

VIRULENCE FACTORS AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ACINETOBACTER SPECIES IN A TERTIARY CARE HOSPITAL IN BANGLADESH

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Abstract

Acinetobacter species are aerobic Gram variable coccobacilli that are now emerging as an important nosocomial pathogen. Infections caused by them are difficult to control due to multidrug resistance. The purpose of this study was to detect virulence factors namely gelatinase production, biofilm formation and antibiotic susceptibility of *Acinetobacter* species. Two hundred fifty six clinical samples collected from Bangabandhu Sheikh Mujib medical University (BSMMU) and from burn unit of Dhaka Medical College Hospital were included in the study. Gelatinase production was seen on Luria Bertani agar media containing gelatin (30 gm/l) and biofilm formation was detected in microtiter plate assay. Out of 256 clinical samples, 52 (20.3%) were *Acinetobacter* species. Out of 52 *Acinetobacter* isolates, none were gelatinase producer but 39 (75%) were found biofilm producers. *Acinetobacter* isolates were 100% resistant to ceftazidime, cefotaxime cefuroxime and ceftriaxone. High level of resistance was also recorded for amoxicillin (98.1%), aztreonam (98.1%), gentamicin (90.4%), ciprofloxacin (73.1%), amikacin (57.6%), netilmicin (53.8%) and imipenem (44.2%). Susceptibility to colistin was maximum (96.2%). The present study demonstrated a high propensity of biofilm formation by the clinical isolates of *Acinetobacter* species and most of the *Acinetobacter* were multidrug resistant.

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Key words: *Acinetobacter*, biofilm, gelatinase

Introduction

Acinetobacter species are Gram negative, strongly aerobic, catalase positive, oxidase negative, non motile encapsulated coccobacilli. *Acinetobacter* are widely distributed in nature and are commonly found as a part of normal flora of human skin and occasionally in the respiratory tract, genitourinary tract, gastrointestinal tract and conjunctiva.^{1,2}

Acinetobacter species is an important opportunistic pathogen responsible for a variety of nosocomial infections, including bacteremia, urinary tract infections, secondary meningitis, surgical- site infections, and ventilator- associated pneumonia, especially in patients in the intensive care units (ICU).^{3,4}

Although *Acinetobacter* species are considered to be relatively of low virulence pathogens, certain

characteristics of these organisms may enhance the virulence of the strains involved in infections. These characteristics include (i) the property of adhesion to human epithelial cells in the presence of fimbriae and / or capsular polysaccharide, (ii) lipopolysaccharide component of the cell wall and the presence of lipid A, (iii) biofilm formation and (iv) gelatinase production. Gelatinase is a protease that is capable of hydrolyzing gelatin, collagen, casein, haemoglobin, and other bioactive peptides.⁵ The potential ability of *Acinetobacter baumannii* to form biofilms might also explain its outstanding antibiotic resistance, survival properties and increased virulence and further dissemination in the hospital setting.^{6,7} The presence of indwelling medical devices increases the risk for biofilm formation and subsequent infection especially in the ICU.

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Infections due to *A. baumannii* often prove difficult to treat due to high level of resistance to multiple antibiotics as a result of both intrinsic and acquired mechanisms. According to Rao *et al.*, *A. baumannii* isolates showed 100% resistance to imipenem, followed by cephotoxime (89%), amikacin (80%) and ciprofloxacin (73%).⁷

Therefore, the aim of the present study was to detect gelatinase production, biofilm formation and antimicrobial susceptibility of *Acinetobacter* species isolated from various clinical specimens.

Material and Methods

Clinical samples were collected from patients admitted in BSMMU and from patients admitted in the Burn unit of Dhaka Medical College Hospital. Samples included were tracheal aspirate, blood from central venous catheter (CVC), peripheral blood, urine from catheterized patients, pus, ascitic fluid, cerebrospinal fluid, sputum, catheter tip, and wound throat and aural swabs. Laboratory works were performed in the department of Microbiology & Immunology, BSMMU, Dhaka between January 2010 to December 2010.

Microbiological methods

a. Identification of *Acinetobacter*: Suspected colonies of *Acinetobacter* species were identified by colony

morphology, 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

b. Gelatinase activity: Gelatinase production was detected by inoculating *Acinetobacter* isolates on the Luria Bertani agar media containing gelatin (30 g/l). After inoculation the plates were incubated overnight at 37°C and then cooled for 5 hours at 4°C. The appearance of a turbid halo around the colonies was considered positive for gelatinase production.⁵ *Serratia* spp. was used as positive control and *E. coli* was used as negative control in this test.

c. Biofilm assay: Qualitative production of biofilm by *Acinetobacter* was assayed by tissue culture plate method as described by Toledo *et al.*, 2001.⁹ Biofilm formations is considered positive when a visible film lines the wall and bottom of the tube. In this method biofilm formation is detected by measuring optical density with ELISA reader.¹⁰ Isolates were grown overnight at 37°C in Brain Heart Infusion Broth (BHIB) with 0.25 % glucose. The culture was diluted at a ratio of 1:20 in fresh BHIB with 0.25 % glucose. 200 µl of this suspension was inoculated in a sterile 96 well flat bottomed polystyrene microtiter plate. Then the plate was incubated at 37°C for 24 hours. Wells were washed with phosphate buffered solution (PBS) three times. Non-adherent cells were removed by washing with PBS. Then the microtiter plate was dried in an inverted position. After drying, the plate was stained with 0.5% Crystal violet (CV) for 15 minutes at room temperature. CV allowed visualization of the attachment pattern of bacteria in microtiter plate. Wells were rinsed once more. Then 200 µl of ethanol/acetone (80:20, v/v) was added in each well to solubilize CV. The optical density (OD) at 550 nm was determined using a microtiter reader. Each assay was performed in triplicate and repeated twice. *Staphylococcus. epidermidis* was taken as a positive control and *Salmonella typhi* was taken as a negative control. The cut off value (ODc) was established and was defined as mean OD + 3xSD of the negative controls. Final OD value of a tested strain was expressed as mean OD value of the triplicate assays. Any OD above the cut off value was indicative of biofilm production.

Table-1: Biofilm production of isolated *Acinetobacter* species from different clinical samples

Samples	Total Number	Positive for <i>Acinetobacter</i> Sp N (%)	Positive for biofilm production N(%)
Tracheal aspirate	35	19 (54.3)	16 (84.2)
Blood CVC	11	4 (36.4)	4 (100.0)
Peripheral blood	26	5 (19.2)	5 (100.0)
Urine	33	7 (21.2)	6 (85.7)
Wound swab	32	8 (25.0)	4 (50.0)
Pus	36	5 (13.9)	2 (40.0)
Endotracheal tube	3	3 (100.0)	2 (66.7)
Throat swab	9	1 (11.1)	0 (0.0)
Others	71	0 (0.0)	0 (0.0)
Total	256	52(20.3)	39(75.0)

Figure within parentheses indicate percentage

*Others: ascitic fluid, sputum, aural swab, oral swab, burn samples, cerebrospinal fluid and catheter tip

d. Antimicrobial susceptibility tests: All the isolated *Acinetobacter* species were tested for antimicrobial susceptibility testing by disc diffusion method using the Kirby-Bauer technique¹¹ and as per recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).¹² Amoxicillin (10µg), ciprofloxacin (5 µg), gentamicin (10µg), ceftriaxone (30µg), ceftazidime (30µg), cefuroxime (30µg), cefotaxime (30µg), amikacin (30µg), aztreonam (30µg), imipenem (10µg), netilmicin (30µg) and colistin (10µg) discs were used.

Results

In the present study, *Acinetobacter* species were predominantly isolated from tracheal aspirate (54.3%) followed by CVC blood (36.4%), peripheral blood (19.2%), urine (21.2%), wound swab (25.0%), pus, 5 (13.9%). 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. All the 52 *Acinetobacter* isolates were negative for gelatinase production but 39 (75.0%) *Acinetobacter* isolates showed biofilm production (Table-1). The rate of biofilm production by isolated *Acinetobacter* from different clinical samples is shown in Table-1. All the *Acinetobacter* isolates (100%) were resistant to ceftazidime, cefotaxime, cefuroxime and ceftriaxone. Higher level of resistance was also recorded for amoxicillin (98.1%), aztreonam (98.1%), gentamicin (90.4%), ciprofloxacin (73.1%), amikacin (57.6%), netilmicin (53.8%) and imipenem (44.2%). Only 3.8% of isolated *Acinetobacter* were resistant to colistin.

Discussion

Acinetobacter, once considered to be a low virulent pathogen, is currently a major problem in hospital especially in ICU. Their emergence as significant pathogen seems to be related to their survival ability and to their ability to develop resistance rapidly to the major groups of antibiotics.

In this current study, out of 52 *Acinetobacter* isolates, none produced gelatinase while 75% was positive for biofilm production. Sechi *et al* found no gelatinase activity and biofilm formation by 80% *Acinetobacter* isolates.¹³ Cevahir *et al* detected gelatinase activity in 14.0% and biofilm formation in 74.4% *Acinetobacter*

isolates.¹⁴ *Acinetobacter* isolated from CVC blood, peripheral blood and tracheal aspirates showed higher biofilm production (84-100%). The presence of indwelling medical devices increases the risk for biofilm formation and subsequent infection especially in the ICU.⁷ Biofilm production in *Acinetobacter species* might promote increased colonization and persistence leading to higher rate of device related infections.

This study showed that 100% of the isolated *Acinetobacter* species were resistant to ceftazidime, cefotaxime, cefuroxime and ceftriaxone. High level of resistance was recorded for amoxicillin, aztreonam, gentamicin, ciprofloxacin, amikacin and netilmicin (53.8%). However, low resistance to imipenem (44.2%) and colistin (3.8%) was observed. Lone *et al*¹⁵ reported high level of resistance of *Acinetobacter* isolates to gentamicin (61.5%), cefotaxime (65.8%), ceftriaxone (61.5%) and ciprofloxacin (69.2%). Amikacin, cefoperazone+sulbactam and imipenem showed maximum susceptibility with an overall resistance rate of 17% 17%, 11.5%, and 1.5% respectively. Most of the *Acinetobacter* species were multidrug resistant. Multidrug resistant isolates are increasing day by day.

A greater understanding of the nature of biofilm production by *Acinetobacter spp*, their role in pathogenicity and in serious infections will help in development of more effective treatment for *Acinetobacter* infections.

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