

**GENOMIC CHARACTERISTICS OF *Klebsiella pneumoniae*
ISOLATES IN KENYA: SEROTYPES, SEQUENCE TYPES,
ANTIMICROBIAL RESISTANCE, VIRULENCE, AND
PHYLOGENY**

ANGELA WANJUGU MURAYA

**MASTER OF SCIENCE IN
MOLECULAR BIOLOGY AND BIOINFORMATICS**

**JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY**

2026

**Genomic Characteristics of *Klebsiella pneumoniae* Isolates in Kenya:
Serotypes, Sequence Types, Antimicrobial Resistance, Virulence, and
Phylogeny**

Angela Wanjugu Muraya

**A Thesis Submitted in Partial Fulfilment of the Requirements for the
Degree of Master of Science in Molecular Biology and Bioinformatics
of the Jomo Kenyatta University of Agriculture and Technology**

2026

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature.....Date.....

Angela Wanjugu Muraya

This thesis has been submitted for examination with our approval as University Supervisors

Signature.....Date.....

Dr. Josephine Kimani, PhD
JKUAT, Kenya

Signature.....Date.....

Dr. Lilian Musila, PhD
KEMRI, Kenya

Signature.....Date.....

Dr. Caleb Kibet, PhD
IAVI, Kenya

DEDICATION

To my parents, Muraya Ngetha and Esther Muraya for their constant love and support.

To my siblings for their encouragement and prayers.

ACKNOWLEDGEMENT

I wish to express my sincere appreciation to everyone who supported me during my Master's journey. Their collective contributions made this achievement possible.

My deepest gratitude goes to Dr. Musila, whose comprehensive support—ranging from the research idea, lab space, and financial backing to guidance in lab work, data analysis, and writing—was the foundation of this thesis.

I am especially thankful to Dr. Kimani for her valuable input in proposal and thesis writing, as well as her support in navigating university requirements and administrative processes.

To Dr. Kibet, I am sincerely grateful for his expert guidance in the bioinformatic analysis of genomic data, and for introducing me to Dr. Musila, which enabled this research opportunity. I also thank Shahiid Kiyaga for his significant assistance in my bioinformatic analysis.

Special thanks to Cecilia Kyany'a for training me in molecular biology techniques including DNA extraction, library prep, sequencing, and PCR. I further acknowledge Eric Odoyo, Fred Tiria, and Martin Georges for their excellent instruction in microbiology.

I am grateful to the Walter Reed Army Institute of Research – Multidrug-Resistant Organism Repository and Surveillance Network (WRAIR-MRSN) team for performing the Illumina sequencing, and to the Institut Pasteur teams for curating and maintaining the BIGSdb-Pasteur databases used in this study.

Lastly, I extend my heartfelt thanks to all who contributed in any way to this academic journey.

TABLE OF CONTENTS

DECLARATION.....	ii
DEDICATION.....	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF APPENDICES	xi
ABBREVIATIONS AND ACRONYMS	xii
ABSTRACT	xiv
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background Information	1
1.2 Problem Statement	3
1.3 Justification	4
1.4 Hypothesis	5
1.5 Research Questions	5
1.6 Objectives	6

1.6.1 General Objective	6
1.6.2 Specific Objectives	6
1.7 Significance of the Study	7
CHAPTER TWO	8
LITERATURE REVIEW.....	8
2.1 <i>Klebsiella pneumoniae</i> Phylogroups	8
2.2 Genomic Structure of <i>Klebsiella pneumoniae</i>	8
2.3 Multilocus Types of <i>Klebsiella pneumoniae</i>	9
2.4 Antimicrobial Resistance in <i>Klebsiella pneumoniae</i>	10
2.5 Virulence in <i>Klebsiella pneumoniae</i>	12
2.6 Mobile Genetic Elements in <i>K. pneumoniae</i> Isolates.....	15
CHAPTER THREE	16
MATERIALS AND METHODS	16
3.1 Study Site	16
3.2 Study Samples	16
3.3 Ethical Approval, Confidentiality and De-identification	17
3.4 Bacterial Identification and Antimicrobial Susceptibility Testing.....	17
3.5 DNA Extraction and Sequencing	18

3.6 <i>De novo</i> Assembly of Raw Reads and Database Querying.....	19
3.7 Statistical Analysis of the AMR and Virulence Genes between Community-Acquired Infection and Healthcare-Associated Infection Isolates	20
3.8 Global Phylogenetic Context of Study Isolates.....	21
3.9 Mobile Genetic Element Analysis.....	22
3.10 Data Visualization	22
CHAPTER FOUR.....	23
RESULTS	23
4.1 Population Structure and Genomic Features of <i>Klebsiella pneumoniae</i>	23
4.1.1 Distribution and Population Structure of <i>Klebsiella pneumoniae</i>	23
4.1.2 Genomic Characteristics of <i>K. pneumoniae</i> Isolates	25
4.1.3 Serotypes of <i>K. pneumoniae</i> Isolates.....	26
4.1.4 Multilocus Sequence Types and Distribution of <i>K. pneumoniae</i> Isolates	26
4.1.5 Antimicrobial Resistance in the <i>K. pneumoniae</i> Isolates	28
4.1.6 Virulence Factors Associated with the <i>K. pneumoniae</i> Isolates.....	32
4.2 Comparison of the Distribution of the AMR Genes and Virulence Genes among the Community-Acquired and the Healthcare-Acquired Isolates of the Clinical Samples	36
4.3 Analysis of the Phylogenetic Relationship of the Whole Genome Sequences of the Study Isolates and Isolates from Other Selected Regions of the World	36

4.4 Assessment of the Risk of Spread of Antimicrobial Resistance and Virulence Genes by Investigating their Genomic Location.....	38
CHAPTER FIVE.....	41
DISCUSSION	41
5.1 Discussion	41
CHAPTER SIX	48
CONCLUSION AND RECOMMENDATION	48
6.1 Conclusion.....	48
6.2 Recommendations	49
REFERENCES.....	51
APPENDICES	72

LIST OF TABLES

Table 3.1: The Categories of <i>K. pneumoniae</i> Virulence Genes used for Frequency Analysis using Fisher's Exact Test.....	20
Table 3.2: The <i>K. pneumoniae</i> Antimicrobial Resistance Genes used for Frequency Analyses using Fisher's Exact Test	21
Table 4.1: Allelic Profiles of <i>K. pneumoniae</i> Isolates with Novel Multilocus Sequence Types	28
Table 4.2: Antimicrobial Resistance and Virulence genes Identified in the Plasmids of the <i>K. pneumoniae</i> Isolates (ID – identity)	35
Table 4.3: <i>K. pneumoniae</i> Isolates with the Highest Risk for AMR Genes Dissemination	38

LIST OF FIGURES

Figure 2.1: Bacterial Antimicrobial Resistance Mechanisms (Peterson & Kaur, 2018)	12
Figure 2.2: Virulence Factors of <i>K. pneumoniae</i> (Podschun & Ullmann, 1998)	14
Figure 4.1: Circular Cladogram Shows Population Structure and Genomic Features of <i>K. pneumoniae</i> Isolates	24
Figure 4.2: The Types and Number of Plasmid Replicons Identified in the Kenyan <i>K. pneumoniae</i> Isolates	25
Figure 4.3: Distribution of the Most Abundant Sequence Types of the <i>K. pneumoniae</i> Isolates by County	27
Figure 4.4: A Heatmap of the Phenotypic and Genotypic Profiles and Plasmid Replicon Abundance of <i>K. pneumoniae</i> Isolates	31
Figure 4.5: A Heatmap of the Multidrug Resistance Status, ST, Serotype, and Virulence Gene Content of <i>K. pneumoniae</i> Isolates	33
Figure 4.6: Phylogenetic Tree showing the Phylogenetic Relationship between the Kenyan <i>K. pneumoniae</i> Isolates with that of Other Isolates from Other Parts of the World	37
Figure 4.7: Heatmap Displaying AMR Genes (Columns) Found in Plasmids of <i>K. pneumoniae</i> Isolates (Rows)	40

LIST OF APPENDICES

Appendix I: Genomic and Epidemiological Characteristics of <i>K. pneumoniae</i> Isolates	72
Appendix II: Plasmids Replicons Present in the <i>K. pneumoniae</i> Isolates	76
Appendix III: Phenotypic Antimicrobial Susceptibility Test (AST) Results of the <i>K. pneumoniae</i> Isolates	79
Appendix IV: Antimicrobial Resistance Genes in the <i>K. pneumoniae</i> Isolates	82
Appendix V: Virulence Genes Identified in the <i>K. pneumoniae</i> Isolates	86
Appendix VI: Fluoroquinolone Mutations Identified in the <i>K. pneumoniae</i> Isolates	90
Appendix VII: Contingency Table of the Fisher's Exact Test for Virulence Gene Loci	91
Appendix VIII: Contingency Table of the Fisher's Exact Test for AMR Genes.....	92
Appendix IX: The Mating Pair Formation Genes and the Relaxase Genes in the <i>K. pneumoniae</i> Isolates	93
Appendix X: Publication - The Antimicrobial Resistance and Virulence Characteristics of <i>K. pneumoniae</i> Isolates in Kenya by Whole-Genome Sequencing.....	97

ABBREVIATIONS AND ACRONYMS

AME	Aminoglycoside Modifying Enzyme
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
CAI	Community-acquired Infection
CARD	Comprehensive Antibiotic Resistance Database
CRE	Carbapenemase Resistant <i>Enterobacteriaceae</i>
DNA	Deoxyribonucleic Acid
ESBL	Extended Spectrum Beta Lactamase
HAI	Health-acquired Infection
KEMRI	Kenya Medical Research Institute
LPS	Lipopolysaccharide
MDR	Multidrug Resistant
MLST	Multilocus Sequence Type
MRSN	Multidrug Resistant Organism Repository and Surveillance Network
NCBI	National Center for Biotechnology Information
NDM	New Delhi Metallo beta lactamase
ONT	Oxford Nanopore Technology

PMQR	Plasmid-mediated Quinolone Resistance
SSTI	Skin and Soft Tissue Infection
ST	Sequence Type
UTI	Urinary Tract Infection
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
XDR	Extensively Drug Resistant

ABSTRACT

Klebsiella pneumoniae is a globally important opportunistic pathogen associated with both healthcare-associated and community-acquired infections. In Kenya, limited knowledge of its genomic characteristics hinders efforts to detect, treat, and control multidrug-resistant strains. This study analyzed 89 *K. pneumoniae* isolates collected over six years from eight hospitals in five counties (Kisumu, Kericho, Kilifi, Kisii, and Nairobi) to characterize their phenotypic antimicrobial susceptibility, multidrug resistance, geographic distribution, and genomic features, including serotypes, sequence types, antimicrobial resistance and virulence genes, and phylogenetic relationships. Isolates were obtained from community-acquired infections (62/89) – infections occurring within 48 hours of patient admission, healthcare-associated infections (21/89) – infections occurring after 48 hours of patient admission or among healthcare workers, and the hospital environment (6/89) – isolates obtained from hospital high touch surfaces and analyzed using Illumina and Nanopore whole-genome sequencing. The genomes were screened for several gene features: antimicrobial resistance genes (CARD), virulence genes (Kaptive), multilocus sequence types (mlst) K-locus and O-antigen serotypes (Kaptive) analyzed, plasmid replicons (Plasmidfinder) and plasmid classification (Plascard). The phylogenetic tree of the 89 study isolates alone (parsnp) and when combined with 6286 global isolate representatives from the BV-BRC database (Mash) was generated and the trees were edited using iTOL. A total of 38 sequence types were identified, including globally disseminated high-risk clones (ST14, ST15, ST147, and ST307) and the regionally important ST17. The community-acquired infection isolates have a higher antibiotic burden than the healthcare-associated infection isolates ($p = < 0.05$) while the healthcare-associated infection isolates have a slightly higher virulence burden than the healthcare-associated isolates ($p = > 0.05$). Phylogenetic analysis showed that Kenyan isolates were interspersed with global strains, suggesting either long-term global circulation or recent introduction from external sources. Genomic analysis revealed the presence of carbapenemase genes (*bla*NDM-1 and *bla*OXA-181), pan-aminoglycoside resistance genes (*armA* and *rmtF*), and extended-spectrum beta-lactamase genes, predominantly *bla*CTX-M-15 (36/89), *bla*TEM (35/89), and *bla*OXA (18/89). A mobile colistin resistance gene (*mcr*-8) was identified in one isolate, and fluoroquinolone resistance-associated mutations in *gyrA* and *parC* were detected. Key virulence factors included regulators of hypervirulence (*rmpA/A2* and *magA*) and siderophores such as yersiniabactin, salmochelin, and aerobactin. Notably, two potential hypervirulent isolates and two community-acquired ST147 high-risk clones carried both carbapenemase genes and virulence determinants. Plasmids played a central role in the dissemination of antimicrobial resistance and virulence genes through conjugation. Overall, these findings demonstrate that Kenyan *K. pneumoniae* isolates harbor diverse resistance and virulence profiles, including high-risk clones with the potential for rapid spread. This underscores the need for expanded national surveillance integrating genomic and phenotypic data across clinical and environmental settings to better monitor and control emerging threats.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Klebsiella pneumoniae is a globally significant Gram-negative, encapsulated, opportunistic pathogen and ranks among the leading causes of healthcare-associated infections, particularly in low-resource settings (Irek et al., 2018). It is a major cause of both healthcare-associated infections (HAIs) such as pneumonia, ventilator-associated infections, urinary tract infections, wound infections, and surgical site infections (Paczosa & Meccas, 2016) and community-acquired infections (CAIs) such as liver abscess, particularly affecting neonates and immunocompromised individuals. Community-acquired infections are defined as those occurring at or within 48 hours of hospital admission, while healthcare-associated infections arise after 48 hours of admission and have not been present before the admission of the patient to the healthcare facility, or occupational infections occurring among healthcare workers (Horan et al., 2008; WHO, 2002).

K. pneumoniae poses a growing global public health challenge due to the emergence of multidrug-resistant (MDR) strains and hypervirulent variants. The World Health Organization (WHO) lists *K. pneumoniae* as among the highest-priority bacterial pathogens due to the limited treatment options available for these infections and their substantial contribution to antimicrobial resistance worldwide to antibiotics such as penicillins, cephalosporins, and quinolones (WHO, 2017). Infection outbreaks have occurred worldwide due to several high-risk sequence types (STs), including ST14, ST15, ST17, ST147, ST307, and ST607 (Mathers et al., 2015). These successful lineages readily acquire and disseminate antimicrobial resistance determinants, persist in healthcare settings, and spread between patients, enabling them to become established as endemic high-risk clones in some regions (Tolentino et al., 2019; Wyres & Holt, 2016).

The major transmission route for *K. pneumoniae* infections in healthcare facilities is patient-to-patient transmission via the hands of healthcare workers, particularly for multidrug-resistant strains (Tsioutis et al., 2021). High-touch hospital surfaces such as sinks, doorknobs, drains, and medical equipment can serve as reservoirs for resistant strains and facilitate indirect transmission through contact with contaminated surfaces and medical devices (Odooyo et al., 2023). The strains also colonize medical devices such as urinary catheters, ventilators and central venous catheters (Chang et al., 2021). Molecularly, *K. pneumoniae* readily acquires and disseminates antimicrobial resistance and virulence determinants through horizontal gene transfer mediated by plasmids and other mobile genetic elements (Wyres & Holt, 2018). Its highly plastic genome and frequent colonization of the gastrointestinal tract facilitate genetic exchange with other members of the gut microbiota, contributing to the emergence and spread of multidrug-resistant and hypervirulent lineages (Wyres et al., 2020).

K. pneumoniae species can be identified in clinical laboratories using automated systems such as the VITEK® 2 platform and Gram-negative identification cards (GN-ID). However, whole-genome sequencing enables higher-resolution characterization of isolates, including phylogenetic classification through multilocus sequence typing (MLST), antigenic classification through capsular (K) and lipopolysaccharide (O) serotyping, and assignment to the currently recognized phylogroups (Holt et al., 2015). These analyses can be performed using bioinformatics tools such as Kleborate (Lam et al., 2021) and comparison against curated reference genome databases (Olson et al., 2023).

Understanding genomic diversity, antimicrobial resistance determinants, virulence factors, and phylogenetic lineages is crucial for surveillance, outbreak detection, and infection control.

1.2 Problem Statement

K. pneumoniae is a major cause of both healthcare-associated and community-acquired infections worldwide, contributing to morbidity and mortality, particularly in low- and middle-income countries. It causes several clinical outcomes such as bloodstream infections (Girometti et al., 2014), urinary tract infections, pneumonia, skin and soft tissue infections and sepsis (Aiken et al., 2011), with reported mortality of 16 to 47.6% in neonates in low- and middle-income countries (Khaertynov et al., 2018; Liu et al., 2012; Zea-Vera & Ochoa, 2015).

Globally, *K. pneumoniae* is responsible for approximately 790,000 deaths each year, including 124,000 neonatal deaths in sub-Saharan Africa (Murray et al., 2022). In Kenya, a substantial share of infant mortality is attributed to *K. pneumoniae* (Verani et al., 2024). CHAMPS surveillance data indicate that around 30% of deaths in infants aged 1–11 months involve this pathogen (Kumar et al., 2023; Verani et al., 2024).

In Kenya, *K. pneumoniae* and *Escherichia coli* are among the leading causes of bacterial infections in Kenya, especially in children under five years (Irek et al., 2018) (Karanja et al., 2024). The prevalence of multidrug-resistant *K. pneumoniae* (MDR-*K. pneumoniae*) in Kenya is rising, driven by factors such as prolonged hospital stays, overuse of antibiotics—particularly cephalosporins (Girometti et al., 2014)—and inadequate infection prevention and control practices (Maina et al., 2017). Limited diagnostic capacity further impedes effective surveillance (Girometti et al., 2014; Moremi et al., 2016).

This burden is worsened by the rapid emergence of antimicrobial resistance, which limits treatment options and worsens patient outcomes. In Kenya, *K. pneumoniae* has been reported with high levels of resistance to commonly used antibiotics, including third-generation cephalosporins (e.g., ceftriaxone resistance rates of 47–91.3%) (Girometti et al., 2014; Henson et al., 2017) and fluoroquinolones (37.6% of 92 ciprofloxacin-resistant commensal gut *Klebsiella* spp. isolates) (Kariuki et al., 2023), largely driven by extended-spectrum β -lactamase (ESBL) production. Resistance to carbapenems, which are often

considered last-resort antibiotics, has also been documented, with carbapenemase-producing isolates including *bla*NDM-1 reported at frequencies of 15% in one Kenyan clinical series and other carbapenemase genes detected in genomic studies (Henson et al., 2017; Muraya et al., 2022; Poirel et al., 2010).

Although carbapenem resistance remains a significant concern globally, its low detection rate in Kenya may not reflect a true absence of these determinants but rather the existence of systemic gaps in nationwide surveillance.

While several studies in Kenya have examined *K. pneumoniae*, they have typically been limited in scope—analyzing small sample sizes from single hospitals. As such, these studies may not accurately represent the national epidemiological landscape. Most focus primarily on antibiotic susceptibility profiles and multidrug resistance. However, there remains a limited understanding of the underlying resistance and virulence genes, their potential for horizontal gene transfer, or the phylogenetic relationships among the circulating isolates.

1.3 Justification

Multidrug-resistant *K. pneumoniae* has emerged as a major public health concern globally due to its increasing resistance to critically important antibiotics and its association with severe healthcare-associated and community-acquired infections. In Kenya, genomic data describing the circulating *K. pneumoniae* strains remain limited, with most prior studies relying mainly on phenotypic antimicrobial susceptibility testing disc diffusion antibiotic susceptibility test (Apondi et al., 2016; Maina et al., 2011, 2017) and involving isolates from single healthcare facilities or limited geographic regions (Henson et al., 2017; Taitt et al., 2017). Consequently, there is insufficient information on the national distribution of high-risk clones, antimicrobial resistance genes, virulence factors, and their associated mobile genetic elements.

This study utilized clinical and environmental *K. pneumoniae* isolates collected from selected healthcare facilities in Kenya to provide a broader understanding of the genomic epidemiology of circulating strains. Characterization of serotypes (Stanton et al., 2025), multilocus sequence types (Maiden et al., 2013), antimicrobial resistance genes (Lam et al., 2021), and virulence factors (Hsu et al., 2011; Khaertynov et al., 2018) will provide important information on the genetic diversity and pathogenic potential of Kenyan *K. pneumoniae* isolates. In addition, evaluating the genomic context of antimicrobial resistance genes will help assess their potential for horizontal gene transfer and dissemination.

Comparative genomic and phylogenetic analyses will further improve understanding of the relationship between Kenyan isolates and globally circulating strains, including the presence of internationally recognized high-risk clones associated with antimicrobial resistance and infection outbreaks. These findings will contribute valuable genomic surveillance data to support antimicrobial stewardship, infection prevention and control strategies, and public health decision-making in Kenya. Furthermore, the study will strengthen the existing knowledge base on multidrug-resistant *K. pneumoniae* in low- and middle-income countries and contribute to global efforts aimed at monitoring and controlling the spread of antimicrobial resistance.

1.4 Hypothesis

The *Klebsiella pneumoniae* isolates circulating in Kenya harbor diverse antimicrobial resistance and virulence genes, with distinct genomic patterns between healthcare-associated and community-acquired infections, and a high potential for horizontal gene transfer.

1.5 Research Questions

- i. What serotypes, multilocus sequence types and antimicrobial resistance and virulence genes are in the *Klebsiella pneumoniae* isolates?

- ii. What are the differences in the distribution of the antimicrobial resistance and virulence genes between the community-acquired and the healthcare-associated isolates of the clinical samples?
- iii. What is the phylogenetic relationship of the whole genome sequences of the study isolates and isolates from other regions of the world?
- iv. What is the genomic location of antimicrobial resistance in the *K. pneumoniae* isolates that will enable the assessment of the risk of their spread?

1.6 Objectives

1.6.1 General Objective

To determine the genomic characteristics of *Klebsiella pneumoniae* isolates in Kenya: serotypes, sequence types, antimicrobial resistance, virulence, and phylogeny.

1.6.2 Specific Objectives

- i. To determine the serotypes, multilocus sequence types and antimicrobial resistance and virulence genes of the *Klebsiella pneumoniae* isolates using whole genome sequence analysis
- ii. To compare the distribution of the antimicrobial resistance genes and virulence genes among the community-acquired and the healthcare-associated isolates of the clinical samples
- iii. To analyze the phylogenetic relationship of the whole genome sequences of the study isolates and isolates from other regions of the world
- iv. To assess the risk of spreading antimicrobial resistance genes in the *K. pneumoniae* isolates by investigating their genomic location.

1.7 Significance of the Study

Understanding the genomic landscape of *K. pneumoniae* in Kenya will help in mapping its resistance and virulence potential, support clinical decision-making, and guide infection prevention strategies. Insights into phylogenetic patterns and gene mobility mechanisms will also contribute to the global understanding of *K. pneumoniae* evolution and dissemination, supporting more informed public health interventions and AMR surveillance.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Klebsiella pneumoniae* Phylogroups

Klebsiella pneumoniae belongs to the phylum *Proteobacteria*, class γ -*Proteobacteria*, family *Enterobacteriaceae*, and genus *Klebsiella* (Krieg Noel R. et al., 2015). Clinically significant members include *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *ozaenae*, *K. pneumoniae* subsp. *rhinoscleromatis*, *K. oxytoca*, and *K. granulomatis* (Podschun & Ullmann, 1998). A pan-genome-wide association study by (Holt et al., 2015) has further classified *K. pneumoniae* commonly associated with human infections into three distinct phylogroups: KpI – *Klebsiella pneumoniae* subsp. *pneumoniae*, KpII-A - *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*, KpII-B – *Klebsiella quasipneumoniae* subsp. *similipneumoniae* and KpIII - *Klebsiella variicola* (Holt et al., 2015).

2.2 Genomic Structure of *Klebsiella pneumoniae*

The genomic sequence of *Klebsiella pneumoniae* varies from 5.3 Mbp to 5.8 Mbp and the numbers and sizes of plasmid sequences vary widely. For instance, the multiple-drug resistant strain, HS11286 (accession CP003200), has one circular chromosome of 5 Mbp and six plasmids ranging from 1.3 kb to 122.8 kb. The chromosome contains a GC content of 57.5%, 5316 protein-coding, 87 tRNAs and 8 copies of the rRNA operon. The strain also has two integrative conjugative elements *ICEpnHS11286-1* and *ICEKnHS11286-2* and plasmid-bound AMR genes - *blaCTX-M-14*, *blaTEM-1*, *blaKPC-2*, *tetG*, *aa(3)-Ia* and *sul1* (B. Li et al., 2012). Understanding the genome structure is essential for determining the location and transmission potential of antimicrobial resistant and virulence genes.

2.3 Multilocus Types of *Klebsiella pneumoniae*

Multilocus sequence typing is a sequence-based method of characterizing the genetic relationship of bacterial isolates (Maiden et al., 2013). The sequences are analyzed either in the laboratory by amplifying and sequencing the genes or by querying the isolate genomes against the reference database. *Klebsiella* MLST scheme comprises of seven housekeeping genes: *gapA*—glyceraldehyde 3-phosphate dehydrogenase, *infB*—translation initiation factor 2, *mdh*—malate dehydrogenase, *pgi*—phosphoglucose isomerase, *phoE*—phosphoporine E, *rpoB*—beta-subunit of Ribonucleic acid polymerase B, *tonB*—periplasmic energy transducer (Diancourt et al., 2005) which is stored in the *Klebsiella* MLST BIGSdb hosted and maintained at the Institut Pasteur (<https://bigsd.bpasteur.fr/klebsiella/>).

MLST has been widely used to study the population structure and global dissemination of high-risk *K. pneumoniae* clones associated with antimicrobial resistance and hospital outbreaks. Bacterial clones are isolates that are closely related phylogenetically (Baquero et al., 2015). High-risk clones are pathogenic bacteria clones that possess traits that make them highly invasive (hypervirulent) and readily acquire antimicrobial resistance genes to increase their chances of survival in the host (Mathers et al., 2015). In addition, they readily disseminate these virulence and antimicrobial resistance genes through mobile genetic elements to other cells of the same or different species.

Several high-risk global clones like ST14, ST15, ST17, ST37, ST147, ST258, ST307, ST340, and ST607 are associated with resistance dissemination (Baquero et al., 2015; Tolentino et al., 2019). They have been linked to multidrug resistance and clinically important β -lactam resistance determinants. ST14 and ST15 are widely distributed high-risk clones - and are frequently associated with ESBL production and carbapenem resistance, including *bla*CTX-M-15 and carbapenemase genes such as *bla*NDM and *bla*OXA-48-like. ST307 is an emerging global lineage strongly associated with multidrug resistance, nosocomial transmission, and genes including *bla*CTX-M-15, *bla*KPC, *bla*NDM, and *bla*OXA-48-like. ST17 is now well supported as a global problem clone associated with

multidrug-resistant hospital infections, including neonatal outbreaks in Kilifi (Henson et al., 2017). ST607 is also documented as a multidrug-resistant outbreak lineage, including a neonatal intensive care unit outbreak linked to *bla*CTX-M-15 and plasmid-mediated quinolone resistance determinants.

2.4 Antimicrobial Resistance in *Klebsiella pneumoniae*

K. pneumoniae is intrinsically resistant to ampicillin and readily acquires a high diversity of antimicrobial-resistant genes. It acts as a key disseminator of AMR determinants to other Enterobacteriaceae (Wyres et al., 2020). These determinants are grouped according to their antibiotic resistance modes of action, as summarized in Figure 2.1.

Enzymatic inactivation mechanisms include the modification of aminoglycosides and macrolides through the addition of acetyl, phosphate, and adenylyl groups. This is mediated by N-acetyltransferases (*aac*), e.g. chloramphenicol acetyltransferase (*cat*) and bleomycin N-acetyltransferase (*BlmB*), O-phosphotransferases (*aph*), and O-adenylyltransferases (*ant*) enzymes (Domingues et al., 2012). β -lactam antibiotics—penicillins and cephalosporins—are inactivated by β -lactamase enzymes (Rawat & Nair, 2010). MDR *K. pneumoniae* strains often produce extended-spectrum β -lactamases (ESBLs) such as TEM, SHV, CTX-M and OXA. Carbapenems, a last-resort drug for MDR strains, are inactivated by carbapenemases, notably *bla*KPC, *bla*NDM-1, and *bla*OXA-48-like genes.

Target modification mechanisms include polymyxins such as colistin, which disrupt the bacterial cell membrane, being blocked by extracellular surface modifications on peptide binding proteins (PBPs) and polysaccharides (Andrade et al., 2020). Macrolides, which target ribosomes essential for protein synthesis, are blocked by methylation of ribosomal RNA, while quinolones are resisted through mutations in target enzymes such as DNA gyrase and topoisomerase IV (Endimiani et al., 2009).

Reduced intracellular drug accumulation is mediated by the overexpression of efflux pumps, which actively transport antibiotics out of the bacterial cytosol (X. Z. Li & Ninkaido, 2009). These efflux pumps are often non-antibiotic-specific, allowing bacteria to resist a wide range of antibiotics, in the presence or absence of antibiotic-specific resistance mechanisms (Blair et al., 2014). Other mechanisms involve antibiotic sequestration in proteins to prevent them from reaching their target (Rudolf et al., 2015) or the use of alternative substrate target sites that are not compatible with antibiotic binding (Schmutz et al., 2003).

As a result of the accumulation of these resistance mechanisms, multidrug-resistant (MDR) bacteria resist at least one agent in three or more antibiotic classes; extensively drug-resistant (XDR) strains are non-susceptible to all but one or two classes; and pan-drug-resistant (PDR) strains resist all classes (Magiorakos et al., 2012).

At the regional level, Kenyan studies have identified ESBL-producing *K. pneumoniae* in Nairobi, Kisumu, Kisii, Homabay, Migori, Kilifi, and Eldoret, with genes such as *bla*CTX-M-15, *bla*SHV, *bla*LEN-1, and *bla*TEM-1 commonly detected (Apondi et al., 2016; Henson et al., 2017; Maina et al., 2017). Carbapenemase genes *bla*NDM-1 and *bla*SPM were identified in 3.7% of urine samples at a Nairobi tertiary hospital (Maina et al., 2017), while Henson et al. (2017) reported a pNDM-mar-like plasmid carrying *bla*NDM in 12.8% of blood samples, with ESBL prevalence in 94% of HAIs and 44% of CAIs (Apondi et al., 2016; Henson et al., 2017; Maina et al., 2017).

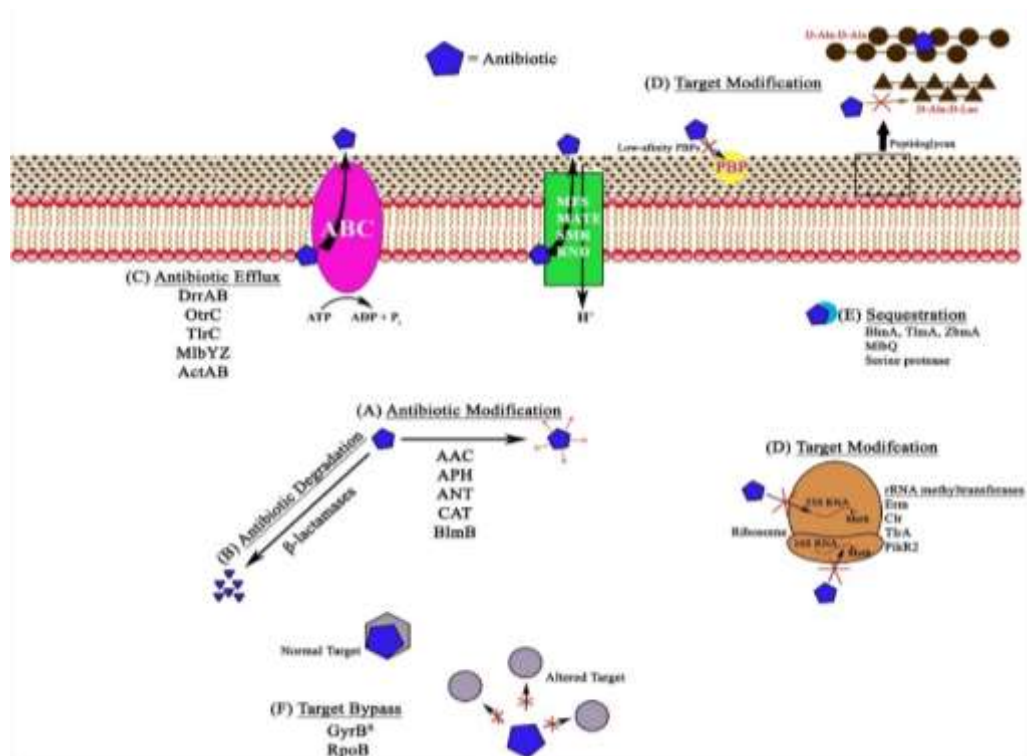


Figure 2.1: Bacterial Antimicrobial Resistance Mechanisms (Peterson & Kaur, 2018)

Antibiotic modification (A) Antibiotic degradation (B) Antibiotic removed via efflux pumps (C) Antibiotic target modification (D) Antibiotic sequestering (E) Antibiotic target bypass (F).

2.5 Virulence in *Klebsiella pneumoniae*

Klebsiella pneumoniae is an opportunistic pathogen that attacks mostly when the host immune system is compromised or impaired. More than one virulence determinant or mechanism is used by invasive bacteria to cause an infection. *K. pneumoniae* bacteria utilize adhesins to attach themselves to the mucosal cell walls of the host whereby they penetrate and localize the tissues from where they obtain nutrients from the cells for growth. (Figure 2.2). FimA genes encode the fimbriae adhesins, and they form the first step of invasion by adhering to the mannose molecule of the glycoprotein located on the host epithelial cell surface.

A few mechanisms have been determined which aid *K. pneumoniae* to avoid host defenses. One of them is the use of a hydrophilic polysaccharide capsule that prevents phagocytic engulfment, opsonization induced by the host antibody or complementary system, and in some cases to mimic host polysaccharide moieties (Huynh et al., 2017).

Serotyping is a bacterial classification method based on variation in surface antigens, particularly capsular (K) polysaccharides and lipopolysaccharide (O) antigens (C. R. Lee et al., 2017; Wick et al., 2018; Wyres & Holt, 2016). In *K. pneumoniae*, these antigenic differences are key determinants of virulence and epidemiological diversity. Serotyping can be performed phenotypically using immunoassays or inferred genomically by comparing whole genome sequence data against curated databases such as Kaptive (Wick et al., 2018).

MLST genes are highly conserved and are not horizontally transferred hence this typing is best for studying evolutionary and transmission patterns of strains (Wick et al., 2018). Meanwhile, serotyping gene markers are constantly mutating and recombining and being transferred horizontally making them ideal for studying the host and phage interactions, immune evasion and virulence patterns (Stanton et al., 2025; Wick et al., 2018).

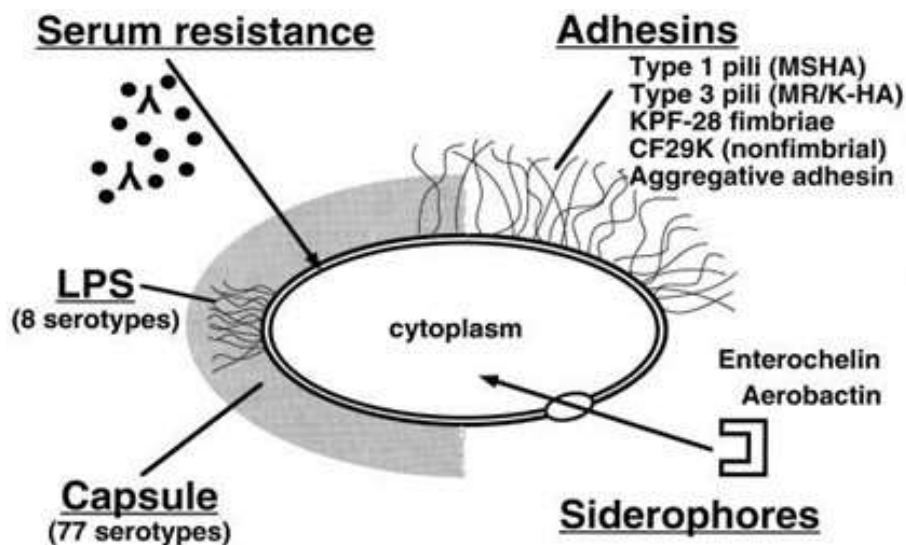


Figure 2.2: Virulence Factors of *K. pneumoniae* (Podschun & Ullmann, 1998)

The core lipopolysaccharides (LPS) embedded on the pathogen's outer membrane are antigenic triggers of the host immune system, especially the complementary system (Figure 2.2). Invasive bacteria have smooth "O" side chains on the LPS which deters binding of the complement components to mark them for destruction by macrophages. The pathogens presenting these "O" side chains are more resistant than the "rough" type without the side chains. The LPS is encoded by the *wabG* gene (C. R. Lee et al., 2017).

For invasive *K. pneumoniae* to survive and multiply in the host, it requires host iron molecules which are tightly bound to iron-binding proteins, *lactoferrin* and *transferrin*. To overcome this hurdle, *K. pneumoniae* has adapted the use of siderophores which are iron-chelating protein complexes: aerobactin, salmochelin, yersiniabactin and colibactin (Figure 2.2) (Podschun & Ullmann, 1998). The hypervirulent *Klebsiella pneumoniae* (*hvKP*) variants are associated with the presence of the hypermucoviscous phenotype in capsule k1 and k2 serotypes, aerobactin and salmochelin siderophores and the *magA* and *rmpA* genes that encode for regulator mucoid phenotype (Cloeckaert et al., 2021; Parrott et al., 2021). The *rmpA* genes are plasmid-mediated virulence genes that regulate capsule synthesis and are also associated with community-acquired pyogenic liver abscess (Hsu et al., 2011).

2.6 Mobile Genetic Elements in *K. pneumoniae* Isolates

Mobile genetic elements (MGEs) are DNA molecules that exist either chromosomally or extra-chromosomally within bacterial genomes. They include plasmids, integrative and conjugative elements (ICEs), integrons, transposons, gene cassettes, and pathogenicity islands (Han et al., 2025). Plasmids can be classified as conjugative, mobilizable, or non-mobilizable. Conjugative plasmids contain all the necessary genes for self-transmission, enabling them to transfer a copy of themselves to another bacterial cell via conjugation. These include the origin of transfer (*oriT*) and relaxase genes (e.g., *mob*, *traI*) the mating pair formation (MPF) system (encoded by the *tra* operon), which constitutes a type of Type IV Secretion System (T4SS), and T4SS coupling proteins (T4CP) (Han et al., 2025; Smillie et al., 2010). The *oriT* acts as the recognition site of the relaxosome (expressed by relaxase & helicase genes) which nicks and unwinds one strand of the double stranded plasmid, leaving the second single strand as template of DNA polymerase to regenerate the double strand (C. A. Lee & Grossman, 2007). The nicked single strand is transferred to the recipient cell via the channel formed by the T4SS (Guasch et al., 2003). Mobilizable plasmids possess the *oriT* sequence and relaxase genes which but lack the MPF system. Consequently, they depend on the MPF machinery provided by a co-resident conjugative plasmid for their transfer (Smillie et al., 2010). Unmobilizable plasmids lack relaxase genes and cannot be transferred via conjugation, thus accessory genes such as AMR genes within them cannot be transferred via conjugation. However, under special conditions, they can be transferred through co-integration of ummobilizable with conjugative plasmids or transposition (Smillie et al., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The bacterial culture, DNA extraction, quantification, and Oxford Nanopore sequencing were conducted in the Kenya Medical Research Institute (KEMRI) - Center for Microbiology Research. Illumina sequencing was performed at the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN), Walter Reed Army Institute of Research (WRAIR). The bioinformatic analysis was conducted using the high-performance computing server at the Department of Emerging Infectious Diseases, WRAIR -Africa in KEMRI, Nairobi.

3.2 Study Samples

The study included all archived bacterial isolates that were phenotypically confirmed as *K. pneumoniae* resulting in a final sample of 89 isolates from an initial collection of approximately 2,000 archived isolates. Accordingly, the study analyzed the total population of available *K. pneumoniae* isolates recovered during the study period rather than a purposively selected subset. The isolates were collected between May 2015 and March 2020 from eight hospitals (Musila et al., 2021) across five counties (Nairobi, Kilifi, Kisii, Kisumu and Kericho) in Kenya, as part of ongoing antimicrobial resistance surveillance (KEMRI 2767/WRAIR 2089) and a hospital environmental study (KEMRI 3482/WRAIR 2416).

The isolates were unevenly distributed across locations, with most originating from more urbanized areas with higher human traffic (Appendix 1), Kisumu (35), Nairobi (23), Kisii (15), Kilifi (9), and Kericho (7). Clinical samples included urine (26), and wound swabs and pus (57) collected from consenting patients with suspected bacterial infections. Environmental samples (6) were obtained via swabs from high-touch surfaces within participating hospitals.

Samples obtained from infections that occurred at or within 48 hours of hospital admission were labelled as community-acquired infection isolates while samples obtained from infections arising after 48 hours of admission and had not been present before the admission of the patient to the healthcare facility, or occupational infections occurring among healthcare workers were labelled as healthcare-associated infections (Horan et al., 2008; WHO, 2002).

More details on the sources and distribution of the isolates, infection type and MDR status are provided in Appendix 1.

3.3 Ethical Approval, Confidentiality and De-identification

This study involves no human or animal subjects. Use of the archived bacterial isolates in this retrospective analysis was previously approved by the Scientific Ethics Review Unit (SERU) at KEMRI under parent protocol KEMRI SSC# 2767. The isolates were de-identified to preserve patient privacy. In accordance with confidentiality requirements, hospital names are not reported to protect participating institutions and minimize stigmatization risk. De-identified sub-cultures and associated metadata were used throughout the analysis.

3.4 Bacterial Identification and Antimicrobial Susceptibility Testing

Klebsiella pneumoniae identification and antimicrobial susceptibility testing (AST) were performed on the Vitek2[®] system (bioMérieux, France) using the GN-ID and XN05-AST cards. The AST panel consisted of penicillins (piperacillin and ticarcillin/clavulanic acid), monobactam (aztreonam), cephalosporins (cefuroxime, cefuroxime axetil, cefixime, ceftriaxone, and cefepime), carbapenems (meropenem), fluoroquinolones (levofloxacin and moxifloxacin), tetracyclines (tetracycline and minocycline), glycylicycline (tigecycline), phenicol (chloramphenicol) and trimethoprim. The raw AST results (Appendix III) were interpreted according to CLSI guidelines (2018), and isolates were

classified as either multidrug-resistant (resistant to 3 or more drug classes) or non-multidrug resistant and ESBL positive or negative (Appendix 1).

3.5 DNA Extraction and Sequencing

Forty isolates (out of 89) were previously sequenced on an Illumina MiSeq platform at MRSN-WRAIR as previously described (Musila et al., 2021). The remaining 49 samples were sequenced in this study using the MinION platform (Oxford Nanopore Technology). Forty-nine *K. pneumoniae* isolates were inoculated on Mueller Hinton Agar plates and incubated for 24 hours at 37°C. *Klebsiella pneumoniae* JH930422.1 was included as a positive control. Approximately 14×10^8 cells/mL at OD600 overnight bacterial cells were resuspended in nuclease-free water and centrifuged at 5,400g for 10 minutes to pellet the cells. Total DNA was then extracted using the DNeasy® UltraClean Microbial Kit (QIAGEN Inc., Netherlands) according to the manufacturer's instructions. Briefly, bacterial cells were harvested and subjected to mechanical and chemical lysis to disrupt the cell wall and release genomic DNA. Cell debris, proteins, and other contaminants were removed through inhibitor-removal and purification steps. The released DNA was then selectively bound to a silica membrane spin column, washed to remove residual impurities, and finally eluted in nuclease-free elution buffer. The concentration and purity of the extracted DNA were subsequently assessed prior to downstream whole-genome sequencing analyses. DNA purity was determined on the Nanodrop One (ThermoFisher Scientific) and quantified on the Qubit dsDNA fluorometer, following the manufacturer's instructions.

For library preparation, the extracted DNA was end-repaired using the NEBNext® UltraII End Repair/dA-Tailing kit using the manufacturer's instructions. LSK 109 ligation kit, EXP-NBD 104 (1-12), and EXP-NBD 114 (13-24) Native Barcoding kits were used to barcode each DNA sample and adapters added. The DNA library was loaded onto a FLO-MIN106 R9.4.1 flowcell for sequencing based on the standard Oxford Nanopore Technology (ONT) 1D sequencing protocol launched in the MinKNOW v20.10.3 software. A quality control experiment was conducted to evaluate the Nanopore workflow

by sequencing the lambda phage DNA on the FLO-MIN106 R9.4.1 flowcell for 6 hours as per the Lambda DNA control experiment protocol, and the sequences compared with the reference sequence (NC_001416.1). Guppy v4.4.2 (Wick et al., 2019) was used to basecall and trim the barcodes and adapters from both ends of the reads, using default parameters.

3.6 *De novo* Assembly of Raw Reads and Database Querying

The fastQ files were filtered to retain only those with a Q-score ≥ 7 . The previously sequenced paired-end Illumina reads were retrieved from the in-house server for downstream analysis. The adapters in the short pair-ended reads were trimmed using *Trimmomatic* v0.39 (Bolger et al., 2014). The trimmed Illumina reads were assessed for quality using *FastQC* v0.11.9 (Andrews, 2010) before *de novo* assembly using the default *Shovill* v1.1.0 pipeline settings (Seaman, 2016). Next, the draft assemblies were polished using *pilon* v1.24 (Walker et al., 2014). Finally, the ONT long-reads were de-novo assembled using *flye* assembler v2.8.1 (Kolmogorov et al., 2019) with the plasmid option, followed by one polishing round using *medaka* v1.3.2. All polished draft assemblies from Illumina and ONT sequencing were analyzed in the same way. First, the quality was assessed using *QUAST* (Gurevich et al., 2013). Then, the draft assemblies were queried using the *ABRicate* pipeline v1.0.1 against *CARD* (Alcock et al., 2020) to identify AMR genes, *VFDB* (Chen et al., 2005) to identify virulence factors, and *PlasmidFinder* (Carattoli et al., 2014) to identify plasmids. The assemblies were queried against the *Klebsiella* *MLST* database using the command line *mlst* v2.19 pipeline to determine the sequence types, while the capsule (K) and O types were determined using the *Kleborate* pipeline (Lam et al., 2021) against the *Kaptive* database (Wick et al., 2018). Next, a new ybt typing scheme updated in the *Kleborate* pipeline was explored that assigned allelic profiles to yersiniabactin genes (Lam et al., 2021). Finally, a maximum-likelihood phylogenetic tree was generated using *Parsnp* v1.2 (Treangen et al., 2014) and NC_009648.1 as the reference genome. The tables were created using *flextable* (R

package), while the circular tree and heatmaps were generated using Interactive Tree of Life (*iToL*) v6.3.2 tree annotator (Letunic & Bork, 2019).

3.7 Statistical Analysis of the AMR and Virulence Genes between Community-Acquired Infection and Healthcare-Associated Infection Isolates

The 83/89 community-acquired infection and healthcare-associated infection isolates were selected. The relative proportions of the antimicrobial resistance and virulence genes was compared between the isolates obtained from the community-acquired infections and the healthcare-associated infections. The frequencies of the AMR and virulence genes were compared using the Fisher's exact test and the statistical significance was set at a P value of <0.05. The virulence genes under comparison were grouped based on gene function/locus as shown in Table 3.1 while each antimicrobial resistance gene was given one score (Table 3.2). The siderophores genes were carried in genetic clusters/loci as also observed by Lam et al (Lam et al., 2018).

Table 3.1: The Categories of *K. pneumoniae* Virulence Genes used for Frequency Analysis using Fisher's Exact Test

Gene function/locus	Genes	Score
Yersiniabactin	<i>fyuA, irp1, irp2, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX</i>	1
Aerobactins	<i>iucA, iucB, iucC, iucD, iutA</i>	1
Salmochelins	<i>iroB, iroC, iroD, iroE, iroN</i>	1
Regulation of mucoid phenotype	<i>rmpA/rmpA2</i>	1
Enterotoxin	<i>astA</i>	1
Enterobactins	<i>entD</i>	1
Allantoin utilization	<i>allA, allB, allC, allD, allR, allS</i>	1

The analysis was performed to determine if the *K. pneumoniae* isolates from community-acquired infections have more virulence genes than isolates from hospital-acquired infections. The hypothesis states that the community-acquired infection isolates have the same number of virulence genes as the healthcare-associated infection isolates.

The study then performed analysis to determine if *K. pneumoniae* isolates from healthcare-associated infections have more antimicrobial resistance genes than isolates from community-acquired infections. The hypothesis states that the healthcare-associated infection isolates have the same number of antimicrobial resistance genes as the community-acquired infection isolates.

Table 3.2: The *K. pneumoniae* Antimicrobial Resistance Genes used for Frequency Analyses using Fisher's Exact Test

Antibiotic class	AMR genes (all genes per isolate have one score)
Beta-lactams	<i>blaCTXM15, blaOXA181, blaNDM1, blaDHA</i>
Aminoglycosides	<i>rmtF, armA, aph.3.ib, aac.3.iid, aac.3.iie, aac.3.iv, aac.6.ib.cr4, aac.6.ib.cr6, aac.6.ib10, aadA16, aadA2, aadA5, ant.3.iia, aph.3.ia, aph.3.vi, aph.4..ia, aph.6.id</i>
Quinolones	<i>qnrB4, qnrB17, qnrB20, qnrS1, blaLAP2</i>
Tetracyclines	<i>tetA, tetD</i>
Sulphonamides	<i>sul1, sul2, sul3</i>
Trimethoprim	<i>dfrA14, dfrA12, dfrA5, dfrA15, dfrA27, dfrA17, dfrA16</i>
Colistin	<i>mcr-8</i>
Chloramphenicols	<i>catB3, cmlA1, floR, catII, cmlA5, catII</i>
Rifampins	<i>arr2, arr3</i>
Macrolides	<i>mphA, mphE, msrE</i>
Antiseptics	<i>qacEΔ1</i>

3.8 Global Phylogenetic Context of Study Isolates

All available whole-genome sequences of *Klebsiella* spp. and related metadata were downloaded from the Bacterial and Viral Bioinformatics Resource Center database (v20230303) and the sequences were filtered to include only those belonging to the major *Klebsiella* Species Complex. Specifically, they comprised of *K. pneumoniae* subsp. *pneumoniae*, *K. quasipneumoniae* subsp. *quasipneumoniae*, *K. quasipneumoniae* subsp. *similipneumoniae*, *K. variicola* subsp. *variicola*, and *K. quasivariicola*. The 6,286 genomes were subsequently combined with the 89 isolates of this study and analyzed using multilocus sequence typing (*mlst* v2.23.0). Phylogenomic analysis of isolates was

performed using MASH v2.3. In brief, pairwise ANI was calculated based on the MASH distance, and a UPGMA-based tree was created using phangorn libraries in R, while tree handling and exporting were performed using *ape*.

3.9 Mobile Genetic Element Analysis

Platon was used to identify and extract plasmid sequences from the draft assemblies of the 89 *K. pneumoniae* isolates (Schwengers et al., 2020). The extracted plasmid sequences were then classified using *PlasCard* (Che et al., 2021) as conjugative, mobilizable, or unmobilizable based on the presence or absence of self-transmissible genes. Plasmids were classified as conjugative if they had the origin of transfer (*oriT*) and relaxase genes (e.g., *mob*, *traI*) the mating pair formation system (Han et al., 2025; Smillie et al., 2010). Plasmids were mobilizable if they lacked the mating pair formation system while unmobilizable plasmids lacked self-transmissible genes. Additionally, AMR genes present within the plasmid sequences were identified (Che et al., 2021).

3.10 Data Visualization

The phylogenomic trees and associated meta-data (Appendices 1-5) for Figure 4.1, Figure 4.4, Figure 4.5, Figure 4.6 and Figure 4.7 were visualized and generated using iTOL v461 (Letunic & Bork, 2019). The heatmap of the AMR genes and plasmid mobilizability, Figure 4.7, was generated using *ComplexHeatmap* R package.

CHAPTER FOUR

RESULTS

4.1 Population Structure and Genomic Features of *Klebsiella pneumoniae*

4.1.1 Distribution and Population Structure of *Klebsiella pneumoniae*

Clinical samples were collected from skin and soft tissue infections (SSTIs) (64%, 57/89) and urinary tract infections (UTIs) (29%, 26/89), and the hospital environment (7%, 6/89) from five counties in Kenya: Kisumu (39%, 35/89), Nairobi (26%, 23/89), Kisii (17%, 15/89), Kilifi (10%, 9/89), and Kericho (8%, 7/89). Sixty-two isolates were isolated from community-acquired infections, while 21 were from healthcare-associated infections.

Of the four members of the *K. pneumoniae* Species Complex, *K. pneumoniae subsp. pneumoniae* represented the largest proportion of all isolates at 79% (70/89), with 44 recovered from SSTIs, 20 recovered from UTIs, and 6 from the hospital environment. This phylogroup had the largest number of MDR isolates (39%). The second most represented phylogroup was *K. variicola subsp. variicola* (18%, 17/89) with 15 isolates recovered from SSTIs and 2 isolated from UTIs. There was only one MDR isolate (kkp059) in this category. The least represented phylogroups were *K. quasipneumoniae subsp. quasipneumoniae* (2%, 2/89) and *K. quasipneumoniae subsp. similipneumoniae* (1%, 1/89) (Figure 4.1). Among the isolates from these less abundant subspecies, kkp022 and kkp034 were isolated from a UTI, while kkp036, the only MDR isolate in this category, was recovered from an SSTI. All *K. quasipneumoniae subsp. quasipneumoniae*, *K. quasipneumoniae subsp. similipneumoniae*, and *K. variicola subsp. variicola*, except for one isolate (kkp078), were from community-acquired infections from different geographical locations (Figure 4.1, (Appendix 1)). There was no evident geographical clustering of all the phylogroups by county or infection types.

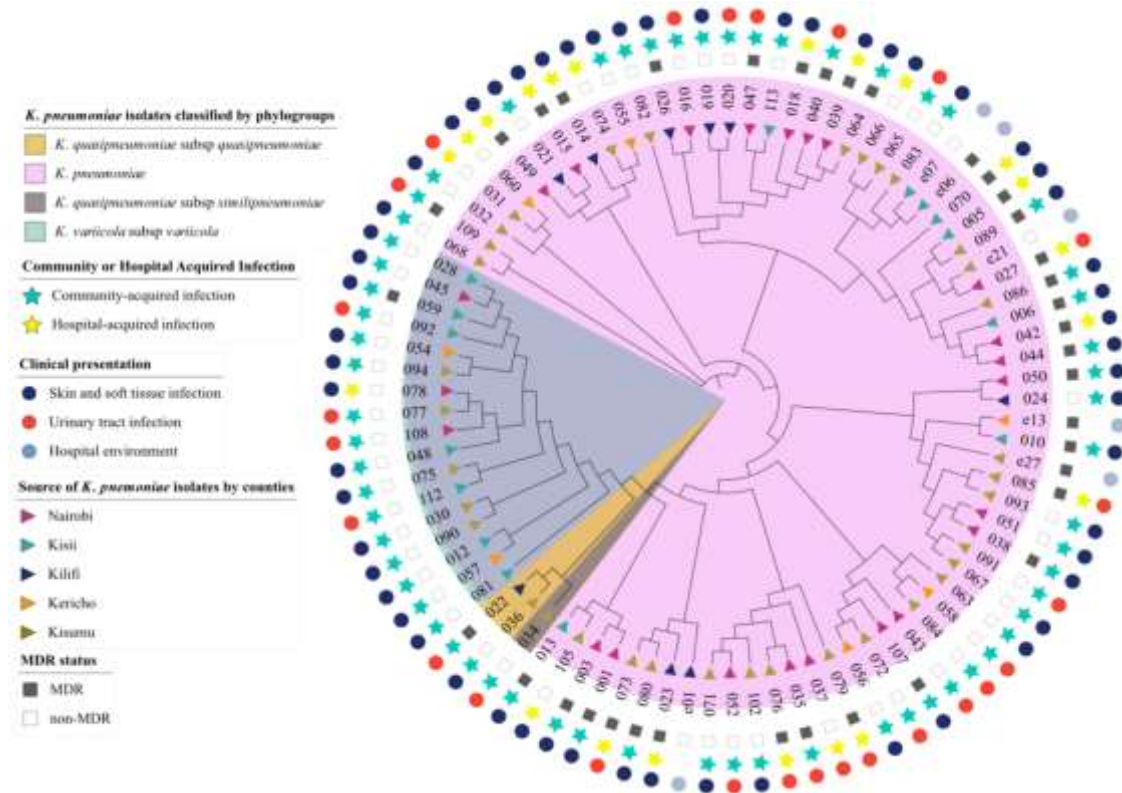


Figure 4.1: Circular Cladogram Shows Population Structure and Genomic Features of *K. pneumoniae* Isolates

The color shading indicates the clustering of the isolates by phylogroups: *K. pneumoniae* (pink), *Klebsiella quasipneumoniae* subsp. *similipneumoniae* (brown), *K. quasipneumoniae* subsp. *quasipneumoniae* (tan), and *K variicola* subsp. *variicola* (blue). The triangle symbols in the innermost ring represent the geographic source of the isolates: Nairobi (purple), Kisii (teal), Kilifi (blue), Kericho (orange), and Kisumu (brown). The star symbols represent the isolates recovered from community-acquired (blue) or healthcare-associated infections (yellow). The square symbols represent the multidrug resistance status of the isolates as either multidrug-resistant (black) or non-multidrug-resistant (white). The circle symbols in the outermost ring represent the clinical presentation of the isolates, i.e., skin and soft tissue infections (dark-blue), urinary tract infection (red), and the hospital environment (light-blue).

4.1.2 Genomic Characteristics of *K. pneumoniae* Isolates

Draft genomes were generated from the 89 isolates: 40 via Illumina short-read sequencing and 49 via MinION-based long-read sequencing. The sizes of the draft genomes ranged from 5.2 to 5.9 Mb with an average GC content of 57.27%, typical of *K. pneumoniae* genomes (Liu et al., 2012). The average N50 for the short and long reads was 226,402 and 5,147,285 base pairs, respectively. The isolates had 0 - 8 plasmid replicons, averaging 3 per isolate. The highest number of plasmid replicons were in genomes kkp001 (8), kkp018 (8), and kkp0e21 (8) (Appendix 2), whereas five genomes had no predicted replicons: kkp012, kkp030, kkp070, kkp102, and kkp112.

The most abundant plasmids replicon types identified belonged to the Col and Inc family (particularly the IncF type) (Figure 4.2). The other Inc-like plasmid replicons identified were IncR, IncH, IncX, IncC, IncN, IncM, and IncY. Seven types of Col plasmid replicons, the second most represented type, were identified, dominated by Col (pHAD28) (Figure 4.2). Other plasmid types identified included pKP1433 and rep_KLEB_VIR in kkp056 in kkp043 genomes, respectively.

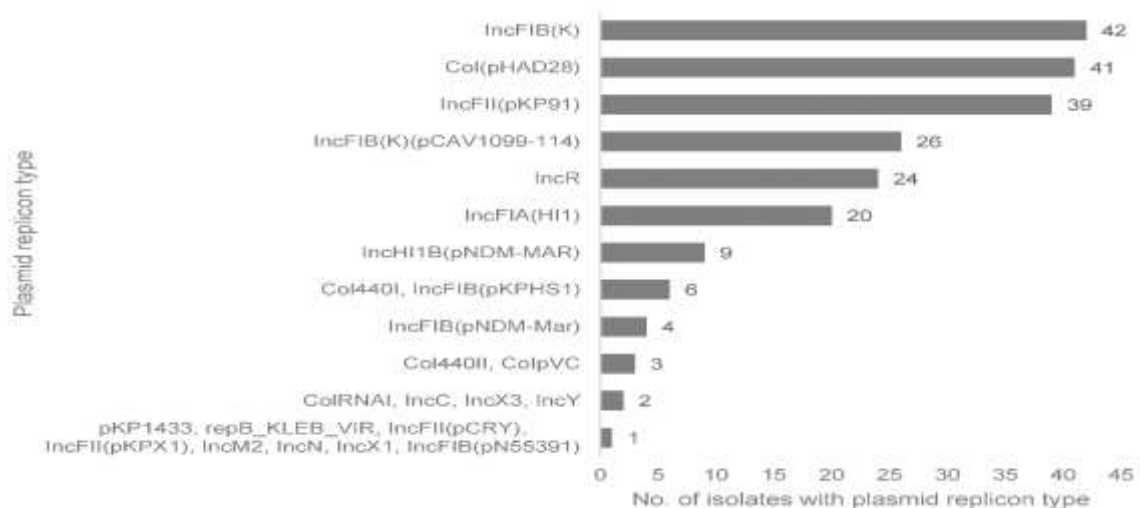


Figure 4.2: The Types and Number of Plasmid Replicons Identified in the Kenyan *K. pneumoniae* Isolates

The most abundant plasmids replicon types identified belonged to the Col and Inc family (particularly the IncF type) (Figure 4.2). The other Inc-like plasmid replicons identified were IncR, IncH, IncX, IncC, IncN, IncM, and IncY. Seven types of Col plasmid replicons, the second most represented type, were identified, dominated by Col(pHAD28) (Figure 4.2). Other plasmid types identified included pKP1433 and rep_KLEB_VIR in kkp056 in kkp043 genomes, respectively.

4.1.3 Serotypes of *K. pneumoniae* Isolates

This study identified no evident clustering of capsule and lipopolysaccharide types based on geographical locations or clinical presentation. There were 9 different O-loci types identified (O1, O2, O3a, O3b, O4, O5, OL101, OL103, and OL104) in the 57 assigned isolates, whereby O1 (46%, 26/57) and O2 (19%, 11/57) were the most common and clinically significant. There were 24 different K-types in the 45 assigned isolates, and the most abundant were K2 (11%, 5/45), K25 (11%, 5/45), K102 (9%, 4/45), K62 (9%, 4/45), and K24 (7%, 3/45).

4.1.4 Multilocus Sequence Types and Distribution of *K. pneumoniae* Isolates

The STs could only be assigned to 57% (51/89) of the isolates. The 38 unassigned isolates were not typeable due to assembling errors or inadequate genome coverage. The multilocus sequence types (STs) of the *K. pneumoniae* isolates were diverse, with thirty-eight different STs identified. STs that were represented by more than one isolate were ST15 (4/89), ST17 (3/89), and ST607 (3/89 each); and two isolates each represented the ST14, ST37, ST39, ST48, ST147, and ST307 lineages (Figure 4.3).

The remaining 29 singleton sequence types (76%) were geographically distributed as follows: Kisumu (n=17) - ST20, 45, 101, 198, 336, 391, 751, 1927, 2010, 3717, 3397, 3609, 5594, 5595, 5596, 5598, 5599; Nairobi (n=6) - ST55, 219, 966, 1786, 3692, 5600; Kisii (n=4) - ST25, 711, 3717, 5597 and Kilifi (n=2) - ST364 and 5593. Eight isolates had

novel allelic profiles assigned and deposited in the *K. pneumoniae* MLST database (<https://bigsdbs.web.pasteur.fr/>) (Table 4.1).

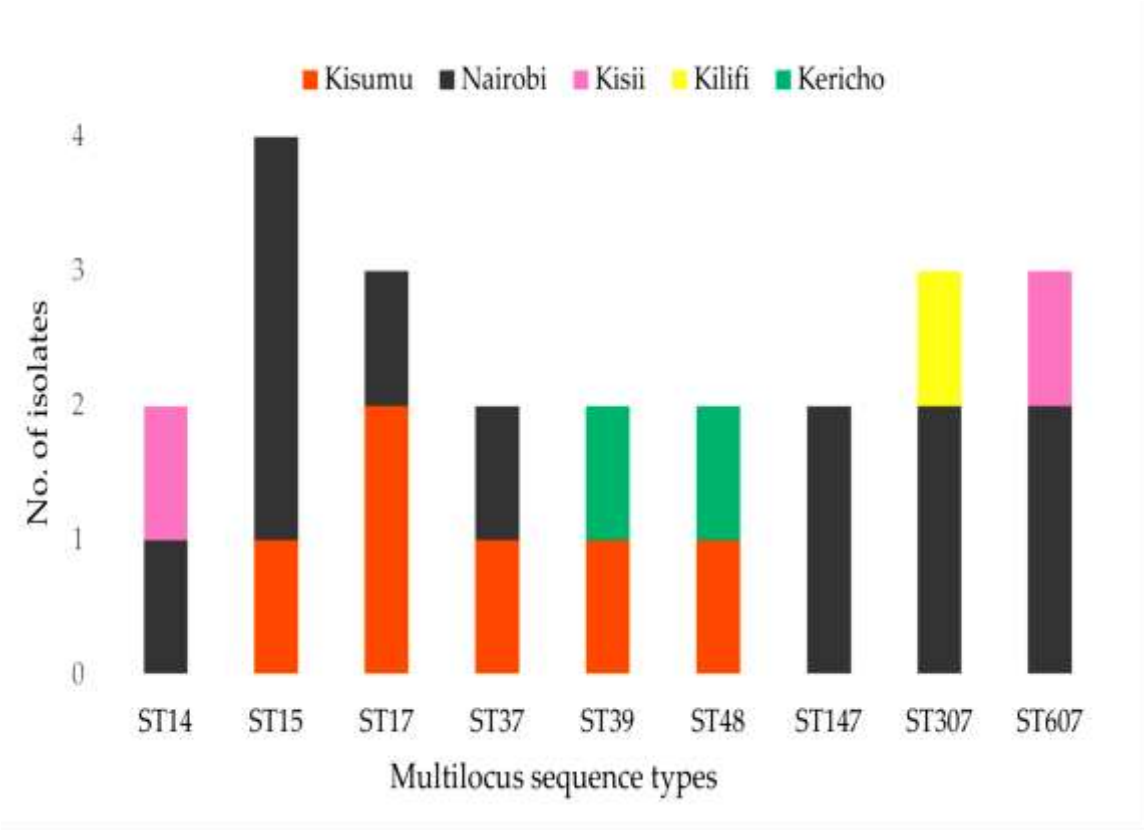


Figure 4.3: Distribution of the Most Abundant Sequence Types of the *K. pneumoniae* Isolates by County

Table 4.1: Allelic Profiles of *K. pneumoniae* Isolates with Novel Multilocus Sequence Types

Isolate ID	BIGSdb ID	Species	MLST	<i>gapA</i>	<i>infB</i>	<i>mdh</i>	<i>pgi</i>	<i>phoE</i>	<i>rpoB</i>	<i>tonB</i>
kkp022	16011	<i>K. quasipneumoniae</i>	5593	17	19	39	39	552	21	262
kkp034	16017	<i>K. quasipneumoniae</i>	5594	18	22	56	162	556	13	51
kkp075	16032	<i>K. variicola</i>	5595	45	18	21	105	455	22	786
kkp076	16033	<i>K. pneumoniae</i>	5596	2	9	2	1	13	1	787
kkp081	16037	<i>K. variicola</i>	5597	16	24	21	40	106	17	67
kkp090	16041	<i>K. variicola</i>	5598	16	18	21	33	55	17	341
kkp091	16042	<i>K. pneumoniae</i>	5599	294	3	1	1	4	331	4
kkp108	16051	<i>K. variicola</i>	5600	306	24	21	27	47	22	188

ID—identity; MLST—multilocus sequence type, the seven housekeeping genes for *K. pneumoniae* strain typing (*gapA*—glyceraldehyde 3-phosphate dehydrogenase, *infB*—translation initiation factor 2, *mdh*—malate dehydrogenase, *pgi*—phosphoglucose isomerase, *phoE*—phosphoporine E, *rpoB*—beta-subunit of Ribonucleic acid polymerase B, *tonB*—periplasmic energy transducer (Diancourt, 2005); BIGSdb—*K. pneumoniae* MLST database.

There was no evident clustering of STs by infection type or ESBL and MDR status. Kisumu had the highest number of distinct STs (23), followed by Nairobi (12), Kisii (6), Kilifi (3), and Kericho (2). The high-risk lineages, ST14, ST15, ST17, ST307, and ST607, were concentrated in the biggest cities – Kisumu and Nairobi. Global high-risk strains ST14, ST15, ST307, and ST607, were predominantly from the *Klebsiella pneumoniae* *subsp. pneumoniae*.

4.1.5 Antimicrobial Resistance in the *K. pneumoniae* Isolates

To assess the phenotypic resistance profiles of the *K. pneumoniae* isolates, antibiotic susceptibility tests (AST) performed on the VITEK2® platform (Appendix 3) identified high levels of non-susceptibility to trimethoprim (57%, 51/89), ticarcillin/clavulanate (49%, 44/89), cefuroxime axetil (49%, 44/89), cefuroxime (48%, 43/89), cefixime (48%, 43/89), ceftriaxone (47%, 42/89), cefepime (46%, 41/89), aztreonam (47%, 42/89), tetracycline (37%, 33/89) and minocycline (34%, 31/89); moderate non-susceptibility to

levofloxacin (19%, 17/89), moxifloxacin (20%, 18/89) and chloramphenicol (19%, 17/89); and low levels of non-susceptibility to tigecycline (4%, 4/89) and meropenem (2%, 2/89). All isolates were non-susceptible to piperacillin (Figure 4.4). Among the dataset, 41% (37/89) were classified as MDR, i.e., resistant to 3 or more drug classes. A large proportion (47%, 42/89) were ESBL-producing isolates (Figure 4.4), of which 30 isolates were MDR. One isolate, kkp001, was pan-resistant (resistant to all 16 antibiotics tested in the panel).

In the beta-lactam antibiotic class, genes encoding penicillinases, ESBLs, and carbapenemases were identified and are summarized in Appendix 4. Each phylogroup exhibited a distinct chromosomal penicillinase, with *K. pneumoniae* harboring *blaSHV*, *K. quasipneumoniae* carrying *blaOKP*, and *K. variicola* possessing *blaLEN*, consistent with previous findings (Wyres et al., 2016). However, some isolates carried a different additional penicillinase which was observed to have been acquired via a plasmid, e.g., kkp036 (*K. quasipneumoniae*) obtained a *blaSHV*-134 from an IncN plasmid (Table 4.2). There was a high diversity of plasmid-mediated ESBL genes in the isolates: *blaCTX-M*-15 (40%, 36/89), *blaCTX-M*-98 (1%, 1/89), *blaCTX-M*-14 (1%, 1/89), *blaTEM*181 (39%, 35/89), *blaOXA* (18/89), and the less common *blaLAP2* and *blaSCO1*. Two MDR isolates had carbapenemase genes: *blaNDM1* (kkp003) and *blaOXA181* (kkp001). Isolates kkp013 and kkp063 did not demonstrate any β -lactamase genes, and they were unsurprisingly ESBL-negative and non-MDR. Most isolates possessed at least one and at most six β -lactamase genes (Appendix 4). There were 42 ESBL-positive isolates, and 38 of them had a *blaCTX-M* co-harbored with two or more other types of ESBL genes (Figure 4.4).

For the non-beta-lactamase resistance genes, a previously described colistin resistant isolate (kkp018) (Kyany'a & Musila, 2020), carried *mcr-8*, *blaDHA1*, and 20 additional antimicrobial resistance (AMR) genes (Appendix 4). Additionally, 67% of the isolates (60/89) harbored aminoglycoside-modifying enzyme (AME) genes, including *ant*(3') (or *aadA*) encoding aminoglycoside nucleotidyltransferases; *aph*(3'), *aph*(4), and *aph*(6)

encoding aminoglycoside phosphotransferases; *rmtF* and *armA* encoding 16S rRNA methyltransferases; and *aac(3)* and *aac(6')-Ib-cr* encoding aminoglycoside acetyltransferases (Figure 4.4, Appendix 4). The *aac(6')-Ib-cr* gene also induces fluoroquinolone resistance. The genes associated with pan-aminoglycoside resistance were found in two isolates: *armA* (kkp018) and *rmtF* (kkp001). The genes for efflux pumps associated with aminoglycoside resistance (*arnT*, *crcB*, *acrD*, *baeR*, *cpxA*) were well-conserved among all the isolates. In addition, the common multidrug resistance-associated efflux pump genes were identified: *LptD*, *CRP*, *H-NS*, *KpnEFGH*, *acrAB*, *marA*, *mdtBC*, *msbA*, and *ramA*.

Three mechanisms for quinolone resistance were detected. First, plasmid-mediated quinolone resistance (PMQR) genes (*qnrB* or *qnrS1*) identified in 26 isolates. Second, chromosomal-encoded efflux pumps (*emrR*, *oqxA*, and *oqxB*) genes constitutive in all isolates.

bar plot represents the number of plasmid replicons found in the isolates; CAE—cefuroxime axetil; TCC—ticarcillin/clavulanate.

Third, gene mutations were detected in gyrase A (*gyrA*) - Ser83Phe, Asp87Asp, and Ser83Ile - and Deoxyribonucleic acid topoisomerase IV subunit A (*parC*) - Ser80Ile (Appendix 6), which are involved in DNA synthesis. Twenty-nine isolates (32%, 28/89) had the tetracycline-resistance genes, *tetA* or *tetD*. Fifteen isolates (17%, 15/89) carried the chloramphenicol resistance genes *cat1*, *catII*, and *catB3* (encoding chloramphenicol acetyltransferases) as well as *floR* and *cmlA* (encoding chloramphenicol efflux pumps). Resistance to sulphonamide and trimethoprim, administered as co-trimoxazole, was mediated by the *dfrA* trimethoprim resistance gene and *sul* sulphonamide resistant gene, found in 54 and 55 isolates, respectively. Resistance to other drug classes was conferred by: fosfomycins - *fosA*; macrolides - *mphA*, *mphE*, *msrE*, and *ereA2*; rifamycins - *arr2* and *arr3*; and even antiseptics - *qacEΔ1* and *qacL*.

4.1.6 Virulence Factors Associated with the *K. pneumoniae* Isolates

Known virulence factors involved in adherence, biofilm formation, capsule synthesis regulation, mucoid phenotype regulation, immune evasion, secretion system, serum resistance, siderophores expression (enterobactin, yersiniabactin, aerobactin, and salmochelin), efflux pump expression, allantoin utilization, and enterotoxin generation were detected among the isolates (Figure 4.5). The most ubiquitous were the chromosomal genes *fim*, *mrk*, and *ecp* for adherence and biofilm formation, which were present in all isolates except kkp043 which lacked the *fim* genes. In addition, some isolates carried multiple copies of the *mrk* genes in plasmids (Table 4.2). Other genes identified in all the isolates were those for serum resistance factors that determine the 'O-antigen' lipopolysaccharide serotype, the immune evasion factors which determine the polysaccharide capsule (*K antigen*) type, capsule synthesis regulation (*rcs*), efflux pump expression (*acrAB*), and enterobactin (*ent*, *fep*) (Appendix 5). In addition, all isolates possessed type VI secretion system loci genes except kkp034.

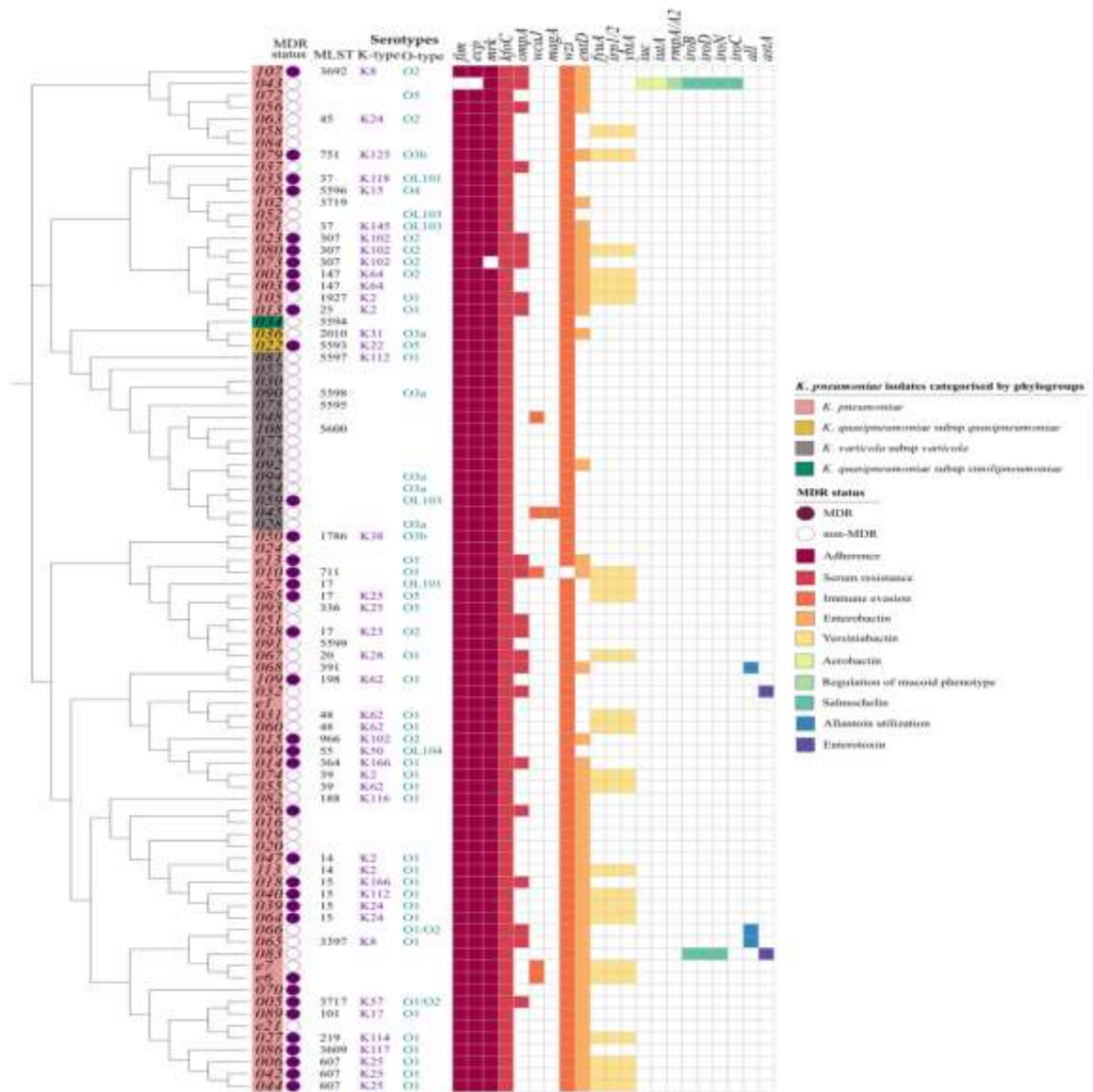


Figure 4.5: A Heatmap of the Multidrug Resistance Status, ST, Serotype, and Virulence Gene Content of *K. pneumoniae* Isolates

A heatmap of the multidrug resistance status, ST, serotype, and virulence gene content of *K. pneumoniae* isolates (n = 89). The cladogram on the left with colored labels on the edge indicates the clustering of the isolates by phylogroups: *K. pneumoniae* (pink), *K. quasipneumoniae* subsp. *similipneumoniae* (blue), *K. quasipneumoniae* subsp. *quasipneumoniae* (gold), and *K. variicola* subsp. *variicola* (green). The circular symbols represent the multidrug resistance status of the isolates as either multidrug-resistant (purple) or non-multidrug-resistant (white). The serotypes assigned to the isolates are

indicated as follows: multilocus sequence type (MLST) (black), capsule type (purple), and O type (teal). The virulence profile is represented as a gene present (color) or absent (white) for factors: adherence (purple), serum resistance (blue), immune evasion (pink), enterobactin (green), yersiniabactin, aerobactin, salmochelin, regulation of mucoid phenotype, enterotoxin and allantoin utilization; blank spaces indicates unassigned serotypes.

The *ybt* loci, identified in 24/89 isolates, encode for yersiniabactin siderophores found within conjugative transposons in the chromosome. Based on a new typing scheme (Lam et al., 2018) updated in the kleborate pipeline (Lam et al., 2021), this study identified four distinct *ybt* types: *ybt14* found within ICEKp5, *ybt15* in an ICEKp11, *ybt16* in an ICEKp12, and *ybt9* within an ICEKp3. In addition, the analysis revealed three isolates with chromosomally encoded genes for allantoin utilization and two isolates (kkp012 and kkp045) with *magA* and K2 capsule types linked to hypervirulence (Fang et al., 2004) (Figure 4.5).

Several isolates were unique in having plasmid-encoded virulence genes. For example, a hypervirulent isolate (hvKP), kkp043, was identified bearing the repB_KLEB_VIR plasmid containing *rpmA* and *rpmA2* genes, which regulate the expression of the mucoid phenotype, salmochelin (*iroBCDN*), and aerobactin (*iucABCD*, *iutA*). In addition, kkp083 also demonstrated an *iroBDEN* cluster and a heat-stable enterotoxin (*astA*) gene carried in an IncF(K)_1 plasmid, in contrast to the chromosomally bound *astA* gene in kkp032. This study did not identify MDR hypervirulent isolates with AMR and hypervirulent genes (Hennequin & Robin, 2016). Furthermore, the MDR isolates did not carry factors associated with hypervirulence, while the hypervirulent isolates were susceptible to most antibiotics, i.e., they were non-MDR and ESBL-positive (Figure 4.4).

Table 4.2: Antimicrobial Resistance and Virulence Genes Identified in the Plasmids of the *K. pneumoniae* Isolates (ID – identity)

Isolate ID	Plasmid ID	Plasmid Replicon	AMR Genes	Virulence Genes
kkp001	kkp001_p002	IncFII(pKPX1), IncR	aph(3'')-ib, aph(6)-id, CTX-M-15, OXA-181, TEM-181, dfrA, sul2	
kkp005	kkp005_p002	IncFIA(HI1), IncR	aac(60)-ib-cr, aph(3'')-ib, aph(6)-id, CTX-M-15, qnrB, TEM-181, aadA, arr3, CatII, dfrA, qacEdelta1, sul1, sul2	
kkp006	kkp006_p003	IncFIA(HI1), IncR	aac(60)-ib-cr, aph(3'')-ib, aph(6)-id, qnrB, aadA, arr3, CatII, dfrA, qacEdelta1, sul1, sul2, TetD	
kkp019	kkp019_p001	IncFIA(HI1)	aph(3'')-ib, aph(6)-id	mrkABCDFJ
kkp020	kkp020_p001	IncFIA(HI1)	aph(3'')-ib, aph(6)-id	mrkABCDFJ
kkp024	kkp024_p002	IncFIA(HI1)		mrkABCDFJ
kkp026	kkp026_p001	IncFIB(K), IncFII(pKP91)	aac(3)-iie, aac(60)-ib-cr, aph(3'')-ib, aph(6)-id, CTX-M-15, OXA-1, qnrB, TEM-181, dfrA, sul2, TetA	
kkp032	kkp032_p001	IncFIB(K)	aph(3'')-ib, aph(6)-id	mrkABCDFJ
kkp034	kkp034_p002	IncY	TEM-181, aadA, dfrA, qacEdelta1, sul1, sul2	
kkp036	kkp036_p002	IncN	SHV-134	
kkp037	kkp037_p001	IncC, IncFIB(K), IncFII(pKP91)	aac(60)-ib, aph(3'')-ib, aph(30)-ia, aph(6)-id, floR, mphA, qacEdelta1, sul1, sul2	
kkp037	kkp037_p002	IncX3	qnrS, SHV-134	
kkp039	kkp039_p001	IncFIB(K), IncFII(pKP91)	aadA, dfrA, mphA, qacEdelta1, sul1	
kkp043	kkp043_p001	repB_KLEB_VIR		iroBCDN, iucABCD, iutA, rmpA, rmpA2
kkp043	kkp043_p002	IncFIA(HI1)	dfrA, qacEdelta1, sul1	
kkp051	kkp051_p001	IncFIB(K)(pCAV1099114), Col(pHAD28), IncR	aph(3'')-ib, aph(6)-id	
kkp052	kkp052_p003	IncR	aph(3'')-ib, aph(6)-id, dfrA, qacEdelta1, sul1, sul2	mrkABCDFJ
kkp066	kkp066_p001	IncFIB(K), IncFII(pKP91)	aph(3'')-ib, aph(6)-id, SHV-120, sul2, TetD	
kkp068	kkp068_p001	IncFIB(K), IncFII(pKP91)	aph(3'')-ib, aph(6)-id, TEM-181, dfrA, mphA, sul2	
kkp083	kkp083_p001	IncFIB(K)		astA, iroBDEN
kkp090	kkp090_p001	IncFIB(K), IncFII(pKP91)	OXA-926	
kkp094	kkp094_p002	IncFIA(HI1)	ant(3'')-ia, dfrA, qacEdelta1, sul1, TetA	
kkp108	kkp108_p002	IncFII(pKP91)	dfrA, qacEdelta1, sul1	
kkp0e13	kkp0e13_p001	IncFII(pKP91)	aac(3)-iie, aac(60)-ib-cr, aph(3'')-ib, aph(6)-id, CTX-M-15, OXA-1, qnrB, TEM-181, dfrA, sul2, T	
kkp0e13	kkp0e13_p002	IncM2	aac(3)-IId, CTX-M-15, TEM-181, mphA	
kkp0e21	kkp0e21_p004	Col(pHAD28)	aph(6)-id, dfrA	
kkp0e27	kkp0e27_p001	IncFIB(K), IncFII(pKP91)	aph(3'')-ib, aph(6)-id, CTX-M-15, qnrB, TEM-181, dfrA, sul2, TetA	
kkp0e7	kkp0e7_p001	IncFIB(K), IncFII(pKP91)	aac(3)-iie, CTX-M-15, TEM-181	

4.2 Comparison of the Distribution of the AMR Genes and Virulence Genes among the Community-Acquired and the Healthcare-Acquired Isolates of the Clinical Samples

In line with our hypothesis, community-acquired infection isolates harbored more virulence genes than healthcare-associated infection isolates, while healthcare-associated infection isolates carried a greater number of AMR genes. However, Fisher's exact test comparing the frequency of virulence gene loci between community-acquired infection and healthcare-associated infection isolates yielded a statistic of 0.5868 with a p-value > 0.05 , indicating no statistically significant difference (Appendix VII). In contrast, the AMR gene frequency comparison showed a statistically significant difference, with healthcare-associated infection isolates carrying more AMR genes than community-acquired infection isolates (statistic < 0.00001 , $p < 0.05$; Appendix VIII).

4.3 Analysis of the Phylogenetic Relationship of the Whole Genome Sequences of the Study Isolates and Isolates from Other Selected Regions of the World

The clustering of these global isolates by phylogroups of the *K. pneumoniae* Species Complex resembles that in Figure 4.6. Notably, there was no clear association between isolates and specific continents, which demonstrates the global distribution of these strains. The wide dispersion of the Kenyan isolates across the phylogenetic tree indicates that they are widely distributed and not regionally clustered. There is apparent regional clustering of clonal complexes.

The isolates cluster according to their phylogroups and corresponding clonal complexes. Certain clonal complexes seem to be more prevalent in specific regions, such as CC11 and CC16 in Asia, CC512 and CC307 in Europe, and CC258 in North and South America, as highlighted by Wyres et al. (2020).

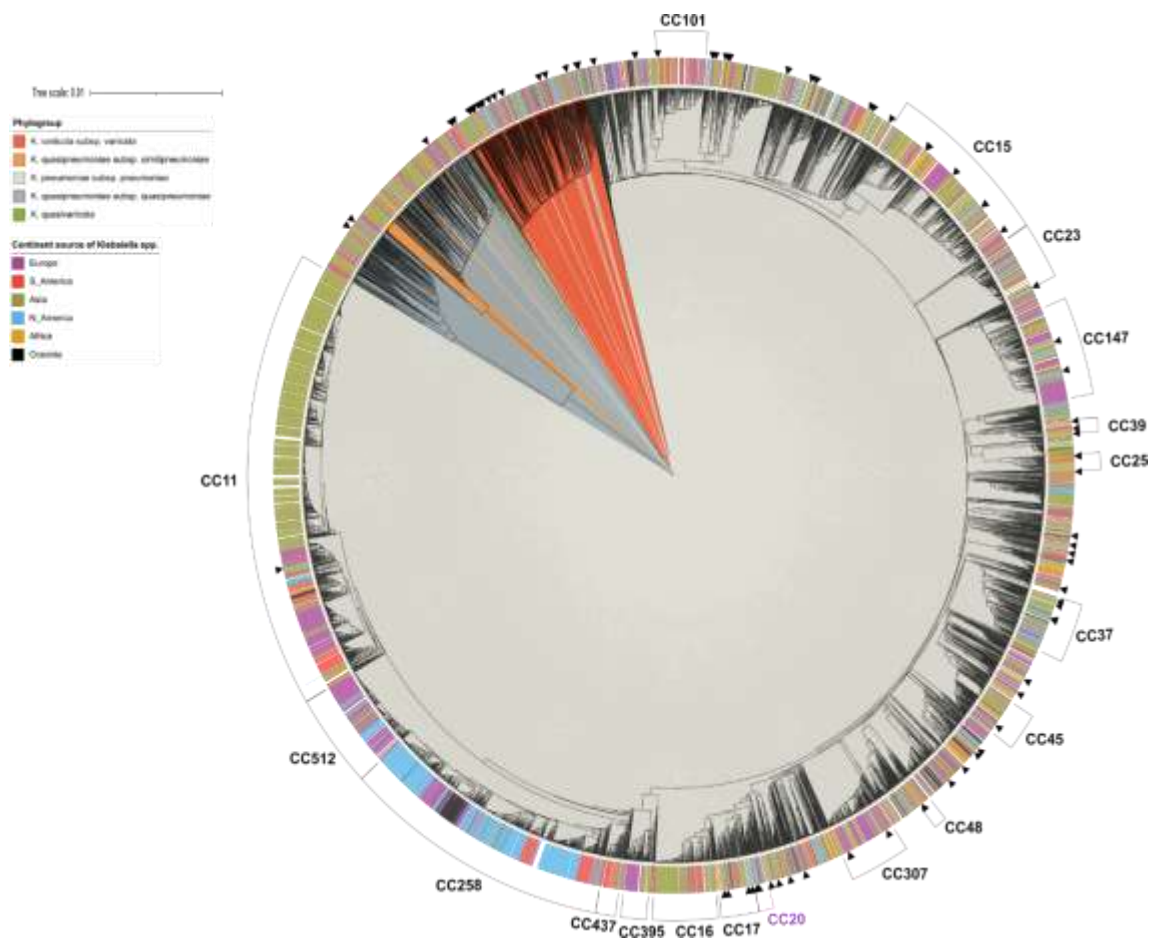


Figure 4.6: Phylogenetic Tree showing the Phylogenetic Relationship between the Kenyan *K. pneumoniae* Isolates with that of Other Isolates from Other Parts of the World

The colors in the inner ring represent the clustering of the *K. pneumoniae* isolates based on the phylogroups.: *K. pneumoniae* (tan), *K. quasipneumoniae* subsp. *similipneumoniae* (orange), *K. quasipneumoniae* subsp. *quasipneumoniae* (grey), *K. variicola* subsp. *variicola* (red), and *K. quasivariicola* (green). The outer ring represents the continent from which the isolate originated. The black triangles show the position of the 89 isolates of this study in the tree. The square brackets (with labels e.g. CC11) show the clonal complex of the enclosed isolates.

4.4 Assessment of the Risk of Spread of Antimicrobial Resistance and Virulence Genes by Investigating their Genomic Location

As highlighted in Figure 4.7 and Table 4.3, 17 out of 89 isolates pose the highest risk for antimicrobial resistance (AMR) gene dissemination, as their resistance genes are located on conjugative plasmids. The presence of a complete conjugation system is a key factor in transmissibility. For instance, kkp001, which carries the *bla*OXA-181 carbapenemase gene on a conjugative plasmid, presents a greater risk of horizontal gene transfer compared to kkp003, which carries *bla*NDM on a non-mobilizable plasmid. In Appendix 9, only isolates whose plasmids encode both mating pair formation (MPF) system genes and relaxase genes, along with other essential components such as T4CP and oriT, are classified as harboring conjugative plasmids.

Table 4.3: *K. pneumoniae* Isolates with the Highest Risk for AMR Genes Dissemination

Isolate code	Species	Location	Clinical ...Presentation	Isolate type	MDR status	ESBL status	Plasmid Mobilizability
kkp001	<i>K. pneumoniae</i>	Nairobi	Skin soft tissue	CAI	MDR	POS	conjugative
kkp019	<i>K. pneumoniae</i>	Kilifi	Skin soft tissue	CAI	non-MDR	NEG	conjugative
kkp020	<i>K. pneumoniae</i>	Kilifi	Urinary tract	CAI	non-MDR	NEG	conjugative
kkp026	<i>K. pneumoniae</i>	Kilifi	Skin soft tissue	CAI	MDR	POS	conjugative
kkp034	<i>K. quasipneumoniae</i> subsp. <i>similipneumoniae</i>	Kisumu	Urinary tract	CAI	non-MDR	NEG	conjugative
kkp036	<i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i>	Kisumu	Skin soft tissue	CAI	non-MDR	POS	conjugative
kkp037	<i>K. pneumoniae</i>	Nairobi	Urinary tract	HAI	non-MDR	POS	conjugative
kkp066	<i>K. pneumoniae</i>	Kisumu	Skin soft tissue	HAI	non-MDR	NEG	conjugative
kkp068	<i>K. pneumoniae</i>	Kisumu	Skin soft tissue	CAI	non-MDR	NEG	conjugative
kkp081	<i>K. variicola</i> subsp. <i>variicola</i>	Kisii	Skin soft tissue	CAI	non-MDR	POS	conjugative
kkp094	<i>K. variicola</i> subsp. <i>variicola</i>	Kisumu	Skin soft tissue	CAI	non-MDR	NEG	conjugative

Isolate code	Species	Location	Clinical ...Presentation	Isolate type	MDR status	ESBL status	Plasmid Mobilizability
kkp0e13	<i>K. pneumoniae</i>	Kericho	hospital environment	ENV	MDR	POS	conjugative
kkp0e27	<i>K. pneumoniae</i>	Kisumu	hospital environment	ENV	MDR	POS	conjugative
kkp0e6	<i>K. pneumoniae</i>	Kisii	hospital environment	ENV	MDR	POS	conjugative
kkp0e7	<i>K. pneumoniae</i>	Kisii	hospital environment	ENV	non-MDR	POS	conjugative
kkp108	<i>K. variicola subsp. variicola</i>	Nairobi	urinary tract	CAI	non-MDR	NEG	conjugative
kkp109	<i>K. pneumoniae</i>	Kisumu	urinary tract	CAI	MDR	NEG	conjugative

CAI – community-acquired infection, HAI – healthcare-associated, ENV – hospital environment

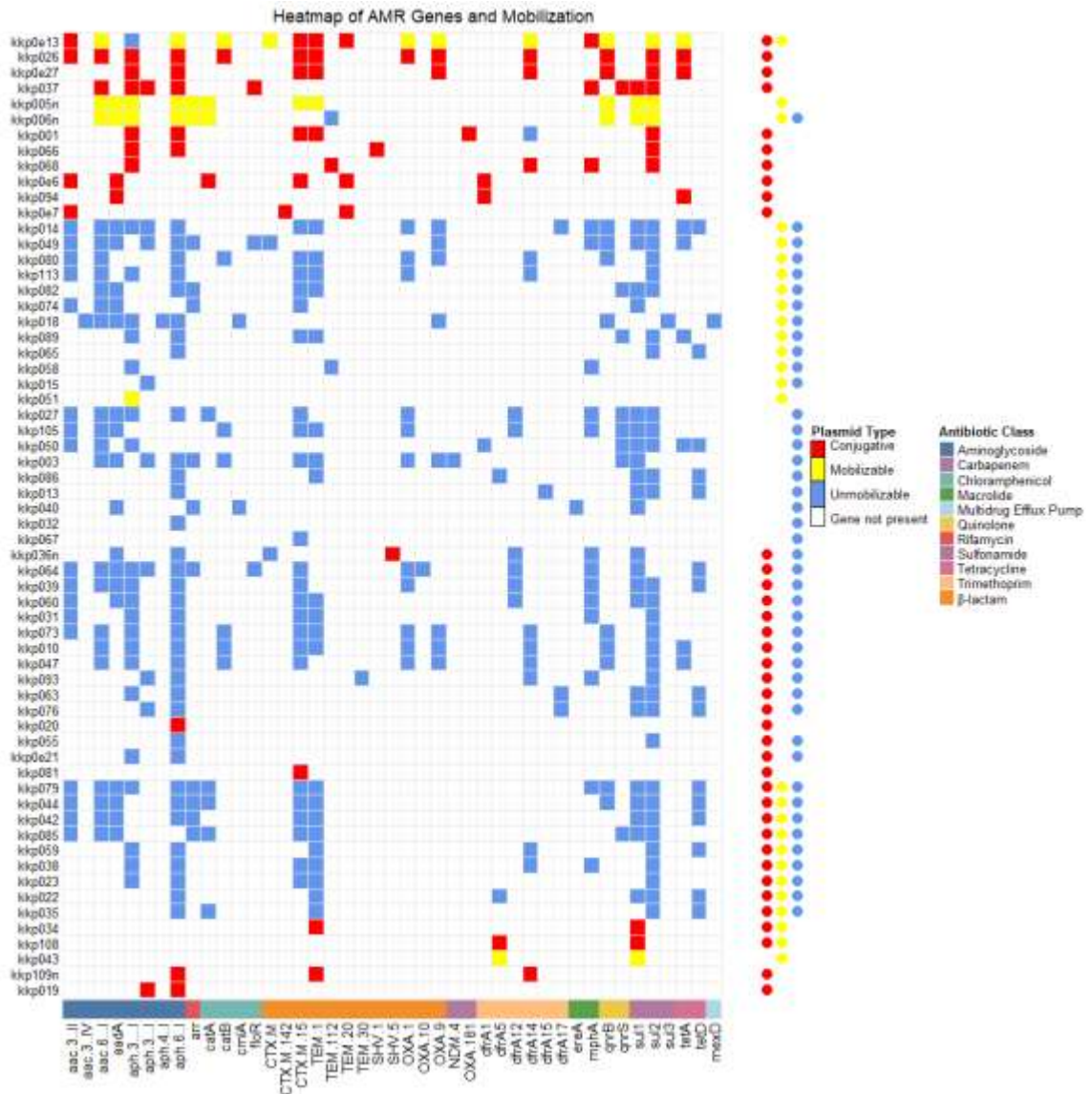


Figure 4.7: Heatmap Displaying AMR Genes (Columns) Found in Plasmids of *K. pneumoniae* Isolates (Rows)

The colors indicate the mobility type of the plasmid carrying the gene: conjugative (red), mobilizable (yellow), and unmobilizable (blue). The dot annotations show the types of plasmids in the isolates regardless of whether they carry AMR genes and highlight isolates with AMR genes in mobilizable plasmids but also harbor conjugative plasmids that could facilitate gene mobilization. The bottom annotation represents the antibiotic class to which the AMR genes confer resistance.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

Klebsiella pneumoniae is a significant opportunistic pathogen listed among the WHO critical priority pathogens (WHO, 2017). This study aimed to characterize the phenotypic and genotypic features of Kenyan *K. pneumoniae* strains that contribute to their risk and transmissibility within both healthcare and community settings. A total of 89 isolates were analyzed for phenotypic antibiotic susceptibility, multidrug resistance, geographic distribution, and genomic characteristics, including serotype, sequence type, antimicrobial resistance and virulence genes, and phylogenetic relationships both locally and in a global context.

All four recognized phylogroups were identified, with *Klebsiella pneumoniae* subsp. *pneumoniae* (76%, 69/89) being the most prevalent—consistent with previous reports (Heinz et al., 2019; Holt et al., 2015; Musicha et al., 2019). Some differences between phylogroups were observed. For example, while *K. variicola* subsp. *variicola* (19%, 16/89) is typically associated with bloodstream infections and UTIs (Imai et al., 2019; Maatallah et al., 2014), the isolates identified in this study were mostly linked to SSTIs and were largely antibiotic-susceptible (Figures 4.1 and 4.5). Similarly, although *K. quasipneumoniae* subsp. *similipneumoniae* (2%, 2/89) has been associated with nosocomial UTIs (Brisse et al., 2014; Elliott et al., 2016; Nicolás et al., 2018), we identified a community-acquired SSTI caused by k034, suggesting a potentially broader ecological distribution, as reported in Brazil and China where isolates were identified in sewage and from animals (Furlan et al., 2020; C. F. Li et al., 2018)).

Healthcare-associated infection isolates (HAI) carried a greater number of AMR genes than community-acquired infection isolates (CAI), while CAI isolates harbored more virulence genes than HAI isolates. The higher AMR burden in HAI isolates is likely due

to the higher antibiotic pressure in healthcare settings, which causes the accumulation of AMR genes in HAI isolates (Caneiras et al., 2019). Similarly, previous studies have found that HAI isolates are more multidrug-resistant than CAI isolates (Caneiras et al., 2019; Kang et al., 2006; Le et al., 2021; Mustafa et al., 2025), often having resistance to last-line antibiotics such as tigecycline and carbapenems (Mustafa et al., 2025), such as carbapenem-resistant kkp001 and kkp003 isolates which carry the *bla*OXA181 and *bla*NDM, respectively. The HAI isolates are generally less virulent and are commonly associated with high-risk clones such as ST15, ST11, and ST101, ST147 (Hullur et al., 2022; Sharma et al., 2024), for example ST147 kkp001. In contrast, CAI are typically more virulent because they carry more virulent biomarkers linked to siderophore production and the mucoid phenotype (Hullur et al., 2022; Sharma et al., 2024), and are associated with hypervirulent clones like ST23 and ST65 (8), like in the case of kkp043 that has *ompA* mucoid phenotype gene. They also cause community acquired infections such as pyogenic liver abscess (Chang et al., 2021; Choby et al., 2019). Some community-acquired infection isolates lack MDR genes altogether (Monteiro et al., 2025; Sharma et al., 2024). Virulent strains notably, isolates exhibiting both high virulence and multidrug resistance are more frequent among healthcare-associated infection cases (Caneiras et al., 2019; Kang et al., 2006; Monteiro et al., 2025; Sharma et al., 2024).

The identified *Klebsiella pneumoniae* lineages were highly diverse. Approximately 62% (56/89) appeared to be locally circulating strains not previously reported elsewhere, as shown in Figure 4.6 (indicated by black triangles outside clonal complex brackets). However, globally disseminated high-risk clones—including ST14, ST15, ST307, and ST607—were also identified (Figure 4.6). These lineages are known for their capacity to rapidly acquire and spread antimicrobial resistance (AMR) genes (de Lagarde et al., 2021). Notably, ST14, ST15, and ST147 have been implicated in the global dissemination of carbapenemase genes (Giske et al., 2012; C. R. Lee et al., 2017). In this study, two extensively drug-resistant (XDR) ST147 strains were found to carry *bla*OXA-181 (kkp001) and a *bla*NDM gene (kkp003), respectively. ST14 and ST15 isolates carried multiple extended-spectrum beta-lactamase (ESBL) genes. For instance, one ST15 strain

(kkp018) harbored *mcr-8*, and an AmpC beta-lactamase gene, *blaDHA* (Figure 4.4). These genes were identified in six multidrug-resistant (MDR) isolates derived from skin and soft tissue infections (SSTIs) and urinary tract infections (UTIs) (Figures 4.2 and 4.6) (Henson et al., 2017; Magobo et al., 2023; Peirano et al., 2020). ST17, a regional lineage, has previously been implicated in outbreaks in Kilifi (Henson et al., 2017), Mwanza (Mshana et al., 2013), and Kilimanjaro (Sonda et al., 2018). In this study, three MDR and ESBL-positive ST17 isolates were detected: one from a community-acquired SSTI, another from a nosocomial UTI, and one from a hospital environmental swab (Figure 4.1).

Beyond sequence type, the presence of functional antimicrobial resistance genes plays a critical role in determining the multidrug resistance and phenotypic characteristics of an isolate (Paczosa & Mecsas, 2016). These genetic determinants were analyzed and compared with their corresponding phenotypic antibiotic susceptibility profiles revealing a high level of concordance, particularly for trimethoprim, beta-lactams, and tetracyclines (Figure 4.4). Strong agreement was observed between the presence of ESBL-CTX-M genes and resistance to beta-lactams and cephalosporins, *dfrA* genes and trimethoprim resistance, and *tet* genes and tetracycline resistance (Figure 4.4). Long-read sequencing enabled the identification of circularized plasmids carrying AMR genes (Table 4.2). Notably, multidrug-resistant isolates harbored more plasmid replicons than non-MDR isolates, which typically carried between zero and three replicons (Figure 4.4; Appendix 2). These findings highlight the role of mobile genetic elements in shaping resistance profiles across diverse lineages, underscoring the importance of considering both genetic determinants and clonal background when assessing the epidemiological risk of *K. pneumoniae* isolates.

At the population level, high-risk clones are also associated with specific capsular serotypes (Figure 4.1, 4.4 and 4.5). For example, ST607-K25 was responsible for a nosocomial outbreak in a neonatal intensive care unit in France (Peltier et al., 2019). Significantly, three MDR and ESBL-positive ST607-K25 strains were identified in this study, all linked to SSTIs (Figure 4.6). Outbreaks involving CC15 and CC258 clones have

been reported across several African countries, including Morocco, Senegal, Côte d'Ivoire, Cameroon, and Madagascar (Breurec et al., 2013). The identification of such high-risk clones in Kenya underscores their growing clinical and public health significance. This risk was particularly elevated in Kisumu, and Nairobi (Figure 4.3), where 79% (11/14) of the global lineages were detected. These cities, being travel and referral hubs, likely promote the introduction and circulation of diverse strains—including novel alleles and international clones—compared to other regions like Kisii, Kilifi, and Kericho.

The Kenyan isolates are globally distributed in all phylogroups (Figure 4.6). This global interspersed distribution could indicate two scenarios. It might reflect recent introduction of strains from multiple external sources (Rodrigues et al., 2022), or it could be the result of long-term global circulation (S. Li et al., 2026), where these lineages have been established across diverse regions over an extended period. Elucidating these possibilities would require integrating additional epidemiological data—such as precise collection dates, detailed geographic origins, patient travel histories, and outbreak information—with molecular clock analyses.

Approximately 41% of isolates were multi-drug resistant and carried resistance to extended-spectrum beta-lactams and other antibiotic classes (Figure 4.4 and 4.6). Many belonged to clonal complexes linked to past outbreaks, suggesting a latent risk for future epidemics. Their close phylogenetic relatedness to global strains could also facilitate bi-directional gene flow of virulent or resistant traits between Kenya and other regions.

Hypervirulent *K. pneumoniae* (hvKP) strains are typically antibiotic-susceptible (Surgers et al., 2019) and harbor *rmpA/A2*, *magA*, and the K2 capsule type (C. R. Lee et al., 2017). This study identified a potentially hypervirulent isolate (kkp043) carrying *rmpA*, *rmpA2*, aerobactin, and salmochelin genes on a repB_KLEB_VIR plasmid (Table 4.2). Interestingly, it was ESBL-negative and non-MDR, suggesting a trade-off between virulence and resistance. Two additional non-MDR isolates (kkp012 and kkp045) carried *magA* but lacked ESBL genes. Similarly, *ybt*-positive K2 isolates were non-MDR,

whereas MDR K2 isolates lacked *ybt* (Figure 4.5). These findings echo global concerns about the emergence of MDR-hvKP hybrids (Banerjee et al., 2021; Cejas et al., 2019; Lam et al., 2018)), which are notoriously difficult to treat and emphasize the need for ongoing surveillance.

Plasmids were the predominant carriers of AMR genes, particularly the IncFIB (K) and IncFII(pKP91) replicons (Table 4.2). These plasmids—part of the diverse Inc family (Carattoli, 2009)—are primarily found in *Enterobacterales* and often occur as large multi-replicon plasmids (Rozwandowicz et al., 2018; Villa et al., 2012). The abundance of multi-replicon IncF plasmids (e.g., FII, FIA, FIB) likely accounts for their widespread presence (Figure 4.2, Table 4.2 and Appendix 2). Predictably, ESBL-negative and non-MDR isolates such as kkp012, kkp030, kkp102, and kkp112 had few or no plasmids. One exception was kkp070, an MDR strain with no plasmid replicons but several chromosomally integrated AMR genes. Such integration was also seen in other isolates (e.g., *bla*CTX-M-15 in kkp005 and kkp006, *dfr*A14 in kkp109, and multiple genes in kkp001), raising concern, as chromosomal resistance is clonally transmitted and enhances the fitness and spread of resistant strains.

While col-like plasmids were the second most prevalent group, most did not harbor AMR or virulence genes—except for colE1-like Col(pHAD28), known to carry *qnr*S1 and others (Rozwandowicz et al., 2018), as seen in kkp0e21 (Table 4.2). These plasmids may also provide competitive advantages by producing bacteriocins lethal to rival bacteria. Another notable plasmid, pK1433 (kkp056), has been associated with *bla*KPC2 carriage (Papagiannitsis et al., 2016). Isolates harboring *bla*CTX-M often co-carried *bla*OXA, *bla*SHV, or *bla*TEM, suggesting co-transfer (e.g., kkp081 and kkp059 with *bla*CTX-M-15, *bla*SHV-134, and *bla*TEM-181). Curiously, three ESBL-positive isolates (kkp015, kkp070, kkp107) carried only *bla*SHV, raising the possibility of efflux pump-mediated resistance. Efflux pumps can non-specifically confer resistance and may cause discrepancies between genotypic and phenotypic profiles (Appendix IV).

Given that most AMR genes in *K. pneumoniae* are plasmid-borne, the mobilizability of these plasmids is critical in determining their potential for spread. The 17 isolates carrying conjugative plasmids with AMR genes represent the highest risk for AMR dissemination (Figure 4.7, Table 4.3 and Appendix 9). Notably, these isolates are linked to a wide range of characteristics: they include both MDR and non-MDR strains, originate from community-acquired and healthcare-associated infections, are associated with diverse infection types such as urinary tract and soft tissue infections, and were isolated from multiple regions across Kenya—including Nairobi, Kilifi, Kisumu, Kisii, and Kericho. This diversity suggests that the risk of AMR gene spread from these isolates is broad and multifaceted, with implications for both local and widespread transmission.

Virulence gene analysis also revealed notable phylogroup differences. *K. quasipneumoniae* subsp. *similipneumoniae* lacked the Type VI Secretion System (T6SS) found in other phylogroups and instead possessed Type II Secretion (T2SS) (Appendix 5), consistent with prior studies (Perlaza-Jiménez et al., 2020). Among virulence factors, siderophores were the most prominent. While enterobactin is ubiquitous, its effect is neutralized by human lipocalin-2 (Martin & Bachman, 2018). In response, *K. pneumoniae* uses the more virulent yersiniabactin to bind iron and other heavy metals like copper to avoid metal toxicity and phagocytosis using reactive oxygen species (Paczosa & Meccas, 2016). According to (Holt et al., 2015), acquiring yersiniabactin is usually the first step in accumulating more potent siderophores to make them more invasive. Yersiniabactin genes were found among the *K. pneumoniae* subsp. *pneumoniae* isolates causing both urinary tract and skin and soft tissue infections and from across the five geographical locations. Most of the isolates were MDR and ESBL positive, making their infections persistent and antibiotic-resistant and likely contributing to their dominance over the other phylogroups (Figure 4.5).

This study had limitations. Differences between sequencing platforms (long vs. short reads) may introduce bias. Long reads provided better contiguity but lower depth, while short reads gave higher depth but more fragmented assemblies (Appendix IV). The

antimicrobial susceptibility testing, performed via Vitek2®, used a limited panel and lacked confirmatory phenotypic assays. Nevertheless, results were largely concordant with genotypic findings.

The isolates are unevenly distributed across different locations. Those from larger cities with greater human traffic and diversity had higher chances of obtaining multidrug resistant strains belonging to high-risk international clones. Lastly, virulence assessments were based on predicted gene presence, as *in-vivo* validation was not performed.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Objective 1

- Whole genome sequencing revealed a highly diverse population of isolates, including globally recognized high-risk clones such as ST14, ST15, ST147, ST307, and ST607, as well as local lineages not previously reported.
- A wide range of antimicrobial resistance genes was identified, including *bla*CTX-M, *bla*SHV, *bla*TEM *bla*OXA-181 and *bla*NDM along with *mcr*-8 and *bla*DHA.
- Virulence-associated genes such as yersiniabactin, aerobactin, and various adhesins were also detected, highlighting the pathogenic potential of these strains.

Objective 2

- Healthcare-associated infection isolates harbored a higher burden of resistance genes.
- Community-acquired infection isolates, while generally carrying fewer resistance genes, still encoded important virulence factors.

Objective 3

- Phylogenetic analysis placed Kenyan isolates into both globally recognized clades and unique local clusters.
- Approximately 62% of the isolates were found to be novel or region-specific, suggesting ongoing local adaptation.
- Several isolates showed close genetic relatedness to strains from other African countries, Europe, and Asia, implying regional and possibly international transmission.

- The detection of high-risk clones in urban centers that serve as travel and referral hubs reinforces the potential for cross-border spread and emphasizes the need for coordinated surveillance efforts at both national and regional levels.

Objective 4

- Genomic context analysis indicated that key resistance genes were predominantly plasmid-borne, particularly on conjugative and mobilizable plasmids such as IncF and IncX3.
- Plasmids carrying similar resistance determinants were found in both clinical and environmental isolates, suggesting that the hospital environment plays a role in the maintenance and spread of resistance.

6.2 Recommendations

- To ensure more comprehensive antimicrobial resistance surveillance, national surveillance programs should expand sampling to include isolates from healthcare, community, and environmental settings across diverse geographic regions, including both urban and rural areas.
- Surveillance should be continuous through sustained longitudinal monitoring to detect outbreaks, identify their sources, monitor the dissemination of associated strains within and across national borders, and evaluate the effectiveness of infection control interventions.
- The sustainability of genomic surveillance should be strengthened by investing in local capacity for genomic epidemiology through training, bioinformatics expertise, and laboratory infrastructure.
- National surveillance programs should be designed to foster regional and global collaboration and facilitate timely data sharing, which is crucial for tracking the movement of antimicrobial-resistant and virulent strains across borders and informing coordinated public health responses.

- Targeted surveillance of high-risk clones should be prioritized in major urban centers such as Nairobi and Kisumu, which serve as important referral centers and potential hubs for the dissemination of multidrug-resistant *Klebsiella pneumoniae*.
- Future research should focus on integrating genotypic data with phenotypic assays to validate the expression and clinical significance of resistance and virulence genes. Studies should also characterize the genetic context of antimicrobial resistance determinants, including their association with plasmids, transposons, integrons, and other mobile genetic elements that facilitate horizontal gene transfer.
- Genomic findings should inform and strengthen infection prevention and control (IPC) strategies, particularly in healthcare facilities with high burdens of multidrug-resistant (MDR) and extended-spectrum β -lactamase (ESBL)-producing *Klebsiella pneumoniae*.

REFERENCES

- Aiken, A. M., Mturi, N., Njuguna, P., Mohammed, S., Berkley, J. A., Mwangi, I., Mwarumba, S., Kitsao, B. S., Lowe, B. S., Morpeth, S. C., Hall, A. J., Khandawalla, I., & Scott, J. A. G. (2011). *Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study. The Lancet*, 378(9808), 2021–2027. [https://doi.org/10.1016/S0140-6736\(11\)61622-X](https://doi.org/10.1016/S0140-6736(11)61622-X)
- Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A. L. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H. K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., ... McArthur, A. G. (2020). *CARD 2020: antibiotic resistance surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Research*, 48(D1), D517–D525. <https://doi.org/10.1093/NAR/GKZ935>
- Andrade, F. F., Silva, D., Rodrigues, A., & Pina-Vaz, C. (2020). *Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges. Microorganisms*, 8(11), 1716. <https://doi.org/10.3390/MICROORGANISMS8111716>
- Andrews. (2010). *Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data*. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc>
- Apondi, O. E., Oduor, O. C., Gye, B. K., & Kipkoech, M. K. (2016). *High Prevalence of Multi-Drug Resistant Klebsiella pneumoniae in a Tertiary Teaching Hospital in Western Kenya. African Journal of Infectious Diseases*, 10(2), 89–95. <https://DOI.org/10.21010/Ajid.V10i2.3>

- Banerjee, T., Wangkheimayum, J., Sharma, S., Kumar, A., & Bhattacharjee, A. (2021). *Extensively Drug-Resistant Hypervirulent Klebsiella pneumoniae From a Series of Neonatal Sepsis in a Tertiary Care Hospital, India. Frontiers in Medicine*, 8. <https://doi.org/10.3389/FMED.2021.645955>
- Baquero, F., Lanza, V. F., Cantón, R., & Coque, T. M. (2015). *Public health evolutionary biology of antimicrobial resistance: priorities for intervention. Evolutionary Applications*, 8(3), 223–239. <https://doi.org/10.1111/EVA.12235>
- Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2014). *Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology* 2014 13:1, 13(1), 42–51. <https://doi.org/10.1038/nrmicro3380>
- Bolger, A. M., Lohse, M., & Usadel, B. (2014). *Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics (Oxford, England)*, 30(15), 2114–2120. <https://doi.org/10.1093/BIOINFORMATICS/BTU170>
- Breurec, S., Guessennnd, N., Timinouni, M., Le, T. A. H., Cao, V., Ngandjio, A., Randrianirina, F., Thiberge, J. M., Kinana, A., Dufougeray, A., Perrier-Gros-Claude, J. D., Boisier, P., Garin, B., & Brisse, S. (2013). *Klebsiella pneumoniae resistant to third-generation cephalosporins in five African and two Vietnamese major towns: Multiclonal population structure with two major international clonal groups, CG15 and CG258. Clinical Microbiology and Infection*, 19(4), 349–355. <https://doi.org/10.1111/j.1469-0691.2012.03805.x>
- Brisse, S., Passet, V., & Grimont, P. A. D. (2014). *Description of Klebsiella quasipneumoniae sp. nov., Isolated from human infections, With two subspecies, Klebsiella quasipneumoniae subsp. quasipneumoniae subsp. nov. and Klebsiella quasipneumoniae subsp. similipneumoniae subsp. nov., And demonstration that Klebsiella singaporensis is a junior heterotypic synonym of Klebsiella variicola.*

International Journal of Systematic and Evolutionary Microbiology, 64(Pt_9), 3146–3152. <https://doi.org/10.1099/IJS.0.062737-0/CITE/REFWORKS>

Caneiras, C., Lito, L., Melo-Cristino, J., & Duarte, A. (2019). *Community- and Hospital-Acquired Klebsiella pneumoniae Urinary Tract Infections in Portugal: Virulence and Antibiotic Resistance*. *Microorganisms*, 7(5), 138. <https://doi.org/10.3390/MICROORGANISMS7050138>

Carattoli, A. (2009). *Resistance Plasmid Families in Enterobacteriaceae*. *Antimicrobial Agents and Chemotherapy*, 53(6), 2227–2238. <https://doi.org/10.1128/AAC.01707-08>

Carattoli, A., Zankari, E., García-Fernández, A., Larsen, M. V., Lund, O., Villa, L., Aarestrup, F. M., & Hasman, H. (2014). *In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing*. *Antimicrobial Agents and Chemotherapy*, 58(7), 3895–3903. <https://doi.org/10.1128/AAC.02412-14>

Cejas, D., Elena, A., Guevara Nuñez, D., Sevilla Platero, P., De Paulis, A., Magariños, F., Alfonso, C., Berger, M. A., Fernández-Canigia, L., Gutkind, G., & Radice, M. (2019). *Changing epidemiology of KPC-producing Klebsiella pneumoniae in Argentina: Emergence of hypermucoviscous ST25 and high-risk clone ST307*. *Journal of Global Antimicrobial Resistance*, 18, 238–242. <https://doi.org/10.1016/J.JGAR.2019.06.005>

Chang, D., Sharma, L., Dela Cruz, C. S., & Zhang, D. (2021). *Clinical Epidemiology, Risk Factors, and Control Strategies of Klebsiella pneumoniae Infection*. *Frontiers in Microbiology*, 12, 750662. <https://doi.org/10.3389/FMICB.2021.750662/TEXT>

Che, Y., Yang, Y., Xu, X., Brinda, K., Polz, M. F., Hanage, W. P., & Zhang, T. (2021). *Conjugative plasmids interact with insertion sequences to shape the horizontal*

transfer of antimicrobial resistance genes. Proceedings of the National Academy of Sciences of the United States of America, 118(6), e2008731118. https://doi.org/10.1073/PNAS.2008731118/SUPPL_FILE/PNAS.2008731118.SD07.XLSX

Chen, L., Yang, J., Yu, J., Yao, Z., Sun, L., Shen, Y., & Jin, Q. (2005). *VFDB: a reference database for bacterial virulence factors. Nucleic Acids Research*, 33(Database issue). <https://doi.org/10.1093/NAR/GKI008>

Choby, J. E., Howard-Anderson, J., & Weiss, D. S. (2019). *Hypervirulent Klebsiella pneumoniae – clinical and molecular perspectives. Journal of Internal Medicine*, 287(3), 283. <https://doi.org/10.1111/JOIM.13007>

Cloeckaert, A., Dong, N., Rosen, D. A., Veeraraghavan, B., Du, H., Zhu, J., Wang, T., & Chen, L. (2021). *Virulence Factors in Hypervirulent Klebsiella pneumoniae. Frontiers in Microbiology*, 12, 642484. <https://doi.org/10.3389/FMICB.2021.642484>

de Lagarde, M., Vanier, G., Arsenault, J., & Fairbrother, J. M. (2021). *High Risk Clone: A Proposal of Criteria Adapted to the One Health Context with Application to Enterotoxigenic Escherichia coli in the Pig Population. Antibiotics 2021, Vol. 10, Page 244, 10(3), 244. https://doi.org/10.3390/ANTIBIOTICS10030244*

Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A. D., & Brisse, S. (2005). *Multilocus Sequence Typing of Klebsiella pneumoniae Nosocomial Isolates. Journal of Clinical Microbiology*, 43(8), 4178. <https://doi.org/10.1128/JCM.43.8.4178-4182.2005>

Domingues, S., Harms, K., Fricke, W. F., Johnsen, P. J., da Silva, G. J., & Nielsen, K. M. (2012). *Natural Transformation Facilitates Transfer of Transposons, Integrons and Gene Cassettes between Bacterial Species. PLOS Pathogens*, 8(8), e1002837. <https://doi.org/10.1371/JOURNAL.PPAT.1002837>

- Elliott, A. G., Ganesamoorthy, D., Coin, L., Cooper, M. A., & Cao, M. D. (2016). *Complete Genome Sequence of Klebsiella quasipneumoniae subsp. similipneumoniae Strain ATCC 700603*. *Genome Announcements*, 4(3), 438–454. <https://doi.org/10.1128/GENOMEA.00438-16>
- Endimiani, A., DePasquale, J. M., Forero, S., Perez, F., Hujer, A. M., Roberts-Pollack, D., Fiorella, P. D., Pickens, N., Kitchel, B., Casiano-Colón, A. E., Tenover, F. C., & Bonomo, R. A. (2009). *Emergence of blaKPC-containing Klebsiella pneumoniae in a long-term acute care hospital: a new challenge to our healthcare system*. *Journal of Antimicrobial Chemotherapy*, 64(5), 1102. <https://doi.org/10.1093/JAC/DKP327>
- Furlan, J. P. R., Gallo, I. F. L., De Campos, T. A., & Stehling, E. G. (2020). *Genomic Characterization of a Multidrug-Resistant and Hypermucoviscous/Hypervirulent Klebsiella quasipneumoniae subsp. similipneumoniae ST4417 Isolated from a Sewage Treatment Plant*. *Microbial Drug Resistance*, 26(11), 1321–1325. https://doi.org/10.1089/MDR.2019.0417/ASSET/IMAGES/MDR.2019.0417_FIGURE1.JPG
- Girometti, N., Lewis, R. E., Giannella, M., Ambretti, S., Bartoletti, M., Tedeschi, S., Tumietto, F., Cristini, F., Trapani, F., Gaibani, P., & Viale, P. (2014). *Klebsiella pneumoniae Bloodstream Infection: Epidemiology and Impact of Inappropriate Empirical Therapy*. *Medicine*, 93(17), 298–309. <https://doi.org/10.1097/MD.0000000000000111>
- Giske, C. G., Fröding, I., Hasan, C. M., Turlej-Rogacka, A., Toleman, M., Livermore, D., Woodford, N., & Walsh, T. R. (2012). *Diverse sequence types of Klebsiella pneumoniae contribute to the dissemination of bla NDM-1 in India, Sweden, and the United Kingdom*. *Antimicrobial Agents and Chemotherapy*, 56(5), 2735–

2738. <https://doi.org/10.1128/AAC.06142-11/ASSET/E5B2414C-7664-4B6B-93E3-5C4A7B0A5E39/ASSETS/GRAPHIC/ZAC9991008350001.JPEG>

Guasch, A., Lucas, M., Moncalián, G., Cabezas, M., Pérez-Luque, R., Gomis-Rüth, F. X., De La Cruz, F., & Coll, M. (2003). *Recognition and processing of the origin of transfer DNA by conjugative relaxase TrwC*. *Nature Structural & Molecular Biology* 2003 10:12, 10(12), 1002–1010. <https://doi.org/10.1038/nsb1017>

Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. (2013). *QUAST: quality assessment tool for genome assemblies*. *Bioinformatics (Oxford, England)*, 29(8), 1072–1075. <https://doi.org/10.1093/BIOINFORMATICS/BTT086>

Han, B., Feng, C., Jiang, Y., Ye, C., Wei, Y., Liu, J., & Zeng, Z. (2025). *Mobile genetic elements encoding antibiotic resistance genes and virulence genes in Klebsiella pneumoniae: important pathways for the acquisition of virulence and resistance*. *Frontiers in Microbiology*, 16, 1529157. <https://doi.org/10.3389/FMICB.2025.1529157/FULL>

Heinz, E., Brindle, R., Morgan-McCalla, A., Peters, K., & Thomson, N. R. (2019). *Caribbean multi-centre study of Klebsiella pneumoniae: whole-genome sequencing, antimicrobial resistance and virulence factors*. *Microbial Genomics*, 5(5). <https://doi.org/10.1099/MGEN.0.000266>

Hennequin, C., & Robin, F. (2016). *Correlation between antimicrobial resistance and virulence in Klebsiella pneumoniae*. *European Journal of Clinical Microbiology & Infectious Diseases : Official Publication of the European Society of Clinical Microbiology*, 35(3), 333–341. <https://doi.org/10.1007/S10096-015-2559-7>

Henson, S. P., Boinett, C. J., Ellington, M. J., Kagia, N., Mwarumba, S., Nyongesa, S., Mturi, N., Kariuki, S., Scott, J. A. G., Thomson, N. R., & Morpeth, S. C. (2017). *Molecular epidemiology of Klebsiella pneumoniae invasive infections over a decade at Kilifi County Hospital in Kenya*. *International Journal of Medical*

Microbiology : *IJMM*, 307(7), 422–429.
<https://doi.org/10.1016/J.IJMM.2017.07.006>

Holt, K. E., Wertheim, H., Zadoks, R. N., Baker, S., Whitehouse, C. A., Dance, D., Jenney, A., Connor, T. R., Hsu, L. Y., Severin, J., Brisse, S., Cao, H., Wilksch, J., Gorrie, C., Schultz, M. B., Edwards, D. J., Van Nguyen, K., Nguyen, T. V., Dao, T. T., Thomson, N. R. (2015). *Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proceedings of the National Academy of Sciences of the United States of America*, 112(27), E3574–E3581.
https://doi.org/10.1073/PNAS.1501049112/SUPPL_FILE/PNAS.1501049112.SD01.XLSX

Horan, T. C., Andrus, M., & Dudeck, M. A. (2008). *CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. American Journal of Infection Control*, 36(5), 309–332. <https://doi.org/10.1016/J.AJIC.2008.03.002>

Hsu, C. R., Lin, T. L., Chen, Y. C., Chou, H. C., & Wang, J. T. (2011). *The role of Klebsiella pneumoniae rmpA in capsular polysaccharide synthesis and virulence revisited. Microbiology (Reading, England)*, 157(Pt 12), 3446–3457.
<https://doi.org/10.1099/MIC.0.050336-0>

Hullur, M. S., Natarajan, A., & Sreeramulu, P. N. (2022). *Phenotypic Characterization of Virulence Factors and Antibigram of Klebsiella pneumoniae Isolates from Various Clinical Samples – A Cross Sectional Study. Journal of Pure and Applied Microbiology*, 16(3), 1783–1791.
<https://doi.org/10.22207/JPAM.16.3.21>

Huynh, D. T. N., Kim, A. Y., & Kim, Y. R. (2017). *Identification of Pathogenic Factors in Klebsiella pneumoniae Using Impedimetric Sensor Equipped with Biomimetic*

Surfaces. Sensors (Basel, Switzerland), 17(6), 1406.
<https://doi.org/10.3390/S17061406>

Imai, K., Ishibashi, N., Kodana, M., Tarumoto, N., Sakai, J., Kawamura, T., Takeuchi, S., Taji, Y., Ebihara, Y., Ikebuchi, K., Murakami, T., Maeda, T., Mitsutake, K., & Maesaki, S. (2019). *Clinical characteristics in blood stream infections caused by Klebsiella pneumoniae, Klebsiella variicola, and Klebsiella quasipneumoniae: a comparative study, Japan, 2014-2017. BMC Infectious Diseases, 19(1), 1–10.* <https://doi.org/10.1186/S12879-019-4498-X/TABLES/5>

Irek, E. O., Amupitan, A. A., Obadare, T. O., & Aboderin, A. O. (2018). *A systematic review of healthcare-associated infections in Africa: An antimicrobial resistance perspective. African Journal of Laboratory Medicine, 7(2), 796.* <https://doi.org/10.4102/AJLM.V7I2.796>

Kang, C. I., Kim, S. H., Bang, J. W., Kim, H. Bin, Kim, N. J., Kim, E. C., Oh, M. D., & Choe, K. W. (2006). *Community-Acquired versus Nosocomial Klebsiella pneumoniae Bacteremia: Clinical Features, Treatment Outcomes, and Clinical Implication of Antimicrobial Resistance. Journal of Korean Medical Science, 21(5), 816.* <https://doi.org/10.3346/JKMS.2006.21.5.816>

Kariuki, K., Diakhate, M. M., Musembi, S., Tornberg-Belanger, S. N., Rwigy, D., Mutuma, T., Mutuku, E., Tickell, K. D., Soge, O. O., Singa, B. O., Walson, J. L., Pavlinac, P. B., & Kariuki, S. (2023). *Plasmid-mediated quinolone resistance genes detected in Ciprofloxacin non-susceptible Escherichia coli and Klebsiella isolated from children under five years at hospital discharge, Kenya. BMC Microbiology 2023 23:1, 23(1), 129-.* <https://doi.org/10.1186/S12866-023-02849-2>

Khaertynov, K. S., Anokhin, V. A., Rizvanov, A. A., Davidyuk, Y. N., Semyenova, D. R., Lubin, S. A., & Skvortsova, N. N. (2018). *Virulence factors and antibiotic*

resistance of Klebsiella pneumoniae strains isolated from neonates with sepsis. Frontiers in Medicine, 5(AUG), 347945. <https://doi.org/10.3389/FMED.2018.00225/TEXT>

Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). *Assembly of long, error-prone reads using repeat graphs. Nature Biotechnology 2019 37:5, 37(5), 540–546. <https://doi.org/10.1038/s41587-019-0072-8>*

Krieg Noel R., Staley James T., Brown Daniel R., Hedlund Brian P., Paster Bruce J., Ward Naomi L., Ludwig Wolfgang, & Whitman William B. (2015). *Systematic Bacteriology Second Edition Volume Four.*

Kumar, C. K., Sands, K., Walsh, T. R., O'Brien, S., Sharland, M., Lewnard, J. A., Hu, H., Srikantiah, P., & Laxminarayan, R. (2023). *Global, regional, and national estimates of the impact of a maternal Klebsiella pneumoniae vaccine: A Bayesian modeling analysis. PLOS Medicine, 20(5), e1004239. <https://doi.org/10.1371/JOURNAL.PMED.1004239>*

Kyany'a, C., & Musila, L. (2020). *Colistin Resistance Gene mcr-8 in a High-Risk Sequence Type 15 Klebsiella pneumoniae Isolate from Kenya. Microbiology Resource Announcements, 9(39). <https://doi.org/10.1128/MRA.00783-20>*

Lam, M. M. C., Wick, R. R., Watts, S. C., Cerdeira, L. T., Wyres, K. L., & Holt, K. E. (2021). *A genomic surveillance framework and genotyping tool for Klebsiella pneumoniae and its related species complex. Nature Communications, 12(1). <https://doi.org/10.1038/S41467-021-24448-3>*

Lam, M. M. C., Wyres, K. L., Duchêne, S., Wick, R. R., Judd, L. M., Gan, Y. H., Hoh, C. H., Archuleta, S., Molton, J. S., Kalimuddin, S., Koh, T. H., Passet, V., Brisse, S., & Holt, K. E. (2018). *Population genomics of hypervirulent Klebsiella pneumoniae clonal-group 23 reveals early emergence and rapid global*

dissemination. *Nature Communications*, 9(1). <https://doi.org/10.1038/S41467-018-05114-7>

Le, T., Wang, L., Zeng, C., Fu, L., Liu, Z., & Hu, J. (2021). *Clinical and microbiological characteristics of nosocomial, healthcare-associated, and community-acquired Klebsiella pneumoniae infections in Guangzhou, China. Antimicrobial Resistance and Infection Control*, 10(1), 1–11. <https://doi.org/10.1186/S13756-021-00910-1/FIGURES/1>

Lee, C. A., & Grossman, A. D. (2007). *Identification of the origin of transfer (oriT) and DNA relaxase required for conjugation of the integrative and conjugative element ICEBs1 of Bacillus subtilis. Journal of Bacteriology*, 189(20), 7254–7261. <https://doi.org/10.1128/JB.00932-07/ASSET/37B03906-8AE6-4105-A65F-B84805E669AA/ASSETS/GRAPHIC/ZJB0200772030002.JPEG>

Lee, C. R., Lee, J. H., Park, K. S., Jeon, J. H., Kim, Y. B., Cha, C. J., Jeong, B. C., & Lee, S. H. (2017). *Antimicrobial resistance of hypervirulent Klebsiella pneumoniae: Epidemiology, hypervirulence-associated determinants, and resistance mechanisms. Frontiers in Cellular and Infection Microbiology*, 7(NOV), 483. <https://doi.org/10.3389/FCIMB.2017.00483/FULL>

Letunic, I., & Bork, P. (2019). *Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Research*, 47(W1). <https://doi.org/10.1093/NAR/GKZ239>

Li, B., Yi, Y., Wang, Q., Woo, P. C. Y., Tan, L., Jing, H., Gao, G. F., & Liu, C. H. (2012). *Analysis of drug resistance determinants in Klebsiella pneumoniae isolates from a tertiary-care hospital in Beijing, China. PloS One*, 7(7). <https://doi.org/10.1371/JOURNAL.PONE.0042280>

Li, C. F., Tang, H. L., Chiou, C. S., Tung, K. C., Lu, M. C., & Lai, Y. C. (2018). *Draft genome sequence of CTX-M-type β -lactamase-producing Klebsiella*

quasipneumoniae subsp. *similipneumoniae* isolated from a Box turtle. *Journal of Global Antimicrobial Resistance*, 12, 235–236. <https://doi.org/10.1016/J.JGAR.2017.12.012>

Li, S., Yu, Y., Liu, S., Zhang, M., Li, G., Tong, Q., Sun, Q., Jia, H., Wang, Z., Kong, Y., Zhang, Y., Pedro, J., Furlan, R., Draz, M. S., Dong, N., Xu, Y., & Ruan, Z. (2026). *Genomic dissection of the clonal background and global dissemination of hypervirulent Klebsiella pneumoniae CG23-KL57 lineage*. *Nature Communications* 2026 17:1, 17(1), 1451-. <https://doi.org/10.1038/s41467-025-68184-4>

Li, X. Z., & Nikaido, H. (2009). *Efflux-Mediated Drug Resistance in Bacteria: an Update*. *Drugs*, 69(12), 1555. <https://doi.org/10.2165/11317030-0000000000-00000>

Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., Rudan, I., Campbell, H., Cibulskis, R., Li, M., Mathers, C., & Black, R. E. (2012). *Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000*. *The Lancet*, 379(9832), 2151–2161. [https://doi.org/10.1016/S0140-6736\(12\)60560-1](https://doi.org/10.1016/S0140-6736(12)60560-1)

Maatallah, M., Vading, M., Kabir, M. H., Bakhrouf, A., Kalin, M., Nauc  r, P., Brisse, S., & Giske, C. G. (2014). *Klebsiella variicola Is a Frequent Cause of Bloodstream Infection in the Stockholm Area, and Associated with Higher Mortality Compared to K. pneumoniae*. *PLOS ONE*, 9(11), e113539. <https://doi.org/10.1371/JOURNAL.PONE.0113539>

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., & Monnet, D. L. (2012). *Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim*

standard definitions for acquired resistance. *Clinical Microbiology and Infection*, 18(3), 268–281. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>

Magobo, R. E., Ismail, H., Lowe, M., Strasheim, W., Mogokotleng, R., Perovic, O., Kwenda, S., Ismail, A., Makua, M., Bore, A., Phayane, R., Naidoo, H., Dennis, T., Ngobese, M., Wijnant, W., & Govender, N. P. (2023). *Outbreak of NDM-1– and OXA-181–Producing Klebsiella pneumoniae Bloodstream Infections in a Neonatal Unit, South Africa. Emerging Infectious Diseases*, 29(8), 1531. <https://doi.org/10.3201/EID2908.230484>

Maiden, M. C. J., Van Rensburg, M. J. J., Bray, J. E., Earle, S. G., Ford, S. A., Jolley, K. A., & McCarthy, N. D. (2013). *MLST revisited: the gene-by-gene approach to bacterial genomics. Nature Reviews. Microbiology*, 11(10), 728–736. <https://doi.org/10.1038/NRMICRO3093>

Maina, D., Revathi, G., Kariuki, S., & Ozwara, H. (2011). *Genotypes and cephalosporin susceptibility in extended-spectrum beta-lactamase producing enterobacteriaceae in the community. The Journal of Infection in Developing Countries*, 6(06), 470–477. <https://doi.org/10.3855/jidc.1456>

Maina, D., Revathi, G., & Whitelaw, A. (2017). *Molecular characterization of multidrug-resistant Klebsiella pneumoniae and Escherichia coli harbouring extended spectrum beta-lactamases and carbapenemases genes at a tertiary hospital, Kenya. Microbiologia Medica*, 32(4). https://ecommons.aku.edu/eastafrica_fhs_mc_pathol/176

Martin, R. M., & Bachman, M. A. (2018). *Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Frontiers in Cellular and Infection Microbiology*, 8(JAN), 314961. <https://doi.org/10.3389/FCIMB.2018.00004/PDF>

- Mathers, A. J., Peirano, G., & Pitout, J. D. D. (2015). *The role of epidemic resistance plasmids and international high- risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clinical Microbiology Reviews*, 28(3), 565–591. <https://doi.org/10.1128/CMR.00116-14/ASSET/528CDC89-4C0B-4F4D-A4BF-6885E30053A4/ASSETS/GRAPHIC/ZCM0031525120005.JPEG>
- Monteiro, A. de S. S., Silva, M. de O., Galvão, V. S., Bomfim, A. P., de Araújo, L. G., Silva, C. M. P., Barberino, M. G., Gouveia, E. L., Cordeiro, S. M., & Reis, J. N. (2025). *High proportions of multidrug-resistant Klebsiella pneumoniae isolates in community-acquired infections, Brazil. Scientific Reports*, 15(1), 1–12. <https://doi.org/10.1038/S41598-025-92549-W>;SUBJMETA=1434,22,326,631;KWRD=ANTIMICROBIAL+RESISTANCE,MICROBIOLOGY
- Moremi, N., Claus, H., & Mshana, S. E. (2016). *Antimicrobial resistance pattern: A report of microbiological cultures at a tertiary hospital in Tanzania. BMC Infectious Diseases*, 16(1), 1–7. <https://doi.org/10.1186/S12879-016-2082-1/FIGURES/1>
- Mshana, S. E., Hain, T., Domann, E., Lyamuya, E. F., Chakraborty, T., & Imirzalioglu, C. (2013). *Predominance of Klebsiella pneumoniae ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. BMC Infectious Diseases*, 13(1), 1–8. <https://doi.org/10.1186/1471-2334-13-466/FIGURES/2>
- Muraya, A., Kyany'a, C., Kiyaga, S., Smith, H. J., Kibet, C., Martin, M. J., Kimani, J., & Musila, L. (2022). *Antimicrobial Resistance and Virulence Characteristics of Klebsiella pneumoniae Isolates in Kenya by Whole-Genome Sequencing. Pathogens (Basel, Switzerland)*, 11(5). <https://doi.org/10.3390/PATHOGENS11050545>
- Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta,

- M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., ... Naghavi, M. (2022). *Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet*, 399(10325), 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- Musicha, P., Msefula, C. L., Mather, A. E., Chaguzza, C., Cain, A. K., Peno, C., Kallonen, T., Khonga, M., Denis, B., Gray, K. J., Heyderman, R. S., Thomson, N. R., Everett, D. B., & Feasey, N. A. (2019). *Genomic analysis of Klebsiella pneumoniae isolates from Malawi reveals acquisition of multiple ESBL determinants across diverse lineages. Journal of Antimicrobial Chemotherapy*, 74(5), 1223–1232. <https://doi.org/10.1093/JAC/DKZ032>
- Musila, L., Kyany'a, C., Maybank, R., Stam, J., Oundo, V., & Sang, W. (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). <https://doi.org/10.1371/JOURNAL.PONE.0246937>
- Mustafa, A. M., Salih, R. Q., Yaseen, H. A., Hamadameen, W. A., Kakamad, S. H., Abdullah, F., Mohammed, K. K., Hamad, D. Q., Ismaeel, B. H., Abdullah, H. S., Ahmed, S. H., Abdalla, B. A., Kakamad, F. H., Salih, M. O., & Abdalla, S. S. (2025). *Journal home page. Barw Medical Journal* |, 3(1), 14–22. <https://doi.org/10.58742/bmj.v2i4.138>
- Nicolás, M. F., Ramos, P. I. P., de Carvalho, F. M., Camargo, D. R. A., Alves, C. de F. M., de Moraes, G. L., Almeida, L. G. P., Souza, R. C., Ciapina, L. P., Vicente, A. C. P., Coimbra, R. S., & de Vasconcelos, A. T. R. (2018). *Comparative genomic analysis of a clinical isolate of Klebsiella quasipneumoniae subsp. similipneumoniae, a KP -2 and OKP-B-6 beta-lactamases producer harboring two drug-resistance plasmids from Southeast Brazil. Frontiers in Microbiology*, 9(FEB), 317281. <https://doi.org/10.3389/FMICB.2018.00220/BIBTEX>

- Odoyo, E., Matano, D., Tiria, F., Georges, M., Kyanya, C., Wahome, S., Mutai, W., & Musila, L. (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). <https://doi.org/10.1186/S13756-023-01227-X>
- Olson, R. D., Assaf, R., Brettin, T., Conrad, N., Cucinell, C., Davis, J. J., Dempsey, D. M., Dickerman, A., Dietrich, E. M., Kenyon, R. W., Kuscuoglu, M., Lefkowitz, E. J., Lu, J., Machi, D., Macken, C., Mao, C., Niewiadomska, A., Nguyen, M., Olsen, G. J., ... Stevens, R. L. (2023). *Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. Nucleic Acids Research*, 51(D1), D678–D689. <https://doi.org/10.1093/NAR/GKAC1003>
- Paczosa, M. K., & Mecsas, J. (2016). *Klebsiella pneumoniae: Going on the Offense with a Strong Defense. Microbiology and Molecular Biology Reviews*, 80(3), 629–661. <https://doi.org/10.1128/MMBR.00078-15/ASSET/BC53380B-7897-453E-8D3A-11A15859C177/ASSETS/GRAPHIC/ZMR0031624270006.JPEG>
- Papagiannitsis, C. C., Di Pilato, V., Giani, T., Giakkoupi, P., Riccobono, E., Landini, G., Miriagou, V., Vatopoulos, A. C., & Rossolini, G. M. (2016). *Characterization of KPC-encoding plasmids from two endemic settings, Greece and Italy. Journal of Antimicrobial Chemotherapy*, 71(10), 2824–2830. <https://doi.org/10.1093/JAC/DKW227>
- Parrott, A. M., Shi, J., Aaron, J., Green, D. A., Whittier, S., & Wu, F. (2021). *Detection of multiple hypervirulent Klebsiella pneumoniae strains in a New York City hospital through screening of virulence genes. Clinical Microbiology and Infection*, 27(4), 583–589. <https://doi.org/10.1016/j.cmi.2020.05.012>

- Peirano, G., Chen, L., Kreiswirth, B. N., & Pitouta, J. D. D. (2020). *Emerging antimicrobial-resistant high-risk klebsiella pneumoniae clones ST307 and ST147. Antimicrobial Agents and Chemotherapy*, 64(10). <https://doi.org/10.1128/AAC.01148-20/ASSET/DBF54EC9-96F5-438D-9A7D-0429A7A78FF3/ASSETS/GRAPHIC/AAC.01148-20-F0003.JPEG>
- Peltier, F., Choquet, M., Decroix, V., Adjidé, C. C., Castelain, S., Guiheneuf, R., & Pluquet, E. (2019). *Characterization of a multidrug-resistant klebsiella pneumoniae st607-k25 clone responsible for a nosocomial outbreak in a neonatal intensive care unit. Journal of Medical Microbiology*, 68(1), 67–76. <https://doi.org/10.1099/JMM.0.000884/CITE/REFWORKS>
- Perlaza-Jiménez, L., Wu, Q., Torres, V. V. L., Zhang, X., Li, J., Rocker, A., Lithgow, T., Zhou, T., & Vijaykrishna, D. (2020). *Forensic genomics of a novel klebsiella quasipneumoniae type from a neonatal intensive care unit in China reveals patterns of colonization, evolution and epidemiology. Microbial Genomics*, 6(10), 1–10. <https://doi.org/10.1099/MGEN.0.000433/CITE/REFWORKS>
- Podschun, R., & Ullmann, U. (1998). *Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical Microbiology Reviews*, 11(4), 589–603. <https://doi.org/10.1128/CMR.11.4.589/ASSET/52D837AD-C851-4111-A83C-DE7329F17AE7/ASSETS/GRAPHIC/CM0480028002.JPEG>
- Poirel, L., Revathi, G., Bernabeu, S., & Nordmann, P. (2010). *Detection of NDM-1-Producing Klebsiella pneumoniae in Kenya. Antimicrobial Agents and Chemotherapy*, 55(2), 934. <https://doi.org/10.1128/AAC.01247-10>
- Rawat, D., & Nair, D. (2010). *Extended-spectrum β -lactamases in Gram Negative Bacteria. Journal of Global Infectious Diseases*, 2(3), 263. <https://doi.org/10.4103/0974-777X.68531>

- Rodrigues, C., Desai, S., Passet, V., Gajjar, D., & Brisse, S. (2022). *Genomic evolution of the globally disseminated multidrug-resistant Klebsiella pneumoniae clonal group 147. Microbial Genomics*, 8(1), 000737. <https://doi.org/10.1099/MGEN.0.000737/CITE/REFWORKS>
- Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, B., Mevius, D. J., & Hordijk, J. (2018). *Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy*, 73(5), 1121–1137. <https://doi.org/10.1093/JAC/DKX488>
- Rudolf, J. D., Bigelow, L., Chang, C., Cuff, M. E., Lohman, J. R., Chang, C. Y., Ma, M., Yang, D., Clancy, S., Babnigg, G., Joachimiak, A., Phillips, G. N., & Shen, B. (2015). *Crystal Structure of the Zorbamycin-Binding Protein ZbmA, the Primary Self-Resistance Element in Streptomyces flavoviridis ATCC21892. Biochemistry*, 54(45), 6842–6851. <https://doi.org/10.1021/ACS.BIOCHEM.5B01008>
- Schmutz, E., Mühlenweg, A., Li, S. M., & Heide, L. (2003). *Resistance genes of aminocoumarin producers: Two type II topoisomerase genes confer resistance against coumermycin A1 and chlorobiocin. Antimicrobial Agents and Chemotherapy*, 47(3), 869–877. <https://doi.org/10.1128/AAC.47.3.869-877.2003;WGROU:STRING:PUBLICATION>
- Schwengers, O., Barth, P., Falgenhauer, L., Hain, T., Chakraborty, T., & Goesmann, A. (2020). *Platon: Identification and characterization of bacterial plasmid contigs in short-read draft assemblies exploiting protein sequence-based replicon distribution scores. Microbial Genomics*, 6(10), 1–12. <https://doi.org/10.1099/MGEN.0.000398/CITE/REFWORKS>
- Seaman. (2016). *GitHub - tseemann/shovill: ♠️ Assemble bacterial isolate genomes from Illumina paired-end reads · GitHub*. <https://github.com/tseemann/shovill>

- Sharma, A., Vats, N., Rati, R., Sharma, L., Chatterjee, N., & Jaggi, N. (2024). *Genotypic diversity and virulence factors of Klebsiella pneumoniae in a North Indian tertiary care hospital. BMC Infectious Diseases*, 24(1), 1477. <https://doi.org/10.1186/S12879-024-10012-Z>
- Smillie, C., Garcillán-Barcia, M. P., Francia, M. V., Rocha, E. P. C., & de la Cruz, F. (2010). *Mobility of Plasmids. Microbiology and Molecular Biology Reviews : MMBR*, 74(3), 434. <https://doi.org/10.1128/MMBR.00020-10>
- Sonda, T., Kumburu, H., van Zwetselaar, M., Alifrangis, M., Mmbaga, B. T., Lund, O., Kibiki, G. S., & Aarestrup, F. M. (2018). *Molecular epidemiology of virulence and antimicrobial resistance determinants in Klebsiella pneumoniae from hospitalised patients in Kilimanjaro, Tanzania. European Journal of Clinical Microbiology and Infectious Diseases*, 37(10), 1901–1914. <https://doi.org/10.1007/S10096-018-3324-5/FIGURES/1>
- Stanton, T. D., Hetland, M. A. K., Löhr, I. H., Holt, K. E., & Wyres, K. L. (2025). *Fast and Accurate in silico Antigen Typing with Kaptive 3. BioRxiv*, 2025.02.05.636613. <https://doi.org/10.1101/2025.02.05.636613>
- Surgers, L., Boyd, A., Girard, P. M., Arlet, G., & Decré, D. (2019). *Biofilm formation by ESBL-producing strains of Escherichia coli and Klebsiella pneumoniae. International Journal of Medical Microbiology: IJMM*, 309(1), 13–18. <https://doi.org/10.1016/J.IJMM.2018.10.008>
- Taitt, C. R., Leski, T. A., Erwin, D. P., Odundo, E. A., Kipkemai, N. C., Ndonye, J. N., Kirera, R. K., Ombogo, A. N., Walson, J. L., Pavlinac, P. B., Hulseberg, C., & Vora, G. J. (2017). *Antimicrobial resistance of Klebsiella pneumoniae stool isolates circulating in Kenya. PLOS ONE*, 12(6), e0178880. <https://doi.org/10.1371/JOURNAL.PONE.0178880>

- Tolentino, F. M., Bueno, M. F. C., Francisco, G. R., Barcelos, D. D. D. P., Lobo, S. M., Tomaz, F. M. M. B., Da Silva, N. S., De Andrade, L. N., Casella, T., Darini, A. L. D. C., Polotto, M., De Oliveira Garcia, D., & Nogueira, M. C. L. (2019). *Endemicity of the High-Risk Clone Klebsiella pneumoniae ST340 Coproducing QnrB, CTX-M-15, and KPC-2 in a Brazilian Hospital. Hhttps://Home.Liebertpub.Com/Mdr, 25(4), 528–537. https://doi.org/10.1089/MDR.2018.0006*
- Treangen, T. J., Ondov, B. D., Koren, S., & Phillippy, A. M. (2014). *The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biology, 15(11). https://doi.org/10.1186/S13059-014-0524-X*
- Tsioutis, C., Eichel, V. M., & Mutters, N. T. (2021). *Transmission of Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae: the role of infection control. Journal of Antimicrobial Chemotherapy, 76(Supplement_1), i4–i11. https://doi.org/10.1093/JAC/DKAA492*
- Verani, J. R., Blau, D. M., Gurley, E. S., Akelo, V., Assefa, N., Baillie, V., Bassat, Q., Berhane, M., Bunn, J., Cossa, A. C. A., El Arifeen, S., Gunturu, R., Hale, M., Igunza, A., Keita, A. M., Kenneh, S., Kotloff, K. L., Kowuor, D., Mabunda, R., ... Breiman, R. F. (2024). *Child deaths caused by Klebsiella pneumoniae in sub-Saharan Africa and south Asia: a secondary analysis of Child Health and Mortality Prevention Surveillance (CHAMPS) data. The Lancet. Microbe, 5(2), e131–e141. https://doi.org/10.1016/S2666-5247(23)00290-2*
- Villa, L., Poirel, L., Nordmann, P., Carta, C., & Carattoli, A. (2012). *Complete sequencing of an IncH plasmid carrying the blaNDM-1, blaCTX-M-15 and qnrB1 genes. Journal of Antimicrobial Chemotherapy, 67(7), 1645–1650. https://doi.org/10.1093/JAC/DKS114*

- Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., & Earl, A. M. (2014). *Pilon: An Integrated Tool for Comprehensive Microbial Variant Detection and Genome Assembly Improvement*. *PLOS ONE*, 9(11), e112963. <https://doi.org/10.1371/JOURNAL.PONE.0112963>
- WHO. (2002). *Prevention of hospital-acquired infections A practical guide 2nd edition*. <http://www.who.int/emc>
- WHO. (2017). *Nation Action Plan on Prevention and Containment of Antimicrobial Resistance 2017-2022 | WHO | Regional Office for Africa*. <https://www.afro.who.int/countries/kenya/publication/nation-action-plan-prevention-and-containment-antimicrobial-resistance-2017-2022>
- Wick, R. R., Heinz, E., Holt, K. E., & Wyres, K. L. (2018). *Kaptive Web: User-Friendly Capsule and Lipopolysaccharide Serotype Prediction for Klebsiella Genomes*. *Journal of Clinical Microbiology*, 56(6). <https://doi.org/10.1128/JCM.00197-18>
- Wick, R. R., Judd, L. M., & Holt, K. E. (2019). *Performance of neural network basecalling tools for Oxford Nanopore sequencing*. *Genome Biology*, 20(1), 129. <https://doi.org/10.1186/S13059-019-1727-Y>
- Wyres, K. L., & Holt, K. E. (2016). *Klebsiella pneumoniae Population Genomics and Antimicrobial-Resistant Clones*. *Trends in Microbiology*, 24(12), 944–956. <https://doi.org/10.1016/j.tim.2016.09.007>
- Wyres, K. L., & Holt, K. E. (2018). *Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria*. *Current Opinion in Microbiology*, 45, 131–139. <https://doi.org/10.1016/j.mib.2018.04.004>

- Wyres, K. L., Lam, M. M. C., & Holt, K. E. (2020). *Population genomics of Klebsiella pneumoniae*. *Nature Reviews. Microbiology*, 18(6), 344–359. <https://doi.org/10.1038/S41579-019-0315-1>
- Wyres, K. L., Wick, R. R., Gorrie, C., Jenney, A., Follador, R., Thomson, N. R., & Holt, K. E. (2016). *Identification of Klebsiella capsule synthesis loci from whole genome data*. *Microbial Genomics*, 2(12), e000102. <https://doi.org/10.1099/MGEN.0.000102>
- Zea-Vera, A., & Ochoa, T. J. (2015). *Challenges in the diagnosis and management of neonatal sepsis*. *Journal of Tropical Pediatrics*, 61(1), 1. <https://doi.org/10.1093/TROPEJ/FMU079>

APPENDICES

Appendix I: Genomic and Epidemiological Characteristics of *K. pneumoniae* Isolates

HAI: healthcare-associated infection. CAI: community-acquired infection, ENV: hospital environment

Isolate	Species	Location	Clinical presentation	Infection type	MDR status	ESBL status	Plasmids	Sequencing technology
kkp001	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	MDR	Positive	8	Nanopore
kkp003	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	MDR	Positive	3	Illumina
kkp005	<i>K pneumoniae</i>	Kisii	skin & soft tissue	HAI	MDR	Positive	4	Nanopore
kkp006	<i>K pneumoniae</i>	Kisii	skin & soft tissue	CAI	MDR	Positive	5	Nanopore
kkp010	<i>K pneumoniae</i>	Kisii	skin & soft tissue	CAI	MDR	Positive	3	Illumina
kkp012	<i>K variicola subsp. variicola</i>	Kisii	skin & soft tissue	CAI	non-MDR	Negative	0	Nanopore
kkp013	<i>K pneumoniae</i>	Kisii	skin & soft tissue	CAI	MDR	Negative	2	Illumina
kkp014	<i>K pneumoniae</i>	Kilifi	skin & soft tissue	HAI	MDR	Positive	4	Illumina
kkp015	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	HAI	MDR	Positive	2	Illumina
kkp016	<i>K pneumoniae</i>	Nairobi	urinary tract	CAI	non-MDR	Negative	3	Nanopore
kkp018	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	HAI	MDR	Negative	7	Illumina
kkp019	<i>K pneumoniae</i>	Kilifi	skin & soft tissue	CAI	non-MDR	Negative	1	Nanopore
kkp020	<i>K pneumoniae</i>	Kilifi	urinary tract	CAI	non-MDR	Negative	1	Nanopore
kkp021	<i>K pneumoniae</i>	Kilifi	skin & soft tissue	CAI	non-MDR	Negative	2	Nanopore
kkp022	<i>K quasipneumoniae subsp. quasipneumoniae</i>	Kilifi	urinary tract	CAI	MDR	Negative	4	Illumina
kkp023	<i>K pneumoniae</i>	Kilifi	skin & soft tissue	HAI	MDR	Positive	4	Illumina
kkp024	<i>K pneumoniae</i>	Kilifi	skin & soft tissue	CAI	non-MDR	Negative	3	Nanopore
kkp026	<i>K pneumoniae</i>	Kilifi	skin & soft tissue	CAI	MDR	Positive	3	Nanopore
kkp027	<i>K pneumoniae</i>	Nairobi	urinary tract	HAI	MDR	Positive	2	Illumina
kkp028	<i>K variicola subsp. variicola</i>	Kisii	skin & soft tissue	CAI	non-MDR	Negative	3	Nanopore

Isolate	Species	Location	Clinical presentation	Infection type	MDR status	ESBL status	Plasmids	Sequencing technology
kkp030	<i>K variicola subsp. variicola</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	0	Nanopore
kkp031	<i>K pneumoniae</i>	Kisumu	urinary tract	HAI	non-MDR	Positive	3	Illumina
kkp032	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	2	Nanopore
kkp034	<i>K quasipneumoniae subsp. similipneumoniae</i>	Kisumu	urinary tract	CAI	non-MDR	Negative	2	Nanopore
kkp035	<i>K pneumoniae</i>	Nairobi	urinary tract	CAI	MDR	Negative	4	Illumina
kkp036	<i>K quasipneumoniae subsp. quasipneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Positive	3	Nanopore
kkp037	<i>K pneumoniae</i>	Nairobi	urinary tract	HAI	non-MDR	Positive	5	Nanopore
kkp038	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	MDR	Positive	3	Illumina
kkp039	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	HAI	MDR	Positive	6	Nanopore
kkp040	<i>K pneumoniae</i>	Nairobi	urinary tract	CAI	MDR	Negative	4	Illumina
kkp042	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	HAI	MDR	Positive	4	Illumina
kkp043	<i>K pneumoniae</i>	Nairobi	urinary tract	CAI	non-MDR	Negative	2	Nanopore
kkp044	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	MDR	Positive	3	Illumina
kkp045	<i>K variicola subsp. variicola</i>	Nairobi	skin & soft tissue	CAI	non-MDR	Negative	1	Nanopore
kkp047	<i>K pneumoniae</i>	Nairobi	urinary tract	CAI	MDR	Positive	2	Illumina
kkp048	<i>K variicola subsp. variicola</i>	Kisii	skin & soft tissue	CAI	non-MDR	Negative	2	Nanopore
kkp049	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	HAI	MDR	Positive	3	Illumina
kkp050	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	MDR	Positive	2	Illumina
kkp051	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	non-MDR	Negative	3	Nanopore
kkp052	<i>K pneumoniae</i>	Nairobi	urinary tract	CAI	non-MDR	Negative	2	Nanopore
kkp054	<i>K variicola subsp. variicola</i>	Kericho	skin & soft tissue	CAI	non-MDR	Negative	1	Nanopore
kkp055	<i>K pneumoniae</i>	Kericho	skin & soft tissue	CAI	non-MDR	Positive	2	Illumina
kkp056	<i>K pneumoniae</i>	Kericho	skin & soft tissue	CAI	non-MDR	Negative	1	Nanopore

Isolate	Species	Location	Clinical presentation	Infection type	MDR status	ESBL status	Plasmids	Sequencing technology
kkp057	<i>K variicola subsp. variicola</i>	Kericho	skin & soft tissue	CAI	non-MDR	Negative	2	Nanopore
kkp058	<i>K pneumoniae</i>	Kericho	urinary tract	CAI	non-MDR	Positive	2	Nanopore
kkp059	<i>K variicola subsp. variicola</i>	Kisii	skin & soft tissue	CAI	MDR	Positive	5	Illumina
kkp060	<i>K pneumoniae</i>	Kericho	skin & soft tissue	HAI	non-MDR	Positive	3	Illumina
kkp063	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	2	Illumina
kkp064	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	MDR	Positive	7	Illumina
kkp065	<i>K pneumoniae</i>	Kisumu	urinary tract	CAI	non-MDR	Negative	2	Illumina
kkp066	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	HAI	non-MDR	Negative	2	Nanopore
kkp067	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Positive	3	Illumina
kkp068	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	2	Nanopore
kkp070	<i>K pneumoniae</i>	Kisii	skin & soft tissue	HAI	MDR	Positive	0	Nanopore
kkp071	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Positive	2	Illumina
kkp072	<i>K pneumoniae</i>	Kisumu	urinary tract	CAI	non-MDR	Negative	3	Nanopore
kkp073	<i>K pneumoniae</i>	Kisumu	urinary tract	HAI	MDR	Positive	4	Illumina
kkp074	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	HAI	non-MDR	Positive	3	Illumina
kkp075	<i>K variicola subsp. variicola</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	1	Nanopore
kkp076	<i>K pneumoniae</i>	Kisumu	urinary tract	HAI	MDR	Negative	2	Illumina
kkp077	<i>K variicola subsp. variicola</i>	Kisumu	urinary tract	CAI	non-MDR	Negative	2	Nanopore
kkp078	<i>K variicola subsp. variicola</i>	Nairobi	skin & soft tissue	HAI	non-MDR	Negative	2	Nanopore
kkp079	<i>K pneumoniae</i>	Kisumu	urinary tract	HAI	MDR	Positive	5	Illumina
kkp080	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	MDR	Positive	3	Illumina
kkp081	<i>K variicola subsp. variicola</i>	Kisii	skin & soft tissue	CAI	non-MDR	Positive	2	Illumina
kkp082	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Positive	3	Illumina
kkp083	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	1	Nanopore

Isolate	Species	Location	Clinical presentation	Infection type	MDR status	ESBL status	Plasmids	Sequencing technology
kkp084	<i>K pneumoniae</i>	Kisumu	urinary tract	CAI	non-MDR	Negative	3	Nanopore
kkp085	<i>K pneumoniae</i>	Kisumu	urinary tract	HAI	MDR	Positive	5	Illumina
kkp086	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	MDR	Negative	2	Illumina
kkp089	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	MDR	Positive	3	Illumina
kkp090	<i>K variicola subsp. variicola</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	2	Nanopore
kkp091	<i>K pneumoniae</i>	Kisumu	urinary tract	CAI	non-MDR	Negative	2	Nanopore
kkp092	<i>K variicola subsp. variicola</i>	Kisii	urinary tract	CAI	non-MDR	Negative	3	Nanopore
kkp093	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Positive	3	Illumina
kkp094	<i>K variicola subsp. variicola</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	4	Nanopore
kkp0e1	<i>K pneumoniae</i>	Kilifi	hospital_environment	ENV	non-MDR	Negative	5	Nanopore
kkp0e13	<i>K pneumoniae</i>	Kericho	hospital_environment	ENV	MDR	Positive	4	Nanopore
kkp0e21	<i>K pneumoniae</i>	Kisumu	hospital_environment	ENV	non-MDR	Negative	5	Nanopore
kkp0e27	<i>K pneumoniae</i>	Kisumu	hospital_environment	ENV	MDR	Positive	2	Nanopore
kkp0e6	<i>K pneumoniae</i>	Kisii	hospital_environment	ENV	MDR	Positive	3	Nanopore
kkp0e7	<i>K pneumoniae</i>	Kisii	hospital_environment	ENV	non-MDR	Positive	2	Nanopore
kkp102	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	CAI	non-MDR	Negative	0	Nanopore
kkp105	<i>K pneumoniae</i>	Kisumu	skin & soft tissue	HAI	non-MDR	Positive	2	Illumina
kkp107	<i>K pneumoniae</i>	Nairobi	skin & soft tissue	CAI	MDR	Positive	1	Illumina
kkp108	<i>K variicola subsp. variicola</i>	Nairobi	urinary tract	CAI	non-MDR	Negative	3	Nanopore
kkp109	<i>K pneumoniae</i>	Kisumu	urinary tract	CAI	MDR	Negative	1	Nanopore
kkp112	<i>K variicola subsp. variicola</i>	Kisii	urinary tract	CAI	non-MDR	Negative	0	Nanopore
kkp113	<i>K pneumoniae</i>	Kisii	skin & soft tissue	CAI	non-MDR	Positive	1	Illumina

Appendix II: Plasmids Replicons Present in the *K. pneumoniae* Isolates

Isolate	Plasmid replicons	Total number of replicons identified per isolate
kkp001	Col(pHAD28) (2), Col8282, ColpVC, IncFIB(pNDM-MAR), IncFII(pKPX1), IncHI1B(pNDM-MAR), IncR	8
kkp018	Col(pHAD28) (3), IncFIA(HI1), IncFIB(K)(pCAV1099-114), IncFIB(pKPHS1), IncHI1B(pNDM-MAR), IncR	8
kkp0e21	Col(pHAD28) (3), ColRNAI, IncFIA(HI1), IncFII(pKP91) (2), IncR	8
kkp064	Col(pHAD28), ColpVC, IncC, IncFIA(HI1), IncFIB(K), IncFII(pKP91), IncR	7
kkp014	Col(pHAD28), Col440II, Col440I, IncFIB(K), IncFII(pKP91) (2)	6
kkp039	Col(pHAD28), ColpVC, IncFIA(HI1), IncFIB(K), IncFII(pKP91), IncR	6
kkp006	IncFIA(HI1), IncFIB(K), IncFIB(pKPHS1), IncFII(pKP91), IncR	5
kkp022	Col(pHAD28), IncFIA(HI1), IncFIB(K)(pCAV1099-114), IncHI1B(pNDM-MAR)	5
kkp037	Col(pHAD28), IncC, IncFIB(K), IncFII(pKP91), IncX3	5
kkp049	Col(pHAD28) (3), IncFIB(K)(pCAV1099-114), IncFII(pCRY)	5
kkp059	IncFIB(K), IncFIB(pNDM-MAR), IncFII(pKP91), IncHI1B(pNDM-MAR), IncR	5
kkp073	Col(pHAD28) (2), IncFIB(K), IncFII(pKP91), IncR	5
kkp079	Col440I, IncFIA(HI1), IncFIB(K), IncFII(pKP91), IncR	5
kkp085	Col(pHAD28), IncFIA(HI1), IncFIB(K), IncFII(pKP91), IncR	5
kkp0e1	Col(Ye4449), Col(pHAD28), IncFIB(K)(pCAV1099-114), IncFIB(pKPHS1), IncHI1B(pNDM-MAR)	5
kkp005	Col(pHAD28), IncFIA(HI1), IncFIB(pKPHS1), IncR	4
kkp023	IncFIB(K), IncFIB(pNDM-MAR), IncFII(pKP91), IncHI1B(pNDM-MAR)	4
kkp024	Col(pHAD28) (2), IncFIA(HI1), IncFIB(K)	4
kkp035	Col(pHAD28), IncFIB(K), IncFII(pKP91), IncHI1B(pNDM-MAR)	4
kkp040	Col(pHAD28), IncFIA(HI1), IncR, IncX3	4
kkp042	IncFIA(HI1), IncFIB(K), IncFII(pKP91), IncR	4
kkp074	Col(pHAD28), IncFII(pKP91), IncR	4
kkp094	Col(pHAD28), Col440I, IncFIA(HI1), IncFIB(K)(pCAV1099-114)	4
kkp0e13	Col(pHAD28), IncFII(pKP91), IncM2, IncR	4
kkp003	Col(pHAD28), IncFIB(pQil), IncR	3
kkp010	Col440I, IncFIB(K), IncFII(pKP91)	3
kkp013	Col(pHAD28) (2), IncFIB(K)(pCAV1099-114)	3
kkp016	IncFIA(HI1), IncFIB(K), IncFII(pKP91)	3
kkp026	IncFIB(K), IncFIB(pKPHS1), IncFII(pKP91)	3
kkp028	Col(pHAD28), IncFIB(K)(pCAV1099-114), IncFIB(pKPHS1)	3
kkp031	Col(pHAD28), IncFIB(K), IncFII(pKP91)	3
kkp036	Col(pHAD28), IncFIB(K)(pCAV1099-114), IncN	3
kkp038	IncFIA(HI1), IncFIB(K), IncFII(pKP91)	3
kkp044	IncFIB(K), IncFII(pKP91), IncR	3
kkp048	Col(pHAD28) (2), IncFIB(K)(pCAV1099-114)	3

Isolate	Plasmid replicons	Total number of replicons identified per isolate
kkp051	Col(pHAD28), IncFIB(K)(pCAV1099-114), IncR	3
kkp060	Col(pHAD28), IncFIB(K), IncFII(pKP91)	3
kkp067	Col440I, IncFIB(K)(pCAV1099-114), IncY	3
kkp071	Col(pHAD28) (2), IncFIB(K)	3
kkp072	Col(pHAD28), Col440II, IncFIB(K)(pCAV1099-114)	3
kkp080	Col(pHAD28), IncFIB(K), IncFII(pKP91)	3
kkp082	Col440I, IncFIA(HI1), IncR	3
kkp084	Col440II, ColRNAI, IncFIB(K)(pCAV1099-114)	3
kkp089	Col(pHAD28), IncFIB(K), IncFII(pKP91)	3
kkp092	IncFIB(K)(pCAV1099-114), IncFII(pKP91), IncR	3
kkp093	Col(pHAD28), IncFIB(K), IncFII(pKP91)	3
kkp0e6	IncFIB(K), IncFII(pKP91), IncR	3
kkp108	Col(pHAD28), IncFIB(K)(pCAV1099-114), IncFII(pKP91)	3
kkp015	IncFIB(K)(pCAV1099-114), IncHI1B(pNDM-MAR)	2
kkp021	IncFIB(K)(pCAV1099-114), IncHI1B(pNDM-MAR)	2
kkp027	Col(pHAD28), IncFIB(K)(pCAV1099-114)	2
kkp032	IncFIB(K), IncR	2
kkp034	IncFIB(K)(pCAV1099-114), IncY	2
kkp043	IncFIA(HI1), repB_KLEB_VIR	2
kkp047	IncFIB(K), IncFII(pKP91)	2
kkp050	Col(pHAD28), IncFIB(K)	2
kkp052	IncFIB(K)(pCAV1099-114), IncR	2
kkp055	IncFIB(K), IncFII(pKP91)	2
kkp057	Col(pHAD28), IncFIB(K)(pCAV1099-114)	2
kkp058	IncFIB(K), IncFII(pKP91)	2
kkp063	IncFIB(K), IncFII(pKP91)	2
kkp065	IncFIB(K), IncFII(pKP91)	2
kkp066	IncFIB(K), IncFII(pKP91)	2
kkp068	IncFIB(K), IncFII(pKP91)	2
kkp076	IncFIB(K), IncFII(pKP91)	2
kkp077	Col(pHAD28), IncFIB(K)(pCAV1099-114)	2
kkp078	Col(pHAD28), IncFIB(K)(pCAV1099-114)	2
kkp081	IncFIB(pN55391), IncX1	2
kkp086	IncFIB(K), IncR	2
kkp090	IncFIB(K), IncFII(pKP91)	2
kkp091	Col(pHAD28), IncFIB(K)(pCAV1099-114)	2
kkp0e27	IncFIB(K), IncFII(pKP91)	2
kkp0e7	IncFIB(K), IncFII(pKP91)	2
kkp105	Col(pHAD28), IncFIB(K)(pCAV1099-114)	2
kkp019	IncFIA(HI1)	1
kkp020	IncFIA(HI1)	1
kkp045	IncFIB(K)	1

Isolate	Plasmid replicons	Total number of replicons identified per isolate
kkp054	Col(pHAD28)	1
kkp056	pKP1433	1
kkp075	IncFIB(pNDM-MAR)	1
kkp083	IncFIB(K)	1
kkp107	IncFIB(K)(pCAV1099-114)	1
kkp109	IncFIB(K)	1
kkp113	IncFIA(HI1)	1
kkp012		0
kkp030		0
kkp070		0
kkp102		0
kkp112		0

Appendix III: Phenotypic Antimicrobial Susceptibility Test (AST) Results of the *K. pneumoniae* Isolates

MIC values were interpreted according to CLSI breakpoints; corresponding susceptibility categories are provided in .ATM, Aztreonam; FEP, Cefepime; CRO, Ceftriaxone; CXM, Cefuroxime; LVX, Levofloxacin; MEM, Meropenem; TET, Tetracycline; TGC, Tigecycline; CFM, Cefixime; CHL, Chloramphenicol; MIN, Minocycline; MXF, Moxifloxacin; PIP, Piperacillin; TIM, Ticarcillin-Clavulanate; TMP, Trimethoprim.

Isolate	ATM	FEP	CRO	CXM	CXM_AX	LVX	MEM	TET	TGC	CFM	CHL	MIN	MXF	PIP	TIM	TMP
kkp001	>=64	>=64	>=64	>=64	>=64	>=8	>=16	>=16	>=8	>=4	32	>=16	>=8	>=128	>=128	>=16
kkp003	>=64	>=64	>=64	>=64	>=64	>=8	>=16	8	4	>=4	16	>=16	>=8	>=128	>=128	<=0.5
kkp005	>=64	>=64	>=64	>=64	>=64	1	<=0.25	2	<=0.5	>=4	>=64	4	2	>=128	>=128	>=16
kkp006	>=64	4	>=64	>=64	>=64	1	<=0.25	>=16	2	>=4	>=64	>=16	2	>=128	>=128	>=16
kkp010	>=64	32	>=64	>=64	>=64	1	<=0.25	>=16	2	>=4	4	>=16	2	>=128	>=128	>=16
kkp012	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	4	<=0.25	8	<=8	<=0.5
kkp013	<=1	<=1	<=1	2	2	<=0.12	<=0.25	>=16	<=0.5	<=0.25	<=2	>=16	<=0.25	>=128	16	>=16
kkp014	>=64	>=64	>=64	>=64	>=64	1	<=0.25	>=16	2	>=4	4	>=16	2	>=128	>=128	>=16
kkp015	2	2	32	>=64	>=64	1	<=0.25	>=16	<=0.5	>=4	8	>=16	4	>=128	>=128	>=16
kkp016	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	<=1	<=0.25	8	<=8	<=0.5
kkp018	2	<=1	<=1	>=64	>=64	>=8	<=0.25	8	4	>=4	>=64	4	>=8	>=128		>=16
kkp019	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	<=1	<=0.25			<=0.5
kkp020	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	<=1	<=0.25		<=8	<=0.5
kkp021	<=1	<=1	<=1	<=1	<1	<=0.12	<=0.25	<=1	<=0.25	<=0.25	4	2	<=0.25	16	<=8	<=0.5
kkp022	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	>=16	<=0.5	<=0.25	4	>=16	<0.25	>=128	16	>=16
kkp023	>=64	2	>=64	>=64	>=64	4	<=0.25	2	<=0.5	>=4	8	4	>=8	>=128	>=128	>=16

Isolate	ATM	FEP	CRO	CXM	CXM_AX	LVX	MEM	TET	TGC	CFM	CHL	MIN	MXF	PIP	TIM	TMP
kkp024	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	<=1	<=0.25	8	<=8	<=0.5
kkp026	16	2	>=64	>=64	>=64	1	<=0.25	>=16	<=0.5	>=4	<=2	4	2	>=128	64	>=16
kkp027	>=64	>=64	>=64	>=64	>=64	>=8	<=0.25	8	2	>=4	>=64	>=16	>=8	>=128	>=128	>=16
kkp028	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	2	<=0.25	<=4	<=8	<=0.5
kkp030	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	8	<=8	<=0.5
kkp031	>=64	>=64	>=64	>=64	>=64	<=0.12	<=0.25	<=1	<=0.5	>=4	<=2	2	<=0.25	>=128	>=128	<=0.5
kkp032	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	8	<=8	<=0.5
kkp034	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	4	<=0.25	<=128	16	>=16
kkp035	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	>=16	<=0.5	<=0.25	>=64	>=16	<=0.25	<=128	64	<=0.5
kkp036	>=64	2	>=64	>=64	>=64	1	<=0.25	<=1	<=0.5	>=4	<=2	2	2	>=128	64	>=16
kkp037	>=64	<=1	8	32	32	1	<=0.25	<=1	<=0.5	>=4	>=64	4	2	>=128	32	<=0.5
kkp038	16	2	>=64	>=64	>=64	<=0.12	<=0.25	<=1	1	>=4	4	4	<=0.25	>=128	16	>=16
kkp039	>=64	>=64	>=64	>=64	>=64	>=8	<=0.25	>=16	2	>=4	16	>=16	>=8	>=128	>=128	>=16
kkp040	<=1	<=1	<=1	4	4	>=8	2	2	1	<=0.25	>=64	4	>=8	>=128	32	>=16
kkp042	16	2	>=64	>=64	>=64	<=0.12	<=0.25	>=16	2	>=4	8	>=16	<=0.25	>=128	64	>=16
kkp043	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	<=1	<=0.25	8	<=8	>=16
kkp044	>=64	>=64	>=64	>=64	>=64	>=8	<=0.25	>=16	<=0.5	>=4	<=2	<=1	>=8	>=128	>=128	>=16
kkp045	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	2	<=0.25	8	<=8	<=0.5
kkp047	>=64	32	>=64	>=64	>=64	1	<=0.25	>=16	1	>=4	4	8	2	>=128	>=128	>=16
kkp048	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	8	<=8	<=0.5
kkp049	2	<=1	32	>=64	>=64	4	<=0.25	1	1	>=4	>=64	>=16	>=8	>=128	<=8	>=16
kkp050	<=1	<=1	>=64	>=64	>=64	1	<=0.25	>=16	2	1	4	>=16	2	>=128	16	>=16
kkp051	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	16	<=8	<=0.5
kkp052	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	<=1	<=0.25	8	<=8	>=16
kkp054	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	4	<=0.25	8	<=8	<=0.5
kkp055	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=1	4	<=1	<=0.25			<=0.5
kkp056	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	<=1	<=0.25	8	<=8	<=0.5
kkp057	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	1	<=0.25	4	2	<=0.25	8	<=8	<=0.5
kkp058	16	2	>=64	>=64	>=64	<=0.12	<=0.25	<=1	<=0.5	>=4	4	2	<=0.25	>=128	>=128	>=16
kkp059	16	<=1	8	4	4	<=0.12	<=0.25	>=16	<=0.5	>=4	>=64	>=16	<=0.25	>=128	16	>=16
kkp060	16	32	>=64	>=64	>=64	<=0.12	<=0.25	<=1	<=0.5	>=4	<=2	2	<=0.25	>=128	>=128	>=16
kkp063	<=1	<=1	<=1	2	2	<=0.12	<=0.25	>=16	<=0.5	<=0.25	4	>=16	<=0.25	>=128	16	>=16
kkp064	>=64	>=64	>=64	>=64	>=64	>=8	<=0.25	>=16	2	>=4	>=64	>=16	>=8	>=128	>=128	>=16
kkp065	<=1	<=1	<=1	2	2	<=0.12	<=0.25	>=16	1	<=0.25	8	>=16	<=0.25	>=128	32	<=0.5
kkp066	<=1	<=1	<=1	2	2	<=0.12	<=0.25	>=16	1	<=0.25	8	>=16	<=0.25	>=128	16	<=0.5
kkp067	4	<=1	16	>=64	>=64	<=0.12	<=0.25	2	<=0.5	>=4	4	4	<=0.25	>=128	<=8	<=0.5
kkp068	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	<=1	<=0.25	>=128	>=128	>=16
kkp070	16	4	>=64	>=64	>=64	1	<=0.25	>=16	1	>=4	8	>=16	2	>=128	>=128	>=16
kkp071	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	8	<=8	<=0.5
kkp072	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	<=1	<=0.25	8	<=8	<=0.5
kkp073	32	4	>=64	>=64	>=64	>=8	<=0.25	>=16	>=8	>=4	>=64	>=16	>=8	>=128	>=128	>=16
kkp074	>=64	>=64	>=64	>=64	>=64	<=0.12	<=0.25	<=1	<=0.5	>=4	<=2	<=1	<=0.25	>=128	32	>=16
kkp083	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	<=4	<=8	<=0.5

Isolate	ATM	FEP	CRO	CXM	CXM_AX	LVX	MEM	TET	TGC	CFM	CHL	MIN	MXF	PIP	TIM	TMP
kkp084	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	2	<=0.25	8	<=8	<=0.5
kkp085	>=64	>=64	>=64	>=64	>=64	2	<=0.25	<=1	1	>=4	>=64	4	4	>=128	>=128	>=16
kkp086	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	>=16	<=0.5	<=0.25	4	>=16	<=0.25	>=128	16	>=16
kkp089	16	2	>=64	>=64	>=64	1	<=0.25	>=16	1	>=4	4	8	2	>=128	16	>=16
kkp090	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	8	<=8	<=0.5
kkp091	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	2	<=0.25	8	<=8	<=0.5
kkp092	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	2	<=0.25	<=4	<=8	<=0.5
kkp093	16	2	>=64	>=64	>=64	<=0.12	<=0.25	<=1	<=0.5	>=4	<=2	4	<=0.25	>=128	>=128	>=16
kkp094	<=1	<=1	<=1	2	2	<=0.12	<=0.25	>=16	1	<=0.25	<=2	8	<=0.25	8	<=8	>=16
kkp102	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	<=1	<=0.25	<=4	<=8	<=0.5
kkp105	16	2	>=64	>=64	>=64	1	<=0.25	<=1	<=0.5	>=4	<=2	<=1	2	>=128	16	>=16
kkp107	4	2	>=64	>=64	>=64	>=8	<=0.25	>=16	<=0.5	>=4	8	4	>=8	>=128	<=8	>=16
kkp108	<=1	<=1	<=1	<=1	<=1	<=0.12	<=0.25	<=1	<=0.5	<=0.25	<=2	2	<=0.25	<=4	<=8	>=16
kkp109	<=1	<=1	<=1	2	2	<=0.12	<=0.25	>=16	<=0.5	<=0.25	4	>=16	<=0.25	>=128	16	>=16
kkp112	<=1	<=1	<=1	2	2	<=0.12	<=0.25	<=1	<=0.5	<=0.25	4	4	<=0.25	8	<=8	<=0.5
kkp113	16	2	>=64	>=64	>=64	<=0.12	<=0.25	<=1	<=0.5	>=4	4	2	<=0.25	>=128	64	>=16

Appendix IV: Antimicrobial Resistance Genes in the *K. pneumoniae* Isolates

Isolate	Beta-lactamase genes	Aminoglycoside-modifying enzymes	Other genes
kkp001	<i>bla</i> CTXM15, <i>bla</i> TEM181	<i>bla</i> SHV11,aac.6.ib9, aph.3.ib, aph.6.id, amT, rmtF, acrD, baeR, cpxA	dfrA14, arr.2, sul2, <i>bla</i> OXA181
kkp003	<i>bla</i> CTXM15, <i>bla</i> OXA9, <i>bla</i> TEM181	<i>bla</i> OXA1,aac.6.ib.cr4, ant.3.iiia, aph.3.vi, amT, acrD, baeR, cpxA	catB3, qnrS1, arr.3, sul1, <i>bla</i> NDM
kkp005	<i>bla</i> CTXM15, <i>bla</i> TEM181	<i>bla</i> SHV187,aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA16, acrD, catII, baeR, cpxA	qnrB17, dfrA27, arr.3, sul1, sul2
kkp006	<i>bla</i> CTXM15, <i>bla</i> TEM181	<i>bla</i> SHV1,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA16, tetD, acrD, baeR, cpxA	catII, qnrB17, dfrA27, arr.3, sul1, sul2
kkp010	<i>bla</i> CTXM15, <i>bla</i> SHV187, <i>bla</i> TEM181	<i>bla</i> OXA1,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, baeR, tetA, cpxA	qnrB17, dfrA14, sul2
kkp012	<i>bla</i> LEN11	amT, acrD, baeR, cpxA	
kkp013		aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	tetD, dfrA15, sul1, sul2
kkp014	<i>bla</i> CTXM15, <i>bla</i> SHV120, <i>bla</i> TEM181	<i>bla</i> OXA1,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.3.ia, aph.6.id, amT, arcB, aadA5, acrD, baeR, cpxA	tetD, qnrB17, dfrA14, dfrA17, sul1, sul2, mphA
kkp015	<i>bla</i> SHV41	aph.3.ia, amT, baeR, cpxA	
kkp016	<i>bla</i> SHV187	amT, arcB, acrD, baeR, cpxA	
kkp018	<i>bla</i> SHV106	aac.3.iv, aac.6.ib.cr6, ant.3.iiia, aph.3.ib, aph.3.ia, cmlA1, floR, qnrB20, qnrB4, dfrA27, aph.4.ia, aph.6.id, amT, aadA16, aadA2, acrD, armA, arr.3, baeR, cpxA	sul1, sul3, mphE, msrE, <i>bla</i> DHA, mcr8
kkp019	<i>bla</i> SHV42	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	
kkp020	<i>bla</i> SHV42	aph.3.ib, aph.6.id, amT, baeR, cpxA	
kkp021	<i>bla</i> SHV61	amT, baeR, cpxA	
kkp022	<i>bla</i> OKPA5, <i>bla</i> TEM181	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	tetD, dfrA5, sul1, sul2
kkp023	<i>bla</i> CTXM15, <i>bla</i> SHV106, <i>bla</i> TEM181	<i>bla</i> OXA1,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	dfrA14, sul2
kkp024	<i>bla</i> SHV187	amT, acrD, baeR, cpxA	
kkp026	<i>bla</i> CTXM15, <i>bla</i> SHV187, <i>bla</i> TEM181	<i>bla</i> OXA1,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, arcB, tetA, acrD, baeR, cpxA	qnrB17, dfrA14, sul2
kkp027	<i>bla</i> CTXM15, <i>bla</i> SHV223	<i>bla</i> OXA1,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA2, catII, acrD, baeR, cpxA	qnrS1, dfrA12, sul1, sul2, mphA
kkp028	<i>bla</i> LEN16	amT, acrD, baeR, cpxA	
kkp030	<i>bla</i> LEN17	amT, acrD, baeR, cpxA	
kkp031	<i>bla</i> CTXM15, <i>bla</i> SHV172, <i>bla</i> TEM181	<i>bla</i> SCO1,aac.3.iie, aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	sul2, mphA
kkp032	<i>bla</i> SHV187	aph.3.ib, aph.6.id, amT, baeR, cpxA	
kkp034	<i>bla</i> OKPB2, <i>bla</i> TEM181	amT, aadA2, acrD, baeR, cpxA	dfrA16, sul1, sul2
kkp035	<i>bla</i> SHV187, <i>bla</i> TEM181	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	tetD, catII, sul2
kkp036	<i>bla</i> CTXM15, <i>bla</i> SHV134	<i>bla</i> OKPA9,aph.3.ib, aph.3.ia, aph.6.id, amT, aadA2, acrD, baeR, cpxA	qnrS1, dfrA12, sul1, sul2, mphA

Isolate	Beta-lactamase genes	Aminoglycoside-modifying enzymes	Other genes
kkp037	<i>blaSHV110, blaSHV134</i>	aac.6.ib10, aph.3.ib, aph.3.ia, aph.6.id, amT, acrD, floR, qnrS1, sul1, sul2, mphA baeR, cpxA	
kkp038	<i>blaCTXM15, blaTEM181</i>	<i>blaSHV215</i> , aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	dfrA14, sul2, mphA
kkp039	<i>blaCTXM15, blaSHV106</i>	<i>blaOXA1</i> , aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA2, tetD, acrD, baeR, cpxA	dfrA12, dfrA14, sul1, sul2, mphA
kkp040	<i>blaSHV106</i>	ant.3.ia, amT, acrD, baeR, cpxA	cmlA5, dfrA14, arr.2, sul1, EreA2
kkp042	<i>blaCTXM15, blaSHV1, blaTEM181</i>	<i>blaSCO1</i> , aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA16, tetD, acrD, baeR, cpxA	dfrA27, arr.3, sul1, sul2
kkp043	<i>blaSHV187</i>	amT, acrD, baeR, cpxA	dfrA5, sul1
kkp044	<i>blaCTXM15, blaSHV1, blaTEM181</i>	<i>blaSCO1</i> , aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA16, tetD, acrD, baeR, cpxA	catII, qnrB17, dfrA27, arr.3, sul1, sul2
kkp045	<i>blaLEN13</i>	amT, acrD, baeR, cpxA	
kkp047	<i>blaCTXM15, blaSHV106</i>	<i>blaOXA1</i> , aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA, tetA	qnrB17, dfrA14, sul2
kkp048	<i>blaLEN55</i>	amT, acrD, baeR, cpxA	
kkp049	<i>blaCTXM98, blaSHV187</i>	aac.3.iid, aac.6.ib.cr6, aph.3.ib, aph.3.ia, aph.6.id, tetA, amT, aadA16, acrD, baeR, cpxA	floR, qnrB20, qnrS1, dfrA27, arr.3, sul1, sul2, mphA
kkp050	<i>blaCTXM14, blaSHV178</i>	<i>blaLAP2</i> , aac.3.iid, aph.3.ib, aph.6.id, amT, baeR, cpxA	tetA, tetD, qnrS1, dfrA1, sul1, sul2
kkp051	<i>blaSHV215</i>	aph.3.ib, aph.6.id, amT, baeR, cpxA	
kkp052	<i>blaSHV110</i>	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	dfrA5, sul1, sul2
kkp054	<i>blaLEN16</i>	amT, acrD, baeR, cpxA	
kkp055	<i>blaSHV120, blaSHV187</i>	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	sul2
kkp056	<i>blaSHV187</i>	amT, acrD, baeR, cpxA	
kkp057	<i>blaLEN13</i>	amT, acrD, baeR, cpxA	
kkp058	<i>blaCTXM15, blaTEM181</i>	<i>blaSHV1</i> , aac.3.iie, aph.3.ib, aph.6.id, amT, baeR, cpxA	dfrA14, sul2, mphA
kkp059	<i>blaLEN16, blaTEM181</i>	<i>blaSHV134</i> , aph.6.id, amT, acrD, baeR, cpxA	tetD, catI, dfrA14, sul2
kkp060	<i>blaCTXM15, blaSHV172, blaTEM181</i>	<i>blaSCO1</i> , aac.3.iie, aph.3.ib, aph.6.id, amT, aadA2, acrD, baeR, cpxA	dfrA12, sul1, sul2, mphA
kkp063		aph.3.ib, aph.3.ia, aph.6.id, amT, acrD, baeR, cpxA	tetD, dfrA7, sul1, sul2
kkp064	<i>blaCTXM15, blaOXA10, blaSHV106</i>	<i>blaOXA1</i> , aac.3.iie, aac.6.ib.cr6, ant.3.ia, aph.3.ib, aph.3.ia, tetD, amT, aadA2, acrD, baeR, cpxA	floR, dfrA12, dfrA14, arr.2, sul1, sul2, mphA
kkp065	<i>blaSHV33</i>	aph.3.ib, amT, crcB, baeR, cpxA	tetD, sul2
kkp066	<i>blaSHV120, blaSHV33</i>	aph.3.ib, aph.6.id, amT, crcB, acrD, baeR, cpxA	tetD, sul2
kkp067	<i>blaCTXM15, blaSHV187</i>	amT, acrD, baeR, cpxA	
kkp068	<i>blaSHV187, blaTEM181</i>	aph.3.ib, aph.6.id, amT, baeR, cpxA	dfrA14, sul2, mphA
kkp070	<i>blaCTXM15, blaSHV187, blaTEM181</i>	<i>blaOXA320</i> , aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, crcB, baeR, cpxA, tetA	qnrB17, dfrA14, sul2
kkp071	<i>blaSHV110</i>	amT, acrD, baeR, cpxA	
kkp072	<i>blaSHV187</i>	amT, acrD, baeR, cpxA	

Isolate	Beta-lactamase genes	Aminoglycoside-modifying enzymes	Other genes
kkp073	<i>blaCTXM15</i> , <i>blaSHV106</i> , <i>blaTEM181</i>	<i>blaOXA1</i> ,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, acrD,qnrB17, dfrA14, sul2 baeR, cpxA	
kkp074	<i>blaCTXM15</i> , <i>blaSHV187</i>	aac.3.iid, aac.6.ib.cr6, amT, aadA16, acrD, baeR, cpxA	dfrA27, arr.3, sul1
kkp075	<i>blaLEN13</i>	amT, acrD, baeR, cpxA	
kkp076	<i>blaSHV120</i> , <i>blaSHV187</i>	aph.3.ib, aph.3.ia, aph.6.id, amT, acrD, baeR, cpxA	tetD, dfrA7, sul1, sul2
kkp077	<i>blaLEN32</i>	amT, acrD, baeR, cpxA	
kkp078	<i>blaLEN32</i>	amT, acrD, baeR, cpxA	
kkp079	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV182</i> ,aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA16,tetD, catII, qnrB17, dfrA14, dfrA27, acrD, baeR, cpxA	arr.3, sul1, sul2, mphA
kkp080	<i>blaCTXM15</i> , <i>blaSHV106</i> , <i>blaTEM181</i>	<i>blaOXA1</i> ,aac.3.iie, aac.6.ib.cr6, aph.6.id, amT, acrD, baeR, cpxA	qnrB17, dfrA14, sul2
kkp081	<i>blaCTXM15</i> , <i>blaLEN16</i>	amT, acrD, baeR, cpxA	
kkp082	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV37</i> ,aac.6.ib.cr6, aph.3.ib, aph.6.id, amT, aadA16, acrD,qnrS1, dfrA27, arr.3, sul1, sul2 baeR, cpxA	
kkp083	<i>blaSHV33</i>	amT, crcB, baeR, cpxA	
kkp084	<i>blaSHV1</i>	amT, baeR, cpxA	
kkp085	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV215</i> ,aac.3.iie, aac.6.ib.cr6, amT, aadA16, baeR, cpxA	catI, catII, qnrS1, dfrA27, arr.3, sul1, sul2
kkp086	<i>blaSHV212</i> , <i>blaTEM181</i>	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	tetD, dfrA5, sul1, sul2
kkp089	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV212</i> ,aph.3.ib, aph.6.id, amT, baeR, cpxA	tetA, qnrS1, dfrA14, sul2
kkp090	<i>blaLEN11</i> , <i>blaOXA926</i>	amT, acrD, baeR, cpxA	
kkp091	<i>blaSHV187</i>	amT, acrD, baeR, cpxA	
kkp092	<i>blaLEN2</i>	amT, acrD, baeR, cpxA	
kkp093	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV215</i> ,aph.3.ib, aph.3.ia, aph.6.id, amT, baeR, cpxA	dfrA14, sul2, mphA
kkp094	<i>blaLEN2</i>	ant.3.iiia, amT, acrD, baeR, cpxA	tetA, dfrA1, sul1
kkp0e1	<i>blaSHV187</i>	amT, acrD, baeR, cpxA	
kkp0e13	<i>blaCTXM15</i> , <i>blaSHV187</i> , <i>blaTEM181</i>	<i>blaOXA11</i> ,aac.3.iid, aac.3.iie, aac.6.ib.cr6, aph.3.ib, aph.6.id,tetA, qnrB17, dfrA14, sul2, mphA amT, acrD, baeR, cpxA	
kkp0e21	<i>blaSHV223</i>	aph.6.id, amT, acrD, baeR, cpxA	dfrA14
kkp0e27	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV215</i> ,aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	tetA, qnrB17, dfrA14, sul2
kkp0e6	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV33</i> ,aac.3.iie, ant.3.iiia, amT, crcB, acrD, baeR, cpxA	catII, dfrA1, sul1
kkp0e7	<i>blaCTXM15</i> , <i>blaTEM181</i>	<i>blaSHV33</i> ,aac.3.iie, amT, crcB, baeR, cpxA	
kkp102	<i>blaSHV110</i>	amT, acrD, baeR, cpxA	
kkp105	<i>blaCTXM15</i> , <i>blaSHV110</i> , <i>blaTEM181</i>	<i>blaOXA1</i> ,aac.3.iie, aac.6.ib.cr6, amT, aadA2, acrD, baeR, cpxA	qnrS1, dfrA12, sul1, sul2, mphA
kkp107	<i>blaSHV187</i>		
kkp108	<i>blaLEN32</i>	amT, acrD, baeR, cpxA	dfrA5, sul1
kkp109	<i>blaSHV77</i> , <i>blaTEM181</i>	aph.3.ib, aph.6.id, amT, acrD, baeR, cpxA	tetD, dfrA14, sul2

Isolate	Beta-lactamase genes	Aminoglycoside-modifying enzymes	Other genes
kkp112	<i>bla</i> LEN13	arnT, acrD, baeR, cpxA	
kkp113	<i>bla</i> CTXM15, <i>bla</i> SHV106, <i>bla</i> TEM181	<i>bla</i> OXA1, aac.3.iie, aac.6.ib.cr6, arnT, acrD, baeR, cpxA	dfrA14, sul2

Appendix V: Virulence Genes Identified in the *K. pneumoniae* Isolates

Isolate	siderophores	serum resistance	immune evasion	allantoin metabolism
kkp001	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
kkp003	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
kkp005		glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wzi		
kkp006	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wzi		
kkp010	fyuA, irp1, ybtAEPQSTUX	irp2, glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wcaGHIJ		
kkp012		ompA	cpsACP, galF, gnd, magA, wzy_K1, manBC, ugd, wcaGHIJ, wclY, wcsST, wza, wzb, wzc, wzi, wzx	
kkp013		glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
kkp014		glf, kfoC, ompA, cpsACP, galF, gnd, manB, ugd, wzi		
kkp015		glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wzi		
kkp016		glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wzi		
kkp018		glf, kfoC, ompA, cpsACP, galF, gnd, manB, ugd, wzi		
kkp019		glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wzi		
kkp020		glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wzi		
kkp021		glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wzi		
kkp022		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp023		glf, kfoC, ompA, cpsACP, galF, gnd, ugd, wzi		
kkp024		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp026		glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wzi		
kkp027	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wzi		
kkp028		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp030		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp031	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wzi		

Isolate	siderophores	serum resistance	immune evasion	allantoin metabolism
kkp032		glf, kfoC, ompA,cpsACP, galF, gnd, manBC, ugd, wzi wbbMNO, wzm, wzt		
kkp034		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp035		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp036		ompA	cpsACP, galF, gnd, manC, ugd, wzi	
kkp037		glf, kfoC, ompA,cpsACP, galF, gnd, manBC, ugd, wzi wbbMNO, wzm, wzt		
kkp038		glf, kfoC, ompA,cpsACP, galF, gnd, ugd, wzi wbbMNO, wzm, wzt		
kkp039	fyuA, irp1, ybtAEPQSTUX	irp2,glf, ompA, wbbMNO,cpsACP, galF, gnd, manBC, ugd, wza, wzi wzm, wzt		
kkp040	fyuA, irp1, ybtAEPQSTUX	irp2,glf, ompA, wbbM,cpsACP, galF, gnd, manBC, ugd, wzi wbbN, wzm, wzt		
kkp042	fyuA, irp1, ybtAEPQSTUX	irp2,glf, ompA, wbbMNO,cpsACP, galF, gnd, ugd, wzi wzm, wzt		
kkp043	iucABCD, iroBCDN	iutA,glf, kfoC, ompA,cpsACP, galF, gnd, manBC, ugd, wzi wbbMNO, wzm, wzt		
kkp044	fyuA, irp1, ybtAEPQSTUX	irp2,glf, ompA, wbbMNO,cpsACP, galF, gnd, ugd, wzi wzm, wzt		
kkp045		ompA	cpsACP, galF, gmd, gnd, magA.wzy_K1, manBC, ugd, wcaGHII, wclY, wcsST, wza, wzb, wzc, wzi, wzx	
kkp047		glf, ompA, wbbMNO,cpsACP, galF, gnd, manBC, ugd, wza, wzi wzm, wzt		
kkp048		ompA	cpsACP, galF, gmd, gnd, manBC, ugd, wcaGHII, wzi	
kkp049		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp050		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp051		glf, kfoC, ompA,cpsACP, galF, gnd, ugd, wzi wbbMNO, wzm, wzt		
kkp052		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp054		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp055	fyuA, irp1, ybtAEPQSTUX	irp2,glf, ompA, wbbMNO,cpsACP, galF, gnd, manBC, ugd, wzi wzm, wzt		
kkp056		glf, kfoC, ompA,cpsACP, galF, gnd, manBC, ugd, wzi wbbMNO, wzm, wzt		
kkp057		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp058	fyuA, irp1, ybtAEPQSTUX	irp2,ompA	cpsACP, galF, gnd, ugd, wzi	
kkp059		ompA	cpsACP, galF, gnd, ugd, wza, wzi	
kkp060	fyuA, irp1, ybtAEPQSTUX	irp2,glf, ompA, wbbMNO,cpsACP, galF, gnd, manBC, ugd, wzi wzm, wzt		
kkp063		glf, ompA, wbbMNO,cpsACP, galF, gnd, manBC, ugd, wza, wzi wzm, wzt		

Isolate	siderophores	serum resistance	immune evasion	allantoin metabolism
kkp064	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
kkp065		wzm, wzt		
		glf, kfoC, ompA, cpsACP, galF, gnd, ugd, wzi		allABCDRS
		wbbMNO, wzm, wzt		
kkp066		glf, kfoC, ompA, cpsACP, galF, gnd, ugd, wzi		allABCDRS
		wbbMNO, wzm, wzt		
kkp067	fyuA, irp1, ybtAEPQSTUX	irp2, glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
		wbbMNO, wzm, wzt		
kkp068		glf, kfoC, ompA, cpsACP, galF, gnd, manBC, ugd, wzi		allABCDRS
		wbbMNO, wzm, wzt		
kkp070		glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wzi		
		wzm, wzt		
kkp071		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp072		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp073		glf, kfoC, ompA, cpsACP, galF, gnd, ugd, wzi		
		wbbMNO, wzm, wzt		
kkp074	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
		wzm, wzt		
kkp075		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp076		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp077		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp078		ompA	cpsACP, galF, gnd, ugd, wzi	
kkp079	fyuA, irp1, ybtAEPQSTUX	irp2, ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp080	fyuA, irp1, ybtAEPQSTUX	irp2, glf, kfoC, ompA, cpsACP, galF, gnd, ugd, wzi		
		wbbMNO, wzm, wzt		
kkp081		ompA	cpsACP, galF, gnd, manB, ugd, wzi	
kkp082		glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wzi		
		wzm, wzt		
kkp083	iroBDN	glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wzi		
		wzm, wzt		
kkp084		glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wza, wzi		
		wzm, wzt		
kkp085	fyuA, irp1, ybtAEPQSTUX	irp2, ompA	cpsACP, galF, manBC, ugd, wzi	
kkp086		glf, ompA, wbbMNO, cpsACP, galF, gnd, manBC, ugd, wzi		
		wzm, wzt		
kkp089		glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wza, wzi		
		wzm, wzt		
kkp090		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp091		glf, ompA, wbbMNO, cpsACP, galF, gnd, ugd, wzi		
		wzm, wzt		

Isolate	siderophores	serum resistance	immune evasion	allantoin metabolism
kkp092		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp093		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp094		ompA	cpsACP, galF, gnd, manBC, ugd, wza, wzi	
kkp0e1		glf, ompA, wbbMNO,	cpsACP, galF, gnd, manBC, ugd, wza, wzi	
		wzm, wzt		
kkp0e13		glf, kfoC, ompA,	cpsACP, galF, gnd, manBC, ugd, wza, wzi	
		wbbMNO, wzm, wzt		
kkp0e21		glf, ompA, wbbMNO,	cpsACP, galF, gnd, ugd, wza, wzi	
		wzm, wzt		
kkp0e27	fyuA, irp1, ybtAEPQSTUX	irp2, ompA	cpsACP, galF, gnd, ugd, wzi	
kkp0e6	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO,	cpsACP, galF, gmd, gnd, manBC, ugd, wcaGHII, wzi	
		wzm, wzt		
kkp0e7	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO,	cpsACP, galF, gmd, gnd, manBC, ugd, wcaGHII, wzi	
		wzm, wzt		
kkp102		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp105	fyuA, irp1, ybtAEPQSTUX	irp2, glf, kfoC, ompA,	cpsACP, galF, gnd, manBC, ugd, wza, wzi	
		wbbMNO, wzm, wzt		
kkp107		glf, kfoC, ompA,	cpsACP, galF, gnd, ugd, wzi	
		wbbMNO, wzm, wzt		
kkp108		ompA	cpsACP, galF, gnd, manBC, ugd, wza, wzi	
kkp109		glf, ompA, wbbMNO,	cpsACP, galF, gnd, manBC, ugd, wzi	
		wzm, wzt		
kkp112		ompA	cpsACP, galF, gnd, manBC, ugd, wzi	
kkp113	fyuA, irp1, ybtAEPQSTUX	irp2, glf, ompA, wbbMNO,	cpsACP, galF, gnd, manBC, ugd, wza, wzi	
		wzm, wzt		

Appendix VI: Fluoroquinolone Mutations Identified in the *K. pneumoniae* Isolates

Isolate ID	Enzymes			
	<i>gyrase A (gyrA)</i>		<i>DNA topoisomerase 4 subunit A (parC)</i>	
kkp001	-	-	<i>Ser83Ile</i>	<i>Ser80Ile</i>
kkp003	-	-	<i>Ser83Ile</i>	<i>Ser80Ile</i>
kkp018	<i>Ser83Phe</i>	<i>Asp87Aspn</i>	-	<i>Ser80Ile</i>
kkp023	-	-	<i>Ser83Ile</i>	<i>Ser80Ile</i>
kkp039	<i>Ser83Phe</i>	<i>Asp87Aspn</i>	-	<i>Ser80Ile</i>
kkp040	<i>Ser83Phe</i>	<i>Asp87Aspn</i>	-	<i>Ser80Ile</i>
kkp064	<i>Ser83Phe</i>	<i>Asp87Aspn</i>	-	<i>Ser80Ile</i>
kkp073	-	-	<i>Ser83Ile</i>	<i>Ser80Ile</i>
kkp079	-	-	<i>Ser83Ile</i>	<i>Ser80Ile</i>
kkp080	-	-	<i>Ser83Ile</i>	<i>Ser80Ile</i>

Appendix VII: Contingency Table of the Fisher's Exact Test for Virulence Gene Loci

	Isolates from CAI (n=62)	Isolates from HAI (n=21)
Isolates with virulence gene loci (≥ 1)	44 (71%)	13 (62%)
Isolates without virulence gene loci (0)	18 (29%)	8 (38%)

Appendix VIII: Contingency Table of the Fisher's Exact Test for AMR Genes

Contingency table of the Fisher's exact test for AMR genes	Isolates from CAI (n=62)	Isolates from HAI (n=21)
Isolates with AMR genes (≥ 1)	39 (63%)	20 (95%)
Isolates without AMR genes (0)	23 (37%)	1 (5%)

Appendix IX: The Mating Pair Formation Genes and the Relaxase Genes in the *K. pneumoniae* Isolates

Bacteria ID	Mobilizability	Mating Pair Formation system genes	Relaxase genes
kkp001	conjugative	MPFI_ATPase, MPFI_T4CP_1, MPFI_T4CP_2, MPFI_traI, MPFI_traK, MPFI_traL, MPFI_traM, MPFI_traN, MPFI_traP, MPFI_traQ, MPFI_traR, MPFI_traW, MPFI_traY	MOBP, MOBQ, MOBPB, MOBV, MOBH
kkp019	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBC
kkp020	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp022	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBF, MOBPB
kkp023	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBH, MOBC
kkp026	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp031	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp034	conjugative	MPFT_ATPase, MPFG_T4CP_2, MPFT_virB3, MPFT_virB6, MPFT_virB8, MPFT_virB9	MOBF, MOBH
kkp035	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBH
kkp036n	conjugative	MPFT_ATPase, MPFT_T4CP_1, MPFF_T4CP_1, MPFT_virB3, MPFT_virB6, MPFT_virB8, MPFT_virB9	MOBF
kkp037	conjugative	MPFG_ATPase, MPFF_ATPase, MPFG_T4CP_1, MPFF_traC, MPFF_traK, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW, MPFT_ATPase, MPFT_T4CP_1, MPFT_virB3, MPFT_virB6, MPFT_virB8, MPFT_virB9	MOBF
kkp038	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBC, MOBH
kkp039	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBH

kkp042	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp044	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBH, MOBV
kkp047	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBQ
kkp055	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp059	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBC
kkp060	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp063	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBC
kkp064	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW, MPFG_ATPase, MPFF_ATPase, MPFG_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traK, MPFF_traL, MPFF_traN,	MOBF
kkp066	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBP, MOBPB, MOBH
kkp068	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBH
kkp073	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp076	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBC
kkp079	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF, MOBH, MOBC
kkp081	conjugative	MPFT_ATPase, MPFT_T4CP_1, MPFT_virB3, MPFT_virB6, MPFT_virB8, MPFT_virB9	MOBF

kkp085	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp093	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traH, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBH, MOBF
kkp094	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp0e13	conjugative	MPFI_ATPase, MPFI_T4CP_2, MPFI_T4CP_1, MPFI_traL, MPFI_traK, MPFI_traL, MPFI_traM, MPFI_traN, MPFI_traP, MPFI_traQ, MPFI_traR, MPFI_traW, MPFI_traY	MOBF
kkp0e21	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW, MPFF_ATPase, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW,	MOBF
kkp0e27	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp0e6	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW, MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp0e7	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp108	conjugative	MPFF_ATPase, MPFF_T4CP_1, MPFF_T4CP_2, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW, MPFF_ATPase, MPFF_T4CP_1, MPFI_traK, MPFI_traN, MPFI_traP, MPFI_traR	MOBF, MOBC
kkp109n	conjugative	MPFF_ATPase, MPFF_T4CP_2, MPFF_T4CP_1, MPFF_traC, MPFF_traE, MPFF_traH, MPFF_traK, MPFF_traL, MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBF
kkp006n	conjugative	MPFF_traN, MPFF_traU, MPFF_traV, MPFF_traW	MOBC
kkp005n	mobilizable		MOBV
kkp014	mobilizable		MOBF, MOBC

kkp018	mobilizable		MOBF
kkp043	mobilizable		MOBF
kkp049	mobilizable		MOBF
kkp051	mobilizable		MOBP, MOBF
kkp058	mobilizable		MOBF, MOBF
kkp065	mobilizable		MOBF
kkp074	mobilizable		MOBF, MOBF
kkp080	mobilizable		MOBF
kkp082	mobilizable		MOBF, MOBH
kkp089	mobilizable		MOBF
kkp113	mobilizable		MOBF

Appendix X: Publication - The Antimicrobial Resistance and Virulence Characteristics of *K. pneumoniae* Isolates in Kenya by Whole-Genome Sequencing



Article

Antimicrobial Resistance and Virulence Characteristics of *Klebsiella pneumoniae* Isolates in Kenya by Whole-Genome Sequencing

Angela Muraya¹, Cecilia Kyany'a^{2,3}, Shahid Kiyaga⁴, Hunter J. Smith^{2,5}, Caleb Kibet^{1,5}, Melissa J. Martin⁶, Josephine Kimani¹ and Lillian Musila^{2,3,*}

- ¹ Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi P.O. Box 62000-00200, Kenya; angelimuraya@gmail.com (A.M.); kipkuruis@hotmail.com (C.K.); jkimani@juat.ac.ke (J.K.)
² United States Army Medical Research Directorate-Africa, Village Market, Nairobi P.O. Box 606-00621, Kenya; cc.katunge@gmail.com (C.K.); hunterjsmith11@gmail.com (H.J.S.)
³ Kenya Medical Research Institute, Nairobi P.O. Box 54840-00200, Kenya
⁴ Department of Immunology and Molecular Biology, School of Biomedical Sciences, College of Health Sciences, Makerere University, Kampala P.O. Box 7072, Uganda; ashakykiyaga91@gmail.com
⁵ International Center for Insect Physiology and Ecology, Nairobi P.O. Box 30772-00100, Kenya
⁶ Multidrug-Resistant Organism Repository and Surveillance Network (MRSN), Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA; melissa.j.martin28.ctr@mail.mil
* Correspondence: lillian.musila@usamru-k.org; Tel.: +254-7288-17872



Citation: Muraya, A.; Kyany'a, C.; Kiyaga, S.; Smith, H.J.; Kibet, C.; Martin, M.J.; Kimani, J.; Musila, L. Antimicrobial Resistance and Virulence Characteristics of *Klebsiella pneumoniae* Isolates in Kenya by Whole-Genome Sequencing. *Pathogens* 2022, 11, 545. <https://doi.org/10.3390/pathogens11050545>

Academic Editors: Raspaël Carrel Fournou Zanque and Malik Aydin

Received: 26 March 2022

Accepted: 28 April 2022

Published: 5 May 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: *Klebsiella pneumoniae* is a globally significant opportunistic pathogen causing healthcare-associated and community-acquired infections. This study examined the epidemiology and the distribution of resistance and virulence genes in clinical *K. pneumoniae* strains in Kenya. A total of 89 *K. pneumoniae* isolates were collected over six years from five counties in Kenya and were analyzed using whole-genome sequencing and bioinformatics. These isolates were obtained from community-acquired (62/89) and healthcare-associated infections (21/89), and from the hospital environment (6/89). Genetic analysis revealed the presence of *bla*_{NDM-1} and *bla*_{OXA-18} carbapenemase genes and the *armA* and *rmtF* genes known to confer pan-aminoglycoside resistance. The most abundant extended-spectrum beta-lactamase genes identified were *bla*_{CTX-M-15} (36/89), *bla*_{TEM} (35/89), and *bla*_{OXA} (18/89). In addition, one isolate had a mobile colistin resistance gene (*mcr-8*). Fluoroquinolone resistance-conferring mutations in *gyrA* and *parC* genes were also observed. The most notable virulence factors were those associated with hyper-virulence (*mmpA/A2* and *magA*), yersiniabactin (*ybt*), salmochelin (*iro*), and aerobactin (*iuc* and *iutA*). A total of 38 distinct sequence types were identified, including known global lineages ST14, ST15, ST147, and ST307, and a regional clone ST17 implicated in regional outbreaks. In addition, this study genetically characterized two potential hypervirulent isolates and two community-acquired ST147 high-risk clones that contained carbapenemase genes, yersiniabactin, and other multidrug resistance genes. These results demonstrate that the resistance and virulence of Kenyan clinical and hospital environmental *K. pneumoniae* isolates are diverse. The reservoir of high-risk clones capable of spreading resistance, and virulence factors have the potential to cause unmanageable infection outbreaks with high morbidity and mortality.

Keywords: antimicrobial resistance; virulence; whole-genome sequencing; Kenya; *Klebsiella pneumoniae*

1. Introduction

Klebsiella pneumoniae is a Gram-negative, rod-shaped ubiquitous bacterium that inhabits soil, water, and sewage ecosystems. It is also found on various human body sites and organ systems, including skin, nose, throat, and intestinal tract, as part of the natural microflora [1]. *K. pneumoniae* is a prominent member of the *Klebsiella pneumoniae* species complex that consists of seven species that include *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*, *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, *Klebsiella variicola* subsp.