Characterization of colonizing *Acinetobacter baumannii* strains isolated from select hospitals and communities in Kenya

ROBERT MAINA MUGOH

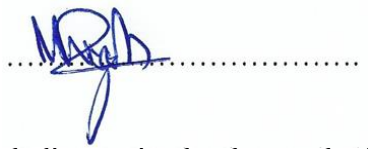
H56/34079/2019

A research dissertation submitted to the Department of Medical Microbiology in partial fulfillment of the requirement for the award of a Master of Science degree in Medical Microbiology at the University of Nairobi

DECLARATION

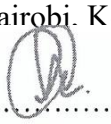
1. I declare that this dissertation is my original work and has not been submitted elsewhere for examination, award of a degree, or publication. Where other people's work or my work has been used, this has adequately been acknowledged and referenced per the University of Nairobi's requirements.
2. I have not sought or used the services of any professional agencies to produce this work.
3. I have not allowed and shall not allow anyone to copy my work to pass it off as his/her work.
4. I understand that any false claim regarding this work shall result in disciplinary action according to the University's Plagiarism Policy.

MSc Student

Signature:  Date…08.12.2023……

This research dissertation has been submitted with our approval as University Supervisors.

Dr. Winnie Mutai (BSc, MSc, PhD),
Lecturer, Department of Medical Microbiology and Immunology,
University of Nairobi, Kenya.

Signature:  Date…08.12.2023……

Moses Musyoki (BSc, MSc, PhD - FL),
PhD Fellow, Department of Medical Microbiology and Immunology,
University of Nairobi, Kenya.

Signature: …… Date……08.12.2023……

Dr. Sylvia Omulo (BSc, MPH, PhD),
Assistant Professor, Paul G. Allen School for Global Health,
Washington State University, USA.

Signature:  Date……08.12.2023……

DEDICATION

I dedicate this research thesis to my entire family, my wife who has been my greatest source of motivation and inspiration to work hard. To God, I dedicate this work, as this journey began with faith.

TABLE OF CONTENTS

ACKNOWLEDGMENT

I am deeply grateful to my supervisors Dr. Sylvia Omulo, Dr Winnie Mutai, and Mr. Moses Musyoki for their unwavering support and guidance throughout my master's program. Their expertise and patience have been invaluable to me and have played a crucial role in the success of this thesis.

I am grateful to Washington State University Global Health–Kenya (WSU GH-K), CDC, and UNITID for providing me with the opportunity to conduct my research and for all the resources and support they provided. I would like to extend a special thanks to Susan Kiiru (KEMRI-Center for Microbiology Research), who went above and beyond to help me with my molecular work.

I am deeply thankful to my WSU GH-K (ARCH study) colleagues and family for their love and support during this process. Without their encouragement and motivation, I would not have been able to complete this journey.

Finally, I would like to thank God, for letting me through all the difficulties. I have experienced your guidance day by day. You are the one who let me finish my master's degree. I will keep on trusting you for my future.

LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
ARCH	Antimicrobial Resistance in Communities and Hospitals
AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
CRE	Carbapenem-resistant Enterobacterales
DNA	Deoxyribonucleic acid
ERC	Ethics Review Committee
ESBL	Extended-spectrum beta-lactamase
ICU	Intensive care unit
KNH	Kenyatta National Hospital
KEMRI	Kenya Medical Research Institute
MBL	Metallo-β-lactamase
MDR	Multidrug-resistant
MIC	Minimum Inhibitory Concentration
NDM	New Delhi metallo-β-lactamase
PCR	Polymerase chain reaction
PDR	Pan-drug resistance
RNA	Ribonucleic acid
UNITID	University of Nairobi Institute of Tropical and Infectious Diseases
UoN	University of Nairobi
WHO	World Health Organization
XDR	Extensively drug-resistant

DEFINITION OF TERMS

Antimicrobial resistance: The ability of a microorganism (bacteria, virus, fungi, parasite) to change over time and no longer respond to the effects of a drug, making its infection challenging to treat and increasing its disease severity and spread.

Antibiotic resistance: The ability of microbes, specifically bacteria, and fungi, to evolve with time and circumstances, rendering the effects of drugs to which they were once sensitive lesser or completely ineffective.

Colonization: Presence of a microorganism on an internal (gastrointestinal, respiratory, or genitourinary tract) or external (skin) surface of the host without causing disease.

ESKAPE pathogens: A group of disease-causing bacteria that include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumanii, Pseudomonas aeruginosa, and Enterobacter* spp.

Extensively drug-resistant organism: Bacteria and other organisms that are resistant to almost all or multiple approved antimicrobial agents.

Hospitalization: Inpatient care given to a patient whose condition requires admission to a hospital.

Isolate: A pure strain of microorganisms obtained after culture.

Mechanism of resistance: The pathway by which a microorganism achieves tolerance e.g., by enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, or by acquiring changes in membrane permeability to antibiotics.

Multi-drug resistant organisms: Bacteria and other microorganisms that are resistant to more than two antibiotic classes.

Pan-drug-resistant organisms: Resistant to all antibiotic classes available for empirical treatment

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Background: Colonization with drug-resistant *Acinetobacter baumannii* increases the risk of infection with similar organisms and multidrug-resistant pathogens. Human colonization with resistant *A. baumannii* in hospitals and communities is scantly studied. We tested antibiotic susceptibility profiles of *A. baumannii* isolated from hospitals and community settings in Kenya between January 2019 and March 2020.

Methods: A laboratory-based cross-sectional study characterized presumptively identified and archived strains of *A. baumannii*. The VITEK®2 System was used for confirmatory identification and antimicrobial susceptibility testing. Conventional polymerase chain reaction (PCR) was used to detect extended-spectrum beta-lactamases and carbapenemases genes coding for cephalosporin and carbapenem resistance.

Results: In total, 125 isolates were revived from rural and urban sources. Of the 18 rural hospital isolates, 72% showed resistance to sulfamethoxazole-trimethoprim (SXT). However, between 78 to 89% were susceptible to ampicillin-sulbactam (SAM), cefepime (FEP), ciprofloxacin (CIP), gentamycin (GEN), imipenem (IMP), meropenem (MEM), and tobramycin (TOB). On the other hand, 48% of the 21 urban hospital isolates were resistant to SXT, but there was a 62-100% susceptibility rate to TOB, SAM, IMP, MEM, CIP, GEN, tigecycline (TGC), and FEP. Of the 32 rural community isolates, 38% were resistant to SXT. Twenty-six percent of the 54 urban community isolates were resistant to SXT. Phenotypically, two multidrug-resistant isolates and two extensively drug-resistant isolates were identified in rural hospitals, while three and five isolates, respectively, were identified in urban hospitals. The multidrug-resistant and extensively drug-resistant isolates subjected to polymerase chain reaction revealed the presence of the suspected resistance genes.

Conclusion: Resistance among *A. baumannii* strains varied by setting. Hospitals had more resistant strains than community sites. Genes linked to antimicrobial resistance were identified in these hospitals. Hence, healthcare facilities should plan and execute antimicrobial stewardship programs effectively to reduce the rate of resistance development.

CHAPTER 1

1. INTRODUCTION

1.1. Background

The levels of antimicrobial resistance (AMR) are escalating to concerning heights throughout the world as new resistance mechanisms emerge, posing a risk in the treatment of infectious diseases (WHO, 2020). World Health Organization (WHO) classifies *A. baumanii,* an ESKAPE pathogen, under critical priority pathogens, highlighting its serious threat to public health (WHO, 2017). ESKAPE pathogens—*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumanii, Pseudomonas aeruginosa, and Enterobacter* species—cause healthcare-associated infections globally, particularly among critically ill persons and immunocompromised individuals (WHO, 2020).

Two to 10% percent of Gram-negative bacterial infections in hospitals are caused by *A. baumannii,* which is an opportunistic nosocomial pathogen (Antunes, Visca, and Towner, 2014). In healthcare settings, it causes bacteremia, meningitis, urinary tract infections, and wound infections. Due to its capacity to tolerate wide temperature ranges and extended periods on surfaces, *A. baumanii* is considered an endemic healthcare-associated pathogen implicated in hospital outbreaks (Maragakis and Perl, 2008).

Acinetobacter-associated infections are rare in communities and occur among individuals living in tropical and sub-tropical regions, and those with underlying comorbidities (Farrugia *et al.*, 2013). The risk factors that are commonly associated with community-acquired *A. baumanii* infections are diabetes mellitus, alcoholism, smoking, lung disease, and renal failure (Sharma *et al.,* 2021). Several strains of *A. baumanii* have been confirmed to be resistant to a variety of antibiotic groups, including aminoglycosides, cephalosporins, carbapenems quinolones, and penicillins (Gootz and Marra, 2008).

Extended-spectrum β-lactamases (ESBLs) are a major cause of cephalosporin resistance, which occurs through mutation of penicillin-binding proteins, alteration of membrane permeability, or plasmid or chromosomal-borne cephalosporinases induced by β-lactamases bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ (Smiline, Vijayashree and Paramasivam, 2018). Reduced permeability, efflux pump, and formation of carbapenemases (metallo-β-lactamases or carbapenem hydrolyzing oxacillinases) all contribute to *A. baumanii* resistance to carbapenems (Bertini *et al.*, 2007). The plasmid-borne carbapenem resistance genes $bla_{OXA\text{-}23}$ and $bla_{OXA\text{-}58}$ play a key role in resistance (Héritier *et al.*, 2005).

A. baumanii has developed resistance to most or all groups of antimicrobial agents, decreasing the number of effective drugs available for treatment. Therefore, many hospitals have faced challenges in treating infections caused by these microbes (Bertini *et al.*, 2007). Additionally, many LMICs lack the diagnostic capacity in their laboratories for the effective isolation and identification of the microbe in different settings to facilitate protocols like AST and eventual AMS.

Colonization with ESBL *A. baumanii* is asymptomatic, increasing the risk of multidrug-resistant infections resulting in unexpected transmission to non-colonized individuals. Though *A. baumanii* can colonize both hospitalized patients and healthy people, it is not clear to what degree colonizing *A. baumanii* is comparable to *A. baumanii* which causes infections (Alyamani *et al.*, 2015). Studies of travelers returning to the United States or Europe from Sub-Saharan Africa and South Asia were colonized with ESBLs even when they were not exposed to healthcare facilities or antibiotics, showing that communities are under significant colonization pressure (Sharma *et al.*, 2021).

Though *A. baumanii* may colonize healthy people and inpatients, it's unclear how similar colonizing *A. baumanii* is to infection-causing *A. baumanii*. This study, therefore, aimed to establish the antimicrobial susceptibility profile of colonizing *A. baumanii* from selected hospitals and communities in Kenya between January 2019 and March 2020. Also, the proportion of cephalosporin and carbapenem resistance strains and genes coding for this resistance was investigated within this cohort.

1.2. Rationale

AMR has become a global epidemic because of the escalating evolution of resistance combined with a reduced antibiotic pipeline. According to a recent survey, if the current situation persists unabated, 10 million people will die each year from AMR-associated infections by 2050 (Xie *et al.*, 2018).

Colonization with drug-resistant *A. baumanii* increases the risk of infection with multidrug-resistant pathogens, exacerbating the resistance problem. Once colonized, immunosuppression, invasive procedures, ICU stays, alcoholism, smoking, and chronic lung disease are known risk factors for hospital and community infections. Colonized individuals not only face an increased risk of illness as the prevalence of resistant *A. baumanii* colonization increases in communities and hospitals, but they may also spread these germs to other people in the community and hospital settings.

Multidrug resistance in *A. baumanii* not only raises treatment costs but also makes infection control difficult. Colonizing strains of *A. baumanii* can be resistant to last-resort antimicrobials like cephalosporins and carbapenems. This renders the infections caused by such strains difficult to treat using conventional antibiotics due to the evasive mechanisms that they have developed over the years to help them survive and persist in their niche.

Despite the increased risk of colonization with multidrug-resistant organisms, there is limited information on *A. baumanii* colonization in hospitals and communities globally, particularly in Africa. There is little evidence on the distribution and antimicrobial susceptibility patterns of colonizing *A. baumanii* from fecal samples in community and hospital setups in Kenya. Also, the genomic diversity of carbapenemases and beta-lactamases genes harbored by *A. baumanii* is not well described in our settings.

The results from this study will provide data on colonizing *A. baumanii* in hospital and community setups and provide a baseline on the heterogeneity of the resistant genes compared to those isolated globally.

1.3. Study questions

i. What are the antimicrobial susceptibility profiles of hospital and community isolates of *A. baumanii* in rural and urban sites in Kenya?
ii. Do the *A. baumanii* that colonize hospitalized patients differ phenotypically from those that colonize community residents?
iii. Which genes encode resistance to beta-lactams among *A. baumanii* strains?

1.4. Study objectives

1.4.1. Broad objective

To phenotypically and genotypically characterize colonizing *A. baumanii* strains isolated from urban and rural hospitals and communities between January 2019 and March 2020.

1.4.2. Specific objectives

i. To determine the antimicrobial susceptibility profiles of *A. baumanii* isolates from inpatients and community residents in rural and urban sites in Kenya.
ii. To evaluate differences in the distribution of multidrug resistance phenotypes among *A. baumanii* isolated from different settings.
iii. To characterize and analyze the distribution of genes that encode beta-lactam resistance among the isolated *A. baumanii* strains.

CHAPTER 2

2. LITERATURE REVIEW

2.1. Classification and morphology of *A. baumanii*

The genus *Acinetobacter* belongs to the order Gammaproteobacteria, and the family Moraxellaceae which comprises at least 21 known species, the most important being *A. baumanii* (Abbott et al., 2013; A. Evans, Hamouda, and G.B. Amyes, 2013). The *A. calcoaceticus–A. baumanii* complex (ABC) includes *A. calcoaceticus* (genomic species 1), *A. baumanii* (genomic species 2), *A. pittii* (genomic species 3), and *A. nosocomialis* (genomic species 13TU), which are phenotypically different but genetically related (Nowak and Paluchowska, 2016).

2.2. Prevalence of *A. baumanii*

A. baumanii is among the six common multidrug-resistant (MDR) bacteria in hospitals globally, as per the Infectious Diseases Society of America (Antunes, Visca, and Towner, 2014). It has emerged as a significant nosocomial pathogen, with 12,000–46,000 cases reported annually in the United States and up to a million cases reported worldwide (Schmier and Hulme Lowe, 2016). The prevalence of *Acinetobacter* spp. varies depending on the patient's socioeconomic status and geographic location. According to an international study of intensive care units (ICUs), *Acinetobacter* spp. infections were found in 19% of Asia, 17% of Eastern Europe, 14% in Central and South America, 6% in Western Europe, 4% in Oceania, 4% in North America, and 15% in Africa (Uwingabiye et al., 2016). In East Africa where Kenya lies, the average prevalence of *A. baumanii* is 5% (Wangai *et al*., 2019). In Kenya, community-acquired *A. baumanii* accounts for 10% of hospital bacteremia (Chaudhary and Payasi, 2012).

2.3. Colonization with *A. baumanii*

Colonization with *A. baumanii* on mucosal surfaces and wounds is asymptomatic, but it increases the risk of multi-drug resistance, resulting in the unintended transfer of resistant strains to non-colonized people. Though resistant strains colonize hospitalized patients and healthy individuals, evidence is still unclear on the extent to which colonizing resistant strains are identical to resistant strains causing infections (Sharma *et al*., 2021). Colonization is a precursor for infection, risk factors for colonization include prolonged hospitalization, immunosuppression, invasive processes, age, pneumonia, and ICU stay (Thorne *et al*., 2019).

2.3.1. Risk factors associated with hospital colonization.

Long-term hospitalization, ICU admission, mechanical breathing, antimicrobial agent use, surgery, and invasive procedures are colonization risk factors with multidrug-resistant *A. baumanii* (Maragakis and Perl, 2008).

2.3.2. Risk factors associated with community colonization.

Those who live in tropical and sub-tropical climates and have underlying co-morbidities such as chronic obstructive pulmonary disease, diabetes mellitus, renal illness, excessive alcohol use, and smoking are at risk for *A. baumanii* colonization (A. Evans, Hamouda, and G.B. Amyes, 2013).

2.4. Antimicrobial resistance among *A. baumanii* strains

A. baumanii resistance has escalated in the last decade. AMR in *A. baumanii* may be due in part to the organism's impermeable outer membrane and its exposure to varied resistance genes in the environment (Maragakis and Perl, 2008).

Different terminologies are used to describe the degree of AMR in *A. baumanii*, including multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR). According to the World Health Organization, MDR organisms have developed resistance to more than two classes of antimicrobials. For instance, an MDR in this case of *A. baumanii* can be resistant to cephalosporins, carbapenems, ampicillin-sulbactam combinations, fluoroquinolones, and aminoglycosides (Almasaudi, 2018). XDR strains, however, are resistant to at least one agent in three or more classes of antibiotics, whereas PDR is a phenomenon in bacteria that confers non-susceptibility to all antimicrobial agents from all classes.

2.4.1. Resistance mechanisms

A. baumanii uses a variety of mechanisms to evade antibiotic destruction, such as enzyme-mediated degradation, mutations, and efflux pumps (Asif, Alvi, and Ur Rehman, 2018).

2.4.1.1. β-Lactamases

A. baumanii produces a broad range of β -lactamases that hydrolyze penicillin, cephalosporin, and carbapenems, conferring resistance. AmpC cephalosporinases, which are chromosomally encoded, provide broad-spectrum cephalosporin resistance, whereas carbapenem resistance is conferred by oxacillinases (Maragakis and Perl, 2008; Gordon and Wareham, 2010).

2.4.1.2. Efflux Pumps

Efflux pumps are capable of actively excreting β-lactams, quinolones, and aminoglycosides. It is made up of three parts: the cytoplasmic membrane pump, porins, and lipoprotein. A resistance-nodulation-division (RND)-type efflux pump has been described in *A. baumanii* (Sharma *et al*., 2021). The efflux pump is responsible for resistance to aminoglycosides, quinolones, tetracyclines, chloramphenicol, erythromycin, and trimethoprim via inactivating *adeB* (Bonomo and Szabo, 2006).

The resistance-nodulation-division superfamily, the multidrug and toxic chemical extrusion family, the major facilitator superfamily, and the small multidrug resistance family transporters are efflux pumps associated with AMR in *A. baumanii* (Lee *et al.,* 2017). Overexpression of the *adeABC* gene, which is regulated by a two-component regulator system produced by the *adeRS* genes, causes resistance to aminoglycosides, B-lactams, chloramphenicol, erythromycin, and tetracyclines (Gordon and Wareham, 2010).

2.4.1.3. Permeability effects

AMR may be influenced by changes in outer membrane permeability. Porins influence membrane permeability and are important in the resistance process. CarO, Omp 33–36, and OprD homolog are three porins that have been linked to carbapenem resistance when their expression is lowered (Abbott *et al*., 2013; Almasaudi, 2018).

2.4.2. Resistance to specific antibiotics

2.4.2.1. Quinolone resistance

A change in the structure of DNA gyrase or topoisomerase IV produced by mutations in the quinolone resistance-determining regions of the *gyrA* and *parC* genes is the most common cause of *A. baumanii*'s resistance to quinolones. The quinolones' affinity for binding to the enzyme-DNA complex is reduced because of these changes. Quinolone resistance is also mediated by efflux systems, such as the RND-type pump AdeABC and the multi-antimicrobial extrusion proteinMATE pump AbeM, which reduce intracellular drug accumulation (Almasaudi, 2018).

2.4.2.2. Tetracycline and Glycylcycline resistance

Tetracycline resistance and its derivatives can be mediated by efflux or ribosomal protection. Tetracycline efflux pumps are encoded by genes ranging from *tetA* through *tetE*. Tigecycline resistance is acquired by *A. baumanii* through the AdeABC pump, which is caused by insertional inactivation of the *adeB* gene. (Peleg, Seifert, and Paterson, 2008).

2.4.2.3. Aminoglycoside resistance

Aminoglycoside resistance is obtained by adding an amino or hydroxyl group to aminoglycoside-modifying enzymes. All kinds of aminoglycoside-modifying enzymes have been found in *A. baumanii* (adenylases, acetylases, methyltransferases, and phosphotransferases). Other processes implicated in aminoglycoside resistance include reduced drug entry, efflux pump, and changes in target ribosomal protein (Asif, Alvi, and Ur Rehman, 2018).

2.4.2.4. Cephalosporin resistance

Cephalosporin resistance in *A. baumanii* is mediated through increased breakdown by beta-lactamases, changes in penicillin-binding proteins, changes in outer membrane porins for reduced permeability, and antibiotic expulsion from the cell via efflux pump. Resistance to broad-spectrum cephalosporins is conferred by AmpC cephalosporinases, which are chromosomally encoded and overexpressed due to insertion of insertion sequence (IS) ISAba1 (Maragakis and Perl, 2008; Asif, Alvi and Ur Rehman, 2018). This kind of resistance has been confirmed as being present in all *A. baumanii* strains, rendering cephalosporins that have this single mechanism of action like cefazolin ineffective against them.

A, B, C, and D are different classes of β-lactamases. Class A ESBLs are mutant plasmid-mediated hydrolyzing penicillins and cephalosporins. Major genes that confer resistance in *A. baumanii* include bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$. All β-lactam drugs, including carbapenems, are hydrolyzed by Class B metallo- β - lactamases (MBLs), which require zinc for catalysis. Class C, which is encoded by the *bla* genes provides therapeutic challenges as they impart resistance to cephamycins, penicillins, cephalosporins, and β-lactamase inhibitor combinations (Perez *et al*., 2007; Lee *et al*., 2017).

2.4.2.5. Carbapenem resistance

The most common mechanism of carbapenem resistance in *A. baumanii* is enzymatic degradation by carbapenemases, especially OXA type, and metallo-β-lactamases. The main efflux mechanism associated with carbapenem resistance is AdeABC, a chromosomally encoded tripartite efflux pump. (Abbott *et al*., 2013).

Carbapenem resistance in *A. baumanii* is primarily caused by the horizontal acquisition of genes for carbapenem hydrolyzing enzymes from either class D (oxacillinases) or class B (MBLs) (Hamidian and Nigro, 2019). *A. baumanii*'s *OXA* carbapenemase genes are classified into phylogenetic groupings including $bla_{OXA\text{-}23}$-like, $bla_{OXA\text{-}24/40}$-like, $bla_{OXA\text{-}51}$-like, $bla_{OXA\text{-}58}$-

like, *bla*$_{OXA-143}$-like, and *bla*$_{OXA-235}$-like. The genes that code for the five sets of genes are presumed to have come from outside sources (plasmid-mediated), while the genes that encode the OXA-51-like enzymes are thought to be intrinsic or chromosomally encoded (A. Evans, Hamouda, and G.B. Amyes, 2013; Li *et. al*., 2019). Class B genes encode the imipenemase metallo-β-lactamase, the Verona integron-encoded metallo-β-lactamase, and the New Delhi metallo-β-lactamase which confer carbapenem resistance in *A. baumanii* via horizontal gene transfer (Aruhomukama *et. al*., 2019; Li *et. al*., 2019).

2.5. Isolation and identification of *A. baumanii*

A. baumanii can be grown on blood, chocolate, and MacConkey agar. After 18–24 hours of incubation at 37°C, aerobically, it develops 1–2 mm colorless, non-hemolytic mucoid colonies on blood agar. Convex, colorless non-lactose fermenters, and mucoid colonies are produced on MacConkey agar (Asif, Alvi, and Ur Rehman, 2018).

Phenotypic tests used in routine diagnostic laboratories to identify bacterial genera to species level are relatively costly for *A. baumanii* identification which is not a common infectious agent in many LMICs and hence is not prioritized for tests like the Analytical Profile Index 20E (API 20E) that uses biochemical paneling to identify notorious pathogens in select contexts. Hence, automated analytic systems such as the VITEK®2 Compact system and Microscan Walk-Away are used to identify the *A. baumanii* isolates but are not preferred for identifying sub-species of *A. baumanii* complex (Peleg, Seifert, and Paterson, 2008) due to their low sensitivity and specificity as compared to systems like the API 20E and molecular approaches like 16S rRNA specific gene PCR.

High-resolution fingerprint analysis by amplified fragment length polymorphism, tRNA spacer fingerprinting, amplified 16S rRNA gene restriction analysis, restriction analysis of the 16S–23S rRNA intergenic spacer sequences, ribotyping, sequencing of the *rpoB* (RNA polymerase β-subunit) gene and its flanking spacers, and sequence analysis of the 16S–23S rRNA intergenic space are advanced molecular diagnostic methods used for *Acinetobacter* spp. identification to sub-species level (Howard *et al*., 2012).

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Study design

This was a laboratory-based cross-sectional study that characterized isolates that were collected under the Antimicrobial Resistance in Communities and Hospitals (ARCH) in Kenya study, approved by the KNH ERC (P164/03/2018).

3.2. Study site

The study used archived isolates obtained from stool/rectal samples of inpatients and communities enrolled in the ARCH study. For the ARCH study, study participants were enrolled at hospital and community sites in Nairobi and Siaya counties. In Nairobi, the study was conducted in Kibera. This is an urban informal settlement located in the southwest of Nairobi. It's characterized by inferior-quality housing, unsanitary environments, and a high population density (~87,000 people/km^2). Sampling was done in two villages (Gatwekera and Soweto). Asembo was selected as the study site in Siaya county. It is a rural community that is organized into dispersed villages and residents primarily practice subsistence farming and fishing. Sampling was done in 33 villages.

Study hospitals were selected based on proximity to community sites and the availability of inpatient facilities. Mbagathi County Hospital, which serves Kibera and neighboring communities, is a 300-bed hospital located in Nairobi County with ~11,000 annual admissions (2019 hospital estimates). For the Asembo site, hospitals included Siaya County Referral Hospital, Bondo sub-county Hospital, and St. Elizabeth (Lwak) Hospital, which had 9,000, 2,700, and 1,300 admissions in 2019, respectively. Isolates were recovered from cultured stool samples of community residents and hospitalized patients who consented to participate in the ARCH study between January 2019 and March 2020. The following eligibility criteria were applied for community residents had to be less than 5 years or over 18 years, old and must not have had an ongoing fever, diarrhea, or cough at the time of enrollment and specimen collection. Hospitalized patients must not have documented severe neutropenia (i.e., absolute neutrophil count < 500 cells/µL) or gastrointestinal bleeding.

3.3. Sample selection

3.3.1. Inclusion criteria

All presumptive *Acinetobacter* spp. isolates collected between Jan 2019 and Mar 2020.

3.3.2. Exclusion criteria

Isolates lacking proper or insufficient information and labelling were excluded from the study.

3.3.3. Sample size

The sample size was calculated using Fisher's formula (Fisher., 1998).

$$\text{Fisher's formula: } N = \frac{Z^2 PQ}{d^2}$$

Where:

N = Sample size

Z = Normal deviation at the desired confidence interval. At 95%, Z value = 1.96

P = Proportion of 10% Based on a study conducted in Kenyan hospitals and community-acquired *Acinetobacter* spp. bacteremia (Chaudhary and Payasi, 2012).

Q = 1 - P

d = Degree of precision; desired to be 5%

$$\text{Thus: } N = \frac{1.96^2(0.1)(1-0.1)}{0.05^2} = 138 \text{ samples}$$

3.4. Study procedures

3.4.1. Workflow

The workflow (Figure 1) highlights the steps taken from sample processing, isolation, archiving, identification/AST, and PCR.

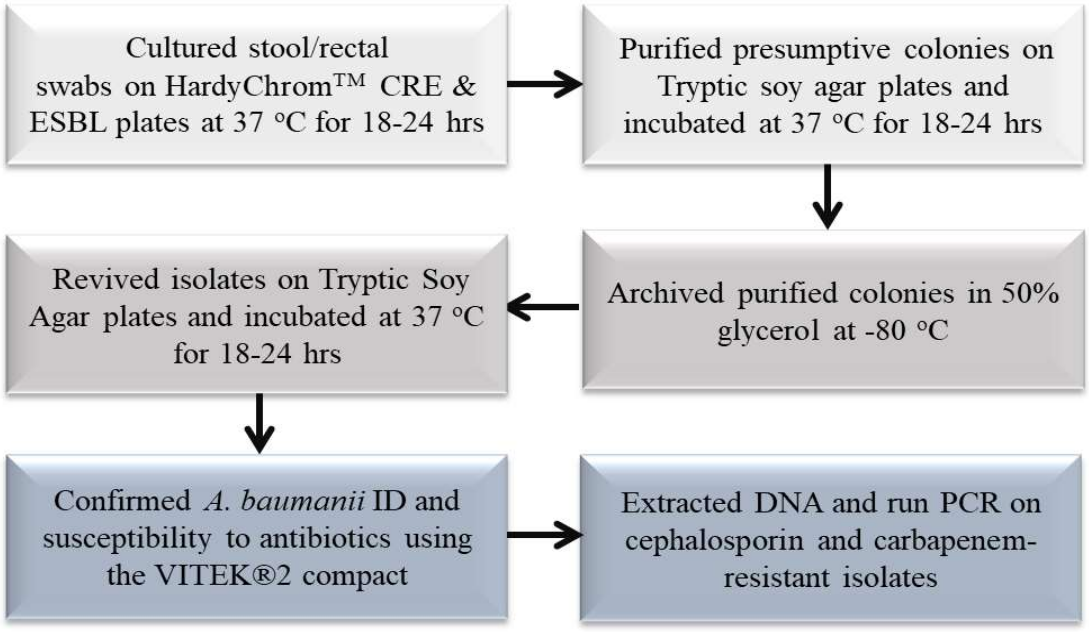


Figure 1. Sample handling workflow

3.4.2. Initial isolation and archiving of *Acinetobacter* sp. Isolates

Stool/rectal samples were cultured on HardyCHROM™ CRE and HardyCHROM™ ESBL agar plates and incubated at 37 °C overnight (18–24 hrs). A single off-white colony, presumptively identified as *Acinetobacter* spp., was picked from positive ESBL and CRE plates and sub-cultured on TSA agar at 37 °C overnight. Purified colonies were then collected and emulsified in 50% glycerol with phosphate-buffered saline, then archived at -80 °C.

3.4.3. Reviving *Acinetobacter* spp. isolates

Laboratory analyses were conducted at the UNITID Bacteriology Laboratory. Archived isolates were revived by thawing at -20 °C overnight, then at 4 °C for 1 hour before processing at 25 °C. Isolates were cultured on Tryptic soy agar and incubated overnight at 37 °C.

3.4.4. Confirmation of *Acinetobacter* spp. isolates using the VITEK®2

Bacterial suspensions were prepared from each archived isolate by emulsifying single colonies in 3 mL of 0.45% saline and then adjusted to 0.5 McFarland standard using a densitometer (DensiCHEK plus bioMerieux, France). Samples were then loaded into a VITEK®2 automated system (bioMérieux, France) for identification using VITEK®2 Gram-negative ID cards.

3.4.5. Antibiotic susceptibility testing

Isolates were prepared for antibiotic susceptibility testing by transferring 145 µL of the 0.5 McFarland standard from the identification tube into 3 mL of 0.45% saline. The suspension was loaded and tested using VITEK®2 AST-GN71 cards which contain a panel of 11 antibiotics: ampicillin-sulbactam ≤ 4/2µg/ml, cefazolin ≤ 4µg/ml, cefepime ≤ 2µg/ml, ceftriaxone ≤ 1µg/ml, ciprofloxacin ≤ 0.5µg/ml, gentamicin ≤ 4µg/ml, imipenem ≤ 1µg/ml, meropenem ≤ 0.5µg/ml, tigecycline ≤ 0.75µg/ml, tobramycin ≤ 8µg/ml and sulfamethoxazole-trimethoprim ≤ 1/19µg/ml. Minimal inhibitory concentration (MIC) values were interpreted following the Clinical Laboratory Standard Institute (CLSI) 2020 guidelines.

3.4.6. Selection of isolates for DNA extraction

Isolates that were resistant to ≥ 1 third or fourth-generation cephalosporin with the following MIC values were presumed to harbor ESBLs: cefepime, MIC ≥ 32 µg/ml; ceftriaxone, MIC ≥ 64 µg/ml; and/or ceftazidime, MIC ≥ 32 µg/ml. Even though *A. baumanii* has been established to have intrinsic resistance to cefazolin with a chromosomal origin, this additional testing was conducted to establish any potential presence of ESBLs or other genes that may encode acquired resistance mechanisms that may otherwise render the organism susceptible to the

antibiotic agent. Isolates that were resistant to ≥1 μg/ml carbapenem with the following MIC values were presumed to have carbapenemases: ertapenem, MIC ≥ 8 μg/ml; meropenem, MIC ≥8 μg/ml and/or imipenem, MIC ≥ 8 μg/ml.

3.4.7. Quality control/assurance

K. pneumoniae ATCC 700603 was used as a positive control for phenotypic and genotypic assays. Nuclease-free water was used as a negative control for molecular assays.

3.4.8. DNA extraction

DNA was extracted using the DNeasy ultra clean kit (Qiagen, Maryland, USA), a bead-based method for the isolation of genomic DNA, following the manufacturer's instructions. Bacterial cells were suspended in a bead solution-containing tube and beads were added along with the lysis solution. On vortexing, microorganisms were lysed by a combination of heat, mechanical energy, and detergent. DNA from lysed cells is bonded to a silica spin filter, washed, and recovered in DNA-free Tris buffer. The extracted DNA was stored at -80°C until analyzed using conventional PCR.

3.4.9. Detection of resistance genes

The primers listed in Table 1 were used to screen isolates for resistance genes encoding resistance to cephalosporins (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$) and carbapenems (*bla*$_{NDM1}$, *bla*$_{OXA-23}$, and *bla*$_{OXA-48}$) (Ranjbar, Zayeri, and Mirzaie, 2020).

Table 1. Primers for detection of antimicrobial resistance genes in *A. baumanii*

Target class†	Gene	Primer sequence	Size (bp)	Temp (°C)
Class A	*bla*$_{CTX-M}$	F5'-ATGTGCAGYAACAGTAARRGTKATGGC-3' R5'-TGGGTRAARTARGTSAACAGAAYCAGC GG-3'	593	60
	bla$_{SHV}$	F5'-TTCGCCTGTGTATTATCTCCCTG-3' R5'-TTAGCGTTGCCAGTGYTCG-3'	854	50
	bla$_{TEM}$	F5'-ATGAGTATTCAACATTTCCG-3' R5'-CCAATGCTTAATCAGTGACG-3'	867	50
Class B	*bla*$_{NDM1}$	F5'-GAGATTGCCGAGCGACTTG-3' R5'-CGAATGTCTGGCAGCACACTT-3'	813	61
	bla$_{OXA23}$	F5'-CTTGCTATGTGGTTGCTTCTC-3' R5'-ATCCATTGCCCAGTC-3'	650	50
Class D	*bla*$_{OXA48}$	F5'-TGTTTTTGGTGGCATCGAT-3' R5'-GTAAMRATGCTTGGTTCGC-3'	177	60

†Extended-spectrum β-lactamases **Class A**; metallo-β-lactamases Ambler **Class B**, and **Class D** carbapenemases. F, forward primer; R, reverse primer; bp: base pairs for amplicon size.

3.4.9.1. Polymerase chain reaction

A singleplex assay consisting of 25 µL reaction mix was prepared by mixing 12 µL of PCR master-mix, (Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), dATP 400 µM, dGTP 400 µM dCTP 400 µM, dTTP 400 µM, MgCl2 23 mM) (Promega, Madison, USA) with pairs of specific primers (10 pmol) at 1µL each both forward and reverse primers (Table 1) and 1µL DNA template and 12 µL nuclease-free water.

PCR was conducted using the Gene AMP 9700 PCR system (Applied Biosystems, USA) in 0.2ml PCR tubes. Amplification was done by initial heating at 95 °C for 5 minutes followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at specified primer temperature (Table 1) for 30 seconds, extension at 72 °C for 2 minutes with a final extension of 72 °C for 7 minutes. The PCR products were kept at -20 °C for gel electrophoresis.

3.4.9.2. Gel electrophoresis

Visualization of resistant genes in the amplified PCR products was performed using agarose gel electrophoresis. A 1.5% agarose gel with SYBR Green dye was loaded with samples, along with a 1kb molecular ladder. In a gel electrophoresis tank, the gel was run for 60 minutes in 5X Tris-acetate Ethylenediaminetetraacetic acid buffer at 90 volts, 65 mA, and 6 watts. Bands corresponding with respective sequence amplicon sizes were visualized under a UV trans-illuminator.

3.5. Ethical considerations

Ethical approval was sought from the KNH-UON Ethics and Research Committee and approved under Ref# P504/06/2022. The archived isolates were obtained from stool samples from a study previously approved by KNH/ UoN ERC Ref# P164/03/2018. This study, therefore, requested a waiver from ERC for informed consent.

3.6. Data management and analysis

Data from VITEK® 2 were exported to Excel for cleaning and management and saved in a password-protected computer. Data were analyzed using Stata v. 17 frequency distributions of susceptible, intermediate, and resistant strains used to show antimicrobial susceptibility profiles of *A. baumanii* isolated from hospitalized patients and community residents in rural and urban sites. Tested antibiotics were categorized into classes and the distribution and proportion of isolates were classified as MDR, XDR, or PDR. Finally, we looked at what genes

encode resistance to beta-lactam resistance, and how they are distributed in rural and urban sites in community and hospital settings.

CHAPTER 4

4. RESULTS

4.1. Revived isolates

A total of 138 archived and presumptively identified *A. baumanii* isolates were revived from previously collected samples from the rural and urban hospital and community settings. However, 13 of these isolates were other species of *Acinetobacter* and were hence excluded from the study. Out of the 125 isolates, approximately 60% (75) were from the urban settings while 40% (50) were from the rural (Siaya County)

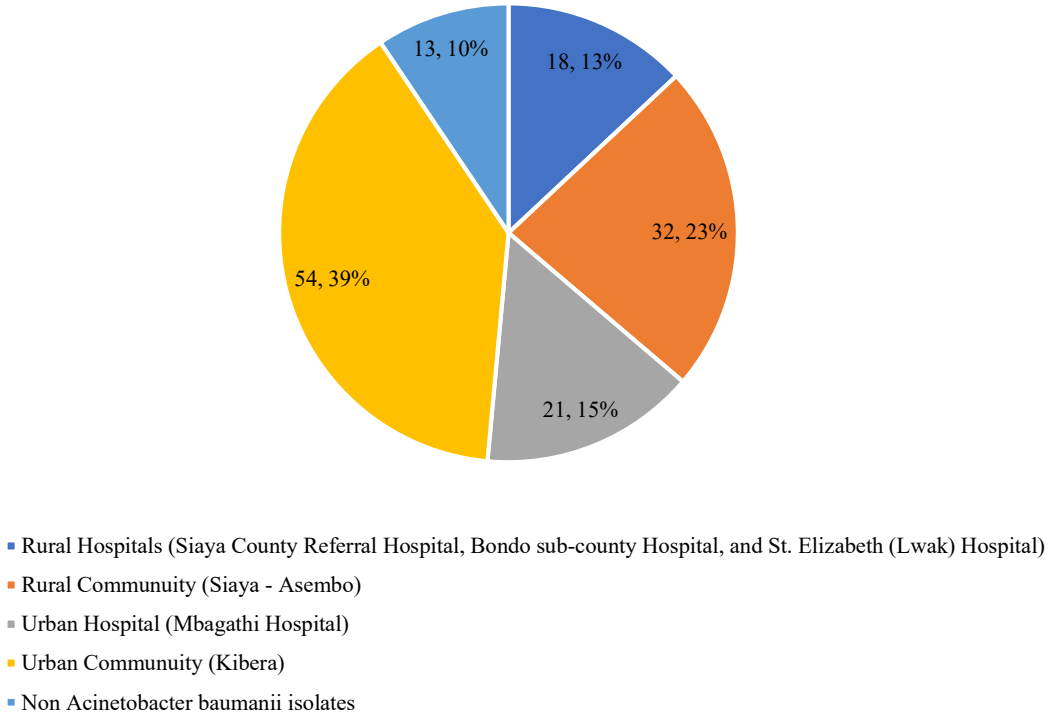


Figure 2: Distribution of revived isolates from different settings for use in the study

4.2. Antimicrobial susceptibility profiles of *A. baumanii* isolates

Rural isolates showed resistance to cefazolin and sulfamethoxazole/trimethoprim with hospital cefazolin rates at 100% and sulfamethoxazole-trimethoprim at 72%, while community rates at 100% and 38%, respectively. Rural hospital isolates were susceptible to the aminoglycosides, fourth-generation cephalosporin, fluoroquinolone, glycylcycline, carbapenems, and the beta-lactam/beta-lactamase inhibitor combination. The community samples from the rural setting showed a similar AST pattern (Table 2).

Table 2. AST patterns of *A. baumanii* isolates across the different study sites

Site			Antibiotic										
			CEZ	CIP	CRO	FEP	GEN	IMP	MEM	SAM	SXT	TGC	TOB
Hospital	**AST result**												
Rural (N = 18)	S	n (%)	0 (0)	16 (89)	3 (17)	14 (78)	14 (78)	16 (89)	16 (89)	15 (83)	5 (28)	18 (100)	14 (78)
	I	n (%)	0 (0)	0 (0)	10 (56)	1 (6)	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (17)
	R	n (%)	18 (100)	2 (11)	5 (28)	3 (17)	3 (17)	2 (11)	2 (11)	3 (17)	13 (72)	0 (0)	1 (6)
Urban (N = 21)	S	n (%)	0 (0)	14 (67)	3 (14)	13 (62)	14 (67)	15 (71)	15 (71)	15 (71)	11 (52)	14 (67)	21 (100)
	I	n (%)	0 (0)	0 (0)	9 (43)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	7 (33)	0 (0)
	R	n (%)	21 (100)	7 (33)	9 (43)	8 (38)	7 (33)	6 (29)	6 (29)	5 (24)	10 (48)	0 (0)	0 (0)
Community													
Rural (N = 32)	S	n (%)	0 (0)	32 (100)	6 (19)	31 (97)	32 (100)	32 (100)	32 (100)	32 (100)	19 (59)	32 (100)	32 (100)
	I	n (%)	0 (0)	0 (0)	26 (81)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)
	R	n (%)	32 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	12 (38)	0 (0)	0 (0)
Urban (N = 54)	S	n (%)	0 (0)	54 (100)	13 (24)	53 (98)	54 (100)	54 (100)	54 (100)	53 (98)	40 (74)	54 (100)	52 (96)
	I	n (%)	2 (4)	0 (0)	40 (74)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
	R	n (%)	52 (96)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	14 (26)	0 (0)	1 (2)

Key: Antibiotics: CEZ, cefazolin; CIP, ciprofloxacin; CRO, Ceftriaxone; FEP, Cefepime; GEN, Gentamicin; IMP, Imipenem; MEM, Meropenem; SAM, Ampicillin/Sulbactam; SXT, Sulfamethoxazole/Trimethoprim; TGC, Tigecycline; TOB, Tobramycin. **AST result:** S, susceptible; I, intermediate resistance; R, resistant.

In the urban setting, isolates from Mbagathi Hospital showed the highest resistance (100%) to cefazolin followed by sulfamethoxazole/trimethoprim at 48%. A similar trend was noted in the community isolates, reporting relatively lower resistance rates for cefazolin (96%) and sulfamethoxazole/trimethoprim (26%). Most hospital isolates from the urban cohort showed susceptibility to gentamycin, ciprofloxacin, and tigecycline, each at 14 (67%), tobramycin (21, 100%), cefepime (13, 62%), and ampicillin-sulbactam, imipenem, meropenem each at 15 (71%). In the urban community, susceptibility was demonstrated towards gentamycin, imipenem, meropenem, ciprofloxacin, and tigecycline at 54 (100%) each, tobramycin at 52 (96%), and cefepime and ampicillin sulbactam at 53 (98%) each. None of the isolates from the urban setting were susceptible to cefazolin as they showed 100% resistance in both hospital and community isolates.

The patterns indicate that most hospital isolates (both rural and urban) were highly susceptible to the aminoglycosides gentamicin and tobramycin (68% combined), indicating low resistance rates to these agents by the bacteria. However, higher resistance rates were observed in the three cephalosporins (cefazolin (intrinsic), ceftriaxone, and cefepime) as well as in sulfamethoxazole/trimethoprim, each with resistance rates at 48% and 72%, respectively, in the hospital setting and 33% and 38%, respectively, in the community setting.

4.3. Diversity in the Distribution of MDR and XDR *A. baumanii* Isolates.

The phenotypes of the strains of *A. baumanii* were established based on their resistance profiles against the 11 selected antibiotic agents. MDR and XDR strains were observed in various isolates from rural and urban settings (Table 3). MDR organisms were defined as *A. baumanii* isolates with resistance to at least one agent from three or more categories of antimicrobials. On the other hand, XDR strains are those resistant to at least one of the drugs in all but two or fewer of the classes of antimicrobials (World Health Organization, 2021).

The phenotypic characterization process shows that there were two MDR isolates from the rural hospitals, accounting for 11.1% of all the samples. Similarly, only two of the isolates from the rural hospitals showed extra drug resistance (11%). On the other hand, the urban isolates from the hospital demonstrated multidrug resistance in three cases (14%) but had five XDR isolates (24%). In the community setting, however, none of the isolates from both rural and urban locations showed resistance to more than two classes of antibiotics, hence there were no MDR or XDR organisms from the samples revived from community settings (Figure 3). Also, the characterization process revealed that urban hospitals face higher risks of developing MDR

and XDR strains as compared to their rural counterparts as the single urban facility (Mbagathi Hospital) had more MDR and XDR strains (19%) than the three rural hospitals that had 11%.

Table 3. Distribution of the *A. baumanii* phenotypes across the study sites

Phenotype	Hospital (n(%))			Community (n(%))		
	Rural	Urban	Total	Rural	Urban	Total
CEZ	5 (28)	11 (52)	16 (41)	20 (63)	38 (72)	58 (68)
CEZ-SXT	8 (44)	1 (5)	9 (23)	12 (37)	13 (24)	25 (29)
CEZ-CIP-CRO-FEP-GEN-IMP-MEM-SAM-SXT	1 (6)	5 (24)	6 (15)	0 (0)	0 (0)	0 (0)
CEZ-CIP-CRO-FEP-GEN-IMP-MEM-SAM-SXT-TOB	1 (6)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
CEZ-CIP-CRO-FEP-GEN-SXT	0 (0)	2 (10)	2 (5)	0 (0)	0 (0)	0 (0)
CEZ-CRO	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (1)
CEZ-CRO-FEP-IMP-MEM-SXT	0 (0)	1 (5)	1 (3)	0 (0)	0 (0)	0 (0)
CEZ-CRO-FEP-SAM-SXT	1 (6)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
CEZ-CRO-GEN-SXT	1 (6)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
CEZ-CRO-SXT	1 (6)	1 (5)	2 (5)	0 (0)	0 (0)	0 (0)
SXT-TOB	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (1)
Total	**18**	**21**	**39**	**32**	**53**	**85**

Key: CEZ, cefazolin; CIP, ciprofloxacin; CRO, Ceftriaxone; FEP, Cefepime; GEN, Gentamicin; IMP, Imipenem; MEM, Meropenem; SAM, Ampicillin/Sulbactam; SXT, Sulfamethoxazole/Trimethoprim; TOB, Tobramycin.

Hospital isolates showed higher resistance since their resistance profiles cut across different drug classes used in the study. None of the isolates were resistant to all seven antibiotic classes. Only 1% of community isolates were susceptible to all drugs.

Community isolates showed high resistance to one drug class (69%), while only 41% of hospital isolates were resistance to at least one class of antibiotics. Resistance to two drug classes was similar between community and hospital isolates (30% and 28% respectively) but none of the community isolates were resistant to more antibiotic classes, hence no MDR or XDR isolates were identified from these sites.

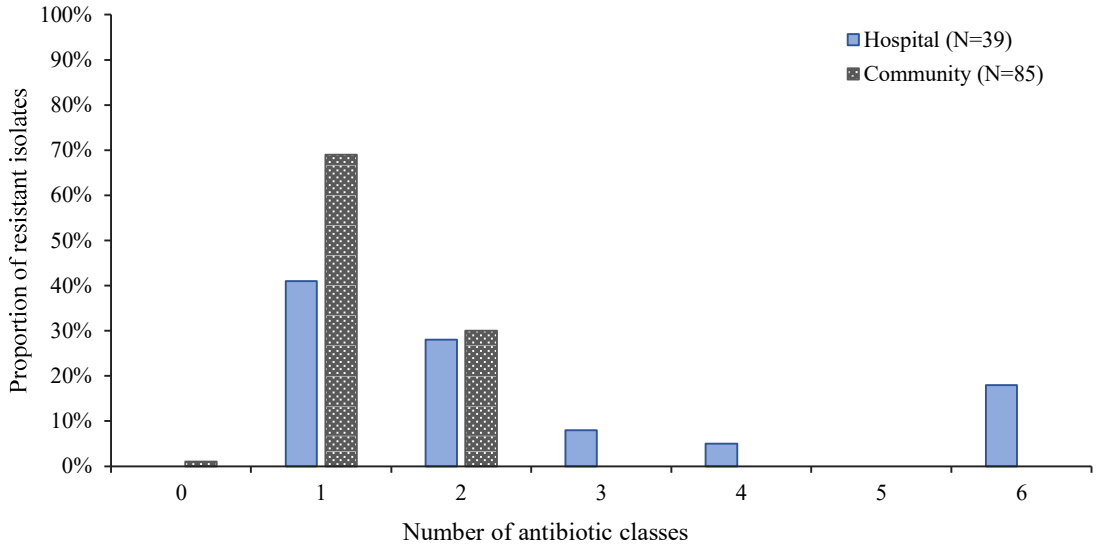


Figure 3: Number of antibiotic classes to which *A. baumanii* isolates were resistant.

4.4. Distribution of genes that encode resistance in *A. baumanii* strains.

Six genes responsible for beta-lactam and carbapenem resistance were detected in the five rural isolates and 10 urban isolates that had MDR and XDR traits. These isolates were presumed to harbor genes that mediate the production of enzymes that inhibit antibiotic action. The genes include bla_{TEM}, bla_{SHV}, bla_{OXA23}, $bla_{CTX\text{-}M}$, bla_{OXA48}, and bla_{NDMI} genes. Table 4 shows the proportion of isolates that were positive for each genes while figure 4 shows a representation of the amplicons.

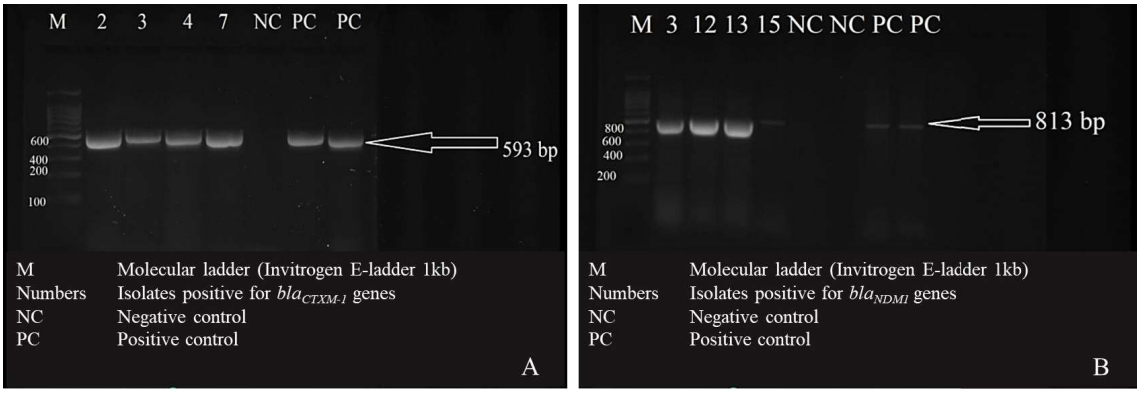


Figure 4. Gel electrophoresis for $bla_{CTXM\text{-}1}$ (A) and bla_{NDMI} (B) gene confirmation.

The $bla_{CTX\text{-}M}$ gene and the bla_{NDMI} gene were found in the isolates, with the former being more prevalent in urban hospitals and the latter in isolates from rural hospitals. No community isolates had the target resistance genes (Table 4)

Table 4. Resistance genes identified among *A. baumanii* isolates

Genes	Hospital	
	Rural (N=3) n (%)	Urban (N=9) n (%)
bla$_{TEM}$	0 (0)	0 (0)
bla$_{SHV}$	0 (0)	0 (0)
bla$_{OXA23}$	0 (0)	0 (0)
bla$_{CTX-M}$	0 (0)	4 (44)
bla$_{OXA48}$	0 (0)	0 (0)
bla$_{NDM1}$	2 (67)	1 (11)

Based on the findings shown in the figures, two resistance genes were positively identified in the hospital isolates, that is, the *bla*$_{CTX-M}$ (Figure 3A) and the *bla*$_{NDM1}$ (Figure 3B) genes. Gel electrophoresis demonstrated that seven of the 15 selected isolates had positive matches for the two genes (3 for *bla*$_{NDM1}$ and 4 for *bla*$_{CTX-M}$). Thus, the AMR genes mostly occurred in isolates from the hospitals (rural and urban) and not in the community. Community isolates were neither MDR nor XDR.

CHAPTER 5

5. DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1. Discussion

This study explored the characteristics of colonizing strains of *A. baumanii* from different settings to determine their antibiotic susceptibility and the genes that encode resistance. The study results show that antimicrobial resistance was higher in urban settings compared to rural settings. Urban hospitals around the world face a similar risk (Kariuki *et al.,* 2022). According to a survey conducted in LMICs around the world, urban areas are exposed to a wider array of antibiotics compared to rural areas (Rizk, Elwakil, and Attia, 2021). Kariuki *et al.* (2022) also reported a similar trend among other Kenyan healthcare settings, attributing this to factors such as overuse of over-the-counter antibiotics in urban settings, among others.

The AST patterns for the isolates in this study show that *A. baumanii* strains from the hospital samples were highly susceptible to aminoglycosides more than any other antimicrobial agent tested. This indicates that the bacteria in these settings are yet to acquire significant antimicrobial resistance previously reported against common agents like gentamicin. An investigation by Mirzaei *et al*. (2020) in Iran reported that *A. baumanii* strains in many urban hospital settings are progressively acquiring resistance at an unexpectedly high rate against various drug classes and the process is gradual. Similarly, an exploration linked to the evolution of carbapenem-resistant *A. baumanii* through whole-genome sequencing techniques in Southeast Asia was done by Li *et al.* (2015). It showed that the development of resistance in this microbe has been spreading to different drug classes over time Li *et al.* (2015). Additionally, the AST patterns for the hospital isolates indicate that newer agents like tobramycin are more effective against common nosocomial microbes (Mwale *et al*., 2023). However, cephalosporins showed a low effect on the hospital isolates, aligning with the tenet that their frequent use in Kenyan hospitals has exposed the bacteria to the agents, resulting in the induction of chromosomal β-lactamases (Muyu, 2020). This induction process is mediated by mutations that occur in different ways, with horizontal gene transfer being a major route. Hung *et al.* (2012) also reported instances of *A. baumanii* heteroresitance to cephalosporins and other drug classes as demonstrated by two patient cases in Taiwan (Hung *et al.,* 2012).

As expected, no rural or urban hospital isolate was susceptible to cefazolin due to the microbe's chromosomally induced intrinsic resistance. This outcome may therefore confirm that the *A. baumanii* isolates used in this characterization process have not gone through any potential

genetic mutation process that may otherwise render the bacteria susceptible to the antimicrobial agent. Only cefepime, a fourth-generation cephalosporin, showed significant activity against the hospital isolates in this study. This phenomenon is similar in sulfamethoxazole-trimethoprim whereby various mobile genetic elements like plasmids and transposons as well as integrons have been implicated in the spread of resistance to this drug combination in Kenyan hospitals (Mwale *et al*., 2023). The resistance to sulfamethoxazole-trimethoprim, however, being higher in rural settings, indicates that rural hospital patients receive the antibiotic more compared to patients in urban hospitals (Sharma *et al*., 2022).

In the community, rural isolates showed 100% susceptibility to aminoglycosides, carbapenems, the beta-lactam/beta-lactamase inhibitor combination, the glycylcycline, and the fluoroquinolone. The fourth-generation cephalosporin also demonstrated activity (97%) against the isolates. These drugs have remained effective in the community against *A. baumanii* because of their lack of frequent use as most are second or third-line prescription options and are barely accessible to rural communities (Cambaco *et al.,* 2020). However, 1st and 2nd-generation cephalosporins are commonly used, as well as the recently recommended sulfamethoxazole-trimethoprim combination, which has since been prescribed to outpatients frequently and is even being sold as over-the-counter products in drug outlets in Kenya (Morombaye, 2018). A similar trend was reported by Gebeyehu, Bantie, and Azage (2015) where rural settings in Northwest Ethiopia were implicated for inappropriate use of first-line and readily-available antibiotics. The authors reported that inappropriate antimicrobial use (AMU) was rampant in about 30% of these communities. However, Wang *et al*'s (2022) study conducted in Eastern China showed a gradual improvement in the behaviors of antibiotic use in rural areas of the country. This difference is attributable to the socioeconomic factors in different countries around the globe, including literacy levels among rural populations. More literate rural populations, like those in China and other developed countries, exhibit better antibiotic use behaviors as compared to the rural populations of LMICs (Wang *et al*., 2022).

On the other hand, people living in urban settings have more access to antibiotics as compared to their rural counterparts (Agyepong, Fordjour, and Owusu-Ofori, 2023a). Hence, the findings show higher levels of resistance to various drug classes in the urban community of Kibera. For instance, the 100% susceptibility to tobramycin, an aminoglycoside, the beta-lactam/beta-lactamase inhibitor combination (ampicillin-sulbactam), and the fourth-generation cephalosporin (cefepime) in rural communities of Asembo were reduced to 96% and 98% respectively in the urban setting of Kibera.

However, some drug classes like glycylcyclines, carbapenems, and fluoroquinolones have maintained integral action against *A. baumanii* because most members of the urban community settings are faced with various life stressors, mainly financial constraints, that may not allow them to be able to purchase most second and third-line antibiotics that are more expensive and rarely available over the counter (Kariuki *et al.,* 2022). Therefore, most people living in urban areas like Kibera that have a high bacterial infection rate tend to resort to cheaper and readily available over-the-counter options which are mostly first-line antibiotics (Rizk, Elwakil and Attia, 2021). The progressively increasing rates of resistance shown to the cephalosporins (3rd and 4th generation) and beta-lactams, as well as newer alternatives like sulfamethoxazole/trimethoprim combinations across all the isolates, is a clear indication of the advancing menace of AMR in the country prevalent in various disease-causing organisms like *A. baumanii* strains that have for a long time been associated with nosocomial infections but have now spread to the community (Agyepong, Fordjour and Owusu-Ofori, 2023a). This spread carries along the resistant genes that can potentially be transmitted to other strains and microbes, both horizontally and vertically.

The distribution patterns of MDR and XDR phenotypes found in this study indicate that hospitals are key areas of interest in the development and spread of AMR (Stephen *et al*., 2020). The MDR isolates reported in this study showed specific resistance to three of the following classes: aminoglycosides, cephalosporins (3rd and 4th generation), the beta-lactams/beta-lactamase inhibitor combination, and the sulfonamide/trimethoprim combination. The common sulfamethoxazole-trimethoprim resistance in the MDR phenotypes may be attributed to an increased prescription practice for this antimicrobial agent since its introduction to the market as an ideal antibiotic with a wide therapeutic window and low rates of adverse effects (Mwale *et. al*., 2023). The drug has widely been used by HIV patients and patients on mechanical ventilation who are immunocompromised. Hospitalization of such patients exposes them to these nosocomial pathogens which then develop various resistance mechanisms.

Also, cephalosporins have continued to be inappropriately prescribed in rural hospitals as evidenced by the development of resistance to *A. baumanii* strains, despite the known intrinsic resistance of the bacteria to the first- and second-generation agents of this drug class (Abdar *et al*., 2019). In Kenya, according to the national guidelines provided by the technical working group (TWG) on AMR, the prescription of antibiotics for conditions like low-grade fever should be prohibited to slow down the rates of resistance development (Ministry of Health, 2020). The cost of cephalosporins also contributes to their extensive misuse since cheaper

drugs are more readily available and hence used more often, predisposing them to resistance, especially in low and middle-income countries like Kenya (Odoyo *et al*., 2023). In the wake of improving care accessibility by providing more drug classes for use in the treatment of infections (Agyepong *et al*., 2023), healthcare providers and the government have also facilitated the development of XDR organisms as seen in the two rural hospital isolates. Previously effective drug classes like carbapenems have now been inculcated into the resistance profile of organisms like *A. baumanii* (Naomi *et al*., 2020). Advanced drugs like tobramycin, which are deemed one of the most effective aminoglycosides, have also been compromised by the resistance that has now spread to rural hospitals due to their increased availability (Agyepong *et al*., 2023). In the community, however, the lack of MDR and XDR strains of *A. baumannii* is common as reported by Zaidan, Hornak, and Reynoso, (2021). The community setting has not been exposed to these strains which generally thrive in hospital settings.

Urban hospitals remain a hotspot and a big concern as cultivators of MDR and XDR strains in the *A. baumanii* nosocomial debacle (Odoyo *et al*., 2023). This study's findings show that these settings have a significantly higher prevalence of MDR and XDR organisms. The MDR phenotypes were 14.3%, 3% higher in the urban hospital compared to the rural hospitals. The XDR phenotypes show a similar trend considering the 23.8% prevalence, which was 12% higher than the prevalence in rural settings. Urban hospitals encounter more patients exposed to different strains; hence they face a higher risk of developing drug resistance (Musyoki *et al*., 2019). Even more advanced drug classes like fluoroquinolones have been exposed to organisms often, hence they have developed resistance.

The distribution patterns for resistance show that single-drug resistance is more prevalent in community settings compared to the hospital context (Musyoki *et al*., 2019). This phenomenon backs up the fact that professionals give hospital antibiotic prescriptions. There is a minimal risk of uninformed and unnecessary use of drugs from different classes as compared to the community setting where instances of self-prescribed over-the-counter antibiotics are common (Musila *et al*., 2021).

Out of the 15 samples that exhibited MDR and XDR phenotypes, seven isolates exclusively from the hospital setup harbored two of the six genes, with none detected in isolates from the community. The genes of interest, the $bla_{CTX\text{-}M}$, and the *NMD1* genes were present only in hospital isolates, with two from the rural setting and five from the urban setting. While rural

communities may not have the same level of exposure to antibiotics, they still harbor risks for AMR development and spread (Ibrahim *et al*., 2021) as seen in the presence of the NMD1 gene in two of the three rural hospital isolates. While the percentage is 67% and higher than that of the urban hospital (55%), the absolute values still show that microbes with ESBLs and carbapenemases are more prevalent in urban hospitals.

The $bla_{CTX\text{-}M}$ gene was not present in rural hospital isolates, indicating that cephalosporin resistance may be lower in these contexts as posited by Ranaei *et al*. (2020) in their report in Saudi Arabia, another LMIC. This gene, therefore, is majorly responsible for the development and spread of resistance to beta-lactams in hospital settings (Benamrouche *et al*., 2020). However, resistance to carbapenems mediated by the *NMD1* gene has also spread across these hospital settings (Zahra, Zeshan, and Ishaq, 2022). Urban hospitals, on the other hand, had isolates with both genes. The extensive use of multiple antibiotics in hospitals is influenced by myriad factors including the prevalence of MDR and XDR strains that necessitate increased antibiotic use and further fuel the development of resistance (Omulo *et al*., 2021). The genotypes (containing these two genes) of *A. baumanii* strains, therefore, are linked to the AST profiles where the bacteria develop into an MDR or XDR phenotype. From these findings, it can be established that these phenotypes are common in hospital settings, solidifying the increasing menace of *A. baumanii* as a key nosocomial microbe.

Overall, AST patterns among *A. baumanii* strains indicate that urban settings pose higher risks of developing resistance to various antibiotics due to predisposing factors. For instance, the high patient population and the wide range of infections create a pool for the spread of resistance across different strains. Additionally, the readily accessible and available antibiotics from different classes play a pivotal role in the development of MDR and XDR phenotypes. While rural hospitals have developed these traits, they are minimal and may have spread from the occasional urban to rural movements. The exploration of the genes responsible for MDR and XDR traits also showed that these genes are more prevalent in urban hospital settings and their lack of occurrence in community settings shows that hospitals remain a key area of focus for AMR. The $bla_{CTX\text{-}M}$ gene was established as the main culprit responsible for the development of resistance against beta-lactam antibiotics.

The main limitation of this study lies in the use of archived isolates from the ARCH study which was conducted in 2019. These isolates may lack the genetic diversity of the current

pathogen population and hence the findings of the study may not represent the prevailing antibiotic resistant strains.

5.2.Conclusion

This study has established the following: (i) colonizing *A. baumannii* can proliferate and develop antimicrobial resistance in different settings. These variations may be attributed to high patient populations, access to antibiotics, and prescription practices in urban hospital settings that have demonstrated higher risks and exposure to the bacteria. (ii) the occurrence of MDR and XDR *A. baumanii* phenotypes is an issue in hospitals as isolates from community settings showed resistance only to two drug classes. Therefore, hospitals, as aforementioned, harbor the highest risks for the development and spread of drug resistance. (iii) two of the six selected genes were found in seven of the 15 identified MDR and XDR strains of *A. baumanii*. The $bla_{CTX\text{-}M}$ gene was the predominant resistant gene responsible for the development of resistance to beta-lactams like cephalosporins. However, the prevalence of this gene in hospital settings is disproportionately higher in urban hospitals, priming them as platforms for ESBL microbes.

5.3.Recommendations

A. baumanii has long been in the spotlight as a significant agent for nosocomial infections, as such to decrease the acquisition and spread of *A. baumanii* in hospitals, the study recommends that: (i) since hospitals harbor the development of drug-resistant *A. baumannii,* efficient implementation of AMS programs is key to the prevention of their emergence and dissemination. These programs should strengthening AMR surveillance specifically targeting MDR and XDR *A. baumanii*; (ii) as urban settings like informal settlements are susceptible to exposure to MDR and XDR strains that have previously been absent in community settings, it is crucial to implement effective control measures to prevent hospital to community spread of these strains; (iii) advanced research including genetic manipulation techniques like clustered regularly interspaced short palindromic repeats (CRISPR) should be explored to investigate ways in which gene-targeted therapy can help mitigate MDR and XDR *A. baumannii.*

REFERENCES

A. Evans, B., Hamouda, A., and G.B. Amyes, S. (2013) 'The Rise of Carbapenem-Resistant *Acinetobacter baumannii*', *Current Pharmaceutical Design*, 19(2), pp. 223–238.

Abbott, I. *et al*. (2013) 'Carbapenem resistance in *Acinetobacter baumannii*: Laboratory challenges, mechanistic insights, and therapeutic strategies', *Expert Review of Anti-Infective Therapy*, 11(4), pp. 395–409.

Abdar, M.H. *et al*. (2019) 'Prevalence of extended-spectrum beta-lactamase genes in *Acinetobacter baumannii* strains isolated from nosocomial infections in Tehran, Iran', *GMS Hygiene and Infection Control*, 14, p. Doc02.

Agyepong, N., Fordjour, F. and Owusu-Ofori, A. (2023) 'Multidrug-resistant *Acinetobacter baumannii* in healthcare settings in Africa', *Frontiers in Tropical Diseases*, 4, p. 15.

Almasaudi, S.B. (2018) '*Acinetobacter* spp. as nosocomial pathogens: Epidemiology and resistance features', *Saudi Journal of Biological Sciences*, 25(3), pp. 586–596.

Alyamani, E.J. *et al*. (2015) 'Molecular characterization of extended-spectrum beta-lactamases (ESBLs) produced by clinical isolates of *Acinetobacter baumannii* in Saudi Arabia', *Annals of Clinical Microbiology and Antimicrobials*, 14(1), pp. 1–9.

Antunes, L.C.S., Visca, P. and Towner, K.J. (2014) '*Acinetobacter baumannii*: Evolution of a global pathogen', *Pathogens and Disease*, 71(3), pp. 292–301.

Aruhomukama, D. *et al*. (2019) 'Bla VIM- And bla OXA-mediated carbapenem resistance among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates from the Mulago hospital intensive care unit in Kampala, Uganda', *BMC Infectious Diseases*, 19(1), pp. 1–8.

Asif, M., Alvi, I.A. and Ur Rehman, S. (2018) 'Insight into *Acinetobacter baumannii*: Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities', *Infection and Drug Resistance*, 11, pp. 1249–1260.

Benamrouche, N. *et. al*. (2020) 'Phenotypic and genotypic characterization of multidrug-resistant *Acinetobacter baumannii* isolated in Algerian hospitals', *The Journal of Infection in Developing Countries*, 14(12), pp. 1395–1401.

Bertini, A. *et al*. (2007) 'Multicopy blaOXA-58 gene as a source of high-level resistance to carbapenems in *Acinetobacter baumannii*', *Antimicrobial Agents and Chemotherapy*, 51(7), pp. 2324–2328.

Bonomo, R.A. and Szabo, D. (2006) 'Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*', *Clinical Infectious Diseases*, 43(Suppl. 2), pp. 49–56.

Cambaco, O. *et al.* (2020) Community knowledge and practices regarding antibiotic use in rural Mozambique: Where is the starting point for prevention of antibiotic resistance?' *BMC Public Health*, *20*(1), 1-15.

Chaudhary, M. and Payasi, A. (2012) 'Molecular characterization and antimicrobial susceptibility study of *Acinetobacter baumannii* clinical isolates from the middle east, African and Indian patients', *Journal of Proteomics and Bioinformatics*, 5(11), pp. 265–269.

Dexter, C. *et al*. (2015) '*Acinetobacter baumanii* Comunidad Er 2015', *Expert Review of Anti-infective Therapy*, pp. 567–573.

Farrugia, D.N. *et. al*. (2013) 'The Complete Genome and Phenome of a Community-Acquired *Acinetobacter baumannii*', *PLoS One*, 8(3): e58628..

Gootz, T.D. and Marra, A. (2008) '*Acinetobacter baumannii*: an emerging multidrug-resistant threat', *Expert Review of Anti-infective Therapy*, 3(3), pp. 5–7.

Gordon, N.C., and Wareham, D.W. (2010) 'Multidrug-resistant *Acinetobacter baumannii*: mechanisms of virulence and resistance', *International Journal of Antimicrobial Agents*, 35(3), pp. 219–226.

Hung, K. *et al.* (2012). 'Heteroresistance to cephalosporins and penicillins in Acinetobacter baumannii.' *Journal of clinical microbiology*, *50*(3), 721-726.

Hamidian, M. and Nigro, S.J. (2019) 'Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*', *Microbial Genomics*, 5(10), pp. 1–12.

Handal, R. *et. al*. (2017) 'Characterization of Carbapenem-Resistant *Acinetobacter baumannii* Strains Isolated from Hospitalized Patients in Palestine', *Hindawi*, 2017(2006), pp. 1–8.

Héritier, C. *et al*. (2005) 'Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*', *Antimicrobial Agents and Chemotherapy*, 49(8), pp. 3198–3202.

Howard, A. *et al*. (2012) '*Acinetobacter baumannii* an emerging opportunistic pathogen', *Virulence*, 3(3), p. 5.

Ibrahim, M.E. *et al*. (2021) 'Emergence of *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{SHV,}$ and *bla*$_{OXA}$ genes in multidrug-resistant Enterobacteriaceae and *Acinetobacter baumannii* in Saudi Arabia', *Experimental and Therapeutic Medicine*, 22(6), pp. 1–11.

Kariuki, S., *et al.* (2022) 'Antimicrobial resistance rates and surveillance in sub-Saharan Africa: where are we now?' *Infection and drug resistance*, 3589-3609.

Lee, C.R. *et al*. (2017) 'Biology of *Acinetobacter baumannii*: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment options', Frontiers in Cellular and Infection Microbiology, 7(Mar), p. 55.

Li, H. *et al.* (2015) 'Evolution of carbapenem-resistant Acinetobacter baumannii revealed through whole-genome sequencing and comparative genomic analysis.' *Antimicrobial agents and chemotherapy*, *59*(2), 1168-1176.

Li, S. *et al*. (2019) 'Molecular characteristics of carbapenem-resistant *Acinetobacter* spp. from clinical infection samples and fecal survey samples in Southern China,' *BMC Infectious Diseases*, 19(900), p. 1-12

Maragakis, L.L., and Perl, T.M. (2008) '*Acinetobacter baumannii*: Epidemiology, antimicrobial resistance, and treatment options', *Clinical Infectious Diseases*, 46(8), pp. 1254–1263.

Ministry of Health. (2020). 'Antimicrobial stewardship guidelines for health care settings in Kenya. Available at: https://www.momanyink.com/wp-content/uploads/2020/07/National-AMS-Guidelines-for-Healthcare-Settings-in-Kenya.pdf

Mirzaei, B. *et al*. (2020) 'Prevalence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran', *BMC Research Notes*, 13(1).

Morombaye, S.M. (2018) 'In vitro evaluation of the antimicrobial effect of Nepeta cataria and Basella alba against clinically resistant *Acinetobacter baumannii*', *Advances in Microbiology*, 08(10), p. 790-803

Musila, L. *et al*. (2021) 'Detection of diverse carbapenem and multidrug resistance genes and high-risk strain types among carbapenem non-susceptible clinical isolates of target gram-negative bacteria in Kenya', *PLoS One*, 16(2), p. e0246937.

Musyoki, V.M. *et al*. (2019) 'Antimicrobial susceptibility pattern of *Acinetobacter* isolates from patients in Kenyatta National Hospital, Nairobi, Kenya', The Pan African Medical Journal, 33, p. 233.

Muyu, M.G. (2020) 'Antimicrobial Use Practices in Mbagathi Hospital, Nairobi-Kenya: A Point Prevalence Survey' (Thesis: BPharm).

Mwale, J. *et al*. (2023) 'Phenotypic and genetic extended-spectrum beta-lactamase profiles of bacterial isolates from ICU in tertiary level hospital in Kenya', *F1000Research*, 12:469, 12, p. 469.

Naomi, W. *et al*. (2020) 'Carbapenemase genes among multidrug-resistant *Acinetobacter baumannii* in the critical care unit of Kenyatta National Hospital, Kenya', *East African Medical Journal*, 95(12), pp. 2196–2202.

Nowak, P. and Paluchowska, P. (2016) '*Acinetobacter baumannii*: Biology and drug resistance — role of carbapenemases', *Folia Histochemica et Cytobiologica*, 54(2), pp. 61–74.

Odoyo, E. *et al*. (2023) 'Environmental contamination across multiple hospital departments with multidrug-resistant bacteria pose an elevated risk of healthcare-associated infections in Kenyan hospitals', *Antimicrobial Resistance and Infection Control*, 12(1), p. 22.

Omulo, S. *et. al*. (2021) '1277. Colonization Rates for Antimicrobial-resistant Bacteria in Kenya: An Antibiotic Resistance in Communities and Hospitals (ARCH) Study', *Clinical Forum Infectious Diseases*, 8(Supplement_1), pp. S727–S727.

Peleg, A.Y., Seifert, H., and Paterson, D.L. (2008) '*Acinetobacter baumannii*: Emergence of a successful pathogen', *Clinical Microbiology Reviews*, 21(3), pp. 538–582.

Perez, F. *et al*. (2007) 'Global challenge of multidrug-resistant *Acinetobacter baumannii*', *Antimicrobial Agents and Chemotherapy*, 51(10), pp. 3471–3484.

Ranaei, M.A. *et al*. (2020) 'Prevalence of the $bla_{CTX\text{-}M}$ and antibiotic resistance pattern among clinical isolates of *Acinetobacter baumannii* isolated from Zahedan, Southeast Iran', *Gene Reports*, 19, p. 100626.

Ranjbar, R., Zayeri, S., and Mirzaie, A. (2020) 'Development of multiplex PCR for rapid detection of metallo-β-lactamase genes in clinical isolates of Acinetobacter baumannii.' *Iranian Journal of Microbiology*, *12*(2), 107.

Rizk, S.S., Elwakil, W.H. and Attia, A.S. (2021) 'Antibiotic-Resistant *Acinetobacter baumannii* in Low-Income Countries (2000–2020): Twenty-One Years and Still below the Radar, Is It Not There or Can They Not Afford to Look for It?', *Antibiotics* 2021, Vol. 10, Page 764, 10(7), p. 764.

Safari, M. *et al*. (2015) 'Prevalence of ESBL and MBL encoding genes in *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU)', *Saudi Journal of Biological Sciences*, 22(4), pp. 424–429.

Schmier, J.K., and Hulme Lowe, C.K. (2016) 'Economic Burden and Healthcare Resource Utilization Associated with Multi-Drug Resistant *Acinetobacter baumannii*: A Structured Review of the Literature', *Journal of Pharmaceutical Care & Health Systems*, 3(2), p. 1.

Sharma, A. *et al*. (2021) 'Multi-country cross-sectional study of colonization with multidrug-resistant organisms: protocol and methods for the Antibiotic Resistance in Communities and Hospitals (ARCH) studies', *BMC Public Health*, 21(1), pp. 1–9.

Sharma, A. *et al*. (2022) 'Menace of antimicrobial resistance in LMICs: Current surveillance practices and control measures to tackle hostility', *Journal of Infection and Public Health*, 15(2), pp. 172–181.

Smiline, A.S.G., Vijayashree, J.P. and Paramasivam, A. (2018) 'Molecular characterization of plasmid-encoded bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ among extended-spectrum β-lactamases [ESBLs] producing *Acinetobacter baumannii*', *British Journal of Biomedical Science*, 75(4), pp. 200–202.

Stephen, Njoroge, *et al*. (2020) 'Carbapenemase genes among multidrug-resistant *Acinetobacter baumannii* in the critical care unit of Kenyatta National Hospital, Kenya', *East African Medical Journal*, 95(12), pp. 2196–2202.

Thorne, A. *et al*. (2019) 'Risk factors for endemic *Acinetobacter baumannii* colonization: A case–case study', *American Journal of Infection Control*, 47(11), pp. 1294–1297.

Uwingabiye, J. *et al*. (2016) 'Acinetobacter infections prevalence and frequency of the antibiotic resistance: Comparative study of intensive care units versus other hospital units', *Pan African Medical Journal*, 23, pp. 1–10.

Wangai, F. K. *et al.* (2019) 'Bridging antimicrobial resistance knowledge gaps: the East African perspective on a global problem.' *PLoS One*, *14*(2), e0212131.

World Health Organization. (2017). WHO publishes a list of bacteria for which new antibiotics are urgently needed. [Online]. Available at: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

World Health Organization. (2021) 'Antibiotic resistance', Fact sheet, [online] Available at: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.

Woodford, N. *et. al*. (2006) 'Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp.', *International Journal of Antimicrobial Agents*, 27(4), pp. 351–353.

Xie, R. *et al*. (2018) 'Analysis of global prevalence of antibiotic resistance in *Acinetobacter baumannii* infections disclosed a faster increase in OECD countries,' *Emerging Microbes and Infections*, 7(1), pp. 1–10.

Zahra, N., Zeshan, B. and Ishaq, M. (2022) 'Carbapenem resistance gene crisis in *A. baumanii*: a computational analysis', *BMC Microbiology*, 22(1), pp. 1–17.