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Bacteriophage PEV20 and ciprofloxacin combination treatment enhances removal of *P. aeruginosa* biofilm isolated from cystic fibrosis and wound patients.

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Abstract

Antibiotic resistance in *Pseudomonas aeruginosa* biofilms necessitates the need for novel antimicrobial therapy with anti-biofilm properties. Bacteriophages (phages) are recognized as an ideal biopharmaceutical for combating antibiotic-resistant bacteria especially when used in combination with antibiotics. However, previous studies primarily focused on using phages against of *P. aeruginosa* biofilms of laboratory strains. In the present study, biofilms of six *P. aeruginosa* isolated from cystic fibrosis and wound patients, and one laboratory strain were treated singly and with combinations of anti-Pseudomonas phage PEV20 and ciprofloxacin. Of these strains, three were highly susceptible to the phage, while one was partially resistant and one completely resistant. Combination treatment with PEV20 and ciprofloxacin enhanced biofilm eradication compared to single treatment. Phage and ciprofloxacin synergy was found to depend on phage-resistance profile of the target bacteria. Furthermore, phage and ciprofloxacin combination formulation protected the lung epithelial and fibroblast cells from *P. aeruginosa* and promoted cell growth. The results demonstrated that thorough screening of phage-resistance is crucial for designing phage-antibiotic formulation. The addition of highly effective phage could reduce the ciprofloxacin concentration required to combat *P. aeruginosa* infections associated with biofilm in cystic fibrosis and wound patients.

Keywords

Bacteriophage (phage); PEV20; ciprofloxacin; biofilm; *Pseudomonas aeruginosa*; combination treatment

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Introduction

Cystic fibrosis (CF) patients suffer from multi-species bacterial colonization in the lung, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenza*, *Burkholderia cenocepae* and others (1). Of these, *P. aeruginosa* is the predominant species responsible for up to 80% morbidity and mortality cases worldwide (2). Similarly, multiple bacterial species are found in wound infection sites (*Staphylococcus epidermidis*, *P. aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter baumannii* and others) (3) and *P. aeruginosa* is one of the predominant pathogens which readily colonizes in the wounds (4, 5). Eradication of chronic *P. aeruginosa* infection is thought to be extremely challenging due to antibiotic resistance, both naturally occurring and acquired (6). It readily colonizes on surfaces, and becomes embedded in self-secreted molecules, including polysaccharides, proteins, extracellular DNA, pyocyanin. These molecules contribute to the biofilm matrix and structural integrity of biofilm. Biofilms can tolerate up to 1000 times higher antibiotic concentrations than planktonic cells and act as barricades from chemical, physical and biological challenges (7). With rapidly growing number of antibiotic-resistant bacteria, a novel antimicrobial therapy is urgently needed for treatment of chronic infections associated with persistent biofilms.

The use of bacteriophages (phage) against bacterial infection is regaining attention due to its ability to kill antibiotic-resistant bacteria (8), and to penetrate and disrupt biofilms (9, 10). Phages can move through biofilm matrixes and promote degradation of extracellular polymeric substances by producing depolymerizing enzymes (11, 12). Additionally, phages can infect persister cells in the biofilm, and start the lytic infection cycle once the bacteria become metabolically active (11). Combined use of phages with other antimicrobial agents such as antibiotics is being recognized as a potential therapeutic regimen. Reports have shown antibiotic and phage synergism against biofilms of *P. aeruginosa* laboratory strains (13–15). In another study, Nouraldin *et al.* have shown that amikacin and phage combination could remove *P. aeruginosa* biofilms in 50% of clinical isolates (16). Recently, we have demonstrated the feasibility of delivering anti-*Pseudomonas* phage PEV20 and ciprofloxacin combination using nebulizers to kill *P. aeruginosa* (17). This current study is a continuation from the published work, where we aim to examine the efficacy of phage PEV20 and ciprofloxacin combination against *P. aeruginosa* biofilms from CF and wound patients. Furthermore, we assessed the anti-biofilm effect of the combination treatment in *in vitro* infection model using human lung epithelial and fibroblast cells infected with *P. aeruginosa*.

Materials and methods

Bacterial strains and phage

A total of six clinical and one laboratory *P. aeruginosa* strains were used in this study. CF isolates: the Australian Epidemic Strains AES-1R and AES-2 Liverpool Epidemic Strain LESB58 and Manchester strain MANC3733. Australian wound isolates: PA 365707 (left ankle) and PA 364077 (scalp). Laboratory isolate: ATCC25619. Phage PEV20 used in this study was supplied by AmpliPhi Biosciences AU at a high titre of 10^{10} pfu/mL in phosphate buffered saline. These phages were originally isolated by the Kutter Lab (Evergreen Phage Lab) from a sewage treatment plant in Olympia (WA, USA). Phage PEV20 can kill over

60% of clinical antibiotic-resistant *P. aeruginosa* strains (18) with demonstrated *in vivo* efficacy in a mouse lung infection model (19).

Minimum inhibitory concentration and phage susceptibility

Ciprofloxacin and tobramycin sulphate were purchased from Sigma-Aldrich Inc. The minimum inhibitory concentrations (MIC) of antibiotics against seven *P. aeruginosa* strains were determined using a microtiter plate method (17). Briefly, 10 μ L of antibiotics (0.25, 0.5, 1, 2, 4, 5, 7, 10, 20 and 25 μ g/mL) or PEV20 (10^{10} pfu/mL) were added to 190 μ L of early-log phase bacterial culture ($\sim 10^6$ cfu/mL). The treated culture was incubated for 24 h at 37 °C with continuous shaking. Optical density at 600 nm (OD₆₀₀) was measured using a microplate reader (Tecan infinite M1000 pro). Four independent biological replicates were performed.

Minimum biofilm inhibitory concentration

P. aeruginosa isolates were grown in TSB medium (24 h, 37 °C, 150 rpm) and harvested by centrifugation (5000 g, 5 min). Bacterial cell pellet was suspended in PBS with OD₆₀₀ adjusted to 0.5 ± 0.05 . Bacterial culture (250 μ L) was added to the wells of 96-well plate (Corning Corp. USA) and incubated at 37 °C for 2 h at 150 rpm. After 2 h, the wells were gently rinsed with PBS to remove any loosely adhered bacteria. Then, 200 μ L of TSB was added, followed by further incubation at 37 °C for 48 h (150 rpm) to initiate biofilm growth. In antibiotic-treated groups, biofilms were grown in the presence of ciprofloxacin or tobramycin. After 48 h incubation, the biofilms were rinsed twice with PBS and then stained with 0.1 % (w/v) crystal violet (200 μ L), followed by further incubation at room temperature for 1 h. The wells were rinsed three times with PBS to remove excess crystal violet. Stained biofilms were dissolved in 70% ethanol and then transferred into a new 96-well plate for biomass quantification at OD₅₅₀ using a microplate reader (Infinite M1000 pro, Sydney Australia). Biofilm inhibitory concentrations were determined using multiple replicates (n=4) for each condition.

Biofilms viability

Biofilms were prepared as per our previous study (20). Briefly, *P. aeruginosa* strains were grown in TSB medium for 24 h at 37 °C with continuous shaking (150 rotations per minute, rpm). Overnight bacterial culture was diluted with fresh TSB to a final density of OD₆₀₀ = 0.2 ± 0.02 . To initiate biofilm growth, diluted culture was aliquoted into 96-well plates (Corning Corp. USA) and incubated at 37 °C for 48 h at 150 rpm. Biofilm was washed with PBS and then treated with either PEV20 (10^8 pfu/mL), ciprofloxacin (range, 0.25 – 15 μ g/ml) or combination of PEV20 (10^8 pfu/mL) + ciprofloxacin (range, 0.13 – 10 μ g/ml). Control biofilms were treated with PBS. After 24 h treatment, biofilm supernatant was replaced with 200 μ L of PBS. To each well, 15 μ L of 0.05% w/v resazurin solution (Sigma-Aldrich) was added, followed by further incubation for 24 h at 37 °C with continuous shaking. Resazurin is an indicator dye that measures oxidation-reduction reactions, which principally occur in live cells. Weakly fluorescent blue resazurin dye is irreversibly reduced to highly fluorescent pink in the presence of metabolically active cells. The fluorescent intensity of the biofilm was determined at Ex_{544nm} and Em_{590nm} (Tecan infinite M1000 pro). Four independent biological replicates were performed.

Quantification of biofilm biomass using crystal violet staining

P. aeruginosa isolates (AES-1R, MAN3733, PA365707, PA364077 and ATCC 25619) biofilms were grown for 48 h as per above protocol. Biofilms (48 h-old) were rinsed with PBS, and then treated with either PEV20 (10^8 pfu/mL), ciprofloxacin (MIC or 3MIC) or combination formulation containing PEV20 (10^8 pfu/mL) and ciprofloxacin (MIC or 2MIC) and incubated for 24 h at 37 °C at 150 rpm. After 24 h treatment, the biofilm biomass was washed twice with PBS, and then stained with 0.1 % (w/v) crystal violet (200 μ L). The biofilm biomass was quantified using a microplate reader as mentioned above. Four independent biological replicates were performed for each condition.

Biofilm architecture of PA365707 using confocal microscopy

To initiate biofilm growth, 500 μ L of planktonic culture of PA365707 ($OD_{600} = 0.5 \pm 0.05$) was added to microscopic glass slides and incubated at 37 °C for 2 h in a static incubator. The glass slides were rinsed twice with PBS to remove all loosely adhered bacteria. Then, 1 ml of TSB was added to the glass slide and further incubated for 48 h at 37 °C in a static incubator to trigger biofilm formation. After 48 h incubation, the biofilms were washed with PBS, followed treatment with: ciprofloxacin (3MIC), combination formulation containing PEV20 (10^8 pfu/mL) and ciprofloxacin (MIC or 2MIC), or PBS (control). The treated biofilms were incubated for 24 h at 37 °C in a static incubator. After 24 h, the biofilms were washed three times with PBS to remove planktonic or loosely adhered bacterial cells. Biofilms were stained with Live/Dead Stain (Bacterial viability kit, Life Technologies) for 60 min in the dark. Confocal scanning laser microscopy (Olympus FV1200, Australia) was used to visualize the biofilms at 40x magnification. Green syto-9 (Ex_{473nm} and Em_{500nm}) was used to stain live cells and red propidium iodide (Ex_{559nm} and Em_{637nm}) was used to stain dead cells. ImageJ software was employed for image processing. Three independent biological replicates were performed for each treatment conditions and control.

Human cell lines

Human lung epithelial (BEAS-2B) and fibroblast (HFF-1) cells were cultured in DMEM supplemented with 10% (v/v) FBS, penicillin (100 IU/mL) and streptomycin (100 μ g/mL). Cells were grown in a T-25 cell culture flask (Corning, USA) at 37 °C with 5% (v/v) CO₂ and harvest at 90% confluence using 0.12% v/v trypsin-EDTA. Cells were collected by quenching trypsin (1:1, v/v) with supplemented DMEM media and transferred to conical centrifuge tubes, followed by centrifugation (5 min, 200 x g, 20 °C). The cell pellet was suspended in supplemented DMEM media for further experiments.

In vitro efficacy

The effect of selected *P. aeruginosa* CF (AES-1R) and wound (PA365707) isolates were examined using BEAS-2B and HFF-1 cell lines, respectively. BEAS-2B and HFF-1 cells were cultured and harvested as above. Harvested cells were plated to a density 6×10^5 cells/mL into six-well plates (Corning) and allowed to incubate for 72 h at 37 °C with 5% (v/v) CO₂ to a confluence of 90%. Media was replaced with fresh DMEM, and then 100 μ L of *P. aeruginosa* ($OD_{600} = 0.1$, suspended in PBS) was added, followed by further incubation for 24 h.

Statistical analysis

Student t test was used to examine the statistical significance of the data (GraphPad, Unpaired t test). The null hypothesis was rejected if the P value was <0.05. Percentage biofilm viability of the treated groups (antibiotic only, phage only and combination of antibiotic and phage) were compared to non-treated control. Antibiotic-phage synergy was defined if the biofilm viability of the combination formulation-treated group was statistically lower than the single treatment groups combined (antibiotic only or phage only).

Results and discussions

MIC of ciprofloxacin ranged from 0.25 – 5 µg/mL for CF and wound isolates (Table I). CF isolates were less susceptible to tobramycin (MIC range, 15 – 20 µg/mL) compared to ciprofloxacin (MIC range, 2 – 5 µg/mL). Wound infection isolates were susceptible to both ciprofloxacin and tobramycin at low concentration with MIC of 0.25 µg/mL. The minimum biofilm inhibitory concentrations of ciprofloxacin (1 – 5 µg/mL) were similar to MICs across all seven isolates (Table II). For tobramycin, AES-1R and LESB58 exhibited intermediate resistance at 60 µg/ml (3MIC) and all other isolates were resistant at 5MIC. Five *P. aeruginosa* isolates, including AES-1R, PA365707, PA364077, AES-2 and ATCC25619 were highly susceptible to PEV20, MANC3733 was partially resistant and LESB58 was completely resistant (Table I). All seven isolates were assessed for anti-biofilm activity of phage and antibiotic combination treatment.

AES-1R biofilm viability was reduced by 47% and 74% using combination treatment with PEV20 and ciprofloxacin at ½MIC and MIC, respectively (Figure 1). Treatment with phage or ciprofloxacin alone at MIC could not reduce the biofilm viability. Similar results were observed for the two wound infection strains PA365707 and PA364077. Combination treatment with PEV20 and ciprofloxacin at MIC reduced 98% of biofilms for both strains, whilst individual antimicrobial agents failed to reduce the biofilm viability (Figure 1). ATCC25619 biofilm viability was reduced by 67% using ciprofloxacin alone at MIC. In the presence of phage, 90% biofilm reduction was observed with only ½MIC of ciprofloxacin. Although AES-2 planktonic cells were highly susceptible to PEV20, the combination treatment did not induce synergistic anti-biofilm effect (Figure 1). This could be due to entrapment of phage particles in the extracellular matrix, production of phage-inactivating enzymes and/or lowering of surrounding pH by the bacteria (21). Independently performed crystal violet assay further validated the synergistic anti-biofilm effect of PEV20 and ciprofloxacin against AES-1R, ATCC25619, PA365707 and PA364077 (Figure 2A). PA365707 was further investigated using confocal microscopy. Treatment with combination formulation containing PEV20 and ciprofloxacin (MIC or 2MIC) facilitated biofilm disruption and removal (Figure 2B, red stain: dead cells; green stain: live cells). Furthermore, the combination formulation enhanced bacterial killing within the biofilm as compared with ciprofloxacin treatment alone at 3MIC or untreated control biofilms. A study by Walters *et al.* showed that ciprofloxacin action is limited to areas adjacent to the air-biofilm interface and not the interior of the biofilm (22). Bacterial filamentation was observed on the air-biofilm interface of ciprofloxacin-treated biofilm, while those residing in the interior were spared. Antibiotic tolerance in the mid-layer of the biofilm is likely due to

lack of oxygen, which decreases bacterial metabolic activity. The presence of phage could help reduce the integrity of extracellular matrix, thereby exposing the metabolically inactive bacteria to surrounding nutrients in the media (23). Once these bacteria become metabolically active, both ciprofloxacin and phage could induce antimicrobial effect. Furthermore, phages can diffuse across biofilm, amplify and remain viable within the complex biofilm matrix (21, 24). In fact, close proximity of bacterial cells within the biofilm is favourable for the phages to multiply resulting in high local titres and rapid spread of phage infections (25).

Although AES-1R, PA365707, PA364077 and ATCC25619 planktonic cells were highly susceptible to PEV20 (Table I), phage treatment alone was ineffective against biofilms. It is likely that phage monotherapy has led to emergence of phage-resistant bacteria and subsequent increase in bacterial and biofilm density over time (26). Use of a phage cocktail (a mixture of two or more phages) could help reduce emergence of phage-resistant bacteria, cover a wide host range, and enhance the antibacterial and antibiofilm activities (27, 28). Fu *et al.* showed that a cocktail of five lytic phages significantly reduced *P. aeruginosa* biofilm formulation on catheters compared a single phage treatment (29). Thus, phage cocktail may be preferred for the treatment of wound infection and CF patients, who suffer from multi-species bacterial colonization. However, Fu *et al.* reported that emergence of phage-resistant bacterial sub-population was inevitable, with one isolate being completely resistant to all five phages in the mixture (29).

CF strain MANC3733 formed persistent biofilm that could not be removed even at high ciprofloxacin concentration of 3MIC (Figure 3). In contrast, the addition of PEV20 (partially resistant to MANC3733) reduced the biofilm viability by half, regardless of the ciprofloxacin concentration used ($\frac{1}{2}$ MIC to 3MIC). The percentage of viable bacterial cells in LESB58 biofilm was negligible when treated with ciprofloxacin at 2MIC. Addition of PEV20 (completely resistant to LESB58) did not enhance the anti-biofilm effect, but rather it had an antagonistic effect with only 55% reduction in the biofilm viability. CF strain AES-2 also formed persistent biofilm. The results demonstrated the importance of phage-resistance profile of target bacterial strain when designing combination formulation against biofilms.

Combination treatment of tobramycin and PEV20 did not induce synergistic antimicrobial effect against 48-h biofilm in all seven strains (Supplemental 1). This agrees with previous results using phage PB-1 and NP1/NP3 against 48-h old biofilm from PAO1 and PA14, (13, 14). Ciprofloxacin and other fluoroquinolones are known to readily penetrate *P. aeruginosa* biofilms compared to aminoglycosides, such as tobramycin (22, 30). Aminoglycosides slowly diffuse across the biofilm as the drug tend to bind to extracellular matrix (31). This could explain the lack of biofilm dispersant effect using tobramycin compared to ciprofloxacin.

Treatment with combination formulation facilitated the growth of lung epithelial (BEAS-2B) and fibroblast cells (HFF-1), while inhibiting bacterial growth and biofilm formation (Figure 4A and B). BEAS-2B and HFF-1 cells exhibited 100% pre-confluence in control groups (no bacteria and no treatment) with complete covering of the culture dish by these adherent cells

(Figure 4Aii **and** Bii). Infection with AES-1R or PA365707 isolates resulted in bacterial colonization and consequently, the mammalian cells lost adherence and died (Figure 4Aiii **and** Biii). Treatment with ciprofloxacin or PEV20 improved the cell confluence to 60%, while the combination formulation rescued these mammalian cells from bacterial colonization with 100% confluence (Figure 4Aiv–vi **and** Biv–vi). Furthermore, lung epithelial and fibroblast cells remained viable (100% confluence) after 24 h incubation with the combination formulation, demonstrating the safety *in vitro*. Chaudhry *et al* infected human nasopharyngeal cells with *P. aeruginosa* PA14 for 8 h to establish biofilm, followed by treatment with antibiotic and mixture of NP1 and NP3 phages (14). Synergy was observed only with ceftazidime-phage combination, whereas ciprofloxacin-phage showed additive effect. In another study, Sillankorva *et al*. reported antagonism between phage and amikacin (32) whereas Nouraldin *et al*. reported synergism between phage and amikacin for controlling *P. aeruginosa* biofilms (16). Hence, synergism between phage and antibiotics is largely phage- and/or strain-dependent. Chronic infections often associated with polymicrobial biofilms are recalcitrant to antibiotic treatment. Hence, future studies should involve the effect of phage and antibiotic combination therapy against mixed cultures.

Conclusion

Our study showed synergistic antibacterial activities using a combination of PEV20 and ciprofloxacin against biofilms from clinical *P. aeruginosa* strains isolated from wounds and sputum of CF patients. Phage and antibiotic combination formulation can enhance biofilm eradication and at the same time facilitate host cell growth. Addition of phage could potentially lower the antibiotic concentration required to treat *P. aeruginosa* infections in CF and wound patients. This indicates the potential for implementing lower dosage regimen to help circumvent the side effects often associated with administration of high doses of antibiotics. However, it is essential to select phages that are highly effective against the target bacteria to avoid antagonistic effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation:

CF cystic fibrosis

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Figure 1.

Percentage biofilm viability of 48-h old biofilm of bacteria that are highly susceptible to PEV20 (AES-1R, PA365707, PA364077 and ATCC25619) after 24-h treatment with ciprofloxacin (CIP) alone (MIC, 2MIC and 3MIC), PEV20 alone (10^8 PFU/mL), or antibiotics ($\frac{1}{2}$ MIC, MIC and 2MIC) combined with PEV20 (10^8 PFU/mL). Error bars represent standard deviations from multiple cultures ($n=4$). * indicate statistically significant differences ($P<0.05$) in percentage biofilm viability of the treated groups in comparison to non-treated control. # indicate statistically significant ($P<0.05$) phage-antibiotic synergy.

Figure 2.

(A) Percentage biofilm biomass after 24-h treatment with ciprofloxacin (CIP) alone (MIC and 3MIC), PEV20 alone (10^8 PFU/mL), or antibiotics (MIC and 2MIC) combined with PEV20 (10^8 PFU/mL). Crystal violet assay was used to measure the biofilm biomass of *P. aeruginosa* isolates. Error bars represent standard deviations from multiple cultures (n=4). Asterisks indicate statistically significant differences (P<0.05) in percentage biofilm biomass of the treated groups in comparison to non-treated control. (B) Representative images showing the effect of PEV20 and ciprofloxacin on PA365707 biofilm architecture. Confocal microscopy in conjunction with Live/Dead bacterial viability kit showed marked disruption of biofilm architecture and increased dead biofilm after 24 h treatment with combination formulation containing PEV20 and ciprofloxacin. Scale Bar = 50 μ m. Green: live cells; red: dead cells; yellow: mix of live and dead cells. The experiment was performed in biological replicates (n=3).

Figure 3.

Percentage biofilm viability of 48-h old biofilm of bacteria that are partially (MANC3733) or completely (AES-2 and LESB58) resistant to PEV20 after 24-h treatment with ciprofloxacin (CIP) alone (MIC, 2MIC and 3MIC), PEV20 alone (10^8 PFU/mL), or antibiotics ($1/2$ MIC, MIC and 2MIC) combined with PEV20 (10^8 PFU/mL). Error bars represent standard deviations from multiple cultures ($n=4$). Asterisks indicate statistically significant differences ($P<0.05$) in percentage biofilm viability of the treated groups in comparison to non-treated control.

Figure 4.

(A) Lung epithelial cells BEAS-2B treated with *P. aeruginosa* Australian CF isolate (AES-1R): **i:** 72 hr (100% confluence) before treatment. **ii:** 24 hr (control/No bacterial treatment, 100% confluence). **iii:** 24 hr incubation with bacteria (No adherent BEAS-2B found, complete AES-1R colonization). **iv:** 24 hr incubation with bacteria and PEV20 (BEAS-2B cells adhered with AES-1R colonization). **v:** 24 hr incubation with bacteria and ciprofloxacin (10 μ g/ml) (BEAS-2B cells adhered with AES-1R colonization). **vi:** 24 hr incubation with bacteria and PEV20 + ciprofloxacin (5 μ g/ml) (BEAS-2B cells adhered with 75% confluence with lower AES-1R colonization) **vii:** BEAS-2B cells with addition of PEV20 + ciprofloxacin (5 μ g/ml) showed complete confluence. B) Human foreskin Fibroblast cells HFF-1 treated with *P. aeruginosa* wound isolate (PA365707): **i:** 72 hr (100%

confluence) before treatment. **ii:** 24 hr (control/No bacterial treatment, 100% confluence). **iii:** 24 hr incubation with bacteria (No adherent HFF-1 found, 100% PA365707 colonization). **iv:** 24 hr incubation with bacteria and PEV20 (HFF-1 cells adhered and no PA365707 colonization). **v:** 24 hr incubation with bacteria and ciprofloxacin (0.5µg/ml) (HFF-1 cells adhered and no PA365707 colonization) **vi:** HFF-1 cells with PEV20 + ciprofloxacin (0.25µg/ml) (HFF-1 cells adhered and no PA365707 colonization) **vii:** HFF-1 cells with addition of PEV20 + ciprofloxacin (0.25µg/ml) showed complete confluence. Four independent biological replicates were performed. Scale Bar = 20 µm.

Table I.

Minimum inhibitory concentrations of ciprofloxacin and tobramycin, and phage PEV20 susceptibility against seven *P. aeruginosa* isolates.

		Minimum Inhibitory Concentration (µg/ml)						
		AES-1R	LESB58	MANC3733	PA365707	PA364077	AES-2	ATCC25619
Ciprofloxacin	5	5	2	0.25	0.25	2	1	
	20	20	15	0.25	0.25	20	1	
Tobramycin								
PEV20	Susceptible	Resistant	Partially resistant	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible

Minimum biofilm inhibitory concentrations of ciprofloxacin and tobramycin against seven *P. aeruginosa* isolates.

Table II.

		Minimum Biofilm Inhibitory Concentration (µg/ml)					
		AES-IR	LESB58	MANC3733	PA365707	PA364077	AES-2 ATCC25619
Ciprofloxacin	5 (S)	5 (S)	2 (S)	1 (S)	1 (S)	5 (S)	1 (S)
	60 (I)	60 (I)	45 (R)	1.5 (R)	1.5 (R)	60 (R)	45 (R)
Tobramycin							

NOTE: S = Susceptible with 90% decrease in biofilm biomass; I = Intermediate 25-50% decrease in biofilm biomass. R = Resistance > 50% decrease in biofilm biomass compare to untreated biofilm/control. Antibiotic concentrations of up to 5 times the MIC were used to assess biofilm inhibitory concentration.