Section: Microbiology

Original Research Article

PHENOTYPIC DETECTION OF METALLO-β-LACTAMASE PRODUCTION IN *ACINETOBACTER* BAUMANNII ISOLATED FROM VARIOUS CLINICAL SPECIMENS

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ABSTRACT

Background: *Acinetobacter baumannii* has emerged as an important opportunistic Gram-negative bacterium in health care institutions globally, as it resists desiccation, is hard to eradicate and has numerous intrinsic and acquired mechanisms of drug resistance. Among the resistance mechanisms, the ability to produce carbapenemase enzymes such as metallo-beta-lactamases (MBLs) is one of the most important and frequently observed mechanisms exhibited by *A. baumannii*. In addition to penicillins, cephalosporins and monobactams (aztreonam), carbapenamases hydrolyse carbapenems, the drugs with high efficacy and broad spectrum of activity against this organism and this has been a cause of worry both to the clinicians as well as the microbiologists.

Materials and Methods: This study was conducted in the Department of Microbiology at Jagannath Gupta Institute of Medical Sciences & Hospital, Budge Budge, Kolkata. The duration of study was over a period of one year. Of the various samples from the clinical departments processed for culture in the microbiology laboratory, a total of 400 positive culture isolates were selected for further processing (identification, antibiotic sensitivity test) by standard manual methods. The IMP-EDTA combined disk synergy test was performed on *A. baumannii* isolates for detection of MBL production, as per CLSI guidelines.

Results: Of the 400 isolates included in this study, maximum isolates were from the samples of patients in the age group 21-30 years (160) followed by 31–40 years (102), while the lowest number of isolates (6) were found in the age group of 71–80 years. Of the total isolates, 267 were from male patients and the remaining (133) were from female patients. A total of 145 isolates were identified as *Acinetobacter baumannii* (ACB). Samples of pus (62), sputum (20), urine (33), endotracheal aspirate (23), blood (4) and others (3) together accounted for all the ACB isolates. Among the 145 isolates of *A. baumannii*, 50 (34.48%) showed resistance to imipenem and 48 (33.10%) to meropenem, followed by high resistance rates to piperacillin (131; 90.34%), piperacillin/tazobactam (132; 91.03%), ceftazidime (129; 88.96%), cefepime (131; 90.34%), aztreonam (117; 80.68%), gentamicin (118; 81.37%), tobramycin (119; 82.06%), amikacin (107; 73.79%), ciprofloxacin (113; 77.93%), and levofloxacin (113; 77.93%). However, the isolates exhibited 100% sensitivity to tigecycline and polymyxin B. The overall prevalence of metallo-beta-lactamase (MBL) production by *Acinetobacter baumannii* was found to be 80% in this study.

Conclusion: *Acinetobacter baumannii* has a significant role in nosocomial infections especially due to its propensity to develop antibiotic resistance. It invariably shows a high level of antimicrobial resistance, with a substantial proportion of isolates producing metallo-beta-lactamase enzyme (MBL). This study emphasizes the need for early detection of MBL and stringent infection control practices which act as the best defenses against this organism.
Keywords: Metallo-β-lactamase, *Acinetobacter baumannii*, carbapenamases, ESKAPE, combined disk synergy test.

INTRODUCTION

Acinetobacter baumannii is a gram-negative coccobacillus characterized by its pleomorphic aerobic nature, encapsulation, inability to ferment lactose and lack of motility. [1, 2] It is an opportunistic bacterial pathogen primarily associated with hospital-acquired infections (HAIs). It is known to cause a diverse array of infections including skin and soft tissue infections, wound and bloodstream infections, meningitis, urinary tract infections and ventilator-associated pneumonia. Classified among the ESKAPE organisms alongside *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* species, *A. baumannii* is recognized as a significant pathogen responsible for nosocomial infections worldwide. [3, 4, 5] The infections caused by *Acinetobacter baumannii* are particularly dangerous due to the pathogen's ability to resist the action of currently used antimicrobial agents. [3, 6] The treatment of *A. baumannii* infections has recently become increasingly challenging due to the acquisition of resistance to numerous antibiotics, including fluoroquinolones, through both acquired and intrinsic mechanisms. [4] Beta-lactam antibiotics have traditionally been the drug of choice for treating *A. baumannii* infections. [4,7] However, bacterial resistance to beta-lactam antibiotics poses a significant problem globally, affecting both developing and developed countries. The emergence of metallo-β-lactamases (MBLs), such as NDM-1 (New Delhi metallo-β-lactamase), represents one of the latest and most important resistance mechanisms identified in *A. baumannii*. [8, 9] Among the resistance mechanisms, the ability to produce carbapenemase enzymes such as metallo-beta-lactamases (MBLs) (classified as Ambler class B) is also one of the most frequently observed mechanisms exhibited by *A. baumannii*. [3, 10] Four molecular classes of beta-lactamases (A, B, C, and D) have been identified in *A. baumannii*. Class B beta-lactamases, specifically the metallo-beta-lactamases, possess the ability to hydrolyze beta-lactam antibiotics and require zinc at their active site for this function. [11]

Acinetobacter baumannii has become a significant opportunistic Gram-negative bacterium in healthcare facilities worldwide, owing to its ability to withstand desiccation, its resilience to eradication efforts, and its plethora of intrinsic and acquired mechanisms for drug resistance. Of particular concern to clinicians and microbiologists is the production of carbapenemases, enzymes capable of hydrolyzing carbapenems in addition to penicillins, cephalosporins and monobactams (aztreonam). Carbapenems are highly effective drugs with a broad spectrum of activity against *A. baumannii*, making the emergence of carbapenemase production a worrisome development in the battle against this pathogen. [12]

Production of metallo-beta-lactamase (MBL) is commonly associated with resistance to aminoglycosides and fluoroquinolones, further limiting therapeutic options. Among the seven types of MBL genes identified worldwide, bla-IMP and bla-VIM are the most prevalent, with reports of their presence also from India. [13, 14] The genes responsible for MBL production can be either chromosomal or plasmid-mediated, posing a risk of horizontal transfer to other Gram-negative bacteria. [15]

Various authors have described different phenotypic techniques for detecting MBL producers using various substrates; however, no standard guidelines exist, and no single method has been universally effective. Discordant results have been observed depending on factors such as the methodology employed, the beta-lactam substrate and/or MBL inhibitors used, and the bacterial genus tested. [16, 17]

The objective of this study was to detect phenotypically the production of metallo-beta-lactamase (MBL) enzyme in *Acinetobacter baumannii* isolated from various clinical specimens.

MATERIALS AND METHODS

Study area: This study was conducted in the Department of Microbiology at Jagannath Gupta Institute of Medical Sciences & Hospital, Budge Budge, Kolkata.

Study Duration: The duration of study was over a period of one year.

Sample size: The sample size in this study was 400.

Methods: Of the variety of samples received from various clinical departments and processed for culture in the microbiology laboratory, a total of 400 positive culture isolates were selected for further processing (identification, antibiotic sensitivity test) by standard manual methods.

The urine, pus, respiratory and other types of samples (except blood/body fluids) were directly inoculated on Blood agar and MacConkey agar media. The blood and other sterile fluid samples received in the bacteriology laboratory were inoculated into in-house prepared blood culture

bottles. The inoculated bottles were incubated for a maximum period of 5 days followed by subculture on Blood agar and MacConkey agar media and incubated at 37°C. Growth was examined after overnight incubation.

Identification of all isolates was done as per standard methods (colony characteristics, gram staining & by using various biochemicals). *A. baumannii* (ACB) also was presumptively identified from colony characteristics and gram staining. Further confirmation was done on the basis of biochemical tests such as Catalase and IMVIC tests (Indole, MR, VP, citrate, urease & TSI). Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method, as per CLSI guidelines.

The IMP-EDTA combined disk synergy test was performed on *A. baumannii* isolates for detection of MBL production, as described by Yong et al. [18] Overnight cultures of the test isolates (0.5 McFarland opacity standard) were inoculated on to plates with Mueller Hinton agar media, as per CLSI guidelines. Two discs (HiMedia) - imipenem (10µg) and imipenem-EDTA (10µg + 750µg) were placed on the plate. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of aerobic incubation at 37°C. The zone difference between the imipenem and imipenem / EDTA discs of diameter 7 mm or more was interpreted as positive for MBL production. [19]

Data Analysis: Data was analyzed by using Microsoft Excel.

RESULTS

A total of 400 positive culture isolates of samples from various clinical departments, encompassing patients across different age groups ranging from 1 to ≥ 80 years, were included in this study. The age group of 21–30 years had the highest number of isolates (160), followed by the age group of 31–40 years (102), while the lowest number of isolates (6) were found in the age group of 71–80 years (Table 1). No isolates were obtained from patients older than 80 years. Of the total isolates, 267 were from male patients and the remaining (133) were from female patients (Figure 1).

Out of the 400 isolates included in this study, 145 were of *Acinetobacter baumannii* (Table 2). *Acinetobacter baumannii* (ACB) isolates were obtained from pus (62), sputum (20), urine (33), endotracheal aspirate (23), blood (4) and 3 from other samples (Figure 2). It was observed that among ACB isolates, 50 (34.48%) showed resistance to imipenem and 48 (33.10%) to meropenem, followed by high resistance rates to piperacillin (131; 90.34%), piperacillin/tazobactam (132; 91.03%), ceftazidime (129; 88.96%), cefepime (131; 90.34%), aztreonam (117; 80.68%), gentamicin (118; 81.37%), tobramycin (119; 82.06%), amikacin (107; 73.79%), ciprofloxacin (113; 77.93%), and levofloxacin (113; 77.93%). However, the isolates exhibited 100% sensitivity to tigecycline and polymyxin B (Table 3). The overall prevalence of metallo-beta-lactamase (MBL) production by *Acinetobacter baumannii* was found to be 80% in this study (Table 4).

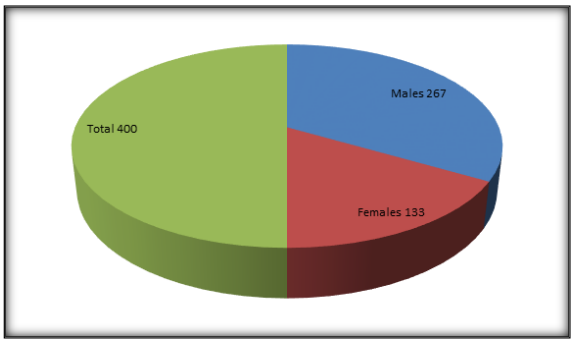

Figure 1: Distribution according to gender

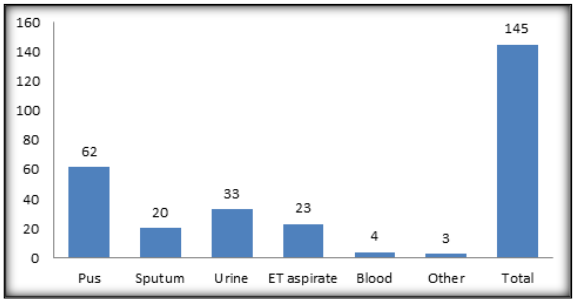

Figure 2: Sample wise distribution of *Acinetobacter baumannii* isolates

Table 1: Distribution according to age group

Age Group (years)	Number of Isolates	Percentage (%)
1-10	08	2%
11-20	29	7.25%
21-30	160	40%
31-40	102	25.5%
41-50	46	11.5%
51-60	38	9.5%
61-70	11	2.75%
71-80	06	1.5%
≥81	00	0%
Total	400	100%

Table 2: Distribution of isolates according to organisms identified

Name of the organism	Number of isolates	Percentage (%)
Others	255	63.75%
Acinetobacter baumannii	145	36.25%
Total	400	100%

Table 3: Results of Antibiotic susceptibility test in ACB isolates

Antimicrobial class	Name of Antibiotic	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)
Carbapenems	Imipenem	87(60%)	08(5.51%)	50(34.48%)
	Meropenem	85(58.62%)	12(8.27%)	48(33.10%)
Penicillin	Piperacillin	14(9.65%)	-	131(90.34%)
β-lactamase inhibitor combination	Piperacillin/Tazobactam	13(8.96%)	-	132(91.03%)
Cephalosporins	Ceftazidime	16(11.03%	-	129(88.96%)
	Cefepime	14(9.65%)	-	131(90.34%)
Monobactam	Aztreonam	28(19.31%)	-	117(80.68%)
Aminoglycosides	Gentamicin	21(14.48%)	06(4.13%)	118(81.37%)
	Tobramicin	26(17.93%)	-	119(82.06%)
	Amikacin	38(26.20%)	-	107(73.79%)
Fluoroquinolones	Ciprofloxacin	32(22.06%)	-	113(77.93%)
	Levofloxacin	32(22.06%)	-	113(77.93%)
Polymyxin	Polymyxin B	145(100%)	-	-
Glycylcyclines	Tigecycline	145(100%)	-	-

Table 4: Prevalence of MBL among the ACB isolates

Acinetobacter baumannii MBL producer	Number of isolates	Percentage (%)
Yes	116	80%
No	29	20%
Total	145	100%

DISCUSSION

Infections affecting the gastrointestinal and urinary tracts, peritonitis, pneumonia, sepsis, meningitis and infections associated with medical devices are commonly attributed to Enterobacteriaceae. These bacteria are recognized as among the most prevalent pathogens responsible for both community-acquired and hospital-acquired infections. [20]

Our study comprised 400 culture positive isolates in total. Based on our observations, the age group between 21 and 30 years had the highest percentage of isolates at 40 % followed by the age group between 31 and 40 years (25.5 %). Jitendra et al (2017) also found that the maximum percentage of infections was in the 21-30 years age group (23.17 %), which is equivalent to the numbers found in this study. [21]

According to Benachinmardi KK et al (2014), samples from patients in the age group of 21 to 50 years produced the majority of NFGNB isolates, while Jayapriya et al (2014) found that the majority of NFGNB isolates were obtained from samples of patients in the 21 to 40 years age group. [22, 23] All these findings are in concordance with the observations of our study. However, according to Kalidas et al (2013), 72% of NFGNB isolates were from samples of individuals over the age of 45. [24]

In this study, 267 (67%) isolates were from male patients and the rest 133 (33%) isolates were from female patients, which is comparable with the study of Jitendra et al (2017) who reported 226 (56.92 %) isolates from male patients and 171 (43.07 %) isolates from female patients. [21] Male predominance was also reported by various other studies including Anirudh et al (2021) (77%), EL-Behery & E M et al (2016) (88.2%) , Maniyan G et al (2016) (65.4%) and Esther J et al (2017) (67%), which is comparable to the findings of this study. [25-28]

This study found that of the 400 isolates, 145 were of *Acinetobacter baumannii* (36.25%) and the rest were isolates identified as other organisms (255, 63.75%). A study conducted by Goel et al (2013) and Kakati B et al (2017) reported *Acinetobacter* species in 48.78% and 63.63% of samples respectively, which is more than the findings of our study.[29, 30]

In this study, *Acinetobacter baumannii* showed maximum resistance to piperacillin / tazobactam (132, 91.03%) followed by piperacillin (131, 90.34%), cefepime (131, 90.34%), ceftazidime (129, 88.96%), tobramicin (119, 82.06%), gentamicin (118, 81.37%), aztreonam (117, 80.68%), ciprofloxacin (113, 77.93%), levofloxacin (113, 77.93%) and amikacin (107, 73.79%), whereas relatively less resistance was observed among the isolates to imipenem (50, 34.48%) and meropenem (48, 33.10%).

A study done by Sumana et al (2017) reported high levels of resistance to ciprofloxacin (78.46%), gentamicin (76%), imipenem (70.46%) cefepime (65.23%), meropenem (63.60%), cefoperazone/sulbactam (59%) and piperacillin/tazobactam (62.15%).[31] However, colistin and tigcycline showed lower resistance and maximum activity with an overall susceptibility of

74.46% and 57.85% respectively. [31] Another study by Kaur et al (2013) also reported high levels of resistance for imipenem (64.7%) followed by lower rates of resistance for tobramycin (24.95%), amikacin (26%), gentamicin (22.83%), ceftazidime (23.05%), ceftizoxime (18.19%) and carbenicillin (21.14%). [32] The resistance pattern of antibiotics as found in our study (except imipenem and meropenem) is comparable to the findings of these studies. However, we found lower rates of resistance for imipenem as well as meropenem (both < 35%) as against the much higher resistance rates for these two antibiotics (> 60%) as found in these studies. [31, 32]

In our study, the prevalence of MBL was found to be 80%. The results of the present study correlate well with the studies by Kaur et al (2013), Agrawal et al (2015) and Massik et al (2018) who reported MBL positivity rates as 80%, 87.5% and 82% respectively.[32, 33, 34] The results obtained in the present study, however, are higher than the findings of Kabbaj et al (2013), Yadav et al (2019) and Parajuli et al (2017) who reported MBL positivity rates as 74%, 67.7% and 50.2% respectively. [35, 36, 37] However, studies by Anwar et al (2016), Szejbach et al (2013), Safari et al (2015) and Guzel et al (2018) reported MBL production in *A. baumannii* isolates to be 95.4%, 94.4%, 99% and 100% respectively, which are higher as compared to the present study. [1, 38, 39, 40]

CONCLUSION

This study highlights the significant role of *Acinetobacter baumannii* in nosocomial infections and its propensity to develop antibiotic resistance. It also underscores a high level of antimicrobial resistance seen in this organism, particularly with a substantial proportion of isolates producing metallo-beta-lactamase enzyme (MBL). The study emphasizes the importance of early detection of MBL and stringent infection control measures which act as the best defenses against such isolates. Additionally, it suggests the implementation of antimicrobial restriction policies in healthcare settings to prevent the injudicious use of antibiotics and thus minimize the emergence of drug-resistant organisms.

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