ASSESSMENT OF ANTIBIOTIC SENSITIVITY PATTERNS OF *Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia*e ISOLATES FROM BARN SWALLOW DROPPINGS IN ISHAKA TOWN, BUSHENYI DISTRICT, UGANDA

BY

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BPH/0036/133/DU

A RESEARCH REPORT SUBMITTED TO THE SCHOOL OF PHARMACY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF PHARMACY DEGREE OF KAMPALA INTERNATIONAL UNIVERSITY

DECEMBER, 2017

DECLARATION

I, CLAUDIA JANICE LATABU, declare that this research report titled "**ASSESSMENT OF ANTIBIOTIC SENSITIVITY PATTERNS OF *Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumonia* ISOLATES FROM BARN SWALLOW DROPPINGS IN ISHAKA TOWN,BUSHENYI DISTRICT,UGANDA**", is my work and has never been presented to any other academic institution elsewhere for any award.

Signature.....

Date.15/DEC/2017.....

CLAUDIA JANICE LATABU

BPH/0036/133/DU

APPROVAL

This is to certify that this research report has been prepared under my supervision and has never been presented anywhere for other purposes and is now ready for submission to the school of pharmacy of Kampala International University – Western Campus.

CONRAD ONDIEKI MIRUKA, BSc, MSc.

Signature..... Date..20/12/2017.....

DEDICATION

I dedicate this research to my mother, Mrs. Constance Ajok Oloya for her tireless effort and support in my education. My brother Gerald Ocamker for the selfless sacrifice and financial support offered during my education.

ACKNOWLEDGEMENT

I would like to give thanks to my supervisor Mr. Conrad Ondieki for his good work and supervision during the project development period. Not forgetting the staff and management of school of pharmacy for the assistance rendered at the right time.

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ABSTRACT

Introduction: It has been reported that the main factors behind the emergence of drug resistance is the use and misuse of antimicrobial drugs during the past few decades, but there is also the aspect of epidemic spread of drug-resistant bacteria as a factor. This has caused a major concern with serious implications in human and animal health. It's noted that as much as man's action contribute to the development of antimicrobial drug resistance, it is also seen with domestic animals, wild life and wild birds. Wild birds and other migratory species have been linked to the spread of pathogens.

Materials and Methods: The study design was experimental where samples from Barn Swallow droppings were collected and taken to the microbiology laboratory for analysis. The samples were inoculated in an enriched broth media for 24 hours and sub-cultured on MacConkey agar Deoxycholate citrate agar. Cultural characteristics, morphology (Gram reaction) and identification (biochemical test) was done to determine the identity of the bacterial isolates.
Results: The study findings showed that bacteria species isolated from the 51 Barn Swallow droppings of were *Klebsiella species* 33(64.7%), *Salmonella species* 11(21.5%), *Pseudomonas species* 0%, others 3(5.9%) and no growth isolated from 4(7.8%) of the samples.

The susceptibility test of showed that *Klebsiella* isolates were sensitive to Imipenem (93.9%) streptomycin (75.8%), Perfloxacin (42%), Nalidixic acid (12.1%) and Amikacin (9%) respectively while *Salmonella* isolates were sensitive to Imipenem (81.8%), Streptomycin (36.4%), Nalidixic acid (36.4%) and Perfloxacin (18.2%) respectively.

Klebsiella spp isolates were found to be 100% resistant to Gentamycin, Erythromycin, Piperacillin, Oxacillin, Augmentin and (90.9%) to Chloramphenicol while (87.9%) to Nalidixic acid, Ciprofloxacin and 84.8% to Amikacin.

Salmonella species were also found to be 100% resistant to Gentamycin, Erythromycin, Piperacillin, Oxacillin, Augmentin and Amikacin; (81.8%) Chloramphenicol, Ciprofloxacin, while (63%) Nalidixic acid and Perfloxacin.

Conclusion: This study found that Barn Swallow droppings contained bacteria (Salmonella sp. and Klebsiella sp.) that may be a risk to human infection and are found to be resistant to most of

the commonly used antibiotics. The recurrence of human infection with Salmonella species may as a result of frequent contact with the pathogen which contaminates the environment and water as a result of the Barn Swallow droppings.

LIST OF ABBREVIATIONS:

ASPs:	Antimicrobial Stewardship Programs.
AST:	Antimicrobial Susceptibility Testing.
CDC:	Centre for Disease Control
ECDC:	European Centre for Disease Prevention and Control
PD:	Pharmacodynamic.
PK:	Pharmacokinetic.
WHO:	World Health Organization.
SPP	Species

CHAPTER ONE:

1.0 INTRODUCTION

1.1 BACKGROUND

Antibacterial agents have been used to treat bacterial infections in human and animals as way from the time of world war II (Shobrak & Abo-Amer, 2014b).They either inhibit or destroy the vital functions of bacteria. This has caused bacteria to develop defensive mechanisms, which enables them to survive and they do this by; hydrolysis or chemical modification of the antibiotic through the production of an enzyme; alteration of the antibiotic target site; changes in membrane permeability and efflux of the antibiotic (Howard, 2015).

Bacteria are one of the causative agents of infections in man and animals both wild and domestic in ecology (Report & Yewale, 2014).They are either gram negative or gram positive in nature. Bacteria have a very unique ability to adapt to changes in their environment, and develop mechanisms of resistance to compounds that are toxic to them like antibacterial agents (Saga & Yamaguchi, 2009).

Bacteria do benefit from the possession of an antibacterial resistance gene when the corresponding antibacterial agent is present, however, in the absence of antibiotic, resistant genotypes can have lower growth rates than their sensitive counterparts, because mutations that confer resistance do so by disrupting some normal physiological processes in the cell (Lenski, 1998).

Different reports shows that the main factors behind the emergence of drug resistance is the use and misuse of antimicrobial drugs during the past few decades, but there is also the aspect of epidemic spread of drug-resistant bacteria as a factor (Bonnedahl & Järhult, 2014).This has caused a major concern with serious implications in human and animal health. It's noted that as much as man's action contribute to the development of antimicrobial drug resistance, it is also seen with domestic animals, wild life and wild birds. Wild birds and other migratory species have been linked to the spread of pathogens (Carroll, Wang, Fanning, & Mcmahon, 2015). With urban expansion, interface between wild animals and humans has increased dramatically with

some species, such as small rodents, flocking birds which thrive both in rural and human environments acting as potential reservoirs for antimicrobial resistant bacteria potentially transferring pathogens between these two environments (Sellin *et al.,* 2000; Mallon et al, 2002; Walderstro M et al, 2005, Fisichella et al, 2017).

Wild birds have been part of the circle in antibiotic resistance because of their diverse ecological niches and the ease with which they pick up human and environmental bacteria during the course of migration, act as reservoir for antibiotic resistant genes. This has been confirmed with a study carried out in Chile, which shows that there was high prevalence of ESBL-producing E. coli among Franklin's gulls (*Leucophaeuspipixcan*) that is more than twice as high as in humans in the same area (Bonnedahl & Järhult, 2014). Unrestricted use of antimicrobial drugs in feed for domestic birds and the spread of resistance genes to the large bird reservoir in Bangladesh are growing problems (Hasan et *al.*, 2012). A study in Bangladesh showed a 22.7% multidrug resistance in *Escherichia coli* isolates from bird samples; 30% produced extended-spectrum β-lactamases, including clones of CTX-M genes among wild and domestic birds.

1.2 PROBLEM STATEMENT

Despite the massive gain from the discoveries and use of antibiotics from 1910 to date, the emergence of antibiotic resistant pathogens in humans and animals has remained a critical healthcare issue threatening the management of infectious diseases, causing an international concern in terms of available treatment options and cost incurred (Sarmah, Meyer, & Boxall, 2006); Jose Luis Martinez, 2009). Guidelines on antimicrobial use such as antimicrobial stewardship programs (ASPs), various data like pharmacokinetic (PK) and pharmacodynamic (PD) properties of antibiotics, antimicrobial susceptibility testing (AST), and new antibiotic development have been put in place as measures to combat the development of antibiotic resistant strains in bacteria but to no avail (Lee, Cho, Jeong, & Lee, 2013). The emergence of antibiotic resistant microbes/pathogens have remained a threat up to date (UNAS, 2015)

1.3 GENERAL OBJECTIVE

To determine the prevalence of antibiotic sensitivity patterns of *Salmonella typhimurium,Pseudomonas aeruginosa and Klebsiella pneumonia* from Barn Swallow droppings in Ishaka Town, Uganda.

1.4 SPECIFIC OBJECTIVE

1) To isolate the three species of bacteria (*Salmonella typhimurium, Pseudomonas aeruginosa and Klebsiella pneumoniae*) from Barn swallow droppings in Ishaka Town, Uganda.
2) To determine the antimicrobial susceptibility patterns of the isolates.
3) To determine whether the isolates are resistant to the antibacterial agents available for their control.

1.5 JUSTIFICATION OF THE STUDY

The global view on antimicrobial resistance indicates that, it's one of the few examples of evolution that can be addressed experimentally focusing on the network that regulate its acquisition and its effect on bacterial physiology (Jose Luis Martinez et al, 2009).There is evidence and indication that wild birds can carry antibiotic-resistant bacteria. Spread and transmission of such antibiotic resistance does occur between humans and wild birds and vice versa (Tsiodras, Kelesidis, Kelesidis, Bauchinger, & Falagas, 2008). Such wild birds are known to be important reservoirs and vectors of transmission of resistant strains in the environment (Shobrak & Abo-Amer, 2014b).

It's also noted that not much research on this particular subject has been carried out, in Uganda and Africa at large, and limited information is available focusing on other bacteria and domestic animals, hence it's important that studies on antibiotic drug resistance in different natural habitat of wild birds are necessary (Lozano, Gharsa, Slama, Zarazaga, & Torres, 2016; Blomberg et al, 2007).

This research will therefore help in creating better understanding about the dissemination of antibiotic resistance in the environment, and potentially increasing knowledge about antibiotic resistant microbes in wild birds in Uganda. It will also help to design mitigation strategies to deal with the spread of such antibiotic resistance from wild birds to the human population.

CHAPTER TWO:

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

This chapter review relevant articles to the study areas and it is done according to the objectives of the study.

Antibiotics are a common class of drugs that are used in the prevention and treatment of bacterial infection in human, animal husbandry and agriculture. They are either bacteriostatic or bactericidal found in a variety of dosage form with different routes of administration, i.e. oral, parenteral, topical route. The majority of antibiotics being used since the 1910's for therapy and prophylaxis in food, animals and humans do have an environmental origin, e.g. from fungi and bacteria as a natural source(Davies & Davies, 2010). A case in place being penicillin, the first antibiotic that was discovered and produced from fungus *penicillium chrysogenum*, although most antibiotics used currently are of semi-synthetic or fully synthetic nature (Saga & Yamaguchi, 2009) and are classified based on their chemical nature , spectrum and mode of action (Jose L Martinez, 2009). An antibiotic exerts it's therapeutic effect by inhibition of any of the following; cell wall, protein, nucleic acid synthesis and disruption of cell wall, and its sensitivity is determined by knowing the Minimum Inhibitory Concentration (Orsolya, 2014).

Antimicrobial resistance occurs when the causative agents of infection or disease like bacteria, viruses are no longer susceptible to the common medicine used to treat infections caused by them, which is a natural response to threat by the causative agents (WHO, 2012). Resistance can be acquired where there is gene mutation in the existing DNA or intrinsic, achieved by enzymatic inactivation, decreased permeability, efflux, alteration of target site and overproduction of the target (Howard, 2015).

The emergence and re-emergence of pathogens are on the rise ,including the emergence of multidrug resistant bacteria which has made the development of antibiotic resistance among bacteria a concern in both human and animal medicine (Woolhouse,2002).There are a number of factors that have contributed to the increased development of antibiotic resistance; resulting from genetic changes in host population, addition of antibiotic for growth promotion in livestock feed. misuse (Marrow, Whittington, Mitchell, Hoyer, & Maddox, 2009) and the day to day

inappropriate choices, inadequate dosing ,poor adherence to treatment, substandard antimicrobials playing an important role in this phenomenon (Levy & Marshall, 2004).

It is noted too that antibiotic resistance does not only occur due to direct exposure to the drug itself but also due to exposure to horizontal mobile elements like viruses, wild birds that carry antibiotic resistant genes which is easily transferred from one organism to another(Middleton & Ambrose, 2005). Although wild birds do not have direct contact with anti –bacterial agents ,they can acquire or be colonized by resistant bacteria through contaminated water and food which is a major route of transmission of human and veterinary resistant bacteria to wild birds, hence they become reservoirs of resistant bacteria and genetic determinants of antibacterial resistance (Radhouani, Gonc, Pacheco, Sargo, & Igrejas, 2017).The birds have a diverse ecological niche ,with the ability to migrate long distances ,while picking bacteria and transmitting them to human and animals (Bonnedahl & Järhult, 2014).This has been demonstrated by a study done in Sweden, isolating antibiotic resistant Salmonella strains from black-headed gulls (*Chroicocephalus ridibundus*) which arrived from west and southwest Europe (Hatanaka et al., 2003).

The increasing importance of zoonotic diseases and the need to understand the emerging resistant pathogens has made the origin of anti bacterial resistance in wildlife important to human health for the fact that it presents with difficulty in medical treatment of wildlife (Igrejas, 2014) and the management of such cases becomes considerably costly with prolonged hospital stays which necessitate additional doctor visits and health care use in human with greater risk of complications, higher mortality rates and economic burden resulting into loss of productivity due to time being spent by family with the patient (CDC, 2013). Analysis from the European centre for Disease Prevention and Control (ECDC) in 2009 estimated that each year at least 2 million human infections caused by a subset of resistant bacteria are responsible for about 25000 deaths with a healthcare cost and productivity losses of at least 1.5 billion.

An important change in resistance prevalence rates has occurred with the shift from Gram-positive to multi-resistant Gram-negative bacteria, for which treatment options are limited or entirely lacking with particular attention given to a gene coding metallo-lactamase 1 (NDM–1) which makes Gram-negative enterobacteria resistant to last line antibiotics, such as carbapenems. Owing to the fact above, *Salmonella typhimurium* , *Pseudomonas aeruginosa* and *Klebshiella pneumoniae* infections have become areas of interest. A survey done in Europe in 2011 to determine the prevalence of the above infections and results shows great presence of resistant strains(Levy & Marshall, 2004).

Figure 1: % of resistant *Klebshiella pneumoniae* to third generation cephalosporin, aminoglycosides and floroquinones,in Europe 2012.

Source; EARS-NET, 2013.

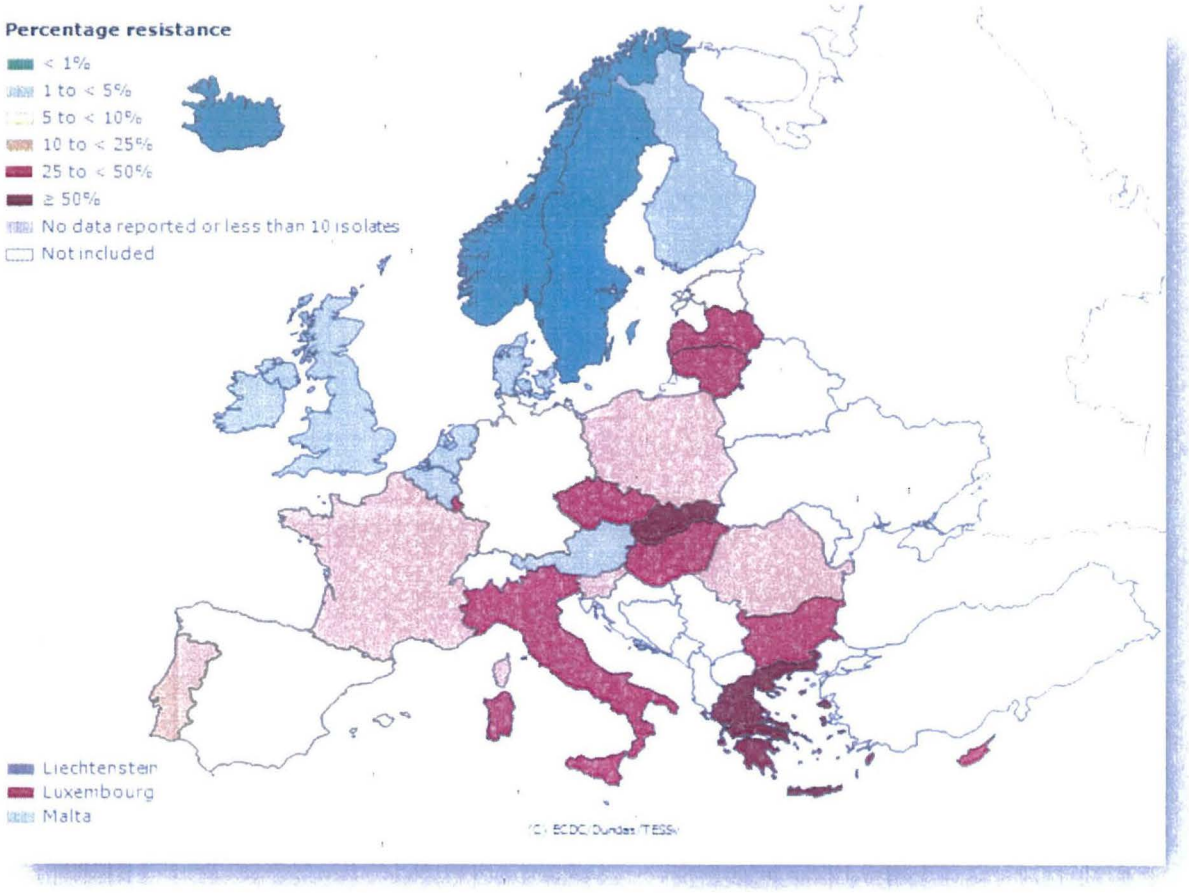
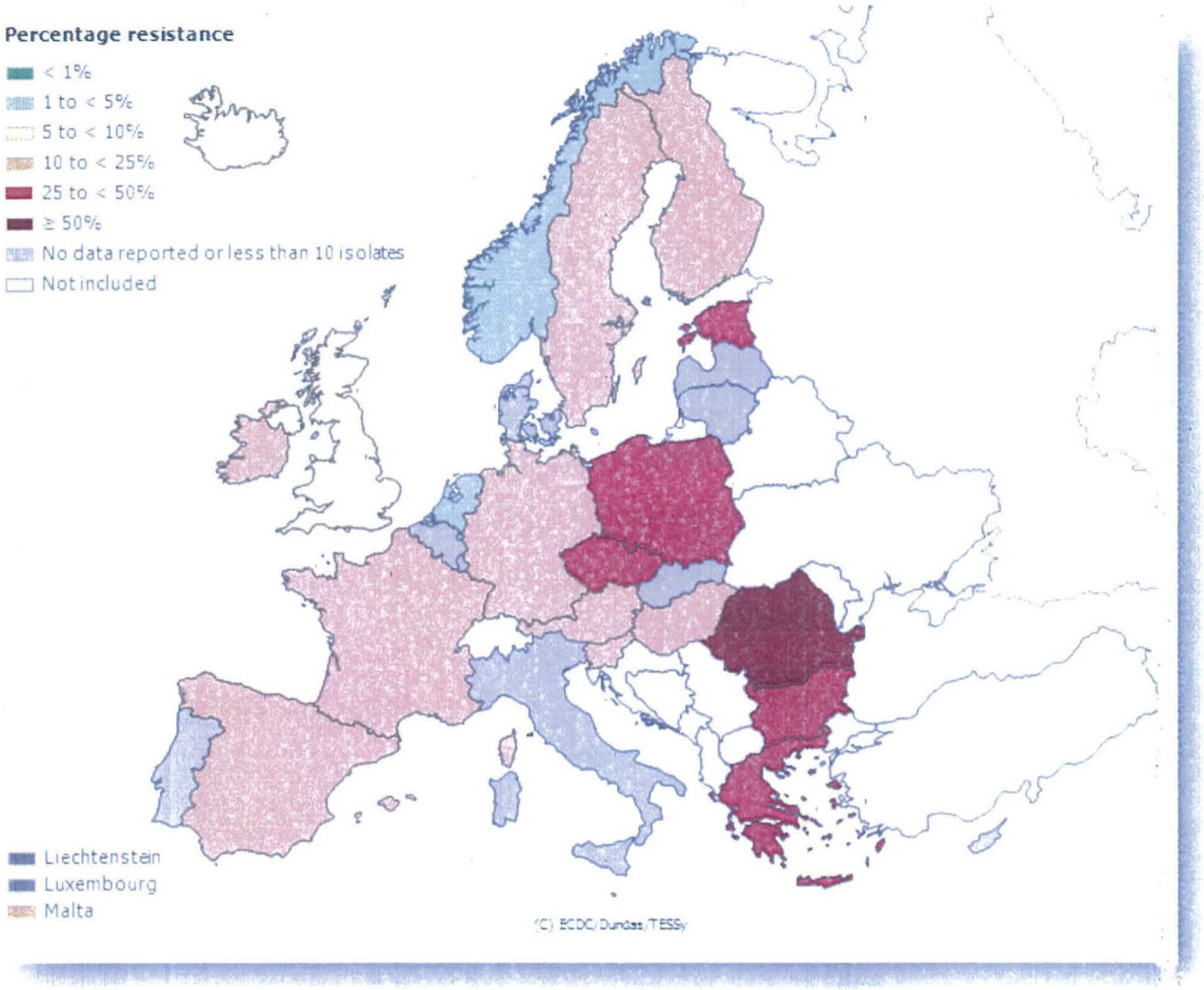


Figure 2: % of resistant *pseudonmonas aeruginosa* to Carbepenem in Europe, 2012. Source; EARS-NET, 2013.



An enteric systemic infection in human known as typhoid fever is caused *salmonella typhimurim* and *Salmonella paratyphimurim A* (Sudeepa et al., 2013) which is characterized by continuous fever for 3-4 weeks, relative bradycardia, weakness, headache, loss of appetite with involvement of lymphoid tissue (Crump, Gordon, & Parry, 2015).The source of infection are attributed to infected and healthy carriers, with the"five Fs" (food, fingers, flies, fomites and faeces) playing a major factor (Marineli, Tsoucalas, Karamanou, & Androutsos, 2013).

Each year, the world over, there are at least 13-17 million cases of typhoid fever, resulting in 600,000 deaths. 80% of these cases and deaths occur in Asia alone. In South East Asian nations, 5% or more of the strains of the bacteria may already be resistant to several antibiotics like chloramphenicol which has been a gold standard for therapy since its introduction in 1948. Resistance has also been seen with other antibiotics like Ampicillin and Cotrimoxazole and floroquinolones which has posed a major setback in management of the disease (Prajapati et al., 2008).

A study carried out in United States and Canada on the role of wildlife in epidemiology of antimicrobial resistance shows prevalence of *Salmonella typhimurium* in 7-65% of the raccoon feaces (Bondo et al., 2016). Another study done in Kampala –Uganda shows greater diversity of sources of *Salmonella* entering the human population due to continuous evolution in the human environment suggesting *Salmonella* and associated resistance from humans may not be wholly derived from a common population (Sur, 2012).

Various studies have shown the existence of multidrug resistant strains of bacteria with *salmonella typhimurium* inclusive on the surface of water bodies ,drinking water source, rivers and the black- headed gull (*Larusridibundus*) being a migrating species that nests in colonies on water have been identified as reservoirs for antibiotic resistant bacteria mainly *Salmonella typhimurium* (Literak et al., 2010). A study conducted in Eastern Uganda on *E.coli* suggests opportunities for gastrointestinal bacterial exchange among humans and non human primates and wildlife living closer to dense human settlements are more likely to carry resistant bacteria (Sur, 2012).

Pseudomonas aeruginosa is an opportunistic pathogen which causes severe, acute and chronic nosocomial infections with infections mainly in immunocompromised patients with respiratory disease,cancer and burns(Lutz & Lee, 2011).These makes the patient more susceptible to infection with the organism presenting with difficulties in treatment due to emergence of multidrug resistance especially in developing countries leaving carbepenem class of antibiotic as one of the drugs used for its management(Lambert, 2002),although an antibiotic susceptibility study result showed 99.2% resistance to carbenicillin, 98.4% to ticarcillin, 96.2% to ciprofloxacin, 95.4% to co-trimoxazole, 94.7% to imipenem and meropenem, 93.9% to piperacillin,

93.2% to azetronam, 92.4% to tobramycin, 91.7% to cefepime, 89.4% to amikacin and ceftazidime, and 87.2% to piperacillin-tazobactam,with all isolates resistant to at least three different classes of antibiotics, i.e., carbapenems, quinolones, and aminoglycosides is a recent concern (Moazami-goudarzi & Eftekhar, 2013). Results from another study showed the presence of *Pseudomonas aeruginosa*is in swimming pool and other water bodies (Zhang, Zhang, & Fang, 2009), hence still relating the possible path of transmission to water.

Klebsiella Pneumoniae a world wide spread disease causing bacteria mainly nosocomial infections has been ranked the leading cause of death and mortality and still being the primary cause of respiratory and urinary tract infections (Podschun & Ullmann, 1998).They are carbapenemases producing enzymes that are disseminating worldwide rapidly (Gene et al., 2010) ,which has been seen with *Pseudomonas aeruginosa* and *salmonella typhimurium* as well (Hirsch & Tam, 2010).A study conducted in India to determine the prevalence of resistant strain of *Klebsiella pneumonia* showed that all samples were resistant to carbenicillin and over 60% resistance to chloramphenicol and tetracycline and cephalosporins but susceptible to amikacin only (Sikarwar & Batra, 2011).

Carbapenems are powerful broad-spectrum antibiotics that are often the last line of effective treatment for patients with multidrug-resistant infections, including those caused by ESBL-producing bacteria. In recent years bacteria have been isolated that produce carbapenemase enzymes. These bacteria are resistant to carbapenems and also many other drugs, which creates significant treatment problems(Moazami-goudarzi & Eftekhar, 2013)

Salmonella typhi and paratyphi A, Pseudomonas aeruginosa infections are both associated with water and wild birds, and with the fact that contaminated water is an important factor in the spread of resistant bacteria, wild birds associated with aquatic environment are indicators for environmental pollution (Bonnedahl & Järhult, 2014). It's therefore important to understand the transmission pathways of antibiotic resistant strains of bacteria to be able to determine the methods of prevention (Hatanaka et al., 2003) .

The Barn Swallow (*Hirundo rustica*) is the most wide spread species of Barn swallow in the world, with a distinctive passerine bird with blue upperparts ,a long deeply forked tail and curved, pointed wings. It is found in Europe, Asia, Africa and the different parts of America (Turner *et al*, 1989).

There are six subspecies of Barn Swallow, which breeds across the Northern Hemisphere. Four are strongly migratory, and their wintering grounds cover much of the southern Hemisphere as far South as central Argentina, the cape province of South Africa and northern Australia. This huge range means that the Barn Swallow is not endangered; although there may be local population declines due to specific threats (Cocker *et al*, 2005).The preferred habitat for the barn swallow is open country with low vegetation, such as pasture, meadows and farm land, preferably near water. This Swallow avoids heavily wooded or precipitous areas and densely built- up locations. The presence of accessible open structures such as barns,stables,culverts,provide nesting sites and exposed locations such as wires, roof ridges or bare branches for perching ,are also important in the bird's selection of its breeding range (Barlow *et al*, 1997).

Antimicrobial resistance is a global problem that must be addressed in all countries, due to the importation of drug-resistant micro-organisms through international travel and trade (Shobrak & Abo-Amer, 2014a). The World Health Organization (WHO) chose antimicrobial resistance as the theme for World Health Day since 2011. On the 7th day of April every year an international call for concerted action to halt the spread of antimicrobial resistance and recommendation of a six-point policy package for governments is always issued by World Health Organization. one of the 2015-2020 national strategy to combat the rise of antibiotic resistance is to improve knowledge of what drives the development and spread of antibiotic resistance (Strategy, 2015). This study is therefore in line with the strategy, because it will widen the knowledge gap on the spread of antibiotic resistance.

CHAPTER THREE:

3.0 METHODOLOGY

3.1 Study area

The study was carried out in Ishaka –Bushenyi District, western Uganda.

3.2 Study design

The study design was experimental where samples from Barn Swallow droppings were collected and taken to the microbiology laboratory for analysis.

3.3 Materials and Methods

3.3.1 Collection of Bird Faecal Samples

51 convenient faecal samples was collected using sterile cotton swabs wetted with sterile normal saline from residential areas and educational institutions, housing nests of Barn Swallows in Ishaka town, Uganda. Individual faecal samples were transported in a sterile specimen container to Microbiology laboratory of Kampala International University-Western Campus, Ishaka.

3.3.2 Culturing and Isolation

The samples collected were cultivated for *Salmonella typhimurium, Pseudomonas aeruginosa and Klebsiella pneumoniae* on selective media Xylose lysine deoxycholate (XLD) and MacConkey agar plates as described by Sikarwar & Batra,(2011).

3.3.3 Identification

Cultural characteristics of the isolates were observed for texture, color appearance, size of colonies, and elevation.

Morphologically, Gram stain was done with the colonies to identify the bacteria colour reaction if they are gram negative (red or pink) or gram positive (purple or blue) and morphology (if they

are rods, cocci, club shape or coma shape). The gram negative rods organisms were subjected further for biochemical test to identify the species.

3.3.4 Biochemical tests

The colonies identified by Gram stain were inoculated in triple sugar iron agar (TSI) for carbohydrate utilization that will aid in their identification. TSI has three (3) sugars (sucrose, glucose and lactose), the fermentation of any of the sugars will result in acid production that will change the colour of the media yellow or red when there is no fermentation and some organism can produce hydrogen sulphide which will be observed with black coloration of the media.

Other biochemical tests are urea utilization, citrate utilization, oxidase test and indole test all with combination aid in the identification of the species of bacteria isolates. The details of the procedures found in appendix.

3.3.5 Antimicrobial susceptibility testing

Colonies from each plate were tested for susceptibility to antimicrobial agents by Kirby's Bauer's disc diffusion method in accordance with the Clinical and Laboratory Standards Institute

A total of 12 antimicrobial discs Chloramphenicol (30 mcg), Streptomycin (10 mcg), Perfloxacin (5 mcg), ciprofloxacin (5mcg), Amoxicillin+ Clavulanic acid (25 mcg), Piperacillin (100 mcg), Gentamycin (10mcg), Erythromycin (15mcg), Nalidixic acid (30mcg), Amikacin (5mcg), Imipenem (10mcg), Oxacillin (1mcg) were used. This antimicrobial panel were chosen to include antibiotics with potential efficacy in treating infections against *Salmonella typhimurium,Pseudomonas aeruginosa and Klebsiella pneumoniae* in human and animals infections in addition to their use as feed additives to promote growth in animals and the diversity for which they represent among the different antimicrobial classes (Shobrak & Abo-Amer, 2014a).

3.4 Ethical considerations

a) A letter of permission and introduction was obtained from the dean school of pharmacy.
b) A letter of permission to do the study was obtained from the executive director of KIU TH to conduct the study in the hospital Laboratory.

c) Permission from the different sites for sample collection was sought out before collection of sample, especially from the residential homes.

CHAPTER FOUR:

PRESENTATION OF RESULTS

4.0 Introduction

This chapter presents the findings of the research according to the stated objectives in graphs, tables and charts, discussion of the results, conclusion and recommendations to study outcome.

4.1 Presentation of findings

Table 1: Bacteria species isolated from Barn Swallow droppings.

The table below shows the bacteria species isolated from the Barn Swallow droppings

With *Klebsiella* species the highest with a total of 33(64.7%) isolates, *Salmonella* species 11(21.5%), *Pseudomonas* species 0%, others 3(5.9%) and no growth isolated from 4(7.8%) of the samples.

Bacteria isolated	Number	%
Klebsiella sp	33	70
Salmonella sp	11	23
Pseudomonas sp	0	0
Others	3	5.9
Total	51	98.9

4.2 Antimicrobial susceptibility patterns of the isolates.

Table 2: Susceptibility test for *Klebsiella sp* (33).

The susceptibility test of Klebsiella isolates indicated Imipenem is the most sensitive (93.9%) streptomycin (75.8%), Perfloxacin (42%), Nalidixic acid (12.1%) and Amikacin (9%) respectively as seen below.

ANTIBIOTICS	% SENSITIVE	% INTERMEDIATE	% RESISTANT
STREPTOMYCIN	75.7575	15.1515	9.0909
AMIKACIN	9.0909	6.0606	84.8484
IMIPENEM	93.9393	0	6.0606
PERFLOXACIN	42.4242	9.0909	48.4848
CIPROFLOXACIN	0	12.1212	87.8787
NALIDIXIC ACID	12.1212	0	87.8787
AUGMENTIN	0	0	100
OXACILLIN	0	0	100
PIPERACILLIN	0	0	100
ERYTHROMYCIN	0	0	100
GENTAMYCIN	0	0	100
CHLORAMPHENICOL	6.0606	3.0303	90.909

Figure 3: Susceptibility test for *Klebsiella sp*

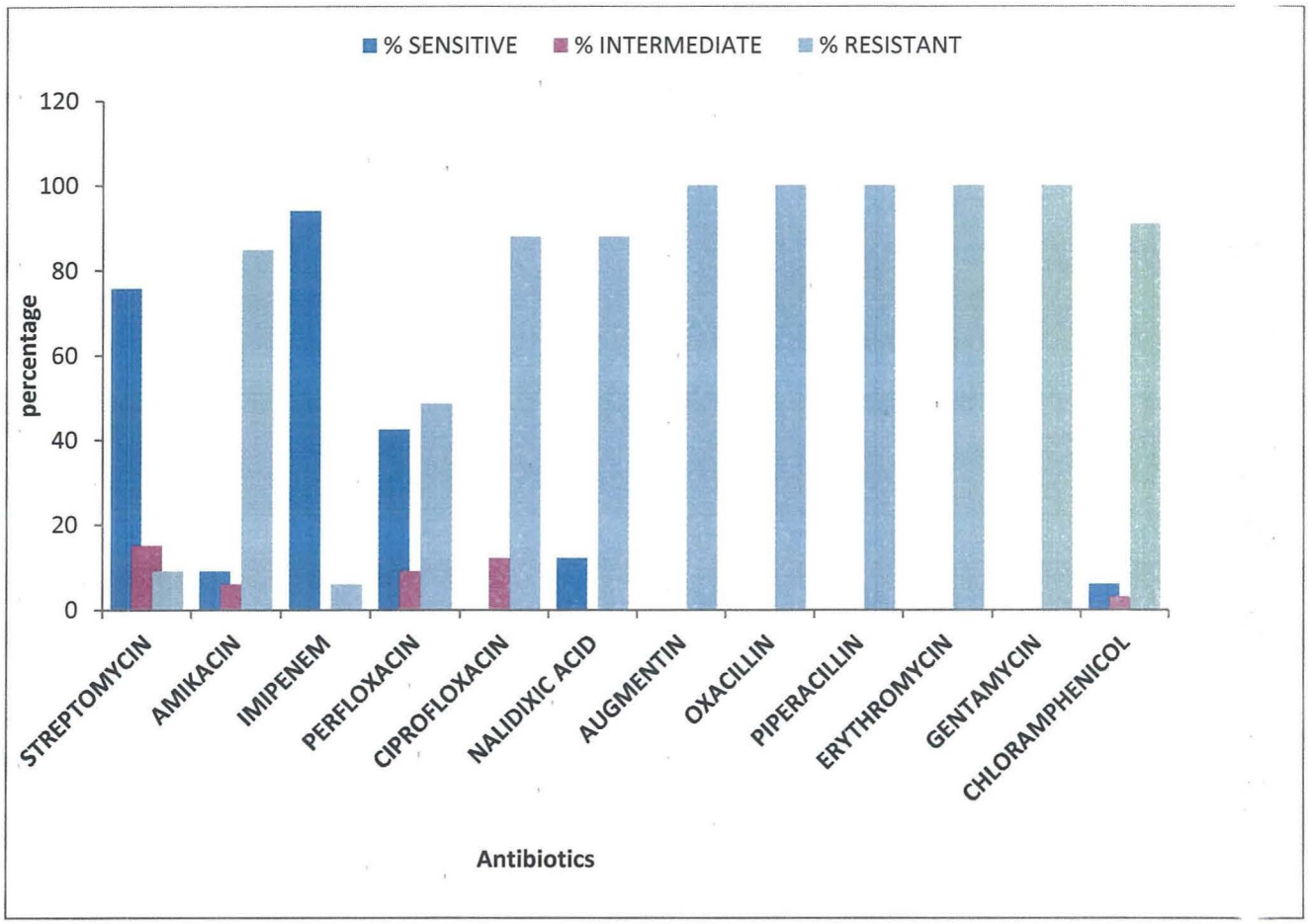


Table 3: Susceptibility test for *Salmonella sp* (11).

The susceptibility test of Salmonella isolates indicated Imipenem is the most sensitive (81.8%) Streptomycin (36.4%), Nalidixic acid (36.4%) and Perfloxacin (18.2%) respectively.

ANTIBIOTICS	% SENSITIVE	% INTERMEDIATE	% RESISTANT
STREPTOMYCIN	36.3636	9.0909	54.5454
AMIKACIN	0	0	100
IMIPENEM	81.8181	0	18.1818
PERFLOXACIN	18.1818	18.1818	63.6363
CIPROFLOXACIN	0	18.1818	81.8181
NALIDIXIC ACID	36.3636	0	63.6363
AUGMENTIN	0	0	0
OXACILLIN	0	0	100
PIPERACILLIN	0	0	100
ERYTHROMYCIN	0	0	100
GENTAMYCIN	0	0	100
CHLORAMPHENICOL	0	18.1818	81.8181

Figure 4: Susceptibility test for *Salmonella sp*

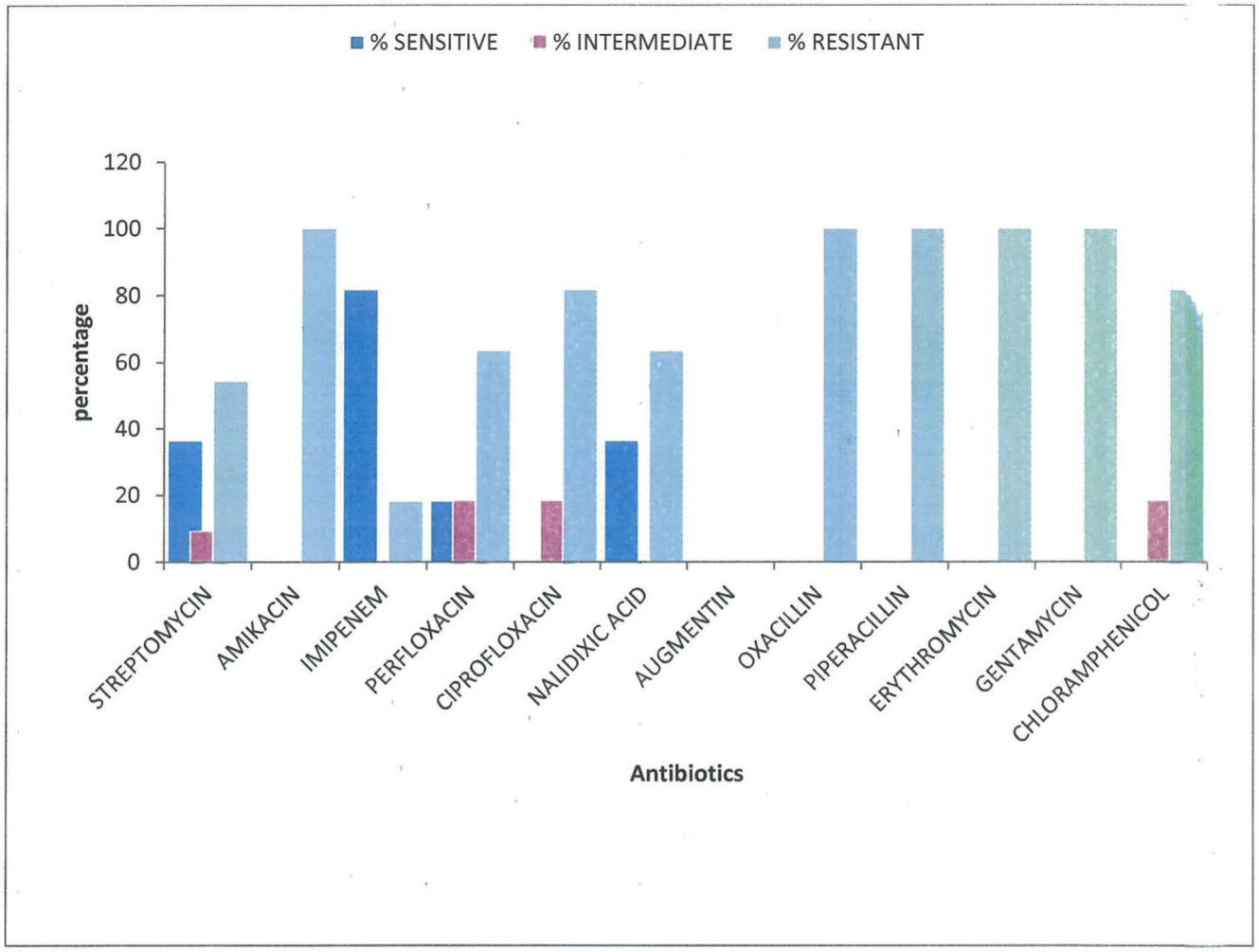
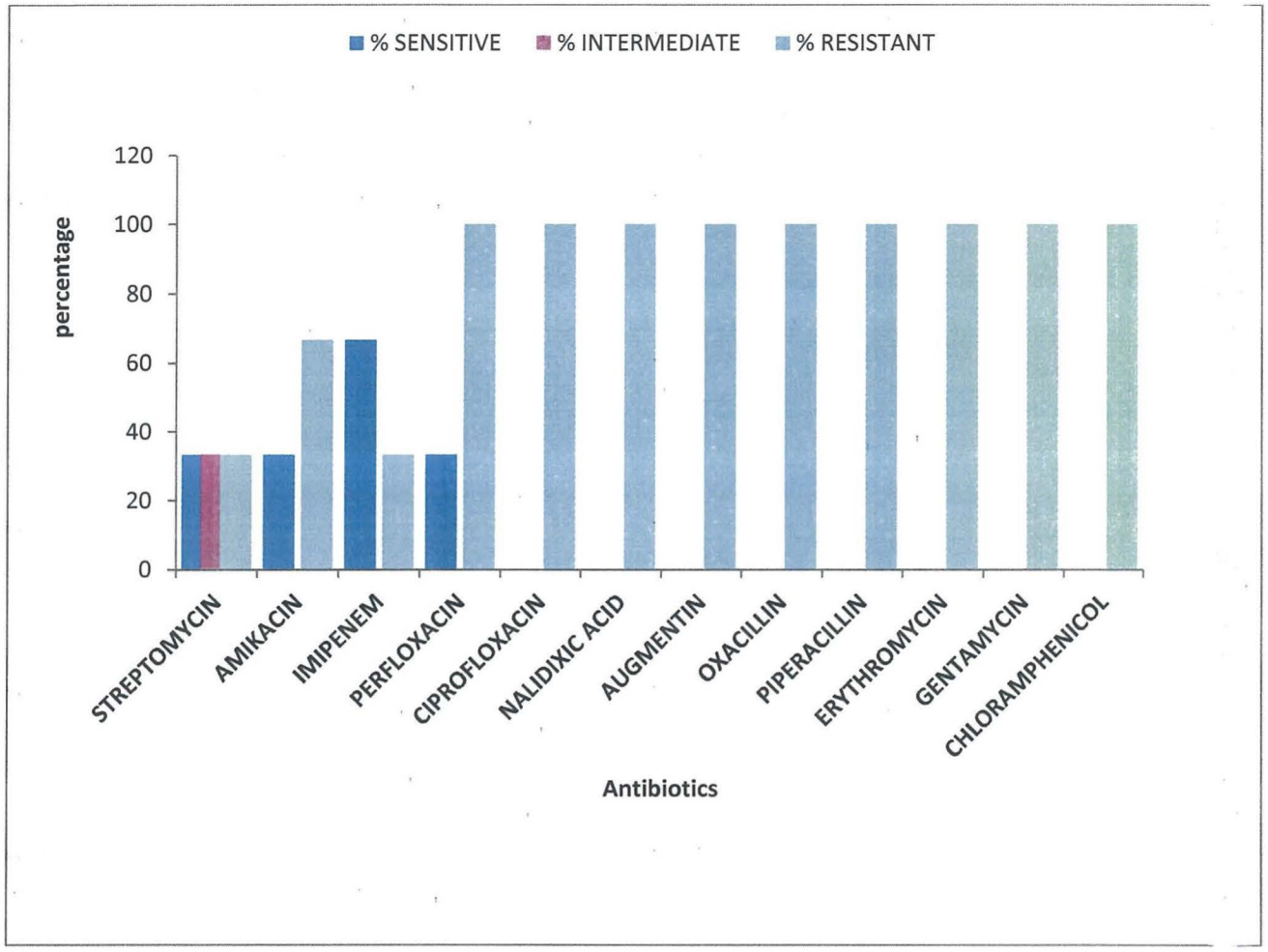


Table 4: Susceptibility test for other microorganisms (*Proteus spp*, 3).

The table below indicates Proteus species to be 66.6% susceptibity to Imipenem, 33% to Streptomycin,Amikacin,Perfloxacin,with 100% resistance to Ciprofloxacin,Nalidixic acid,Augmentin,Oxacillin,Piperacillin,Erythromycin,Gentamycin,Chloramphenicol,66.6% to Amikacin,Perfloxacin and 33.3% to Streptomycin,Imipenem.

ANTIBIOTICS	% SENSITIVE	% INTERMEDIATE	% RESISTANT
STREPTOMYCIN	33.3333	33.3333	33.3333
AMIKACIN	33.3333	0	66.6666
IMIPENEM	66.6666	0	33.3333
PERFLOXACIN	33.3333	0	66.6666
CIPROFLOXACIN	0	0	100
NALIDIXIC ACID	0	0	100
AUGMENTIN	0	0	100
OXACILLIN	0	0	100
PIPERACILLIN	0	0	100
ERYTHROMYCIN	0	0	100
GENTAMYCIN	0	0	100
CHLORAMPHENICOL	0	0	100

Figure 5: Susceptibility test for other microorganisms (*Proteus spp)*



CHAPTER FIVE:

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 Introduction

This chapter discussed the findings of the research report according to each objective and compared results to other relevant research areas, also gives a conclusion on the findings and recommendations accordingly.

5.1 Discussion of findings

The study findings showed that bacteria species isolated from the 51 Barn Swallow droppings were *Klebsiella species* with highest total of 33(64.7%) isolates, *Salmonella species* 11(21.5%), *Pseudomonas species* 0%, others, *Proteus species* 3(5.9%) and contaminant growth 4(7.8%) of the samples. This concers with a study done in Portugal,on wild birds, common buzzard (*Buteo buteo*) faecal sample, where multi- resistant *E. coli* and enterococci isolates were found (Radhouani, Gonc, Pacheco, Sargo, & Igrejas, 2017).

The microorganisms isolated were seen to be only gram negative bacteria, hence confirming that there is an important change in resistance prevalence rates with a shift from Gram-positive to multi-resistant Gram-negative bacteria, for which treatment options are limited (CDC,2013).

The susceptibility test of *Klebsiella* isolates indicated Imipenem is the most sensitive (93.9%) Streptomycin (75.8%), Perfloxacin (42%), Nalidixic acid (12.1%) and Amikacin (9%) respectively. The susceptibility test of *Salmonella* isolates indicated Imipenem is the most sensitive (81.8%) Streptomycin (36.4%), Nalidixic acid (36.4%) and Perfloxacin (18.2%) respectively.

Klebsiella species isolates were found to be 100% resistance to Gentamycin, erythromycin, Piperacillin, Oxacillin, Augmentin and Chloramphenicol (90.9%), while Nalidixic acid and Ciprofloxacin (87.9%) resistance respectively. Amikacin was 84.8% resistance.

Salmonella species were also found to be 100% resistance to Gentamycin, Erythromycin, Piperacillin, Oxacillin, Augmentin and Amikacin; Chloramphenicol (81.8%), Ciprofloxacin (81.8%) resistance respectively, while Nalidixic acid and Perfloxacin are (63%) resistance. This data has been demonstrated in figure 3 and 4. This therefore confirms that antibiotic resistance does not only occur due to direct exposure to the drug itself, but also due to exposure to horizontal mobile elements like viruses, wild birds that carry antibiotic resistant genes which is easily transferred from one organism to another (Fisichella *et al,* 2017). A study carried out in sUnited States and Canada on the role of wildlife in epidemiology of antimicrobial resistance also showed prevalence of *Salmonella typhimurium* in 7-65% of the raccoon feaces (Bondo *et al.*, 2016).Another study conducted in India to determine the prevalence of resistant strain of *Klebsiella pneumonia* showed that all samples were resistant to carbenicillin and over 60% resistance to chloramphenicol and tetracycline and cephalosporins but susceptible to amikacin only (Sikarwar & Batra, 2011) as relatively seen with this study too.

5.2 Conclusion

These studies conclude that the pathogens causing infection among the population are also found in zoonotics among other places of occurrence as seen in this study were the bacteria isolated are commonly *Klebsiella species* and *Salmonella species* which are known to be the common causes of infection among the populace. Looking at the recurrence of Salmonella infection, the Bird droppings may be a significant etiology of the persistence of the infection and lack of performing susceptibility test before treatment may be the cause of high resistant of commonly used antibiotics.

5.3 Recommendations

This study recommends that populace should be made aware of the existence of antibiotic resistant strains of bacteria in wild birds. Strategies to stop the continuous transfer of bacteria between man and zoonotics should be adopted this includes, boiling water used for drinking as they may contain the Barn Swallow droppings which according to this study has potential pathogens like *Salmonella* and *Klebsiella species*, good hygiene habbits of washing hands with

clean water and detergents after cleaning their droppings found on the veranda and use of personal protective equipments like gloves.

Looking at the high resistance to antibiotics which are most commonly used for treatment of bacterial infection, this study found out that most antibiotics used are not really susceptible to the said organism leading to more resistance to many antibiotics in the market therefore, all suspected bacterial infection should be tested for antimicrobial susceptibility test before prescription for used.

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Appendix 1: Biochemical test

a) Indole Test

Principle: Indole test is performed to determine the ability of the organism to split tryptophan molecule into Indole. Indole is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with the production of Indole, Pyruvic acid and ammonia.

This test is performed to help differentiate species of the family *Enterobacteriaceae.* Tryptone broth contains tryptophan. Kovac's reagent—contains hydrochloric acid, dimethylaminobenzaldehyde, and amyl alcohol—yellow in color.

Procedure

Inoculate tryptone broth with the test organism and incubate for 18- 24hrs at 37 degree Celsius. Add 15 drops of Kovac's reagent down the inner wall of the tube.

Interpretation.

Development of bright red color at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of Indole and is a positive test

b) Tripple Sugar Iron Agar

TSI agar is used to determine whether a gram negative rod utilizes glucose and lactose or sucrose fermentative and forms hydrogen sulphide (H2S). TSI contains 10 parts lactose: 10 parts sucrose: 1 part glucose and peptone. Phenol red and ferrous sulphate serves as indicators of acidification and H2S formation, respectively. The formation of CO2 and H2S is indicated by the presence of bubbles or cracks in the agar or by separation of the agar from the sides or bottom of the tube. The production of H2S requires an acidic environment and is indicated by blackening of the butt of the medium in the tube.

Method

With a straight inoculating wire, touch the top of a well isolated colony.

Inoculate TSI by first stabbing through center of the medium to the bottom of the tube and then streaking the surface of the agar slant.

Leave the cap on loosely and incubate the tube for 18-24 hours at 35oC in an incubator.

Interpretation

Alkaline slant/no change in the butt (K/NC) = Glucose, lactose and sucrose non-utilizer (alkaline slant/alkaline butt.

Alkaline slant/acid butt (K/A) = Glucose fermentation only

Acid slant/acid butt (A/A), with gas production = Glucose, sucrose, and/or lactose fermenter.

Alkaline slant/acid butt (K/A), H2S production = Glucose fermentation only.

Quality control:

a. A/A with gas = E. coli
b. K/A, H2S = Salmonella typhi
c. K/NC: *Pseudomonas aeruginosa*

c) Urease Test

Property it tests for: This test is done to determine a bacteria's ability to hydrolyze urea to make ammonia using the enzyme urease.

Media and Reagents Used: Stuarts Urea broth (pH 6.8) contains a yeast extract, monopotassium phosphate, disodium phosphate, urea, and phenol red indicator.

Principle

To determine the ability of the organism to split urea forming 2 molecules of ammonia by the action of the enzyme Urease with resulting alkalinity

Method: Inoculate Urea broth with inoculating loop.

Interpretation

Urea broth is a yellow-orange color. The enzyme urease will be used to hydrolyze urea to make ammonia. If ammonia is made, the broth turns a bright pink color, and is positive. If test is negative, broth has no color change and no ammonia is made.

d) Citrate Utilization Test

This test is one of several technique used to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only sole source of carbon and ammonia as its only source of nitrogen.

Principle

The test organism is cultured in a medium which contains sodium citrate, an ammonium salt and the indicator bromothymol blue. Growth in the medium is shown by turbidity and a change in color of the indicator from light green to blue, due to alkaline reaction following citrate utilization.

Procedure

Inoculums is streaked over the slant of Simmon's citrate agar in a tube and incubated for 24-48hrs

Interpretation :

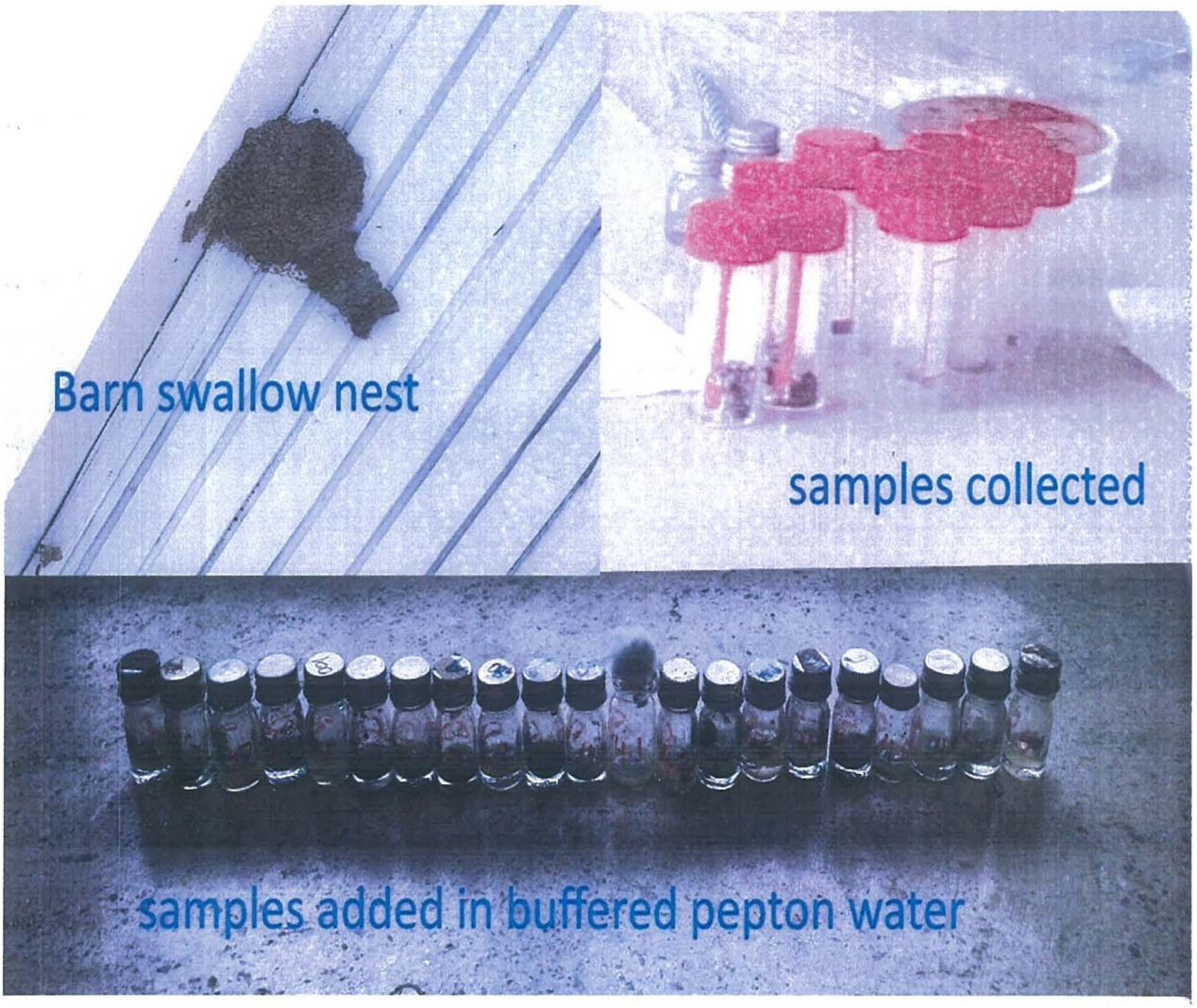
Growth on slant and change in colour to blue of the medium indicates positive result.

Positive: Klebsiella species

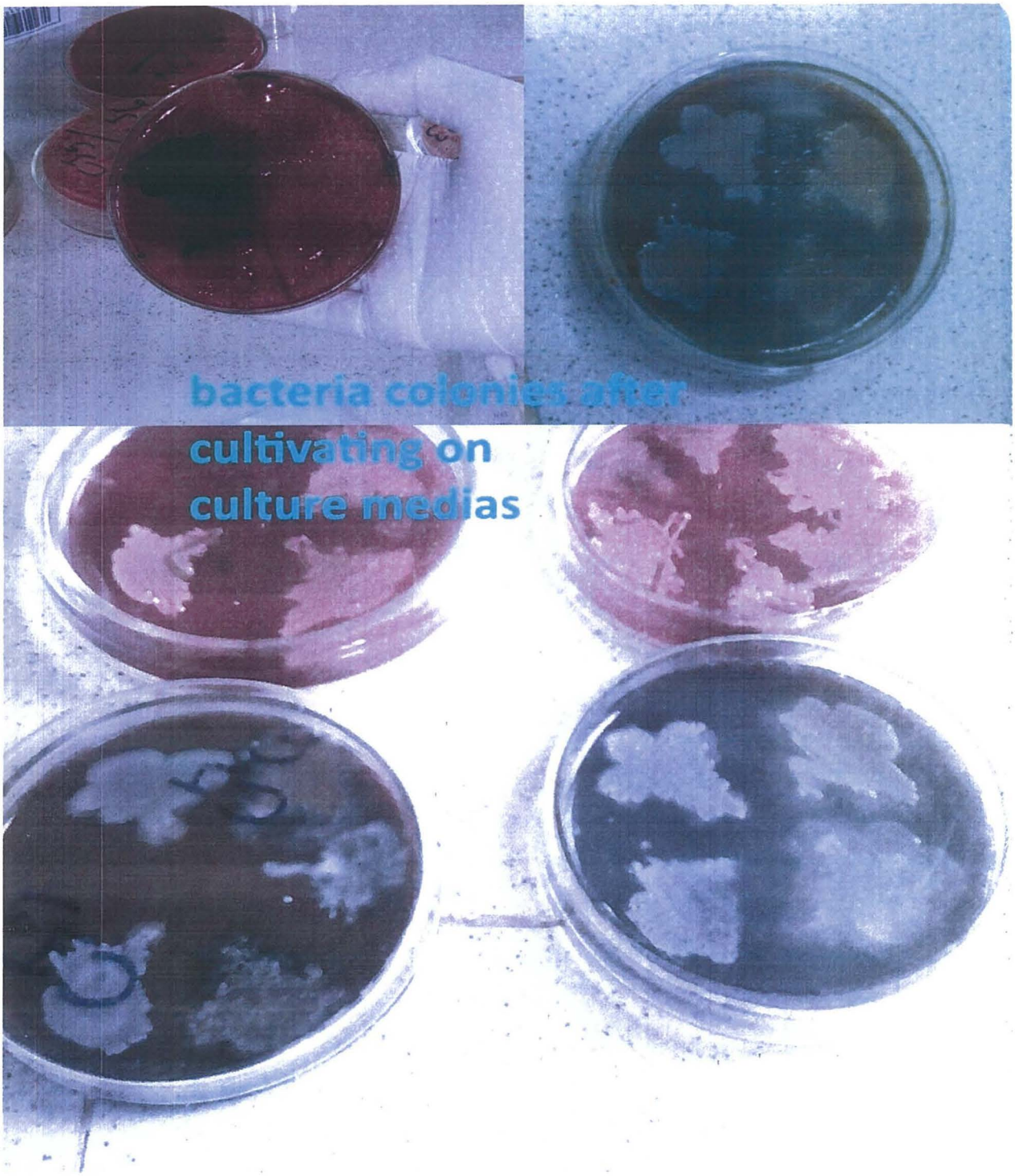
Negative: *E. coli*

Appendix 2: Photographs of the study.

Step 1and 2



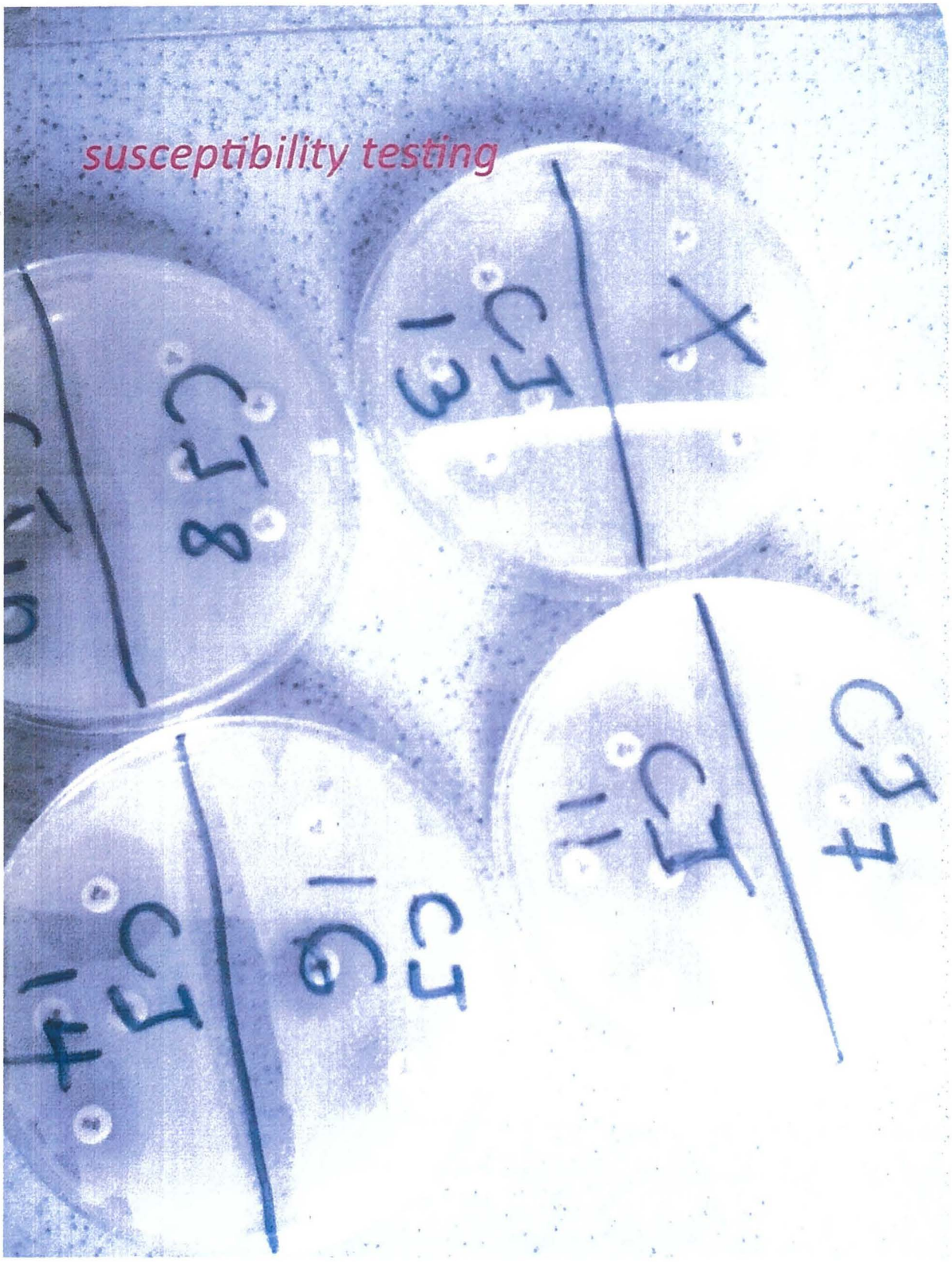
Step 3:



Step 4:

carrying out gram stain

Step 5:


susceptibility testing

Appendix 3: Antibiotic reference.

Interpretation of zones of inhibition (in mm) for Kirby-Bauer antibiotic susceptibility test.

Antibiotic	Disk Conc.	Diameter of zone of inhibition (ZOI)		
		Resistant	Intermediate	Susceptible
Amikacin	10 μg	≤11	12-13	≥14
Ampicillin	10 μg	≤11	12-13	≥14
Bacitracin	10 units	≤8	9-11	≥13
Cephalothin	30 μg	≤14	15-17	≥18
Chloramphenicol	30 μg	≤12	13-17	≥18
Clindamycin	2 μg	≤14	15-16	≥17
Erythromycin	15 μg	≤13	14-17	≥18
Gentamicin	10 μg	≤12	13-14	≥15
Kanamycin	30 μg	≤13	14-17	≥18
Lincomycin	2 μg	≤9	10-14	≥15
Methicillin	5 μg	≤9	10-13	≥14
Nalidixic acid	30 μg	≤13	14-18	≥19
Neomycin	30 μg	≤12	13-16	≥17
Nitrofurantoin	0.3 mg	≤14	15-16	≥17
Penicillin				
vs. staphylococci	10 units	≤20	21-28	≥29
vs. other organisms	10 units	≤11	12-21	≥22
Polymyxin	300 units	≤8	9-11	≥12
Streptomycin	10 μg	≤11	12-14	≥15

Appendix 4: Antibiotic diameter readings for:

a) Klebsiella spp

Antibiotics disc	**Sensitive**	**Intermediate**	**Resistance**	**% sensitive**
Streptomycin	S (25)	I (5)	R (3)	75.8
Amikacin	S (3)	I (2)	R (28)	9.0
Imipenem	S (31)	I (0)	R (2)	93.9
Pefloxacin	S (14)	I (3)	R (16)	42.0

Ciprofloxacin	S (0)	I (4)	R (29)	0
Nalidixic acid	S (4)	I (0)	R (29)	12.1
Augmentin	S (0)	I (0)	R (33)	0
Oxacillin	S (0)	I (0)	R (33)	0
Piperalcillin	S (0)	I (0)	R (33)	0
Erythromycin	S (0)	I (0)	R (33)	0
Gentamicin	S (0)	I (0)	R (33)	0
Chloramphenicol	S (2)	I (1)	R (30)	6.1

KEY: S= sensitive; I= intermediate; R= resistance

b) Salmonella spp

Antibiotics disc	**Sensitive**	**Intermediate**	**Resistance**	**% sensitive**
Streptomycin	S (4)	I (1)	R (6)	36.4
Amikacin	S (0)	I (0)	R (11)	0
Imipenem	S (9)	I (0)	R (2)	81.8
Pefloxacin	S (2)	I (2)	R (7)	18.2
Ciprofloxacin	S (0)	I (2)	R (9)	0
Nalidixic acid	S (4)	I (0)	R (7)	36.4
Augmentin	S (0)	I (0)	R (0)	0
Oxacillin	S (0)	I (0)	R (11)	0
Piperalcillin	S (0)	I (0)	R (11)	0
Erythromycin	S (0)	I (0)	R (11)	0
Gentamicin	S (0)	I (0)	R (11)	0
Chloramphenicol	S (0)	I (2)	R (9)	0

KEY: S= sensitive; I= intermediate; R= resistance