Molecular Characterization of Carbapenem Resistant *Acinetobacter baumannii* isolated from Clinical Samples obtained from In-patients of Two Selected Hospitals in Minna, Nigeria

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ABSTRACT

Carbapenem resistant *Acinetobacter baumannii* (CRAB) are fast rising threat to the global medical health care system. This study characterized carbapenem resistant *A. baumannii* isolated from clinical samples obtained from in-patients of two selected hospitals in Minna, Niger State. One-hundred and five samples (105) comprising of urine (35), sputum (35) and wound swab (35) were collected from in-patients of each hospital. The samples were inoculated on MacConkey agar for the isolation of Gram negative bacteria. *Acinetobacter baumanii* isolates were identified using biochemical tests and polymerase chain reaction (PCR). The resistance profile of the isolates were determined using the Kirby Bauer's disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute guidelines. Carbapenemase production was detected using simplified carbapenem inactivation method, while the presence of β-lactamase encoding genes (blaOXA-23 and blaOXA-51) were detected using PCR. The results indicated that 7(5.65%) *A. baumanii* isolates were isolated from all the samples. The isolates were identified as strain Ab7 (42.86%), NCTC_7364 (28.57%), Ab21 (14.29%) and NCTC_7412 (14.29%). Resistance against meropenem and imipenem shown by the isolates were 71.5% and 85.7% respectively. Total (100%) resistance to Trimethoprim-sulphamethaxole, amoxicillin clavulanic acid, fosfomycin and tetracycline was also observed. Whereas, they were highly susceptible to ciprofloxacin (85.7%), gentamycin (85.7%), colistin (71.4%) and ceftriaxone (71.4%). The results also indicated that 100% and 42.86% of the isolates were positive for blaOXA-51 and blaOXA-23 genes respectively. High prevalence of CRAB among patients was recorded. This is of concern, routine surveillance and comprehensive infection control measures are needed to minimize the spread of this pathogen.

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Acinetobacter baumannii is undoubtedly one of the most successful pathogens responsible for hospital acquired nosocomial infections in the modern healthcare system. *Acinetobacter baumannii* has been found to be a normal inhabitant of human skin, throat, respiratory and intestinal tract of hospitalized patients while other reservoirs include the medical equipment within the hospital environment as well as the patients and staff (Towner, 2019). The organism has the ability to accumulate diverse mechanisms of resistance, leading to the emergence of strains that are resistant to all commercially-available antibiotics including the carbapenem class of antibiotics (Lolans *et al.,* 2016).

Carbapenem resistant *Acinetobacter baumannii* (CRAB) is a perilous nosocomial pathogen causing substantial morbidity and mortality (Lee *et al.,* 2017). Healthy people usually do not acquire CRAB infections or colonization and most CRAB infections are healthcare-associated infections (HAIs) occurring in people who have underlying medical conditions or certain types of healthcare exposures, such as immunocompromising conditions, recent prolonged stays in health care settings, invasive medical devices such as breathing tubes, feeding tubes, and central venous catheters, open wounds from surgery or a history of taking certain antibiotics for long periods of time (Towner, 2019).

Due to the prevalence of infections and out breaks caused by carbapenem resistant *A. baumannii* which are also usually multidrug- resistant, very few antibiotics are effective for treating infections caused by this pathogen (Lee *et al.,* 2017). Current treatment options for CRAB are limited and suffer from pharmacokinetic limitations, such as high toxicity and low

plasma levels (Isler *et al.,* 2018). As a result, CRAB is declared as the top priority pathogen by the World Health Organisation for the investment in new drugs (Piperaki *et al*., 2019).

1.2 Statement of the Research Problem

The estimated global incidence of *A. baumannii* infections is approximately 1,000,000 cases annually, of which 50% are resistant to multiple antibiotics including carbapenems (Piperaki *et al*., 2019). Nigeria as a developing country is seriously challenged in the area of accurate data collection and records keeping which is an essential step in the fight against diseases, especially the management of highly resistant strains with limited therapeutic options (Odewale *et al.,* 2016).

The hospital environment is one in which continuous close contact and interactions between in-patients, health workers and the environment is almost unavoidable and as such, poses a major challenge directly responsible for the rising rates of infections from carbapenem resistant *Acinetobacter baumannii* worldwide.

The emergence and spread of multidrug-resistant *Acinetobacter baumannii* strains in healthcare settings is a growing public health concern made worse by the limited information on the prevalence and antibiotic resistance patterns of these isolates (Odewale *et al.,* 2016).

1.3 Aim and Objectives of the Study

The aim of this study was to determine the molecular characteristics of carbapenem resistant *Acinetobacter baumannii* isolated from clinical samples from two selected hospitals in Minna, Nigeria.

The objectives of the study were to:

i. isolate and identify *Acinetobacter baumannii* from clinical samples obtained from in-patients.

ii. determine the antibiotic susceptibility patterns of isolated *Acinetobacter baumannii*.

iii. phenotypically detect the production of carbapenemase by carbapenem resistant *Acinetobacter baumannii* isolates

iv. identify genes encoding carbapenem resistance using molecular techniques.

1.4 Justification for the Study

Acinetobacter baumannii has shown high levels of resistance to multiple antibiotics, including carbapenems, which are the last resort drugs for treating multidrug-resistant bacterial infections. Understanding the prevalence and resistance patterns of *A. baumannii* in Niger State, Nigeria can help healthcare providers develop effective treatment strategies and implement infection control measures to prevent the spread of this bacterium. Additionally, this study can contribute to the global body of knowledge on *A. baumannii* and its antibiotic resistance patterns, which can inform future research and treatment strategies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Carbapenem Class of Antibiotics

Carbapenems are antimicrobial agents that are important in the treatment of severe and complicated nosocomial infections which may be invasive or life-threatening since they have the most comprehensive spectrum of activity as well as the most prominent potency against Gram-positive and Gram-negative bacteria (Nguyen and Joshi, 2021). Carbapenems are used for a wide range of infections, including those affecting the respiratory tract, skin, soft tissue, central nervous system, urinary tract, joints, muscles, gynecologic, obstetric, abdominal infections, febrile neutropenia, and cystic fibrosis (Nguyen and Joshi, 2021). This has led to an increase by 45% in the consumption rate of carbapenems globally from 2000 to 2010 (*Patrier and Timsit, 2020*).

Carbapenems belong to the beta-lactam class of antibiotics reserved for the treatment of high-risk or severe bacterial infections caused by suspected or confirmed multidrug-resistant bacteria (MDR) (Krisztina *et al.,* 2011). Other active antibiotics that belong to the beta-lactam group with a lesser spectrum of activity compared to carbapenems include the cephalosporins and penicillins. The mechanism of action of carbapenem involves killing bacteria by preventing cell wall synthesis (Zhanel, 2017). It works by binding to penicillin-binding proteins (PBP) which are a normal component of many bacteria and are vital for the cell wall synthesis of bacteria as they catalyse the cross linking of the bacterial cell wall.

Originally, the carbapenem group of antibiotics were developed from thienamycin a natural product of the Gram-positive bacterium *Streptomyces cattleya* which produces penicillin, cephamycin and thienamycin. This thienamycin eventually became the parent compound for the over 80 carbapenem compounds available today (Poirel *et al.,* 2017).

Carbapenems are the third most commonly used antibiotic worldwide for community-acquired infections in intensive care units and the most commonly used antibiotic for nosocomial infections due to their good safety profile and generally accepted tolerance (*Patrier and Timsit, 2020*). Carbapenems have a concentration-independent bacteria-killing effect, making them ideal for critically ill patients (Nguyen and Joshi, 2021). Against Gram negative bacteria, Carbapenems exhibit a wide range of activity while having a narrower spectrum against Gram positive bacteria (Poirel *et al.,* 2017). Common examples of carbapenems include imipenem, doripenem and meropenem. These three examples are especially effective against *Pseudomonas aeruginosa* and *Acinetobacter* species which are usually resistant to many other antibiotic groups (Bassetti *et al.,* 2019). Carbapenems are less harmful than other last-resort drugs such as polymyxins, making them more commonly prescribed (Nguyen and Joshi, 2021). They also have good stability against many β-lactamases and are usually successful in treating severe nosocomial infections and infections caused by pathogens such as *A. baumannii* (Meletis, 2015).

2.1.1 Groups and structure of carbapenem

In terms of structure, the term "carbapenem" is defined as the 4:5 fused ring lactam of penicillins with a double bond between C-2 and C-3 but with the substitution of carbon for sulfur at C-1 (Breilh *et al.,* 2013). The hydroxyethyl side chain of thienamycin is a radical departure from the structure of conventional penicillins and cephalosporins, all of which have an acylamino substituent on the β-lactam ring (Breilh *et al.,* 2013). In summary, the carbapenems are very similar to the (penams), but the sulfur atom in position 1 of the structure has been replaced with a carbon atom, and an unsaturation has been introduced.

Carbapenems are further broken down into groups with ertapenem being the lone member of group 1, it is though inefficient against non-fermentative Gram-negative bacilli and may be

more suitable for community-acquired infections (Nguyen and Joshi, 2021). Agents from group 2, such as meropenem, imipenem and doripenem, have broad-spectrum actions, are also active against multidrug resistant Gram-negative bacilli such as *Pseudomonas* and *Acinetobacter* species and are effective against nosocomial infections (Yoon *et al.*, 2014). Group 3 carbapenems, such as Tomopenem and Razupenem, are potent against non-fermentative Gram-negative bacilli and *Staphylococcus aureus* which are resistant against methicillin (Nguyen and Joshi, 2021). Figure 2.1 shows the chemical structures of some common carbapenems.

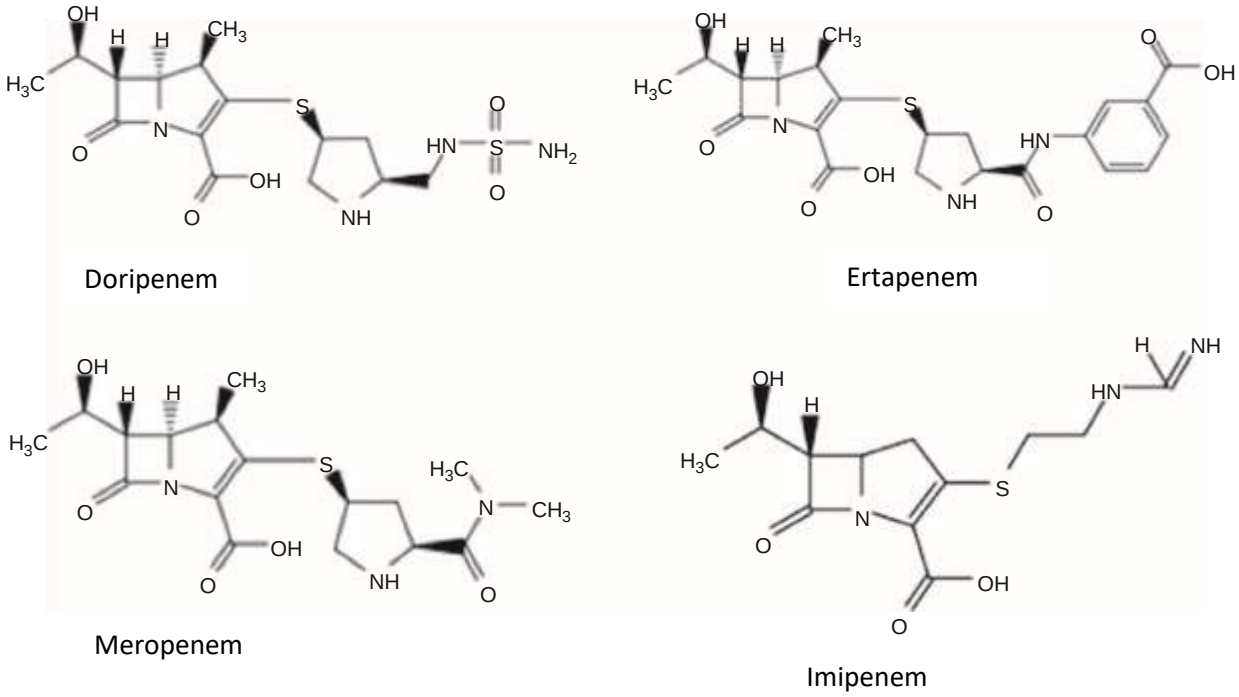

Figure 2.1: Chemical structures of some Carbapenems

Source: Nguyen and Joshi, (2021)

2.2 Antibacterial Activity of Carbapenem

Carbapenems demonstrate an overall broader antimicrobial spectrum *in vitro* than the available penicillins, cephalosporins, and β-lactam/β-lactamase inhibitor combinations (Bassetti *et al.,* 2019). Different carbapenems have different potencies against a variety of Gram-negative organisms and anaerobes and Gram-positive aerobes (Nguyen and Joshi, 2021). Meropenem, biapenem, ertapenem, and doripenem are slightly more effective against Gram-negative organisms while imipenem and doripenem show potent activities against Gram positive bacteria (Bassetti *et al.,* 2019). Important considerations here are the following: (i) ertapenem has a more limited spectrum, because it is not as active as imipenem or meropenem against *P. aeruginosa* (Hugonnet *et al.,* 2019); (ii) meropenem is not as potent as imipenem or doripenem against *Acinetobacter baumannii* (Oliver *et al.,* 2014); (iii) doripenem has a lower Minimum Inhibitory Concentration than imipenem and meropenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Mandell, 2019). 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 (Poirel *et al.,* 2017).

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 that patients are treated with more than one antibiotic (Krisztina *et al.,* 2011). A list of the antibiotic combinations which have been tested *in vitro* against common MDR organisms and their effects is presented in Table 2.1. 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 or additivity and strain dependence.

Table 2.1: *In vitro*-tested carbapenem combination therapies

Drug 1	Drug 2	Bacterium	Effect
Doripenem or imipenem	Vancomycin	MRSA	+
Doripenem	Teicoplanin	MRSA	+
Imipenem	Linezolid	MRSA	+
Imipenem	Teicoplanin	VRSA	+
Meropenem	Levofloxacin	*S. pneumoniae*	+
Meropenem	Rifampin	*S. pneumoniae*	−
Imipenem or meropenem	Clavulanic acid	*Nocardia brasiliensis*	−
Meropenem	Clavulanic acid	*Mycobacterium tuberculosis*	+
Meropenem	Ciprofloxacin	*A. baumannii*	+
Imipenem or meropenem	Colistin (and sulbactam)	*A. baumannii*	+
Meropenem	Sulbactam	*A. baumannii*	+
Imipenem	Azithromycin	*A. baumannii*	+
Imipenem	Rifampin	*A. baumannii*	+
Imipenem	Polymyxin B	*A. baumannii*	−
Imipenem	Amikacin	*A. baumannii*	−
Carbapenem	Fluoroquinolone	*P. aeruginosa*	+
Imipenem	Tachyplesin	*P. aeruginosa*	+
Meropenem or imipenem	Colistin	*P. aeruginosa*	+/−
Carbapenem	Aminoglycoside	*P. aeruginosa*	−
Meropenem	Polymyxin B	*P. aeruginosa*	−
Imipenem or meropenem	Tobramycin-rifampin	*B. cepacia*	+
Imipenem or meropenem	Ciprofloxacin	*B. cepacia*	+
Imipenem	Colistin	MBL *K. pneumoniae*	+
Imipenem	Tigecycline	ESBL *K. pneumoniae* and *E. coli*	−
Imipenem	Gentamicin	ESBL *K. pneumoniae* and *E. coli*	−

Some combinations demonstrate positive effects (+), such as extending spectrum or working additively or synergistically. Adverse effects (−) include increased resistance to one of the drugs used in the combination, as well as lack of synergy or additivity and strain dependence.
Key: MRSA: Methicillin-resistant *S. aureus*, VRSA: Vancomycin resistant *S. aureus*, MBL*:* Metallo-β-lactamase producing, ESBL: Extended Spectrum β-lactamase
Source: Krisztina *et al*. (2011)

`2.3 *Acinetobacter baumannii*

Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. An opportunistic pathogen, *A. baumannii* has a high incidence among immunocompromised individuals, particularly those who have experienced a prolonged stay of 90 days or more in a hospital (Montefour *et al.*, 2018). Commonly associated with aquatic environments (Sebeny *et al.*, 2018), it has been shown to colonize the skin as well as being isolated in high numbers from the respiratory and oropharynx secretions of infected individuals (Nowak and Paluchowska, 2016). In recent years, it has been designated as a "red alert" human pathogen, generating alarm among the medical fraternity, arising largely from its extensive antibiotic resistance spectrum. The genus *Acinetobacter*, as currently defined, comprises Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria with a DNA G + C content of 39% to 47%.

As a pathogen, *A. baumannii* specifically targets moist tissues such as mucous membranes or areas of the skin that are exposed, either through accident or injury. Skin and soft tissues infected with *A. baumannii* initially present with a "peau d'orange" appearance (similar to the skin of an orange) followed by a sandpaper-like presentation which eventually gives way to clear vesicles on the skin (Nowak and Paluchowska, 2016). In areas of skin disruption haemorrhagic bullae can be seen, with a visible necrotizing process followed by bacteraemia (Nowak and Paluchowska, 2016). If left untreated, this infection can lead to septicaemia and death.

Once *A. baumannii* is isolated in a hospital environment, this poses a significant risk, particularly in Intensive Care Unit wards where patients are chronically ill. As most of these patients are immunocompromised and spend a prolonged period of time in hospital, they represent a high risk group for *A. baumannii* infection (Montefour *et al.*, 2018). Patients that

acquire artificial devices such as catheters, sutures, ventilators and those who have undergone dialysis or antimicrobial therapy within 90 days are also at risk of developing *A. baumannii* infections (Montefour *et al.,* 2018). The respiratory tract, blood, pleural fluid, urinary tract, surgical wounds, skin and eyes may be sites for infection or colonization according to Almasaudi (2018).

2.4 Natural Habitats and Epidemiology of *Acinetobacter baumannii*

Acinetobacter genus members are believed to be ubiquitous in nature due to their ability to be recovered from soil, water, animals or humans; however, they are not limited to natural habitats (Sebeny *et al.,* 2018). For example, *A. baumannii* is predominantly found in hospital environment, specifically in ICUs of both adults and neonates, and burn, neurosurgery, surgical, medical and oncology units (Sebeny *et al.,* 2018).

Acinetobacter baumannii is most frequently isolated from wounded skin and tissues, respiratory system (in the pharynx, trachea or bronchi), bloodstream and central nervous system. It is also related to skin and tissue infections at surgical sites and catheter-associated urinary tract infections (Harding *et al.,* 2017). The common factor for those scenarios is a break in the anatomical barrier which allows *A. baumannii* to directly enter the affected area.

Overall, *A. baumannii* is responsible for many hospital acquired infections across several sites in a patient's body but often presents itself as ventilator-associated pneumonia (VAP) or bloodstream infections (Harding *et al.,* 2017). Pneumonia threatens patients dependent on mechanical ventilation since *A. baumannii* can exteriorly create biofilms on endotracheal tube which results in excessive colonization in the lower respiratory tract (Harding *et al.,* 2017).

Once *A. baumannii* strain is introduced to a hospital ward by a patient who is already colonized, it can thrive on dry surfaces under limited nutrient conditions. During an outbreak, it can be recovered from several sites from the patient's environment, such as bed curtains, furniture,

hand washing sinks and other hospital equipment such as ventilator tubing, arterial pressure transducers and humidifiers. Modes of transmission may include air droplets or skin of colonized patients; however, the bacteria most commonly spread from hospital workers via hands.

Infected patients can unknowingly carry it for days or weeks until *A. baumannii* strain is identified in clinical specimens. The overall interaction among *A. baumannii* pathogen, hospital environment (surface and equipment), high-risk patients and hospital staff is summarized in Figure 2.2.

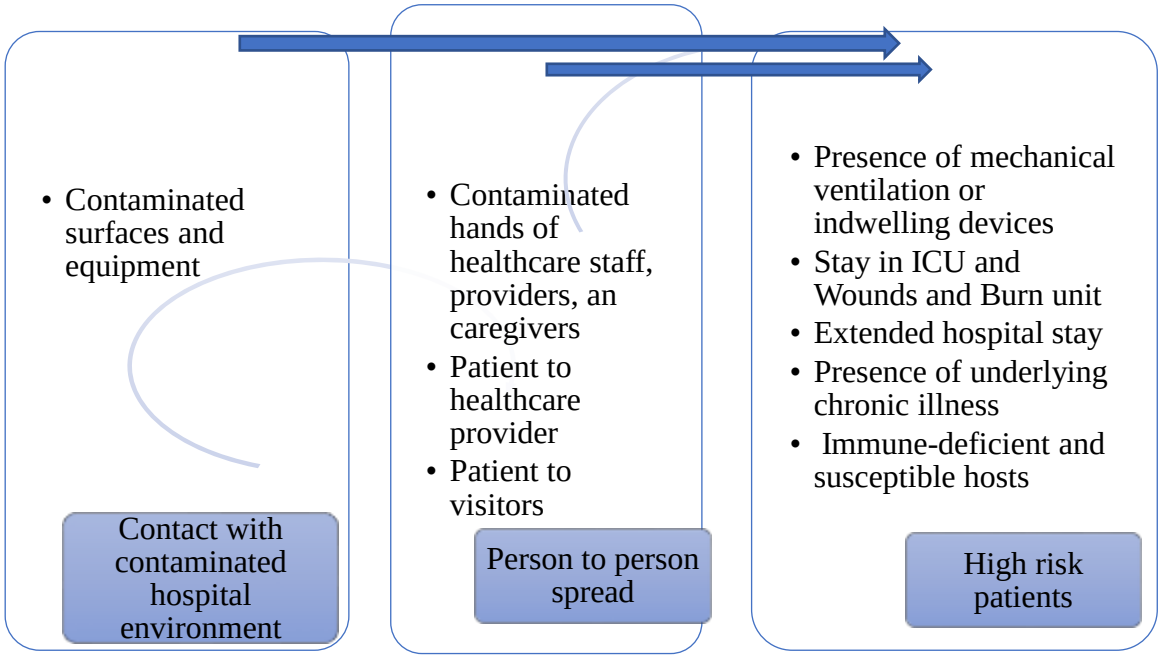

Figure 2.2: Schematic diagram of how epidemic *Acinetobacter baumannii* strains spread in hospitals (adapted from Nguyen and Joshi, 2021).

2.5 Carbepenem Resistance

The rapid emergence of multi- and pan drug-resistant strains of *Acinetobacter* highlights the organism's ability to quickly acclimatize to selective changes in environmental pressures. The up regulation of the organism's innate resistance mechanisms coupled with the acquisition of foreign determinants have played a crucial role in the express route the organism has taken to becoming a multidrug-resistant pathogen (Aoife *et al.*, 2012).

Many non-fermenting Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*., *Acinetobacter* spp., and *Stenotrophomonas* spp.), as well as the *Enterobacteriaceae* (e.g., *Klebsiella* spp., *Escherichia coli*, and *Enterobacter cloacae*.) and Gram-positive bacteria (e.g., *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., and *Nocardia* spp.), are or are becoming resistant to most clinically available carbapenems (Peleg *et al.,* 2018). This distressing pattern poses a major public health threat. Mechanisms of resistance to carbapenems include production of β-lactamases, efflux pumps, and mutations that alter the expression and/or function of porins and PBPs. Combinations of these mechanisms have caused high levels of resistance to carbapenems in *A. baumannii* (Peleg *et al.,* 2018).

2.5.1 Synthesis of carbapenem-hydrolysing β-lactamases (carbapenemases)

Inactivation or enzymatic degradation of carbapenems is the most significant carbapenem resistance mechanism in *A. baumannii* and is usually carried out by carbapenemase enzymes, which are found usually on plasmids and are very transmissible (Poirel *et al.,* 2017). There are four main molecular β-lactamase enzyme categories detected in *A. baumannii* according to their catalytic domain and substrate preference: classes A, B, C and D (Poirel *et al.,* 2017). Carbapenemases are from Class A, B and D, while cephalosporins are hydrolysed by class C enzymes. Metallo-β-lactamases (MBLs) or β-lactamases found in class B need a water molecule and a zinc ion (a divalent cation) to trigger and disrupt the β-lactam ring. On the other

hand, β-lactamases from class A, C and D are non-metallocarbapenemases that require serine for their catalytic activity (Nowak and Paluchowska, 2016). Examples of carbapenemases that are clinically relevant and occur among *A. baumannii* include *K. pneumoniae* carbapenemases (KPC) and Guiana extended-spectrum β-lactamase (GES) from class A; imipenemase (IMP), Verona integrin encoded metallo-β-lactamase (VIM), Seoul imipenemase (SIM) and New Delhi metallo-b-lactamase (NDM) from class B.

Carbapenem-hydrolysing oxacillinases (OXA-type carbapenemases) are more commonly seen in *A. baumannii* than MBLs but the latter demonstrate carbapenem-resistant hydrolytic activities that are 100 – 1000 times deadlier than that of OXA-type carbapenemases (Abouelfetouh *et al.,* 2019). Carbapenem-hydrolysing class D β-lactamases (CHDLs) or OXA hydrolyse isoxazolylpenicillin and are the most frequent cause of rendering *A. baumannii* resistant to carbapenem (Abouelfetouh *et al.,* 2019). There are six main groups in CHDLs: the intrinsic OXA-51-like and the acquired OXA-23-like, OXA-58-like, OXA-24/40-like, OXA-235-like and OXA-143-like β-lactamases (Abouelfetouh *et al.,* 2019).

Among the OXA-group β-lactamase genes mentioned above, overexpression of OXA-23 is the most widespread mechanism of carbapenem resistance in *A. baumannii* isolates (Poirel *et al.,* 2017).

2.5.2 Loss of outer membrane porins

Another carbapenem resistance mechanism in *Acinetobacter* species is related to membrane impermeability due to the reduced expression or mutation in porins. Outer membrane porins (OMPs) are grouped into four large families: general/nonspecific porins, substrate-specific porins, gated porins, and efflux porins (Rodloff *et al.,* 2016). Porins allow the passage of molecules of ≤1,500 Da into the cell (Rodloff *et al.,* 2016). General/nonspecific, substrate-specific, and efflux porins are the main families mediating resistance to carbapenems because

not all carbapenems interact with OMPs the same way; some OMPs are affected by certain carbapenems more than others (Siroy, 2015).

2.5.3 Efflux pumps

Carbapenem resistance due to overexpression of efflux porins, which are a part of a tripartite protein complex, is reported mostly for *A. baumannii* (Giamarellou *et al.,* 2018). Compared to outer membrane porins which are associated with antibiotic uptake, efflux systems are responsible for actively removing a number of antimicrobial agents by pumping them out of the cell which leads to multidrug resistance (Wong *et al.,* 2019). These pumps can extrude some carbapenems but not others. Efflux pumps are grouped into several superfamilies: the small multidrug resistance (SMR) superfamily, the resistance-nodulation-division (RND) superfamily, the major facilitator superfamily (MFS), the ATP-binding cassette (ABC) superfamily, and the multidrug and toxic compound extrusion (MATE) superfamily (Misra and Bavro, 2019).

Efflux pumps that eliminate carbapenems in *A. baumannii* belong to the RND superfamily and are a complex of proteins connecting the cytoplasm to the outside of the cell. These complex protein machines have three major components: a cytoplasmic membrane pump, a peripheral cytoplasmic membrane linker, and an outer membrane-periplasmic channel or efflux porin (Misra and Bavro, 2019). Ligands can enter the efflux system either at the cytoplasm-membrane interface or the periplasm-membrane interface, and a proton motive force can actively extrude the ligand. OprM and OprJ are two efflux porins involved in carbapenem resistance in *P. aeruginosa* (Zhang and Ma, 2010). These efflux porins assemble with MexA, MexC, or MexX, a peripheral cytoplasmic membrane linker, and either MexB, MexD, or MexY, a cytoplasmic membrane pump, to form a complete efflux complex. Resistance to carbapenems is mediated by overexpression of efflux pumps due to mutations in transcriptional regulatory proteins (Pankuch *et al.,* 2018). The true ligands of these efflux pumps are not

known; however, they may be involved in the efflux of quorum sensing auto inducers or their metabolic precursors.

2.5.4 Penicillin binding proteins

Mutations in the Penicillin Binding Protein (PBP) and/or decreases in PBP transcription also results in carbapenem-resistant phenotypes. Expression of PBPs in *A. baumannii*, is therefore reduced, resulting in carbapenem resistance (Krisztina *et al.*, 2011). Even though mutations modifying the production level or the binding affinity of PBPs lead to resistance in β-lactam antimicrobials, PBP's role is associated with only low-level carbapenem resistance in *A. baumannii* (Nowak and Paluchowska, 2016).

2.6 Carbapenem-Resistant *A. baumannii* in Nigeria: Previous Research

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) has emerged as a significant public health concern worldwide, including Nigeria. A few studies over the years have investigated the prevalence and distribution of CRAB in Nigerian healthcare settings thus shedding light on its epidemiological landscape. Research by Bashir *et al.* (2019), conducted in Kano, Nigeria, revealed a CRAB prevalence of approximately 6.5% across multiple healthcare facilities, including teaching hospitals and general hospitals. Odewale *et al.* (2016) investigated CRAB in Osogbo and reported an 8.5% prevalence in a tertiary care hospital. Nwadike *et al.* (2014) conducted research in Ibadan, reporting a 7.1% CRAB prevalence in a teaching hospital. These studies collectively emphasize that CRAB is not confined to specific regions but is distributed nationwide, highlighting the endemic nature of this multidrug-resistant pathogen in various Nigerian healthcare settings.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in General Hospital Minna and Ibrahim Badamasi Babangida Specialist Hospital, Minna, Niger State. Minna is the capital of Niger State which is the twelfth largest state in Nigeria in terms of population and the largest by land mass. Minna metropolis is situated at latitude 9°37' North and longitude 6°33' East. The northeast part of the city has a rock outcrop that acts as a physical constraint to development. Minna has an estimated population of 479,000 as at January 2022 (Macrotrends, 2022). Minna as a city lies in North Central Nigeria, and it is in the Savannah region of the country.

General Hospital Minna was established in 1946 in order to provide quality healthcare services, it is located at the metropolitan part of Minna as shown in Figure 3.1, thereby providing easy access to people around its environment. From inception, the hospital was controlled by the regional and native authorities as well as missionaries but is currently controlled by the Niger state ministry of health where it serves as a medical centre for medical internship (houseman-ship) for medical students from other institutions.

Ibrahim Badamasi Babangida Specialist hospital is a 100 bed capacity tertiary hospital located in Minna, Niger State as shown in Figure 3.2. It is specially equipped for the management of renal, cardiovascular, endocrine diseases, paediatric surgery, ear, nose and throat (ENT) and obstetrics and gynaecology (Usman *et al.*, 2019).

3.2 Study Population

The study was conducted among in-patients of General Hospital Minna and IBB Specialist Hospital, Minna, Niger State, Nigeria. The study population included only patients who had been admitted for 14 days or more. Out-patients and other in-patients who had not been hospitalized for up to 14 days were excluded from this study.

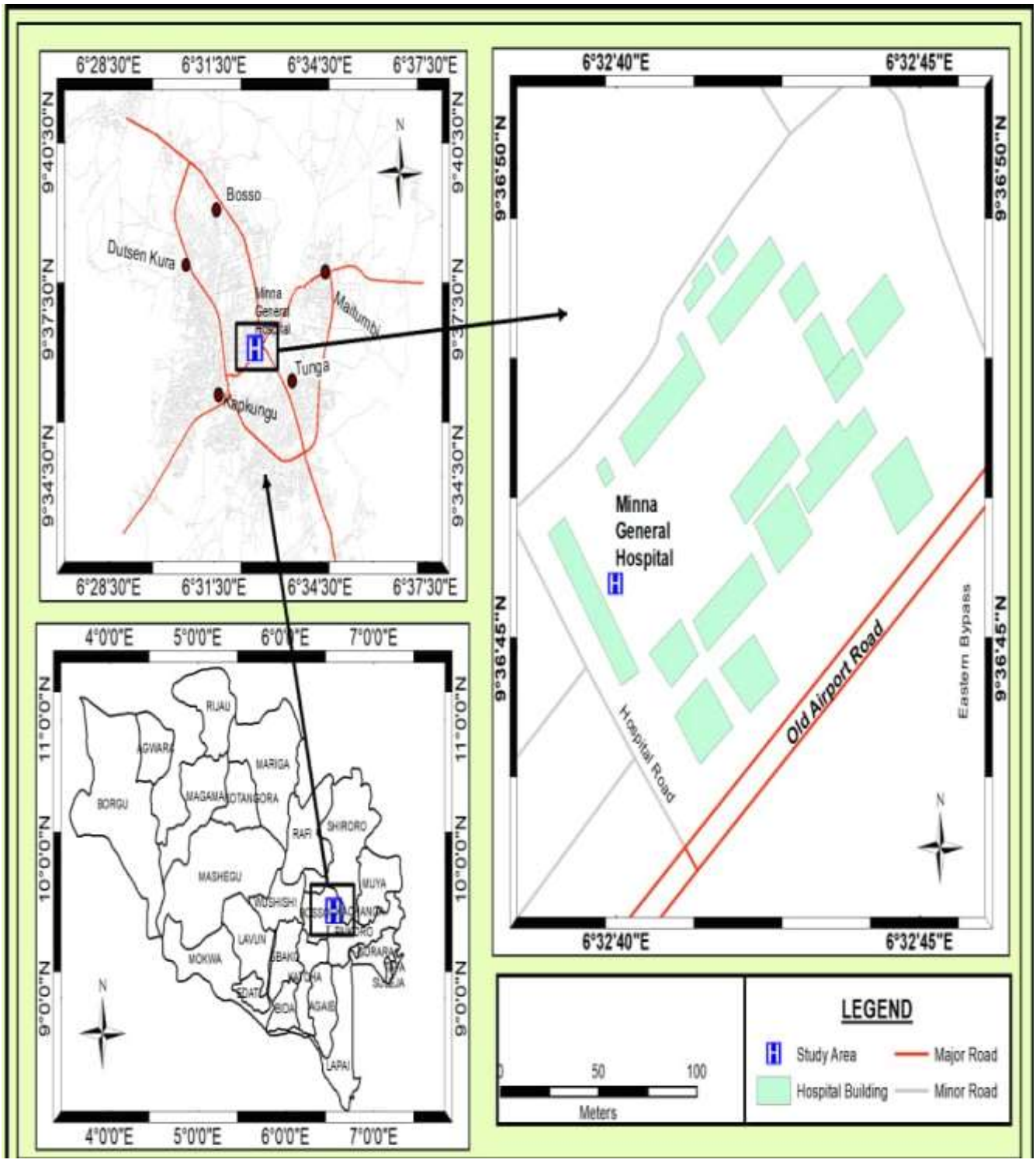

Figure 3.1: Map of Niger State, showing General Hospital, Minna.
Source: Geography Department, Federal University of Technology, Minna.

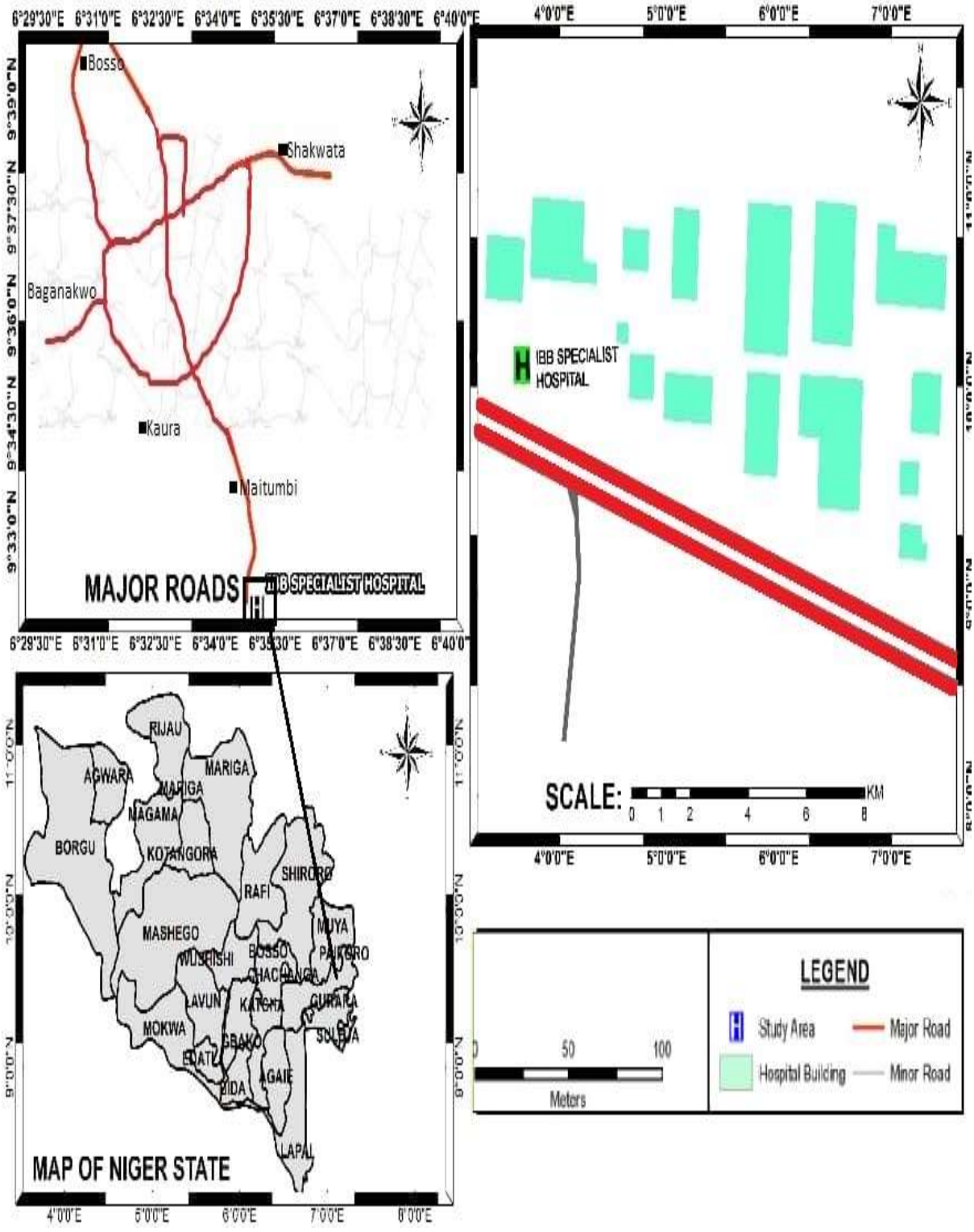

Figure 3.2: Map of Niger State, showing IBB Specialist Hospital, Minna.
Source: Geography Department, Federal University of Technology, Minna.

3.3 Sample Size Determination

The sample size was determined using Equation 3.1 (Fisher's formula). A prevalence of 8.5% was used from the study carried out in Osun state, Nigeria by Odewale *et al.* (2016).

$$n = \frac{z^2 p(1-P)}{d^2} \qquad (3.1)$$

Where: N = Sample Size

Z = 1.96 (constant)

P = Anticipated Population Proportion

d = 0.05 (constant)

Sample size equals to 117.4, total samples collected in this study = 210.
Each hospital provided 105 samples each.

3.4 Ethical Approval

Ethical approval was obtained from the Research, Ethics and Publication Committee of both hospitals (General Hospital, Minna and IBB Specialist Hospital, Minna) while written individual patient's consent was obtained before inclusion in this study. Letters of ethical clearance and consent forms are contained in appendices H and F.

3.5 Administration of Questionnaire

Brief questionnaires for descriptive data were issued to the patients involved in this study.

The questionnaire sample is given as appendix G.

3.6 Collection of Samples

A total of 105 samples comprised of urine (35), sputum (35) and wound swab (35) were collected from each hospital.

3.6.1 Urine sample

Appropriately labelled sterile specimen bottles were given to selected in-patients at the hospitals and the patients were informed on how to collect midstream urine in the sample bottles. The lids of the bottles were then tightly screwed after which samples were transported

to the Centre for Genetic Engineering and Biotechnology laboratory, Federal University of Technology, Minna for analysis (Karah *et al.*, 2020).

3.6.2 Wound swab

Sterile swab sticks were used to collect wound samples. The sterile swab stick was moved across the surface of the wound in a zig zag motion, at the same time being rotated between the fingers. In cases where the wound surface was dry, the swab stick tip was moistened with a physiological saline (0.85% NaCl) to increase the chance of recovering organisms from the surface (Cooper, 2010). On collection of samples, swab sticks were returned to their containers and transported to the Centre for Genetic Engineering and Biotechnology laboratory, Federal University of Technology, Minna for analysis.

3.6.3 Sputum sample

Sterile specimen bottles were given to selected in-patients at the hospitals, the patients were informed on how to collect the sputum in the sample bottles. After sample collection, the bottles were labelled appropriately and the lids of the bottles tightly screwed. Samples were then transported to the Centre for Genetic Engineering and Biotechnology laboratory, Federal University of Technology, Minna for analysis (Karah *et al.*, 2020).

3.7 Isolation of *Acinetobacter baumannii*

Samples collected were inoculated on selective media such as MacConkey agar and the Leeds *Acinetobacter* medium and incubated at 37°C for 24 hours for the isolation of *Acinetobacter baumannii*.

3.7.1 Sample inoculation on MacConkey Agar

MacConkey agar was prepared according to manufacturer's instructions for the selective isolation, cultivation, and differentiation of Gram negative bacteria based on their ability to ferment lactose. Lactose-fermenting organisms appeared as red to pink colonies while

Acinetobacter which is a non-lactose fermenting organism appeared as colourless or transparent colonies after 24 hour of incubation at 37°C.

3.7.2 Sub-culturing

The suspected isolates obtained from the MacConkey Agar were sub-cultured on Leeds *Acinetobacter* selective medium which was prepared according to the manufacturer's instruction using a sterile wire loop and incubated at 37°C for 24 hours. After incubation, distinct colonies which were pink, circular, smooth and mucoid were sub-cultured unto nutrient agar slant, incubated at 37°C for 24 hours and stored in the refrigerator for further analysis.

3.8 Identification of Isolates

Isolates stored on nutrient agar were identified using Gram staining and biochemical tests such as oxidase, motility, methyl red, urease, indole and catalase tests as described by Cheesebrough (2010).

3.8.1 Gram staining

A smear of each isolate was prepared and heat fixed on clean grease free slide. The smear was stained with 0.5% crystal violet for 1 minute rinsed with water and then stained with Grams iodine for 60 seconds. The stain was then washed off with distilled water and decolourised with 95% ethanol. It was then rinsed under running water, counter stained with safranin for a 30 seconds and washed with water. The slide was then blotted dry and examined under a microscope using immersion oil objective lens (x100 objective). Gram positive bacteria appeared purple while Gram negative bacteria appeared pink (Zuhair *et al.*, 2014).

3.8.2 Motility test

Motility test was used to differentiate motile from non-motile organisms using hanging drop techniques. A clean cover slide was held between two fingers and molten vaseline placed at each edge of the cover slip. A drop of bacterial suspension was placed at the centre of the cover slip applied with vaseline. Then quickly and carefully the cover slip was inverted over the centre of the slide. The hanging drop preparation was examined under the microscope using x40 objective (Zuhair *et al.*, 2014).

3.8.3 Methyl red test

The test organism was grown in dextrose phosphate medium at 37°C for 24 hours and after the period of incubation, the broth culture was dispensed into a clear tube and three drops of methyl red was added and allowed to stand for 30 seconds. The pH indicator methyl red was to detect the presence of large concentrations of acid end products. The methyl red indicator in the pH range of 4 turned to a red colour with a ring on top of the solution which was indicative of a positive test while for negative result there was none (Zuhair *et al.*, 2014).

3.8.4 Oxidase test

Using a dropper pipette, one drop of 1% aqueous solution of tetramethyl-p-phyenlene diamine dihydrochloride solution was added to sterile filter paper on to which portion of the isolates were added (using sterile wire loop). A deep purple colour within 15 seconds indicated a positive result, while oxidase negative did not produce this colour. The enzyme in the presence of atmospheric O_2 oxidises colourless substrate tetramethyl- p – phenylenediamine dihydrochloride to form a dark purple patch (Zuhair *et al.*, 2014).

3.8.5 Urease production

Using a sterile inoculating needle, portions of the isolates were stabbed into urea agar. It was incubated at 37°C for 24 hours. Urease positive organisms showed a change in colour from

yellow to red or pink and while no change in colour indicated a negative result (Cheesebrough, 2010).

3.8.6 Catalase test

Catalase production was determined by adding a drop of 3-6% the substrate hydrogen peroxide (H_2O_2) to a smear of the culture on slide (Cheesebrough, 2010). If catalase was present, bubbles of free oxygen gas is produced. This indicated a positive catalase test result and the absence of bubble formation was a negative result for catalase test.

3.8.7 Indole test

The test was used to determine the ability of an organism to split Trytophan (amino acid) which was present in nutrient broth. It was used to differentiate gram negative bacilli. Isolates were grown in nutrient broth for 24 hours after which 5 drops of kovac's indole reagent was added and allowed to stand for 5mins. A red ring at the reagent layer indicated indole production while no changes indicated an indole negative result (Zuhair *et al.*, 2014).

3.9 Molecular Identification of *A. baumannii* Isolates

Suspected *A. baumannii* isolates were identified using Polymerase chain reaction (PCR). To perform PCR, the blaOXA-51-like gene which is intrinsic to *A. baumannii* with 5' AATGATCTTGCTCGTGCTTC 3′ (forward), 5′ CATGTCCTTTTCCCATTCTG 3′ (reverse) primers was used (Saber and Alireza, 2021). PCR was performed according to methods described by Xiaopeng *et al.* (2018).

A total volume of 45 µL was prepared by mixing 25 µL of PCR Master Mix (CWBio, Beijing, China) with 4 µL of forward and reverse primers and adding water. Then, 5 µL of sample lysate was added to the mix after which the PCR program consisting of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, elongation at 72°C for 1 min, and a final extension at 72°C for 5 min. The resulting PCR

products was examined by electrophoresis and visualization by gel documentation system on 1.5% agarose gel containing ethidium bromide. Polymerase chain reaction products were selected for sequencing and sequences were aligned using the BLAST software tool from the GENE bank to determine identities of the isolates.

3.10 Antibiotic Susceptibility Testing

Antibiotic susceptibility test was carried out by using the Kirby Bauer disc diffusion method. Suspension of the isolates adjusted to the turbidity of 0.5 Macfarland standard was prepared and spread onto Mueller-Hinton agar using a sterile swab stick. Single antimicrobial disc of Imipenem (10 µg), Meropenem (10 µg), Colistin sulphate (10 µg), Trimethoprim-sulfamethoxazole (25 µg), Amoxicillin-clavulanic acid (30 µg), Fosfomycin (50 µg), Gentamicin (10 µg) Ciprofloxacin (10 µg), Ceftriaxone (30 µg) and Tetracycline (10 µg) were applied aseptically on the surface of the inoculated plates. The plates were allowed to sit for a while at room temperature and then incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition around the antibiotic discs was measured and interpreted in accordance with the standard criteria and breakpoints (appendix B) recommended by the Clinical Laboratory Standards Institute (CLSI, 2017).

3.11 Multiple Antibiotic Resistance Index

The Multiple Antibiotic Resistance (MAR) index was also determined by following the procedure described by Krumperman (1983). The formula used to calculate the MAR index for each isolate is given below as equation 3.2

$$MAR\ index = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics against which isolate was tested}} \quad (3.2)$$

3.12 Phenotypic Test for Carbapenemase Production

Carbapenemase enzyme production was determined using the simplified carbapenem inactivation method as described by Xiaopeng *et al.* (2018). A prepared bacterial suspension adjusted to the turbidity of 0.5 McFarland standard for *A. baumannii* was diluted 1:10 in normal saline (using the direct colony suspension method) and inoculated onto Mueller Hinton Agar (MHA) plate, following the routine disk diffusion procedure. Plates were allowed to dry for 3–10 min. Then, a 12 hour old colony of the test organism grown on blood agar was smeared onto a Meropenem disk (10 µg; Oxoid, Hampshire, United Kingdom) to allow one side of the disk be evenly coated with the test bacteria; immediately afterward, the side of the disk having bacteria was placed on the MHA plate previously inoculated with *A. baumannii*. All plates were incubated at 35°C for 18 hours.

After incubation, plates were examined for the presence or absence of zones of inhibition around the Meropenem disk. No inhibition zone indicated carbapenemase production by the test organism and the isolate was classified as carbapenemase-positive while the presence of an inhibition zone around the Meropenem disk suggested either no carbapenemase production or very low-level production and the isolate was categorized as carbapenemase-negative.

3.13 Detection and Molecular Characterization of Genes Encoding Carbapenem Resistance

Detection of genes encoding carbapenem resistance (blaOXA-23-like) was performed by conventional polymerase chain reaction as described by Xiaopeng *et al.* (2018). Forward (5'-GATCGGATTGGAGAACCAGA-3') and reverse (5'-ATTTCTGACCGCATTTCCAT-3') primers described by Li *et al.* (2012) were used for the identification. A total volume of 45 µL was prepared by mixing 25 µL of PCR Master Mix (CWBio, Beijing, China) with 4 µL of forward and reverse primers and adding water. Then, 5 µL of sample lysate was added to the

mix after which the PCR program consisting of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 25 s, annealing at 52°C for 40 s, elongation at 72°C for 50s, and a final extension at 72°C for 6 min. The resulting PCR products were examined by electrophoresis and visualization by gel documentation system on 1.5% agarose gel containing ethidium bromide.

3.14 Data Analysis

Data analysis was carried out using Microsoft excel and quantitative data were represented as percentages in this study.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

Majority (n=124) of the samples collected showed positive growth on MacConkey agar. Appendix A shows the recorded results of isolates obtained with preliminary biochemical tests for confirmation.

4.1.1 Distribution and prevalence of Gram negative bacterial isolates in clinical specimens

Table 4.1 shows the distribution of Gram negative bacterial isolates obtained from the clinical samples. *E. coli* was the most prevalent 47 (37.90%) Gram negative bacteria followed by *Pseudomonas aeruginosa* 34 (27.42%), *Klebsiella pneumoniae* 21 (16.94%), *Enterobacter cloacae* 15 (12.09%), while *A. baumannii* was the least prevalent isolate 7 (5.65%). Highest prevalence of *A. baumannii* was observed in wound swabs 4 (57.14%), followed by sputum 2 (28.57%) while urine had the least 1 (14.28%).

Table 4.1: Distribution of Gram Negative Isolates in Different Clinical Specimens (n=124)

Specimen	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*E. coli*	*Acinetobacter baumannii*	*Enterobacter cloacae*	Total
Urine	7 (5.65 %)	8 (6.45%)	31(25.00 %)	1(0.81 %)	8(6.45 %)	55(44.36%)
Wound swab	9(7.26 %)	21(16.94%)	10(8.06 %)	4(3.23 %)	5(4.03 %)	49(39.52%)
Sputum	5(4.03 %)	5(4.03 %)	6(4.84 %)	2(1.61 %)	2(1.61 %)	20(16.12%)
Total	21(16.94%)	34(27.42%)	47(37.90 %)	7(5.65 %)	15(12.09 %)	124(100%)

4.1.2 Molecular identification of *A. baumannii* isolates

Plate 1 shows the agarose results of 16S rRNA PCR amplified products from extracted DNA of *A. baumannii* isolates. The blaOXA-51-like gene primer has a band size of 353 base pairs. All 7 suspected isolates of *A. baumannii* were confirmed as shown in the gel image with horizontal lines across all lanes. Lane (L), DNA molecular size marker (l000 bp ladder), Lane (U17b-S9b) show positive results with positive bands of 353 bp. Lane (N) is the negative control with no DNA template.

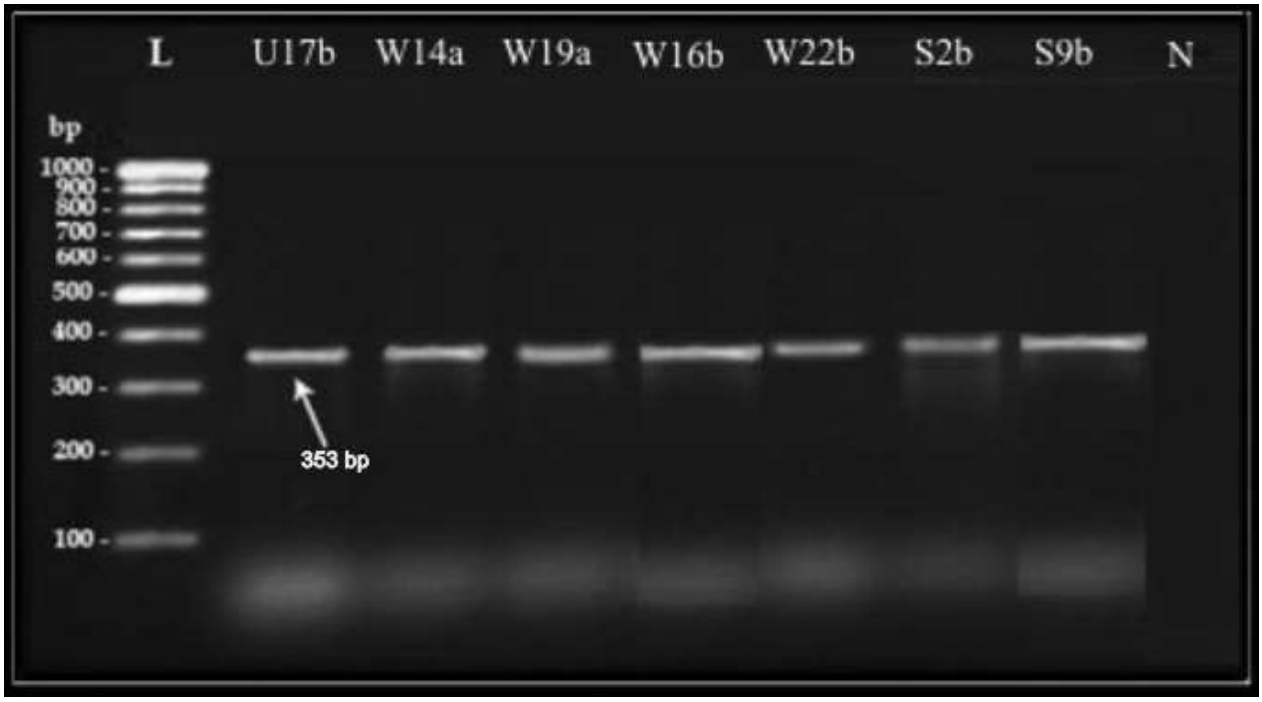

Plate 1: Agarose gel electrograph of blaOXA-51-like gene

4.1.3 Molecular Similarities between *A. baumannii* strains

The dendogram (Figure 4.1) shows the relative similarities between strains of *A. baumannii* isolated in this research. The seven isolates were identified as four different strains with isolates W14a, W19a and W22b showing varying degrees of 99% similarity to *Acinetobacter baumannii* strainAb7 while isolate W16b showed a 99.2% similarity index to *Acinetobacter baumannii* strainAb421. Isolates S9b and U17b showed varying degrees of 99% similarity to *Acinetobacter baumannii* strain NCTC_7364 while isolate S2b showed a 99.9% similarity index to *Acinetobacter baumannii* strain NCTC_7412. Details of *A. baumannii* isolate identification is given as Table 4.2 while the nucleotide sequences for each isolate is given in appendix E.

Figure 4.1: Dendogram of molecular similarity between *A. baumannii* isolates.

Table 4.2: Identity of the selected isolates

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident.	Accession Number
U17b	*Acinetobacter baumannii*	1386	1386	100%	0	99.4%	NZ_LT605059.1
W16b	*Acinetobacter baumannii*	1951	1948	99%	0	99.2%	CP014266.1
S9b	*Acinetobacter baumannii*	1670	1670	99%	0	99.3%	LT605059
W19a	*Acinetobacter baumannii*	2327	2327	97%	0	99.7%	CP002522
S2b	*Acinetobacter baumannii*	1670	1670	99%	0	99.9%	NZ_UFRP00000000
W14a	*Acinetobacter baumannii*	2327	2327	97%	0	99.9%	CP006963.1
W22b	*Acinetobacter baumannii*	2327	2327	97%	0	99.5%	CP006964.1

4.1.4 Distribution and prevalence of *A. baumannii* isolates in the two selected hospitals

The distribution of *A. baumannii* isolates according to age, gender and hospital is showed in Table 4.3. IBB specialist Hospital had the highest distribution of *A. baumannii* (71.40%). Most of the isolates were obtained from patients aged 36-50 years. Higher number of *A. baumannii* was isolated from male patients while the least were from female patients. In General Hospital, the prevalence of *A. baumannii was* 28.57%. All the isolates were obtained from male patients within the age of 51 -75 years.

Table 4.3: Age and gender specific distribution of *A. baumannii* among patients of the two hospitals (n=7)

	General Hospital		IBB Specialist Hospital		Total
Age	**Male**	**Female**	**Male**	**Female**	
0-17	0 (0.00%)	0(0.00%)	0 (0.00%)	0(0.00 %)	0(0.00%)
18-35	0(0.00%)	0(0.00%)	0(0.00%)	0 (0.00%)	0(0.00%)
36-50	0(0.00%)	0(0.00%)	2(28.57%)	1(14.29%)	3(42.86%)
51-75	2 (28.57%)	0(0.00%)	1(14.29%)	1(14.29%)	4(57.14%)
>75	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.0%)
Total	2(28.57%)	0(0.00%)	3(42.86%)	2(28.57%)	7(100%)

4.1.5 Antibiotic susceptibility profile of *A. baumannii* isolates

A. baumannii isolates showed high level of resistance to trimetoprim/sulfamethoxazole 7 (100 %), amoxicillin/clavulanic acid 6(85.7 %) and tetracycline 4 (57.1 %). The isolates were highly susceptible to ciprofloxacin 6 (85.7 %) and gentamycin (85.7%) followed by ceftriaxone (71.4%) and colistin (71.4 %). *A. baumannii* isolates mostly showed intermediate susceptibility to carbapenems, (Meropenem 3(42.9 %) and Imipenem 6 (85.7%)) as presented in Table 4.4.

Table 4.4: Antimicrobial susceptibility profile of *A. baumannii* isolates

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	6 (85.7)	1 (14.3)	0 (0.0)
Ceftriaxone	5 (71.4)	2 (28.6)	0 (0.0)
Gentamycin	6(85.7)	1 (14.3)	0 (0.0)
Tetracycline	0 (0.0)	3 (42.9)	4 (57.1)
Meropenem	2 (28.6)	3(42.9)	2 (28.6)
Imipenem	1 (14.3)	6 (85.7)	0 (0.0)
Fosfomycin	0 (0.0)	5 (71.4)	2 (28.6)
Amoxicillin/Clavulanic Acid	0 (0.0)	1 (14.3)	6 (85.7)
Trimethoprim/sulfamethoxazole	0 (0.0)	0 (0.0)	7 (100)
Colistin sulphate	5 (71.4)	2 (28.6)	0 (0.0)

4.1.6 Carbapenem susceptibility profile of *A. baumannii* isolates

Highest carbapenem resistance was recorded in 2 (28.6%) isolates (W14a and W22b) which showed full resistance to Meropenem but were intermediate to Imipenem. All the isolates except S9b showed intermediate resistance to Imipenem whereas, W19a and W16b exhibited susceptibility to meropenem. This data is represented in table 4.5.

Table 4.5: Susceptibility of *A. baumannii* isolates to carbapenems

Isolate code	Meropenem			Imipenem		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
U17b		+			+	
W14a			+		+	
W19a	+				+	
W16b	+				+	
W22b			+		+	
S2b		+			+	
S9b		+		+		

4.1.7 Multiple Antibiotic Resistance Index

Four *A. baumannii* isolates had a MAR Index of >0.2 and the remaining isolates had a MAR Index ≤ 0.2. The common resistance patterns are Sxt/Amc/Tcn, Sxt/Amc and Sxt/Amc/Tcn/Fos/Mer as presented in Table 4.6.

Table 4.6: Multiple Antibiotic Resistant Index (MARI) of *A. baumannii* isolates

Isolate code	Resistance Pattern	MAR index
U17b	Sxt/Amc/Tcn	0.3
W14a	Sxt/Amc/Tcn/Fos/Mer	0.5
W19a	Sxt/Amc	0.2
W16b	Sxt/Amc	0.2
W22b	Sxt/Amc/Tcn/Fos/Mer	0.5
S2b	Sxt/Amc/Tcn	0.3
S9b	Sxt	0.1

Key:

Sxt= Trimethoprim/sulfamethoxazole, Amc= Amoxicillin/Clavulanic Acid, Tcn= Tetracycline, Fos = Fosfomycin, Mer = Meropenem

4.1.8 Carbapenemase Production

Two out of the seven *A. baumannii* isolates were positive for the production of carbapenemase enzyme as shown in Table 4.7.

Table 4.7: Carbapenemase production by *A. baumannii* isolates.

Isolate code	Carbapenemase Producer
U17b	-
W14a	+
W19a	-
W16b	-
W22b	+
S2b	-
S9b	-

Keys:

+ means positive

- means negative

4.1.9 Molecular identification of carbapenem resistant gene in *A. baumannii* isolates

Plate 2 shows the agarose results of 16S rRNA PCR amplified products from extracted DNA of *A. baumannii* isolates. The blaOXA-23-like gene primer has a band size of approximately 501 base pairs. Lane (L), DNA molecular size marker (l100 bp ladder), Lane W14a, W22b and S2b show positive results with positive bands of 501 bp.

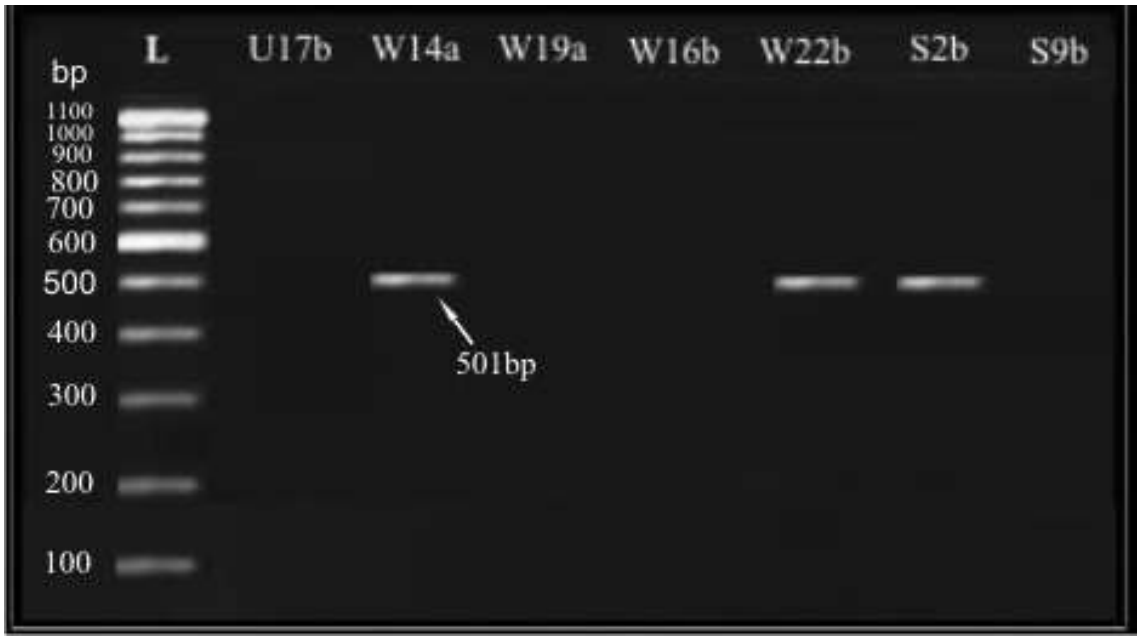

Plate II: Agarose gel electrograph of blaOXA-23-like gene

4.2 Discussion

4.2.1 Distribution and prevalence of *A. baumannii* isolates in clinical specimen

Acinetobacter baumannii is one of the most important bacteria in clinical settings because of its remarkable ability to acquire antibiotic resistance and adaptability to survive in the hospital environment. The prevalence of *A. baumannii* in this present study is 5.7%. This is similar to the prevalence obtained in similar studies carried out by Bashir *et al,* (2019), Odewale *et al.* (2016) and Nwadike *et al.* (2014) with their percentage prevalence being 6.5%, 8.5% and 7.1% respectively. However, higher prevalence of 14.19% and 24.81% have been reported by Agodi *et al.* (2015) in Italy and Koca *et al.* (2018) in Turkey respectively.

Among the various clinical specimen, the highest frequency of *A. baumannii* isolates were from wound swabs. The high prevalence of *A. baumannii* isolates from wounds in this study was predictable due to the fact that majority of the patients with wounds have spent a longer duration on hospitalization compared to others and also due to the fact that wounds are a common site of infection and colonization by this pathogen (Agaba *et al.,* 2017). Studies by Rahimi *et al.* (2019) and Ohadian *et al.* (2020) reported a high prevalence of *A. baumannii* in wound infections, with 32.8% and 26.2% respectively of isolates obtained from wound swabs.

Patients colonized or infected with CRAB can spread the bacteria to other patients via the contaminated hands of health care workers, through contaminated medical equipment, or a contaminated health care environment. This distribution pattern of *A. baumannii* is not uncommon as it is known to be an opportunistic pathogen that can cause infections in patients with weakened immune systems, particularly those who have been hospitalized for an extended period or have undergone invasive medical procedures. In general, the dissemination of *A. baumannii* in clinical samples may be due to its ability to cause different nosocomial infections and resistance to a wide range of antibiotics.

4.2.2 Antibiotic susceptibility profile of *A. baumannii* isolates

Acinetobacter baumannii isolates from this study showed high levels of resistance to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, tetracycline and fosfomycin. The result of findings by Rana and Asmaa, (2020) which reported 75.9% and 89.7% high levels of resistance to Trimethoprim/sulfamethoxazole and Amoxicillin/Clavulanic and Bashir *et al.* (2019) which also reported 85.7% and 78.4% levels of resistance respectively are in line with the results from this study.

The high level of resistance to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, and tetracycline is a concerning finding, as these antibiotics are commonly used to treat infections caused by *A. baumannii* (Castilho *et al.,* 2017). This suggests that alternative treatment options may need to be explored in cases where carbapenems are not effective against *A. baumannii* infections. The high levels of susceptibility to ciprofloxacin, gentamicin, and ceftriaxone and colistin sulphate on the other hand, suggest that these antibiotics may be effective treatment options for infections caused by *A. baumannii* in this particular setting.

High susceptibility levels to ciprofloxacin and gentamycin in this study were in disagreement with previous studies by Nwadike *et al.* (2014) and this difference may be due to varying prior exposure levels of patients to antibiotics.

The susceptibility profile from this study of *A. baumannii* isolates to two carbapenem antibiotics, Meropenem and Imipenem indicate the existence of varying degrees of resistance. All the *A. baumannii* isolates in this present study showed some level of resistance to carbapenems. This is also noteworthy, as carbapenems are often considered as the last resort antibiotics for treating infections caused by multidrug-resistant bacteria such as *A. baumannii*. Resistance to meropenem in this study was at 28.6% which was similar to the result carried out by Nwadike *et al.* (2014) that revealed a 35.7% resistance to the antibiotic. Emergence of CRAB isolates limits the available treatment options for *A. baumannii* infections. It is

important to note that *A. baumannii* is known for its ability to acquire antibiotic resistance mechanisms, including carbapenem resistance, through the acquisition of resistance genes on plasmids or other mobile genetic elements (Peleg *et al.*, 2018).

4.2.3 Multiple antibiotic resistance index (MARI)

Majority of the *A. baumannii* isolates had a MARI ≥ 0.2. This indicates that these isolates originated from sources where antibiotic exposure was high. Specifically, W14a and W22b were resistant to at least one antibiotic in five different classes of antibiotics. Overall, the MARI values suggest that *A. baumannii* isolates from this research exhibit a range of antibiotic resistance patterns. The results highlight the importance of continued monitoring of antibiotic resistance patterns in *A. baumannii* and other bacteria in clinical settings, to guide appropriate antibiotic use and prevent the emergence and spread of multidrug-resistant strains

4.2.4 Carbapenemase production

It was observed that 28.57% of the *A. baumannii* isolates were positive for the production of carbapenemase. This value is lower than the report from the study carried out by Niranjan *et al*. (2018) which was reported as 48.2% but higher than that of Alyousefi *et al.* (2019) which reported a production of 19.4%. The possible differences in the percentages of carbapenemase production may be due to larger sample sizes or geographic locations which could be affected by controlled antibiotic use. Carbapenemase production is a major mechanism of resistance in *A. baumannii,* and it can render the bacteria resistant to most antibiotics, including carbapenems, which are often used as a last resort treatment for infections caused by multidrug-resistant bacteria. The detection of carbapenemase-producing *A. baumannii* is, therefore, an important step in the management of infections caused by these bacteria.

Other *A. baumannii* isolates which were negative for carbapenemase production but exhibited resistance to carbapenems might suggest that their resistance is not due to carbapenemase

production but may be as a result of other mechanisms such as *the overexpression of* efflux pumps, formation of biofilms and mutations that alter the expression and/or function of porins and Penicillin Binding Proteins (PBPs). *Patrice and Laurent (2014) and* Kuo *et al.* (2019) *reported that non-carbapenemase producing carbapenem resistant A. baumannii are less of a threat to the health care system than carbapenemase producing isolates due to the fact that non-carbapenemase mechanisms of resistance are not transferrable thus making infections caused by such isolates easier to treat.*

4.2.5 Carbapenem resistance genes in carbapenem resisant *Acinetobacter baumannii*

The oxacillinase gene (blaOXA-51-like) was detected in all the *A. baumannii isolates obtained in this study.* This finding supports those of other studies like Durmaz *et al.* (2009) and Zhao *et al.* (2010) demonstrating that the detection of blaOXA-51-like gene can be used as a complimentary tool to identify *A. baumannii* at species level. This gene also confers a level of decreased susceptibility of the isolates to carbapenem (Ababneh *et al.,* 2021).

The most common carbapenemase gene a blaOXA-23 that belongs to class D β-lactamases. Two isolates (W14a and W22b) positive for the production of carbapenemase were also observed to harbour the blaOXA-23-like gene in addition to the oxacillinase gene (blaOXA-51-like) that was detected in all the *A. baumannii* isolates in the present study. The presence of both blaOXA-23-like gene and blaOXA-51-like gene in *Acinetobacter baumannii* has significant implications in terms of antimicrobial resistance. Several studies have reported that the co-existence of these two genes is associated with high levels of carbapenem resistance thereby making treatment of infections caused by these strains extremely challenging (Cai *et al.,* 2012; Hamouda *et al.,* 2016; Kuo *et al.,* 2019). Furthermore, the blaOXA-23-like gene is commonly associated with outbreaks of multidrug-resistant *A. baumannii* infections, particularly in healthcare settings (Hamouda *et al.,* 2016). Therefore, the detection of both

blaOXA-23-like gene and blaOXA-51-like gene in *A. baumannii* isolates raises concerns about the potential for the spread of multidrug-resistant strains in clinical settings.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

The prevalence of *A. baumannii* isolated from clinical samples of in-patients attending two selected hospitals in Minna was 5.65%. Other Gram negative bacteria obtained were *E. coli* (37.90%), *K. pneumoniae* (16.94%), *E. cloacae* (12.09) and *P. aeruginosa* (27.42%).

Acinetobacter baumannii isolates were identified as strains from Ab_7, Ab_421, NCTC_7364 and NCTC_7412.

All the *A. baumannii* isolates showed resistance to tetracycline, fosfomycin, trimethoprim/sulfamethoxazole and amoxicillin/clavulanic acid. However, they exhibited high susceptibility against colistin sulphate, ceftriaxone, ciprofloxacin and gentamycin.

Two out of the seven isolates (28.57%) were positive for carbapenemase production.

Carbapenem resistant genes; blaOXA-51-like and blaOXA-23-like were detected in 100% and 42.86% of the carbapenem resistant *A. baumannii* respectively.

5.2 Recommendations

Based on the result of these findings, the following recommendations were made:

1. There is a need for increased surveillance of carbapenem resistant *A. baumannii* infections in hospital settings, especially among the in-patients. This would aid in the early detection of infections and the implementation of appropriate control measures.

2. Antibiotic stewardship programs should be implemented to promote the judicious use of antibiotics and minimize the development of antibiotic resistance. This would help to preserve the efficacy of antibiotics and limit the emergence of multidrug-resistant bacteria.

3. Effective infection control measures, such as proper hand hygiene, isolation precautions, and environmental cleaning, should be implemented to prevent the transmission of *A. baumannii* and other multidrug-resistant bacteria in hospital settings.

4. Further research is needed to determine other molecular mechanisms of carbapenem resistance in *A. baumannii* and develop new treatment strategies.

5.3 Contribution to Knowledge

i. This study has contributed to the knowledge of carbapenem resistant *A. baumannii*, particularly in terms of its antibiotic resistance and prevalence in hospital settings.

ii. This study revealed a higher prevalence of *A. baumannii* in clinical samples obtained from IBB Specialist hospital compared to General Hospital Minna.

iii. The study also provides insight into the molecular mechanisms of resistance, including the presence of the blaOXA-51-like and blaOXA-23-like genes in 100% and 42.86% of the carbapenem resistant *A. baumannii* isolates obtained from clinical samples in the study area.

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Appendix A

Biochemical Test Results of Isolates

Sample code	Shape	L/F	Mot	M/R	Oxi	Urea	Cat	Indole	Suspected Isolate
U1a	Rod	+	+	+	-	-	+	+	*E. coli*
U2a	Rod	+	+	+	-	-	+	+	*E. coli*
U3a	Rod	+	+	+	-	-	+	+	*E. coli*
U4a	Rod	+	+	+	-	-	+	+	*E. coli*
U5a	Rod	+	+	+	-	-	+	+	*E. coli*
U6a	Rod	+	+	+	-	-	+	+	*E. coli*
U7a	Rod	+	+	+	-	-	+	+	*E. coli*
U8a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U9a	Rod	+	+	+	-	-	+	+	*E. coli*
U10a	Rod	+	+	-	-	-	+	-	*E. cloacae*
U11a	Rod	+	+	+	-	-	+	+	*E. coli*
U12a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U13a	Rod	+	+	+	-	-	+	+	*E. coli*
U14a	Rod	+	+	-	-	-	+	-	*E. cloacae*
U15a	Rod	+	+	+	-	-	+	+	*E. coli*
U16a	Rod	+	+	+	-	-	+	+	*E. coli*
U17a	Rod	+	+	+	-	-	+	+	*E. coli*
U18a	Rod	+	+	+	-	-	+	+	*E. coli*
U19a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U20a	Rod	+	+	+	-	-	+	+	*E. coli*
U21a	Rod	+	+	+	-	-	+	+	*E. coli*

U22a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U23a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U24a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U25a	Rod	+	+	-	-	-	+	-	*E. cloacae*
U26a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U27a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U1b	Rod	+	+	+	-	-	+	+	*E. coli*
U2b	Rod	+	+	+	-	-	+	+	*E. coli*
U3b	Rod	+	+	+	-	-	+	+	*E. coli*
U4b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U5b	Rod	+	+	+	-	-	+	+	*E. coli*
U6b	Rod	+	+	+	-	-	+	+	*E. coli*
U7b	Rod	+	+	-	-	-	+	-	*E. cloacae*
U8b	Rod	+	+	+	-	-	+	+	*E. coli*
U9b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U10b	Rod	+	+	+	-	-	+	+	*E. coli*
U11b	Rod	+	+	+	-	-	+	+	*E. coli*
U12b	Rod	+	+	+	-	-	+	+	*E. coli*
U13b	Rod	+	+	+	-	-	+	+	*E. coli*
U14b	Rod	+	+	+	-	-	+	+	*E. coli*
U15b	Rod	+	+	-	-	-	+	-	*E. cloacae*
U16b	Rod	+	+	+	-	-	+	+	*E. coli*
U17b	Rod	-	-	+	-	-	+	-	*A. baumannii*
U18b	Rod	+	+	+	-	-	+	+	*E. coli*
U19b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*

U20b	Rod	+	+	+	-	-	+	+	*E. coli*
U21b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U22b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U23b	Rod	+	+	-	-	-	+	-	*E. cloacae*
U24b	Rod	+	+	-	-	-	+	-	*E. cloacae*
U25b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
U26b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
U27b	Rod	+	+	+	-	-	+	+	*E. coli*
U28b	Rod	+	+	-	-	-	+	-	*E. cloacae*
W1a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W2a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W3a	Rod	+	+	+	-	-	+	+	*E. coli*
W4a	Rod	+	+	+	-	-	+	+	*E. coli*
W5a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W6a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W7a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W8a	Rod	+	+	+	-	-	+	+	*E. coli*
W9a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W10a	Rod	+	+	-	-	-	+	-	*E. cloacae*
W11a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W12a	Rod	+	+	+	-	-	+	+	*E. coli*
W13a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W14a	Rod	-	-	+	-	-	+	-	*A. baumannii*
W15a	Rod	+	+	+	-	-	+	+	*E. coli*
W16a	Rod	+	+	+	-	-	+	+	*E. coli*

W17a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W18a	Rod	+	+	-	-	-	+	-	*E. cloacae*
W19a	Rod	-	-	+	-	-	+	-	*A. baumannii*
W20a	Rod	+	+	+	-	-	+	+	*E. coli*
W21a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W1b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W2b	Rod	+	+	+	-	-	+	+	*E. coli*
W3b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W4b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W5b	Rod	+	+	+	-	-	+	+	*E. coli*
W6b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W7b	Rod	+	+	-	-	-	+	-	*E. cloacae*
W8b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W9b	Rod	+	+	-	-	-	+	-	*E. cloacae*
W10b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W11b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W12b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W13b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W14b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W15b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W16b	Rod	-	-	+	-	-	+	-	*A. baumannii*
W17b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W18b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W19b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W20b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*

W21b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W22b	Rod	-	-	+	-	-	+	-	*A. baumannii*
W23b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W24b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W25b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
W26b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
W27b	Rod	+	+	+	-	-	+	+	*E. coli*
W28b	Rod	+	+	-	-	-	+	-	*E. cloacae*
S1a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
S2a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
S3a	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
S4a	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
S5a	Rod	+	+	+	-	-	+	+	*E. coli*
S6a	Rod	+	+	+	-	-	+	+	*E. coli*
S7a	Rod	+	+	+	-	-	+	+	*E. coli*
S1b	Rod	+	+	+	-	-	+	+	*E. coli*
S2b	Rod	-	-	+	-	-	+	-	*A. baumannii*
S3b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
S4b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*
S5b	Rod	+	+	+	-	-	+	+	*E. coli*
S6b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
S7b	Rod	+	+	-	-	-	+	-	*E. cloacae.*
S8b	Rod	+	+	+	-	-	+	+	*E. coli*
S9b	Rod	-	-	+	-	-	+	-	*A. baumannii*
S10b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*

S11b	Rod	-	+	-	+	-	+	-	*P. aeruginosa*
S12b	Rod	+	+	-	-	-	+	-	*E. cloacae*
S13b	Rod	+	-	-	-	+	+	-	*K. pneumoniae*

Key: L/F: Lactose Fermentation; Mot: Motility; M/R: Methyl Red; Oxi: Oxidase; Urea: Urease; Cat: Catalase;

'U' represents Urine samples collected

'W' represents Wound Swab samples collected

'S' represents Sputum samples collected

Sample codes with 'a' represent General Hospital Minna while sample codes with 'b' represent IBB Specialist Hospital, Minna.

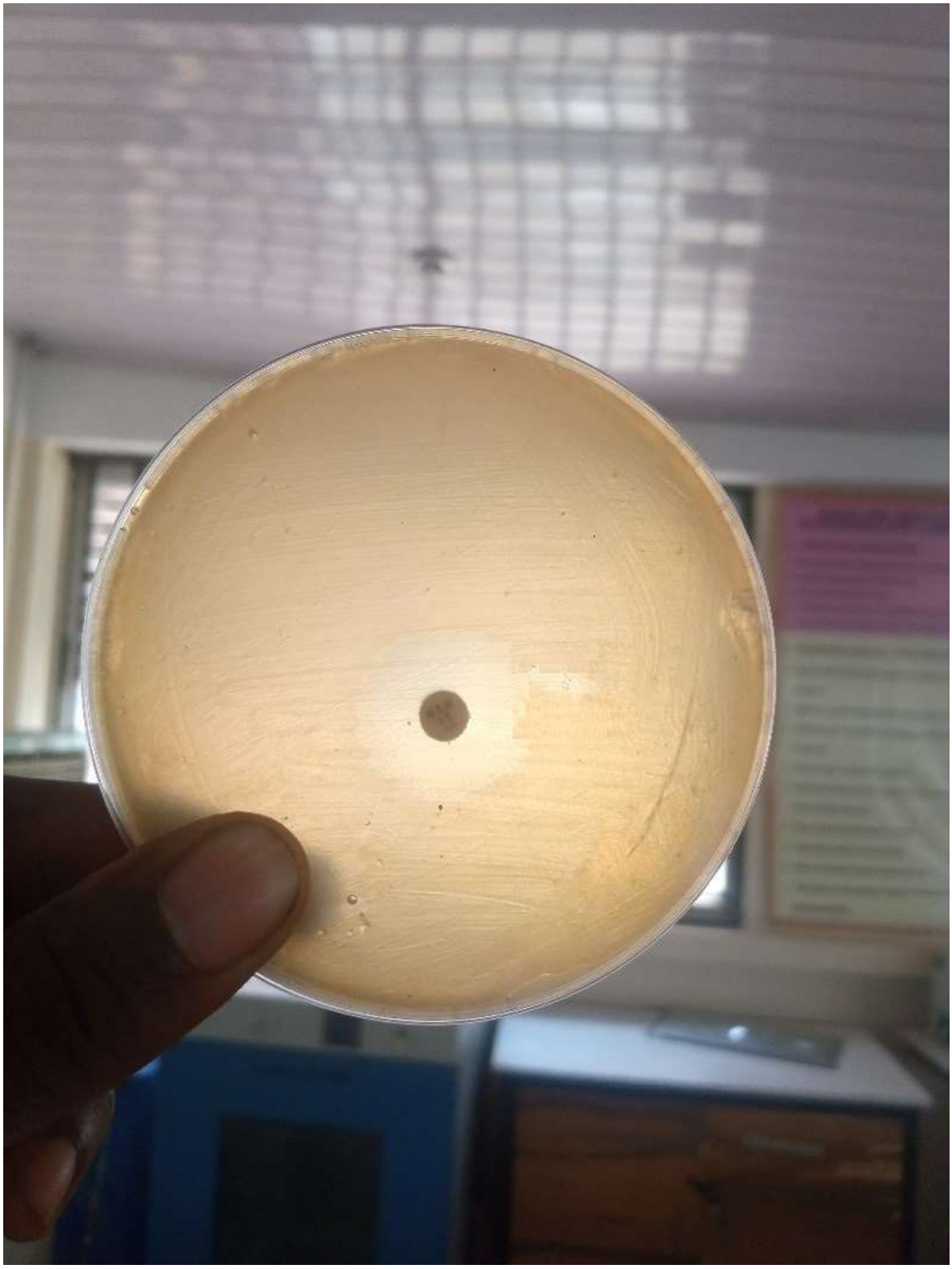
+ represents a standard positive reaction while – represents a standard negative reaction

Appendix B

CLSI Interpretative chart, 2017.

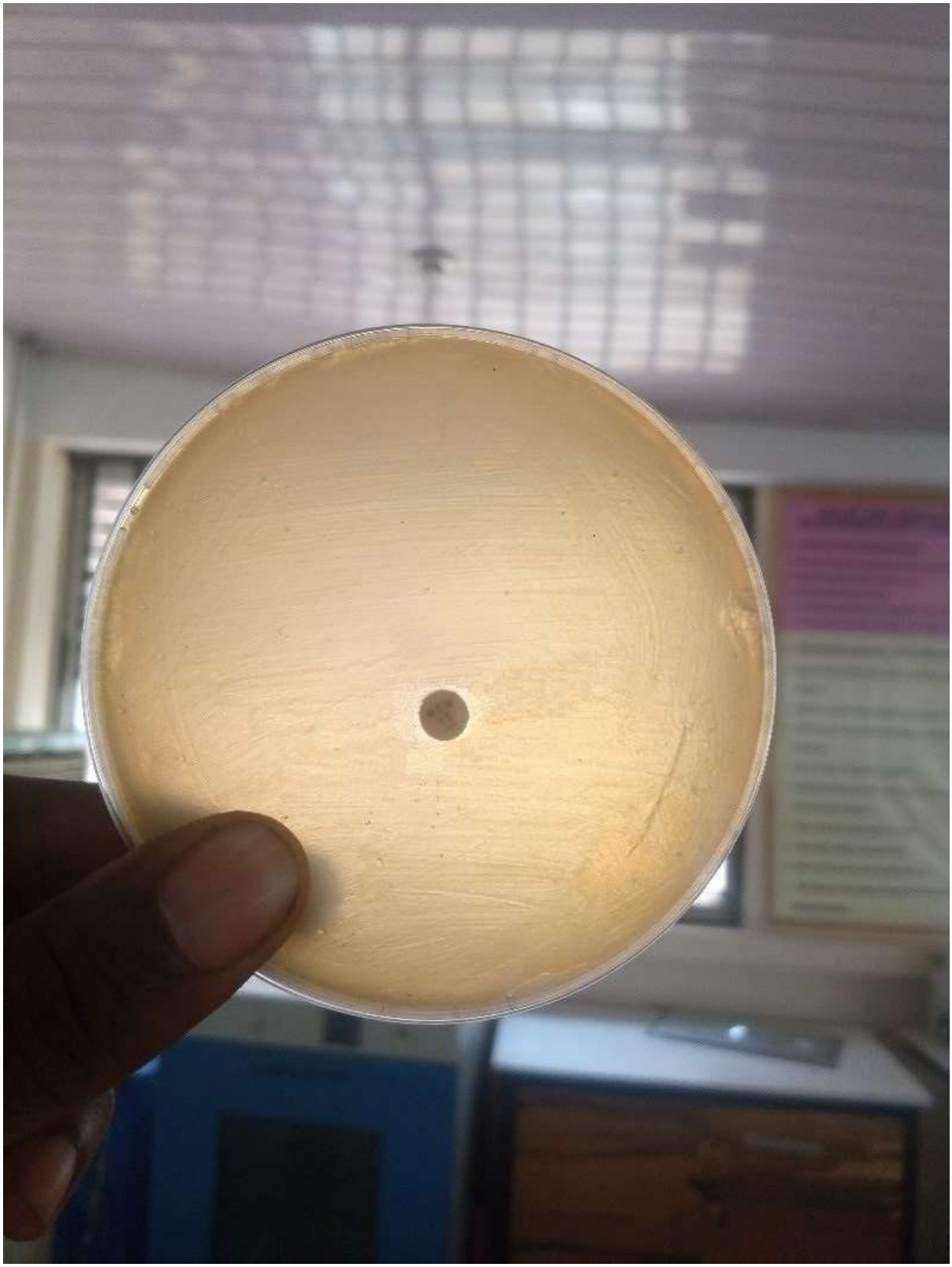
Antibiotics	Disc Content	Susceptible	Intermediate	Resistance
Ciprofloxacin	10µg	≥21	16-20	≤15
Ceftriaxone	30µg	≥21	14-20	≤13
Gentamycin	10µg	≥15	13-14	≤12
Tetracycline	10µg	≥15	12-14	≤11
Meropenem	10µg	≥18	15-17	≤14
Imipenem	10µg	≥22	19-21	≤18
Fosfomycin	50µg	≥16	13-15	≤12
Amoxicillin/Clavulanic Acid	30µg	≥18	14-17	≤13
Trimethoprim/sulfamethoxazole	25µg	≥16	11-15	≤10
Colistin sulphate	10µg	≥18	15-17	≤14

Appendix C



Result from sCIM showing isolate S9b negative for carbapenemase production

Appendix D



Result from sCIM showing isolate W14a positive for carbapenemase production

Appendix E1

Nucleotide Sequence of *A. baumannii* Isolates

Sample W16b

TGTGGATAACTTGGGTAGAATGGCGACCCCTTCTCATCAGGAAGGGTTAATCTTT
AAATGATTTGAATTTAAAACGCAGACATAGGGGATACACATGCTTTGGACAGAC
TGCTTAACTCGCTTGCGACAAGAGCTCTCTGATAACGTCTTTGCGATGTGGATTC
GCCCTTTAGTAGCTGAAGAAGTAGAGGGGATACTACGTCTCTATGCTCCTAATCC
TTATTGGACGCGTTATATTCAAGAGAATCATTTAGAGTTAATTTCTATATTGGCTG
AACAATTGTCAGAAGGGCGGGTGCGTCAGGTTGAAATCTTGGTAGATTCTCGTCC
TGGTAGTATTTTGTCCTCTAGTGAACAGCCTGCAACAACTACAGCAGCTTTACAA
ACTGCCCCTATACCTCAACCTGCTAAGGTTAAAAGAGAACCGGAACCTGTTGCTA
ATACTGCGGTTAGTTCTAAGAGTTCAAAAAAGAAACTATTAAATCCACAATTTAC
TTTTTCACTATTTGTTGAAGGCCGTTCTAACCAAATGGCAGCAGAAACCTGTAGA
AAAGTATTAACACAGTTAGGTGCTTCTCAACATAACCCTTTGTTTTTATATGGCCC
GACAGGTCTTGGTAAGACTCACTTAATGCAAGCAGTTGGTAATGCCTTACTGCAA
GCGAAGCCGAATGCAAGAGTCATGTATATGACTTCAGAAAGTTTTGTACAAGATT
TTGTGAGCTCATTACAAAAAGGAAAGGTAGAAGA

Isolate W16b was 99.2% identical to strain *Acinetobacter baumannii* Ab421

Appendix E2

Sample U17b

ATGCTTTGGACAGACTGCTTAACTCGCTTGCGACAAGAGCTCTCTGATAACGTCT

TTGCGATGTGGATTCGCCCTTTAGTAGCTGAAGAAGTAGAGGGGATACTACGTCT

CTATGCTCCTAATCCTTATTGGACGCGTTATATTCAAGAGAATCATTTAGAGTTA

ATTTCTATATTGGCTGAACAATTGTCAGAAGGGCGGGTGCGTCAGGTTGAAATCT

TGGTAGATTCTCGTCCTGGTAGTATTTTGTCCTCTAGTGAACAGCCTGCAACAAC

TACAGCAGCTTTACAAACTGCCCCTATACCTCAACCTGCTAAGGTTAAAAGAGAA

CCGGAACCTGTTGCTAATACTGCAGTTAGTTCTAAGAGTTCAAAAAAGAAACTAT

TAAATCCACAATTTACTTTTTCACTATTTGTTGAAGGCCGTTCTAACCAAATGGCA

GCAGAAACCTGTAGAAAAGTATTAACACAGTTAGGTGCTTCTCAACATAACCCTT

TATTTTTATATGGTCCGACAGGTCTAGGTAAGACTCACTTAATGCAAGCAGTTGG

TAATGCCTTACTGCAAGCGAAGCCGAATGCAAGAGTCATGTATATGACTTCAGA

AAGTTTTGTACAAGATTTTGTGAGCTCATTACAAAAAGGAAAGGTAGAAGAGTTT

AAGAAAAATTGCCGTTCTTTAGACTTGTTATTAGTAGATGATATTCATCTTTTGGC

AGGAAAAGAAGCAAGTCTTGTTGAATTCTTCTAT

Isolate U17b was 99.4% identical to strain *Acinetobacter baumannii* NCTC_7364

Appendix E3

Sample S9b

ATGCTTTGGACAGACTGCTTAACTCGCTTGCGACAAGAGCTCTCTGATAACGTCT

TTGCGATGTGGATTCGCCCTTTAGTAGCTGAAGAAGTAGAGGGGATACTACGTCT

CTATGCTCCTAATCCTTATTGGACGCGTTATATTCAAGAGAATCATTTAGAGTTA

ATTTCTATATTGGCTGAACAATTGTCAGAAGGGCGGGTGCGTCAGGTTGAAATCT

TGGTAGATTCTCGTCCTGGTAGTATTTTGTCCTCTAGTGAACAGCCTGCAACAAC

TACAGCAGCTTTACAAACTGCCCCTATACCTCAACCTGCTAAGGTTAAAAGAGAA

CCGGAACCTGTTGCTAATACTGCAGTTAGTTCTAAGAGTTCAAAAAAGAAACTAT

TAAATCCACAATTTACTTTTTCACTATTTGTTGAAGGCCGTTCTAACCAAATGGCA

GCAGAAACCTGTAGAAAAGTATTAACACAGTTAGGTGCTTCTCAACATAACCCTT

TATTTTTATATGGTCCGACAGGTCTAGGTAAGACTCACTTAATGCAAGCAGTTGG

TAATGCCTTACTGCAAGCGAAGCCGAATGCAAGAGTCATGTATATGACTTCAGA

AAGTTTTGTACAAGATTTTGTGAGCTCATTACAAAAAGGAAAGGTAGAAGAGTTT

AAGAAAAATTGCCGTTCTTTAGACTTGTTATTAGTAGATGATATTCATCTTTTGGC

AGGAAAAGAAGCAAGTCTTGTTGAATTCTTCTAT

Isolate S9b was 99.3% identical to strain *Acinetobacter baumannii* NCTC_7364

Appendix E4

Sample W19a

CGTAGAGGCTTGCCAAGAGATAACAGTCGCGCCAGAACGTTTACGTCGTTCAAG

TTACTGTTTGTTAGATCAAGGCTATTGCTAAGCGAAGTAATCTGCCCGCTTTGACT

TGAAATCTTGCCCTCGGCTGTTTCCACTCGGCTAGTTAAGTTATTGACCGCGCTTG

AATCGGCTTTGTTTGCCAGTGTTCCATTGATTGAGGTGACGCTATTTTGTAGCGAT

GCAATTGAATCACTTTGATTGGTGATCTTGCCCTCAGCGGTTGCCATGCGTGTCG

AAAGTCCACCAACCGCAGTATTTGTGCTGTTGATATTTCCTTCGGCTGTAGACAT

GCGCGAATTTAGTGACGTAATCGAATCTGTAGCAGTGGTTAGCCGACCATCGATA

TTGTCAACTTTTGTTTGGGTCGTTTGAATTGCAGAAGCATTGGCGTCAATTGCAGC

CTTTGTATCACGAGGGCTTGGACTCCAAGCGGTAGCCTTTGTGCCCGCTTCAATT

TGTAATTTACGAATCGTCGGGATACGGCCAGTTCCATACGTTCCATAGAACTCAA

TTGTCGAAACAGTTGTACTTGCCGTATGCGCTTTTGGACTAACTGTTACTGAATAT

TTGGCAAATTGATTTACGATAATTGCATTAACGGAAGTAACGAATTGGTGAGCAG

AACCATTTGACGAATAAACTTGAACCGGTCCAGCCACAGGAACGCTCATTTCAA

ACGAAATCGTGATTGGCTTCTCAAGGTTTTCGTCATAGAACGCTTTTAATTC

GGCGCTACGTTCATACAGTAAATATTCGCGATTCGTTGCGGC

Isolate W19a was 99.7% identical to strain *Acinetobacter baumannii* Ab7

Appendix E5

Sample S2b

ATGCTTTGGACAGACTGCTTAACTCGCTTGCGACAAGAGCTCTCTGATAACGTCT

TTGCGATGTGGATTCGCCCTTTAGTAGCTGAAGAAGTAGAGGGGATACTACGTCT

CTATGCTCCTAATCCTTATTGGACGCGTTATATTCAAGAGAATCATTTAGAGTTA

ATTTCTATATTGGCTGAACAATTGTCAGAAGGGCGGGTGCGTCAGGTTGAAATCT

TGGTAGATTCTCGTCCTGGTAGTATTTTGTCCTCTAGTGAACAGCCTGCAACAAC

TACAGCAGCTTTACAAACTGCCCCTATACCTCAACCTGCTAAGGTTAAAAGAGAA

CCGGAACCTGTTGCTAATACTGCAGTTAGTTCTAAGAGTTCAAAAAAGAAACTAT

TAAATCCACAATTTACTTTTTCACTATTTGTTGAAGGCCGTTCTAACCAAATGGCA

GCAGAAACCTGTAGAAAAGTATTAACACAGTTAGGTGCTTCTCAACATAACCCTT

TGTTTTTATATGGTCCGACAGGTCTAGGTAAGACTCACTTAATGCAAGCAGTTGG

TAATGCCCTACTGCAAGCGAAGCCGAATGCAAGAGTCATGTATATGACTTCAGA

AAGTTTTGTACAAGATTTTGTGAGCTCATTACAAAAAGGAAAGGTAGAAGAGTTT

AAGAAAAATTGCCGTTCTTTAGACTTGTTATTAGTAGATGATATTCATCTTTTGGC

AGGAAAAGAAGCAAGTCTCGTTGAATTCTTCTAT

Isolate S2b was 99.9% identical to strain *Acinetobacter baumannii* NCTC_7412

Appendix E6

Sample W14a

CGTAGAGGCTTGCCAAGAGATAACAGTCGCGCCAGAACGTTTACGTCGTTCAAG

TTACTGTTTGTTAGATCAAGGCTATTGCTAAGCGAAGTAATCTGCCCGCTTTGACT

TGAAATCTTGCCCTCGGCTGTTTCCACTCGGCTAGTTAAGTTATTGACCGCGCTTG

AATCGGCTTTGTTTGCCAGTGTTCCATTGATTGAGGTGACGCTATTTTGTAGCGAT

GCAATTGAATCACTTTGATTGGTGATCTTGCCCTCAGCGGTTGCCATGCGTGTCG

AAAGTCCACCAACCGCAGTATTTGTGCTGTTGATATTTCCTTCGGCTGTAGACAT

GCGCGAATTTAGTGACGTAATCGAATCTGTAGCAGTGGTTAGCCGACCATCGATA

TTGTCAACTTTTGTTTGGGTCGTTTGAATTGCAGAAGCATTGGCGTCAATTGCAGC

CTTTGTATCACGAGGGCTTGGACTCCAAGCGGTAGCCTTTGTGCCCGCTTCAATT

TGTAATTTACGAATCGTCGGGATACGGCCAGTTCCATACGTTCCATAGAACTCAA

TTGTCGAAACAGTTGTACTTGCCGTATGCGCTTTTGGACTAACTGTTACTGAATAT

TTGGCAAATTGATTTACGATAATTGCATTAACGGAAGTAACGAATTGGTGAGCAG

AACCATTTGACGAATAAACTTGAACCGGTCCAGCCACAGGAACGCTCATTTCAA

ACGAAATCGTGATTGGCTTCTCAAGGTTTTCGTCATAGAACGCTTTTAATTCGGC

GCTACGTTCATACAGTAAATATTCGCGATTCGTTGCGGC

Isolate W14a was 99.9% identical to strain *Acinetobacter baumannii* Ab7

Appendix E7

Sample W22b

CGTAGAGGCTTGCCAAGAGATAACAGTCGCGCCAGAACGTTTACGTCGTTCAAG

TTACTGTTTGTTAGATCAAGGCTATTGCTAAGCGAAGTAATCTGCCCGCTTTGACT

TGAAATCTTGCCCTCGGCTGTTTCCACTCGGCTAGTTAAGTTATTGACCGCGCTTG

AATCGGCTTTGTTTGCCAGTGTTCCATTGATTGAGGTGACGCTATTTTGTAGCGAT

GCAATTGAATCACTTTGATTGGTGATCTTGCCCTCAGCGGTTGCCATGCGTGTCG

AAAGTCCACCAACCGCAGTATTTGTGCTGTTGATATTTCCTTCGGCTGTAGACAT

GCGCGAATTTAGTGACGTAATCGAATCTGTAGCAGTGGTTAGCCGACCATCGATA

TTGTCAACTTTTGTTTGGGTCGTTTGAATTGCAGAAGCATTGGCGTCAATTGCAGC

CTTTGTATCACGAGGGCTTGGACTCCAAGCGGTAGCCTTTGTGCCCGCTTCAATT

TGTAATTTACGAATCGTCGGGATACGGCCAGTTCCATACGTTCCATAGAACTCAA

TTGTCGAAACAGTTGTACTTGCCGTATGCGCTTTTGGACTAACTGTTACTGAATAT

TTGGCAAATTGATTTACGATAATTGCATTAACGGAAGTAACGAATTGGTGAGCAG

AACCATTTGACGAATAAACTTGAACCGGTCCAGCCACAGGAACGCTCATTTCAA

ACGAAATCGTGATTGGCTTCTCAAGGTTTTCGTCATAGAACGCTTTTAATTC

GGCGCTACGTTCATACAGTAAATATTCGCGATTCGTTGCGGC

Isolate W22b was 99.5% identical to strain *Acinetobacter baumannii* Ab7

Appendix F
Research Informed Consent Form

TITLE OF STUDY: Prevalence of Carbapenem Resistant *Acinetobacter baumannii* in Both Medical and Surgical In-Patients Admitted in Two Selected Hospitals in Minna, Niger State

PURPOSE OF STUDY
The aim of this study is to determine the prevalence of carbapenem resistant *Acinetobacter baumannii* within two selected hospitals in Minna metropolis in Niger State.

PROCEDURES
Samples of Urine, Sputum and wound swabs will be collected to determine the prevalence of carbapenem resistant *Acinetobacter baumannii* within two selected hospitals in Minna metropolis in Niger State. After sample collection, the research will be carried out at the Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna.

BENEFITS

This study aims to provide t*he proper documentation of areas confirmed to be reservoirs of carbapenem resistant Acinetobacter baumannii* which is the first step in managing the spread of this important pathogen.

CONFIDENTIALITY

Every effort will be made by the researcher to preserve your confidentiality. Participant data will be kept confidential except in cases where the researcher is legally obligated to report specific incidents. These incidents include, but may not be limited to, incidents of abuse and suicide risk.

VOLUNTARY PARTICIPATION
Your participation in this study is voluntary. It is up to you to decide whether or not to take part in this study. If you decide to take part in this study, you will be asked to sign a consent form. After you sign the consent form, you are still free to withdraw at any time and without giving a reason. Withdrawing from this study will not affect the relationship you have, if any, with the researcher. If you withdraw from the study before data collection is completed, your data will be returned to you or destroyed.

CONSENT
I have read and I understand the provided information and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I voluntarily agree to take part in this study.

Participant's Signature _____ Date _____

Researcher's Signature _____ Date _____

Appendix G

Questionnaire

Kindly underline the relevant answer

Sex: [Male] [Female]

Age: [17 years and below] [18 – 35 years] [36 – 50 years] [51 – 75 years] [>75 years]

Are you currently on antibiotic treatment [Yes] [No]

How long have you been on your current treatment [< 1 week] [1-2 weeks] [>2 weeks]

Appendix H1

NIGER STATE HOSPITALS MANAGEMENT BOARD
GENERAL HOSPITAL MINNA

ADDRESS:
No 1 Hospital Road,
P.M.B 2 Minna
Niger State, Nigeria.
Tel: 08053899102

Our Ref: _____ *Your Ref:* _____ *Date:* _____

22nd February, 2022
HMB/GHM/136/VOL.III/593

The Head of Department,
Microbiology Department,
Federal University of Technology,
Minna.

Sir,

ETHICAL APPROVAL

IN RESPECT OF AYAMS JUDE NDANUSA

(M.TECH/SLS/2019/10291)

The General Hospital Minna Research, Ethics and Publication Committee (REPC) has given approval for the implementation of your research protocol titled: **"Prevalence of Carbapenem Resistant *Acinetobacter baumannii* in Both Medical and Surgical In-Patients Admitted in Two Selected Hospital in Minna, Niger State".**

You are required to submit periodically a review of the study to this committee. On completion of the study, the committee must be informed before your research findings are published and a copy of the published article (s) must be submitted to the committee.

Furthermore, do not hesitate to inform the committee of any difficulties or unwanted effects that might arise in the course of the studies.

Best regards.

23/2/2022.

Dr. Wey George D MBBS, Cert Derm, FMCFM,
Chairman Research, Ethics and Publication Committee

E-mail: genhospminna@yahoo.com genhospminna@gmail.com

Appendix H2

GOVERNMENT OF NIGER STATE

IBRAHIM BADAMASI BABANGIDA SPECIALIST HOSPITAL

MINNA

Ag. Chief Medical Director
Dr. Bala Waziri, MBBS, FMCP, MSc(Wits), PhD(Wits)

Director/Clinical Services
Dr. Eso S. Francis, MB, ChB, FMCS

KM 10, Paiko Road,
Chanchaga P.M.B 169
Minna, Niger State, Nigeria.

Our Ref: IBBSH/SUB/514 Your Ref: Date: 10/02/2022

HUMAN RESEARCH ETHICS CLEARANCE COMMITTEE
APPROVAL CERTIFICATE NUMBER M2022-04

This is to certify that:-

Project Title:

"CARBAPENEN RESISTANT ACINETOBACTER BAUMANNII" FROM SOME HOSPITALS IN MINNA, NIGER STATE.

Principal Investigator:

AYAMS, Jude Ndanusa. MTech/SLS/2019/10291, Department of Microbiology, Federal University of Technology Minna, was considered on the 2/2/2022 and approved unconditionally on 7/2/2022

NOTE:

This approval is valid for 5 years from date of approval.

Annual progress report and any significant change made to the project should be reported to the research ethics committee.

Suleiman N Mohammad
Senior Admin Officer
For the Chairman Ethics Committee

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Website: www.ibbspecialisthospital.org.ng