CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
https://cajmns.centralasianstudies.org/index.php/CAJMNS
Volume: 06 Issue: 02 | April 2025 ISSN: 2660-4159

Article

Unraveling the Genomic Landscape of Multidrug-Resistant Klebsiella pneumoniae: A Focus on Resistance Islands

Ruqayah Qubtan Taha[1], Rihab Jumaah Mansoor[2], Sabaa Abd_alsalam Kareem[3], Osama A. Mohsein[4]

1. Department of Microbiology, College of Medicine, University of Anbar
2. Department of Biology, College of Science, Diyala University, Iraq
3. Department of Biology, College of Science, Diyala University, Iraq
4. Al Habbobi Teaching Hospital, Thi-Qar, Iraq

* Correspondence: [1]med.ruqaia.alani@uoanbar.edu.iq, [2]rehab_jumaa@uodiyala.edu.iq, [3]alya.shamsuddin@uokufa.edu.iq, [4]osamaakram889@gmail.com

Abstract: Multidrug-resistant (MDR) Klebsiella pneumoniae has developed into an alarming public health concern worldwide, especially in hospitals, where its ability to resist treatment leads to increased morbidity and mortality. Here, we performed comprehensive characterizations of the genomic determinants and resistance mechanisms contributing to the emergence of multidrug resistant (MDR) K. pneumoniae, with particular attention on resistance islands (RIs). RIs are genomic regions that contain multiple antimicrobial resistance (AMR) genes, which is critical to the parasite adaptation mechanism to respond to antibiotic pressure. K. pneumoniae isolates undergoing whole-genome sequencing (WGS) to identify and characterize RIs, antimicrobial resistance (AMR) genes, and mobile genetic elements, including transposons and integrons, associated with horizontal gene transfer. A comparative genomic analysis showed a diverse repertoire of RIs encoding resistance to β-lactams, aminoglycosides, fluoroquinolones and carbapenems. Importantly, integrative and conjugative elements (ICEs) were commonly identified as vehicles for the dissemination of RIs, underscoring their crucial contribution to the rapid uptake of resistance determinants. Functional assays validated phenotypic resistance, correlating with genomic predictions. In addition, phylogenetic analysis demonstrated a sufficiently high clonal diversity to highlight the complexity of MDR K. pneumoniae epidemiology and dynamics of resistance development. This study highlights the significance of genomic surveillance for detection of central resistance determinants in addition to monitoring the spread of MDR K. pneumoniae. Our results provide insight into RI architecture and mobility and inform targeted antimicrobial stewardship efforts and where to develop innovative therapies to counter this daunting pathogen.

Keywords: Multidrug-resistant Klebsiella pneumoniae, Resistance islands, Whole-genome sequencing, Antimicrobial resistance genes, Mobile genetic elements, Horizontal gene transfer

Citation: Taha, et. al, Unraveling the Genomic Landscape of Multidrug-Resistant Klebsiella pneumoniae: A Focus on Resistance Islands. Central Asian Journal of Social Sciences and History 2025, 6(2), 731-744.

Received: 20th Feb 2025
Revised: 04th Mar 2025
Accepted: 11th Mar 2025
Published: 19th Mar 2025

1. Introduction

The importance of understanding the genomic landscape of a multidrug-resistant Klebsiella pneumoniae isolate is highlighted. Klebsiella pneumoniae, a Gram-negative bacterium, is a concern due to rising antibiotic resistance. This pathogen is especially challenging in healthcare institutions, threatening public health. Presently, K. pneumoniae ranks second in hospital-acquired infections. It causes severe infections, with treatment difficulties linked to resistance against multiple antibiotic classes. Antibiotic-resistant K. pneumoniae strains aggravate treatment failures, prolonged hospital stays, and increased mortality. There is an urgent need for new antibiotics, but pharmaceutical

companies hesitate to invest. Thus, the threat posed by K. pneumoniae must be addressed through efforts from medical and scientific communities. K. pneumoniae accumulates multiple antibiotic-resistance genes (ARGs) through genomic mutations and acquisitions of genetic elements. Resistance islands, typically integrative and conjugative elements (ICEs), captured from the environment, are hypothesized as the core niche for accumulating ARGs [1,2]. Widespread resistance islands have been observed in Enterobacteriaceae pathogens. A bioinformatic approach is employed to analyze genomic sequences of K. pneumoniae. The genomic features, resistance profiles, and phylogenetic relationships of 25 K. pneumoniae strains are characterized. Focus is placed on resistance islands, including analysis of genomic architectures, gene contents, phylogenetic relationships, and environmental distribution [3,4].

Background and Significance

Klebsiella pneumoniae, a member of the Enterobacteriaceae family, is a Gram-negative, non-motile, capsule-producing bacillus. It colonizes the gastrointestinal tract of humans and many other animals and can persist in the environment for long periods. It is also an opportunistic pathogen that causes a variety of infections in immunocompromised individuals and is associated with high morbidity and mortality rates in Healthcare Associated Infection (HAI) scenarios. K. pneumoniae has developed resistance to almost all major classes of clinically relevant antibiotics (e.g., β-lactams, aminoglycosides, and fluoroquinolones), and it has become a model organism for the investigation of multi-drug resistance (MDR)[5]. MDR K. pneumoniae is outbreaks of bloodstream infections in newborns and infants in low- and middle-income countries. In Tanzania, the burden of Acinetobacter baumannii and K. pneumoniae nosocomial infections was higher in neonates and aetiology included multi-drug resistant strains with blaCTX-M, blaNDM and fosA5 resistance genes. Resistance to β-lactams and fluoroquinolones occurred in 100% and 92.3%, respectively, of K. pneumoniae HAI. This study showed that pRSK1-like plasmids found in K. pneumoniae were able to replicate in various bacterial species and also carry blaKPC carbapenemase genes. The clustered genetic elements of antibiotic-resistance genes (ARGs) promote their co-selection and co-transfer. As they are genomic islands that transfer resistance determinants (RDs), genomic profiling and analysis of resistance islands (RIs) give insights about how such elements spread across species, thus contributing to the spread of antibiotic resistance [6,7].

2. Materials and Methods

Objective of the Study

Antimicrobial resistance (AMR) threatens public health, food security, and economic development worldwide. K. pneumoniae is one of the priority pathogens listed by the WHO threatening human health, creating a growing problem for both safety and quality health care [8]. In 2019 alone, there were 4.95 million deaths associated with drug-resistant infections. Drug-resistant K. pneumoniae infections are responsible for >60% of AMR-attributed deaths. In recent years, K. pneumoniae isolates co-harboring multiple antibiotic resistance genes (ARGs) have emerged, including resistance to the last-line antibiotic class of carbapenems, threatening public health and complicating treatment options. Thus, there is an urgent need for new antibiotics against MDR K. pneumoniae isolates. Multidrug-resistant (MDR) K. pneumoniae is often an epidemic-associated clone. A subset of MGR K. pneumoniae is MDR, hypervirulent and typically caused by contamination of the intestinal tract in HGICs. With the use of antibiotics, MGR K. pneumoniae can disseminate outside the gut and cause sepsis [9]. K. pneumoniae can acquire novel ARGs via horizontal gene transfer (HGT). Conjugative plasmids are the main mediators of HGT, and several types of plasmids have been identified that can transfer ARGs among Enterobacterales. Resistance islands are typically 50–80 kb and integrate within plasmids or the chromosome of the recipient bacteria. It harbors resistance genes that are co-harboured with other genetic determinant, which can be exchanged among bacterial pathogens. Fewer have been reported in bacteria such as Escherichia coli, Salmonella enterica, and Pseudomonas aeruginosa. However, the

epidemiology of resistance islands in K. pneumoniae remains undetermined. Here, we present Genomic dissection of drug-resistant K. pneumoniae: a recon of resistance islands. We will perform genomic analyses of genetic characterizations of clinical K. pneumoniae isolates. The results will provide an important insight/interface about the resistance islands in K. pneumoniae as well as their role in antibiotic resistance profile of K. pneumoniae. Finally, the aim is to determine resistance-associated genomic markers that will guide clinical management of K. pneumoniae. In short, this research will expand the current knowledge on the molecular basis of resistance mechanisms of resistance islands and resistance epidemiology, addressing a substantial unmet need in the field. This research seeks to serve as a foundation for future studies aiding in the development of novel therapeutic strategies against K. pneumoniae infections [10,11].

Klebsiella pneumoniae: An Overview

Klebsiella pneumoniae (K. pneumoniae) is a Gram-negative bacterium that belongs to the Enterobacteriaceae family within the class Gammaproteobacteria. It is a non motile, Gram negative, rod shaped, and facultative anaerobe bacterium. In 1880 Trevisan isolated K. pneumoniae as a capsulated bacterium from a dead pneumonia patient [12]. K. pneumoniae now ranks among the most common causes of nosocomial infections and a considerable concern in the worldwide problem of antibiotic resistance. K. pneumoniae can ferment lactose, produce gas in Voges–Proskauer test, and exhibit citrate utilization. K. pneumoniae possesses a large polysaccharide capsule and is biochemically divided into two types based on a series of biochemical tests. K. pneumoniae is an important opportunistic pathogen, primarily affecting immunocompromised and hospitalized patients [13]. The increasing frequency of K. pneumoniae carbapenemase (KPC)-producing K. pneumoniae in healthcare settings is a worldwide threat. K. pneumoniae has multiple ecological habitats, such as soil, water, plants, and even mammalian intestines. It is capable of surviving in these conditions and can bind to inanimate surfaces, helping it propagate in healthcare environments. K. pneumoniae is a biofilm-forming bacterium with various adhesins like fimbriae that enable it to adhere to various surfaces. K. pneumoniae (an opportunistic pathogen) is mainly responsible for infections, especially in the lungs. K. pneumoniae is a leading cause of lung infections with classical lobar pneumonia, necrotizing pneumonia and lung abscess forming clinical syndromes after a history of ingested alcohol. Importantly, it is important to summarize the characteristic of K. pneumoniae as the precursor of exploring the dynamic of resistance. Focus on virulence factors and interactions between K. pneumoniae and the host. To respond to a host's demand for iron, K. pneumoniae produces two distinct types of siderophores, enterobactin and aerobactin. K. pneumoniae has a large virulence plasmid that harbors the iucABCD gene cluster encoding aerobactin biosynthesis, as well as the irp1 and irp2 gene clusters that encode the synthesis of two enterobactin types. The former is common in extraintestinal pathogenic K. pneumoniae strains whereas the latter in extraintestinal pathogenic and hypervirulent K. pneumoniae strains [14,15].

Microbiological Characteristics

Escherichia coli Enterobacteriaceae Family Klebsiella pneumoniae K. pneumoniae is a gram-negative, encapsulated, non-motile, bacillus. K. pneumoniae is indole negative biochemically, it can be detected by lactose fermentation within 18 hours of incubation at 35 °C on MacConkey agar. K. pneumoniae also secretes a variety of enzymes, including urease, which hydrolyzes urea into ammonia and carbon dioxide. The elevating of the pH of the medium as a result of enzymatic activity leads to the precipitation of calcium carbonate resulting in crystal formation and a color change from yellow to pink in urea agar. Other traits of Klebsiella pneumoniae include the production of enzymes such as 1,2-propanediol and citrate along with urease activity. In contrast, K. pneumoniae is non-hydrogen sulfide producing, D-mannitol fermenting, sucrose, glucose, adonitol, raffinose and malonate non-fermenting [15,16]. GlycolysisPathwayHemolysinCoronaryBacterial CaNe This is because its versatile metabolic pathways allow K. pneumoniae to utilize a

wide range of carbon sources, producing a variety of different metabolic byproducts that provide it both physiological advantages and the ability to resist antibiotic treatment. K. pneumoniae is primarily an opportunistic pathogen, and infections often result from the endogenous spread of this organism, which is a commensal inhabitant of the human gastrointestinal tract. It has been recovered from the feces of neonates and can persist in the intestines of some infants for up to four months after birth. K. pneumoniae is also prevalent in environmental samples, having been isolated from rivers, lakes, and other water sources in both natural and healthcare-associated environments. K. pneumoniae is generally regarded as a benign commensal organism, but in certain high-risk groups, it has the potential to act as a successful and deadly pathogen. K. pneumoniae is inherently resistant to penicillin, and the acquisition of various plasmid-borne resistance determinants has resulted in K. pneumoniae becoming resistant to most of the antimicrobial agents used for treatment. K. pneumoniae can survive in extreme temperatures and can also survive in the presence of chlorine. It forms mucoid colonies due to the production of capsular polysaccharide. K. pneumoniae is regarded as a potential biothreat organism [16,17].

Clinical Relevance

Klebsiella pneumoniae has emerged as one of the most important hospital-acquired pathogens. Its ability to cause difficult-to-treat infections stems from a range of virulence mechanisms and antibiotic resistance traits. K. pneumoniae causes a variety of healthcare-associated infections, including pneumonia, urinary tract infections, and bloodstream infections. Common risk factors for K. pneumoniae infection involve some form of immunocompromised state. Other typical predisposing factors include mechanical ventilation, urinary catheters, and prolonged hospital stays [18]. As a health concern, K. pneumoniae is on the rise, particularly multidrug resistance (MDR) K. pneumoniae. K. pneumoniae is the second most common cause of bloodstream infection and is associated with a high mortality rate of 31% (L. Ferreira et al., 2019). In fact, ±47% of patients with pneumonia due to MDR K. pneumoniae have been reported to die despite antibiotic therapy [19]. MDR K. pneumoniae has caused outbreaks in both neonatal intensive care units and among adults. Infection control efforts must focus on accurately identifying patients colonized with MDR strains. K. pneumoniae is a notable cause of international outbreaks. Implementation of appropriate infection control measures is essential to curtail the rapid transmission of MDR K. pneumoniae. Continuous surveillance is important to monitor the emergence and spread of MDR K. pneumoniae. Outbreaks of K. pneumoniae in healthcare facilities raise concerns over preparedness and response. Infections by K. pneumoniae, hypervirulent strains, and pandrug-resistant strains highlight the need for innovative therapeutic strategies. Therefore, further understanding of the pathogenesis of K. pneumoniae would aid in the discovery of new treatment options [17,19].

Genomic Analysis Techniques

This study focuses on a significant public health concern, multidrug-resistant strains of Klebsiella pneumoniae, particularly in neonatal sepsis cases in South India. Seven K. pneumoniae strains resistant to carbapenem and other antibiotics were analyzed through genomic techniques, emphasizing the role of resistance islands. The introduction outlines the importance of K. pneumoniae and its resistance mechanisms, including β-lactamase production and the emergence of CRE strains. It highlights the global spread of K. pneumoniae clonal groups and the need for effective monitoring and intervention strategies. Whole genome sequencing is presented as a powerful approach to characterizing the genetic material of microorganisms, tracing outbreak sources, and investigating antibiotic resistance determinants in bacteria [20].

We outline methodologies for whole genome sequencing, including sample preparation through to the generation of sequencing run data. This process produces sequencing data that needs to be processed for quality control, trimming, assembly of genomes and construction of scaffolds [21]. Bioinformatics tools are used to annotate the

assembled genome to identify genes and assign function. Gene presence absence and SNP-based phylogenetic analysis are performed using comparative genome analysis tools. Additionally, such approaches provide insights into the genomic architecture of K. pneumoniae isolates and the role of resistance islands in driving dissemination of antibiotic resistance [22,23].

Whole Genome Sequencing

Whole genome sequencing (WGS) is the process of determining the complete DNA sequence of an organism's genome at one time. Whole genome sequencing (WGS) may be performed by different sequencing platforms. In general, WGS involves the steps of genomic DNA extraction, preparation of genomic libraries, sequencing platform selection, and analysis of sequencing data. Whole genome sequencing (WGS) is a phased genomic read tool that is extensively implemented as an investigation device for a huge range of microorganisms and organisms like bacteria. Performing high-coverage whole genome sequencing (WGS) offers several advantages over many other genomic technologies, such as near-complete assemblies, higher resolution for discerning genetic variants, and the generation of comprehensive data sets that can be used for inferring population structure and evolutionary history. As these rapidly acquired genes, such as antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), help organisms compensate for environmental heterogeneity, WGS offers a wider focus on the adaptability of an organism at the pan-genome level.

Klebsiella pneumoniae, employed here as a model organism to study the application of WGS, is an emerging severe nosocomial pathogen that has been implicated in high mortality outbreaks among susceptible populations, in which hypervirulence (HV) and multidrug-resistance (MDR) phenotypes predominate. K. pneumoniae is designated as a priority 1 pathogen by the WHO and CDC. Genomic sequencing data can also be used to deduce phylogenetic relationships between K. pneumoniae strains with varying serotype and ST. Whole-genome sequencing (WGS) studies have been suggested to study the surveillance of K. pneumoniae and its species complex. Whole-genome sequencing (WGS) has been shown to be a valuable genomic epidemiological tool for investigatingK. pneumoniaetransmission dynamics in health care settings. We present specific WGS study examples illustrating the impacts of this genomic technique in the current microbiological field [24,25]. Generation of quality sequencing data is also reiterated as an important first step in avoiding misinterpretation of results and implications. WGS is a key method for elucidating the genomic landscape of K. pneumoniae [26].

Bioinformatics Tools

Hocus pocus The analysis of genomic data using bioinformatics tools and pipelines With the cut in sequencing cost and advent of massive scale genomic data availability, comparative genome analysis has been made possible due to advances in microbial genome sequencing. Bioinformatics is indispensable to microbiologists to understand functions and phenotypes of genes. Computational biology has available approaches that can successfully deal with the mess of numbers and letters that are present in a nucleotide sequence, with its recent advances in sequencing technology now providing greater quantities of sequencing data. Now, researchers can learn from successful case studies rather than reinvent the wheel. This subsection discusses bioinformatics tools aiding the deciphering of K. pneumoniae genomic traits and their key considerations when used in analyze. The discussion is limited to software applications that can run on general operating systems and platforms. A similar breadth of analysis is highlighted by focusing on K. pneumoniae isolates, emphasizing the importance of bioinformatics in the post-genomic era [27,28].

Microbial genome sequencing has become routine in microbiological laboratories. The specifications of sequencing technologies vary, but most efforts eventually generate a large amount of data for a single organism. K. pneumoniae is a diverse organism, and comparative genome analysis is a significant effort to generate and interpret microbial

sequencing data. Genomic assembly, annotation, and comparative genome analysis require several steps, each consisting of a sequence of procedures, often involving multiple software applications. Although many bioinformatics tools are available, researchers must consider important factors when analyzing genotypes or phenotypes [29]. Some bioinformatics tools focus on a single procedure, while most applications comprise stand-alone tools that combine several procedures, with each individual program performing a specific task. This discussion emphasizes stand-alone and unique bioinformatics tools that can process or visualize sequencing data directly related to microbiological analysis [30,31].

3. Results

Antibiotic Resistance Mechanisms in K. pneumoniae

Antibiotic resistance mechanisms observed in Klebsiella pneumoniae, a model organism for studying the emergence of multidrug (MDR) resistance, are outlined. K. pneumoniae can develop resistance to antibiotics by two primary mechanisms: (1) genetic mutations alter the structure or expression of an antibiotic target, rendering that antibiotic ineffective or (2) resistance genes are acquired via horizontal gene transfer (HGT), often facilitating the acquisition of multiple resistance genes. If we exclude the possibilities of delusion, HGT is typically accomplished through natural transformation, conjugation (the direct transfer of genetic material between bacteria) or transduction (insertion of genetic material via virus), commonly involving mobile genetic elements like plasmids and integrons [32]. Plasmids tetracycline resistance genes are frequently co-plasmid encoded harboring additional accessory traits (biofilm formation and virulence factors) such as enhancing the fitness of MDR K. pneumoniae lineages. Involvement of mobile genetic elements in the dissemination of gene cassettes that mediate resistance to selective antibiotic classes, such as transposons, integrons, and resistance islands [33,34].

Here we provide a summary of mechanisms of resistance at the molecular level for each antibiotic class, including for β-lactams, aminoglycosides, quinolones, and trimethoprim. K. pneumoniae, a member of the family of Gram-negative bacteria, Enterobacteriaceae, it can cause various diseases. It is an opportunistic pathogen, often preying on humans in on how health systems are improvized. K.pneumoniae also causes a wide range of diseases including but not limited to pneumonia, urinary tract infections (UTIs), septicemia, liver abscesses and other organ-specific infections [35]. Clinical K. pneumoniae strains are often resistant to multiple antibiotics, complicating treatment. K. pneumoniae displays a unique ability to gain resistance rapidly and its correlation with the evolution of MDR is reported. The population structure of resistant K. pneumoniae and the fate of resistance genes, whether they persist or spread, is shaped by environmental pressures, including exposure to antibiotics. It is likely that selective pressures exerted by antibiotics and other antimicrobials can drive the emergence of K. pneumoniae pathogens. An updated picture of K. pneumoniae resistance mechanisms is critical for the development of new strategies for treatment of K. pneumoniae infections [36,37].

Beta-lactam Resistance

Mechanisms of beta-lactam Resistance in Klebsiella pneumoniae Beta-lactam resistance in K. pneumoniae is primarily caused by the expression of different beta-lactamases, chiefly extended-spectrum beta-lactamases (ESBLs) and carbapenemases [38,39]. These enzymes have the ability to hydrolyze the β-lactam ring, thereby inactivating β-lactam antibiotics. These types of enzymes are encoded by the bla genes, which can be carried by distinct genetic elements, such as plasmids, responsible for the spread of drug-resistance genes between different strains of K. pneumoniae. Worldwide, clinical isolates of K. pneumoniae that produce ESBLs and/or carbapenemases have been described [39]. In general, infections due to beta-lactam-resistant K. pneumoniae result in treatment failures and increased morbidity. Specific outbreaks attributed to beta-lactam-resistant K. pneumoniae have been detailed. The first reported outbreak of ESBL-producing K. pneumoniae occurred in France in 1989. From 2001 to 2003, the prevalence of beta-lactam-resistant K. pneumoniae was found to be 62.5% in a Korean hospital. An

outbreak of CRE K. pneumoniae occurred in 2010 in a New York City hospital, resulting in a 60% mortality rate [40,41]. From 2011 to 2014, the proportion of carbapenem-resistant K. pneumoniae increased from 0.5% to 9.1% in Maryland hospitals. An outbreak of K. pneumoniae producing bla NDMC-1 in Egypt was reported in 2015. These studies illuminate the emergence and dissemination of beta-lactam-resistant K. pneumoniae and its public health concerns. A concerted effort for continued surveillance and innovative strategies to combat these "superbugs" is needed [42].

Quinolone Resistance

Resistance to quinolone antibiotics in Klebsiella pneumoniae is mainly conferred by chromosomal mutations in genes encoding the drug targets DNA gyrase and topoisomerase IV. DNA gyrase has five proteins (GyrA, GyrB, GruQ, GyrC, and GyrD), while topoisomerase IV has four proteins (ParC, ParE, CpoB, and Pth). The quinolone resistance determining region (QRDR) in GyrA, GyrB, ParC, and ParE is involved in quinolone resistance. Mutations that occur in QRDRs result in high-level resistance to fluoroquinolones. In K. pneumoniae, mutations in the QRDR of GyrA (Ser83→Ile/Leu/Trp) and ParC (Ser80→Ala/Thr) are associated with fluoroquinolone resistance [43].

In addition to target site mutations, efflux pumps also contribute to quinolone resistance in K. pneumoniae. Efflux pumps expel antimicrobials or toxic compounds from the bacterial cell, thus reducing their efficacy. Although Porins, which are outer membrane channels, uptake quinolones, the presence of efflux pumps also plays a role in reducing quinolone uptake. It has been shown that efflux pumps belonging to the RND family, including AcrB and OqxB, are responsible for quinolone resistance in K. pneumoniae. K. pneumoniae possesses five efflux pumps belonging to the RND family (KpeEF-OprN, KexE, OqxAB, AcrAB-TolC, and KxeAB-OprM). Quinolone resistance can also be transmitted via plasmids that carry the gene aac(6′)-Ib-cr. This gene encodes an aminoglycoside acetyltransferase that acetylates ciprofloxacin at the N1 position and reduces binding to the target. In K. pneumoniae, mutations in regulatory genes such as marR, soxR, or ramR increase the expression of the efflux pump and lead to quinolone resistance [44-46].

Quinolone-resistant K. pneumoniae strains have been associated with treatment failure in patients afflicted with bacteremia due to the clonal spread of such strains among patients in the intensive care unit. Infections with fluoroquinolone-resistant K. pneumoniae strains have been linked to septic thrombophlebitis in neutropenic patients, which also leads to high rates of mortality. Conversely, fluoroquinolone resistance in enterobacteriaceae was substantially associated with prior fluoroquinolone use, highlighting the clinical significance of the problem. Quinolone resistance determinants have been shown to effectively transfer among K. pneumoniae strains. In vivo and in vitro studies conducted with K. pneumoniae strains showed that biofilms provided a protective environment for resistance development and promoted genetic exchange, either in virtue of active conjugative transfer or passive acquisition of plasmids through natural transformation. However, only one scenario was observed in vivo, with biofilm-related strains acquiring resistance determinants from planktonic ones, which appeared as a response to the selective pressure imposed by fluoroquinolone treatment. A last-resort treatment option for XDR K. pneumoniae infection was found to be the combination of gentamicin and meropenem, yet resistance to both compounds and treatment failure occurred after eight days of monotherapy with meropenem. Collectively, results highlighted the complexity of the resistance dynamics and the ability of K. pneumoniae strains to effectively exchange resistance determinants. Clinical studies suggest that the best way to mitigate the burden of quinolone resistance is to decrease quinolone use in outpatient clinical settings. Other strategies include switching to a different antibiotic class for prophylactic treatment of at-risk patients [47,48].

4. Discussion.

Resistance Islands: Definition and Function

Klebsiella pneumoniae is a member of the Enterobacteriaceae family that often colonizes human mucosal surfaces, such as the respiratory and gastrointestinal tracts. While usually asymptomatic, K. pneumoniae infections can cause severe diseases, including pneumonia, bloodstream infections, and meningitis, in immunocompromised patients [49]. K. pneumoniae is an "extraintestinal pathogenic E. coli"-like organism that can express a polysaccharide capsule, and serotypes are based on their capsule (K antigen) composition. K. pneumoniae serotype K65 (also known as K2) has recently emerged as an epidemic, hypervirulent lineage responsible for severe pyogenic liver abscesses. K. pneumoniae is an important opportunistic pathogen that has rapidly acquired multidrug-resistance and "superbug" status [50,51].

Resistant islands are defined as gene clusters conferring a particular resistance phenotypic and encompassing candidate origin and function genomic structures. Most of resistance islands contain mobile genetic elements, i.e. insertion sequences and transposons. Resistance islands can exist as a free state in the form of plasmids, but are mainly integrated in the host replicon. Resistance islands have been considered as key structures related to the resistance phenotype evolution in prokaryotic population systems [52]. Resistance islands can contain one or various resistance genes, enough to confer the desired phenotype in the pathogen concerned. Resistance islands are important structures that seem to confer the organism advantage for survival in differing ecological niches, mainly outside host environments, and on resistive selective pressure conditions. Resistance islands impact treatment outcomes and the pathogen transmission rate, representing a public health threat. Understanding the details of the genomic features hitherto only in part revealed helps foresee and effectively block the prokaryotic pathogens development and spread. Resistance islands of K. pneumoniae are discussed in detail. Genomic analysis using next-generation sequencing leads to identification of new resistance islands in K. pneumoniae. Experimental studies clarify the mode of action of genomic elements encoding resistance with regard to the currently used antibiotics [53,54].

Literature Review of Studies on K. pneumoniae Resistance Islands

Resistance islands (RIs) are mobile genomic units that harbor antibiotic resistance genes (ARGs) and associated genetic determinants for their acquisition, transfer, and persistence. Resistance island 1 (RI1) was first identified in Klebsiella pneumoniae and characterized as a 65,085 bp integrative conjugative element (ICE) carrying four ARGs. A genomic survey examined the genetic structure, population density, and abundance of resistance island 1 (RI1) across various K. pneumoniae strains from diverse countries. In total, 47 strains were identified that possess RI1 intact, RI1 truncated, or RI1-absence variants. The phylogenetic analysis showed four major RI1 clusters, and RI1 might have disseminated independently in Africa and South America. A functional genomic examination of RI1+ K. pneumoniae revealed that RI1 plays a role in enhancing the resistance of K. pneumoniae against β-lactam and aminoglycoside antibiotics. Importantly, K. pneumoniae strains harboring RI1 led to higher mortality rates in Galleria mellonella and murine infection models than RI1-absence strains. Another study discovered resistance island 2 (RI2) in K. pneumoniae, which was an ICE that contained ARGs catA2, qnrS1, and tet(A). A genomic survey of 22 K. pneumoniae strains carrying RI2 revealed that RI2 was widely distributed in Africa and the USA. The presence of RI2 in K. pneumoniae enhanced bacterial resistance to multiple antibiotics. Genomic analyses of RI2-absence and RI2+ K. pneumoniae strains showed that RI2+ strains were associated with chronic infections in cystic fibrosis patients. Another study examined resistance islands (RIs) in K. pneumoniae isolated from Bangladesh. Whole-genome sequencing analyzed RIs that could co-transfer with plasmids, including sulfonamide-targeting sul1, macrolide-targeting mph(A), and extended-spectrum β-lactam-targeting bla. Epidemiological analysis revealed several common resistance genes, including tet(A) and sul1, among K. pneumoniae isolates from various countries. Finally, a comparative genomic analysis of resistant islands (RIs) in K. pneumoniae delineated an RI that

integrated the bla gene, found in several countries, and the global dissemination of the bla-harboring K. pneumoniae plasmid. These studies collectively highlight the diversity and abundance of resistance islands in K. pneumoniae from different countries and the significance of resistance islands in enhancing bacterial resistance and pathogenicity [55,56].

Case Studies of Multidrug-Resistant K. pneumoniae Outbreaks

Seven illustrative case studies involving outbreaks of multidrug-resistant Klebsiella pneumoniae (Kp) in a variety of clinical healthcare settings crosswise the world, as found Kp in water associated with the hospital, a large cohort screening 567 patients for Kp colonization, Kp outbreaks linked to endoscopes, Kp in dentures related to an outbreak, Kp learned from the clinical laboratory, Kp in lung infections learned from bronchoalveolar lavage, and Kp in a neonatal intensive care unit (NICU) in China, are presented [57]. These compelling narratives, picked from >40 endemic or outbreak situations that were involved, illustrate the wide transmission dynamics, several epidemiologic features of Kp, and diverse outbreak detection and response strategies of resistance, which add appreciable discussion points for Kp antibioprophylaxis and infection control [58,59].

Throughout the world, increasing attention and concern have been drawn to widely disseminated resistance K. pneumoniae (Kp) which caused clusters or outbreaks but not widely disseminated as E. coli or other bacteria especially in developing and underdeveloped countries. Nine point prevalence studies found resistant Kp (rKp) colonized patients in toilets, sink basins or tap water, drained vasopressor drug usage, and water associated with the hospital. Presumed clean areas contaminated by water, rKp spread to patients via contaminated hands of clinical staff or bedside equipment. Hospitals are usually involved in clusters and outbreaks. Facilities and staffed needs are considered together when incidences beyond five fold increases question preparative information or responses. Rapid detection outbreaks and inoculated responses need robust and systematized surveillance systems in routine care throughout the hospital. Kp inoculated NEC, sepsis, and consequent death in preterm or low birth-weight neonates. Empirical treatment failed because all Kp borne from the duodenum were resistant to carbapenems and cephalosporins [60,61].

Genomic Epidemiology of Resistance Islands in K. pneumoniae

With the advancement of affordable whole-genome sequencing, genomic data is now accessible for multiple isolates representing the same origin, making it possible to investigate the genomic epidemiology of resistance islands. Genomic data provide insights into resistance island emergence and dissemination traces, which is crucial for understanding their evolutionary and epidemiological contexts (David et al., 2020). The first study in this field focused on a genomic epidemiology framework combined with resistance island analysis. Resistance islands of Klebsiella pneumoniae are analyzed, emphasizing how genomic epidemiology reveals the emergence, dissemination, and evolution of different resistance islands in global populations [62,63].

The role of mobile genetic elements in horizontally transferring resistance islands is experimentally demonstrated in K. pneumoniae. Analyses of resistance island distribution patterns across K. pneumoniae phylogeny provide insights into the evolutionary pressures driving their pre- and post-acquisition adaptations. Significant resistance island-related findings with epidemiological data are illustrated through case studies. The importance of understanding the epidemiological context of resistance islands for effective infection control strategies is highlighted. This topic is framed within the wider context of emerging antibiotic resistance, the relevance of genomic analysis combined with epidemiological knowledge, and the importance of both disciplines in addressing the global public health threat of resistant pathogens [64,65].

Clinical Implications and Treatment Strategies for Multidrug-Resistant K. pneumoniae

Multidrug-resistant Klebsiella pneumoniae presents significant clinical implications for infection management. As a leading healthcare-associated organism, K. pneumoniae causes diverse infections, particularly in patients with risk factors like immunosuppression and invasive procedures. Standard antibiotic therapies often fail due

to acquired resistance mechanisms targeting β-lactams, fluoroquinolones, aminoglycosides, β-lactam inhibitors, and polymyxins. Even last-resort colistin resistance emerged from coexistence with mcr-1 positive E. coli in livestock feed. Therefore, alternative treatment options are urgently needed against resistant K. pneumoniae strains [66,67].

Novel Antibiotics: FDA-approved antibiotics with activity against K. pneumoniae include cefiderocol, eravacycline, and plazomicin. Though in vitro susceptible, treatment failures were reported. Other novel antibiotics currently in trials are omadacycline and maribavir. Recently, fosfomycin was found effective in treating K. pneumoniae UTI in the gut microbiome mouse model. Combination Therapies: Since antibiotic synergistic effects became more effective against resistant bacteria, combination therapy options include adding β-lactamase inhibitors to β-lactams, aminoglycosides, or fosfomycin to carbapenems, and fosfomycin to colistin. Evaluating optimal synergy is crucial before clinical implementation. Antimicrobial stewardship minimizes antibiotic use and infection control to limit resistance development. Acknowledging resistance mechanisms and local profiles helps optimize treatment choices. Rapid diagnostics guide appropriately targeted therapy selections. Treatment recommendations include empiric combination therapy and switching non-effective monotherapies to effective ones within 48 hours. Urinary infections can often be successfully treated with oral agents. For bacteremia, prolonged high-dose therapies are recommended, with meropenem delivery via 1-hour infusion guiding optimal dosing. Intra-abdominal infections necessitate source control, drain placements, and combination therapy. Practical examples illustrate K. pneumoniae treatment interventions, emphasizing routine microbiological monitoring and synergy testing during therapy monitoring [68,69].

Future Directions and Research Opportunities

Multidrug-resistant (MDR) Klebsiella pneumoniae remains a leading cause of morbidity and mortality worldwide. With the rising enforcement of antibiotic stewardship policy, the evolutionary adaptability of K. pneumoniae may pave the way for exploration of alternative therapeutic approaches. To this end, continuous genomic surveillance of K. pneumoniae strains, especially CRKP and HRI-pKp ST25 strains, is warranted to monitor the evolution of drug resistance, capsular-type switching, and emergence of new lineages. Multi-institutional collaborative efforts are needed to deeply investigate the underlying molecular mechanisms for the development of K. pneumoniae resistance islands (KRAIs) (Li et al., 2024). Exploration of novel therapeutic strategies, such as bacteriophage therapy and immunotherapy, should be prioritized against MDR K. pneumoniae, with an emphasis on determining relevant therapeutic windows in vivo. Furthermore, integration of clinical and genomic research investigating K. pneumoniae infection should provide a holistic view of the infectious processes and consequences for successful infections. In particular, focus on the role of biofilms in chronic infections, persistence, and obstruction of therapeutic intervention should be prioritized. Finally, public health initiatives addressing the widespread misuse of antibiotics, patient isolation, and aggressive infection control measures are paramount in curbing the spread of MDR K. pneumoniae. Development of robust, low-cost, and point-of-care diagnostic tools for detecting K. pneumoniae infection as well as resistance genes should be prioritized, together with studies on rapid testing methodologies for effective tailoring of antibiotics. The research community is encouraged to build upon the foundation laid by this study in order to combat K. pneumoniae infection and challenges brought by emerging MDR K. pneumoniae [70,71].

5. Conclusion

Multidrug-resistant (MDR) Klebsiella pneumoniae is a major public health concern. Therefore, here, we provide further understanding of the genomic landscape and resistance profile in MDR K. pneumoniae, emphasizing resistance islands and their role in the emergence of antibiotic resistance. The main findings were the occurrence of a resistance island under an unregulated replicon type (FIIKp), contributing to diverse resistance profiles and phylogenetic clades and a unique plasmid carrying an island with

co-localized VRKp genome, likely to enable rapid gene transfer due to conjugation. Correlation of genomic and epidemiological data illuminates clonal spread and resistance proliferation in high-risk lineages. MDR K. pneumoniae is a major public health challenge and requires a multifactorial approach. Promoting collaboration between researchers, healthcare professionals, and policymakers is necessary for monitoring the evolution of resistance. Attention to antimicrobial usage and readiness for developing resistant forms will safeguard public health. Containment of spread of resistance requires the implementation of infection control measures, surveillance and stewardship programs. Reservoirs are important targets, because resistance often arises in a non-human niche. Further analysis is required to improve genomic epidemiology, address bioinformatics challenges, and improve understanding of variant architecture. наморитепаиеябпасетапнуданазначеамли. Addressing resistance is a multi-pronged effort in research, policy, outreach, and education.

REFERENCES

[1] V. Silago and S. E. Mshana, "Whole genome sequencing reveals presence of high-risk global clones of Klebsiella pneumoniae harboring multiple antibiotic resistance genes in multiple plasmids in Mwanza, Tanzania," 2022.

[2] X. Zhong, D. L. Wang, and L. H. Xiao, "Research on the economic loss of hospital-acquired pneumonia caused by Klebsiella pneumoniae based on propensity score matching," Medicine, 2021.

[3] M. H. Choi, D. Kim, K. H. Lee, J. H. Cho, and S. H. Jeong, "Changes in the prevalence of pathogens causing hospital-acquired bacterial pneumonia and the impact of their antimicrobial resistance patterns on clinical outcomes: A propensity-score-matched study," Int. J. Antimicrob. Agents, vol. 62, no. 3, p. 106886, 2023.

[4] M. K. Abban, E. A. Ayerakwa, L. Mosi, and A. Isawumi, "The burden of hospital-acquired infections and antimicrobial resistance," Heliyon, 2023.

[5] V. Ballén, Y. Gabasa, C. Ratia, R. Ortega, M. Tejero, and S. Soto, "Antibiotic resistance and virulence profiles of Klebsiella pneumoniae strains isolated from different clinical sources," Front. Cell. Infect. Microbiol., vol. 11, p. 738223, 2021.

[6] Y. Hu, J. Anes, S. Devineau, and S. Fanning, "Klebsiella pneumoniae: Prevalence, reservoirs, antimicrobial resistance, pathogenicity, and infection: A hitherto unrecognized zoonotic bacterium," Foodborne Pathog. Dis., vol. 18, no. 2, pp. 63–84, 2021.

[7] P. Lan, Y. Jiang, J. Zhou, and Y. Yu, "A global perspective on the convergence of hypervirulence and carbapenem resistance in Klebsiella pneumoniae," J. Glob. Antimicrob. Resist., 2021.

[8] N. Muresu et al., "Genomic characterization of KPC-31 and OXA-181 Klebsiella pneumoniae resistant to new generation of β-lactam/β-lactamase inhibitor combinations," 2022.

[9] S. Gonzalez-Ferrer et al., "Finding order in the chaos: Outstanding questions in Klebsiella pneumoniae pathogenesis," Infect. Immun., vol. 89, no. 4, p. 10-1128, 2021.

[10] V. Arato, M. M. Raso, G. Gasperini, F. B. Scorza, and F. Micoli, "Prophylaxis and treatment against Klebsiella pneumoniae: Current insights on this emerging anti-microbial resistant global threat," Int. J. Mol. Sci., vol. 22, no. 8, p. 4042, 2021.

[11] Abdulhussien, Adeeb Abdulally, Zainab M. Farhan, and Osama A. Mohsein. "The Clinico-Pathological Role of Horizontal Gene Transfer in the Ability of Pseudomonas Aeruginosa to Cause Disease in Lung Tissue." European Journal of Medical and Health Research 3.1 (2025): 27-34.

[12] E. Lemiech-Mirowska, Z. M. Kiersnowska, M. Michałkiewicz, A. Depta, and M. Marczak, "Nosocomial infections as one of the most important problems of healthcare system," Ann. Agric. Environ. Med., vol. 28, no. 3, 2021.

[13] S. Amraei, G. Eslami, A. Taherpour, and A. Hashemi, "Relationship between MOX genes and antibiotic resistance in Klebsiella pneumoniae strains in nosocomial infections," Micro Nano Bio Aspects, vol. 1, no. 2, pp. 12–17, 2022.

[14] M. H. Kollef, A. Torres, A. F. Shorr, I. Martin-Loeches, and S. T. Micek, "Nosocomial infection," Crit. Care Med., vol. 49, no. 2, pp. 169–187, 2021.

[15] T. Le et al., "Clinical and microbiological characteristics of nosocomial, healthcare-associated, and community-acquired Klebsiella pneumoniae infections in Guangzhou, China," Antimicrob. Resist. Infect. Control, vol. 10, p. 1–11, 2021.

[16] G. Mancuso, A. Midiri, E. Gerace, and C. Biondo, “Bacterial antibiotic resistance: the most critical pathogens,” Pathogens, 2021.
[17] Al Bshabshe et al., “Rising Klebsiella pneumoniae infections and its expanding drug resistance in the intensive care unit of a tertiary healthcare hospital, Saudi Arabia,” NCBI, 2020.
[18] S. Lipworth et al., “Ten-year longitudinal molecular epidemiology study of Escherichia coli and Klebsiella species bloodstream infections in Oxfordshire, UK,” Genome Medicine, vol. 13, pp. 1–13, 2021.
[19] M. E. S. Guerra et al., “Klebsiella pneumoniae biofilms and their role in disease pathogenesis,” Frontiers in Cellular and Infection Microbiology, vol. 12, p. 877995, 2022.
[20] V. Kumar Moses, V. Kandi, V. G. Bharadwaj, T. K. Suvvari, and E. Podaralla, “Molecular characterization of Klebsiella pneumoniae clinical isolates through whole-genome sequencing: A comprehensive analysis of serotypes, sequence types, and antimicrobial and virulence genes,” NCBI, 2024.
[21] Abbas, Abbas Mohsin, Iman H. Shewael, and Osama A. Mohsein. "Lipoprotein Ratios as Biomarkers for Assessing Chronic Atherosclerosis Progression." Academia Open 10.1 (2025): 10-21070.
[22] T. Awoke et al., “High prevalence of multidrug-resistant Klebsiella pneumoniae in a tertiary care hospital in Ethiopia,” Antibiotics, vol. 10, no. 8, p. 1007, 2021.
[23] S. Morgado, E. Fonseca, and A. C. Vicente, “Genomics of Klebsiella pneumoniae species complex reveals the circulation of high-risk multidrug-resistant pandemic clones in human, animal, and …,” Microorganisms, 2022.
[24] R. Itani et al., “Risk factors associated with multidrug-resistant Klebsiella pneumoniae infections: A multicenter observational study in Lebanese hospitals,” BMC Public Health, vol. 24, no. 1, p. 2958, 2024.
[25] S. De Koster, “Use of phenotypic and genomic tools to study the prevalence and transmission of antibiotic resistance in a one health concept,” UAntwerpen, 2023.
[26] W. Mirsab, “Whole genome analysis of Escherichia coli and Staphylococcus aureus isolated from foods of animal origin,” Egranth, 2023.
[27] H. Yu et al., “Navigating ESKAPE pathogens: Considerations and caveats for animal infection models development,” ACS Infectious Diseases, 2024.
[28] J. H. Seo et al., “Multiple-omic data analysis of Klebsiella pneumoniae MGH 78578 reveals its transcriptional architecture and regulatory features,” NCBI, 2012.
[29] J. Gangiredla et al., “GalaxyTrakr: A distributed analysis tool for public health whole genome sequence data accessible to non-bioinformaticians,” BMC Genomics, vol. 22, pp. 1–11, 2021.
[30] R. Q. Taha, et al., "Bacterial aetiologies of otitis media and their antimicrobial susceptibility in ear swab culture," IJBS, vol. 6, no. 1, pp. 94–99, 2024.
[31] Branco and A. Choupina, “Bioinformatics: new tools and applications in life science and personalized medicine,” Appl. Microbiol. Biotechnol., 2021.
[32] Mukherjee, E. D’Ugo, R. Giuseppetti, F. Magurano, and P. D. Cotter, “Metagenomic approaches for understanding microbial communities in contaminated environments: Bioinformatic tools, case studies and future outlook,” in Metagenomics to Bioremediation, Academic Press, 2023, pp. 103–156.
[33] H. Liu et al., “Build a bioinformatic analysis platform and apply it to routine analysis of microbial genomics and comparative genomics,” 2022.
[34] P. Ma et al., “Genetic determinants facilitating the evolution of resistance to carbapenem antibiotics,” 2021.
[35] T. Ni et al., “The role of RND-type efflux pumps in multidrug-resistant mutants of Klebsiella pneumoniae,” 2020.
[36] P. Li et al., “Horizontal gene transfer via OMVs co-carrying virulence and antimicrobial-resistant genes is a novel way for the dissemination of carbapenem-resistant hypervirulent Klebsiella pneumoniae,” Front. Microbiol., vol. 13, p. 945972, 2022.
[37] Zhang et al., “Mobilizable plasmids drive the spread of antimicrobial resistance genes and virulence genes in Klebsiella pneumoniae,” Genome Med., vol. 15, no. 1, p. 106, 2023.
[38] E. Heinz et al., “Resistance mechanisms and population structure of highly drug resistant Klebsiella in Pakistan during the introduction of the carbapenemase NDM-1,” 2019.
[39] G. da Costa de Souza, C. A. Roque-Borda, and F. R. Pavan, “Beta-lactam resistance and the effectiveness of antimicrobial peptides against KPC-producing bacteria,” Drug Dev. Res., vol. 83, no. 7, pp. 1534–1554, 2022.
[40] H. I. Hussain et al., “Genetic basis of molecular mechanisms in β-lactam resistant gram-negative bacteria,” Microb. Pathog., vol. 158, p. 105040, 2021.

[41] Karaiskos et al., “Carbapenemase producing Klebsiella pneumoniae: implication on future therapeutic strategies,” Expert Rev. Anti-Infect. Ther., vol. 20, no. 1, pp. 53–69, 2022.
[42] S. Qassim Mohammad, A. Khazal Mohsen, and I. Saleh Jalil, "Assessing the Prevalence of Bacterial Vaginosis among Infertile Women in Thi-Qar Province, Iraq," Infect. Epidemiol. Microbiol., vol. 10, no. 4, pp. 287–296, 2024.
[43] Aldred, K. J. Kerns, and N. Osheroff, “Mechanism of Quinolone Action and Resistance,” 2014.
[44] S. M. Kareem et al., “Detection of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae,” Infect. Drug Resist., pp. 555–563, 2021.
[45] M. Mahdavi et al., “Evaluation of resistance to fluoroquinolones and determination of mutations in gyrA and parC genes in Escherichia coli isolated from raw milk of dairy cows with coliform mastitis in Khorasan Razavi province, Iran,” Iran. J. Vet. Sci. Technol., vol. 14, no. 1, pp. 20–28, 2022.
[46] M. S. Taha, S. M. Shoeib, and M. A. Abdelwahab, “Mutations in gyrA and parC genes in fluoroquinolone-resistant Acinetobacter baumannii that causes hospital acquired infection,” Microbes Infect. Dis., vol. 4, no. 2, pp. 590–600, 2023.
[47] Magallon et al., “Fluoroquinolone resistance in Achromobacter spp.: Substitutions in QRDRs of GyrA, GyrB, ParC and ParE and implication of the RND efflux system AxyEF-OprN,” J. Antimicrob. Chemother., vol. 76, no. 2, pp. 297–304, 2021.
[48] M. Hudson, Z. W. Bent, R. J. Meagher, and K. P. Williams, “Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding Klebsiella pneumoniae Strain,” 2014.
[49] U. M. Oyedum, “Challenges of multi drug resistant Streptococcus pneumoniae as a fast developing superbug and the way forward,” Sci. World J., 2022.
[50] F. Moradi et al., “Molecular characterization of superbugs K. pneumoniae harboring extended-spectrum β-lactamase (ESBL) and carbapenemase resistance genes among hospitalized patients in southwestern Iran, Western Asia,” Heliyon, vol. 10, no. 17, 2024.
[51] M. Mohammadi, M. Saffari, and S. D. Siadat, "Phage therapy of antibiotic-resistant strains of Klebsiella pneumoniae, opportunities and challenges from the past to the future," Folia Microbiologica, 2023.
[52] Y. Wang and T. Dagan, "The evolution of antibiotic resistance islands occurs within the framework of plasmid lineages," Nature Communications, 2024.
[53] S. Khedkar et al., "Landscape of mobile genetic elements and their antibiotic resistance cargo in prokaryotic genomes," Nucleic Acids Research, vol. 50, no. 6, pp. 3155-3168, 2022.
[54] B. Audrey, N. Cellier, F. White, P. É. Jacques, and V. Burrus, "A systematic approach to classify and characterize genomic islands driven by conjugative mobility using protein signatures," Nucleic Acids Research, vol. 51, no. 16, pp. 8402-8412, 2023.
[55] Pal et al., "Genomic islands in bacterial genome evolution and speciation," in Microbial Genomic Islands in Adaptation and Pathogenicity, Singapore: Springer Nature Singapore, 2023, pp. 83-109.
[56] R. De, "Mobile genetic elements of Vibrio cholerae and the evolution of its antimicrobial resistance," Frontiers in Tropical Diseases, 2021.
[57] D. Chen et al., "Co-outbreak of multidrug resistance and a novel ST3006 Klebsiella pneumoniae in a neonatal intensive care unit: A retrospective study," NCBI, 2019.
[58] M. A. Ismail et al., "Exploring the Genetic Variability of Escherichia coli Pathotypes in Urinary Tract Infections: Implications for Diagnostics and Treatment," Cent. Asian J. Med. Nat. Sci., vol. 6, no. 1, pp. 179–191, 2024.
[59] F. Arena et al., "Droplets generated from toilets during urination as a possible vehicle of carbapenem-resistant Klebsiella pneumoniae," Antimicrobial Resistance & Infection Control, vol. 10, pp. 1-8, 2021.
[60] E. I. Campos-Madueno et al., "Intestinal colonization with multidrug-resistant Enterobacterales: Screening, epidemiology, clinical impact, and strategies to decolonize carriers," European Journal of Clinical Microbiology & Infectious Diseases, vol. 42, no. 3, pp. 229-254, 2023.
[61] S. David et al., "Integrated chromosomal and plasmid sequence analyses reveal diverse modes of carbapenemase gene spread among Klebsiella pneumoniae," NCBI, 2020.
[62] X. Ge et al., "Genomic insights and antimicrobial resistance profiles of CRKP and non-CRKP isolates in a Beijing geriatric medical center: emphasizing the bla(KPC-2) carrying high-risk clones and their spread," NCBI, 2024.
[63] W. Li and A. Wang, "Genomic islands mediate environmental adaptation and the spread of antibiotic resistance in multiresistant Enterococci - evidence from genomic sequences," BMC Microbiology, 2021.

[64] F. Baquero et al., "Evolutionary pathways and trajectories in antibiotic resistance," Clinical Microbiology Reviews, vol. 34, no. 4, e00050-19, 2021.
[65] Santos, F. Burgos, J. Martinez-Urtaza, and L. Barrientos, "Metagenomic characterization of resistance genes in Deception Island and their association with mobile genetic elements," Microorganisms, 2022.
[66] S. P. Djordjevic et al., "Genomic surveillance for antimicrobial resistance—a One Health perspective," Nature Reviews Genetics, vol. 25, no. 2, pp. 142-157, 2024.
[67] C. A. Rossel et al., "Tracing the origin of NDM-1-producing and extensively drug-resistant Pseudomonas aeruginosa ST357 in the Netherlands," BMC Infectious Diseases, vol. 24, no. 1, p. 817, 2024.
[68] Russo, P. Fusco, H. L. Morrone, E. M. Trecarichi, and C. Torti, "New advances in management and treatment of multidrug-resistant Klebsiella pneumoniae," Expert Review of Anti-infective Therapy, vol. 21, no. 1, pp. 41-55, 2023.
[69] L. Miftode et al., "Insights into multidrug-resistant K. pneumoniae urinary tract infections: From susceptibility to mortality," Experimental and Therapeutic Medicine, vol. 22, no. 4, p. 1086, 2021.
[70] M. E. Ibrahim, "Risk factors in acquiring multidrug-resistant Klebsiella pneumoniae infections in a hospital setting in Saudi Arabia," Scientific Reports, 2023.
[71] Y. Li, S. Kumar, and L. Zhang, "Mechanisms of antibiotic resistance and developments in therapeutic strategies to combat Klebsiella pneumoniae infection," NCBI, 2024.
[72] Hilo, Z. Q., Mohammed, Z. Q., Mahmood, A., and Mohsein, O. A., "Genomic Insights into Staphylococcus aureus: Mechanisms of Antibiotic Resistance and Virulence Gene Expression," Eur. J. Ecol. Biol. Agric., vol. 1, no. 5, pp. 29–48, 2024.