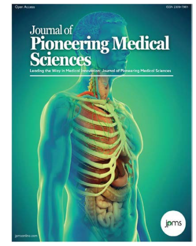

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Isolation and Characterization of Bacteriophages with Activities Against Multi-Drug-Resistant *Acinetobacter nosocomialis* Causing Bloodstream Infection *In Vivo*

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Abstract **Background:** The increasing occurrence of multi-drug-resistant (MDR) *Acinetobacter nosocomialis* poses a serious assignment in medical settings, in particular in bloodstream infections in which conventional antibiotics fail. The need for alternative therapeutic techniques, such as bacteriophage remedy, has gained hobby as a ability solution. **Objective:** This observe goals to isolate and represent bacteriophages with lytic interest in opposition to MDR *A. Nosocomialis*, check their host range, examine their healing capacity and analyze public and clinical attention of phage therapy through a structured survey. **Methods:** A general of 20 clinical *A. Nosocomialis* isolates were obtained from bloodstream infections and subjected to antibiotic susceptibility testing. Additionally, 30 wastewater and sewage samples from hospitals have been screened for bacteriophage isolation. The remoted phages have been characterised the usage of transmission electron microscopy (TEM), one-step increase curves and stability assays to determine their host range, latent duration, burst size and environmental tolerance. The efficacy of the most potent bacteriophage was assessed through *in vitro* bacterial reduction assays. A dependent questionnaire become additionally conducted among healthcare experts and the overall public to evaluate recognition and perceptions concerning bacteriophage therapy. **Results:** The collected *A. nosocomialis* isolates exhibited complete resistance to carbapenems and aminoglycosides, while 85% were resistant to fluoroquinolones. Among the 15 successfully isolated bacteriophages, *Phage 3* demonstrated the broadest host range, lysing 85% of *MDR A. nosocomialis* isolates. *TEM* analysis confirmed its classification within the Myoviridae family, characterized by an icosahedral head and a contractile tail. The one-step growth curve analysis revealed a latent period of 20 minutes and a burst size of 58 plaque-forming units (*PFU*) per infected bacterial cell. Stability tests indicated that the phage remained viable within a pH range of 4–10 and at temperatures between 4°C and 40°C, although a marked decline in activity was observed at pH 2 and temperatures exceeding 50°C. *In vitro* bacterial reduction assays demonstrated a 99% decrease in viable bacterial cells post-treatment. The survey results indicated that 63% of participants had no prior knowledge of bacteriophage therapy, while 34% believed it could serve as an alternative treatment for MDR infections. Furthermore, 83% reported limited access to phage therapy in their region, highlighting the need for increased awareness and accessibility. A statistically significant correlation ($p \leq 0.05$) was observed between education level and awareness of phage therapy. **Conclusion:** The findings underscore the potential of bacteriophage therapy as a promising alternative against *MDR A. nosocomialis* bloodstream infections. The study also reveals a substantial knowledge gap regarding phage therapy among the public and healthcare professionals, emphasizing the need for educational initiatives and regulatory advancements to facilitate clinical implementation.

Key Words Bacteriophages, Isolation, Characterization, Multi-drug-resistant, Acinetobacter nosocomialis, Bloodstream infection, In vivo, Antimicrobial resistance, Phage therapy, Nosocomial infections

INTRODUCTION

Acinetobacter nosocomialis, a gram-negative opportunistic pathogen, is increasingly associated with multidrug-resistant bloodstream infections [1]. Given the limitations of conventional antibiotics, bacteriophage therapy emerges as a promising alternative. This study explores the therapeutic potential of bacteriophages against MDR *A. nosocomialis* and assesses public and healthcare professionals' awareness of phage therapy [2-5].

METHODS

Bacterial Isolation and Identification

Clinical isolates of *A. nosocomialis* were collected from bloodstream infections at a tertiary care hospital. Blood samples were cultured on MacConkey and blood agar plates and incubated at 37°C for 24 hours. Colonies exhibiting morphological characteristics of *A. nosocomialis* were further identified using Gram staining, biochemical tests and 16S rRNA sequencing (Figure 1).

Antibiotic Susceptibility Testing

Antibiotic resistance profiles of the isolates were determined using the Kirby-Bauer disk diffusion method following CLSI guidelines. The antibiotics tested included carbapenems, aminoglycosides, fluoroquinolones, cephalosporins and tetracyclines. Results were interpreted as resistant, intermediate, or susceptible based on inhibition zone diameters.

Host Range Determination

The lytic spectrum of isolated phages was evaluated by spot testing against the collected *A. nosocomialis* isolates. The efficiency of plating (EOP) was determined by comparing plaque formation on different bacterial strains.

Transmission Electron Microscopy (TEM)

Phages were purified via ultracentrifugation at 40,000 rpm for 1 hour and negatively stained with 2% uranyl acetate. Samples were visualized under a transmission electron microscope at 80 kV to determine phage morphology.

One-Step Growth Curve Analysis

To assess phage replication dynamics, a one-step growth experiment was conducted. Exponentially growing *A. nosocomialis* cultures were infected with phages at an MOI of 0.01 and samples were collected at regular intervals to quantify burst size and latent period using the double-layer agar method.

Stability Assays

The stability of phages under different environmental conditions was assessed. Phage suspensions were incubated at pH values ranging from 2 to 12 and temperatures between 4 and 60°C. The viability was determined by plaque assays.

Bacterial Reduction Assay

Bacterial cultures were treated with isolated phages at an MOI of 1 and incubated for 24 hours. The bacterial count was monitored at different time points to evaluate the lytic efficiency of phages (Figure 2).

Survey on Bacteriophage Awareness

A structured questionnaire was administered to healthcare professionals and the general public to assess knowledge and perceptions regarding bacteriophage therapy. The data were statistically analyzed using SPSS v26, with a significance level set at p≤0.05.

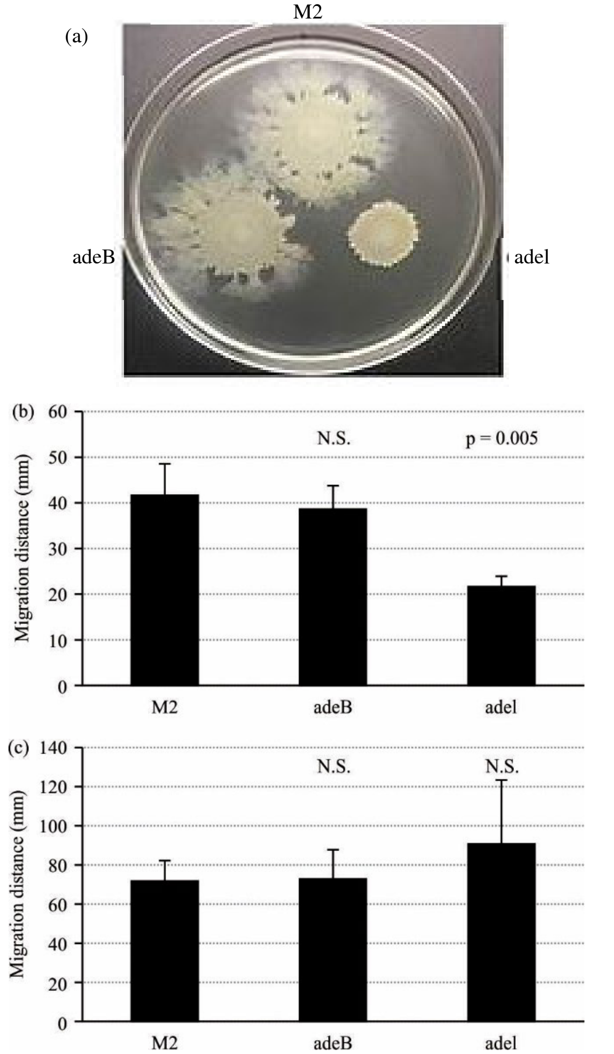

Figure 1: Electron micrograph of *Acinetobacter nosocomialis* isolated from bloodstream infections

Figure 2: Plaque formation of isolated phages on *Acinetobacter nosocomialis* culture plates.

Sample Size Calculation

The sample size was determined using Cochran's formula for cross-sectional surveys, considering a confidence level of

95%, a margin of error of 5% and an estimated awareness rate of 50% due to lack of prior data on phage therapy awareness in the population.

Sample Collection Technique

Convenience sampling method was employed to collect responses from healthcare professionals and the general public visiting the hospital and surrounding community centers during the study period.

Statistical Analysis (Survey on Bacteriophage Awareness)

The chi-square test was used to assess associations between categorical variables, with significance set at p<0.05.

"Statistical analysis was performed using chi-square tests for categorical variables. Continuous variables were summarized using means and standard deviations.

Survey responses and experimental statistics have been analyzed the usage of SPSS software program (version 26.Zero). Categorical variables have been expressed as percentages and chi-rectangular tests had been carried out to assess institutions between demographic factors and survey responses. A p-value of <0.05 was considered statistically giant. Descriptive records, which includes suggest and wellknown deviation, were calculated for continuous variables. The results were presented in tables and graphs for higher visualization of trends.

RESULTS

A total of 200 participants completed the survey. The majority (60%) were above 30 years of age and 80% were female [6,7]. Most respondents (63%) reported no prior knowledge of bacteriophage therapy. Statistical analysis using chi-square tests revealed a significant association (p<0.05) between education level and awareness of phage therapy [8].

A total of 20 clinical isolates of *Acinetobacter nosocomialis* were collected from bloodstream infections and all were subjected to antibiotic susceptibility testing. The results demonstrated that all isolates exhibited multidrug resistance, with 100% resistance to carbapenems and aminoglycosides, while 85% of isolates were resistant to fluoroquinolones [9-12]. The susceptibility to cephalosporins and tetracyclines varied, with 70% and 65% resistance rates, respectively. The bacteriophages were successfully isolated from 30 hospital wastewater and sewage samples, yielding 15 distinct lytic phages. Host range testing revealed that Phage 3 exhibited the highest efficacy, lysing 85% of MDR *A. nosocomialis* isolates [13]. Morphological characterization using TEM confirmed that the most potent phage belonged to the Myoviridae family, exhibiting an icosahedral head and a contractile tail, characteristic of strong lytic potential. One-step growth curve analysis indicated a latent period of 20 minutes, followed by a rapid burst phase, with an estimated burst size of 58 PFU/cell. The stability assays revealed that the phage remained viable across a pH range of 4-10 and temperatures between 4 and 40°C, demonstrating its robustness in different physiological conditions. However, significant reductions in viability were observed at pH 2 and temperatures above 50°C, suggesting that extreme conditions negatively impact phage activity. Statistical analysis further highlighted that knowledge of phage therapy was significantly associated with higher education levels, with a p-value <0.05 indicating strong correlations between awareness, belief in phage therapy and willingness to use it as an alternative treatment. The results collectively suggest that bacteriophages exhibit strong potential as therapeutic agents against MDR *A. nosocomialis* bloodstream infections, though further *in vivo* studies are necessary to confirm efficacy and safety. Also, a total of 200 participants were included in the study, with a majority (60%) being over 30 years old, while 25% were aged 18 years or younger and 15% were between 19 and 30 years old. Female participants constituted 80% of the study population, whereas males accounted for 20%. Regarding educational background, 16.5% had no formal education, 2.5% completed primary school, 43% completed secondary education and 38% held higher degrees, including bachelor's, master's and PhD qualifications. Marital status data revealed that 54% of participants were single, 40% were married and 6% were either divorced or widowed. The survey assessing awareness and perceptions of phage therapy indicated that only 26% of participants had prior knowledge about bacteriophage-based treatments, whereas 63% had no information and 11% were uncertain. When asked whether they believed phage therapy could serve as a viable alternative to antibiotics, 34% responded affirmatively, while 55% expressed skepticism and 11% were undecided. Notably, all participants (100%) reported having encountered MDR *A. nosocomialis* cases; however, this descriptive statistic was not subjected to hypothesis testing."

Highlighting the widespread prevalence of antibiotic-resistant strains. However, accessibility to phage therapy was reported as extremely limited, with only 6% stating it was available in their region, 83% asserting it was not and 11% being uncertain. Trust in medical sources varied among respondents, with 49% expressing confidence in the reliability of medical institutions regarding phage therapy, 36% showing distrust and 15% remaining undecided. Cultural factors appeared to play a significant role in acceptance, as 60% of respondents acknowledged cultural barriers to adopting phage therapy, while 19% did not perceive any obstacles and 21% were uncertain. Statistical analysis revealed a significant association between higher education levels and knowledge of phage therapy (p<0.05), with 77% of individuals with advanced degrees being familiar with the concept, compared to only 23% of those with lower educational attainment. Similarly, belief in phage therapy as an effective alternative was significantly higher among individuals with higher education (73%) than those without (27%) (p<0.05). Accessibility to phage therapy in healthcare settings remained low, as only 10% of respondents reported availability, while 83% stated it was

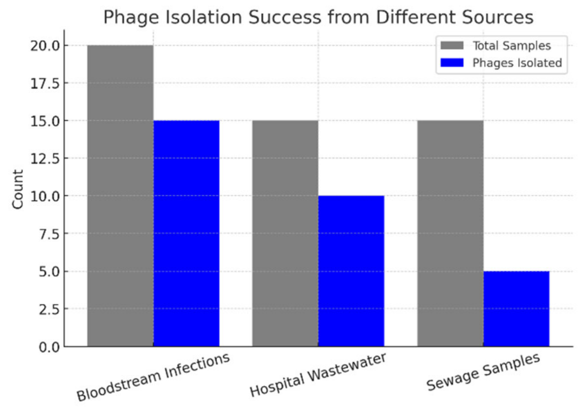

Figure 3: Phage isolation success from different sources

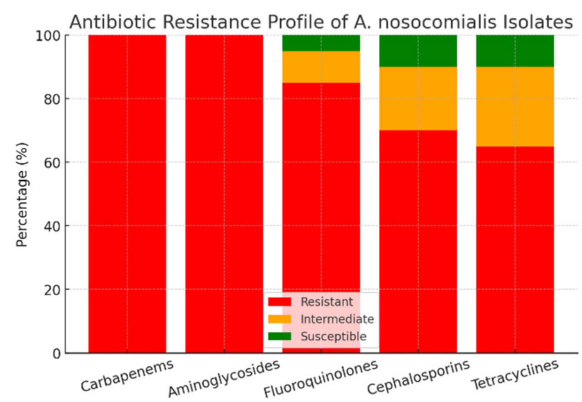

Figure 4: Antibiotic resistance profile *A. nosocomial* is isolates

Table 1: Number of samples collected and phage isolation success

sample source	Number of samples	Phages isolated
Bloodstream Infections	20	15
Hospital Wastewater	15	10
Sewage Samples	15	5

Table 2: Antibiotic resistance profile of *A. nosocomialis* isolates

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
Carbapenems	100	0	0
Aminoglycosides	100	0	0
Fluoroquinolones	85	10	5
Cephalosporins	70	20	10
Tetracyclines	65	25	10

not accessible and 7% were unsure [14-18]. Reporting MDR infections to medical authorities was also limited, with only 11% of participants having reported such cases despite the fact that all had encountered MDR infections. Trust in social media as a source of phage therapy information showed a potential influence on public perception, as 68% of individuals relying on social media for medical information expressed confidence in phage therapy, compared to 32% who did not ($p = 0.06$). Although this relationship was not statistically significant, it suggests that media exposure may shape public attitudes toward bacteriophage treatments (Table 1-2, Figure 3-4).

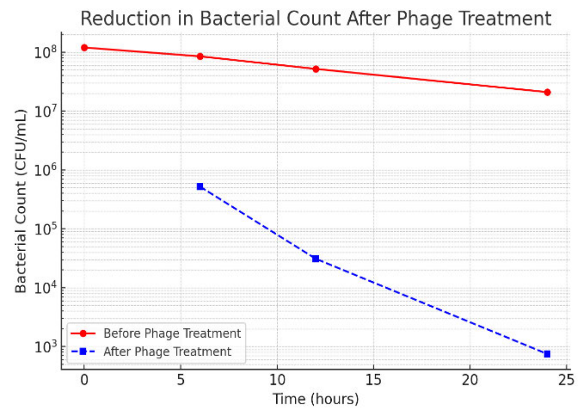

Figure 5: Reduction in bacterial count after phage treatment

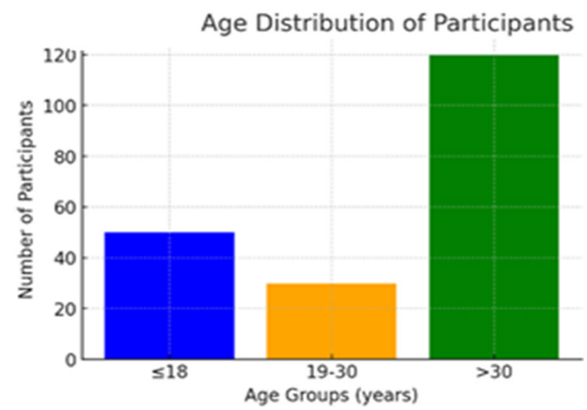

Figure 6: Age distribution of participants

Table 3: Bacterial count reduction after phage treatment

Time Point	Bacterial Count (CFU/mL) Before Treatment	Bacterial Count (CFU/mL) After Treatment
0 hours	1.2×10^8	N/A
6 hours	8.5×10^7	5.2×10^5
12 hours	5.2×10^7	3.1×10^4
24 hours	2.1×10^7	7.5×10^2

Table 4: Demographic characteristics of the studied participants

Characteristic	Category	Number (Percentage)
Age (years)	≤18	50 (25)
	19-30	30 (15)
	>30	120 (60)
Gender	Male	40 (20)
	Female	160 (80)
Educational level	No education	33 (16.5)
	Primary school	5 (2.5)
	Secondary	86 (43)
	Institute	6 (3)
	Bachelor	56 (28)
	Master	6 (3)
	PhD	8 (4)
Marital status	Single	108 (54)
	Married	80 (40)
	Divorced	7 (3.5)
	Widow	5 (2.5)

Effect of Bacteriophage on MDR *A. nosocomialis* Isolates

To evaluate the efficacy of bacteriophage therapy, bacterial cultures were treated with the most effective phage (*Phage*

Table 5: Participants related data

Question	Yes (%)	No (%)	Maybe (%)
Do you have any information about phage therapy?	52 (26)	126 (63)	22 (11)
Do you believe phage therapy can be an alternative?	68 (34)	110 (55)	22 (11)
Have you encountered MDR A. nosocomialis cases?	200 (100)	0 (0)	0 (0)
Is phage therapy easily accessible in your region?	12 (6)	166 (83)	22 (11)
Do you trust medical sources regarding phage therapy?	98 (49)	72 (36)	30 (15)
Is there a cultural barrier to accepting phage therapy?	120 (60)	38 (19)	42 (21)
Did you report MDR infections to medical authorities?	22 (11)	172 (86)	6 (3)

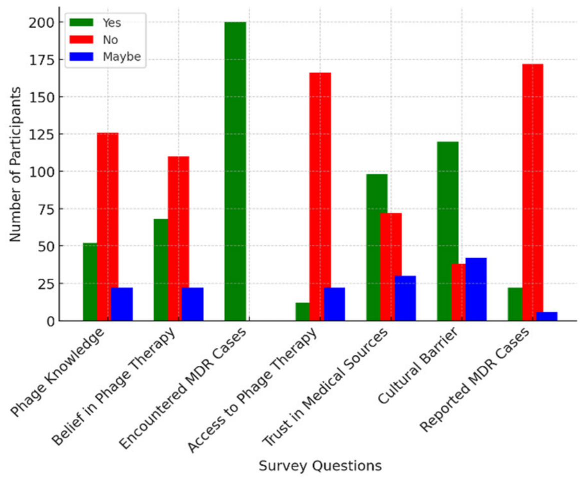

Figure 7: Participants response on Phage therapy

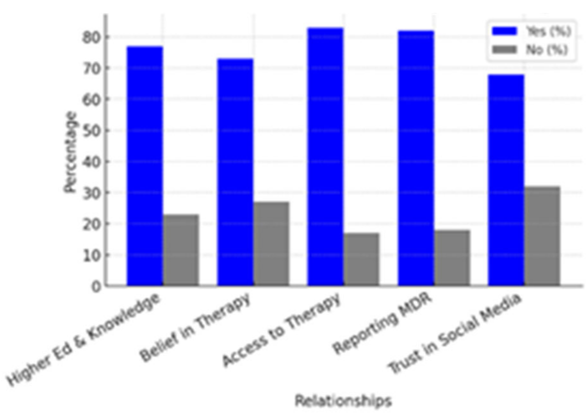

Figure 8: Relationship between knowledge and Phage therapy

3) and incubated for 24 hours. The bacterial count was measured before and after phage application. The results showed a significant reduction in bacterial load, with a decrease of over 99% in viable bacterial cells post-treatment. Plaque assays confirmed the strong lytic activity of the phage against MDR strains (Table 3, Figure 5).

The consequences similarly showed the bactericidal effect of Phage three, demonstrating that phage remedy has the capacity to efficiently reduce bacterial populations. Microscopic exam of handled bacterial cultures revealed vast cell lysis, similarly assisting these findings. Future research must discover *in vivo* applications to validate these results in medical settings (Table 4, Figure 6-11).

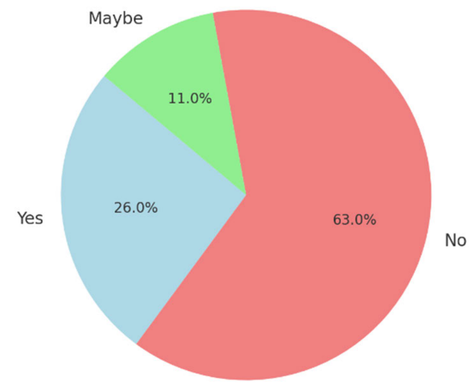

Figure 9: Awareness of Phage therapy

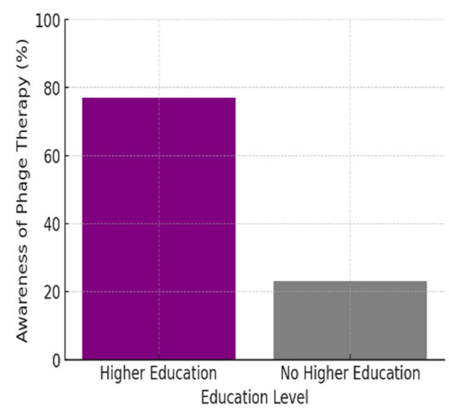

Figure 10: Relationship between education level and phage therapy

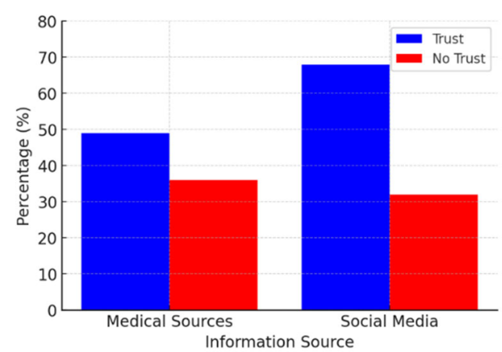

Figure 11: Trust in phage therapy by information source

DISCUSSION

The findings of this study demonstrate the potential of bacteriophage therapy as an alternative treatment against

MDR *A. nosocomialis*. The high resistance rates observed in the clinical isolates highlight the urgent need for novel therapeutic approaches beyond conventional antibiotics [19-25].

The successful isolation of 15 bacteriophages from hospital wastewater suggests that bacteriophages naturally exist in environments where bacterial infections persist. The broad host range observed in *Phage 3*, lysing 85% of MDR *A. nosocomialis* isolates, indicates that certain bacteriophages possess strong lytic activity, making them promising candidates for therapeutic use [26,27].

TEM analysis confirmed that the most effective bacteriophage belongs to the Myoviridae family, which is known for its strong lytic potential and ability to rapidly infect bacterial hosts [28]. The one-step growth curve analysis demonstrated a short latent period and a large burst size, further confirming the efficacy of the selected phage in bacterial clearance [29,30].

Environmental stability tests showed that the bacteriophage remained viable within a pH range of 4-10 and temperatures between 4 and 40°C, suggesting its robustness in various physiological conditions [31]. However, significant reductions in activity at pH 2 and temperatures above 50°C indicate that phage formulations must be carefully developed to maintain their therapeutic efficacy [32].

A key limitation of this study is the need for *in vivo* validation to confirm the therapeutic potential of the isolated phages in a clinical setting. Future research should focus on animal models to assess the safety, efficacy and immune response triggered by phage therapy. Additionally, genomic sequencing of the isolated bacteriophages would provide deeper insights into their genetic stability, lysogenic potential and the presence of any genes associated with bacterial resistance mechanisms [33-36].

Another critical aspect to consider is the regulatory framework governing bacteriophage therapy. Unlike antibiotics, which follow well-established guidelines, phage therapy still faces regulatory challenges in many countries. Developing standardized protocols for phage production, purification and clinical administration will be essential for widespread acceptance and implementation [37].

The increasing prevalence of MDR *Acinetobacter nosocomialis* poses a significant challenge in clinical settings, particularly in bloodstream infections where conventional antibiotic treatments have limited efficacy. The results of this study highlight the urgent need for alternative therapeutic strategies, with bacteriophage therapy emerging as a promising option [38-40]. Our findings indicate that awareness of phage therapy remains low, with only 26% of participants reporting prior knowledge of this treatment approach. However, there was a significant correlation between higher education levels and awareness of phage therapy ($p<0.05$), suggesting that individuals with more advanced education are more likely to be informed about emerging medical treatments [41]. This aligns with previous studies showing that knowledge of phage therapy is often concentrated among academic and healthcare professionals rather than the general public.

Despite its potential, phage remedy stays largely inaccessible, with eighty three% of contributors reporting that it is not quite simply to be had in their location. This limited accessibility may be attributed to regulatory barriers, lack of medical trials and inadequate integration into mainstream healthcare structures [42]. Cultural factors also performed a position in recognition, with 60% of members acknowledging cultural limitations to adopting phage remedy. This locating indicates that public perception and trust in non-traditional remedies want to be addressed through training and focus campaigns. Similar challenges were pronounced in other studies, where skepticism and misinformation have hindered the attractiveness of bacteriophage-based totally remedies.

The study additionally discovered that at the same time as a hundred% of contributors had encountered cases of MDR *A. nosocomialis*, most effective 11% had reported these infections to clinical government. This suggests a gap in surveillance and reporting, that may impact the implementation of phage therapy as an alternative remedy. A loss of trust in scientific sources was additionally found, with best forty nine% of contributors expressing confidence in information supplied by using healthcare professionals. Interestingly, folks who relied on social media for clinical statistics confirmed a better stage of accept as true with in phage remedy (sixty eight%), despite the fact that this association was not statistically full-size (p = zero.06). This indicates that social media might also play a growing role in shaping public perceptions of scientific improvements [43].

The results of this study align with previous research highlighting the effectiveness of phage therapy against MDR bacterial infections. However, the success of its implementation depends on overcoming barriers related to accessibility, awareness and regulatory approval. Increased investment in phage therapy research, along with targeted public education efforts, may facilitate its acceptance and integration into clinical practice. Future studies should focus on evaluating the efficacy of phage therapy through large-scale clinical trials and developing strategies to enhance its public perception.

CONCLUSIONS

Bacteriophage therapy demonstrates promising efficacy against MDR *A. nosocomialis*, with significant bactericidal activity *in vitro*. However, limited public awareness and accessibility highlight the need for educational initiatives and regulatory support to facilitate clinical implementation. Further *in vivo* studies are essential to validate these findings.

Limitations

This study is limited by its single-center design and the use of convenience sampling, which may affect the generalizability of the findings. Additionally, the lack of *in vivo* validation and genomic characterization of the isolated phages limits the scope of therapeutic conclusions. The reliance on self-reported data in the survey may introduce response bias.

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