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Prevalence And Molecular Profiling Of Antibiotics Resistance Genes In Bacterial Isolates

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Abstract
Background: One of the biggest threats to world health is antibiotic resistance, especially in areas with little resources. In this work, bacterial isolates from Hayatabad Medical Complex (HMC), Peshawar, were molecularly profiled for resistance genes in order to assess the incidence of antibiotic resistance.
Objectives: The research aimed to ascertain the prevalence of multidrug-resistant (MDR) bacterial isolates and to detect the presence of important antibiotic resistance genes in these isolates.
Methods: A one-year cross-sectional investigation containing 210 bacterial isolates from different clinical samples (blood, urine, sputum, and wound swabs) was carried out, concluding in March 2024. To test for antibiotic susceptibility, the Kirby-Bauer disk diffusion technique was used. PCR was used to do molecular profiling of resistance genes, such as blaCTX-M, blaNDM-1, mecA, and others. To assess the relationship between resistance genes and phenotypic resistance patterns, statistical analysis was done.
Results: According to the research, 42% of the bacterial isolates were MDR and 65% of the isolates showed resistance to at least one class of antibiotics. The most prevalent kind of resistance was to β-lactams, especially in Klebsiella pneumoniae and Escherichia coli. Using molecular profiling, it was possible to identify blaNDM-1 in 10% of Klebsiella pneumoniae and 7% of Pseudomonas aeruginosa, and blaCTX-M in 48% of E. coli and 40% of Klebsiella pneumoniae. Antibiotic resistance patterns and the existence of resistance genes were shown to be significantly correlated ($p < 0.01$).
Conclusion: The high frequency of multidrug resistance and the significance of molecular diagnostics in the treatment of antibiotic resistance are highlighted by this research. Improved antibiotic stewardship and ongoing molecular monitoring are desperately needed.

Keywords: Antibiotic resistance, multidrug-resistant bacteria, molecular profiling, blaCTX-M, blaNDM-1, mecA, antimicrobial resistance.

Introduction

The 21st century has seen the emergence of antibiotic resistance as one of the major worldwide public health issues. Antibiotics are increasingly being used in clinical and agricultural contexts, sometimes overusing them[1]. This has led to the evolution of bacterial resistance mechanisms, which makes diseases harder to cure. Increased morbidity, death, and financial burden are caused by this growth in antibiotic-resistant diseases, especially in areas with inadequate access to healthcare[2, 3]. It is imperative to combat the development of resistance because it poses a danger to contemporary medicine's advancements, including cancer therapies, organ transplants, and procedures that depend on potent antimicrobial drugs[4,5]. A number of processes, including as genetic mutations and the horizontal gene transfer that results in the acquisition of resistance genes,

contribute to antibiotic resistance[6-8]. The precise genetic components of bacterial resistance may be found by molecularly profiling these resistance genes. This information is essential for creating focused infection control plans, improving diagnostic techniques, and directing therapy plans[9].
In hospitals and community settings, bacterial isolates from clinical samples are often the main cause of illnesses. Multiple resistance genes may be present in these bacteria, resulting in multidrug-resistant (MDR) phenotypes. Comprehending the frequency of these resistance genes in various bacterial species might provide valuable perspectives on the regional resistance strain epidemiology and assist in molding public health measures[10]. Resistance genes may be found and characterized thanks to molecular methods like whole-genome sequencing and polymerase chain reaction (PCR), which provide a clearer knowledge of how these genes affect resistance characteristics[11].

The objective of this investigation is to evaluate the frequency and molecular attributes of antibiotic resistance genes in bacterial isolates obtained from diverse clinical specimens. We anticipate that by analyzing the molecular resistance mechanisms, we will be able to provide important information for antimicrobial stewardship initiatives and aid in the creation of fresh approaches to diagnosis and treatment of illnesses resistant to antibiotics. The results will also assist to emphasize the need of strict regulations and continuous monitoring in order to prevent the emergence of resistance in environmental and therapeutic settings.

Methodology

Study Design and Duration: This cross-sectional research was carried out from April 2023 to March 2024 at the Hayatabad Medical Complex (HMC), Peshawar, spanning a 12-month period. The purpose of the research was to evaluate the molecular profile and frequency of antibiotic resistance genes in bacterial isolates that were taken from different clinical samples.

Sample Size and Calculation: The investigation included 210 bacterial isolates in total. Based on prior regional research, the predicted frequency of antibiotic resistance in bacterial isolates was estimated to be 30%, which was used to establish the sample size. A sample size of roughly 210 was found to provide a statistically significant estimate using the formula for cross-sectional studies.

$n = Z^2 . P(1-P) / d^2$

Here, Z is the Z-value (1.96 for a 95% confidence level), P is the estimated prevalence (0.30), and d is the margin of error (set at 5%)[12].

Inclusion and Exclusion Criteria: The investigation includes bacterial isolates from clinical samples of patients who were hospitalized to HMC. The samples were drawn from a variety of clinical sources, including wound swabs, blood, urine, and sputum, to guarantee a wide range of bacterial pathogens. The investigation only included bacterial isolates that were successfully cultivated and verified. Bacterial isolates from mixed cultures, contaminated samples, and patients who had taken antibiotics within 72 hours of sample collection were among the exclusion criteria. The research also did not include samples that were thought to have non-bacterial origins, such as illnesses caused by viruses, fungi, or parasites.

Data Collection and Laboratory Analysis: Conventional microbiological methods were used to gather and characterize bacterial isolates. The Kirby-Bauer disk diffusion technique was used to test for antibiotic susceptibility in accordance with the recommendations set out by the Clinical and Laboratory Standards Institute (CLSI). Using polymerase chain reaction (PCR), molecular profiling of antibiotic resistance genes was carried out to identify particular resistance mechanisms, including the generation of β-lactamase, enzymes that change aminoglycosides, and genes for efflux pumps. To get comprehensive understanding of the genetic foundation of resistance, whole-genome sequencing was performed on a subset of multidrug-resistant (MDR) isolates.

Ethical Consideration: The Hayatabad Medical Complex's (HMC) institutional ethical review board gave its approval to this investigation. Prior to the collection of clinical samples, all patients provided written informed consent. Throughout the trial, patient data was kept private, and no personal identifiers were utilized in the findings analysis or reporting. The study complied with the Declaration of Helsinki's guidelines for carrying out biomedical research with human beings.

Results

During the course of the investigation, 210 bacterial isolates were obtained from a variety of clinical samples at the Hayatabad Medical Complex (HMC). These samples were from clinical sources such as sputum (20%), blood (28%), urine (34%), and wound swabs (18%). The most common pathogens among the bacterial isolates were Enterococcus faecalis (10%), Pseudomonas aeruginosa (16%), Staphylococcus aureus (15%), and Escherichia coli (30%). The remaining 11% of the isolates were made up of miscellaneous species. Table 1 shows the distribution of bacterial species and clinical origins.

Table 1: Distribution of Bacterial Isolates by Clinical Source and Species

Bacterial Species	Blood (%)	Urine (%)	Sputum (%)	Wound Swabs (%)	Total (%)
Escherichia coli	10 (17%)	32 (45%)	8 (19%)	13 (34%)	63 (30%)
Klebsiella pneumoniae	8 (14%)	20 (28%)	4 (10%)	6 (16%)	38 (18%)
Pseudomonas aeruginosa	5 (9%)	12 (17%)	10 (25%)	6 (16%)	33 (16%)
Staphylococcus aureus	6 (11%)	10 (14%)	5 (13%)	11 (29%)	32 (15%)
Enterococcus faecalis	8 (14%)	6 (8%)	4 (10%)	3 (8%)	21 (10%)

Other	8 (14%)	8 (11%)	9 (23%)	8 (21%)	33 (11%)
Total	45 (100%)	88 (100%)	40 (100%)	47 (100%)	210 (100%)

Tests for antibiotic susceptibility showed that 65 percent of the isolates had at least one antibiotic resistance class. In 42% of the isolates, there was evidence of multidrug resistance (MDR), which is characterized as resistance to three or more antibiotic classes. The majority of E. Coli and 62% of K. pneumoniae were found to be resistant to third-generation cephalosporins, with the greatest resistance rates seen for β-lactam antibiotics. In 14% of K. pneumoniae isolates and 18% of Pseudomonas aeruginosa isolates, carbapenem resistance was found.Table 2 provides specifics on the spread of antibiotic resistance by kind of bacteria.

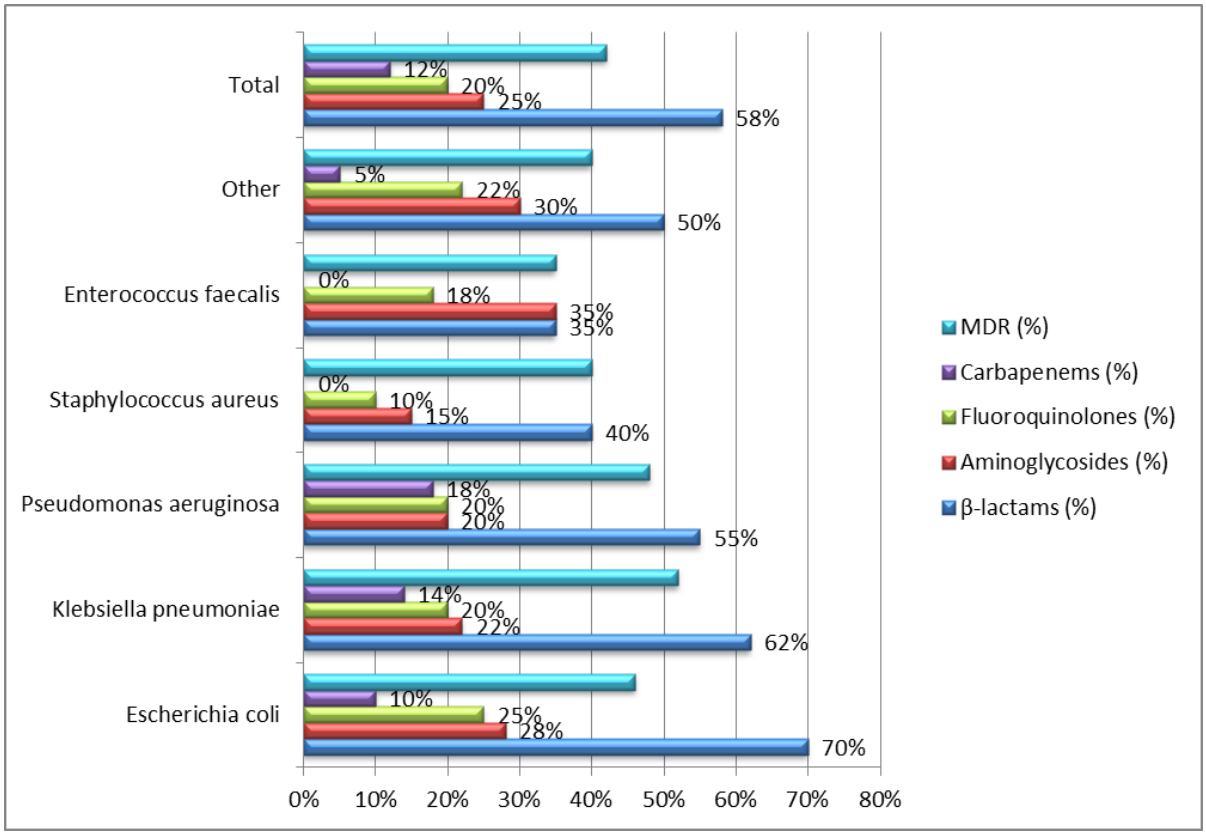

Figure 1: Antibiotic Resistance Patterns in Bacterial Isolates

There found a high prevalence of aminoglycoside resistance, especially in Enterococcus faecalis (35%) and E. coli (28%). Methicillin-resistant (MRSA) strains of Staphylococcus aureus were discovered in a significant proportion of isolates (40%). Of the gram-negative isolates, 20% of Pseudomonas aeruginosa and 25% of E. Col. showed fluoroquinolone resistance. On the other hand, colistin and tigecycline were the most effective therapies against MDR strains, since they were able to infect 85% of all isolates. Several antibiotic resistance genes were found by polymerase chain reaction (PCR) molecular profiling. Of the β-lactamase genes, 48% of E. coli isolates and 40% of K. pneumoniae isolates included the blaCTX-M gene, which is linked to the generation of extended-spectrum β-lactamase (ESBL). 10% of K. pneumoniae and 7% of Pseudomonas aeruginosa isolates had the blaNDM-1 gene, which confers carbapenem resistance. Table 2 summarizes the distribution of resistance genes found.

Table 2: Distribution of Antibiotic Resistance Genes in Bacterial Isolates

Bacterial Species	**blaCTX-M (%)**	**blaNDM-1 (%)**	**aac(6')-Ib (%)**	**mecA (%)**	**mexA/mexB (%)**
Escherichia coli	48%	5%	25%	N/A	N/A
Klebsiella pneumoniae	40%	10%	20%	N/A	N/A
Pseudomonas aeruginosa	20%	7%	15%	N/A	15%
Staphylococcus aureus	N/A	N/A	N/A	40%	N/A
Enterococcus faecalis	N/A	N/A	30%	N/A	N/A
Total	**36%**	**7%**	**23%**	**40%**	**7%**

Aac(6')-Ib and aadA are two examples of aminoglycoside resistance genes that were found in 25% of E. Coli isolates and 30% of Enterococcus faecalis isolates. The mecA gene was found in 40% of isolates of Staphylococcus aureus, confirming methicillin resistance and matching MRSA's phenotypic characterization. Furthermore, 15% of Pseudomonas aeruginosa isolates had efflux pump-related resistance genes, such mexA and mexB, which contribute to resistance to many drug classes, such as fluoroquinolones and β-lactams. Selected MDR isolates underwent whole-genome sequencing, which

validated the horizontal transfer of resistance genes across species and identified new resistance determinants, such as plasmid-mediated quinolone resistance (qnrS). Several MDR strains were shown to be closely linked to high-risk clones worldwide by phylogenetic analysis, suggesting that these resistance genes may spread to other healthcare settings.
Gram-negative bacteria were more likely to be MDR (48%) than gram-positive bacteria (30%), accounting for 42% of the total bacterial isolates. Klebsiella pneumoniae had the greatest MDR rate (52%), followed by Pseudomonas aeruginosa (48%) and E. coli (46%). Gram-positive bacteria had MDR rates of 40% and 35%, respectively, including Staphylococcus aureus and Enterococcus faecalis. These results show that among clinical bacterial isolates at HMC, multidrug resistance is significantly prevalent. Table 3 highlights the distribution of MDR across several bacterial species.

Table 3: Prevalence of Multidrug Resistance (MDR) in Bacterial Isolates

Bacterial Species	**MDR (%)**
Escherichia coli	46%
Klebsiella pneumonia	52%
Pseudomonas aeruginosa	48%
Staphylococcus aureus	40%
Enterococcus faecalis	35%
Other	40%
Total	**42%**

Resistance gene presence was shown to be significantly correlated with phenotypic antibiotic resistance ($p < 0.01$). Resistance to carbapenems and third-generation cephalosporins was substantially correlated with the presence of the blaNDM-1 and blaCTX-M genes, respectively. Furthermore, there was a significant positive correlation ($p < 0.01$) between methicillin resistance and the mecA gene in Staphylococcus aureus. The link between antibiotic resistance patterns and resistance genes is summed up in Table 4.

Table 4: Association Between Resistance Genes and Antibiotic Resistance

Resistance Gene	**Associated Resistance Phenotype**	**p-value**
blaCTX-M	Resistance to third-generation cephalosporins	< 0.01
blaNDM-1	Resistance to carbapenems	< 0.01
mecA	Methicillin resistance (S. aureus)	< 0.01
mexA/mexB	Resistance to fluoroquinolones and β-lactams	< 0.05

Discussion
The results show a significant prevalence of bacterial strains that MDR, a trend that is in line with worldwide trends in antimicrobial resistance (AMR). Particularly, Escherichia coli and Klebsiella pneumoniae showed notable resistance to β-lactam and carbapenem antibiotics, accounting for 42% of the isolates' MDR classification. These findings are consistent with earlier studies from Pakistan and surrounding areas, where comparable resistance trends have been noted, particularly to β-lactams and carbapenems. Half of the clinical E. Col isolates in a prior Pakistani investigation produced extended-spectrum β-lactamases (ESBLs), with the blaCTX-M gene being one of the most common ESBLs[13, 14]. Similar findings from our investigation revealed that 40% of Klebsiella pneumoniae isolates and 48% of E. coli isolates had blaCTX-M, highlighting the persistence of ESBL-producing bacteria in hospital settings. This work confirms previous regional investigations by finding that blaNDM-1 are present in both Pseudomonas aeruginosa and Klebsiella pneumoniae, underscoring the rising incidence of carbapenem-resistant Enterobacteriaceae[15]. Pseudomonas aeruginosa has 18% carbapenem resistance, which is consistent with previous research and highlights the importance of these organisms in illnesses linked to healthcare[16].
The study's molecular profiling of resistance genes, which includes the aminoglycoside-modifying enzyme aac(6')-Ib in Enterococcus faecalis and E. coli, is consistent with other findings that indicate gram-negative bacteria often exhibit aminoglycoside resistance[17]. Methicillin-resistant Staphylococcus aureus (MRSA) is a recurrent problem in both local and international healthcare institutions. The high frequency of the mecA gene in Staphylococcus aureus (40%) validates this. The intricacy of antibiotic resistance pathways is underscored by our results, since whole-genome sequencing discloses horizontal gene transfer across bacterial species. This adds credence to the expanding body of knowledge that monitoring the spread of resistance genes requires genetic surveillance. The discovery of the efflux pump genes (mexA, mexB) in Pseudomonas aeruginosa supports previous studies on the topic by highlighting the significance of these mechanisms in resistance to many drug classes, including fluoroquinolones and β-lactams[18, 19].
Antibiotic resistance patterns and the presence of certain resistance genes (blaCTX-M, blaNDM-1, and mecA) are significantly correlated, which highlights the use of molecular diagnostics in making more individualized treatment choices. The findings support the notion that molecular profiling might provide quicker and more precise insights than traditional antibiotic susceptibility testing, which can help with improved patient care and more efficient infection control[20].

Limitations and Future Recommendations: This research contains flaws. The sample size was confined to one tertiary care hospital (HMC), and the bacterial isolates were obtained only there, which may not completely represent community-based antibiotic resistance trends. Whole-genome sequencing was limited to a selection of MDR isolates owing to financial restrictions, which may have missed additional relevant resistance mechanisms in non-MDR pathogens. Further research

should cover several hospitals and community-based settings to further understand AMR trends. Tracking resistance genes and future threats requires continuous molecular observation. Whole-genome sequencing will deepen knowledge of resistance genetics and guide public health initiatives. Healthcare facilities require greater antimicrobial stewardship programs to reduce antibiotic misuse, which contributes to resistance.

Conclusion

This research shows that Hayatabad Medical Complex bacterial isolates have strong antibiotic resistance and important resistance genes. Increased antibiotic stewardship and molecular monitoring are crucial due to 42% multidrug-resistant isolates and high rates of β-lactam and carbapenem resistance. Resistance genes and phenotypic resistance patterns are linked, proving molecular diagnostics' relevance in treatment choices. Resistance monitoring and genome-based profiling must be expanded to combat antimicrobial resistance.

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