

Prevalence of ESBL and MBL in *Acinetobacter* Species Isolated from Clinical Samples in Tertiary Care Hospital

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Abstract: *Objective:* The emergence of infections by *Acinetobacter* spp causes serious threats and further limits the treatment options. The present study aims to determine prevalence of ESBL and MBL among *Acinetobacter* species. *Materials and methods:* Various clinical specimens and isolates were identified using a standard bacteriological protocol. Antimicrobial sensitivity patterns were determined using Kirby Bauer disc diffusion method. ESBL was detected by phenotypic confirmatory disc diffusion test (PCDDT) using ceftazidime alone and ceftazidime in combination with clavulanic acid. MBL detection was done by Imipenem EDTA combined disc diffusion test. *Result and Conclusion:* The incidence of *Acinetobacter* was more in male (64.17%) than female (35.82). Among the males 16-40 years age group was more prevalent (43.28%). Out of the total 67 *Acinetobacter* spp maximum number of isolates were sensitive to imipenem (90%), followed by meropenem(87.57%) and amikacin (80.21%). Out of total 67 isolates 34(50.70%) were ESBL producer and 16 (23.88%) were MBL producer. Early detection of beta lactamase producing *Acinetobacter* strains would be important for better patient outcome including reduction of mortality rates and spread of multidrug resistant strains.

Keywords: *Acinetobacter* species, Antibiotic sensitivity, ESBL, MBL

1. Introduction

Acinetobacter is an important known pathogen causing nosocomial infections including pneumonia, urinary tract infections, septicaemia, and wound infections. [1] The prevalence of *Acinetobacter* infections ranges from 2% to 10% of all gram negative bacterial infections in Europe [2], about 2.5% in United States, [3] and 71.2% in India. [4]

Acinetobacter spp have ability to acquire antimicrobial-resistance rapidly, leading to multi-drug resistance.[5] One of the main concerns about antimicrobial resistance in *Acinetobacter* species has been the resistance to the last line of antimicrobials through acquisition of carbapenem resistance. [6]

Many kinds of β -lactamases demonstrating resistance to expanded-spectrum cephalosporins have been detected in *Acinetobacter* spp. [7] Extended-spectrum β -lactamases are capable of hydrolyzing extended spectrum cephalosporins with an oxyimino side chain and its activity is well inhibited by clavulanic acid, sulbactam and tazobactam.[8] The overall prevalence of ESBL-producing *Acinetobacter* spp. varies greatly in different geographical areas and from institute to institute. Previous studies from India have reported ESBL production varying from 68%-72%.[9,10] ,5% in Mumbai, [11] 75% in UP. [12]

Carbapenem-resistant *A.baumannii* strains are increasingly recovered from hospitalized patients worldwide. MBL producing strains are frequently resistant to aminoglycosides and fluoroquinolones but remain susceptible to polymyxins.[13] In India, the prevalence of MBLs range from 8% to 79%.[14] Hence the aim of study was to find out

prevalence of ESBL and MBL producer *Acinetobacter* species.

2. Material and Methods

The present study was conducted in Mayo Institute of Medical Sciences and Hospital, Barabanki in the Department of Microbiology, during (2014 September to 2015 June). A total of 67 clinical isolates of *Acinetobacter* species, which were isolated from various samples, were identified by standard procedures. [15] The susceptibility pattern of isolates to various antibiotics was determined by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines. [16]

Detection of extended spectrum beta lactamase

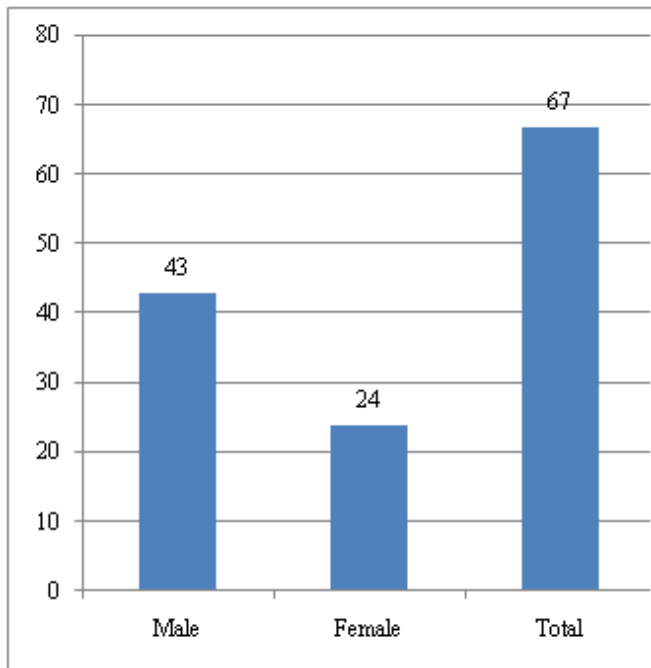
The test was done by CLSI phenotypic disk confirmatory test using disks of ceftazidime (30 μ g) and ceftazidime-clavulanic acid (30 μ g/10 μ g). Both the disks were placed at least 20 mm apart, centre to centre on Mueller-Hinton agar plate and incubated over night at 37°C. A zone difference of ≥ 5 mm around ceftazidime and ceftazidime-clavulanic acid was taken as ESBL positive. [16]

Detection of MBL

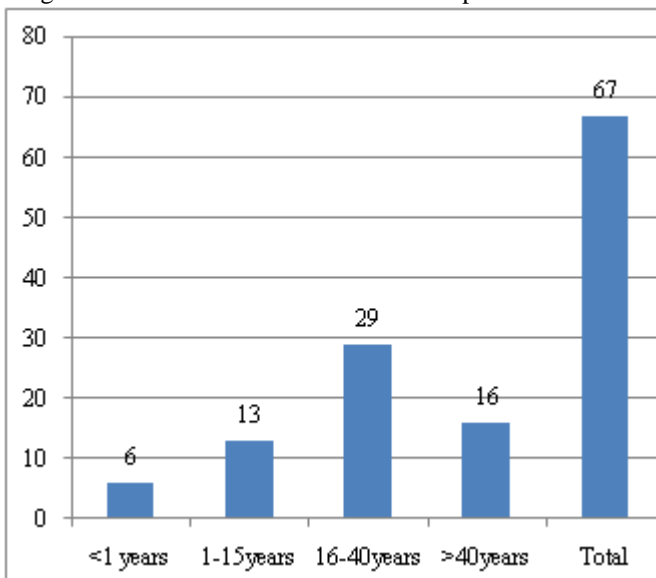
This was performed by Imipenem EDTA combined disc test. Two (10 μ g) imipenem discs were placed on a plate inoculated with the test organism, and 10 μ l of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of 7 mm was interpreted as a positive result for MBL production. [16]

3. Result and Discussions

1. Distribution of *Acinetobacter* isolates among male & female patients . (n=67).



2. Age -wise distribution of *Acinetobacter* species.



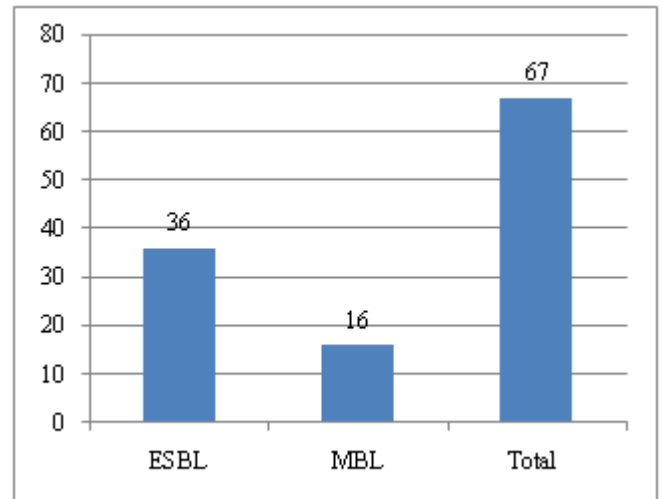
3. Antibiotic sensitivity patterns of *Acinetobacter* species

Antibiotics	Sensitive %
Ampicillin sulbactam	55.23
Ceftazidime	35.45
Ciprofloxacin	50.32
Levofloxacin	50.00
Imipenem	90.12

Meropenem	87.57
Gentamicin	63.45
Amikacin	80.21
Piperacillin	43.81
Piperacillin tazobactam	65.33
Cefepime	25.15
Cefotaxime	33.25
Ceftriaxone	23.67
Tetracycline	25.21
Cotrimoxazole	26.48

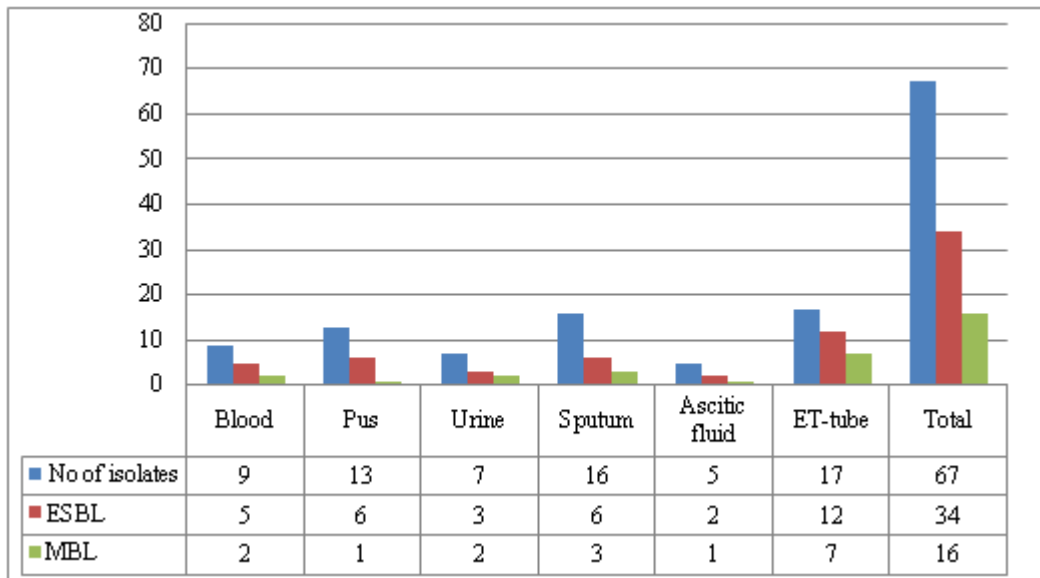
4. ESBL and MBL distribution.

ESBL producer (%)	MBL producer (%)	Total isolates
34 (50.74%)	16 (23.88%)	67



5. Distribution of ESBL and MBL producers in various clinical specimens.

Sample	No of isolates (%)	ESBL producer (%)	MBL producer
Blood	9 (13.43)	5 (14.70)	2 (12.5)
Pus	13 (19.40)	6 (17.64)	1 (6.25)
Urine	7 (10.44)	3 (8.82)	2 (12.5)
Sputum	16 (23.88)	6 (17.64)	3 (18.75)
Ascitic fluid	5 (7.46)	2 (5.88)	1 (6.25)
ET-tubes	17 (25.37)	12 (35.29)	7 (43.75)
Total	67 (100)	34 (100)	16 (100)



Among these isolates, 35.82% was female and 64.17% was male (table 1). *Acinetobacter* infections were more common in patient's age group 16-40 years. (table 2) this study was supported by Hossien Fazeli et al.;[17]. Antibiotic sensitivity patterns of *Acinetobacter* species shows 90% imipenem flowed by 87.57% meropenem and 80.21% amikacin (table 3). This study was similar with Jayapriya Sukumaran et al.,[18] but study by Purti Tripathi [19] shows the most of drug resistance. Out of total 67 isolates 34(50.70%) ESBL producer and 16 (23.88%) was MBL producer (table 4). Similar results were obtained by Vahaboglu et al.[20] Yong et al.[21]., Sinha et al.[22] A recent study introduced a more sensitive procedure for MBL detection in a broad range of host organisms, including carbapenem susceptible isolates.[23] MBL production rate in imipenem resistant *Acinetobacter* ranged from very occasional to as high as 50.00%. Lee et al [24] reported MBL production in imipenem resistant *Acinetobacter* to be 15.10% (range 0-34%). Yong et al [25].The distribution of ESBL and MBL producer in various clinical samples, ET-tubes was highest ESBL 35%, MBL 43.75% producer and least was in Ascitic fluid ESBL 5.88% and MBL 6.25% (table 5). This findings were similar to study by Nachimuthu Ramesh et al.,[26].

4. Conclusions

Observations from the present study showed the *Acinetobacter* species infection is more common in male patients. *Acinetobacter* species were isolated from various clinical samples proved their existence in all sites leading to a range of diseases. Isolates shows 90% imipenem sensitive flowed by 87.57% meropenem and 80.21% amikacin. In above isolates 50.70% ESBL and 23.88% were MBL producer. Hence, the early detection of beta lactamase producing isolates would be important for the reduction of mortality rates and spread of multidrug resistant organism.

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