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Research Article

Biofilm producing multidrug resistant *Acinetobacter* species from a tertiary care hospital: a therapeutic challenge

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ABSTRACT

Background: *Acinetobacter* species are responsible for nosocomial infections which are related to the biofilm forming capacity of this pathogen. Biofilm formation helps the bacteria in surviving stressed environmental conditions and bacteria growing in biofilms are multidrug resistant. The objective was to detect biofilm formation and its relation to drug resistance among *Acinetobacter* spp isolates.
Methods: A total of 75 clinical isolates of *Acinetobacter* species were tested for biofilm production by microtitre plate method and susceptibility to antibiotic discs were determined by Kirby Bayer Method.
Results: On testing by tissue culture plate method, 12 (16%) *Acinetobacter* isolates were weak biofilm producers, 9 (12%) were moderate producers, 30 (40%) were strong biofilm producers, 24 (32%) isolates were nonbiofilm producers and 63 % isolates were multidrug resistant.
Conclusions: This study demonstrates the ability of *Acinetobacter* isolates to form biofilm and biofilm production has strong association with multiple drug resistance.

Keywords: *Acinetobacter baumanii*, Biofilm, Multidrug resistant

INTRODUCTION

Acinetobacter spp are nonfermentative gram negative bacilli, related to outbreaks of nosocomial infections.[1] *Acinetobacter baumanii* is the predominant species responsible for causing infections like pneumonia, urinary tract infections, septicaemia, meningitis among critically ill patients in ICU.[2] Biofilm formation on invasive devices by strains of *Acinetobacter* spp. is considered to be an important virulence factor for such infections. The biofilm formation plays a pivotal role in survival of bacteria under stressed environmental conditions. The infection is difficult to eradicate as *Acinetobacter* spp. growing in biofilm are resistant to most of the antimicrobials thereby limiting therapeutic options. Biofilm formation on surfaces and expression of multidrug resistance favours dissemination of *Acinetobacter* in hospital setting.[3]

So the present study was undertaken to detect biofilm production and its relation to drug resistance among *Acinetobacter* spp isolates.

METHODS

The study was conducted at Department of Microbiology, Gian Sagar Medical College and Hospital Banur, a tertiary care centre of Punjab, India from January 2015 to December 2015. The study was performed on 75 clinical *Acinetobacter* spp. isolates cultured from various clinical samples. Identification was done up to species level as per conventional laboratory methods.[4,5]

Biofilm detection by tissue culture plate method

The quantitative biofilm detection method was performed as described by Christensen et al. Test organisms were inoculated in 10 ml TSB and were incubated at 37 ºC for

24 hours.[6] 20 µl of this overnight culture (equivalent to 0.5 Mcfarland standards) was inoculated in wells of 96 wells flat bottomed polystyrene tissue culture plate. 3 wells were inoculated for each sample. Sterile TSB medium (180 µl) was added to each well. Sterile TSB was used as negative control. The plates were covered with a lid and incubated aerobically for 24 hours at 37 ºC. After incubation wells were washed three times with 0.2 ml of phosphate buffered saline to remove free floating bacteria. 200 µl of 99% methanol was added to the wells to fix adherent bacteria for 15 min. The plates were decanted and dried. Dried plates were stained with 0.2 ml of 2% crystal violet for 7 min followed by washing with distilled water to remove excess stain. After the plates were completely dry, 160 µl of 33% glacial acetic acid was added to the wells to remove excess stain. The optical density of each well was measured using ELISA reader.

According to the absorbance values, the test organisms were classified: none (-), weak (+), moderate (++) and strong (+++) adherent cells. The cut-off absorbance value (ODc) was calculated as three standard deviations above the mean OD of the negative control. Tests were performed in triplicates and results were averaged.

Table 1: Classification of adherence based on microtiter plate method.

Mean OD value	Adherence	Biofilm formation
OD_ODc	None	None
ODc<OD_2 ODc	Weak	Weak
2 ODc<OD_4 ODc	Moderate	Moderate
4 ODc<OD	Strong	High

In the present study, only strongly and moderately adherent isolates were considered as positive for biofilm formation while weakly adherent ones as negative for biofilm production.

Antibiotic sensitivity testing

Antibiotic susceptibility testing was done by Kirby Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CSLI) guidelines.[7] Antibiotics tested were: amikacin (10 µg), aztreonam (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), imipenem (10 µg), ofloxacin (30 µg), pipercillin+tazobactum (100mcg/10mcg), piperacilin (PIP, 100 mg), polymyxin B (PB, 300 mg), using Hi-Media discs .

Statistical analysis

The data was statistically analyzed using the statistical package for social sciences (SPSS)/16.0. Statistical significance was accepted at p <0.001.

RESULTS

Of the 75 *Acinetobacter* spp. isolates, 67 were *Acinetobacter baumanii*, 8 were *Acinetobacter* iwoffii. The isolates were obtained from endotracheal aspirate (42%), sputum (29%), pus (16%), blood and other sterile body fluids (6%), urine (4%), bronchoalveolar lavage (3%).

On testing by tissue culture plate method, 12 (16%) *Acinetobacter* isolates were weak biofilm producers, 9 (12%) were moderate producers, 30 (40%) were strong biofilm producers, 24 (32%) isolates were non-biofilm producers.

Comparison of antibiotic susceptibility results among biofilm producers and biofilm non producers has been shown in Table 2. Maximum resistance by biofilm producers was shown to imipenem and piperacillin tazobactum (89.7%) followed by piperacillin (87.1%), amikacin (79.4%), aztreonam (74.3%) and ciprofloxacin (76.9%). Resistance to antibiotics such as amikacin (79.4% versus 16.6%, P <0.0001), ceftriaxone (82.1% vs 47.2, P<0.001), and ciprofloxacin (76.9% vs 38.8%, P=0.0008) was higher among biofilm producers and this correlation was statistically significant than among non-biofilm producers (Table 2). All isolates were sensitive to polymyxin B.

Table 2: Comparison of antibiotic susceptibility results among biofilm producers and biofilm non producers.

Antibiotics	Resistance (%)	
	Biofilm producers (39 isolates)	Biofilm nonproducers (36 isolates)
Amikacin	79.4	16.6
Aztreonam	74.3	33.3
Ciprofloxacin	76.9	38.8
Ceftriaxone	82.1	47.2
Cefepime	79.4	30.5
Imipenem	89.7	75
Ofloxacin	71.7	38.8
Piperacillin	87.1	47.2
Piperacillin tazobactum	89.7	80.5

DISCUSSION

Acinetobacter spp has emerged as nosocomial pathogen and causes invasive device related infections. This is due to propensity of this organism to form biofilms on devices which further results in decreased penetrability of antibiotics, thus making these infections a clinical challenge. Biofilm formation among clinical isolates of *Acinetobacter* has been reported.[8,9] In the present study 39 (52%) isolates (30 strong and 9 moderate) were biofilm producers by tissue culture plate method. This is

in concordance with the study of Rao SR et al in which 62% isolates of *Acinetobacter* were biofilm producers.[10] This study was also comparable with the other study in which 63% isolates were biofilm producers.[11]

In present study, statistical significant association was observed between antibiotic resistance to ciprofloxacin, amikacin, ceftriaxone and biofilm production. However all the isolates were sensitive to polymyxin B. In this study, biofilm producing isolates of *Acinetobacter* were resistant to imipenem in 89.7%, amikacin in 79.4%, ciprofloxacin in 76.9%, pipercillin+tazobactum (89.7%) and aztreonam in 74.3%. Similar results were reported in other study in which biofilm positive *Acinetobacter* were resistance to ceftazidime (95%), cefepime (95%), aztreonam (85%), ciprofloxacin (85%), amikacin (80%), imipenem (65%) and pipercillin+tazobactum (40%).[12]

In this study 63% isolates were multidrug resistant strains, similar results were seen in previous study in which 79.5% isolates of *Acinetobacter baumanii* were multi-drug resistant (MDR) in which 66 isolates were resistant to imipenem.[13] High imipenem resistance has been reported by other studies however drugs like amikacin, ceftazidime, quinolone have been reported to be effective.[14,15] *Acinetobacter* spp transfer genes horizontally in biofilm and acquire resistance to multiple drugs. Hence combination therapy should be given to avoid treatment failure.

CONCLUSION

Study demonstrated the ability of the clinical isolates of *Acinetobacter* species to produce biofilm. Resistant to commonly used antibiotics such as cephalosporin, aminoglycosides, quinolone, carbapenem was observed among biofilm producers. Polymyxins were the only effective therapeutic agent in the study. This trend of multidrug resistance among *Acinetobacter* species is a matter of concern and combination therapy can be an effective option. So a greater understanding of the antibiogram of *Acinetobacter* species will help in development of effective treatment.

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