

A five year assessment of the emerging sensitivity patterns of the top six pathogenic bacterial isolates at the University Teaching Hospital (UTH) in Lusaka

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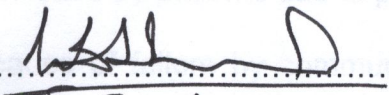
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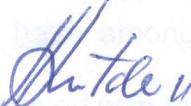
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ABSTRACT

The effect of microbial resistance to antibiotics is growing at a very fast rate all over the world. One of the main reasons for this is the frequency of use of drugs for the treatment of common ailments, some of which may not even require antibiotics. Therefore, there is selective pressure resulting from drug-over use, misuse by patients due to poor health education, use of poor quality drugs because of failure by community members to afford the cost of high efficacy drugs have among other factors contributed to high Anti-Microbial Resistance. Coupled with this is the poor health-seeking behaviour of patients in poor communities who would rather share drugs meant for one patient because they cannot afford the cost.

The irrational prescribing habits of clinicians have also been cited as being contributors to high Anti-Microbial Resistance. The University Teaching Hospital in Lusaka serves as the largest referral hospital in Zambia and therefore is affected by all the factors stated above. Consequently, the problem of AMR is prominent.

This study was therefore carried out to explore the pattern of resistance of pathogenic microorganisms commonly isolated at the hospital against the prescribed classes of drugs. An assessment of which microorganism was most resistant over the entire period was done. In addition, the study identified which, among the antimicrobials tested over the five year period

performed the least with respect to microbial sensitivity profiles.

In order to carry out this study, a retrospective compilation of the data of antibiotic sensitivity testing at UTH was undertaken to cover a five year period stretching from June 2001 to June 2006. Data was entered in the same format used for reporting results (Resistance, Intermediate and Sensitive) and analysed for frequency of each per organism per year.

Data was carried out first by using the Epi Data entry programme. Subsequently, data was exported for final analysis to the Statistical Package for Social Sciences (SPSS). Pearson's Chi square statistics were used in arriving at the associations between the effect of the various drugs and the microbial response as the basis for hypothesis testing.

The results of this study showed that there has been a significant change in the way microorganisms are responding to the various antimicrobials over time ($p < 0.05$). In addition, the most used drug groups, like chloramphenicol and penicillins at UTH were most affected by the problem of AMR ($p = 0.001$ in each case). Microorganisms, however remained highly sensitive to quinolone drugs especially ciprofloxacin and norfloxacin with over 80% organisms testing sensitive. On the other hand, the penicillin group of antibiotics had the worst performance over the entire period of five years. They had a combined failure of 3197 isolates resisting their action. This represented a total of 73.2%

resistance.

Klebsiella was the single most resistant organism against all classes of drugs used, although it was prominently sensitive to the action of ciprofloxacin.

Recognising that the problem of AMR is gradually increasing at the UTH, this study has proposed several interventions. Some of these include the need to uphold and promote the prudent use of antibiotics especially in human beings and revising or amending the policy on use of antimicrobials as growth promoters in animals. The other interventions should take into consideration the need to launch campaigns in communities on the safe and effective use of all medicines including antimicrobials, in similar ways that HIV/AIDS campaigns are done and to institute into routine work the aspects of continuous drug efficacy monitoring.

DEDICATION

I dedicate this dissertation to my wife Mercy Fwambo and my children, Daniel Fwambo (jnr), Twiza Fwambo, Mwaka Fwambo and Chipasha Fwambo for their physical and moral support, knowingly or unknowingly at all times and for the prayers they offered in so many ways. I also praise the good Lord for his abundant blessings and love.

The Lord provided this opportunity and success true to what He has made me believe: ***“I have the strength to face all conditions by the power that Christ gives me”, Philippians 4:13.***

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Working Definitions:

- *Antibiotic*: Naturally occurring (produced by microorganisms) or synthetically produced antimicrobial agents.
- *Anti-infective agent*: Any substance which by virtue of its action on the microbe may cause failure of that microbe to infect or stop further multiplication in a susceptible host.
- *Bactericidal*: Substance that act as antimicrobial agents by causing death of the microbe.
- *Bacteristatic*: Substances that act as antimicrobial agents by causing cessation of growth of microbes. The process may be reversed on removal of the agent.
- *Beta-Lactam Drugs*: Those drugs chemically known to process either a 6-Amino-penicillanic acid or 7-Aminocephalosporanic acid.
- *Isolate*: bacterial isolate
- *Microbe*: Bacteria (unless otherwise explained)
- *Nosocomial infections*: Infections acquired within the hospital
- *Partially sensitive*: isolate testing intermediate (Neither clearly sensitive nor clearly resistant)

Acronyms used

AIDS	Acquired Immunodeficiency Syndrome
AM	antimicrobial
AMP	Ampicillin
AMR	Antimicrobial Resistance/Resistant
AMX	Amoxicillin
AN	Amikacin
β	Beta
C	Chloramphenicol
CAF	Ceffazidine
CC	Clindamycin
CEF	Cefriaxone
CEP	Cephalothin
CIP	Ciprofloxacin
CN	Gentamicin
CTX	Cefotaxime
CZ	Cephalozolin
E	Erythromycin
F	Nitrofurantoin
FR	Furazolidone
HIV	Human Immunodeficiency Virus
K	Kanamycin

MET	Metacillin
MIC	Minimum Inhibitory Concentration(s)
MDR-TB	MultiDrug Resistant Tuberculosis
NA	Nalidixic acid
NN	Tobramycin
NOR	Norfloxacin
OX	Oxacillin
P	Penicillin
PB	Polymixin B
PRSP	Penicillin Resistant <i>Streptococcus</i> Pneumonia
RUM	Rational Use of Medicines
Spp	Species
SXT	Co-trimoxazole
TE	Tetracycline
TMP-SMX	Trimethoprim-sulphamethoxazole
UTI	Urinary Tract Infection
VA	Vancomycin
WHO	World Health Organisation
XDR-TB	Extensively resistant tuberculosis

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Preface

The overuse, misuse of antibiotics and various factors associated with both the patient and health care practices have in the last couple of decades accelerated the development and spread of resistant bacteria and other microorganisms. This has resulted in difficulties in management and treatment of most infections on a global level. The problem is worse in poor countries, which in addition to the emergency of new infectious diseases are also unable to afford the cost of more potent quality antimicrobials. This has resulted in the unfortunate use of sub standard and sub therapeutic drug regimens.

In view of these changing trends, it has become necessary that the approach to antimicrobial use is addressed both at the level of health education of the patient/client as well as at the level of health/medical worker training curriculum. Currently, the health seeking behaviour of patients is economically mediated in the sense that patients would rather spend their meager income on food than spend it on medication. This results in failure to seek curative interventions as they source drugs of low efficacy. In the same vein it has been acknowledged that the current medical curriculum at the University of Zambia requires review to strengthen pharmacology and rational use of antimicrobial agents.

This study was therefore designed to, not only establish the magnitude of

antimicrobial resistance problem at the largest health institution in the country, but to also identify indicators of the problem as well as offer interventions to the existing AMR problem in other health institutions as well. This would be through policy direction that would emanate from findings of this study.

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Chapter One: Introduction and Theory

1.1 Introduction

The discovery of antimicrobial agents in the first half of the 20th century resulted in substantial reduction in the threat that was posed by infectious diseases. The use of these antimicrobial drugs combined with improvements in sanitation, housing, nutrition, coupled with the development of immunization programmes has further led to dramatic drop in death rates arising from diseases that were previously widespread, untreatable and frequently fatal (WHO, 2002). In addition many serious infectious diseases have been controlled using drugs and this has subsequently resulted in major gains in life expectancy that the latter part of the last century experienced.

However, in the last few decades it has been clearly apparent that the gains derived from antimicrobial agent use are now being jeopardized by the emergence of microbes that are resistant to cheap and previously effective first-choice, or “first line” drugs. It has been observed that bacterial infections that tend to contribute to most human diseases are unfortunately also the ones in which antimicrobial resistance is most evident. Consequently, treatment and general management of diseases such as diarrhoea, respiratory infections, meningitis, sexually transmitted infections and hospital acquired infections is no longer as easy as used to be in the past.

Recently there have been increased cases of penicillin resistant *Streptococcus Pneumoniae*, vancomycin resistant *Enterococci*, methicillin resistant *Staphylococcus aureus*, multi-resistant *salmonellae* and multi-resistant *mycobacterium tuberculosis*. Increasingly, infections caused by resistant microorganisms fail to respond to treatment resulting in prolonged illness and greater risk of infirmity and death. As treatment fails, patients will suffer over long periods of time. In addition the situation gets even worse as the population of patients harboring resistant organisms increases in the community.

The disease causing microorganisms have a special ability to adapt themselves in such ways as to acquire and transfer antimicrobial resistance. Globally, this problem has been exacerbated by the fact that there has been excessive and uncontrolled use of antimicrobial agents that in turn favours the growth of resistant microorganisms. Resistance to antimicrobial agents is primarily a biological phenomenon that can be amplified by a variety of factors, including practices. The use of an antimicrobial agent for any infection, real or feared, in any dose and over any period, will tend to force microbes to either adapt or die. This is a phenomenon known as "selective pressure." Under this phenomenon, microbes which adapt and survive will carry genes for resistance. These genes can be passed on as the microbe multiplies itself and resistance to a single drug may spread rapidly through this route. For instance a study on the mechanisms and nature of resistance of *Salmonella* species ampicillin, trimethoprim and tetracyclin found that the resistance differed across species and different

mechanisms were responsible for such variations (Cabrera, Ruiz, Marco, Oliveira, Arroyo, Aladuená, Usera, De Anta, Gascon, and Vila, 2004).

When antibiotics are incorrectly used (as may be; for too short a period, too long a period, use of low potency drugs or for a wrong disease), there is a high likelihood that bacteria and other microorganisms will adapt and even replicate instead of being killed (WHO, 2002).

The combination of drugs may also have an effect on the end result of treatment. Some antibiotics are known to be effective against a wide range of microorganisms while others are not. Drugs that can act on a wide variety of microorganisms have been referred to as broad spectrum antibiotics. The present insights into the determinants of the resistance and the biochemistry of antibiotic “target molecules” in different species have enabled drug molecules to be tailored to perform specific antibacterial roles (Sweetman, 2002).

The need for judicious use of antibiotics, is increasingly becoming necessary in order to control what is currently an ever increasing problem of antibiotic resistant bacteria. In addition, it is also becoming increasingly essential to promote patient education programmes regarding the risk of inappropriate antibiotic use, in the treatment of such conditions as common cold or serous otitis. For that matter, patients must be told of the importance of taking antibiotics exactly as prescribed.

Sometimes, physicians, clinicians and other medical staff are called upon to manage a life-threatening condition. In such cases, it has been within the physician's or clinicians informed judgment to prescribe and administer antibiotics that they think would save the life of a patient at a point in time. Even with this judgment, the need for clinician-laboratory collaboration cannot be over-emphasized because there are many instances where prescribed antibiotics have turned out to be ineffective due to resistance. Often upon realization of this, the change of regimen has at times not only been expensive but would predispose some patients to various forms of drug reactions (Cars & Hakansson , 1995).

Where it is inevitable that antibiotic treatment and prescriptions have to be given before laboratory results are available, it may be necessary for the attending doctor to prescribe antibiotics for a shorter duration while awaiting the culture and sensitivity results from the laboratory. Ideally, the choice of antibiotics should be limited to first-line agents, such as amoxillin, trimethoprim-sulphurmethoxazole or erythromycin-sulphixazole. In the treatment of Streptococcal infections for instance, some experts have advised that broad spectrum, oral antibiotics such as cephalosporins, should be avoided unless symptoms persist or relapse (Harrison & Lederberg, 1998)

1.2 Theories on the emerging resistance patterns

In a study conducted in Britain, it was found that the chronic use of ampicillin for urinary tract infections in women was associated with a multi-drug resistant faecal flora. Similarly another study done in Denmark correlated the amount of erythromycin used in different hospitals with frequency of erythromycin resistance for *Staphylococci* (Apua-Zambia Chapter, 2005).

The nature of the antibiotic is also a factor in assessing causes of antibiotic resistance of organisms. Several classifications of antibiotics are based on the spectrum of bacteria acted on (broad or narrow), route of administration (parenteral versus oral versus topical), or type of activity (bactericidal versus bacteriostatic) and the chemical structure of the drug with which the organisms will interact (Healthline, 2005).

1.2.1 Penicillins

Penicillins are the oldest class of antibiotics with a chemical structure similar to cephalosporins. The two groups are classified as beta-lactam antibiotics. They are generally bactericidal in action (Healthline, 2005). Penicillin group of drugs may be classified into two: the Natural penicillin G which was isolated from a mould; *Penicillium notatum* was the first significantly effective agent developed in this group of drugs. It is an acid labile substance with a characteristic Beta-lactam structure which is readily destroyed by microorganisms such as *Staphylococci*

and Gonococci that have the ability to produce penicillinase (a Beta lactamase) which destroys penicillin (Frohlich, 1993). Others are synthetic derivatives and are based on the original Penicillin G. Some are even known to be less susceptible to degradation by gastric acid, e.g. ampicillin and Amoxicillin, while others like methicillin, oxacillin and nafcillin are penicillinase resistant.

The action of various penicillins may be summarized as:

Narrow spectrum: Beta-lactamase labile penicillins

Benzylpenicillin, phenoxymethylpenicillin) with specific activity against Gram positive bacteria; Beta-lactamase-stable, narrow spectrum drugs such as methicillin (orally inactive) and cloxacillin and flucloxacillin (both are orally active); broad spectrum, Beta lactamase labile penicillins (ampicillin, amoxicillin) which are inactive against *Pseudomonas aeruginosa*; Broad-spectrum, Beta-lactamase labile drugs (e.g. carbenicillin) activity includes that against *Ps aeruginosa*; Pro-drugs, i.e. antibiotics that are themselves inactive but are hydrolysed in-vivo to give the active drug; substituted ampicillins ($R=C_6H_5CH_2(NH_2).CO$, e.g. piperacillin, mezlocillin and azlocillin, which combine the properties of ampicillin and carbenicillin. (Russell and Quenelle 1993).

1.2.2 Cephalosporins

These antibiotics are originally derived from a mould called *Cephalosporium*. They have two side chains. They are similar to penicillin in their possession of beta-lactam nucleus and in their antibacterial action. This group of drugs has

been classified into “generations” due to their varied spectrum of activity. The ‘first’ generation groups are known to be effective against gram-positive organisms. Gram positive organisms are also known to be sensitive to penicillin. The ‘second’ generation is a group that is active against many anaerobes while the ‘third’ include those that are active against Gram-negative organisms.

Most of the cephalosporins are β -lactamase resistant, and are useful in the eradication of penicillinase-producing bacteria including Staphylococci, Neisseria, and Haemophilus influenzae. Most cephalosporins have relatively short plasma half-life, though cefonocid is reported effective with once-daily dosing. The first generation agents, cephalexin, cephradine, and cefadroxil, including the second generation cefaclor, are resistant to acid degradation, thus being orally active. Other cephalosporins are administered parenterally (Frohlich, 1993).

Other less common inhibitors of cell wall synthesis include, clavams such as clavulanic acid, oxacephems, e.g. moxalactam (1-oxacephalosporin derivative), carbapenems e.g. olivanic acids acting as competitive inhibitors of β -lactamases and monobactams such as 3-aminomonobactamic acids, active against Gram negative bacteria.

1.2.3 Membrane active agents

These act against bacteria by causing damage to their cytoplasmic membranes. The commonest among these are the polymixins (Colistin and Polymixin B). They have been known to increase bacterial cell wall permeability. They are best active against gram negative bacteria such as *Pseudomonas aeruginosa*. These agents have been known to damage the nephron hence their limited use. When administered orally, they tend to be poorly absorbed rendering them useful in the elimination of susceptible bacteria in the gastrointestinal tract. They have also been known to induce respiratory failure when used in combination with neuromuscular blocking agents or in persons with myasthenia gravis (one of the type II autoimmune diseases), (British National Formulary,2005).

1.2.4 Inhibitors of Protein Synthesis

In this group are drugs that affect the bacterial protein synthesis at different stages of their normal multiplication phases. The main groups are the aminoglycosides (streptomycin,gentamicin, kanamycin, tobramycin, amikacin, sisomyicin and netimyicin (Russell and Quesnel 1993); tetracyclines with about eight clinically useful subgroups. They include Chlortetracycline, oxytetracycline,tetracycline, 6-demethyltetracycline and demethylchlortetracycline. These are all derived as secondary metabolites from a mould of *Streptomyces* species. Tetracyclines are broad spectrum antibiotics acting at the 30S ribosomal unit of the bacterium leading to bacteristasis if administered in recommended clinical concentrations, (Sweetman 2002).

1.2.5. Inhibitors of Nucleic Acid Synthesis

Drugs such as rifampicin act by binding to the β -subunit of the bacterial RNA polymerase inhibiting attachment to a promoter site. Similarly, actinomycin D inhibits the function of DNA-dependant RNA polymerase as it binds strongly to helical double stranded DNA. Nalidixic acid, a synthetic drug, inhibits the synthesis of DNA gyrase A although it has been reported to have other effects as well (Crumplin et al 1980). Similarly, Staudenbauer (1975), reported that Novobiocin tended to inactivate the action of an enzyme DNA gyrase B that acts by introducing negative super-helical which turns into covalently closed circular double stranded DNA.

1.2.6. Inhibitors of metabolic function

Dihydrofolic acid, a precursor of tetrahydrofolic acid is normally biosynthesized by most bacteria in order for them to produce methionine, purines, and thymine, all of which are nucleic acid constituents. The reaction of dihydrofolic acid synthesis is mediated by the action of an enzyme, dihydrofolate reductase (DHFR), which in effect reduces p-aminobenzoic acid (PAB; 4-aminobenzoic acid), pteridine and glutamic acid in a complex reaction. These reactions have been disturbed by the presence of sulphur drugs. For instance, sulphanilamide and its derivative modifications have been known to competitively inhibit dihydropteroate synthetase, an enzyme that condenses pteridine and PAB. It has been found that the sulphonamides do not have any action on preformed folate. As such they will

only inhibit microbial growth, rendering such drugs bacteriostatic. The most prominent of these drugs have been trimethoprim and tetroxoprim (Stockley, 1998)..

1.3 The Problem

1.3.1 The Global Perspective

The emergence and spread of antimicrobial resistance among bacteria, viruses, and other disease causing microorganisms has created a threat to our ability to combat infectious diseases on a global level (National Academy Press, 2006).

Numerous interconnected factors, many of which are linked to the misuse of antimicrobials are responsible for the emergence and spread of antimicrobial resistance. In addition, the use of antimicrobials is influenced by interplay of the knowledge, expectations, interactions of prescribers and patients, economic perspectives, characteristics of country's health care system and the regulatory environment. Several specific factors contributing to the resistance problem may be cited and include:

Patient-related factors: These have been found to be the major drivers of inappropriate antimicrobial use (National Academy of Sciences, 2006). Many patients believe that new and expensive medications are more efficacious than older drugs. While this perception causes unnecessary health expenditure, it also encourages the selection of

resistance to these new drugs together with those of the older drugs that are in similar pharmacological classification.

Self-medication and non-compliance with recommended treatments:

These have significant contribution to resistance because they are often unnecessary; inadequate dosages are used or may not contain adequate amounts of active ingredients of the drugs. Resistance ensues if the drugs are counterfeit or expired. It has also been noted, particularly in developing countries that antimicrobials are purchased in inadequate dosages and only taken until the patient feels better. This often results in microorganisms adapting against such drugs.

Prescribers' perceptions: patients' expectations and demands may influence practice as they may demand to be prescribed antibiotics even without diagnostic justification. These practices have sometimes been driven by diagnostic uncertainties, lack of opportunity for patient follow-up, lack of knowledge regarding optimal therapies and patient demand (WHO, 2002).

Hospital environment: These are fertile grounds for breeding resistant microbes. Hospitals deal with large numbers of patients (in many cases with those with suppressed immunity) in relatively close proximity to each other. The patients may be under very heavy and prolonged antimicrobial therapy. Large hospitals and teaching hospitals generally experience more

problems with drug resistant microbes, probably because they treat greater numbers of the sickest patients and those at high risk of becoming infected (National Academy of Sciences, 2006).

Use of veterinary drugs: Veterinary prescriptions contribute greatly to the problem of resistance. In North America and Europe, an estimated 50% of all antimicrobial production is used in food-production, animal and poultry. The largest quantities are used as regular supplements for prophylactic and growth promotion, thus exposing a large number of animals, irrespective of their health status to frequent sub-therapeutic concentrations of antibiotics. Consequently resistances in such organisms as Salmonella and campylobacter are high. Ultimately this resistance is passed on to human beings through the normal food chain, thus creating even more problems for clinicians (National Council Research, 1999).

1.3.2 The Zambian perspective

Current evidence shows that the problem of Antimicrobial resistance (AMR) is steadily growing in Zambia. Banda (2006), provides several findings with regard to AMR problem. He states that among other reasons, practitioners have recognized the value of accurate diagnosis in the management of common infections particularly with regard to RUM and AMR. In addition he cites the lack of adequate laboratory facilities including poor staffing as being contributory to failure in attaining accurate diagnosis of patients. The report further recognizes

significant gaps in the current undergraduate medical curriculum and government policy on rational use of drugs. In the curriculum, the report identifies a mismatch between theoretical knowledge and what is actually in practice. These are some of the factors that are contributing greatly to the exacerbation of the problem of AMR.

Mwansa, et al, (2006), in a study of the antibiotic resistance of *Vibrio cholerae* 01 isolated in Zambia during the outbreaks that occurred between 1990 and 2004, report that a low level of resistance (2-3 %) to tetracycline was recorded in the first two cholera outbreaks that occurred during 1990-1991. They further report that due to continued use of therapy and prophylaxis, resistance increased dramatically to tetracycline (95 %) along with chloramphenicol (78 %), doxycycline (70 %) and Trimethoprim-sulphamethoxazole (TMP-SMX) (97 %), in subsequent outbreaks in 1992.

The study further recognizes that there is a significant association between the development of resistance to tetracycline, chloramphenicol, and co-trimoxazole with the large scale use of these antibiotics for the treatment and prophylaxis of cholera.

HIV and AIDS and TB influence

It has been observed that the advent of HIV/AIDS has resulted in heavy use of antibiotics and other drugs in order to fight off opportunistic infections. For instance, in order to try and prolong the lives of children infected with HIV, co-trimoxazole was used to combat secondary infections in HIV infected children at the University Teaching Hospital in Lusaka. The study found that, of the 541 children treated, 42 % (227) of the children who received the placebo died, compared with 53% (285) of the children on co-trimoxazole (Gibb, et al, 2004). It was observed that there was 43 % reduction in mortality over about 20 months for those that were on co-trimoxazole.

In this study, the idea was to give co-trimoxazole to all children diagnosed with AIDS because the drug was cheap (costing an annual amount of \$7 to \$12 per child). This was a bargain drug compared with the \$200 to \$ 300 for even the cheapest antiretroviral. However, Gibb et al admits that this trial was deemed controversial because the study was deliberately held in an area where bacteria were resistant to co-trimoxazole.. The argument against the administration of this drug was that there would eventually be widespread resistance to this drug due to prolonged use as a prophylactic.

Until recently, few people from countries with limited resources had access to HIV antiretroviral (ARV) drugs (WHO,2000). This meant that patients resorted to usage of a lot of drugs for treatment of opportunistic infections. This in turn

caused drug resistances in many patients thus worsening the problem of patient management.

Similarly, Multi-Drug Resistant TB (MDR-TB) (i.e. tuberculosis strains resistant to at least isoniazid and rifampicin), has been identified as a global problem that threatens TB control. Currently, globally, we have to deal with an even greater emerging variant termed *Extensively Drug-Resistant (XDR) TB* (Gandhi 2007).

1.4 Justification and rationale of the study

This study takes cognizance of the fact that the disease patterns continue to change through out the world due to various reasons including globalization (migration). In addition, the advent of the HIV/AIDS pandemic has resulted in the emergence of new diseases caused by what conventionally were commensalistic organisms, which under normal physiological state of the human body were not pathogenic at all. Therefore, there is the possibility that these and true pathogens will react differently to various antibiotics cannot be ruled out.

The study has provided some insight into the magnitude of AMR which has emerged between 2001 and 2006. It is hoped that the findings will contribute to raising the levels of awareness about the performance of antibiotics in relation to the commonly isolated bacteria at UTH. It is expected that the results of this study will be of benefit to clinicians by providing them with information on the

current performance of antimicrobial agents, for them to be able to arrive at evidence-based decisions as they manage their patients.

1.5 Hypothesis:

Over the years there has been a change in the pharmacological action of antibiotics against known groups of commonly isolated pathogenic bacteria at the University Teaching Hospital in the period between 2001 and 2006.

1.6 Main objective

To retrospectively explore over a five year period (2001-2006) the pattern of Antimicrobial Resistance (AMR) of the pathogenic bacteria against prescribed classes of drugs at the University Teaching Hospital (UTH) in Lusaka.

1.7 Specific Objective

1.7.1 To explore the pattern of resistance of particular microorganisms against specific drugs.

1.7.2 To describe the pattern and extent of Antimicrobial resistance over the period under review (2001-2006).

1.7.3 To explain the observed patterns of resistance using drug class, pharmacological action and bacterial type.

1.7.4 To recommend strategies for preventing Antimicrobial Resistance.

1.8 Research questions

1.8.1 Which class(es) of microorganisms have exhibited AMR the most?

1.8.2 Which pharmacological agents have shown the highest inactivity against microorganisms over the last five years (2001-2006)?

1.8.3 What patterns of association have emerged between antibiotics and microorganisms during the last five years?

1.9. Scope of the study

The study reviewed information covering the period between January 2001 and June 2006. All information on the antibiotic results of the listed top eight microorganisms as recorded were extracted and analysed. The study was limited, for purposes of time, to bacteria, excluding other micro-organisms. Therefore, viruses, yeasts and other mycological species shall not form a part of this study although they may be referred to in specific cross-references as and when necessary.

The behaviour of organisms shall be considered with respect to not only the type of species but also from the specimen point of view and the disease caused.

Chapter Two: Literature Review

The effect of an antibiotic is influenced by many factors, principally being the knowledge and experience of the attending physician or clinician. There is a general perception among physicians that if one anti-microbial drug is good, two should be better, and three should cure almost everybody of almost every ailment (Stockley 1998). According to Stockley, this may be true in a number of occasions. However, there is evidence that the opposite could be true. Antibiotic combination may be justified in cases where there is a suspected acute and undiagnosed infection. In this case, the principle is that the use of more than one drug would increase chances of at least one of the administered drugs being actually effective against the microbe, especially in mixed infections.

Generally it is extremely risky to treat patients with more than one drug at a time since there could be an over-reaction. In some cases, in the use of many drugs has been known to interact in some patients (Stockley 1998).

Inappropriate use of antibiotics has been a result of several factors. They include the sheer lack of knowledge on the part of prescribers about the role and function of antibiotics. WHO (2001) reports that in China, it was established that 63 % of antimicrobials selected to treat proven bacterial infections were inappropriate. Similarly, a retrospective study in Viet Nam found that more than 70% of patients were prescribed inadequate dosages of antibiotics (WHO, 2001). According to a study by Gumodoka (1996), one in every four patients reporting for medical

attention who were given antimicrobial injections, 70% of these patients did not need such medication.

Other compounding factors to inappropriate use of antibiotics have included: the lack of access to information needed to make appropriate prescribing decisions; inadequate laboratory diagnostic support; fear of bad clinical outcomes; and patients' perception about what they need or prefer. Studies have further established that prices at which antibiotics are sold and bought are a powerful determinant of how consumers use antibiotics (WHO, 2001). Economic hardships can lead to early cessation of therapy, e.g. antimicrobials are purchased in inadequate dosages in many developing countries and taken until patient is convinced that he or she is feeling better. This practice has the potential of fostering the selection of resistant micro-organisms, and therefore, has a higher likelihood of treatment failure. This is could be the reason for current problems associated with MDR and XDR TB.

A number of serious consequences have resulted from inappropriate use of antibacterial drugs. One such notable consequence is the problem of acquired resistance: this means that an organism which was once known to be sensitive to a particular drug develops the ability to survive in the presence of that particular drug. Sometimes, bacteria may develop spontaneous mutations; and excessive administration of anti-infectives can suppress the growth of susceptible bacteria, providing unopposed opportunity for survival of resistant cells (Frohlich, 1993).

Frohlich further observes that premature termination of anti-bacterial administration also promotes development of drug resistance. For this reason patients or those responsible for administering drugs should ensure that full regimens are taken even though the symptoms of infection would have subsided.

Under normal circumstances, administration of drugs is meant to suppress the growth of susceptible organisms. Other non-susceptible organisms (with either acquired or natural resistance) will be allowed to increase in numbers and induce infections. Sometimes, however, patients have super-infections as a result of antibiotic administration. These risks are increased when broad-spectrum agents are used and if administration of such drugs continues beyond 7 to 10 days.

The elderly and seriously ill people, in whom the immune response is responding at less than optimal, are most susceptible to the development of super-infections. Colitis is a frequent manifestation of typical super-infection. For instance pseudo membranous colitis induced by *Clostridium difficile* causes severe diarrhea with consequent dehydration which in some cases may be fatal.

Another adverse effect of antibiotic use is the risk of allergic reactions (Stockley, 1998). Symptoms can range from skin rashes, to asthma and even anaphylactic shock.

All over the world, clinicians are often faced with the problem of whether to prescribe or not to prescribe antibiotics. It may, therefore, be necessary to establish possible reasons for the differences in prescribing habits among various prescribers. In addition, it is also important to establish the effects and possible consequences of such variations on patient care, particularly with respect to the responses of organisms to prescribed drugs. For instance one study had found that antibiotics were prescribed for 76% of all the patients by the most generous clinician, but only for 21% by the most conservative one (Cars and Hakanson 1995). They further reported that the use of diagnoses suggesting bacterial infection varied in a similar way.

In this study, it was found that the patients seen by different doctors presented with similar signs and symptoms, (and the return visits for the same complaints during the study months) were on average about 5% for all doctors. It was concluded, in this study that doctors tend to have an individual and constant pattern of prescribing antibiotics. It further seemed apparent that diagnoses are given to justify the treatment, rather than the treatment justifying the diagnosis.

Grob (1992), suggests, that, prescribing of antibiotics should take into account the most likely causal pathogens, as well as, the severity of the illness. He also recognizes the that there were few laboratory diagnostic aids in the practice of community medicine that have been available to the clinician. He envisages, however that, the development of databases on the epidemiology of infectious

diseases will lead to the definition of the most probable causal organisms and their sensitivity patterns.

Grob goes further to argue that there is need to recognize the that prescribing habits of a community physician are, to some extent, influenced by patient expectation about health service delivery in general. Patient compliance with antibiotic regimens is poor in the communities. This problem has become increasingly relevant as more patients are discharged early from hospital while still on medication. It has been noted that non-compliance with medication is associated with the negative interaction of four factors:

- The patient
- The physician
- The severity of the disease
- The therapy (its frequency, availability and duration)

Patient counseling has been known to have remarkable improvement on the patients' compliance to take drugs as prescribed. In addition, it has been observed that decreasing the frequency and duration of dosing has improved compliance. Hence, the need for the new generation short-course drugs.

In Africa, cases of resistance have been numerous partly due to the fact that most countries are very poor economically and therefore unable to provide high quality public health services to their citizens. This predisposes communities to episodes of infections. Provision of antibiotics has often not only been erratic but

has in most cases been riddled with sub-standard, poor efficacious drugs on account of their failure to afford the cost of new generation high quality drugs.

The problem is further compounded by inadequate surveillance and monitoring instruments due to lack of research. For instance, Mwansa, Mutela, Zulu, Amadi, Kelly (2002), observe that the evidence base relating to patterns of antimicrobial resistance in Africa is small and that antimicrobial agents are chosen on the basis of availability and cost. They further observe that there is evidence of resistance patterns varying across Africa, with resistance to SXT in nontyphoidal salmonellae of 14% and 83% in Abidjan and Malawi respectively.

2.1 The Antibiotic Resistance problem in Zambia

Attempts have been made by various academicians and professionals in Zambia to establish the magnitude of the bacterial resistance to commonly used antibiotics. Nyeleti et al (2004) in their study to determine the antibiotic and disinfectant resistance of Salmonella isolates collected from the environment of the poultry processing plant and commercial farms in Zambia, found that 18/496 (3.6 %) and 6/220 (2.7 %) samples respectively were positive for Salmonella. In this study, Nyeleti and his colleagues used a selection of strains and tested them for resistance to those antibiotics and disinfectants commonly used in Zambia. They found that the Salmonella isolates tested displayed multiple antibiotic resistance to a number of antibiotics used to treat both human beings and animals. They further established that there was no resistance to antibiotics used

at the manufacturers' recommended rate or dilution. However, they found that the organisms were resistant at lower dilutions, justifying the need to use such disinfectant at correct concentrations.

Similarly, Ngoma, Suzuki, Takashima and Sato (1993), carried out a study on *E. coli* and *Salmonella choleraesuis* from apparently healthy slaughtered cattle and pigs in 1989 in Zambia for antibiotic resistance along with the presence of conjugative R plasmid. The *Salmonella* strains from diseased animals (cattle, chickens and other animals) were similarly tested. The majority of the cattle had been nomadically reared in 'traditional farms' while all the pigs were from commercial farms. It was found that more pigs (39 %; 41/105) harboured drugs resistant *E. coli* than cattle (6.7 %; 7/105). Further, the number of drug resistant *E. coli* was higher among strains from pigs (31.2 %; 49/157) than cattle (4.2 %; 7/167).

The study further established that for both cattle and pigs, drug resistance was more frequently observed against tetracycline, streptomycin, sulphadimethoxine and ampicillin than other antibiotics and the single resistance pattern of *E. coli* strains occurred most frequently, among pigs. It was found that 1 in every 28 (3.6%) cattle that were slaughtered had been infected with drug resistant salmonella species. The study further established that there was very high frequency of the drug resistant, R plasmid carrying *E.coli* from pigs and cattle. Under normal circumstances, the animals that have been subjected to sub

therapeutic drugs for prophylactic purposes will enter the human food chain, making the drug resistance problem even worse.

2.2 Antimicrobial resistance Profile at UTH

The study on enterobacteria conducted by Mwansa, et al (2002), form part of the evidence that bacterial resistance is becoming a serious problem at the University Teaching Hospital. The table below, adapted from this study shows the response of the micro-organisms:

Table 2.1..a. Summary of antimicrobial sensitivity patterns for three enterobacteria isolated from patients with HIV-related persistent diarrhea in Zambia

Antimicrobial agent ^a	No. sensitive (%)		
	Nontyphoidal salmonellae	<i>Shigella flexneri</i>	<i>S. dysenteriae</i>
Tetracycline	37 (23)	2 (6)	3 (16)
Chloramphenicol	36 (23)	7 (23)	8 (42)
Gentamicin	119 (75)	24 (77)	18 (95)
Sulphamethoxazole-trimethoprim	25 (16)	3 (10)	0 (0)
Amoxycillin	74 (47)	9 (29)	7 (37)
Amoxycillin-clavulanic acid	95 (60)	27 (87)	12 (63)
Cephalexin	105 (66)	23 (74)	17 (89)
Cefuroxime	93 (59)	11 (35)	16 (84)
Cefotaxime	149 (94)	28 (90)	19 (100)
Nalidixic acid	107 (68)	31 (100)	19 (100)
Ciprofloxacin	157 (99)	30 (97)	18 (95)
Erythromycin	22 (14)	0 (0)	4 (21)
Azithromycin	64 (93)	9 (100)	19 (100)

One hundred fifty-eight isolates of nontyphoidal salmonellae, 31 isolates of *S. flexneri*, and 19 isolates of *S. dysenteriae* were tested against all these antimicrobial agents except for azithromycin, against which 69, 9, and 19 isolates were tested respectively.

Source: Findings of the study

2.2.1 Criteria for selection of study microorganisms

The study was profiled using drug sensitivity results of all ten top genera microorganisms that are commonly isolated at the University Teaching Hospital Microbiology Laboratory. The study was initially based on records and statistics compiled on *Escherichia*, *Klebsiella*, *Proteus*, *Staphylococci*, *Streptococci*, *Pseudomonas*, *Haemophilus*, *Salmonella*, *Shigella* and *Vibrio*. Later, their frequency of isolation was used as selection criterion to limit the analysis to six on account of huge and voluminous nature of the data. Consequently, this study has excluded *Vibrio*, *Shigella*, *Haemophilus* and *Proteus* in the detailed detailed although they may be referred to from time to time.

Table 2.2.a: Six pathogenic bacteria commonly isolated at the UTH Microbiology Laboratory

Specimen	Microorganism					
	Kleb	Staph	Esch	Strept	Salm	Pseudo
Urine	****		****			
Pus and body fluids	****	****	****	****		****
Stools					****	
Blood	****	****	****			
Cerebro-spinal Fluid				****		
Sputum (Other than for TB)				****		

source: Results of this study

2.2.2 *Streptococcus*

Most community acquired infections are caused by *Streptococcus pneumoniae*. It is responsible for such infections as, otitis media, pneumonia, bacteraemia and meningitis. It has been observed that the increase in resistance to penicillin and other antibiotics is evident in both developed and developing countries and affects all age groups. This, therefore, calls for more concerted efforts to fight this threat to patient management. Resistance to penicillin by penicillin resistant *Streptococcus pneumoniae* (PRSP) is a result of mosaic mutation of the penicillin binding protein (PBP) genes due to interspecies recombination of homologous genes. Penicillin resistant *Pneumococcus* is also resistant to other common antibiotics such as macrolide, tetracycline, co-trimoxazole, chloramphenicol and clindamycin. This picture has further limited the drugs that clinician have at their disposal.

PRSP cases are known to be higher in isolates from hospitalized patients as opposed to those of out-patients. They are also higher in children compared with adults. Resistant strains are mainly confined to a limited number of sero-groups notably 23F, 19F, 14, 6 which account for more than 90 % of all PRSP. In order to stem this occurrence, prudent use of antibiotics is essential, especially, in children and elderly patients where viral infection and chronic obstructive pulmonary disease are common and misuse of antibiotics are a common feature.

As a result of frequent exposure of children to antibiotics (especially those attending day care facilities), it has been reported that they tend to carry penicillin resistant *Pneumococcus* in their nasopharynx (Keller A. M. & Stiehm, 2000).

Streptococcus pneumoniae should be closely monitored due to its ever increasing global problem of resistance.

2.2.3 Coliforms and *Proteus*

Escherichia, *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter*, collectively referred to as coliform bacteria and *Proteus* are essentially opportunistic pathogens that have been implicated in a wide range of infections. Majority of these species belong to the normal intestinal flora. Of these, *E. coli* is the commonly isolated organism in microbiology laboratories. It is also the premier of nosocomial infections. It is the major enteric pathogen in developing countries and also the main cause of Urinary Tract Infections (UTIs), such as prostatitis, and pyelonephritis (Harrison & Lederberg, 1998).

This organism is widely distributed in nature, and present in soil, surface water and almost invariably in faeces of man and animals. Many different types may be differentiated based on biochemical and serological tests. The different morphological effects observed in cultures of *Escherichia coli* K12 grown in the presence of β -lactam antibiotics are dependent on both the particular antibiotic used and its concentration (Spratt, 1977). The process of the β -lactam inhibition

is a fairly complex one, and known to involve inactivation of more than one enzyme. The organism is known to develop resistance fairly rapidly.

Many of the coliform organisms were dismissed as harmless commensals. However, currently, these organisms are known to be responsible for major problems world wide. Species like *E. coli*, *K. pneumoniae*, *Ent. aerogenes*, *Ent. Cloacae*, *S. marcescens* and *P. mirabilis*, are responsible for most infections produced by this group of organisms. The increasing incidence of the coliforms, *Proteus*, and other Gram-negative organisms in diseases reflect in part a better understanding of their pathogenic potential but more importantly the changing ecology of bacterial disease.

The wide spread and often indiscriminate use of antibiotics has created drug - resistant Gram-negative bacilli that rapidly acquire multiple resistance through transmission of drug resistance R plasmids.

2.2.4 Staphylococcus, Streptococcus and Enterococci

Streptococci, Staphylococci, and Enterococci tend to have similar resistance patterns because they share the same resistance genes. For instance, the penicillinase found in Enterococci is identical to that in staphylococci. Similarly the Enterococci has the ability to transfer resistance genes to many other organisms. The vancomycin resistance gene has been transferred to *Staphylococcus* in vitro and in vivo in animal models.

Lalitha, (1997) states that given laboratory conditions, if test organism is either *Staphylococci* or *Enterococci* species, 24 hours of incubation are required for vancomycin and oxacillin; but other agents can be read at 16 to 18 hours. Transmitted light is used to examine the oxacillin and vancomycin zones for light growth of methicillin or vancomycin-resistant colonies, respectively, within apparent zones of inhibition. Any discernible growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

2.2.5 *Pseudomonas*

Pseudomonas is a common human saprophyte that rarely causes disease in healthy persons. Therefore, most infections by this organism occur in compromised hosts. Antimicrobial agents are needed to treat *Pseudomonas* infections. Usually two-drug combination therapy such as anti-pseudomonal β -lactam antibiotic with an aminoglycoside are used. This is advised particularly for patients with neutropaenia, bacterimia, sepsis, and abscesses (Selina, 2006). The choice of antibiotic also depends on the site and extent of the infection and on local resistance patterns.

Pseudomonas should be considered in the differential diagnosis in any suspected gram-negative infections. The effect of this organism tends to generate concern since it can cause severe hospital acquired infections, especially in immunocompromised hosts. In addition, a concomitant antibiotic

resistance is often present. This makes the choice of antibiotics treatment difficult. As such it is advisable that pseudomonas infections should always be treated with two anti-pseudomonal antibiotics, each with different mechanisms of action.

2.2.6 *Haemophilus*

Haemophilus influenzae is sensitive to a wide range of antibiotics. It is easily inhibited by low concentrations of ampicillin, chloramphenicol, tetracycline, sulphonamide and trimethoprim. Previously, it was found that the early cephalosporins were relatively ineffective against this species, but later compounds such as cefuroxime, cefotaxime and ceftazime are highly active. Other antibiotics include ciprofloxacin, aztreonam and co-amoxiclav (Greenwood, 1992).

In case of meningitis as a result of *H. influenzae*, chloramphenicol has been found to be the first line antibiotic since it tends to be bactericidal to the organism. It tends to absorb well through the meninges and the cerebral tissues. On the other hand, while the organism was quite sensitive to ampicillin which even provided a choice over the potentially toxic chloramphenicol, it has now been found that there has been up to 25% resistance from type b strain of the organism in the UK (Greenwood, 1992).

2.2.7 *Salmonella*,

Many infections due to salmonella are usually self limiting and should normally not be treated (Cruikshank, 1973). Treatment may encourage emergence of carrier states for this organism. If, however treatment is unavoidable, trimethoprim-sulfamethoxazole is the drug of choice. Sometimes quinolones are also effective but chloromphenicol may be necessary for life threatening illnesses (Barron et al, 1994). Susceptibility testing are necessary too, since resistant strains have frequently been reported.

Shigella, on the other hand, has been reported resistant to ampicillin and therefore trimethoprim-sulphamethoxazole (TMP-SMX), quinolones and furazolone may be useful agents for the treatment of bacillary dysentery.

The marked and fairly rapid development of resistance as judged by in vitro tests has mirrored the decreasing clinical usefulness of most sulphonamides in the treatment of shigellosis (Cruikshank, 1973).

The management of cholera is primarily by administration of fluid (rehydration). Chemotherapy tends to play a supportive role. The *Vibrio cholerae* species tend to be sensitive to the tetracyclines, chloromphenicol, streptomycin, furazolone and other chemotherapeutic drugs active against most Gram-negative organisms. In areas such as Bangladesh where cholera is endemic, tetracycline has been the drug of choice (Barron, 1994). Barron further contends that the

decision whether to treat the patient with antimicrobial agents depends on demographic, economic and clinical features of the patient's nutritional status. He notes that there have been ampicillin, trimethoprim, sulfamethoxazole and tetracycline resistant isolates reported from the developed world as well. As such susceptibility testing may be indicated in some cases. For instance, resistance to ampicillin, cephalosporin and carbenicillin has been reported.

Chapter Three: Methodology

3.1 Study design , population and setting

This was a retrospective descriptive study based on laboratory data generated at the University Teaching Hospital laboratory. The sensitivity profile records covering the entire period of five years, from June 2001 to June 2006 constituted the study population.

3.2 Study setting

The study was undertaken in the microbiology section of the department of Pathology and Microbiology of the university Teaching Hospital (UTH) in Lusaka. The hospital is the largest referral centre in the country.

Given the large volume of microbial isolations that were compiled, only the first top six of all isolates were selected for detailed analysis. Available data pertaining to the other micro-organisms, was included for specific illustration or cross referencing only.

3.3. Populations and Sampling

The study units were drawn from reports of sensitivity tests. It comprised compilation of bacteriological sensitivity test results on various organisms isolated in urine, pus including associated fluids, stool, blood, cerebrospinal fluid and sputum. A total of five thousand three hundred and sixteen (5316) isolates were compiled over the entire period. Ninety five of these cases were reported

missing on account of being either insufficiently recorded or, in some cases organisms having been identified without their antibiogram profile. The study was a population study since all the isolates had to be enumerated and reported on. However, to allow for statistical treatment, the population had to be treated as though it were a sample.

3.4 Data collection.

Data that had been stored on hard copy files and in electronic form constituted the source of information for this study. Data was collected over a period of one month with the help of two members of the Microbiology laboratory, (a Recorder and a Laboratory Scientist) who closely assisted to ensure that all available data was documented.

In this study, it has been recognized that, ideally population studies do not require tests of significance because they do not require a sample to estimate characteristics of the population. The most ideal statistics would include means of central tendency and deviations. For purposes of statistical treatment, however, the population data in this study, has been treated as though it were a sample in order to allow for analytical triangulation and consequently provide for a more descriptive picture of the data.

3.5 Methods of data Analysis

Data was collected on a specifically designed data sheet and then fed in the Epi Data application package. Subsequently the data was transformed into excel form to allow for final transformation to the Statistical Package for Social Sciences (SPSS).

The bulk of the results was in form of frequencies and percentages of the action of antibiotics against the microorganisms across all specimens used in this study. The analysis further categorized the differential responses of drugs across the specimens and the isolates therein to ascertain whether there was any significant difference in the drug action with time and in different specimens.

3.6 Presentation of results

Results of this study have been presented in form of cross tabulations, charts and graphs. Each chart, graph or table is explained by way of specific narration.

3.7 Materials

Data was collected from existing hard copy and electronic information in the microbiology laboratory at UTH. Data was collected using a suitably designed data collection form for ease of entry later.

3.7 Ethical considerations

The study involved collecting, arranging and compiling data that had been recorded over a five year period. Since this was an audit of the records stored over five years in the Microbiology Laboratory at UTH there was no direct or indirect involvement of human subjects at any stage of the study. Identities of patients and their samples as recorded did not form part of the study except for their results. Each case was assigned a number just for the purpose of the study. The study did not therefore; invade privacy or confidentiality of any person.

Permission, however, was sought and granted by the Head of Department, Pathology and Microbiology, on behalf of the hospital for the researcher to proceed. Consequently, a research ethics waiver was obtained from the Research Ethics Committee.

3.9 Limitations of the study

This study was exclusively a record review of laboratory test results generated in the microbiology laboratory at the University Teaching Hospital, Lusaka. The results, therefore, represent observed interaction of bacteria with drugs impregnated on diffusible paper and not the actual interaction in the human body. Consequently, it is possible that where the discs may not have been quality controlled, it may not have been easy to explain variations in interaction beyond what the laboratory reported. This unfortunately, holds true even for clinicians, who have to interpret the same results. Failure of drug activity under laboratory

conditions may include use of possible expiry of discs and the poor quality of media in which organisms fail to grow.

Chapter Four: Results

4.1 Introduction

This chapter presents quantitative results of this study in both tabular and graphical forms in order to allow for ease of translation of the performance of the various antibiotics against specific genera of microorganisms. A general overall picture of antibiotic performance and trend is first presented to show how the antibiotics have performed. Then, a year by year profile is presented to show how they faired in each year. Eleven generic bacteria were profiled during data collection and are shown in the table below. However, only the six top organisms were selected for discussion because of the huge quantity of data collected.

4.2 Selection of microorganisms

The criterion for selection of the top six microorganisms was based on the frequency of isolation of the organisms across all the specimens (urine, pus, stool, blood, cerebrospinal fluid and sputum). Over the five years, a total number of five thousand three hundred and sixteen (5316) microorganisms were isolated. From this number a total of ninety-five were either not properly entered or had their antibiogram results missing. These have been recorded as missing in the study, This left a total number of five thousand two hundred and twenty-one. Based on this a rank order of frequency of isolations was made in order to select the six Table 4.2.a. below:

Table 4.2.a. : Frequency of isolations of microorganisms over a five year period (2001-06)

Microorganism	Frequency	%	validity %	Cummulative Frequency
<i>Klebsiella</i>	1450	27.3	27.8	27.8
<i>Staphylococcus</i>	1136	21.4	21.7	49.5
<i>Escherichia</i>	997	18.8	19.1	68.6
<i>Streptococcus</i>	535	10.1	10.2	78.8
<i>Salmonella</i>	335	6.3	6.4	85.2
<i>Psuedomonas</i>	257	4.8	4.9	90.1
<hr/>				
Other isolates				
<i>Vibrio</i>	230	4.3	4.4	94.5
<i>Proteus</i>	201	3.8	3.9	98.4
<i>Shigella</i>	60	1.1	1.2	99.6
<i>Haemophilus</i>	20	0.4	0.4	100.0
<hr/>				
Total	5221		100	
Recorded missing	95	1.8		
Grand total	5316	100		

Klebsiella Staphylococcus and Escherichia were the most frequently isolated, while Haemophilus, Shigella and Proteus were the least isolated across all specimens.

4.3 CUMMULATIVE ANTIBIOTIC ACTION OVER A FIVE YEAR PERIOD

Fourteen antibiotics were regularly employed as antimicrobials between 2001 and 2006, table 4.3. a.

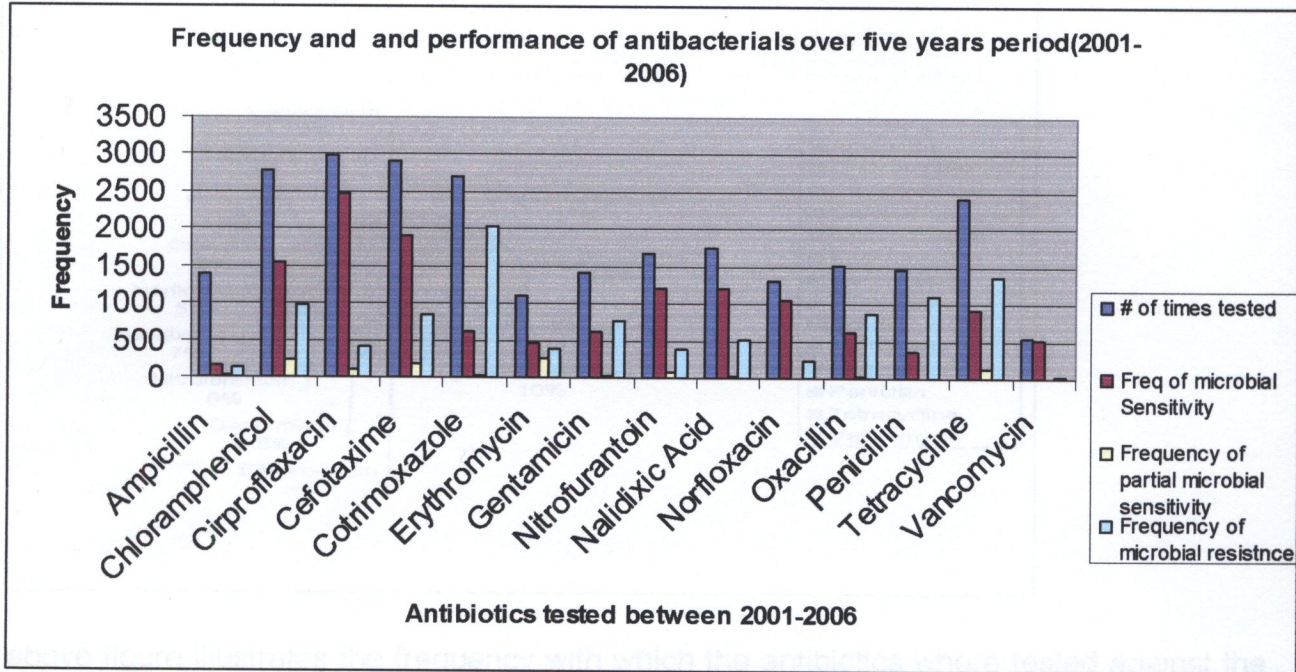
Table 4.3.a. Frequency of antimicrobial use between 2001 to 2006

Antibiotic	Freq of use	Combined desired effect (sensitive microbes) (%)	Intermediate (partial sensitive) (%)	Resistant (%)
Ampicillin	1384	143 (10.3)	20 (1.5)	1221 (88.2)
Chloramphenicol	2769	1455 (52.6)	224 (8.1)	990 (71.5)
Ciprofloxacin	2995	2476 (82.7)	106 (3.5)	413 (13.8)
Cefotaxime	2912	1898 (65.2)	168 (5.8)	846 (29.1)
Cotrimoxazole	2700	623 (23.1)	35 (1.3)	2042 (75.6)
Erythromycin	1096	427 (39.0)	255 (23.3)	384 (35.0)
Genatmicin	1410	616 (43.7)	27 (1.9)	767 (54.4)
Nitrofurantoin	1665	1204 (72.3)	71 (4.3)	390 (23.4)
Nalidixic acid	1757	1197 (68.1)	36 (2.1)	524 (29.8)
Norloxacin	1307	1051 (80.4)	12 (0.9)	244 (18.7)
Oxacillin	1511	624 (41.3)	16 (1.0)	871 (57.6)
Penicillin	1471	354 (24.1)	12 (0.8)	1105 (75.1)
Tetracycline	2413	917 (38.0)	136 (5.6)	1360 (56.4)
Vancomycin	544	511 (94.0)	6 (1.1)	27 (5.0)

It was found that out of these, particularly, penicillin, ampicillin and tetracycline performed quite badly on most organisms subjected to them. Other antibiotics like Chloramphenicol, ciprofloxacin, cefotaxime, nitrofurantoin, nalidixic acid and norflaxacin performed fairly well over the same period and across all specimens. Other antibiotics exhibited border line activity.

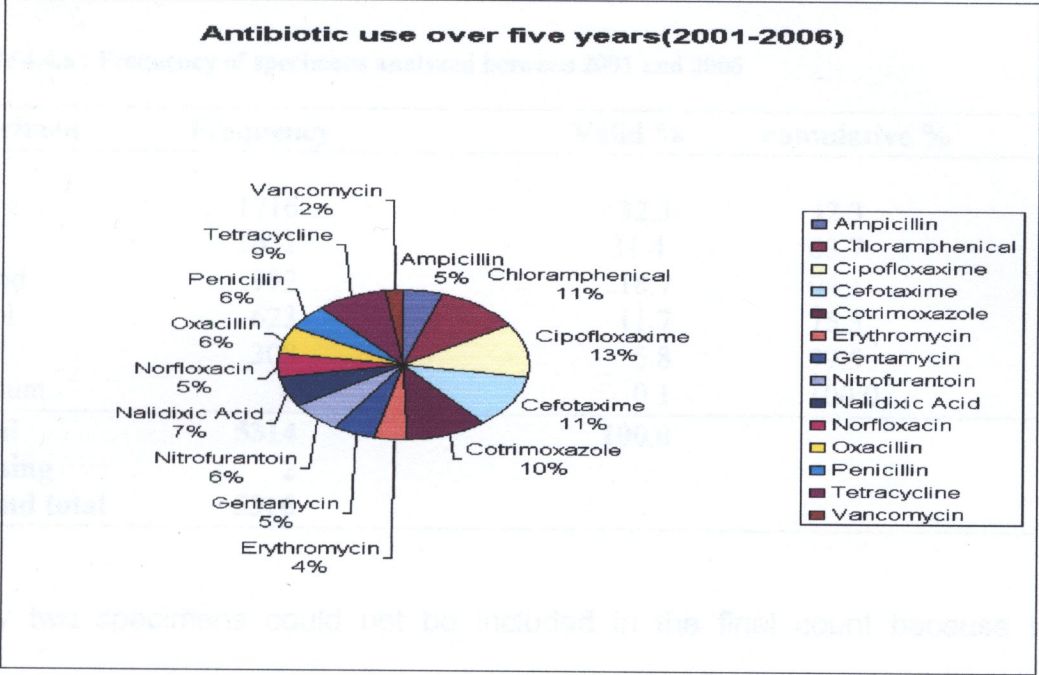
Table 4.3.a is further summarized in the figure below

Fig 4.3 a:



The number of times each antibiotic was used over the five year period has been shown below in figure 4.3.b. It is clear from this pie chart illustration that ciprofloxacin had the largest share with 13% of all the drugs used. Vancomycin had the least with only 2%.

Figure 4.3.b. Relative frequencies (%) of antibiotics



The above figure illustrates the frequency with which the antibiotics were tested against the various organisms. Of all the antibiotics, ciprofloxacin was the most frequently tested (13% all the drugs tested) while the least was vancomycin (2% of all drugs tested).

4.4 Frequency of specimens

The microbiology unit at the UTH receives specimens of varying nature from all medical and surgical departments. The most common was urine from urogenital infections, pus from surgical and other wound infections, stools from suspected gastro-intestinal infections, blood from suspected septicaemias, cerebrospinal fluids from suspected cases of meningitis of bacterial or fungal origins and sputum from patients complaining of chest problems other than Tb.

The frequency of handling and processing of these specimens during and between 2001 and

2006 is shown in table 4.4.a. below:

Table 4.4.a : Frequency of specimens analyzed between 2001 and 2006

Specimen	Frequency	Valid %	cumulative %
Urine	1716	32.3	32.3
Pus	1671	31.4	63.7
Blood	992	18.7	94.1
Stool	623	11.7	75.5
CSF	308	5.8	99.5
Sputum	4	0.1	100.0
Total	5314	100.0	
Missing	2		
Grand total	5316		

Only two specimens could not be included in the final count because they were either improperly entered or had insufficient detail to warrant inclusion. As such they were recorded as missing.

Sputum, in routine microbiology laboratory was mainly processed for detection of such organisms as *Streptococcus pneumoniae* and *Klebsiella* species. As such its frequency was very low, 4 (0.1 %). On the contrary, urine was frequently analysed, 1716 (32.3 %) of all the specimens. Pus totaled 1671 (31.4 %), Blood 992 (18.7), Stool 623 (11.7 %) and CSF 308 (5.8 %).

4.5 Cumulative individual antimicrobial activity over five years

The results presented in tables below represent the performance of each antibiotic against all the organisms listed in table 4.2.a. above.

Table 4.4.b. Microbial response to ampicillin action between 2001-06

		Year						Total
		2001	2002	2003	2004	2005	2006	
Sensitive	Count	29	33	25	25	28	3	143
	% within year	29.9	18.3	8.9	7.3	7.6	2.5	10.3
Intermediate	Count	4	4	1	6	4	1	20
	% within year	4.1	2.2	0.4	1.8	1.1	0.8	1.4
Resistant	Count	64	143	254	310	336	114	1221
	% within year	66.0	79.4	90.7	90.9	91.3	96.6	88.2
Total	Count	97	180	280	341	368	118	1384(N)
	% within year 100	100	100	100	100	100	100	100

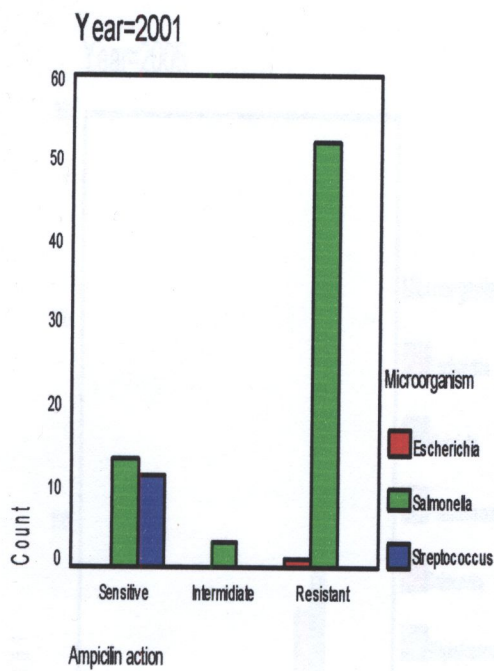
Pearson Chi-Square 77.704(a)
p=0.001

Generally most microorganisms subjected to ampicillin resisted the action of antibiotic. Over the review period across all specimens, 1384 microorganisms were tested for ampicillin sensitivity. only 29(29.9) of the microorganisms were sensitive to ampicillin in 2001. It drastically dropped to 7.6% and 2.5 % in 2005 and 2006 respectively. The over-all picture represents 10.3 % sensitivity and 88.2% resistance of micro-organisms over a period of five years.

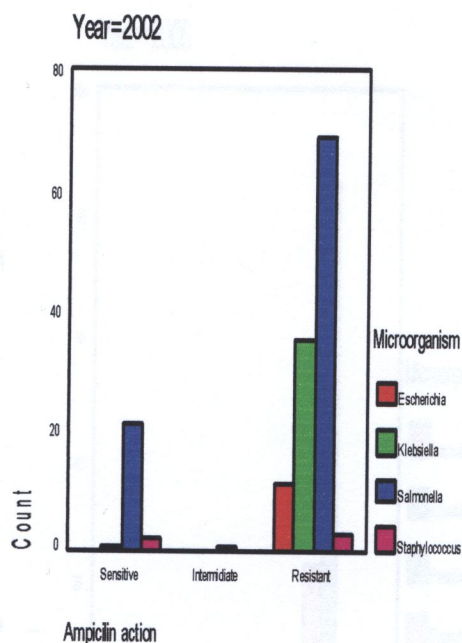
Annual antibiotic action

Given the individual microbial picture, the graphs below show that ampicillin generally performed badly with respect to the commonly isolated bacteria. Salmonella showed a 77% (52/68) resistance to ampicillin in 2001.

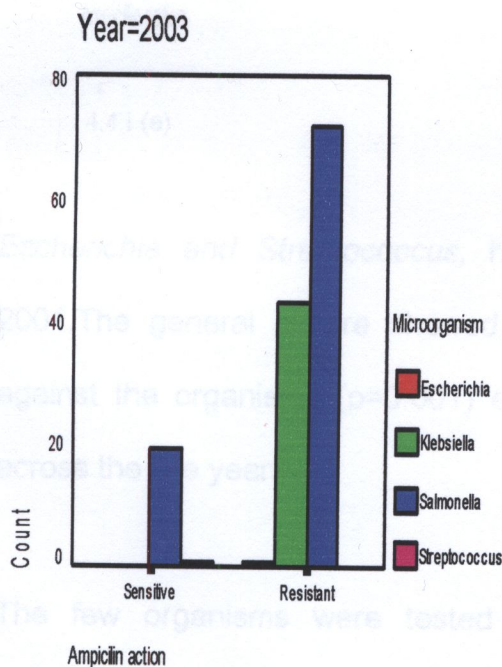
Fig4.4. i: Ampicillin action



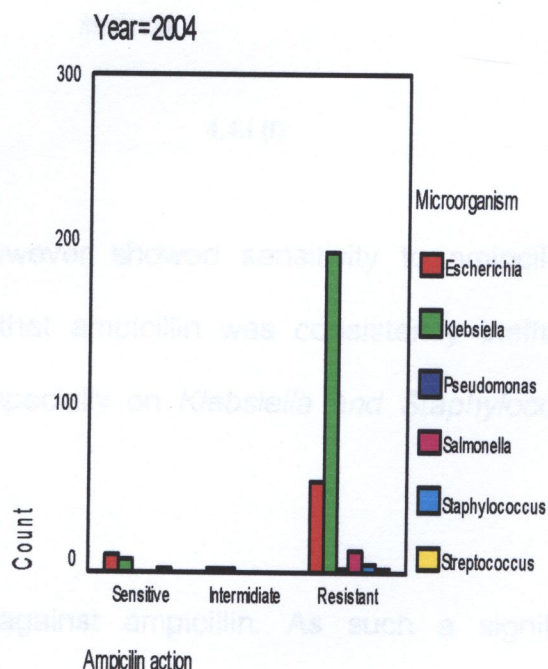
4.4.i. (a)



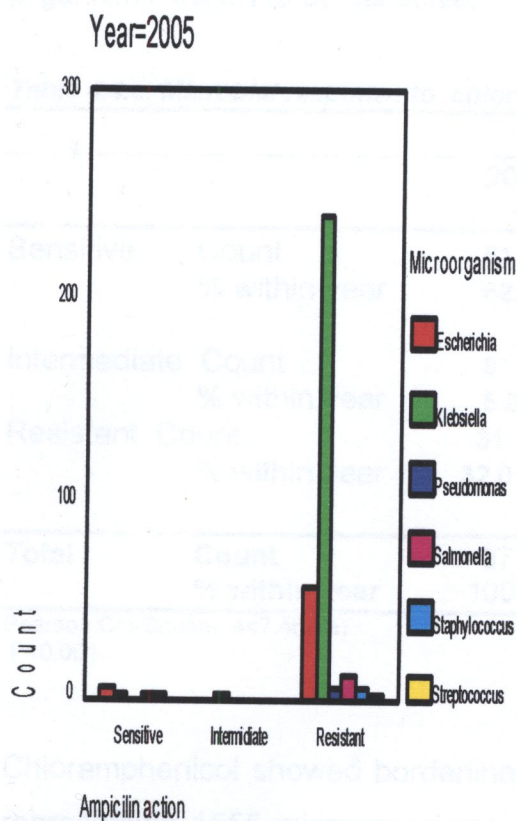
4.4.i (b)



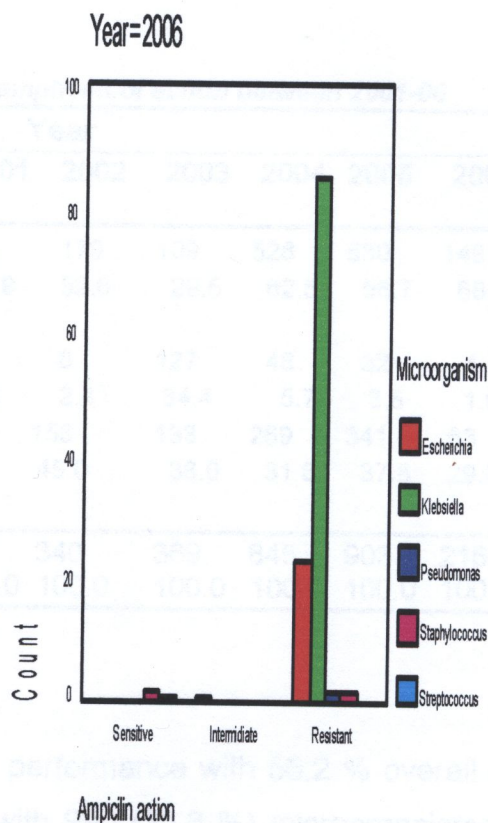
4.4.i (c)



4.4.i (d)



4.4.i (e)



4.4.i (f)

Escherichia and *Streptococcus*, however showed sensitivity to ampicillin in 2001. The general picture showed that ampicillin was consistently ineffective against the organisms ($p=0.001$) especially on *Klebsiella* and *Staphylococcus* across the five years.

The few organisms were tested against ampicillin. As such a significant conclusion of interaction between organisms and the drug could not be drawn. It is normal practice to use antibiotic discs in a cost effective manner and therefore

it is possible that antibiotic discs may have been reserved for use, mainly on organisms known to be sensitive.

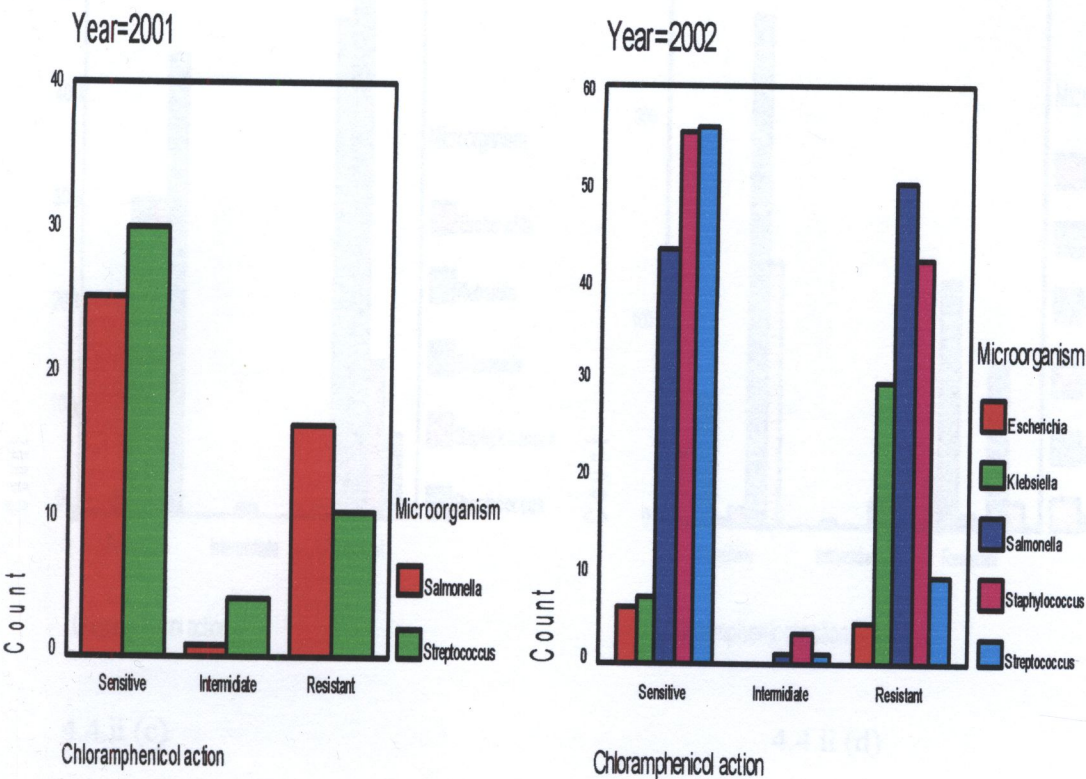
Table 4.4.c: Microbial response to chloramphenicol action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	61	179	109	528	530	148	1555
	% within year	62.9	52.6	29.5	62.5	58.7	68.8	56.2
Intermediate	Count	5	8	127	48	32	4	224
	% within year	5.2	2.4	34.4	5.7	3.5	1.9	8.1
Resistant	Count	31	153	133	269	341	63	990
	% within year	32.0	45.0	36.0	31.8	37.8	29.3	35.8
Total	Count	97	340	369	845	903	215	2769
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 447.464(a)
P=0.001

Chloramphenicol showed borderline performance with 56.2 % overall sensitivity representing 1555 microorganisms with 990 (35.8 %) microorganisms resisting its action, 224 (8.1 %) microorganisms showed partial sensitivity (intermediate), compared to only 1.4 % for ampicillin. The above figures are against a total of 2769 isolates tested.

Figure 4.4.ii: Chloramphenicol action

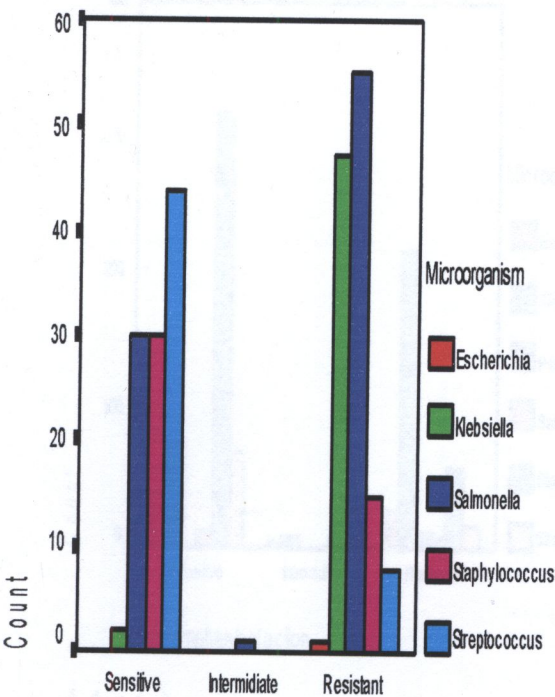


4.4.ii (a)

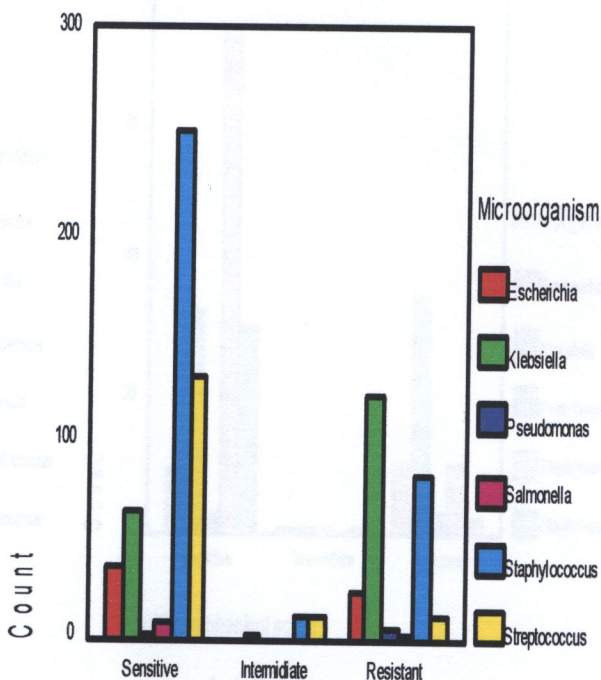
4.4.ii (b)

The performance of chloramphenicol on Salmonella was particularly noteworthy there seemed to be almost as many resistant isolates as there were sensitive ones in both 2001 and 2002. A similar trend, though not to the same extent was apparent for Streptococcus.

Year=2003



Year=2004



Chloramphenicol action

Chloramphenicol action

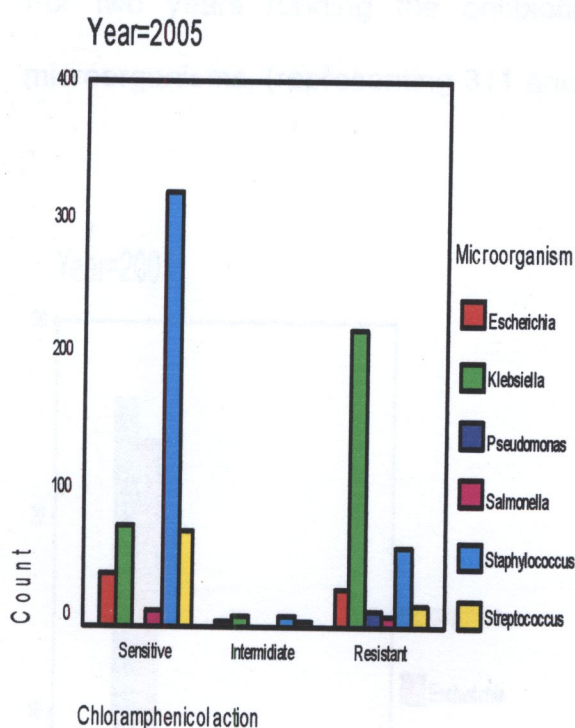
4.4.ii (c)

4.4.ii (d)

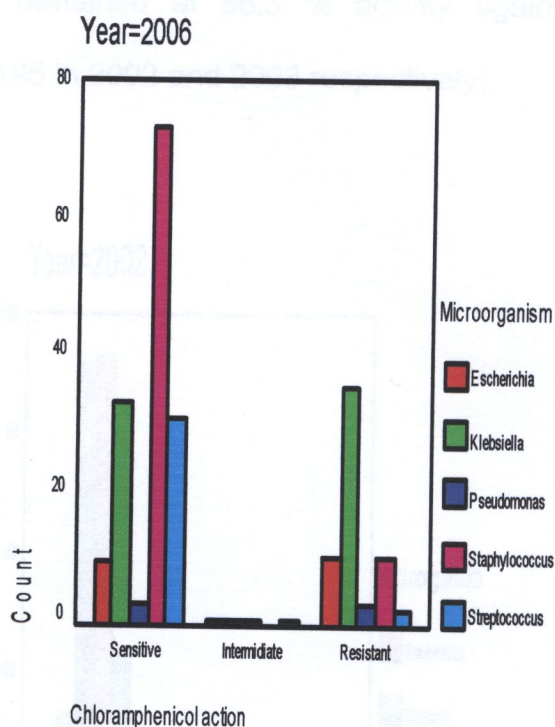
The resistance trend for Salmonella which was beginning to show from 2001 clearly emerged in 2003. There were a lot more resistant isolates than the sensitive ones. Very few isolates were, however tested against this drug in the subsequent years as to be able to show a trend of interaction.

Total	Count	70	358	218	986	1053	308	2995
% w/ in year		100.0	100.0	100.0	100.0	100.0	100.0	100.0
Pearson Chi-Square= 37.429								
P= 0.001								

Out of the 2995 microorganisms subjected to ciprofloxacin, 2476 (82.7 %) were sensitive against only 413 (13.8 %) resistant and 16 (1.5 %) partially sensitive.



4.4.ii (e)



4.4.ii (f)

Table 4.4.d: Microbial response to ciprofloxacin action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	53	311	194	760	911	247	2476
	% within year	75.7	86.6	88.6	77.1	86.5	80.2	82.7
Intermediate	Count	5	6	3	65	16	11	106
	% within year	7.1	1.7	1.4	6.6	1.5	3.6	3.5
Resistant	Count	12	42	22	161	126	50	413
	% within year	17.1%	11.7%	10.0	16.3	12.0	16.2	13.8
Total	Count	70	359	219	986	1053	308	2995
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 67.400^a
P=0.001

Out of the 2995 microorganisms subjected to ciprofloxacin, 2476 (82.7 %) were sensitive against only 413 (13.8 %) resistant and 16(1.5 %) partially sensitive.

For two years running the antibiotic remained at 88.6 % activity against microorganisms, (representing 311 and 195 in 2002 and 2003 respectively).

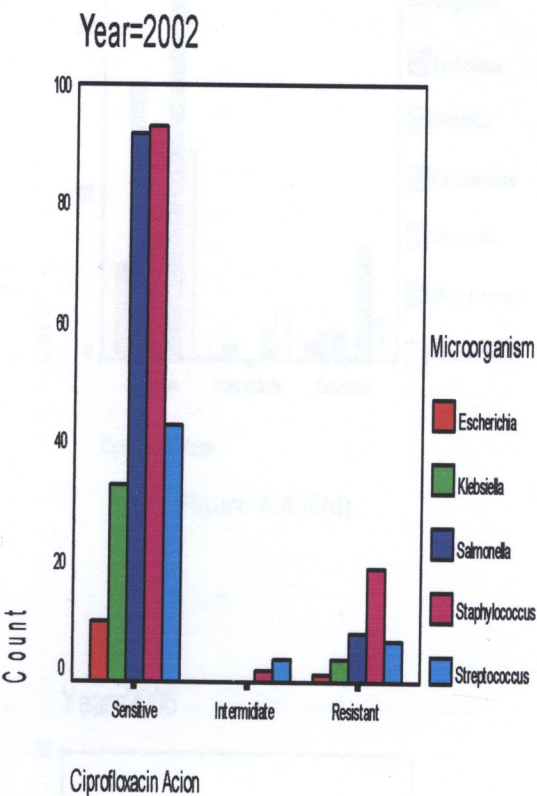
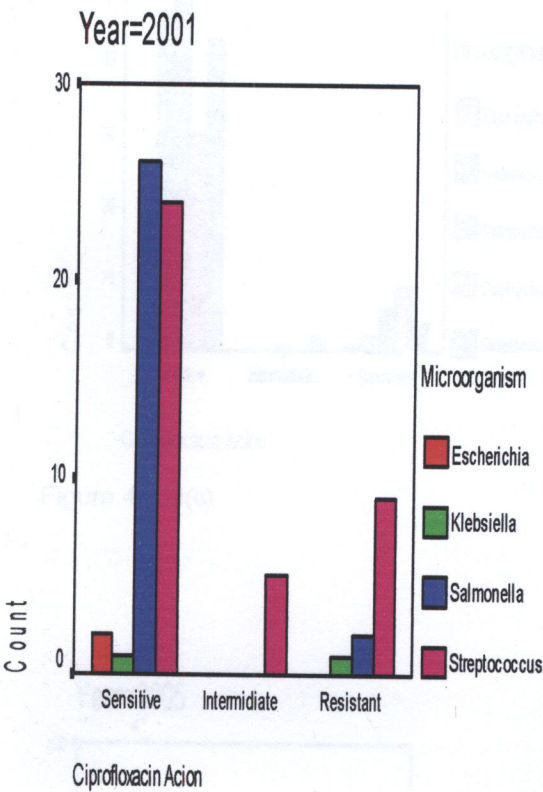
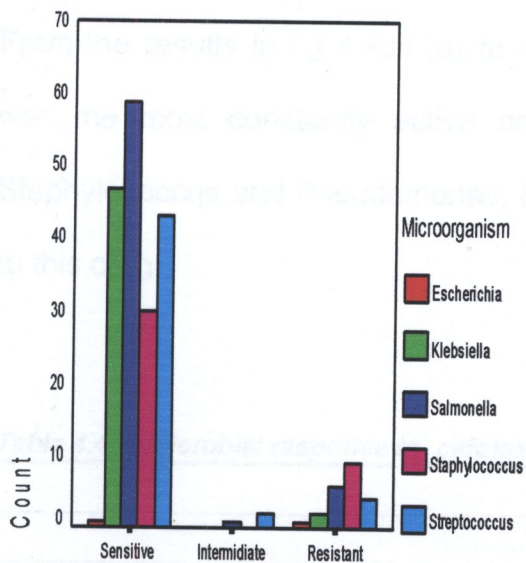


Figure 4.4.iii(a)

Figure 4.4.iii(b)

Both staphylococcus and Streptococcus isolates were generally sensitive to the action of ciprofloxacin across all the years. A similar trend was also evident for Salmonella and Klebsiella. If the results of the laboratory testing were to be true in-vivo, this would be said to be a good drug for both Gram positive and negative organisms.

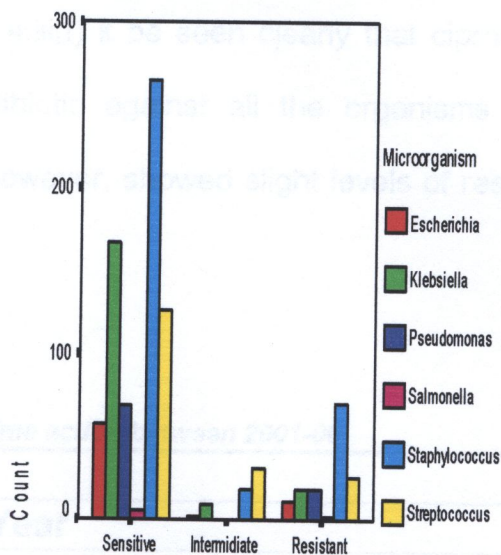
Year=2003



Ciprofloxacin Acion

Figure 4.4.iii(c)

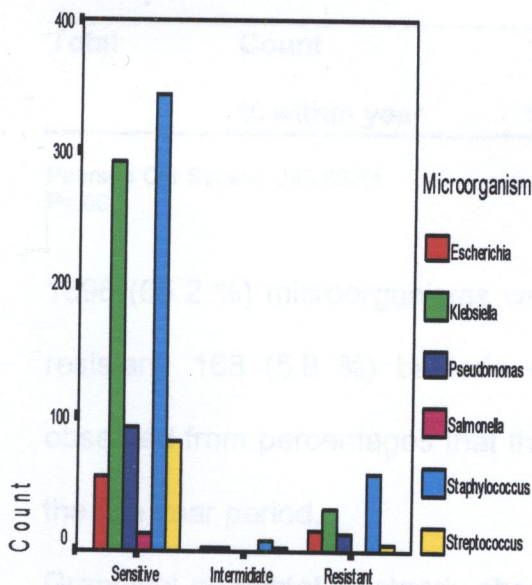
Year=2004



Ciprofloxacin Acion

Figure 4.4.iii(d)

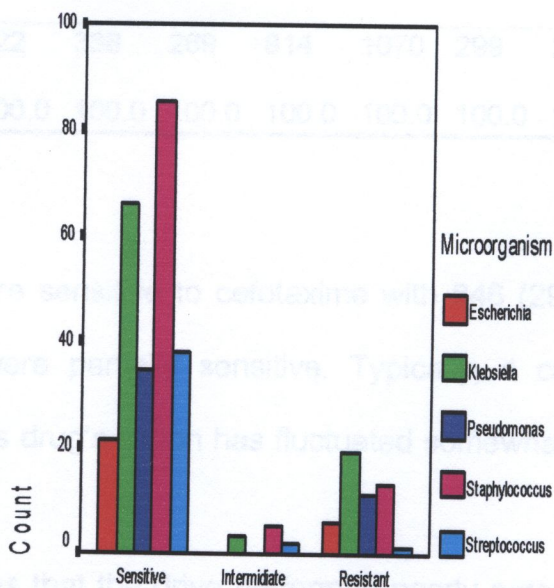
Year=2005



Ciprofloxacin Acion

Figure 4.4.iii(e)

Year=2006



Ciprofloxacin Acion

Figure 4.4.iii(f)

From the results in fig 4.4.iii (a) to 4.4.iii(f) it be seen clearly that ciprofloxacin was the most constantly active antibiotic against all the organisms tested. Staphylococcus and Pseudomonas, however, showed slight levels of resistance to this drug.

Table 4.4.e: Microbial response to cefotaxime action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	106	273	249	607	544	119	1898
	% within year	86.9	80.8	92.6	74.6	50.8	39.8	65.2
Intermediate	Count	5	3	1	56	81	22	168
	% within year	4.1	0.9	0.4	6.9	7.6	7.4	5.8
Resistant	Count	11	62	19	151	445	158	846
	% within year	9.0	18.3	7.1	18.6	41.6	52.8	29.1
Total	Count	122	338	269	814	1070	299	2912
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 385.892(a)
P=.001

1898 (65.2 %) microorganisms were sensitive to cefotaxime with 846 (29.1 %) resistant. 168 (5.8 %) bacteria were partially sensitive. Typically, it can be observed from percentages that this drug's action has fluctuated somewhat over the five year period.

Graphical presentative clearly shows that the drive performed poorly across the five years. Although there were some sensitive organisms, majority were resistant to the drug. The situation was worse in 2005 where it was shown that

Staphylococcus and *Streptococcus* were very resistant to the drug (65%), (59.7%), and (86.1%), (77.3%) respectively. This was significant at 59. (P=0.001)

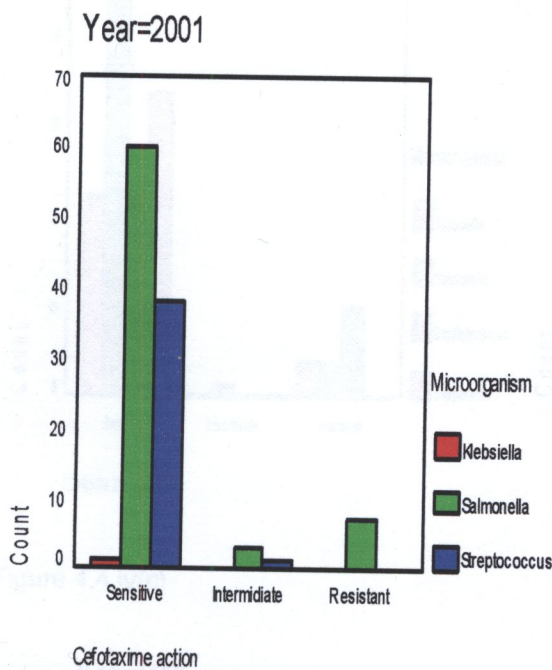


Figure 4.4.iv(a)

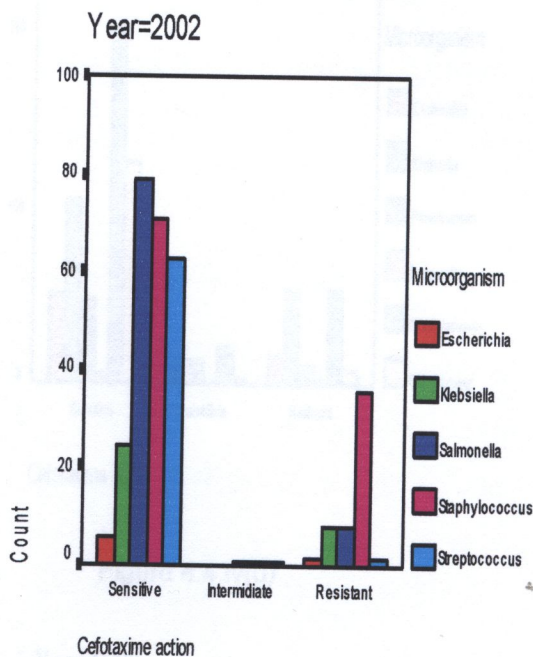


Figure 4.4.iv(b)

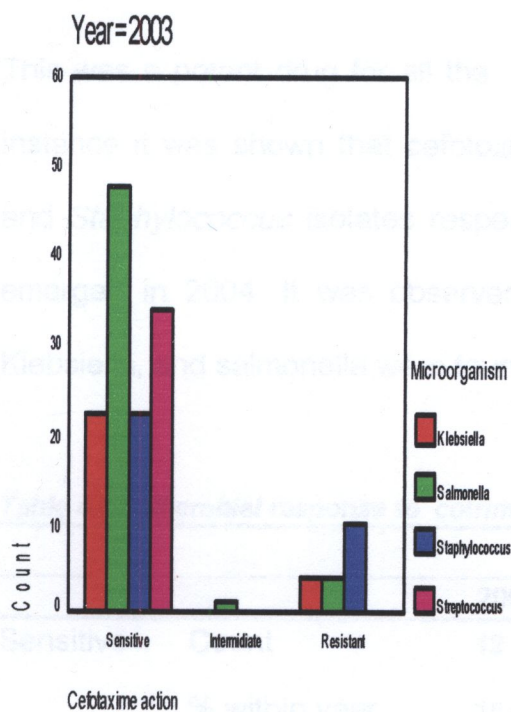


Figure 4.4.iv(c)

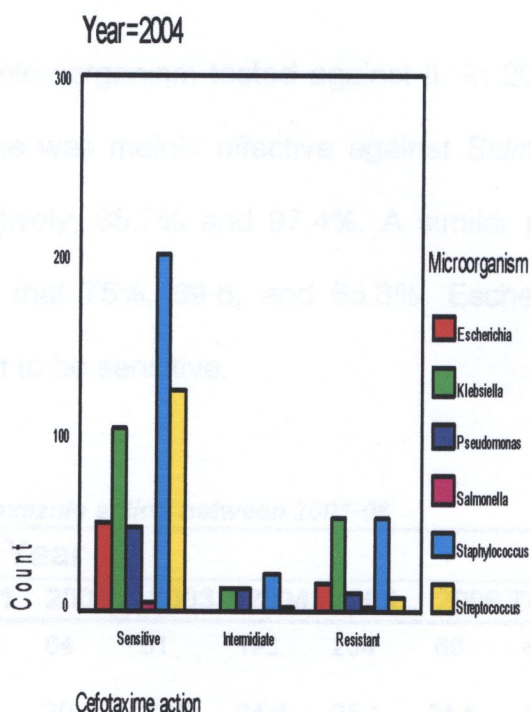


Figure 4.4.iv(d)

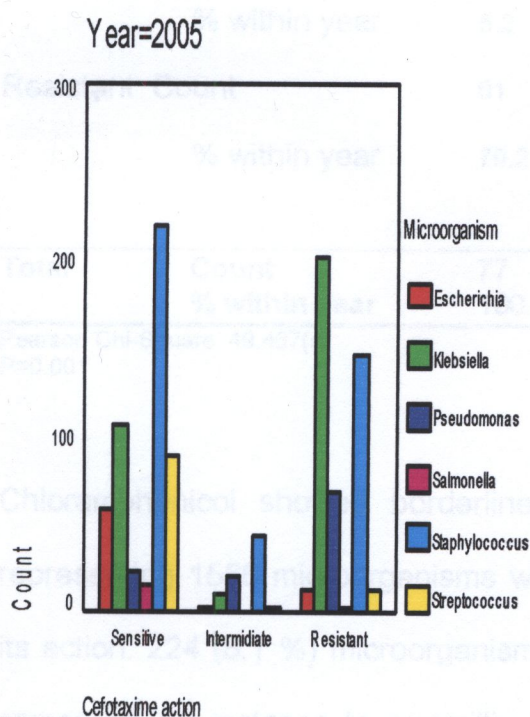


Figure 4.4.iv(e)

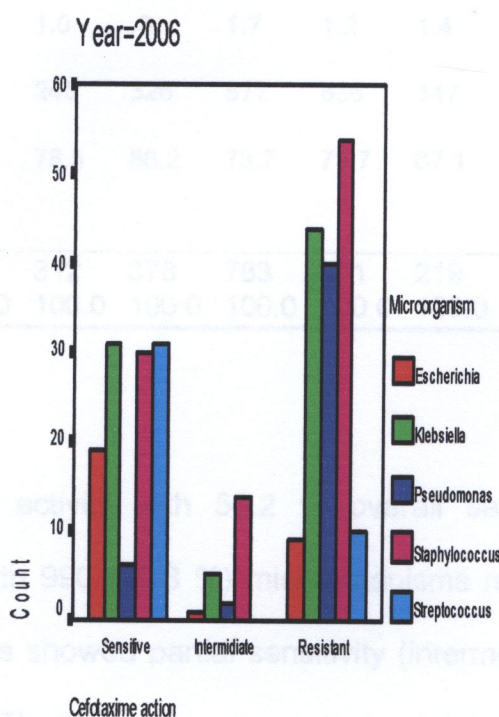


Figure 4.4.iv(f)

This was a potent drug for all the microorganism tested against it. In 2001 for instance it was shown that cefotaxime was mainly effective against *Salmonella* and *Staphylococcus* isolates respectively, 85.7% and 97.4%. A similar picture emerged in 2004. It was observed that 75%, 89.8, and 65.8%, *Escherichia*, *Klebsiella*, and *salmonella* were found to be sensitive.

Table 4.4.f: Microbial response to cotrimoxazole action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	12	64	51	193	234	69	623
	% within year	15.6	20.5	13.5	24.6	25.1	31.5	23.1
Intermediate	Count	4	3	1	13	11	3	35
	% within year	5.2	1.0	.3	1.7	1.2	1.4	1.3
Resistant	Count	61	245	326	577	686	147	2042
	% within year	79.2	78.5	86.2	73.7	73.7	67.1	75.6
Total	Count	77	312	378	783	931	219	2700
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 49.467(a)
P=0.001

Chloramphenicol showed borderline activity with 56.2 % overall sensitivity representing 1555 microorganisms with 990 (35.8 %) microorganisms resisting its action. 224 (8.1 %) microorganisms showed partial sensitivity (intermediate), compared, for instance to ampicillin. These figures are against a total of 2769 micro organisms subjected to this drug.

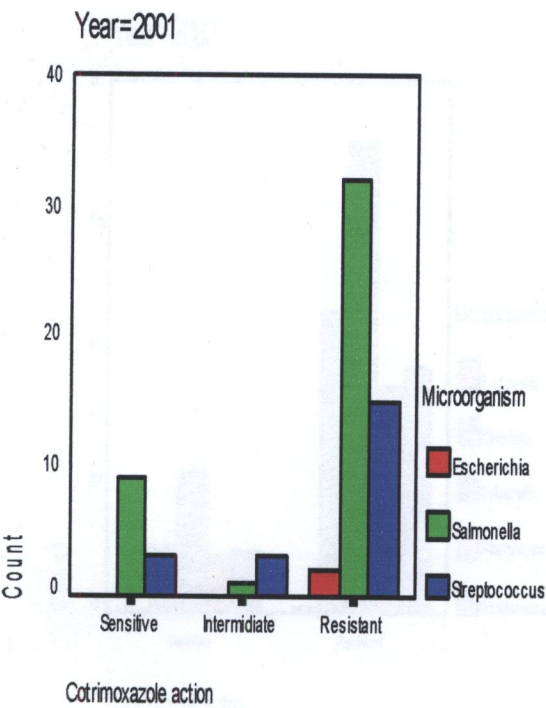


Figure 4.4.v(a)

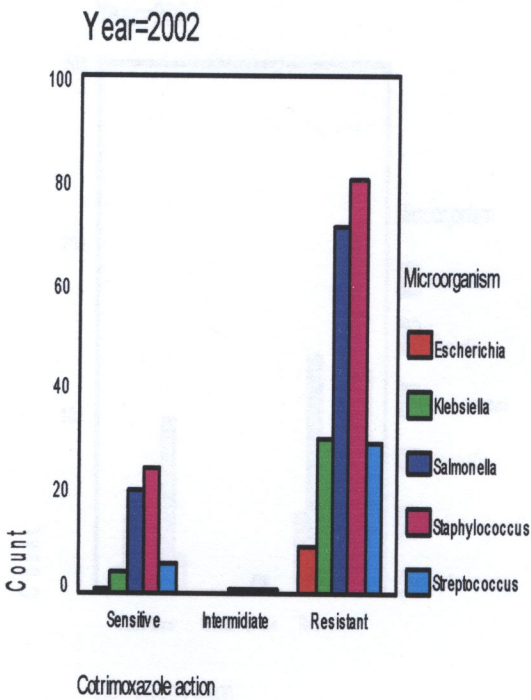


Figure 4.4.v(b)

Out of the organisms tested against cotrimoxazole, Salmonella was strikingly resistant against

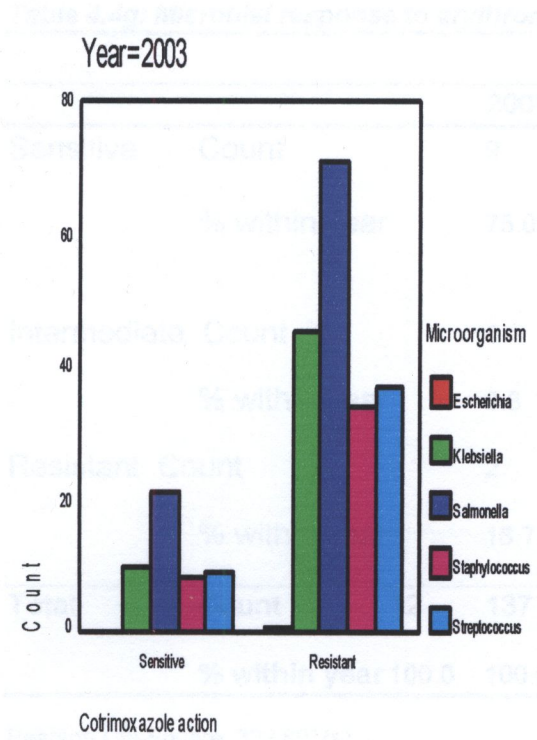


Figure 4.4.v(c)

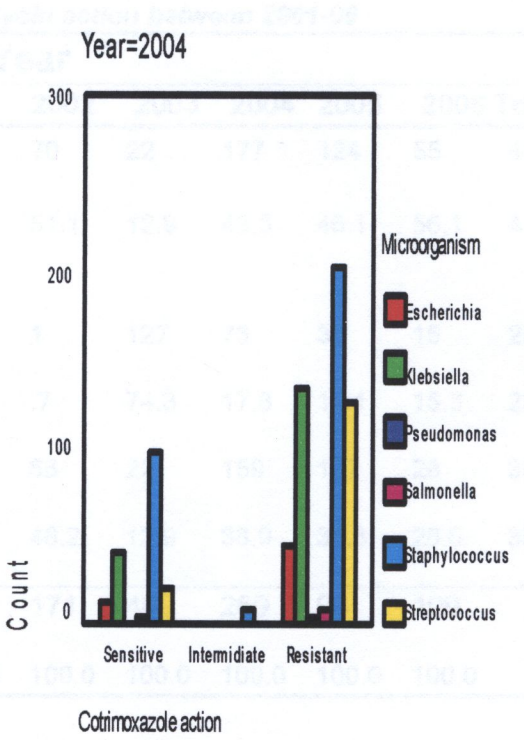


Figure 4.4.v(d)

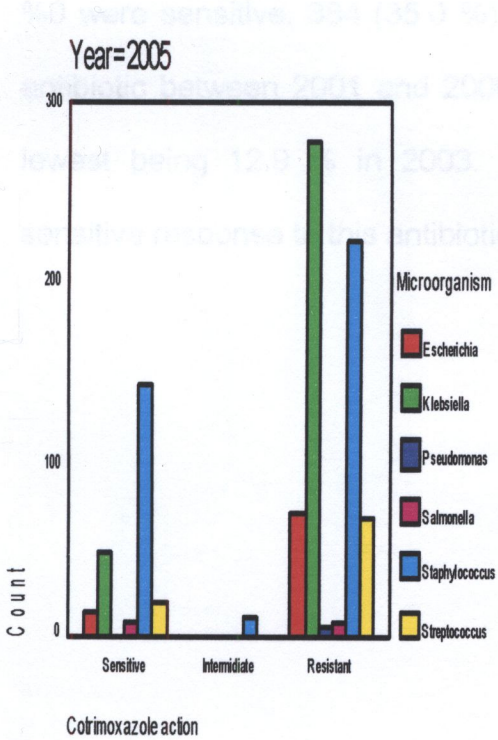


Figure 4.4.v(e)

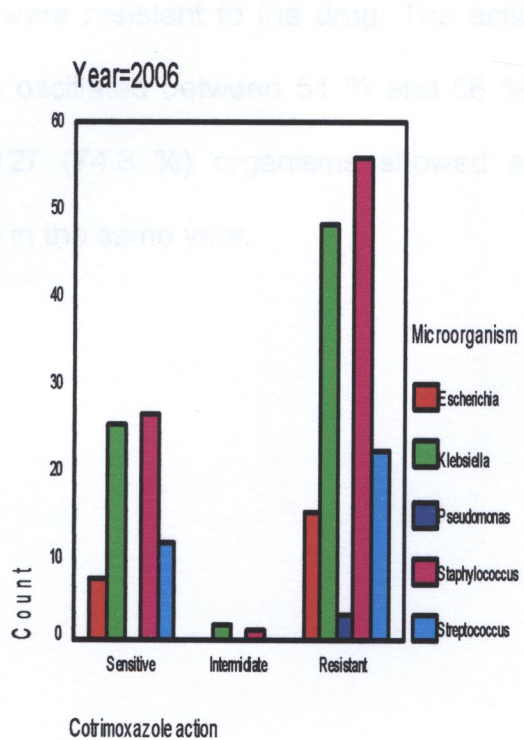


Figure 4.4.v(f)

Table 4.4g: Microbial response to erythromycin action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	9	70	22	177	124	55	457
	% within year	75.0	51.1	12.9	43.3	46.1	56.1	41.7
Intermediate	Count	1	1	127	73	38	15	25
	% within year	8.3	.7	74.3	17.8	14.1	15.3	23.3
Resistant	Count	2	66	22	159	107	28	38
	% within year	16.7	48.2	12.9	38.9	39.8	28.6	35.0
Total	Count	12	137	171	409	269	98	109
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 322.807(a)
P=0.001

255 (23.3 %) microorganisms were partially sensitive to erythromycin. 457 (41.7 %) were sensitive. 384 (35.0 %) were resistant to the drug. The action of this antibiotic between 2001 and 2006 oscillated between 51 % and 56 % with the lowest being 12.9 % in 2003. 127 (74.3 %) organisms showed a partially sensitive response to this antibiotic in the same year.

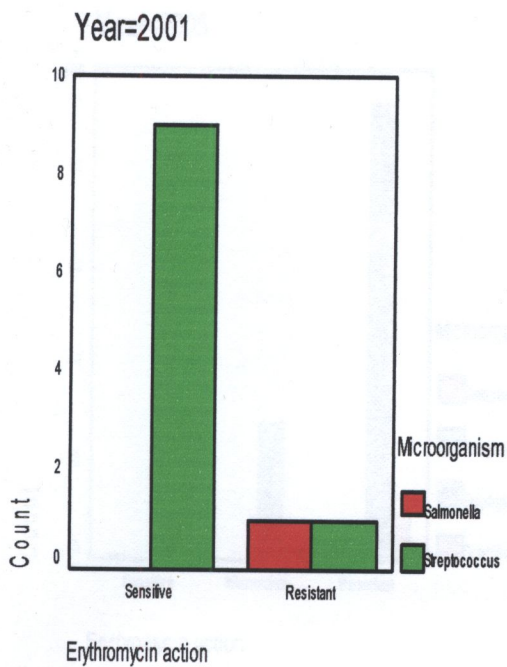


Figure 4.4.v(a)

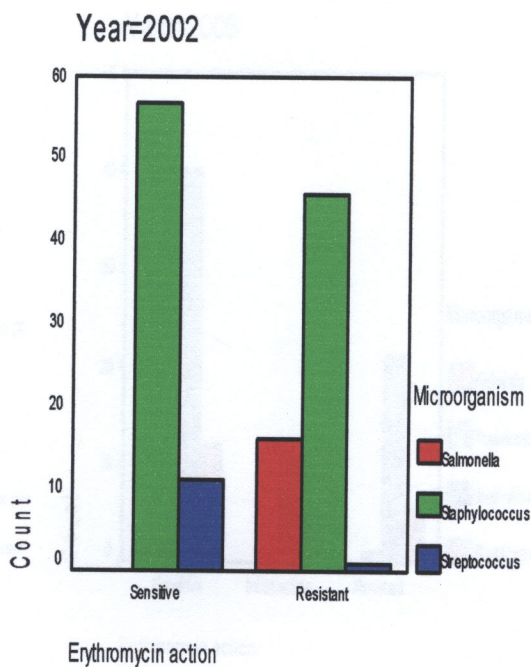


Figure 4.4.v(b)

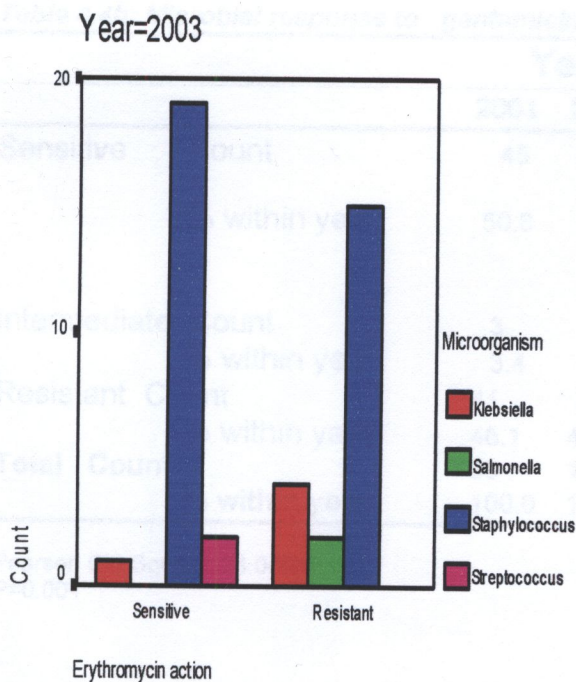


Figure 4.4.v(c)

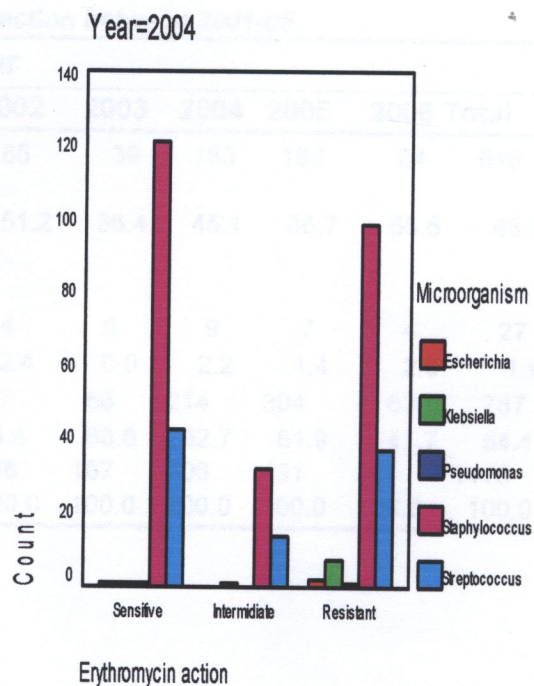


Figure 4.4.v(d)

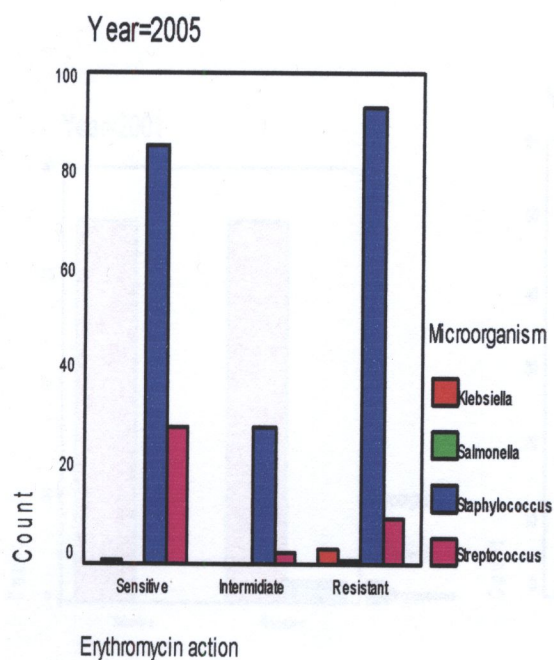


Figure 4.4.v(e)

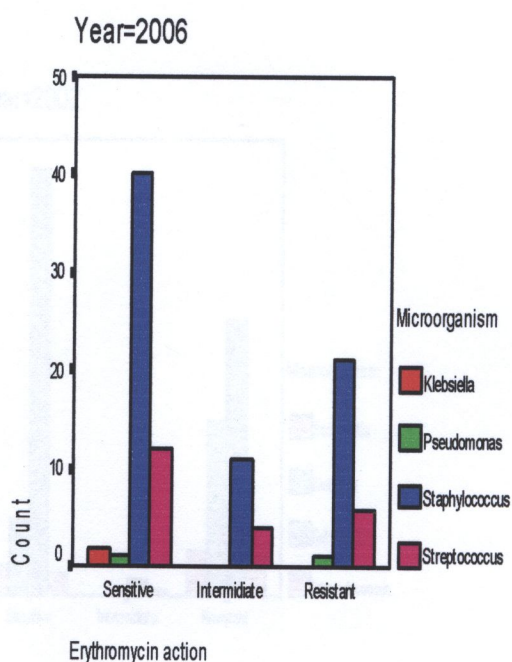


Figure 4.4.v(f)

Table 4.4h: Microbial response to gentamicin action between 2001-06

		Year					
		2001	2002	2003	2004	2005	2006 Total
Sensitive	Count	45	85	39	183	180	84 616
	% within year	50.6	51.2	36.4	45.1	36.7	55.6 43.7
Intermediate	Count	3	4	0	9	7	4 27
	% within year	3.4	2.4	0.0	2.2	1.4	2.6 1.9
Resistant	Count	41	77	68	214	304	63 767
	% within year	46.1	46.4	63.6	52.7	61.9	41.7 54.4
Total	Count	89	166	107	406	491	151 1410
	% within year	100.0	100.0	100.0	100.0	100.0	100.0 100.0

Pearson Chi-Square 34.045(a)
P=0.001

Of the 1410 microorganisms tested against gentamicin, only 616 (43.7 %) were sensitive against 767 resistant. Only 27 of the bacteria were partially sensitive to this antibiotic.

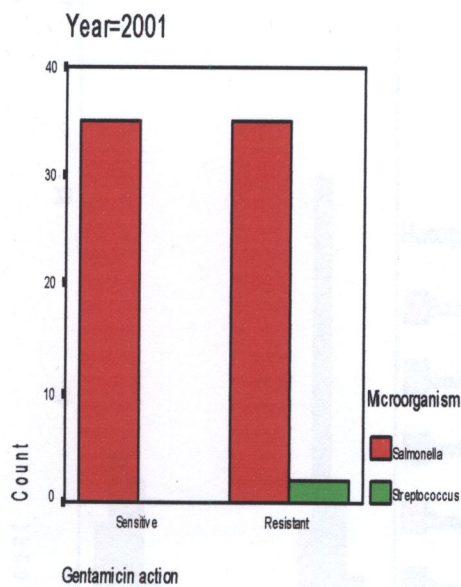


Figure 4.4.vi(a)

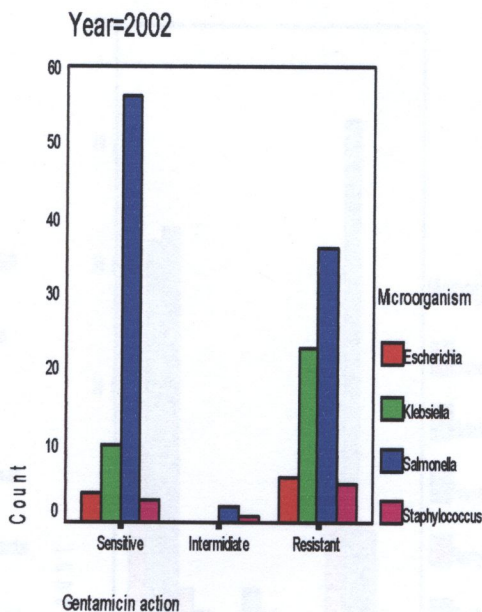


Figure 4.4.vi(b)

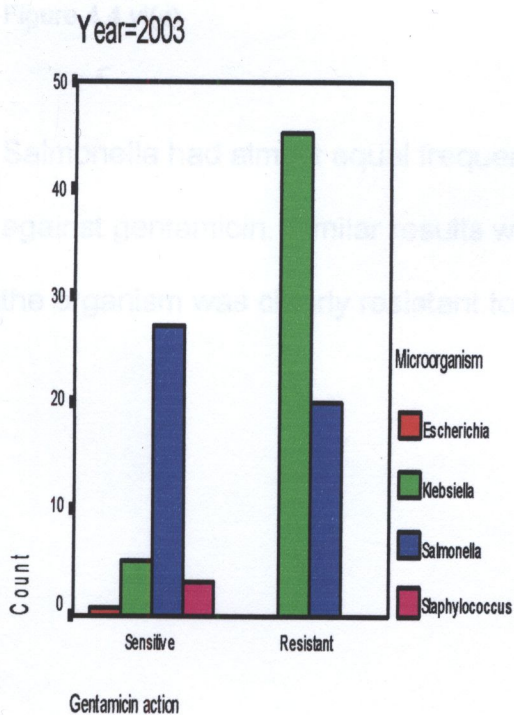


Figure 4.4.vi(c)

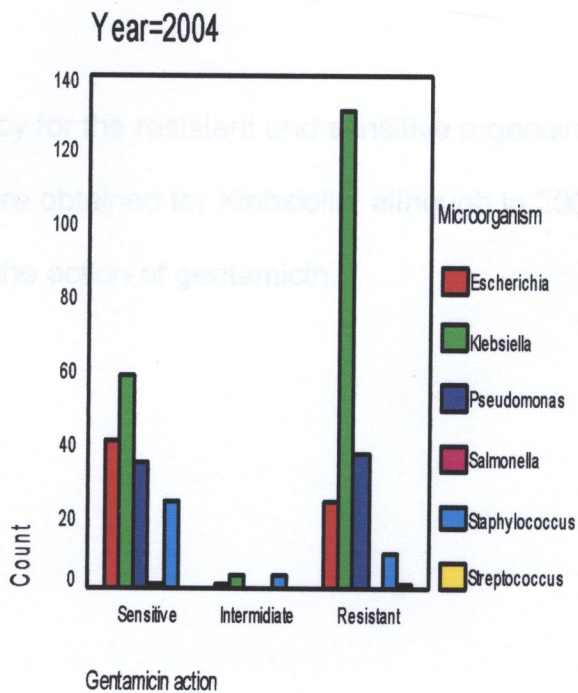


Figure 4.4.vi(d)

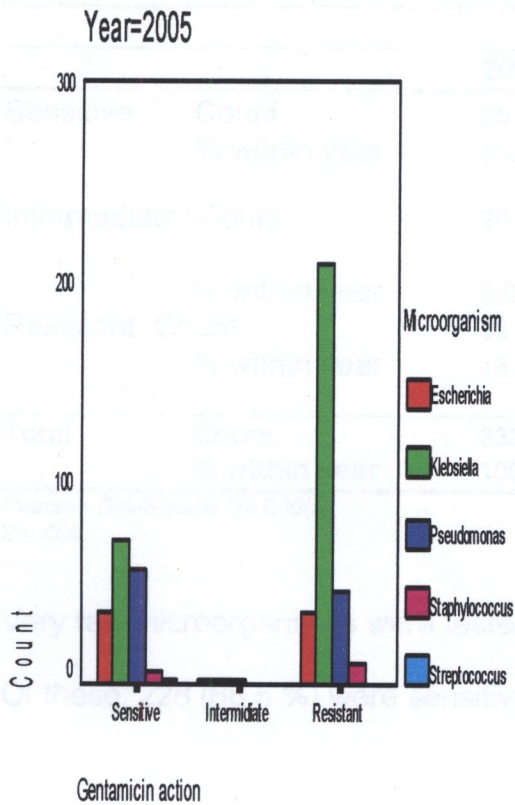


Figure 4.4.vi(e)

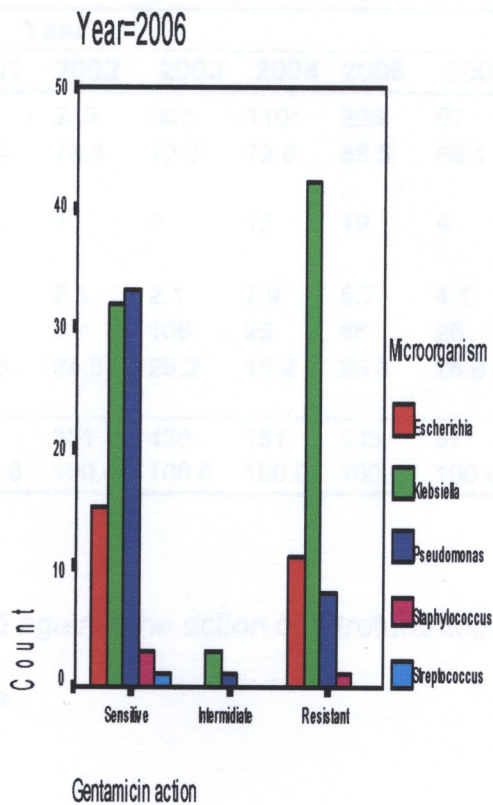


Figure 4.4.vi(f)

Salmonella had almost equal frequency for the resistant and sensitive organisms against gentamicin. Similar results were obtained for Klebsiella, although in 2006, the organism was clearly resistant to the action of gentamicin.

Table 4.4.i: Microbial response to nitrofurantoin action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	251	243	305	110	228	67	1204
	% within year	75.4	73.4	72.6	72.8	68.5	69.1	72.3
Intermediate	Count	20	7	9	12	19	4	71
	% within year	6.0	2.1	2.1	7.9	5.7	4.1	4.3
Resistant	Count	62	81	106	29	86	26	390
	% within year	18.6	24.5	25.2	19.2	25.8	26.8	23.4
Total	Count	333	331	420	151	333	97	1665
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 24.606(a)
P= .006

Very few microorganisms were tested against the action of nitrofurantoin (333).
Of these, 228 (68.5 %) were sensitive.

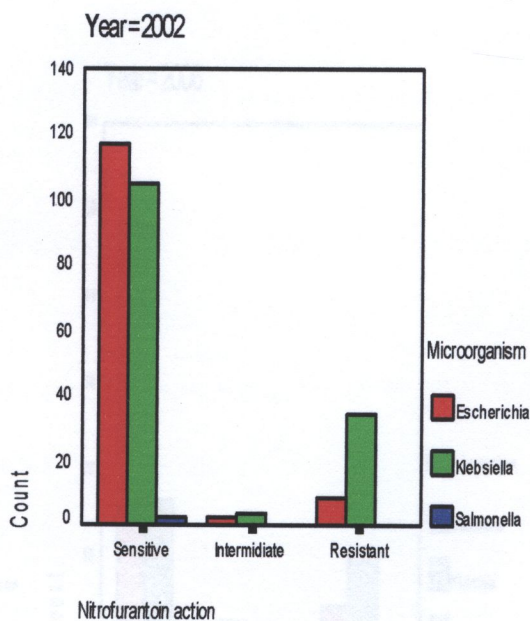
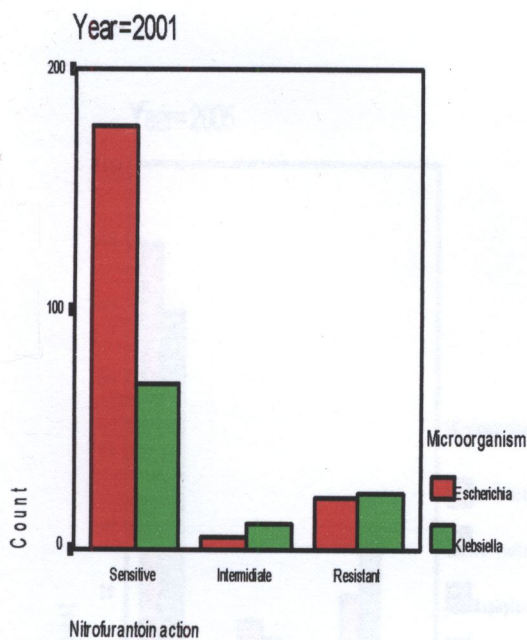


Figure 4.4.vii(a)

Figure 4.4.vii(b)

Table 4.4: Microbial response to nalidixic acid action between 2003-06

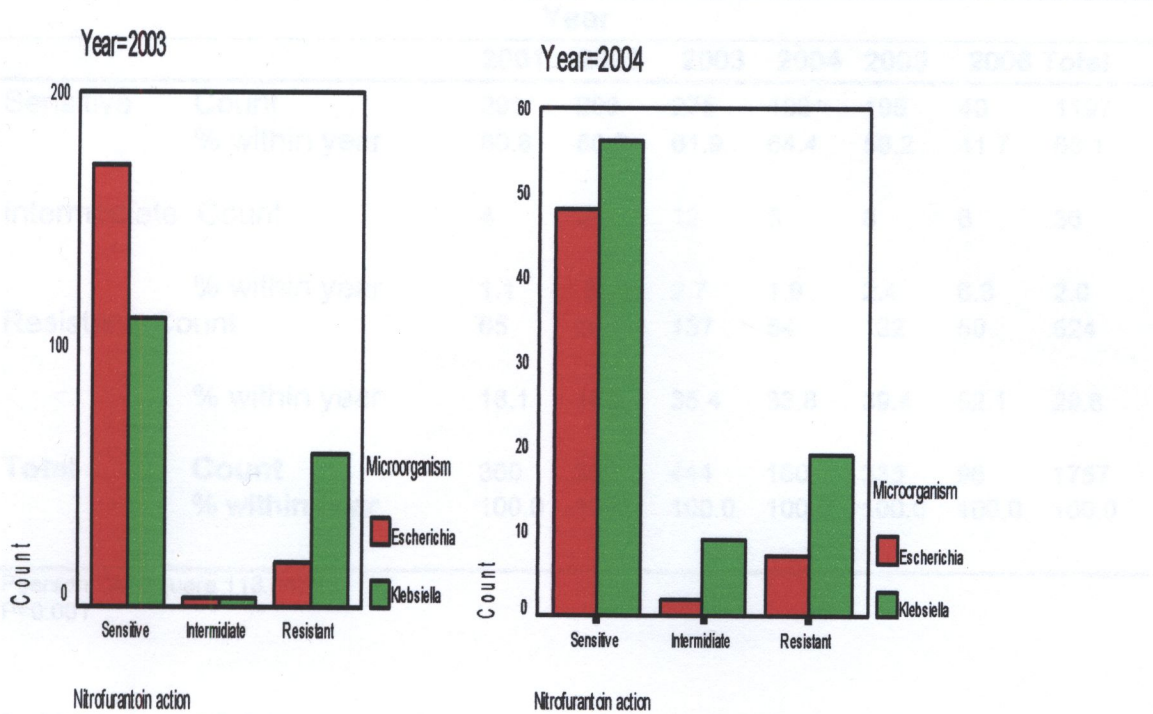


Figure 4.4.vii(c)

Figure 4.4.vii(d)

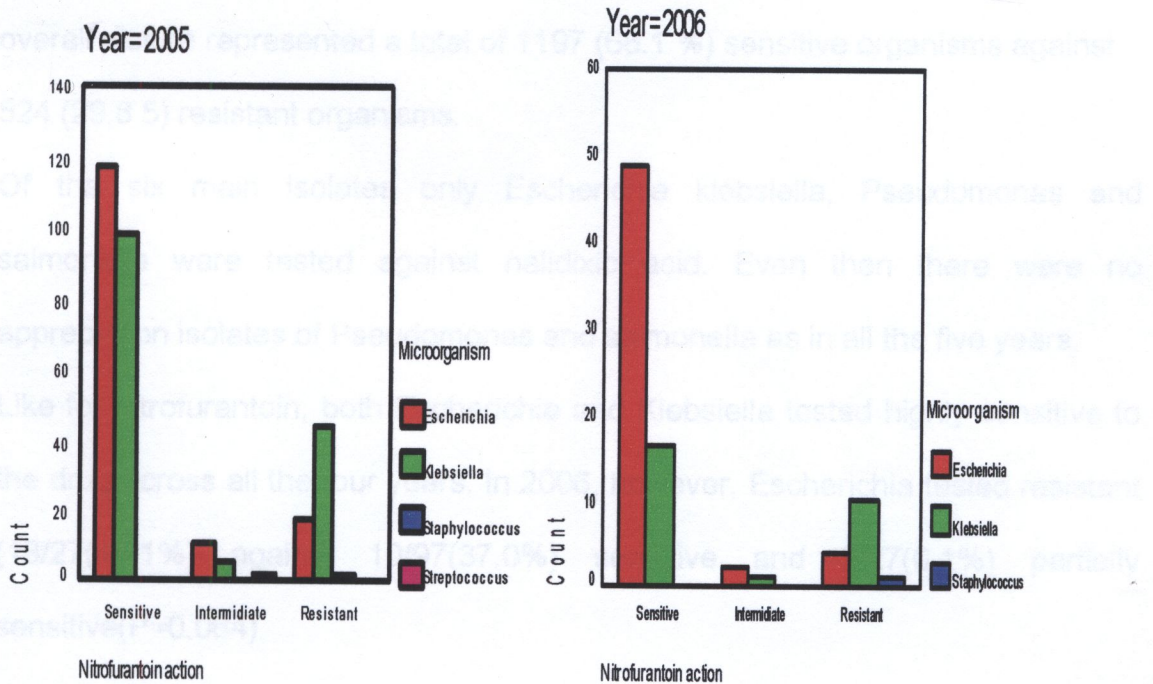


Figure 4.4.vii(e)

Figure 4.4.vii(f)

Table 4.4.j:Microbial response to nalidixic acid action between 2001-06

		Year						Total
		2001	2002	2003	2004	2005	2006	
Sensitive	Count	291	293	275	103	195	40	1197
	% within year	80.8	80.9	61.9	64.4	58.2	41.7	68.1
Intermediate	Count	4	3	12	3	8	6	36
	% within year	1.1	.8	2.7	1.9	2.4	6.3	2.0
Resistant	Count	65	66	157	54	132	50	524
	% within year	18.1	18.2	35.4	33.8	39.4	52.1	29.8
Total	Count	360	362	444	160	335	96	1757
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 113.042(a)
P=0.001

In 2001 up to 80.8 % of the microorganisms tested against nalidixic acid were sensitive and only 18. 1 % was resistant. However, with time (by 2006), the action of this drug reduced to 41.7 % with respect to sensitive organisms. The overall picture represented a total of 1197 (68.1 %) sensitive organisms against 524 (29.8 5) resistant organisms.

Of the six main isolates only Escherichia klebsiella, Pseudomonas and salmonella were tested against nalidixic acid. Even then there were no appreciation isolates of Pseudomonas and salmonella as in all the five years.

Like for nitrofurantoin, both Escherichia and Klebsiella tested highly sensitive to the drug across all the four years. In 2006, however, Escherichia tested resistant (13/27(48.1%) against 10/97(37.0%) sensitive and 3/27(0.1%) partially sensitive(P=0.064).

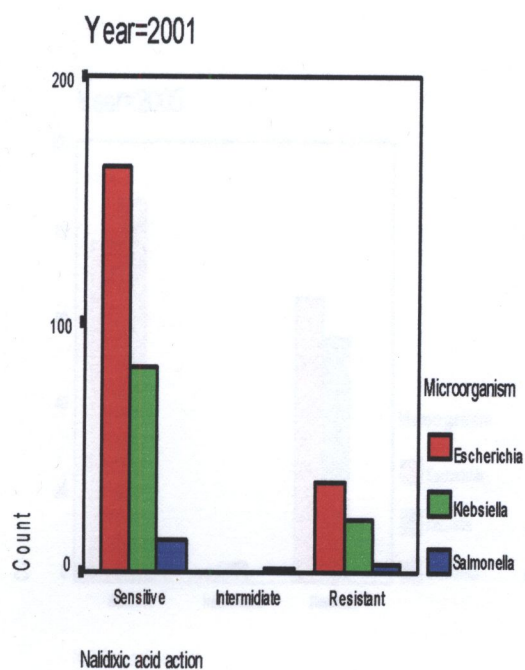


Figure 4.4.viii(a)

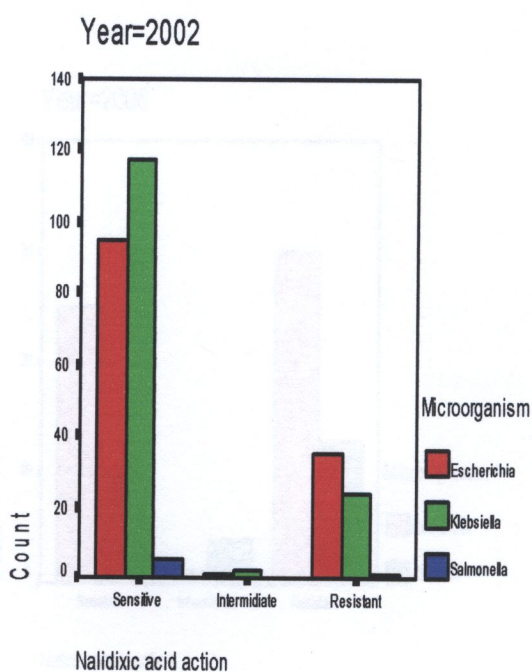


Figure 4.4.viii(b)

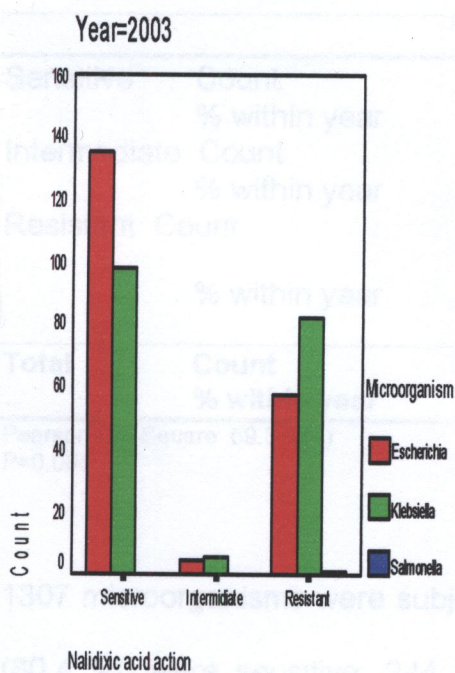


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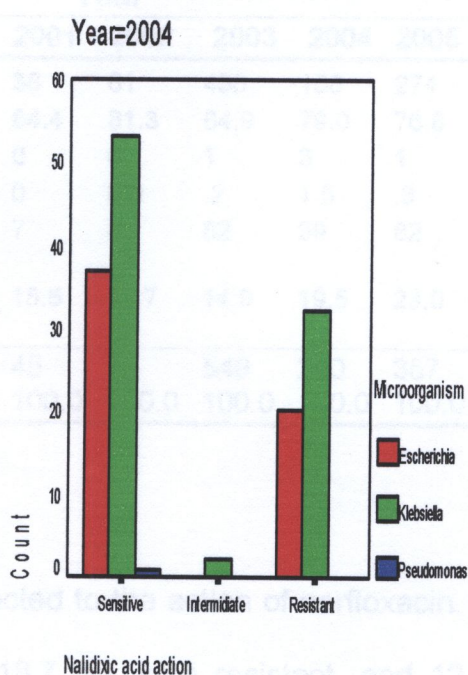


Figure 4.4.viii(d)

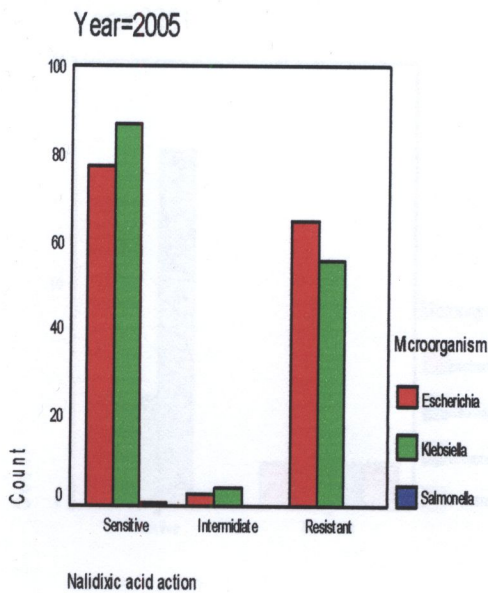


Figure 4.4.viii(e)

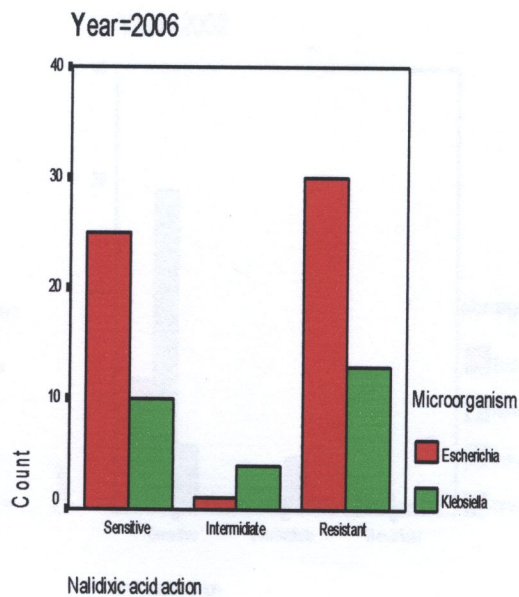


Figure 4.4.viii(f)

Table 4.4 k: microbial response to norfloxacin action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	38	61	466	158	274	54	1051
	% within year	84.4	81.3	84.9	79.0	76.8	66.7	80.4
Intermediate	Count	0	6	1	3	1	1	12
	% within year	0	8.0	.2	1.5	.3	1.2	.9
Resistant	Count	7	8	82	39	82	26	244
	% within year	15.6	10.7	14.9	19.5	23.0	32.1	18.7
Total	Count	45	75	549	200	357	81	1307
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 69.379(a)
P=0.001

1307 microorganisms were subjected to the action of norfloxacin. Of these 1051 (80.4 %) were sensitive, 244 (18.7 %) were resistant, and 12 (0.9 %) were partially sensitive.

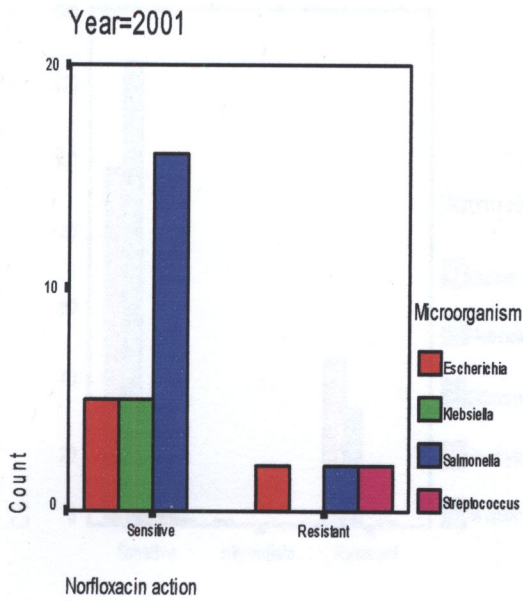


Figure 4.4.ix(a)

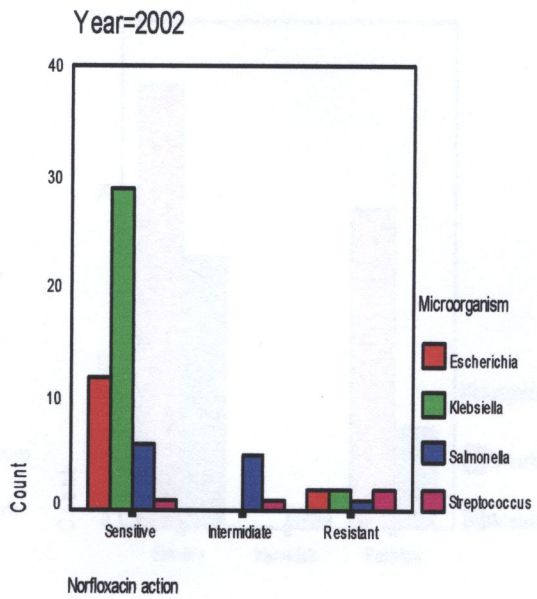


Figure 4.4.ix(b)

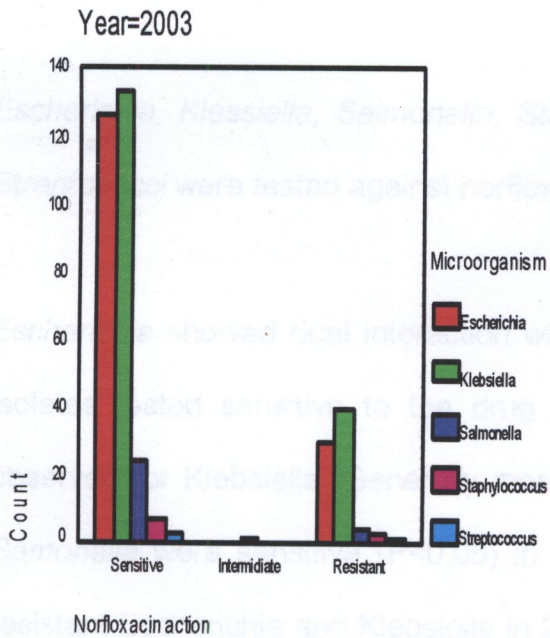


Figure 4.4.ix(c)

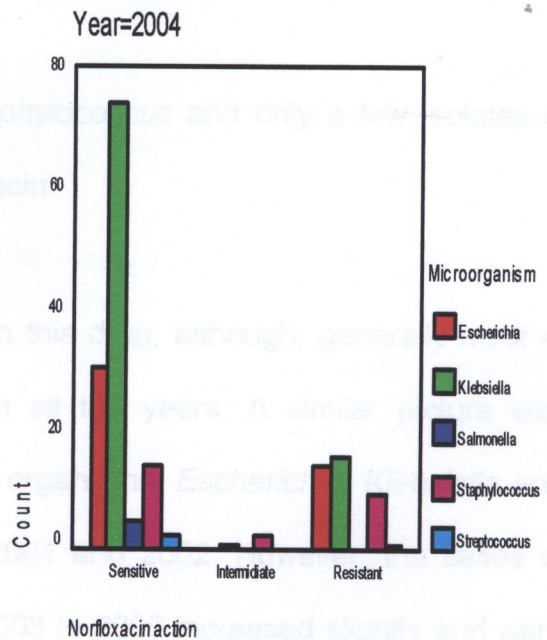


Figure 4.4.ix(d)

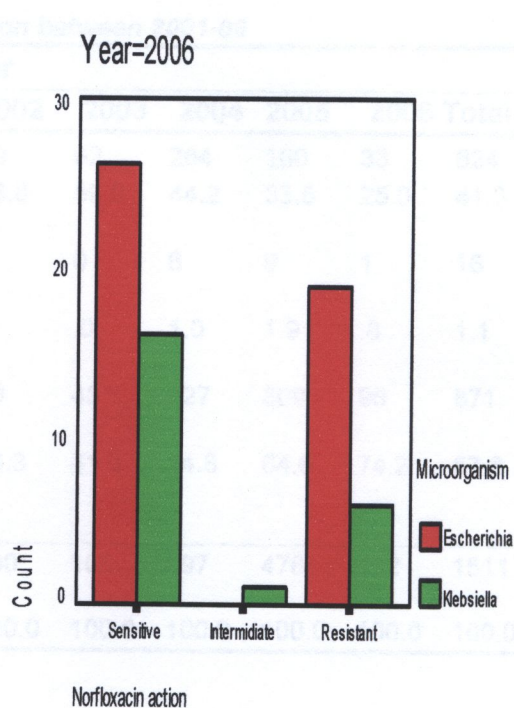
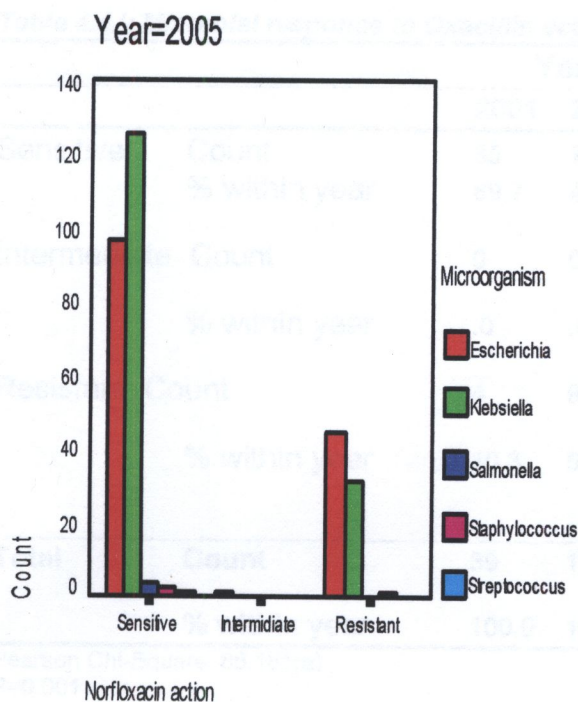


Figure 4.4.ix(e)

Figure 4.4.ix(f)

Escherichia, *Klebsiella*, *Salmonella*, *Staphylococcus* and only a few isolates of *Streptococci* were tested against norfloxacin.

Escherichia showed dual interaction with this drug, although, generally most of isolates tested sensitive to the drug in all the years. A similar picture was observed for *Klebsiella*. Generally most organisms, *Escherichia*, *Klebsiella* and *Samonella* were sensitive ($P < 0.05$) in 2001 and 2002. However, the cases of resistant *Escherichia* and *Klebsiella* in 2003 to 2006 increased slightly and were even more prominent for *Escherichia* in 2006.

Table 4.4.I: Microbial response to Oxacillin action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	35	70	62	264	160	33	624
	% within year	89.7	43.8	59.0	44.2	33.5	25.0	41.3
Intermediate	Count	0	0	0	6	9	1	16
	% within year	.0	.0	.0	1.0	1.9	.8	1.1
Resistant	Count	4	90	43	327	309	98	871
	% within year	10.3	56.3	41.0	54.8	64.6	74.2	57.6
Total	Count	39	160	105	597	478	132	1511
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 85.181(a)
P=0.001

Oxacillin recorded a progressive drop in activity from 89.7 % in 2001 to 25.0 % in 2006. 1511 microorganisms were tested out of which 624 (41.3 %) were sensitive, 871 (57.6 %) resistant and 16 (1.1 %) partially sensitive.

Oxacillin's action was mainly tested on Staphylococcus and Streptococcus in the five years. There were very few isolates of Escherichia, Klebsiella and salmonella (2004 to 2006).

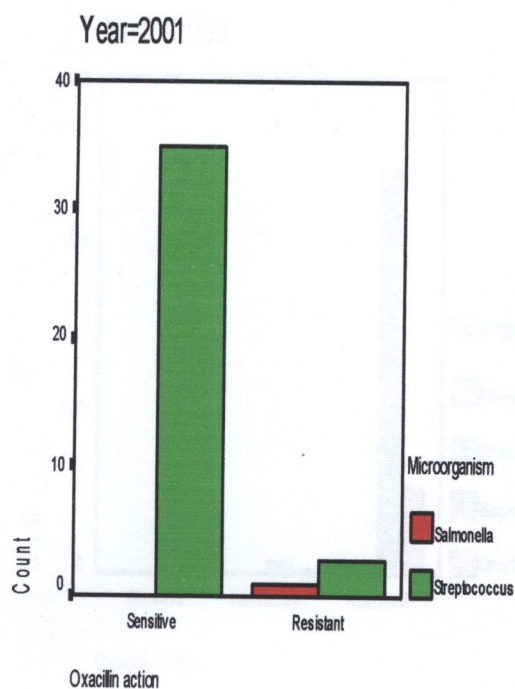


Figure 4.4.x(a)

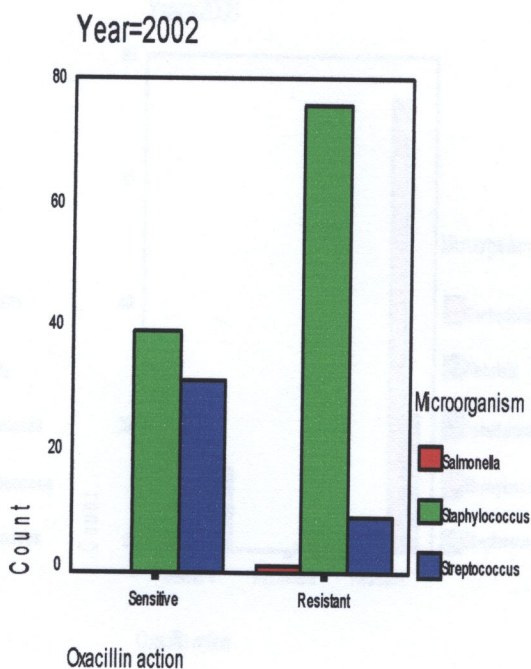


Figure 4.4.x(b)

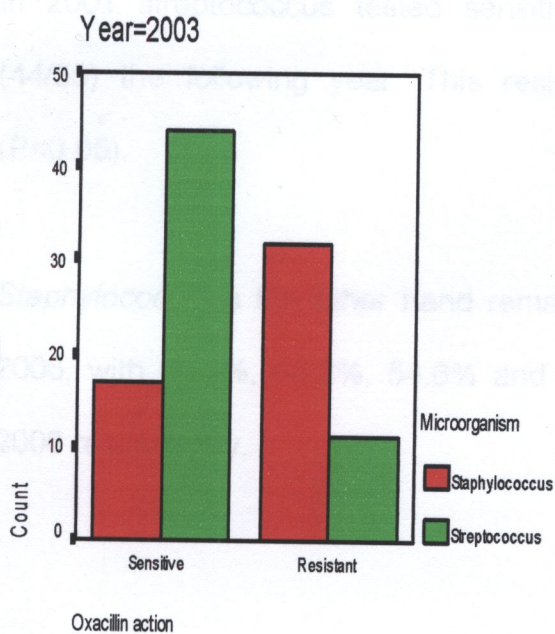


Figure 4.4.x(c)

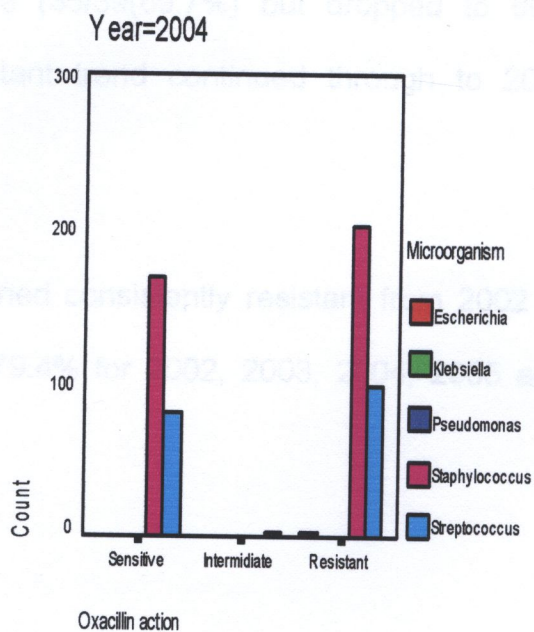


Figure 4.4.x(d)

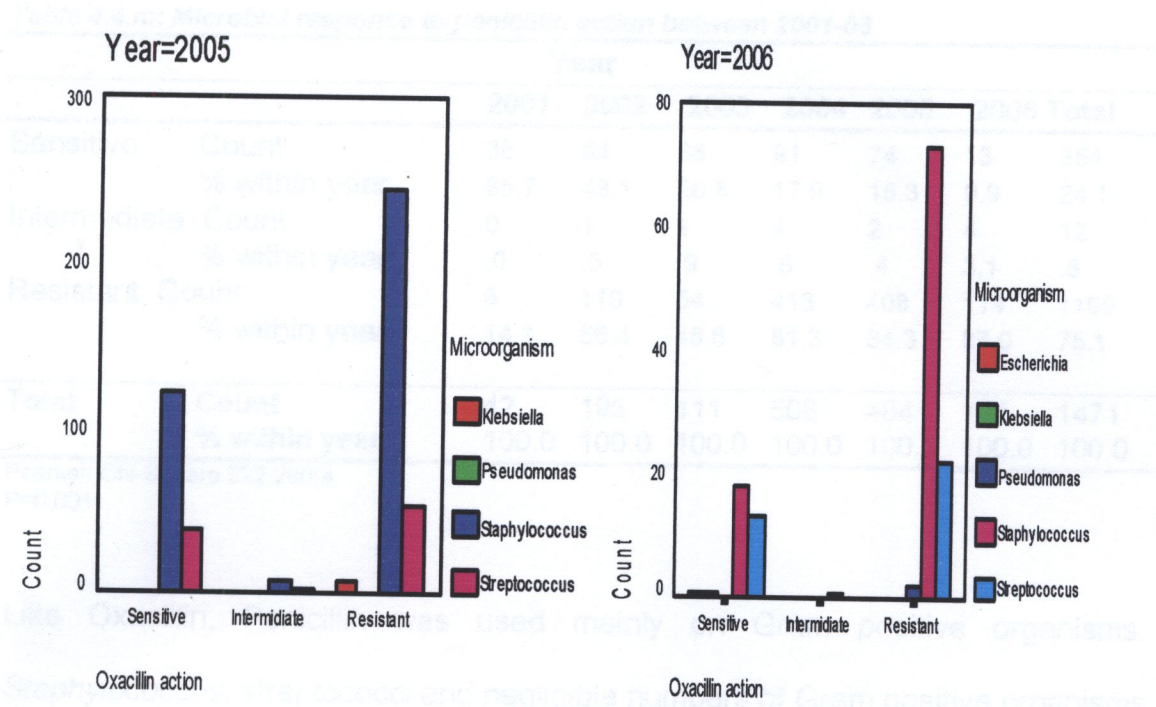


Figure 4.4.x(e)

Figure 4.4.x(f)

In 2001 streptococcus tested sensitive (35/39(89.7%) but dropped to 80% (44/55) the following year. This resistant trend continued through to 2006 (P<0.05).

Staphylococci on the other hand remained consistently resistant from 2002 to 2006, with 66.1%, 65.3%, 54.6% and 79.4% for 2002, 2003, 2004, 2005 and 2006 respectively.

Table 4.4.m: Microbial response to penicillin action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	36	84	56	91	74	13	354
	% within year	85.7	43.1	50.5	17.9	15.3	9.9	24.1
Intermediate	Count	0	1	1	4	2	4	12
	% within year	.0	.5	.9	.8	.4	3.1	.8
Resistant	Count	6	110	54	413	408	114	1105
	% within year	14.3	56.4	48.6	81.3	84.3	87.0	75.1
Total	Count	42	195	111	508	484	131	1471
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 222.286(a)
P=0.001

Like Oxacillin, Penicillin was used mainly on Gram positive organisms. *Staphylococcus*, streptococci and negligible numbers of Gram positive organisms like *Escherichia*, *Pseudomonas*, *Klebsiella*, and salmonella. *Escherichia* was the only Gram negative organisms that tested sensitive in 2006. It was also observed that in 2001 up to 92.1% *Streptococcus* isolates tested sensitive to oxacillin. However, its resistance began to rise steadily in subsequent years up to 2006.

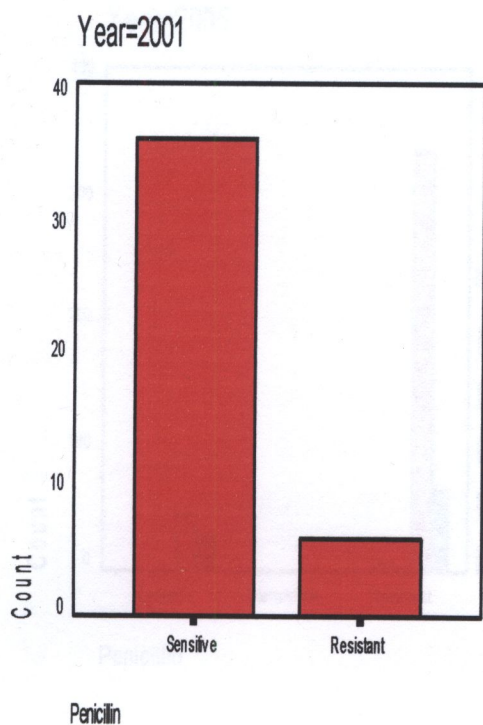


Figure 4.4.xi(a)

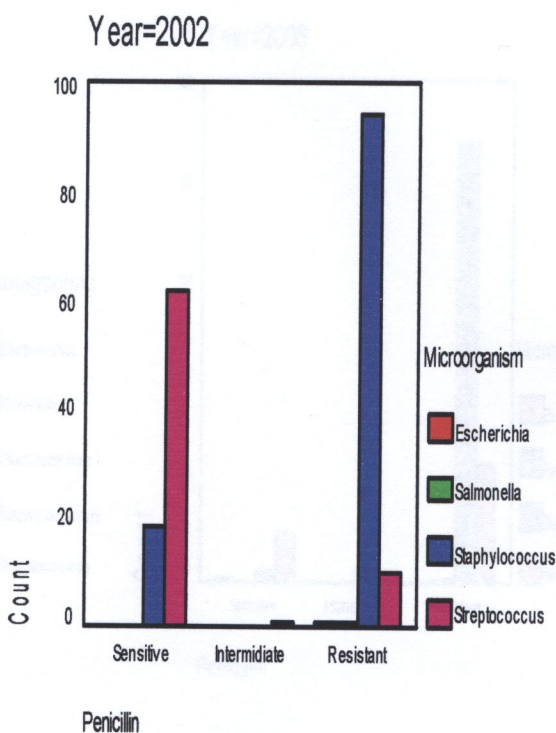


Figure 4.4.xi(b)

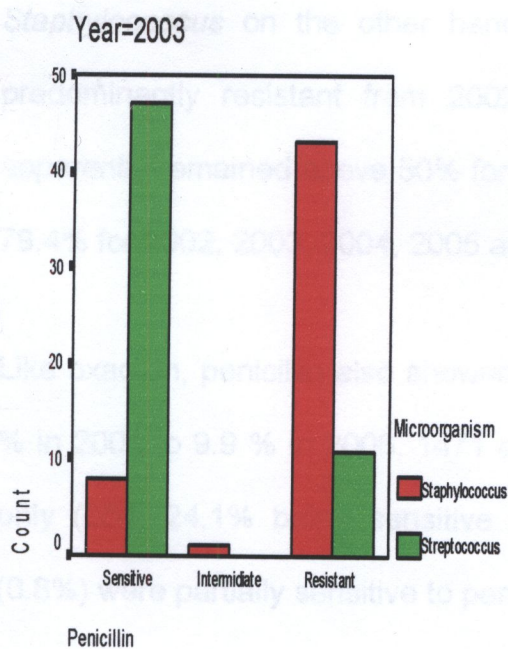


Figure 4.4.xi(c)

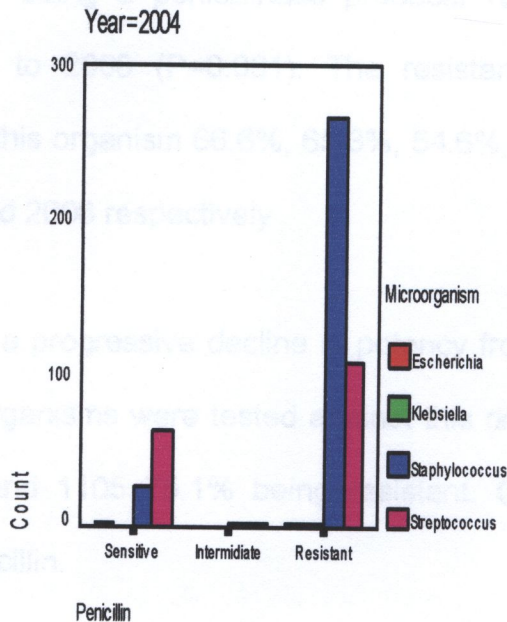


Figure 4.4.xi(d)

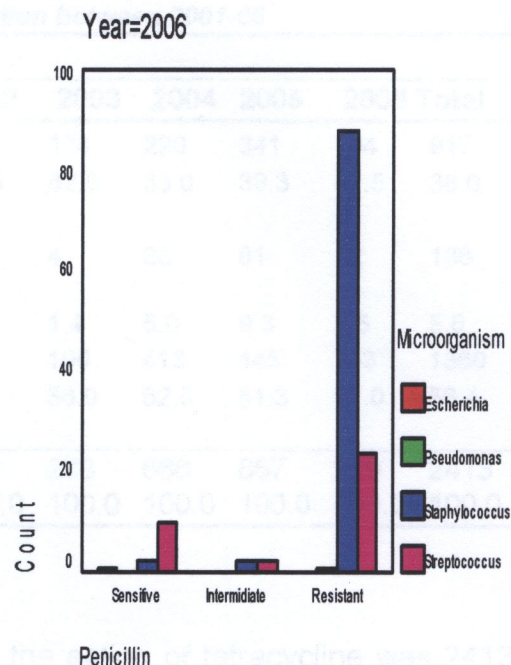
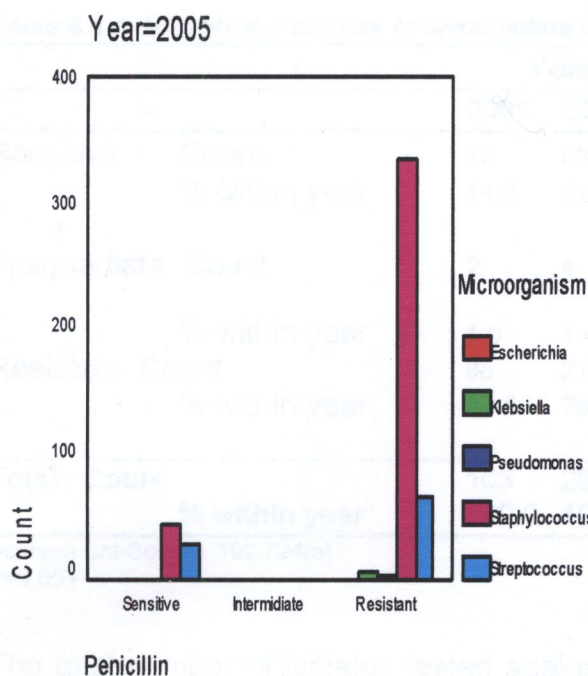


Figure 4.4.xi(e)

Figure 4.4.xi(f)

Staphylococcus on the other hand, being a penicillinase producer remained predominantly resistant from 2002 to 2006 ($P=0.001$). The resistance has apparently remained above 50% for this organism 66.6%, 65.3%, 54.6%, 65.6%, 79.4% for 2002, 2003, 2004, 2005 and 2006 respectively

Like oxacillin, penicillin also showed a progressive decline in potency from 85.7 % in 2001 to 9.9 % in 2006. 1471 organisms were tested against this drug with only (354) 24.1% being sensitive and 1105 75.1% being resistant. Only 12 (0.8%) were partially sensitive to penicillin.

Only *Streptococcus* was subjected to the action of penicillin in 2001 and therefore only two peaks are shown in the first graph below.

Table 4.4.n: Microbial response to tetracycline action between 2001-06

		Year						
		2001	2002	2003	2004	2005	2006	Total
Sensitive	Count	15	63	174	220	341	104	917
	% within year	14.6	22.5	62.6	33.0	39.3	47.5	38.0
Intermediate	Count	2	4	4	33	81	12	136
	% within year	1.9	1.4	1.4	5.0	9.3	5.5	5.6
Resistant	Count	86	213	100	413	445	103	1360
	% within year	83.5	76.1	36.0	62.0	51.3	47.0	56.4
Total	Count	103	280	278	666	867	219	2413
	% within year	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Pearson Chi-Square 192.724(a)
P=0.001

The total number of isolates tested against the action of tetracycline was 2413. Out of these, 317 (38.0%) were sensitive, 1360 (56.4%) resistant and 136 (5.6 %) partially sensitive.

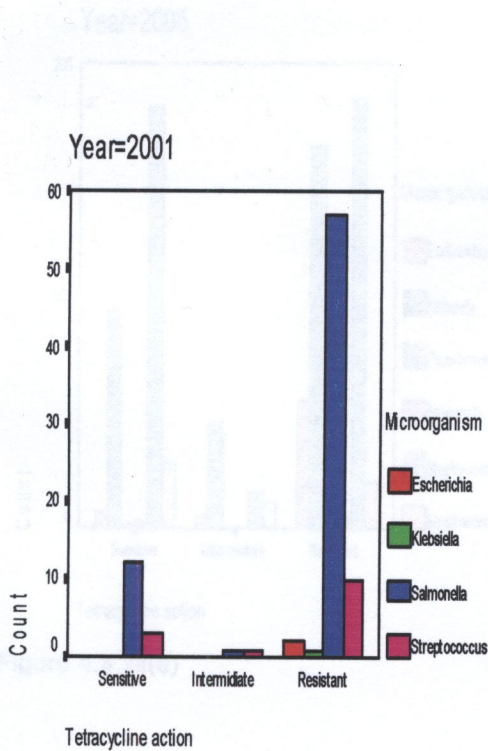


Figure 4.4.xii(a)

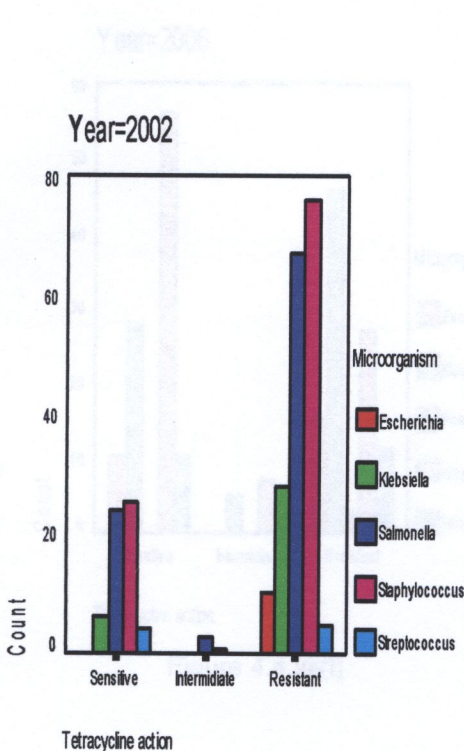


Figure 4.4.xii(b)

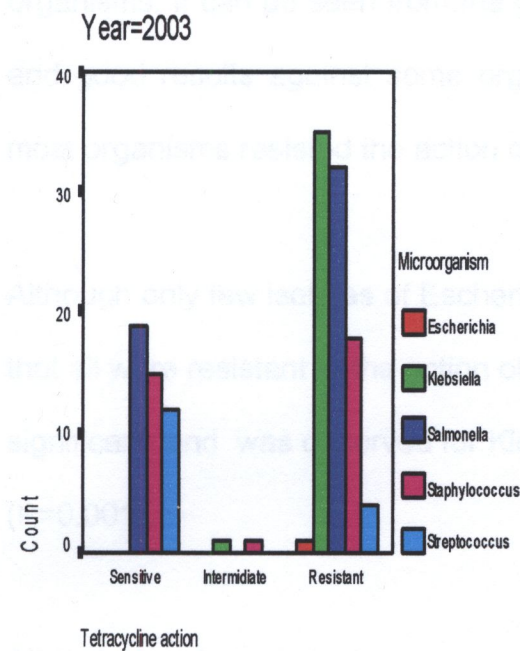


Figure 4.4.xii(c)

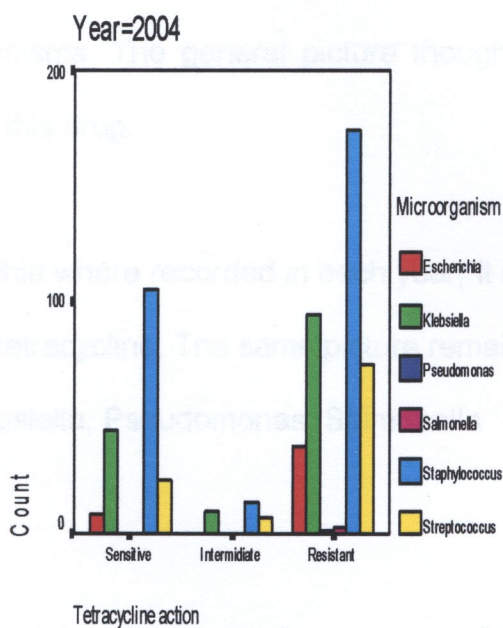


Figure 4.4.xii(d)

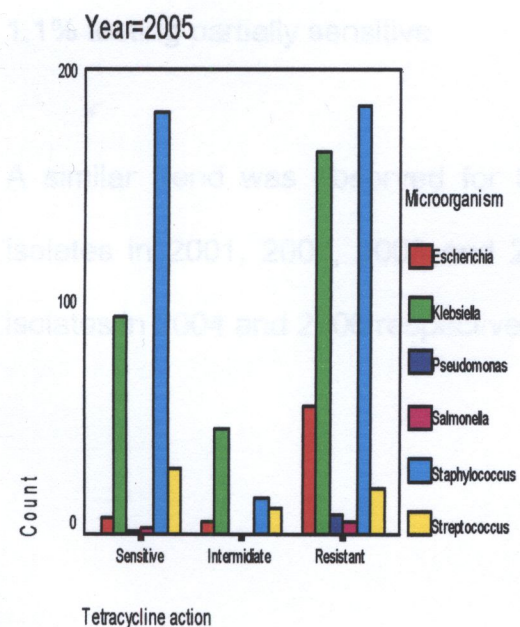


Figure 4.4.xii(e)

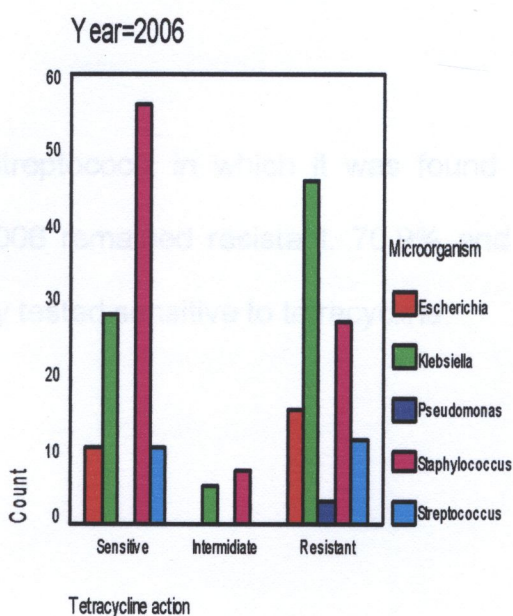


Figure 4.4.xii(f)

Tetracycline, being a broad spectrum antibiotic was tested against all the organisms. It can be seen from the graphs that the drug has produced both poor and good results against some organisms. The general picture though is that most organisms resisted the action of this drug.

Although only few isolates of *Escherichia* were recorded in each year, It is clear that all were resistant to the action of tetracycline. The same picture remained significant and was observed for *Klebsiella*, *Pseudomonas*, *Salmonella* ($P=0.001$).

All isolates of *Staphylococcus* were resistant to tetracycline between 2002 and 2005. In 2006, however 62.2% of *Staphylococcus* isolates were sensitive, with 1.1% testing partially sensitive.

A similar trend was observed for *Streptococci* in which it was found that all isolates in 2001, 2002, 2003 and 2006 remained resistant. 70.9% and 52.4% isolates in 2004 and 2006 respectively tested sensitive to tetracycline.

Table 4.4.o: Microbial response to vancomycin action between 2001-06

		Year						Total
		2001	2002	2003	2004	2005	2006	
Sensitive	Count	11	77	8	371	44	nil	511
	% within year	91.7	92.8	88.9	95.4	86.3	nil	93.9
Intermediate	Count	0	1	0	1	4	nil	6
	% within year	.0	1.2	.0	.3	7.8	nil	1.1
Resistant	Count	1	5	1	17	3	nil	27
	% within year	8.3	6.0	11.1	4.4	5.9	nil	5.0
Total	Count	12	83	9	389	51	nil	544
	% within year	100.0	100.0	100.0	100.0	100.0	nil	100.0

Pearson Chi-Square 25.728(a)
P=0.001

Throughout the five years, vancomycin showed over 85% activity against microorganisms. Noticeably though, the number of microorganisms tested against this drug was rather small over the same period (544). A very large number of isolates 511 (93.9 %) were sensitive to the drug. Only 27 (5.0 %) were resistant and 6 (1.1 %) partially sensitive.

Like for penicillin, out of the six major organisms, only *Streptococcus* was tested against vancomycin in 2001. Similarly only *Staphylococcus* results were available for vancomycin in 20003 and 2005, hence the two peaks shown in each case below.

Similarly in 2003, 8/9 (88.9%) *Staphylococci* isolates were sensitive to vancomycin with only 11.1% remaining resistant.

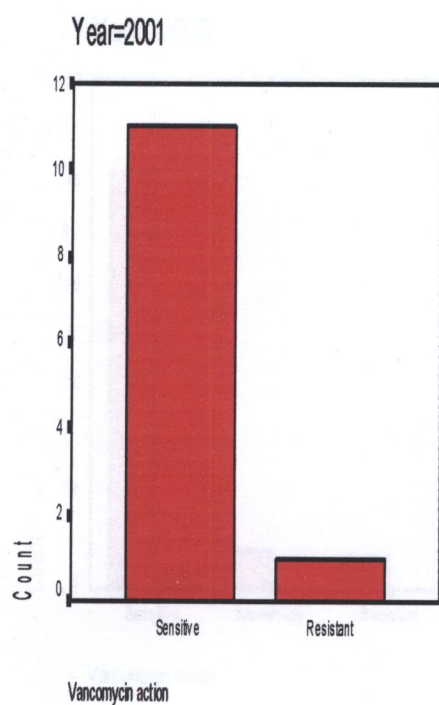


Figure 4.4.xiii(a)

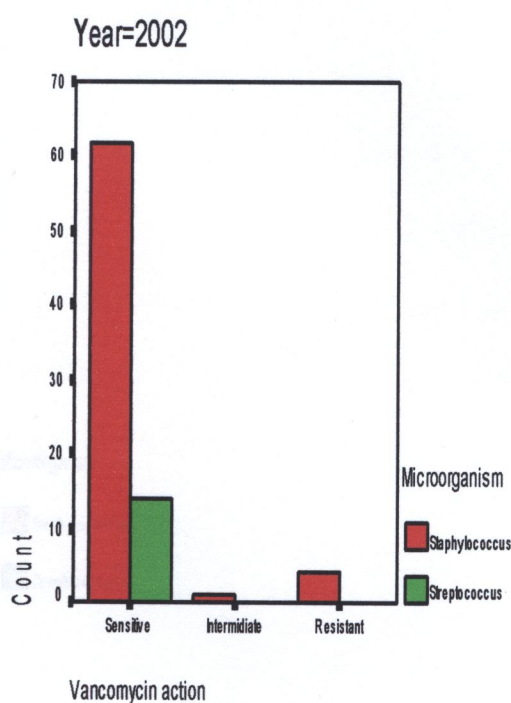


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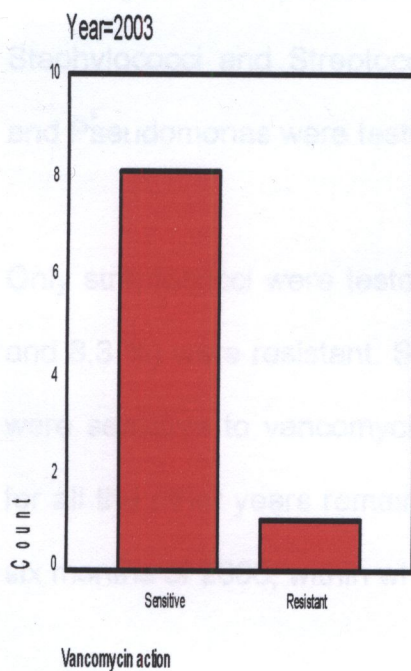


Figure 4.4.xiii(c)

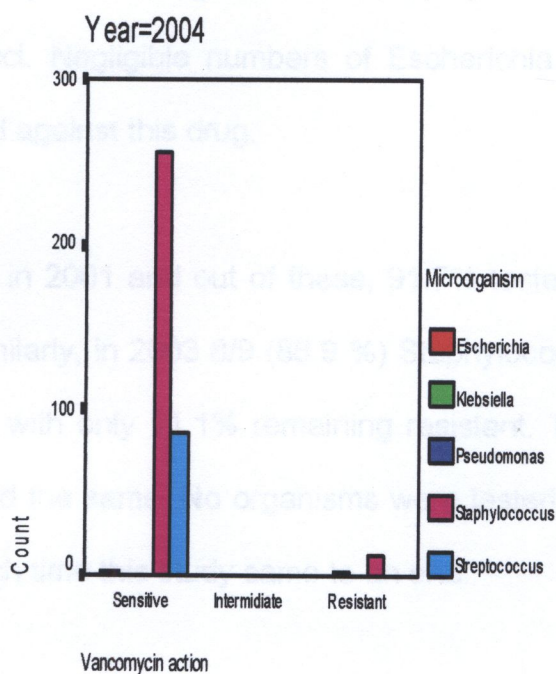


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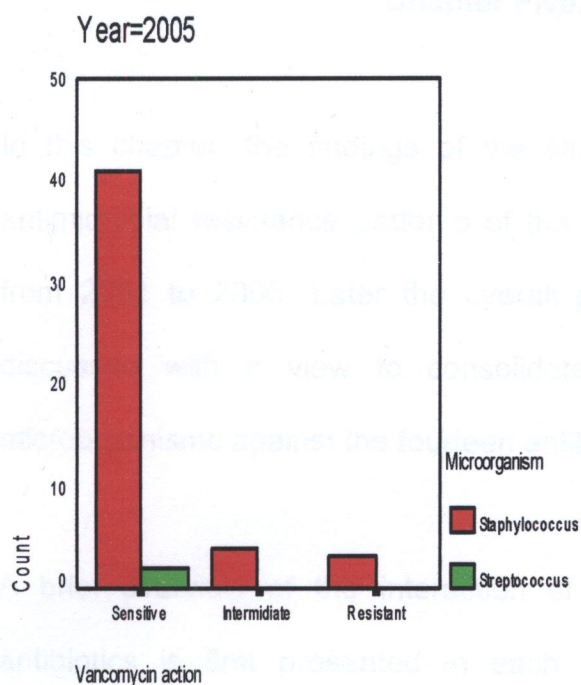


Figure 4.4.xiii(e)

Vancomycin was predominantly tested against two Gram positive isolates, Staphylococci and Streptococci. Negligible numbers of Escherichia, Klebsiella and Pseudomonas were tested against this drug.

Only streptococci were tested in 2001 and out of these, 91.7% tested sensitive and 8.3 % were resistant. Similarly, in 2003 8/9 (88.9 %) Staphylococci isolates were sensitive to vancomycin with only 11.1% remaining resistant. The picture for all the other years remained the same. No organisms were tested in the first six months of 2006, within which time this study came to an end.

Chapter Five: Discussion

In this chapter, the findings of the study are discussed, first by addressing antimicrobial resistance patterns of the six major microorganisms in each year from 2001 to 2006. Later the overall picture of antimicrobial profiles will be discussed with a view to consolidate the pattern of behaviour of these microorganisms against the fourteen antibiotics.

A brief overview of the interaction of the six microorganisms with specific antibiotics is first presented in each case then followed by the observed interaction over the study period to ascertain any deviations in patterns if any.

5.1 Frequency of Antibiotic testing over the study period

Altogether the most prescribed group of antibiotics were the macrolides, with a frequency of 24% of the total (Table 4.3.a). Of these tetracycline contributed 9 %, chloramphenicol 11% and erythromycin only 4 %. Unfortunately it was apparent that the performance of chloramphenicol was poor with 71.5% of organisms resisting its action. Similarly, erythromycin and tetracycline remained on borderline with 35 % and 56.4 % resistance respectively. For each of the two drugs it was found that fairly large numbers of isolates were testing partially sensitive; 5.6% for tetracycline and 23.3% for erythromycin.

The fluoroquinolones constituted a total of 18 % over five years; (13% for Ciproflaxacin and 5 % for Norfloxacin). This frequency is complemented by their relative potency as they faired fairly well against microorganisms. Ciprofloxacin showed 82.7 % activity against all isolates over five years while norfloxacin showed 80.4 % activity.

The next group of prescribed drugs was the Beta-lactam drugs with a combined total of 17 % over the five year period. These included Ampicillin, 5 %, Penicillin , 6 % and oxacillin 6 %. Compared to the fluroquinolones, this group of drugs generally recorded poor activity results against all isolates over the entire period of five years. The resistance for Ampicillin was 88.2 5, Penicillin (75.1 % and 57.6 %).

Cefotaxime, the only cephalosporine used in the period under review constituted a total of 11% of the drugs used. It was only 65.25 active against all isolates. Like the macrolides, this drug had relatively large proportion of partially sensitive isolates (5.8%).

Nitrofurantoin, a nitroimidazole antibiotic, performed fairly well with 72.3% activity against all isolates, with only 23.4% resistant. It constituted 6% of the total antibiotics tested over the five year period.

Nalidixic acid, a quinolone antibiotic, and occupying a total of 7% frequency.. Its performance was 68.1% activity against all isolates against 29.8 % resistant. The others were partially sensitive.

Cotrimoxazole had a total frequency of 11% of all drugs tested. Its performance though was poor in the sense that over 75.6% isolates resisted the action of this antibiotic over the entire period of five years. Thus for such a cheap and commonly used antibiotic, this does not give much hope for its use in the coming future.

Gentamicin with frequency of testing of 5% had poor or borderline performance. Of the isolates tested, it was found that 54.4% were resistant. On the other hand vancomycin, with a test of frequency of 2% had 94% activity against all isolates tested against it.

5.2 Microbial resistance pattern Profiles (Specific Objective 1.6.1)

In this section, the individual organisms are discussed with respect to the major results obtained from their interaction with the fourteen antibiotics to which they were tested in the five year period as presented in the results chapter.

5.2.1 Klebsiella

The results of Klebsiella against ampicillin show that the organism was consistently resistant to the action of ampicillin. Of the isolates tested,

593(97.5%) were resistant. This result was significant ($p=0.002$) for resistance of *Klebsiella* to the drug across the five years. In each year, it was observed that the level of resistance was more than 97%. This finding is further supported by the findings of Kahlmeter (2003) in his survey on the antimicrobial susceptibility of pathogens from uncomplicated UTIs. In this study he found that *Klebsiella* spp were upto 83.5% resistant to the action of ampicillin(fig 4.4.i). It also resisted the action of the other beta-lactam drugs, oxacillin and penicillin though very few isolates were tested against the action of these two drugs.

A similar picture was observed with the action of chloramphenicol. It was observed that out of the 636 *Klebsiella* isolates over the five year period 448 (70.4%) were resistant to the drug (fig 4.4.ii). The result was significant at $p<0.05$ showing that chloramphenicol was ineffective against *Klebsiella*. In each of the five years, the sensitivity of *Klebsiella* to chloramphenicol remained negligible relative to frequency of resistance.

In testing for the action of ciprofloxacin, 698 isolates were exposed to its action. It was observed that this drug was very active against *Klebsiella*, with 607 (87%) of all *Klebsiella* isolates testing sensitive to the drug (fig 4.4.iii). On a yearly basis, the drug performed exceptionally well against the organisms, though negligible isolates of *Klebsiella* were recorded in 2001 and 2002.

Klebsiella resisted the action of cefotaxime too, 626 isolates were tested against its action. 287 (45.7% were sensitive against 310 (49.5%) (fig4.4.iv). This is a borderline result considering the difference. In a study by Zurabian, Ryzhkova, and makarovskaia (1994), it was found that cefotaxime was effective both as a prophylactic and a therapeutic drug when combined with amino glycerides like gentamicin and sisomicin.

Cotrimoxazole was inactive against Klebsiella throughout the five years (4.4.v). The results were more pronounced in 2004 and 2005 in which 176 and 326 isolates were tested respectively. It was found that 134 (76%) of the 176 isolates in 2004 were resistant ($p=0.001$) and 276 (84.7% of the 326 in 2005 were resistant ($p=0.001$). Overall it was found that out of a total of 666, 535 (80.3%) were found resistant at $p < 0.05$. This result was therefore significant to the effect that Klebsiella was highly resistant to cotrimoxazole.

Nitrofurantoin, like cirprofloxacin was one of the consistently active antibiotic against Klebsiella in all the five years (4.4.viii). Of the 681 isolates tested in five years 458 (67.2%) tested sensitive to the drug. 34 (5%) of the isolates, however, tested partially sensitive, indicating that the drug did not perform as well as ciprofloxacin did over the same period.

Klebsiella was sensitive to the action of nalidixic acid (4.4.ix). A large number of isolates (6930 were tested against the drug with 447 (64.5%) testing sensitive. A

fairly large number of the isolates, too tested resistant; 228 (32.9%), $p=0.36$. This was an insignificant result at 5% significance level.

The level of sensitivity of *Klebsiella* to norfloxacin was very high (fig 4.4.x). 477 isolates were tested out of which 382 (80.1%) were sensitive. There were negligible cases of partial sensitivity against this drug. It therefore performed better than nalidixic acid (fig 4.4.ix).

Both gentamicin and tetracycline (fig 4.4.xiii) failed against *Klebsiella*. 635 isolates were tested against gentamicin with a resistance of 450 (70.9%) isolates (fig 4.4.vii). With regard to tetracycline, out of 602 isolates tested, 368 (61.2%) were resistant to the drug.

Very few isolates were exposed to the action of erythromycin, oxacillin, penicillin and vancomycin (figs. 4.4.vi., 4.4.xi., 4.4.xii., 4.4.xiv).

5.2.2 *Staphylococcus*

None of the *Staphylococcus* isolates were tested against the action of nalidixic acid (fig 4.4.ix). Similarly there were very few tested against the action of ampicillin, nitrofurantoin, norfloxacin and gentamicin.

The organism was found to be very sensitive to the action of chloramphenicol. 957 isolates were tested, out of which 727 (76%) were sensitive in the four years period commencing 2002 (fig 4.4.ii). Prescribers would have to remember, though that this drug should not be administered to the patient who is on cefotaxime or ceftiaxone because of the antagonistic effect that these two groups of drugs have on each other Asmar, Prainito and dajani (1988).

A large number of Staphylococcus were subjected to the action of ciprofloxacin (fig.4.4.iii). Out of the 1,021 isolates tested, 818 (80.1%) were sensitive to the action of the drug. The drug was also earlier found to be effective against a Gram negative organism, Klebsiella. The fact that it was active against Staphylococcus, a gram positive is an indication that the drug may be used for both Gram positive and gram negative organisms.

Although Staphylococcus was sensitive to the action of cefotaxime, there was also a good number of isolates that were resistant to the action of the drug between 2002 and 2006 (fig 4.4.iv). For instance, in 2005, out of a total of 407 isolates, 145 representing 35.6% were resistant with 42 (10.3%) testing partially sensitive. There were more resistant isolates than the sensitive ones in 2006 54/98 (55.1%). Overall, 919 isolates were tested and 542 (59%) were sensitive, with $p=0.14$, an insignificant result at 5% level. Considering the 10% partially sensitive results, this drug could be said to have borderline activity against Staphylococcus.

There was 65.2% (593) resistance of staphylococcus to cotrimoxazole. 295 (32.5%) were sensitive over entire five year period (fig. 4.4.v). There was also a fairly large count of partially sensitivity isolates.

There was a significant number of Staphylococcus isolates that tested partially sensitive to erythromycin (fig.4.4.vi). Like those of Streptococcus all the graphs for this drug show that there were almost as many sensitive isolates as there were resistant isolates, $p=0.182$. This result was not significant for any change in the isolate the drug sensitivity.

Except in 2001 when no Staphylococcus isolate was tested against the action of oxacillin, the results show that a large number (1002) of Staphylococcus isolates were tested (fig 4.4.xi). 629 (62.8%) tested resistant to the action of the drug against a total of 364 (36.3) sensitive, $p=0.001$.

Staphylococcus is well known to produce an enzyme penicillinase which breaks down the β -lactam ring of the penicillin, thereby inactivating its action. Together with others, it may be one of the reasons why this drug failed against Staphylococcus. Out of the 928 isolates tested, only 98 (10.7%) were sensitive to the penicillin over the entire period of five years. 824 (88.8%) were resistant (fig.4.4.xii). Noticeably for this organism, resistance kept getting worse with time, unlike other organisms where it would oscillate within the study period.

The performance of tetracycline, tested against *Staphylococcus* was close to borderline considering that 480 (53%) and 382 (42.4%) isolates were resistant and sensitive respectively between 2002 to 2006, $p=.051$. There were no isolates tested in 2001(fig.4.4.xiii).

5.2.3 *Escherichia*

Very few isolates of *Escherichia* were tested against the action of erythromycin and oxacillin in the five years (fig 4.4.vi., and 4.4.xi). There were equally few cases of *Escherichia* tested against chloramphenicol (fig.4.4.ii). It was noted that in 2005, 39 out of 70 isolates tested sensitive to chloramphenicol, while only 27 were resistant. In addition 4 of these isolates were partially sensitive. A very small number of isolates (187) were exposed to the action of ciprofloxacin. There were no notable results except that 157 (78.6%) of these tested sensitive to the drug. Similarly, it was found that *Escherichia* was generally resistant to the action of cotrimoxazole. It was further found that in 2004 only 59 of the 178 were tested against cotrimoxazole while in 2005, only 84 of 178 of *Escherichia* were tested against the drug. This was rather low for an organism that was so frequently isolated in various specimens.

Although only 166 isolates were tested against ampicillin, the levels of resistance were noteworthy (fig.4.4.i). For instance it was found that all the 11 (100%) of

isolates were resistant to the action of ampicillin in 2002 ($P=0.001$). Similarly notwithstanding the small numbers, 23 (95.8%) isolates were found to be resistant in 2006 at $p=0.001$. These results were very significant especially considering that resistance remained very high over the entire period.

The largest number of *Escherichia* was tested against the action of nitrofurantoin (782) and nalidixic acid (786) during the five year period. Evidently (fig 4.4.viii) shows that the organism remained sensitive to nitrofurantoin throughout the five years. 680 (87%) of all isolates remained sensitive, to the drug, $p < 0.05$. This therefore was a successful drug for treatment of this infection.

Similarly nalidixic acid was also very effective against *Escherichia* except in 2006 when 30 of the 56 isolates were resistant (fig. 4.4ix). Overall, 533 (67.8%) isolates were sensitive to the drug while 244(31.0%) tested resistant for the same five year period, $p > 0.05$.

Norfloxacin's action on *Staphylococcus* was above 60% over the five year period. Of the 409 organisms, 246 (60.2%) were found to be sensitive to the drug and 112(27.4%) resistant (fig.4.4.x). On the other hand another fluoroquinolone derivative, ciprofloxacin also performed well against this organism. 187 isolates were tested and out of these, 147(78.6%) tested sensitive and 37 (19.8%) were resistant. This would indicate a general sensitivity of *Escherichia* to the two fluoroquinolones.

5.2.4 Streptococcus

Streptococcus, like Staphylococcus is a Gram positive organism. The antibiogram has conventionally included the broad spectrum antibiotic like ampicillin and amoxicillin. Cephalosporins e.g. cefotaxime which are also known to be effective against Gram positive organisms are expected to be active against these organisms.

However, except for 2001 when all 11 isolates of this organism were found to be sensitive, the organism tested resistant in all the subsequent years. Notwithstanding that, however, the numbers tested were few. On the contrary, cefotaxime was very active against streptococcus (fig.4.4.iv). This is evidenced by the fact that out of the 417 tested, 378 (90.7%) were sensitive compared to only 32 (7.7%) resistant.

Majority of the Streptococcus isolates were sensitive to the action of chloramphenicol (fig.4.4.ii). From the 437 isolates in five years, 360 (82.3%) were sensitive. This justifies its continued use on Streptococcal infections compared to other common drugs like ampicillin.

Although both ciprofloxacin and norfloxacin belong to the fluoroquinone group of drugs, it was found that Streptococcus was resistant to throughout the four years (2001-2004) (fig.4.4.x). Very few isolates were tested against this drug. On the other hand ciprofloxacin had 78% (356) activity from the 456 isolates tested. This

is evidence that drugs of the same group could perform differently on the same organism.

Cotrimoxazole was unsuccessful against *Streptococcus* in each of the years without exception (fig.4.4.v). Of the 368 isolates 295 (80.2%) were resistant, $p=0.001$. Only 18.2% tested sensitive, a significant indication of the drug's inability to treat *Streptococcus* infections.

Streptococcus was sensitive to the action of erythromycin, although only 105 (58%) tested sensitive, $p=0.18$. The number of partially sensitive at 22 (12.3%) contributed to the poor result for erythromycin (fig.4.4.vi). This result is not significant especially considering that the 22 isolates are intermediate and the tendency for such isolates would be to test resistant with time.

It was observed that there was borderline activity of oxacillin and penicillin on *Streptococcal* isolates, (fig.4.4xi). Only 241 (54.7%) of the tested *streptococcus* isolates were sensitive and 196 (44%) resistant to oxacillin. Similarly, for penicillin, only 246 (51.8%) of the 475 isolates were sensitive against 224 (47.2%) resistant. There was a shift of response of *staphylococcus* to oxacillin. The organisms remained sensitive between 2001 to 2003 then converted to resistant from 2004 to 2006. This was a very significant shift in sensitivity profile of this organism, $p=0.001$. Tetracycline also had up to 55% (123) activity against

Streptococcus. Results such as these justify the need for continuous efficacy monitoring in order to detect trends early.

Unlike oxacillin, penicillin and tetracycline whose activity ranged in between 50% and 55%, the action of vancomycin on Streptococcus was overwhelmingly high (fig.4.4.xi). Of the 115 isolates, only one isolate tested resistant in 2001. 114 (99.1%) were sensitive. This is the highest single action of the drug against the microbes as well as the highest recorded single microbial sensitivities in this study.

5.2.5 *Salmonella*

Salmonella, a Gram negative bacilli and non-beta lactamase producer was very resistant to the action of ampicillin throughout the four years (2001-05) (fig.4.4.i). There were no isolates tested in 2006. Out of the 279 tested, 217 (77.8%) were resistant, clearly showing that this drug was inferior for the treatment of Salmonella infections. Chloramphenicol, on the other hand showed 51.8% inactivity compared to 47.1% sensitive organisms (fig.4.4.ii). This was a borderline result. In addition this drug did not show a steady pattern of activity. The organism was sensitive in 2001 (59.5%) but became resistant in 2002/3 (53.2% and 64% respectively), but again showed sensitivity in 2004/5. During this time, it rose to 69.2% and 65% respectively.

There were no *Salmonella* isolates tested against gentamicin in 2005/6 (fig.4.4.vii). However in the years earlier, the organism was only slightly sensitive, 119 (51.1%). With regard to the year 2004, only one *Salmonella* isolate was tested against gentamicin making it difficult to draw valid conclusions.

Cefotaxime performed very well against *Salmonella* considering that out of the 235 isolates 206 (87.7%) were found sensitive to the drug (fig.4.4.iv). There were noticeably very few partially sensitive isolates for this drug. It was also noted that although its efficacy was high, it wasn't tested as often as it should have been in 2004/5. Tetracycline on the other hand remained inactive across all the four years (2001-2005). 164 (72.6%) of the isolates were resistant to the action of the drug. As for cefotaxime, a very few isolates tested partially sensitive throughout the four years.

Cotrimoxazole was ineffective against *Salmonella*. Of the 255 isolates 191 (74.9%) were resistant, $p=0.001$ (fig.4.4.v). This was a significant result across all the years. On the contrary, ciprofloxacin was very active in all the years. It was found that out of the 216 isolates 197 (91.2%) were sensitive to its action.

There were no appreciable numbers of isolates tested against the action of nitrofurantoin, nalidixic acid, norfloxacin, erythromycin, oxacillin and penicillin. No *Salmonella* isolates were tested against the action of vancomycin (fig.4.4.xiv).

5.2.6 *Pseudomonas*

Only two antibiotics were used to any appreciable extent in five years on this organisms, gentamicin (fig.4.4.vii) and cefotaxime (fig.4.4.iv). Even then no *Pseudomonas* isolates were tested between 2001 and 2003 for both drugs, again making it difficult to draw any valid conclusion as to their performance. Between 2004 and 2006, however, 220 isolates were tested against gentamicin, out of which only 125 (56.8%) were sensitive to the drug. Cefotaxime, on the contrary was inactive against *Pseudomonas* with just about 50% (119) testing resistant (fig. 4.4.iv) to the drug.

5.3 *Pattern and Extent of Antimicrobial resistance (Objective 1.6.2)*

The overall pattern of antimicrobial resistance is shown in table 4.3.a. The performance of individual drugs per year, together with their graphical illustrations are shown and discussed below (table 4.4.b to 4.4.o).

The information in table 4.3.a provides a summary of the antibiotics' performance across all the five years. From this it can be seen that the most frequently tested drug, ciprofloxacin, also produced the highest levels of efficacy across all organisms. It can also be concluded from the table that the other two quinolone antibiotics nalidixic acid and norfloxacin also performed well. 68.1% and 80.4% of the organisms were sensitive to nalidixic acid and norfloxacin respectively.

Although the frequency of use of vancomycin on bacteria was not that high (only 544) isolates against the highest of 2995 for ciprofloxacin, the drug nevertheless exhibited very high efficacy on all the organisms with which it was tested. Up to 94% of all the organisms tested were sensitive, therefore making this drug the highest performing drug in the period under study.

The penicillin group of drugs, Ampicillin, oxacillin and penicillin, on the other hand were the second most frequently tested drug in this study (total 4366), compared to quinolones (6059). In spite of this, they emerged as the worst performing group. The level of microbial resistance to ampicillin was as high as 88.2%, oxacillin 57.6% and penicillin 75.1%. This is noteworthy considering the high frequency of use both in the laboratory testing as well as in dispensaries (prescription mediated and otherwise).

5.4. Observed pattern of resistance using drug class, pharmacological action and bacterial type (objective 1.6.3)

5.4.1: Penicillins (Ampicillin, Oxacillin and Penicillin)

The penicillins generally act by inhibiting the cell wall mucopeptide biosynthesis. They are broad spectrum bactericidal drugs, acting on many gram-positive and Gram negative aerobic and anaerobic bacteria. The drugs in this group have a β -lactam ring which is often susceptible to degradation by those organisms that produce an enzyme β -lactamase, for instance *Staphylococcus* group of organisms and some *Escherichia* spp. (APP, 2005).

In the study, under review, very few isolates of *Escherichia*, *Klebsiella*, *Pseudomonas* and *Salmonella* were tested against penicillin and oxacillin. *Staphylococcus* was resistant to the action of both oxacillin and penicillin (62% and 88.8% respectively), while *Streptococcus* was only 54.7% sensitive oxacillin and only 51.8% sensitive to the action of penicillin. Very few isolates of *Pseudomonas*, *Staphylococcus* and *Streptococcus* were tested against the action of ampicillin. *Escherichia*, *Klebsiella* and *Salmonella* tested resistant throughout the five year period. If this were to be an indication of the drug's performance in patients, then it would best be avoided for general routine prescription.

5.4.2: Cephalosporines(*Cefotaxime and chloramphenicol*)

The action of chloramphenicol has not been understood fully. However, it is known to act by inhibiting bacterial protein synthesis thereby inducing actively growing bacterial colonies into static ones (Stockley, 1998). The action was similar to that exhibited by cefotaxime. However, cefotaxime tended to be active even against some β -lactamase producing Gram negative bacteria, thereby eliminating them.

In this study, it was found that the performance of the two drugs varied between Gram positive and Gram negative organisms. For instance *Klebsiella* was resistant (70%) to chloramphenicol. Similarly *Salmonella* was 51.8% resistant to

the same drug yet it was significantly sensitive (87.7%) to cefotaxime. With regard to the action of chloramphenicol, *Staphylococcus*, Gram positive was 76% sensitive to the action of chloramphenicol. *Streptococcus* also tested sensitive to the drug (82.3%). Therefore, chloramphenicol did not demonstrate any particular pattern of activity in respect of Gram positive and Gram negative organisms

5.4.3: Tetracyclines (*Tetracycline*)

These are broad spectrum bacteristatic ribosomal acting agents. They are particularly active against Gram positive and Gram negative bacteria, such as chlamydiae, mycoplasmas, rickettsiae and protozoan parasites (Chopra and Roberts, 2001). Though being one of the most tested drug, its performance on most organisms was not impressive. In general, all the Gram negative organisms were resistant to the drug. The Gram positive organisms, *Staphylococcus* and *Streptococcus*, generally tested sensitive to the drug. This was an example of an apparent difference in the response pattern based on Gram reaction.

5.4.4: Aminoglycosides (*Gentamicin*)

These are also ribosomal reactants when in contact with bacteria (Stockley 1998). They are very active against both Gram positive and negative bacteria like *Escherichia*, *Enterobacter*, *Salmonella*, *Serratia* and *Staphylococcus*.

In the study, *Escherichia*, *Klebsiella*, *Salmonella*, *Staphylococcus* and *Streptococcus* were tested against this drug. It was found that very few gram

positive organisms were tested and the few that were tested were sensitive. In the Gram negative group of organisms, it was found that the sensitivity of *Escherichia*, *Klebsiella*, *Pseudomonas* and *Salmonella* to gentamicin was borderline. However, *Salmonella* showed exceptional difference in that it tested highly sensitive to the drug (83.8%).

5.4.5: Quinolones/Fluoroquinolones (*Ciprofloxacin*, *Nalidixic acid* and *Norfloxacin*)

Quinolones and Fluoroquinolones are a group of drugs that include nalidixic acid, norfloxacin and ciprofloxacin, among others. They act by binding topoisomeraes, the enzymes that govern the twisting and knotting of double stranded DNA. These drugs are widely used to treat otitis media and acute exacerbations of bronchitis caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* especially in adults (Musher 2002).

Of these two organisms *Streptococcus* was tested against these drugs, in this study. It was found that although they both belong to the same class, norfloxacin failed against *Streptococcus* while ciprofloxacin was very active (78%). of Nalidixic acid was not tested for its activity against *Streptococcus*. The drug, however, was very active against the Gram negative bacilli, particularly *Escherichia* and *Klebsiella* (67.8% and 64.5% respectively). It is therefore useful in the treatment of acute and chronic arising from these two organisms (Health digest, 2006).

5.4.6 Sulfonamides

Cotrimoxazole (sulphamethoxazole/trimethoprim) fall within this group of drugs. It has in-vitro activity against coliforms, and *Proteus spp.* It is usually bactericidal in action. It tends to exhibit synergistic antibacterial effect when compared to each of its components when administered as single drugs. This is because trimethoprim and sulfamethoxazole inhibit successive steps in the folate synthesis pathway (Wikipedia 2007). It is specifically indicated for use against Shigellosis, *Pneumocystis carinni*, *Listeria monocytogenes* and *nocardia spp.*

Its performance in this study was extremely poor in the sense that all organisms, Gram positive and negative were resistant to its action. *Escherichia*, *Klebsiella*, and *Streptococcus* isolates showed the highest levels of resistance (79.8%, 80.3%, and 80.2% respectively). Salmonella and Staphylococcus followed with 74.9% and 65.2% respectively. Only 15 Pseudomonas isolates were recorded and even then all were resistant. This is one drug which completely failed against all the six organisms.

5.4.7 Nitrofurantoin

Nitrofurantoin is a strong antibiotic clinically proven for use only for *Escherichia coli* and *Staphylococcus saprophyticus*. However, it has also been known to have in vitro action against coagulase negative staphylococcus (CNS), *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Citobacter spp* and

Klebsiella spp (few spp only. It has no activity against *Pseudomonas*.) (Wikipedia, 2007).

The drug is also known to show antagonistic effects when used with quinolones like nalidixic acid (Stockley, 1998) and (Wikipedia, (2007). In this study, *Klebsiella* spp were very sensitive to this drug although other studies have shown that only few *Klebsiella* spp are sensitive to it. The study has also established that none of the *pseudomonas* spp were tested against this drug, probably for the same reason that it would not be active. The other reason could include stock outs in drugs discs.

Although this strategy may appear too broad and expensive, given the poor Zambian economy, it would be worth investing in for the future.

6.1.2 Realizing that antibiotic use for animal growth promotion is being practiced especially by commercial farmers, there is need to re-examine this policy for possible amendment, strategic realignment or even abolishment in favour of improved animal health and hygiene. Some of the strategies may include:

- Working with veterinarians and other health care experts to establish a herd-health management plan that focuses on preventing disease

Chapter 6: Recommendations and Conclusions

6.1 Recommendations

6.1.1 In order to improve and sustain a favourable situation for AM activity, there is need to uphold and promote the tradition of prudent use of antimicrobial drugs in human beings and animals. This is also supported by a statement issued by the National Hog farmer Staff (200):

Public health officials, veterinarians, government regulators, farmers and makers of animal medicines agree that proper use of antibiotics on the farm is imperative to ensure that antibiotics are effective for both humans and animals. Before a product is ever approved, companies conduct thorough tests to determine the dose needed to maximize effectiveness, which minimizes chances of resistance. Instructions for using the product safely are carefully determined and reviewed by the Food and Drug Administration.

Although this strategy, may appear too broad and expensive, given the poor Zambian economy, it would be worth investing in for the future.

6.1.2 Realising that antibiotic use for animal growth promotion is being practiced especially by commercial farmers, there is need to re-examine this policy for possible amendment, strategic realignment or even abolishment in favour of improved animal health and hygiene. Some of the strategies may include:

- *Working with veterinarians and other health care experts to establish a herd-health management plan that focuses on preventing disease.*

- *Performing drug residue tests to ensure that trace levels of a drug have cleared an animal's system before it is sold as food.*

6.1.3 While the use of drugs should be closely monitored, it should be supplemented by a regular and constant evaluation of pathogen susceptibility to specific drugs. It is well known for instance that under laboratory conditions, antibiograms are obtained by testing the grown colonies of organisms against drugs impregnated on filter paper placed on agarose gel. The process involves observation of an organism's response to the drug usually after further 24 hours incubation. A microorganism is said to be sensitive or resistant according to the diameter of the zone of inhibition of cultural growth, which is then correlated statistically with the minimal inhibitory concentration (MIC).

In their study on the action of antibiotic diffusible discs, Dickert, Machka and Braveny (2005), found that *The degree of correlation depended on both the antibiotic and the species tested; between 71% and 90% of the results of disc diffusion were consistent with the MIC. The expected error distribution could not be reliably predicted, in this study. This was especially so for those bacteria which are classified as having intermediate sensitivity in their inhibition zones, based on their MIC values. Of the 18 substances tested, the inhibition zone determination was found to be least reliable, according to this study, for aminoglycosides, in particular netilmicin and amikacin.*

These findings may be extrapolated to in-vivo drug-bacterial interactions too. Therefore, it may be possible that variations in drug activity different from those

observed in-vitro may occur in-vivo, hence the need for in-vivo drug monitoring too.

6.1.4 There is need to establish a comprehensive antibiotic drug statistics. It is hoped that through this, it will be possible to assign the major efficacious drugs that the country needs in the treatment of routine infections. The data base built up will also help track emerging resistance patterns in organisms, especially considering that UTH is the main national referral hospital.

6.1.5 There is a dire need for the Zambian policy makers to review the current place of laboratory service in both case detection and patient management. This will imply that extra investment in both human resource training and infrastructure recapitalization be made a priority. The current poor state of laboratories coupled with the use, by hospitals, of unqualified laboratory staff have contributed to poor quality and often unreliable laboratory results.

6.1.6 In order to ensure effective application of the principles of prudent use of antibiotics (PUA), a review of the current medical nursing and other related medical and health curricula should be done based on the findings of Training needs Assessment in Banda's report of 2006.

6.1.7 It may be necessary, after a though drug efficacy evaluation, to withdraw certain drugs that may prove to be clearly ineffective for routine use.

6.2 Conclusions

Antimicrobial resistance has no doubt become an important public health subject not only in Zambia but the world as well. The World Health Organization (2001), also recognizes this and even terms it as a global public good for health. This is because it is affecting all human beings without exception. Given poor financial resource base, the third world countries are affected more because they are unable to afford drugs of high quality. The government should increase the overall health budget and improve sourcing and drug procurement procedures.

Inadequate health education especially in poor economies has resulted in poor health seeking behaviours in communities and individuals. This culminates in failure of patients to adhere to clinicians' instructions on antibiotic use. Most do not complete their prescribed course thereby predisposing the emergence of AMR. It is important therefore that government improves primary health care service to its citizens up to community level. People should not only be instructed on drug taking when they are sick and have reported to a health centre, but rather, like the campaigns for HIV and AIDS, malaria and TB, there is need to embed components of antibiotic use as a campaign tool.

Irrational use of drugs has been cited as a prominent contributor to the problem of AMR. Resulting from this it has been found that some organisms are attaining resistance to antibiotics due to the effect of selection pressure. In addition, this study has also established that it is possible that AMRs may have been brought

about as a result of co-administration of antagonistic drugs, e.g nitrofurantoin and nalidixic acid (Wikipedia, 2007). In Zambia, this has also been acknowledged by the Medical school of the University of Zambia. It has proposed a reveal of the curriculum in order to strengthen the teaching of rational antimicrobial use and prescribing procedures.

This study has further established that the problem of AMR is rising at UTH. The beta-lactam drugs (Ampicillin, Oxacillin and penicillin) are most implicated. Chloramphenicol and cotrimoxazole also rate high in the frequency of failure to act against the common microbes.

Of all the isolates, Klebsiella which is the most isolated organism, has shown that it has consistently resisted the action of the most commonly prescribed antimicrobials namely cefotaxime, chloramphenicol, cotrimoxazole and tetracycline. It has however been fairly sensitive to only one commonly prescribe drug, ciprofloxacin.

REFERENCES

- American Pharmaceutical Partnership (2005). *Ampicillin and Sulbactam for injection, USP*. Available from <http://www.appdrug.com/pls/apicillin> and *Subactam 451008*. 12/12/06: 22:25
- APUA-Zambia Chapter (2005). *Antibiotic prescribing practices survey in Lusaka, Zambia*. Alliance for the Prudent Use of Antibiotics PUA, Lusaka
- Banda S.S (2006). *Curriculum Review & Interviews of Experts to identify topics on Antimicrobial use and Antimicrobial Resistance included in the Undergraduate Medicine Training Programme in Zambia*.
- Barron E.J.O. et al (1994). *Bailey & Scott's Diagnostic Microbiology*. Mosby, Toronto.
- Blumer J.L. & Tolzis P. (1995). *Antibiotic-resistant Gram-negative bacteria in the critical care setting*. Paediatric Clin North Amer.
- British National Formulary. (2005). Royal Pharmaceutical Society of Great Britain. British Medical Association. London
- Cabrera R., Ruiz, J. Marco, F. Oliveira, I. Arroyo, M. Aladuena, A. Usera, A.M. De Anta, J. Gascon, J and Vila J. (2004). *Mechanism of Resistance to Several Antimicrobial Agents in Salmonella Clinical isolates causing Traveller's Diarrhea*, American Society of Microbiology.
- Cars H & Hakansson A. (1995). *To prescribe or not to prescribe antibiotics*. District

physicians' habits vary greatly, and are difficult to change. Pubmed.

Available from. Vaxjo. Available from

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed>

&list_uids=77...29/5/2006: 14:32

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve>

[&db=PubMed&list_uids=77.](#)

Cohen S.P. (1988). *MarA locus causes decreased expression of OmpF porin in multiple-antibiotic resistant(Mar) mutants of Escherichia coli.* Journal of Bacteriology. Massachusetts. Available from

<http://jb.asm.org/cgi/content/abstract/170/12/5416...12/12/06: 21:20>

Cruikshank R. (1973). *Medical Microbiology.* English Book Society & Churchill Livingstone, Edinburgh.

Crumplin G.C. et. al (1980). *Mechanism of action of nalidixic acid and its congeners. In topics in antibiotic chemistry ed. Sammes,P.G. Ellis* Horwood.Chichester.

David R. N. Harman, J. Wald ,R.E. and Kellerher, J.K. (2002). *Antibiotic prescribing by primary care physicians for children with upper respiratory tract infections.* Jama, USA.

Dickert H , Machka K and. Braveny I (2005). *The uses and limitations of disc diffusion in the antibiotic sensitivity testing of bacteria* .Available from www.springerlink.com/index/R74635625H013380.pdf -...15/3/07:21:22

Europa (2006). *Overview of public health policy.* Available from

http://ec.europa/health/ph_threats/com/mic_resistance_en.htm...01/01/07: 09:13

Frohlich D.E. (1993). *Rypins' Basic Sciences Review*. J.B Lippincott Company, Philadelphia.

Ghandi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, (2007).

Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention. Available from

[www.scielo.org/scielo.php?script=sci_arttext&pid=S0042-](http://www.scielo.org/scielo.php?script=sci_arttext&pid=S0042-96862007000500016&lng=es&nrm=is&...)

[96862007000500016&lng=es&nrm=is&...](http://www.scielo.org/scielo.php?script=sci_arttext&pid=S0042-96862007000500016&lng=es&nrm=is&...) - 40k...11/08/07: 16:07

Gibb D..M. Chintu C. Bhatt, G.J. Walker, A.S. Mulenga V. Sinyinza, F. Lishimpi, K. Farrelly,L. Kganson, N. Zumla,A. illespie, S.H. and Nunn, A.J. (2004). *Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambia children (CHAP): a double-blinded randomised placebo-controlled trial*. Lancet. London.

Gumodoka B. (1996). *Injection practices in Mwanza region,*

Tanzania:prescriptions, patient demand and sterility. Top Med Int Health.

Greenwood (1992). *Medical Microbiology: .A guide to Microbial Infections:*

Pathogenesis, Immunity, Laboratory Diagnosis and Control. Churchill Livingstone, Edinburgh.

Guentzel N.M (2006) *Escherichia, Klebsiella,Enterobacter,Serratia, Citrobacter and Proteus*. Available from

<http://gsbs.utmb.edu/microbook/ch026.htm>.11/09/06: 19:15

Grob P.R, (1992). *Antibiotic prescribing practices and patient compliance in the*

community. Robens Inst. Of Health and safety, University of

Surrey, Gilford. Available from

[http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed
&list_uids+14...11/09.06:13:10](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids+14...11/09.06:13:10)

Harrison P.F. & Lederberg J. (eds) (1998). *Antimicrobial resistance: issues and options*. Washington, DC: National Academy Press.

Healthline (2005). *Antibiotic information*. Healthline networks.

Kahlmeter G. (2003). *An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS project*. Available from

<http://jac.oxfordjournals.org/cgi/content/abstract/51/1.69...27/10.06:23:00>

Keller A. M. & Stiehm (2000). *Passive immunity in prevention and treatment of infectious diseases*. Clinical microbiology reviews California. Available from
<http://cmr.asm.org/contents/abstract/13/4/602>.

Lalitha M.K, Manyani D.J, Priya L, Jesudason M.V. Thomas K, Steinhoff M.C. (1997). *Manual on antimicrobial susceptibility testing*. NCCS publication, Pennsylvania.

Musher M.D. (2007). *Resistance of Streptococcus pneumoniae to the fluoroquinolones, doxycycline and trimethoprim-sulfamethoxazole*. Available from

http://patients.uptodate.com/topic.asp?file=pulm_inf/7649...02/02.07

Mwansa J.c. Mwaba, J. Lukwesa, C. Bhuiya, N.A. Ansaruzamma, M. Ramamurthy, T. Alam, M and Balakrishna, N.G (2006). *Multiply antibiotic-resistant Vibrio*

cholerae 01 biotype E1 Tor strains emerging during outbreaks in Zambia.

Epidemiology of infection, Cambridge university Press.

Mwansa J. Mutela K. Zulu, I. Amadi,M. and Kelly,P. (2002). Antimicrobial sensitivity in enterobacteria from AIDS patients, Zambia. Dispatches.

Emerging Infectious diseases. Available from

URL:<http://www.cdc.gov/ncidod/EID/vol8no1/01-0018.htm>...06/6/07: 22:31

National Academy of Science (2006). *The resistance phenomenon in Microbes and infectious disease*.

National Academy Press (2006). The Resistance Phenomenon in Microbes and Infectious. Disease Vectors: Implications for Human Health & strategies for Containment. Available from

<http://fermat.nap.edu/books/0339088542/130.html>.

National Council Research. (1999). *The use of Drugs in Food Animals: Benefits and Risks*. National Academy Press, Washington,DC.

National Hog farmer Staff (200). Defining Prudent use of antibiotics. Available from nationalhogfarmer.com/mag/farming_defining_prudent_antibiotics/ - 40k –

Ngoma, M Suzuki, A.Takashima, I. Sato, G. (1993). *Antibiotic Resistance of Escherichia coli and Salmonella from apparently healthy slaughtered cattle and pigs, and diseased animals in zambia*. Availble from

<http://hdl.handle.net/2115/2408> 30/05/06: 21:34

Nyeleti C. Cogan T.A. and Humphrey T.J. (2004). *Effect of sunlight on the*

- survival of Salmonella on surfaces. Available from www.blackwell-synergy.com/doi/pdf/10.1111/j.1365-2672.2004.02335.x....06/06/06: 19:00*
- Russell A. D. & Quesnell B. L. 983). Antibiotics: Assessment of antimicrobial activity and resistance. Academic Press, Toronto.
- Schaberg, D.R. et.al. (1991). Major trends in the microbial etiology of nosocomial infection. Amer Med.
- Selina C. (2006). Pseudomonas infection. Emedicine.Hawaii.
- Spratt B.G. (1977). The Mechanisms of Action of Methicillin. Journal of Antimicrobial Chemotherapy.
- Staudenbauer W.L. (1975). Novobiocin-a specific inhibitor of semiconservative DNA replication in permeabilised Escherichia coli. Journal of molecular biology.
- Stokes E. J. (1975). Clinical bacteriology. Edward Arnold, London.
- Stockley I. (1998). Drug Interactions. Pharmaceutical Press, Nottingham.
- Stuart B. L. (2002). The antibiotic paradox. Perseus publishing, Cambridge. London.
- Sweetman, S C. (2002). *Resistance to Antibiotics & Other Antimicrobial agents: 7th report of Select Committee on.* Pharmaceutical Press QV438. available from www.nbt.nhs.uk/researcheducation/library/Subject%20Resource%20Guides/4g%20medicine%20-%20pharmacy.doc ...13/3/2007:09:15
- WHO. (2000). *WHO Global Strategy for the Containment of Antimicrobial Resistance: Prioritisation and Implementation workshop.* Geneva.

WHO. (2001). *WHO Global strategy for the containment of antimicrobial resistance*, Switzerland.

WHO. (2002). Antimicrobial resistance. Available from
<http://www.who.int/mediacentre/factsheets/fs195/en/...> 18/11/2006: 0950

Wikipedia (2007). Cotrimoxazole. Available from
<http://www.answers.com/topic/cotrimoxazole...> 05/06/07: 15:30

Wikipedia (2007). Nitrofurantoin. Available from
<http://en.wikipedia.org/wiki/nitrofurantoin...> 05/06.07: 17:01

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APPENDICES



THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
ASSISTANT DEAN'S OFFICE

Telephone: 252641
Cell: 097 849302
13th February, 2007

P.O. Box 50110
Lusaka, Zambia

Mr. Daniel Fwambo
Department of Community Medicine
LUSAKA

Dear Mr. Fwambo

Re: GRADUATE PROPOSAL PRESENTATION FORUM

Following the Graduate proposal presentation Forum (GPPF) which was held on Thursday, 1st February, 2007 in the Main Lecture Theatre (UTH) at 14:00 hours, we wish to inform you that your research proposal titled: **"A five year assessment of the emerging sensitivity patterns of the top ten pathogenic bacterial isolates at the University Teaching Hospital (UTH)"** was approved by the Board of Graduate Studies of the School of Medicine. The assessors gave you a mark of 70%.

The overall comments were that:

1. You will need to have a microbiologist help supervision.
2. Ethics waiver is justified.
3. Literature review should focus on the UTH pattern of pathogens.
4. Reduce the number of pathogens and or the duration of study
5. Generally a good research subject which will benefit clinical practice at UTH.

The study is passed. Make above recommended changes and submit to ethics to request ethics waiver.

Yours faithfully

Mr. Kasonde Bowa, MSc (Glasgow) M.Med (UNZA), FRCS (Glasgow)
ASSISTANT DEAN, POSTGRADUATE

CC: Director, Graduate studies
Dean, School of Medicine
Head of Department, Community Medicine



**THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
DEPARTMENT OF COMMUNITY MEDICINE**

Telephone: 252641,
Fax: + 260-1-250753,

P.O. BOX 50110,
Lusaka, Zambia.

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15th February 2007.

Dr. V Mudenda
The Head
Pathology and Microbiology
UTH
LUSAKA

Dear Dr. Mudenda

**RE: REQUEST FOR PERMISSION FOR MPH STUDENT TO COLLECT
INFORMATION FOR DISSERTATION.**

We are writing to kindly request for permission for Mr. Daniel Fwambo who is currently studying for his Masters in Public Health (MPH) to collect information in your Department. The collected information would serve the purpose of dissertation on:

❖ Topic:

"A five year assessment of the emerging sensitivity patterns of the top ten bacterial isolates at the University Teaching Hospital (UTH)".

We appreciate your support to our MPH programme and the student.

Yours Sincerely


Mr. T Glover-Akpey
MPH COORDINATOR



THE UNIVERSITY OF ZAMBIA

RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
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E-mail: unzarec@zamtel.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

15 February, 2007
Ref.: 006-02-07

Mr Daniel Fwambo
Department of Community Medicine
School of Medicine
University of Zambia
LUSAKA

Dear Ms Fwambo,

RE: RESEARCH PROPOSAL: "A FIVE YEAR ASSESSMENT OF THE EMERGING SENSITIVITY PATTERNS OF THE TOP TEN PATHOGENIC BACTERIA ISOLATES AT THE UNIVERSITY TEACHING HOSPITAL (UTH)"

The above research proposal was presented to the Research Ethics Committee Secretariat on 15 February, 2007. The proposal has no ethical problems and is approved. Congratulations!

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a final copy of your results at the end of the study.
- Any serious adverse events must be reported at once to this Committee.

Yours sincerely,

Prof. J. T. Karashani, MB, ChB, PhD
CHAIRMAN

Date of approval: 15 February, 2007

Date of expiry: 14 February, 2008

UTHB/DLS/2.2a

20th February, 2007

Mr T. Glover-Akpey
MPH Coordinator
UNZA-SOM
Department of Community Medicine
P.O. Box 50110
LUSAKA

Dear Sir

**RE: REQUEST FOR PERMISSION FOR MPH STUDENT TO COLLECT
INFORMATION FOR DISSERTATION**

Your letter dated 15th February, 2007 on the above stated subject refers.

Accordingly, we have no objection for Mr Daniel Fwambo to collect information from our department. By copy of this letter, the Unit Head, Bacteriology is hereby requested to allow the student access the laboratory.

Yours faithfully
UNIVERSITY TEACHING HOSPITAL



Dr V.C. Mudenda
DIRECTOR OF LABORATORY SERVICES

c.c. Unit Head, Bacteriology

/mm