*Sahel Journal of Life Sciences FUDMA (SAJOLS)*
**September 2025 Vol. 3(3): 33-36**
**ISSN: 3027-0456 (Print)**
**ISSN: 1595-5915 (Online)**
**DOI: *https://doi.org/10.33003/sajols-2025-0303-05***

***Research Article***

# Evaluation of Bacteriophage Therapy against Multidrug-Resistant *Pseudomonas aeruginosa* Isolated from Wound Infections at Ahmadu Bello University Teaching Hospital, Zaria

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**ABSTRACT**
The global rise of multidrug-resistant (MDR) bacteria has reignited interest in alternative therapeutic options such as bacteriophage therapy. This study evaluates the effectiveness of bacteriophages isolated from hospital sewage in combating MDR *Pseudomonas aeruginosa* strains obtained from wound infections in Ahmadu Bello University Teaching Hospital, Zaria. Fifteen clinical isolates were reconfirmed using conventional biochemical tests, followed by antibiotic susceptibility testing. Eight isolates were identified as MDR. Bacteriophages were isolated using standard enrichment and double-layer agar methods and displayed visible lytic activity against the resistant strains. The findings highlight the potential of bacteriophages as an effective and economical therapeutic alternative.

**Keywords:** Antibiotic resistance; Bacteriophage; Multidrug resistance; Phage therapy; *Pseudomonas aeruginosa*; Wound infection

**Citation:** Mani, S., Mzungu, I., Kilani, A.M. & Ameh, R.E. (2025). Evaluation of Bacteriophage Therapy against Multidrug-Resistant *Pseudomonas aeruginosa* Isolated from Wound Infections at Ahmadu Bello University Teaching Hospital, Zaria. *Sahel Journal of Life Sciences FUDMA*, 3(2): 33-36. DOI: *https://doi.org/10.33003/sajols-2025-0303-05*

## INTRODUCTION

The rapid emergence of antimicrobial resistance (AMR) has rendered several classes of antibiotics ineffective against common pathogens, including *Pseudomonas aeruginosa*, a highly adaptable Gram-negative bacillus responsible for severe nosocomial infections such as pneumonia, urinary tract infections, and wound sepsis (Laith *et al.,* 2024). This resistance crisis is especially exacerbated in resource-limited settings like Africa, where surveillance and control strategies remain inadequate (WHO, 2014).

Among the various alternatives proposed, bacteriophage therapy that was first practiced by Félix d'Hérelle in the early 20th century has resurfaced as a promising solution (Adams, 1959; Summers, 1999). Bacteriophages are viruses that selectively infect and lyse bacteria without harming the host's microbiota due its specificity (Ackermann and Dubow, 1987). Although phage therapy has gained momentum in countries like Georgia and Russia (Kutter *et al*., 2010; Expert Round Table Participants, 2016), its application in Africa is almost nonexistent despite its affordability, specificity, and environmental abundance (Rohde and Wittmann, 2020; Morozova *et al*., 2018).

This study investigates the isolation of bacteriophages from hospital sewage and their activity against MDR *P. aeruginosa* obtained from wound infections of the patients attending Ahmadu Bello University Teaching Hospital, Zaria.

## MATERIALS AND METHODS

### Ethical Approval

Ethical approval for the study was obtained from the institutional review board of ABUTH.

**Funding**

The research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

**Conflict of Interest**

The authors declare no conflict of interest.

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**Sample Collection and Bacterial Isolation**

Wound swab samples were aseptically collected from patients at Ahmadu Bello University Teaching Hospital (ABUTH), Shika. Each swab was enriched in Tryptic Soy Broth and incubated at 37°C for 24 hours before streaking onto Cetrimide agar. Colonies were subjected to conventional biochemical tests including oxidase, citrate utilization, Methyl Red- Voges-Proskauer, indole, motility, Triple Sugar Iron, urease, and sugar fermentation (Josephine *et al.,* 2002).

**Antibiotic Susceptibility Testing**

Antimicrobial susceptibility was tested on the eight best selected isolates using the Kirby-Bauer disk diffusion method on Mŭller-Hinton agar following CLSI, 2024 guidelines. Antibiotics tested included ciprofloxacin(5µg), tetracycline(30µg), gentamicin(10µg), cefepime(15µg), erythromycin(10µg), cefoxitin(10µg), and imipenem(10µg). Multi-drug resistance (MDR) was defined as non-susceptibility to ≥3 different antibiotic classes (Magiorakos *et al*., 2012).

**Bacteriophage Isolation**

Hospital sewage was centrifuged at 5000 rpm for 15 minutes, and the supernatant was filtered through a 0.22 µm membrane. Serial dilutions of $10^{-1}$ to $10^{-10}$ were prepared and tested using the double-layer agar method. A mixture of filtered phage lysate and log-phase identified MDR *P. aeruginosa* isolates (MC/339 and MC/249) were overlaid onto Luria-Bertani (LB) agar and incubated at 37°C for 24 hours (Adams, 1959; Schuch Fischetti, 2013). Clear lytic zones (plaques) were observed and recorded in Plaque Forming Unit (PFU)

**Phage Amplification and Purification**

Plaques were picked using sterile tips and suspended in saline. Chloroform (10%) was added, followed by incubation and centrifugation. The resulting lysate was filtered and concentrated using Amicon centrifugal devices, yielding a high-titer phage stock expressed as PFU/ml (Vipra *et al*., 2013).

## RESULTS

**Biochemical Identification of *P. aeruginosa***

Fifteen isolates were obtained (MC/309, MC/222, MC/249, MC/321, MC/314, MC/399, MC/224, MC/308, MC/221, MC/302, MC/322, MC/305, MC/306, MC/339, MC/307) all of which shared classical *P. aeruginosa* traits on conventional biochemical testing give oxidase-positive, motile, utilize citrate, MR-positive, VP-negative, and non-fermentative for lactose and sucrose. Arginine dihydrolase activity was also detected in all isolate, confirming species identity.

**Antibiotic Resistance Profile**

Among the 15 solates, eight were classified as MDR(Table1). Notably, resistance to erythromycin and cefoxitin was consistent across all MDR strains. Imipenem retained the highest activity, while tetracycline and cefoxitin showed limited efficacy as shown in Table1.

**Bacteriophage Lytic Activity**

Bacteriophages isolated from hospital sewage showed clear lytic zones against the MDR isolates. Notably, isolate MC/339 and MC/249, displayed marked susceptibility to the Bacteriophage with well-defined plaques (Plate 1).

**Table 1**. **The antibiotic susceptibility pattern of the isolate-*Pseudomonas aeruginosa***

| Isolate | CIP 5µg | TE 30µg | GEM 10µg | CPM 15µg | E 10µg | FOX 10µg | IMP 10µg | CLSI Classification (CIP / CPM / IMP) | MDR Status |
|---|---|---|---|---|---|---|---|---|---|
| Mc/249 | 10 (R) | 14 | 7 | 11 (R) | 9 | 0 | 20 (R) | R / R / R | MDR |
| Mc/322 | 20 (I) | 13 | 17 | 12 (R) | 0 | 0 | 31 (S) | I / R / S | MDR |
| Mc/309 | 30 (S) | 10 | 18 | 13 (I) | 0 | 0 | 25 (S) | S / I / S | Not MDR |
| Mc/222 | 22 (S) | 22 | 18 | 12 (R) | 0 | 0 | 28 (S) | S / R / S | MDR |
| Mc/224 | 30 (S) | 14 | 18 | 15 (S) | 12 | 0 | 25 (S) | S / S / S | Not MDR |
| Mc/399 | 20 (I) | 16 | 20 | 13(R) | 10 | 0 | 20(R) | I / R / R | MDR |
| Mc/314 | 29 (S) | 15 | 20 | 26 (S) | 0 | 9 | 27 (S) | S / S / S | Not MDR |
| Mc/321 | 20 (I) | 12 | 16 | 10 (R) | 0 | 0 | 29 (S) | I / R / S | MDR |

Key S = sensitive, I = Intermediate, R = Resistance, MDR = multi drug resistance, CIP =Ciprofloxacin, TE =Tetracycline, GEM =Gemifloxacin, CPM =Cefepime, E =Erythromycin, FOX =Cefoxitin, IMP =Imipenem.

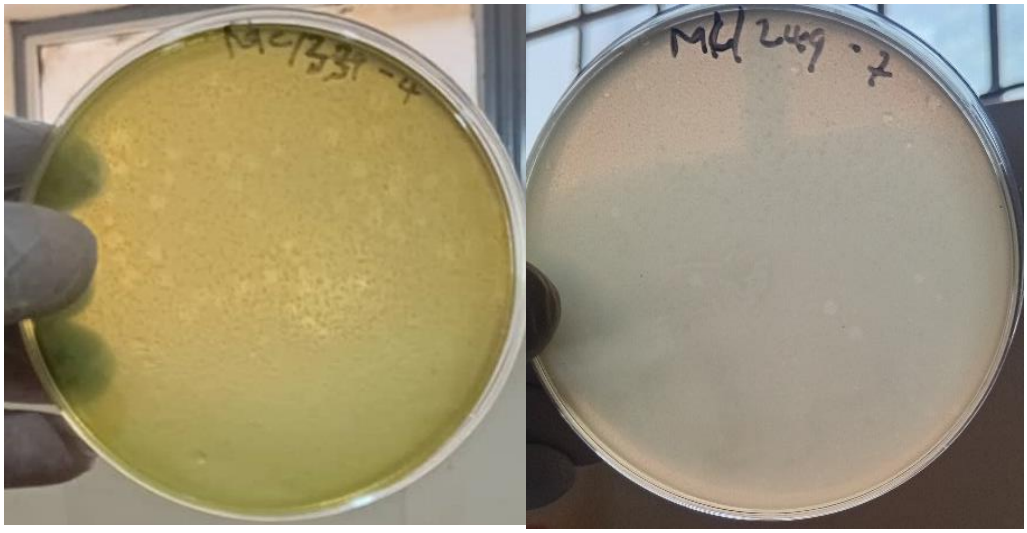

**Plate 1**. **Lytic activity of the Bacteriophage against *P. aeruginosa* isolates**

## DISCUSSION

This study confirms that *P. aeruginosa* remains a persistent nosocomial pathogen exhibiting high rates of resistance to conventional antibiotics as shown in Lopez *et al*., (2018); Suwesu, (2018). The MDR status as classified using Clinical Laboratory Standard institute(CLSI, 2024),is observed among over half of the isolates, with the MC/339 and MC/249 showing extensively resistance to the antibiotics tested which aligns with global trends and highlights the critical need for alternative interventions (Magiorakos *et al*., 2012; WHO, 2014).

The Phage therapy, showed promising results in lysing MDR *P. aeruginosa* isolates (MC/339 and MC/249). These finding is consistent with previous studies conducted in Russia and Georgia, where phage therapy is already in use (Kutter *et al*., 2010). Bacteriophages are naturally abundant, target-specific, and can be rapidly isolated and amplified at low cost (Vipra *et al*., 2013; Rohde & Wittmann, 2020). Moreover, their narrow host range minimizes the risk of dysbiosis, a common complication of broad-spectrum antibiotics (Elbreki *et al*., 2014).

Despite these advantages, the clinical application of phages in Africa remains underexplored. A lack of molecular infrastructure and regulatory guidelines has stifled research and public investment (Expert Round Table Participants, 2016). This study offers an initial step toward demonstrating the feasibility and clinical relevance of phage therapy.

## CONCLUSION

In this study, multidrug-resistant *P. aeruginosa* was isolated from wound sample of the patient attending Ahmadu Bello University Teaching hospital, zaria and was found to be MDR strain which potentially, poses a serious challenge to conventional antimicrobial therapies. The successful isolation and *in vitro* efficacy of bacteriophages against these isolates suggest that phage therapy may offer a viable, low-cost alternative in the fight against MDR isolates. Further clinical evaluation and policy development are necessary to integrate phage therapy into public health frameworks.

Given the evidence of bacteriophage lytic activity against MDR *Pseudomonas aeruginosa* in this study, we recommend molecular characterization of isolated bacteriophages and genome analysis to confirm phage identity, safety, and ensure absence of resistance or virulence genes. There is also need for morphological classification of the phage using transmission electron microscopy (TEM).

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