

Antibiotics Resistance and Plasmid Curing Studies of *Pseudomonas aeruginosa* associated with Wound Infection amongst Patients Accessing a Tertiary Healthcare Facility in Rivers State, Nigeria

Okpukpu, E. N^{*1}., Sampson, T². and Wemedo, S. A².

¹Department of Pharmaceutical Microbiology/Biotechnology, University of Port Harcourt, Rivers State, Nigeria. ²Department of Microbiology, Rivers State University, P.M.B. 5080, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

*Corresponding Author: emeka4nelson@gmail.com.

ABSTRACT

Wound infections present a persistent challenge in healthcare, particularly in developing countries where limited resources affect treatment outcomes. Among causative agents, *Pseudomonas aeruginosa* is of major concern due to its ability to resist multiple antibiotics and survive in hospital settings. The study therefore bothered on Antibiotics Resistance and Plasmid Curing Studies of *Pseudomonas aeruginosa* associated with wound infections amongst patients accessing University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. A total of 150 wound samples were screened using standard microbiological procedures. Antimicrobial susceptibility testing was conducted with the Kirby-Bauer disc diffusion method, as prescribed by CLSI. The study indicted high antibiotics resistance profile, with the percentage of the isolates showing resistance to Cefuroxime (96.3%), Cefixime (92.6%), and Augmentin (92.6%). The isolates were however least resistant to Ciprofloxacin (37.0%). Multiple Antibiotics Resistance (MAR) index values varied, with most of the isolates having MAR index value that exceeded 0.2. Plasmid curing using acidine orange reduced resistance and MAR index values in some strains, demonstrating the role of plasmids in multidrug resistance. The study has confirmed that multidrug resistance in *P. aeruginosa* was plasmid-mediated. Effective treatment should therefore be guided by current laboratory diagnoses. Further research is recommended on the prevalence and mechanisms of *Pseudomonas* species in wound infections to better inform therapeutic approaches.

Keywords: *Pseudomonas aeruginosa*, Antibiotics, Multiple Antibiotic Resistance, Plasmid curing.

Introduction

Wound injuries are serious public health problems worldwide (Ozer *et al.*, 2010). Infection of wounds is a common, often severe and costly complication resulting from bacteria colonization. Traumatic ulcers can become a serious complication resulting from *Pseudomonas* infection. "They are now the most common proximate and nontraumatic causes of leg amputation (Hayat *et al.*, 2011).

Pseudomonas aeruginosa is often preliminarily identified by its pearlescent appearance and grape-like or tortilla-like odour *in vitro*. It can be responsible for a spectrum of presentations from superficial colonization of ulcers to extensive tissue damage, including osteomyelitis, septic arthritis and bacteremia

(Edmond, 2009). *P. aeruginosa* and *Staphylococcus aureus* are the most commonly. *Pseudomonas aeruginosa* is a ubiquitous Gram-negative bacterium belonging to the family *Pseudomonadaceae* that is able to survive in a wide range of environment (Silby *et al.*, 2011). The organism is an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infections and leads to substantial morbidity in immune compromised patients. Despite therapy, the mortality due to nosocomial *Pseudomonas* sp is approximately 70% (Wahab *et al.*, 2013).

Pseudomonas aeruginosa is commonly resistant to antibiotics, and because of this, it is a dangerous and dreaded pathogen (Sivanmaliappan and Sieunaine, 2011). Forty four percent (44%) of *P. aeruginosa* are multi drug resistant (Driver *et al.*, 2010).

In antimicrobial resistance pattern using Mueller Hinton agar-based study of *P. aeruginosa* isolated from wound ulcers of foot, multidrug resistance for about 8 to 11 antibiotics was shown among 55.5% of the strains. There was no single antibiotic which showed 100% sensitivity to all *P. aeruginosa* strains. Cefotaxime showed the least resistance of 16.6% while ciprofloxacin followed with an intermediate resistance of 66.7%. Ciprofloxacin and cefotaxime were observed to be better choices for patients with foot ulcers in this part of the region when compared to gentamicin, imipenem, piperacillin, and other third-generation cephalosporins (Sivanmaliappan and Sieunaine, 2011). The study indicated a high resistance of the isolates to most of the antibiotics used with 96.30% of the isolates resistant to Cefruoximne. The high resistance could be indicative of inappropriate use or abuse of antibiotic by the patients. The resistance could also be plasmid related. *Pseudomonas aeruginosa* develops resistance to most of antibiotics (Okpukpu et al., 2022). However, *Pseudomonas* resistant to imipenem were observed as a new problem mostly in hospitalized patient (Chalya et al., 2011). Imipenem resistance was however observed in (61.2%) of the isolates in Brazil (Khanakar et al., 2008). Studies have revealed that burns are associated with high percentage occurrence of *Pseudomonas aeruginosa*. These could be as a result of the fact that wounds from surgical site are exposed to many ubiquitous environmental pathogens which include *P. aeruginosa* found in unsterile surface, water and soil (Okpukpu et al., 2022). The increasing level of resistance of *Pseudomonas aeruginosa* to antibiotics could be as a result of the presence of plasmids or resistance genes.

The study therefore bothered on Antibiotics Resistance and Plasmid Curing Studies of *Pseudomonas aeruginosa* associated with wound infections amongst patients accessing University of Port Harcourt Teaching Hospital, Rivers State, Nigeria.

Materials and Methods

Study Area

The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), located in Choba, Rivers State, Nigeria.

The hospital is geographically positioned at coordinates 4°53'58"N, 6°55'43"E, serving as a major tertiary healthcare facility in the region.

Study Design and Sample Collection

A cross-sectional study design was adopted to determine the resistance patterns of *Pseudomonas aeruginosa* isolated from wound infections in patients attending UPTH. A total of 150 wound swab samples were collected from patients with diverse wound types using sterile swab sticks. Samples were collected aseptically and transported immediately to the Microbiology Laboratory of Rivers State University for analysis.

Sample Size Determination

The sample size for the study was determined by the formula (Niang et al., 2006).

$N = [Z^2(pq)]/d^2$ (Niang et al., 2006) Where:

N= the desired sample size

Z= Normal standard distribution that corresponds to confidence interval as 1.96 p= Prevalence of *Pseudomonas* species q = 1-p

d= degree of accuracy / precision expected at 0.05

Isolation of *Pseudomonas aeruginosa*

Swab specimens were streaked on sterile Cetrimide agar plates and incubated at 37°C for 24 hours. Colonies with characteristic morphology were sub-cultured repeatedly on freshly prepared nutrient agar to obtain pure isolates (Sampson et al., 2020). The isolates were preserved on nutrient agar slants in Bijou bottles at 4°C.

Preparation of 0.5M Mcfarland Turbidity Standard

To standardize bacterial inoculum density, a 0.5 McFarland turbidity standard was prepared by mixing 1% v/v sulfuric acid with 1% w/v barium chloride solution. Specifically, 0.6 ml of barium chloride solution was added to 99.4 ml of sulfuric acid solution, producing a suspension equivalent to 1.5×10^8 CFU/ml, which was stored in sealed tubes at room temperature (Sampson et al., 2022).

Antibiotic Susceptibility Test by Disc Diffusion Method

A 0.1ml volume of the standardized culture was aseptically inoculated into a 20 ml molten Mueller Hinton agar and gently swirled to effect mixing. The medium was poured aseptically into a sterile Petri dish and allowed to solidify. The plate was labelled appropriately. This was repeated for rest of the isolated culture.

After solidifying, a sterile forcep was used to implant the commercial multi-antibiotics disc onto the surface of the medium aseptically. This culture medium was incubated at 37 °C for 24 hours. After the incubation period, Petri dishes were examined and the zone of inhibition across the various antibiotics was determined (CLSI, 2017)

Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance is the resistance of isolates of *psseudomonas aeruginosa* to three or more antibiotics (Sampson et al., 2022). The Multiple Antibiotics Resistance (MAR) Index was determined using the formulae:

$$A/B$$

Where:

A= The number of antibiotics the organisms were resistant to

B= The number of antibiotics tested or used in the study.

Plasmid Curing

The gene reversal was done as described by Alexander et al., (2023). A 0.1 mg/ml of Acredine orange was added into 100 ml of Nutrient broth. The solution was autoclaved at 121°C at 15 psi for 15 min. An overnight culture of the sample was standardized according to 0.5 McFarland standard and 0.5ml from the standardized solution was pipette using Pasteur pipette into the 100ml sterile Nutrient broth. The solution was incubated at 37°C for 4 hours. After incubation, the isolates were-inoculated into a sterile nutrient broth and incubated for 24 hours (Oyekele et al., 2008).

Results

The result of antimicrobial susceptibility of *Pseudomonas* sp before plasmid curing showed that the population (percentage) of *Pseudomonas* species that showed resistance to standard antibiotics tested ranged from 37.04% - 96.30%. The highest percentage of *Pseudomonas* species resistance was seen in Cefuroxime with 96.30 % of the isolates showing resistance. This was followed by Cefixime and Augmentine with 92.59% each. The study indicated that 88.88% of the isolates showed resistance to Cefixime, Augmentin and Nitrofurantoin. While 51.85% showed resistance to Gentamycin, Ceftazidime and Ofloxacin had 48.15% of the isolates showing resistance. The least percentage resistance by *Pseudomonas* isolates was however seen in Ciprofloxacin with 37.04% (Figure 1).

Multiple Antibiotics Resistance (MAR) Index of *Pseudomonas* sp isolated before Plasmid Curing ranged from 0 – 1. The percentage occurrence of the Mar index was evaluated which ranged from 3.70% - 29.63%. The MAR index 0.9 had the highest percentage occurrence of 29.63%, followed by MAR index 0.5 with percentage occurrence of 25.93%. MAR index 1 had a percentage occurrence of 22.22% and MAR index 0.6 had a percentage occurrence of 11.11%. The least percentage occurrence was 3.70% which was observed in MAR index 0, 0.3, and 0.8 (Figure 2).

The highest percentage of antimicrobial resistance of *Pseudomonas* species after plasmid curing was seen in Augmentin with 96.29% of the isolates showing resistance, followed by Cefixime with 92.59%. Trailing behind Cefixime was Cefuroxime with 92.51% of the isolates resistant to the drug. Nitrofurantoin was associated 74.07%, followed by Ceftazidime with 59.26%. Data reported 51.85% resistance to Ciprofloxacin for *Pseudomonas* isolates. The least percentage resistance for *Pseudomonas* isolates was seen in Gentamycin and Ofloxacin with a percentage resistance of 44.44% each (Figure 3). The multiple antibiotics resistance (MAR) index of *Pseudomonas* sp after plasmid curing for the *Pseudomonas* isolated, the MAR index 0.8 had the highest percentage occurrence of 29.63%, followed by MAR index 0.5 with percentage occurrence of 22.22%. MAR index 1 had a percentage occurrence of

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18.52%, MAR index 0.9 had a percentage occurrence of 14.81%, and MAR index 0.6 had a percentage occurrence of 7.41% for the pseudomonas tested.

The result showed that there was reduction in the mar index after the plasmid curing. The study showed that 0.9 had the highest reduction while there were no changes in 0.3 and 0 (Figure 5).

The least percentage occurrence was 3.70% of the *Pseudomonas* isolates which was observed in MAR index 0, and 0.3 (Figure 4).

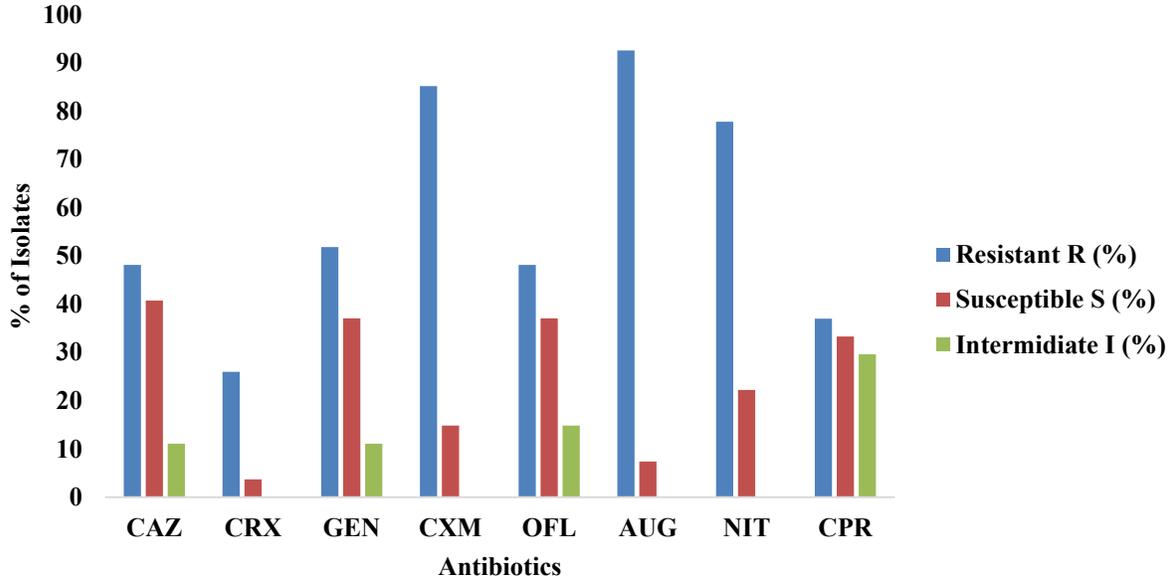


Figure 1: Percentage susceptibility of *Pseudomonas* sp before plasmid

CAZ = Ceftazidime; CRX = Cefuroxime; GEN = Gentamycin; CXM = Cefuroxime; OFL = Ofloxacin AUG = Augmentin; NIT = Nitrofurantoin; CPR = Ciprofloxacin

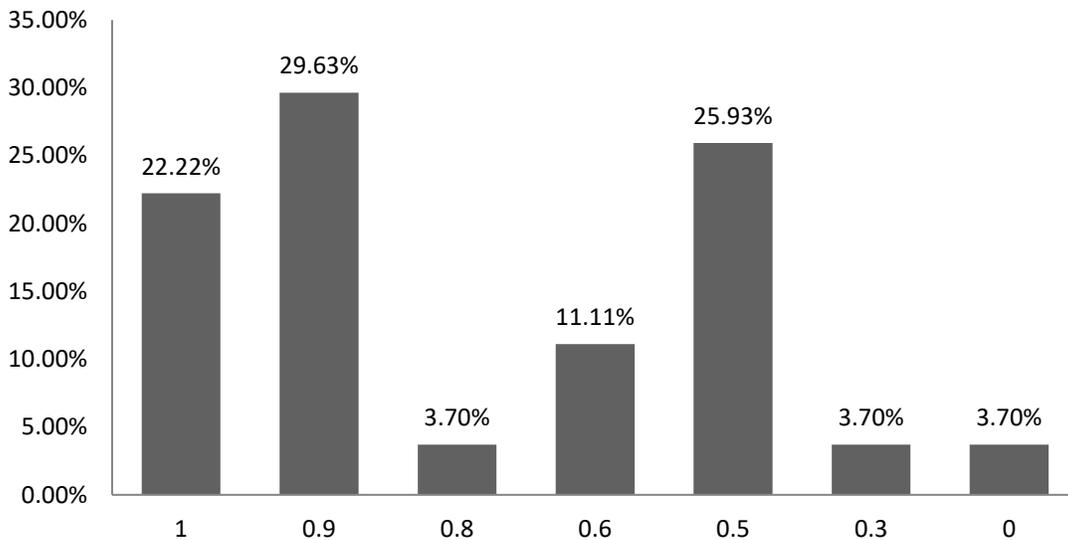


Figure 2: Mar_i of *Pseudomonas* sp isolated from wounds before plasmid curing

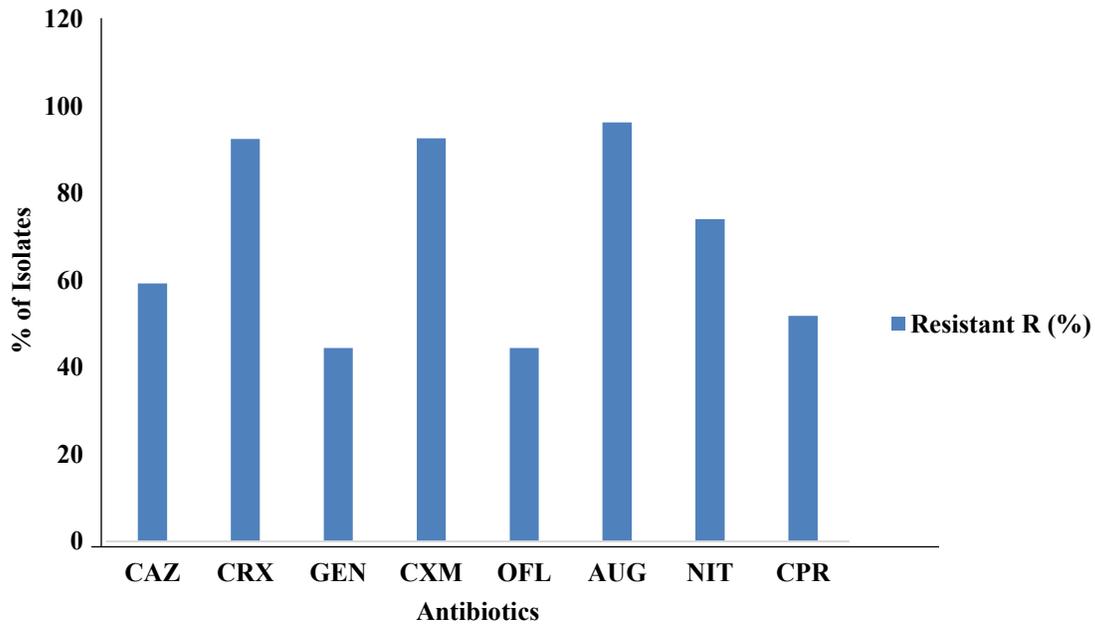


Figure 3: Percentage susceptibility of *Pseudomonas* sp After plasmid

CAZ = Ceftazidime CRX = Cefuroxime GEN = Gentamycin CXM = Cefuroxime OFL = Ofloxacin
 AUG = Augmentin NIT = Nitrofurantoin CPR = Ciprofloxacin

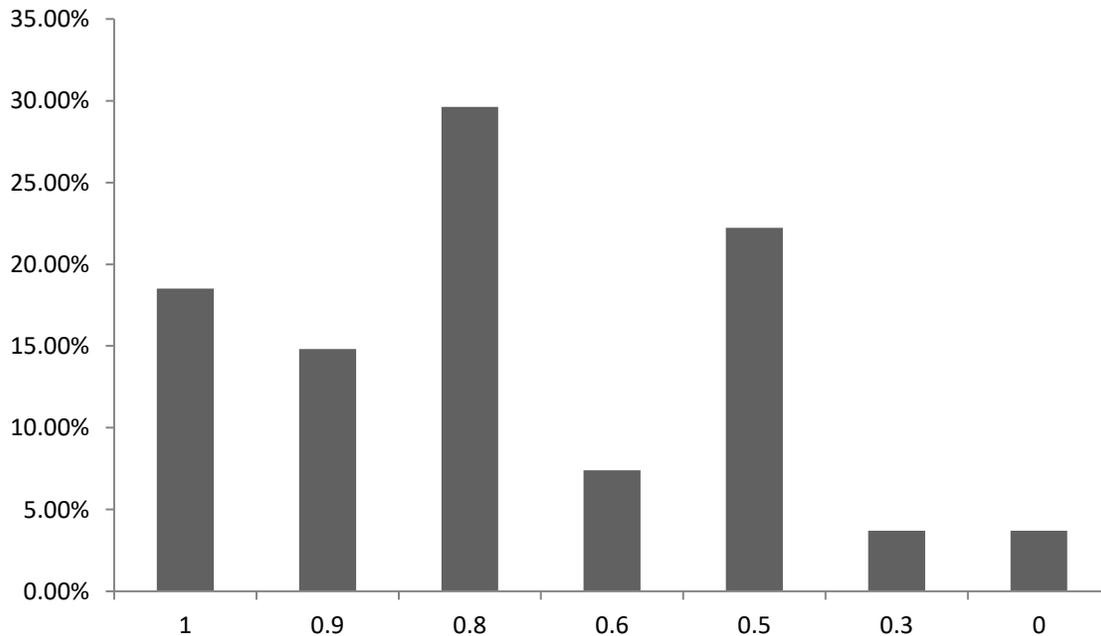


Figure 4: Mar_i of *Pseudomonas* sp isolated from wounds after plasmid curing

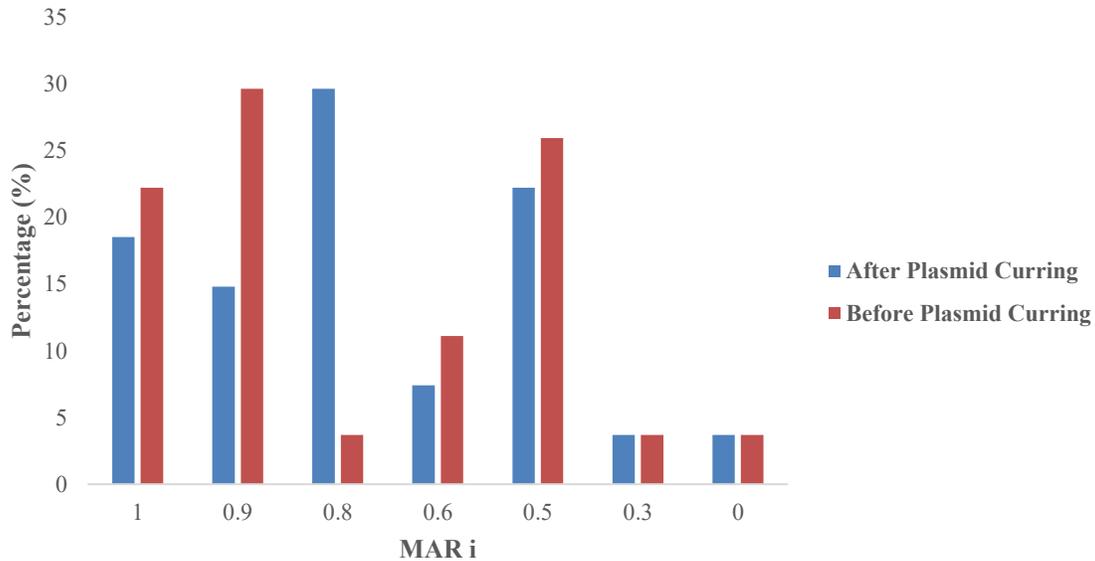


Figure 5: Percentage Occurrence of Mar i. of *Pseudomonas* sp before and after plasmid curing

Discussion

Plasmids in *P. aeruginosa* often carry genes that encode β -lactamases, aminoglycoside-modifying enzymes, and efflux pump components, which together contribute to the bacteria's multidrug resistance (Juan et al., 2017). This plasmid-mediated resistance complicates treatment outcomes and underscores the importance of surveillance and alternative therapeutic strategies (Lister et al., 2009).

A consistently high resistance to cefuroxime was observed in this study, which reaffirms that second-generation cephalosporins offer little or no therapeutic benefit against *P. aeruginosa*. This resistance is largely attributed to the organism's strong β -lactamase activity and reduced cell wall permeability (Oliver et al., 2015). Similar studies have reported resistance rates exceeding 90%, supporting the recommendation that cefuroxime should be excluded from empirical therapy for *P. aeruginosa* infections (Fayyaz et al., 2020). When comparing this antibiogram results with earlier studies, similar patterns is noticed. For example, Walcott et al. (2015) reported that a considerable proportion of *P. aeruginosa* isolates were resistant to fluoroquinolones, while Wathiq et al. (2020) observed varied sensitivity rates, which they attributed to differences in efflux pump gene expression among clinical isolates.

This suggests that resistance in *P. aeruginosa* is influenced by both plasmid-mediated and chromosomal mechanisms, such as efflux pumps like MexAB-OprM (Sadikot et al., 2005).

Importantly, this study showed that plasmid curing with acridine orange reduced resistance levels in *P. aeruginosa*. The decrease in resistance following curing confirms that plasmid-encoded genes play a central role in multidrug resistance (Ranjbar & Farahani, 2019). This finding also highlights plasmid curing or plasmid-targeted approaches as promising strategies for restoring antibiotic susceptibility (Rozwandowicz et al., 2018).

Analysis of the MAR index provided further insights. Post-curing, the MAR index values ranged from 0 to 1. The most common value was 0.8 (29.63%), indicating that substantial resistance persisted, likely due to chromosomal or non-plasmid resistance mechanisms. However, the presence of isolates with lower MAR indices (0 and 0.3, each at 3.70%) showed that plasmid curing was able to restore partial susceptibility.

These results are consistent with recent studies that reported MAR indices between 0.26 and 0.86 in *P. aeruginosa*, with most values above 0.2, reflecting significant multidrug resistance (Onohuean et al., 2021).

Similarly, experimental evidence has confirmed that plasmid curing reduces MAR indices in Gram-negative bacteria, emphasizing the therapeutic potential of targeting plasmid-mediated resistance (Akinjogunla et al., 2021).

Finally, these findings align with those of Wathiq et al. (2020), who observed that some fluoroquinolone-resistant isolates became susceptible after plasmid curing. This suggests that denaturation or removal of plasmid-borne resistance genes by curing agents, such as sodium dodecyl sulfate, can restore antibiotic effectiveness. These results support the broader understanding that *P. aeruginosa*'s success as an opportunistic pathogen lies in both its intrinsic resistance mechanisms and its ability to acquire plasmid-mediated resistance (Cotar, 2013).

Overall, this study demonstrates that plasmid curing can meaningfully reduce resistance in *P. aeruginosa*, although residual resistance mechanisms remain. This underscores the need for continued research into combined strategies that target both plasmid-mediated and chromosomal resistance to improve treatment outcomes in infections caused by this formidable pathogen.

Conclusion

A high MAR index was also observed in the study. Plasmid analysis revealed that the multidrug resistant strains of *P. aeruginosa* were plasmid mediated. Treatment should be based on current laboratory diagnosis. Further studies on the presence of *Pseudomonas* sp in wound infections is recommended.

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