Original Article

Prevalence and Antibiotic Resistance Patterns of *Acinetobacter baumannii* Isolated from Patients at Dhaka Medical College Hospital

Rizwana Sharmin[1], SM Shamsuzzaman[2], M Faruk Hossain[3], Tasnim Sanwar[4], Nargis Akter[5], Dilruba Sharmin[6], Nazmun Nahar[7], Farhana Shamiha Joarder[8], Shahina Parvin[9]

Abstract:

Background: *Acinetobacter baumannii* causes serious nosocomial infections, and is associated with high morbidity and mortality. **Objective:** The objective of this study was to determine the prevalence of *Acinetobacter baumanii* infection in patients at Dhaka Medical College Hospital and to estimate antimicrobial resistance pattern among the *Acinetobacter baumannii* isolates. **Method:** During the study period from January 2022 to December 2022, 400 clinical samples, including endotracheal aspirates, wound swab and pus, blood, sputum, urine were collected and analyzed from patients admitted and visiting outpatient department of various ward in Dhaka Medical College Hospital, Dhaka. **Result:** Out of 400 clinical samples, 275(68.8%) yielded growth. Among them 50 were *Acinetobacter spp*. Thirty-nine samples were confirmed as *Acinetobacter baumannii* through PCR by detection of *bla*OXA -51 like gene, and the prevalence of *Acinetobacter baumannii* was 14.2%. The highest (89.7%) number of *Acinetobacter baumannii* samples showed resistance to amoxiclav, ceftazidime and ciprofloxacin and least to colistin (30.8%). **Conclusion:** The results of this study could help our clinicians create a local antibiogram in the fight against *Acinetobacter baumannii* infections.

Keywords: *Acinetobacter baumannii*; Multi-drug-resistance (MDR); Antibiotic-resistance (ABR), prevalence.

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1. Dr Rizwana Sharmin
   Virologist, Institute of Public Health (IPH)
   Mohakhali, Dhaka
2. Prof. Dr SM Shamsuzzaman
   Former Head, Department of Microbiology
   Dhaka Medical College, Dhaka
3. Prof. Dr Md. Faruk Hossain
   Professor and Head, Department of Psychiatry
   Enam Medical College, Savar, Dhaka
4. Dr Tasnim Sanwar
   Chief Quality Control Officer, Institute of Public Health, Mohakhali, Dhaka
5. Dr Nargis Akter
   Lecturer, Department of Pharmacology & Therapeutics, Comilla Medical College, Cumilla
6. Dr Dilruba Sharmin
   Medical Officer, Daudkandi Upazilla Health Complex, Cumilla
7. Dr Nazmun Nahar
   Clinical Pathologist, Shaheed Suhrawardy Medical College Hospital, Dhaka
8. Dr Farhana Shamiha Joarder
   Lecturer, Department of Pharmacology & Therapeutics, Shaheed Monsur Ali Medical College and Hospital, Uttara, Dhaka
9. Dr Shahina Parvin
   Lecturer, Department of General & Dental Pharmacology, Dhaka Dental College, Dhaka

Address of correspondence:
Dr Rizwana Sharmin
Virologist, Institute of Public Health (IPH)
Mohakhali, Dhaka, Mob no: 01912531727
e-mail: rsharmin43@gmail.com;
ORCID: https://orcid.org/0009-0001-8672-9390

Introduction:

Acinetobacter species are important opportunistic bacterial pathogens that cause serious nosocomial infections worldwide with high morbidity and mortality.[1] This organism is particularly dangerous because its capacity to stick to surfaces, create biofilms and acquire genetic components from unrelated species. *Acinetobacter baumannii* is a widely distributed strictly aerobic, non-fermentative, non-fastidious, catalase positive Gram-negative coccobacillus that is found in both natural and clinical environments.[2] It is also one of the six most significant pathogens of the ESKAPE group, that includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*.[3] Infectious Diseases Society of America recognize it as an alarming global public health threat for its antimicrobial resistance (AMR).[4] Patients with immune deficiencies, the elderly, premature newborns, those undergoing major surgery or trauma, or who had previously been admitted to contaminated critical care units, long-term intubation and tracheal or lung aspiration are at risk.[5] *Acinetobacter baumannii* causes

septicemia, endocarditis, meningitis, skin and soft tissue infections, wound and burn infections, respiratory tract infections and urinary tract infections.[6] Mortality rates from *Acinetobacter baumannii* infections have risen by 30% to 75% in several parts of the world over the past decade.[7] Because it is resistant to many potent antibiotics, such as carbapenems and third-generation cephalosporins, multidrug-resistant *Acinetobacter baumannii* is considered one of the most dangerous microorganisms by the World Health Organization.[8] Though colistin is still one of the few agents effective against multidrug- resistant *Acinetobacter baumannii*, the breakdown of lipopolysaccharide (LPS) and the appearance of lipid A target alterations have reduced its effectiveness.[9]

Method:

Study Setting and Population: It was a cross-sectional study conducted at the Department of Microbiology, Dhaka Medical College, Dhaka from January 2022 to December 2022. The study population was all the patients admitted to different wards, units or visiting OPD of DMCH.

Sample collection procedure: After obtaining written consent from the patients (regardless of age, gender, intake of antibiotics), clinical samples (wound swab/pus, urine, sputum, blood and endotracheal aspirates) were collected for culture and sensitivity testing. Patients who did not provide consent were excluded from the study.

Isolation and Identification of *Acinetobacter baumannii*: Collected samples were processed and the inoculated in blood agar, MacConkey agar madia, incubated aerobically at 37°C for 24 hours. Phenotypic identification of *Acinetobacter spp*. was done by observing colony morphology on blood agar, MacConkey agar and growth at 42°C on agar was also done[10]. Genotypic identification of *Acinetobacter baumannii* was done by *bla*OXA-51 like gene by PCR.[11]

Microscopic examination: Smears were prepared from culture plates and stained with Gram stain according to standard procedures. It is then examined under a microscope to look for the presence of Gram-positive or Gram-negative bacteria.

Biochemical test: Among the isolated Gram-negative cocco-bacilli, *Acinetobacter baumannii* was identify by oxidase test, catalase tests, TSI agar, urease production, indole test, motility and citrate utilization test.[10]

Antimicrobial Susceptibility Testing: The antimicrobial susceptibility test of all *Acinetobacter baumannii* isolates was determined by modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar plates[12]. The inhibition zones were interpreted using the Clinical Laboratory Standard Institute's (CLSI, 2022) guidelines[13]. Criteria of the United States Food and Drug Administration (FDA, 2019) was used for the interpretation of zone of inhibition of tigecycline as such guideline was absent in CLSI guideline.[14] Isolates were categorized as susceptible or resistant to an antibiotic and isolates exhibiting intermediate resistance were categorized as resistant. The antibiotics used were amikacin (30 µg), amoxiclav (30 µg) (amoxicillin 20 µg and clavulanic acid 10 µg), aztreonam (30 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), ceftriaxone (30 µg), colistin (10 µg), gentamicin (10 µg), fosfomycin (200 µg), Piperacillin-tazobactam (100 µg/10 µg), imipenem (10 µg), tigecycline (15 µg).

Amplification and Detection of *bla*OXA-51 Gene: *Acinetobacter baumannii* was confirmed using a traditional PCR. By identifying the *bla*OXA-51-like gene, 39 of the 50 *Acinetobacter species* were identified as *Acinetobacter baumannii*. Using certain primers, the PCR was carried out in culture isolates to detect the *bla*OXA-51-like gene. The boiling method was used to extract DNA from bacterial colonies. A PCR tube was used for the final reaction. After being amplified, the products were electrophoresed in a 1.5% agarose gel.

Statistical analysis: IBM SPSS Statistics for Windows, version 22.0, was used for statistical analysis.

Result:

Among 400 samples 275(68.8%) were culture-positive, of which wound swab & pus were 101(63.1%), urine 81(73.6%), endotracheal aspirates 34(85.0%), blood 39(70.9%) and sputum 20(57.1%) (Figure1). Out of 275 culture-positive samples the predominant bacteria was *Pseudomona aeruginosa* 65(23.6%), followed by Esch. Coli 55(20.0%), *Acinetobacter baumannii* 39(14.2%) and other *Acinetobacter spp*. 11(4%) (Table1). Out of 50 *Acinetobacter spp*., 39(78%) were *Acinetobacter baumannii* confirmed through genotyping identification by the presence of *bla*OXA-51 like gene using PCR and 11(22%) were other species of *Acinetobacter* (Figure 2). Majority 13(92.8%) of *Acinetobacter baumannii* were isolated from endotracheal aspirate (Figure 2). Among the ETA samples containing *Acinetobacter baumannii* 28.20% were from ICU patients.

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All isolates (100%) demonstrated resistance to a minimum of three classes of antibiotics and thus met the MDR criteria. The highest resistance (84.6 to 89.7) was Observed for extended-spectrum cephalosporins. Among *Acinetobacter baumannii* isolates 79.5%, 76.9% and 71.8% were resistant to piperacillin- tazobactum, amikacin and gentamicin. Least resistance was found to colistin (30.8%) and tigecycline (43.6%) (Figure 4).

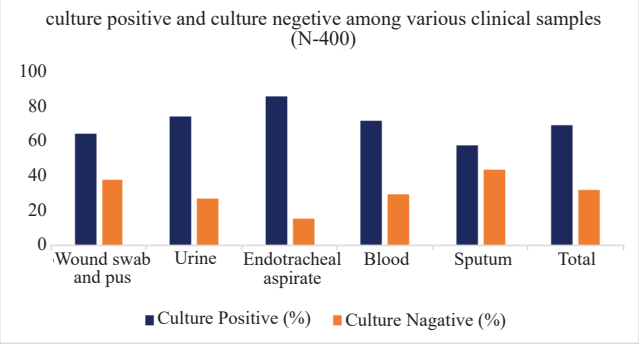

Figure-1: Culture positive among various clinical samples (n=400)

Table-I: Distribution of organisms isolated from different samples (n=275)

Organisms	ETA N=34 n (%)	Blood N=39 n (%)	WS & Pus N=101 n (%)	Urine N=81 n (%)	Sputum N=20 n (%)	Total N=275 N (%)
P. aeruginosa	15 (5.45)	0 (0.00)	33 (11.99)	17(6.18)	0 (0.00)	65 (23.63)
Acinetobacter baumannii	13 (4.72)	9 (3.27)	11 (3.99)	02 (0.72)	4 (1.45)	39 (14.18)
Another Acinetobacterspp	01 (0.36)	3 (1.09)	03 (1.09)	02 (0.72)	2 (0.72)	11 (4.00)
E. coli	0(0.00)	0 (0.00)	10 (3.63)	43 (15.63)	2 (0.72)	55 (20.00)
K. pneumoniae	3(1.09)	2 (0.72)	15 (5.45)	2 (0.72)	9 (3.27)	31 (11.27)
K. oxytoca	0 (0.00)	0 (0.00)	2 (0.72)	0 (0.00)	0 (0.00)	02 (0.72)
S. aureus	01(0.36)	6 (2.18)	17 (6.17)	3 (1.09)	3 (1.09)	30 (10.90)
Salmonella spp	0 (0.00)	19 (6.90)	0 (0.00)	0 (0.00)	0 (0.00)	19 (6.90)
Proteus mirabilis	01(0.36)	0 (0.00)	6 (2.17)	2 (0.72)	0 (0.00)	9 (3.27)
Proteus vulgaris	0 (0.00)	0 (0.00)	1 (0.36)	1 (0.36)	0 (0.00)	2 (0.72)
Enterobacterspp	0 (0.00)	0 (0.00)	2 (0.72)	5 (1.81)	0 (0.00)	7 (2.54)
Citrobacter species	0 (0.00)	0 (0.00)	1 (0.36)	1 (0.00)	0 (0.00)	2 (0.72)
Enterococcus species	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.72)	0 (0.00)	3 (1.09)

ETA = Endotracheal aspirate , WS = wound swab, N = Total number of samples and n = Number of isolates of positive samples.

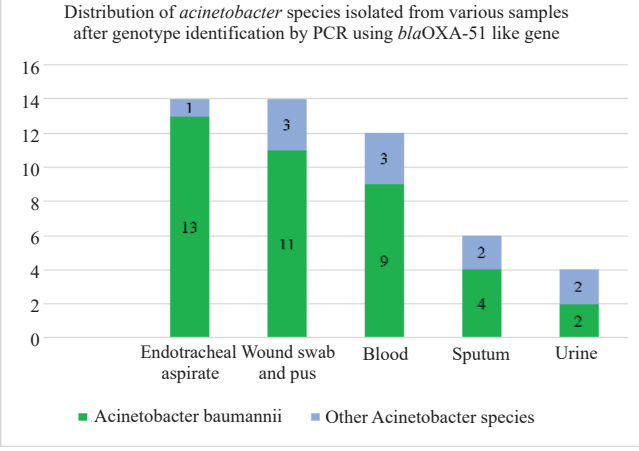

Figure-2: Distribution of *Acinetobacter species* isolated from various samples after genotypic identification by PCR using blaOXA-51-like gene (n=50)

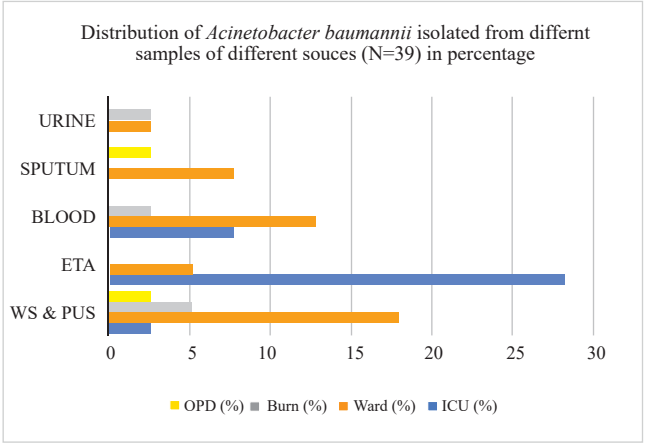

Figure-3: Distribution of *Acinetobacter baumannii* isolates from different samples from different sources (n=39)

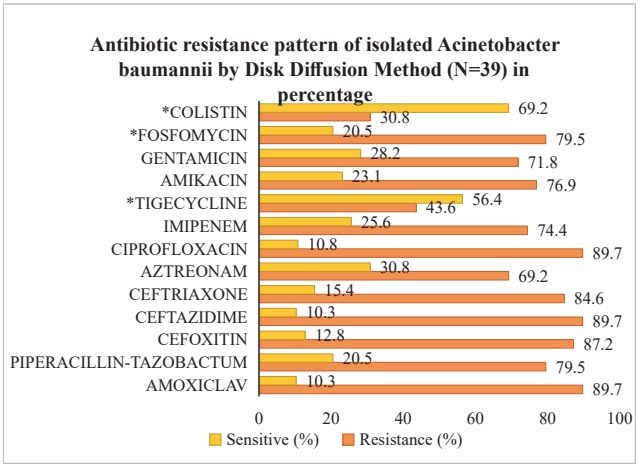

Figure-4: Antibiotic resistance pattern of isolated *Acinetobacter baumannii* (n=39) by disc diffusion method

Discussion:

Acinetobacter baumannii has become a serious bacterial infection acquired in healthcare facilities over the past few decades. Infections of the skin and soft tissues notably in burn units, urinary tract infections, post-surgical endocarditis and meningitis are all linked to *Acinetobacter baumannii*.[15] It resists many classes of antibiotics by virtue of chromosome-mediated genetic elements and also by its ability to persist for a prolonged period in harsh environments (walls, surfaces and medical devices) in the hospital settings.[16] The World Health Organization (WHO) and Center for Disease Control and Prevention (CDC) classified *Acinetobacter baumannii* infection as an urgent threat as it causes over 8,500 hospitalized cases and an estimated 700 deaths each year.[17]

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In this study out of 275 culture-positive samples 39 were confirmed as *Acinetobacter baumannii* through the presence of *bla*OXA-51-like gene (intrinsic oxacillinase), giving the prevalence of *Acinetobacter baumannii* in DMCH as 14.2%. Majority of the isolates (92.8%) were obtained from endotracheal aspirates, of which 28.2% were from ICU patients. A study conducted by Uddin et al.[18] in the same department in 2017 found the prevalence of *Acinetobacter baumannii* as 14.66%. In that study majority of *Acinetobacter baumannii* was isolated from tracheal aspirate which was 75% and highest (75%) was collected from ICU. Different study showed prevalence rate of *Acinetobacter baumannii* in Asian counties - Pakistan 15.17%, Nepal 12.7%.[19,20] According to Antimicrobial Resistance Surveillance in Bangladesh (2016-2020) *Acinetobacter* was the second most common organism identified in the endotracheal aspirate (26%).[21] The reason behind the higher isolation rate of *Acinetobacter baumannii* might be due to collection of majorities of the samples from ICU patients. Resistance to 13 clinical antibiotics was assessed for the 39 *Acinetobacter baumannii* isolates. The drug sensitivity results from this study demonstrated that *Acinetobacter baumannii* isolates in DMCH exhibited high resistance rates to specific drugs and multidrug resistance, warranting serious attention. The highest resistance rate was 89.7% to amoxiclav, ceftazidime and ciprofloxacin, followed by 87.2% to cefoxitin, 84.6% to ceftriaxone, 79.5% to fosfomycin and pipercillin-tazobactum, 74.4% to imipenem, complicating the clinical antibiotic treatment. Fortunately, *Acinetobacter baumannii* demonstrated sensitivity to colistin with the rate of 68.2%. According to Uddin et al[18] 85.71% of *Acinetobacter baumannii* isolates in DMCH were resistant to imipenem, 92.86% to ceftriaxone, cefotaxime, cefepime, amoxiclav, amikacin, and gentamicin, 96.43% to ceftazidime and ciprofloxacin, 14.28% to tigecycline and 7.14% to colistin.

Limitations of the study:

The study was conducted in a single center and the sample size was relatively small.

Recommendations:

Multicenter studies with larger sample sizes should be conducted to obtain a more reliable and authentic picture.

Conclusion:

This study analyzed the prevalence and resistance patterns of *Acinetobacter baumannii* in a tertiary care hospital in Dhaka City. Despite widespread resistance to most of the antibiotics tested, *Acinetobacter baumannii* showed relative sensitivity to colistin. Given the findings of the study, it can be assumed that targeted surveillance and improved infection control measures may help contain *Acinetobacter baumannii* infections. Moreover, the incorporation of sensitivity data into antibiotic prescribing practices can promote effective antibiotic stewardship.

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