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# PLASMID PROFILING AND POST-CURING ANTIBIOTIC SUSCEPTIBILITY EVALUATION OF SELECTED MULTIDRUG-RESISTANT SURGICAL SITE INFECTION BACTERIAL ISOLATES

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## ABSTRACT

**Background:** Multidrug-resistant (MDR) bacterial pathogens associated with surgical site infections (SSIs) represent a major therapeutic challenge due to widespread dissemination of plasmid-mediated antimicrobial resistance. Characterizing plasmid profiles and determining the contribution of plasmids to antibiotic resistance are essential for understanding resistance mechanisms and improving SSI treatment outcomes. **Objective:** This study investigated the plasmid profiles of selected MDR bacterial isolates recovered from patients with surgical site infections and evaluated the effect of plasmid curing on antimicrobial susceptibility. **Materials and Methods:** Multidrug-resistant bacterial isolates obtained from post-operative wound specimens were identified using standard microbiological and biochemical techniques. Antibiotic susceptibility testing was performed before and after plasmid curing. Plasmid DNA was extracted using the alkaline lysis method, profiled by agarose gel electrophoresis, and cured with ethidium bromide. Changes in antibiotic susceptibility following plasmid curing were subsequently evaluated. **Results:** All selected MDR isolates harboured plasmids, with a common plasmid of approximately 3 kb detected in all isolates, while one *Staphylococcus aureus* isolate possessed an additional 5 kb plasmid. Complete plasmid curing resulted in the elimination of both single and multiple plasmids and significantly enhanced susceptibility to selected antibiotics ($P < 0.05$), indicating that resistance was predominantly plasmid mediated. **Conclusion:** This study findings demonstrate that plasmids play a significant role in mediating multidrug resistance among bacterial pathogens associated with surgical site infections.

**KEYWORDS:** Surgical site infections, Antibiotics, Multidrug-resistant bacteria, Plasmids, Susceptibility.

## INTRODUCTION

Surgical site infections remain one of the most frequently reported healthcare-associated infections worldwide and continue to pose significant challenges to patient safety despite remarkable advances in surgical techniques, aseptic practices and antimicrobial prophylaxis.[1,2,3] These infections develop within 30 days after surgery, or within one year when prosthetic materials are implanted, and are associated with prolonged hospitalization, increased healthcare costs, delayed wound healing, higher morbidity and mortality, and reduced quality of life.[2,3] The burden of surgical site infections is disproportionately higher in low- and middle-income countries, where limited infection prevention infrastructure, inappropriate antibiotic use, inadequate surveillance systems and poor antimicrobial stewardship contribute to higher incidence rates than those reported in developed countries.[2,4]

The microbial aetiology of surgical site infections is diverse and is largely influenced by the type of surgical procedure, anatomical location, host immune status and healthcare environment.[4,5] Gram-positive organisms, particularly *Staphylococcus aureus* and coagulase-negative staphylococci, remain among the predominant pathogens, while Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacter* species are increasingly implicated in postoperative wound infections.[6,7] Many of these organisms possess

remarkable adaptive mechanisms that enable survival under antimicrobial pressure and facilitate persistent colonization of surgical wounds.[3,6,7]

The rapid emergence and dissemination of multidrug-resistant bacteria have become major obstacles to the successful treatment of surgical site infections.[1,7] Resistance to β-lactams, fluoroquinolones, aminoglycosides and other commonly prescribed antibiotics has increased substantially over the past two decades, limiting therapeutic options and increasing the likelihood of treatment failure.[8,9,10] The World Health Organization has identified antimicrobial resistance as one of the most serious global public health threats, emphasizing the urgent need for improved surveillance and molecular characterization of resistant pathogens.[11]

A considerable proportion of antimicrobial resistance in clinically important bacteria is mediated by plasmids, which are self-replicating extrachromosomal DNA molecules capable of horizontal transfer between bacterial species.[12] Plasmids frequently carry genes encoding extended-spectrum β-lactamases, carbapenems, aminoglycoside-modifying enzymes, quinolone resistance determinants and numerous other resistance mechanisms.[12,13] Through conjugation, transformation and transduction, these mobile genetic elements facilitate the rapid dissemination of resistance determinants across bacterial populations, thereby accelerating the emergence of multidrug-resistant pathogens within healthcare settings.[14]

Plasmid profiling has become an important molecular epidemiological tool for investigating antimicrobial resistance and determining the genetic relationships among bacterial isolates.[15] The technique enables characterization of the number and approximate sizes of plasmids carried by bacterial pathogens and provides useful information regarding the possible dissemination of resistance genes within hospital environments.[13,14,15] Although plasmid profiling alone does not identify specific resistance genes, it remains a valuable preliminary approach for understanding plasmid diversity and monitoring the spread of resistance-associated mobile genetic elements, particularly in resource-limited laboratories.

Plasmid curing represents another useful experimental approach for evaluating the contribution of plasmids to antimicrobial resistance.[16] Restoration of susceptibility following plasmid elimination provides strong evidence that resistance is plasmid mediated, whereas persistence of resistance suggests chromosomal localization of resistance determinants or the involvement of additional molecular mechanisms.[12,17] Consequently, plasmid curing has become an important laboratory method for distinguishing plasmid-borne resistance from chromosomal resistance and for understanding the genetic basis of multidrug resistance.[12]

Several studies have demonstrated widespread plasmid-mediated resistance among bacterial pathogens isolated from surgical site infections in different regions of the world.[18,19,20] However, information regarding plasmid carriage and post-plasmid curing characteristics of multidrug-resistant surgical site infection pathogens remains limited in many clinical settings in Nigeria. This knowledge gap limits the development of targeted antimicrobial stewardship interventions and effective infection prevention strategies.

The present study therefore investigated the plasmid profiles of multidrug-resistant bacterial isolates recovered from SSI patients and evaluated the effects of plasmid curing on antimicrobial susceptibility. Understanding the extent to which resistance is plasmid mediated will contribute to improved epidemiological surveillance of antimicrobial resistance, enhance infection control programmes and provide valuable evidence for optimizing antibiotic therapy in the management of surgical site infections.

## MATERIALS AND METHODS

### Study Design

This study was a laboratory-based experimental investigation conducted to determine the plasmid profiles of multidrug-resistant bacterial isolates recovered from patients with surgical site infections at 6 specialist hospitals in Calabar-Nigeria. The study evaluated the contribution of plasmid-mediated resistance through post-plasmid curing analysis. The work formed part of a broader investigation by the author, on antimicrobial resistance among bacterial pathogens associated with post-operative wound infections.[7]

### Study Area and Source of Bacterial Isolates

Multidrug-resistant (MDR) bacterial isolates used in this study were previously recovered during the parent study from patients with confirmed surgical site infections (SSIs) attending selected specialist healthcare facilities in Calabar, Cross River State, Nigeria.[7] The participating hospitals included the University of Calabar Teaching Hospital, General Hospital Calabar, Nigerian Navy Reference Hospital Calabar, Nigerian Air Force Clinic Calabar, and Bakor Clinic Calabar. The MDR-SSI isolates were obtained from post-operative wound specimens collected under aseptic conditions and identified using standard microbiological and biochemical techniques, as previously described.[7] Only confirmed multidrug-resistant isolates were selected for plasmid profiling and curing analysis.

### Identification and Confirmation of Test Isolates

A total of eight (8) preserved SSI multidrug-resistant isolates from the species mentioned above, were revived on Nutrient Agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and incubated aerobically at 37°C for 18–24 h. Pure colonies were sub-cultured on appropriate selective media to ensure purity as previously reported.[7] Identification was confirmed using conventional

phenotypic studies including colonial morphology, Gram staining and biochemical characterization. The SSI isolates were further confirmed by VITEK 2 microbial ID/AST system based on standard protocols as reported in the parent study.[7]

**Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility testing was performed on the SSI-MDR isolates using the Kirby–Bauer disc diffusion method on Mueller–Hinton Agar according to Clinical and Laboratory Standards Institute guidelines before plasmid curing as previously reported.[7] Fresh bacterial suspensions were adjusted to 0.5 McFarland turbidity standard (approximately 1.5 × 10^8 CFU/mL) before inoculation.

Commercial antimicrobial discs (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) representing commonly prescribed antibiotics for surgical site infections were aseptically placed on inoculated Mueller–Hinton agar plates. Following incubation at 37°C for 18–24 h, inhibition zone diameters were measured to the nearest millimetre and interpreted using CLSI breakpoints, as reported in the parent study.[7]

**Selection of Multidrug-Resistant Isolates**

Bacterial isolates demonstrating resistance to at least three or more antibiotic classes were classified as multidrug-resistant according to internationally accepted criteria as previously described.[7] Representative MDR and clinically relevant SSI isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Proteus mirabilis*, *Acinetobacter baumannii* and *Staphylococcus epidermidis* were selected for plasmid profiling as reported in the parent study.[7]

**Plasmid DNA Extraction**

Plasmid DNA was extracted from overnight cultures using alkaline lysis procedure as previously described.[13,14,15] Briefly, each selected SSI-MDR isolate was cultured in 5 mL Luria–Bertani broth at 37°C overnight with continuous agitation. Approximately 1.5 mL of each bacterial culture was transferred into sterile microcentrifuge tubes and centrifuged at 12,000 × g for 2 min. The supernatant was discarded and the pellet was resuspended in 100 µL of ice-cold resuspension buffer containing Tris-HCl, ethylenediaminetetraacetic acid and ribonuclease A. Subsequently, 200 µL of freshly prepared alkaline lysis solution containing sodium hydroxide and sodium dodecyl sulphate was added and mixed gently by inversion. Neutralization was achieved by adding 150 µL of potassium acetate solution. Following centrifugation at 12,000 × g for 10 min, the clear supernatant containing plasmid DNA was carefully transferred into sterile tubes. Plasmid DNA for each test isolate was precipitated using chilled absolute isopropanol, washed with 70% ethanol, air dried and finally resuspended in nuclease-free water. DNA samples were stored at −20°C until electrophoretic analysis.

**Agarose Gel Electrophoresis and Plasmid Profiling**

Extracted plasmid DNA from the eight (8) SSI-MDR isolates were analysed by horizontal agarose gel electrophoresis following previously described procedures.[13,14,15] Agarose (1.0%, w/v) was prepared in 1× Tris–Acetate–EDTA buffer containing GelRed® nucleic acid stain. Five microlitres of plasmid DNA mixed with loading dye was loaded into each well alongside a molecular weight DNA marker. Electrophoresis was performed at 90–100 V for approximately 60–90 min. DNA bands were visualized using an ultraviolet gel documentation system, photographed and analysed. Approximate plasmid sizes were estimated by comparison with the DNA molecular weight marker.

**Plasmid Curing Procedure**

Plasmid curing was carried out using ethidium bromide following the method previously described.[16] Overnight bacterial cultures of the eight (8) SSI-MDR isolates were inoculated into Mueller–Hinton broth containing sub-inhibitory concentrations of ethidium bromide. The cultures were incubated aerobically at 37°C and 200 rpm for 18-24 h. Following incubation, cultures were serially diluted and sub-cultured on Nutrient Agar plates without curing agent to obtain isolated colonies. Individual colonies were purified and screened for plasmid loss by repeat plasmid extraction and agarose gel electrophoresis. Colonies showing absence of previously detected plasmid bands were regarded as cured derivatives.

**Post-Plasmid Curing Antimicrobial Susceptibility Testing**

Plasmid-cured SSI-MDR isolates were subjected to repeat antibiotic susceptibility testing using selected conventional antibiotics and the Kirby–Bauer disc diffusion method, under identical experimental conditions.[16] Changes in susceptibility patterns before and after curing were compared to determine the contribution of plasmid-borne resistance determinants. Restoration of susceptibility following plasmid elimination was interpreted as evidence of plasmid-mediated antimicrobial resistance, whereas persistence of resistance suggested chromosomal or other non-plasmid-mediated resistance mechanisms.

**Quality Control**

Quality control procedures were implemented throughout the study. Sterility of media and reagents was routinely monitored, and all experiments were performed to ensure precision and reproducibility.

**Data Analysis**

Experimental data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 25.0. Differences in antimicrobial susceptibility before and after plasmid curing were evaluated using appropriate parametric or non-parametric statistical tests and statistical significance was accepted at $P < 0.05$.

**Ethical Considerations**

Ethical approval was obtained from the Cross River State Health Research Ethics Committee, Cross River State Ministry of Health, Nigeria (REC No. CRSMOH/RP/REC/2021/181). Written informed consent was obtained from all participants prior to sample collection and enrolment in the study, and all procedures involving human participants were conducted in accordance with established ethical standards and the principles of the Declaration of Helsinki.

## RESULTS AND DISCUSSION

**Plasmid Profiling and Post-Plasmid Curing of MDR-SSI Infection Bacterial Isolates**

Plasmid profiling of the 8 selected multidrug-resistant (MDR) bacterial isolates recovered from surgical site infections patients demonstrated that all isolates harboured plasmid DNA. Agarose gel electrophoresis of the extracted plasmid DNA revealed a common plasmid band with an estimated molecular size of approximately 3 kb in all 8 MDR isolates examined (Figure 1). The electrophoretic migration pattern showed distinct plasmid bands and the distance travelled by the plasmids following electrophoresis confirmed successful plasmid extraction and profiling. Among the analysed isolates, one multidrug-resistant *Staphylococcus aureus* isolate (lane 6) possessed an additional plasmid band of approximately 5 kb, indicating the presence of multiple plasmids within the same bacterial cell (Table 1; Figure 1).

The widespread occurrence of plasmids among all MDR-SSI isolates in this study, strongly suggests that plasmid-mediated genetic determinants contributed substantially to the multidrug-resistant phenotype observed in bacterial pathogens associated with surgical site infections, which correlates with findings from other related studies.[14,15,16,17] Furthermore, the presence of multiple plasmids within the *S. aureus* isolate indicates the possible acquisition of multiple resistance determinants through horizontal gene transfer, thereby facilitating dissemination of multidrug resistance within healthcare settings. Similar observations have been reported by Badger-Emeka et al.[14], who demonstrated widespread plasmid carriage in multidrug-resistant clinical isolates among diabetic wound patients. The relatively small molecular sizes of the plasmids detected in this study are comparable with previous reports demonstrating that low-molecular-weight plasmids frequently harbour antimicrobial resistance determinants capable of rapid dissemination among both Gram-positive and Gram-negative bacteria.[14,15,16,17] Although plasmid size alone cannot identify the resistance genes present, the consistent detection of the 3 kb plasmid among all isolates suggests that this plasmid may contain conserved resistance determinants contributing to the multidrug-resistant phenotype. The additional 5 kb plasmid detected in *S. aureus* may represent supplementary resistance or adaptive genetic elements, highlighting the genetic diversity of SSI pathogens.

**Table 1: Plasmid profiles of 8 selected MDR surgical site infection isolates.**

| Lane | Bacterial isolates | Number of plasmids detected | Estimated plasmid size (kb) |
|---|---|---|---|
| 1 | *E. cloacae* | 1 | 3 |
| 2 | *E. coli* | 1 | 3 |
| 3 | *K. pneumoniae* | 1 | 3 |
| 4 | *P. mirabilis* | 1 | 3 |
| 5 | *A. baumannii* | 1 | 3 |
| 6 | *S. aureus* | 2 | 3 and 5 |
| 7 | *P. aeruginosa* | 1 | 3 |
| 8 | *S. epidermidis* | 1 | 3 |

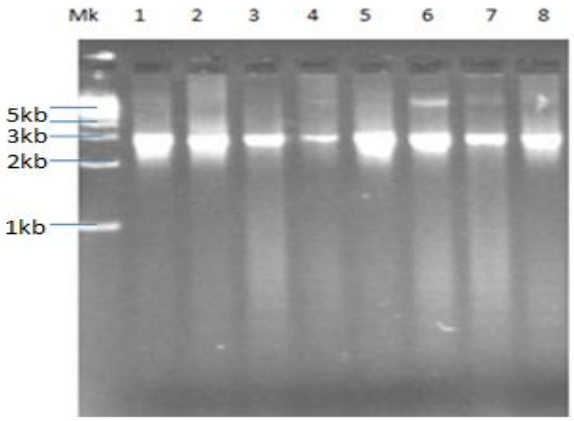


**Fig. 1: Agarose gel electrophoresis showing plasmid profiles of 8 SSI-MDR bacterial isolates. All isolates contained 3 kb plasmid, while Lane 6 (*S. aureus*) shows an additional 5 kb plasmid. The bands migration distance confirmed plasmid extraction and molecular weight estimation.**

Following treatment with ethidium bromide, complete plasmid curing was achieved in all 8 MDR-SSI bacterial isolates. Agarose gel electrophoresis of the cured isolates demonstrated complete disappearance of both the common 3 kb plasmid and the additional 5 kb plasmid previously detected in the *S. aureus* isolate (Figure 2; Table 2). The absence of detectable plasmid DNA confirmed successful elimination of both single and multiple plasmids from the bacterial cells, indicating that the plasmid curing protocol effectively disrupted plasmid replication and maintenance. These findings provide strong evidence that the resistance plasmids harboured by the 8 MDR-SSI isolates were susceptible to chemical curing. Similar observations have been reported in previous studies, where successful elimination of resistance plasmids was observed, following treatment with plasmid-curing agents.[12,16] Plasmid curing therefore remains a valuable experimental approach for differentiating plasmid-mediated resistance from chromosomal resistance and for investigating the molecular basis of antimicrobial resistance.

**Table 2: Plasmid curing outcome among 8 MDR-resistant surgical site infection isolates.**

| Lane | Isolates | Plasmid(s) before curing | Plasmid(s) after curing | Curing outcome |
|---|---|---|---|---|
| 1 | *E. cloacae* | 3 kb | None detected | Complete curing |
| 2 | *E. coli* | 3 kb | None detected | Complete curing |
| 3 | *K. pneumoniae* | 3 kb | None detected | Complete curing |
| 4 | *P. mirabilis* | 3 kb | None detected | Complete curing |
| 5 | *A. baumannii* | 3 kb | None detected | Complete curing |
| 6 | *S. aureus* | 3 kb and 5 kb | None detected | Complete curing |
| 7 | *P. aeruginosa* | 3 kb | None detected | Complete curing |
| 8 | *S. epidermidis* | 3 kb | None detected | Complete curing |

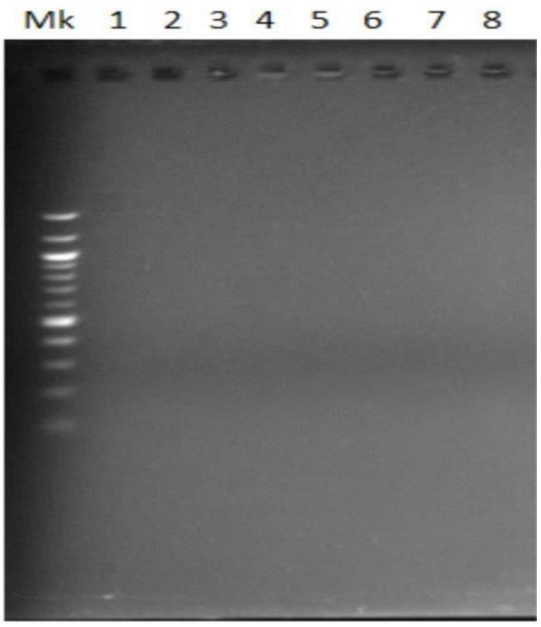


**Fig. 2: Agarose gel electrophoresis of plasmid-cured MDR- SSI isolates showing complete elimination of plasmid DNA following ethidium bromide treatment. No plasmid bands were detected in the cured isolates, confirming complete curing of both single and multiple plasmids.**

**MDR Profiles of SSI Isolates and Antimicrobial Susceptibility Following Plasmid Curing**

Prior to plasmid curing, the MDR-SSI bacterial isolates exhibited resistance to at least three classes of antibiotics, confirming their multidrug-resistant phenotype (Table 3). In contrast, the post-plasmid-cured isolates demonstrated a marked increase in susceptibility to three selected conventional antibiotics compared with their pre-curing susceptibility profiles (Table 4).

Notably, susceptibility to ciprofloxacin, tetracycline, and chloramphenicol increased significantly following plasmid elimination. Among the antibiotics evaluated, ciprofloxacin produced the largest zones of inhibition and exhibited significantly greater antibacterial activity than tetracycline and chloramphenicol ($P < 0.05$). These findings suggest that resistance to these antibiotics was predominantly plasmid-mediated and that removal of the resistance plasmids effectively restored bacterial susceptibility.

Similar observations have been reported about significant reductions in antimicrobial resistance following plasmid curing of MDR bacterial isolates.[12,17,18.19] Collectively, these results highlight the pivotal role of plasmids in the dissemination of multidrug resistance among SSI pathogens. There is also need for routine surveillance of resistance plasmids and continued investigation of complementary sources of antimicrobial agents for the treatment of multidrug-resistant surgical site infections.[4,11,20]

**Table 3: Multi-drug resistance profile of 8 SSI-MDR test isolates to selected before plasmid curing.**

| S/N | MDR-SSI Isolates | Antibiotics resistance profile of isolates | Number of antibiotics resisted (a) | Total number of antibiotics tested (b) | MAR index $(a/b)$ |
|---|---|---|---|---|---|
| 1 | *S. aureus* | PIP, AMP, OXA, AK, LEV, CIP, SXT, CLN, MEM | 9 | 13 | 0.7 |
| 2 | *S. epidermidis* | PIP, AMP, OXA, TOB, TE, CIP, SXT | 7 | 10 | 0.7 |
| 3 | *E. cloacae* | CRO, CEF, CAZ, PIP, AMP, TOB, TE, LEV, CIP | 9 | 15 | 0.6 |
| 4 | *E. coli* | CRO, CZN, CEF, PIP, AMP, MEM, TOB, GM, TE, LEV, AMS | 11 | 15 | 0.7 |
| 5 | *K. pneumoniae* | CZN, CEF, PIP, AMP, TOB, GM, TE, LEV, SXT | 9 | 15 | 0.6 |
| 6 | *P. mirabilis* | CEF, CAZ, AMP, TOB, AK, TE, CIP, SXT | 8 | 15 | 0.5 |
| 7 | *A. baumannii* | CEF, CAZ, PIP, TOB, TE, CIP, AMS | 7 | 13 | 0.5 |
| 8 | *P. aeruginosa* | CZN, CEF, CAZ, MEM, AK, CIP | 6 | 10 | 0.6 |

Keys: CRO-Ceftriaxone, CEF-Cefepime, CAZ-Ceftazidime, PIP-Piperacillin, AMP-Ampicillin, TOB-Tobramycin, TE-Tetracycline, LEV-Levofloxacin, CIP-Ciprofloxacin, AMS-Ampicillin-Sulbactam, MEM-Meropenem, GM-Gentamicin, AK-Amikacin, OXA-Oxacillin, SXT-Trimethoprim-sulfamethoxazole, CZN-Cefazolin, CLN-Clindamycin, MAR index ≤ 0.2: Low risk resistant isolates, MAR index ≥ 0.2: High risk resistant isolates, Key: SAUTHC: SSI-Associated Unique Test Hospital-Isolate Code.

**Table 4: Antimicrobial susceptibility profile of SSI-MDR isolates to 3 selected conventional antibiotics before and after plasmid curing.**

| MDR-SSI test isolates | Antibiotic activity/inhibition zone diameter (mm). | | | | | |
|---|---|---|---|---|---|---|
| | Before plasmid curing | | | Post-plasmid curing | | |
| | Ciprofloxacin | Tetracycline | Chloramphenicol | Ciprofloxacin | Tetracycline | Chloramphenicol |
| *E. cloacae* | 18.0 | 12.0 | 14.0 | 38.0 | 25.0 | 29.0 |
| *E. coli* | 16.0 | 10.0 | 13.0 | 36.0 | 22.0 | 25.0 |
| *K. pneumoniae* | 10.0 | 0.0 | 10.0 | 34.0 | 15.0 | 17.0 |
| *P. mirabilis* | 18.0 | 11.0 | 14.0 | 38.0 | 24.0 | 29.0 |
| *A. baumannii* | 14.0 | 10.0 | 11.0 | 35.0 | 21.0 | 24.0 |
| *S. aureus* | 15.0 | 11.0 | 12.0 | 32.0 | 25.0 | 27.0 |
| *P. aeruginosa* | 18.0 | 10.0 | 13.0 | 39.0 | 24.0 | 28.0 |
| *S. epidermidis* | 15.0 | 11.0 | 12.0 | 35.0 | 23.0 | 26.0 |

Keys: SSI – Surgical-site infection, MDR – Multi-drug resistance, IZD – Inhibition zone diameter.

## CONCLUSION

This study demonstrated that multidrug-resistant bacterial isolates associated with surgical site infections harboured plasmids that played a significant role in mediating antimicrobial resistance. Complete plasmid curing resulted in enhanced susceptibility of the isolates to conventional antibiotics, confirming the contribution of plasmid-borne resistance determinants to the multidrug-resistant phenotype. These findings underscore the importance of plasmid surveillance in antimicrobial resistance monitoring and support the continued investigation for complementary therapeutic agents for the management of MDR surgical site infections.

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