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(RESEARCH ARTICLE)

Antimicrobial resistance in clinical wound infections: A study on *Escherichia coli* and *Pseudomonas aeruginosa* isolates from patients in Imo state, Nigeria

Chioma S. Mark-Ogu *, Enuma E. Mike-Anosike, and Chinwe I. Chikwendu

Department of Microbiology, Federal University of Technology, Owerri, Nigeria.

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Abstract

Background: The growing resistance of *Escherichia coli* and *Pseudomonas aeruginosa* to antimicrobial treatments poses a major public health concern, particularly in the treatment of hospital-acquired wound infections.

Aim: This study investigated the antimicrobial resistance profile of *Escherichia coli* and *Pseudomonas aeruginosa* isolated from patients with wound infections in Imo State, Nigeria.

Study Design: A total of 76 bacterial isolates, comprising *Escherichia coli* and *Pseudomonas aeruginosa*, were recovered from patients with clinically infected wounds and subjected to antimicrobial susceptibility testing against a panel of antibiotics representing multiple antibiotic classes, to determine their antimicrobial resistance profiles.

Methodology: Antimicrobial susceptibility testing was performed using the disc diffusion method, as outlined in the Clinical and Laboratory Standards Institute CLSI (2017) guidelines. The isolates were tested against cephalosporins, carbapenems, polymyxins, and quinolones, which include ceftazidime (10µg), ceftriaxone (30µg), cefepime (30µg), imipenem (10µg), meropenem (10µg), colistin (10µg), polymyxin B (30µg), ciprofloxacin (5µg), and levofloxacin (5µg), to determine susceptibility profiles.

Results: Findings revealed a high prevalence of multidrug resistance (MDR) among the gram-negative bacterial isolates, with a substantial proportion of isolates exhibiting resistance to three or more antibiotic classes. Specifically, 43.9% (18) of *Pseudomonas aeruginosa* and 45.71% (16) of *Escherichia coli* isolates were identified as MDR.

Notably, a significant number of *Escherichia coli* and *Pseudomonas aeruginosa* isolates exhibited resistance to various antibiotic classes, including cephalosporins, carbapenems, polymyxins, and quinolones. However, carbapenems demonstrated remarkable efficacy, with a significant majority of isolates showing susceptibility — 92.68% of *Pseudomonas aeruginosa* and 90% of *Escherichia coli* isolates.

Conclusion: The study highlights the urgent need for robust antimicrobial stewardship and infection control practices to preserve the efficacy of available antibiotics in the treatment of clinical wound infections.

Keywords: *Pseudomonas aeruginosa*; *Escherichia coli*; Wound infection; Multidrug resistance; Antimicrobial resistance; Carbapenems

* Corresponding author: Mark-Ogu, C.S

1. Introduction

Antibiotic resistance has emerged as a significant challenge in healthcare, particularly in the management of wound infections. The overuse and misuse of antibiotics have contributed to the proliferation of multidrug-resistant bacteria, complicating the treatment of wounds and compromising patient outcomes (Magiorakos et al., 2012). Among the most concerning are multidrug-resistant gram-negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*, which are notorious for their ability to cause severe and difficult-to-treat infections (Gajdács & Urbán, 2021).

These multidrug-resistant pathogens pose unique challenges in wound management due to their resistance to multiple classes of antibiotics. Traditional treatment approaches often fail to eradicate these bacteria, leading to prolonged healing times, recurrent infections, and increased morbidity and mortality rates (Gajdács & Urbán, 2021). Furthermore, the presence of multidrug-resistant gram-negative bacteria in wounds complicates surgical procedures and increases the risk of systemic infections, further exacerbating the clinical burden (Magiorakos et al., 2012).

These pathogens frequently form biofilms, which reduce antibiotic penetration and increase tolerance (Oliveira et al., 2018). Both species are on the WHO priority list and are part of the ESKAPE group— (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* spp.—so named for their ability to "escape" many antibiotics (Zhen et al., 2019; Antonelli et al., 2021).

Pseudomonas aeruginosa and *Escherichia coli* are also among the most common colonizers of infected wounds and are prolific biofilm formers. Biofilms are a major problem in infections due to their increasingly difficult control and eradication, and tolerance to multiple prescribed drugs. (Oliveira et. al., 2018).

In Nigeria, where wound infections are prevalent, understanding the antimicrobial resistance of these bacteria is crucial for effective wound management. Therefore, this study aims to investigate the antimicrobial resistance of *Escherichia coli* and *Pseudomonas aeruginosa* isolates from patients with wound infections in Imo State.

2. Methodology

2.1. Study area

The research was conducted in Imo State, Nigeria, across the Owerri, Orlu, and Okigwe zones. Hospitals and health centers in these zones served as collection centers; analyses were performed at the Federal University Teaching Hospital, Owerri (FUTHO).

Inclusion criteria were wounded patients who provided informed consent. The study purpose was explained to participants or guardians; confidentiality was assured.

2.2. Sample Collection

A total of 210 wound specimens were collected from accident, orthopedic, and surgical wards across three hospitals: Federal University Teaching Hospital, Owerri (FUTHO; n = 159), Imo State University Teaching Hospital, Orlu (n = 28), and General Hospital, Okigwe (n = 23). Wound swabs were aseptically collected and transported to the FUTHO Microbiology Laboratory in 0.5 mL sterile normal saline.

2.3. Isolation of Organisms

The Cetrimide agar and MacConkey Agar (Titan Biotech Ltd, India) were prepared according to the manufacturer's instructions for the primary isolation of *Pseudomonas aeruginosa* and *Escherichia coli*, respectively, by streaking the wound swabs onto the surface of the prepared media (Cheesbrough, 2006). Colonies were sub-cultured onto nutrient agar; pure cultures were obtained by repeated streaking and incubated at 37°C for 24 hours.

2.4. Identification of Isolates:

Identification and confirmation were based on morphology, cultural characteristics, and biochemical tests (Cheesbrough, 2006).

Pseudomonas aeruginosa colonies were large, flat, and blue-green; Gram-negative and oxidase-positive. *Escherichia coli* colonies on MacConkey agar were lactose-fermenting (reddish-pink); Gram-negative, catalase-positive, oxidase-negative.

2.5. Antimicrobial Resistance Testing:

Kirby–Bauer disc diffusion was performed on Mueller–Hinton agar. Inocula were standardized to 1.5 × 108 CFU/mL using 0.5 McFarland standards; 0.1 mL was spread per plate. Antibiotic discs included ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), colistin (10 µg), and polymyxin B (300 µg). Zones were interpreted per CLSI (2017).

2.6. Data Analysis and Interpretation

Means were analyzed using SPSS v23.0 and Microsoft Excel. ANOVA was applied for multi-group comparisons, followed by Duncan's test to determine significant group differences.

3. Results

3.1. Occurrence (%) of bacterial isolates recovered from wound specimens

Out of 210 wound specimens, 76 (36.2%) were positive for either *Pseudomonas aeruginosa* (n = 41) or *Escherichia coli* (n = 35). Owerri recorded the highest occurrence (20.48%), followed by Orlu (10.48%) and Okigwe (5.24%).

By organism: Owerri showed the highest occurrence of *Pseudomonas aeruginosa* (11.43%), while Okigwe had the lowest (2.86%). For *Escherichia coli*, Owerri recorded 9.53% and Orlu 2.38%. No significant differences were observed across zones. These are shown in Table 1.

3.2. Antimicrobial susceptibility profile of *P. aeruginosa* isolates

Among *Pseudomonas aeruginosa* isolates, resistance was highest to ceftriaxone (95.12%; P = 0.0122), followed by cefepime (90.24%) and ceftazidime (87.80%). Susceptibility was highest to meropenem (95.12%) and imipenem (90.24%). ANOVA indicated resistant isolates were significantly more frequent (P = 0.0160). These are shown in Table 2.

3.3. Antimicrobial susceptibility profile of *E. coli* isolates

Escherichia coli isolates showed high resistance to colistin and polymyxin B (94.29% each). Ceftriaxone resistance was 51.43%. Imipenem showed the highest susceptibility (91.43%; P = 0.0092). See Table 3 for details.

3.4. Multi-drug resistance profiles of the Gram-negative bacterial isolates

Among Gram-negative isolates, 89.61% were MDR; 10.39% were susceptible to all four antibiotic classes. Of MDR isolates, 43.9% were *Pseudomonas aeruginosa* (n = 18) and 45.71% were *Escherichia coli* (n = 16). (Figure 1).

Table 1 Total distribution (%) of wound infections among zones studied

ZONES	No. of Samples (n=210)	No. of persons infected (n=76)	No. Infected with *P. aeruginosa* (n=41)	No. Infected with *E. coli* (n=35)
Okigwe	23 (11.0)	11 (5.24)	6 (2.86)	5 (2.38)
Orlu	28 (13.3)	22 (10.48)	11 (5.24)	10 (4.76)
Owerri	159 (75.7)	43 (20.48)	24 (11.43)	20 (9.53)
Total	210 (100)	76 (36.20)	41 (19.53)	35 (16.67)

Table 2 Antibiotic susceptibility profile (%) of *Pseudomonas aeruginosa*

ANTIBIOTICS	*PSEUDOMONAS AERUGINOSA* (n=41) Sensitive	Resistant

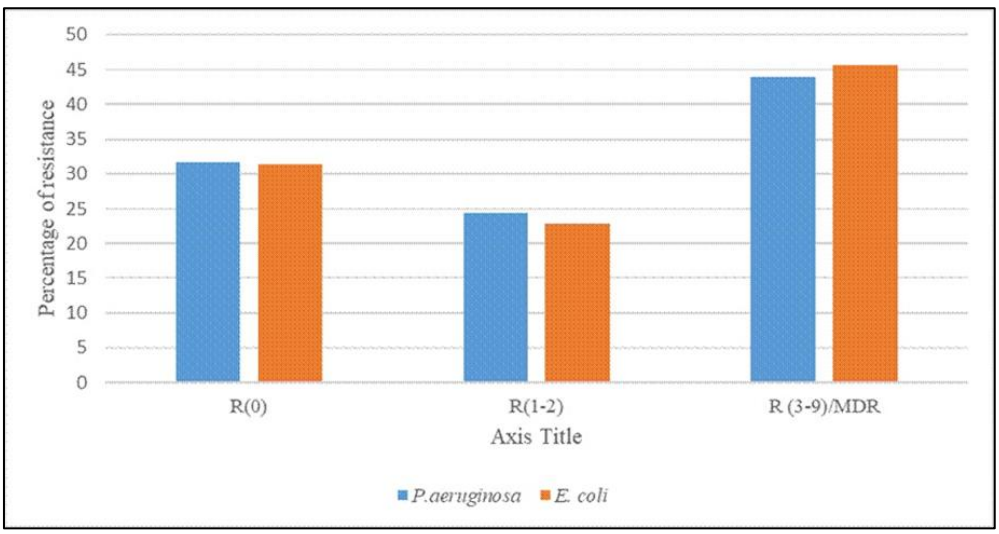
CRO	2 (4.88)	39 (95.12)
FEP	4 (9.76)	37 (90.24)
CAZ	5 (12.2)	36 (87.80)
LVX	24 (58.54)	17 (41.46)
CPX	25 (60.98)	16 (39.02)
CST	36 (87.80)	5(12.2)
POL	36 (87.80)	5 (12.2)
MEM	37 (90.24)	4 (9.76)
IPM	39 (95.12)	2 (4.88)

Key: MEM: Meropenem; POL: Polymyxin B; CPX: Ciprofloxacin; CAZ: Ceftazidime; CRO: Ceftriaxone; IPM: Imipenem; CST: Colistin; FEP: Cefepime; LVX: Levofloxacin

Table 3 Antibiotic susceptibility profile (%) of *Escherichia coli*

Antibiotics	***Escherichia coli* n=35 Sensitive**	**Resistant**
POL	2 (5.71)	33 (94.29)
CST	2 (5.71)	33 (94.29)
CRO	17 (48.57)	18 (51.43)
FEP	20 (57.14)	15 (42.86)
CAZ	21 (60.00)	14 (40.00)
LVX	26 (74.29)	9 (25.71)
CPX	28 (80.00)	7(20.00)
MEM	32 (91.43)	3 (8.57)
IPM	32 (91.43)	3 (8.57)

Key: MEM: Meropenem; POL: Polymyxin B; CPX: Ciprofloxacin; CAZ: Ceftazidime; CRO: Ceftriaxone; IPM: Imipenem; CST: Colistin; FEP: Cefepime;



Key: R0: Sensitive against all selected antimicrobial classes; R1-2: Resistant to one to two antimicrobial classes; R3-9: Resistant to three to four antimicrobial classes. MDR: Resistant to more than three antimicrobial classes.

Figure 1 Multi-Drug Resistance (MDR) Profiles of Gram-Negative *Pseudomonas aeruginosa* and *Escherichia co*li Isolated from Wound Infections

4. Discussion

This study found that *Pseudomonas aeruginosa* and *Escherichia coli* wound infections are widespread in Imo State, with an overall prevalence of 36.2% (76/210). *P. aeruginosa* and *E. coli* had infection rates of 19.53% and 16.67%, respectively. However, the type and level of infection varied across zones, with Owerri showing the highest prevalence (75.7%), followed by Orlu (28%) and Okigwe (23%). These findings are consistent with a previous study by Ohalete et al. (2019) in Owerri, which reported infection rates of 22.3% for *P. aeruginosa* and 13.1% for *E. coli*, with prevalence rates of 78.7% in Owerri, 14% in Orlu, and 7.3% in Okigwe. The higher prevalence in Owerri may be attributed to factors such as urbanization, migration, and greater access to healthcare, but the difference was not statistically significant ($P > 0.05$). The observed prevalence of these infections may also be influenced by factors such as poor hygiene, overcrowding, antibiotic misuse, and inadequate public health awareness.

The present study observed a slightly lower prevalence of *E. coli* (16.67%) compared to *P. aeruginosa* (19.53%), which contrasts with findings from Tom et al. (2019) in Bauchi, Nigeria, where *E. coli* prevalence (23.64%) exceeded that of *P. aeruginosa* (19.12%). Similarly, this differs from the results of Ohalete et al. (2019) in Imo State, who reported a prevalence of 13.1% for *E. coli* and 22.3% for *P. aeruginosa*. Such discrepancies highlight the variability of microbial prevalence across regions and emphasize the need for healthcare facilities to identify their predominant pathogens and associated resistance patterns (Pondei et al., 2013). Despite these variations, our findings confirm the involvement of both *P. aeruginosa* and *E. coli* in wound infections, consistent with the report by De Oliveira et al. (2020).

Pseudomonas spp. are frequently implicated in nosocomial infections, including pneumonia, urinary tract infections, surgical site infections, burns, and infections among patients undergoing chemotherapy or prolonged antibiotic therapy (Spagnolo et al., 2021). *P. aeruginosa* exhibits intrinsic resistance to multiple antibiotics due to its low outer membrane permeability, inducible cephalosporinase production, active efflux mechanisms, and reduced affinity for DNA gyrase (Umoru et al., 2018). The present study revealed high resistance to cephalosporins, with 95.12% resistance to ceftriaxone and 87.80% to ceftazidime, aligning with previous reports (Yatollahi et al., 2018). This resistance pattern is likely associated with the production of extended-spectrum beta-lactamases (ESBLs). Conversely, *P. aeruginosa* demonstrated high sensitivity to carbapenems, with 95.12% susceptibility to imipenem and 90.24% to meropenem, consistent with earlier findings (Javiya et al., 2008). This sensitivity may be attributed to the ability of imipenem to penetrate the outer membrane and inhibit cell wall synthesis, resulting in bacterial death (Javiya et al., 2008).

E. coli was also isolated in significant numbers alongside *Pseudomonas aeruginosa*. As a normal gastrointestinal inhabitant, *E. coli* can cause skin and wound infections, particularly in individuals with poor hygiene or fecal contamination, as well as in postoperative patients (Sahu et al., 2011). Antimicrobial susceptibility testing showed high resistance to colistin and polymyxin B (94.29%), followed by ceftriaxone (51.43%). This resistance is consistent with previous studies (Olaitan et al., 2014), which demonstrated that *E. coli* employs several mechanisms to evade polymyxin action, including lipopolysaccharide modification, efflux pump activation, capsule formation, and outer membrane protein alteration. However, imipenem demonstrated strong activity (91.43% sensitivity), consistent with reports by Joly-Guillou et al. (2010), where imipenem and meropenem achieved 100% susceptibility against *E. coli*. This may be due to their strong affinity for penicillin-binding proteins that disrupt bacterial cell wall synthesis (Nicolau, 2008).

Multidrug resistance (MDR) among Gram-negative bacteria presents a major public health challenge, limiting treatment options and increasing the risk of clinical failure. In this study, 34 (89.61%) isolates exhibited MDR, underscoring the urgent need for enhanced antimicrobial stewardship and infection control. Among the MDR isolates, *P. aeruginosa* and *E. coli* accounted for 43.9% (18) and 45.71% (16), respectively, confirming their significant contribution to the burden of antimicrobial resistance in wound infections. The emergence of MDR strains highlights the importance of continuous surveillance of resistance trends to guide empirical therapy and infection prevention strategies. The finding that only 10.39% of isolates were susceptible to all antibiotic classes emphasizes the need for judicious antibiotic use and development of novel therapeutic alternatives.

4.1. Cephalosporin Resistance

Cephalosporins are β-lactam antibiotics commonly used against Gram-negative infections. However, this study recorded high resistance levels, especially among *P. aeruginosa* isolates—95.12% resistant to ceftriaxone, 90.24% to cefepime, and 87.80% to ceftazidime. *E. coli* isolates exhibited moderate resistance—51.43% to ceftriaxone, 42.86% to cefepime, and 40% to ceftazidime. The higher resistance in *P. aeruginosa* suggests that cephalosporins may be less effective against this pathogen, highlighting the need for careful antibiotic selection and improved stewardship practices.

4.2. Polymyxin Resistance

Polymyxins (colistin and polymyxin B) are often reserved for MDR infections, yet our study observed significant resistance among *E. coli* isolates (94.29%). In contrast, *P. aeruginosa* showed relatively low resistance (12.2%). Although polymyxins remain active against *P. aeruginosa*, their nephrotoxic and neurotoxic effects limit their therapeutic use (Falagas & Kasiakou, 2006). The high resistance rate in *E. coli* underscores the urgent need for novel antimicrobials and reinforced stewardship programs to slow resistance spread.

4.3. Quinolone Resistance

Quinolones are synthetic antibiotics widely used against Gram-negative bacteria. In this study, 40.24% of *P. aeruginosa* and 22.86% of *E. coli* isolates were resistant to quinolones. While these rates are moderate, they still pose a treatment challenge. Nevertheless, quinolones could be considered secondary options after carbapenems due to their comparatively lower resistance levels. Continuous monitoring of quinolone efficacy is necessary to guide rational antibiotic use.

4.4. Carbapenem Resistance

Carbapenems are considered the last line of defense against MDR Gram-negative infections due to their stability against most β-lactamases. Our findings showed low resistance levels—7.32% in *P. aeruginosa* and 10% in *E. coli*—consistent with Martinez et al. (2018), who reported 8% and 12% resistance, respectively. These findings reaffirm the continued clinical relevance of carbapenems but also warn against complacency, as carbapenem-resistant strains are emerging globally.

5. Conclusion

In conclusion, the findings of this study, consistent with previous research, emphasize the continued effectiveness of carbapenems as a treatment option for gram-negative bacterial wound infections, particularly in the context of multidrug resistance. These antibiotics play a crucial role as a last line of defense against MDR strains and remain essential in preserving patient outcomes in clinical settings. Nevertheless, concerted efforts are required to address the underlying factors contributing to the emergence and spread of MDR, including antibiotic misuse, weak infection control practices, and limited development of alternative antimicrobial options.

Compliance with ethical standards

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Disclosure of conflict of interest:

The authors have declared that no competing interests exist.

Statement of ethical approval:

In accordance with international standards, written ethical approval was obtained and preserved by the author(s).

Statement of informed consent:

As per international standards and university standards, patient(s) written consent was collected and preserved by the author(s).

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