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Isolation and Identification of Multi-Drug Resistant *Acinetobacter baumannii* from a tertiary health care centre of Bangladesh

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Abstract: *Acinetobacter baumannii has emerged over the last decade as a significant opportunistic pathogen and primarily associated with hospital-acquired infections. Increasing multidrug resistance pattern of A. baumannii makes it among the most difficult antimicrobial-resistant Gram-negative bacilli to control and treat. The aim of this study was to isolate and identify A. baumannii from clinical samples and to determine their antimicrobial resistance pattern to commonly prescribed drugs to find out Multi-Drug Resistant A. baumannii (MDRAB). Nine different clinical samples were collected from patients admitted to Comilla Medical College Hospital (CoMCH). A. baumannii were isolated and identified based on their growth, physiological, and biochemical characteristics. Their antibiograms were studied through standard disk diffusion method, and antibiotic susceptibility patterns were interpreted. Meropenem, Cephalexin, Ampicillin, Gentamycin, Tetracycline, and Chloramphenicol were used to evaluate the sensitivity of the isolates. Three A. baumannii isolates were recovered from different clinical samples. Though the isolates showed similar growth and physiological characteristics along with similar biochemical profiles, they differ considerably in their sensitivity against several antibiotics. With an exception to tetracycline, A. baumannii W2 found to exhibit remarkable resistance against all the test antibiotics including meropenem. On the other hand, both A. baumannii C2 and A. baumannii C3 showed similar resistance pattern. Both were also MDRAB showing resistance to cephalexin, ampicillin, and tetracycline. The recovery of MDRAB including meropenem-resistant A. baumannii from different clinical specimens, and their antibiotic resistance pattern hint emergence of a formidable pathogen of nosocomial origin. The findings of the study urge revision and up-gradation of current patient maintenance practices in health care providing centres of our country to limit the prevalence of antibiotic resistant A. baumannii.*

Keywords: *Acinetobacter baumannii, Multi-Drug Resistant, Nosocomial Infection*

I. Introduction

Acinetobacter spp. is a group of Gram-negative, non-fermentative, non-motile, and oxidase-negative bacilli. The most clinically significant species of the *Acinetobacter* genus is *A. baumannii* causing over 90% of infections. Infections due to this bacterium are colligated with significant mortality [1]. *A. baumannii* is a commensal bacteria in healthcare facilities and has become one of the most significant microorganisms causing nosocomial infections and hospital outbreaks in last few decades [2]. It is an important infectious agent that is responsible for nosocomial infections among immune-suppressed patients accounting 5% of Gram-negative infections [3]. Its environmental resiliency and abroad range of resistance factors' combinations facilitate to be a successful nosocomial pathogen [4]. A worldwide statistical study on *A. baumannii* remarked significant rise in antimicrobial resistance between 2004 and 2009 [5]. Antimicrobial resistant *A. baumannii* has become a global problem [6]. Some clinicians believe that the recovery of *A. baumannii* from the hospitalized patient bespeaks serious sickness, with an associated mortality of around 30% [7]. *A. baumannii* strains resisting minimum three antibiotic classes are considered as multidrug-resistant A. baumannii (MDRAB) [8]. The main mechanisms for confabbing resistance to different antibiotic classes in *A. baumannii* include multidrug efflux pumps, β-lactamases, permeability defects, aminoglycoside-modifying enzymes, and target sites' alteration [9, 10]. Variety and coexistence of resistance mechanisms in this bacterium consequence in multiple drug resistance and can create troubles in treatment [9, 11-13]. Immunosuppressed patients, patients with severe inherent diseases, and those gone through invasive processes and subjected to broad-spectrum antibiotics are vulnerable to MDRAB infections [14]. Completely working antibiotic choices available to treat hospital-acquired infections with MDRAB are critically limited [15]. The rapid distribute of MDRAB in hospitals has been authenticated in various reports [16, 17].

Also in Bangladesh, *A. baumannii* is a major concern among the doctors, researchers and practitioners. Different researches have been conducted about *A. baumannii* infections and its resistance to different antimicrobials. Recovery of MDRAB isolates from different clinical samples has been reported in several

reports in Bangladesh [18-22]. In a study conducted at the Dhaka Medical College Hospital (DMCH) reported by Khatun [21], 25 *A. baumannii* isolates were recovered from endotracheal aspirate samples of patients admitted to the ICU of DMCH. 96 % of them (24 out of 25) showed multi-drug resistance. In another analysis carried in Square Hospitals Ltd. (SHL) reported by Mannan [22], 210 specimens were collected from the patients with lower respiratory tract infections hospitalized in the ICU of SHL. They reported *A. baumannii* as the most dominant pathogen among recovered isolates and 90 % of the *A. baumannii* strains were MDRAB.
The goal of this investigation was isolation and identification of MDRAB from different types of patients with prolonged hospitalization account in different units of CoMCH. Three *A. baumannii* isolates were recovered from wound swab, blood, urine, and sputum samples; and their resistance pattern to some prescribed antimicrobials had also been ascertained.

II. Materials And Methods

2.1 Sampling

Clinical Samples were collected from CoMCH during August 2016. All the nine samples (3 wound swab, 3 sputum, 2 blood, and 1 urine samples)were collected from patients of different units with long (~3 months) history of hospitalization. Swabs from wounds were collected after removing gauge bandage roll for the dressing of wounds. Then the swab sticks' tips were placed into the tubes containing sterile buffer peptone water. Blood, urine, and sputum samples were also obtained in the sterile buffer peptone water tubes according to their respective collection procedures, aseptically capped, placed in the cooler box, and immediately transferred to the laboratory [23, 24].

2.2 Isolation and identification of *A. baumannii*

One loopful culture from each tube of the enriched buffer peptone water was streaked on MacConkey agar surface. Plates were incubated at 37°C for 24 hours. White color non-lactose fermenter colonies from incubated plates were isolated and purified. Suspected isolates were subjected to catalase and oxidase test as presumptive tests. Catalase positive and oxidase negative presumptive *A. baumannii* were then subjected to physiological and biochemical profiling to confirm their identity.

2.3 Antibiotic susceptibility test

The disk diffusion technique of Kirby-Bauer [25] was employed to assess antibiotic susceptibility patterns of the selected isolates. We selected commercially available standardized antibiotic discs of Meropenem (10 µg), Cephalexin (30 µg), Ampicillin (10 µg), Gentamycin (10 µg), Tetracycline (30 µg), and Chloramphenicol (30 µg). The bacterial suspensions were prepared with nutrient broth from subcultures, and the McFarland standard 0.5 was used for adjusting the turbidity of the suspensions. A cotton swab was dipped into the culture preparation and streaked across the Muller-Hinton agar surface to get uniform inocula. Discs of the test antibiotics were then set on the seeded plates' surface using sterile forceps through the appropriate procedure and kept for 30 minutes at 4°C to diffuse antibiotic on the media surface. Then the plates were incubated for 24 hours at 37°C. The diameter of the clear zones of inhibition were measured after incubation.Values were recorded, and susceptibility status was interpreted from the standard tables of the Clinical and Laboratory Standards Institute (CLSI) [26].

III. Result And Discussion

A. baumannii is an emerging pathogen causing legion worldwide outbreaks [27]. *A. baumannii* is spotted among the most problematic nosocomial pathogenic micro-organisms [6, 28]. Several studies reported recognizing different health care facilities in Bangladesh as breeding grounds of pathogenic *A. baumannii* [21, 22]. Several authors of Bangladesh cited about the recovery of MDRAB strains from clinical samples from Bangladesh [18-22].

In this study, the presence of MDRAB from few patients admitted to different units of CoMCH was investigated. The isolation of bacterial strains from the samples and streaking them on the MacConkey agar surfaces was the first step on isolation. Ten white color non-lactose fermenter colonies were suspected as *A. baumannii*. However, several enteric bacteria like Salmonella *spp.*, Proteus *spp.*, Yersinia *spp.*, Shigella *spp.*, and *Pseudomonas aeruginosa* produce similar colonies on MacConkey agar [29]. For differentiation between the target organism and enteric pathogens with similar growth characteristics on MacConkey, ten suspected isolates (4 from wound swabs, 4 from sputum, 1 from blood, and 1 from urine) were subjected to presumptive catalase and oxidase test. Only three (designated as W2, C2, and C3) of them were presumed as *A. baumanni* based on their hallmark catalase-positive and oxidase-negative reactions. Decisive morphological, cultural and biochemical behaviour of the three isolateswere investigated for identification purpose that involved comparing

and contrasting the recorded characteristics (Table 1) with the standard description provided in Bergey's Manual of Determinative Bacteriology, 8th edition [30].

Table 1: Physiological and Biochemical behaviour of *A. baumannii* isolates

Category	**Test**		**Behavior of the isolates**		
			A. baumannii W2	*A. baumannii* C2	*A. baumannii* C3
Biochemical characteristics	Indole		-	-	-
	Citrate		+	+	+
	H_2S production		-	-	-
	Nitrate reduction		-	-	-
	Oxidase		-	-	-
	Urease		-	-	+
	Catalase		+	+	+
	Starch hydrolysis		-	-	-
	Gelatin hydrolysis		-	-	-
	Esculin hydrolysis		-	-	-
	Acid from:	Arabinose	-	-	-
		Sorbitol	-	-	-
		Xylose	+	+	+
		Mannitol	-	-	-
		Fructose	-	-	-
		Sucrose	-	-	-
		Mannose	-	-	-
		Maltose	-	+	-
Physiological characteristics	Motility		-	-	-
Colony characteristics	White colony on MacConkey Agar		+	+	+
	Fluorescent pigmentation		-	-	-

"+" indicates positive result; "-" indicates negative result

The presence of *A. baumannii* in clinical samples indicates the suboptimal condition of healthcare facilities as it is an opportunistic pathogen [31]. Moreover, it is a grievous sign as detrimental effects have been found in nearly every study evaluating the contribution of *A. baumannii* to final consequences of patients [9]. Most MDRAB outbreaks have been attributed to environmental origins; hence, recovery of *A. baumannii* from our samples suggest the hospital lacks standard operating facilities that allows the growth of the serious pathogen. Significant effort should be devoted, without any delay, to the identification of reservoir, the introduction of rigorous hygienic practices, proper isolation of the infected individuals, prevention of cross contamination and regular surveillance of the pathogens' presence and drug resistance patterns.

We studied the susceptibility of the target isolates against some significant antibiotics. Among three *A. baumannii* isolates, *A. baumannii* W2 exhibited resistance to all the representative antibiotics including Meropenem, except Tetracycline (Figure 1a and Table 2).

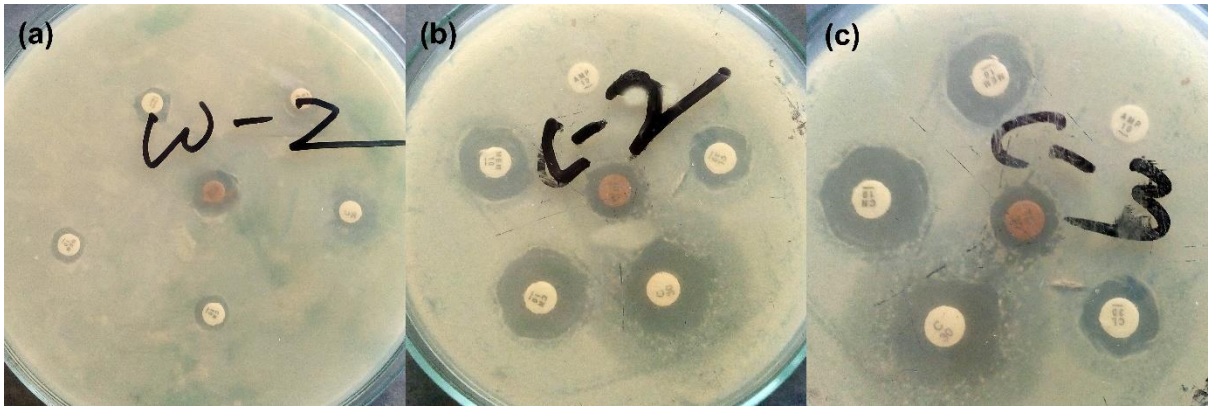

Fig. 1: Antibiotic susceptibility patterns of *A. baumannii* W2 (a), *A. baumannii* C2 (b), and *A. baumannii* C3 (c) against Meropenem (10 µg), Cephalexin (30 µg), Ampicillin (10 µg), Gentamycin (10 µg), Tetracycline (30 µg), and Chloramphenicol (30 µg)

Table 2: Antibiotic sensitivity patterns of the *A. baumannii* isolates against test antibiotics

Antibiotic	Antibiotic Code	Susceptibility								
		A. baumannii W2		*A. baumannii* C2		*A. baumannii* C3		Reference Value (mm)		
		Pattern	*Zone of Inhibition (mm)*	*Pattern*	*Zone of Inhibition (mm)*	*Pattern*	*Zone of Inhibition (mm)*	*R[1]*	*I[2]*	*S[3]*
Meropenem (10 µg)	MEM	R	13	S	16	S	17	≤13	14-15	≥16
Cephalexin (30µg)	CL	R	14	R	13	R	12	≤14	15-17	≥18
Ampicillin (10µg)	AMP	R	13	R	0	R	0	≤13	14-16	≥17
Gentamicin (10 µg)	CN	R	12	S	19	S	20	≤12	13-14	≥15
Tetracycline (30 µg)	TC	S	15	R	10	R	11	≤11	12-14	≥15
Chloramphenicol (30µg)	C	R	11	S	21	S	21	≤12	13-17	≥18

[1]R = Resistant; [2]I = Intermediate; [3]S = Susceptible

A. baumannii W2 was an MDRAB strain showing $MEM^{R}CL^{R}AMP^{R}CN^{R}TC^{S}C^{R}$ antibiotype. On the other hand, *A. baumannii* C2 and *A. baumannii* C3 was found to exhibit almost same antibiogram with the resistance to cephalexin, ampicillin, and tetracycline (Figure 1b, Figure 1c, and Table 2). The both isolates were also designated as MDRAB of $MEM^{S}CL^{R}AMP^{R}CN^{S}TC^{R}C^{S}$ antibiotype.

The resistance to β-lactams and cephalosporins showed by *A. baumannii* has been well documented [8, 15, 32-34]. Yang [8] attributed the resistance to β-lactams as a result including the production of β-lactamases, changes in penicillin-binding proteins that prevent activities of β-lactam drugs, alterations of porin proteins that result in decreased permeability to antibiotics, and the activity of efflux pumps that decreases the concentration of antibiotics within the bacteria. Previous studies have shown that a wide range of extended spectrum beta-lactamases could be found in *A. baumannii* clinical isolates [9]. Resistance to aminoglycosides was cited in several studies [15, 32, 35, 36].Similarly, tetracycline resistant *A. baumannii* is also not a new finding in clinical research [15, 37, 38].

The overall findings of susceptibility assay are significant regarding prevention and control perspective, and onerous. The isolates' resistance to different antibiotics of multiple groups is an appalling finding of this investigation as totally working antibiotic options available to treat nosocomial infections with MDRAB are censoriously limited [15]. Moreover, MDRAB nosocomial infections are associated with a significant mortality percentage [7].

The presence of MDRAB among patients in our sampling healthcare set up further bolster the urgency of revision of hospital management system and implementation of necessary practices to limit the presence, cross-contamination and possible outbreaks of severe *A. baumanii* infections.

IV. Conclusion

These findings alarm the onset of more devastating antimicrobial resistant catastrophe. The study also urges adequate hygienic practices in health care practices to curb prevalence and subsequent antimicrobial resistance of the formidable emerging pathogen.

Declaration of conflict

Kartik Dhar and Tareque Mahmud contributed equally.

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