OCCURRENCE OF EXTENDED –SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE AND BACTERIAL LOADS IN DUST FROM POULTRY FARMS IN LUSAKA-ZAMBIA

BY

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH ANALYTICAL EPIDEMIOLOGY

UNIVERSITY OF ZAMBIA

DECLARATION

I**, Linda Basikolo,** do hereby declare that this dissertation is a result of my own original efforts and work, and that to the best of my knowledge, the findings have never been previously presented to the University of Zambia or any other university for the award of any academic qualification. Where assistance was sought, it has been accordingly acknowledged. It has been presented in accordance with the guidelines for MSc dissertation of the University of Zambia.

Signature ……………………….   Date………………….

CERTIFICATE OF APPROVAL

The University of Zambia approves the dissertation submitted by **LINDA BASIKOLO,** as fulfilling the partial requirements for the award of the Master of Science Degree in One Health Analytical Epidemiology by the University of Zambia.

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Examiner 2 Signature Date

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Examiner 3 Signature Date

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Chairperson Signature Date
(Board of Examiner)

ABSTRACT

Poultry houses accommodate substantial amounts of microorganisms which are carried by the dust present in these premises and the effects of this dust on humans and animal health is detrimental when inhaled. Extended Spectrum beta lactamases are a universal public health alarm which are frequently identified in humans and animals including poultry. Poultry dust is a possible source of drug resistant microorganisms including Extended spectrum beta lactamases which confer antimicrobial resistance to different classes of antimicrobials including beta lactam, cephalosporins and fruoloquinolones. The concentration of microorganisms in dust from poultry houses in Lusaka-Zambia is not known and in order to know the risks for birds, environment and people working in these houses, it is important to have information on the microbial composition of this dust, therefore, this study is conducted to determine poultry dust bacterial composition in farms in Lusaka, Zambia.

A cross sectional study with quantitative data collection approach was conducted in Kafue, Lusaka and Chongwe districts of Lusaka province where 351 dust samples from intensive poultry production were collected from 29 poultry farms (10 kafue,12 Lusaka and 7 Chongwe). 4 farms were for indigenous chickens, 15 layer farms and 10 broiler farms. They were analyzed for total viable bacteria counts (cfu/g), the occurrence of extended-spectrum beta- lactamases (ESBL)-producing Enterobacteriacea, antimicrobial resistance for ESBL-E isolates. The association between the sociodemographic issues and the occurrence of the pathogens in dust from poultry houses in Lusaka- Zambia was also determined.

The total viable bacterial counts was 2.599 x10^8 cfu/g. For Enterobacteriaceae was 2.296x10^8 cfu/g. 62 Positive ESBL samples occurred in 16 farms (n=29) where ESBL samples was 4.842x10^4cfu/g. E.Coli showed highest resistant rate amongst other isolates. All ESBL- E isolates showed 100% resistance rate to penicillin followed by 56.5% for cefotaxime and 48.4% for ciprofloxacin. There was no association between the sociodemographic issues and the occurrence of the pathogens in dust from poultry houses.

The results obtained would help that ensures poultry farms comply to safety rules by providing protective clothing for their workers and also close monitoring of antibiotic usage to poultry to avoid dust transmitted antimicrobial organisms to humans, animals and the environment.

DEDICATION

To my dear husband, Godfrey and our daughter and son Favour and Faith, respectively for financial and moral support they rendered to me during the entire study period of this degree. Their continuous prayers and encouragement made me complete this degree successfully. To them I say, thank you and I love you all. Above all, I thank God Almighty for His sufficient grace upon my life.

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To my lovely kids, Favour and Faith Elisha Munkhondya, your prayers worked the magic. I say thank you.

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ABBREVIATIONS AND ACRONYMS

ARB	Antimicrobial Resistance Bacteria
ARGs	Antimicrobial resistance genes
BHI	Brain Heart Infusion
CFU	Colony Forming Unit
CHL	Chloramphenicol
CLSI	Clinical and Laboratory Standard Institute
E. coli	*Escherichia coli*
ESBL	Extended Spectrum Beta Lactamases
ESBL-E	Extended Spectrum Beta Lactamase Enterobacteriaceae
GDP	Gross Domestic Product
GEN	Gentamicin
K. pneumoniae	*Klebsiella pneumonia*
LA-MRSA	Livestock associated methicillin-resistant *Staphylococcus aureus*
MacC	MacConkey
MacC+CTX	MacConkey and Cefotaxime
MRSA	Methicillin Resistant *Staphylococcus aureus*
NCCLS	National Committee for CLINICAL Laboratory Standards
NHRA	National Health Research Authority
ODTS	Organic Dust Toxic Syndrome
PEN	Penicillin
PHA	Poultry House Attendants
PPE	Personal Protective Equipment
SD	Standard Deviation
TET	Tetracycline
UNZABREC	University of Zambia Biomedical Research Ethics Committee
USDA	United States, Department of Agriculture
XLD	Xylose Lysine Desoxycholate
β	Beta
DR	Doctor

CHAPTER ONE

INTRODUCTION

1.1 Background

The phenomenon of organic dust particles on poultry farms, which are formed of both viable and non-viable particles, can cause a serious risk to bird production and stockmen health (David et al., 2015; Banhazi et al., 2018). Many studies have shown that the dust which is present in laying birds can be the origin of potential pathogens, zoonotic agents, antimicrobial resistant bacteria and toxic compounds (Schwarz et al, 2017). Livestock housing systems, for poultry in particular, form and produce large amounts of dust (David et al., 2015; Kim and Ko, 2020).

Airborne dust or settled dust from surfaces in livestock structures could include plant particles, particles from food, fecal particles, epithelia from birds, bacterial cells and spores, fungi and fungal spores, viruses, endotoxins, mycotoxins, and antibiotic agents (Kim and Ko, 2020). The amount and the constitution of particles in poultry houses are influenced by factors such as housed species, stocking density, age and fattening period of the birds, feeding system, antibiotic treatment, bedding material, humidity, ventilation system, and implementation of hygienic measures (Schulz et al., 2019). The health sequel of dust particles on animals is dependent on the nature of the particles (organic, inorganic and non-specific), the composition of the particles carried and the diameter of the particles (Ostović et al., 2016).

The different levels of the effective transmission of airborne infection are dependent on the production of the pathogenic agents from the origin and the arrival of the adequate numbers of viable organisms to cause infections in a secondary host. Environmental exposure is a common threat for all such organisms such as Staphylococcus species during transmission between hosts. Transmission through air has been alluded as another possible way for Staphylococcus aureus and MRSA, as contaminated dust particles may disseminate in the air in shelters, and subsequently be inspired by people working in these barns (Masclaux et al., 2013). Increased dust levels may irritate the mucous membranes of the respiratory system and causing inflammatory and allergic reactions and reduce animal resistance. Therefore, this direct contact with colonized animals and frequent contact with contaminated surfaces and inhalation of contaminated air are assumed to contribute to nasal colonization (Cuny et al, 2015; Bos et al., 2016).

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1.2 Statement of the problem

Zambia as a developing country has in the recent past years seen a steady rise in poultry production which plays a vital role to Zambia's economy as a source of food and employment. However, within poultry houses, the sources of dust are food, feathers, manure, bedding and drugs (Gržinić et al., 2023). Depending on their concentration, aerosol pollutants can be dangerous to the respiratory health of birds within the regulated areas and may also be harmful to the well-being of workers or dwellers living close to poultry production areas. The studies addressing dust in animal houses have been put in a summary in a review from Zhao et al (2014). To my knowledge, there is no information available in the scientific literature regarding viable and non- viable particles in dust from poultry farms in Zambia. Since most studies about farm dust composition were conducted in temperate zones, this research is aimed at gaining more information on the situation in ventilated barns under tropical conditions.

There is a threat of increased Antimicrobial resistant organisms in the poultry environment which can cause danger to human lives (Agyare et al., 2018).An increased number of various antimicrobials are used in poultry production for therapeutic treatment as well as growth promoters in most countries including Zambia ((Paintsil et al., 2021)) and increased number of such antimicrobials are also used as essential drugs in human medicine. Therefore, this result in elimination of susceptible bacterial strains leaving the resistant ones and the consequences be treatment failure, economic losses and this can act as a genetic pool for transmission to humans. The prevalence of the ESBL-E in poultry dust in Zambia is not known. Some studies were done in slaughtered broiler chickens where Enterobacteriaceae prevalence of isolates was determined (Mwansa et al., 2022), as well as ESBL-E prevalence in poultry litter (Brower et al., 2017) but not in the dust which is produced in these houses and inhaled by both poultry and humans working in the houses. If this dust cannot be assessed, farmers might be colonized with these inhaled resistant bacteria and become carriers (Dahms et al., 2014) as well be a probable long-term reservoir of these resistant bacteria and zoonotic agents. For example, there is evidence that resistant Escherichia coli are capable of surviving for a considerable period of time in dust from livestock structures, (Schulz et al., 2016). Therefore, knowledge of the composition of these dusts is very significant to understanding their zoonotic potential.

1.3 Justification of the study

The Zambia poultry industry has been one of the fastest growing subsectors of the livestock industry which generates about 5% of the national Gross Domestic Product (GDP) and an estimated 47% of the livestock GDP (Ministry of Fisheries and Livestock, 2019). In Zambia, poultry is currently considered as the main source of meat consumed by the population, totaling an estimated 50% of the total meat consumption in the country (Ministry of Fisheries and Livestock, 2019). As a result, a lot of people have found business opportunities in poultry production because of demand for meat and also jobs that are being created on these farms. However, little is known about the risks these people who come in contact with poultry have and therefore, the need for the research to be done to assess the factors that contribute to the occurrence of these pathogens. Farmers might be colonized and become carriers (Dahms et al,2014). This means that the colonized individuals can transmit these pathogens to their family members and the community. It can also be a possible reservoir for resistant bacteria and zoonotic agents. Resistant *E. coli* are capable of surviving for a considerable period of time in dust from livestock structures (Schulz et al, 2016) thereby indicating that they can be easily transmitted to human working in these structures. High dust burden can distinctly influence the health of animals kept in confined buildings as well as workers since dust exerts adverse effects on the structure and protective functions of the mucous membranes of the respiratory system and conjunctiva which leads to allergies and inflammation (Joubert et al., 2020). Therefore, the personnel responsible in the management of the animals need to understand that dust from poultry houses carry dangerous particles. This knowledge may reduce the occupational hazards thereby reducing dust related infections. This study aimed at generating evidence on whether dust from poultry houses contain biological materials that are hazardous to the health of workers working in these houses in Lusaka, Zambia.

1.4 Study Objectives

1.4.1 Main Objective

To assess the occurrence of extended–spectrum beta-lactamase-producing *Enterobacteriaceae* and bacterial loads and factors associated with occurrence of these pathogens in dust from poultry houses in Lusaka province.

1.4.2 Specific Objectives

a) To determine the bacterial load in dust from Poultry houses.
b) To determine the antimicrobial susceptibility of *Enterobacteriaceae* isolated from dust in poultry houses.
c) Assessing the association between the sociodemographic issues and the occurrence of the pathogens in dust from poultry houses in Lusaka- Zambia.

1.5 Research Question

Does extended spectrum beta lactamase producing *Enterobacteriaceae* occur in dust from poultry houses in Lusaka?

CHAPTER TWO

LITERATURE REVIEW

2.1 Global Poultry production

Poultry products including meat and eggs make a substantial contribution to global food production and as a source of protein (Mottet and Tempio, 2017a). It was reported by the United States Department of Agriculture (USDA) in 2016, that on approximation, egg production was at 70 million tonnes and poultry meat production was more than 100 million tonnes accounting to more than one third of global meat production (Pawar et al., 2016).

Furthermore, poultry production is the fastest growing agricultural sub sector, especially in the developing countries due to its short production cycle and its ability to convert a broad variety of agriculture food by-products and wastes into eggs and meat consumed by humans. The global poultry production is expected to increase its expansion as demands for meat and eggs are pushed by the rising incomes, urbanization and increasing population growth. However, poultry production poses a threat to human health mainly as a vector of infectious diseases and the role it plays in antimicrobial resistance (Mottet and Tempio, 2017b).

Foodborne transmission of pathogenic microorganisms has been recognized as a hazard in the past few decades and a source of bacterial pathogens including *E.coli* which has the antibiotic-resistant strains of zoonotic origin (Gregova and Kmet, 2020). The development of antibiotic resistant bacteria and the existence of such bacteria on poultry farms in any country is of global importance due to the likelihood of their local or even worldwide spreading by animals, humans, insect vectors, agricultural products or surface water (Singer et al., 2016). Poultry litter might grow into a possible source of infectious agents and *E. coli* is used as an indicator bacterium to detect their probable existence (Mwansa et al., 2022).

Antibiotics are occasionally used as growth promoters on poultry farms so it is not surprising that resistance of *E. coli* to antibiotics has broaden (Brower et al., 2017) and mostly these antimicrobials are partially absorbed in the chicken gastrointestinal tract and up to 90% of the given dose can be discharged in faeces (Ribeiro et al., 2023). It is well known that usage of increased quantities of antibiotics in poultry farming may end up in an escalated load of resistant bacteria in poultry, their excretory products and consequently in the litter and environment (Polianciuc et al., 2020).

Resistant bacteria and resistant genes from poultry and their production environment can be transferred to humans by direct contact between poultry and humans ,indirectly via the food chain or as a result of fertilizer use (Marshall and Levy, 2011). Poultry litter can amplify the expansion of antibiotic resistance in soil bacterial groups and could be a playground for antibiotic resistance genes from selfish genetic elements. When poultry litter which has antibiotic residues and antibiotic resistant bacteria strain is used as fertilizer, the extent and sequel of these antibiotics and antimicrobial resistant bacteria in the environment are of public health importance because they end up being transmitted to humans (Kyakuwaire et al., 2019).

2.2 Poultry production in Zambia

The Zambian poultry industry has been one of the fastest growing subsectors of the livestock sector which generates about 5% of the national Gross Domestic Product (GDP) and an estimated of 47% of the livestock GDP (Ministry of Fisheries and Livestock,2019). In Zambia, poultry is currently considered as the main source of meat consumed by the population, totaling an estimated of 50% of the total meat consumption in the country. This industry has helped Zambia to move from an importing country to self-reliant and self-sufficient country. The poultry production trend shows that the total growth was at 89% in 2018 compared to 75% recorded in 2017 (Ministry of Fisheries and Livestock, 2019).

2.3 Microbial contaminations of poultry farms and health effects of dust in poultry farm houses on poultry attendants

Stocking density in poultry houses is very high especially in intensive production systems hence making it hard to keep up optimal microclimate and sanitary conditions. Therefore, the health status of both birds and personnel is affected by air microflora. High temperature, humidity and particulate pollution levels sustain the growing and developing microorganisms (Kumar et al., 2021).

In poultry farms an increased load of fecal excretion is produced therefore, feces, feed, dander and beddings contribute to the microbial contamination of poultry houses. The contribution of feed to microbial contamination is because of the addition of the therapeutic doses of antimicrobial agents which are used as growth promoters.

Respiratory diseases of both humans and animals such as bronchitis, pneumonia, pulmonary fibrosis and asthma have multiple sources, and the amount and characteristics of airborne dust take part in a dominant role (May et al, 2012).

High levels of dust, micro-organisms as well as gases present in the respiratory pathway can lead to reduced resistance and can induce inflammation and infections of animals (Roque et al., 2015). This can happen when animals and humans inhale large amounts of dust from poultry houses which can lead to overloading of clearance mechanisms in the respiratory tract and mechanical irritation which facilitates beginning of infection (Viegas et al., 2013a).

In addition to that, poultry houses dust can act as a reservoir for zoonotic agents and resistant bacteria. It has shown that Zoonotic *Salmonella* species can occur and survive in dust from layer houses and can be transmitted horizontally through air pathway (Hossain et al., 2021). This might also increase the spread within the flock, and that can result into an increased probability of contaminated eggs (Gole et al., 2014). A further risk is the transmission of probable dangerous microbes from animals to farmers. For example, livestock- associated methicillin-resistant *Staphylococcus aureus (*LA-MRSA*)* colonize farmers who frequently come into contact with these bacteria and they are at an increased risk than those people who do not come into contact with livestock (Cuny et al., 2015). Direct contact with colonized animals and frequent contact with contaminated air are assumed to contribute to nasal colonization (Bos et al., 2016).

Previous studies have shown that Extended-spectrum B-lactamase (ESBL)-producing bacteria have been reported worldwide in poultry (Blaak et al., 2015).Ahmed et al,(2020) did the study on occurrence of ESBL-E in poultry dust from laying hens in Egypt. This study involved the laying hens only leaving out broiler and local chickens found and Mwansa et al,(2023) concentrated their study on antimicrobial resistance patterns from farm workers in broiler poultry production.

Extended-spectrum β-lactamase (ESBL) are enzymes responsible for the hydrolysis of oxyimino-beta lactam antibiotics like penicillins, cepharosporins and monobactams (Paterson and Bonomo, 2005), which are significant medicinal agents for the treatment of serious human and animal diseases. Extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* are a real threat to human health. They were responsible for 1700 deaths in the USA in 2013 due to therapeutic failure in severe infections (Adeolu et al., 2016). These bacteria secrete enzymes, β-lactamase, which confers resistance to β- lactam antimicrobials, including penicillins, cephalosporins and monobactams. The β-lactamases are often located on extra chromosomal mobile genetic elements, such as plasmids (Shaikh et al., 2015). Since plasmids harbouring resistance genes can be transferred between bacteria of the same species or different genera, such exchanges increase the development of reservoirs of resistance genes in animals and the environment (Lerminiaux and Cameron, 2019). The presence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* in several ecological niches such as commensals in humans and animals as environmental contaminants, is reported worldwide (Tseng et al, 2023). However, in the last decades a niche that has raised a great concern, for being able to function as a reservoir and vehicle of transmission and dissemination of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* is the production sites of animals due to their direct connection with the food chain (Madec et al., 2017; Palmeira and Ferreira, 2020). This circulation of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* within our ecosystem creates a consensual concern to the scientific community and to the authorities involved in the one health approach (Robinson et al., 2016).

There is epidemiological evidence that the health of people working in animal houses may be harmed by constant exposure to air contaminants like ammonia, microorganisms, dust and endotoxins (Steinfeld et al., 2013; Sethi et al., 2019). Many studies have indicated links between dust and ill health in a number of livestock associated industries (Sigsgaard et al., 2020). Providing a safe and health working environment for workers is of great importance for any industry as well as animal production (Schmitt et al, 2018). A collection of acute respiratory symptoms have been described by workers following exposure to their work environment, but not connected to the generalized clinical syndrome (de Perio et al, 2020). A range of clinical symptoms in people include occupational asthma due to allergens in the air space, chronic bronchitis, chronic airway obstruction, allergic alveolitis and organic dust toxic syndrome(ODTS) (Baur, 2012). Studies in livestock have shown that due to prolonged influence of endotoxins, chronic bronchitis and deficiency of lung function might develop and there is a significant likelihood that both acute and chronic effects are caused by inflammatory responses in the lungs where alveolar macrophages play a role (Baur, 2012). Figure 2.1 below shows the conceptual framework of dust inhalation that might results into disease and reduced productivity.

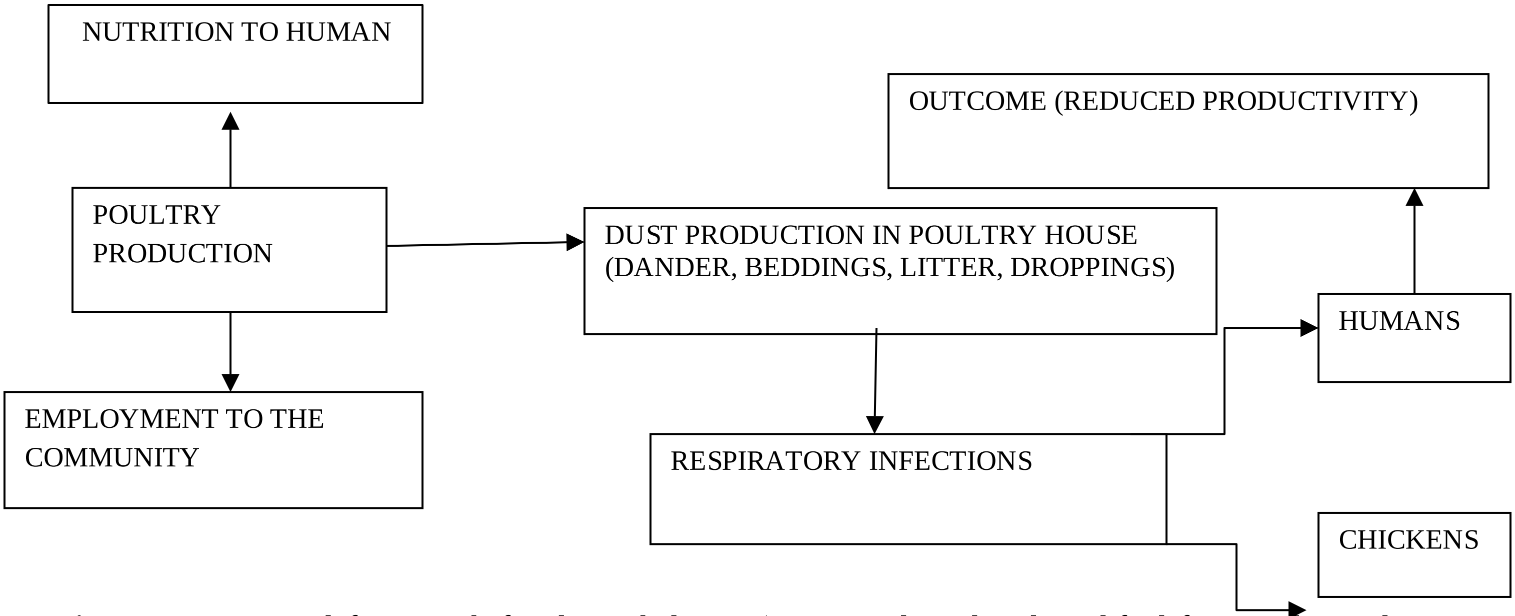

Fig 2. 1: Conceptual framework for dust inhalation (Source; adopted and modified from Sethi et al, 2019).

2.4 reservoirs of ESBL infections

The primary reservoir of zoonotic pathogens are the food-producing animals and the discovery of extended spectrum beta-lactamase (ESBL) producers among *Escherichia coli* and *Salmonella* strains has increased in recent years (Hammerum et al., 2014). They are not restricted to animal reservoir, ESBL producing organisms can be noticed in the fecal flora of children and they exist on extremely transmissible plasmids (Birgy et al., 2012). Infections in the community with ESBL-producing organisms are connected with animal consumption of antibiotics, foodstuffs and regular contact with patients (Paterson and Bonomo, 2005).A study conducted in Germany proved that CTX-M genes were high in health broilers which after some time disseminate ESBLs from the environment to the food chain (Reich et al, 2013). Samples of excreta obtained from various environmental places in Spain like livestock farms, animal meat products and effluent (sewage) also showed extensive existence of ESBL producing bacteria carriages (Dorado-García et al., 2018).

Some researchers recognized an extensive spread of ESBL producing *E. coli* in health food producing animals like meat, raw milk and fish (Geser et al, 2012). Contamination of meat products when preparing the meat which include slaughtering, dressing and evisceration pose another risk which can lead to increased extent of widespread of resistant ESBLs genes in the human population (Reich et al., 2013).

2.5 Antimicrobial resistance in ESBL producing organisms

Substantial usage of antibiotics has been established to be a risk factor for acquisition for ESBL-producing organisms (Shaikh et al., 2015). ESBL-producing strains of *Enterobacteriaceae* have become a leading worry in hospitals and communities (Guzmán-Blanco et al., 2014). Cases of treatment failure with cephalosporins antibiotics have been shown with these ESBL producing bacteria like *K. pneumoniae* and *E. coli* (Park et al., 2022). Increased trend of resistance to most used antibiotics like co-trimoxazole, ampicillin, tetracycline, erythromycin, gentamycin as well as the third generation cephalosporins has been revealed in published from Democratic Republic of Congo, Zambia, Mozambique and Tanzania (Mshana et al, 2013).

There is a rise in multidrug antimicrobial resistance mostly in developing countries caused by *E. coli* (Ayukekbong et al, 2017a). The majority of ESBL-producing isolates have an increased resistant rate to gentamycin, chloramphenicol, streptomycin, ampicillin, tetracycline and cefotaxime which limit the therapeutic alternatives (Santos et al., 2021). Possible reservoirs for multidrug resistance for gram negative bacteria are food animals as well as the environment (Manyi-Loh et al., 2018). Food animals t o a large extent contribute to a significant proportion of the *E. coli* in the human digestive tract which includes drug resistant strains and are fairly host specific with a revealed potential animal origin drug-resistant strain like fluoroquinolone-resistant *E. coli* from chickens with a likelihood of colonizing or causing human infections (Collignon et al., 2016).

Usage of antibiotics as growth promoters uncontrollably in food animals has highly contributed to the antibiotic resistance status development (Manyi-Loh et al., 2018). Additionally, bacteria that habours ESBLs has shown the added resistance to other antimicrobial classes like co-trimoxazole, quinolones, trimethoprim and aminoglycosides which cause difficulties in therapeutic treatment (Ruppé et al, 2015). Furthermore, horizontal gene transfer has an important role in spreading genes that are linked to antimicrobial resistance amongst bacteria species located on plasmids (Sun et al., 2019). Tetracyclines have been used as livestock feed additives because it stimulates weight gain in some domestic animals

(Chattopadhyay, 2014; Granados-Chinchilla and Rodríguez, 2017). Routinely, tetracyclines have been fed to calves, chickens, turkeys, sheep and pigs (Granados-Chinchilla and Rodríguez, 2017). Food industry is the significant user of antibiotics and it was reported that in many industrialized countries, the total amount of antibiotic usage for food production surpasses the amount used in human medicine and thus a significant factor in exposing bacteria to antibiotics (Founou et al., 2016; Ayukekbong et al., 2017; Manyi-Loh et al., 2018). Emergency and spread of Salmonella enterica (non-typhi), *Escherichia coli* and other gut bacteria resistant to third generation cephalosporins have been linked by some authors to ceftiofur use in veterinary practice and animal husbandry (Carson et al., 2019).

2.6 Treatment and control of antimicrobial resistant infections

Nearly all infections caused by ESBL- producing *Enterobacteriaceae* are significant in clinical practice because they are mostly connected with high morbidity and mortality (Shamsrizi et al., 2020). Difficulties in treating ESBL-*Enterobacteriaceae* infections are related to co-resistance which is perhaps the effect of the ESBL plasmid harbouring a number of antibiotic resistance determinants which makes treatment hard and lead to co-selection of various resistant bacterial strains (Shaikh et al., 2015). Increased mortalities have been reported in patients diagnosed with these ESBL-*Enterobacteriaceae* who were not given proper antibiotic medication than those patients who had non-ESBL-*Enterobacteriaceae* of the similar species (Paterson and Bonomo, 2005). Several outbreaks of infections resulting from ESBL-*Enterobacteriaceae* were reported in South Africa and North African countries (Storberg, 2014). Other outbreaks were reported in Nigeria and Kenya because of *Klebsiella* infections which had strains resistant to third generation cephalosporins (Paterson and Bonomo, 2005).

There are narrow therapeutic choices for ESBL-producing organisms because a number of ESBLs confer resistance to a variety of ß-lactam antibiotics but not cephamycins and carbapens. Conducted studies have demonstrated that carbapens are higher to quinolones for treatment of critical infections caused by ESBL-producing organisms (Karaiskos and Giamarellou, 2020).

2.7 Treatment and Resistance to Tetracyclines and cephalosporins

Cephalosporins and tetracyclines are used in both animals and humans to treat a variety of bacterial diseases. Ceftiofur, a third generation cephalosporins has been used in beef cattle to treat bovine respiratory disease and acute bovine interdigital necrobacillosis and also in preventing early mortality infections in day old chicks and turkey poults (Awosile et al., 2018). Ceftiofur is classified as a highly significant antimicrobial by the world Health Organization (Collignon et al., 2016). Ceftriaxone and ceftiofur both belong to the same class of antibiotics which are valuable in human medicine as far as treating serious and life threatening infections is concerned (Velazquez-Meza et al., 2022).

Tetracycline have been extensively used because they have low toxicity and broad spectrum of activity and are used as antibacterial drugs since they are agents with activity against a broad range of Gram positive and Gram negative anaerobic and aerobic bacteria (Grossman, 2016). They have been used broadly for treatment in man for bacterial respiratory and urogenital tract diseases. Tetracyclines are broad spectrum, relatively safe and have low cost, therefore, have been extensively used throughout the world and they come second after penicillins in total tons used each year (Fair and Tor, 2014; Xu et al., 2021). In the last 10 years, there has been a reduction in the use of tetracyclines in human medicine as bacterial resistance has become more widespread (Fair and Tor, 2014; Ventola, 2015) A number of reports have been produced on chromosomal mutations that cause resistance to multiple antibiotics including tetracyclines (Grossman, 2016). Multiple antibiotic resistance in Gram negative enteric bacteria has also been reported (Exner et al., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted in Lusaka, Chongwe and Kafue districts of Lusaka province in Zambia. This study was purposively conducted in Lusaka Province because of familiarity, proximity, cost and predominance of poultry farms. It is the smallest province in Zambia, with an area of 21,986 km2. It is the most densely populated and most urbanized province in Zambia, with an estimated population of about 3,079,964 people and population density of 140.1km2 whereby chongwe has a population of 313389,Lusaka 2,204059 and Kafue 219574(CSO, 2022).The province has the literacy rate of 83% and unemployment rate of 31%. The most spoken languages in Lusaka are Nyanja (Nsenga, Chewa, Chikunda, Kunda and Ngoni) which make 30.5% and Bemba make up 20.2%. There are 51.7% Females and 48.3% Males. The province has lower number of other livestock species compared to other provinces but it has the highest broiler production concentration due to small scale and commercial poultry clusters .Lusaka has around 20000 livestock farming households with about 8000 households which have mixed enterprises and nearly 4000 households which are purely poultry farmers. (CSO,2010).The area has small scale, semi commercial and commercial poultry farmers where some small scale farmers practice free range especially for indigenous birds and some practice intensive farming.

3.2 Research design

Cross sectional research design was employed, with quantitative data collection approach (questionnaire survey).Convenient sampling technique was used to poultry farms involved in poultry production (indeginous chicken farms, broiler farms and layer farms) located within the study area. The study was conducted between November, 2021 and February, 2022.

The study included both broiler and layer farms as well as caged and floored birds. Small scale, semi commercial and commercial farms were included. The questionnaires to be answered were consented to individuals 18 years and above of either gender. The non- consenting adults and poultry farms on free range were excluded.

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3.3 Sampling and sample collection

3.3.1 Sample size calculation

Sample size was calculated using the formular for determining the sample size for infinite population since the exact number of poultry farms within the study area was unknown.

The following Cochrans (1977) sample size formula was used:

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$$

Where **n** is the sample size, **$Z_{1-\alpha/2}$** is standard normal variate (at 5% type 1 error (*P*=0.050) =1.96, **p** is expected prevalence and **d** is absolute error or precision.

$$n=1.96^2 (1-0.5)^2) /0.05^2$$

$$=385$$

Therefore, a total of 351 samples were collected from the selected farms.

3.4 Sampling method

Simple random sampling was used. A total of 29 farms representing 12, 7, and 10 farms for Lusaka, Chongwe and Kafue were selected of which 37 poultry houses were sample. 145 samples were collected from Lusaka, 85 samples from Chongwe and 121 samples from Kafue. The chickens in all barns were housed on either deep litter system or battery cage system where 4 indigenous chicken farms, 15 layers farms and 10 broiler farms were included.

3.4.1 Dust collection

From each farm, samples of settled dust was collected from the house. Where more than one house was sampled, random sampling was done. The settled dust was collected from different elevated surfaces inside the poultry house, including the drinking system line, feeding system line and ventilation opening. Samples from different location within the poultry house was treated as a different sample. Samples were collected by brushing settled dust from all sampling points into a sterile container using a sterile swab. 4-6g of settled dust was collected from each location. The collected dust samples were put in a sealed pack and taken to the laboratory where they were stored at -20ºC until analysis.

3.4.2 Questionnaire

A total of 60 questionnaires were administered to 60 respondents (owners, spouses or workers where owner was not available) on each of the sampled farms to collect information on the association between the sociodemographic issues and the occurrence of the pathogens in dust from poultry farms in Lusaka province-Zambia. Between one and three questionnaires were administered at a farm depending on number of poultry houses. The demographic data, biosecurity, administration of medicines, usage of protective equipment at the farm, illnesses they have had in a specified period and if they think that dust is the source were part of the information collected. The questionnaire was translated to Njanja and respondents who were not understanding English or have low literacy level, they responded in Nyanja. The questionnaire was pretested at the University of Zambia, school of Agriculture farm to ascertain the validity of the questions and research objectives.

3.5 Microbiological analysis of dust samples

3.5.1 Isolation and enumeration of the total viable mesophilic bacteria

The total viable counts of aerobic mesophilic bacteria in dust samples were examined by plating dilution series from dust suspensions. Dust suspensions were prepared by weighing 0.1g of dust which was added to 9.9ml of normal saline. Then the suspension was shaken for 30 minutes in a water bath at 25°C. Afterwards, the suspension was vortexed for 5 minutes. Aliqouts (0.1ml) of suspensions and serial dilutions from these suspensions from 10^{-1} to 10^{-4} were made where 10^{-3} and 10^{-4} were plated in duplicates on nutrient agar plates for counting colonies of mesophilic bacteria colonies. After incubation at 37°C for 24 hours, colony forming unit (cfu/g) were counted, and the results were expressed in cfu/g of dust.

3.5.2 Isolation and enumeration of *Enterobacteriaceae* and ESBL-producing *Enterobacteriaceae*

Aliquots of 0.1ml from the original dust suspension and serial dilutions from these suspensions from 10^{-1}

to 10^{-4} were made where dilutions of 10^{-3} and 10^{-4} were plated in duplicates on MacConkey agar with 25mg/L cefotaxime and on MacConkey agar without any antibiotic to determine the count of ESBL-producing *Enterobacteriaceae* and also non-resistant *Enterobacteriaceae*, respectively. Plates were incubated at 37°C for 24 hours. Suspected ESBL producing *Enterobacteriaeae* and *Enterobacteriaceae* colonies were counted and the cfu/g of dust were recorded.

3.5.3 Isolation of *Salmonella*

About 1g of dust was suspended into 9ml of selenite /tetrathionate broth and was incubated for 24hrs at 37°C.After incubation, it was streaked on Xylose Lysine Desoxycholate (XLD) agar and incubated for a further 24hrs at 37°C. Results were recorded with respect to the appearance of colonies on the plate. *Salmonella* had red colonies with black centers while *Shigella* had red colonies, *E. coli* had large flat yellow colonies, *Enterobacter* had mucoid yellow colonies while *Klebsiella*, had pink flat colonies. *Proteus* had yellow colonies while *Citrobacter* had yellow colonies with weak black centres).

5.5.4 Identification of Bacteria Using Sugars

The cultured *Enterobacteriaceae* were isolated on MacConkey agar and then put in four different sugars to identify the different Enterobacteriaceae using Biochemical reactions according to the Bergey's Manual Determination Bacteriology (Buchanan and Gibbons, 1974). The sugars used were Triple Sugar Iron (TSI), Simmons Citrate, Urease and Sulphur Indole Motility (SIM).

5.5.5Antibacterial Susceptibility Test of Bacterial Isolates

The Kirby-Bauer disc diffusion antibacterial sensitivity test method was used to test antibacterial resistance of the bacterial isolates based on the Clinical Laboratory Standard Institute (CLSI) guideline (Kiehlbauch et al., 2000). Ten antibacterial agents (HiMedia from Mumbai,India). A total of 10 antibacterial agents belonging to 7 antibacterial classes (Beta- lactam penicillins, macrolides, tetracyclines, aminoglycosides and sulphonamides, fluoroquinolones and cephalosporins) were used to determine the antibiograms of the isolates. The 10 antimicrobial drugs used included; gentamycin (10µg), nalidixic acid (30µg), streptomycin (300 µg), cefotaxime (CTX 30µg), ciprofloxacin (CIP 5µg), enrofloxacin (5µg), tetracycline (30µg), sulfamethoxazole/trimethoprim (25 µg), doxycycline (30 µg), azithromycin (15 µg), penicillin G (10µg)

Mueller-Hinton agar was prepared according to the manufacturer's protocol (HiMedia Laboratory Pvt, Mumbai, India). The organisms were purified on nutrient agar by replating distinct single colonies from MacConkey agar which contained cefotaxime 25mg/L and each isolate was suspended in sterile normal saline at a concentration of McFarland 0.5, vortexed and then spread with a sterile cotton swab on the surface of Muller-Hinton agar plate. Using the sterile forceps, the antibiotic discs were placed on the agar surface. The discs were placed at equidistant from each other with only five antibiotic discs placed per plate. Then plates were placed upside down and incubated for 24 hours at 37°C (Kiehlbauch et al., 2000). The sensitivity of each isolate was read by measuring the clear, circular diameter around each disc (zone diameter measurement) measured with a vernier caliper and end points were determined based on the areas showing no bacterial growth visible to the naked eye. Results were recorded in millimeters and were recorded as susceptible, intermediate and resistant using the CLSI guideline (Kiehlbauch et al., 2000).

3.6 Statistical analyses

Laboratory data and questionnaires was entered into Microsoft Excel and transferred to SPSS version 20.0 (IBM, USA) for analysis according to the objectives of the study. The laboratory data was merged with the questionnaire. To explore the data, frequency of distribution tables, means and standard deviation were calculated for descriptive purposes. The Chi square (Fishers Exact Test) was used for ESBL- *Enterobacteriacea* bacteria(cfu/g) (dependent variable) against the independent variables (e.g. demographic characteristics) to assess the association. Fishers Exact test was used for variable screening, to identify those variables that are associated with ESBL- *Enterobacteriacea* positivity so that the variable associated with ESBL-E positivity could be included in the multivariate analysis to check if the variable is a true risk or not. All variables with p-values less than or equal to 0.250 were included in the model. Probability and partial plots were used to determine whether the model fitted the data. All statistics were considered significant at $p \leq 0.050$.

3.7 Ethical Consideration

The participants were free to participate or decline to participate and also no incentives was provided for participating in the study.

Ethical approval was sought from the University of Zambia Biomedical Research and Ethics Committee (UNZABREC) (REF.NO.2269-2021) and National Health Research Authority

(REF.NO.0000002/28/02/2022). Introduction letter was given to Managers/Farm owners of the poultry farms before samples were collected and workers were interviewed. Informed consent was also obtained from each participant prior to the interview. Farmers who opted not to participate were not included in the study.

CHAPTER FOUR

RESULTS

4.1 Detection of microbial load, *Enterobacteriaceae* and ESBL *Enterobacteriaceae*

A total of 351 samples were collected from 29 farms (37 poultry houses). From these houses, 291 samples of Enterobacteriaceae were determined. From these 291 samples of Enterobacteriaceae, 62 ESBL- *Enterobacteriacea* were isolated and these 62 samples were subjected to Antimicrobial Susceptibility.

All samples (351) from farms showed the growth of mesophilic bacteria in settled dust samples. The total mesophilic bacterial cfu/g was 2.6x10^8 with SD = 7.36x105cfu/g while the total *Enterobacteriaceae* was 2.296x10^8 cfu/g, with SD = 5.6x10^5cfu/g (Table 4.13).

The detected frequency of *Enterobacteriaceae* from the 351 samples analyzed was 82.9%. When comparing *Enterobacteriacea* results and ESBL- *Enterobacteriacea,* ESBL-producing *Enterobacteriaceae* were less frequently detected. The prevalence of ESBL was at 21.7%.out of the 291 samples, 62 samples were ESBL- *Enterobacteriacea* positive and the total cfu/g was 4.842x10^4 /g with SD = 4.3x$10^{2.}$cfu/g. Out of 62 ESBL positive samples, 32.3% were *E. coli*, 27.4% were *Enterobacter spp*, 24.2% were *Klebsiella spp* and other bacteria *spp* were between 3.23 to 8.06 % (Table 4.1 and Table 4.2).

Table 4. 1: Summary of microbial load, *Enterobacteriaceae* and ESBL *Enterobacteriaceae* from the dust samples analysed

	Standard deviation (SD)	**Total cfu/g**
Mesophilic bacteria	$7.36x10^5$	$2.599x10^8$
Enterobacteriaceae	$5.6x10^5$	$2.296x10^8$
ESBL *Enterobacteriaceae*	$4.3x10^2$	$4.842x10^4$

CFU= Colony Forming Unit, g= gram, SD =standard Deviation

Table 4. 2: Summary of resistant ESBL- *Enterobacteriaceae* isolates from poultry house dust samples to MacConkey and Cefotaxime

Variable	**Frequency(n=62)**	**Percentage resistant**	**95% CI**
Klebsiella spp	62	24.2	14.6-37.0
Enterobacter spp	62	27.4	17.2-40.4
E.Coli	62	32.3	21.3-45.5
Proteus spp	62	8.1	3.0-18.6
Citrobacter spp	62	4.8	1.3-14.4
Shigella spp	62	3.2	0.6-12.2

CI= Confidence interval; n=number of ESBL positive

4.2 Antimicrobial susceptibility in ESBL Producing *Enterobacteriaceae* isolates from dust samples from poultry farms using Kirby-Bauer Disc Diffusion

All isolates (60) showed high resistance to penicillin (100%), followed by cefotaxime (56.5%) and then ciprofloxacin (48.4%). Resistance rates to other tested antibiotics were between 9.7% and 35.5%. *E. coli* showed higher rates of antimicrobial resistance to the drugs used seconded by *Enterobacter spp* and then *Klebsiella spp* with 32.3%, 27.4% and 24.2%, respectively.

Table 4.3: Antimicrobial susceptibility in ESBL producing *Enterobacteriaceae* isolates

ANTIBIOTIC	CLASS OF ANTIBIOTIC	S	I	R	RESISTANCE RATE (%)
Ciprofloxacin	Fluoroquinolones	18	14	30	48.4
Penicillin	Beta lactam	0	0	62	100
Azithromycin	Macrolides	39	1	22	35.5
Enrofloxacin	Fluoroquinolones	46	9	7	11.3
Streptomycin	Aminoglycosides	50	6	6	9.7
Cefotaxime	Cephalosporins	14	13	35	56.5
Nalidixic acid	Fluoroquinolones	42	8	12	19.4
Doxycycline	Tetracyclines	46	7	9	14.5
Gentamycin	Aminoglycosides	51	5	6	9.7
Trimethoprim/sulpha	Sulphonamides	47	6	9	14.5

S = susceptible, I =Intermediate, R =Resistant, n=62

4.3 Demographic characteristics of participants

This study recruited 60 participants of which 75.0% were males, while 25.0% were females. Among the respondents 86.7% were poultry house attendants (PHA), 8.33% were the spouses to the farm owners and 5.0% were the farm owners (Table 4.1).

Table 4.4: Socio-demographic characteristics of study participants

Variable	**Category**	**Frequency (*n*=60)**	**Proportion**	**95% CI**
Gender	Male	45	75.0	61.8-84.9
	Female	15	25.0	15.1-38.1
Age	20-30	26	43.3	30.8-56.7
	31-40	20	33.3	22.0-46.9
	41-50	10	16.7	8.7-29.0
	≥51	4	6.7	2.2-17.0
Education	≤G12	32	53.3	40.1-66.1
	G12	20	33.3	22.0-46.8
	Tertiary	8	13.3	6.3-25.1
Respondent	PHA	52	86.7	74.8-93.7
	Spouse	3	5.0	1.3-14.8
	Owner	5	8.3	3.1-19.1

CI= Confidence interval; n=number of respondents; G12=Grade 12; PHA=Poultry House Attendant;

4.4 Exposure Risk Factors to poultry dust

The duration of time someone has been working in a poultry house, daily contact time, biosecurity measures taken before entering the poultry house and the observations they make upon entering the poultry house are shown in Tables 4.2 to Table 4.4.

On the duration of employment from their respective working places, 48.3% of the PHAs had worked for more than three years (Table 4.2). Majority of the workers (68.3%) were not practicing any measure of biosafety, whilst 13.33% were able to bath and disinfect both overalls and boots before entering the poultry house (Table 4.3). Most respondents (41.7%) reported that they had observed dust particles in the air and some respondents representing 31.7 % reported that they do not observe anything.

Table 4.5: Duration of employment, daily contact time and length of each contact time

Variable	**Category**	**Frequency (*n*=60)**	**Proportion**	**95% CI**
Number of working years	≤ 1 year	3	5.0	1.30-14.8
	1 year	5	8.3	3.1-19.1
	2years	10	16.7	8.7-29.0
	3 years	13	21.7	12.5-34.5
	≥3years	29	48.3	45.4-61.5
Daily contact time	3 Times	18	30.0	19.2-43.4
	4 Times	13	21.7	12.5-34.5
	5 Times	17	28.3	17.8-41.6
	≥5 Times	12	20.0	11.2-32.7
Estimated time for each contact	10 Min	16	26.7	16.5-40.0
	20 Min	13	21.7	12.5-34.5
	30Min	17	28.3	17.8-41.6
	≥30Min	14	23.3	13.8-36.3

CI= Confidence interval; n=number of respondents

Table 4.6: Biosafety measures before entering the poultry house

Variable	Category	Frequency(n=60)	Proportion	95%CI
Biosafety measures	Bathing and changing clothes	10	16.7	8.7-29.0
	Bathing, disinfecting the overalls and boots	8	13.3	6.3-25.1
	Disinfecting on the entrance	1	1.67	0.9-10.1
	Nothing	41	68.3	54.9-79.4

CI= Confidence interval; n=number of respondents

Table 4.7: Observations made by respondents upon entering the poultry house

Variable	Category	Frequency(n =60)	Proportion	95% CI
Observation description	Dust particles	25	41.7	29.3-55.1
	Birds are active	5	8.3	3.1-19.1
	Look for dead birds	6	10.0	4.1-21.2
	Check for water, feed and general conditions of chickens	12	20.0	11.2-32.7
	Nothing	19	31.7	20.6-45.1

CI= Confidence interval; n=number of respondents

Respondents who reported that they used sawdust were 45.0% and 16.7% used rice husks and the other bedding types were between 1.7% and 13.3%. Most of the respondents (60%) reported that they had problems with the type of litter they used, while 26.7% reported that they had no problems with the litter (Table 4.8).

Table 4.8: Type of bedding used in poultry houses

Variable	**Category**	**Frequency *(n=60)***	**Percentage**	**95% CI**
Bedding type	Sawdust	27	45.0	32.3-58.3
	Cages are used	8	13.3	6.4-3.1
	Rice husks/bran	10	16.7	8.70-29.0
	Grass	9	15.0	7.5-27.1
	Wood shavings	5	8.3	3.1-19.1
	Soil	1	1.7	0.9-10.1
Problems with the bedding used	Yes	36	60.0	46.5-72.2
	No	16	26.7	16.5-39.9
	Cages are used	8	13.3	6.3-25.1

CI= Confidence interval; n=number of respondents

Most respondents (86.7%), reported that they changed litter frequently. The most reported reason for litter change was cleaning and disinfecting the house in readiness to the new batch (48.3%) (Fig4.1).

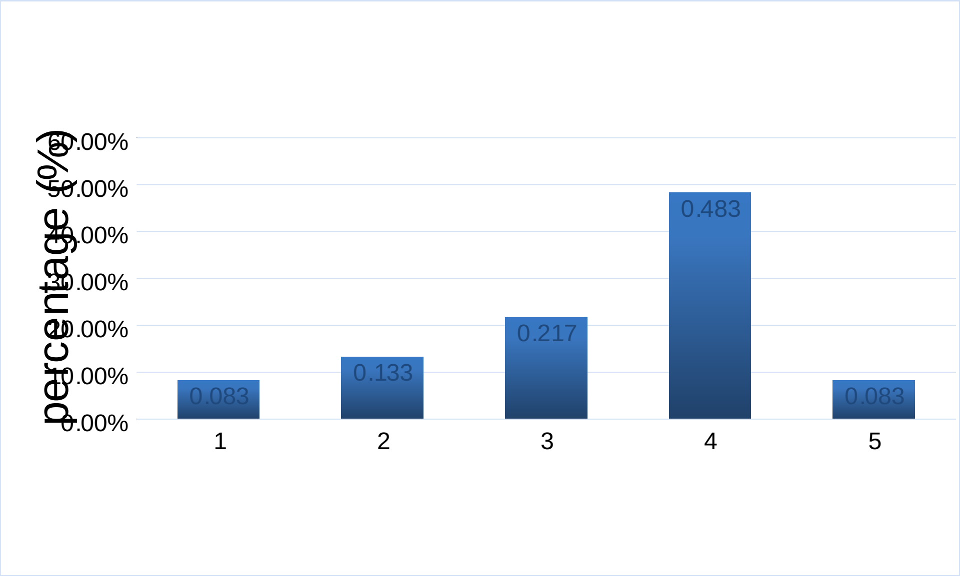

Figure 4.1: Reasons for litter change in poultry houses.

Table 4.9 shows the activities that were reported to cause dust in the poultry houses by the respondents. Chicken activities were reported to cause most dust. For instance, movement (60%) while feeding the birds by workers caused the least dust (8.3 %).

Table 4.9: Activities causing dust production in poultry houses

Variable	**Category**	**Frequency(*n=60)***	**Percentage**	**95% CI**
Activities causing dust in poultry house	Chicken movements s	36	60.0	46.5-72.2
	Cleaning	19	31.7	20.6-45.1
	Giving feed	5	8.3	3.1-19.1

CI= Confidence interval; n=number of respondents

4.5 Source of potential dust exposure, PPE utilization among poultry farm workers and prevalence of respiratory symptoms among PHA

Litter had the highest percentage with 31.7% as the source of potential dust exposure while other sources of potential settled dust exposure from poultry house like beddings, feed and drugs showed the least potential dust exposure and they were between 11.7% and 16.7% as presented in Figure 4.2.

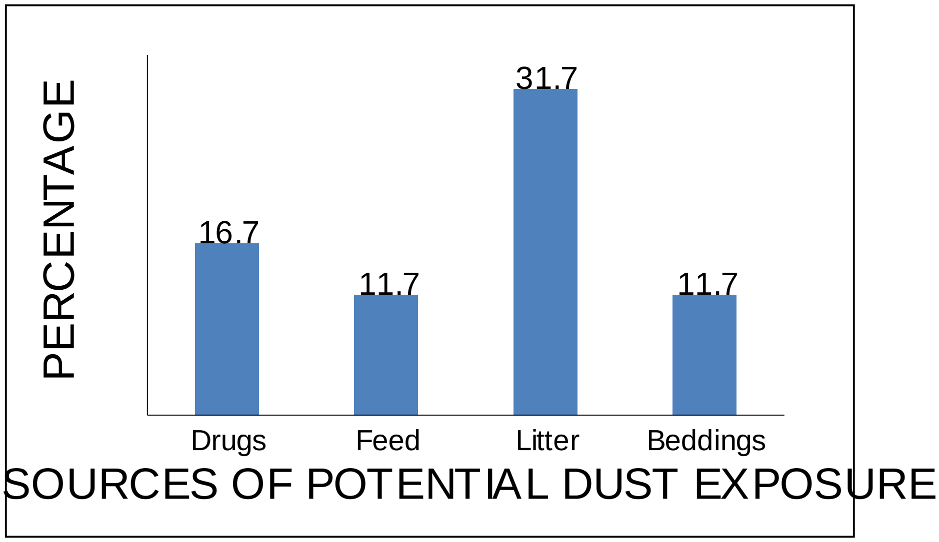

Figure 4. 2: Sources of potential dust exposure to poultry dust in poultry farms.

Table 4.10 shows the types of PPE used by the respondents. The results show that 83.3% use a certain type of PPE. Boots and overalls were the most used PPE (50.0%), while 16.7% of the respondents reported working without any PPE.

TABLE 4.10: Types of PPEs used among poultry farm workers

Variable	Category	Frequency(*n=60*)	Percentage	95% CI
protective clothing	Boots and overalls	30	50.0	37.7-62.3
	Boots ,overall and mask	4	6.7	2.2-17.0
	mask only	3	5.0	1.30-14.8
	Mask, gloves and boots	10	16.7	0.9-10.1
	Boots only	3	5.0	1.30-14.8
	nothing	10	16.7	8.7-29.0

CI= Confidence interval; n=number of respondents

Prevalence of self-reported respiratory symptoms experienced by the respondents is shown in Figure 4.3. The symptoms included itchy and watery eyes (66.7%), cough (75%), sore throat (16.7%), wheezing (1.7%), shortness of breath (1.7%), flu-like symptoms (38.8%), chest tightness (1.7%) and phlegm production (11.7%). No mixed infections were captured.

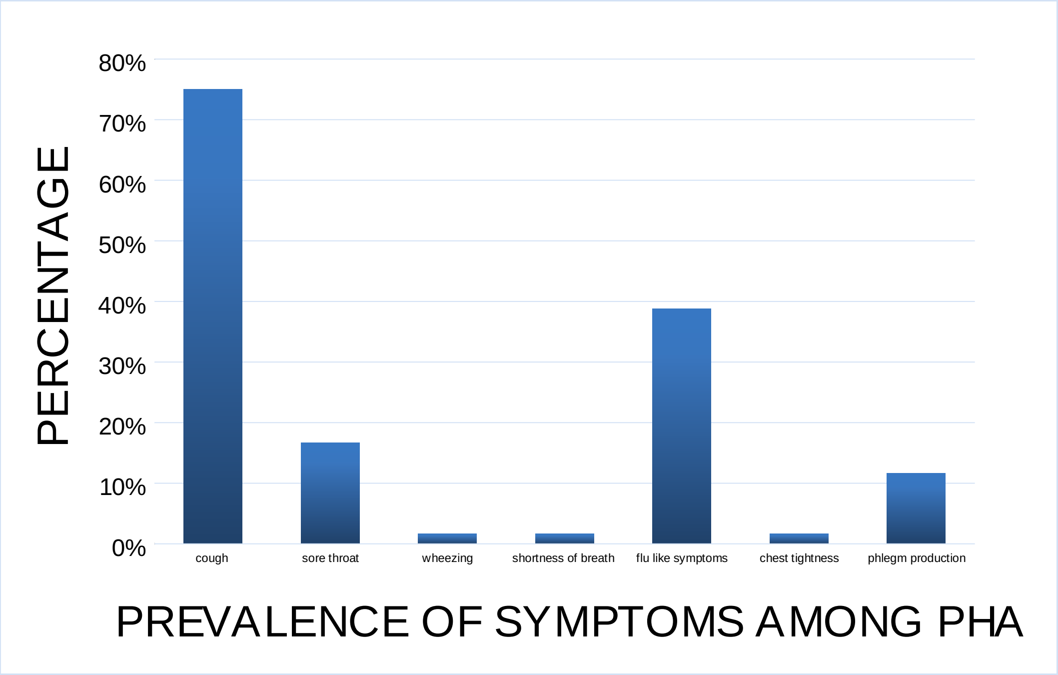

Figure 4. 3: Prevalence of symptoms among PHA

About 66.7%, of the respondents who reported these symptoms did not do anything about them and the conditions resolved on their own. Five percent used home remedies, while 25 % went to clinic to get some treatment. About 78.3% of the respondents reported their ailments to their supervisors while, and 21.7 % did not report (Table 4.11).

Table 4.11: Health welfare of respondents

Variable	**Category**	**Frequency*(n=60)***	**Proportion**	**95% CI**
Action taken when you were sick	Resolved on its own	40	66.7	53.2-78.0
	Went to the clinic	15	25.0	15.1-38.1
	I used home remedies	5	8.3	3.1-19.1
Was the boss aware that you were sick	yes	47	78.3	65.5-87.5
	no	13	21.7	12.5-34.5

CI= Confidence interval; n=number of respondents

4.6 Antibiotic usage in poultry houses

The poultry farms under the study were all under intensive feeding and they were purchasing the poultry drugs from veterinary shops (pharmacies). The results show that the prophylactic use of antibiotics was higher than for treatments with the reported percentage use of 70% and 30%, respectively. The commonly used antibiotics were Tetracyclines, gentamycin, doxycycline, sulfadimidine, Limoxin, ciplofloxacin, Amprolium and Tylosin. These drugs were bought from Agrovets and pharmacies. The respondent admitted to observe the withdrawal periods of each drug they use. Antibiotics were most frequently administered by the supervisors (50%), owners and spouses (20%), veterinarians and para veterinarians (16.7%) and any other worker (13.3%) (Figure 4.4)

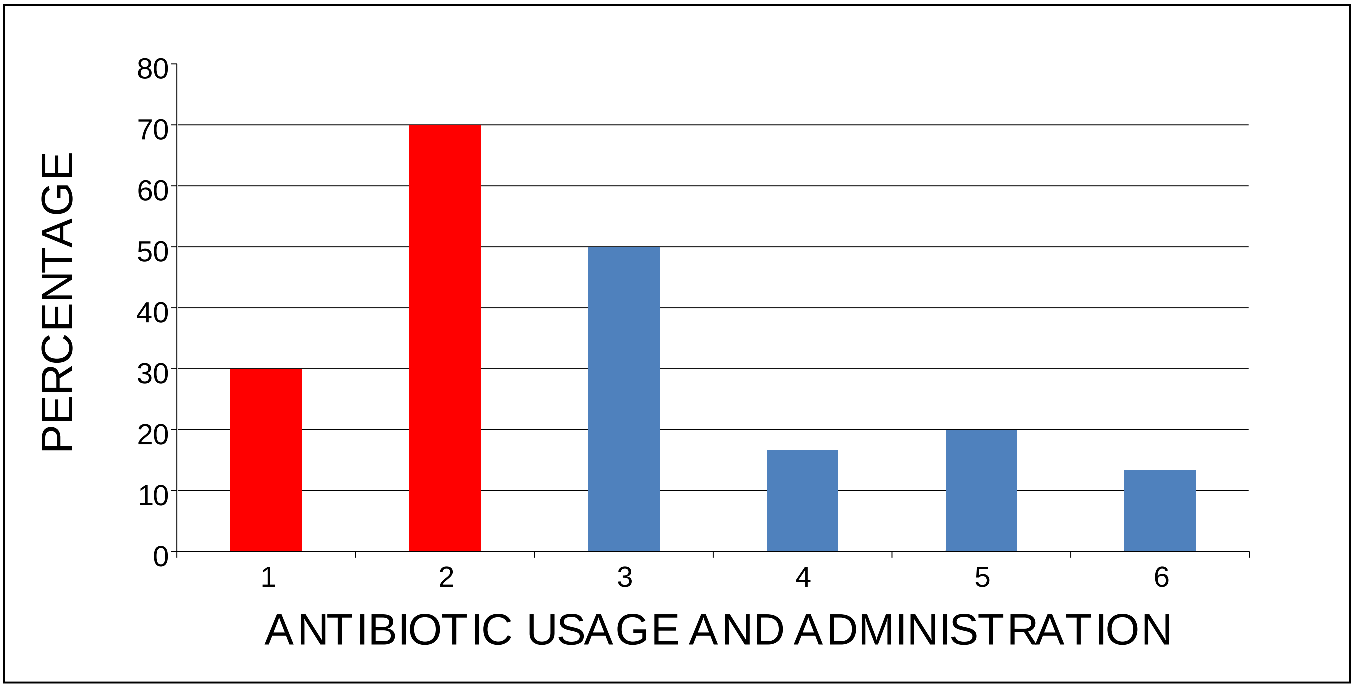

Figure 4.4: The graphical presentation of antibiotic usage and administration in poultry house.

The table 4.12 shows the assessment of the association between the sociodemographic issues (Independent variables) and the occurrence of the pathogens (ESBL-E) in dust from poultry houses. The results show that there is no association between variables and the occurrence of ESBL-E in poultry dust.

Table 4.12: The association of ESBL-E positivity with independent variables

Variable	Category	*n*	Positive for ESBL (%)	95% CI	p-value
Gender of attendant	Male	45	7 (15.6)	7.8-28.8	0.543
	Female	15	8 (53.2)	30.1-75.2	
Education	< Grade 12	32	19 (59.4)	42.3-74.5	0.761
	Grade 12	20	13 (65.0)	43.3-81.9	
	Tertiary	8	4 (50.0)	21.5-78.5	
Medication	Yes	59	35 (59.3)	46.6-70.9	1.00[a]
	No	1	1 (100.0)	20.7-100.0	
Who administers the medication	Supervisor	30	19 (63.3)	45.5-78.1	0.872
	Veterinarian	10	6 (60.0)	31.3-83.2	
	Worker	12	6 (50.0)	25.4-74.6	
	Owner	8	5 (62.5)	30.8-86.3	
Biosecurity	Bathing and changing clothes	20	9 (45.0)	25.8-65.8	0.234
	Bathing and disinfect the overalls and rain boots	26	18 (69.2)	50.0-83.5	
	Disinfect on the entrance	14	9 (64.3)	38.8-83.7	
Frequency of litter change	Use cage	8	6 (75.0)	40.9-92.9	0.103
	Once after two months	7	2 (28.6)	8.2-64.1	
	Once in a month	15	11 (73.3)	48.1-89.1	
	After selling the batch	11	4 (36.4)	15.2-64.6	
	When receiving another batch	3	3 (100.0)	43.9-100.0	
	Every two weeks	6	5 (83.3)	43.7-97.0	
	Every week	10	5 (50.0)	23.7-76.3	
Respondent	Worker	52	30 (57.7)	44.2-70.1	0.438
	Spouse	3	3 (100.0)	43.9-	

				100.0	
	Owner	5	3 (60.0)	23.1-88.2	
What type of beddings do you use in the poultry house	Sawdust	27	18 (66.7)	47.8-81.4	0.343
	Cages	8	6 (75.0)	40.9-92.9	
	Rice husks/bran	10	3 (30.0)	10.8-60.3	
	Grass	9	5 (55.6)	26.7-81.1	
	Wood shaving	5	3 (60.0)	23.1-88.2	
	Soil	1	1 (100.0)	20.7-100.0	
How long have you been working in poultry houses	Less than 1 year	3	2 (66.7)	20.8-93.9	0.649
	1 year	5	2 (40.0)	11.8-76.9	
	2 years	10	5 (50.0)	23.7-76.3	
	3 years	13	7 (53.9)	29.1-76.8	
	More than 3 years	29	20 (69.0)	50.8-82.7	

CHAPTER FIVE

DISCUSSION

A number of studies on airborne dust from layer hen, turkey and broiler houses have been done in Europe (Ahmed et al., 2020). These studies reported increased microbial loads concentrations in poultry farms than that generated on pig and cattle farms (Lawniczek-Walczyk et al., 2013). This is the first study to report on dust from poultry houses in Zambia. The results of this study are similar to those reported by (Hartung and Saleh, 2008), who found between 2.0×10^8 and 1.7×10^9 bacteria (cfu/g) in three different laying hen systems in Germany. Similar studies also found the total bacterial count of 3.2×10^9 (Skóra et al., 2016) as well as 7.10×10^8cfu/g (Ahmed et al., 2020).

Very few studies have been done to show the *Enterobacteriaceae* concentration in settled poultry dust. Amador et al., (2019) reported the *Enterobacteriaceae* counts ranging from 1.4×10^7 to 7.6×10^9 cfu/g. ESBL-E concentration in poultry settled dust was higher than the concentration which was reported by Ahmed et al., (2020), which was 6.98x103 cfu/g. The detection of ESBL-E in poultry dust is of great concern in the human, food animal population and their environment since these microorganisms can easily be acquired via excessive bacterial growth and also cross contamination due to poor sanitation. Substantial antibiotic use is one of the contributing factors for elevated levels of ESBL-E producing organisms (Shakya et al., 2013) Circulation of ESBL-E. interfere with the activity of broad-spectrum antibiotics building up significant therapeutic failure with a remarkable consequence on the treatment outcome for patients (Dierikx et al., 2013; Adler et al., 2016).

Occurrence of bacterial resistant to anti-microbial agents were reported in this study. Antibiotics usage in Zambian poultry industry is prevalent (Mwansa et al., 2022). *E. coli* has the highest resistance rate (32.3%) amongst other isolates in this study and is of great concern due to its ubiquitous nature in the environment and human and animal hosts and also the relatively easy acquirement and transmission of genetic determinants that confer resistance to most classes of antibiotics (Singh et al., 2022). ESBL-*E. coli* occurred with prevalence of 34% in 2010 and 54% in 2011 in Swedish broiler flocks to tetracyclines and sulfamethoxazole which suggested that the co selection is a source of high prevalence (Börjesson et al., 2013). The increased antimicrobial resistance rate was highly shown in beta lactams 100%, cephalosporins (56.5%) and fluoroquinolones (48.8%) and this emergency of resistance to widely used antimicrobials such as cephalosporins and fluoroquinolones reduces the efficacy of treating enteric, urinary tract and skin infection. This result in extended and more serious illness. Co-existence exists amongst different antibiotic classes, for example, ESBL-E can have a co resistance to fluoroquinolones, tetracyclines as well as trimethoprim/sulphonamide (Singh et al., 2022). The resistance of Enterobacteriaceae to third generation cephalosporins are as a result of the overuse in poultry production and are associated with the increased human deaths in Europe (Collignon et al., 2013).

Unrestricted and excessive use of antibiotics in agriculture, animal husbandry, and humans worldwide has a significant role in the emergence of antimicrobial resistance (Singh et al., 2018; Almakki et al., 2019). Overuse of antibiotics has escalated the buildup of active antimicrobial compounds in the environment and favoured the selection of antimicrobial resistance genes (ARGs) and antimicrobial resistance bacteria (ARBs) (Almakki et al., 2019). Poultry farmers and drugstore owners do not follow the procedure the government had put in place to purchase the antibiotics from the drugstores. Due to inadequate monitoring of these drugstores, farmers have easy access to livestock antibiotics thereby increasing the risk of ESBL in food animals.

In this study, there were more male workers working in the poultry farms than female workers. This finding is consistent with the a previous study that attributed the hiring of male workers in poultry farmers (Viegas, et al., 2013).This could be because the job is physically demanding (Ngajiro,2014).The majority of the respondents were between 20 to30 years old, with just a few of them above 50 years. This could have been as a result of employers opting to hire young energetic people than older one who could have been physically week and/or in poor general health. Such a physical state could have made them not to present themselves for employment or made them to go on early retirement from employment (Shah, 2009; Chowdhury et al, 2017). The high proportions of respondents had not reached Grade 12 in education. The majority of the respondents were Poultry House Attendants (PHA) which can be explained in line with the nature of the job which is unskilled labour because most of them had low education levels.

Materials used for poultry litter can also contribute to dust production. In this study we found out that the beddings used were sawdust, rice husks/hulls, grass, wood shavings, soil and some farms used cages. Commonly, most people use different materials as bedding or poultry litter including pine shavings, sawdust, chopped straws, shredded paper, oil seed haulms, rice husks or hulls, hay and grass (Viegas, et al., 2013). Ambient air pollution tends to increase the risk of the lower respiratory infections since exposure to air pollutants like particulate matter leading to oxidative stress.(Smit et al., 2017).

The results of this study are in line with those from previous ones that showed that exposure to dust vary with particular jobs and tasks (Crook et al., 2018) such as those undertaken by broiler and layer farm workers. The activities like poultry inspection and handling, vaccination, routine house maintenance and cleaning during growth or production periods, laying down litter, population and depopulating of the birds, litter/manure removal, catching or removal of birds are well known to increase the exposure to poultry dust (Crook et al., 2018). However, activities such as sweeping, brushing down surfaces and spreading litter have been associated with increased exposures (Whyte, 2002).

García-Cobos et al., (2015) detected ESBLs in both dust and air from pig farms. The occurrence of ESBL producing bacteria in dust particles contributes to the prevalence of ESBL producing bacteria in farm animals as well as human (Dohmen et al., 2017). Frequently exposure enhances the transmission of ESBL producing bacteria between humans and livestock (Dohmen et al., 2017). This is so because of inhalation of this dust by poultry workers in poultry farms as they spend considerable period of time in the poultry houses (Ngajiro,2014; Jerez et al,2014).

This study found that the most used PPE were boots and overalls with a few of workers were not using any protective wear. In accordance with Kearney et al., (2015) PPE have an absolute role in the prevention and curtailing of the infinite of health hazards (diseases) that poultry workers are exposed to at work. Appropriate respiratory equipment should be used so that poultry workers can ably prevent work related respiratory diseases as work safety climate is associated with use of job appropriate PPE. Minnesota poultry farmers reported that 26% of poultry workers used nose mask and 51% used gloves (Odo et al., 2015). A study on the use of PPE among poultry workers in England reported that 81% used protective coveralls, 82% protective footwear, 67% disposable gloves, 51% facemasks and 19% used protective goggles (Morgan et al., 2009). Studies on Nepal poultry workers reported the use of PPE at facemask 27.1%, gloves 30.2%, boots 7.3% and coverall 3.1% (Jha et al., 2021) and use of these PPE especially face masks help in reducing inhalable dust.

In this study, the most prevalent self-reported respiratory symptom experienced by the respondents was coughing. In a study which was conducted in France on prevalent respiratory symptoms among poultry farmers cough was reported by 79% of the respondents (Guillam et al., 2013). Similarly, another study reported less than 10% of wheezes among poultry workers (Jaiyesimi and Agbaje, 2015). These symptoms have been attributed to poultry dust that irritates the cough receptors and causes inflammation of the mucous membrane in the upper respiratory tract leading to cough and phlegm production mostly due to immune response against pathogenic biological agents (Viegas, et al., 2013).

In this study, Fisher's exact test showed that none of the variables were associated with the risk of ESBL- E. therefore there was no significant association between the variables and the ESBL- E. occurrence in the poultry dust. Dahms et al., (2015) found that Age, sex and daily working hours were not significant risk factors for ESBL-E occurrence in livestock and farm workers in Germany. Some researchers found that, biosecurity and hygienic measures play a role in the reduction of ESBL-*E. coli* on the pig farm (Gay et al., 2018).The assumption was made by that it is because of insufficient cleaning and disinfection which cause high incidences of ESBL-E due to contaminated barns (Hiroi et al ., 2012) and this can be supported by the finding of ESBL-E in the poultry dust. Age of the animal and antibiotic use were also associated with the increased risk of ESBL-E occurrence in beef animals (Liebana et al., 2013; Gay et al., 2018).On medication, the positive findings of ESBL-E in poultry dust can be supported by the 98% respondents who admitted that they give medication to their chickens

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

This study examined the microbial loads and the occurrence of extended –spectrum beta-lactamase-producing *Enterobacteriaceae* in dust from poultry farms in Lusaka-Zambia. The following conclusions were derived:

1 Poultry dust are the reservoir for ESBL-Producing *Enterobacteriaceae* which could expose farmers and workers to these bacteria.
2 The poultry dust carries bacteria which are resistant to some antibiotics.
3 None of the risk factors which is associated with the occurrence of these bacteria (ESBL-E) in a poultry house.

6.2 RECOMMENDATION

1. Poultry farm owner have to ensure that poultry workers are given the proper protective equipment.
2. Close monitoring of antibiotic usage in poultry farms to avoid unmanaged and overuse use of antibiotics in veterinary sector.
3. Factors linked to the expansion or spread of ESBL-E are not known and remain the serious issue and require further research to determine the relationship between these factors.

6.3 LIMITATIONS OFTHE STUDY

1. The data was collected between Nov (2021) and Feb (2022) where rains had started falling and we failed to sample some farms since the dust was wet or cold making it difficult to be scrapped.
2. Due to rains, some hard to reach areas were impassable therefore some farms were not sampled
3. Lack of database from Agricultural offices contributed to the challenge of locating poultry farmers to include in our study hence failed to meet the targeted sample size.
4. Some farms were cleaned and disinfected despite telling them that we are going for sample

collect dust samples hence failure to collect.

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APPENDICES

ANNEX 1

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 256067 Ridgeway Campus

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INFORMED CONSENT TEMPLATE FOR INTENDING RESEARCHERS

Title of the proposed study: OCCURRENCE OF EXTENDED –SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE AND MICROBIAL LOADS IN DUST FROM POULTRY FARMS IN LUSAKA-ZAMBIA

Investigators:

Linda Basikolo, UNZA, Samora Machel school of Veterinary Medicine.

Background and rationale for the study:

The phenomenon of organic dust particles on poultry farms, which are formed of both viable and non-viable particles, can cause a serious risk to bird production and stockmen health (Banhazi et al., 2018; David et al., 2015). Many studies have shown that the dust which is present in laying birds can be the origin of potential pathogens, zoonotic agents, resistant bacteria and not forgetting toxic compounds (Just et al., 2009).Livestock housing systems,for poultry in particular, form and produce large amounts of dust (David et al., 2015;Cambra-López et al., 2009; Just et al., 2009).

Airborne dust or settled dust from surfaces in livestock structures could include plant particles, particles from food, fecal particles, epithelia, bacterial cells and spores, fungi and fungal spores, viruses, endotoxins, mycotoxins, and antibiotic agents (Hartung and Saleh, 2008).Dust particles are frequent carriers of disease agents, dangerous stable gases and unpleasant odours (Matković, Vučemilo and Vinković, 2012).

The amount and the constitution of particles in poultry houses are influenced by factors such as housed species, stocking density, age and fattening period of the birds, feeding system, antibiotic treatment, bedding material, humidity, ventilation system, and implementation of hygienic measures (Schulz *et al.*, 2019).

The health sequel of dust particles on animals is dependent on the nature of the particles (organic, inorganic and non-specific),the composition of the particles carried and the diameter of the particles (Ostović *et al.*, 2016).

The different levels of the effective transmission of airborne infection are dependent on the production of the pathogenic agent from the origin and the arrival of the adequate numbers of viable organisms to cause infections in a secondary host. Environmental exposure is a common threat for all such organisms during transmission between hosts. Transmission through air has been alluded as another possible way for *staphylococcus aureus* and MRSA, as contaminated dust particles may disseminate in the air in shelters, and subsequently be inspired by people working in these barns (Masclaux et al., 2013) .Increased dust levels may irritate the mucous membranes of the respiratory system and causing inflammatory and allergic reactions and reduce animal resistance. Therefore, this direct contact with colonized animals and frequent contact with contaminated surfaces and inhalation of contaminated air are assumed to contribute to nasal colonization (Cuny, Wieler and Witte, 2015; Bos*et al.*, 2016) .

Purpose:

This study aims at finding out if dust in poultry houses has the potential of transmitting Extended Spectrum Beta lactamase producing Enterobacteriaceae and for the participants, i just want to find out if they have been adversely affected with this dust in their day to day lives when working in these houses. The dust will be collected and analysed in the lab if they contain the said Enterobacteriaceae.

Procedures:

It is a cross sectional study and the Questionnaire has been developed for the participants to respond to by ticking or writing short answers.

The dust will be collected and analysed in the lab if they contain the said Enterobacteriaceae.

Who will participate in the study?

Owners of the farms, workers, spouses are the intended participants.196 participants will be required and be active in the study in one month duration.

Risks/Discomforts:

No any risks or discomforts for participants but Participant identification is private and the information collected from the participants will be used strictly for research purposes

Benefits:

- This study will help in bridging the knowledge gap which is there in terms of dust contents and bacterial load in dust from poultry houses which may reduce the occupation hazard thereby reducing dust related infections thereby give poultry farmers, policy makers and relevant authorities to use this work as reference point in the establishment of prevention and control measures.

Alternatives:

Participating in this study is voluntary.

Cost:

You will not incur any costs by participating in this study.

Compensation for participation in the study:

There are no monetary or other forms of compensation for participating in the study. But the information you will provide will be highly beneficial to our communities.

Reimbursement:

You will not incur expenses, travel or otherwise and therefore, no reimbursement will be provided.

Questions:

If you have any questions regarding the study, feel free to contact the researcher using the details included above.

Questions about participants rights:

If you have questions concerning your rights as a research participant, you can contact the UNZABRECS

Statement of voluntariness:

You reserve the right to refuse to disclose any information and refusal to participate in this study at any point in time. If you however do consent to this study, we require you to sign the consent provided.

Confidentiality:

The results of this study will be kept strictly confidential, and used only for research purposes. My identity will be concealed in as far as the law allows. My name will not appear anywhere on the coded forms with the information. Paper and computer records will be kept under lock and key and with password protection respectively.

The interviewer has discussed this information with me and offered to answer my questions. For any further questions, I may contact the Chairperson, UNZABREC on the following details__Telephone: 256067 Ridgeway Campus

Telegrams: UNZA, LUSAKA P.O. Box 50110

Telex: UNZALU ZA 44370 Lusaka, Zambia

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E-mail: unzarec@unza.zm

Assurance No. FWA00000338

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STATEMENT OF CONSENT/ASSENT

.... has described to me what is going to be done, the risks, the benefits involved and my rights regarding this study. I understand that my decision to participate in this study will not alter my usual medical care. In the use of this information, my identity will be concealed. I am aware that I may withdraw at any time. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name:………………………….Signature of participant ……………………..Age..……………

Date (DD/MM/YY)……………………………

Name of Witness……………………………….. Signature of Witness…………………………

Date (DD/MM/YY)…………………………………………….

Name…………………………Signature of parent or guardian for minors ……………………

Date (DD/MM/YY)…………………………

Name……………………………………..Signature of Interviewer Date:

ANNEX 2

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF DISEASE CONTROL

BOX 32379

LUSAKA, ZAMBIA

A cross- sectional study on the occurrence of extended spectrum beta lactamase producing *Enterobacteriaceae* and microbial loads in dust from poultry houses in Lusaka- Zambia.

NAME: LINDA BASIKOLO

AN INTERVIEW GUIDE FOR INDIVIDUAL PARTICIPANTS

Introduction

Dear participant, we are conducting this questionnaire survey in order to explore and assess the risks of dust in poultry houses to the lives of workers. You have been purposively selected to participate in this study. Your input in this research, will enable us to generate the much-needed baseline information for developing alternative disease control strategies for the infections which this dust cause and also help duty holders to make an informed and valid judgement concerning the hazards of the dust and to assess the risks of health in the workplace during the course of rearing these chickens. Your participation is voluntary, your personal information will be treated strictly confidential and will be used for study purposes only. Your name or any other identifying information will not appear in any report from this study. Although you could choose not to participate, we are sincerely hopeful that you will opt to as this will provide very valuable information. If you consent to participate, please sign below:

Interviewer's Name: …………………………………. Signature: …………………………

Date: ……………………..

Participant's Name: ………………………………….. Signature: …………………………..

Date: …………………….

BACKGROUND INFORMATION

Province: ………………………………………………………………………………….....

District: ……………………………………………………………………………….

Gender of participant: Male: ……………….. Female: ……………………….........................

Age of participant: ……………………………………………………………………

Level of education of participant: ……………………………………………………………………..

Phone number on which you can be contacted: ……………………………………………………