Effects of lysozyme, proteinase K, and cephalosporins on biofilm formation by clinical isolates of *Pseudomonas aeruginosa*

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INTRODUCTION

•***Pseudomonas aeruginosa*** **is an environmental pathogen that exhibits metabolic versatility. It is one of the major opportunistic pathogens associated with various infections, including respiratory tract infections, implant infections, burns, wounds, and nosocomial infections.**

•Biofilms are sessile microbial territories covered by an extracellular polysaccharide material, which facilitates irreversible attachment of microbial cells to the substructure or each other.

•Multidrug resistance is higher among biofilm producers than among biofilm non-producers. Antibiotics at concentrations 50 times their minimum inhibitory concentrations (MICs) may decrease the number of colony-forming units of some biofilm-producing species. Lysozyme and proteinase K were reported to exhibit antibiofilm activities. Proteinase K resembles naturally produced proteases and may be used to facilitate biofilm dissemination by breaking surface proteins. Antibiofilm activity of lysozyme is associated with the protective function of the innate immune system against infections with biofilms.

OBJECTIVE

- **To evaluate the effect of different media; Tryptic soya broth supplemented with 1% anhydrous glucose (TSBG) , muller hinton both, brain heart infusion and luria-Bertani upon biofilm formation in *P. aeruginosa* isolates.**
- **To study the effect of different concentrations of lysozyme, proteinase k and cephalosporin against biofilm of *P. aeruginosa*.**

METHODS

•Identification of *P. aeruginosa* isolates was done by suitable biochemical test according to standard bacteriological techniques.

• Qualitative and quantitative detection of biofilm production among *P. aeruginosa* isolates by tube method and tissue culture plate method respectively.

•Evaluation of lysozyme to affect viability of planktonic cells.

•Determination of MIC by microbroth dilution method and study the effect of 50x MIC against pseudomnal biofilm.

RESULTS

•Thirty six isolates were from urine, 16 from wound, 13 from eye, 13 from sputum, 10 from burn, 9 from urinary catheter, 4 from contact lenses and 2 from blood i.e. totally 103 clinical isolate.

•As shown in Table 1, Strong biofilm formation of *P. aeruginosa* clinical isolates (45 isolates preliminary) was 44% in TSBG, while the % of strong biofilm-producing isolates was lower in BHIB (28.8%), LB (15.5%), and MHB (13.3%) . Hence, TSBG medium was selected for further quantitative screening of all clinical isolates.

•Among all isolates biofilm production assessed by tube method revealed that 81.36 % of isolates were biofilm producing, On contrast 100% of isolates were biofilm producers by tissue culture plate method using TSBG medium (Table 2). The most strongly adherent 16 isolates were selected for antibiofilm studies.

•The highest reduction (19%) was reported at concentration of 30 µg/mL. The effect of proteinase K was biphasic, i.e. it promoted or inhibited biofilms at different concentration (Table 3).

•There was a significant reduction ($P < 0.01$) in the biofilm mass of *P. aeruginosa* clinical isolates in the presence of either lysozyme (30 µg/mL) or 50 times the MICs of ceftazidime or cefepime. The combination of lysozyme (30 µg/mL) and 50 times MIC of ceftazidime or cefepime significantly inhibited ($P < 0.01$) biofilm formation as compared with any of them alone (Figure 4).

Biofilm classification (45 isolates)	Number of isolates (%) according to biofilm formation			
	LB	BHIB	MHB	TSBG
Strongly adherent	7 (15.5%)	13 (28.8%)	6 (13.3%)	20 (44.4%)
Moderately adherent	13 (28.8%)	20 (44.4%)	22 (48.8%)	18 (40%)
Weakly adherent	25 (55.5%)	12 (26.6%)	17 (37.7%)	7 (15.5%)
Non-adherent	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table (1): Quantitative assay of the effect of different media on biofilm formation by *P. aeruginosa* via tissue culture plate method.

Biofilm classification (103 isolates)	Number of isolates (%) according to biofilm formation	
	`Tube method (Qualitative method)	Tissue culture plate method (Quantitative method)
Strongly adherent	28 (27.1%)	61 (59.2%)
Moderately adherent	23 (22.3%)	34 (33.0%)
Weakly adherent	33 (32.0%)	8 (7.7%)
Non-adherent	19 (18.4%)	0 (0%)

Table (2): Screening of the isolates for biofilm formation using tissue culture plate and tube method

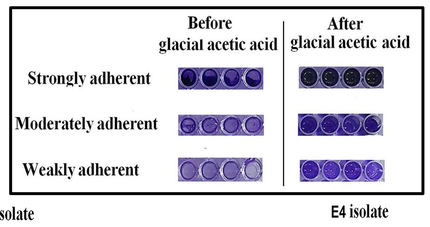


Figure (1): Detection of biofilm by tissue culture plate method

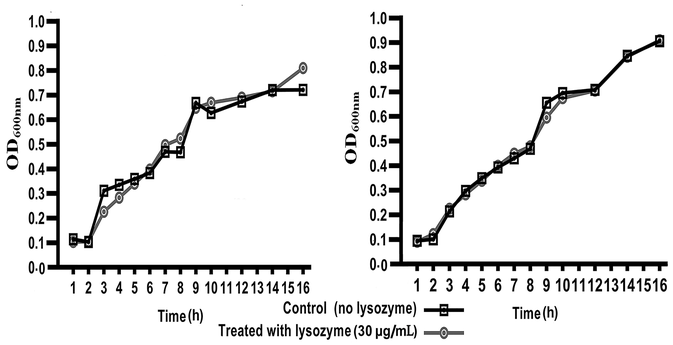


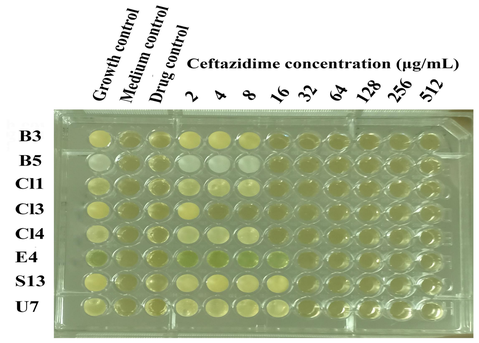
Figure (2): Effect of lysozyme (30 µg/mL) on the viability of planktonic *P. aeruginosa* cells.

Isolate code	Lysozyme concentration (µg/mL)			Proteinase K concentration (µg/mL)		
	5	10	30	2	5	10
B3	16.9	18.1	18.4	3.5	20.2	8.8
Cl3	17.5	25.8	30.6	1.3#	2.5#	4.2#
E4	25.8	34.3	44.8	2.5#	1.3#	3.3#
U7	8.7	10.4	12.9	53.4	69.8	55.4
U16	0.8	0.9	1.5	33.2	26.9	41.8
U19	5.1	6.5	7.4	10.4	10.2	5.6
W10	10.5	11.2	12	5.2#	6.1#	7.3#
W12	3.4	6.9	8.2	15.4	15.4	15.4
W14	28.2	30	35.9	28	30.5	15.9

#Enhancement of biofilm formation indicated as %.

- B: burn, Cl: contact lens, E: eye, U: urine, W: wound

Table (3): Biofilm reduction (%) at different concentrations of lysozyme and proteinase K



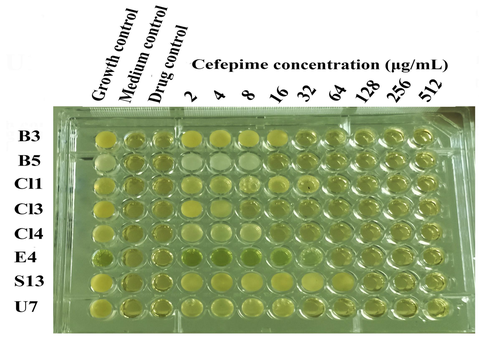
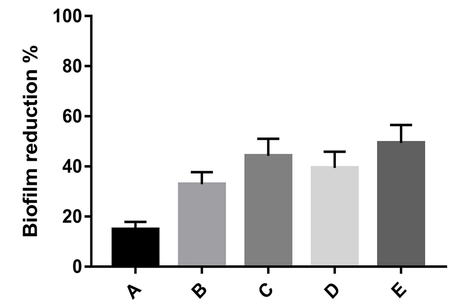


Figure (3): Determination of MIC for ceftazidime and cefepime by microbroth dilution method

Effect on biofilm of 16 clinical isolates	Lysozyme 30 µg/mL	Ceftazidime 50 x MIC	Cefepime 50 x MIC	Lysozyme + ceftazidime (50 x MIC)	Lysozyme + cefepime (50 x MIC)
Average ± SE	14.8 ± 0.75	32.8 ±1.2	44.2 ± 1.7	39.3 ± 1.61	49.3 ± 1.79



A: Biofilm treated with lysozyme (30 µg/mL).
B & C: 24h-old biofilm treated with 50 times MIC of ceftazidime and cefepime respectively.
D & E: 24h-old biofilm treated with lysozyme (30 µg/mL) followed by addition of 50 times MIC of ceftazidime or cefepime respectively.
- Error bars represent standard error

Fig. (4): Effect of lysozyme (30 µg/mL) and 50 times MIC of ceftazidime or cefepime alone or in combinations on the average reduction in percent biofilm mass of *P. aeruginosa* clinical isolates.

Discussion

- ***P. aeruginosa*** **is an opportunistic human pathogen associated with chronic lung infections and cystic fibrosis. Some studies have evaluated the effect of medium composition on biofilm formation.**
- **TSBG was considered to enhance biofilm formation as compared with other media such as LB and BHI.**
- **The tube method is considered to be less sensitive than the tissue culture plate method as it was associated with a higher strong adherence.**
- **The highest antibiofilm activity of lysozyme was noted at a concentration 30 µg/mL and without affecting cells viability. Moreover, the effect of proteinase K was biphasic. A reason for such unexpected result could be the relatively unspecific cleavage of this protease.**
- **The promising antibiofilm strategy was the combination of 50 times cephalosporin "ceftazidime or cefepime" with lysozyme (30 µg/mL).**

References

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