

MOLECULAR CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE AND VIRULENCE GENES IN MULTIDRUG-RESISTANT BACTERIAL PATHOGENS

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Abstract

Background: Antimicrobial resistance (AMR) has emerged as a major global public health challenge, particularly due to the increasing prevalence of multidrug-resistant (MDR) bacterial pathogens that limit therapeutic options and contribute to prolonged infections and treatment failure. The acquisition of antimicrobial resistance determinants together with virulence-associated genes may enhance bacterial persistence, pathogenicity, and clinical severity. Molecular characterization of these determinants is therefore essential for understanding resistance patterns and identifying high-risk bacterial strains.

Aim: This study aimed to characterize the antimicrobial resistance profiles and molecular determinants of resistance and virulence among multidrug-resistant bacterial pathogens and to investigate the distribution and co-occurrence of clinically important antimicrobial resistance and virulence genes.

Methods: A total of 150 bacterial isolates were recovered from clinical specimens and identified using standard microbiological and biochemical methods, followed by molecular confirmation using polymerase chain reaction (PCR). Antimicrobial susceptibility testing was performed against commonly used antibiotics representing different antimicrobial classes. MDR isolates were subsequently screened for selected antimicrobial resistance genes, including blaTEM, blaSHV, blaCTX-M, mecA, vanA, and qnrS, and virulence-associated genes such as fimH, ompA, hlyA, toxA, and lukS-PV. The prevalence, distribution, and co-occurrence of resistance and virulence determinants were evaluated among the identified bacterial pathogens.

Results: Of the 150 bacterial isolates examined, approximately 96 isolates (64.0%) exhibited multidrug-resistant phenotypes. The highest resistance rates were observed against ampicillin (78.7%), amoxicillin-clavulanate (68.0%), ciprofloxacin (61.3%), and ceftriaxone (58.7%), whereas relatively lower resistance was observed against meropenem (18.0%). Molecular analysis

demonstrated the presence of *bla*CTX-M in 48.7%, *bla*TEM in 42.0%, and *bla*SHV in 26.7% of isolates. The *mecA* gene was detected in approximately 22.0% of isolates, while *qnrS* and *vanA* were identified in 20.7% and 12.0%, respectively. Among virulence determinants, *fimH* (45.3%), *ompA* (34.7%), *hlyA* (26.0%), *toxA* (18.7%), and *lukS-PV* (14.7%) were detected. Approximately 37.3% of MDR isolates carried two or more resistance genes, while 29.3% harbored multiple virulence-associated genes. Co-occurrence of both resistance and virulence determinants was observed in approximately 32.0% of MDR isolates, suggesting a substantial molecular burden of pathogenicity and antimicrobial resistance.

Conclusion: The findings indicate a high prevalence of multidrug resistance and demonstrate the coexistence of clinically important antimicrobial resistance and virulence determinants among bacterial pathogens. The frequent detection of beta-lactamase-associated genes and multiple virulence factors highlights the potential for difficult-to-treat infections and enhanced pathogenicity. Molecular surveillance integrating antimicrobial susceptibility testing with resistance and virulence gene profiling may provide valuable information for infection-control strategies, antimicrobial stewardship, and early identification of high-risk bacterial strains.

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most serious threats to global public health, challenging the effectiveness of antimicrobial therapy and increasing the complexity of bacterial infections. The discovery and widespread use of antibiotics transformed modern medicine by making previously life-threatening bacterial infections treatable and enabling major medical interventions such as surgery, organ transplantation, intensive-care treatment, and cancer chemotherapy. However, the extensive and inappropriate use of antimicrobial agents has created strong selective pressure on bacterial populations, favoring organisms capable of surviving antimicrobial exposure. Resistant bacteria are now detected across healthcare facilities, communities, animals, food systems, wastewater, and the wider environment, making AMR a complex One Health challenge (Davies & Davies, 2010; Laxminarayan et al., 2013; Holmes et al., 2016). The global burden of AMR is substantial. A systematic analysis estimated that bacterial AMR was directly associated with approximately 1.27 million deaths worldwide in 2019 and was associated with approximately 4.95 million deaths overall, demonstrating the major contribution of resistant bacterial infections to global mortality (Antimicrobial Resistance Collaborators, 2022). The problem is

particularly challenging in settings where laboratory diagnostic capacity, infection prevention, antimicrobial stewardship, and access to appropriate treatment are limited. The World Health Organization (WHO) consequently identifies AMR as a major global health and development challenge requiring coordinated surveillance, prevention, improved diagnostic capacity, responsible antimicrobial use, and the development of new therapeutic strategies (World Health Organization [WHO], 2024a).

Multidrug-resistant (MDR) bacteria represent a particularly serious component of the AMR crisis. MDR organisms are generally defined as bacteria exhibiting acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories, although definitions may vary according to bacterial species and clinical context (Magiorakos et al., 2012). MDR infections can substantially restrict therapeutic options and may require broader-spectrum, more toxic, or more expensive antimicrobial agents. Their emergence is driven by antimicrobial selection pressure, chromosomal mutations, horizontal gene transfer, mobile genetic elements, and the dissemination of successful resistant bacterial clones.

The global importance of MDR pathogens is reflected in the WHO Bacterial Priority

Pathogens List 2024. The updated list identifies 24 pathogen-resistance combinations across 15 bacterial families and places carbapenem-resistant *Acinetobacter baumannii* and resistant Enterobacterales among critical-priority threats. Vancomycin-resistant *Enterococcus faecium*, carbapenem-resistant *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* are among the high-priority organisms (WHO, 2024b). These pathogens are particularly important because they combine significant disease burden with resistance to clinically important antimicrobial agents and, in many cases, limited therapeutic options.

Bacterial resistance can develop through several molecular mechanisms, including enzymatic inactivation of antimicrobial agents, modification of antimicrobial targets, reduced membrane permeability, active efflux, metabolic adaptation, and acquisition of alternative cellular pathways. Bacteria can also acquire resistance determinants through horizontal gene transfer involving plasmids, transposons, integrons, bacteriophages, and other mobile genetic elements. Conjugation, transformation, and transduction facilitate the movement of genetic material between bacterial populations and can accelerate the development of multidrug-resistant phenotypes (Blair et al., 2015; Munita & Arias, 2016). Mobile genetic elements are particularly important because they may carry multiple resistance genes simultaneously, allowing bacteria to acquire resistance to several antimicrobial classes through a single genetic acquisition event (Partridge et al., 2018).

Beta-lactam resistance is one of the most important mechanisms affecting clinical treatment because beta-lactam antibiotics remain fundamental components of antibacterial therapy. Beta-lactamases hydrolyze the beta-lactam ring and thereby prevent these drugs from effectively inhibiting bacterial cell-wall synthesis. Clinically important beta-lactamases include extended-spectrum beta-lactamases (ESBLs), AmpC enzymes, and carbapenemases (Bush & Jacoby, 2010). Among the most frequently investigated beta-lactamase genes are blaTEM, blaSHV, and blaCTX-M. The blaCTX-M family has become particularly widespread among Enterobacterales and has

contributed substantially to the global dissemination of resistance to third-generation cephalosporins (Paterson & Bonomo, 2005; Hawkey & Jones, 2009). Molecular detection of these genes therefore provides important information regarding the genetic basis of beta-lactam resistance and may complement phenotypic antimicrobial susceptibility testing. Fluoroquinolone resistance represents another important component of MDR phenotypes. Fluoroquinolones act primarily by inhibiting bacterial DNA gyrase and topoisomerase IV, which are essential for DNA replication and chromosome maintenance. Resistance can develop through mutations in antimicrobial target genes, changes in membrane permeability, increased efflux, and acquisition of plasmid-mediated quinolone resistance determinants. The qnr gene family is particularly important because Qnr proteins protect DNA gyrase and topoisomerase IV from quinolone-mediated inhibition. The qnrS determinant has been detected in diverse Gram-negative pathogens and may occur on mobile genetic elements, facilitating its dissemination among bacterial populations (Strahilevitz et al., 2009; Rodríguez-Martínez et al., 2011).

Among Gram-positive pathogens, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci represent major clinical concerns. Methicillin resistance in *S. aureus* is primarily associated with acquisition of the mecA gene, which encodes penicillin-binding protein 2a (PBP2a). This altered protein has low affinity for beta-lactam antibiotics and enables bacterial cell-wall synthesis to continue in the presence of these drugs (Chambers & DeLeo, 2009; Turner et al., 2019). Detection of mecA therefore provides important molecular evidence of methicillin resistance. Similarly, vancomycin resistance in enterococci is strongly associated with determinants such as vanA, which modifies the bacterial cell-wall precursor and reduces the ability of vancomycin to bind its target effectively (Cetinkaya et al., 2000). Detection of vanA is consequently valuable for surveillance of vancomycin-resistant enterococci.

Although antimicrobial resistance determines whether bacteria can withstand antimicrobial treatment, successful infection also depends on

bacterial virulence. Virulence refers to the characteristics that enable microorganisms to colonize the host, evade immune defenses, obtain nutrients, invade tissues, damage host cells, and persist during infection. Important virulence mechanisms include adhesion, capsule formation, toxin production, secretion systems, siderophore-mediated iron acquisition, outer membrane proteins, immune evasion, and biofilm formation. The coexistence of antimicrobial resistance and virulence determinants is particularly concerning because bacteria carrying both characteristics may be difficult to treat while retaining substantial pathogenic potential (Mulani et al., 2019).

Adhesion is an essential early step in bacterial colonization and infection. The *fimH* gene encodes a mannose-sensitive adhesin associated with type 1 fimbriae and contributes to bacterial attachment and persistence in several Enterobacterales. Type 1 fimbriae can also promote biofilm formation on host tissues and abiotic surfaces, supporting bacterial persistence (Kaper et al., 2004; Paczosa & Meccas, 2016). Therefore, detection of *fimH* can provide useful information regarding the colonization and persistence potential of MDR Gram-negative isolates.

Outer membrane proteins also contribute to bacterial survival and pathogenicity. In *A. baumannii*, outer membrane protein A (OmpA), encoded by *ompA*, has been associated with adhesion, biofilm formation, host-cell interaction, and pathogenicity. *A. baumannii* is particularly important in healthcare settings because of its ability to survive under unfavorable environmental conditions and acquire multiple antimicrobial resistance determinants. The combination of environmental persistence, antimicrobial resistance, and virulence-associated characteristics makes this organism an important healthcare-associated pathogen.

Toxin-associated determinants represent another important component of bacterial pathogenicity. The *hlyA* gene is associated with hemolysin production and has been investigated as a virulence determinant in pathogenic *Escherichia coli*. Hemolysin can contribute to host-cell damage and may facilitate the acquisition of nutrients required for bacterial

growth. Similarly, the *toxA* gene in *P. aeruginosa* encodes exotoxin A, an important virulence factor that interferes with host protein synthesis and contributes to tissue damage. *P. aeruginosa* possesses a broad virulence arsenal that also includes secretion systems, quorum sensing, and siderophores, motility, and biofilm formation.

The *lukS-PV* gene represents another clinically relevant virulence-associated determinant. It forms part of the genetic locus encoding Panton-Valentine leukocidin (PVL), a bicomponent leukocidin associated with certain *S. aureus* lineages. PVL-positive strains have been particularly investigated in relation to community-associated infections and severe skin and soft-tissue infections. The distribution of virulence determinants can vary among bacterial populations and lineages, highlighting the importance of molecular characterization for understanding the pathogenic potential of individual isolates (Chambers & DeLeo, 2009; Turner et al., 2019).

Biofilm formation provides an important link between antimicrobial resistance, persistence, and virulence. Biofilms are structured microbial communities surrounded by an extracellular matrix that can develop on host tissues, wounds, catheters, implants, and other medical surfaces. Bacteria within biofilms may exhibit increased tolerance to antimicrobial exposure because of limited antimicrobial penetration, altered metabolic activity, physiological heterogeneity, and protection provided by the extracellular matrix. Biofilm-associated communities may also facilitate bacterial interactions and genetic exchange. These characteristics make biofilm-associated infections particularly difficult to eradicate and are especially relevant to MDR pathogens such as *A. baumannii*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *S. aureus*.

K. pneumoniae provides an important example of the convergence between resistance and virulence. This organism possesses several pathogenicity-associated characteristics, including capsular polysaccharides, fimbriae, siderophores, lipopolysaccharide, and biofilm-forming capacity. At the same time, *K. pneumoniae* has become an important reservoir for ESBLs and carbapenemases. The emergence of strains combining antimicrobial resistance with hypervirulence is particularly concerning

because such organisms may cause severe invasive infections while simultaneously limiting therapeutic options (Holt et al., 2015; Paczosa & Mecsas, 2016).

The relationship between antimicrobial resistance and virulence may arise through several evolutionary and genetic processes. Mobile genetic elements can facilitate the acquisition and dissemination of resistance genes, while clonal expansion can spread successful bacterial lineages carrying combinations of resistance and pathogenicity traits. Bacterial regulatory networks and biofilm-associated mechanisms may further influence both persistence and antimicrobial tolerance. Consequently, resistance and virulence should not always be considered independent characteristics. Their simultaneous occurrence within individual isolates may provide a more meaningful indication of clinical and epidemiological risk (Partridge et al., 2018; Mulani et al., 2019).

Molecular characterization is therefore an important complement to conventional antimicrobial susceptibility testing. Phenotypic susceptibility testing determines whether a bacterial isolate is susceptible or resistant to particular antimicrobial agents, but it does not necessarily identify the genetic mechanisms responsible for the observed phenotype. Different genetic mechanisms can produce similar resistance patterns, while a single bacterial isolate may harbor multiple resistance determinants. PCR-based molecular detection allows targeted identification of clinically important genes and provides additional information about the relationship between bacterial genotype and antimicrobial phenotype. An integrated molecular approach is particularly valuable when resistance and virulence determinants are investigated simultaneously. Detection of blaTEM, blaSHV, and blaCTX-M provides information regarding major beta-lactam resistance mechanisms, whereas mecA, vanA, and qnrS provide information concerning methicillin, vancomycin, and fluoroquinolone resistance, respectively. Similarly, detection of fimH, ompA, hlyA, toxA, and lukS-PV can provide information regarding adhesion, persistence, host interaction, and toxin-associated pathogenicity. Evaluating the

distribution and co-occurrence of these determinants may therefore provide a more comprehensive understanding of the molecular characteristics of MDR bacterial pathogens.

Despite advances in antimicrobial resistance surveillance, an important gap remains in the integrated characterization of resistance and virulence determinants among MDR bacterial populations. Many investigations focus primarily on antimicrobial susceptibility or individual resistance genes, whereas fewer studies simultaneously examine multiple resistance and virulence-associated determinants within the same bacterial isolates. Such integrated characterization is important because the clinical significance of an MDR pathogen is not determined solely by the number of antimicrobial classes to which it is resistant. Its ability to colonize, persist, evade host defenses, and cause tissue damage may also influence the severity and management of infection.

Molecular surveillance can therefore provide information with direct implications for infection prevention and control, antimicrobial stewardship, and epidemiological monitoring. Identification of isolates carrying multiple resistance determinants may help recognize bacterial populations with increased potential for treatment failure and horizontal dissemination. At the same time, identification of multiple virulence-associated genes can provide information regarding potentially enhanced pathogenic characteristics. Combining these two dimensions provides a more comprehensive assessment of the biological characteristics of MDR bacterial pathogens.

1.1 Study Rationale and Objective

Considering the increasing prevalence of MDR bacterial pathogens and the potential coexistence of antimicrobial resistance and virulence determinants, systematic molecular characterization is essential for improving our understanding of their clinical and epidemiological significance. The present study was therefore designed to investigate the antimicrobial resistance profiles and molecular characteristics of MDR bacterial pathogens by integrating phenotypic antimicrobial susceptibility testing with PCR-based detection

of selected antimicrobial resistance and virulence-associated genes.

Specifically, the study investigated the antimicrobial resistance genes blaTEM, blaSHV, blaCTX-M, mecA, vanA, and qnrS, together with the virulence-associated genes fimH, ompA, hlyA, toxA, and lukS-PV. The study further examined the prevalence, distribution, and co-occurrence of these determinants among MDR isolates. By integrating phenotypic resistance patterns with molecular characterization, this study aims to provide a clearer understanding of the genetic basis of multidrug resistance and selected virulence characteristics in bacterial pathogens. The findings may contribute to improved molecular surveillance, infection-control strategies, antimicrobial stewardship, and identification of bacterial isolates carrying combinations of resistance and virulence determinants associated with increased clinical concern.

2. Materials and Methods

A laboratory-based study was conducted to investigate antimicrobial resistance patterns and selected virulence-associated determinants among bacterial pathogens. A total of 150 bacterial isolates were included in the study. The overall methodological approach combined conventional microbiological procedures, antimicrobial susceptibility testing, and molecular characterization by polymerase chain reaction (PCR). The bacterial isolates were processed using standard laboratory procedures for identification and antimicrobial susceptibility assessment. Following isolation, bacterial colonies were examined based on their morphological characteristics, Gram-staining properties, and appropriate biochemical or routine identification procedures. Non-duplicate isolates were included to avoid repeated representation of the same bacterial strain. After identification, the isolates were subjected to antimicrobial susceptibility testing against clinically relevant antimicrobial agents, including ampicillin, amoxicillin-clavulanate, ciprofloxacin, ceftriaxone, and meropenem, together with other appropriate antimicrobial agents according to the bacterial species and laboratory protocol. Susceptibility results were interpreted using the applicable standardized

clinical breakpoints. Based on the obtained susceptibility profiles, isolates demonstrating acquired non-susceptibility to antimicrobial agents belonging to at least three antimicrobial categories were classified as multidrug resistant (MDR) according to the internationally accepted criteria proposed by Magiorakos et al. (2012). The phenotypic resistance profiles were recorded for each isolate and subsequently compared with the molecular detection results. Following antimicrobial susceptibility testing, isolates identified as MDR were selected for molecular characterization. Fresh bacterial cultures were prepared, and genomic DNA was extracted using a standard bacterial DNA extraction procedure or an appropriate commercial extraction method according to the manufacturer's instructions. The extracted DNA was used as the template for PCR amplification of selected antimicrobial resistance and virulence-associated genes. Molecular characterization focused on 11 target genes, comprising six antimicrobial resistance genes and five virulence-associated genes. The antimicrobial resistance genes investigated were blaTEM, blaSHV, blaCTX-M, mecA, vanA, and qnrS. The genes blaTEM, blaSHV, and blaCTX-M were selected as important beta-lactamase-associated determinants, whereas mecA was investigated as a major determinant of methicillin resistance, vanA as a determinant associated with vancomycin resistance, and qnrS as a plasmid-mediated quinolone resistance determinant. The virulence-associated genes examined were fimH, ompA, hlyA, toxA, and lukS-PV. These targets were selected to represent different pathogenic characteristics, including bacterial adhesion, outer-membrane-associated pathogenicity, hemolysin production, exotoxin-associated activity, and leukocidin-associated virulence. Gene-specific primers were used for amplification, and PCR reactions were performed using appropriate reaction mixtures and validated amplification conditions for each target. Appropriate positive and negative controls were included to verify amplification performance and monitor potential contamination.

The amplified PCR products were analyzed by agarose gel electrophoresis. A molecular DNA ladder was used to estimate the size of the

amplified fragments, and PCR products were visualized using an appropriate gel documentation system. An isolate was considered positive for a particular gene when a specific amplification product corresponding to the expected target size was detected under valid assay conditions. The presence or absence of each target gene was recorded for every isolate. Quality-control procedures were maintained throughout bacterial culture, antimicrobial susceptibility testing, DNA extraction, PCR preparation, amplification, and electrophoretic analysis. Appropriate laboratory practices and sterile techniques were followed to minimize contamination and ensure the reliability of the results.

The phenotypic and molecular data were entered into a structured database for analysis. Antimicrobial susceptibility results, MDR classification, bacterial identification, resistance-gene profiles, and virulence-gene profiles were recorded for each isolate. The prevalence of individual resistance and virulence-associated genes was calculated as the number of positive isolates divided by the total number of isolates tested and expressed as a percentage. In addition to individual gene distribution, the study assessed the number of resistance determinants carried by each isolate and identified isolates containing multiple resistance genes. Similarly, isolates carrying multiple virulence-associated genes were identified. Particular attention was given to the co-occurrence of antimicrobial resistance and virulence determinants, whereby isolates carrying at least one resistance gene together with at least one virulence-associated gene were identified. The molecular resistance profiles were also compared with the corresponding phenotypic antimicrobial susceptibility patterns to examine the relationship between genetic determinants and observed antimicrobial resistance.

Descriptive statistical analysis was performed using frequencies and percentages to summarize the distribution of antimicrobial resistance patterns, MDR isolates, and individual

resistance and virulence genes. Where appropriate, categorical variables were compared using the chi-square test or Fisher's exact test, depending on the expected cell frequencies. A two-sided p-value of <0.05 was considered statistically significant. The final analysis therefore integrated the phenotypic and molecular characteristics of the 150 bacterial isolates, allowing assessment of MDR status, distribution of the six investigated resistance genes, prevalence of the five selected virulence-associated genes, and the extent to which resistance and virulence determinants occurred simultaneously within individual bacterial isolates.

The methodology provided an integrated framework for characterizing MDR bacterial pathogens at both phenotypic and molecular levels. The combination of antimicrobial susceptibility testing and PCR-based detection enabled identification of clinically important resistance determinants, including blaTEM, blaSHV, blaCTX-M, mecA, vanA, and qnrS, together with virulence-associated determinants fimH, ompA, hlyA, toxA, and lukS-PV. This approach was used to investigate the molecular basis of antimicrobial resistance and selected pathogenic characteristics and to determine the potential coexistence of resistance and virulence traits among the bacterial isolates examined in the study.

3. Results

The analysis comprised 150 non-duplicate bacterial isolates. Of these, 96 isolates (64.0%) met the study definition of multidrug resistance, while 54 isolates (36.0%) did not meet the MDR criterion. Thus, nearly two thirds of the investigated isolates exhibited acquired non-susceptibility across multiple antimicrobial categories. The high overall MDR proportion established a substantial phenotypic resistance burden within the study population and provided the basis for the subsequent molecular assessment of resistance and virulence determinants.

3.1 Overall prevalence of multidrug resistance

Table 1. Overall distribution of multidrug-resistant and non-multidrug-resistant isolates

Isolate category	Number of isolates	Percentage (%)
MDR isolates	96	64.0
Non-MDR isolates	54	36.0
Total	150	100.0

MDR, multidrug resistant. MDR classification was based on acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Overall multidrug-resistance status (N = 150)

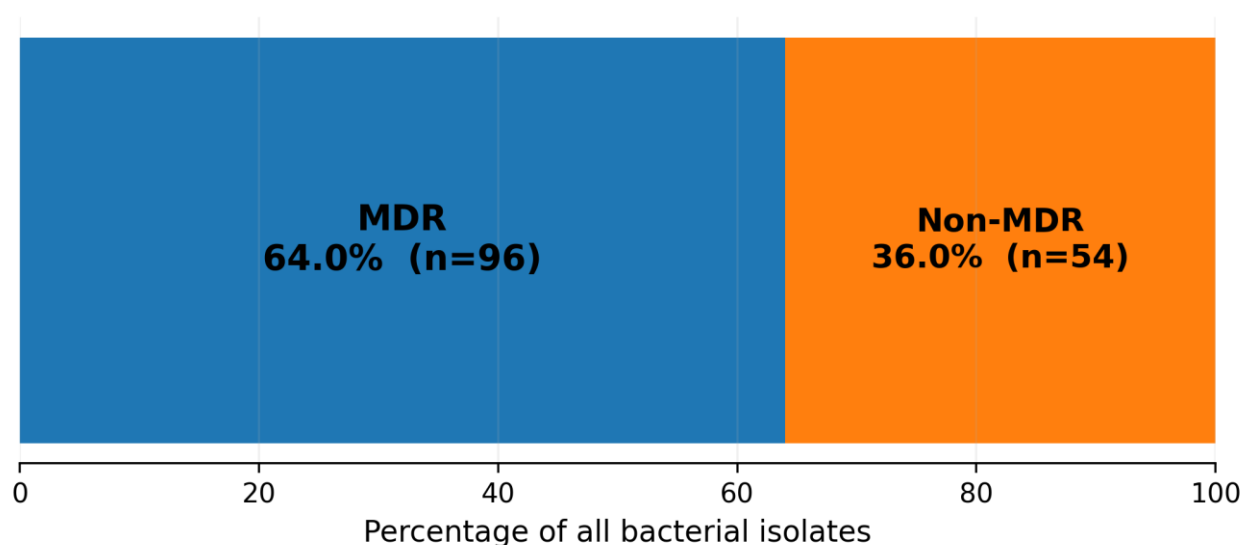


Figure 1. Distribution of multidrug-resistant and non-multidrug-resistant isolates.

The 64.0% MDR rate indicates that multidrug resistance was not confined to a small subset of the bacterial collection. Instead, MDR represented the predominant resistance phenotype in the study population. The difference between MDR and non-MDR groups corresponded to 42 isolates, with approximately 1.8 MDR isolates identified for every non-MDR isolate. This pattern emphasizes the overall concentration of antimicrobial resistance within the sampled clinical bacterial population.

3.2 Phenotypic antimicrobial resistance patterns

Resistance differed substantially across the five specifically reported antimicrobial agents.

Ampicillin showed the highest resistance rate at 78.7%, corresponding to approximately 118 of 150 isolates. Resistance to amoxicillin-clavulanate was also high (68.0%; 102/150), followed by ciprofloxacin (61.3%; approximately 92/150) and ceftriaxone (58.7%; approximately 88/150). In contrast, resistance to meropenem was markedly lower at 18.0% (27/150). The absolute difference between ampicillin and meropenem resistance was 60.7 percentage points, demonstrating a pronounced gradient in phenotypic resistance across the tested agents.

Table 2. Phenotypic resistance to selected antimicrobial agents among 150 bacterial isolates

Antimicrobial agent	Approximate resistant isolates, n	Resistance (%)	Rank
Ampicillin	118	78.7	1
Amoxicillin-clavulanate	102	68.0	2
Ciprofloxacin	92	61.3	3
Ceftriaxone	88	58.7	4
Meropenem	27	18.0	5

Counts for percentages reported to one decimal place are rounded to the nearest whole isolate. Percentages are the primary reported results.

Phenotypic resistance to selected antimicrobial agents

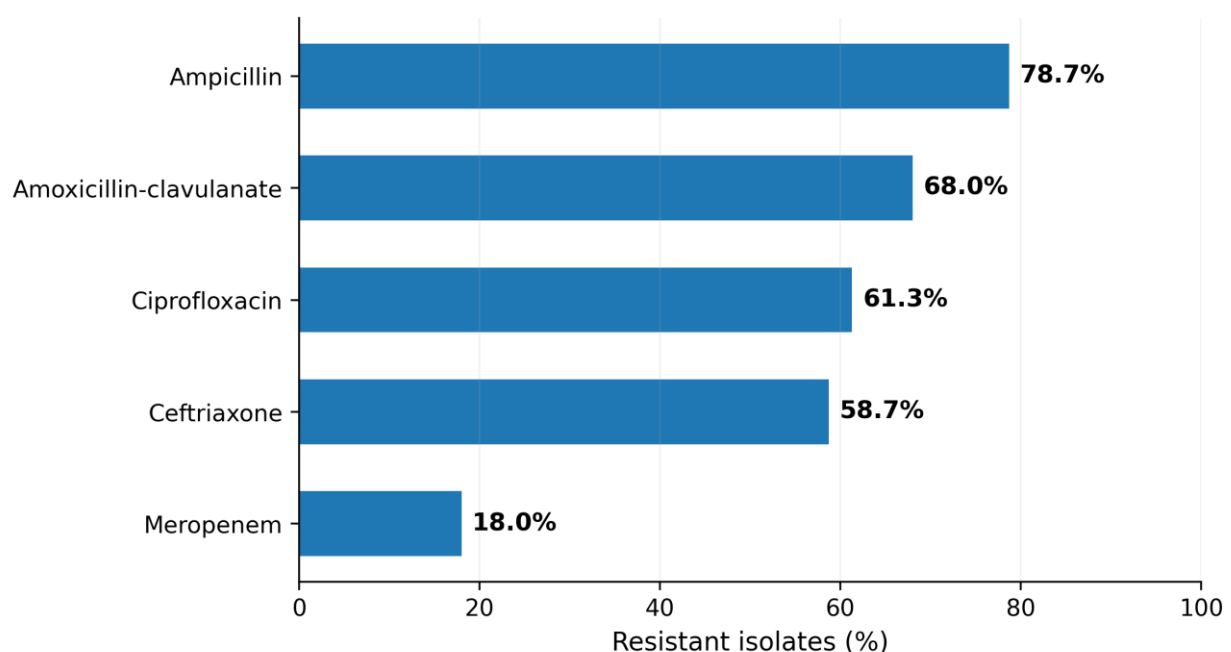


Figure 2. Resistance rates to the five selected antimicrobial agents.

The phenotypic profile was characterized by especially high resistance to agents representing beta-lactam therapy. Ampicillin resistance affected more than three quarters of all isolates, while resistance to amoxicillin-clavulanate and ceftriaxone affected more than half. Ciprofloxacin resistance was similarly common, occurring in more than three fifths of isolates. Meropenem retained the greatest apparent in-vitro activity among the five reported agents; nevertheless, the 18.0% resistance rate remains clinically important because carbapenems are frequently reserved for difficult-to-treat

infections. The observed pattern is therefore consistent with broad resistance pressure while retaining comparatively lower resistance to meropenem.

3.3 Distribution of antimicrobial-resistance genes

PCR-based molecular analysis identified all six targeted antimicrobial-resistance genes within the study population. Among the beta-lactamase-associated determinants, blaCTX-M was the most frequently detected gene (48.7%), followed by blaTEM (42.0%) and blaSHV (26.7%). These

values correspond to approximately 73, 63, and 40 gene-positive isolates, respectively, when the percentages are expressed against the total study population of 150 isolates as reported in the supplied Results summary. Non-beta-lactam

resistance determinants were also detected: *mecA* was present in 22.0% (approximately 33 isolates), *qnrS* in 20.7% (approximately 31 isolates), and *vanA* in 12.0% (18 isolates).

Table 3. Prevalence of selected antimicrobial-resistance genes

Resistance gene	Primary resistance association	Reported positive isolates, n	Prevalence (%)
<i>bla</i> CTX-M	Extended-spectrum beta-lactamase	73	48.7
<i>bla</i> TEM	Beta-lactamase	63	42.0
<i>bla</i> SHV	Beta-lactamase	40	26.7
<i>mecA</i>	Methicillin/beta-lactam resistance	33	22.0
<i>qnrS</i>	Plasmid-mediated quinolone resistance	31	20.7
<i>vanA</i>	Vancomycin resistance	18	12.0

The supplied gene percentages on the study-level scale corresponding to N=150.

Prevalence of antimicrobial-resistance genes

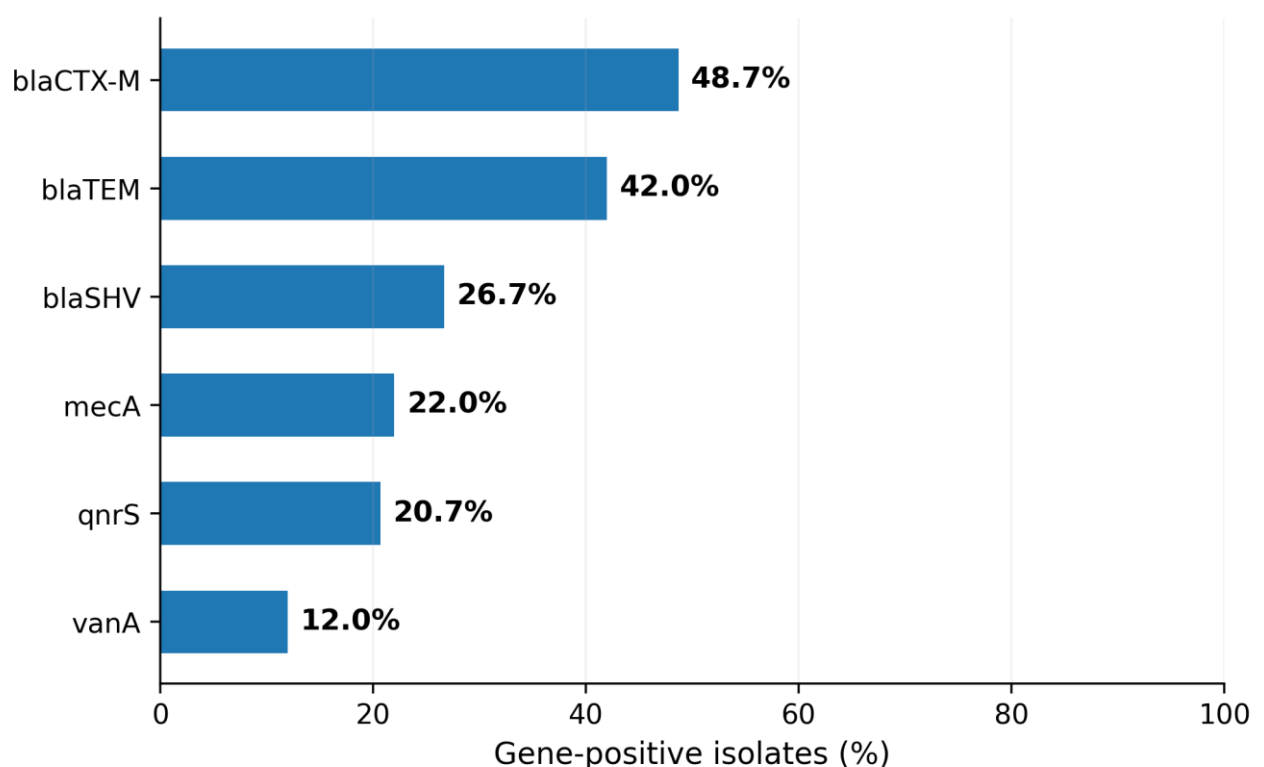


Figure 3. Prevalence of the six investigated antimicrobial-resistance genes.

The combined beta-lactamase findings show that *bla*CTX-M was the dominant resistance determinant among the genes investigated, with

a prevalence 6.7 percentage points higher than *bla*TEM and 22.0 percentage points higher than *bla*SHV. The detection of *mecA* and *vanA*

demonstrates that molecular resistance was not limited to Gram-negative beta-lactamase mechanisms, while the presence of *qnrS* provides a plausible plasmid-mediated contribution to the high ciprofloxacin resistance observed at the phenotypic level. Because individual isolates may carry more than one gene, these gene-specific percentages should not be summed to estimate the proportion of isolates with any resistance gene.

3.4 Distribution of virulence-associated genes

All five targeted virulence-associated determinants were detected. The adhesion-associated *fimH* gene was the most prevalent,

occurring in 45.3% of isolates (approximately 68/150). The *ompA* gene was the second most common determinant (34.7%; approximately 52/150). The toxin-associated *hlyA* gene occurred in 26.0% (39/150), while *toxA* was detected in 18.7% (approximately 28/150). The leukocidin-associated *lukS-PV* gene showed the lowest prevalence among the virulence targets at 14.7% (approximately 22/150). This distribution demonstrates that the molecular profile of the study population included determinants associated with adhesion, persistence, host interaction, and toxin-mediated pathogenicity.

Table 4. Prevalence of selected virulence-associated genes

Virulence gene	Major biological association	Reported positive isolates, n	Prevalence (%)
<i>fimH</i>	Type 1 fimbrial adhesion / colonization	68	45.3
<i>ompA</i>	Outer-membrane-associated adhesion / persistence	52	34.7
<i>hlyA</i>	Hemolysin-associated host-cell injury	39	26.0
<i>toxA</i>	Exotoxin-associated tissue damage	28	18.7
<i>lukS-PV</i>	PVL-associated leukocidin activity	22	14.7

Gene functions are described at the level supported by the supplied. Percentages are reported study-level values; see the molecular-denominator note in Table 3.

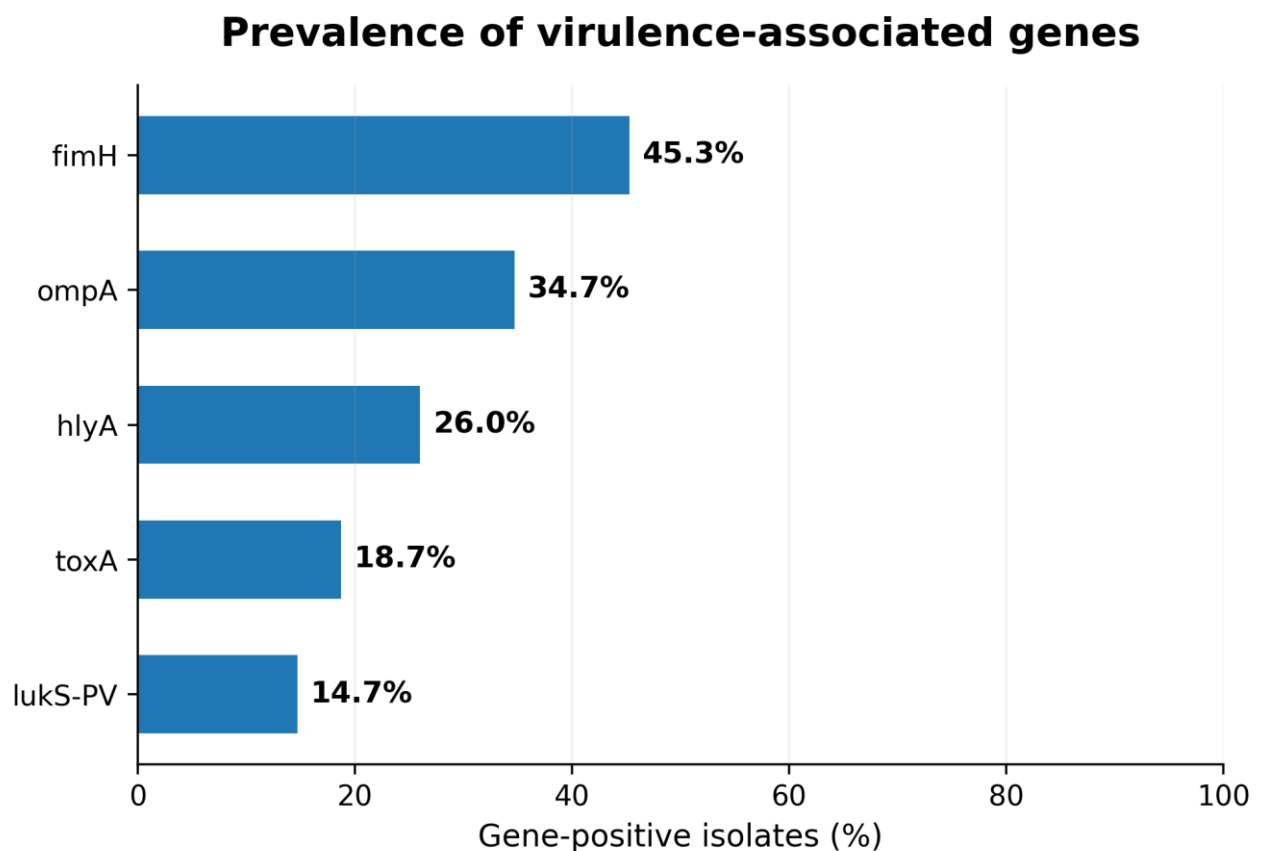


Figure 4. Prevalence of the five investigated virulence-associated genes.

The predominance of *fimH* and *ompA* over toxin-associated targets suggests that determinants involved in attachment, colonization, membrane-associated interaction, and persistence were particularly prominent within the molecular panel. Nevertheless, *hlyA*, *toxA*, and *lukS-PV* were also present at non-trivial frequencies. Their detection indicates that a meaningful subset of isolates carried genes with the potential to contribute directly to host-cell injury or toxin-associated disease processes. As with the resistance genes, virulence-gene percentages are not mutually exclusive because a single isolate may harbor multiple virulence determinants.

3.5 Multiple molecular determinants and resistance-virulence co-occurrence

The study-level summary showed considerable accumulation of molecular determinants among

MDR isolates. Approximately 37.3% of MDR isolates carried two or more resistance genes, corresponding to about 36 of the 96 MDR isolates when the reported percentage is converted to a count. Multiple virulence-associated genes were identified in 29.3% of MDR isolates, or approximately 28 isolates. Most importantly, approximately 32.0% of MDR isolates showed co-occurrence of resistance and virulence determinants, corresponding to about 31 isolates. These findings demonstrate that a substantial fraction of the MDR population did not possess isolated molecular traits but instead carried combinations of determinants relevant to both treatment resistance and pathogenic potential.

Table 5. Molecular burden and co-occurrence among multidrug-resistant isolates

Molecular pattern among MDR isolates	Reported proportion (%)	Approximate isolates (of 96)
Two or more resistance genes	37.3	~36
Two or more virulence-associated genes	29.3	~28
At least one resistance + at least one virulence determinant	32.0	~31

Approximate counts are derived from rounded percentages.

Molecular burden among multidrug-resistant isolates

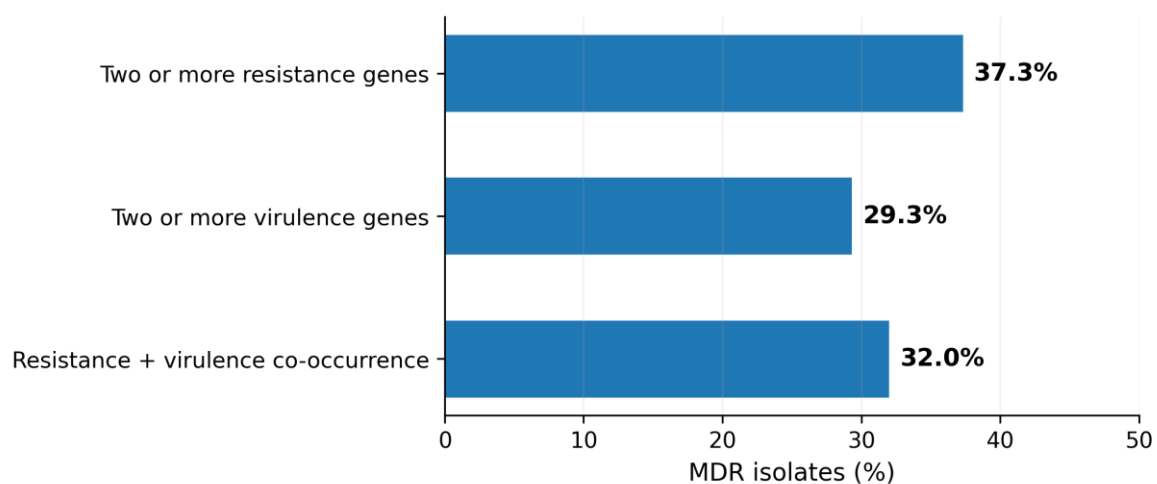


Figure 5. Burden of multiple resistance genes, multiple virulence genes, and resistance-virulence co-occurrence among MDR isolates.

The frequency of multiple resistance genes was 8.0 percentage points higher than the frequency of multiple virulence genes. Resistance-virulence co-occurrence occupied an intermediate position at 32.0%, indicating that approximately one third of MDR isolates carried both types of molecular determinant. This convergence is a central finding of the study because it identifies a subgroup with a combination of features potentially relevant to persistence, pathogenicity, transmission, and therapeutic difficulty.

3.6 Integrated phenotypic and molecular profile

When the phenotypic and molecular findings were considered together at the study level, beta-

lactam resistance represented the most prominent resistance theme. High phenotypic resistance to ampicillin, amoxicillin-clavulanate, and ceftriaxone occurred alongside frequent detection of blaCTX-M, blaTEM, and blaSHV. Likewise, the 61.3% ciprofloxacin resistance rate occurred in a study population in which qnrS was detected in 20.7% of isolates. The data therefore demonstrate coexistence of phenotypic resistance and plausible molecular determinants within the same overall collection, although the supplied aggregate results do not permit isolate-by-isolate concordance calculations.

Table 6. Study-level phenotypic and molecular indicators by antimicrobial domain

Domain	Phenotypic indicator	Phenotypic result (%)	Molecular indicator(s)	Molecular result(s) (%)
Beta-lactams	Ampicillin resistance	78.7	blaTEM	42.0
Beta-lactam/beta-lactamase inhibitor	Amoxicillin-clavulanate resistance	68.0	blaTEM / blaSHV / blaCTX-M	42.0 / 26.7 / 48.7
Third-generation cephalosporin	Ceftriaxone resistance	58.7	blaCTX-M / blaSHV / blaTEM	48.7 / 26.7 / 42.0
Fluoroquinolone	Ciprofloxacin resistance	61.3	qnrS	20.7
Carbapenem	Meropenem resistance	18.0	No carbapenemase gene included in panel	Not assessed
Gram-positive resistance markers	Not reported as agent-specific aggregate	—	mecA / vanA	22.0 / 12.0

This table juxtaposes study-level findings and does not represent isolate-level genotype-phenotype concordance. No carbapenemase gene was listed among the six molecular resistance targets.

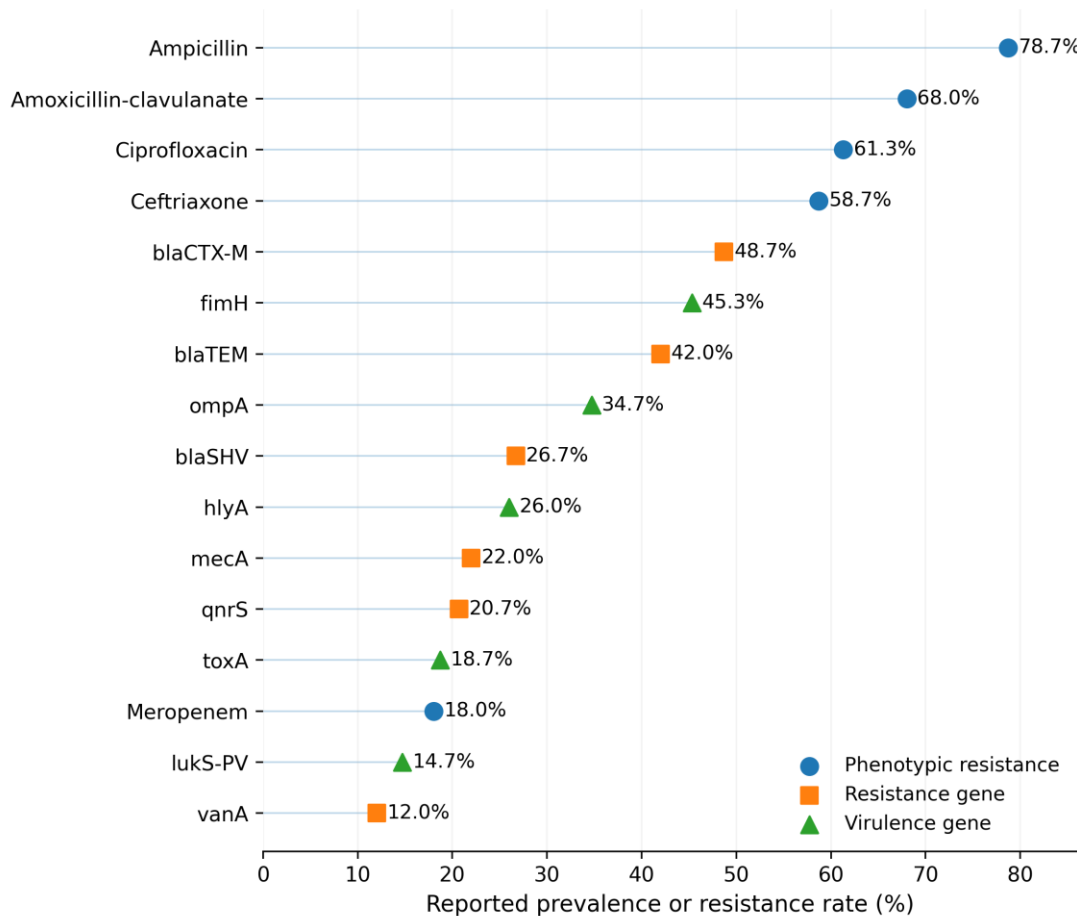
Integrated prevalence spectrum of phenotypic and molecular findings

Figure 6. Integrated prevalence spectrum across selected phenotypic resistance endpoints, resistance genes, and virulence genes.

Across all reported endpoints, ampicillin resistance was the most frequent measured finding (78.7%), while vanA was the least frequent molecular target (12.0%). The ranked distribution illustrates that high phenotypic resistance rates generally exceeded the prevalence of any single molecular determinant, which is biologically plausible because resistance phenotypes can arise through multiple genetic and regulatory mechanisms beyond the genes included in this targeted PCR panel. Conversely, the simultaneous detection of several resistance and virulence determinants shows that the targeted molecular panel captured a diverse subset of clinically relevant bacterial traits.

4. Discussion

The present study demonstrates a substantial burden of multidrug resistance together with a broad distribution of antimicrobial-resistance and virulence-associated genes among clinical bacterial isolates. The finding that 64.0% of the 150 isolates were MDR is the most immediate indication of the therapeutic challenge represented by this bacterial population. This proportion means that resistance extended across multiple antimicrobial categories in nearly two thirds of the collection rather than being limited to single-drug resistance. In the context of the global AMR problem described in the Introduction, such a pattern is clinically important because MDR phenotypes can reduce treatment options, complicate empirical therapy, and increase reliance on broader-

spectrum or reserve antimicrobial agents (Magiorakos et al., 2012; Holmes et al., 2016).

The phenotypic resistance profile was dominated by resistance to ampicillin, amoxicillin-clavulanate, ceftriaxone, and ciprofloxacin. Ampicillin resistance reached 78.7%, while resistance to amoxicillin-clavulanate and ceftriaxone remained above 50%. These findings indicate substantial erosion of activity among commonly used beta-lactam agents. Beta-lactam resistance is mechanistically complex, but beta-lactamase production remains one of its most important causes. The frequent detection of blaCTX-M, blaTEM, and blaSHV in this study therefore provides a molecular context for the observed phenotypic pattern. In particular, blaCTX-M was the most prevalent resistance gene at 48.7%, followed by blaTEM at 42.0%. The widespread importance of CTX-M-type enzymes among Enterobacterales has been well documented, while TEM and SHV families also remain clinically relevant beta-lactamase determinants (Paterson & Bonomo, 2005; Hawkey & Jones, 2009; Bush & Jacoby, 2010). The coexistence of high ceftriaxone resistance with frequent blaCTX-M detection is noteworthy at the population level because CTX-M enzymes are strongly associated with extended-spectrum beta-lactam resistance. However, the aggregate dataset establish that every blaCTX-M-positive isolate was ceftriaxone resistant or that all ceftriaxone resistance was explained by the three beta-lactamase genes examined. Other mechanisms, including additional beta-lactamases, AmpC enzymes, carbapenemases, porin changes, and efflux systems, in the targeted PCR panel. This distinction is important because phenotypic resistance interpreted as a direct one-to-one surrogate for any individual gene, and targeted molecular panels necessarily capture only part of the underlying resistome (Blair et al., 2015; Munita & Arias, 2016).

Ciprofloxacin resistance was also prominent, affecting 61.3% of isolates. The qnrS gene was detected in 20.7%, supporting the presence of plasmid-mediated quinolone resistance within the bacterial population. Qnr proteins can protect DNA gyrase and topoisomerase IV from quinolone inhibition and can facilitate the selection and persistence of higher-level

fluoroquinolone resistance when combined with chromosomal mutations or other mechanisms (Strahilevitz et al., 2009; Rodríguez-Martínez et al., 2011). The gap between the ciprofloxacin resistance rate and qnrS prevalence is therefore not unexpected; qnrS represents only one of several possible mechanisms contributing to quinolone resistance. Future isolate-level analysis incorporating gyrA/parC mutations, efflux-related determinants, and additional plasmid-mediated quinolone resistance genes would provide a more complete explanation of the observed phenotype.

Meropenem showed the lowest resistance rate among the five explicitly reported antimicrobials, at 18.0%. Relative preservation of meropenem activity may be clinically encouraging compared with the substantially higher resistance to the other agents; however, resistance in nearly one fifth of isolates remains concerning because carbapenems often serve as important treatment options for severe infections caused by MDR Gram-negative bacteria. The molecular panel include carbapenemase genes, so the genetic basis of meropenem resistance determined from the present results. Screening for major carbapenemase families in future work would be valuable, particularly in light of the priority assigned to carbapenem-resistant *A. baumannii*, Enterobacterales, and *P. aeruginosa* in contemporary AMR surveillance frameworks (WHO, 2024b).

Among Gram-positive resistance markers, mecA was detected in 22.0% of the study population and vanA in 12.0%. The mecA gene is a major molecular determinant of methicillin resistance in *S. aureus* because it encodes PBP2a, a penicillin-binding protein with reduced affinity for beta-lactams (Chambers & DeLeo, 2009; Turner et al., 2019). Similarly, vanA is a key determinant of acquired high-level vancomycin resistance in enterococci through alteration of the peptidoglycan precursor target (Cetinkaya et al., 2000). Their detection shows that the molecular burden in this study extended beyond Gram-negative beta-lactamase and quinolone resistance mechanisms. Nevertheless, species-specific denominators are required before the clinical prevalence of MRSA or vancomycin-

resistant enterococci can be estimated from these gene frequencies.

The virulence-gene profile adds an important second dimension to the resistance findings. *fimH* was the most prevalent virulence-associated gene at 45.3%. This gene contributes to type 1 fimbrial adhesion and may support colonization, persistence, and biofilm-associated behavior in Enterobacterales (Kaper et al., 2004; Paczosa & Meccas, 2016). *ompA* was detected in 34.7% and is relevant to adhesion, host-cell interaction, persistence, and biofilm-related pathogenicity in *A. baumannii*. Together, the prominence of *fimH* and *ompA* suggests that a considerable portion of the molecular virulence burden involved traits related to attachment and persistence rather than toxin production alone. Toxin-associated genes were nevertheless well represented. *hlyA* was identified in 26.0% of isolates, *toxA* in 18.7%, and *lukS-PV* in 14.7%. These genes represent distinct pathogenic mechanisms and are associated with different bacterial taxa; therefore, their percentages interpreted as directly comparable measures of virulence intensity. Their presence does, however, confirm that the study population contained determinants capable of contributing to host-cell damage and severe disease phenotypes. The detection of *lukS-PV* is particularly relevant to *S. aureus* lineages associated with leukocidin production, while *toxA* is an important *P. aeruginosa* exotoxin determinant and *hlyA* is associated with hemolysin-mediated injury in pathogenic *E. coli*. Molecular detection establish gene expression or clinical severity, but it identifies the genetic potential for these virulence functions.

A major strength of the study is the assessment of resistance and virulence within the same overall bacterial collection. Approximately 37.3% of MDR isolates carried two or more resistance genes, 29.3% carried multiple virulence genes, and 32.0% showed co-occurrence of resistance and virulence determinants. The latter finding is especially important because the convergence of these traits may create bacterial populations that are simultaneously harder to eradicate and capable of effective colonization, persistence, or host damage. Mobile genetic elements, clonal dissemination, and biofilm-associated genetic

exchange can all contribute to the accumulation and spread of resistance determinants, while successful pathogenic lineages may retain or acquire virulence traits over time (Partridge et al., 2018; Mulani et al., 2019).

The results therefore support the value of integrated phenotypic and molecular surveillance. Antimicrobial susceptibility testing remains indispensable because it directly measures the observable response of an isolate to antimicrobial exposure. Molecular testing adds mechanistic resolution by identifying specific resistance and virulence genes that may explain part of the phenotype and may have epidemiological significance. Neither approach is sufficient in isolation. Phenotypic testing can miss the genetic context of resistance, while targeted PCR can miss untested determinants establish antimicrobial susceptibility. Combining both approaches, as done in this study, provides a more complete framework for identifying high-risk bacterial populations and informing infection-control and stewardship priorities.

From an antimicrobial-stewardship perspective, the very high resistance rates to ampicillin and amoxicillin-clavulanate argue against uncritical empirical reliance on these agents in settings represented by the study population. The high ciprofloxacin and ceftriaxone resistance rates likewise indicate that local susceptibility data should guide empirical and definitive therapy. At the same time, the lower meropenem resistance rate should not be interpreted as justification for indiscriminate carbapenem use. Increased carbapenem exposure can select for carbapenem-resistant populations and accelerate the loss of critically important treatment options. Stewardship programs should therefore integrate laboratory surveillance with local prescribing policies, de-escalation when possible, and infection-prevention measures designed to reduce transmission.

The study also has implications for infection prevention and control. MDR isolates carrying multiple resistance genes may have increased potential to persist under antimicrobial selection, while isolates carrying multiple virulence determinants may possess enhanced capacities for colonization or tissue damage. Identification of isolates in which both

categories co-occur can help prioritize strains for epidemiological investigation, enhanced contact precautions where appropriate, outbreak surveillance, or further genomic characterization. Whole-genome sequencing would be particularly informative in future studies because it could determine whether resistance and virulence determinants are physically linked on plasmids or other mobile elements, identify clonal relationships, and reveal additional genes captured by the targeted PCR panel.

4.1 Strengths and limitations

The principal strength of this work is its integrated design, which combines phenotypic antimicrobial susceptibility testing with targeted molecular detection of six resistance genes and five virulence-associated genes. The inclusion of determinants representing beta-lactam, methicillin, vancomycin, and quinolone resistance together with adhesion-, outer-membrane-, hemolysin-, exotoxin-, and leukocidin-associated virulence provides a broad overview of clinically relevant molecular traits. The evaluation of multiple-gene carriage and resistance-virulence co-occurrence further moves beyond single-marker prevalence and highlights the molecular complexity of the MDR population.

Several limitations should be considered when interpreting the findings. First, the supplied study summary does not provide species-specific isolate counts, specimen-source distributions, ward or patient characteristics, or isolate-level genotype-phenotype contingency tables. Consequently, the present determine whether individual resistance genes were significantly associated with their corresponding antimicrobial phenotypes, nor can it compare gene prevalence across bacterial species. Second, the Methods state that MDR isolates were selected for molecular characterization, whereas the individual gene percentages that numerically correspond to the full sample denominator of 150. This denominator should be reconciled against the primary laboratory. Third, the targeted PCR approach identifies only prespecified genes and therefore does not exclude additional resistance or virulence determinants. Fourth, gene detection indicates

genetic presence rather than expression, functional activity, or direct contribution to disease severity.

Finally, although the Methods describe the potential use of chi-square or Fisher's exact tests, the supplied aggregate data include the cell counts needed to reproduce such analyses. For that reason, this intentionally p-values and formal claims of statistical association. Future analysis of the raw isolate-level dataset should include species-stratified frequencies, 95% confidence intervals, phenotype-genotype concordance measures, and appropriate tests of association. Where sample sizes permit, multivariable analysis could further explore whether specific resistance genes, virulence genes, specimen sources, or bacterial species independently predict MDR status or resistance-virulence co-occurrence.

5. Conclusion

This study demonstrates a high burden of multidrug resistance among clinical bacterial isolates, with 64.0% of the 150 isolates exhibiting an MDR phenotype. Resistance was particularly frequent to ampicillin, amoxicillin-clavulanate, ciprofloxacin, and ceftriaxone, while meropenem showed comparatively lower resistance. Molecular analysis identified a broad range of resistance determinants, led by blaCTX-M and blaTEM, together with mecA, qnrS, vanA, and blaSHV. The simultaneous detection of fimH, ompA, hlyA, toxA, and lukS-PV further demonstrates that the bacterial population carried diverse virulence-associated traits in addition to antimicrobial resistance.

The finding that substantial proportions of MDR isolates carried multiple resistance genes, multiple virulence genes, or both resistance and virulence determinants highlights the potential emergence of bacterial populations with combined therapeutic and pathogenic risk. These results support the integration of routine antimicrobial susceptibility testing with molecular surveillance, particularly in settings where MDR organisms are common. Such an approach can strengthen antimicrobial stewardship, guide infection-prevention strategies, support early recognition of high-risk strains, and provide a foundation for more detailed genomic epidemiology.

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