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Phenotypic and Genotypic Identification of *Acinetobacter baumannii* with their Antibiotic Sensitivity Pattern Isolated from Different Specimens at a Tertiary Care Hospital in Dhaka City of Bangladesh

Rizwana Sharmin[1], SM Shamsuzzaman[2], Md Faruk Hossain[3], Muhammed Imran Uddin[4], Farmin Sultana[5], Sanzida Rahman[6]

[1]Virologist, Institute of Public Health, Dhaka, Bangladesh; [2]Former Head & Professor of Microbiology, Dhaka Medical College, Dhaka, Bangladesh; [3]Associate Professor and Head, Department of Psychiatry, Enam Medical College, Savar, Dhaka, Bangladesh; [4]Bacteriologist, Institute of Public Health, Dhaka, Bangladesh; [5]Clinical Pathologist, Kurmitola General Hospital, Dhaka Cantonment, Dhaka, Bangladesh; [6]Junior Food Chemist, Institute of Public Health Nutrition, Dhaka, Bangladesh

Abstract

Background: *Acinetobacter baumannii* is an important opportunistic pathogen that causes serious nosocomial infections. Its ability to adhere to surfaces, forming biofilms, acquiring genetic materials from unrelated genera is making it a dangerous organism causing high morbidity and mortality. **Objective:** The goal of this study was to isolate and identify *Acinetobacter baumannii* from various samples and to identify the pattern of antibiotic resistance. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh over the course of January 2022 to December 2022. Collected samples were processed and inoculated in media, incubated aerobically at 37°C for 24 hours. Phenotypic identification of *Acinetobacter* species was done by observing colony morphology on media and growth at 42°C on agar was also done. Genotypic identification of *Acinetobacter baumannii* was done by blaOXA-51-like gene by PCR. The antimicrobial susceptibility test of all *Acinetobacter baumannii* isolates was determined by modified Kirby-Bauer disc diffusion technique using Mueller-Hinton agar plates. **Results:** Out of 275 culture positive clinical samples 14.2% isolates were *Acinetobacter baumannii*. Among the 39 *Acinetobacter baumannii* isolates 53.9% were multidrug-resistant, 33.3% were extended drug-resistant and 12.8% were pan drug-resistant. The highest (89.7%) number of *Acinetobacter baumannii* showed resistance to amoxiclav, ceftazidime and ciprofloxacin and least to colistin (30.8%). **Conclusion:** In conclusion *Acinetobacter baumannii* has been isolated frequently form the clinical specimens, and mostly are multi-drug resistant.

Keywords: *Acinetobacter baumannii*; antibiotic-resistant (ABR); multidrug resistance (MDR)

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Introduction

Acinetobacter species are non-fermentative, non-fastidious, catalase positive, oxidase negative, non-motile and strictly aerobic gram-negative coccobacilli[1]. Among the *Acinetobacter species*, *Acinetobacter baumannii* is the most important member associated with hospital-acquired infections worldwide[2]. *Acinetobacter baumannii* causes serious infections associated with high morbidity and mortality[3]. Infection with *Acinetobacter baumannii* can lead to death if left untreated or treated ineffectively particularly if the strain is antibiotic-resistant[4]. Patients with immune deficiencies, the elderly, premature newborns, who have undergone major surgery or trauma or who have previously been admitted to contaminated critical care units are at risk[5].

The global rise of multidrug-resistant (MDR) *Acinetobacter baumannii* has been attributed to the

Correspondence: Dr. Rizwana Sharmin, Virologist, Institute of Public Health (IPH), Mohakhali, Dhaka-1212, Bangladesh; Email: rsharmin43@gmail.com; Cell No.: +8801912-531727; ORCID: https://orcid.org/0009-0001-8672-9390

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indiscriminate use of antibiotics over the past few decades[6]. According to the Antimicrobial Resistance Surveillance in Bangladesh (2016-2020), *Acinetobacter* species displayed less than 50.0% sensitivity to all of the used antibiotics[7]. Currently there are fewer antibiotics available for the treatment of *Acinetobacter baumannii* infections due to its numerous resistance mechanisms and ability to acquire resistance genes from other gram-negative bacteria[2]. The goal of this study was to isolate and identify *Acinetobacter baumannii* from various samples and to identify the pattern of antibiotic resistance.

Methodology

Study Settings and Population: This cross-sectional study was conducted at the Department of Microbiology, Dhaka Medical College, Dhaka from January 2022 to December 2022 for a period of one year. The study population was all the patients admitted to different wards, burn unit, ICU unit or visiting OPD of Dhaka Medical College Hospital (DMCH).

Sample Collection Procedure: After obtaining written consent from the patient regardless of age, gender, intake of antibiotics, clinical samples like wound swab and pus, urine, sputum, blood and endotracheal aspirates were collected from patients admitted to different wards, burn unit, ICU unit or visiting OPD of DMCH for culture and sensitivity testing. Patient who did not provide consent were excluded from this study.

Isolation and Identification of *Acinetobacter baumannii*: Collected samples were processed and the inoculated in blood agar, MacConkey agar media, incubated aerobic at 37ºC for 24 hours. Phenotypic identification of *Acinetobacter* species was done by observing colony morphology on blood agar, MacConkey agar and growth at 42ºC on agar was also done[8]. Genotypic identification of *Acinetobacter baumannii* was done by blaOXA-51 like gene by PCR[9]. 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. 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[8].

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[10]. The inhibition zones were interpreted using the Clinical Laboratory Standard Institute's guidelines[11]. Criteria of the United States Food and Drug Administration[12] was used for the interpretation of zone of inhibition of tigecycline as such guideline was absent in CLSI guideline. 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 blaOXA-51 Gene: Conventional polymerase chain reaction (PCR) was done for confirmation of *Acinetobacter baumanni*. Among 50 *Acinetobacter* species. About 39 were confirmed as *Acinetobacter baumannii* by identification of the blaOXA-51-like gene. The PCR was performed in culture isolates using specific primers for detection of blaOXA-51-like gene (Applied biosystems, Thermofisher scientific, USA). DNA was extracted from bacterial colonies by boiling method (Incublock, Denville scientific inc. USA). PCR was performed in a final reaction in a PCR tube (applied biosystems, Thermofisher scientific, USA). The amplified products were subjected to electrophoresis in 1.5% agarose gel.

Statistical Analysis: Statistical analysis was done with SPSS, version 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were summarized as the mean, standard deviation, median, minimum, maximum and number of observations. Categorical or discrete data were summarized in terms of frequency and percentage. In case of missing values, the denominator was stated. Chi-square test was used to compare categorical variables. Every effort was made to collect missing data. A two-sided P value of less than 0.05 was considered statistically significant.

Ethical Approval: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethical approval was granted by the Research Review Committee (RRC) of Dhaka

Medical College, Dhaka, Bangladesh (Reference number: ERC-DMC/ECC/2022/92). Participants in the study were informed about the procedure and purpose of the study and confidentiality of information was maintained. All participants consented willingly to participate in the study during the data collection period. All data were collected anonymously and analyzed using the coding system.

Results

Among 400 samples 275(68.7%) isolates were culture positive and of the positive 50 were *Acinetobacter* species (Table 1 and Table 2).

Table 1: Culture Positive Among Various Clinical Samples (n=400).

Specimens	Culture		Total
	Positive	Negative	
Wound swab and pus	101(63.1%)	59(36.9%)	160(100.0%)
Urine	81(73.6%)	29(26.4%)	110(100.0%)
Endotracheal aspirate	34(85.0%)	6(15.0%)	40(100.0%)
Blood	39(70.9%)	16(29.1%)	55(100.0%)
Sputum	20(57.1%)	15(42.9%)	35(100.0%)
Total	**275(68.7%)**	**125(31.3%)**	**400(100.0%)**

Among them 39(78.0%) were *Acinetobacter baumannii* which was confirmed by the presence of blaOXA-51 like gene using the PCR and 11(22.0%) were other species of *Acinetobacter*. The prevalence of *Acinetobacter baumannii* was 14.2% among the isolates. Majority (92.9%) of *Acinetobacter baumannii* positive samples were taken from endotracheal aspirate followed by 78.6% from wound swab & pus, 75.0% from blood samples, 66.7% from sputum and 50% from urine (Table 2).

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

Samples	Isolated *Acinetobacter* species	*Acinetobacter baumannii*	Other *Acinetobacter* species
Endotracheal aspirate	14	13 (92.9%)	1 (7.1%)
Wound swab and pus	14	11 (78.6%)	3 (21.4%)
Blood	12	9 (75.0%)	3 (25.0%)
Sputum	6	4 (66.7%)	2 (33.3%)
Urine	4	2 (50.0%)	2 (50.0%)
Total	**50**	**39 (78.0%)**	**11 (22.0%)**

Among the ETA samples 11 (28.2%) were collected from ICU (Table 3).

The antibiotic resistance patterns against amoxiclav (AMC), amikacin (AK), aztreonam (AZ), ceftriaxone (CTR), ceftazidime (CAZ), cefoxitin (CX), ciprofloxacin (CIP), colistin (CL), gentamicin (CN), imipenem (IMP), piperacillin-tazobactam (PIT), tigecycline (TGC), fosfomycin (FOS) were recorded (Table 4).

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

Samples	ICU	Ward	Burn	OPD
WS & Pus	1 (2.6%)	7 (17.9%)	2 (5.1%)	1 (2.6%)
ETA	11 (28.2%)	2 (5.1%)	0 (0.0%)	0 (0.0%)
Blood	3 (7.7%)	5 (12.8%)	1 (2.6%)	0 (0.0%)
Sputum	0 (0.00%)	3 (7.7%)	0 (0.0%)	1 (2.6%)
Urine	0 (0.00%)	1 (2.6%)	1 (2.6%)	0 (0.0%)
Total	**15 (38.5%)**	**18 (46.1%)**	**4 (10.3%)**	**2 (5.2%)**

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

Antimicrobial drugs	Resistance
Amoxiclav	35 (89.7%)
Piperacillin-Tazobactum	31 (79.5%)
Cefoxitin	34 (87.2%)
Ceftazidime	35 (89.7%)
Ceftriaxone	33 (84.6%)
Aztreonam	27 (69.2%)
Ciprofloxacin	35 (89.7%)
Imipenem	29 (74.3%)
*Tigecycline	17 (43.6%)
Amikacin	30 (76.9%)
Gentamicin	28 (71.8%)
*Fosfomycin	31 (79.5%)
*Colistin	12 (30.8%)

Colistin = Antimicrobial susceptibility test for colistin was determined by agar dilution method.; Fosfomycin = Antimicrobial susceptibility test for fosfomycin was determined by agar dilution method.; Tigecycline = Antimicrobial susceptibility test for tigecycline was determined by agar dilution method.

Acinetobacter baumannii isolates were more resistant to AMC, CTR, CAZ, CIP, FOX, FOS, IPM and PIT, and more sensitive to TGC and CL. Fifty-three-point nine percent of *Acinetobacter baumanni* were multidrug-resistant (MDR), 33.3% extensively drug-resistant (XDR) and 12.8% pan drug-resistant (PDR). All isolates (100.0%) demonstrated resistance to a minimum of three classes of antibiotics and thus met the MDR criteria (Table 5).

Table 5: Types of antibiotic resistance patterns among the isolated *Acinetobacter baumannii* (n =39)

Types of resistance	Frequency	Percentage
MDR	21	53.9%
XDR	13	33.3%
PDR	5	12.8%
Total	**39**	**100.0%**

Discussion

Acinetobacter baumannii is one of the "ESKAPE" organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter*) recognized by the Infectious Diseases Society of America as an alarming threat to global public health because of their antimicrobial resistance[13-14]. 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[15].

In the study out of 275 culture-positive samples 39 were confirmed as *Acinetobacter baumannii* through the presence of blaOXA-51-like gene (intrinsic oxacillinase), giving the prevalence of 14.2%. A study in Pakistan showed prevalence of *Acinetobacter baumannii* 15.2%[16] & another study conducted in Nepal reported 12.7%[17]. In this study 92.9% of isolates were obtained from endotracheal aspirates of which 28.2% were from ICU patients. According to Antimicrobial Resistance Surveillance in Bangladesh (2016-2020) *Acinetobacter* was the second most common organism identified in the endotracheal aspirate (26%)[7]. The reason behind the higher isolation rate of *Acinetobacter baumannii* might be due to collection of majorities of the samples from ICU patients.

In this study, the resistance of *Acinetobacter baumannii* isolates was found as follows: 89.7% were resistant to amoxiclav, ceftazidime and ciprofloxacin, 87.2% to cefoxitin, 84.6% to ceftriaxone, 79.5% to fosfomycin and pipercillin-tazobactum, 74.3% to imipenem, 43.6% to tigecycline and 30.8% to colistin. A study done in critical care center (CCC) of Combined Military Hospital (CMH) by Mamun[18] reported the following resistance rates: Ceftazidime (91.3%), ceftriaxone (91.3%), amikacin (52.2%), gentamicin (33.3%), and ciprofloxacin (65.2%). According to Uddin et al19 *Acinetobacter baumannii* was found resistant to imipenem (85.7%), ceftriaxone, gentamicin, amoxiclav, amikacin (92.8%), ceftazidime and ciprofloxacin (96.4%), tigecycline (14.2%) and to colistin (7.1%). Highest resistance (84.6 to 89.7) was found to extended spectrum of cephalosporins in this study. We also observed increased resistance to colistin, that is 30.8%. This study demonstrated that 53.9% of *Acinetobacter baumannii* were multidrug-resistant, 33.3 % extensively drug-resistant and 12.8% pan drug-resistant.

Conclusion

Acinetobacter baumannii infection poses a major public health risk due to its rising prevalence, increasing rates of antibiotic resistance and lack of any effective treatment options. In the present study 14.2% of the culture positive samples were *Acinetobacter baumannii* and they were resistant to most of the commonly used antibiotics. Increasing resistance to the last-resort antibiotics like colistin (30.8%) was also observed in this study which is alarming.

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Conflict of Interest
The authors have no conflicts of interest to disclose.

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Authors' contributions
The authors confirm their contribution to the paper as follows: study conception and design: Sharmin R, Shamsuzzaman SM. Data collection: Sharmin R. Analysis and interpretation of results: Sharmin R, Shamsuzzaman SM. Draft manuscript preparation: Hossain MF,Uddin MI, Sultana F, Rahman S. All authors reviewed the results and approved the final version of the manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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ORCID
Rizwana Sharmin: https://orcid.org/0009-0001-8672-9390
SM Shamsuzzaman: https://orcid.org/0000-0003-2886-8840
Md Faruk Hossain: https://orchid.org/0009-0002-2517-6259
Muhammed Imran Uddin: https://orcid.org/0009-0006-1177-9701
Farmin Sultana: https://orcid.org/0009-0007-2214-2711
Sanzida Rahman: https://orchid.org/0009-0000-0582-8948

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