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PHENOTYPIC DETECTION OF METALLO-β-LACTAMASE AND CARBAPENEMASE AMONG IMIPENEM RESISTANT *ACINETOBACTER BAUMANNII*

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ABSTRACT

Background: Carbapenems are the emperical and last resort of treatment used for Gram negative bacilli including Acinetobacter spp. However; an increase in carbapenem resistance among *A. baumannii* due to Ambler class B metallo-beta-lactamases (MBL) or class D OXA carbapenamases in recent years is of global concern. So this study was undertaken to detect antimicrobial susceptibility pattern and the presence of carbapenemase and metallo-β-lactamase among the carbapenemase resistant clinical isolates of Acineto*b*acter *b*aumannii by phenotypic method.**Material and Methods:** A total of 130 non duplicate clinical isolates of *A. baumannii* from various clinical specimens were included in this study. Antimicrobial susceptibility test was carried out for all 130 isolates by Kirby Bauer disk diffusion method. Carbapenemase and metallo- β-lactamase production was tested among 76 imipenem resistant isolates of *A. baumannii* by modified Hodge test and combined disk test respectively. **Result:** In our study, resistance pattern of *A. baumannii* was as follows: ampicillin (91.53%), amikacin (63.07%), ceftazidime (73.07%), ciprofloxacin (73.84%), co-trimoxazole (60%), gentamicin (59.23%), imipenem (58.46%), ofloxacin (64.61%), and Piperacillin/Tazobactam(50%). Colistin and tigecycline are 93.84% and 100% sensitive respectively by disk diffusion method. Among 76 imipenem resistant isolates carbapenemase and MBL was detected in 54 (71.05%) isolates and 15 (19.73%) isolates respectively.

KEYWORDS: *Acinetobacter baumannii*, Carbapenemase, Metallo-β-lactamase, Modified Hodge Test.

Increasing isolation of multidrug-resistant *Acinetobacter baumannii* (MDR-Ab) has been reported worldwide, and it is now one of the most difficult nosocomially acquired gram-negative pathogens to control and treat.[1–3] Its ability to survive under a wide range of environmental conditions makes it a frequent cause of outbreaks of infection and an endemic health care associated pathogen.[4] It has emerged as a major cause of healthcare-associated infections including pneumonia, urinary tract infection, and septicemia.[5] Carbapenems were the remaining choice of drug to treat these superbug in late 90s, but carbapenem resistant clones have already been emerged.[6] The efficacy of carbapenems against multidrug-resistant Acinetobacter spp. has been undermined by the emergence of Ambler class B and class D carbapenemase-hydrolyzing β-lactamases.[7]

Carbapenem resistance in *A. baumannii* is due to a variety of combined mechanisms such as hydrolysis by beta-lactamases, alterations in the outer membrane protein and penicillin-binding proteins and increased activity of efflux pumps. Acquired resistance to carbapenems, mediated by the Ambler class D beta-lactamases or OXA type carbapenamases and Ambler class B metallo-β- lactamases are of greatest concern as they are encoded by genes which are transmissible and account for most of the resistance to carbapenems.[8, 9] MDR- *A. baumannii* is usually treated with broad-spectrum antibiotics, including carbapenems. Carbapenems such as imipenem and meropenem are of the antibiotics of last resort for such infection, and resistance against them is considered an alarming situation.[10, 11]

Thus this study was undertaken to know the antimicrobial susceptibility pattern of *A. baumannii* and to evaluate carbapenemase and MBL production among the imipenem resistant isolates of *A. baumannii.*

MATERIALS AND METHODS

A total of 130 clinical isolates of *A. baumannii* were collected from various patients from SRM Medical College Hospital, Kattankulathur, Tamil Nadu from from Dec 2013 to April 2015. The isolates were obtained from different specimens including tracheal aspirate, wound, blood, urine, abscess, and CSF. The isolates were

identified by colony morphology on Mac Conkey agar, Gram stain, catalase test, oxidase test, oxidative and fermentative properties, citrate test, growth at 44^0C, production of acid from lactose 1% and 10% and utilization of malonate and aspartic acid.

Antibiotic susceptibility test was performed by using Kirby Bauer disk diffusion method as per Clinical Laboratory Standards institute (CLSI) guidelines 2014.[12] Pseudomonas aeruginosa ATCC 27853 was used as control strain.

The antibiotics tested were ampicillin (10µg), amikacin (30 µg), ceftazidime (30 µg), Ceftazidime /Clavulanic acid (30/10 µg), meropenem (10µg), ciprofloxacin (5µg), co-trimoxazole (25 µg), gentamicin (10µg), imipenem (10µg), cefepime (30 µg), ofloxacin (5µg), Piperacillin/Tazobactam (100/10 µg), colistin (10 µg) and tigecycline (5µg) from Himedia Laboratories (Mumbai, Maharashtra, India).

Detection of Carbapenemase

The carbapenem resistant isolates were tested for carbapenemase production by modified Hodge test as per CLSI guidelines. First 0.5 Mc Farland standard of *Escherichia coli* ATCC 25922 was prepared and carpet culture done on well dried Mueller Hinton agar. An imipenem disc was placed at the center of the plate after 5 minutes. Test organism was heavily streaked in a straight line from edge of imipenem disk towards the edge of the plate. Four test isolates were inoculated in each plate at an angle of 90^0 to each other. Then the plates were incubated at 37^0C for 24 hours. *Klebsiella pneumoniae* ATCC 1705 was used as quality control for carbapenemase production.

Positive test showed a clover leaf like indentation of *E. coli* 25922 along test organism growth streak within the disk diffusion zone. No clover leaf like pattern near to test organism was regarded as negative.

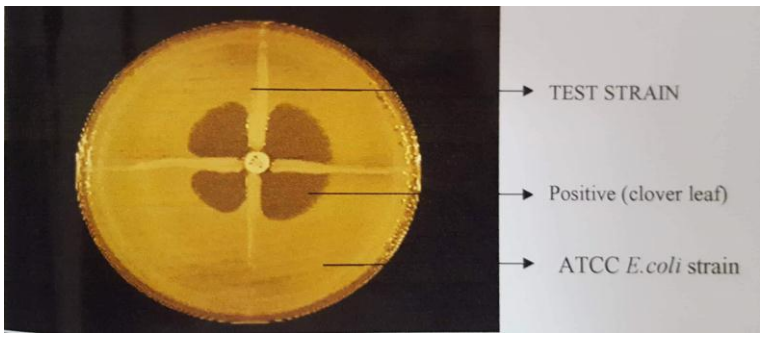


Figure 1: Modified Hodge Test for Detection of Carbapenemase.

Metallo-β-lactamase Screening

All the isolastes of *A. baumannii* showing resistance to imipenem, meropenem, etrapenem and third generation cephalosporin by Kirby Bauer disk diffusion method as per CLSI guidelines were considered screening positive and taken for evaluation of metallo-β-lactamase.

Detection of Metallo-β-lactamase

Imipenem - EDTA combined disk synergy test was employed for evaluation of metallo-β-lactamase. 0.5 Mc Farland standard of test organism was prepared and inoculated on well dried Mueller Hinton agar plate by carpet culture method. Two imipenem disks (10 µg) and two ceftazidime disk were taken and placed on the Mueller Hinton agar plate. 10 µl of EDTA solution was added to one of imipenem and ceftazidime disks to obtain a desired concentration of 750 µg. The plates were incubated at 37^0C for 16-18 hours. Zone of inhibition was measured for all for disks and an increase in zone of inhibition of 7 mm or more around imipenem+EDTA or ceftazidime+EDTA compared to imipenem or ceftazidime disks without EDTA was considered as MBL positive.

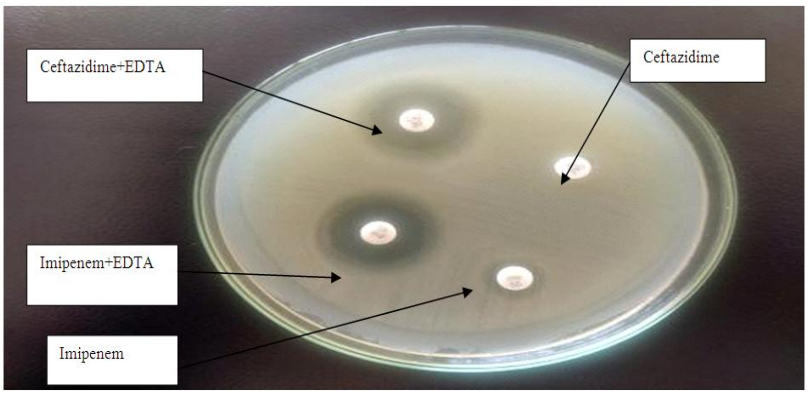


Figure 2: Imipenem-EDTA Comined Disk Synergy test.

RESULT

In a total of 7685 specimens collected for culture; a total of 565 Non-fermenting Gram negative bacilli were isolated. Out of which 130 *Acinetobacter baumannii* strains were isolated from patients admitted and attending SRM Medical College Hospital during the study period.

Acinetobacter baumannii	130
Non-fermentors other than *Acinetobacter baumannii*	435
Total number of non-fermentors	565

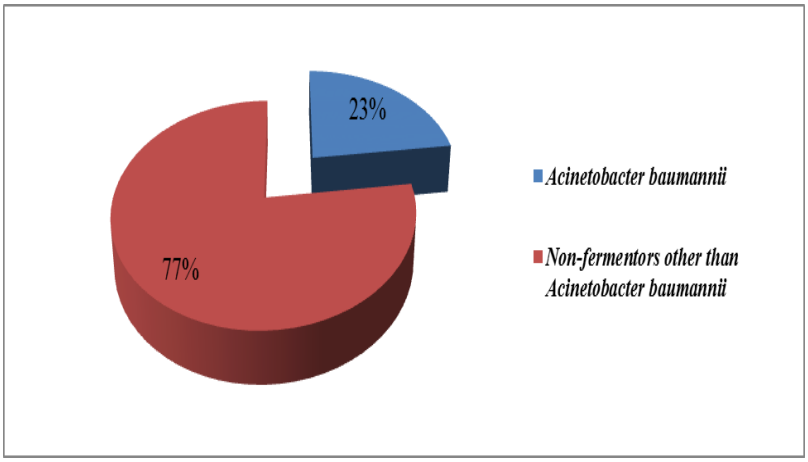
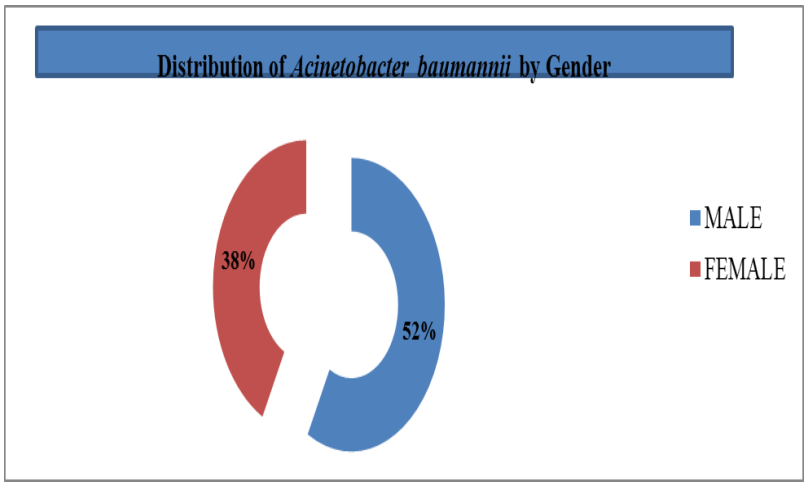


Figure 3: Percentage of *Acinetobacter baumannii* and other Non-fermentors.

DISTRIBUTION OF ACINETOBACTER BAUMANNII BY GENDER

Out of 130 *Actinetobacter baumannii* isolates, 80 were from male patients and 50 were from female patients with a male:female ratio of 4:2.5.



AGE WISE DISTRIBUTION OF ACINETOBACTER BAUMANNII

Age in Years	No. of Patients
0-10	1
11-20	8
21-30	9
31-40	18
41-50	19
51-60	31
61-70	33
71-80	9
81-90	2

RESISTANCE PATTERN OF ACINETOBACTER BAUMANNII BY KIRBY BAUER DISC DIFFUSION METHOD

Antibiotics	Percentage of isolates sensitive to the drug (number)	Percentage of isolates intermediate to the drug (number)	Percentage of isolates resistance to the drug (number)
Ampicillin (10µg)	6.92% (9)	1.53% (2)	91.53% (119)
Amikacin (30 µg)	36.92% (48)		63.07% (82)
Ceftazidime (30 µg)	15.38% (20)	11.53% (15)	73.07% (95)
Ceftazidime /Clavulanic acid (30/10 µg)	20% (26)	3.07% (4)	76.93% (100)
Cefotaxime (30 µg)	13.07% (17)	4.61% (6)	82.3% (107)
Cefepime (30 µg)	30.76% (40)	2.30% (3)	66.92% (87)
Ciprofloxacin (5µg)	25.38% (33)	0.76% (1)	73.84% (96)
Colistin (10 µg)	93.84% (122)		6.15% (8)
Co-trimoxazole (25 µg)	20.76% (27)	19.23% (25)	60% (78)
Gentamicin (10µg)	34.61% (45)	6.15% (8)	59.23% (77)
Imipenem (10µg)	38.46% (50)	3.07% (4)	58.46% (76)
Meropenem (10µg)	36.15% (47)	3.84% (5)	60% (78)
Ofloxacin (5µg)	33.84% (44)	1.53% (2)	64.61% (84)
Piperacillin/Tazobactam (100/10 µg)	46.15% (60)	3.84% (5)	50% (65)
Tigecycline (5µg)	100% (130)		

DOUBLE DISC SYNERGY TEST FOR DETECTION OF METALLO-β LACTAMASE (MBL)

Out of 76 Imipenem resistant isolates, only 15 (19.73%) gave positive MBL production by Imipenem-EDTA Double Disk Synergy Test.

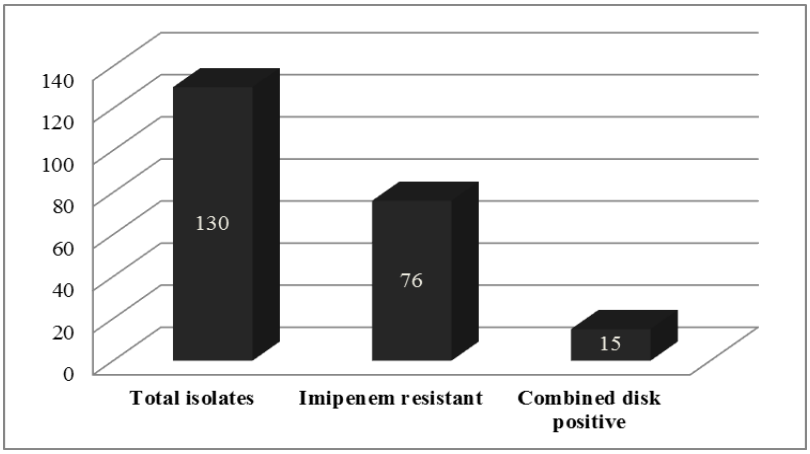


Figure 4: Number of imipenem resistant and metallo-β-lactamase positive *Acinetobacter baumannii* isolates.

COMPARISION OF CARBAPENEMASE PRODUCTION IN IMIPENEM RESISTANT IN STRAINS

Out of 76 imipenem resistance strains, 54 (71.05%) were carbapenemase producer.

IMIPENEM RESISTANCE (n= 76)		
CARBAPENEMASE PRODUCTION ONLY	METALLO-β LACTAMASE PRODUCTION ONLY	PRODUCTION OF BOTH
44 (57.89%)	5 (6.57%)	10 (13.51%)

Distribution of Metallo-β-lactamase isolates

Mean age of patient from which MBL was isolated was found to be 56.66 (range 0-80 years). Highest number of cases were from age group 51-60 years 5 (33.33%), followed by age group 61-70 years 4 (26.66%), 31-40 years 3 (20%), 41-50 years 2 (13.33%) and 71-80 years 1 (6.66%) respectively.

Of 15 MBL isolates, 8 were from male and 7 were from female which was not stastically significant ($p>0.05$) when compared to gender distriution of MBL negative

isolates; whereas ward wise distribution showed 8 isolates were from intensive care unit (ICU), 4 from general ward, 2 from surgical ward and 1 from nephrology ward respectively. MBL isolates were isolated predominantly from endotracheal tip 7 (46.66%), sputum 4(26.66%), pus 3 (20%) and urine 1 (6.66%) respectively.

DISCUSSION

Acinetobacter baumannii is one of the most common causative agents of nosocomial infections in hospitals. Ability to survive for an extended period of time in hospital environment and development of resistance to multiple drug agents possess a great challange in management of *A. baumannii* infections. Besides *A. baumannii* causes serious infection, especially in immune supressed hosts, patients in ICUs and those with critical disorders.[13, 14]

130 isolates of *Acinetobacter baumannii* was isolated from various clinical samples in the present study. More isolates of *A. baumannii* were obtained from males (62%) than females (38%).

Sen B. et al. also found that the incidence was higher in males (66.96%) than that of females (33.04%) which is similar to our study. In another study done by Nahid H Ahmed, Kamaldeen Baba et al. the results were also similar to our study.

In our study, lowest incidence was of age group (0-10) and (71-80) years old group whereas the highest incidence was of age group (61-70), followed by (51-60) and (41-50). Similar results were seen in study done by Nahid H Ahmed et al. where highest incidence was seen in age group 31-59. In another study of Harit Jabbar Fahad, the lowest incidence was of age range (71-80) year old group whereas the highest incidence was of age range (31-40) year old group.

Resistance pattern of *Acinetobacter baumannii* showed 91.53%, 63.07%, 73.07%, 73.84%, 60%, 59.23%, 58.46%, 64.61%, and 50% to ampicillin, amikacin, ceftazidime, ciprofloxacin, co-trimoxazole, gentamicin, imipenem, ofloxacin and Piperacillin/Tazobactam. Colistin and tigecycline were 93.84% and 100% sensitive respectively by disk diffusion method.

In study of Ahmed S. Attia, Wael M. Tawakkol, the resistance level to other antibiotics was 100% for each of meropenem, ceftazidime, cefepime, ciprofloxacin, and piperacillin/tazobactam, and 80% or more to amikacin, aztreonam, gentamicin, and tobramycin which was higher than our study.

Resistance pattern to ampicillin, amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, and Piperacillin/Tazobactam was 100%, 50%, 66.7%, 83.3% , 40%, 73.3% and 70% in study done by Nermin H. Ibrahim.

In study done by Abhisek Routray, high level of resistance was seen for ampicillin 94%, cefepime - 83.07%, amikacin-76.3%, gentamycin-81.53%, ciprofloxacin-78.46%, imipenem-64.61% and colistin-3.1%. Resistance rate was slightly higher for ampicillin, amikacin, cefepime and ciprofloxacin than our study. While resistance to imipenem and colistin was lower than our study.

Resistance to ampicillin-sulbactam was least. 36.1% isolates were resistant to imipenem, 66.6% to ceftazidime, 72.2% to ciprofloxacin, 80.5% to amikacin and 84.7% to piperacillin in study done by Nachiket D. Vaze, Christopher L. Emery.

Nahar et al. has reported even 100% resistance to amoxicillin, ceftriaxone, ceftazidime, cefuroxime, and aztreonam in biofilm forming Acinetobacter species which was higher than in our study.

Jeetendra Gurung et al. all isolates were resistant to ticarcillin, ticarcillin/clavulanic acid, Ceftazidime and trimethoprim/sulfamethoxazole. Twenty-seven (27) isolates were resistant to ciprofloxacin, 27 to amikacin, 20 to Gentamycin, 15 to imipenem, 10 to tobramycin and finally all isolates were susceptible to colistin.

In study of R Srinivasa Rao, R Uma Karthika et al. *A. baumannii* isolates showed 100% resistance to imipenem, 89% resistance to cephotaxime, 80% to amikacin and 73% to ciprofloxacin. Resistance to imipenem and cefotaxime was higher than out study while resistance to ciprofloxacin was similar to our study.

Out of 76 imipenem resistant isolates 15 (19.73%) were MBL producer and 54 (71.05%) carbapenemase producer in our study. However MBL production and carbapenemase production was lower 13.3% and 26.6% respectively in study done by Anu Madanan Sunu Kumari.

Of 116 *A. baumannii*, the modified Hodge test was positive for 113 (97.4%) of isolates and the metallo-beta-lactamase screening test with EDTA was positive in 92 (79.3%) isolates in study done by Amudhan SM. In another study done by Anurag Paysi and Manu Chaudhary, MBL production was 66.8%.

Out of 21 meropenem resistant strains 14/21 (66.66%) were found to be carbapenemase positive. 21.42% (3/14) were found to be MBL producers phenotypically in study done by Sivasankari S, Senthamarai, Anitha.C.

Mojtaba Moosavian et al. among imipenem resistant *A. baumannii* isolates, 53 (53%) showed carbapenemase production by MHT.

96.30%, 94.7%, 80% and 100% were MBL positive by CDST-IPM in study reported by Nirav P. Pandya et al., Galani et al., Picao et al. and Franklin et al. respectively.

High degree of resistance was shown by MBL positive isolates to all β-lactam antibiotics, aminoglycosides, tetracycline, and fluoroquinolones. However they remain sensitive to tigecycline (100%) and colistin (60%).

This study shows that MHT and combined disk synergy test could be used in clinical laboratories for monitoring emergence of carbapenemase and Metallo-β-lactamase in *A. baumannii*, especially MDR isolates.

CONCLUSION

The early detection of MBL and other carbapenemase is of utmost importance in deciding the most appropriate therapeutic regimen for treatment of carbapenem resistant non-fermenters, the reduction of mortality rates for patients with MBL and carbapenemase producing isolates and also to check the intrahospital dissemination of such strains. Imipenem-EDTA combined disk test (CDST-IPM) and modified Hodge test is the most sensitive method for detection of MBL production in Gram negative bacilli. Intravenous colistin with rifampin and imipenem is recommended for the treatment of carbapenem resistant isolates lacking metallobetalactamase where as the combination of colistin and rifampicin (with or without tigecyclin) was recommended for treatment of metallobetalactamase producing imipenem resistant isolates (Maragakis Ll, Perl et al.2008) (Perez F et al 2007).

Controlling antibiotic use, particularly aminoglycosides, cephalosporins, may be an important component of strategies to limit the spread of Carbapenem-resistant Acinetobacter spp.

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