OCCURRENCE OF CARBAPENEMASE AMONG *KLEBSIELLA PNEUMONIAE* ISOLATES FROM SELECTED HOSPITALS IN ZARIA,

KADUNA STATE, NIGERIA

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

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AHMADU BELLO UNIVERSITY,

ZARIA

OCTOBER, 2016

DECLARATION

I declare that the work in this dissertation entitled "OCCURRENCE OF CARBAPENEMASE AMONG *KLEBSIELLA PNEUMONIAE* ISOLATES FROM SELECTED HOSPITALS IN ZARIA, KADUNA STATE, NIGERIA" has been carried out by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at this or any other Institution.

_____ _____

Ibrahim Mohammed HUSSAINI Date

(P13SCMC8071)

CERTIFICATION

This dissertation entitled "OCCURRENCE OF CARBAPENEMASE AMONG *KLEBSIELLA PNEUMONIAE* ISOLATES FROM SELECTED HOSPITALS IN ZARIA, KADUNA STATE, NIGERIA" by Ibrahim Mohammed HUSSAINI (P13SCMC8071) meets the requirement for the award of Master of science degree in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The spread of carbapenem-resistant bacteria has caused grave concern due to the limited choice in antibiotics for treating infections caused by Gram negative bacilli. The study was aimed to determining the prevalence of Carbapenem resistant *Klebsiella pneumoniae* at some hospitals in Zaria, Kaduna State. A total of 150 clinical samples comprising of urine (68), wound swab (44) and sputum (38) were collected and subjected to cultural isolation followed by biochemical identification of the isolated organisms among which 19 *Klebsiella pneumoniae* were identified. Antibiotic susceptibility testing was carried out on all the isolates by disc diffusion method. All the isolates were resistant to at least two of the eight different antibiotics tested. All (100%) the isolates were resistant to Ampicillin, 18 isolates (94.7%) were resistant to Cefotaxime, while 15 (78.9%) and 11 (57.9%) isolates were resistant to Cotrimoxazole and Tetracycline respectively. Low level of resistance was observed against Chloramphenicol (36.8%), Ciprofloxacin (26.3%) and Gentamicin (21.1%). The Multiple Antibiotic Resistance indices of the isolates were between 0.25 and 0.88. Out of the 19 isolates screened, only one was intermediately resistant to Imipenem. This isolate was screened for *Klebsiella pneumoniae* carbapenemase production using the Modified Hodge Test. The isolate was a non KPC producer, suggesting the resistance to Imipenem is likely due to other mechanism such as decreased outer membrane permeability, over expression of β-lactamases, production of cephalosporinase and porin loss. The prevalence of carbapenems resistant *Klebsiella pneumoniae* in Zaria as seen in this study is 5.26% and that of KPC producing *Klebsiella pneumoniae* is 0%. Even though the level of carbapenem resistance was low and none of the isolates was a KPC producer, most of the isolates were multidrug resistant isolates and this is alarming.

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ABBREVIATIONS AND SYMBOLS

ATCC	American Type Culture Collection.
CDC	Centers for Disease Control and Prevention.
CLSI	Clinical and Laboratory Standards Institute.
CRE	Carbapenem Resistant Enterobacteriacae.
CRKP	Carbapenem Resistant *Klebsiella pneumoniae.*
CTX-M	Cefotaximase-Munich β-lactamases.
df	degree of freedom.
DHFR	Dihydrofolate reductase.
ESBL	Extended Spectrum Beta-lactamases.
ICU	Intensive Care Unit.
KPC	*Klebsiella pneumoniae* Carbapenemase.
MAR I	Multiple Antibiotic Resistance Index.
MDR	Multi Drug Resistant.
MIC	Minimum Inhibitory Concentration.
MTH	Modified Hodge Test.
OMP	Outer Membrane Protein.
PBP	Penicillin Binding Protein.
SHV	Sulphydryl variable β-lactamases.
UTI	Urinary Tract Infection.
WHO	World Health Organisation.
β	Beta

χ^2	Chi square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Antimicrobial resistance is now part of the everyday vocabulary. It is a current problem and also one which will not reduce in importance, but rather increase. In order to ensure that the daily challenges of bacterial infections are tackled there is need for antibiotics. However, the march of antibiotic resistance continues and increases with each year passing (Bosso, 2005).

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines. In the recent years, *Klebsiella pneumoniae* has become important pathogen in nosocomial infections. It is most frequently recovered from clinical specimens and can cause a classic form of primary pneumonia as well as a variety of extrapulmonary infections, including enteritis and meningitis in infants, urinary tract infections in children and adults and septicaemia. In the United States, *Klebsiella* accounts for 3-7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals. Klebsiellae have a tendency to *harbour* antibiotic resistance plasmids; thus, infections with multiple antibiotic-resistant strains can be anticipated (Sarathbabu *et al*., 2012). It has been a known human pathogen since it was first isolated in the late nineteenth century by Edwin Klebs (Yu and Chuanng, 2013).

Klebsiella pneumoniae is second only to *Escherichia coli* as a urinary tract pathogen. *Klebsiella* infections are encountered far more often now than in the past. It may be due to the bacterium's

antibiotic resistance property. *Klebsiella pneumoniae* is recognized as a resident of the intestinal tract in about 40% of humans and animals. It is considered to be an opportunistic human pathogen meaning that under certain conditions it may cause disease. For example, nosocomial infections are those that hospitalized patients pick up because they are in a weakened state (Eickhoff, 1999; Akpan *et al*., 2011). *Klebsiella pneumoniae* causes recurrent cough and acute exacerbations of chronic obstructive pulmonary disease, defined as presence of increased sputum volume, sputum purulence and dyspnoea and is responsible for causing 30-50% of exacerbations (Martin *et al.*, 1971; Madhavi *et al*., 2012).

Carbapenems are a class of β-lactam, broad spectrum antibiotic which act by inhibiting the cell wall synthesis and are known to be most effective against Gram negative infections. Carbapenem in combination with other agents, remain a mainstay of therapy in patients with serious hospital acquired infections. The introduction of carbapenem into clinical practice represents a great advancement for the treatment of β-lactam resistant bacteria. Due to their broad spectrum of activity and stability to hydrolysis by most beta lactamases, the carbapenem have been the drug of choice for treatment of infections caused by penicillin or cephalosporin resistant Gram negative bacilli (Jesudason *et al*., 2005).

Carbapenems exhibit their bacteriocidal activity by binding to the penicillin binding proteins (PBP), thus preventing the linking of peptidoglycan strands and further synthesis of the bacterial cell wall (Kimbery, 2008). Resistance to carbapenem emerged through 3 mechanisms: reduced permeability, efflux and synthesis of carbapenem β-lactamases (James, 2008).

Until recently, carbapenems were the choice for the therapeutic management of multidrug-resistant Gram-negative bacterial infections. Currently, the spread of carbapenem-resistant

bacteria has caused grave concern due to the limited choice in antibiotics for treating infections caused by Gram negative bacilli (Walsh, 2010). Resistance in bacteria to carbapenems mainly is due to the production of carbapenem hydrolyzing enzymes called carbapenemases. These bacteria have the potential to spread rapidly within the hospital environment and also across continents (Cornaglia and Rossolini, 2010).

In 2001, the first KPC-producing *K. pneumoniae* isolate was reported in North Carolina, USA (Yigit *et al*., 2001). The enzyme (KPC-1), an Ambler class A beta-lactamase, was not the first carbapenemase to be detected in *K. pneumoniae*, as isolates *harbour*ing Ambler class B metallo-beta-lactamases capable of hydrolyzing carbapenems had previously been reported in Japan as early as 1994 (Paterson and Bonomo, 2005).

Klebsiella pneumoniae carbapenemase production is an important mechanism of resistance for an increasingly wide range of Gram-negative bacteria and is no longer limited to *K. pneumoniae*. KPC-producing bacteria are often misidentified by routine microbiological susceptibility testing and incorrectly reported as sensitive to carbapenems; however, resistance to the carbapenem antibiotic ertapenem is common and a better indicator of the presence of KPCs. Carbapenem antibiotics are generally not effective against KPC-producing organisms. The common drugs of choice based on *in vitro* susceptibility testing are the polymyxins, tigecycline, and less frequently the aminoglycosides (Arnold *et al.*, 2011).

In 2012, Oshun and Ogunsola conducted a study on carbapenem resistant *Klebsiella pneumoniae* isolated from in-patients at the Lagos University Teaching Hospital over a period of 6 months. Out of the 153 *Klebsiella pneumoniae* isolates from in-patients, 8 were resistant to carbapenem

while only 4 of the 8 CRKP were recognized by Modified Hodge Test as carbapenemase producers (Osun and Ogunsola, 2012).

1.2 Statement of Research Problem

Antimicrobial resistance is a growing threat worldwide. Resistance mechanisms have been found for every class of antibiotics (Umadevi *et al.,* 2011). The metallo β-lactamase in Gram negative bacilli is becoming a therapeutic challenge, as this enzyme usually possesses a broad hydrolysis profile that includes the carbapenems and other β-lactam antibiotics (Galani *et al.,* 2008). In Nigeria there have been reports of carbapenemase producing clinical isolates of enteric bacteria particularly among *E. coli* and *Klebsiella* spp (Akinduti *et al*., 2012).

Infections caused by KPC-producing *K. pneumoniae* have been associated with increased cost and length of stay as well as frequent treatment failures and death (CDC, 2009). Risk factors for infection include advanced age (Nadkarni *et al*., 2009), being severely ill (Gasink *et al*., 2009), previous treatment with antibiotics (Bratu *et al*., 2005), organ or stem-cell transplantation, mechanical ventilation, and long hospital stays (Patel *et al*., 2008). In addition to the infection control challenges that have arisen, infections caused by these organisms present clinicians with serious treatment challenges, due to limited antibiotic options (Arnold et *al.,* 2011).

KPC-producing bacteria present a significant problem in clinical situations where administration of effective empiric antibiotics is essential to preventing mortality. This applies to serious infections such as bacteremia, but also extends to other infections in patients undergoing organ transplants and cancer treatment, where the immunocompromised status of patients requires effective empiric antibiotics (Arnold et *al*., 2011). Mathers *et al.* (2009) reported two cases of orthotopic liver transplant recipients that died as a result of infections caused by KPC-producing

K. pneumoniae. Both patients were initially treated with meropenem based on the results of routine susceptibility testing.

1.3 Justification

Accurate and timely detection of these resistance mechanisms (i.e. carbapenemase production) is very important in deciding the appropriate treatment schedule. Detection of the resistance mechanisms is always a serious challenge to the clinical laboratories (Valsan *et al.,* 2013). The treatment of patients with serious Gram negative infection must be both prompt and correct. Numerous studies have demonstrated that mortality risk is significantly increased when the antibiotic regimen does not adequately cover the infecting pathogen (James, 2008).

Enterobacteriaceae are among the leading causes of nosocomial infections (Hidron *et al*., 2008). Early identification of KPC-producing bacteria through *in vitro* testing is of paramount importance to the success of infection control efforts (CDC, 2009). In the appropriate setting, active surveillance can improve infection control by detecting colonization and preventing horizontal spread (Kochar *et al*., 2009).

CRE are known to *harbour* additional drug-resistance genes to other antimicrobial drug classes, which may also be carried on mobile genetic elements. *K. pneumoniae* sequence type 258 strains are KPC-producing clones that harbour Tn4401-bearing plasmids. These clones are highly effective in plasmid transfer across bacteria and are known to carry other plasmid-based antimicrobial drug resistance genes such as those that encode resistance to trimethoprim/sulfamethoxazole, aminoglycosides, and fluoroquinolones (Munoz-price and Quinn, 2009)

On 23rd August 2011, the Disease Daily reported an outbreak of an infection caused by a KPC producing *Klebsiella pneumoniae* among patients at Panama's Social Security Hospital. The death toll rose to 50 with 71 patients infected within a month (Pinheiro, 2011). In the same year, U.S National Institutes of Health Clinical Center experienced an outbreak of Carbapenem Resistant *Klebsiella pneumoniae* that affected 18 patients, 11 of whom died. Integrated genomic and epidemiological analysis traced the outbreak back to three independent transmissions from a single patient who was discharged 3 weeks before the next case became clinically apparent (Evan *et al.*, 2012).

1.4 Aim and Objectives

1.4.1 Aim: The aim of this study was to determine the occurrence of carbapenemase among *Klebsiella pneumoniae* isolates from selected hospitals in Zaria, Kaduna State, Nigeria

1.4.2 Objectives:

The objectives were to:

1. isolate and characterize *Klebsiella pneumoniae* from clinical samples in selected hospitals in Zaria.
2. determine the antibiotic susceptibility pattern of the *Klebsiella pneumoniae* isolates.
3. screen the isolates that are resistant to Carbapenem (Imipenem) for the production of carbapenemase using the Modified Hodge Test.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Genus *Klebsiella*

Klebsiella [kleb−see−ell−uh] is a type of Gram-negative bacteria that can cause different types of healthcare associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems. *Klebsiella* bacteria are normally found in the human intestines (where they do not cause disease). They are also found in human stool (faeces). In healthcare settings, *Klebsiella* infections commonly occur among sick patients who are receiving treatment for other conditions. Patients whose care requires devices like ventilators (breathing machines) or intravenous (vein) catheters, and patients who are taking long courses of certain antibiotics are most at risk for *Klebsiella* infections. Healthy people usually do not get *Klebsiella* infections. To get a *Klebsiella* infection, a person must be exposed to the bacteria. For example, *Klebsiella* must enter the respiratory (breathing) tract to cause pneumonia, or the blood to cause a bloodstream infection (CDC, 2012).

In healthcare settings, *Klebsiella* bacteria can be spread through person-to-person contact (for example, from patient to patient via the contaminated hands of healthcare personnel, or other persons) or, less commonly, by contamination of the environment. The bacteria are not spread through the air. Patients in healthcare settings also may be exposed to *Klebsiella* when they are on ventilators (breathing machines), or have intravenous (vein) catheters or wounds (caused by injury or surgery). Unfortunately, these medical tools and conditions may allow *Klebsiella* to enter the body and cause infection (CDC, 2012).

Three species in the genus *Klebsiella* are associated with illness in humans: *Klebsiella pneumoniae, Klebsiella oxytoca,* and *Klebsiella granulomatis*. Organisms previously known as *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are considered non lactose fermenting subspecies of *K. pneumoniae* that have characteristic clinical manifestations. With those exceptions, strains within this genus ferment lactose, most produce highly mucoid colonies on plates because of the production of a luxuriant polysaccharide capsule and all are nonmotile. In recent years, klebsiellae have become important pathogens in nosocomial infections (Qureshi, 2014).

2.1.1 *Klebsiella* antigens

Members of the genus *Klebsiella* like other members of the family *Enterobacteriaceae* express different types of cell surface antigens. *Klebsiella* typically express 2 types of antigens on their cell surface. The first is Somatic (O antigen) which is a component of lipopolysaccharide and the second is a capsular polysaccharide (K antigen). Both of these antigens contribute to pathogenicity of the members of this genus. About 77 K antigens and 9 O antigens exist. The structural variability of these antigens form the basis for classification into various serotypes. The virulence of all serotypes appears to be similar (Qureshi, 2014).

2.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative, non-motile, lactose fermenting, rod-shaped facultative anaerobic organism. It is encapsulated, which increases its virulence by acting as a physical barrier to evade the host's immune response. This capsule also protects the cell from dessication. It can be found in the mouth, skin, and intestinal tract, where it initially does not cause disease. Although found in the microbiota, *K. pneumoniae* can progress into severe

bacterial infections leading to pneumonia, bloodstream infections, wound infections, urinary tract infections, and meningitis. Patients who require equipment such as catheters or ventilators are at high risk for infections. Also, a patient administered a course of broad-spectrum antibiotic treatment is at an even high risk due to the disruption of the normal flora of the bacteria in the body, deeming it more susceptible to pathogens (Microbewiki, 2014).

Klebsiella pneumoniae was named after Edwin Klebs (1834-1913), a 19th century German microbiologist (CDC, 2012). Although he was not the first to isolate *K. pneumoniae*, naming a genus after him honoured his work with *Corynebacterium diptheriae*. Around the same time, Hans Christian Gram (1853-1938), created a microbiological technique known as the Gram stain in 1884 to differentiate between *K. pneumoniae* and *S. pneumoniae* (Microbewiki, 2014).

Klebsiella pneumoniae has historically been, and currently remains, a significant cause of human disease. It is a frequent cause of urinary tract infections and pneumonia, and subsequent systemic infections can have mortality rates as high as 60%. *Klebsiella pneumoniae* is ubiquitously found in the environment and is often found as a commensal resident of the human gastrointestinal tract (Seaton, 2000).

Klebsiella pneumoniae, an inhabitant of the gastrointestinal tract, skin, and nasopharynx, can cause infection in many parts of the body, including urinary tract infections, hospital acquired pneumonia, intra-abdominal infections, wound infections, and primary bacteremia (Landman *et al*., 2012; Tzouvelekis *et al*., 2012; Shilo *et al*., 2013).

2.2.1 Taxonomy of *Klebsiella pneumoniae.*

Domain = Bacteria

Phylum = Proteobacteria

Class = Gammaproteobacteria

Order = Enterobacteriales

Family = *Enterobacteriaceae*

Genus = *Klebsiella*

Species = *K. pneumoniae* (Microbewiki, 2014)

2.2.2 Pathogenesis

The ability of *Klebsiella pneumoniae* to cause disease depends on some bacterial factors that contribute to it pathogenesis. The pattern of the pathogenic factors found in isolates from different body sites depends on the host defence mechanism of that body site. The search for the pathogenic mechanisms of *Klebsiella* infections has identified a number of bacterial factors that contribute to the pathogenesis of these bacteria. These factors are:

2.2.2.1 Capsular Antigens

The capsules of *Klebsiella pneumoniae* composed of complex acidic polysaccharides made up of repeating subunits of four to six sugars and very often, uronic acids (as negatively charged components). Capsules are essential to the virulence of *Klebsiella*. The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers (Podschun and Ullman, 1998). This protects the bacterium from phagocytosis by polymorphonuclear

granulocytes, on the one hand, and prevents killing of the bacteria by bactericidal serum factors, on the other. The molecular mechanism presumably consists of inhibiting the activation or uptake of complement components, especially C3b. Apart from their antiphagocytic function, *Klebsiella* capsule polysaccharides have been reported to inhibit the differentiation and functional capacity of macrophages *in vitro* (Edelman *et al*., 1994).

2.2.2.2 Pili (Fimbriae)

Pili (otherwise known as fimbriae) are nonflagellar, filamentous projections on the bacterial surface. They are critical in infectious process, since they help in the adherence of microorganisms to host mucosal surfaces and in maintenance of this proximity. These structures are up to 10 mm long and have a diameter of 1 to 11 nm ; they consist of polymeric globular protein subunits (pilin) with a molecular mass of 15 to 26 kDa. Pili are demonstrated mainly on the basis of their ability to agglutinate erythrocytes of different animal species. Depending on whether the reaction is inhibited by D-mannose, these adhesins are designated as mannose-sensitive or mannose-resistant hemagglutinins (MSHA and MRHA), respectively. There are two predominant types in *Klebsiella* spp (Matsumoto *et al*., 1990; Podschun and Ullman, 1998).

Type 1 (common) pili

The relevance of these pili to bacterial virulence is thought to arise mainly from binding of the bacteria to mucus or to epithelial cells of the urogenital, respiratory, and intestinal tracts. Their role in the pathogenesis of UTI was clarified mostly in studies on *E. coli* but has also been described for *K. pneumoniae* in animal models (Podschun and Ullman, 1998).

In pathogenic microorganisms, colonization of the mucous membrane is followed by invasion of the underlying tissue, with all of the subsequent events of infectious pathogenesis. Once in the

host tissue, however, the type 1 pili are no longer of use to the bacteria, since they trigger an opsonin-independent leukocyte activity known as lectinophagocytosis. The repulsion forces separating bacterium and leukocyte are weakened by the hydrophilic character of these pili, thus enabling the adhesins to bind to specific mannose-containing receptors on the leukocyte surface. Adhesin-binding triggers stimulation of the leukocyte, which ultimately leads to phagocytosis and intracellular killing of the bacterium. The bacterium counters this form of host defense by switching off the expression of type 1 pili in tissue. Thus, while type 1 pili are important for host colonization, their contribution to subsequent steps of pathogenesis is less clear (Podschun and Ullman, 1998).

Type 3 pili

Unlike other fimbriae, type 3 pili agglutinate only erythrocytes that have been treated with tannin. Originally described as the adhesion organelles of *Klebsiella* inhabiting plant roots, these pili were later found to be capable of binding to various human cells. Strains of *K. pneumoniae* expressing type 3 pili adhere to endothelial cells, epithelia of the respiratory tract, and uroepithelial cells. In the kidneys, these pili mediate bacterial adhesion to tubular basement membranes, Bowman's capsules, and renal vessels. Binding to tannic acid-treated erythrocytes is inhibited by spermidine, a polyamine that is also secreted in urine (Podschun and Ullman, 1998).

The role of this fimbrial type in the pathogenetic process is largely unknown. So far, the only evidence of a correlation between the type 3 MrkD hemagglutinin and disease has been the observation of expression of type 3 pili in *Providencia stuartii* in catheter-associated bacteriuria. This species is not a common cause of UTI in short-term-catheterized or noncatheterized persons but has a much higher prevalence in the urine of patients with long-term indwelling catheters. In

the above-mentioned study, it was demonstrated that the higher prevalence of *P. stuartii* in catheter-associated bacteriuria was due to its ability to adhere and persist to the catheter in the catheterized urinary tract by expression of the MR/K hemagglutinin. Unfortunately, so far, no experimental animal model has been established to investigate the role of these pili in infection. The structure of the corresponding host receptors is unknown (Podschun and Ullman, 1998).

2.2.2.3 Serum Resistance and Lipopolysaccharide

The bactericidal effect of serum in addition to phagocytosis by polymorphonuclear granulocytes forms part of the first line of defense by the host against invading microorganisms. The serum bactericidal activity is mediated primarily by complement proteins. After their cascade-like activation, these proteins accumulate as membrane attack complex on the surface of the microorganism. This complex consists of the terminal complement proteins C5b–C9, which produce a transmembranous pore in the outer membrane of Gram-negative bacteria, leading to an influx of Na^{+1} and subsequent osmotic lysis of the bacteria. The complement cascade can be activated by two different mechanisms: the classic complement pathway, which typically requires specific antibodies to be activated, and the alternative complement pathway, which can be activated even in the absence of antibodies. The alternative pathway is also regarded as an early defense system of innate immunity, which enables the host to react to invading microorganisms even before specific antibodies are formed. Both complement pathways lead, via the activation of C3, to the formation of the opsonin C3b, which ultimately results in formation of the terminal C5b–C9 complex and thus plays a key role in this defense system (Albert *et al.*, 1996; Podschun and Ullman, 1998).

In response to this host defense, pathogenic microorganisms have developed strategies to counter the serum bactericidal effect. Most commensal Gram-negative bacteria are sensitive to the bactericidal effect of human serum, whereas pathogenic strains often exhibit serum resistance properties. Thus, clinical isolates of enterobacteria often show resistance to serum, and the feature "serum resistance" has been correlated with the onset of infection and severity of symptoms. Since the main role of the serum bactericidal system is thought to prevent microorganisms from invading and persisting in the blood, even differences in the degree of bacterial serum susceptibility may determine whether a strain is able to infect as well as the length of time it takes the organisms to establish the infection (Williams and Tomas, 1990; Podschun and Ullman, 1998).

2.2.2.4 Siderophores

Supply of available iron also limits the growth of bacteria in host tissue. Iron is an essential factor in bacterial growth, functioning mainly as a redox catalyst in proteins participating in oxygen and electron transport processes. The supply of free iron available to bacteria in the host milieu is extremely low, since this element is bound intracellularly to proteins such as hemoglobin, ferritin, hemosiderin, and myoglobin and extracellularly to high-affinity iron-binding proteins such as lactoferrin and transferrin. The level of free, bioavailable iron is several thousand fold too low for normal bacterial growth. The marked effect of the iron supply in the host body on the pathogenesis of infections has been demonstrated for *Klebsiella* (Podschun *et al.*, 1992; Podschun and Ullman, 1998).

Many bacteria attempt to secure their supply of iron in the host by secreting high-affinity, low-molecular-weight iron chelators, called siderophores. Siderophores are capable of competitively

taking up iron bound to host proteins (Podschun and Ullman, 1998). Under iron-deficient conditions, e.g., in the host milieu, enterobacteria synthesize a variety of siderophores, which belong to two different chemical groups, one consisting of the phenolate-type siderophores (e.g. enterobactin also known as enterochelin - a cyclic trimer of 2,3-dihydroxy- benzoyl-serine) and one consisting of the hydroxamate-type siderophores (e.g. ferrichromes [which are synthesized only by fungi], the ferrioxamines, and aerobactin) (Podschun *et al*., 1992).

2.2.3 Transmission

Klebsiella infection is spread through exposure to the bacteria via respiratory tract, which causes pneumonia, or the blood to cause an infection in the bloodstream. *Klebsiella* infections are most well-known in hospitals spread through person-to-person contact by contaminated hands of surrounded people in the hospitals, whether it is an employee or a patient. *Klebsiella* is spread very easily and rapidly, but not through the air. Healthcare settings are most vulnerable to *Klebsiella* infections due to the nature of procedures that allow easy access of bacteria into the body. Patients who are on ventilators, catheters, or surgery wounds are highly prone to getting this deadly infection (Boston Medical Research Occupational Health Program, 2012).

2.2.4 Incubation period/Infectious Dose/Colonization

In humans, the infectious dose is not known. As for the incubation period, it is also not fully understood but possibly arises within a number of days (Boston Medical Research Occupational Health Program, 2012).

Klebsiella bacteria are found widely through nature in soil and water. In regards to human, *K. pneumoniae* is prevalent in normal microbiota of the intestinal tract and colon, but not in

alarmingly high numbers. They may also be found in the mouth and the skin (Department of Health and Hospital, 2010). Infection of *K. pneumoniae* occurs in the lungs, where they cause necrosis, inflammation, and hemorrhage within the lung tissue. This is caused by aspirating oropharyngeal microorganisms into the lower respiratory tract. Hospital-acquired infections rely on the urinary tract, lower respiratory tract, biliary tract, and surgical wounds to set up colonization (Qureshi, 2014).

2.2.5 Epidemiology

Klebsiella species are ubiquitous in nature. Klebsiellae probably have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. In this respect, the genus *Klebsiella* is like *Enterobacter* and *Citrobacter* but unlike *Shigella* spp. or *E. coli*, which are common in humans but not in the environment (Podschun and Ullmann, 1998).

In humans, *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract. Carrier rates differ considerably from study to study. The detection rate in stool samples ranges from 5 to 38%, while rates in the nasopharynx range from 1 to 6%. Because Gram-negative bacteria do not find good growth conditions on the human skin, *Klebsiella* spp. are rarely found there and are regarded simply as transient members of the skin flora (Podschun and Ullman, 1998).

Klebsiella pneumoniae is responsible for 6-17% of Urinary Tract Infections, 7-14% of pneumonia, 4-15% of septicemia, 2-4% of wound infections, 4-17 nosocomial infections in intensive care units, and 3-20% of all neonatal septicemia cases. In humans, *K. pneumoniae*

resides in the nasopharynx and in the intestinal tract. Since Gram-negative bacteria do not have good growth on human skin, they are rarely found there in comparison to internal parts of the body. Reported carrier rates are quire the opposite of this fact, when in a hospital environment (Podschun and Ullmann, 1998).

In 2011, an investigation of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* was conducted in hospitals among patient with short and long-term stays. Over a 1-year period, KPC-producing *Enterobacteriaceae* was found throughout 4 counties in Indiana and Illinois. The source of the problem was found to be within long-term facilities and patients (Qureshi, 2014). KPC has been found in a total of 44 states thus far (Rockwood and Childress, 2013).

Fourteen percent of bacteremia cases are caused by *K. pneumoniae*, which places it in second place next to *Escherichia coli* for origins of Gram-negative sepsis. Outbreaks of neonatal septicemia and *K. pneumoniae* can be found worldwide (Gupta *et al*., 2011).

2.3 Carbapenems

The term "carbapenem" is defined as the 4:5 fused lactam ring of penicillins with a double bond between C-2 and C-3 and substitution of carbon for sulfur at C-1. The stereochemistry of the hydroxyethyl side chain of carbapenem is a key contributor of carbapenems and is important for activity (Papp-Wallace *et al*., 2011).

Carbapenems are often used as "last-line agents" or "antibiotics of last resort" when patients with infections become gravely ill or are suspected of harbouring resistant bacteria because carbapenems posses the broadest spectrum of activity among hundreds of different β-lactams and

greatest potency against Gram positive and Gram negative bacteria (Paterson, 2002; Paterson and Bonomo, 2005).

Carbapenems are unique because they are relatively resistant to hydrolysis by most β-lactamases, in some cases act as "slow substrates" or inhibitors of β-lactamases and still target Penicillin Binding proteins (PBPs) (Papp-Wallace *et al*., 2011).

2.3.1 Chemistry of carbapenems

The carbon atom at C-1 position plays a major role in the potency, spectrum of carbapenems and in their stability against β-lactamases. Also the strategically positioned hydroxyethyl side chain aids in resistance to hydrolysis by β-lactamases. The *trans* configuration of the β-lactam ring at C-5 and C-6 results in the stability against β-lactamases (Papp-Wallace *et al*., 2011).

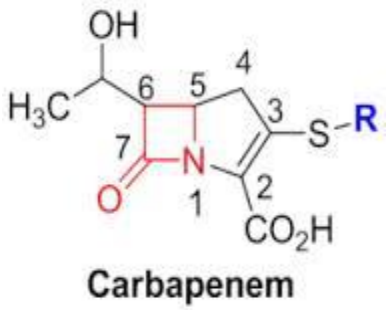


Figure 2. 1: Structure of carbapenem. Source: https://quizlet.com/10760751/antibiotics-flash-cards/

2.3.2 Synthesis of carbapenems

Several chemical approaches were developed for the synthesis of carbapenems since fermentation was not an efficient method for production. Natural products such as L-Cystine, L-Valine, L-α- amino adipic acid, and *S*-adenosyl-Methionine were often used as starting material for production of carbapenems, and the synthetic approach was largely influenced by the desired

stereochemistry of the final compound. In addition, once a carbapenem is developed which has an *R* configuration at C-8, is *trans* about the C-5OC-6 bond, and has a methyl at C-1 and a hydroxyethyl at C-6, most modifications are at the R1 side chain (at position C-2). Thus, carbapenems are unique compared to other β-lactams, which tend differ in both R1 and R2 side chains (Papp-Wallace *et al*., 2011).

2.3.3 Mechanism of action

Carbapenems like other members of β-lactams, do not easily diffuse through the bacterial cell wall (Martinez-Martinez, 2008). Carbapenems enter Gram negative bacteria through outer membrane proteins (OMPs), also known as porins. After transversing the periplasmic space, carbapenems "permanently" acylate the PBPs. PBPs are enzymes (e.g. transglycolases, transpeptidases and carboxypeptidases) that catalyze the formation of peptidoglcan in cell wall of bacteria (Papp-Wallace *et al*., 2011).

Carbapenems act as mechanism-based inhibitors of the peptidase domain of PBPs and can inhibit peptide cross linking as well as other peptidase reactions. A key factor to the efficacy of carbapenems is their ability to bind to multiple different PBPs. Since cell formation is a dynamic "3-dimentional process" with formation and autolysing occurring at the same time, when PBPs are inhibited, autolysing continues (Van Dam *et al*., 2009). Eventually the peptidoglycan weakens and the cell bursts due to osmotic pressure (Papp-Wallace *et al*., 2011).

2.3.4 Microbiological activity

Carbapenems demonstrate an overall broader antimicrobial spectrum *in vitro* than the available penicillins, cephalosporins, and β-lactam/β-lactamase inhibitor combinations. In general,

Imipenem, panipenem, and doripenem are potent antibiotics against Gram-positive bacteria. Meropenem, biapenem, ertapenem, and doripenem are slightly more effective against Gram-negative organisms. Important considerations here are the following:

(i) Ertapenem has a more limited spectrum, because it is not as active as Imipenem or meropenem against *Psuedomonas aeruginosa*.

(ii) Meropenem is not as potent as Imipenem or doripenem against *Acinetobacter baumannii.*

(iii) Doripenem has lower MICs than do Imipenem and meropenem versus *P. aeruginosa* and *A. baumannii*. In addition, doripenem is the carbapenem least susceptible to hydrolysis by carbapenemases; hydrolysis of doripenem is 2 to 150 fold slower than that of Imipenem.

(iv) A unique application of meropenem is that when combined with clavulanic acid, it is potent at killing MDR *Mycobacterium tuberculosis*, a bacterium that typically is not susceptible to β-lactams due to a chromosomally expressed β-lactamase. This ability to inhibit or kill *M. tuberculosis* is likely to be a property of other carbapenems as research in this area grows.

Carbapenems can also be combined with other antimicrobials to treat serious infections. Combination therapy is a subject of intense interest, since the emergence of MDR pathogens often requires us to treat patients with more than one antibiotic. Some combinations demonstrate positive effects, such as extending the spectrum or working additively or synergistically. Adverse effects include increased resistance to one of the drugs used in the combination, as well as a lack of synergy and strain dependence (Papp-Wallace *et al*., 2011).

2.3.5 Pharmacology

All clinically available carbapenems have low oral bioavailability and thus do not cross gastrointestinal membranes readily and must be administered intravenously; Imipenem-cilastatin and ertapenem can also be delivered intramuscularly (Nix *et al.*, 2004; Papp-Wallace *et al*., 2011).

As with other β-lactams, all carbapenems are eliminated predominantly by renal excretion. A carbapenem is often combined with an antibiotic that targets Gram positive bacteria when used for empirical treatment of patients with serious nosocomial infections of unidentified origin (Papp-Wallace *et al*., 2011).

2.3.6 Safety and tolerability

Some of the side effects of carbapenems include: nephrotoxicity, neurotoxicity, and immunomodulation. Thus predisposing factors should be considered when administering any carbapenem. In addition, the use of carbapenems can alter the intestinal microflora and select for carbapenem-resistant isolates (Papp-Wallace *et al*., 2011).

2.3.7 Mechanisms of resistance to carbapenems

Mechanisms of resistance to carbapenems include production of β-lactamases (carbapenemases), efflux pumps and mutations that alter the expression and/or function of porins and PBPs. Combination of these mechanisms can cause levels of resistance to carbapenems in certain bacteria species such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Limansky *et al*., 2002; Rodriguez-Martinez *et al*., 2009).

A distinction exists between resistance to carbapenems in Gram positive cocci and Gram negative rods. In Gram positive cocci, carbapenem resistance is typically as a result of substitution of amino acid sequences of PBPs or acquisition/production of a new carbapenem resistant PBP (Katayama *et al*., 2004; Papp-Wallace *et al*., 2011).

Expression of β-lactamases and efflux pumps as well as porin loss and alteration in PBP are all associated with carbapenem resistance in Gram negative rods (Pearson *et al*., 1999; Papp-Wallace *et al*., 2011).

2.3.6 Specific anti-Methicin Resistant *Staphylococcus aureus* (MRSA) carbapenems

Several carbapenems were design to target MRSA while maintaining activity against most Gram negative bacteria. The anti-MRSA activity is related to the high affinity of these compounds for PBP2a of MRSA. The R_2 side chains present on these compounds are important affinity determinants for interactions with PBP2a of MRSA (Papp-Wallace *et al*., 2011).

2.3.7 Oral carbapenems

Oral carbapenems are given as prodrugs to increase intestinal absorption (Kattan *et al.*, 2008; Mammeri *et al.*, 2010 and Papp-Wallace *et al*., 2011). These prodrugs get activated by host enzymes in the intestinal wall or liver. An advantage for these compounds is the ability to treat patients in a nonhospital setting and to avoid the disadvantages of intravenous (IV) administration (i.e., inability of patients to self-administer, need for strict asepsis, and inconvenience). The world first oral carbapenem is Tebipenem-pivoxil which is active against MDR *S. pneumoniae* (MIC = 0.06 mg/liter) and other Gram-positives, as well as the *Enterobacteriaceae*. Tebipenem-pivoxil's spectrum does not include MRSA and *P. aeruginosa*.

It is absorbed well in the intestine, with a half-life of 0.3 to 0.5 h in humans with otolaryngological infections. It is also useful in treating pneumonia in children (Papp-Wallace *et al*., 2011).

2.4 Carbapenemase

Klebsiella pneumoniae carbapenemase is an enzyme first found in *Klebsiella pneumoniae* isolates (hence, the name). However, it can be produced by other organisms including *Serratia* spp., *Enterobacter* spp., *E. coli* and *Salmonella enterica*. The global spread of KPC-producing organisms appears to have been rapid.

The most common mechanism of carbapenem resistance among *Enterobacteriaceae* in the United States is the production of the *Klebsiella pneumoniae* carbapenemase (KPC). KPC-producing *Enterobacteriaceae* are widespread in the United States and other countries.

The production of these enzymes results in resistance to all penicillins, cephalosporins (i.e., cefepime, ceftriaxone), carbapenems (i.e., meropenem, ertapenem), and aztreonam. Treating infections caused by KPC-producing organisms is very difficult and very few antibiotics are effective. Often, these organisms are only susceptible to tigecycline and colistin. These antibiotics have significant side effects, are potentially inferior to more conventional therapies and can be costly (Department of Health and Hospital, 2010).

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is resistant to almost all antimicrobial agents, is associated with substantial morbidity and mortality, and poses a serious threat to public health. The ongoing worldwide spread of this pathogen emphasizes the need for immediate intervention (Saidel-Odes and Borer, 2014).

2.4.1 Characterization of carbapenemases

Carbapenemases include enzymes from the classes A, B and D of the Ambler classification of β-lactamases (Queenan and Bush, 2007). The Ambler classification scheme separates β-lactamases into four major classes (A–D) based on amino acid sequence homology (Paterson and Bonomo, 2005; Queenan and Bush, 2007). Classes A, C and D are β -lactamases with serine at their active site, while class B (also known as metallo- β -lactamases) have zinc at their active site (Paterson, 2006).

Klebsiella pneumoniae Carbapenemase (KPC) enzymes falls under Ambler class A and Bush functional group 2f enzymes (Bush *et al*., 1995). KPC enzymes differ from the other 2f enzymes by two specific characteristics: (i) they are found on transferable plasmids; and (ii) they are able to hydrolyse the aminothiazoleoxime cephalosporins such as cefotaxime (Queenan and Bush, 2007).

KPCs are predominantly found in *K. pneumoniae*; however, they have also been found in many other *Enterobacteriaceae* including *Escherichia coli, Enterobacter* species, *Salmonella enterica, Proteus mirabilis* and *Citrobacter freundii* (Bush *et al.,* 1995; Paterson, 2006; Queenan and Bush, 2007). The identification of a KPC enzyme outside the *Enterobacteriaceae* family was first reported in 2007 in *Pseudomonas aeruginosa* (Villegas *et al*., 2007) and most recently in an *Acinetobacter baumannii* strain from Puerto Rico. The KPC family has a great potential for spreading due to the location of KPC genes on plasmids (Queenan and Bush, 2007).

2.4.2 *Klebsiella pneumoniae* carbapenemase (KPC) producers

Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and

mortality. Once confined to outbreaks in the northeastern United States (US), they have spread throughout the US and most of the world. KPCs are an important mechanism of resistance for an increasingly wide range of Gram-negative bacteria and are no longer limited to *K. pneumoniae*. Carbapenem antibiotics are generally not effective against KPC-producing organisms. The best therapeutic approach to KPC-producing organisms has yet to be defined; however, common treatments based on *in vitro* susceptibility testing are the polymyxins, tigecycline, and less frequently aminoglycoside antibiotics (Arnold *et al*., 2011).

KPCs are encoded by the gene bla_{KPC}, whose potential for inter-species and geographic dissemination is largely explained by its location within a Tn3-type transposon, Tn4401. This transposon is a genetic element which is capable of inserting into diverse plasmids of Gram-negative bacteria. Plasmids carrying bla_{KPC} are often also associated with resistance determinants for other antibiotics. Although *K. pneumoniae* remains the most prevalent bacterial species carrying KPCs, the enzyme has been identified in several other Gram-negative bacilli. Of all bacteria species that have been found to produce *Klebsiella pneumoniae* carbapenemase (KPC) resistance enzymes. *Klebsiella pneumoniae* remains the most common organism to harbour KPCs, however relative frequency in other organisms has not been reported (Munoz-Price and Quinn, 2009).

2.4.3 Predictors of Carbapenem Resistant *Klebsiella pneumoniae* (CRKP) colonization

Several studies have evaluated predictors for colonization by carbapenem resistant *Klebsiella pneumoniae*. These predictors are as follows:

i. Kwak *et al.* (2005) reported that previous use of carbapenem and cephalosporin predict CRKP colonization. In this study, prior treatment with fluoroquinolones was associated with decreased risk for the emergence of CRKP.

ii. In a study by Schwaber *et al.* (2008), the predictors of CRKP colonization were Poor functional status, intensive care unit (ICU) stay and the use of antibiotics. In this study exposure to fluoroquinolones was independently predictive of CRKP isolation.

iii. Borer *et al*. (2012) in their study demonstrated that nursing home residency before hospital admission, bedridden status and previous antibiotic therapy were predictors of CRKP colonization.

2.4.4 Risk factors for Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) infection

Several risk factors have been associated with CRKP infection in previous studies. These risk factors are as follows:

i. Diabetes mellitus (Borer *et al.,* 2012; Schechner *et al.,* 2013), Intensive Care Unit (ICU) admission (within 2 weeks) (Wu *et al.,* 2011; Hussein *et al.,* 2013; Shilo *et al.,* 2013; Schechner *et al.,* 2013), solid tumors, previous invasive procedures, tracheostomy (Borer *et al.,* 2012), urinary catheter insertion (Borer *et al.,* 2012; Shilo *et al.,* 2013), central venous catheterization (Hussein *et al*., 2013; Schechner *et al*., 2013), prior exposure to antibiotics especially carbapenems, colistin or glycopeptides (Wu *et al.,* 2011; Shilo *et al.,* 2013; Hussein *et al.,* 2013; Schechner *et al*., 2013) and antipseudomonal penicillin therapy (Borer *et al.,* 2012).

ii. ICU admission (within 2 weeks) or prior exposure to carbapenems or glycopeptides (Wu *et al*., 2011).

iii. Antibiotic use, especially colistin, presence of a urinary catheter, surgery, invasive procedures, and ICU admission (Shilo *et al.,* 2013).

iv. Prior use of macrolides and any antibiotic exposure ≥14 days, hematological malignancy, chronic renal failure, chronic liver disease, previous bone marrow transplantation, longer length of stay before the onset of bacteremia, receipt of mechanical ventilation, central venous catheterization, dialysis, and stay in the ICU or hematology department (Hussein *et al*., 2013).

v. Independent predictors of subsequent carbapenem-resistant *Enterobacteriaceae* (CRE) infection were admission to the ICU, having a central venous catheter, receipt of antibiotics, and diabetes mellitus (Schechner *et al*., 2013).

2.4.5 Outbreak of Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) around the globe.

During the last decade, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has spread throughout the world, becoming a matter of great concern. After the first report of CRKP in North Carolina, United States in 2001 CRKP has been reported in other parts of the world. The table below summarises some of the out breaks of CRKP around the world (Yigit *et al*., 2001).

Table 2. 1: Outbreaks of Carbapenem-Resistant *Klebsiella pneumoniae* CRKP around the world.

Country	**Year**	**Number of cases**	**Reference**
Saudi Arabia	2010	20	Balkhy *et al*., 2012
Germany	2010	7	Steinmann *et al*., 2011
Spain	2009	7	Robustillo Rodela *et al.*, 2012
France	2009	13	Carbonne *et al*., 2010
Israel	2009	16	Wiener-Well *et al*., 2010
Colombia	2008	84	Lopez *et al.*, 2011
Puerto Rico	2008	26	Gregory *et al*., 2010
Greece	2007-2008	50	Souli *et al*., 2010
People's Republic of China	2007	28	Zhang *et al*., 2011
Israel	2006	90	Samra *et al*., 2007

Source: Saidel-Odes and Borer (2014).

2.4.6 Treatment options for infections caused by CRKP

With the emergence of carbapenem resistant *Klebsiella pneumoniae* and carbapenem resistant *Enterobacteriaceae* generally, few treatment options were left for the treatment of infection caused by these organisms. Polymyxins, some aminoglycosides, and tigecycline generally retain *in vitro* activity against CRE, and these are the most commonly used "drugs of last resort" (van Duin *et al*., 2013). Some experts advocate the use of high-dose prolonged-infusion carbapenem therapy as part of a combination regimen in infections with CRE with carbapenem MICs ≤4 mg/L (Daikos and Markogiannakis, 2011). So far, reports evaluating the efficacy and safety of double-carbapenem therapy in the treatment of human infection are scarce. For now, colistin, tigecycline, and aminoglycosides remain the "mainstay of treatment" for invasive CRE infections (van Duin *et al*., 2013).

2.4.6.1 Polymyxins

Polymyxins are cyclic peptides which differ by 1 amino acid and possess targeted Gram-negative activity. Their mechanism of action is through an electrostatic interaction between the cationic polypeptide antimicrobial and the anionic lipopolysaccharides of the bacterial outer membrane leading to a leakage of cellular contents and, ultimately, bacterial cell lysis. Examples of polymyxins are polymyxin E (also known as colistimethate) and polymyxin B (Falagas and Kasiakou, 2005; van Duin *et al*., 2013).

Colistin is given intravenously as a mix of various colistin methanesulphonate (CMS) or colistimethate compounds. Knowledge of colistimethate and colistin pharmacokinetics is currently evolving due to the advent of reliable assays that can distinguish colistimethate from colistin in biological fluids (van Duin *et al*., 2013).

Colistin demonstrates nonlinear protein binding with bothalbumin and alpha-1-acid glycoprotein, an acute phase plasma protein. Colistimethate, the inactive prodrug, is hydrolyzed to active colistin and inactive sulfomethylated derivatives (van Duin *et al*., 2013).

Colistimethate primary undergoes renal clearance and has a short elimination half-life (2–3 h), whereas colistin has a longer half-life (9–13 h) and is minimally excreted in the urine due to extensive renal tubular reabsorption (van Duin *et al*., 2013).

Data describing the pharmacokinetics of polymyxin B are less available. The volume of distribution was 47 L, and the elimination half-life was 13 h in 1 small study (Jin *et al*., 2008). For both polymyxins, the pharmacodynamics are best described by area under the curve to minimum inhibitor concentration (AUC/MIC) ratio (van Duin *et al*., 2013).

The 2 major safety concerns with polymyxins are nephrotoxicity and neurotoxicity. The exact mechanism of nephrotoxicity is not well understood, and incidence in the contemporary literature varies from 14% to 53% (Falagas *et al*., 2005; van Duin *et al*., 2013). Total daily dose and longer durations of therapy correlate with increased risk of renal dysfunction; however, it is typically reversible after discontinuation of the drug (van Duin *et al*., 2013). Animal studies suggest possible attenuating effects with the use of melatonin and ascorbic acid (van Duin *et al*., 2013).

Neurotoxicity can manifest as a spectrum from paresthesias to ataxia to neuromuscular blockade and is reported in approximately 4–6% of patients (van Duin *et al*., 2013).

2.4.6.2 Tigecycline

Tigecycline is a glycylcycline antimicrobial which was modified to overcome 2 major mechanisms of tetracycline resistance. Tigecycline is bacteriostatic and exerts its mechanism by binding to the 30S ribosomal subunit thereby inhibiting protein synthesis. In the USA, tigecycline is approved by Food and Drug Administration (FDA) for the treatment of skin and skin structure infections, complicated intra abdominal infections, and community-acquired pneumonia (van Duin *et al*., 2013).

Tigecycline has a large volume of distribution and extensive tissue penetration in skin, gallbladder, bowel, and intracellular pulmonary tissue. In contrast, the plasma concentrations of tigecycline are relatively low, maximum concentrations of 0.62 μg/ mL at steady state dosing with 50 mg every 12 h (van Duin *et al*., 2013). Elimination primarily occurs through the feces via biliary excretion (59%) with a minority (15–22%) excreted in the urine as unchanged drug. Serum concentrations of tigecycline are generally deemed not adequate to treat blood stream infections, and tigecycline is not approved for this indication (Nix and Matthias, 2010; Tarchini, 2010). Tigecycline has a long elimination half-life, 42 h after multiple doses, and clearance is not affected by renal impairment or mild hepatic impairment (van Duin *et al*., 2013).

Of greatest concern regarding the use of tigecycline as a single agent in the treatment of invasive infections are data which support an increased mortality in those patients treated with tigecycline as compared to other agents. In 2010, tigecycline received an FDA warning regarding increased mortality risk. Four recent meta-analyses have used different analytical tools and have come to different conclusions about this issue. However, one is struck by the notion that a small but

significant increased mortality risk exists with tigecycline, which is most likely secondary to decreased efficacy (van Duin *et al*., 2013).

The most common adverse effects associated with tigecycline therapy are nausea and vomiting. Although generally mild, these gastrointestinal complaints have been reported in up to 30% of patients in some studies and lead to more drug discontinuation in clinical trials versus the comparator antimicrobials (van Duin *et al*., 2013).

2.4.6.3 Fosfomycin

Fosfomycin, a phosphonic acid derivative, is bactericidal against a broad spectrum of Gram positive and Gram-negative organisms. The bacterial enzyme pyruvyl transferase is inactivated by fosfomycin leading to inhibition of bacterial cell wall synthesis. It is currently available in the USA as an oral powdered sachet of 3 g of fosfomycin (equivalent to 5.61 g of fosfomycin tromethamine). In many European countries, both oral (as either the tromethamine or the calcium salt) and intravenous (as fosfomycin disodium) are available (Wisher, 2012; van Duin *et al*., 2013).

Oral bioavailability is improved with the tromethamine salt (37% in fasting conditions) compared to the calcium salt (12%) due to poor solubility and acid degradation. Fosfomycin has a low molecular weight and almost negligible protein binding, affording it good distribution into tissues including kidney, bladder wall, prostate, lung, soft tissues, bone, and cerebrospinal fluid (especially with inflamed meninges) (van Duin *et al*., 2013). Serum concentrations are not equivalent between oral and intravenous formulations, and the use of the oral formulation should generally be restricted to treatment of cystitis.

Primarily excreted unchanged in the urine (38%), high concentrations of fosfomycin tromethamine persist in the urine for up to 48–72 h. The elimination half-life is 2–3 h for fosfomycin disodium and 6 h for fosfomycin tromethamine in subjects with normal renal function. In patients with renal impairment, the urine recovery is lower and the half-life is significantly prolonged, up to 50 h (van Duin *et al*., 2013).

Fosfomycin is generally well tolerated. The most common adverse effects of the oral formulations are gastrointestinal which are usually mild and transient. For the intravenous product, the most common adverse effects include phlebitis and allergic reactions. Laboratory alterations, including fluctuations in white blood cell count, eosinophils, bilirubin, and liver function, have been reported; however, the changes are generally transient and not clinically significant (van Duin *et al*., 2013).

2.4.6.4 Aminoglycosides

The aminoglycosides act by inhibiting protein synthesis. They do so by binding to the 30S subunit of the ribosome. Plazomicin (ACHN-490), a new sisomicin-derived aminoglycoside currently in development, joins currently available aminoglycosides (amikacin, gentamicin, and tobramycin) and has demonstrated *in vitro* activity against CRE. Primarily utilized for Gram-negative activity, aminoglycosides exhibit concentration dependent activity and a prolonged post-antibiotic affect. The pharmacodynamic target for aminoglycosides is peak concentration to MIC ratios of 8–12:1. Close monitoring of levels is recommended to ensure sufficient therapeutic levels while minimizing toxicity (van Duin *et al*., 2013).

Pharmacokinetic properties are similar among all agents in the class (Edson and Terrell, 1999). They have a volume of distribution that approximates extracellular space. They distribute well

into bone, peritoneal fluid, and urine, but have limited penetration into the cerebral spinal fluid and prostrate. The elimination half-life is highly dependent on age and renal function, but for adults with normal renal function it is around 2 h. Excretion is primarily in the urine through glomerular filtration. Aminoglycosides are often used as part of combination regimens (van Duin *et al*., 2013).

Nephrotoxicity is one of the major adverse effects of aminoglycosides due to drug accumulation in the proximal renal tubular cells. Typically, nephrotoxicity related to aminoglycoside use is reversible. Ototoxicity is another adverse effect which is often irreversible and can manifest as vestibular or cochlear damage. For longer treatment courses, baseline and periodic follow-up audiology evaluations can be beneficial. Another serious, but less common adverse effect is neuromuscular blockade (van Duin *et al*., 2013).

2.4.6.5 Temocillin

Temocillin, a semisynthetic derivative of ticarcillin, is primarily available in the United Kingdom but is not available in the USA. As with other beta-lactams, its mechanism is to bind to penicillin-binding proteins and inhibit cell wall synthesis. This drug displays time dependent activity. Temocillin's spectrum of activity is limited to *Enterobacteriaceae*, but it is stable against a variety of beta-lactamases. In a small *in vitro* study, the MIC50 and MIC90 were both 32 μg/mL for 33 KPC-producing organisms (mostly *K. pneumoniae*). Temocillin MIC breakpoints as per the British Society of Antimicrobial Chemotherapy are ≤8 and ≤32 μg/mL, for systemic and urinary infections, respectively. In a larger *in vitro* study, 4/81 and 26/81 CRE were susceptible at the ≤8 and ≤32 μg/mL breakpoints, respectively. Clinical studies are not available for its use in CRE (van Duin *et al*., 2013).

Pharmacokinetics are similar to ticarcillin. Temocillin distributes well to most tissues and has relatively high protein binding (70–85%). It is primarily eliminated in the urine through glomerular filtration and tubular secretion. Half-life is normally 4–6 h but can be prolonged in renal insufficiency (Boelaert *et al*., 1983). Temocillin is generally well tolerated but as with other beta-lactams, allergic reactions such as rash can occur (van Duin *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Zaria. It is a major city in Kaduna State in northern Nigeria, as well as a Local Government Area. It was a formerly known as Zazzau and also one of the original seven Hausa city-states. Zaria lies within the coordinates $11^{o}04'N\ 7^{o}42'E$ / $11.067^{o}N\ 7.700^{o}E$ (htts://en.m.wikipedia.org/wiki/zaria).

The selected hospitals were Hajiya Gambo Sawaba General Hospital, located at Zaria, Zaria Clinic located at Tudun Wada, Juma'a Hospital located at Samaru and University Medical Center, Ahmadu Bello University, Main Campus.

3.2 Sample Size

The sample size of the study was determined using the prevalence rate of carbapenem resistance of 9.3% (Motayo *et al*., 2013) and the Kish Leisle formula (Mshana *et al*., 2009).

$N=Z^2\ P\ (1-P)/d^2$

Where N = sample size

P = prevalence of carbapenem resistance.

Z = confidence interval (1.96)

D = allowable error (5%)

$N=Z^2\ P\ (1-P)/d^2$

$N = 1.96^2 x 0.093 \times (1-0.093)/ (0.05)^2$

N= 130 samples.

A total of 150 clinical samples were collected to increase statistical precision and minimize error.

3.3 Inclusion criteria

Patients sent to the Microbiology laboratory for suspected cases of Urinary Tract Infection, Respiratory Tract Infection or wound infection and who consented.

3.4 Exclusion criteria

Patients sent to the Microbiology laboratory for suspected cases other than Urinary Tract Infection, Respiratory Tract Infection or wound infection and those who did not consented.

3.5 Ethical approval

Ethical approval was obtained from the ethical committee of Kaduna State Ministry of Health.

3.6 Sample Collection

A total of 150 samples of sputum (38), urine (68) and wound swab (44) were collected from patients sent to Microbiology laboratory of the selected hospitals in Zaria using convenience sampling technique. For sputum collection, the patients were given clean, dry, wide-necked, leak-proof containers and requested to cough deeply to produce a sputum specimen. Each container was then labeled appropriated. As for urine collection, the patients were given sterile, dry, wide-necked, leak-proof container and requested to provide 10–20 ml of midstream urine. The containers were also labeled appropriately. Wound swabs were collected by using a sterile

swab stick to swab the wound surface. Each swab stick was then labeled appropriately (Cheesbrough, 2006).

3.7 Isolation and Characterization

3.7.1 Isolation

The samples were inoculated on MacConkey agar and incubated for 24hours at 37ºC. The isolates were identified by their morphological characteristics on MacConkey agar. Isolates that appeared as pink mucoid colonies on MacConkey agar after incubation at 37^0C for 18 - 24hours were considered as presumptive *Klebsiella pneumoniae.* These isolates were then Gram stained (Akter *et al.,* 2014).

3.7.2 Gram Staining

A smear was prepared from the 18 - 24 hours culture of the pink mucoid colonies, a pinch of well separated colony (pure culture) was placed directly into a drop of normal saline on a clean, dried, grease-free slide, smeared and then allowed to air-dry. The smear was then heat-fixed by passing the slide over a Bunsen flame for three quick successions. The slide was flooded with crystal violet solution and allowed to stand for one minute, then washed with slow-running tap water and then flooded with Gram's iodine (mordant) and allowed to act for also one minute. The slide was washed with tap water and decolourize with 95% alcohol for 30seconds. The slide was rinsed with tap water and then counter-stained with safranin for another 30seconds. The slide was finally rinsed with slow-running tap water, allowed to air-dry and then examined microscopically under oil immersion objective lens after adding a drop of oil immersion (Cowan and Steel, 2003). Isolates showing Gram negative short, plump, straight rods were subcultured

on nutrient agar slant and then stored in a refrigerator before they were further characterized biochemically.

3.7.3 Biochemical Characterization

The isolates were characterized using the following biochemical tests:

Indole produciton

Indole test was carried out by inoculating the suspected colonies of *Klebsiella pneumoniae* into 1% peptone water and then the inoculated peptone water was incubated at 37^{o}C for 48hours. After 48hours incubation, 0.5ml of Kovacs reagent was added and shaken. A positive reaction was indicated by the development of a red colour in the reagent layer above the broth. Negative reaction was indicated by a yellow colour (Cowan and Steel, 2003). Isolates that were indole negative were targeted as presumptive *Klebsiella pneumoniae*.

Methyl Red - Voges-Proskauer test:

This test was carried out by inoculating 5ml of MR-VP broth with the suspected colonies and then the inoculated broth was incubated at 37^{o}C for 48 hrs. After 48 hrs of incubation, about 1ml of the cultured broth was transferred to a test tube to which 2 drops of methyl red solution was added and then shaken. Formation of red colour on addition of the indicator signifies a positive methyl red test and an orange or yellow colour signifies a negative test.

To the rest of the broth, 6 drops of 5% α-Naphthol solution was added followed by 2 drops of 40% potassium hydroxide. The tube was shaken and placed in a slope. Development of a red colour starting from the liquid – air interface within 15minutes to 1 hour indicates a VP positive

test. No colour change indicates VP negative test (Cowan and Steel, 2003). Isolates that were MR negative and VP positive were targeted as presumptive *Klebsiella pneumoniae.*

Citrate utilisation test

This test was carried out by inoculating the suspected colonies of *Klebsiella pneumoniae* on Simmons' citrate agar slant and the inoculated slant was then incubated at 37^{o}C for 72 hrs. Development of a deep blue colour indicates a positive reaction while if the original green colour maintained it means citrate was not utilized (Cowan and Steel, 2003). Isolates that were positive for citrate utilization test were targeted as presumptive *Klebsiella pneumoniae*.

Urease test

Urease test was carried out by inoculating Christensen's urea agar slant with the suspected colonies of *Klebsiella pneumoniae* and then the inoculated Christensen's urea agar slant was incubated at 35^{0}C for 48 hrs. The development of bright pink or red colour indicates a positive reaction (Cowan and Steel, 2003). Isolates that were urease positive were targeted as presumptive *Klebsiella pneumoniae*.

Sugar Fermentation Test

Tubes of Triple Sugar Iron Agar (TSI Agar) were inoculated by stabbing the butt and streaking the slope. The tubes were then incubated at 37^{o}C for 24hous. After incubation, the tubes were observed for colour change (from red to yellow), gas production and blackening of the butt. Red slant (Alkaline) indicates lactose or sucrose not fermented, yellow slant (acidic) indicates lactose or sucrose fermented, red butt indicates that glucose was not fermented and yellow butt indicates glucose fermentation. While bubbles or cracks in the medium indicate gas production,

blackening of the butt indicates H_2S production. Isolates that gave acidic slant and butt, produces gas but not H_2S were targeted as presumptive *Klebsiella pneumoniae* (Cowan and Steel, 2003).

Motility

This test was carried out by stab-inoculating the tubes of motility medium with the suspected *Klebsiella pneumoniae* colonies. A fine stab with a needle was made to a depth of about one third the total volume of the medium. The medium was then incubated at 37^{o}C for 24hrs. If the medium turns cloudy (turbid) after incubation, it means the organism is motile but if growth is restricted to the line of inoculation and the rest of the medium remains clear, then the organism is non motile (Cowan and Steel, 2003). Isolates that were non motile were targeted as presumptive *Klebsiella pneumoniae*.

Aesculin hydrolysis

Suspected colonies of *Klebsiella pneumoniae* were inoculated on bile aesculin agar and incubate at 37^{o}C 24hrs. A positive test was indicated by a dark brown to black colouration of the whole medium after 24hrs incubation. A negative reaction was indicated by lack of colour change throughout the medium. (Cowan and Steel, 2003). Isolates that were able to hydrolyze arsculin were targeted as presumptive *Klebsiella pneumoniae*.

3.8 Antibiotic Susceptibility Test

Biochemically identified isolates of *Klebsiella pneumoniae* were standardized by using a sterilized wire to pick four or five isolated colonies of the test organism and then suspending the organism in 2ml sterile normal saline. The suspension was then mixed properly and the turbidity was adjusted to a 0.5 McFarland standard. The standardized inocula were then subjected to

antibiotics susceptibility test on Mueller Hinton agar by modified Kirby-Bauer disc diffusion technique using the following antibiotic discs: Tetracycline (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Cefotaxime (30µg), Ampicillin (30µg), Cotrimoxazole [Trimethoprim-Sulfamethoxazole] (1.25/23.75 µg), Gentamicin (10µg) and Imipenem (10µg).

Briefly, a sterile swab was dipped into the inoculum tube and then excess fluid removed by rotating the swab against the side of the tube. The Mueller Hinton agar was then inoculated by swabbing the swab stick three times over the surface of the agar, rotating the plate approximately 60^{o} each time to ensure even distribution of the inocula. The plates were kept at room temperature for 3-5minutes for the surface of the agar to dry (Acharya, 2013).

Using a sterile forcep the disks were placed one at a time on the plates and pressed gently to ensure complete contact with the agar surface. The plates were then kept at room temperature for 5minutes before incubation at 37^{o}C for 24hours. The sizes of the zone of inhibition were measured with the aid of a ruler to the nearest millimetre (Acharya, 2013). Using the published CLSI guidelines, the susceptibility or resistance of the organism to each of the drug tested was determined (CLSI, 2015).

Isolates resistant to Imipenem were screened for carbapenemase production by the Cloverleaf test/Modified Hodge Test as recommended by the Clinical Laboratory Standards Institute (CLSI, 2015).

3.9 Determination of the MAR Index of the *Klebsiella pneumoniae* Isolates

The MAR index of the isolates was calculated using the formula below as described by Olonitola *et al*. (2007).

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which the isolate is resistant}}{\text{Total number of antibiotics used}}$$

3.10 Detection of carbapenemase production using Cloverleaf test or Modified Hodge Test (MHT)

This test is based on the inactivation of a carbapenem by either whole cells or cell extracts of the test organisms, which enables a carbapenem susceptible indicator strain (*Escherichia coli* ATCC 25922) to extend growth towards a carbapenem disk, along the streak of inoculum of the test strain.

A 0.5 McFarland standard suspension of the indicator organism (*Escherichia coli* ATCC 25922) was prepared in normal saline and then a 1:10 dilution of it in normal saline was inoculated on Mueller Hinton Agar plate as a lawn. The plate was allowed to dry for 3 to 10 minutes. Imipenem disk was then placed at the middle of the inoculated Mueller Hinton Agar plate. Using a sterile loop, 3 to 5 colonies of test isolates and the MHT positive control strain (*Klebsiella pneumoniae* ATCC BAA-1705) grown overnight were picked and inoculated in a straight line out from the edge of the disk. Following incubation at 37^{o}C for 16 to 20 hours, the MHA plate was examined for enhanced growth of the indicator organism around the test isolates at the intersection of the streak and the zone of inhibition. Enhanced growth of the indicator organism (*Escherichia coli* ATCC 25922) means the test isolate was positive for carbapenemase production while no enhanced growth of the indicator organism means the isolate was negative for carbapenemase production (CLSI, 2015).

3.11 Data Analysis

The data obtained were analyzed statistically using Graphpad QuickCals (www.graphpad.com). Chi square test was used to determine the statistical association between *Klebsiella pneumoniae* and factors such as clinical sample, gender and age group. $P \leq 0.05$ was considered statistically significant

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence of *Klebsiella pneumoniae* in Zaria

Out of the 150 clinical samples collected from selected hospitals in Zaria comprising of 68 urine samples, 44 wound swabs and 38 sputum samples, 19 *Klebsiella pneumoniae* were isolated giving a prevalence of 12.67% (Figure 4.1).

4.2 Prevalence of *Klebsiella pneumoniae* isolates according to clinical samples

Amongst the 68 urine samples screened, 11 were positive for *Klebsiella pneumoniae* giving a prevalence of 16.18%. Out of the 44 wound swabs screened 2 were positive giving a prevalence of 4.55% while 6 samples out of the 38 sputum samples screened were positive with a prevalence of 15.79% (Figure 4.2). The difference observed was not statistically significant (χ^2 = 3.716, df = 2, p = 0.1560).

4.3 Prevalence of *Klebsiella pneumoniae* by gender

Figure 4.3 shows the prevalence of *Klebsiella pneumoniae* among male and female patients attending the selected hospitals. Out of the 90 females screened, 12 were positive for *Klebsiella pneumoniae* giving a prevalence of 13.33%. Seven (7) out of the 60 males screened were positive giving a prevalence of 11.67%. The difference observed was not statistically significant (χ^2 = 0.090, df = 1, p = 0.7642).

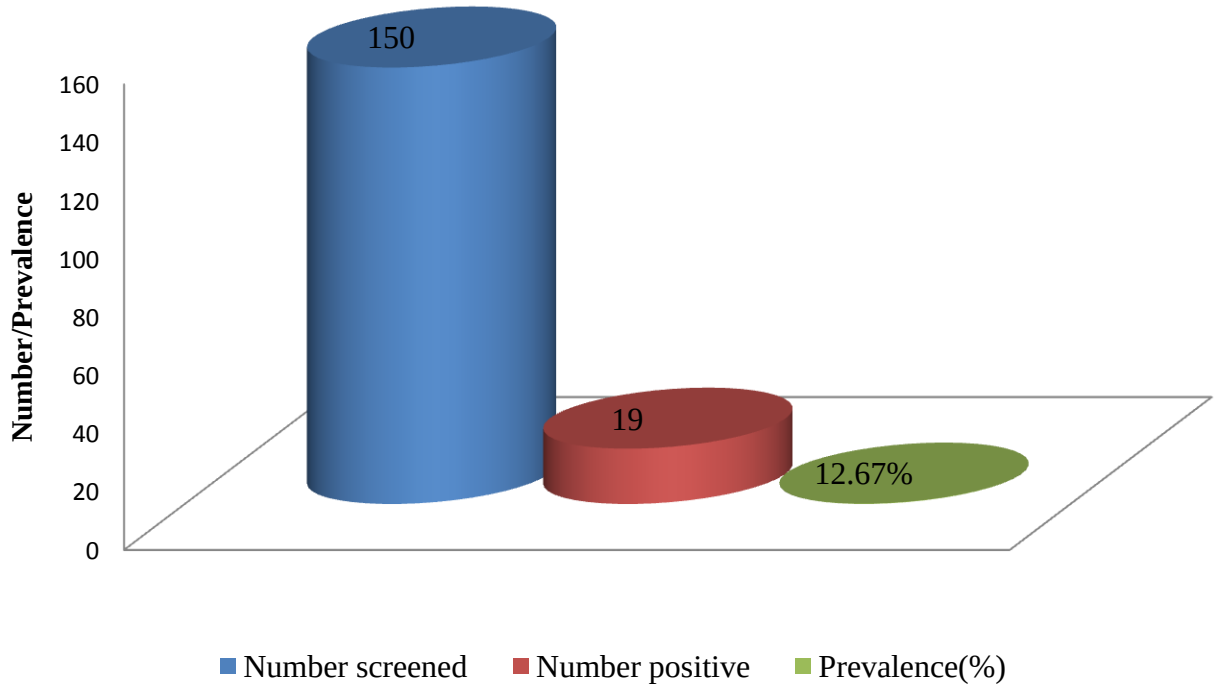
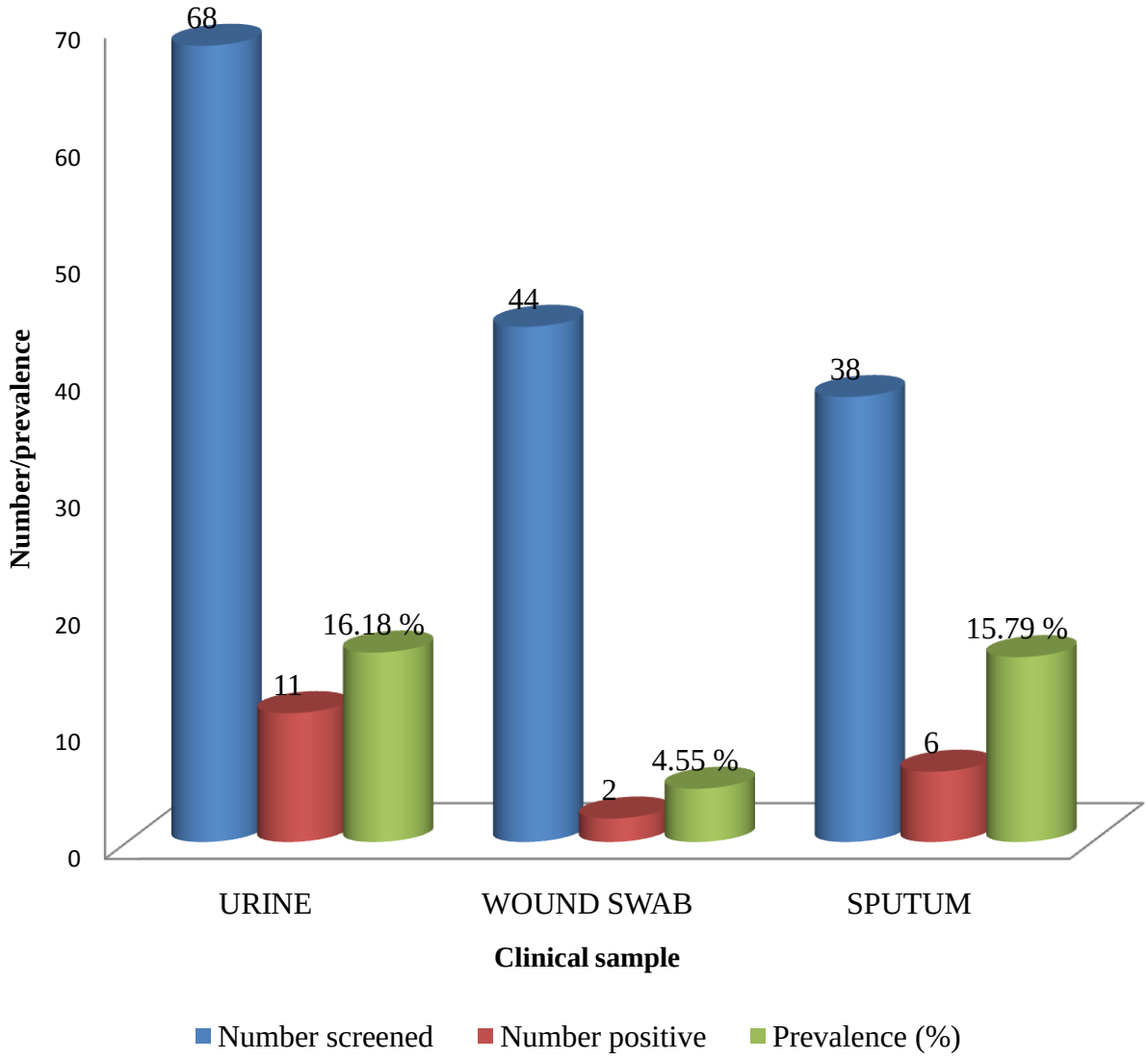
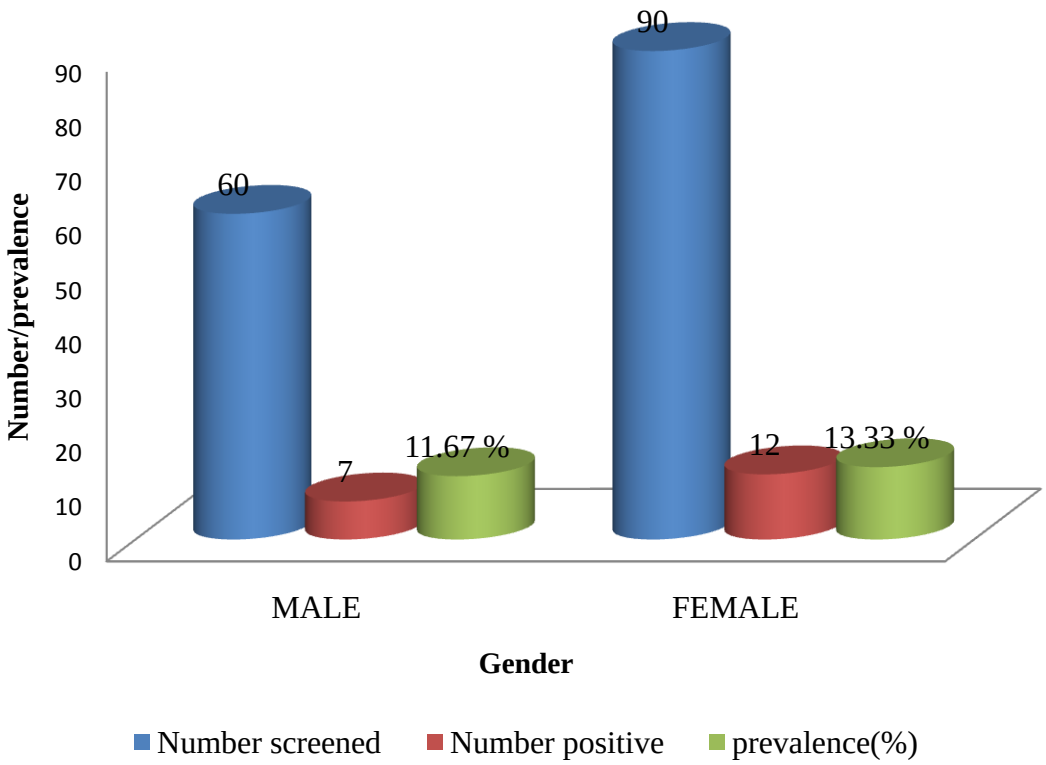


Figure 4. 1: Prevalence of *Klebsiella pneumoniae* in Zaria.



$\chi^2 = 3.716$, df = 2, p = 0.1560.

Figure 4. 2: Prevalence of *Klebsiella pneumoniae* according to the different clinical samples.



$\chi^2 = 0.090$, df = 1, p = 0.7642

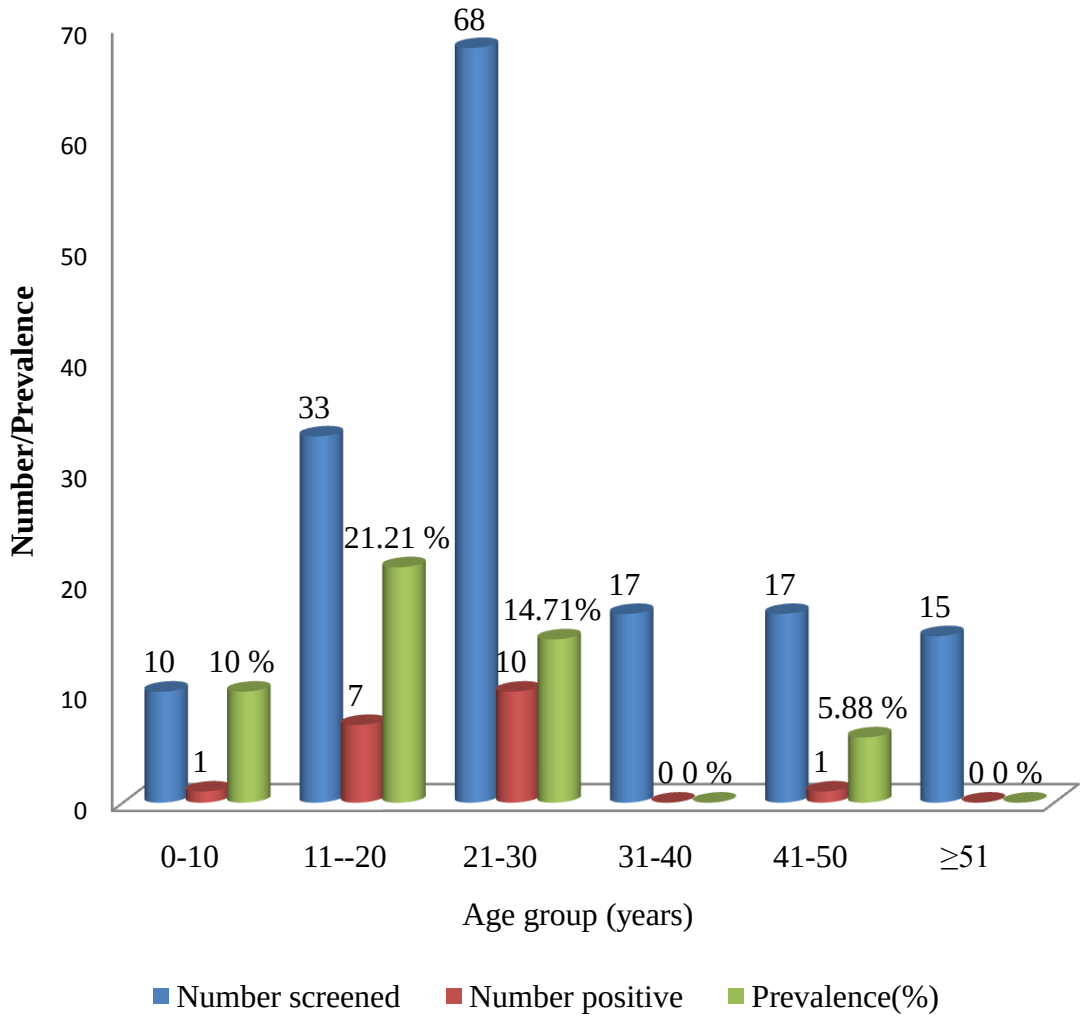
Figure 4. 3: Prevalence of *Klebsiella pneumoniae* according to gender of patients.

4.4 Prevalence of *Klebsiella pneumoniae* by age group

The age group prevalence of *Klebsiella pneumoniae* is presented in Figure 4.4. The age group with the highest prevalence was 11-20 year (21.21%) followed by age group 21-30 years (14.71%). Zero (0%) prevalence was observed for age groups 31-40 and ≥50. The difference observed was not statistically significant ($\chi^2 = 3.183$, df = 5, p = 0.6718).

4.5 Antibiotic susceptibility pattern of *Klebsiella pneumoniae*

Result of the antibiotic susceptibility pattern of the isolates is presented in Table 4.1. All the isolates (100%) screened were resistant to Ampicillin. As for Cefotaxime, 94.7% resistance was recorded. So also 94.7%, 78.9%, 63.2% and 57.9% of the isolates were susceptible to Imipenem, Gentamicin, Ciprofloxacin and Chloramphenicol respectively. A moderately high and high resistance to Tetracycline (57.9%) and Cotrimoxazole (78.9%) respectively were recorded in this study.



$\chi^2 = 3.183$, df = 5, p = 0.7856.

Figure 4. 4: Prevalence of *Klebsiella pneumoniae* according to age group.

Table 4. 1: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolates from selected hospitals in Zaria.

Antibiotic (disk content)	Number (%) of isolates		
*n = 19	Susceptible	Intermediate	Resistant
Imipenem (10 µg)	18(94.7)	1(5.3)	0(0.0)
Tetracycline (30 µg)	8(42.1)	0(0.0)	11(57.9)
Ciprofloxacin (5 µg)	12(63.2)	2(10.5)	5(26.3)
Chloramphenicol (30 µg)	11(57.9)	1(5.3)	7(36.8)
Cefotaxime (30 µg)	0(0.0)	1(5.3)	18(94.7)
Ampicillin (10 µg)	0(0.0)	0(0.0)	19(100.0)
Gentamicin (10 µg)	15(78.9)	0(0.0)	4(21.1)
Cotrimoxazole (1.25/23.75 µg)	4(21.1)	0(0.0)	15(78.9)

***** Total number of isolates screened (n = 19)

4.6 Antibiotic Resistance Pattern of the Isolates According to Source of Isolates.

Of the 19 *Klebsiella pneumoniae* isolates screened, 11 were from urine, 6 from sputum and 2 from wound swab. One (9.09%) out of the 11 isolates from urine was resistant to Imipenem, 6 (54.55%) out of the 11 isolates from urine were resistant to Tetracycline, Ciprofloxacin and Chloramphenicol. All the 11(100%) isolates from urine were resistant to Cefotaxime and Ampicillin. Nine (81.82) and four (36.36) of the isolates from urine were resistant to Cotrimoxazole and Gentamicin (Table 4.2).

None of the isolates from sputum was resistant to Imipenem and Gentamicin, however all the 6 (100%) isolates from sputum were resistant to Cefotaxime and Ampicillin. One (16.67%) out the 6 were resistant to Ciprofloxacin and Chloramphenicol while 4 (66.67%) were resistant to Tetracycline and Cotrimoxazole (Table 4.2).

In the case of isolates from wound swab, none of the 2 isolates was resistant to Imipenem, Ciprofloxacin and Gentamicin. Whereas 1 (50%) of the two isolates were resistant to Tetracycline and Chloramphenicol. All of the isolates resisted Cefotaxime, Ampicillin and Cotrimoxazole (Table 4.2). The difference observed was not statistically significant at $p \leq 0.05$.

Table 4. 2: Frequency of isolates resistant to the antibiotic with regards to the source of isolate.

Antibiotic (disk content)	Number (%) of isolates resistant				
Sample:	Urine (n = 11)	Sputum (n = 6)	Wound swab (n = 2)	χ^2	p value *
Imipenem (10 µg)	1(9.09)	0(0.00)	0(0.00)	0.775	0.6788
Tetracycline (30 µg)	6(54.55)	4(66.67)	1(50.00)	0.296	0.8624
Ciprofloxacin (5µg)	6(54.55)	1(16.67)	0(0.00)	3.709	0.1565
Chloramphenicol (30 µg)	6(54.55)	1(16.67)	1(50.00)	2.352	0.3085
Cefotaxime (30 µg)	11(100.00)	6(100.00)	2(100.00)	0.000	1.0000
Ampicillin (10 µg)	11(100.00)	6(100.00)	2(100.00)	0.000	1.0000
Gentamicin (10 µg)	4(36.36)	0(0.00)	0(0.00)	3.668	0.1598
Cotrimoxazole (1.25/23.75 µg)	9(81.82)	4(66.67)	2(100.00)	1.138	0.5661

* the differences observed in resistance to the antibiotics by the isolates from different clinical sources was not statistically significant.

4.7 Multiple Antibiotic Resistance (MAR) Indices, sources and resistance patterns of the isolates.

Table 4.3 shows the source, antibiotic resistance pattern and the Multiple Antibiotic Resistance Index of the *Klebsiella pneumoniae* isolates. All the isolates screened were resistant to 2 or more antibiotics. The MAR index of the isolates was between 0.25 and 0.88. Seven of the 19 isolates were resistant to 4 out of the 8 antibiotics tested with a MAR index of 0.50. Five of the 19 isolates screened were resistant to 6 out of the 8 antibiotics tested (MAR index = 0.75). Only one of the isolates was resistant to 7 out of the 8 antibiotics tested (0.88). None of the isolates was resistant to all the 8 antibiotics tested, likewise none was resistant to only one antibiotic. The most common resistance pattern observed was TE, CTX, AMP, SXT (five times) followed by CTX, AMP (three times).

4.8 Distribution of the isolates screened by number of antibiotics resisted and MAR index.

Figure 4.5 represents the distribution of the isolates with respect to the number of antibiotics resisted and their MAR index. A large proportion (37%) of the isolates have a MAR index of 0.50 and are resistant to four antibiotics.

4.9 Prevalence of carbapenem resistant *Klebsiella pneumoniae* isolated from clinical samples in Zaria.

Prevalence of carbapenem resistant *Klebsiella pneumoniae* in Zaria is shown is Figure 6. Out of 19 *Klebsiella pneumoniae* isolates from 150 clinical samples, 1 was intermediately resistant (non-susceptible) to Imipenem giving a prevalence of 5.26%.

Table 4. 3: Multiple Antibiotic Resistance Indices (MARI), sources and resistance patterns of *Klebsiella pneumoniae* isolated from clinical samples in Zaria.

Isolate code	Source	Number of antibiotics Resisted	Resistance pattern	MAR index
JUF1	urine	2	CTX, AMP.	0.25
S'S"M17	sputum	2	CTX, AMP.	0.25
S'S"F19	sputum	2	CTX, AMP.	0.25
SUF16	urine	3	CIP, CTX, AMP,	0.38
GWF22	wound swab	4	C, CTX, AMP, SXT.	0.50
GWM24	wound swab	4	TE, CTX, AMP, SXT.	0.50
JUF18	urine	4	C, CTX, AMP, SXT.	0.50
ZUF58	urine	4	TE, CTX, AMP, SXT.	0.50
S'S"F2	sputum	4	TE, CTX, AMP, SXT.	0.50
S'S"M3	sputum	4	TE, CTX, AMP, SXT.	0.50
S'S"M14	sputum	4	TE, CTX, AMP, SXT.	0.50
ZUF57	urine	5	IMP, TE, CTX, AMP, SXT.	0.63
SUM15	urine	5	TE, C, CTX, AMP, SXT.	0.63
S'S"F30	sputum	6	TE, CIP, C, CTX, AMP, SXT.	0.75
SUF1	urine	6	CIP, C, CTX, AMP, CN, SXT.	0.75
SUF3	urine	6	TE, CIP, C, CTX, AMP, SXT.	0.75
JUF4	urine	6	TE, CIP, CTX, AMP, CN, SXT.	0.75
SUM12	urine	6	CIP, C, CTX, AMP, CN, SXT.	0.75
SUM32	urine	7	TE, CIP, C, CTX, AMP, CN, SXT.	0.88

Key: IMP = Imipenem, TE - Tetracycline, CIP = Ciprofloxacin, C = Chloramphenicol, CTX = Cefotaxime, AMP = Ampicillin, CN = Gentamicin, SXT = Cotrimoxazole, J = juma'a, S' Sa'adah, S = Sickbay, G = Gambo Sawaba, Z = Zaria clinic, U = urine, W = wound swab, M = male, F = female, S" = sputum.

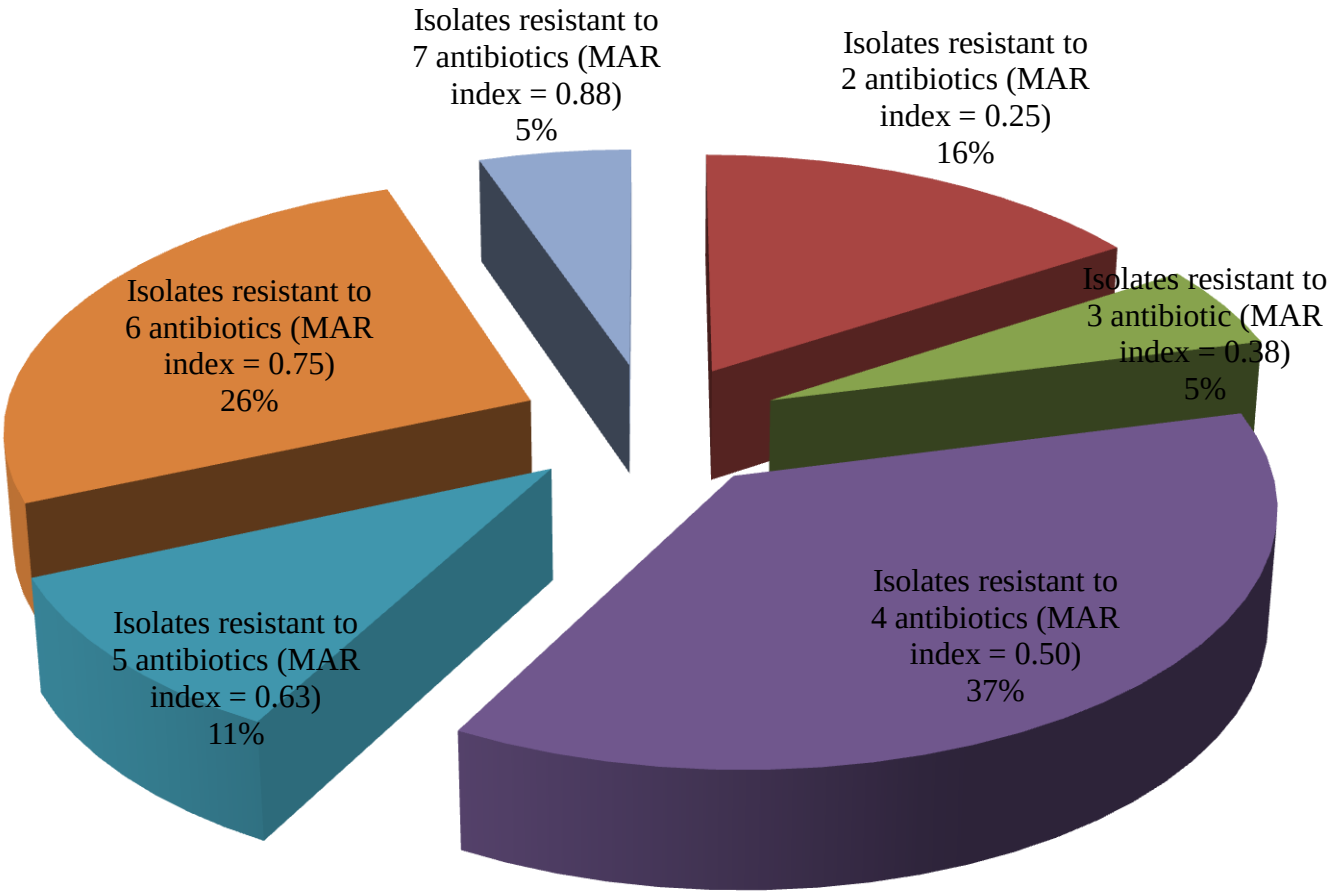


Figure 4. 5: Distribution of the isolates screened according to the number of antibiotics resisted and MAR index.

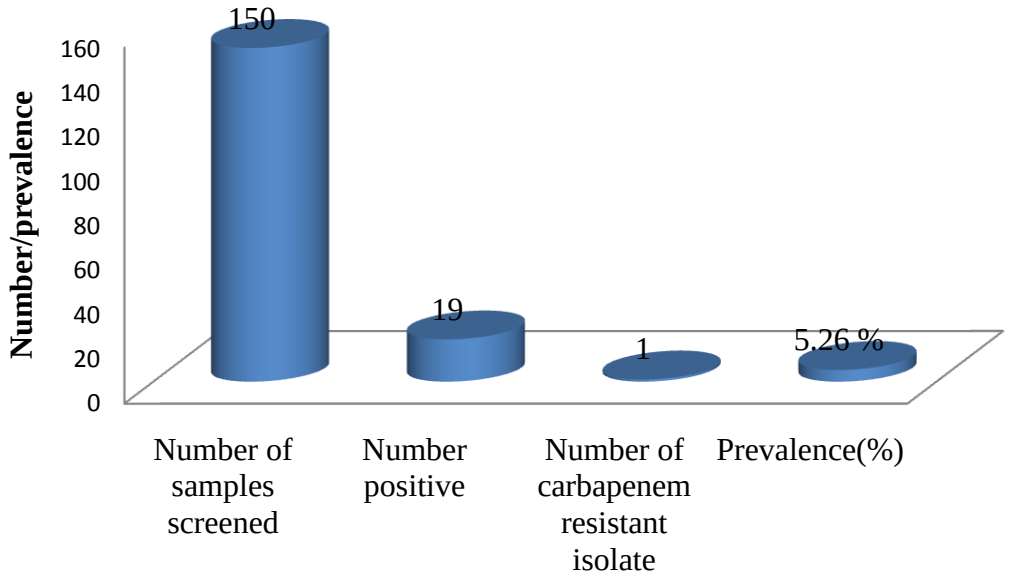


Figure 4. 6: Prevalence of carbapenem resistant *Klebsiella pneumoniae* isolated from clinical samples in Zaria.

4.10 Occurrence of *Klebsiella pneumoniae* carbapenemase (KPC) among *Klebsiella pneumoniae* isolates from selected hospitals in Zaria.

The Occurrence of *Klebsiella pneumoniae* carbapenemase (KPC) among *Klebsiella pneumoniae* isolates from selected hospitals in Zaria is presented in Figure 4.7. Only one out of the 19 isolates screened was intermediately resistant to Imipenem. This isolate gave a negative Modified Hodge Test.

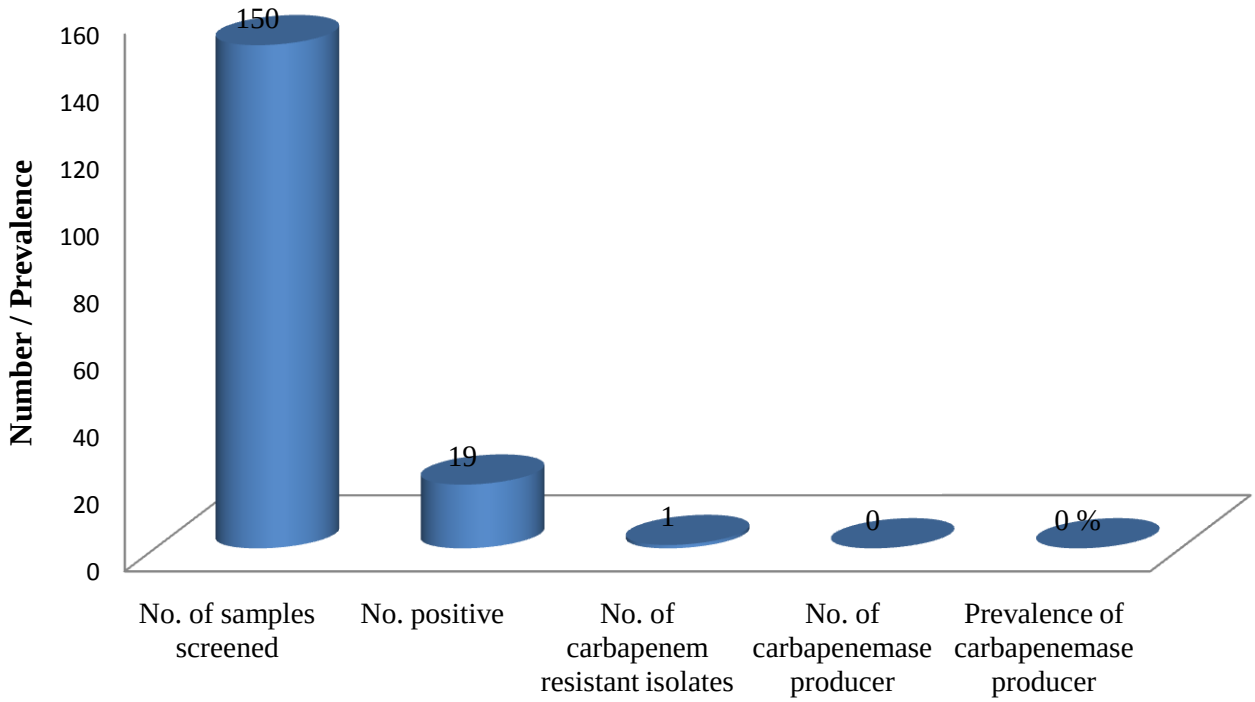


Figure 4. 7: Occurrence of *Klebsiella pneumoniae* carbapenemase (KPC) among *Klebsiella pneumoniae* isolates from selected hospitals in Zaria.

CHAPTER FIVE

5.0 DISCUSSION

Klebsiella pneumoniae is a major cause of community and a hospital acquired infections in humans and therefore poses a great challenge to the public health. It is an opportunistic human pathogen that causes infections such as pneumonia, septicemia, liver abscesses, urinary tract infections, soft tissue infections, and bacterial meningitis.

The overall prevalence of *Klebsiella pneumoniae* isolated from clinical samples in this study was found to be 12.67%. The overcrowded nature of some of the hospital wards and poor personal hygiene practice of the patients might be the reasons for the relatively high prevalence rate observed in this study. This is similar to that reported by Gangoue-Pieboji *et al.* (2005), who reported a prevalence of 12.0% in Younde, Cameroon. Higher prevalence of 36.4% and 20.0% were reported by Yusuf *et al*. (2011) in Kano and Aibinu *et al*. (2003) in Lagos respectively. However a lower prevalence of 9.3% was recorded by Yusha'u *et al*. (2007) in Kano. The difference in the prevalence is likely due to differences in geographical locations, category of patients (study population) involved and the type of samples collected.

The fact that *Klebsiella pneumoniae* is second only to *E. coli* as a urinary tract pathogen and that it constitutes a large proportion of the urinary tract flora argue in favour of the high prevalence observed in urine (16.18%) compared to other clinical samples since with low hygienic practices, *Klebsiella pneumoniae* will be isolated more in urinary tract infection. This finding is in agreement with that of Akter *et al.* (2014) where they reported high prevalence of *Klebsiella pneumoniae* in urine compared to other clinical samples.

In this study, a higher prevalence (13.33%) of *Klebsiella pneumoniae* was found in females compared to males (11.67%). This is likely due to differences in the anatomy of the females; short urethra and small distance between anal and vaginal opening hence easy invasion and colonization. This is similar to the reports of Anjun and Mir (2010) and Chickwendu *et al.* (2010), of a higher prevalence of *Klebsiella pneumoniae* in females compared to males.

A higher number of *Klebsiella pneumoniae* was isolated from age group 11-20 years (21.21%) and 21-30years (14.71%). This disagrees with the finding of Akter *et al.* (2014).

The development of antimicrobial resistance is a natural phenomenon. However, certain human actions accelerate its emergence and spread. The inappropriate use of antimicrobial drugs, including in animal husbandry, favours the emergence and selection of resistant strains, poor infection prevention and control practices contribute to further emergence and spread of antimicrobial resistance (WHO, 2015).

High level of resistance to Ampicillin (100%), Cefotaxime (94.7%) and Cotrimoxazole (78.9%) was demonstrated by the isolates screened. High level of resistance to Cefotaxime and Ampicillin observed in this study is likely due to emergence and spread of β-lactamases especially Cephalosporinases and penicillinases. The resistance mechanism of *Klebsiella* species to β-Lactam antibiotics involves the production of inactivating enzymes such as Sulphydryl variable (SHV) β-lactamases, Cefotaximase-Munich (CTX-M) β-lactamases and other extended spectrum β-lactamases (ESBLs) (Liu *et al*., 1992) and Cephalosporinases such as Amp C type β-lactamases. All these β- lactamases are constitutively produced at low levels, but sufficient to protect against β-lactam antibiotics. Routine use of Cotrimoxazole as prophylaxis, overproduction of the host dihydrofolate reductase (DHFR), mutations in the structural gene for

DHFR, and acquisition of a gene (dfr) encoding a resistant DHFR enzyme which is the most resistant mechanism in clinical isolates (Thomson, 1993) could be the reason for the high level of resistance observed in this study. The high level of resistance observed to these antibiotics is in accordance with the finding of Aruna and Mobashshera (2012) where they reported a high level of resistance to Ampicillin, Cefotaxime and Cotrimoxazole. Osundiya *et al*. (2013) also reported a high level of resistance to Cefotaxime.

Moderate level of susceptibility to Chloramphenicol (57.9%) and Tetracycline (42.1%) was seen in this study. These two antibiotics are used in clinical and veterinary medicine as prophylaxis and for growth promotion in animal foods in developing countries because of their availability and low cost as well as low toxicity and broad spectrum of activity. Misuse and overuse of these drugs have resulted in the widespread resistance determinants in different microorganisms, limiting their utility in treating infections (Aminov *et al.*, 2004). Decreased outer membrane permeability or active efflux could also be responsible for the resistance observed to these antiobiotics (Butaye *et al*., 2003).

High level of susceptibility was observed in response to Imipenem (94.7%), Gentamicin (78.9%) and Ciprofloxacin (63.2%) in this study. The high level of susceptibility to these antibiotics is likely due to low usage and probably because of less prescription of these antibiotics. Gentamicin and Imipenem are administered intramuscularly or intravenously hence they are not likely abused. Ciprofloxacin resistance in *Enterobactericeae* is majorly by the mutations in the molecules targeted by fluoroquinolones namely, DNA gyrase and DNA topoisomerase IV. Generally, multiple mutations are required to achieve clinically important resistance to fluoroquinolones in *Enterobacteriaceae* (Kocsis and Szabó, 2013). This argues in favour of the low level of resistance observed to Ciprofloxacin. The high level of susceptibility demonstrated

to Imipenem, Gentamicin and Ciprofloxacin in this study agrees with the study of Osundiya *et al*. (2013), Prado *et al.* (2007) and Chikwendu *et al.* (2010). High susceptibility level (94.7%) to Imipenem recorded in this study is lower than that recorded by Enwuru *et al*. (2012) where they reported a 100% susceptibility of *Klebsiella pneumoniae* to Imipenem in Lagos. Yusuf *et al.* (2012) reported 6-9% resistance of *Klebsiella pneumoniae* to Imipenem in Kano.

The high rate of resistance observed in the isolates can be attributed to antimicrobial abuse, poor hygiene, over use and misuse of drugs. All these human factors results in a "selective pressure" for the survival of resistant strains of bacteria. In the presence of an antibiotic agent, the susceptible bacteria are killed while those that carry the resistance gene naturally will survive in the presence of the antibiotic agent, replicate and their progeny will become the dominant type thorough out the microbial population (NIAID, 2015). Looking at the relationship between antibiotic usage and enhanced development of resistance to antibiotics, this study attests to the probable increase in the usage of antibiotics in the form of self medication, inappropriate antibiotics prescription, incorrect dose or duration of use, adulteration of drugs and use of antibiotics without prescription.

Differences in antimicrobial usage and infection control practices bring about the great variation observed in the prevalence of antibiotic resistance from one geographical area to another as well as between hospitals.

Multiple Antibiotic Resistance (MAR) index is used as a tool to analyze health risk and it is also helpful in monitoring the spread of bacterial resistance in a given population (Christopher *et al.,* 2013). It is considered as a good tool for risk assessment. Isolates with MAR index value higher than 0.2 are considered to have originated from high risk sources where antibiotics are often used

(Hemen *et al.,* 2012). The MAR index gives an insight on the number of isolates showing antibiotic resistance and the consequence risk zone in routine susceptibility testing.

The high level of drug resistance and MAR indices (0.25-0.88) observed in the isolates screened is worrisome because 84.21% of the *Klebsiella pneumoniae* isolates were resistant to 3 or more antibiotics while the remaining 15.79% of the isolates were resistant to only two antibiotics. Analysis of the MAR index of the isolates showed that 84.21% of the isolates had MAR index great than 0.3. High MAR index values are indicative of environment with high endemic disease potential and risk sources where drugs are often used. MAR index $\geq$ 0.2 indicates that isolates were recovered from individuals who have had contact with significantly contaminated or high risk site. The result of the 84.21% of the *Klebsiella pneumoniae* isolates that were resistant to 3 or more antibiotics observed in this study is higher than that of Osundiya *et al*. (2013) where they reported that 66.7% of the *Klebsiella pneumoniae* isolates were resistant to 3 or more antibiotics. So also the high percent of the isolates with MAR index greater than 0.3 observed disagrees with the work of Olonitola *et al*. (2007) where they reported that 50% of ESBL positive *Klebsiella pneumoniae* had MAR index greater than 0.3. The difference in the MAR index may be due to differences in the antibiotics used or it may be as result of increase in the emergence of multidrug resistant strains.

Isolates that were resistant to $\geq$3 antimicrobial agents were considered to be multidrug resistant (MDR) isolates. The antibiotic susceptibility test revealed that 84.21% (16/19) of the *Klebsiella pneumoniae* isolates screened were multidrug resistant (MDR) isolates. The high rate of MDR isolates in this study calls for concern. Among the MDR-enterobacteria, *Klebsiella* seems to acquire the greatest variety of genes encoding for resistance to most drugs of choice in the treatment of infection caused by Gram negative bacteria and it produced the greatest variety of

resistance enzymes such as Extended Spectrum Beta Lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemsases (KPC) (Paterson *et al*., 2003; Nobrega *et al*., 2013). The multidrug resistance observed may be due to either accumulation of multiple genes, each coding for resistance to a single drug, within a single cell or increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs (Nikaido, 2009). Carbapenem-resistant organisms often carry additional plasmid-borne genes against other antimicrobial drug classes, rendering them multidrug resistant (MDR) (Logan *et al.*, 2015).

Emergence and spread of carbapenemases account for the resistance of bacteria to carbapenems which were the "drug of last resort" in the treatment of infection caused by multidrug resistant Gram negative bacteria. The only treatment option that remains potentially toxic to carbapenems resistant bacteria is polymyxin B and colistin (Behera *et al*., 2008) and Tigecycline (Yusuf *et al.*, 2015). Due to the increase and spread of carbapenems resistant bacteria, in a 2013 Threat Report on Antimicrobial Resistance, the CDC prioritized CRE as an urgent threat (the highest level), requiring concerted commitment and action, and noted that ≈50% of hospitalized patients with bloodstream infection caused by CRE die from the infection (CDC, 2013a; 2013b).As such it is necessary to know the prevalence of carbapenem resistance in the clinical isolates. Failure to identify them may lead to inappropriate therapy, treatment failure, spread of KPC producing organisms among patients and may result in increased mortality.

The prevalence of carbapenem resistant *Klebsiella pneumoniae* in this study was 5.26%. The low prevalence of carbapenem resistant *Klebsiella pneumoniae* and low level of resistance (5.3%) demonstrated by the isolates to Imipenem in this study could be due to the fact that (i) Imipenem (and other carbapenems) usage is still low in Nigeria (ii) They are expensive as such they are not subjected to abuse. (iii) They are not readily available and are reserved for life threatening Gram

negative infections (iv) They are administered intravenously (v) They are not frequently prescribed.

Antibiotic resistance has no border to cross especially in setting where proper infection control is not in practice. It doesn't discriminate between the specimen from which the organism was isolated or even the gender and age of the patient from which it was isolated as demonstrated by the isolates screened in this study.

Klebsiella pneumoniae carbapenemase (KPC) production is one of the mechanisms of resistance used by *Klebsiella pneumoniae* to resist Imipenem. In this study the isolate that was resistant to Imipenem and Cefotaxime (a third generation Cephalosporin) was screened for carbapenemase production using the Modified Hodge Test as recommended by CLSI (2015). This isolate was found to be a non *Klebsiella pneumoniae* carbapenemase (KPC) producer, giving a prevalence of 0.0% for KPC producing *Klebsiella pneumoniae*. This suggests that the resistance is either due to porin loss which limits the entry of the carbapenems into the cell thereby resulting in decreased outer membrane permeability or over expression of β-lactamases (such as AmpC β-lactamases and ESBLs) possessing low-level carbapenemase activity (CLSI, 2015).

The prevalence of KPC producing *Klebsiella pneumoniae* observed in this study (0.0%) is lower than a prevalence of (29.9%) reported by Yusuf *et al*. (2015) among patients in intensive care unit and surgical wards of tertiary health care centers in Kano. The reason for the higher prevalence in their study is because they were dealing with hospitalized patients who form one of the populations at risk of habouring KPC organisms. Mohammed *et al*. (2015) reported that 10.2% of the 28 carbapenem resistant isolates screened for carbapenemase production by MHT were MHT positive (*Klebsiella pneumoniae* carbapenemase (KPC) producer) in Maiduguri.

Carbapenems resistance traits such as decreased outer membrane permeability, over expression of β-lactamases, production of cephalosporinase and porin loss are not transferable, unlike most of the carbapenemase genes. This explains why carbapenem-resistant isolates that do not produce carbapenemases are considered to be much less important from a public health perspective than carbapenemase producers. The spread of carbapenemase producers is by far the most important current clinical issue in antibiotic resistance in Gram-negatives, and must be strictly controlled (Nordmann *et al*., 2012).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, *Klebsiella pneumoniae* was isolated from clinical isolates in Zaria at the prevalence rate of 12.67%. Most of the isolates were resistant to Ampicillin, Cefotaxime and Cotrimoxazole while only one was intermediately resistant to Imipenem. Most of the isolates originate from high risk sources and that antibiotic resistance is on the increase as indicated by MAR index analysis.

Carbapenem resistance among clinical isolates of *Klebsiella pneumoniae* in Zaria occurs at the prevalence rate of 5.26%. Amongst which none was a *Klebsiella pneumoniae* carbapenemase producer. From the result of the antibiotic susceptibility test, we can conclude that 84.21% of the isolates were multidrug resistant isolates since they were resistant to more than three antibiotics.

Antibiotic resistance is on the increase in Nigeria and worldwide. The low level resistance recorded for Imipenem is an indication that it still remains one of the antibiotics of "last resort" for infections caused by multidrug resistant pathogens. The occurrence of carbapenem resistant organism is on the increase even though it remains low and is less common when compared to the occurrence of extended-spectrum β-lactamase (ESBL) and AmpC producing organisms.

The Modified Hodge Test is a readily available confirmatory test for carbapenemase production. It can be perform in many clinical microbiology laboratories since it is less expensive and requires less expertise. Studies have shown that MHT is very sensitive and reliable for the detection of carbapenemases.

6.2 Recommendations

1. Carbapenems should be reserved for life threatening Gram negative infections. Uncontrolled sales of antibiotics in the streets, markets and pharmacy shops should be stopped.
2. All isolates resistant to any of the carbapenems and/or third generation cephalosporin should be screened for carbapenemase production to prevent the spread of KPC producing organisms.
3. Initiation of the actions necessary in the prevention of antimicrobial drug resistance for all patient populations and implementation of infection control practices are necessary to limit spread of KPC producers.

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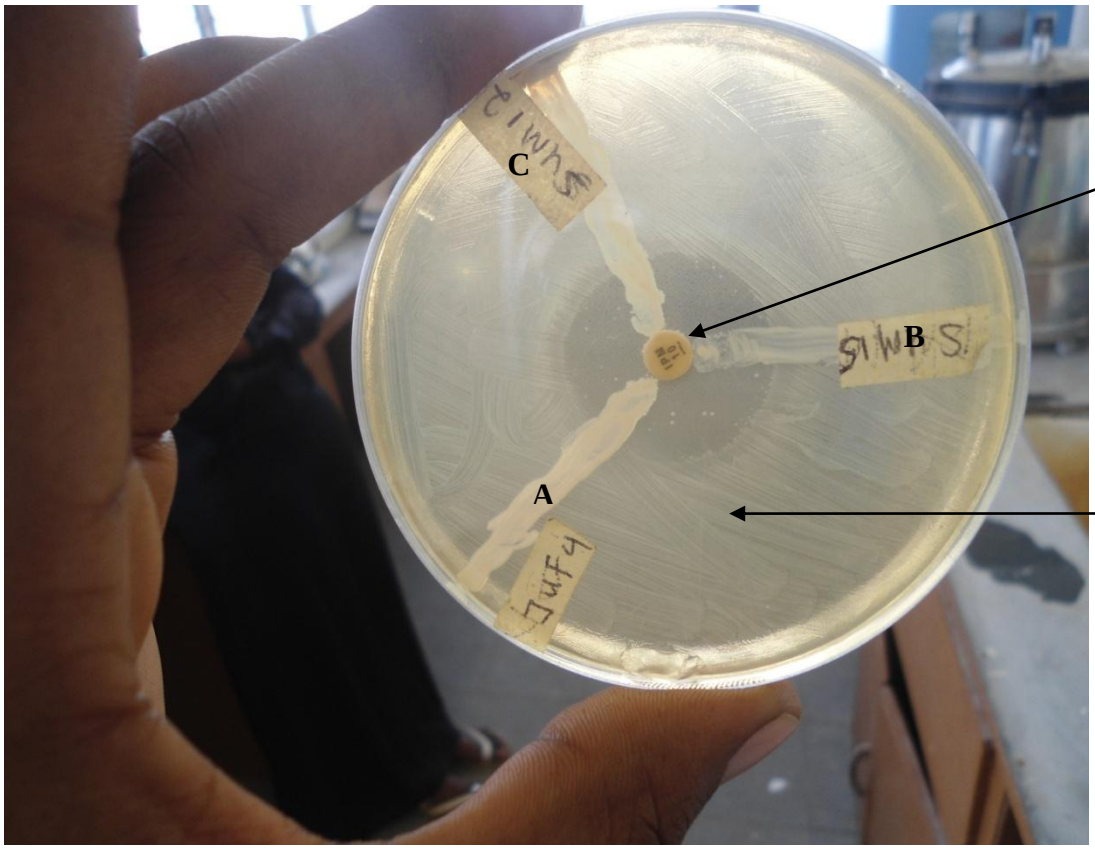
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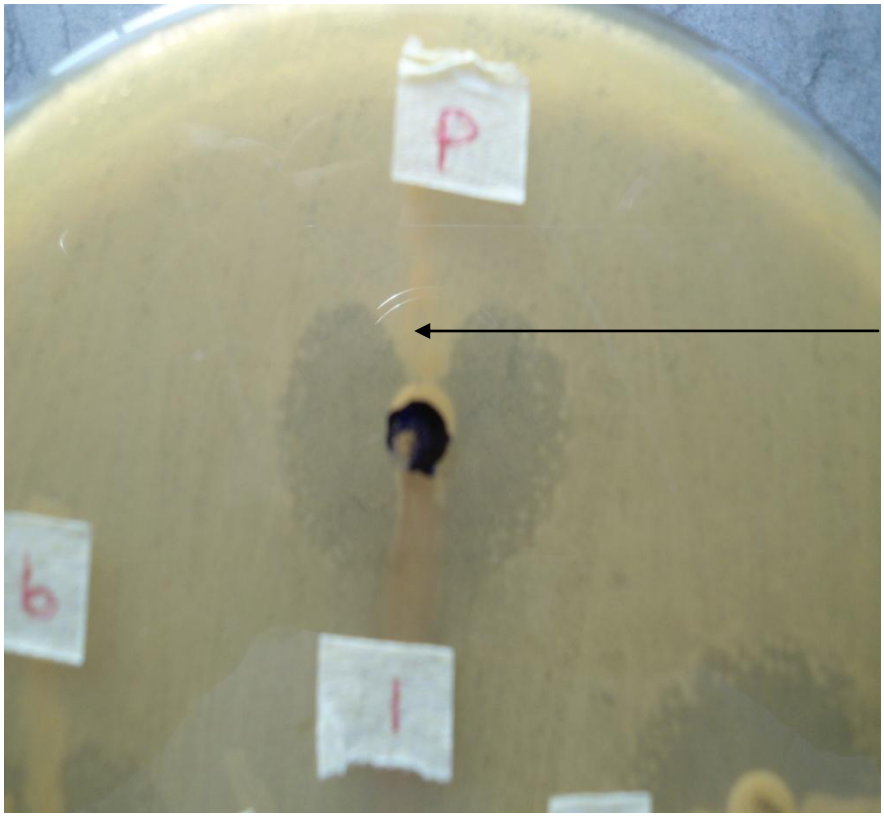
APPENDICES



Appendix I: Antibiotic susceptibility test on Mueller Hinton agar using Kirby-Bauer disc diffusion technique.



Appendix II: Negative result of test for the detection of *Klebsiella pnuemoniae* carbapenemase production using Modified Hodge Test (Cloverleaf test). A, B and C are the test isolates.



MHT positive *Klebsiella pneumonia* ATCC BAA-1705.

Appendix III: Positive result of test for the detection of *Klebsiella pneumoniae* Carbapenemase production using MHT showing enhanced growth of the indicator strain in to the zone of inhibition along the streak of the MHT positive *Klebsiella pneumoniae*.

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.
Head of Department: Prof S.A Ado; B.Sc, M.Sc, PhD **E-mail: microbiology@abu.edu.ng**

INFORMED CONSENT FORM (ICF)

This Informed Consent Form is for patients with suspected causes of urinary tract infection, respiratory tract infection or wound infection attending some selected hospitals in Zaria, Kaduna State. We are inviting you to participate in this research work titled **"Prevelance of Carbapenem Resistant *Klebsiella pneumoniae* in Clinical Samples From Some Selected Hospitals in Zaria."**

Principal Investigator:	Hussaini, Ibrahim Mohammed
Collaborating Investigators:	Prof. O.S. Olonitola
	Dr A.B. Suleiman
Name of Organization:	Department of Microbiology
	Ahmadu Bello University, Zaria - Nigeria
Name of Sponsor:	Parents
Name of Proposal:	Postgraduate Research Proposal

This Informed Consent Form has two parts:

- Information Sheet (to share information about the research with you)
- Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: INFORMATION SHEET

Introduction

I am Hussaini, Ibrahim Mohammed, a postgraduate student of the Department of Microbiology, Ahmadu Bello University, Zaria, carrying out a research work under the supervision of Prof. O.S. Olonitola and Dr A.B. Suleiman.

We are conducting a research work on **"Prevelance of Carbapenem Resistant *Klebsiella pneumoniae* in Clinical Samples From Some Selected Hospitals in Zaria."**I will give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to pause as we go through the information and I will take time to explain. If you have questions, you can ask me, the study doctor or the staff.

Purpose of the research

The purpose of this research is to determine the prevelance of carbapenem resistant *Klebsiella pneumoniae* in clinical samples from some selected hospitals in Zaria.

Participant selection

We are inviting all patients with suspected causes of urinary tract infection, respiratory tract infection or wound infection attending some selected hospitals in Zaria to participate in research.

- *Do you know why we are asking you to take part in this study? YES.....NO……..*
- *Do you know what the study is about? YES..... NO. .....*

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/hospital. You may change your mind later and stop participating even if you agreed earlier.

- *If you decide not to take part in this research study, do you know what your options are? YES…… NO……*
- *Do you know that you do not have to take part in this research study, if you do not wish to? YES…… NO …….*
- *Do you have any questions? YES……. NO. ……*

Procedures and Protocol

When you have agreed to participate in this research, your urine, sputum or wound swab will be collected and transported to the laboratory for processing. At the end of the research leftover samples will be discarded.

Risks

There is a risk that you may share some personal or confidential information by chance, or that you may feel uncomfortable talking about some of the topics. However, we do not wish for this to happen. You do not have to answer any question or take part in the research if you feel the question(s) are too personal or if talking about them makes you uncomfortable.

Benefits

If you participate in this research, you will have the following benefits:

- ✓ Your will be screen for *Klebsiella pneumoniae.*
- ✓ *Antibiotic susceptibility testing will be carried out to determine the drug to be used.*
- ✓ *Isolates resistant to carbapenem will be screen for carpanemase enzyme.*

Confidentiality

With this research, it is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a study number on it in place of your name. Only the principal investigator will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except the principal investigator.

- *Did you understand the procedures that we will be using to make sure that any information that we as researchers collect about you will remain confidential?.....*
- *Do you have any questions about them?.....*

Sharing the Results

The knowledge that we get from doing this research will be shared with you through clinic meetings with your health care provider before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so, refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Who to Contact

If you have any questions you may ask your health care provider now or later even after the study has begun. If you wish to ask questions later, you may contact any of the following:

Hussaini, Ibrahim Mohammed 08142446864, 08076420648.

- *Do you know that you do not have to take part in this study if you do not wish to? ……*
- *Do you know that you can ask me questions later, if you wish to? ……*
- *Do you know that I have given the contact details of the person who can give you more information about the study?.....*
- *You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?.....*

PART II: CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read and translated to me in a language that I understand. I have also talked it over with my health care provider to my satisfaction. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional sheet to keep for myself. I therefore consent voluntarily to participate as a participant in this research.

Name of Participant: .....

Signature of Participant: .....

Date: .....
Day/month/year

Statement by Witness

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness: ..... AND Thumb print of participant

Signature of witness: _____

Date _____
Day/month/year

Statement by the Researcher/Person Taking Consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Urine, sputum or wound swab sample will be taken

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent: .....

Signature of Researcher /person taking the consent: _____

Date _____
Day/month/year

Appendix IV: Copy of the consent form used in the study.



MINISTRY OF HEALTH AND HUMAN SERVICES

KADUNA STATE OF NIGERIA

ADDRESS: Independence Way, P.M.B. 2014, Kaduna.

MOH/ADM/744/VOL.1/333

19th OCT, 2015

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

PREVALENCE OF CARBAPENEM RESISTANT KLEBSIELLA PNEUMONIAE IN CLINICAL SAMPLES FROM SOME SELECTED HOSPITALS IN ZARIA

Name of Principal Investigator:	HUSSEINI IBRAHIM MOHAMMED
Address of Principal Investigator:	Dept. of Microbiology Ahmadu Bello University Zaria, Kaduna State.
Date of receipt of Application	20th Aug, 2015
Date of Ethical Approval	29th Sept, 2015

This is to inform you that the Research described in the submitted Protocol, the Consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

TUMAN DANIEL ALI ESQ
SECRETARY
FOR: CHAIRMAN

KADUNA STATE MINISTRY OF HEALTH
OPEN REGISTRY
DESPATCHED
SIGN DATE 19/10/15

Phone No.: 08184078693 Website:ttp://www/moh:kd.gov.ng. Email: health@kdsg.gov.ng.

Appendix V: Ethical approval from Ministry of health and human resources, Kaduna state of Nigeria.