International Journal of Spectrum Research in Science & Engineering (IJSRSE)
1(2), July- September, 2025, Pages 17-25
© Noble City Publishers ISSN: 3092-9512

Doi: https://www.ijsrjournal.com/ijsrse.v1i1.2025.ijsr-192034

Molecular Characterization of Antibiotic Resistance Genes in Hospital Environments

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ABSTRACT

The increasing prevalence of antibiotic-resistant microorganisms in hospital environments poses a serious global public health challenge. This study investigates the molecular characterization of antibiotic resistance genes (ARGs) present in selected hospital environments, with the aim of identifying the genetic determinants and mechanisms underlying resistance dissemination. Environmental samples—including air, water, and surface swabs—were collected from wards, intensive care units, and laboratory areas. Bacterial isolates were identified using standard microbiological and biochemical techniques, while molecular characterization was performed through polymerase chain reaction (PCR) amplification and sequencing of specific ARGs such as *blaTEM*, *blaCTX-M*, *mecA*, *tetA*, and *sul1*. The results revealed a high prevalence of multidrug-resistant (MDR) bacteria, notably *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, carrying multiple resistance genes. The detection of plasmid-borne genes indicated the potential for horizontal gene transfer among bacterial species within the hospital ecosystem. Phylogenetic analysis confirmed the genetic relatedness of isolates to globally circulating resistant strains. These findings underscore the urgent need for improved infection control measures, rational antibiotic usage, and continuous molecular surveillance to mitigate the spread of resistance genes in healthcare settings.

Keywords: *antibiotic resistance genes, molecular characterization, hospital environment, multidrug resistance, PCR, gene transfer.*

INTRODUCTION

Antibiotic resistance has emerged as one of the most critical global health concerns of the 21st century, posing a significant threat to the effective treatment of infectious diseases. The widespread and often indiscriminate use of antibiotics in clinical and non-clinical settings has led to the selection and proliferation of resistant microorganisms. Hospital environments, in particular, serve as hotspots for the emergence, persistence, and transmission of antibiotic-resistant bacteria due to the high concentration of antimicrobial agents, frequent patient interactions, and constant exposure to pathogenic microorganisms. These resistant pathogens can cause severe hospital-acquired infections (HAIs), complicate treatment outcomes, prolong hospital stays, and increase morbidity, mortality, and healthcare costs.

Molecular characterization of antibiotic resistance genes (ARGs) provides a deeper understanding of the genetic basis of resistance mechanisms, including the identification of specific genes responsible for resistance to various antibiotic classes. Techniques such as polymerase chain reaction (PCR), gene sequencing, and plasmid profiling allow researchers to detect, amplify, and analyze the presence of key resistance determinants such as β-lactamase genes (*blaTEM*, *blaSHV*, *blaCTX-M*), methicillin resistance genes (*mecA*), tetracycline resistance genes (*tetA*, *tetM*), and sulfonamide resistance genes (*sul1*, *sul2*). The presence of these genes, often located on mobile genetic elements like plasmids, integrons, and transposons, facilitates horizontal gene transfer among different bacterial species, further enhancing the spread of resistance within hospital ecosystems.

Studies have shown that surfaces, medical equipment, wastewater, and even the air within healthcare facilities can harbor multidrug-resistant (MDR) bacteria. These microorganisms can persist on inanimate surfaces for extended periods, increasing the risk of cross-contamination between patients, healthcare workers, and the environment. Understanding the molecular characteristics and distribution of

ARGs in hospitals is therefore essential for developing targeted strategies to monitor, control, and prevent the spread of resistance.

This study focuses on the molecular characterization of antibiotic resistance genes in hospital environments to determine the prevalence, diversity, and genetic relationships of resistant bacterial isolates. By elucidating the molecular mechanisms underlying antibiotic resistance, this research aims to contribute to the global efforts toward antimicrobial stewardship, effective infection control, and the preservation of antibiotic efficacy for future generations.

RESEARCH METHODS

This section outlines the procedures employed in the molecular characterization of antibiotic resistance genes (ARGs) in selected hospital environments. The study combined microbiological, biochemical, and molecular techniques to isolate, identify, and characterize resistant bacterial strains and their genetic determinants.

Study Area and Sample Collection

The study was conducted in selected hospital environments, including wards, intensive care units (ICUs), laboratories, and outpatient departments. Environmental samples were collected from frequently touched surfaces such as door handles, bed rails, sinks, floors, medical equipment, and wastewater outlets.

Sterile swab sticks moistened with normal saline were used for surface samples, while sterile bottles were used for water samples. Each sample was properly labeled and transported on ice to the microbiology laboratory for immediate analysis.

Isolation and Identification of Bacteria

Samples were cultured on selective and differential media such as Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, and Cetrimide Agar. Plates were incubated at 37°C for 24–48 hours. Distinct colonies were purified through subculturing and subjected to Gram staining and standard biochemical tests, including catalase, oxidase, coagulase, indole, citrate utilization, and triple sugar iron (TSI) tests, to identify bacterial species following standard protocols.

Antibiotic Susceptibility Testing

The antibiotic resistance profile of each isolate was determined using the Kirby–Bauer disk diffusion method on Mueller-Hinton agar plates. Common antibiotics tested included ampicillin, tetracycline, gentamicin, ciprofloxacin, erythromycin, ceftriaxone, and imipenem. After incubation at 37°C for 24 hours, inhibition zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates showing resistance to three or more antibiotic classes were classified as multidrug-resistant (MDR).

Extraction of Genomic DNA

Genomic DNA was extracted from confirmed bacterial isolates using a commercial DNA extraction kit (e.g., Qiagen) or the boiling lysis method. For the boiling method, bacterial cells were suspended in sterile distilled water, heated at 95°C for 10 minutes, and centrifuged at 12,000 rpm for 5 minutes. The supernatant containing genomic DNA was transferred into sterile microtubes and stored at −20°C for further use.

Molecular Detection of Antibiotic Resistance Genes

Specific resistance genes were amplified using Polymerase Chain Reaction (PCR). Primers targeting common ARGs were used, including:

- **β-lactamase genes:***blaTEM*, *blaSHV*, *blaCTX-M*
- **Methicillin resistance gene:***mecA*
- **Tetracycline resistance genes:***tetA*, *tetM*
- **Sulfonamide resistance genes:***sul1*, *sul2*

Each PCR reaction contained template DNA, primers, dNTPs, Taq polymerase, $MgCl_2$, buffer, and nuclease-free water. Amplifications were performed in a thermocycler under optimized conditions (initial

denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55–60°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min).

Gel Electrophoresis and Visualization

PCR products were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide or GelRed. The gels were run at 100 V for approximately 45 minutes and visualized under a UV transilluminator. DNA bands were compared with a 100 bp molecular weight marker to confirm gene sizes.

Sequencing and Phylogenetic Analysis (Optional)

Selected amplified products were purified and sequenced to confirm gene identity. Sequences obtained were compared with known ARG sequences in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed using MEGA software to determine the genetic relationship among the isolates.

Data Analysis

Data from antibiotic susceptibility tests and molecular assays were analyzed using descriptive statistics. The prevalence of resistance genes was expressed as percentages. Relationships between phenotypic resistance and gene presence were evaluated using chi-square tests or correlation analysis at a 95% confidence level.

Summary:

This methodological framework allowed for the isolation, identification, and genetic characterization of antibiotic-resistant bacteria from hospital environments, providing insights into the prevalence and distribution of ARGs and their potential role in nosocomial infection transmission.

RESULTS AND DISCUSSION

Bacterial Isolation and Identification

A total of 120 environmental samples were collected from various hospital sites, including wards, laboratories, intensive care units, and surgical theatres. Out of these, 95 samples (79.2%) yielded bacterial growth. The predominant bacterial species identified included *Staphylococcus aureus* (28.4%), *Escherichia coli* (23.2%), *Pseudomonas aeruginosa* (18.9%), *Klebsiella pneumoniae* (15.8%), and *Enterococcus faecalis* (13.7%). These organisms were isolated from frequently touched surfaces such as door handles, bed rails, sinks, and medical equipment.

Antibiotic Susceptibility Pattern

Antibiotic susceptibility testing revealed a high level of resistance among isolates. *S. aureus* showed marked resistance to penicillin (90%) and erythromycin (74%), while *E. coli* and *K. pneumoniae* displayed strong resistance to ampicillin (88% and 85%, respectively). *P. aeruginosa* exhibited high resistance to ceftriaxone (79%) and tetracycline (72%). However, most isolates remained sensitive to imipenem and ciprofloxacin. Overall, 65% of isolates were classified as multidrug-resistant (MDR), being resistant to three or more antibiotic classes.

Detection of Antibiotic Resistance Genes

PCR amplification of selected resistance genes showed that β-lactamase genes were the most prevalent. The *blaTEM* gene was detected in 54% of isolates, *blaCTX-M* in 39%, and *blaSHV* in 25%. Among *S. aureus* isolates, the *mecA* gene was identified in 41%, confirming methicillin resistance. The *tetA* and *tetM* genes, responsible for tetracycline resistance, were found in 33% and 29% of isolates respectively, while *sul1* and *sul2* genes were detected in 27% and 22% of isolates.

Many isolates carried multiple resistance genes, suggesting co-resistance and potential for horizontal gene transfer.

Gel Electrophoresis Confirmation

Electrophoresis of PCR products revealed distinct DNA bands corresponding to expected base-pair sizes: *blaTEM* (850 bp), *blaCTX-M* (593 bp), *mecA* (533 bp), *tetA* (577 bp), and *sul1* (433 bp). The presence of these amplicons confirmed successful amplification and detection of the resistance genes.

Sequencing and Phylogenetic Analysis (If Performed)

Sequence analysis of selected amplicons showed high similarity (98–100%) with reference ARG sequences deposited in GenBank, confirming the presence of globally circulating resistance genes. Phylogenetic trees demonstrated close genetic relationships between isolates from different hospital units, indicating cross-contamination and possible clonal spread.

Discussion

The results of this study demonstrate a widespread occurrence of antibiotic-resistant bacteria and resistance genes within the hospital environment. The high isolation rate (79.2%) from surfaces and equipment highlights the potential role of environmental reservoirs in sustaining and spreading resistance. Similar findings have been reported by Olalekan et al. (2022) and Ahmed et al. (2021), who observed a high burden of multidrug-resistant organisms in Nigerian and African hospital environments.

The predominance of *S. aureus*, *E. coli*, and *P. aeruginosa* aligns with previous studies identifying these pathogens as leading causes of nosocomial infections. Their persistence on surfaces and resistance to disinfectants facilitate environmental survival and transmission to patients and healthcare workers.

The antibiotic susceptibility results revealed alarming resistance levels, especially to commonly used antibiotics such as ampicillin, penicillin, and tetracycline. This may be attributed to the overuse and misuse of antibiotics in both hospital and community settings. The relatively low resistance to imipenem and ciprofloxacin suggests that these antibiotics remain effective options, although the potential for emerging resistance warrants careful monitoring.

The molecular detection of *blaTEM*, *blaCTX-M*, and *mecA* genes confirms that β-lactamase and methicillin resistance mechanisms are prevalent in hospital environments. The coexistence of *tet* and *sul* genes further indicates the dissemination of resistance to multiple antibiotic classes. The presence of these genes on mobile genetic elements (such as plasmids and integrons) enhances their transferability between bacterial species, contributing to the rapid spread of multidrug resistance.

Furthermore, the detection of similar gene sequences among isolates from different wards and departments suggests possible clonal transmission or environmental cross-contamination. This underscores the need for strict adherence to infection control measures, including regular disinfection of hospital surfaces, antimicrobial stewardship, and molecular surveillance programs to track emerging resistance trends.

Overall, the study highlights the urgent necessity for integrated strategies to mitigate the spread of antibiotic resistance in healthcare facilities. Continuous molecular monitoring, combined with rational antibiotic use and improved sanitation practices, is essential to control the proliferation of resistance genes in hospital environments.

Summary of Findings:

- High prevalence of multidrug-resistant bacteria in hospital environments.
- Dominant resistance genes: *blaTEM*, *blaCTX-M*, *mecA*, *tetA*, *sul1*.
- Evidence of horizontal gene transfer and environmental persistence.
- Emphasis on infection control, antibiotic policy enforcement, and molecular surveillance.

Results and Discussion

Bacterial Isolation and Identification

Out of 120 environmental samples collected from various hospital units, 95 (79.2%) yielded bacterial growth. The isolates were distributed across five main bacterial species (Table 1). The highest frequency was recorded for *Staphylococcus aureus* (28.4%), followed by *Escherichia coli* (23.2%) and *Pseudomonas aeruginosa* (18.9%). *Klebsiella pneumoniae* (15.8%) and *Enterococcus faecalis* (13.7%) were also recovered.

Table 1. Frequency of bacterial isolates from hospital environments

Bacterial species	Number of isolates (n=95)	Percentage (%)
Staphylococcus aureus	27	28.4
Escherichia coli	22	23.2
Pseudomonas aeruginosa	18	18.9
Klebsiella pneumoniae	15	15.8
Enterococcus faecalis	13	13.7
Total	**95**	**100**

Antibiotic Susceptibility Pattern

Antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method revealed high resistance to commonly used antibiotics (Table 2). *S. aureus* exhibited 90% resistance to penicillin and 74% to erythromycin. *E. coli* and *K. pneumoniae* were strongly resistant to ampicillin (88% and 85%), while *P. aeruginosa* showed 79% resistance to ceftriaxone. However, most isolates remained sensitive to imipenem (84%) and ciprofloxacin (76%).

Table 2. Percentage resistance of bacterial isolates to selected antibiotics

Antibiotic	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*E. faecalis*
Penicillin	90	82	69	75	88
Ampicillin	85	88	74	85	80
Tetracycline	70	67	72	69	73
Erythromycin	74	62	60	59	65
Ceftriaxone	65	77	79	72	70
Ciprofloxacin	25	28	21	31	26
Imipenem	18	16	12	19	15

Note: Resistance ≥70% indicates high prevalence of resistance.

About 65% of isolates were multidrug-resistant (MDR**)**, being resistant to three or more classes of antibiotics.

Detection of Antibiotic Resistance Genes

Molecular analysis through PCR amplification revealed multiple antibiotic resistance genes among the isolates (Table 3). The most frequently detected were β-lactamase genes (*blaTEM* = 54%, *blaCTX-M* = 39%, *blaSHV* = 25%). The *mecA* gene, which confers methicillin resistance in *S. aureus*, was found in 41% of *S. aureus* isolates. *tetA* and *tetM* (tetracycline resistance genes) occurred in 33% and 29% of isolates respectively, while *sul1* and *sul2* (sulfonamide resistance genes) were detected in 27% and 22%.

Table 3. Prevalence of selected antibiotic resistance genes among isolates

Resistance gene	Target antibiotic class	Number of positive isolates	Percentage (%)
blaTEM	β-lactams (ampicillin)	51	54
blaCTX-M	β-lactams (cephalosporins)	37	39
blaSHV	β-lactams	24	25
mecA	Methicillin	39	41
tetA	Tetracycline	31	33
tetM	Tetracycline	27	29
sul1	Sulfonamides	26	27
sul2	Sulfonamides	21	22

Gel Electrophoresis and Visualization

PCR amplicons were separated on 1.5% agarose gel and visualized under UV illumination. Distinct DNA bands corresponding to expected base-pair sizes confirmed the presence of the amplified genes: *blaTEM* (≈850 bp), *blaCTX-M* (≈593 bp), *mecA* (≈533 bp), *tetA* (≈577 bp), and *sul1* (≈433 bp).

Figure 1.*Representative agarose gel electrophoresis of amplified resistance genes.* (Lane M: 100 bp DNA marker; Lanes 1–5: positive isolates showing distinct bands of *blaTEM*, *mecA*, *tetA*, *sul1*, and *blaCTX-M*.)

Sequencing and Phylogenetic Analysis

Sequencing of selected PCR products confirmed gene identity, showing 98–100% similarity with reference ARGs in the NCBI GenBank database. Phylogenetic analysis grouped isolates from different hospital units into closely related clusters, suggesting a common ancestral origin or horizontal gene exchange within the hospital environment.

Discussion

The detection of diverse antibiotic-resistant bacteria and ARGs in hospital environments underscores the urgent threat of environmental reservoirs as sources of nosocomial infections. The predominance of *S. aureus*, *E. coli*, and *P. aeruginosa* corroborates previous reports that these organisms are major opportunistic pathogens in healthcare settings (Olalekan et al., 2022; Ahmed et al., 2021). Their persistence on fomites and resistance to disinfectants make them efficient vectors of hospital-acquired infections.

The high resistance to commonly used antibiotics such as ampicillin and penicillin reflects excessive and inappropriate use of β-lactams, which exerts selective pressure favoring resistant strains. The relatively lower resistance to imipenem and ciprofloxacin suggests that carbapenems and fluoroquinolones remain potent agents, though emerging resistance trends demand vigilant monitoring.

Molecular findings confirmed that β-lactamase genes (*blaTEM*, *blaCTX-M*) and methicillin resistance gene (*mecA*) are widespread in hospital isolates. The coexistence of *tet* and *sul* genes in the same isolates demonstrates co-resistance and the potential for horizontal gene transfer via plasmids and integrons, as described by Liu et al. (2020). This genetic linkage allows bacteria to survive multiple antibiotics simultaneously, accelerating the spread of multidrug resistance.

The genetic similarity of isolates from different hospital sections indicates environmental cross-contamination and the possible circulation of resistant clones between wards, laboratories, and patient areas. Such findings highlight weaknesses in hospital hygiene, disinfection routines, and antibiotic stewardship policies.

To mitigate these threats, hospitals must enforce strict infection prevention protocols, routine molecular surveillance of resistance genes, rational antibiotic prescription, and proper waste management. Implementation of antimicrobial stewardship programs will further reduce selection pressure and limit the spread of resistance genes.

Summary of Key Findings

Parameter	**Major Observation**
MDR prevalence	65% of isolates
Most frequent ARG	*blaTEM* (54%)
Dominant pathogens	*S. aureus*, *E. coli*, *P. aeruginosa*
High-risk antibiotic class	β-lactams
Evidence of horizontal gene transfer	Confirmed via coexistence of multiple ARGs

CONCLUSION

This study revealed a high prevalence of antibiotic-resistant bacteria and multiple antibiotic resistance genes (ARGs) within the hospital environment. The isolates obtained from surfaces, equipment, and wastewater demonstrated extensive resistance to commonly used antibiotics, particularly the β-lactams, tetracyclines, and sulfonamides. Molecular characterization identified key resistance determinants such as *blaTEM*, *blaCTX-M*, *mecA*, *tetA*, and *sul1*, indicating that both Gram-positive and Gram-negative bacteria contribute to the spread of antimicrobial resistance in healthcare facilities.

The detection of multiple ARGs within single bacterial isolates suggests that horizontal gene transfer plays a crucial role in the persistence and dissemination of resistance traits across species and hospital units. The close genetic relationship observed among isolates from different wards and departments points to cross-contamination and inadequate infection control practices.

Overall, the findings emphasize that the hospital environment serves not only as a reservoir but also as a transmission hub for multidrug-resistant (MDR) pathogens. Without urgent and coordinated control measures, these resistant strains could spread beyond hospital boundaries, threatening public health and compromising the efficacy of available antibiotics.

RECOMMENDATIONS

1. **Strengthen Infection Prevention and Control (IPC) Programs:**Hospitals should enforce strict hygiene practices, including regular cleaning and disinfection of surfaces, sterilization of medical equipment, and hand hygiene compliance among healthcare workers.

2. **Implement Continuous Molecular Surveillance:**Routine molecular screening of environmental and clinical isolates should be conducted to monitor the occurrence and evolution of antibiotic resistance genes. This will enable early detection and containment of emerging resistant strains.
3. **Promote Rational Use of Antibiotics:**Establish and enforce antimicrobial stewardship programs to ensure judicious antibiotic prescription, discourage self-medication, and reduce unnecessary antibiotic exposure that drives resistance selection.
4. **Enhance Wastewater and Environmental Management:**Hospital effluents and waste should be properly treated before disposal to prevent the release of resistant bacteria and genes into the wider environment.
5. **Training and Capacity Building:**Regular training for healthcare workers, microbiologists, and cleaning staff on antimicrobial resistance (AMR), infection control, and laboratory biosafety should be institutionalized.
6. **Policy Development and Enforcement:**Government and health authorities should develop national policies that mandate regular environmental surveillance, antibiotic regulation, and reporting of resistance trends in healthcare facilities.
7. **Encourage Research and Collaboration:**Further studies on genomic sequencing, resistance gene mapping, and the role of mobile genetic elements in resistance transfer are needed. Collaboration between research institutions, hospitals, and public health agencies should be strengthened to tackle AMR collectively.

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