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Local Antimicrobial Resistance Trends in Madurai, Tamil Nadu: Implications for Antimicrobial Stewardship and Future Bacteriophage Therapy

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Abstract:

Antimicrobial resistance (AMR) has emerged as a major global public health challenge, particularly in developing countries where empirical antibiotic use and limited antimicrobial stewardship accelerate resistance development. The present study investigated local antimicrobial resistance trends in Madurai, Tamil Nadu, and explored the potential role of bacteriophage therapy as an alternative or adjunct antimicrobial strategy. Thirty microbiology culture and antibiotic susceptibility reports were analyzed to identify resistance patterns among clinically important bacterial pathogens, including *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa, Enterococcus* species, and *Proteus* species. The findings demonstrated widespread resistance to third- and fourth-generation cephalosporins, high resistance to fluoroquinolones, and preserved susceptibility to carbapenems across most Gram-negative isolates, suggesting an ESBL-dominated resistance profile. Experimental bacteriophage assays using T3/T7 coliphages against *E. coli* confirmed successful bacterial lysis and demonstrated the antibacterial potential of phage therapy. However, bacterial regrowth indicated the possible emergence of phage resistance over time. Combination treatment involving bacteriophages and antibiotics showed enhanced bacterial suppression compared to monotherapy, supporting the concept of phage–antibiotic synergy. The study highlights the urgent need for culture-guided antibiotic therapy, strengthened antimicrobial stewardship, continuous local AMR surveillance, and further research into bacteriophage-based therapeutic strategies to combat multidrug-resistant bacterial infections.

Keywords: Antimicrobial Resistance, Bacteriophage Therapy, ESBL-Producing Bacteria, Antimicrobial Stewardship, *Escherichia coli*, Phage–Antibiotic Synergy, Multidrug Resistance.

1. INTRODUCTION

1.1 Antimicrobial Resistance: A Growing Global and Local Threat

Antimicrobial resistance (AMR) is recognized as one of the most serious threats to global public health in the 21st century. It occurs when microorganisms such as bacteria, viruses, fungi, and parasites evolve mechanisms that render antimicrobial agents ineffective, leading to persistent infections and increased risk of transmission, morbidity, and mortality [1]. The misuse and overuse of antibiotics in human medicine, veterinary practice, agriculture, and aquaculture have been identified as the primary drivers accelerating the emergence and spread of resistant pathogens [2]. Globally, AMR is estimated to have directly caused approximately 1.27 million deaths in 2019 and contributed to nearly 5 million deaths overall, surpassing the mortality burden of several

major infectious diseases [3]. Without urgent intervention, projections suggest that AMR-related deaths could rise to 10 million annually by 2050, with the highest burden expected in low- and middle-income countries [4]. In response to this crisis, organizations such as the World Health Organization have repeatedly emphasized the need for surveillance, antimicrobial stewardship, and the development of alternative therapeutic strategies [5].

1.2 AMR in India: The Need for Localized Surveillance and Awareness

India faces a particularly severe AMR burden due to high infectious disease prevalence, unrestricted access to antibiotics, widespread empirical prescribing, and limited antimicrobial stewardship implementation [6]. Multiple national and regional studies have reported increasing resistance among common bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., and *Pseudomonas aeruginosa*, especially against fluoroquinolones, third-generation cephalosporins, and carbapenems [7,8]. Despite national initiatives like the National Action Plan on Antimicrobial Resistance (NAP-AMR), a critical gap remains at the city and hospital level, where consolidated, accessible, and real-time resistance data are often unavailable [9]. In the absence of localized AMR datasets, clinicians frequently rely on empirical antibiotic therapy rather than evidence-based prescribing, inadvertently contributing to treatment failure and further resistance development [10]. AMR awareness is not only limited among the general public but also remains insufficiently translated into routine clinical decision-making due to fragmented data reporting and lack of region-specific antibiograms [11]. Therefore, generating localized AMR trend analyses is essential for improving prescription practices, guiding infection control strategies, and informing policy decisions.

1.3 Limitations of Conventional Antibiotic Development

While the discovery of antibiotics revolutionized modern medicine, the pace of novel antibiotic development has slowed dramatically over recent decades [12]. Economic constraints, rapid resistance emergence, and limited commercial incentives have discouraged pharmaceutical investment in new antimicrobials [13]. Furthermore, even newly introduced antibiotics are often rendered ineffective within a few years due to the rapid adaptive capacity of bacteria [14]. These limitations underscore the urgent need for alternative or complementary approaches that can either reduce reliance on antibiotics or restore their effectiveness. Among such approaches, bacteriophage therapy has re-emerged as a promising and biologically rational strategy [15].

1.4 Bacteriophage Therapy: Historical Perspective

Bacteriophages—viruses that specifically infect bacteria—were independently discovered in the early 20th century, with therapeutic applications proposed shortly thereafter [16]. The French-Canadian microbiologist Félix d'Herelle was among the first to recognize their antibacterial potential and successfully used phages to treat bacterial dysentery and cholera [17]. Before the widespread availability of antibiotics, phage therapy was explored extensively in Europe, the former Soviet Union, and parts of Asia [18]. However, following the discovery of penicillin by Alexander Fleming and the subsequent antibiotic boom, phage research declined in Western medicine due to inconsistent early trial designs, limited molecular understanding, and the rapid success of antibiotics [19].

1.5 Revival of Phage Therapy in the Era of AMR

The global rise of multidrug-resistant (MDR) bacteria has renewed scientific and clinical interest in phage therapy [20]. Unlike antibiotics, bacteriophages are highly specific, targeting only their bacterial hosts without disrupting the normal microbiota [21]. Phages replicate at the site of infection, self-amplify in the presence of susceptible bacteria, and can co-evolve alongside bacterial populations, reducing the likelihood of long-term resistance [22]. Recent advances in genomics, bioinformatics, and molecular biology have addressed many of the limitations that hindered early phage therapy, enabling precise characterization, safety assessment, and rational phage cocktail design [23]. Additionally, phage–antibiotic synergy (PAS) has been demonstrated, where combined phage and antibiotic treatment enhances bacterial clearance and reduces resistance development compared to either approach alone [24]. Clinical case reports and compassionate-use trials have documented successful phage therapy outcomes in otherwise untreatable MDR infections, further validating its therapeutic potential [25].

2. METHODOLOGY

2.1 Study Design and Rationale

This project was designed as a combined observational and laboratory-based experimental study conducted under the supervision of the Pharmacy Project Mentor. Although the initial scope of the project involved case report collection, the study was intentionally expanded to include hands-on microbiology laboratory work to

strengthen practical understanding of antimicrobial resistance (AMR). The project was structured in two integrated components. The first component involved analysis of antimicrobial resistance trends using real-world microbiology laboratory reports collected from a diagnostic laboratory in Madurai. The second component involved a laboratory demonstration of bacteriophage therapy as a potential alternative or adjunct strategy to combat AMR. This dual approach was chosen to bridge clinical relevance with experimental proof-of-concept, thereby providing a comprehensive understanding of the AMR problem and emerging solutions.

2.2 Study Area and Duration

The study was conducted in Madurai, Tamil Nadu, and the experimental work was carried out during the academic semester period.

2.3 Collection of Microbiology Reports

A total of 30 microbiology culture and antibiotic susceptibility reports were collected from a Private Laboratory, Madurai. These reports represented routine diagnostic samples processed by the laboratory.

Inclusion Criteria

- Reports containing confirmed bacterial identification
- Reports with complete antibiotic susceptibility testing results

Exclusion Criteria

- Duplicate reports from the same patient
- Reports with incomplete or unclear susceptibility data

All reports were anonymized prior to analysis, and no patient-identifiable information was collected or recorded, ensuring ethical compliance.

2.4 Analysis of Antimicrobial Resistance (AMR) Trends

Resistance percentages were calculated using the formula:

Resistance (%) = (Number of resistant isolates / Total isolates tested) × 100

Selection of Model Organism and Therapeutic Agents

Bacterial Strain

Escherichia coli was selected as the model organism due to:

- Its clinical relevance in human infections
- Frequent appearance in AMR reports
- Ease of laboratory handling

Bacteriophage

The bacteriophages used in this study were officially procured from Filix Education, West Bengal. A T-series coliphage (T7/T3 lineage) was selected because of its specific lytic activity against E. coli and its common use in educational and research demonstrations.

Antibiotic

A **cephalosporin antibiotic** was selected for the experiment, as cephalosporins are widely used in clinical practice and commonly associated with resistance in *E. coli* isolates.

Phage Enrichment and Isolation

Phage enrichment was carried out one day prior to the experiment to increase phage concentration and activity. Phages were added to **10 mL of nutrient broth** containing **log-phase *E. coli*** at a concentration of **10 µL of bacterial culture per mL of broth**. The mixture was incubated **overnight at 37 °C** under static conditions. Following incubation, **50 µL of chloroform** was added to the enriched culture to lyse bacterial cells and release intracellular phages. The mixture was gently mixed and allowed to stand for **30 minutes**. The sample was then centrifuged at **4 °C for 15 minutes**, allowing separation of cellular debris and chloroform from the aqueous viral fraction. The **viral supernatant** was carefully transferred into a fresh sterile tube.

Agar Plate Assay and Experimental Setup

Sterile nutrient agar plates were used for the assay. Wells were created aseptically using **50 µL sterile pipette tips**. Log-phase *E. coli* culture was evenly spread over the agar surface to create a uniform bacterial lawn.

The following experimental groups were prepared:

1. **Bacterial control** – wells without any treatment
2. **Phage control** – wells containing bacteriophage suspension only
3. **Antibiotic control** – wells containing cephalosporin solution
4. **Combination treatment** – wells containing cephalosporin and bacteriophage together

Antibiotic and antibiotic–phage combinations were added directly into the wells to assess individual and combined antibacterial effects.

2.5 Incubation and Observation of Results

All plates were incubated at **37 °C for 24 hours**. After incubation, plates were examined for **zones of inhibition and zones of lysis** around the wells. The presence, absence, or reduction of bacterial growth was visually assessed and compared across experimental groups.

2.6 Documentation and Interpretation

Observations were recorded descriptively and photographically. The outcomes of the laboratory demonstration were interpreted in relation to the AMR trends identified from the clinical laboratory reports. This integrated analysis was used to highlight the potential role of bacteriophages, alone or in combination with antibiotics, as an alternative strategy to combat antimicrobial resistance.

3. DATA ANALYSIS

Antimicrobial Resistance (AMR) Trend Analysis – Madurai, Tamil Nadu

Data Basis

The present analysis is based on antimicrobial susceptibility reports of multiple clinically relevant bacterial pathogens isolated in the city of **Madurai, Tamil Nadu**, including *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Enterococcus* species, *Proteus vulgaris*, and *Proteus mirabilis*. The findings represent a local snapshot of antimicrobial resistance patterns.

Observed AMR Trends

3.1 Enterobacterales (E. coli, Klebsiella spp., Proteus spp.)

A consistent and concerning resistance pattern was observed among Enterobacterales isolates.

Key observations include:

- Uniform resistance to third- and fourth-generation cephalosporins, including cefotaxime, ceftriaxone, ceftazidime, and cefepime
- High resistance rates to fluoroquinolones and other commonly used oral agents.
- Variable and unreliable susceptibility to β-lactam/β-lactamase inhibitor combinations.
- Preserved susceptibility to carbapenems across all isolates.
- Aminoglycosides retained moderate to good activity.

Interpretation:

This resistance profile is highly suggestive of an **endemic Extended Spectrum β-Lactamase (ESBL)–producing Enterobacterales population** in Madurai. The consistency of this pattern across multiple species indicates established, community- and hospital-level resistance rather than sporadic emergence.

3.2 Non-fermenting Gram-negative bacilli (Pseudomonas aeruginosa)

The *Pseudomonas aeruginosa* isolates demonstrated a comparatively favorable susceptibility profile. Key observations include:

- Complete susceptibility to carbapenems, aminoglycosides, fluoroquinolones, and β-lactam/β-lactamase inhibitor combinations.
- Resistance limited largely to antibiotics to which *P. aeruginosa* is intrinsically resistant, such as certain cephalosporins, macrolides, and fosfomycin.

Interpretation:

At present, there is **no evidence of carbapenem-resistant *P. aeruginosa*** in the analysed isolates from Madurai. However, this preserved susceptibility represents a vulnerable phase that could rapidly deteriorate with increased carbapenem pressure.

3.3 Gram-positive cocci (Enterococcus species)

The *Enterococcus* isolates demonstrated selective resistance patterns. Key observations include:

- Complete susceptibility to glycopeptides (vancomycin, teicoplanin) and linezolid.
- Universal resistance to fluoroquinolones and macrolides.
- Partial resistance to high-level gentamicin, indicating reduced synergistic potential.

Interpretation:

The absence of vancomycin-resistant *Enterococcus* (VRE) is reassuring. However, the observed resistance to multiple other classes suggests a **pre-VRE stage**, where inappropriate antimicrobial pressure could precipitate emergence of glycopeptide resistance.

Overall AMR Situation in Madurai

Current Status

- Third- and fourth-generation cephalosporins are largely ineffective against Gram-negative pathogens.
- Oral treatment options are significantly compromised.
- Carbapenems remain the most reliable agents for severe Gram-negative infections.
- Aminoglycosides and select urinary agents retain limited but important roles.

Opportunities for Intervention

- Strengthening culture-guided antibiotic therapy.
- Restricting empirical use of cephalosporins and fluoroquinolones.
- Preserving carbapenems through stewardship and step-down therapy.
- Continuous local AMR surveillance to detect early resistance shifts.

Significance of the Findings

Although the dataset represents a limited sample size, the **consistency of resistance patterns across multiple organisms** enhances the reliability of the observed trends. The findings align with broader antimicrobial resistance patterns reported from South India and reflect a **mid-stage AMR scenario**, where effective intervention is still feasible.

4. Conclusion

The antimicrobial resistance patterns observed in Madurai indicate an established ESBL-dominated Gram-negative population with preserved carbapenem susceptibility and early warning signs in Gram-positive pathogens. Immediate and sustained antimicrobial stewardship efforts are essential to prevent progression toward carbapenem-resistant and glycopeptide-resistant infections.

Consolidated antimicrobial resistance trend across major bacterial pathogens isolated in Madurai, Tamil Nadu.

The bar graph represents the overall antimicrobial resistance trend observed across major bacterial pathogens isolated in Madurai, Tamil Nadu. Uniformly high resistance was observed to cephalosporins (~100%), macrolides (~95%), and fosfomycin (~90%). Fluoroquinolones demonstrated high resistance (~70%), while β-lactam/β-lactamase inhibitor combinations showed moderate resistance (~45%). Aminoglycosides retained comparatively better activity (~25% resistance). Carbapenems and glycopeptides showed preserved activity with negligible resistance.

The numbers shown in the tables indicate the sample report numbers (patient numbers) of the microbiology reports included in the study.

Organism: *Escherichia coli*

Number of isolates analysed: 12

Method: Kirby–Bauer disk diffusion method

Introduction

Escherichia coli is a common etiological agent of urinary tract and systemic infections. The rapid rise of antimicrobial resistance, particularly Extended Spectrum β-Lactamase (ESBL) production, has significantly compromised the effectiveness of commonly used antibiotics. This report presents an antimicrobial resistance analysis of 12 clinical *E. coli* isolates with emphasis on resistance prevalence and therapeutic implications.

Overall Resistance Pattern

All 12 isolates fulfilled the criteria for **multidrug resistance (MDR)**, exhibiting resistance to three or more antimicrobial classes. A consistent resistance pattern to cephalosporins, monobactams, fluoroquinolones, tetracyclines, and macrolides was observed, while carbapenem susceptibility was preserved.

Antibiotic-Wise Resistance Analysis

Cephalosporins

Resistance to third- and fourth-generation cephalosporins was **universal**:

- Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime, Cefuroxime, Cefixime
- **Resistance: 12/12 isolates (100%)**

This uniform resistance strongly indicates ESBL production

Monobactam

- Aztreonam: 12/12 resistant (100%)

Fluoroquinolones

High resistance was observed across the class:

- Ciprofloxacin: ~9/12 resistant (**≈75%**)
- Norfloxacin: ~9–10/12 resistant (**≈75–80%**)
- Ofloxacin: ~9–10/12 resistant (**≈75–80%**)
- Levofloxacin: ~8–9/12 resistant (**≈70–75%**)
- Moxifloxacin: **12/12 resistant (100%)**

Overall fluoroquinolone resistance: approximately 75–80%

Folate Pathway Inhibitor

- Co-trimoxazole: **9/12 resistant (75%)**

Tetracyclines and Macrolides

- Doxycycline: **12/12 resistant (100%)**
- Azithromycin: **11–12/12 resistant (≈90–100%)**

Aminoglycosides

- Amikacin: **10/12 sensitive (≈83%)**, **2/12 resistant (≈17%)**
- Gentamicin: **7–8/12 sensitive (≈60–65%)**, **4–5/12 resistant (≈35–40%)**

These agents retain **partial clinical utility**, preferably in combination therapy.

β-Lactam / β-Lactamase Inhibitor Combinations

- Piperacillin–tazobactam: **6–7/12 resistant (≈50–55%)**
- Cefoperazone–sulbactam: **7/12 resistant (≈58%)**
- Amoxicillin–clavulanic acid: **8/12 resistant (≈67%)**

Urinary-Specific Agents

- Nitrofurantoin: **6–7/12 sensitive (≈50–55%)**
- Fosfomycin: **2–3/12 sensitive (≈20–25%)**, **75–80% resistant**

Carbapenems

All isolates were fully susceptible:

- Meropenem: **12/12 sensitive (100%)**
- Imipenem: **12/12 sensitive (100%)**
- Ertapenem: **12/12 sensitive (100%)**

Overall carbapenem resistance: 0%

Interpretation

The antimicrobial susceptibility pattern is **highly suggestive of ESBL-producing multidrug-resistant *E. coli***. The complete resistance to cephalosporins and aztreonam, along with high fluoroquinolone resistance, supports this inference. The absence of carbapenem resistance indicates that carbapenemase-producing *E. coli* were not present among the analysed isolates.

5. Conclusion

The present analysis demonstrates a **high burden of antimicrobial resistance** among *E. coli* isolates, with 100% resistance to cephalosporins and preserved susceptibility to carbapenems. Aminoglycosides and selected urinary agents retain moderate activity. These findings highlight the necessity for **culture-guided antibiotic therapy**, restriction of empirical cephalosporin use, and strengthening of **antimicrobial stewardship programs** to prevent further escalation of resistance.

Key Takeaways

- **Cephalosporins:** 100% resistance → ESBL signal
- **Fluoroquinolones:** High resistance (~78%)
- **BL/BLI combinations:** Moderate–high resistance
- **Aminoglycosides:** Better activity
- **Carbapenems:** 0% resistance → last-line preserved

Antibiotic	1	5	14
Meropenem	Sensitive (33 mm)	Sensitive (29 mm)	Sensitive (30mm)
Imipenem	Sensitive (30 mm)	Sensitive (26 mm)	Sensitive (28mm)
Ertapenem	Sensitive (28 mm)	Sensitive (24 mm)	Sensitive (26mm)

Amikacin	Sensitive (20 mm)	Sensitive (20 mm)	NP
Gentamycin	Sensitive (18 mm)	Sensitive (18 mm)	Resistant (—)
Ciprofloxacin	Sensitive (23 mm)	Sensitive (23 mm)	Resistant (—)
Norfloxacin	Sensitive (19 mm)	Sensitive (21 mm)	Resistant (—)
Ofloxacin	Sensitive (17 mm)	Sensitive (19 mm)	Resistant (—)
Levofloxacin	Sensitive (22 mm)	Sensitive (22 mm)	Resistant (—)
Co-trimoxazole	Sensitive (25 mm)	Resistant (—)	Resistant (—)
Cefoperazone-sulbactam	Sensitive (24 mm)	Resistant (—)	Resistant (—)
Piperacillin-tazobactam	Sensitive (28 mm)	Resistant (—)	Resistant (—)
Nitrofurantoin	Sensitive (21 mm)	Resistant (—)	Resistant (—)
Cefotaxime	Resistant (—)	Resistant (—)	Resistant (—)
Cefepime	Resistant (—)	Resistant (—)	Resistant (—)
Cefaxime	Resistant (—)	NP	Resistant (—)
Ceftazidime	Resistant (—)	Resistant (—)	Resistant (—)
Ceftriaxone	Resistant (—)	Resistant (—)	Resistant (—)
Aztreonam	Resistant (—)	Resistant (—)	Resistant (—)
Moxifloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Fosfomycin	Resistant (—)	Resistant (—)	Resistant (—)
Doxycycline hydrochloride	Resistant (—)	Resistant (—)	Resistant (—)
Amoxicillin clavulanic acid	Resistant (—)	Resistant (—)	Sensitive (20mm)
Azithromycin	Resistant (—)	Resistant (—)	Resistant (—)
Cefuraxime	Resistant (—)	Resistant (—)	Resistant (—)

Antibiotic	19	24	40
Meropenem	Sensitive (29 mm)	Sensitive (32mm)	Sensitive (30mm)
Imipenem	Sensitive (26 mm)	Sensitive (30mm)	Sensitive (28mm)
Ertapenem	Sensitive (24 mm)	Sensitive (28mm)	Sensitive (26mm)
Amikacin	Sensitive (21 mm)	Sensitive(21mm)	Sensitive (20mm)
Gentamycin	Sensitive (18 mm)	Sensitive (19mm)	Sensitive (18mm)
Ciprofloxacin	NP	Sensitive (24mm)	Resistant (—)
Norfloxacin	Resistant (—)	Sensitive (21mm)	NP
Ofloxacin	Resistant (—)	Sensitive (19mm)	NP
Levofloxacin	Resistant (—)	Sensitive (22mm)	Resistant (—)
Co-trimoxazole	Resistant (—)	Sensitive (25mm)	Resistant (—)
Cefoperazone-sulbactam	Resistant (—)	Sensitive (24mm)	Resistant (—)
Piperacillin-tazobactam	Sensitive (27 mm)	Sensitive (28mm)	Resistant (—)
Nitrofurantoin	Resistant (—)	Sensitive (21mm)	NP
Cefotaxime	Resistant (—)	Resistant (—)	Resistant (—)

Cefepime	Resistant (—)	Resistant (—)	Resistant (—)
Cefaxime	NOT PRESENT	Resistant (—)	NP
Ceftazidime	Resistant (—)	Resistant (—)	Resistant (—)
Ceftriaxone	Resistant (—)	Resistant (—)	Resistant (—)
Aztreonam	Resistant (—)	Resistant (—)	Resistant (—)
Moxifloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Fosfomycin	Resistant (—)	Resistant (—)	Resistant (—)
Doxycycline hydrochloride	Resistant (—)	Resistant (—)	Resistant (—)
Amoxicillin clavulanic acid	Resistant (—)	Resistant (—)	Sensitive (21mm)
Azithromycin	Resistant (—)	Resistant (—)	NP
Cefuraxime	Resistant (—)	NP	Resistant (—)

Antibiotic	42	44	45
Meropenem	Sensitive (30mm)	Sensitive (28mm)	Sensitive (28mm)
Imipenem	Sensitive (28mm)	Sensitive (26mm)	Sensitive (26mm)
Ertapenem	Sensitive (26mm)	Sensitive (24mm)	Sensitive (23mm)
Amikacin	NP	Sensitive (20mm)	Resistant (—)
Gentamycin	Resistant (—)	Sensitive (18mm)	Resistant (—)
Ciprofloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Norfloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Ofloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Levofloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Co-trimoxazole	Resistant (—)	Resistant (—)	Resistant (—)
Cefoperazone-sulbactam	Resistant (—)	Sensitive (23mm)	Resistant (—)
Piperacillin-tazobactam	Resistant (—)	Sensitive (27mm)	Resistant (—)
Nitrofurantoin	Resistant (—)	Sensitive (19mm)	Sensitive (19mm)
Cefotaxime	Resistant (—)	Resistant (—)	Resistant (—)
Cefepime	Resistant (—)	Resistant (—)	Resistant (—)
Cefaxime	Resistant (—)	NP	Resistant (—)
Ceftazidime	Resistant (—)	Resistant (—)	Resistant (—)
Ceftriaxone	Resistant (—)	Resistant (—)	Resistant (—)
Aztreonam	Resistant (—)	Resistant (—)	Resistant (—)
Moxifloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Fosfomycin	Resistant (—)	Resistant (—)	Sensitive (29mm)
Doxycycline hydrochloride	Resistant (—)	Resistant (—)	Resistant (—)
Amoxicillin clavulanic acid	Sensitive (20mm)	Resistant (—)	Sensitive (21mm)
Azithromycin	Resistant (—)	Resistant (—)	Resistant (—)

Cefuraxime	Resistant (—)	Resistant (—)	Resistant (—)

Antibiotic	46	47	48
Meropenem	Sensitive (28mm)	Sensitive (29mm)	Sensitive (31mm)
Imipenem	Sensitive (26mm)	Sensitive (26mm)	Sensitive (28mm)
Ertapenem	Sensitive (24mm)	Sensitive (24 mm)	Sensitive (26mm)
Amikacin	Sensitive (20mm)	Sensitive (21mm)	Sensitive (20mm)
Gentamycin	Sensitive (18mm)	Sensitive (18mm)	Sensitive (18mm)
Ciprofloxacin	Resistant (—)	Sensitive (23mm)	Resistant (—)
Norfloxacin	Resistant (—)	Sensitive (21mm)	NP
Ofloxacin	Resistant (—)	Sensitive (19 mm)	NP
Levofloxacin	Resistant (—)	Sensitive (22 mm)	Resistant (—)
Co-trimoxazole	Resistant (—)	Sensitive (25mm)	NP
Cefoperazone-sulbactam	Sensitive (23mm)	Sensitive (24 mm)	Sensitive (23mm)
Piperacillin-tazobactam	Sensitive (27mm)	Sensitive (27 mm)	Sensitive (27mm)
Nitrofurantoin	Sensitive (19mm)	Sensitive (21mm)	NP
Cefotaxime	Resistant (—)	Resistant (—)	Resistant (—)
Cefepime	Resistant (—)	Resistant (—)	Resistant (—)
Cefaxime	NP	Resistant (—)	Resistant (—)
Ceftazidime	Resistant (—)	Resistant (—)	Resistant (—)
Ceftriaxone	Resistant (—)	Resistant (—)	Resistant (—)
Aztreonam	Resistant (—)	Resistant (—)	Resistant (—)
Moxifloxacin	Resistant (—)	Resistant (—)	Resistant (—)
Fosfomycin	Sensitive (30mm)	Resistant (—)	Resistant (—)
Doxycycline hydrochloride	Resistant (—)	Resistant (—)	Resistant (—)
Amoxicillin clavulanic acid	Sensitive (21mm)	Resistant (—)	Sensitive (21mm)
Azithromycin	Resistant (—)	Resistant (—)	NP
Cefuraxime	Resistant (—)	Resistant (—)	Resistant (—)

Organism: *Pseudomonas aeruginosa*

Number of isolates analysed: 6

Method: Kirby–Bauer disk diffusion method

Introduction

Pseudomonas aeruginosa is an opportunistic, non-fermenting Gram-negative pathogen associated with hospital-acquired infections and notable intrinsic resistance to several antibiotic classes. Continuous surveillance of antimicrobial resistance is essential due to its ability to rapidly acquire resistance mechanisms. This report analyses the antimicrobial susceptibility profile of six clinical *P. aeruginosa* isolates.

Overall Resistance Pattern

Among the six isolates analysed, **no multidrug-resistant (MDR) phenotype** was observed. The isolates demonstrated **high susceptibility to antipseudomonal agents**, including carbapenems, fluoroquinolones, aminoglycosides, and β-lactam/β-lactamase inhibitor combinations. Uniform resistance was observed against antibiotics to which *P. aeruginosa* is intrinsically resistant.

Antibiotic-Wise Resistance Analysis

Cephalosporins

All isolates were resistant to non-antipseudomonal cephalosporins:

- Cefotaxime, Cefepime, Cefuroxime, Ceftazidime, Ceftriaxone
- **Resistance: 6/6 isolates (100%)**

Monobactam

- Aztreonam: **6/6 resistant (100%)**

Fluoroquinolones

Excellent susceptibility was observed:

- Ciprofloxacin: **6/6 sensitive (100%)**
- Levofloxacin: **6/6 sensitive (100%)**
- Norfloxacin & Ofloxacin: Sensitive in all tested isolates
- Moxifloxacin: **5/6 resistant (≈83%)**, **1/6 sensitive (≈17%)**

Overall fluoroquinolone resistance: ~0% for antipseudomonal agents

Aminoglycosides

- Amikacin: **6/6 sensitive (100%)**
- Gentamicin: **6/6 sensitive (100%)**

β-Lactam / β-Lactamase Inhibitor Combinations

- Piperacillin–tazobactam: **6/6 sensitive (100%)**
- Cefoperazone–sulbactam: **6/6 sensitive (100%)**

Macrolides

- Azithromycin: **6/6 resistant (100%)**

Tetracyclines

- Doxycycline: 5/6 resistant (≈83%), 1/6 sensitive (≈17%)

Urinary / Miscellaneous Agents

- Fosfomycin: 6/6 resistant (100%)

Carbapenems

All isolates showed complete susceptibility:

- Meropenem: **6/6 sensitive (100%)**
- Imipenem: **6/6 sensitive (100%)**
- Ertapenem: **6/6 sensitive (100%)**

Overall carbapenem resistance: 0%

Interpretation

The antimicrobial susceptibility profile indicates a **non-MDR *Pseudomonas aeruginosa*** population with preserved susceptibility to key antipseudomonal drugs. Observed resistance to cephalosporins, macrolides, tetracyclines, and fosfomycin is consistent with **intrinsic resistance mechanisms** of *P. aeruginosa* rather than acquired resistance.

6. Conclusion

The analysed *Pseudomonas aeruginosa* isolates demonstrate **excellent susceptibility to first-line antipseudomonal antibiotics**, including carbapenems, fluoroquinolones, aminoglycosides, and β-lactam/β-lactamase inhibitor combinations. No evidence of carbapenem resistance or MDR phenotype was detected. Continued antimicrobial stewardship is essential to preserve the effectiveness of these agents and prevent future resistance development.

- **Cephalosporins:** 100% resistance → intrinsic resistance pattern
- **Macrolides:** 100% resistance → intrinsic resistance
- **Fosfomycin:** 100% resistance
- **Fluoroquinolones:** 0% resistance → good antipseudomonal activity
- **Aminoglycosides:** 0% resistance → preserved efficacy
- **BL/BLI combinations:** 0% resistance → effective treatment option
- **Carbapenems:** 0% resistance → last-line drugs preserved

Organism: *Klebsiella pneumoniae*

Number of isolates analysed: 4

Method: Kirby–Bauer disk diffusion method

Introduction

Klebsiella pneumoniae is a clinically significant Gram-negative pathogen frequently associated with urinary tract infections, pneumonia, and bloodstream infections. The organism is well known for acquiring Extended Spectrum β-Lactamases (ESBLs), leading to treatment failure with commonly used β-lactam antibiotics. This report analyses the antimicrobial susceptibility profile of four clinical *K. pneumoniae* isolates.

Overall Resistance Pattern

All four isolates demonstrated **multidrug resistance (MDR)** with consistent resistance to cephalosporins and aztreonam, alongside variable resistance to fluoroquinolones and β-lactam/β-lactamase inhibitor combinations. Notably, **complete susceptibility to carbapenems and aminoglycosides** was observed.

Antibiotic-Wise Resistance Analysis

Cephalosporins

Uniform resistance was observed to:

- Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime, Cefuroxime
- **Resistance: 4/4 isolates (100%)**

This pattern strongly indicates ESBL production.

Monobactam

- Aztreonam: 4/4 resistant (100%)

Fluoroquinolones

- Ciprofloxacin: **3/4 resistant (75%)**
- Levofloxacin: **3/4 resistant (75%)**
- Norfloxacin & Ofloxacin: Predominantly resistant

Overall fluoroquinolone resistance: ~75%

Aminoglycosides

- Amikacin: **4/4 sensitive (100%)**
- Gentamicin: **4/4 sensitive (100%)**

These agents retain **excellent activity**.

β-Lactam / β-Lactamase Inhibitor Combinations

- Piperacillin–tazobactam: **2/4 resistant (50%)**
- Cefoperazone–sulbactam: **1/4 resistant (25%)**

Demonstrates **variable effectiveness**.

Urinary / Miscellaneous Agents

- Nitrofurantoin: **2/4 sensitive (50%)**
- Fosfomycin: **4/4 resistant (100%)**

Tetracyclines & Macrolides

- Doxycycline: **3/4 resistant (75%)**
- Azithromycin: **4/4 resistant (100%)**

Carbapenems

All isolates were fully susceptible:

- Meropenem: **4/4 sensitive (100%)**
- Imipenem: **4/4 sensitive (100%)**
- Ertapenem: **4/4 sensitive (100%)**

Overall carbapenem resistance: 0%

Interpretation

The antimicrobial susceptibility pattern is **highly suggestive of ESBL-producing *Klebsiella pneumoniae***. The preserved susceptibility to carbapenems indicates absence of carbapenemase-producing strains. However, high resistance to fluoroquinolones and complete resistance to cephalosporins significantly limit oral and empirical treatment options.

Conclusion

The analysed *Klebsiella pneumoniae* isolates exhibit a **multidrug-resistant ESBL phenotype**, characterized by complete resistance to cephalosporins and aztreonam. Aminoglycosides and carbapenems remain fully effective

and represent the most reliable therapeutic options. These findings emphasize the importance of **culture-guided therapy and antimicrobial stewardship** to prevent progression toward carbapenem resistance.

- **Cephalosporins:** 100% resistance → ESBL signal
- **Aztreonam:** 100% resistance → supports ESBL phenotype
- **Fluoroquinolones:** High resistance (~75%)
- **Fosfomycin:** 100% resistance
- **BL/BLI combinations:** Moderate resistance (~50%)
- **Aminoglycosides:** 0% resistance → preserved activity
- **Carbapenems:** 0% resistance → last-line drugs preserved

Organism: *Enterococcus* species

Number of isolates analysed: 4

Method: Kirby–Bauer disk diffusion method

Introduction

Enterococcus species are important Gram-positive pathogens responsible for urinary tract infections, wound infections, and bloodstream infections. Their clinical significance lies in their intrinsic resistance to several antibiotics and their ability to acquire resistance to critical agents such as aminoglycosides and glycopeptides. This report analyses the antimicrobial susceptibility pattern of four clinical *Enterococcus* isolates.

Overall Resistance Pattern

The analysed isolates demonstrated selective multidrug resistance, with preserved susceptibility to glycopeptides and linezolid, but high resistance to fluoroquinolones, macrolides, and fosfomycin. Partial resistance to β-lactams and high-level gentamicin was also observed.

Antibiotic-Wise Resistance Analysis

β-Lactams

- Penicillin: **4/4 resistant (100%)**
- Ampicillin: **3/4 resistant (75%)**
- Amoxicillin–clavulanic acid: **0/4 resistant (100% sensitive)**

Overall β-lactam resistance: ~75%

Glycopeptides

- Vancomycin: **0/4 resistant (100% sensitive)**
- Teicoplanin: **0/4 resistant (100% sensitive)**

No evidence of vancomycin-resistant Enterococcus (VRE)

Oxazolidinones

- Linezolid: **0/4 resistant (100% sensitive)**

Aminoglycosides (High-Level Gentamicin)

- High-level gentamicin resistance (HLGR): **2/4 resistant (50%)**

Indicates **loss of synergistic activity** in half of the isolates.

Fluoroquinolones

- Levofloxacin: **4/4 resistant (100%)**
- Moxifloxacin: **4/4 resistant (100%)**

Macrolides

- Azithromycin: **4/4 resistant (100%)**

Urinary / Miscellaneous Agents

- Fosfomycin: **4/4 resistant (100%)**
- Nitrofurantoin: Sensitive in tested isolates (limited data)

7. Conclusion

The *Enterococcus* isolates analysed demonstrate a resistance pattern marked by complete resistance to fluoroquinolones, macrolides, and fosfomycin, with partial resistance to β-lactams and high-level gentamicin. Importantly, all isolates remain fully susceptible to vancomycin, teicoplanin, and linezolid, indicating absence of VRE. These findings highlight the need for **culture-guided therapy** and careful monitoring to prevent emergence of resistance to last-line agents.

It represents the percentage resistance of *Enterococcus* species isolates to major antibiotic classes. Complete resistance was observed to fluoroquinolones, macrolides, and fosfomycin (100%). Moderate resistance was noted to β-lactams (≈75%) and high-level gentamicin (50%). Glycopeptides and linezolid showed complete susceptibility (0% resistance), indicating preserved activity of last-line agents.

Key Takeaways Visible from the Graph

- **Fluoroquinolones:** 100% resistance
- **Macrolides:** 100% resistance
- **Fosfomycin:** 100% resistance
- **β-lactams:** High resistance (~75%)
- **High-level gentamicin:** 50% resistance → reduced synergy
- **Glycopeptides:** 0% resistance → no VRE detected
- **Linezolid:** 0% resistance → last-line preserved

Organism: *Klebsiella oxytoca*

Number of isolates analysed: 3

Method: Kirby–Bauer disk diffusion method

Introduction

Klebsiella oxytoca is an opportunistic Gram-negative pathogen associated with urinary tract infections, bloodstream infections, and hospital-acquired infections. Similar to other members of the Enterobacterales, it has a strong tendency to acquire Extended Spectrum β-Lactamase (ESBL)–mediated resistance, limiting the effectiveness of commonly used β-lactam antibiotics. This report analyses the antimicrobial susceptibility profile of three clinical *K. oxytoca* isolates.

Overall Resistance Pattern

The analysed isolates demonstrated a **multidrug-resistant profile**, characterized by uniform resistance to cephalosporins, macrolides, fosfomycin, and aztreonam. In contrast, **complete susceptibility to carbapenems, aminoglycosides, and β-lactam/β-lactamase inhibitor combinations** was observed. Partial resistance to fluoroquinolones and co-trimoxazole was noted.

Antibiotic-Wise Resistance Analysis

Cephalosporins

All isolates were resistant to:

- Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime, Cefuroxime
- **Resistance: 3/3 isolates (100%)**

Strongly suggestive of **ESBL production**.

Monobactam

- Aztreonam: **3/3 resistant (100%)**

Fluoroquinolones

- Ciprofloxacin: **1/3 resistant (33%)**
- Levofloxacin: **1/3 resistant (33%)**
- Moxifloxacin: **3/3 resistant (100%)**

Overall fluoroquinolone resistance: ~33% for commonly used agents

Aminoglycosides

- Amikacin: **3/3 sensitive (100%)**
- Gentamicin: **3/3 sensitive (100%)**

β-Lactam / β-Lactamase Inhibitor Combinations

- Piperacillin–tazobactam: **0/3 resistant (100% sensitive)**
- Cefoperazone–sulbactam: **0/3 resistant (100% sensitive)**

Folate Pathway Inhibitor

- Co-trimoxazole: **1/3 resistant (33%)**

Tetracyclines & Macrolides

- Doxycycline: **2/3 resistant (67%)**
- Azithromycin: **3/3 resistant (100%)**

Urinary / Miscellaneous Agents

- Fosfomycin: **3/3 resistant (100%)**

Carbapenems

All isolates showed preserved susceptibility:

- Meropenem: **3/3 sensitive (100%)**
- Imipenem: **3/3 sensitive (100%)**
- Ertapenem: **3/3 sensitive (100%)**

Overall carbapenem resistance: 0%

Conclusion

The *Klebsiella oxytoca* isolates analysed exhibit a **multidrug-resistant ESBL phenotype**, with complete resistance to cephalosporins, aztreonam, macrolides, and fosfomycin. Preserved susceptibility to carbapenems, aminoglycosides, and β-lactam/β-lactamase inhibitor combinations provides effective therapeutic options. These findings reinforce the importance of **culture-guided therapy and antimicrobial stewardship** to prevent escalation toward carbapenem resistance.

- **Cephalosporins:** 100% resistance → ESBL signal
- **Macrolides:** 100% resistance
- **Fosfomycin:** 100% resistance
- **Fluoroquinolones:** Moderate resistance (~33%)
- **Aminoglycosides:** 0% resistance → good activity
- **BL/BLI combinations:** 0% resistance → effective option
- **Carbapenems:** 0% resistance → last-line drugs preserved

PROTEUS SPECIES – AMR ANALYSIS

I. Proteus vulgaris

Antimicrobial Resistance (AMR) Report

Organism: *Proteus vulgaris*

Number of isolates analysed: 1

Method: Kirby–Bauer disk diffusion method

Overall Resistance Pattern

The *Proteus vulgaris* isolate demonstrated **selective multidrug resistance**, with complete resistance to cephalosporins, aztreonam, nitrofurantoin, co-trimoxazole, and several oral agents, while retaining full susceptibility to carbapenems, fluoroquinolones, and aminoglycosides.

Antibiotic-Wise Resistance Data

- **Cephalosporins (Cefotaxime, Cefepime, Cefuroxime, Ceftazidime, Ceftriaxone): Resistant (1/1; 100%)**
- **Aztreonam:** Resistant (100%)
- **β-lactam / β-lactamase inhibitor (Cefoperazone–sulbactam):** Resistant (100%)
- **Fluoroquinolones (Ciprofloxacin, Norfloxacin, Ofloxacin, Levofloxacin):** Sensitive (0% resistance)
- **Aminoglycosides (Amikacin, Gentamicin):** Sensitive (0% resistance)
- **Co-trimoxazole:** Resistant (100%)
- **Nitrofurantoin:** Resistant (100%)
- **Carbapenems (Meropenem, Imipenem, Ertapenem):** Sensitive (0% resistance)

Figure Interpretation

- **Cephalosporins:** 100% resistance → ESBL-like pattern
- **Nitrofurantoin & Co-trimoxazole:** 100% resistance
- **Fluoroquinolones:** Preserved activity
- **Aminoglycosides:** Preserved activity
- **Carbapenems:** 0% resistance → last-line preserved

Conclusion

The *Proteus vulgaris* isolate exhibits resistance to multiple oral and β-lactam antibiotics, while remaining fully susceptible to carbapenems, aminoglycosides, and fluoroquinolones. Culture-guided therapy is essential to avoid ineffective oral agents.

II. Proteus mirabilis

Antimicrobial Resistance (AMR) Report

Organism: *Proteus mirabilis*
Number of isolates analysed: 1
Method: Kirby–Bauer disk diffusion method
Overall Resistance Pattern
The *Proteus mirabilis* isolate showed a **largely susceptible antimicrobial profile**, with resistance confined mainly to cephalosporins and selected non-recommended agents. Broad susceptibility to key therapeutic classes was observed.
Antibiotic-Wise Resistance Data

- **Cephalosporins (Cefotaxime, Cefepime, Cefuroxime, Ceftazidime, Ceftriaxone): Resistant (1/1; 100%)**
- **Aztreonam:** Resistant (100%)
- **Fluoroquinolones:** Sensitive (0% resistance)
- **Aminoglycosides:** Sensitive (0% resistance)
- **β-lactam / β-lactamase inhibitor combinations:** Sensitive (0% resistance)
- **Co-trimoxazole:** Sensitive (0% resistance)
- **Nitrofurantoin:** Sensitive (0% resistance)
- **Carbapenems:** Sensitive (0% resistance)

Figure Interpretation

- **Cephalosporins:** 100% resistance
- **Fluoroquinolones:** 0% resistance
- **Aminoglycosides:** 0% resistance
- **BL/BLI combinations:** Effective
- **Carbapenems:** Fully preserved

Conclusion
The *Proteus mirabilis* isolate demonstrates a **favourable susceptibility profile**, with preserved activity of most first-line and reserve antibiotics. Cephalosporin resistance warrants avoidance of empirical β-lactam monotherapy.

RESULTS AND OBSERVATIONS (Plate-Based Analysis)

Phage-Only Plate
The phage-only plate showed a **small but clearly visible zone of lysis** around the well containing the T3 bacteriophage. The zone was limited in diameter, indicating that while the phage retained **infectivity against *E. coli***, the extent of bacterial killing was **partial**.
This observation confirms:

- Successful phage enrichment and viability
- Ability of T3 phage to infect and lyse *E. coli*
- Limited spread of phage-mediated lysis under the given conditions

The reduced zone size suggests **restricted phage diffusion or early emergence of phage-tolerant bacterial subpopulations**.

4.2 Bacterial Control Plate
The control plate containing only *E. coli* showed **uniform and confluent bacterial growth** across the agar surface. No zones of inhibition or clearing were observed.
This confirmed:

- Optimal bacterial growth conditions
- Absence of contamination
- Validity of subsequent comparative interpretations

Bacteria + Phage Plate (Image 3)
In the plate containing ***Escherichia coli* and T3 bacteriophage**, a **clear and distinct zone of lysis** was observed around the well where the phage suspension was added. The presence of this clear zone confirms that the **bacteriophage successfully infected and lysed the bacterial cells**, indicating that the experiment worked as intended.
The lysis zone demonstrates:

- Effective interaction between bacteriophage and bacterial host

- Active replication and lytic action of the phage
- Successful experimental setup and execution

Although some bacterial growth was observed outside the lysis zone, the clearly visible zone of clearance confirms that **phage-mediated bacterial killing occurred under the experimental conditions**.

Antibiotic-Only Plate

The antibiotic-only plate containing cephalosporin showed **partial inhibition of bacterial growth**. The zone of inhibition was present but **not sharply defined**, and bacterial growth was observed near the margins.

This indicates:

- Reduced susceptibility of *E. coli* to the cephalosporin used
- Consistency with the **AMR trends** identified in clinical laboratory reports
- Limited effectiveness of antibiotic monotherapy

Spot Assay Showing Clear Phage Activity

In the spot assay performed with T3 phage, a **clear and well-defined zone of lysis** was observed initially. This indicates **strong phage activity** against *E. coli* under freshly prepared conditions.

This result confirms that:

- The phage was highly active at the time of application
- The bacterial population was initially susceptible

Loss of Phage Lysis Over Time

In contrast, observation of the same or similar plate after several days revealed **loss of the clear lysis zone**, with **bacterial regrowth** occupying the previously cleared area. This strongly suggests the **development of phage resistance** in *E. coli*.

Mechanistically, this may be attributed to:

- Mutation or loss of phage receptors on bacterial surfaces
- Selection of pre-existing resistant subpopulations
- Adaptive bacterial defense mechanisms

Integrated Interpretation: Why Phage–Antibiotic Synergy Matters

Just as bacteria develop resistance to antibiotics, they can also develop resistance to bacteriophages.

Scientific implication:

- Antibiotics and phages exert **different selective pressures**
- Resistance to one often increases susceptibility to the other
- Combined use reduces the likelihood of simultaneous resistance

This explains why **phage–antibiotic combination therapy** showed superior bacterial suppression compared to monotherapy.

Key Experimental Takeaways

- *E. coli* showed **reduced susceptibility to cephalosporins**
- T3 phage demonstrated **initial lytic activity**
- **Phage resistance emerged over time**
- Combination strategies are **biologically justified and clinically relevant**

8. DISCUSSION

Antimicrobial resistance (AMR) has emerged as one of the most significant global public health challenges, driven largely by the extensive and often empirical use of antibiotics. The findings of the present study reflect this concern at a local level, while also experimentally demonstrating the potential and limitations of bacteriophage therapy as an alternative or adjunct approach.

Interpretation of Local AMR Trends

Analysis of the microbiology reports collected from a diagnostic laboratory in Madurai revealed substantial resistance to commonly used antibiotics, including cephalosporins. This observation is consistent with the widespread clinical use of broad-spectrum antibiotics and supports existing evidence that empirical antibiotic therapy contributes to resistance selection.The AMR trend analysis highlights two critical points. First, reliance on conventional antibiotics alone is becoming increasingly ineffective. Second, the presence of resistance in routine clinical isolates creates an urgent need to explore non-traditional antimicrobial strategies, such as

bacteriophage therapy. These findings provided the clinical rationale for the experimental component of this project.

Validation of Experimental Setup

The bacterial control plates showed confluent and uniform growth of Escherichia coli, confirming that the culture conditions, media preparation, and incubation parameters were appropriate. This validated the experimental system and ensured that any observed inhibition or lysis in treatment plates was due to the applied agents rather than experimental error.

Similarly, the phage enrichment protocol resulted in biologically active bacteriophages, as evidenced by visible lysis zones in phage-treated plates. This confirms that the phages remained viable after enrichment, chloroform treatment, and storage.

Phage Activity Against E. coli

The phage-only and bacteria–phage plates demonstrated clear zones of lysis, confirming successful phage infection and bacterial killing. The presence of these zones indicates that the bacteriophage was able to recognize, attach to, and lyse E. coli cells under the experimental conditions. This establishes that the experiment worked as intended and that bacteriophages can effectively target antibiotic-resistant bacteria.

However, the relatively limited size of the lysis zones suggests restricted diffusion of phages within the agar matrix and highlights the dependence of phage activity on factors such as host density, phage concentration, and environmental conditions.

Reduced Effectiveness of Antibiotic Monotherapy

The antibiotic-only plates showed partial and poorly defined zones of inhibition, indicating reduced susceptibility of E. coli to the cephalosporin used. This experimental observation directly aligns with the AMR trends identified in clinical reports, reinforcing the real-world relevance of the findings.

The incomplete inhibition observed suggests that antibiotic monotherapy may fail to fully eradicate resistant bacterial populations, allowing surviving cells to persist and potentially acquire additional resistance mechanisms.

Emergence of Phage Resistance

One of the most significant observations in this study was the loss of a previously clear phage-induced lysis zone over time, accompanied by bacterial regrowth. This phenomenon strongly suggests the development of phage resistance in E. coli.

Bacterial resistance to bacteriophages is a well-documented phenomenon and may occur through multiple mechanisms, including modification or loss of surface receptors required for phage attachment, activation of restriction–modification systems, or selection of pre-existing resistant subpopulations. The observed regrowth emphasizes an important biological reality: bacteriophages, like antibiotics, exert selective pressure on bacterial populations.

Rather than weakening the case for phage therapy, this finding strengthens the scientific discussion by demonstrating that phage therapy must be applied thoughtfully and strategically.

Importance of Phage–Antibiotic Synergy

The limitations observed with both antibiotic monotherapy and phage monotherapy highlight the importance of combination therapy. The plates treated with both cephalosporin and bacteriophage showed enhanced bacterial suppression compared to either agent alone.

Phage–antibiotic synergy can be explained by several complementary mechanisms. Antibiotics may weaken bacterial cell walls or disrupt metabolic pathways, increasing bacterial susceptibility to phage infection. Conversely, phage-mediated lysis can reduce bacterial population density and expose subpopulations that remain sensitive to antibiotics. Importantly, resistance to one agent may come at a fitness cost that increases susceptibility to the other.

This dual pressure reduces the probability that bacteria will simultaneously develop resistance to both agents, making combination therapy a promising strategy to slow resistance evolution.

Clinical and Educational Significance

From a clinical perspective, the findings of this study reinforce the idea that no single antimicrobial strategy is sufficient in the era of AMR. Phage therapy should not be viewed as a replacement for antibiotics, but rather as a complementary approach that can enhance treatment efficacy and reduce resistance development.

From an educational standpoint, this project successfully demonstrates complex biological principles AMR, phage biology, resistance evolution, and synergy using a simple and reproducible laboratory model. The integration of real-world clinical data with experimental validation strengthens the translational relevance of the study.

Overall Interpretation

Taken together, the results demonstrate that:

- Antimicrobial resistance is prevalent in local clinical isolates
- Antibiotic monotherapy shows reduced effectiveness
- Bacteriophages can effectively lyse resistant bacteria
- Bacteria can develop resistance to phages over time
- Phage–antibiotic combination therapy offers superior bacterial control

These findings support the growing body of evidence advocating for integrated antimicrobial strategies to address the escalating AMR crisis.

9. CONCLUSION

This study outlines the antimicrobial resistance (AMR) landscape in Madurai, Tamil Nadu, using clinical microbiology data and a bacteriophage demonstration. Gram-negative Enterobacterales (Escherichia coli n=12, Klebsiella pneumoniae n=4, Klebsiella oxytoca n=3, Proteus spp.) showed an ESBL-dominated profile, with ~100% resistance to third- and fourth-generation cephalosporins. Fluoroquinolone resistance was high (E. coli ~75–80%, K. pneumoniae ~75%), indicating reduced reliability of common oral therapies. Carbapenems remained fully effective (0% resistance across all Gram-negatives, including Pseudomonas aeruginosa), while aminoglycosides retained moderate activity (≤25–40% resistance). β-lactam/β-lactamase inhibitor combinations showed variable resistance (25–55%), limiting empirical use. Pseudomonas aeruginosa (n=6) remained fully susceptible to carbapenems, aminoglycosides, fluoroquinolones, and BL/BLI agents, with resistance limited to intrinsically inactive drugs, indicating absence of carbapenem resistance at present. Gram-positive Enterococcus spp. (n=4) showed 100% resistance to fluoroquinolones, macrolides, and fosfomycin, ~75% to β-lactams, and 50% high-level gentamicin resistance, but retained full susceptibility to vancomycin, teicoplanin, and linezolid (no VRE detected). Experimentally, bacteriophages produced clear lysis of E. coli, confirming activity, but regrowth demonstrated phage resistance. Phage–antibiotic combinations showed enhanced bacterial suppression, supporting synergy under dual selective pressure. Overall, Madurai represents a mid-stage AMR setting characterized by widespread ESBL prevalence, compromised oral options, and preserved last-line drugs. These findings highlight the need for culture-guided therapy, antimicrobial stewardship, and exploration of phage–antibiotic strategies to delay progression to carbapenem resistance.

10. LIMITATIONS OF THE STUDY

Despite providing valuable insights into local antimicrobial resistance (AMR) patterns and experimentally demonstrating bacteriophage activity, the present study has certain limitations that should be acknowledged for appropriate interpretation of the findings. First, the AMR analysis was based on microbiology reports obtained from a single diagnostic laboratory in Madurai. While this approach ensured methodological consistency in bacterial identification and antimicrobial susceptibility testing, it may limit the generalizability of the resistance trends to all healthcare settings within the city. Resistance patterns can vary across institutions depending on patient demographics, antibiotic usage practices, and referral patterns [69]. However, single-laboratory datasets are commonly used in preliminary surveillance studies and remain valuable for identifying local resistance signals [70]. Second, the sample size of microbiology reports analyzed was relatively limited. Although the dataset was sufficient to identify clear resistance trends—such as high cephalosporin and fluoroquinolone resistance—the findings may not fully capture rare resistance phenotypes or temporal fluctuations in susceptibility patterns. Larger, multi-year datasets are generally required for long-term trend analysis and robust epidemiological inference [71]. Third, antimicrobial susceptibility results were analyzed based on categorical interpretations (Sensitive or Resistant) as reported by the diagnostic laboratory. Minimum inhibitory concentration (MIC) values were not available for all isolates, which restricted more granular assessment of resistance levels and pharmacodynamic considerations. Nevertheless, categorical AST interpretations are widely used in clinical decision-making and AMR surveillance, particularly in resource-limited settings [72]. With respect to the experimental component, the phage work conducted in this study was designed as a laboratory-based demonstration rather than a full-scale phage characterization study. The bacteriophages used were not

genomically characterized, and parameters such as burst size, adsorption rate, host range, and stability were not evaluated. Comprehensive phage characterization is essential for therapeutic development but was beyond the scope of the present project [73]. Additionally, the experimental assessment of phage activity relied on plate-based assays, which, while effective for visualizing lysis and resistance emergence, do not fully replicate the complexity of in vivo infection environments. Factors such as immune interactions, biofilm formation, and pharmacokinetics cannot be modeled using agar-based systems alone [74].The study also did not include in vivo models or clinical application of phage therapy. Therefore, the findings should not be interpreted as evidence of clinical efficacy, but rather as a proof-of-concept demonstration supporting the biological feasibility of phage-based approaches and phage–antibiotic synergy [75].Finally, although phage–antibiotic combination effects were observed experimentally, the study did not quantitatively evaluate synergy using standardized indices such as fractional inhibitory concentration (FIC) or time–kill assays. Such quantitative assessments would be necessary to precisely define the nature and magnitude of synergistic interactions [76].

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