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Targeting MDR *E. coli* with Bacteriophage-CNP Synergy: Antibiotic-Free Therapeutics Strategy in UTI Infections

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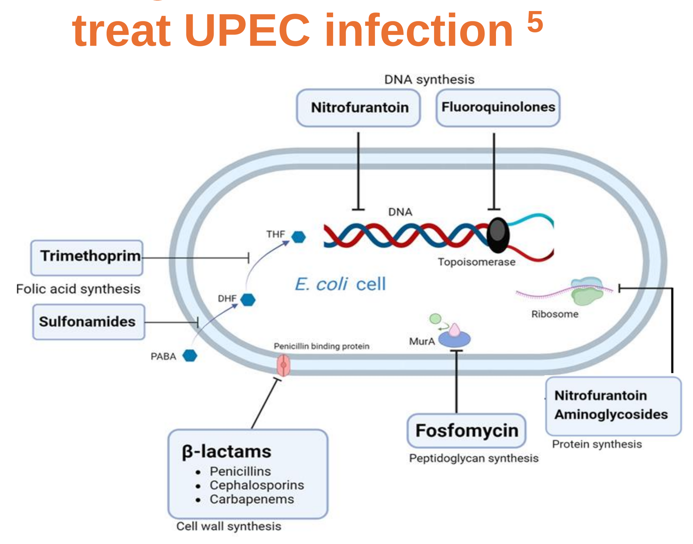
Abstract

Background: The rising prevalence of multidrug-resistant (MDR) *E. coli* in UTIs in India poses a serious challenge to infection control. Due to the pan drug resistance, antibiotics are increasingly ineffective against pathogens. Bacteriophages offer targeted antibacterial activity, and when combined with carbon nanoparticles (CNPs), may enhance therapeutic efficacy through synergistic action. **Methods:** MDR E. coli strains were isolated female urine sample. Identification was performed using MALDI-TOF MS, followed by antibiotic susceptibility testing. Bacteriophages were isolated using a three-step enrichment and $ZnCl_2$ precipitation method. The lytic activity and host specificity of phages were assessed via plaque assays. Combination therapy using phage and CNPs was evaluated through in vitro growth inhibition assays (96-well plate checkerboard method), one-step growth curves, and electron microscopy. **Results:** Phages demonstrated potent lytic activity against MDR E. coli, with no effect on non-target bacteria. Phage stability and viability at different pH and temperature was found remarkable. Combination therapy with CNPs significantly enhanced bacteriolytic effects and delayed regrowth in vitro. Scanning electron microscopy confirmed phage integrity and host interaction. The phage-CNP combination showed greater efficacy than individual treatments, indicating synergistic potential. Mice gut experiment suggest the MDR E. coli is totally killed by phage. **Conclusion**: This study underscores the potential of phage-CNP combination therapy as a novel and translational approach to controlling MDR E. coli. The findings support the development of phage-based interventions along with CNP application as an adjuvant. The mice-based experiment suggests to control the MDR E. coli without the antibiotics.

Introduction

UTI causes in women [7]

Cellular targets of antibiotics used to treat UPEC infection [5]



Treatment as per the European Association of Urology for UTI [6]

UTI Type	Recommended First-Line Treatment	Recommended Empirical Treatments
Uncomplicated cystitis	Fosfomycin trometamol (Oral) Nitrofurantoin Cephalosporins (alternative) Pivmecillinam	Trimethoprim Trimethoprim/sulfamethoxazole
Uncomplicated pyelonephritis	Fluoroquinolones	Ciprofloxacin Levofloxacin Trimethoprim/sulfamethoxazole Cefpodoxime or Ceftibuten
Complicated UTIs	Amoxicillin plus aminoglycoside 2nd generation cephalosporin plus an aminoglycoside	3rd generation cephalosporin used intravenously

Methodology

1. Urinary tract infection (UTI) samples were collected from patients showing clinical symptoms

1A. Bacteriophages were isolated from sewage samples using the MDR/XDR E. coli as hosts through enrichment [1]

2. E. coli isolate were using EMB agar and confirmed by colony morphology and biochemical tests

2A. The phages was examined using modified acid-fast staining under a phase-contrast microscope and confirmed by electron microscopy.

3. Isolates were subjected to antibiotic sensitivity testing to identify MDR and XDR strains.

3A. The host-phage interaction was assessed using an in vitro one-step growth curve to determine latent period and burst size.

4. WHONET software was used for analysis and interpretation of antimicrobial resistance patterns.

4A. Bacteriophages Typing with Pathogens and Probiotics bacteria

5. Carbon nanoparticles (CNPs) were prepared and characterized for their physicochemical properties.

6. The synergistic potential of phage & CNPs was evaluated *in vitro* against MDR *E. coli* for enhanced lytic efficiency

7. The combined phage–CNP therapy was tested in vivo in a mouse gut infection model to assess its effectiveness in reducing MDR E. coli colonization.

8. Evaluation of phage as an adjuvant with antibiotic and CNPs and development of probiotics against E. coli using Lactic acid bacteria with Phage and CNP.

Result

1. UTI Sampling | 2. Culturing and Isolation | 3. Identification | 4. Antimicrobial sensitivity [2]

Antibiotic Class	Antibiotic(s) Tested	Sensitivity
Beta-lactams	Ampicillin, Amoxicillin-Clavulanate, Cefotaxime, Ceftazidime, Ceftriaxone, Cefixime	Resistant to all
Carbapenems	Imipenem, Meropenem	Resistant to all
Aminoglycosides	Amikacin, Gentamicin, Tobramycin, Netilmicin	Intermediate/Resistant to all
Fluoroquinolones	Ciprofloxacin, Pefloxacin, Ofloxacin	Resistant to all
Tetracyclines	Doxycycline, Minocycline	Intermediate/Resistant to all
Phenicols	Chloramphenicol	Sensitive

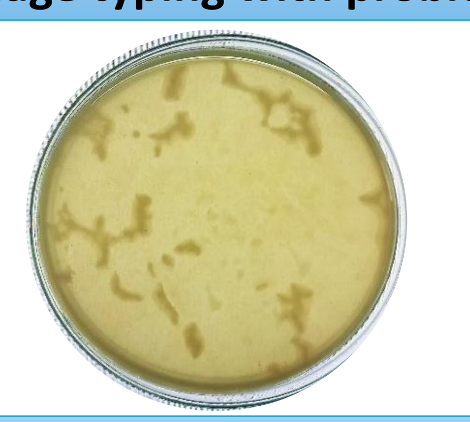
Six Bacteriophage were isolated from difference samples. B3 was further analyzed

Isolation of bacteriophage by $ZnCl_2$ precipitation method with enrichment from B3 Sample

Bacteriophages	Location of Sample for isolation
B1	Ganga river, India
B2	Purna river, India
B3	Patan City Untreated Sewage, India
B4	Unava STP, Surat, India
B5	Railway drainage, Surat, India
B6	Human stool from Healthy Donor

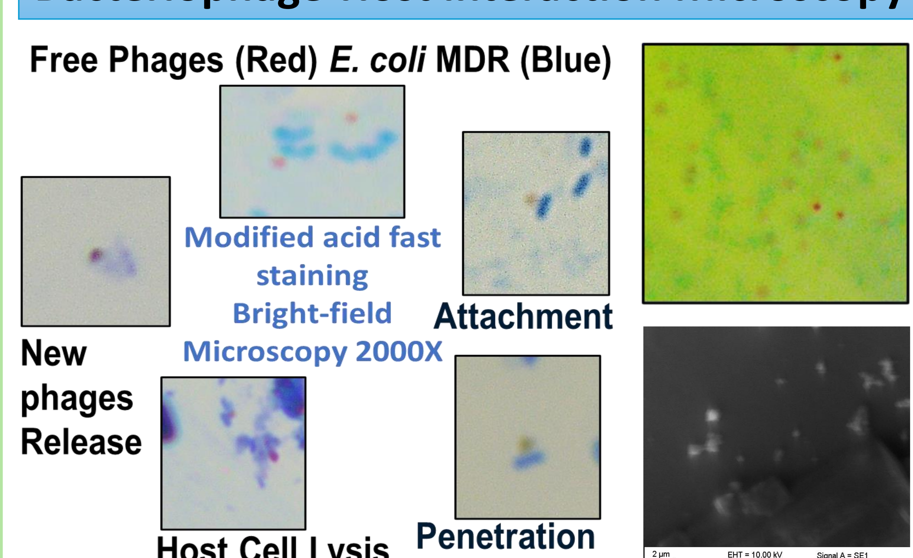
Bacteriophage typing with probiotic bacteria and pathogens

B3 was infected only Non-MDR and MDR *E. coli* did not infected any beneficial or pathogenic species



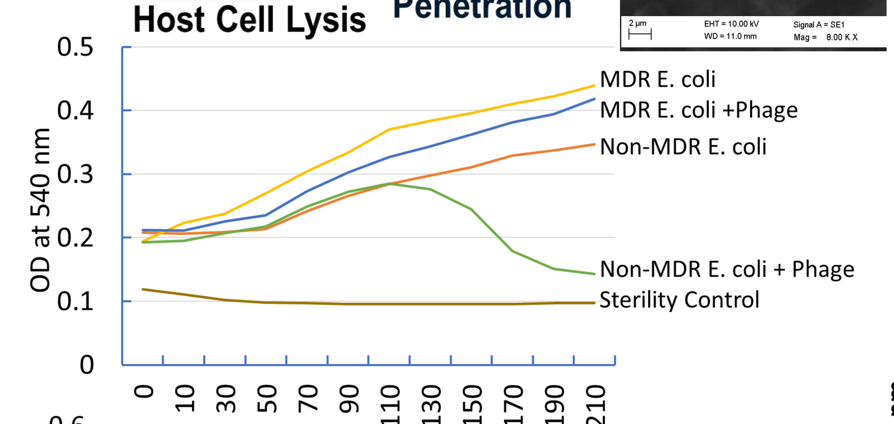
Bacteria	B3 (PFU)
Lacticaseibacillus paracasei	No plaque
Weissella paramesenteroides	No plaque
Lactobacillus casei	No plaque
Lactobacillus zeae	No plaque
Leuconostoc mesenteroides	No plaque
Enterococcus faecalis	No plaque
bacillus cereus	No plaque
Escherichia coli (NON -MDR)	TNTC
Pseudomonas aeruginosa	No plaque
Stenotrophomonas maltophilia	No plaque
Klebsiella variicola	No plaque
Micrococcus luteus	No plaque
Aeromonas caviae	No plaque
Acinetobacter pittii	No plaque

Bacteriophage-Host interaction Microscopy [3]

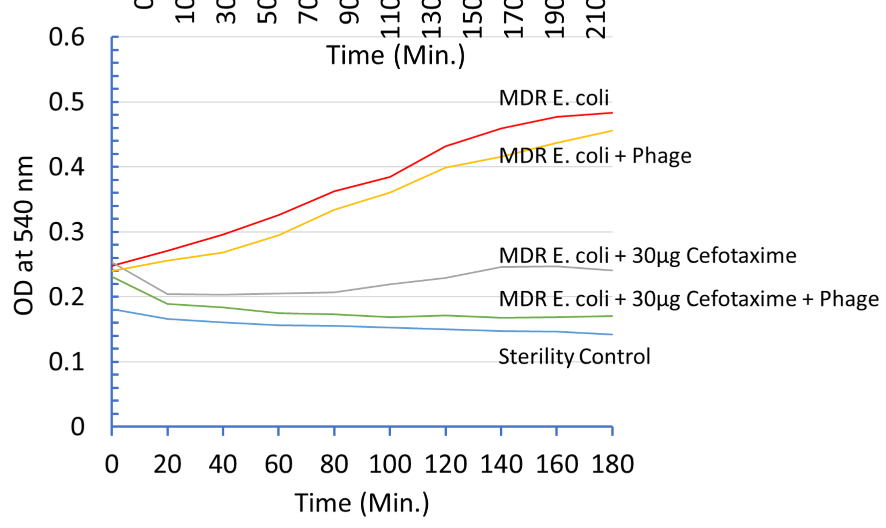


Modified flagella staining observed under phase contrast microscope. *E. coli*: Blue-greenish, Reddish: Phages

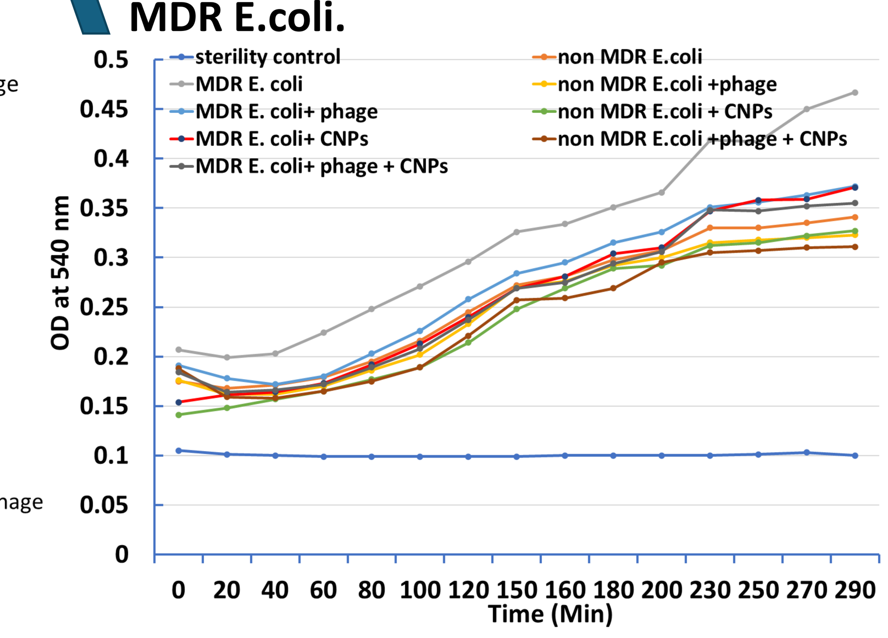
Purified Phages observed under SEM 8000 X



Bacteriophage (B3) inhibits the growth of non-MDR E.coli and marginally MDR E.coli.

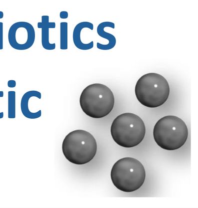


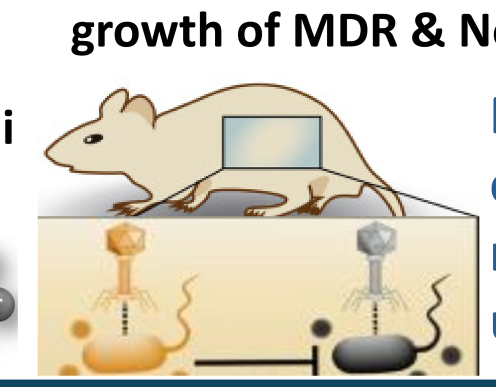
The combination of Antibiotics (Cefotaxime) and bacteriophage (B3) inhibits growth of MDR & Non MDR E. coli



The combination of CNPs and bacteriophage (B3) inhibits growth of MDR & Non MDR E. coli

Prototype formulation of Probiotics for UTI containing Phage + Lactic acid bacteria + CNP [4]





MDR E. coli control in mice gut using phage

Conclusion

- Lytic bacteriophages were isolated using a three-step enrichment and $ZnCl_2$ precipitation method and exhibited strong host specificity and high lytic activity against MDR E. coli.
- Combination therapy with carbon nanoparticles (CNPs) significantly enhanced the bacteriolytic effect of the phages and delayed bacterial regrowth in vitro, demonstrating clear synergistic action between phages and CNPs.
- Microscopy validated phage adsorption and bacterial cell lysis, confirming the mechanism of synergistic action at the structural level.
- *in vivo* mice gut experiment revealed that phage treatment alone could completely eliminate MDR E. coli
- Phage + LAB + CNPs hybrid probiotic formulation offers a sustainable, antibiotic-free strategy for UTI management by combining biological precision, microbial balance, and nanomaterial support.

Reference

(1) https://doi.org/10.1371/journal.pone.0004944 (2). https://doi.org/10.1111/j.1469-0691.2011.03570.x (3) https://doi.org/10.1128/jb.53.6.781-792.1947 (4) https://doi.org/10.1128/AAC.49.7.2874-2878.2005 (5) doi: 10.3390/microorganisms11092169 (6) https://doi.org/10.1016/j.eururo.2024.03.035 (7) doi: 10.5114/pm.2021.105382