# Title: Development and Evaluation of Bacteriophage Cocktail to Eradicate Biofilms Formed by an Extensively Drug-Resistant (XDR) *Pseudomonas aeruginosa*

Authors: Medhavi Vashisth [1,2], Anu Bala Jaglan [1,3], Shikha Yashveer [2], Priya Sharma [1], Priyanka Bardajatya [1], Nitin Virmani [1], Bidhan Chand Bera [1], Rajesh Kumar Vaid [1] and Taruna Anand [1,*]

Supplementary material for the article: The material has been provided in the order of appearance of the reference in the text.

**Supplementary Table S1**: Details of the bacterial strains used for bacteriophage isolation and characterization.

| **Sr. No.** | **NCVTC/ MTCC/ATCC -Accession no./ Lab ID** | **Bacterial strain** | **Source of isolation** |
|---|---|---|---|
| 1 | VTCCBAA237 | *Pseudomonas aeruginosa* | Gangrenous Mastitis |
| 2 | VTCCBAA238 | *Pseudomonas aeruginosa* | Canine faecal Swab |
| 3 | VTCCBAA239 | *Pseudomonas aeruginosa* | Hog Deer Nasal Swab |
| 4 | VTCCBAA325 | *Pseudomonas aeruginosa* | Sheep Lung |
| 5 | VTCCBAA333 | *Pseudomonas aeruginosa* | Camel |
| 6 | VTCCBAA563 | *Pseudomonas aeruginosa* | Sheep |
| 7 | VTCCBAA574 | *Pseudomonas aeruginosa* | Dog |
| 8 | VTCCBAA632 | *Pseudomonas* species | Bovine cervical swab |
| 9 | VTCCBAA785 | *Pseudomonas aeruginosa* | Sheep |
| 10 | VTCCBAA789 | *Pseudomonas aeruginosa* | Canine pus from ear |

| | | | |
|---|---|---|---|
| 11 | VTCCBAA843 | *Pseudomonas aeruginosa* | Turtle cloaca |
| 12 | VTCCBAA844 | *Pseudomonas aeruginosa* | Mastitis |
| 13 | VTCCBAA845 | *Pseudomonas aeruginosa* | Canine |
| 14 | VTCCBAA846 | *Pseudomonas aeruginosa* | Human/Urine |
| 15 | VTCCBAA848 | *Pseudomonas aeruginosa* | Canine gingival swab |
| 16 | VTCCBAA849 | *Pseudomonas aeruginosa* | Canine |
| 17 | VTCCBAA951 | *Pseudomonas* species | Sheep liver tissue |
| 18 | VTCCBAA956 | *Pseudomonas aeruginosa* | Cattle milk from mastitis |
| 19 | VTCCBAA1047 | *Pseudomonas aeruginosa* | Human/pus swab |
| 20 | VTCCBAA1057 | *Pseudomonas aeruginosa* | Milk sample of bovine |
| 21 | VTCCBAA1061 | *Pseudomonas aeruginosa* | Canine ear swab |
| 22 | VTCCBAA1096 | *Pseudomonas aeruginosa* | Sheep Lung swab |
| 23 | VTCCBAA1097 | *Pseudomonas aeruginosa* | Goat lung swab |
| 24 | VTCCBAA1216 | *Pseudomonas aeruginosa* | Cattle faecal matter |
| 25 | RR/ 2021/ 112 | *Pseudomonas aeruginosa* | Buffalo milk |
| 26 | Fop 416A | *Pseudomonas aeruginosa* | Community sewage |
| 27 | Fop 426A | *Pseudomonas aeruginosa* | Sewage water |
| 28 | Fop 489B | *Pseudomonas aeruginosa* | Biohazard waste, Dental ward |
| 29 | Fop 507C | *Pseudomonas aeruginosa* | Human urine |

**Supplementary Table S2:** Efficiency of plating (EOP) of *P. aeruginosa* phages against different *Pseudomonas* strains.

| **Bacteriophage** | **φPA170** | | **φPA172** | | **φPA173** | | **φPA176** | |
|---|---|---|---|---|---|---|---|---|
| ***Pseudomonas* strains (n=29)** | EOP value | Productivity | EOP value | Productivity | EOP value | Productivity | EOP value | Productivity |
| **VTCCBAA237** | 0.007 | Low | 0.008 | Low | 0.000085 | Inefficient | 0.61 | High |
| **VTCCBAA238** | 0.06 | Low | 0.004 | Low | 0.000005 | Inefficient | 0.68 | High |
| **VTCCBAA239** | - | - | - | - | - | - | - | - |
| **VTCCBAA325** | 0.0002 | Inefficient | 0.0005 | Inefficient | 0 | Inefficient | 0.58 | High |
| **VTCCBAA333** | 0.0006 | Inefficient | 1E-07 | Inefficient | 0.0046 | Low | 0.81 | High |
| **VTCCBAA563** | 0.000002 | Inefficient | - | - | - | - | - | - |
| **VTCCBAA574** | 0.72 | High | 0.85 | High | 4.2E-06 | Inefficient | 0.51 | High |
| **VTCCBAA632** | 0.85 | High | 0.72 | High | - | - | 0.68 | High |
| **VTCCBAA785** | 0.12 | Medium | 0.00095 | Inefficient | 3.3E-06 | Inefficient | 0.85 | High |
| **VTCCBAA789** | 0.11 | Medium | - | - | 0.0062 | Inefficient | 0.27 | Medium |
| **VTCCBAA843** | 0.006 | Low | 0.00016 | Inefficient | 8.9E-07 | Inefficient | 0.81 | High |
| **VTCCBAA844** | 0.03 | Low | 0.00029 | Inefficient | 0.16 | Medium | 0.37 | Medium |
| **VTCCBAA845** | 0.00081 | Inefficient | 0.0107 | Low | - | - | 0.61 | High |
| **VTCCBAA846** | 0.25 | Medium | 0.029 | Low | 0.00125 | Low | 0.58 | High |
| **VTCCBAA848** | 0.93 | High | 0.0072 | Low | 1.42E-06 | Inefficient | 0.71 | High |
| **VTCCBAA849** | 0.56 | High | 0.64 | High | 2.5E-06 | Inefficient | 0.85 | High |
| **VTCCBAA951** | - | - | - | - | - | - | - | |
| **VTCCBAA956** | 0.015 | Low | 0.000079 | Inefficient | 0.000107 | Inefficient | 0.0126 | Low |
| **VTCCBAA1047** | 0.06 | Low | 0.0059 | Low | 0.00125 | Low | 0 | Inefficient |
| **VTCCBAA1057** | 0.00012 | Inefficient | 0.0057 | Low | 5.3E-06 | Inefficient | 0.0064 | Low |
| **VTCCBAA1061** | 0.15 | Medium | 0.0073 | Low | 0.000037 | Inefficient | 0.00133 | Low |
| **VTCCBAA1096** | 2.7E-06 | Inefficient | 0.0029 | Low | 2.6E-06 | Inefficient | 0.0014 | Low |

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|---|---|---|---|---|---|---|---|---|
| **VTCCBAA1097** | 0.56 | High | - | - | 6E-07 | Inefficient | 0.98 | High |
| **VTCCBAA1216** | - | - | - | - | - | - | - | - |
| **RR/2021/112** | 0.81 | High | 0.33 | Medium | 1.6E-06 | Inefficient | 0.66 | High |
| **Fop416A** | 1 | High | 1 | High | 1 | High | 1 | High |
| **Fop426A** | 0.0003 | Inefficient | 0.00034 | Inefficient | - | - | 2.73E-05 | Inefficient |
| **Fop489B** | 0.089 | Low | 0.0002 | Inefficient | - | - | 0.109 | Medium |
| **Fop507C** | - | - | 0.00076 | Inefficient | - | - | - | - |

| **Bacteriophage** | **φPA177** | | **φPA178** | | **φPA180** | |
|---|---|---|---|---|---|---|
| ***Pseudomonas* strains (n=29)** | EOP value | Productivity | EOP value | Productivity | EOP value | Productivity |
| **VTCCBAA237** | 0.74 | High | 0.0391 | Low | 0.78 | High |
| **VTCCBAA238** | 0.024 | Low | 0.026 | Low | 0.85 | High |
| **VTCCBAA239** | - | - | - | - | - | - |
| **VTCCBAA325** | 0.56 | High | 0.2 | Medium | 0.57 | High |
| **VTCCBAA333** | 0.7 | High | 0.02 | Low | 0.78 | High |
| **VTCCBAA563** | - | - | - | - | 0.17 | Medium |
| **VTCCBAA574** | - | - | - | - | 1.14 | High |
| **VTCCBAA632** | 0.81 | High | - | - | 0.85 | High |
| **VTCCBAA785** | 0.041 | Low | 3.91E-07 | Inefficient | 0.025 | Low |
| **VTCCBAA789** | 0.029 | Low | 0.023 | Low | 1.28 | High |
| **VTCCBAA843** | 0.017 | Low | 1.56E-08 | Inefficient | 0.42 | Medium |
| **VTCCBAA844** | 0.0046 | Low | 0.0126 | Low | 0.92 | High |
| **VTCCBAA845** | 0.5 | High | 2.17E-05 | Inefficient | 0.57 | High |
| **VTCCBAA846** | 0.065 | Low | 1.39E-07 | Inefficient | 0.135 | Medium |
| **VTCCBAA848** | 0.83 | High | 3E-07 | Inefficient | 0.57 | High |
| **VTCCBAA849** | 0.048 | Low | 9.1E-07 | Inefficient | 1 | High |
| **VTCCBAA951** | - | - | - | - | - | - |
| **VTCCBAA956** | - | - | - | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| **VTCCBAA1047** | 0.0058 | Low | 2.6E-07 | Inefficient | 0.78 | High |
| **VTCCBAA1057** | 0.00037 | Inefficient | 1.04E-05 | Inefficient | 0.185 | Medium |
| **VTCCBAA1061** | 0.069 | Low | 5.2E-10 | Inefficient | 0.607 | High |
| **VTCCBAA1096** | - | - | - | - | 0.71 | High |
| **VTCCBAA1097** | 0.081 | Low | 0.0173 | Low | 0.78 | High |
| **VTCCBAA1216** | - | - | - | - | - | - |
| **RR/2021/112** | 2 | Low | 0.78 | High | - | - |
| **Fop416A** | 1 | High | 1 | High | 0.85 | High |
| **Fop426A** | 8.1E-07 | Inefficient | - | - | 1 | High |
| **Fop489B** | - | - | - | - | - | - |
| **Fop507C** | - | - | - | - | - | - |

Interpretation for Phage productivity on the basis of EOP values:

High productivity > 0.5

Medium productivity 0.5 - 0.1

Low productivity 0.001 - 0.1

Inefficient productivity < 0.001

EOP values of bacteriophages on their respective original hosts are highlighted in yellow.

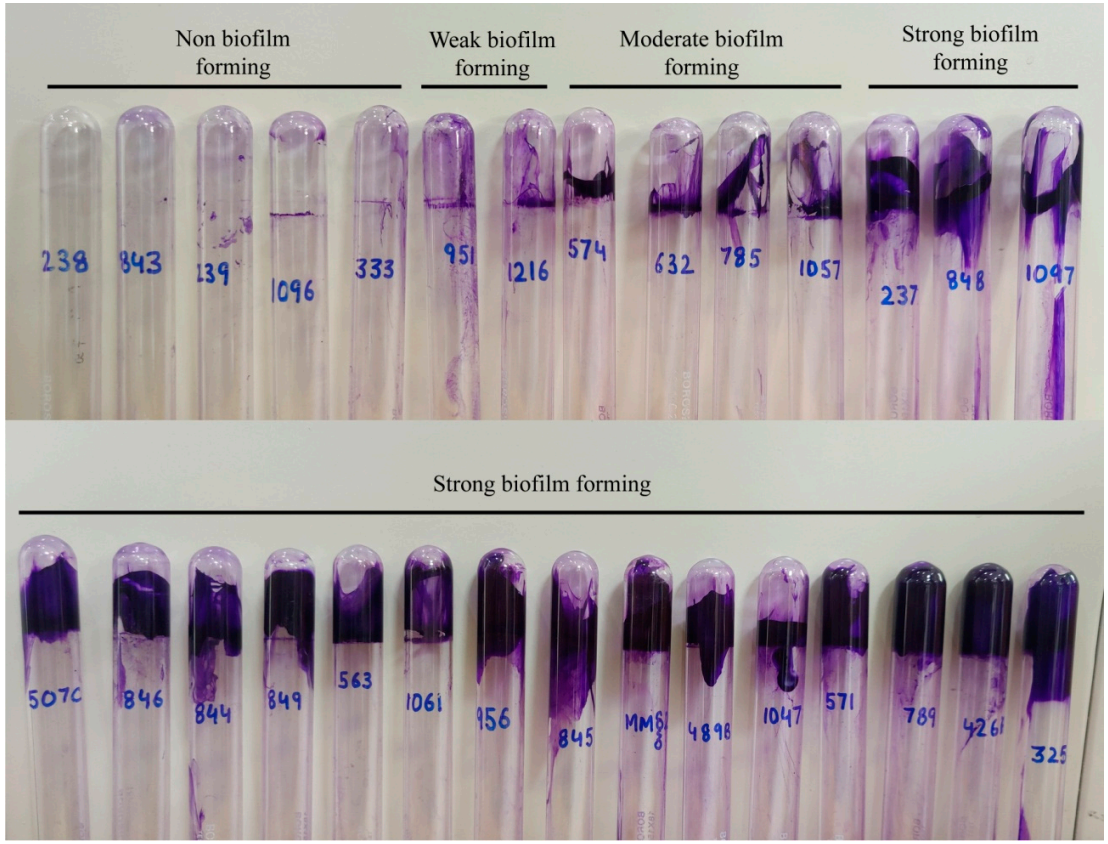
Blank cells represent absence of bacteriophage lytic activity

**Supplementary Table S3**. Antibiogram of *Pseudomonas* strains.

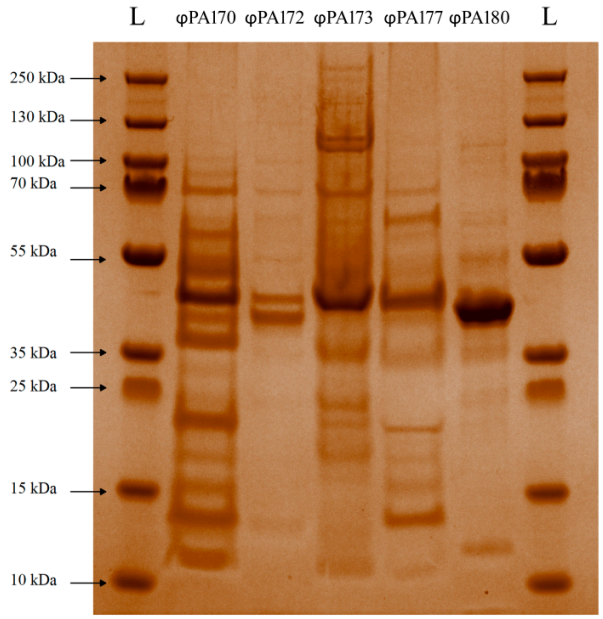
| Antibiotic class | | Penicillins | Monobactams | Cephalosporins | | | | Cephalosporins + β lactamase inhibitors | | Carbapenems | | Polymyxins | | Fluoroquinolones | | | | Aminoglycosides | | | Macrolides |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Strains*. (n=29) | Status | PI 100 | AT 30 | CAZ 30 | CPM 30 | CTX 30 | CEP 30 | CAC 30/10 | CEC 30/10 | IPM 10 | MRP 10 | CL 10 | PB 300 | CIP 5 | LE 5 | NX 10 | OF 5 | AK 30 | GEN 10 | TOB 10 | AZM 30 |
| VTCCBAA237 | MDR | S | S | S | S | R | R | I | S | S | S | S | S | S | S | S | S | S | S | S | R |
| VTCCBAA238 | MDR | S | S | S | S | R | R | I | S | R | S | S | S | S | S | S | S | S | S | S | R |
| VTCCBAA239 | Sensitive | S | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S |
| VTCCBAA325 | MDR | I | S | S | S | R | R | S | R | S | S | S | S | S | S | S | S | I | S | S | R |
| VTCCBAA333 | MDR | R | I | R | R | R | R | R | R | S | S | S | S | S | S | S | S | I | S | S | R |
| VTCCBAA563 | MDR | I | S | I | I | R | R | S | R | R | S | S | S | R | R | R | R | S | R | S | S |
| VTCCBAA574 | MDR | S | I | S | S | R | R | S | R | R | R | S | S | S | R | I | R | S | S | S | R |
| VTCCBAA632* | Sensitive | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | R |
| VTCCBAA785 | MDR | S | S | S | S | S | R | S | S | R | S | S | S | S | S | S | S | S | S | S | R |
| VTCCBAA789 | MDR | S | R | I | I | R | R | I | R | R | I | S | S | S | R | R | R | S | S | S | R |
| VTCCBAA843 | Sensitive | S | S | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | S | S | S |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VTCCBAA844** | **Sensitive** | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| **VTCCBAA845** | **Sensitive** | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | I |
| **VTCCBAA846** | **MDR** | S | I | S | R | R | R | S | R | S | S | S | S | S | S | S | S | S | S | S | S | I |
| **VTCCBAA848** | **Sensitive** | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | R |
| **VTCCBAA849** | **MDR** | S | S | S | S | S | R | S | S | R | S | S | S | S | S | S | S | S | S | S | S | R |
| **VTCCBAA951*** | **MDR** | S | R | R | S | R | R | I | S | R | S | S | S | S | S | S | S | S | S | S | S | R |
| **VTCCBAA956** | **Sensitive** | S | S | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | I |
| **VTCCBAA1047** | **XDR** | I | I | S | S | R | R | I | I | R | R | S | S | S | R | R | R | S | R | S | S | R |
| **VTCCBAA1057** | **MDR** | S | S | S | R | R | R | I | R | R | S | S | S | S | S | S | S | S | S | S | S | R |
| **VTCCBAA1061** | **MDR** | S | S | S | R | R | R | S | R | R | R | S | S | S | S | S | S | S | S | S | S | I |
| **VTCCBAA1096** | **MDR** | S | S | S | I | R | R | S | S | S | S | R | S | S | S | S | S | S | S | S | S | R |
| **VTCCBAA1097** | **MDR** | I | S | S | S | R | I | S | S | S | S | S | S | S | S | S | S | S | S | S | S | I |
| **VTCCBAA1216** | **MDR** | S | I | S | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S | I |
| **RR/ 2021/ 112** | **MDR** | S | I | S | R | I | R | S | S | R | S | S | S | R | R | R | R | R | R | R | R | R |
| **Fop416A** | **MDR** | I | I | S | I | I | R | S | I | R | S | S | S | S | S | S | S | S | S | S | S | R |
| **Fop 507C** | **MDR** | I | I | S | S | S | S | I | S | S | S | R | S | S | S | S | S | I | S | S | S | R |
| **Fop 426A** | **MDR** | I | R | S | R | R | R | I | R | R | R | S | S | I | R | R | R | S | S | S | S | I |
| **Fop 489B** | **MDR** | S | S | S | S | I | I | S | S | R | S | S | S | S | S | S | S | S | S | S | S | R |

***** indicates 2 *Pseudomonas* sp. and remaining 27 strains are *P. aeruginosa*; R - Resistant, I - Intermediately Resistant, S - Susceptible, MDR - Multiple drug resistant, XDR - Extensively drug resistant. Antibiotics are abbreviated as: PI100- Penicillin 100µg, AT30- Aztreonam 30µg, CAZ30- Ceftazidime 30µg, CPM30- Cefepime 30µg, CTX30- Cefotaxime 30µg, CEP30- Cephalothin 30µg, CAC 30/10- Ceftazidime-clavulanic acid 30/10µg, CEC30/10- Cefotaxime-clavulanic acid 30/10µg, IPM10- Imipenem 10µg, MRP10- Meropenem 10µg, CL10- Colistin 10µg, PB300- Polymyxin 300U, CIP5- Ciprofloxacin 5µg, LE5- Levofloxacin 5µg, NX10- Norfloxacin 10µg, OF5- Ofloxacin 5µg, AK30- Amikacin 30µg, GEN10- Gentamicin 10µg, TOB10- Tobramycin 10µg, AZM10- Azithromycin 10µg.




**Supplementary Figure S1:** Biofilm formation ability of *Pseudomonas* strains (qualitative measurement by crystal violet staining using tube method).




**Supplementary Figure S2:** SDS- Polyacrylamide Gel Electrophoresis of phages *viz.* φPA170, φPA172, φPA173, φPA177, and φPA180. The samples were prepared using manufacturer's protocol. Briefly, 20µl of the phage ($1\times10^{10}$ PFU/ml) was mixed with 15µl NuPAGE LDS Sample Buffer (4X), and 6µl of sample reducing agent and were heated for 15 minutes at 90°C in a dry bath. The samples were cooled to room temperature and were loaded into the wells of the NuPAGE 4-12% bis-tris gels and were electrophoresed at 80V for 3 hr till the bands of the protein marker were separated. The gel was then stained using Simply Blue Safe Stain (Invitrogen) for 1 hr on a gel rocker and was de-stained using distilled water and visualized in BioRad Gel Doc XR+ Gel Documentation System (BioRad, USA). Structural protein size was determined by comparing with PageRuler™ Plus Prestained Protein Ladder (26619), (Thermo Scientific) as standard using Image Lab software using (Bio Rad).