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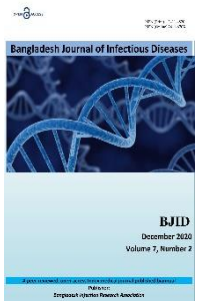

Biofilm Formation and Its Association with Antimicrobial Resistance among Clinical Isolates of *Acinetobacter baumanii* at a Tertiary Care Hospital in Dhaka City of Bangladesh

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Abstract

Background: *Acinetobacter baumanii* is responsible for nosocomial infections which are related to the biofilm forming capacity of this pathogen. **Objective:** The purpose of the present study was to detect biofilm formation in clinical isolates of *Acinetobacter baumannii* and to observe relationship between biofilm formations with its antimicrobial resistance. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College and Hospital, Dhaka, Bangladesh from July 2015 to June 2016. *Acinetobacter baumannii* was isolated from different specimens and was identified and were screened for biofilm production by tissue culture plate method. Antimicrobial susceptibility test was done by disc diffusion method. **Results:** A total 300 samples were studied of which 26(8.7%) were *Acinetobacter baumannii*. From 26 isolated *Acinetobacter baumannii*, 16(61.5%) were biofilm producers. Biofilm producing *Acinetobacter baumannii* were 100% resistant to ceftriaxone, ceftazidime, amoxiclav, amikacin and ciprofloxacin. Resistance to imipenem, meropenem, cephotaxime, cefepime and gentamicin were also higher among biofilm producing *Acinetobacter baumannii* isolates than non-biofilm producers. **Conclusions:** In conclusion the ability of *Acinetobacter baumannii* forms biofilm and biofilm production has strong association with antimicrobial resistance. [*Bangladesh Journal of Infectious Diseases, December 2021;8(2):82-86]*

Keywords: *Acinetobacter baumannii*; biofilm; tissue culture plate method; antimicrobial resistance

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Introduction

Multidrug resistant *Acinetobacter baumannii* is a rapidly emerging opportunistic pathogen associated with a variety of nosocomial infection, including ventilator-associated pneumonia, bacteremia, surgical site infections, secondary meningitis and urinary tract infections[1-2]. Artificial ventilation and other invasive procedures, exposure to antibiotics, colonization pressure, environmental contamination in ICU and underlying illness facilitate the spread of these multidrug-resistant species in ICU[3]. *Acinetobacter baumannii* is the most common cause of device-related nosocomial infection. Biofilm formation is thought to be a key pathogenic feature, especially in relation to intravascular line infections and ventilator associated pneumonia[4].

Biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface[5]. Generally, two properties are often associated with biofilm producing bacteria, namely, the increased synthesis of exopolysaccharide (EPS) and the development of antibiotic resistance[6]. Mechanisms responsible for antimicrobial resistance in organisms producing biofilms may be delayed penetration of the antimicrobial agents through the biofilm matrix, altered growth rate of biofilm organisms and other physiological changes due to the biofilm mode of growth. The ability of bacterial cells to transfer genes horizontally is enhanced within biofilm communities, thereby facilitating the spread of antibiotic resistance[7].

Infections due to *Acinetobacter baumanni* is difficult to eradicate as *Acinetobacter baumaanni* 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 baumannii* in hospital setting[8]. Therefore, the present study was undertaken on clinical isolates of *Acinetobacter baumannii* to determine biofilm formation and to observe relationship between biofilm formation and antimicrobial resistance among *Acinetobacter baumannii* isolates.

Methodology

This cross-sectional study was carried out at Department of Microbiology in Dhaka Medical College (DMC), Dhaka, Bangladesh over a period of one year which was from July 2015 to June 2016. Tracheal aspirate, blood, urine and wound swab samples were collected from all recruited patients for microscopy, culture and sensitivity testing. Samples were collected from patients of all age groups, both sexes, who were critically ill and suspected for pneumonia, urinary tract infection, septicaemia, skin and soft tissue infection. Samples were inoculated on Blood Agar and MacConkey Agar plates under strict aseptic conditions. Plates were incubated at 37^0 C for 24 to 48 hours. *Acinetobacter baumannii* was identified and confirmed by Gram staining as Gram negative coccobacilli or cocci in pairs, non-motile, oxidase negative, Alkaline/Alkaline (K/K) reaction in Triple Sugar Iron (TSI) slant, catalase positive, Indole negative, Citrate utilization test positive, urease test negative. It showed Oxidative–Fermentative (O/F) test –oxidative[9-11]. Susceptibility to antimicrobial agents of all isolates was done by Kirby Bauer modified disc diffusion technique using Mueller Hinton agar plates and zones of inhibition were interpreted according to CLSI guidelines (2015)[12]. Biofilm formation was determined by Tissue Culture Plate (TCP) method. Organisms isolated from fresh agar plates were inoculated in 10 ml of brain heart infusion broth with 1.0% glucose. Broths were incubated at 37°C for 24 hours. Then the cultures were diluted 1:100 with fresh broth. Individual wells of sterile 96 wells flat bottom polystyrine tissue culture plates were filled with 200 µl of the diluted cultures. The control organisms were treated the same way as the test organisms also incubated, diluted and added to tissue culture plates. Negative control wells contained inoculated sterile broth. The plates were incubated at 37°C for 24 hours. After incubation, the contents of each well were removed by gentle tapping. The wells were washed with 0.2 ml of phosphate buffer saline (PH 7.2) four times. The adhered biofilm formed by bacteria was fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed with distilled water and plates were kept for drying. The optical density (OD) of stained adherent biofilm was obtained by using micro ELISA autoreader at wavelength of 570 nm. The experiment was performed in triplicate and repeated three times[13-14]. The interpretation of biofilm production was done according to the criteria of Stepanovic et al[15] (Table 1). The average OD values were calculated for all tested strains and negative controls, since all tests were performed in triplicate and repeated three times. Second, the cut off value (ODc) was established. It was defined as three standard deviate (SD) above the mean OD of the negative control: ODc = average OD of negative controls + (3 X SD of negative control). 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.

Table 1: Interpretation of biofilm production

Average OD value	Adherence	Biofilm production
OD ≤ ODc	None	None
ODc < OD ≤ 2ODc	Weak	Weak
2ODc < OD ≤ 4ODc	Moderate	Moderate
4ODc < OD	Strong	High

Results

Total 300 samples were studied. Of which 130 were wound swabs, 80 were urine, 50 were endotracheal aspirates and 40 were blood samples. From 300 samples, 26 (8.7%) were *Acinetobacter baumannii*. Maximum number of *Acinetobacter baumannii* were isolated from endotracheal aspirate (38.0%) followed by (5.0%) from blood, (3.1%) from wound swab and (1.3%) from urine samples (Table 2). On testing by tissue culture plate method, from 26 isolated *Acinetobacter baumannii*, 16 (61.5%) were biofilm producers. The rate of biofilm production by isolated *Acinetobacter baumannii* from different clinical samples is recorded (Table 2).

Table 2: Biofilm production of isolated *Acinetobacter baumannii* from different clinical samples

Type of Specimens	Positive for *Acinetobacter baumannii*	Positive for production of biofilm
Wound swab	4 (3.1%)	1 (25.0%)
Urine	1 (1.3%)	1 (100.0%)
Endotracheal aspirate	19 (38.0%)	13 (68.4%)
Blood	2 (5.0%)	1 (50%)
Total	**26 (8.7%)**	**16 (61.5%)**

For antibiotic resistance pattern among both positive and negative biofilm producing *Acinetobacter baumannii* isolates, higher antibiotic resistance pattern was observed among biofilm producers *Acinetobacter baumannii* isolates compared to the non-biofilm producers' isolates. 100% resistance pattern was observed among biofilm producing *Acinetobacter baumannii* isolates for ceftriaxone, ceftazidime, amoxiclav, amikacin and ciprofloxacin, compared to 80%, 80%, 80%, 60%% and 60% resistance pattern for the same antibiotics among the non-biofilm producing *Acinetobacter baumannii* isolates. Higher level of resistance for other antibiotics was also recorded (Table 3).

Table 3: Antibiotic Resistance Pattern of Biofilm and Non-Biofilm Producers of *Acinetobacter baumannii* Isolates

Antimicrobial agent	Biofilm positive resistant isolates (n=16)	Biofilm negative resistant isolates (n=10)	Resistance of all isolates (n=26)
Imipenem	15(93.8%)	6(60.0%)	21(80.8%)
Meropenem	15(93.8%)	6(60.0%)	21(80.8%)
Ceftriaxone	16(100.0%)	8(80.0%)	24(92.3%)
Ceftazidime	16(100.0%)	8(80.0%)	24(92.3%)
Cefotaxime	15(93.8%)	9(90.0%)	24(92.3%)
Cefepime	15(93.8%)	9(90.0%)	24(92.3%)
Amoxiclav	16(100.0%)	8(80.0%)	24(92.3%)
Amikacin	16(100.0%)	6(60.0%)	22(84.6%)
Gentamicin	15(93.8%)	7(70.0%)	22(84.6%)
Ciprofloxacin	16(100.0%)	6(60.0%)	22(84.6%)
Piperacillin-Tazobactam	14(87.5%)	9(90.0%)	23(88.5%)
Colistin	2(12.5%)	1(10.0%)	3(11.5%)
Tigecycline	4 (25.0%)	2 (20.0%)	6 (23.1%)

Discussion

Acinetobacter baumannii infections present a global medical challenge. They are opportunistic pathogens and are particularly successful at colonizing and persisting in the hospital environment. They are able to resist desiccation and survive on inanimate surfaces for years[16-19]. Interest in this organism has been growing rapidly because of the emergence of multi-drug-resistant strains,

some of which are pan-resistant to antimicrobial agents[16,19-20]. It is also among the most common causes of device-related nosocomial infection that results when the organism is able to resist physical and chemical disinfection, often by forming a biofilm[5]. Biofilm exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes[21].

In the current study, maximum number of *Acinetobacter baumannii* have been isolated from endotracheal aspirate (38.0%) followed by (5.0%) from blood, (3.1%) from wound swab and (1.3%) from urine samples. In India, a study reported that, the high isolation rate of *Acinetobacter baumannii* of about 42% were from tracheal aspirates, 29.0% from sputum, 16.0% from pus, 6.0% from blood and other body fluids, 4% from urine and 3.0% from bronchoalveolar lavage[8].

In the present study, 61.5% isolates are biofilm producers by tissue culture plate method. This is in concordance with the study of Rao et al[4] in which 62.0% isolates of *Acinetobacter* are biofilm producers. This study is also comparable with the other study in which 63.0% isolates are biofilm producers[22].

In this study, *Acinetobacter baumannii* showed 100% biofilm formation in urine, 68.4% in tracheal aspirate, 50.0% in blood and 25.0% in wound swab. Another study found that, biofilm formation by *Acinetobacter baumannii* were 76.4% in tracheal aspirate, 80.0% in wound swab, 75.0% in blood, 50.0% in sputum, 50.0% in pleural fluid, 75.0% in urine, 80.0% in cerebrospinal fluid[23].

This study shows association of biofilm formation with antibiogram of *Acinetobacter baumannii* isolates. Biofilm forming *Acinetobacter baumannii* isolates from different clinical sources are 100% resistant to ceftriaxone, ceftazidime, amoxiclav, amikacin and ciprofloxacin. Nahar et al[24] has also reported 100.0% resistance to amoxicillin, ceftriaxone, ceftazidime, cefuroxime, and aztreonam in biofilm forming *Acinetobacter* species. In this study, higher level of resistance also seen in imipenem, meropenem, cephotaxime, cefepime, gentamicin and piperacillin-tazobactum. Resistance to most of the antibiotics is becoming common, and very few therapeutic options remain. A study from India showed biofilm producers of *Acinetobacter* isolates were 100% resistant to imipenem, amikacin (82.0%), cephotaxime (88.0%), ciprofloxacin (70.0%) and aztreonam (38.0%)[4]. Study in South India showed, biofilm positive *Acinetobacter* showed resistance to ceftazidime (95.0%), cefepime (95.0%), aztreonam (85.0%), ciprofloxacin (85.0%), amikacin (80%), gentamicin (70.0%), imipenem (65.0%), pipercillin+tazobactum (40.0%) and netilmicin (20.0%)[25].

Conclusion

In conclusion, the data obtained in the present work showed that most of the clinical isolates of *Acinetobacter baumannii* are biofilm producers especially from device in ICU samples and they are multidrug resistant. All biofilm producing *Acinetobacter baumannii* are resistant to clinically achievable levels of most commonly used antibiotics such as penicillin, cephalosporin, aminoglycosides, quinolone, carbapenem and monobactam group of drugs. Colistin and tigecycline remain the only agent that may be consistently active in vitro against *Acinetobacter baumannii*. However, colistin and tigecycline resistant *Acinetobacter baumannii* isolates are slowly emerging. This is very alarming for us that biofilm forming multidrug resistant *Acinetobacter baumannii* represents a severe threat in the treatment of hospitalized patients. Combination therapy can be an effective option. So a greater understanding of the antibiogram of *Acinetobacter baumannii* will help in development of effective treatment.

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