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PHENOTYPIC DETECTION OF METALLO-BETA LACTAMASES (MBL) IN ACINETOBACTER BAUMANNII ISOLATES IN TERTIARY CARE HOSPITAL

Medical Microbiology

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ABSTRACT

Background: *A.baumannii* is an important emerging human pathogen and is among one of the important pathogens causing hospital acquired infections as well as community infections. The present study was conducted with the aim to know resistance pattern of *A.baumannii* and to detect the production of Metallo beta lactamase enzyme in Carbapenem resistant *A.baumanni*. **Material and Methods:** During six months prospective study, all isolates of *Acinetobacter* obtained from various clinical samples like respiratory, pus, blood and others were included. Antimicrobial susceptibility testing was done by Vitek 2 compact system. MBL detection was done phenotypically by imipenem-EDTA combined disk method. **Results:** Out of 1336 culture positive samples, 146 isolates were of *A.baumannii*. Majority of the isolates were isolated from ET tubes/ ET secretions (45.27%). *A.baumannii* isolates were highly resistant towards various antimicrobials tested including, carbapenems. However isolates of *A.baumannii* showed high level of sensitive towards Colistin (94.2%) and Minocycline (82.2%). Out of total 124 carbapenem resistant isolates of *A.baumannii* MBL production was observed in (86.2%) isolates. **Conclusion:** In the present study high rate of resistance was observed towards most of the antimicrobials tested. The present study also revealed high proportion of MBL producing *Acinetobacter baumannii*. Therefore early detection and infection control practices are the best defenses against these organisms; therefore, systematic surveillance to detect MBL producers is necessary. It is most important to follow antibiotic restriction policies to avoid excessive use of carbapenem and other broad spectrum antibiotics in order to prevent going towards the era with no antibiotics.

KEYWORDS

Acinetobacter baumannii, MBL, Carbapenem, Antimicrobial resistance, imipenem-EDTA combined disk method

INTRODUCTION

Acinetobacter baumannii (A.baumanni) It is a gram negative, polymorphic aerobic, encapsulated, non-lactose fermenting, non-motile cocobacili[1, 2] *A.baumanni* causes wide range of Hospital acquired infections (HAIs) including skin and soft tissue infections, wound and blood stream infections, meningitis, urinary tract infections and ventilator associated pneumonia. *A.baumanni* belongs from the dangerous ESKAPE organisms. [*Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, A. baumannii, Pseudomonas aeruginosa* and *Enterobacter* species]. [3] A.baumanni is the common pathogens responsible for nosocomial infections, worldwide. [4, 5] The infections are particularly dangerous due to the ability of pathogens to resist the action of currently used antimicrobial agents. [3,6]

The treatment of *A.baumanni* infections have recently been becoming challenging due to acquisition of resistance to numerous antibiotics by acquired and intrinsic mechanisms.[4] Beta-lectam and fluoroquinolone antibiotics have been the drug of choice for treatment of A.baumanni infections. [4,7] Bacterial resistance to beta-lactam antibiotics is a major problem in both developing and developed countries. [8] The emergence of the metallo-ß-lactamase (MBL) named NDM-1 (New Delhi metallo-ß-lactamase) is one of the latest and most important resistance mechanisms identified in *A.baumanni*.[9]

The ability to produce carbapenemases enzymes such as metallo-beta Lactamases (MBL) (Amber class B) are most frequent resistant mechanism shown by *A.baumanni*. [3, 10] Four molecular beta-Lactamases classes (A, B, C, and D) have been detected in *A.baumanni*. Class B beta-lactams are the enzymes (Metallo beta-lactamases) that have ability to hydrolyze beta-lactam antibiotics which require zinc at active site. [11]

The early detection and identification of MBL-producing are of crucial importance for prevention of nosocomial. As genetic methods for detection of production of MBL by polymerase chain reaction (PCR) usually give highly accurate and reliable results but due to cost and labor constrain this method is of limited practical use for daily use. [9,12]

CLSI have been recommended various methods for phenotypic detection of carbapenemases which are of low cost, convenient and sensitive procedures. The technique is very easy and economic and can be incorporated into routine testing of microbiology laboratory. Various inhibitinin-based tests such as the double-disk synergy test, combined disk test and the modified Hodge test (MHT) are used. [2,13]

MATERIALS AND METHODS

The Six months prospective study was conducted in the Department of Microbiology at tertiary care institute. Various clinical samples like ET tubes/ ET secretions, respiratory, pus, blood, urine and others were processed according to the standard procedures.

Identification of Isolates:

The urine, pus and respiratory samples were directly inoculated on Blood agar and MacConkey agar media .The blood and other sterile body fluid samples received in the bacteriology laboratory were first inoculated into the Bact /Alert standard aerobic bottles. The inoculated bottles were loaded into the Bact /Alert and were incubated for a maximum period of 5 days.[15] Positive samples were further sub-cultured on Blood agar and MacConkey agar media and incubated at 37°C. Growth was examined after overnight incubation.

A.baumannii was identified from colony characteristic and gram Staining morphology and motility [14]. Further confirmation was done by biochemical tests such as Catalase, IMVIC tests (Indole, MR, VP, and citrate), Urease, TSI, and Oxidase. [15]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by VITEK 2 compact using N281 Card as recommended by clinical and laboratory standards institute (CLSI) guidelines. [16]

MBL detection

The IMP-EDTA combined disk test was performed as described by Yong et al [17] Test organisms was inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two discs (HIMEDIA) - imipenem 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 > 7mm will be interpreted as positive for MBL production. [18]

RESULTS

The total samples processed were 4724 out of which 1336 showed isolates growth. Out of 1336 growth positive samples 146 isolates *A.baumanni* were isolated. Therefore the isolation rate of *A.baumannii* from total processed samples came out to be 3.09% (146/4724) and from total 1336 total samples with growth isolation rate of A.baumannii came out to be 10.92% (146/1336). Form total 146 isolates of A.*baumannii*, 89 (60.95%) were obtained from male patients and 57 (39.04%) were obtained from female patients.

Maximum number 55 (37.67%) of A.*baumannii* were obtained from age group of 41-60years followed by age group of 21-40 years; 61-80 years; 0-20 years and minimum isolates were obtained from age group >80 years as shown in Table 1.

Table 1: Age group wise distribution of A.*baumannii* isolates

Age group in years	A.baumannii isolates (N=146)
0-20	16 (10.9%)
21-40	35(23.9%)
41-60	55 (37.8%)
61-80	35(23.9%)
>80	5(3.5%)

Out of total 146 isolates of *A.baumannii* 105 (70.9%) isolates were obtained from ICU patients, Surgery 18 (12.1%), medicine 5(3.4%), emergency 12 (8.2%) gynecology 7(4.8%) and ENT 1 (0.6%) respectively as shown in Table 2..

Table 2: Department wise distribution of A.*baumannii* isolates

Department	Number of isolates (N=148)
ICU's	105 (70.90%)
Surgery	18(12.10%)
Emergency	12(8.20%)
Gynecology	7(4.80%)
Medicine	5 (3.40%)
ENT	1(0.60%)

In the present study maximum isolates of A.*baumannii* were from ET tubes/ET secretions 67(45.3%) followed by urine 24(16.4%), Pus/wound swab 21(14.4%), blood 16(10.9%), and minimum from body fluids 9(6.2%) and other respiratory samples 9 (6.8%) as shown in Table-3.

Table 3: Respective isolation rates of A.baumannii from various samples

Specimen	A.baumannii (N=146)
ET tube/ ET secretions	67(45.9%)
Urine	24(16.4%)
Pus/wound swab	21(14.4%)
Blood	16(10.9%)
Body fluids	9(6.2%)
Other respiratory samples (sputum, BAL)	9(6.2%)

Antibiogram of A.*baumannii*

A.*baumannii* isolates were equally resistant 132(90.6%) towards Ticarcillin/ Clavulanic and acid Piperacillin/ Tazobactum Resistance towards Ceftazidime was 133 (91.1%), Amikacin 134 (91.8 %), Ciprofloxacin 135 (92.5%), Levofloxacin 132 (90.6%), Gentamicin 126 (86.4%) and Trimethroprim / sulfamethoxazole 134 (91.8%) resistance towards Cefoperazone/Sulbactum was 122(83.6%), Cefepime 133(91.1%), equal resistance was seen towards Doripenem 124(85.0%) ,Imipenem 124(85.0%) and Meropenem 124(85.0%). However isolates of A.*baumannii* were highly sensitive to Colistin 136 (94.2%) followed by Minocycline 120(82.2%) as shown Table 4

Table 4: Antibiogram of A.*baumannii* (N=146)

Antibiotic tested	No. of sensitive isolates (% sensitivity)	No. of resistant isolates (% Resistance)
Amikacin	12 (8.2%)	134 (91.8 %)
Gentamicin	20 (14.0%)	126 (86.4%)
Ciprofloxacin	11(7.5%)	135 (92.5%)
Levofloxacin	14(9.4%)	132 (90.6%)
Ceftazidime	13 (8.9%)	133(91.1%)
Cefepime	13(8.9%)	133(91.1%)
Cefoperazone/Sulbactum	24 (16.4%)	122(83.6%)
Ticarcillin/ Clavulanic acid	14 (9.4%)	132(90.6%)
Piperacillin/ Tazobactum	14 (9.4%)	132(90.6%)
Imipenem	22 (15.0%)	124 (85.0%)
Doripenem	22(15.0%)	124(85.0%)
Meropenem	22 (15.0%)	124 (85.0%)
Trimethroprim / sulfamethoxazole	12 (8.2%)	134 (91.8%)
Minocycline	120(82.2%)	26 (17.8%)
Colistin	136 (94.2%)	10 (6.8%)

MBL production in *A.baumannii*

Out of total 124 carbapenem resistant isolates of *A.baumannii* MBL production was observed in 107 (86.2%) isolates.

DISCUSSION

In the present study the total samples processed were 4724 Out of which 1336 showed positive growth, among 1336 positive isolates, 146 isolates were of A. *baumannii.* The isolation rate of *A.baumannii* from total processed samples came out to be 3.09% (146/4724) and from total 1336 growth positive samples isolation rate of A.*baumannii* came out to be *10.92*% (146/1336). The prevalence correlates with the studies done by Alotaibi **et al (2021)** with total samples of 5864 and reported 198 (3.37%) isolates *A. baumannii.* [19] And Kettan **et al (2017)** reported that out of 4232 samples, 2402 (56.8%) were positive among which 220 (9.2%) were *A.baumannii.* [20]

Prevalence of A.*baumannii* from various samples

In the present study maximum isolates of A.*baumannii* were from ET 67(45.3%) followed by urine 24(16.4%), Pus/wound swab 21(14.4%), blood 16(10.9%), and minimum from body fluids 9(6.2%) and other respiratory samples 9 (6.8%). A study was done by **Carvalho et al. (2013**) which reported that the most frequent site of isolation of A.*baumannii* was tracheal secretion (56.3%), followed by the catheter tip (16.9%), blood (7%), and urine (7%). [21] **Kaur et al (2014) reported** (50.6%) isolates of A.*baumannii* from respiratory samples, (21.8%) from pus, (15.6%) from blood, (8.6%) from other clinical samples and 33 (3.2%) from urine samples. [22] A study done by **Nesa et al (2018) reported**, maximum (45.9%) *A.baumannii* from tracheal aspirate, (15.3%) from wound swab, (5.9%) from pus, (21.2%) from blood, 9 (10.6%) from urine, 1 (1.1%) from pleural fluid. [23] **Namiganda et al (2019)** reported 36.84% *A.baumannii* strains from pulmonary samples followed by catheter 31.58% and 15.79% from blood as well as pus. [24] The result of present study are similar to de Carvalho et al. (2013), Kaur et al 2014 reported and Nesa et al 2018 however the results of present study are slightly higher as compared to prevalence reported by Namiganda et al 2019.

Gender wise Prevalence of *A.baumannii.*

In a present study out of total 146 isolates of A.*baumannii*, 89 (60.95%) were obtained from male patients and 57 (39.04%) were obtained from female patients. The present study is quite similar with the following studies who. **Anwar et al (2016),** reported that Out of 66 t isolates of *A.baumannii*, 49 (74.2%) were collected male patients and 17 (25.8%) from female patients. [1] **Yadav** et **al (2020)**, in a study reported out of total 161 isolates, 93 (58.3%)were isolated from male and 68 (41.7%) were from female patients. [25]

Prevalence of *A.baumannii* in ICU.

In a present study out of total 146 isolates of *A.baumannii* 105 (70.9%) isolates were obtained from ICU patients The present study is quite similar with the a study by **Hans et al(2015)** [26], **Dimple *et al* (2016)** [27] and **Massik et al (2021)** [28] who reported the prevalence to be 74%, 58.5% and 81% from ICU patients respectively.**Antimicrobial resistance/sensitivity of *A.baumannii***

In the present study the resistance towards Imipenem by *A. baumannii* is 85.0%, meropenem 85.0%Ceftazidime 91%, Gentamicin 86.4% Cefepime 91.1%, ciprofloxacin 92.5%, Ticarcillin Clavulanic acid 90.6%, where as isolates were highly sensitive to Colistin 94.2%%.**Kettan et al (2017)** reported that *A. baumannii* strains were resistant to: imipenem 75 %. [29] **Nesa et al (2018)** in their study reported that *A.baumannii* showed, , resistance to Ceftazidime 82.4%,, Cefepime 87.1%, ciprofloxacin82.4%, gentamicin63.5%,, amikacin88.2%,, imipenem 75.3%, meropenem85.9%,, Ticarcillin Clavulanic acid 82.4%, Colistin 5.9%, respectively. [23] **Sultan (2021)** reported the resistance by *A. baumannii* towards imipenem and meropenem resistance to be 81 and 84%.87%, Ceftazidime (85.4%), ciprofloxacin (88.2 %), and Gentamicin (93.4%). [30] **Nikibakhsh et al (2021)** reported that *A. baumannii* strains were resistant to imipenem (100%), ciprofloxacin (95.3%). [31]Sensitivity of *A. baumannii* towards Colistin has been reported by various authors, **Bashir et al (2014)** reported 99 % sensitivity similar was reported by **Tojo et al (2015)** and **Kettan et al (2017)**. **Ghasemian et al (2016)** reported 92 % sensitivity towards Colistin; similar 93.1 %was reported by **Maraki et al (2016)**. **Pawar et al (2016)** reported 89.1 % sensitivity for Colistin. However **Gupta et al 2016** reported low level of sensitivity towards Colistin 56.9% which does not co relate with our present study. [32,33,29,34,35,36,37]

MBL production in A.*baumannii*

In present study a total of 146 *A.baumannii* isolates were obtained out if which 124 (86.2 %.) were carbapenem resistant.

The prevalence of MBL was found to be 86.2 %.The results of the present study almost correlate with the studies by, **Kaur et al (2013)** [22], **Agrawal et al (2015)** [38] and **Massik et al (2018)** [28] who reported MBL positive rate as **80%, 87.5%, 82%.**

The results obtained in the present study are slightly higher than **Kabbaj et al (2013)** [39], **Yadav** et **al (2019)** [25], **Parajuli et al (2017)** [40] **and Anwar et al (2015)**[1] **who reported** MBL positive rate as 74%, 67.7%, 78% and 74.3%.

However studies by **Szejbach et al (2013)** [41] , **Safari et al (2015)** [42], **and Guzel et al (2018)**[43] reported MBL production in *A.baumannii* isolates to be 94.4% and 100% which are high as compared to the present study.

CONCLUSION:

The present study indicates major role of Acinetobacter *baumannii* in nosocomial infections with high potential to develop antibiotic resistance. The study also revealed high level of antimicrobial resistance with high proportion of MBL producing in isolates of *Acinetobacter baumannii*. Thus indicating early detection and infection control practices as best defenses against isolates. The study also suggests following up of antimicrobial restriction policies in order to avoid excessive antibiotics.

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