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Association of Biofilm Formation with Antimicrobial Resistance Among the *Acinetobacter* Species in A Tertiary Care Hospital in Bangladesh

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Abstract

Purpose: *The purpose of this study was to detect biofilm formation in clinical isolates of* Acinetobacter *species and to observe correlation between biofilm formation and antimicrobial resistance among* Acinetobacter *isolates.*

Methods: *Two hundred fifty six clinical samples collected from patients who were admitted in Intensive Care Unit (ICU) and on device, patients from Surgery, Medicine, Gynae & Obs and Urology department of Bangabandhu Sheikh Mujib Medical University (BSMMU) and from Burn unit of Dhaka Medical College Hospital were included in this study. Biofilm formation and antibiotyping were performed for the isolates of* Acinetobacter *species recovered from clinical samples including tracheal aspirates, blood, urine, wound swab, pus, throat swab, endotracheal tubes, burn samples, ascitic fluid, sputum, aural swab, oral swab, cerebrospinal fluid, and catheter tip. Correlation of biofilm formation with antimicrobial resistance pattern among* Acinetobacter *isolates were also observed in this study.*

Result: *A total of 256 various specimens were studied of which 95 Intensive Care Unit (ICU) and 161 Non ICU samples. Out of 95 ICU and 161 Non ICU samples,* Acinetobacter *species were isolated from 32 (33.7%) and 20(12.4%) respectively. From 32 ICU and 20 Non ICU* Acinetobacter *isolates, 28 (87.5%) and 11 (55%) were biofilm producers. Biofilm forming capacity of* Acinetobacter *species was significantly (p<0.008) greater in ICU than in Non ICU isolates. In both ICU and Non ICU isolates, biofilm forming* Acinetobacter *species were 100% resistant to amoxicillin, ceftriaxone, ceftazidime, cefotaxime, cefuroxime, and aztreonam. Resistance to antibiotics such as gentamicin, amikacin, netilmicin, ciprofloxacin and imipenem was higher among biofilm forming* Acinetobacter *isolates in ICU than Non ICU isolates. Susceptibility to colistin was 100% in Non ICU isolates but in ICU it showed 7.1% resistance.*

Conclusions: *This investigation showed that most of the clinical isolates of* Acinetobacter *species were biofilm producers especially from ICU samples and they were multidrug resistant. Even polymixin resistant* Acinetobacter *isolates are slowly emerging. This is very alerming for us that biofilm forming multidrug resistant* Acinetobacter *species represents a severe threat in the treatment of hospitalized patients. So, antibiotic policy and guidelines are essential to eliminate major outbreak in future.*

Keywords: Acinetobacter, *biofilm, multidrug resistance.*

Introduction

Acinetobacter species has emerged as an important nosocomial pathogen as it is the causative agent of several types of infections including pneumonia, meningitis, septicaemia, and urinary tract infections and also responsible for causing intermittent outbreaks especially in ICU. They ranked second after *Pseudomonas aeruginosa* among the nosocomial, aerobic, non- fermentative, gram negative bacilli pathogens. [1,2] Infections caused by *Acinetobacter* are associated with medical devices, e.g. vascular catheter, CSF shunt, Foley catheter, surgical interventions etc.[3-5] The presence and duration of invasive procedures, as well as exposure to broad spectrum antibiotics, have been identified as risk factors for acquisition of *Acinetobacter* in numerous studies. [6] Infections caused by them are difficult to control due to multidrug resistance, which limit therapeutic options in critically ill and debilitated patients especially from the intensive care unit, where its prevalence is most noted.[2]

Acinetobacter infections are associated closely with surgery or the use of artificial devices. Patients become infected following initial colonization. This process is influenced by various risk factors, particularly in ICUs, where multiple manipulations following surgery, as well as the use of endotracheal tubes and intravascular, ventricular or urinary catheters, can result in colonization by opportunistic bacteria such as *Acinetobacter*. [6]

Acquisition of the ability to form biofilm could be a good strategy to enhance a microorganism's survival under

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stressed conditions, e.g., during host invasion or following antibiotic treatment. This is because cells growing in biofilms are highly resistant to numerous types of antimicrobial agent. In addition, the ability of horizontal gene transfer of bacterial cells is enhanced within biofilm communities, thereby facilitating the spread of antibiotic resistance. The high colonizing capacity of *A. baumannii*, combined with its resistance to multiple drugs, contribute to the organism's survival and further dissemination in the hospital setting.[7] The present study was undertaken on clinical isolates of *Acinetobacter* species to determine biofilm formation and to observe correlation between biofilm formation and antimicrobial resistance among *Acinetobacter* species.

Material and methods

Clinical samples were collected from patients admitted in Bangabandhu Sheikh Mujib Medical University (BSMMU) and from patients admitted in Burn unit of Dhaka Medical college. Two hundred fifty six clinical samples included tracheal aspirates, blood from central venous catheter (CVC), peripheral blood, urine, wound swab, pus, throat swab, endotracheal tubes, burn samples, ascitic fluid, sputum, aural swab, oral swab, cerebrospinal fluid, and catheter tip were collected. Out of 256 samples 95 Intensive Care Unit (ICU) samples were collected from patient admitted in ICU and on device and 161 Non ICU samples were collected from Medicine, Surgery, Gynaecology & Obstetrics, Urology department of BSMMU and from patients admitted in the Burn unit of Dhaka Medical College Hospital. Laboratory works were performed in the department of Microbiology & Immunology, BSMMU, Dhaka between January 2010 to December 2010.

Isolation and identification of *Acinetobacter*

Typical colonies were enumerated, selected and examined further. *Acinetobacter* was identified by Gram staining, motility, oxidase, catalase, citrate utilization, indole and urease tests, glucose oxidation in Krigler Iron Agar (KIA) media and biochemical tests in oxidation and fermentation media (OF media). These identification schemes were done as per standard techniques. [8, 9]

Antimicrobial susceptibility tests

All the *Acinetobacter* isolates from BSMMU & Dhaka medical college hospital were tested for antimicrobial susceptibility testing by disc diffusion method using the Kirby-Bauer technique[10] and as per recommendations of the National Committee for Clinical laboratory Standards (NCCLS).[11] Antimicrobial disks used for sensitivity tests were amoxicillin(10mg), ciprofloxacin(5mg), gentamicin (10mg), ceftriaxone (30mg), ceftazidime (30mg), cefuroxime(30mg), cefotaxime(30mg), amikacin(30mg), aztreonam(30mg), imipenem(10mg), netilmicin(30mg) and colistin(10mg) were used respectively.

Detection of biofilm

The ability of *Acinetobacter* isolates to form biofilm was performed as described by Toledo *et al*., 2001.[12] Isolates were grown over night at 37^0C in Brain Heart Infusion Broth (BHIB) with 0.25 % glucose. The culture was diluted at a ratio of 1:20 in fresh Brain Heart Infusion broth (BHIB) with 0.25% glucose. 200 µl of this suspension was used to inoculate in sterile 96 well flat bottomed polystyrene microtiter plate. Then the plate was incubated at 37^0C for 24 hours. Wells were washed with Phosphate buffer solution (PBS) three times. Non-adherent cells were removed by washing with phosphate buffer. Then the microtiter plate was dried in an inverted position. After that plate was stained with 0.5% Crystal violet (CV) for 15 minutes. Wells were rinsed once more. Then 200 µl ethanol/ acetone (80: 20, v/v) were added in each well to solubilize CV. The optical density (OD) was determined using a microtiter reader. Each assay was performed in triplicate & repeated twice. 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 deviations (SD) above the mean OD of the negative control: ODc=average OD of negative controls + (3XSD of negative control). Final OD value of a tested strain was expressed as average OD value of the strain reduced by ODc value (OD= average OD of a strain - ODc). ODc value was calculated for each microtiter plate separately. Any negative value was presented as zero, while any positive value was indicated biofilm production.[13]

Results

A total of 256 various specimens were studied which included 95 ICU and 161 Non ICU samples. Out of 95 ICU and 161 Non ICU samples, *Acinetobacter* species were isolated from 32 (33.7%) and 20(12.4%) respectively. In ICU, *Acinetobacter* species were predominantly isolated from endotracheal tube (100.0%) followed by tracheal aspirate (54.3%), blood from central venous catheter blood (36.4%), peripheral blood (13.6%) and urine (12.5%). In Non ICU, *Acinetobacter* species were isolated from wound swab (25.0%), pus (13.9%), peripheral blood (50%), urine (44.4%) and throat swab (11.1%). No growth of *Acinetobacter* species were detected in other samples namely ascitic fluid, sputum, aural swab, oral swab, burn samples, cerebrospinal fluid and catheter tips. From 32 ICU and 20 Non ICU *Acinetobacter* isolates 28 (87.5%) and 11 (55.0%) were biofilm producers. Biofilm forming capacity of *Acinetobacter* species was significantly ($p<0.008$) higher in ICU than Non ICU isolates. The rate of biofilm production by isolated *Acinetobacter species* from different clinical samples is shown in Table-1.

From ICU and Non ICU samples all the biofilm forming *Acinetobacter* isolates were 100% resistant to amoxicillin, ceftriaxone ceftazidime, cefotaxime, cefuroxime and

aztreonam. Higher level of resistance was also recorded in Table-2. Only colistin showed 7.1% resistance in biofilm forming *Acinetobacter* isolates in ICU and 100% sensitivity in Non ICU isolates.

Table-I

Biofilm production of isolated Acinetobacter species from different clinical samples

ICU samples	Total No. samples	Positive for *Acinetobacter* species N (%)	Positive for production biofilm N (%)
Tracheal aspirate	35	19 (54.3)	16(84.2)
Blood CVC	11	4 (36.4)	4(100.0)
Peripheral blood	22	3 (13.6)	3(100.0)
Urine	24	3 (12.5)	3(100.0)
Endotracheal tube	3	3 (100.0)	2(66.7)
Total	95	32 (33.7)	28(87.5)
Non ICU samples			
Wound swab	32	8(25.0)	4(50.0)
Pus	36	5(13.9)	2(40.0)
Peripheral blood	4	2(50.0)	2(100.0)
Urine	9	4(44.4)	3(75.0)
Throat swab	9	1(11.1)	0(0.0)
Others	71	0(0.0)	0(0.0)
Total	161	20(12.4)	11(55.0)

Table-II

The antibiotic resistance patterns of biofilm producing Acinetobacter isolates

Antibiotics	Biofilm forming *Acinetobacter* isolates showing antibiotic resistance (%)	
	ICU	Non ICU
Amoxycillin	100.0	100.0
Ceftriaxone	100.0	100.0
Ceftazidime	100.0	100.0
Cefotaxime	100.0	100.0
Cefuroxime	100.0	100.0
Gentamicin	100.0	88.9
Amikacin	85.7	55.6
Netilmicin	85.7	11.1
Ciprofloxacin	82.1	54.4
Imipenem	81.0	22.2
Aztreonam	100.0	100.0
Colistin	7.1	0.0

Discussion

Acinetobacter infections present a global medical challenge. They are opportunistic pathogens and are particularly successful at colonizing and persisting in the hospital environment.[3-5] Biofilm formation is thought to be a key pathogenic feature, especially in relation to intravascular line infections and ventilated associated pneumonia. Generally, two properties are often associated with biofilm producing bacteria, namely, the increased synthesis of exopolysaccharide (EPS) and the development of antibiotic resistance. [14] One can assume that increased production of EPS in *Acinetobacter* is likely to create a protective environment leading to difficulty in antibiotic penetration leading to development of resistance. In addition, there appears to be some differences in the cellular physiology of cells within the biofilm that also results in increased drug resistance.[15] Thus infections due to bacteria that form biofilm is a tenacious clinical problem. In this work, biofilm formation by *Acinetobacter* isolates were tested and tried to correlate them with antimicrobial resistance.

In this current study, the high isolation rates of *Acinetobacter* species of about 100% from endotracheal tube, followed by 54.3% from tracheal isolates, 36.4% from central venous catheter blood in ICU and 50% from peripheral blood, 44.4% from urine and 25% from wound swab and 13.9% from pus in non ICU samples. In India, a study reported that, the high isolation rate of *Acinetobacter* species of about 24% were from tracheal aspirates, 16% from sputum, 12% from endotracheal tube, 12% from wound swab and 6% from blood.[16]

Our study showed 28 (87.5%) and 11 (55.0%) isolates were biofilm producers from 32 ICU and 20 Non ICU *Acinetobacter* species. Higher rate of biofilm production was found in patients on device in ICU. Present results showed that biofilm plays a role in the pathogenesis of some device-associated *Acinetobacter* infections. Other study showed that, more than 60% of *A. baumannii* isolates from clinical samples formed biofilm, and these isolates were associated mainly with device-associated infections. [17]

In this study, *Acinetobacter* species showed higher biofilm production in central venous catheter blood 100%, peripheral blood 100%, urine 100% and tracheal aspirates 84.2% but endotracheal tube showed 66.7% biofilm production in ICU. In Non ICU isolates, *Acinetobacter* showed 100% biofilm formation in peripheral blood, 75% in urine, 50% in wound swab and 40% in pus. Another study found that, biofilm formation by *A. baumannii* were 76.47% 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. [18]

This study shows association of biofilm formation with antibiogram of *Acinetobacter* isolates in ICU and in Non ICU. From both ICU and Non ICU samples, all the biofilm forming *Acinetobacter* isolates from different clinical sources were 100% resistant to amoxicillin, ceftriaxone ceftazidime, cefotaxime, cefuroxime and aztreonam. In ICU highest resistance was seen in gentamicin 100% followed by amikacin 85.7%, netilmicin 85.7%, ciprofloxacin 82.1% and imipenem 81.0% respectively. In Non ICU, antibiotic resistance was seen in gentamicin 88.9%, amikacin 55.6%, ciprofloxacin 54.4%, netilmicin 11.1% and imipenem 22.2%. Biofilm forming *Acinetobacter* isolates showed 7.1% colistin resistant in ICU isolates and 100.0% sensitivity in Non ICU isolates.

Resistance to most of the antibiotics is becoming common, and very few therapeutic options remain. A study from Pandicherry India showed biofilm producers of *Acinetobacter* isolates were 100% resistant to imipenem, amikacin 82%, cephotaxime 88%, ciprofloxacin 70% and aztreonam 38%.[19] Study in South India showed, biofilm positive *Acinetobacter* showed resistance to ceftazidime 95%, cefepime 95%, aztreonam 85%, ciprofloxacin 85%, amikacin 80%, gentamicin 70%, imipenem 65%, pipercillin+tazobactum 40% and netilmicin 20%. [16] Another study was conducted in USA showed, 79.5% were multi- drug resistant (MDR) *A. baumannii*. Among these, 62 were resistant to ceftazidime and 66 were resistant to imipenem. The imipenem resistant isolates were also resistant to amikacin, gentamicin, streptomycin, tetracycline, ciprofloxacin and nalidixic acid.[20]

Conclusion

In conclusion, the data obtained in the present work showed that most of the clinical isolates of *Acinetobacter* species were biofilm producers especially from device in ICU samples and they are multidrug resistant. All biofilm producing *Acinetobacter* species were found to be resistant to clinically achievable levels of most commonly used antibiotics such as penicillin, cephalosporin, aminoglycosides, quinolone, carbapenem and monobactam group of drugs. Polymyxins remain the only agent that may be consistently active in vitro against *Acinetobacter* species. However, polymixin resistant *Acinetobacter* isolates are slowly emerging. This is very alerming for us that biofilm forming multidrug resistant *Acinetobacter* species represents a severe threat in the treatment of hospitalized patients. Combination therapy may be the only therapeutic option to preserve the clinical utility of the polymixins against *Acinetobacter*. So, antibiotic policy and guidelines are essential to eliminate major outbreak in future.

Conflict of Interest : None

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