvens	-1.826	0.068
Anaerofilum spp.	-1.000	0.317
Anaerolinea spp.	-2.214	0.027
Anaeromusa sp.	-2.000	0.046
Anaeromyxobacter dehalogenans	-0.950	0.342
Anaeromyxobacter spp.	-1.826	0.068
Anaerophaga spp.	-1.588	0.112
Anaerosinus selenomonadaceae sb90	-1.633	0.102
Ancalomicrobium spp.	-1.633	0.102
Angustibacter aerolatus	-1.841	0.066
Anoxybacillus spp.	-1.342	0.180
Aquabacterium sp.	-1.000	0.317
Aquabacterium spp.	-1.619	0.105
Aquaspirillum putridiconchylium	-1.342	0.180
Aquaspirillum sp.	-1.841	0.066
Aquicella siphonis	-2.070	0.038
Aquicella spp.	-2.668	0.008
Aquimonas sp.	-2.401	0.016
Aquimonas spp.	-2.060	0.039

Aquitalea magnusonii	-1.585	0.113
Arcicella sp.	-3.925	0.000
Arcicella spp.	-1.195	0.232
Arenimonas daechungensis	-2.536	0.011
Arenimonas sp.	-3.573	0.000
Arenimonas spp.	-1.484	0.138
Arhodomonas sp.	-1.000	0.317
Aridibacter acidobacteria bacterium	-0.781	0.435
Aromatoleum aromaticum	-1.342	0.180
Arsenicicoccus sp.	-1.633	0.102
Arsenophonus spp.	-1.604	0.109
Arthrobacter agilis	-3.928	0.000
Arthrobacter chlorophenolicus	-1.890	0.059
Arthrobacter monumenti	-2.680	0.007
Arthrobacter nicotianae	-2.384	0.017
Arthrobacter protophormiae	-3.200	0.001
Arthrobacter ramosus	-2.371	0.018
Arthrospira platensis	-1.000	0.317
Asticcacaulis biprosthecium	-1.604	0.109
Asticcacaulis excentricus	-1.841	0.066
Atopostipes sp.	-1.000	0.317
Atopostipes spp.	-1.633	0.102
Aureimonas ferruginea	-2.536	0.011

Austwickia chelonae	-1.000	0.317
Azoarcus sp.	-2.060	0.039
Azoarcus spp.	-0.137	0.891
Azonexus sp.	-3.450	0.001
Azospira dechlorosoma sp.	-3.654	0.000
Azospira oryzae	-2.384	0.017
Azospirillum lipoferum	-2.456	0.014
Azospirillum oryzae	-2.366	0.018
Azospirillum picis	-1.000	0.317
Azospirillum spp.	-1.604	0.109
Azovibrio spp.	-2.689	0.007
Bacillus alcalophilus	-3.832	0.000
Bacillus andreesenii	-3.310	0.001
Bacillus badius	-2.677	0.007
Bacillus cellulosilyticus	-2.032	0.042
Bacillus chandigarhensis	-3.920	0.000
Bacillus clausii	-1.633	0.102
Bacillus flexus	-3.828	0.000
Bacillus horikoshii	-3.622	0.000
Bacillus longiquaesitum	-3.922	0.000
Bacillus nealsonii	-3.923	0.000
Bacillus pocheonensis	-3.920	0.000
Bacillus simplex	-1.841	0.066

Bacillus vireti	-3.736	0.000
Bacillus weihenstephanensis	-3.522	0.000
Bacteriovorax marinus	-1.000	0.317
Bacteriovorax sp.	-2.032	0.042
Bacteriovorax spp.	-0.718	0.473
Bacteroides coprocola	-1.000	0.317
Bacteroides luti	-0.368	0.713
Barnesiella viscericola	-1.826	0.068
Bauldia consociate	-1.633	0.102
Bdellovibrio bacteriovorus	-1.015	0.310
Bdellovibrio exovorus	-1.633	0.102
Bdellovibrio sp.	-1.229	0.219
Bdellovibrio spp.	-1.477	0.140
Beggiatoa sp.	-2.060	0.039
Beggiatoa spp.	-2.689	0.007
Beijerinckia spp.	-0.814	0.415
Bellilinea spp.	-2.809	0.005
Belnapia spp.	-1.732	0.083
Blastomonas spp.	-2.054	0.040
Blastopirellula marina	-2.232	0.026
Blastopirellula spp.	-0.898	0.369
Blautia product	-0.962	0.336
Borrelia carolinensis	0.000	1.000

Bosea thiooxidans	-1.000	0.317
Brachybacterium paraconglomeratum	-3.346	0.001
Brachybacterium zhongshanense	-1.000	0.317
Brachymonas denitrificans	-3.829	0.000
Brevibacillus thermoruber	-1.000	0.317
Brevibacterium daeguense	-1.342	0.180
Brevundimonas abyssalis	-1.826	0.068
Brevundimonas bacteroides	-1.342	0.180
Buchnera aphidicola	-3.936	0.000
Burkholderia xenovorans	-3.108	0.002
Butyricimonas synergistica	-1.000	0.317
Butyrivibrio clostridium proteoclasticum	-3.828	0.000
Byssovorax spp.	-1.000	0.317
Caedibacter spp.	-2.041	0.041
Caenispirillum bisanense	-1.000	0.317
Caloramator spp.	-2.060	0.039
Candidatus accumulibacter sp.	-1.892	0.059
Candidatus acetothermum candidatus acetothermus autotrophicum	-1.000	0.317
Candidatus alysiosphaera europeae	-2.207	0.027
Candidatus aquiluna rubra	-0.625	0.532
Candidatus babela delta proteobacterium	-0.534	0.593
Candidatus carsonella ruddii	-0.524	0.600

Candidatus chloroploca chloroflexi bacterium	-1.604	0.109
Candidatus cloacimonas acidaminovorans	-1.000	0.317
Candidatus cloacimonas uncultured candidatus cloacamonas sp.	-1.342	0.180
Candidatus desulforudis audaxviator	-1.000	0.317
Candidatus halomonas phosphatis	-1.604	0.109
Candidatus lumbricincola sp.	-1.342	0.180
Candidatus macropleicola muticae	-1.342	0.180
Candidatus magnetobacterium uncultured magnetobacterium sp.	-2.032	0.042
Candidatus magnetoovum mohavensis	-1.000	0.317
Candidatus metachlamydia lacustris	-0.677	0.498
Candidatus mycoplasma ravipulmonis	-1.000	0.317
Candidatus nardonella endosymbiont of scyphophorus yuccae	-1.000	0.317
Candidatus nardonella endosymbiont of sphenophorus levis	-0.368	0.713
Candidatus nasuia deltocephalinicola	-1.342	0.180
Candidatus nitrotoga arctica	-1.000	0.317
Candidatus nucleicultrix amoebiphila	-1.000	0.317
Candidatus odyssella thessalonicensis	-1.342	0.180
Candidatus paenicardinium endonii	-1.342	0.180
Candidatus paraholospora nucleivisitans	-1.000	0.317

Candidatus pelagibacter uncultured pelagibacter sp.	-1.000	0.317
Candidatus phytoplasma mexican potato purple top phytoplasma	-1.604	0.109
Candidatus planktoluna difficilis	-3.827	0.000
Candidatus planktophila limnetica	-3.381	0.001
Candidatus planktothricoides rosea	-1.000	0.317
Candidatus protochlamydia amoebophila	-1.342	0.180
Candidatus protochlamydia protochlamydia naegleriophila	-1.604	0.109
Candidatus protochlamydia sp.	-3.642	0.000
Candidatus rhabdochlamydia porcellionis	-1.979	0.048
Candidatus rhabdochlamydia rhabdochlamydia crassificans	-1.000	0.317
Candidatus rhabdochlamydia sp.	-1.633	0.102
Candidatus rhodoluna lacicola	-2.502	0.012
Candidatus rhodoluna planktonica	-1.313	0.189
Candidatus rhodoluna rhodoluna sp.	-3.512	0.000
Candidatus saccharimonas aalborgensis	-2.201	0.028
Candidatus soleaferrea massiliensis	-1.633	0.102
Candidatus thioglobus singularis	-1.633	0.102
Candidatus trichorickettsia mobilis	1.841	0.066
Candidatus zinderia insecticola	-0.315	0.753
Carboxydocella sp.	-1.000	0.317

Carboxydothermus islandicus	-1.000	0.317
Catalinimonas alkaloidigena	-2.041	0.041
Catellatospora yuxiensis	-2.121	0.034
Catenibacterium mitsuokai	-1.000	0.317
Cellulomonas chitinilytica	-2.754	0.006
Cellulomonas terrae	-2.877	0.004
Cellulosilyticum ruminicola	-1.342	0.180
Cellulosilyticum spp.	-0.365	0.715
Cellvibrio gandavensis	-1.342	0.180
Cellvibrio ostraviensis	-2.938	0.003
Chitinibacter tainanensis	-1.342	0.180
Chitinimonas koreensis	-2.264	0.024
Chitinimonas taiwanensis	-2.414	0.016
Chitinophaga flexibacter sancti	-1.342	0.180
Chitinophaga pinensis	-1.414	0.157
Chitinophaga spp.	-0.095	0.924
Chlamydia ibidis	-1.000	0.317
Chlorobium sp.	-1.342	0.180
Chlorobium spp.	0.000	1.000
Chloroflexus spp.	-0.061	0.951
Chloronema giganteum	-1.000	0.317
Chondromyces crocatus	-1.000	0.317
Chondromyces pediculatus	-3.319	0.001

Chondromyces spp.	-0.756	0.450
Chromatium okenii	-2.023	0.043
Chromohalobacter spp.	-2.388	0.017
Chryseobacterium anthropi	-2.023	0.043
Chryseobacterium bovis	-3.129	0.002
Chryseobacterium kwangyangense	-1.342	0.180
Chryseobacterium soldanellicola	-2.527	0.012
Chryseobacterium sp.	-2.527	0.012
Chryseobacterium taiwanensis	-1.342	0.180
Chryseomicrobium sp.	-1.604	0.109
Chthoniobacter flavus	-1.342	0.180
Cloacibacterium sp.	-1.604	0.109
Cloacibacterium spp.	-0.806	0.420
Clostridium aminobutyricum	-2.060	0.039
Clostridium bovipellis	-1.342	0.180
Clostridium bowmanii	-2.384	0.017
Clostridium cavendishii	-3.448	0.001
Clostridium cellulovorans	-1.633	0.102
Clostridium disporicum	-3.192	0.001
Clostridium enrichment	-2.060	0.039
Clostridium frigidicarnis	-2.384	0.017
Clostridium magnum	-2.410	0.016
Clostridium quinii	-1.342	0.180

Clostridium ruminantium	-3.922	0.000
Clostridium scatologenes	-1.604	0.109
Clostridium tunisiense	-2.232	0.026
Cobetia marina	-1.732	0.083
Cohnella sp.	-2.388	0.017
Comamonas guangdongensis	-3.519	0.000
Comamonas koreensis	-3.627	0.000
Compostimonas spp.	-0.990	0.322
Conexibacter sp.	-1.342	0.180
Conexibacter spp.	-2.467	0.014
Congregibacter litoralis	-1.633	0.102
Coprococcus catus	-1.342	0.180
Coprococcus eutactus	-1.000	0.317
Corynebacterium appendicis	-3.636	0.000
Corynebacterium lipophiloflavum	-1.604	0.109
Corynebacterium maris	-2.207	0.027
Corynebacterium matruchotii	-3.852	0.000
Cosenzaea proteus myxofaciens	-1.342	0.180
Couchioplanes caeruleus	-3.629	0.317
Coxiella cheraxi	-1.000	0.317
Craurococcus spp.	-1.000	0.317
Crenothrix polyspora	-1.604	0.109
Criblamydia sequanensis	-1.890	0.059

Crocinitomix spp.	-1.342	0.180
Cryobacterium spp.	-3.937	0.000
Cryocola spp.	-0.933	0.351
Cryptosporangium japonicum	-1.633	0.102
Curvibacter sp.	-0.747	0.455
Curvibacter spp.	-1.867	0.062
Cyanothece spp.	-1.841	0.066
Cycloclasticus spp.	-1.342	0.180
Cystobacter spp.	-2.388	0.017
Cystobacter violaceus	-2.060	0.039
Cytophaga aurantiaca	-1.633	0.102
Cytophaga sp.	-1.841	0.066
Cytophaga spp.	-3.524	0.000
Dactylosporangium spp.	-1.604	0.109
Daeguia caeni	-3.633	0.000
Dechloromonas denitrificans	-2.536	0.011
Dechloromonas spp.	-1.381	0.167
Dehalobacterium spp.	-0.970	0.332
Dehalococcoides spp.	-2.501	0.012
Dehalogenimonas spp.	-0.238	0.812
Deinococcus alpinitundrae	-1.604	0.109
Deinococcus deserti	-2.521	0.012
Deinococcus geothermalis	-1.342	0.180

Deinococcus hohokamensis	-1.841	0.066
Deinococcus navajonensis	-1.342	0.180
Deinococcus radiodurans	-1.826	0.068
Deinococcus radiophilus	-1.000	0.317
Deinococcus sp.	-3.923	0.000
Deinococcus spp.	-3.200	0.001
Deinococcus xinjiangensis	-1.826	0.068
Delftia spp.	-3.929	0.000
Demequina aestuarii	-2.751	0.006
Demequina lutea	-1.000	0.317
Denitratisoma sp.	-2.388	0.017
Denitratisoma spp.	-1.342	0.180
Denitrobacterium detoxificans	-2.807	0.005
Derxia sp.	-2.121	0.034
Desemzia incerta	-2.032	0.042
Desertibacter roseus	-1.841	0.066
Desulfatibacillum alkenivorans	-1.000	0.317
Desulfatiglans desulfobacterium anilini	-1.000	0.317
Desulfatitalea tepidiphila	-1.000	0.317
Desulfitobacterium hafniense	-0.877	0.380
Desulfitobacterium sp.	-1.342	0.180
Desulfitobacterium spp.	-1.857	0.063
Desulfobacter spp.	-1.000	0.317

Desulfobacterium sp.	-1.890	0.059
Desulfobacterium spp.	-0.530	0.596
Desulfobulbus spp.	-0.183	0.855
Desulfocapsa spp.	-3.831	0.000
Desulfococcus biacutus	-1.000	0.317
Desulfococcus spp.	-1.604	0.109
Desulfofaba fastidiosa	-1.342	0.180
Desulfofaba spp.	-1.841	0.066
Desulfofrigus oceanense	-1.633	0.102
Desulfomonile spp.	-1.604	0.109
Desulfomonile tiedjei	-3.836	0.000
Desulfonatronum thiosulfatophilum	-0.647	0.518
Desulfonema limicola	-1.000	0.317
Desulforegula spp.	-0.490	0.624
Desulforhopalus spp.	-1.377	0.168
Desulfosarcina spp.	-1.342	0.180
Desulfosporomusa spp.	-1.342	0.180
Desulfosporosinus meridiei	-2.414	0.016
Desulfosporosinus spp.	-2.032	0.042
Desulfotignum sp.	-1.000	0.317
Desulfotomaculum acetoxidans	-1.342	0.180
Desulfotomaculum solfataricum	-1.000	0.317
Desulfotomaculum sp.	-2.447	0.014

Desulfotomaculum spp.	-1.604	0.109
Desulfovibrio mexicanus	-1.342	0.180
Desulfovibrio oxyvorans	-1.342	0.180
Desulfovibrio putealis	-1.633	0.102
Desulfurobacterium spp.	-1.000	0.317
Desulfuromonas spp.	-1.000	0.317
Desulfuromusa spp.	-0.535	0.593
Dethiosulfatibacter spp.	-1.000	0.317
Devosia insulae	-3.300	0.001
Devosia soli	-2.844	0.004
Devosia sp.	-3.201	0.001
Devosia spp.	-2.986	0.003
Devosia subaequoris	-1.604	0.109
Dissulfuribacter thermophilus	-1.604	0.109
Dokdonella spp.	-2.887	0.004
Dongia spp.	-1.604	0.109
Dorea spp.	-1.342	0.180
Draconibacterium orientale	-1.841	0.066
Duganella sp.	-3.925	0.000
Duganella zoogloeoides	-3.927	0.000
Dyadobacter beijingensis	-1.048	0.295
Dyadobacter psychrophilus	-1.000	0.317
Dyadobacter sp.	-1.841	0.066

Dyadobacter spp.	-1.841	0.066
Ectothiorhodospira imhoffii	-1.342	0.180
Ectothiorhodospira magna	-3.140	0.002
Ectothiorhodospira sp.	-1.342	0.180
Edaphobacter spp.	-1.000	0.317
Elusimicrobium spp.	-2.264	0.024
Emticicia oligotrophica	-3.439	0.001
Emticicia spp.	-1.000	0.317
Enhydrobacter aerosaccus	0.000	1.000
Ensifer adhaerens	-3.827	0.000
Enteractinococcus sp.	-1.633	0.102
Epulopiscium sp.	-1.342	0.180
Erythrobacter gaetbuli	-2.060	0.039
Erythrobacter litoralis	-1.342	0.180
Erythrobacter piscidermidis	-2.371	0.018
Erythrobacter sp.	-3.725	0.000
Erythrobacter spp.	-3.083	0.002
Ethanoligenens cellulosi	-1.000	0.317
Ethanoligenens spp.	-1.000	0.317
Eubacterium coprostanoligenes	-1.000	0.317
Eubacterium oxidoreducens	-2.384	0.017
Exiguobacterium indicum	-1.606	0.108
Exiguobacterium lactigenes	-2.524	0.012

Exiguobacterium panipatensis	-3.921	0.000
Exiguobacterium profundum	-1.633	0.102
Faecalibacterium prausnitzii	-1.000	0.317
Ferrimicrobium spp.	-1.890	0.059
Ferrithrix spp.	-1.000	0.317
Ferrovum spp.	-1.000	0.317
Ferruginibacter sp.	-1.000	0.317
Fibrobacter spp.	-1.841	0.066
Filibacter spp.	-3.921	0.000
Filomicrobium sp.	-1.000	0.317
Flavihumibacter sp.	-2.524	0.012
Flavisolibacter flavosolibacter sp.	-2.456	0.014
Flavisolibacter ginsengisoli	-3.069	0.002
Flavisolibacter sp.	-1.176	0.240
Flavisolibacter spp.	-3.416	0.001
Flavobacterium aciduliphilum	-2.207	0.027
Flavobacterium columnare	-2.264	0.024
Flavobacterium indicum	-1.841	0.066
Flavonifractor clostridium orbiscindens	-1.342	0.180
Flectobacillus spp.	-0.214	0.831
Flexibacter flexilis	-1.633	0.102
Flexibacter spp.	-0.536	0.592
Flexithrix dorotheae	-1.000	0.317

Flexivirga spp.	-2.232	0.026
Fluviicola spp.	-2.093	0.036
Fluviicola taffensis	-1.890	0.059
Fluviimonas pallidilutea	-2.818	0.005
Fluviimonas sp.	-3.269	0.001
Fonticella clostridiaceae bacterium	-1.633	0.102
Formivibrio citricus	-1.604	0.109
Frankia sp.	-2.032	0.042
Frankia spp.	-2.032	0.042
Frateuria aurantia	-1.000	0.317
Frigoribacterium sp.	-1.342	0.180
Fusibacter spp.	-0.320	0.749
Gaiella occulta	-1.000	0.317
Gaiella spp.	-2.692	0.007
Gallaecimonas sp.	-2.023	0.043
Gallionella spp.	-2.207	0.027
Gelria spp.	-1.342	0.180
Geminicoccus roseus	-1.604	0.109
Gemmata sp.	-1.342	0.180
Gemmata spp.	-3.186	0.001
Gemmobacter catellibacterium sp.	-3.926	0.000
Gemmobacter rhodobacter changlaii	-3.103	0.002
Gemmobacter sp.	-3.922	0.000

Geoalkalibacter spp.	-1.404	0.160
Geobacter spp.	-1.456	0.145
Geobacter thiogenes	-1.604	0.109
Geodermatophilus obscurus	-3.832	0.000
Geodermatophilus spp.	-3.476	0.001
Geopsychrobacter electrodiphilus	-1.342	0.180
Georgenia muralis	-1.841	0.066
Georgenia sp.	-1.342	0.180
Georgenia spp.	-2.950	0.003
Geothermobacter spp.	-2.032	0.042
Geothrix spp.	-2.030	0.042
Geovibrio ferrireducens	-1.841	0.066
Gloeobacter spp.	0.000	1.000
Gluconacetobacter spp.	-1.342	0.180
Gordonibacter spp.	-1.743	0.081
Gottschalkia eubacterium angustum	-1.000	0.317
Gracilibacillus halotolerans	-1.342	0.180
Gracilibacillus sp.	-1.633	0.102
Gracilibacter spp.	-1.342	0.108
Gracilimonas sp.	-2.751	0.006
Granulicella spp.	-1.000	0.317
Gulosibacter sp.	-2.333	0.020
Haematobacter missouriensis	-1.342	0.180

Halalkalibacillus halophilus	-1.841	0.066
Haliangium spp.	-2.692	0.007
Haliea mediterranea	-2.807	0.005
Haliea sp.	-1.604	0.109
Haliscomenobacter hydrossis	-1.633	0.102
Haliscomenobacter spp.	-2.703	0.007
Haloanella sp.	-1.342	0.180
Halobacillus hunanensis	-1.342	0.180
Halochromatium spp.	-1.000	0.317
Halospirulina sp.	-3.865	0.000
Halothiobacillus kellyi	-1.342	0.180
Halothiobacillus sp.	-2.344	0.019
Herbiconiux spp.	-1.000	0.317
Hirschia sp.	-2.032	0.042
Hoeflea sp.	-2.565	0.010
Holdemania spp.	-1.000	0.317
Holophaga foetida	-1.604	0.109
Holophaga sp.	-0.447	0.655
Holophaga spp.	-2.812	0.005
Hydrogenophaga palleronii	3.753	0.000
Hydrogenophaga sp.	-3.826	0.000
Hydrogenophaga spp.	-3.920	0.000
Hydrogenophilus thermoluteolus	-1.000	0.317

Hymenobacter gelipurpurascens	-1.000	0.317
Hymenobacter sp.	-3.103	0.002
Hymenobacter xinjiangensis	-1.342	0.180
Hyphomicrobium spp.	-1.831	0.067
Hyphomonas neptunium	-1.841	0.066
Hyphomonas oceanitis	-1.000	0.317
Hyphomonas spp.	-1.841	0.066
Iamia majanohamensis	-1.826	0.068
Iamia spp.	-2.585	0.010
Ideonella sp.	-3.518	0.000
Ideonella spp.	-3.205	0.001
Idiomarina loihiensis	-1.342	0.180
Idiomarina sp.	-2.333	0.020
Idiomarina spp.	-2.121	0.034
Ignavibacterium sp.	-1.000	0.317
Ignavibacterium spp.	-0.736	0.462
Ilumatobacter fluminis	0.000	1.000
Ilumatobacter spp.	-1.725	0.084
Inhella inkyongensis	-0.892	0.373
Insolitispirillum insolitospirillum peregrinum	-3.246	0.001
Intestinimonas butyriciproducens	-1.000	0.317
Isoptericola spp.	-2.032	0.042
Jannaschia sp.	-2.041	0.041

Jatrophihabitans endophyticus	-1.342	0.180
Jeotgalicoccus psychrophilus	-2.121	0.034
Jonesia sp.	-2.070	0.038
Kaistia hirudinis	-1.826	0.068
Kaistia sp.	-1.633	0.102
Kaistobacter spp.	-1.133	0.257
Kallotenue chloroflexi bacterium	-1.342	0.180
Kineococcus radiotolerans	-1.826	0.068
Kineococcus sp.	-1.890	0.059
Kineosporia aurantiaca	-2.214	0.027
Kitasatospora cystarginea	-2.226	0.026
Kitasatospora spp.	-1.633	0.102
Klugiella spp.	-3.216	0.001
Knoellia sinensis	-2.533	0.011
Knoellia subterranea	-3.552	0.000
Kocuria carniphila	-3.828	0.000
Kopriimonas spp.	-1.342	0.180
Kouleothrix aurantiaca	-1.604	0.109
Kouleothrix spp.	-1.604	0.109
Ktedonobacter spp.	-1.342	0.108
Labilithrix luteola	-1.732	0.083
Labrenzia aggregata	-1.433	0.152
Lachnoclostridium clostridium phytofermentans	-1.000	0.317

Lachnoclostridium clostridium xylanolyticum	-1.414	0.157
Lacibacter cauensis	-3.928	0.000
Lacibacter sp.	-3.105	0.002
Lacibacter spp.	-1.000	0.317
Lacibacterium rhodospirillaceae bacterium	-0.041	0.968
Lactobacillus farciminis	-2.410	0.016
Lactobacillus gallinarum	-1.890	0.059
Lactobacillus graminis	-1.000	0.317
Lactobacillus helveticus	-1.342	0.180
Lactobacillus kunkeei	-1.000	0.317
Lactobacillus mali	-1.000	0.317
Lactobacillus pentosus	-1.342	0.180
Lactobacillus reuteri	-1.000	0.317
Lactobacillus rossiae	-1.000	0.317
Larkinella sp.	-1.414	0.157
Leadbetterella sp.	-1.294	0.196
Leeia oryzae	-1.841	0.066
Legionella dresdeniensis	-1.604	0.109
Legionella geestiana	-1.342	0.180
Lentzea spp.	-2.060	0.039
Leptolinea sp.	-1.841	0.066
Leptolinea spp.	-1.342	0.180
Leptolyngbya frigida	-1.000	0.317

Leptolyngbya saxicola	-1.265	0.206
Leptolyngbya sp.	-2.535	0.011
Leptolyngbya spp.	-2.410	0.016
Leptospirillum ferrodiazotrophum	-1.000	0.317
Leptospirillum spp.	-1.342	0.180
Leptothrix sp.	-2.460	0.014
Leptothrix spp.	-1.083	0.279
Leucobacter sp.	-0.210	0.833
Levilinea spp.	-1.795	0.073
Lewinella sp.	-1.342	0.180
Lewinella spp.	-1.342	0.180
Limnobacter litoralis	-1.841	0.066
Limnobacter spp.	-1.951	0.051
Limnohabitans curvus	-2.277	0.023
Limnohabitans spp.	-0.205	0.837
Loktanella salsilacus	-1.841	0.066
Longilinea spp.	-2.207	0.027
Luteimonas composti	-2.388	0.017
Luteimonas sp.	-2.129	0.033
Luteimonas spp.	-3.920	0.000
Luteolibacter algae	-1.000	0.317
Luteolibacter pohnpeiensis	-1.633	0.102
Luteolibacter sp.	-2.032	0.042

Luteolibacter spp.	-2.264	0.024
Luteolibacter yonseiensis	-2.565	0.010
Lutibaculum baratangense	-1.000	0.317
Lutispora spp.	-1.000	0.317
Lutispora thermophila	-1.342	0.180
Lysinibacillus sphaericus	-3.624	0.000
Lysobacter deserti	-3.215	0.001
Lysobacter enzymogenes	-2.988	0.003
Lysobacter sp.	-1.007	0.314
Lysobacter spp.	-3.530	0.000
Lyticum sinuosum	-1.342	0.180
Magnetococcus spp.	-1.841	0.066
Magnetospirillum sp.	-2.524	0.012
Magnetospirillum spp.	-1.414	0.157
Magnetovibrio blakemorei	-1.633	0.102
Malikia spp.	-1.962	0.050
Maribacter sp.	-1.000	0.317
Marinilactibacillus sp.	-1.604	0.109
Marinimicrobium koreense	-1.000	0.317
Marininema halotolerans	-1.890	0.059
Marinithermus spp.	-1.841	0.066
Marinobacter sp.	-1.342	0.180
Marinobacter spp.	-0.990	0.322

Marinobacter zhanjiangensis	-1.000	0.317
Marinobacterium spp.	-1.342	0.180
Marisediminicola spp.	-2.456	0.014
Marispirillum spp.	1.000	0.317
Marmoricola sp.	-3.225	0.001
Meniscus spp.	-1.000	0.317
Merismopedia spp.	-1.342	0.180
Methylibium petroleiphilum	-3.062	0.002
Methylobacillus flagellatus	-1.890	0.059
Methylobacillus spp.	-0.280	0.779
Methylobacter sp.	-1.000	0.317
Methylobacter spp.	-3.923	0.000
Methylocaldum sp.	-2.908	0.004
Methylocaldum spp.	-3.429	0.001
Methylocella sp.	-3.133	0.002
Methylococcus mobilis	-1.604	0.109
Methylococcus spp.	-2.070	0.038
Methylocystis parvus	-3.429	0.001
Methylocystis spp.	-1.633	0.102
Methylomicrobium spp.	-1.633	0.102
Methylomonas fodinarum	-1.000	0.317
Methylomonas methanica	-2.207	0.027
Methylomonas sp.	-1.614	0.107

Methylomonas spp.	-1.759	0.079
Methylophaga sp.	-1.604	0.109
Methylophaga spp.	-0.170	0.865
Methylophilus spp.	-3.562	0.000
Methylopila capsulata	-1.342	0.180
Methylopila sp.	-2.041	0.041
Methylosinus sp.	-1.841	0.066
Methylosinus sporium	-1.633	0.102
Methylosinus spp.	-3.525	0.000
Methylosoma sp.	-1.000	0.317
Methylotenera mobilis	-3.084	0.002
Methylotenera spp.	-3.737	0.000
Methylotenera versatilis	-0.654	0.513
Methylothermus spp.	-1.604	0.109
Methyloversatilis spp.	-1.342	0.180
Methylovulum miyakonense	-3.140	0.002
Microbacterium sediminicola	-1.000	0.317
Microbispora rosea	-1.857	0.063
Microcella putealis	-3.826	0.000
Microcella spp.	-2.264	0.024
Microcoleus spp.	-2.060	0.039
Microcystis sp.	-3.923	0.000
Micromonospora sp.	-1.826	0.068

Micromonospora spp.	-1.874	0.061
Microvirga spp.	-1.352	0.176
Miniimonas arenae	-2.264	0.024
Mitsuaria spp.	-3.659	0.000
Modestobacter spp.	-3.308	0.001
Mogibacterium pumilum	-2.060	0.039
Moorella humiferrea	-1.342	0.180
Moorella spp.	-2.527	0.012
Moorella thermoacetica	-1.342	0.180
Mucilaginibacter sp.	-0.943	0.345
Mucilaginibacter spp.	-2.032	0.042
Mucilaginibacter ximonensis	-1.841	0.066
Mycoplana sp.	-0.220	0.825
Mycoplasma crocodyli	-1.344	0.179
Mycoplasma phocidae	-1.000	0.317
Mycoplasma zalophi	-3.572	0.000
Myxococcus spp.	-1.000	0.317
Nafulsella turpanensis	-1.342	0.180
Nannocystis spp.	-1.734	0.083
Natranaerovirga hydrolytica	-1.732	0.083
Natranaerovirga pectinivora	-1.604	0.109
Natronoanaerobium salstagnum	-2.955	0.003
Neptunomonas spp.	-1.604	0.109

Nevskia soli	-1.000	0.317
Niabella sp.	-1.342	0.180
Niastella sp.	-2.716	0.007
Niastella spp.	-3.072	0.002
Nitratireductor spp.	-1.633	0.102
Nitrobacter spp.	-1.000	0.317
Nitrosococcus spp.	-1.000	0.317
Nitrosomonas spp.	-1.000	0.317
Nitrosospira spp.	-1.033	0.302
Nitrosovibrio spp.	-1.228	0.219
Nitrospina spp.	-1.342	0.180
Nitrospira sp.	-1.342	0.180
Nitrospira spp.	-2.104	0.035
Nitrospirillum azospirillum amazonense	-1.000	0.317
Nocardioides furvisabuli	-1.826	0.068
Nocardioides hankookensis	-2.214	0.027
Nocardioides iriomotensis	-0.201	0.840
Nocardioides maritimus	-3.423	0.001
Nocardioides sp.	-1.345	0.179
Nocardioides spp.	-1.903	0.057
Nonomuraea sp.	-1.342	0.180
Nonomuraea turkmeniaca	-2.264	0.024
Nordella spp.	-1.633	0.102

Nosocomiicoccus ampullae	-1.342	0.180
Noviherbaspirillum malthae	-1.841	0.066
Novosphingobium capsulatum	-2.375	0.018
Novosphingobium mathurensis	-3.920	0.000
Novosphingobium sp.	-1.755	0.079
Novosphingobium spp.	-3.211	0.001
Novosphingobium stygium	-1.906	0.057
Novosphingobium subarcticum	-3.828	0.000
Novosphingobium subterraneum	-2.913	0.004
Nubsella sp.	-1.000	0.317
Nubsella zeaxanthinifaciens	-1.826	0.068
Oceanibaculum pacificum	-1.342	0.180
Oceanibaculum spp.	-1.000	0.317
Oceanimonas smirnovii	-2.060	0.039
Oceanobacillus luteolus	-2.524	0.012
Oceanobacillus sp.	-3.095	0.002
Oculatella coburnii	-1.000	0.317
Ohtaekwangia koreensis	-1.604	0.109
Ohtaekwangia spp.	-2.023	0.043
Oleiphilus messinensis	-1.000	0.317
Oleiphilus spp.	-1.066	0.286
Oleispira spp.	-1.633	0.102
Oleomonas sp.	-1.000	0.317

Opitutus sp.	-1.292	0.196
Opitutus spp.	-0.429	0.688
Opitutus terrae	-2.913	0.004
Oribacterium sinus	-1.604	0.109
Oribacterium sp.	-1.000	0.317
Ornatilinea apprima	-1.633	0.102
Ornithinicoccus hortensis	-2.121	0.034
Ornithinimicrobium sp.	-1.742	0.081
Oscillatoria sp.	-1.000	0.317
Oscillatoria spp.	-3.197	0.001
Oscillospira spp.	-1.342	0.180
Owenweeksia spp.	-2.060	0.039
Oxalicibacterium faecigallinarum	-2.388	0.017
Oxobacter pfennigii	-1.342	0.180
Paenibacillus cellulosilyticus	-2.032	0.042
Paenibacillus chitinolyticus	-1.826	0.068
Paenibacillus contaminans	-1.633	0.102
Paenibacillus favisporus	-1.604	0.109
Paenibacillus graminis	-2.032	0.042
Paenibacillus konsidanse	-1.342	0.180
Paenibacillus nanensis	-1.342	0.180
Paenibacillus stellifer	-1.000	0.317
Paenibacillus wynnii	-1.890	0.059

Palleronia sp.	-1.342	0.180
Paludibacter propionicigenes	-2.751	0.006
Paludibacter sp.	-0.716	0.474
Paludibacter spp.	-1.738	0.082
Paludibacterium sp.	-1.000	0.317
Pannonibacter sp.	-1.000	0.317
Parabacteroides distasonis	-1.633	0.102
Paracoccus marcusii	-3.267	0.001
Paracoccus pantotrophus	-3.624	0.000
Paracoccus spp.	-1.633	0.102
Parasegetibacter luojiensis	-2.060	0.039
Parvibaculum spp.	-1.000	0.317
Parvimonas micra	-1.000	0.317
Pediococcus lactobacillus plantarum	-3.922	0.000
Pedobacter cryoconitis	-3.084	0.002
Pedobacter glucosidilyticus	-1.890	0.059
Pedobacter heparinus	-2.032	0.042
Pedobacter lentus	-1.342	0.180
Pedobacter metabolipauper	-1.000	0.317
Pedobacter sp.	-3.062	0.002
Pedobacter spp.	-3.236	0.001
Pedobacter steynii	-2.207	0.027
Pedobacter wanjuense	-2.530	0.011

Pedomicrobium australicum	-1.000	0.317
Pedomicrobium spp.	-2.692	0.007
Pedosphaera parvula	-2.023	0.043
Pedosphaera spp.	-0.315	0.752
Pelagibacterium halotolerans	-1.841	0.066
Pelagibius litoralis	-1.000	0.317
Pelagibius spp.	-1.000	0.317
Pelagicoccus mobilis	0.000	1.000
Pelobacter carbinolicus	-2.319	0.020
Pelobacter spp.	-2.070	0.038
Pelomonas sp.	-3.926	0.000
Pelomonas spp.	-1.792	0.073
Pelosinus sp.	-1.000	0.317
Pelotomaculum spp.	-0.166	0.868
Peptoclostridium clostridium bifermentans	-2.585	0.010
Peptoclostridium clostridium difficile	-3.312	0.001
Peptoclostridium clostridium sticklandii	-1.000	0.317
Peptococcus sp.	-1.000	0.317
Peredibacter starrii	-2.371	0.018
Perlucidibaca piscinae	-1.633	0.102
Perlucidibaca spp.	-2.677	0.007
Persicirhabdus sediminis	-2.060	0.039
Petrimonas spp.	-1.342	0.180

Phaeospirillum fulvum	-1.633	0.102
Phascolarctobacterium sp.	-2.636	0.008
Phaselicystis spp.	-1.342	0.180
Phenylobacterium sp.	-2.619	0.009
Phenylobacterium spp.	-2.670	0.008
Phycicoccus sp.	-0.739	0.460
Phycisphaera spp.	-1.000	0.317
Phyllobacterium sp.	-2.969	0.003
Pirellula sp.	-1.925	0.054
Pirellula spp.	-1.386	0.166
Planctomyces maris	-1.841	0.066
Planctomyces spp.	-2.155	0.031
Planktothricoides spp.	-1.841	0.066
Planococcus maitriensis	-3.313	0.001
Planococcus sp.	-2.677	0.007
Planomicrobium chinense	-1.342	0.180
Planomicrobium koreense	-3.920	0.000
Planomicrobium mcmeekinii	-3.728	0.000
Plantactinospora sp.	-2.530	0.011
Plasticicumulans lactativorans	-1.342	0.180
Pleomorphomonas spp.	-1.342	0.180
Polaribacter gangjinensis	-2.585	0.010
Polyangium sp.	-1.342	0.180

Polymorphospora rubra	-1.604	0.109
Polynucleobacter cosmopolitanus	-1.105	0.269
Polynucleobacter necessarius	-3.825	0.000
Polynucleobacter rarus	-2.214	0.027
Polynucleobacter spp.	-1.841	0.066
Pontibacter korlensis	-2.032	0.042
Pontibacter populi	-1.000	0.317
Pontibacter sp.	-2.041	0.041
Ponticoccus sp.	-2.940	0.003
Porphyrobacter sp.	-2.692	0.007
Porphyrobacter spp.	-2.459	0.014
Porphyrobacter tepidarius	-3.070	0.002
Porticoccus spp.	-2.699	0.007
Prevotella amnii	-0.736	0.461
Prevotella spp.	-1.248	0.212
Prolixibacter spp.	-0.915	0.360
Propionigenium spp.	-1.940	0.052
Propionivibrio spp.	-2.849	0.004
Prosthecobacter spp.	-1.612	0.107
Prosthecobacter vanneervenii	-1.000	0.317
Prosthecomicrobium spp.	-1.826	0.068
Proteiniphilum acetatigenes	-1.633	0.102
Proteiniphilum spp.	-2.751	0.006

Proteinivorax tanatarense	-1.342	0.180
Pseudoalteromonas sp.	-1.633	0.102
Pseudoalteromonas spp.	-1.342	0.180
Pseudoalteromonas tetraodonis	-1.342	0.180
Pseudoclavibacter spp.	-2.049	0.040
Pseudohongiella sp.	-2.536	0.011
Pseudolabrys sp.	-2.680	0.007
Pseudolabrys spp.	-1.826	0.068
Pseudomonas luteola	-2.790	0.005
Pseudomonas savastanoi	-3.920	0.000
Pseudomonas straminea	-2.207	0.027
Pseudomonas taiwanensis	-3.921	0.000
Pseudomonas tuomuerense	-1.604	0.109
Pseudomonas umsongensis	-1.000	0.317
Pseudomonas veronii	-3.728	0.000
Pseudonocardia spp.	-3.066	0.002
Pseudorhodobacter sp.	-3.921	0.000
Pseudospirillum spp.	-1.000	0.317
Pseudoxanthomonas koreensis	-3.114	0.002
Pseudoxanthomonas sp.	-1.414	0.157
Pseudoxanthomonas taiwanensis	-3.921	0.000
Psychrobacillus bacillus psychrodurans	-1.000	0.317
Psychrobacter aquaticus	-1.342	0.180

Psychrobacter sanguinis	-1.000	0.317
Pullulanibacillus sp.	-2.680	0.007
Puniceicoccus vermicola	-1.342	0.180
Pusillimonas sp.	-2.716	0.007
Pusillimonas spp.	-1.000	0.317
Quadrisphaera sp.	-2.585	0.010
Ramlibacter spp.	-3.724	0.000
Ramlibacter tataouinensis	-3.844	0.000
Rathayibacter tritici	-1.633	0.102
Reyranella massiliensis	-2.214	0.027
Reyranella soli	-1.342	0.180
Reyranella sp.	-1.000	0.317
Rheinheimera aquimaris	-3.570	0.000
Rheinheimera chironomi	-1.857	0.063
Rheinheimera sp.	-1.775	0.076
Rheinheimera texana	-0.803	0.422
Rhizobium leguminosarum	-1.826	0.068
Rhizobium mongolense	-3.590	0.000
Rhizobium tropici	-1.000	0.317
Rhizomicrobium electricum	-1.000	0.317
Rhodanobacter fulvus	-1.414	0.157
Rhodanobacter sp.	-2.821	0.005
Rhodobacter capsulatus	-2.000	0.046

Rhodobacter gluconicum	-2.940	0.003
Rhodobacter sp.	-1.232	0.218
Rhodobacter sphaeroides	-3.833	0.000
Rhodobacter spp.	-1.493	0.135
Rhodobacter vinaykumarii	-1.942	0.052
Rhodobium spp.	-2.060	0.039
Rhodococcus kroppenstedtii	-1.000	0.317
Rhodococcus yunnanensis	-1.342	0.180
Rhodocyclus tenuis	-1.513	0.130
Rhodocytophaga aerolata	-2.060	0.039
Rhodocytophaga spp.	-1.342	0.180
Rhodoferax albidiferax sp.	-1.381	0.167
Rhodoferax antarcticus	-3.475	0.001
Rhodomicrobium sp.	-1.841	0.066
Rhodomicrobium spp.	-2.941	0.003
Rhodomicrobium vannielii	-1.342	0.180
Rhodopila globiformis	-1.633	0.102
Rhodopirellula baltica	-1.000	0.317
Rhodopirellula spp.	-2.489	0.013
Rhodopseudomonas spp.	-2.581	0.010
Rhodothermus spp.	-1.000	0.317
Rhodovastum spp.	-1.342	0.180
Rhodovibrio spp.	-1.000	0.317

Rhodovulum marinum	-1.000	0.317
Rhodovulum sulfidophilum	-1.000	0.317
Rickettsia canadensis	-1.000	0.317
Rickettsiella grylli	-3.316	0.001
Rikenella sp.	-0.978	0.328
Rikenella spp.	-1.186	0.236
Rivibacter sp.	-2.121	0.034
Robiginitomaculum antarcticum	-1.890	0.059
Roseburia faecis	-1.342	0.180
Roseburia spp.	-1.633	0.102
Roseibaca ekhonensis	-1.342	0.180
Roseibacillus spp.	-2.825	0.005
Roseicyclus spp.	-2.032	0.042
Roseiflexus spp.	-3.140	0.002
Roseinatronobacter sp.	-3.321	0.001
Roseobacter sp.	-1.342	0.180
Roseococcus sp.	-0.405	0.686
Roseomonas lacus	-1.633	0.102
Roseomonas ruber	-1.890	0.059
Roseomonas stagni	-2.225	0.026
Roseovarius sp.	-0.564	0.573
Rothia sp.	-3.636	0.000
Rubellimicrobium mesophilum	-3.572	0.000

Rubellimicrobium spp.	-3.632	0.000
Rubrimonas sp.	-1.000	0.317
Rubrivivax gelatinosus	-3.045	0.002
Rubrobacter spp.	-1.233	0.217
Rudaea cellulosilytica	-1.342	0.180
Rudanella sp.	-1.826	0.068
Rufibacter sp.	-3.226	0.001
Ruminiclostridium clostridium aldrichii	-1.826	0.068
Ruminiclostridium clostridium cellobioparum	-2.714	0.007
Ruminiclostridium clostridium josui	-2.536	0.011
Ruminiclostridium clostridium papyrosolvens	-2.232	0.026
Ruminococcus callidus	-1.841	0.066
Rummeliibacillus pycnus	-2.953	0.003
Runella slithyformis	-2.232	0.026
Runella spp.	-3.844	0.000
Saccharibacter spp.	-1.362	0.173
Saccharofermentans acetigenes	-2.410	0.016
Saccharomonospora azurea	-1.000	0.317
Saccharophagus spp.	-2.341	0.019
Saccharospirillum sp.	-1.000	0.317
Saccharospirillum spp.	-1.604	0.109
Saccharothrix xinjiangensis	-1.000	0.317
Salinicoccus roseus	-3.537	0.000

Salinicoccus sp.	-2.410	0.016
Salinimicrobium sp.	-1.000	0.317
Sandaracinus amylolyticus	-1.000	0.317
Sandaracinus spp.	-2.751	0.006
Sandarakinorhabdus sp.	-1.342	0.180
Sandarakinorhabdus spp.	-2.410	0.016
Sanguibacter antarcticus	-1.826	0.068
Schlegelella spp.	-1.342	0.180
Sedimentibacter spp.	-1.000	0.317
Sediminibacterium salmoneum	-1.826	0.068
Sediminibacterium sp.	-2.540	0.011
Sediminibacterium spp.	-0.825	0.409
Segetibacter spp.	-0.085	0.933
Sejongia spp.	-3.635	0.000
Seohaeicola saemankumensis	-3.921	0.000
Serinicoccus sp.	-3.520	0.000
Shimazuella kribbensis	-1.000	0.317
Shimazuella sp.	-1.342	0.180
Shinella spp.	-2.989	0.003
Shinella zoogloeoides	-1.000	0.317
Sideroxydans spp.	-2.810	0.005
Silanimonas sp.	-1.633	0.102
Simplicispira sp.	-2.060	0.039

Singulisphaera sp.	-2.264	0.024
Singulisphaera spp.	-2.041	0.041
Sinorhizobium ensifer fredii	-1.342	0.180
Sinorhizobium sp.	-1.604	0.109
Skermanella sp.	-2.366	0.018
Skermanella spp.	-1.604	0.109
Smaragdicoccus niigatensis	-2.232	0.026
Sneathiella sp.	-1.342	0.180
Solimonas soli	-2.375	0.018
Solirubrobacter spp.	-2.380	0.017
Solitalea canadensis	-0.905	0.366
Solitalea spp.	-1.633	0.102
Sorangium cellulosum	-2.060	0.039
Sphaerobacter spp.	-1.852	0.064
Sphaerobacter thermophilus	-2.214	0.027
Sphaerotilus natans	-3.633	0.000
Sphaerotilus spp.	-3.920	0.000
Sphingobacterium siyangensis	-1.566	0.117
Sphingobium chlorophenolicum	-1.633	0.102
Sphingobium chungbukensis	-1.342	0.180
Sphingobium faniae	-1.000	0.317
Sphingobium xenophagum	-2.950	0.003
Sphingobium yanoikuyae	-3.921	0.000

Sphingomonas faeni	-1.633	0.102
Sphingomonas melonis	-3.066	0.002
Sphingomonas wittichii	-3.069	0.002
Sphingomonas yunnanensis	-1.000	0.317
Sphingopyxis chilensis	-2.032	0.042
Sphingopyxis sp.	-3.421	0.001
Sphingopyxis spp.	-1.342	0.180
Sphingosinicella spp.	-1.890	0.059
Spiribacter sp.	-1.342	0.180
Spirobacillus cienkowskii	-1.604	0.109
Spirochaeta aurantia	-1.732	0.083
Spirochaeta bajacaliforniensis	-1.342	0.180
Spirochaeta sp.	-1.604	0.109
Spirochaeta spp.	-3.157	0.002
Spirosoma linguale	-1.000	0.317
Spongiibacter sp.	-1.604	0.109
Sporichthya sp.	-1.888	0.059
Sporichthya spp.	-0.325	0.725
Sporobacter termitidis	-2.456	0.014
Sporomusa spp.	-0.709	0.478
Stappia spp.	-1.841	0.066
Stella spp.	-1.841	0.066
Stenotrophomonas acidaminiphila	-2.897	0.004

Steroidobacter spp.	-3.626	0.000
Sterolibacterium sp.	-3.422	0.001
Sterolibacterium spp.	-0.447	0.655
Streptomyces glaucescens	-3.921	0.000
Streptomyces macrosporus	-1.000	0.317
Streptomyces phaeopurpureus	-1.342	0.180
Streptomyces scabrisporus	-1.633	0.102
Streptomyces werraensis	-2.275	0.023
Streptomyces yokosukanensis	-3.078	0.002
Streptosporangium vulgare	-1.342	0.180
Subdoligranulum spp.	-2.060	0.039
Sulfuricurvum kujiense	-2.524	0.012
Sulfuricurvum spp.	-0.261	0.794
Sulfurimonas autotrophica	-1.048	0.295
Sulfurimonas paralvinellae	-1.841	0.066
Sulfurimonas spp.	-2.838	0.005
Sulfurisoma sediminicola	-1.915	0.056
Sulfurospirillum deleyianum	-3.732	0.000
Sulfurospirillum spp.	-1.307	0.191
Sulfurovum spp.	-1.000	0.317
Sunxiuqinia faeciviva	1.342	0.180
Sunxiuqinia sp.	-1.342	0.180
Symbiobacterium spp.	-1.301	0.193

Synechococcus sp.	-1.604	0.109
Synechococcus spp.	-0.318	0.750
Synechocystis sp.	-1.342	0.180
Syntrophobacter sp.	-1.890	0.059
Syntrophobacter spp.	-2.041	0.041
Syntrophomonas sp.	-1.857	0.063
Syntrophomonas spp.	-1.021	0.307
Syntrophus sp.	-2.060	0.039
Syntrophus spp.	-2.137	0.033
Tannerella spp.	-1.342	0.180
Telmatobacter spp.	-0.962	0.336
Tepidimonas spp.	-2.881	0.004
Tepidiphilus petrobacter sp.	-3.321	0.001
Teredinibacter sp.	-1.000	0.317
Terrabacter sp.	-3.300	0.001
Terribacillus halophilus	-1.342	0.180
Terribacillus saccharophilus	-2.264	0.024
Terriglobus roseus	-1.000	0.317
Terrimonas sp.	-0.190	0.849
Terrimonas spp.	-0.081	0.936
Tetrasphaera spp.	-3.628	0.000
Thalassobacillus devorans	-3.421	0.001
Thalassobaculum sp.	-2.636	0.008

Thalassolituus sp.	-2.236	0.025
Thalassolituus spp.	-3.235	0.001
Thauera mechernichensis	-2.264	0.024
Thauera phenylacetica	-1.213	0.225
Thauera selenatis	-3.183	0.001
Thauera spp.	-2.282	0.023
Thermacetogenium spp.	-1.000	0.317
Thermaerobacter spp.	-3.194	0.001
Thermincola spp.	-1.342	0.180
Thermoanaerobacter uzonensis	-1.000	0.317
Thermobacillus sp.	-1.342	0.180
Thermodesulfobacterium spp.	-0.948	0.343
Thermodesulfobium spp.	-1.000	0.317
Thermodesulfovibrio spp.	-2.023	0.043
Thermoleophilum album	-1.000	0.317
Thermoleophilum spp.	-2.539	0.011
Thermomicrobium spp.	-2.207	0.027
Thermomonas brevis	-3.624	0.000
Thermomonas fusca	-2.032	0.042
Thermomonas haemolytica	-2.533	0.011
Thermomonas sp.	-1.633	0.102
Thermomonas spp.	-3.103	0.002
Thermosporothrix spp.	-1.342	0.180

Thermovum composti	-1.000	0.317
Thermus sp.	-1.604	0.109
Thermus spp.	-2.201	0.028
Thermus thiopara	-1.890	0.059
Thioalkalibacter halophilus	-0.771	0.441
Thioalkalivibrio nitratireducens	-0.578	0.563
Thioalkalivibrio spp.	-3.494	0.000
Thiobaca spp.	-1.000	0.317
Thiobacillus sp.	-0.184	0.854
Thiobacillus spp.	-1.861	0.063
Thiobacter spp.	-1.000	0.317
Thiocystis violacea	-1.342	0.180
Thiodictyon bacillosum	-2.530	0.011
Thiohalophilus spp.	-1.342	0.180
Thiomicrospira halophilus	-0.137	0.891
Thiomicrospira sp.	-2.848	0.004
Thioprofundum hispidum	-1.342	0.180
Thioprofundum spp.	-1.000	0.317
Thiorhodococcus bheemlicus	-3.114	0.002
Thiorhodospira spp.	-2.552	0.011
Thiorhodovibrio winogradskyi	-2.060	0.039
Thiothrix caldifontis	-2.555	0.011
Thiothrix disciformis	-1.604	0.109

Thiothrix spp.	-1.342	0.180
Thiovirga spp.	-0.597	0.550
Thorsellia spp.	-1.414	0.157
Tissierella spp.	-2.032	0.042
Tistrella spp.	-1.102	0.270
Tolumonas auensis	-2.869	0.004
Tolumonas spp.	-2.410	0.016
Treponema primitia	-1.000	0.317
Treponema zuelzerae	-1.342	0.180
Trichococcus pasteurii	-3.930	0.000
Truepera spp.	-1.342	0.180
Tumebacillus ginsengisoli	-1.841	0.066
Tumebacillus permanentifrigoris	-1.633	0.102
Tumebacillus sp.	-2.232	0.026
Tumebacillus spp.	-1.841	0.066
Turicibacter spp.	-0.949	0.342
Uliginosibacterium gangwonense	-2.214	0.027
Uliginosibacterium sp.	-2.333	0.020
Uncultured candidatus brocadia sp.	-1.604	0.109
Uncultured candidatus competibacter sp.	-1.342	0.180
Uncultured candidatus microthrix sp.	-2.214	0.027
Uncultured candidatus odyssella sp.	-2.384	0.017
Uncultured candidatus pelagibacter sp.	-1.000	0.317

Uncultured candidatus planktophila sp.	-0.523	0.601
Uncultured candidatus protochlamydia sp.	-1.000	0.317
Uncultured candidatus rhabdochlamydia sp.	-2.226	0.026
Uncultured candidatus solibacter sp.	-2.032	0.042
Undibacterium sp.	-3.361	0.001
Undibacterium spp.	-2.891	0.004
Vallitalea guaymasensis	-1.000	0.317
Verrucomicrobium sp.	-1.633	0.102
Verrucomicrobium spp.	-1.422	0.155
Vibrio aestuarianus	-2.887	0.004
Vibrio orientalis	-2.226	0.026
Victivallis spp.	-2.060	0.039
Victivallis vadensis	-2.333	0.020
Virgibacillus halodenitrificans	-3.825	0.000
Virgisporangium ochraceum	-1.604	0.109
Vitreoscilla filiformis	-3.825	0.000
Vogesella indigofera	-3.924	0.000
Vogesella sp.	-0.411	0.681
Vogesella spp.	-3.307	0.001
Weissella cibaria	-1.785	0.074
Weissella fabalis	-3.653	0.000
Woodsholea maritima	-1.857	0.063
Xanthobacillum maris	-3.104	0.002

Xanthobacter spp.	-2.264	0.024
Xenorhabdus nematophila	0.000	1.000
Xenorhabdus vietnamensis	-3.929	0.000
Xylanimonas cellulosilytica	-2.264	0.024
Zavarzinella spp.	-1.000	0.317
Zoogloea oryzae	-2.724	0.006
Zoogloea ramigera	-2.677	0.007
Zoogloea resiniphila	-2.829	0.005
Zoogloea spp.	-2.524	0.012
Zymophilus spp.	-1.342	0.180