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Original Research Article

Plasmid-Associated Antibiotic-Resistant Characteristics of Pseudomonas Species Isolated From Wound Infections

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Abstract

Background: Pseudomonas aeruginosa is a major cause of wound infections and often shows multidrug resistance (MDR), frequently mediated by plasmids that enable horizontal gene transfer. Data from Kerala during 2013 were limited.

Aim and Objectives: To characterize plasmid-mediated antibiotic resistance in Pseudomonas isolates from wound infections.

Materials and Methods: Ten wound swab samples collected in Kerala between May and September 2013 were cultured on selective media and identified using standard biochemical tests. Antibiotic susceptibility was assessed using the disc diffusion method. Plasmid DNA was extracted by the alkaline lysis method and visualised by agarose gel electrophoresis. Plasmid curing was performed using 1% sodium dodecyl sulphate (SDS) at 37°C. The ability of plasmids to transfer resistance was evaluated by conjugation experiments with competent Escherichia coli DH5α.

Results: Five Pseudomonas isolates were recovered (50% positivity). All isolates showed 100% resistance to ampicillin, amoxicillin, gentamicin, cloxacillin, and ciprofloxacin, while remaining susceptible to chloramphenicol and penicillin G. Plasmids were detected in all resistant isolates. After plasmid curing, susceptibility to the five antibiotics was completely restored and plasmid bands disappeared. Conjugation experiments demonstrated successful transfer of ampicillin resistance to E. coli.

Conclusion: The study shows that plasmids play an important role in transferable multidrug resistance in wound-derived Pseudomonas isolates from Kerala. Continuous resistance surveillance and effective antimicrobial stewardship are necessary to control plasmid-mediated MDR.

Keywords: Pseudomonas species, wound infections, plasmid-mediated resistance, antibiotic susceptibility, plasmid curing, horizontal gene transfer, multidrug resistance.

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Introduction

Pseudomonas species, particularly Pseudomonas aeruginosa, are important opportunistic pathogens responsible for a wide range of healthcare-associated infections, including wound infections. These infections are commonly observed in surgical wounds, burn injuries, and traumatic wounds. [1] Wound infections caused by Pseudomonas are clinically significant because they are often associated with delayed healing, increased hospital stay, and higher treatment costs.

These Gram-negative bacteria possess several intrinsic resistance mechanisms and are also capable of acquiring additional resistance determinants through mobile genetic elements such as plasmids. Plasmid-mediated resistance plays a crucial role in horizontal gene transfer (HGT), facilitating the rapid spread of multidrug-resistant (MDR) strains in hospital environments. In India, during the early 2010s, P. aeruginosa emerged as one of the predominant bacterial isolates from postoperative wound infections, with prevalence rates reported between 18% and 30% in various tertiary care centres. [1,2] High levels of resistance were frequently observed against commonly used antibiotics including β-lactams (such as ampicillin and amoxicillin), aminoglycosides (such as gentamicin), and fluoroquinolones (such as ciprofloxacin). Many of these resistance traits were associated with plasmid-borne resistance genes. [3]

Previous studies have also demonstrated that plasmid curing could reverse antibiotic resistance phenotypes, indicating that several resistance

determinants are located on extrachromosomal plasmids. [3,4] In addition, experimental evidence has shown that plasmids carrying resistance genes can be transferred from Pseudomonas species to Escherichia coli, highlighting the potential for interspecies dissemination of antibiotic resistance under selective antibiotic pressure. [5]

Despite the growing concern regarding plasmid-mediated resistance in Pseudomonas, limited studies have specifically investigated the plasmid-associated antibiotic resistance patterns in wound isolates in clinical settings. Therefore, the present study was undertaken to characterise plasmid-associated antibiotic resistance in Pseudomonas isolates obtained from wound infections

Objectives:

Primary Objective: To characterise plasmid-associated antibiotic resistance in Pseudomonas species isolated from wound infections in a Kerala setting during 2013.

Secondary Objectives:

1. To isolate Pseudomonas species from clinical wound swab specimens using selective and differential culture media.
2. To confirm isolate identity as Pseudomonas species by Gram staining, motility testing, cultural characteristics, and standard biochemical tests.
3. To determine antibiotic susceptibility profiles of the isolates using the disc diffusion method.
4. To isolate and visualise plasmid DNA from resistant isolates by alkaline lysis and agarose gel electrophoresis.
5. To perform plasmid curing on resistant isolates using SDS treatment and confirm plasmid loss by agarose gel electrophoresis.
6. To re-evaluate antibiotic susceptibility of plasmid-cured derivatives to assess reversal of resistance.
7. To evaluate conjugative transfer of resistance plasmids to competent E. coli DH5α cells.

Materials and Methods:

Study Design: This study was a descriptive, laboratory-based cross-sectional investigation conducted to evaluate plasmid-associated antibiotic resistance in Pseudomonas isolates obtained from wound infections.

Study Setting and Sample Collection: The investigation was carried out at Unibiosys Biotech Research Laboratories, Kerala, India, over a period of five months from May to September 2013. A total of ten consecutive wound swab samples, including surgical wounds, burn wounds, and traumatic wounds, were collected from patients attending local healthcare facilities.

All samples were transported to the laboratory in sterile Stuart's transport medium and processed immediately upon arrival to ensure sample integrity. No exclusion criteria were applied other than the availability of suitable clinical samples.

Study Variables:

The primary variables assessed in this study included:

- Identity of bacterial isolates
- Antibiotic susceptibility profile
- Presence of plasmids
- Antibiotic susceptibility after plasmid curing
- Ability of plasmids to transfer antibiotic resistance through conjugation

All laboratory measurements were performed according to standard microbiological protocols commonly used during the study period (2013).

Bias Control: To minimise potential bias, consecutive sampling was used and all specimens were processed promptly after collection. Standardised microbiological procedures were followed throughout the study to reduce measurement variability.

Study Size: Ten wound swab samples were initially processed. Among these, five isolates were identified as Pseudomonas species and were included in the final analysis.

Quantitative Variables: Antibiotic resistance patterns were expressed as percentages. Plasmid DNA was detected through agarose gel electrophoresis and recorded qualitatively based on the presence or absence of plasmid bands.

Statistical Analysis: Data were recorded and analysed using Microsoft Excel 2007. Descriptive statistics were applied, and isolation rates as well as antibiotic resistance frequencies were calculated as percentages. Due to the small sample size (n = 5), inferential statistical analysis was not performed. Changes in antibiotic susceptibility following plasmid curing and evidence of plasmid transfer were evaluated qualitatively.

Isolation and Identification of Bacteria: Clinical specimens were inoculated onto nutrient agar, blood agar, MacConkey agar, Pseudomonas F agar, and Pseudomonas P agar. The plates were incubated aerobically at 37°C for 24–48 hours.

Presumptive Pseudomonas colonies were identified based on colony morphology, Gram staining, hanging-drop motility test, and standard biochemical tests.

Antibiotic Susceptibility Testing: Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar. The antibiotic discs used included

ampicillin (10 µg), amoxicillin (30 µg), chloramphenicol (30 µg), penicillin G (10 units), gentamicin (10 µg), cloxacillin (1 µg), and ciprofloxacin (5 µg).

The inhibition zones were measured and interpreted according to the standard guidelines available at the time of the study.

Plasmid Isolation and Agarose Gel Electrophoresis: Plasmid DNA was extracted from overnight cultures grown in Luria–Bertani (LB) broth supplemented with 50 µg/mL ampicillin using the alkaline lysis method. The extracted plasmid DNA was separated on 1% agarose gel electrophoresis run at 100 V for approximately 80 minutes. DNA bands were visualised under ultraviolet (UV) illumination.

Plasmid Curing: To determine the role of plasmids in antibiotic resistance, plasmid curing was performed by exposing bacterial cultures to 1% sodium dodecyl sulphate (SDS) at 37°C for 24 hours. Colonies obtained after treatment were screened for plasmid loss using agarose gel electrophoresis and were subsequently re-tested for antibiotic susceptibility.

Gene Transfer Experiments: Competent Escherichia coli DH5α cells were prepared using calcium chloride ($CaCl_2$) treatment. These competent cells were co-incubated with Pseudomonas donor cultures in LB broth at 37°C for three days to facilitate plasmid transfer. Tran's conjugant colonies were selected on LB agar plates containing ampicillin (50 µg/mL).

Ethical Considerations: The study was conducted in accordance with standard ethical guidelines for research involving clinical samples. Informed consent was obtained where required, and the study procedures were carried out under institutional research oversight.

Results:

Participants and Descriptive Data: A total of ten wound swab samples were analysed during the study period. Among these, five samples yielded confirmed Pseudomonas isolates, giving a positivity rate of 50%. The demographic characteristics of patients and the corresponding isolate details are presented in Table 1. The identified isolates were obtained from different wound types, including general wound infections, burn wounds, and surgical wounds. The ages of patients ranged from 38 to 68 years, and both male and female patients were represented.

Table 1: Patient Demographics and Isolate Details

No.	Strain No.	Sample Type	Age (Years)	Sex
1	PM01	Wound	46	F
2	PM02	Wound	68	M
3	PM03	Burn wound	38	F
4	PM04	Surgical wound	38	M
5	PM05	Surgical wound	44	M

All isolates showed characteristic biochemical properties consistent with Pseudomonas species, including Gram-negative rod morphology, positive motility, oxidase positivity, and catalase positivity, while tests for indole, methyl red, and Voges–Proskauer were negative. The biochemical confirmation results are summarized in Table 2.

Table 2: Biochemical Confirmation of Isolates

Test	Result (All Isolates)
Gram stain	Negative rods
Motility	Positive
Oxidase	Positive
Catalase	Positive
Indole	Negative
Methyl Red	Negative
Voges-Proskauer	Negative

Outcome Data

Antibiotic Resistance Profile: Antibiotic susceptibility testing demonstrated a high level of resistance among the isolates. All five isolates showed 100% resistance to ampicillin, amoxicillin, gentamicin, cloxacillin, and ciprofloxacin. In contrast, complete susceptibility (0% resistance) was observed for chloramphenicol and penicillin G. The detailed resistance profile is presented in Table 3.

Plasmid Detection: Agarose gel electrophoresis revealed the presence of plasmid DNA in all antibiotic-resistant isolates, suggesting that the observed resistance patterns may be associated with plasmid-mediated mechanisms.

Table 3: Initial Antibiotic Resistance Profile (n=5)

Antibiotic	Resistant (%)
Ampicillin	100
Amoxicillin	100
Gentamicin	100
Cloxacillin	100
Ciprofloxacin	100
Chloramphenicol	0
Penicillin G	0

Effect of Plasmid Curing: Following plasmid curing using sodium dodecyl sulphate (SDS), all previously resistant isolates demonstrated complete restoration of antibiotic susceptibility. Agarose gel electrophoresis confirmed the disappearance of plasmid bands after curing, indicating successful plasmid elimination. The comparison of resistance patterns before and after curing is presented in Table 4.

Table 4: Susceptibility Restoration Post-Curing (n=5)

Antibiotic	Resistant Before Curing (%)	Resistant After Curing (%)
Ampicillin	100	0
Amoxicillin	100	0
Gentamicin	100	0
Cloxacillin	100	0
Ciprofloxacin	100	0

Gene Transfer Experiments: Conjugation experiments demonstrated successful transfer of ampicillin resistance from Pseudomonas isolates to Escherichia coli DH5α recipient cells. The appearance of resistant E. coli colonies on selective media confirmed the ability of plasmids to mediate horizontal transfer of antibiotic resistance.

Discussion

The present study showed a 50% isolation rate of Pseudomonas species from wound swab samples (5 out of 10 samples). This rate is higher than many studies from India during the same period, where the prevalence of P. aeruginosa in wound or postoperative infections was usually 18%–30% in tertiary care hospitals. [1,2,5] Ranjan et al. reported 29.6% prevalence in postoperative wounds in Haryana. [1] Similarly, Chaudhari et al. reported 18–30% prevalence in central India. [2] Studies from southern India also showed somewhat lower rates ranging from 14.5% to 21.8%. [6,7]

The higher isolation rate in this study may be due to the different types of wounds included, such as burn wounds, surgical wounds, and traumatic wounds. In addition, local environmental conditions and the selection of severe clinical cases may have influenced the results.

Another important finding of this study was that all isolates showed 100% resistance to ampicillin, amoxicillin, gentamicin, cloxacillin, and ciprofloxacin. Similar multidrug resistance (MDR) patterns have been reported in many Indian studies during the early 2010s. [1,2,4,5,8] .The high resistance to these antibiotics is often linked to frequent and uncontrolled use of antibiotics in clinical settings, which creates strong selection pressure for resistant bacteria.

Plasmid analysis showed that all resistant isolates contained plasmids, and after plasmid curing, the isolates became fully susceptible to the antibiotics tested. The disappearance of plasmid bands after curing confirms that the resistance genes were plasmid-mediated. Similar findings have been reported in earlier studies where R-plasmids played a major role in antibiotic resistance among wound and burn isolates of Pseudomonas. [3,4] In addition, the transfer of ampicillin resistance to Escherichia coli during conjugation experiments shows that these plasmids can move between bacteria. This indicates the possibility of horizontal gene transfer (HGT), which allows antibiotic resistance genes to spread rapidly between different bacterial species. [9] In the Kerala and southern India region, these findings suggest that plasmid-mediated multidrug resistance may be an important factor in wound infections caused by Pseudomonas.10 Limited surveillance data from this region during the study period makes it difficult to fully understand the resistance trends, but later studies have also reported continued resistance to aminoglycosides and fluoroquinolones in Pseudomonas. [6,7]

Interestingly, the isolates in this study were susceptible to chloramphenicol and penicillin G, which is unusual for P. aeruginosa. This may be due to strain variation or methodological variation in disc interpretation, or limitations of phenotypic

identification, but it does not reduce the overall concern about multidrug resistance

Limitations: This study has several limitations. The sample size was small (n = 5), which limits the ability to generalize the findings. Only phenotypic methods were used, and no molecular techniques were performed for species confirmation or resistance gene detection. The observed susceptibility to penicillin G, which is uncommon for Pseudomonas aeruginosa, may require further investigation. In addition, the frequency of plasmid transfer during conjugation was not quantified

Generalizability: Despite these limitations, the findings highlight the potential role of plasmid-mediated multidrug resistance (MDR) in wound-associated Pseudomonas infections. The results support the need for broader surveillance and antimicrobial stewardship programmes across India to monitor and control plasmid-mediated resistance.

Conclusion

This study demonstrates that plasmids contribute to transferable multidrug resistance in wound-derived Pseudomonas isolates from Kerala during 2013. Plasmid curing restored antibiotic susceptibility, and conjugation experiments confirmed the ability of resistance plasmids to transfer between bacterial species. These findings emphasize the importance of continuous resistance surveillance and responsible antibiotic use in clinical settings.

Recommendations: Routine microbiology laboratories should consider including plasmid surveillance in diagnostic workflows**.** Further research is needed to explore strategies that target plasmids and limit the spread of plasmid-mediated antibiotic resistance**.**

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request**.**

References

1. Ranjan KP, et al. Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana, India. Indian J Pathol Microbiol. 2010;53(3):495-498.
2. Chaudhari VL, Gunjal SS, Mehta M. Antibiotic resistance patterns of Pseudomonas aeruginosa in a tertiary care hospital in Central India. Int J Med Sci Public Health. 2013;2(2):386-389.
3. Shahid M, et al. Multidrug-resistant Pseudomonas aeruginosa strains harbouring R-plasmids and AmpC β-lactamases isolated from hospitalised burn patients in a tertiary care hospital of North India. FEMS Microbiol Lett. 2003;228(2):181-186.
4. Joseph NM, Devi S, Shashikala P, Kanungo R. Changing trend in the antibiotic resistance pattern of Pseudomonas aeruginosa isolated from wound swabs of out-patients and in-patients of a tertiary care hospital. J Clin Diagn Res. 2013;7(10):2170-2172.
5. Patel J, Javiya V, Ghatak S, Patel K. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian J Pharmacol. 2008;40(5):230-234.
6. Senthamarai S, et al. Resistance pattern of Pseudomonas aeruginosa in a tertiary care hospital of Kanchipuram, Tamilnadu, India. J Clin Diagn Res. 2014;8(5):DC30-DC32.
7. Bangera D, et al. Clinico-microbiological study of Pseudomonas aeruginosa in wound infections and the detection of metallo-β-lactamase production. Int Wound J. 2015;12(6):705-711.
8. Rizvi MF, Hasan Y, Memon AR, et al. Pattern of antimicrobial resistance of Pseudomonas aeruginosa in a tertiary care hospital of North India. J Ayub Med Coll Abbottabad. 2009;21(1):80-82.
9. Hasegawa H, et al. Horizontal plasmid transfer by transformation in Escherichia coli: environmental factors and possible mechanisms. Front Microbiol. 2018; 9:2369. (Laboratory context)
10. Antibiotic Susceptibility Pattern of Pseudomonas aeruginosa Isolates from Wound Infections in a Tertiary Care Centre in South Kerala, India. Int J Infect Dis Ther. 2018;3(2):39-43.