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Original Research Article

Phenotypic characterization of *Acinetobacter* species and their resistance pattern in a tertiary care hospital

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ABSTRACT

Background: *Acinetobacter* is an emerging opportunistic pathogen and increasingly implicated in hospital acquired infection especially in intensive care unit. The infection caused by *Acinetobacter* spp. is difficult to control due to multi drug resistance which limits its therapeutic options. This study was undertaken to isolate *Acinetobacter* from various clinical samples and to speciate *Acinetobacter* isolates. Efforts were made to study the antimicrobial susceptibility pattern of *Acinetobacter* species and to detect metallo beta lactamase producing *Acinetobacter* strains.

Materials and Methods: Clinical samples blood, urine, sputum, pus, body fluids and ETT sample received in microbiology laboratory were processed according to the standard laboratory procedure. Identification and antimicrobial susceptibility testing was done by VITEK-2 automated method. All the isolates that showed resistance to Imipenem were tested for Metallo – Beta – Lactamase (MBL) production by phenotypic test, Imipenem-EDTA-combined disc test method.

Results: Out of total 100 *Acinetobacter* spp isolated from clinical samples, 80 were isolated from critical care units. *Acinetobacter* baumannii was common spp 88 (88%), followed by A.complex 10 (10%). Isolation rate of *Acinetobacter* species was highest from blood (27%) specimens followed by ETT (19%). 44% strains of *Acinetobacter* were isolated from respiratory samples. 84(84%) *Acinetobacter* spp, were carbapenem resistant. Out of 84 carbapenems resistant strains 48 (57%) were MBL positive strains by combined disc test.

Conclusion: Emergence of MBLs producing strains of *Acinetobacter* is alarming and reflects excessive use of carbapenems. Therefore early detection and prompt infection control measures is important to prevent further spread of MBLs to other Gram negative bacilli.

1. Introduction

The genus *Acinetobacter* which comprises nonfermentative gram negative bacilli of low pathogenicity has attracted recent clinical and research interest as members of the genus are implicated increasingly as nosocomial pathogens.Although associated primarily with nosocomial infection, it has also been involved in cases of community acquired infection.[1]

The number of nosocomial infections caused by *Acinetobacter* species has increased in recent years and is of increasing concern in critically ill patients and the risk factors for this infection are not well established.[2]

Acinetobacter is an emerging opportunistic pathogen and increasingly implicated in hospital acquired infection especially in intensive care unit.[3,4]The infection caused by Acinetobacter spp. is difficult to control due to multi drug resistance which limits its therapeutic options.

A wide spectrum of antimicrobial resistance mechanisms is exhibited by *A. baumannii*. Apart from its intrinsic

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resistance mainly due to the low permeability of the outer membrane to certain antibiotics as well as constitutive expression of certain efflux pumps, *A. baumannii* is able to easily acquire and incorporate genetic elements such as plasmids, transposons and integrons.[5,6]

Acinetobacter spp. have been implicated in a range of nosocomial infections, including bacteremia, urinary tract infection (UTI), and secondary meningitis, but their predominant role is as agents of nosocomial pneumonia, particularly ventilator-associated pneumonia in patients confined to hospital intensive care units (ICUs).[5]

Carbapenems, which used to be the antimicrobials of choice, have been increasingly compromised and no longer constitute salvage therapy for *A. baumannii* infections.[5]

Intensive care units of hospitals are the epicenters for MBL-positive strain isolations. These are the commonest agents of ventilator associated pneumonia. But sadly now, studies show that isolates from outside the intensive care units are also developing multi drug resistance due to MBL production.

This study was undertaken to isolate, identify and to speciate Acinetobacter from various clinical samples based on phenotypic characters. Efforts were made to study the antimicrobial resistance pattern of *Acinetobacter* species and to detect metallo beta lactamase producing *Acinetobacter* strains.

2. Materials and Methods

This was a prospective observational study, which was done in department of microbiology and involved 100 isolates of *Acinetobacter* species from March 2017 to September 2018. A total 100 isolates from clinical specimens collected from the patients suffering from various infections attending outdoors and indoors of Hospital were followed in the laboratory for the isolation *Acinetobacter* species. The study was approved by ethical committee of Medical College and Hospital.

Clinical samples blood, urine, sputum, pus, body fluids and ETT sample received in microbiology laboratory were processed according to the standard laboratory procedure. Identification and antimicrobial susceptibility testing was done by VITEK-2automated method.

All the isolates that showed resistance to Imipenem were tested for Metallo – Beta – Lactamase (MBL) production by phenotypic test, Imipenem-EDTA-combined disc test method.

EDTA being a chelating agent removes Zinc ions from the active site of the MBL enzyme. This makes the enzyme inactive and thus the organism becomes sensitive to Carbapenems.The difference of $\geq$7 mm between the inhibition zone diameter of the IPM-EDTA disc and that of IPM only disk was considered to be a positive for the presence of MBLs.[7]

3. Results

A total of 100 Acinetobacter isolates were obtained from clinical specimens collected from the patients suffering from various infections

Table 1: Age-wise distribution of *Acinetobacter* isolates

Age Group (years)	No. of isolates (100)
0 – 10	3(3%)
11 – 20	5(5%)
21 – 30	10(10%)
31 – 40	11(11%)
41 – 50	16(16%)
51 – 60	22(22%)
61 – 70	15(15%)
71 - 80	18(18%)

Maximum number of *Acinetobacter* spp. Were isolated from 51-60 yr. and 71-80 yr. age group patients followed by 41-50 yr.

Table 2: Location of patients from whose samples *Acinetobacter* Spp. Were isolated

Unit	No. of isolates 100
ICU	59 (59%)
NICU	12 (12%)
PICU	9(9%)
OPD	3(3%)
Medicine ward	3(3%)
HDU	4(4%)
Ortho ward	2(2%)
Oncology ward	4(4%)
Peditric	4(4%)

80 *Acinetobacter* species were isolated from critical care units. Only three strains were isolated from OPD patients

Table 3: *Acinetobacter* spp isolated from various clinical samples

Species	No. of isolate (100)
A.baumanni	88(88%)
A.complex	10(10%)
A.lowffi	02(2%)

Among 100 *Acinetobacter* species isolated common species isolated from clinical samples was Acinetobacter baumannii (88%).

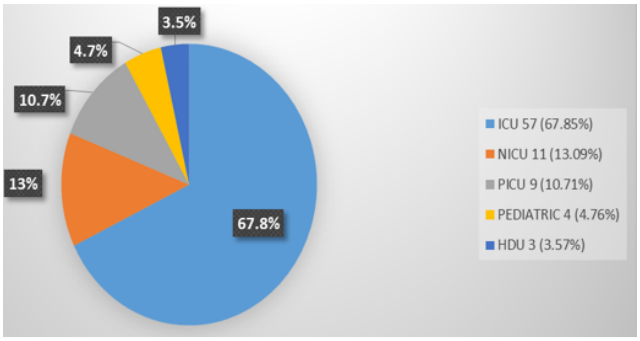
Table 4: Number of *Acinetobacter* Species Isolated from various clinical samples

Sample	No. of *Acinetobacter* spp. 100
Blood	29(29%)
ETT	23(23%)
PUS	18(18%)
Sputum	15(15%)
Urine	09(9%)
BAL	05(5%)
Pleural fluid	01(1%)

Table 5: Antimicrobial susceptibility pattern of *acinetobacter* species

Antibiotic	Sensitive%	Resitance %
Piperacillin/Tazobactam	(5)5%	(95)95%
Ceftriaxone	-	(100)100%
Cefoperazone/Sulbactam	(31)31%	(69)69%
Cefepime	(4)4%	(96)96%
Imipenem	(16)16%	(84)84%
Meropenem	(16)16%	(84)84%
Gentamicin	(7)7%	(93)93%
Ciprofloxacin	(15)15%	(85)85%
Tigecycline	(85)85%	(15)15%
Colistin	(100)100%	-
Trimethoprim/Sulfamethoxazole	(23)23%	(77)77%

Acinetobacter isolates were resistant to Ceftriaxone followed by Cefepime, Piperacillin/Tazobactam, Meropenem, Imipenem and Gentamicin. *Acinetobacter* strains were susceptible to Colistin and Tigecycline



Graph 1: Carbapenem resistant Acienetobacter isolates from different unint

4. Discussion

Acinetobacter are opportunistic pathogens and are frequently present on normal skin. Though widely prevalent in nature and generally regarded as commensals of human skin and respiratory and genitourinary tracts,they have been implicated as the cause of serious infectious diseases such as meningitis, pneumonia, tracheobronchitis, endocarditis, wound infections, and septicemia, mostly involving patients with impaired host defences.[8]

Acinetobacter baumannii is intrinsically less susceptible to antibiotics than Enterobacteriaceae; moreover, it has propensity to acquire resistance. Because of frequent resistance to aminoglycosides, flouroquinolones, ureidopenicillins and third –generation Cephalosporins, Carbapenems are important agents for managing Acinetobacter infections.[8–10]

Carbapenems have remained as drugs of choice for treatment of Acinetobacter infection. Over usage of carbapenems has increased the development of Metallo beta lactamase (MBL) producing bacteria.[11] Treatment of

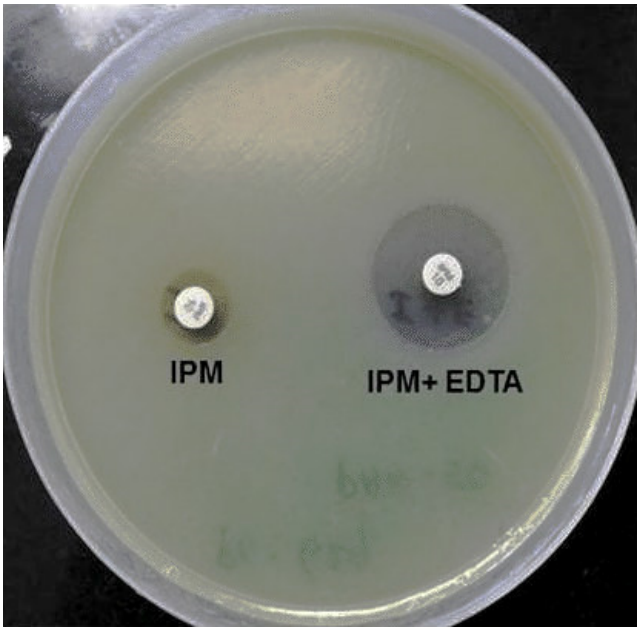


Fig. 1: Metallo – Beta – Lactamase (MBL) production by phenotypic test, Imipenem-EDTA-combined disc test method. Out of 100 *Acinetobacter* strains, 84 were carbapenem resistant strains. 48 (57%) were MBL positive strains by combined disc method.

infections caused by MBL producing *Acinetobacter* has become difficult as few antimicrobial agents remain active against these pathogens.[8,12]

A total of 100 *Acinetobacter* isolates were obtained from clinical specimens collected from the patients suffering from various infections .Out of 100 isolates maximum number of *Acinetobacter* spp. were isolated from 51-60 yr(22%). and 71-80 yr(18%) age group patients followed by 41-50 yr (15%). 61 isolates of *Acinetobacter* were from male patient and 39 isolates from female patient.

In this study rate of isolation was maximum from ICU (59%) followed by NICU (12%) and PICU (9%). 80 *Acinetobacter* species were isolated from critical care units. Out of 100 isolates 97 were isolated from IPD patients and only three isolates were from OPD patients.

Kulkarni Vishal et al (2016), reported maximum rate of isolation from the cases of burn department (38%) followed by ICUs (23%) and was least from Dept. of Obstetrics and Gynecology.[8]

In this study isolation rate of *Acinetobacter* species was highest from blood (29%) specimens followed by ETT (23%), pus (18%), sputum (15%), urine (9%), BAL(5%) and pleural fluid(1%). Out of 100 isolates, 44 isolates of Acinetobacter were from respiratory samples. *Acinetobacter* spp isolated from urine samples of patients which were having urinary catheter, most of these were catheter coloniser.

Jaggi et al (2012) have reported the predominance of *Acinetobacter* strains in broncho-pulmonary samples.[13]

Amarjeet Kaur et al (2014), reported that among 1017 isolates, 515(50.6%) isolates were from respiratory samples, 222 (21.8%) from pus, 159 (15.6%) from blood, 88 (8.6%) from other clinical samples and 33 (3.2%) from urine samples.[7]

Out of 100 *Acinetobacter* species isolated, rate of *Acinetobacter* baumannii was higher 88 (88%), followed by A.complex 10 (10%) and A.lowffi 2 (2%).

Amarjeet Kaur et al (2014), reported that Out of total isolates, 964 (94.7%) were identified as *A. baumannii,* 48(4.7%) *A. lwoffii* and 5 (0.4%) *A. hemolyticus*.[7]

Kulkarni Vishal et al (2016),[8] reported Out of 101 *Acinetobacter* isolates, *Acinetobacter* baumanii (71) was the commonest species isolated amongst the total followed by *A. lowfii* (18) and *A. haemolyticus*.[12]

Chuang et al.(2011), have also observed that among *Acinetobacter* species, *A. baumannii* was the main cause of *Acinetobacter* infections with very high antimicrobial resistance rate which is also responsible for more serious infections than other *Acinetobacter* species.[14]

In this study Acinetobacter isolates were most resistant to ceftriaxone (100%) followed by Cefepime (96%), Piperacillin/tazobactam (95%), Meropenem (84%), Imipenem (84%), Gentamicin (93%), Ciprofloxacin (85%), Trimethoprim/sulfamethoxazole (77%).

Acinetobacter isolates were more susceptible to Colistin (100%) followed by Tigecycline (85%).

Venkata sarada et al (2014),[15] reported out of 102 isolates of *Acinetobacter* baumannii, 83.3% were resistant to Gentamicin, 94% to Amikacin, 99% to Ceftazidime, 98% to Ciprofloxacin, 27% to Gatifloxacin, 93% to Cefepime, 91.% to Piperacillin/ tazobactam, 98% to Aztreonem, 84% to Amphicillin/ sulbactam,100% to Meropenum and 95% to Imipenum. Of the total isolates maximum number isolates were susceptible to Colistin, Polymyxin and Tigecycline at 2 μg/ml, 1 μg/ml and 0.5 μg/ml respectively.

Amarjeet Kaur et al (2014)[7] reported that *A. baumannii* showed high level of resistance to cephalosporins, cotrimoxazole and piperacillin. Majority of *A. baumannii* (71%) were susceptible to piperacillin-tazobactam. Among aminoglycosides, netilmicin showed lesser resistance (46.9%) than amikacin (64.9%) and gentamicin (88.1%). *A. lwoffii* and *A. hemolyticus* showed lesser resistance to all antibiotics as compared to *A. baumannii*. All isolates of *A. lwoffii* and *A. hemolyticus* were susceptible to Imipenem whereas 389 (40.3%) isolates of *A. baumannii* were found to be Imipenem resistant.

Kulkarni Vishal (2017).[8] have found that the *Acinetobacter* isolates were highly resistant to cefotaxime (100%), Co-trimoxazole (87%), Ceftazidime (85%), Ciprofloxacin (78%), Cefepime (77%), Gentamicin (73%). Low resistance was noted against Tetracycline (35%). All isolates were susceptible to Colistin.

In our study out of 100 *Acinetobacter* strains, 84 were Carbapenem resistant. The highest isolation rate of carbapenem resistant *Acinetobacter* spp 57(67.85%) was from ICU followed by NICU11 (13.09%), PICU 09(10.71%) (Critical care units).

Out of 84 Carbapenems resistant strains 48 (57%) were MBL positive strains by combined disc test. Carbapenems have remained as drugs of choice for treatment of *Acinetobacter* infection. Over usage of Carbapenems has led to development of Metallo beta lactamase (MBL) producing bacteria.

Irfan et al.(2008), have reported 97% of the MBL producing *Acinetobacter* species by Disc potentiation technique.[16] Umaa et al(2009) stated that 70.9% of their isolates were positive for production by metallo beta lactamase by Disc potentiation technique**,** while another study reported from Kerala, by Anil et al(2011), mentioned 21% of the *Acinetobacter* baumannii isolates were found to be metallo-β-lacatmase producers.[17,18]

Class B Metallo-beta-lactamases is characterized by the ability to hydrolyze carbapenems and by its resistance to the commercially available beta-lactamase inhibitors, but susceptibility to inhibition by metal ion chelators. Most of these enzymes hydrolyze cephalosporins and penicillins but lack the ability to hydrolyze aztreonam.

The other reason for carbapenem resistance other than MBL production is due to impermeability, which occurs due to loss of the opr D porin and due to the upregulation of an effective efflux system present in these organisms. So, detection of both MBL and carbapenemase is necessary in deciding the treatment option.

5. Conclusion

The alarming increase in the frequency of MBLs producing *Acinetobacter* spp presents an emerging threat of complete resistance to all therapeutic drugs in practice, leaving the potentially toxic polymyxin B and colistin as the only options among antibiotics.

Therefore, it is essential to rapidly screen and detect MBLs producing *Acinetobacter* species to prevent the spread of drug resistance. Implementation of strict infection control practices and antimicrobial stewardship will help to prevent further dissemination.

6. Conflict of Interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

7. Source of Funding

None.

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