Whole-Genome Sequencing-Based Resistome Analysis of a Clinical *Klebsiella pneumonia*

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Abstract

Introduction: *Klebsiella pneumoniae* is an important cause of healthcare-associated infections and a major carrier of antimicrobial resistance (AMR) determinants. Whole-genome sequencing (WGS) provides a comprehensive approach for characterizing bacterial resistomes and supporting genomic AMR surveillance. This study aimed to characterize the resistome of a clinical *K. pneumoniae* isolate recovered from a lower respiratory tract specimen using WGS and bioinformatic analyses.

Methods: Genomic DNA extracted from the selected *K. pneumoniae* isolate was sequenced using the Illumina NovaSeq 6000 platform. Following quality control, high-quality reads were assembled de novo using SPAdes version 4.1.0, followed by reference-based read mapping against the *K. pneumoniae* RefSeq genome (GCF_000240185.1). The resulting genomic data were analyzed using ShortBRED in combination with the Comprehensive Antibiotic Resistance Database (CARD) to identify and quantify antimicrobial resistance-associated markers. Identified markers were categorized according to their resistance mechanisms and associated antimicrobial classes.

Results: A total of 347 antimicrobial resistance markers with a cumulative abundance of 16,124.9 RPKM were identified. The resistome was dominated by β-lactamase-associated markers, particularly blaOXA, blaCTX-M, blaSHV, and blaTEM, together with multidrug efflux-associated markers, including acrA, oqxA, mdfA, and tolC. The polymyxin resistance-associated marker arnT was also detected. Overall, β-lactam antibiotic inactivation and multidrug efflux represented the predominant resistance-associated mechanisms identified in the investigated isolate.

Conclusion: Integration of de novo genome assembly, reference-based read mapping, and ShortBRED- and CARD-based resistome profiling provided a comprehensive genomic characterization of antimicrobial resistance-associated markers in the investigated *K. pneumoniae* isolate. This approach may serve as a complementary tool for genomic AMR surveillance and support further phenotypic and genomic investigation of clinically relevant resistance determinants.

Keywords: Antimicrobial resistance; CARD; *Klebsiella pneumoniae*; ShortBRED; Whole-genome sequencing

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1- Introduction

Antimicrobial resistance (AMR) has emerged as one of the greatest threats to global public health, compromising the effectiveness of antimicrobial therapy and increasing the

burden of infectious diseases worldwide [1,2]. The continuous emergence and dissemination of multidrug-resistant (MDR) bacteria have resulted in prolonged hospitalization, increased healthcare costs, treatment failure, and higher mortality rates. Global estimates have demonstrated a substantial burden of bacterial AMR-associated mortality, highlighting the importance of genomic surveillance and evidence-based antimicrobial stewardship in limiting the emergence and spread of resistant pathogens [1]. Accordingly, the World Health Organization (WHO) has identified antimicrobial resistance as a major global health priority and emphasized the need for coordinated surveillance and control strategies to address resistant bacterial pathogens [2].

Among Gram-negative pathogens, *Klebsiella pneumoniae* is one of the most clinically important opportunistic bacteria and a prominent member of the ESKAPE group because of its substantial contribution to healthcare-associated infections and antimicrobial resistance [3–6]. The organism is associated with a broad spectrum of infections, including pneumonia, bloodstream infections, urinary tract infections, surgical site infections, and sepsis, particularly among hospitalized, critically ill, and immunocompromised patients [3–5]. Its remarkable capacity to acquire and disseminate antimicrobial resistance determinants through mobile genetic elements, including plasmids, transposons, integrons, and insertion sequences, has contributed to the global expansion of multidrug-resistant and carbapenem-resistant lineages [5,6].

The molecular basis of antimicrobial resistance in *K. pneumoniae* is complex and involves several complementary mechanisms. β-Lactamase-mediated hydrolysis is a major mechanism of resistance to β-lactam antibiotics, with extended-spectrum β-lactamases (ESBLs) and carbapenemases substantially reducing the activity of clinically important β-lactam agents [7,8]. OXA-type carbapenemases, together with other β-lactamase families, have disseminated internationally among clinically important Gram-negative pathogens and represent a major concern for the treatment of severe infections [8]. In parallel, multidrug efflux systems contribute to resistance by reducing intracellular antimicrobial concentrations. Major efflux systems in Gram-negative bacteria can affect susceptibility to multiple antimicrobial classes and may enhance bacterial persistence under antimicrobial pressure [9].

Resistance to polymyxins, particularly colistin, represents an additional clinical concern because these agents have historically been used as therapeutic options for infections caused by extensively drug-resistant and carbapenem-resistant Gram-negative bacteria [10]. Polymyxin resistance can arise through chromosomal alterations affecting lipid A modification pathways or through acquisition of plasmid-mediated *mcr* genes, which can reduce the binding of polymyxins to the bacterial outer membrane [10,11]. The emergence of plasmid-mediated colistin resistance has further increased concern regarding the dissemination of polymyxin resistance among clinically important Enterobacterales [11].

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Recent advances in next-generation sequencing (NGS), particularly whole-genome sequencing (WGS), have substantially expanded the capacity to characterize bacterial pathogens at the genomic level. WGS can provide information on antimicrobial resistance determinants, virulence-associated genes, mobile genetic elements, and genomic relationships within a single analytical framework, thereby supporting molecular epidemiology, outbreak investigation, genomic surveillance, and infection-control strategies [12]. In addition, genome-wide approaches can complement conventional antimicrobial susceptibility testing and contribute to the molecular surveillance of antimicrobial resistance [12].

Despite these advances, characterization of resistance determinants from complex sequencing datasets requires analytical approaches capable of accurately identifying and quantifying resistance-associated markers. In particular, marker-based resistome profiling can provide a quantitative overview of resistance determinants across different molecular mechanisms and antimicrobial classes. Such approaches are valuable for integrating genomic information with functional resistance profiles and for identifying dominant resistance mechanisms within bacterial populations.

In the present study, whole-genome sequencing data from a single *Klebsiella pneumoniae* isolate were analyzed to characterize its antimicrobial resistome using a marker-based computational workflow. The study aimed to identify antimicrobial resistance-associated markers, quantify their relative abundance, classify the detected determinants according to their resistance mechanisms and associated antimicrobial classes, and examine relationships between resistance determinants and antimicrobial categories. This approach was used to provide a genomic overview of the antimicrobial resistance determinants identified in the investigated *K. pneumoniae* isolate and to evaluate the utility of genome-based resistome analysis for antimicrobial resistance surveillance.

2. Materials and Methods

2.1. Sample Collection and Whole-Genome Sequencing

A total of approximately 240 clinical specimens were examined for bacterial isolation, of which approximately 47 yielded positive cultures. Among the recovered bacterial isolates, a single *Klebsiella pneumoniae* isolate with a comparatively high antimicrobial resistance profile was selected for whole-genome sequencing (WGS). The antimicrobial susceptibility profile of the recovered isolates was determined and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial culture and isolation were performed according to the procedures described in the *Manual of Clinical Microbiology* of the American Society for Microbiology (ASM) and the *UK Standards for Microbiology Investigations (UK SMI)*. The selected isolate was obtained from a pure

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bacterial culture. Genomic DNA was extracted using a commercial bacterial genomic DNA extraction kit according to the manufacturer's instructions. DNA concentration and purity were assessed prior to library preparation. Paired-end whole-genome sequencing was performed using the Illumina NovaSeq 6000 platform. Raw sequencing reads were subjected to initial quality assessment using FastQC (v0.12.1) [13]. Adapter sequences and low-quality bases were subsequently removed using Cutadapt (v5.1) [14]. The resulting quality-filtered reads were used for downstream genomic and resistome analyses.

2.2. De Novo Genome Assembly and Quality Assessment

Quality-filtered paired-end reads were assembled de novo using SPAdes (v4.1.0) [15] to reconstruct the draft genome directly from the sequencing reads without relying on a reference genome. This approach was used to independently assess the genomic content and assembly characteristics of the selected *Klebsiella pneumoniae* isolate. The quality of the resulting draft genome assembly was assessed using QUAST (v5.3), including evaluation of assembly size, contiguity, number of contigs, and N50 statistics [16]. Reference-based mapping was subsequently performed as a complementary analysis.

2.3. Genome Annotation

The assembled draft genome was subjected to structural and functional annotation using Prokka (v1.14.6) [17] and Bakta (v1.11) [18]. Protein-coding sequences and other genomic features, including transfer RNA (tRNA) and ribosomal RNA (rRNA) genes, were identified during the annotation process. The resulting annotated genome was subsequently used as the genomic basis for downstream antimicrobial resistance analysis.

2.4. Marker-Based Resistome Profiling

Antimicrobial resistance profiling was performed using the ShortBRED marker-based approach [19]. ShortBRED was used to identify and quantify resistance-associated protein families from the sequencing-derived genomic data using specific antimicrobial resistance markers. ShortBRED-derived marker abundance was expressed as reads per kilobase of marker sequence per million mapped reads (RPKM). A total of 347 antimicrobial resistance markers were identified, with a cumulative abundance of 16,124.9 RPKM. The detected markers were subsequently grouped according to their associated antimicrobial resistance mechanisms and antimicrobial classes.

2.5. CARD-Based Functional Annotation of Resistance Determinants

The identified resistance-associated markers were functionally interpreted using the Comprehensive Antibiotic Resistance Database (CARD) [20]. CARD annotations were used to classify resistance determinants according to their molecular mechanisms, including antibiotic inactivation, multidrug efflux, target alteration, target protection, target

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replacement, reduced permeability, and lipid A modification. The resistance determinants were further categorized according to their associated antimicrobial classes, including β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, macrolides, polymyxins, sulfonamides, phenicols, and multidrug resistance.

2.6. Reference-Based Mapping and Species-Specific Sequence Extraction

Following de novo genome assembly, reference-based mapping was performed using CLC Genomics Workbench v20. The assembled genomic sequences were evaluated against the *Klebsiella pneumoniae* reference genome GCF_000240185.1 using the Map Reads to Reference workflow. Genomic sequences showing sufficient similarity and alignment to the reference genome were retained as *K. pneumoniae*-specific sequences and used for subsequent resistome analysis.

2.7. Assembly-Derived Resistome Profiling

The based read mapping obtained following reference-based mapping were subsequently subjected to antimicrobial resistance profiling using ShortBRED. ShortBRED was used to identify and quantify antimicrobial resistance-associated protein families based on specific marker sequences. The abundance of identified resistance markers was expressed as RPKM. A total of 347 antimicrobial resistance markers were identified, with a cumulative abundance of 16,124.9 RPKM. The identified markers were subsequently classified according to their associated molecular resistance mechanisms and antimicrobial classes. Functional interpretation and classification of resistance determinants were performed using the Comprehensive Antibiotic Resistance Database (CARD).

2.8. ARG–Antimicrobial Class Network Analysis

A bipartite network was constructed to investigate the functional relationships between antimicrobial resistance determinants and their associated antimicrobial classes. Resistance markers and antimicrobial classes were represented as two distinct node types, while edges represented documented or CARD-associated relationships between individual resistance determinants and antimicrobial categories. Network visualization was used to identify highly connected resistance determinants and antimicrobial classes and to assess the degree of functional overlap among resistance mechanisms.

2.9. Abundance and Statistical Analysis

Resistance-marker abundance was expressed as RPKM. The cumulative abundance of the detected resistance markers was calculated, and resistance determinants were ranked according to their relative abundance. Descriptive analyses were used to summarize the distribution of resistance markers across molecular mechanisms and antimicrobial classes.

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Figures and network visualizations were generated using appropriate computational and graphical analysis tools.

3. Results.

3.1 Genome assembly and annotation

Whole-genome sequencing was performed on a single *Klebsiella pneumoniae* isolate recovered from a clinical lower respiratory tract specimen. Following quality control, the sequencing reads were mapped to the *K. pneumoniae* reference genome using CLC Genomics Workbench v20. The resulting mapping data were subsequently used for resistome characterization using the ShortBRED-based workflow in conjunction with the Comprehensive Antibiotic Resistance Database (CARD). Because the present study included a single isolate, abundance values are reported for the investigated isolate and are not presented as averages across multiple isolates.

3.2 Overview of the ARG dataset

Comprehensive resistome profiling of the investigated *Klebsiella pneumoniae* isolate identified 347 antimicrobial resistance markers, with a cumulative abundance of 16,124.9 RPKM. The identified resistance markers represented multiple antimicrobial resistance mechanisms and antibiotic classes, demonstrating the complex genomic resistance profile of the investigated isolate. The most abundant resistance determinants were predominantly associated with β-lactamases, including blaOXA, blaCTX-M, blaSHV, and blaTEM, together with multidrug efflux-associated determinants such as acrA, oqxA, mdfA, and tolC. Table 1 summarizes the overall resistome composition,

Table 1. Summary statistics of the Klebsiella pneumoniae genomic and ARG dataset.

Parameter	Value
Total ARG markers	347
Total raw hits	22,920
Total normalized abundance (RPKM)	16,124.9
Median RPKM per marker	12.4
Top ARG family	OXA β-lactamases

whereas Table 2 presents the ten most abundant resistance markers identified in the investigated isolate.

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Table 2. Top 10 ARG markers ranked by normalized abundance (RPKM).

Rank	ARG Marker	Classification	Resistance To	Abundance (RPKM)
1	OXA β-lactamase	β-lactamase	Penams, cephalosporins, carbapenems	2822.8
2	AcrA	Efflux pump	Multidrug	899.7
3	CTX-M β-lactamase	β-lactamase	Cephalosporins	782.1
4	OqxA	Efflux pump	Fluoroquinolones, MDR	778.0
5	ArnT	Lipid A transferase	Polymyxins	707.3
6	MdfA	Efflux pump	Multidrug	646.8
7	SHV β-lactamase	β-lactamase	Penams, cephalosporins	547.5
8	TEM β-lactamase	β-lactamase	Penams	432.1
9	TolC	Efflux pump	Multidrug	389.7
10	MexB	Efflux pump	Multidrug	365.5

3.2 Dominant resistance mechanisms

β-lactamases were the most abundant resistance mechanism category, with a total abundance of 4,584.5 RPKM. Within this group, OXA β-lactamases contributed 2,822.8 RPKM, followed by CTX-M (782.1 RPKM) and SHV (547.5 RPKM). These resistance determinants are associated with β-lactam resistance, although their specific phenotypic effects cannot be established from genomic data alone. (Figure 1)

Groups ARG abundance by resistance mechanism, allowing comparison of the relative contributions of β-lactamases, efflux pumps, and lipid A transferases (Table 3).

Table 3. ARG abundance grouped by resistance mechanism.

Resistance mechanism	Total abundance (RPKM)
β-lactamases	4584.5
Efflux pumps	3079.7
Lipid A transferase	707.3

Depicts the top 10 resistance mechanisms by normalized abundance, visually demonstrating the dominance of β-lactamases and efflux pumps (Figure 1).

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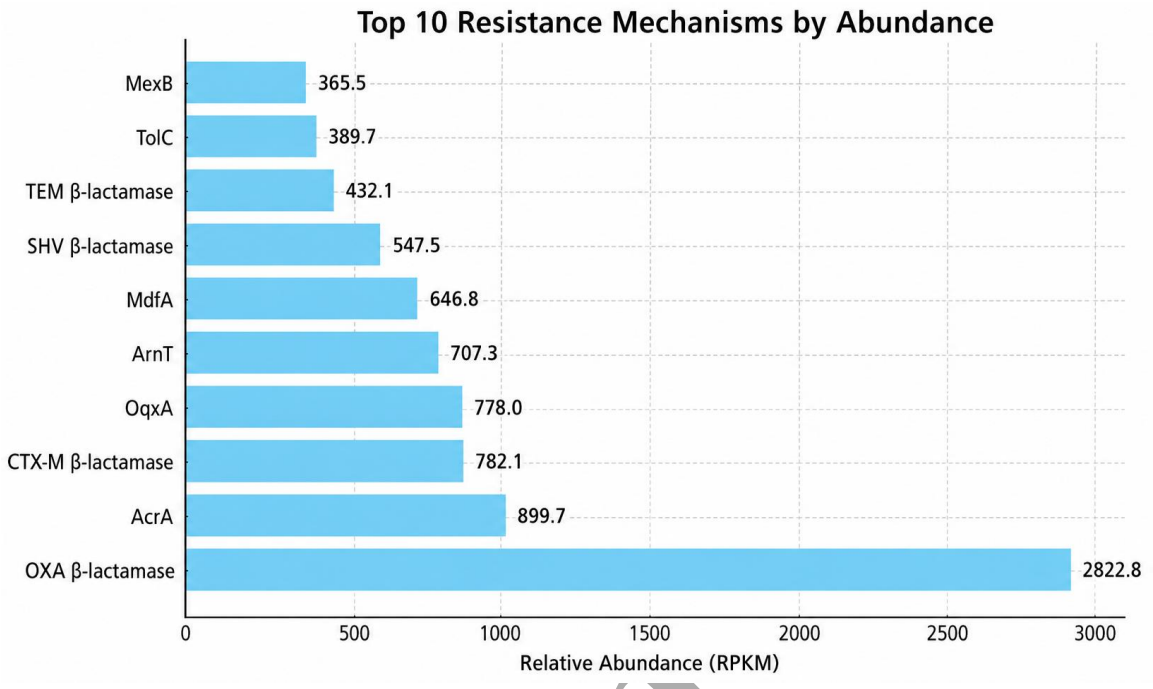


Figure 1. Top 10 resistance mechanisms by abundance. Bar chart showing normalized abundance (RPKM) of the ten most prevalent antibiotic resistance gene (ARG) mechanisms detected in Klebsiella pneumoniae isolate.

Efflux pumps were the second most abundant mechanism, with AcrA (899.7 RPKM), OqxA (778.0 RPKM), and MdfA (646.8 RPKM) among the top contributors. Efflux systems confer broad-spectrum resistance and promote persistence under antimicrobial pressure.

A particularly concerning finding was ArnT (707.3 RPKM), associated with polymyxin resistance. Given polymyxins' role as last-resort antibiotics, the presence of ArnT highlights potential therapeutic challenges.

3.3 Antibiotic class distribution

Grouping by antibiotic class revealed highest abundance for cephalosporins (5,560 RPKM), penams (4,383 RPKM), and carbapenems (3,953 RPKM). Resistance determinants against polymyxins (2,200 RPKM) and tetracyclines (907 RPKM) were also prevalent. This distribution underscores resistance to both clinically important and agriculturally used antibiotics. Figure 2 shows the distribution of antibiotic classes associated with resistance markers, linking ARG profiles to drug categories.

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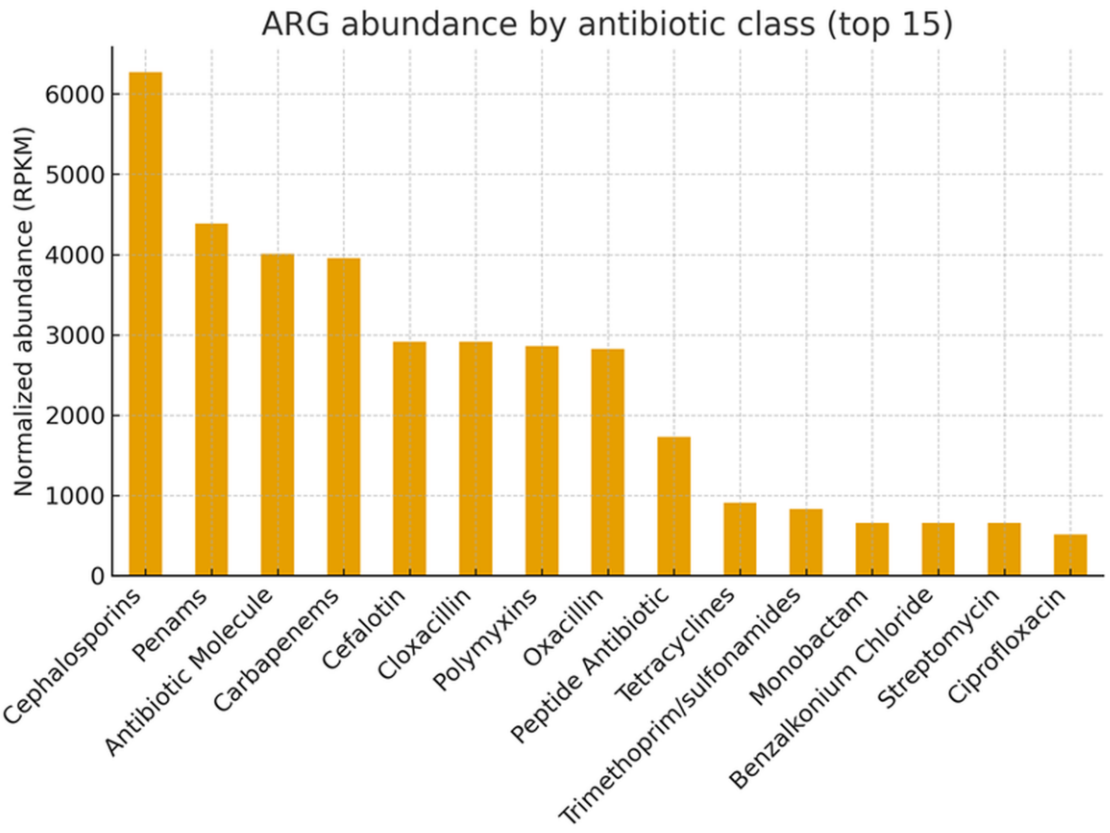


Figure 2. Distribution of antibiotic classes associated with resistance markers. Bar chart showing the number of resistance markers linked to each antibiotic class.

Groups ARG abundance by antibiotic class, illustrating resistance burden across clinically and agriculturally relevant drugs (Table 4).

Table 4. ARG abundance grouped by antibiotic class.

Antibiotic class	**Proportion (%)**
β-lactams	40
Fluoroquinolones	15
Polymyxins	10
Macrolides	8
Aminoglycosides	7
Tetracyclines	5
Others	15

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3.4 Network of ARG–drug associations

The bipartite ARG–antimicrobial class network revealed clustering of β-lactamase-associated determinants linked to multiple β-lactam subclasses, including penams, cephalosporins, carbapenems, cephamycins, and monobactams. Efflux-associated determinants were connected to multiple antimicrobial classes, including fluoroquinolones, tetracyclines, and macrolides. ArnT and related lipid A modification determinants were associated with the polymyxin resistance category. These patterns indicate that individual resistance determinants may be associated with multiple antimicrobial classes within the reference database. Figure 3 presents the ARG–antimicrobial class association network and illustrates the documented relationships between identified resistance determinants and their associated antimicrobial classes (Figure 3).

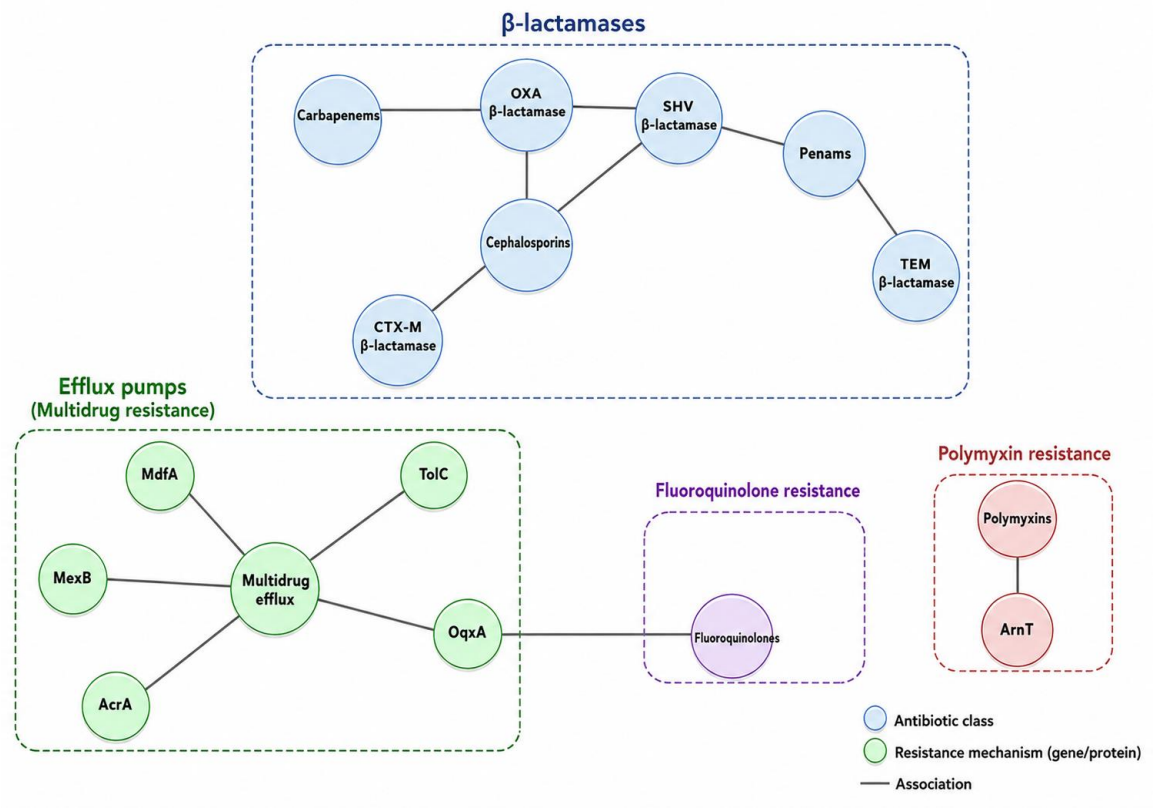


Figure 3. ARG–drug association network. Bipartite network visualization of ARG markers and their associated antibiotic classes, highlighting multidrug resistance connections.

3.5. Resistome composition and functional classification

The resistome profile demonstrated a broad distribution of antimicrobial resistance determinants across multiple molecular resistance mechanisms (Figure 4). Antibiotic inactivation represented the most prominent functional category, with β-lactamase-associated determinants, particularly OXA-, SHV-, TEM-, and CTX-M-type β-lactamases, forming major components of this group. Multidrug efflux systems constituted another substantial component of the resistome and included determinants such as AcrA, OqxA, MdfA, OmpA, and TolC, reflecting the potential contribution of active efflux to resistance against multiple antimicrobial classes. Additional resistance mechanisms included gene-altering cell-wall pathways, antibiotic target modification, and antibiotic target replacement, with determinants such as ArnT, RmrC, Sul1/Sul2, and other resistance-associated markers detected in the profile. The outer ring further illustrates the diversity of individual resistance markers contributing to each functional category, demonstrating that the *K. pneumoniae* resistome comprised multiple overlapping mechanisms rather than being dominated by a single resistance pathway. Overall, the functional distribution highlights the complex and multifactorial nature of antimicrobial resistance in the investigated isolate**.**

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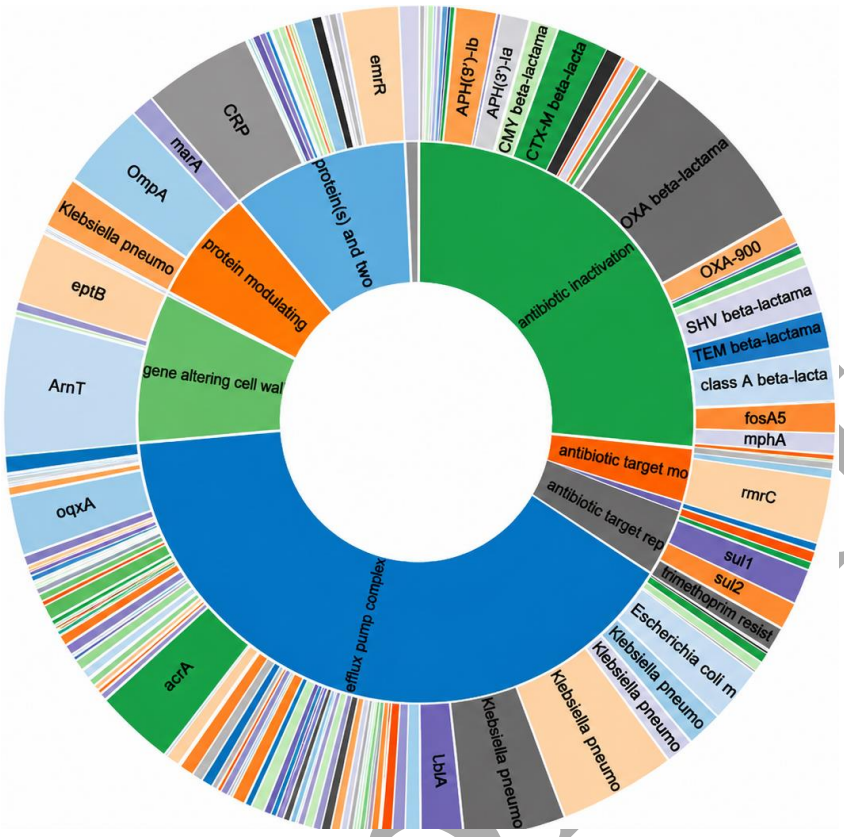


Figure 4. Distribution of antibiotic resistance gene markers by classification. Circular plot showing relative abundance of resistance mechanisms (e.g. inactivation enzymes and efflux pumps) across isolates.

4. Discussion.

In the present study, whole-genome sequencing combined with **reference-based read mapping and ShortBRED- and CARD-based antimicrobial resistance profiling** was used to characterize the resistome of a *Klebsiella pneumoniae* isolate recovered from a clinical specimen. The analysis identified **347 antimicrobial resistance markers with a cumulative abundance of 16,124.9 RPKM**, demonstrating the presence of multiple resistance-associated determinants involving several molecular mechanisms and antimicrobial classes. The predominance of β-lactamase-associated determinants, together with the substantial representation of multidrug efflux-associated markers and lipid A modification-related determinants, indicates a complex antimicrobial resistance profile in the investigated isolate.

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The predominance of β-lactam-associated resistance determinants is consistent with the well-established importance of β-lactam resistance in *K. pneumoniae*. β-Lactamases constitute major mechanisms of resistance to β-lactam antimicrobial agents among Enterobacterales, and the dissemination of clinically important β-lactamase families has contributed substantially to the global burden of resistant *K. pneumoniae* infections [7,8]. In the present resistome, **blaOXA, blaCTX-M, blaSHV, and blaTEM** represented prominent resistance-associated markers. Their high abundance is consistent with the established role of β-lactamase-mediated antibiotic inactivation in the resistance architecture of *K. pneumoniae*. However, because the present study was based on genomic profiling, the detection and abundance of these markers should be interpreted as **genotypic resistance potential rather than direct evidence of phenotypic antimicrobial resistance**.

The high representation of multidrug efflux-associated determinants provides an additional component of the broad resistance potential observed in the isolate. Efflux systems can reduce intracellular concentrations of antimicrobial compounds and contribute to reduced susceptibility across multiple antimicrobial classes [9]. In the present analysis, **acrA, oqxA, and mdfA** were among the abundant resistance-associated markers. Their presence highlights the potential contribution of active transport mechanisms to the overall resistome architecture. Nevertheless, the abundance of an efflux-associated marker alone does not establish that the corresponding system is transcriptionally active or phenotypically responsible for resistance. Functional expression studies and antimicrobial susceptibility testing would therefore be required to determine the clinical contribution of these determinants.

The detection of **arnT**, a determinant associated with lipid A modification, is also noteworthy. Modification of lipid A can decrease the affinity of polymyxins for the bacterial outer membrane and represents an important mechanism of reduced polymyxin susceptibility [10]. In addition, the emergence and dissemination of plasmid-mediated *mcr* genes have increased concerns regarding transferable colistin resistance among Gram-negative bacteria [11]. Although the present study identified an *arnT*-associated resistance marker, genomic detection alone should not be interpreted as confirmation of colistin resistance. Phenotypic susceptibility testing and investigation of the complete genetic context of polymyxin resistance determinants would be necessary to establish the clinical significance of this finding.

The integration of de novo assembly with subsequent reference-based mapping provided complementary information for characterization of the selected Klebsiella pneumoniae isolate. De novo assembly enabled reconstruction of the draft genome directly from the sequencing reads without dependence on a reference genome, whereas reference-based mapping against the K. pneumoniae reference genome was subsequently used to support species-specific genomic analysis. The combined approach therefore provided an

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independent assembly assessment together with reference-guided characterization of the investigated isolate.

The integration of reference-based genomic analysis with **ShortBRED and CARD-based resistance profiling** provided a structured framework for characterizing the resistance-associated markers identified in the investigated *K. pneumoniae* isolate. The resulting profile demonstrated that resistance-associated markers were not restricted to a single antimicrobial class but involved multiple mechanisms, including antibiotic inactivation, multidrug efflux, and lipid A modification. This pattern is consistent with the recognized genomic complexity of *K. pneumoniae* and its capacity to harbor diverse antimicrobial resistance determinants within its accessory genome [3–6]. Importantly, the present analysis characterized resistance-associated markers at the genomic level and did not experimentally establish their genomic location, mobility, expression, or phenotypic effect.

The ARG–antimicrobial class network further illustrated the functional relationships among the detected resistance determinants. The high connectivity of β-lactamase-associated markers with several β-lactam subclasses emphasizes the broad potential impact of these determinants, whereas the connectivity of efflux-associated markers across multiple antimicrobial categories highlights the potential for individual resistance mechanisms to contribute to multidrug resistance. Importantly, this network represents **known or database-supported associations between resistance determinants and antimicrobial classes**, rather than experimentally demonstrated interactions in the investigated isolate. Therefore, the network should be interpreted as a functional representation of the resistome rather than direct evidence of synergistic or simultaneous phenotypic resistance.

The quantitative nature of the resistome analysis also provides an important perspective. The identification of **347 resistance markers and a cumulative abundance of 16,124.9 RPKM** indicates substantial diversity and representation of resistance-associated markers within the analyzed genomic dataset. Nevertheless, RPKM values reflect **sequence-derived marker abundance** and should not be directly equated with gene expression, bacterial fitness, or clinical resistance levels. Quantitative estimates may also be influenced by marker characteristics, sequence similarity, mapping behavior, and database representation. Consequently, the observed abundance ranking is most appropriately interpreted as a **comparative genomic measure of resistance-marker representation** rather than a direct measure of resistance phenotype.

Several limitations should be considered when interpreting these findings. First, the study analyzed a **single *K. pneumoniae* isolate**; therefore, the results cannot be generalized to the broader *K. pneumoniae* population or used to estimate resistance prevalence. Second, the analysis was based primarily on genomic evidence and did not include phenotypic antimicrobial susceptibility testing. Consequently, the presence of resistance-associated

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markers indicates potential resistance mechanisms but does not establish their expression or clinical effect. Third, the use of short-read sequencing and reference-based mapping does not provide complete resolution of the genomic context of every resistance determinant, particularly with respect to plasmid structure, transposable elements, and complex genomic rearrangements. Finally, the identified resistance-associated markers were not experimentally validated at the expression or phenotypic level.

Future studies should therefore integrate WGS-based resistome profiling with **phenotypic antimicrobial susceptibility testing**, long-read sequencing, plasmid characterization, and, where appropriate, transcriptomic or functional analyses. Comparative analysis of multiple *K. pneumoniae* isolates from human, animal, and environmental sources would also help determine whether the resistance profile observed here represents an isolate-specific pattern or part of a broader epidemiological trend.

Despite these limitations, the present study demonstrates the utility of integrating WGS, de novo assembly, **CLC Genomics Workbench v20-based reference mapping**, and **ShortBRED- and CARD-based resistome characterization** for investigating antimicrobial resistance in *K. pneumoniae*. The predominance of β-lactamase-associated determinants, together with multidrug efflux and lipid A modification-associated markers, highlights the multifactorial nature of the resistance profile. The findings support the use of genomic resistome analysis as a **complementary tool** for antimicrobial resistance surveillance and provide a foundation for subsequent phenotypic and genomic investigations of clinically important resistance determinants.

5. Conclusions.

This study demonstrates that the K. pneumoniae resistome was characterized by a high abundance of β-lactamase-associated markers together with multidrug efflux determinants and lipid A modification-associated markers. WGS combined with de novo assembly, reference-based read mapping, and ShortBRED- and CARD-based resistome profiling provided a comprehensive genomic overview of antimicrobial resistance determinants across multiple antimicrobial classes. The findings highlight the multifactorial nature of the resistance profile and support the use of genomic resistome analysis as a complementary approach for antimicrobial resistance surveillance. Further studies incorporating phenotypic antimicrobial susceptibility testing and additional genomic analyses are required to determine the clinical and functional significance of the identified resistance determinants.

Author Contributions:

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RK: Supervision, Project administration, Validation, Writing – Review & Editing, Final approval, Accountability.
SAC: Resources, Data curation, Visualization, Writing – Review & Editing, Final approval, Accountability.
MhD: Final approval,
MV: Software, Methodology, Formal analysis, Visualization, Writing – Review & Editing, Final approval, Accountability.

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Data availability statement

The whole-genome shotgun sequencing project has been deposited in the DDBJ/ENA/GenBank databases under accession number **JBVMPV000000000**. The version described in this manuscript is the first version. Raw sequencing reads and the assembled genome are publicly available through the NCBI GenBank repository under the same accession number. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval

Clinical isolates were collected as part of routine diagnostic testing, and no additional procedures involving human participants were performed. Formal ethical approval was therefore not required.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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