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ORIGINAL RESEARCH

Phenotypic Characterization of Multidrug Resistance *Acinetobacter sppwith* special reference to metellobetalactamase production from various clinical isolate in tertiary care Hospital in Western U.P

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Abstract

Introduction: *Acinetobacter species* is one of the most frequent opportunistic pathogen responsible for serious infection in intensive care unit. *Acinetobacter species* most often multidrug resistant is a difficult to treat pathogen particularly in ICU. The aim of our study to identified phenotypic characterization of multidrug resistance *Acinetobacter species* with special reference to metellobetalactamase production from various clinical isolates in tertiary care hospital.

Methods: The Present study was conducted in the department of Microbiology on 210 isolates of *Acinetobacter* species recovered from various clinical samples. This study was approved by institutional ethical committee. The isolates were identified as per standard conventional method. Antimicrobial susceptibility test were processed as per standard CLSI guideline. The resistance isolates were screened and confirmed by modified hodge test and double disk synergy test.

Results: Out of 210 *Acinetobacter* species isolated from various clinical sample 92.8% was *A.baumanii* followed by *A. lwoffi* (5.8%), *A Haemolyticus* (1.4%). 84% species were carbapenem resistance. Out of 84% carbapenem resistance strain 58 were positive by modified hodge test and 49 were MBL positive by IMP-IMP double disk synergy test.

Conclusion: In this study can be concluded that emergence of *Acinetobacter spp.* alarming threat and excessive use of carbapenem drug therefore early detection and prompt infection control measures is important to prevent spread of MBL to other gram negative bacteria.

Keywords: *Acinetobacter species,* Multidrug Resistance, Metellobetalactamase, Carbapenemase.

Introduction

Acinetobacter species is the most frequent opportunistic pathogen responsible for serious infection in intensive care unit. The *Acinetobacter* species are gram-negative, non flagellated coco bacillus bacteria. This opportunistic pathogen causes infections that are acquired in hospital and the public health. *Acinetobacter baumanii* is responsible for 7.8% to 23% of mortality by acquired pneumonia in the hospital and 10-43% in ICU (1). One of the most important microbial resistant to beta lactams antibiotic (penicillin, cephalosporin, monobactums, and carbapenemase) is hydrolysis by Betalactamase Gene coding for beta lactamases enzymes mutate continuously in response to the excess use of antibiotics leading to development of newer Betalactamase with a broad spectrum of activity (2). Resistance to carbapenemase could evolve by the development of efflux pumps decreased cell permeability and by the production of intrinsic or acquired carbapenemase belonging to either the class B or Class D oxacillinases (3).The MDR (Multi-drug resistant) strains of the *A. baumannii* are behind for higher number of aggressive infections in hospitals. The *Acinetobacter spp*. have been responsible for the range of nosocomial infections, including urinary tract infection, bacteraemia and secondary meningitis, but their principal role is as agents of nosocomial pneumonia, particularly ventilator associated pneumonia in patients confined to hospital intensive care units (4).

There are many technique act together to contribute to the problem of MDR including reduced access to microbial targets through loss of porin channels, possession of efflux pumps that are capable of actively withdraw a broad range of antimicrobial agents from the bacterial cell, and possession of a wide group of beta-lactamases that hydrolyzed and give resistance to penicillins, cephalosporins and carbapenems (5,6).

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Materials and methods

The present current study was conducted in the department of microbiology at Rohilkhand Medical College & Research Bareilly U.P. after taking approval from the Institutional Ethical Committee. In our study we used clinical isolate of the *Acinetobacter species* recovered consecutively from 210 clinical sample that include blood, pus, urine, sputum, body fluid, ET Tip and biopsy samples to the department of microbiology laboratory. The all samples were processed for culture by standard conventional methods and susceptibility testing was determined by Kirby Bauer's disc diffusion.

Antimicrobial Susceptibility Testing and determination of MIC

The sensitivity of different classes of antimicrobial agents was determined using disk diffusion method according to CLSI guidelines (2019). The following antibiotics were used; Amikacin(AMK:30µg, Cefepime (FEP: 30 µg), Ceftazidime (CAZ :30 µg), Colsitin (Col:110 µg),Levofloxacin(LEV:5 µg), Imipenem (Imp :10 µg)**,** meropenem (MER: 10 µg), ciprofloxacin (CIP: 5 µg), ceftriaxone (CRO:30 µg), piperacillin-tazobactam (PTZ: 100/10 µg),Gentamycin (GM: 10 µg),Cefoperazone-sulbactum (CFS:75/30 µg) and Netilmicin, PolymixinB. Minimum Inhibitory Concentrations (MICs) were determined by E strips test (Himedia Ezy MIC TM strip). *Escherichia coli* ATCC25922 were used as negative control strain. MBL-producing *P. aeruginosa* and carbapenemase-producing *A. baumannii* were used as positive control strains. In addition the concentration ranges for the *E*-test was 0.002–32 µg/ ml for the Imipenem (Himedia, Ezy MIC TM strip).

Screening for the Carbapenemase Production

All the carbapenemase resistant *Acinetobacter* isolates were screened for carbapenemase activity by Modified Hodge test (MHT). An overnight culture suspension of *Escherichia coli* ATCC 25922 adjusted to 0.5 Mc Farland standard was inoculated using a sterile cotton swab on the surface of a Muller Hinton agar plate (Himedia, Mumbai, India) After drying 10 mcg meropenem disk (Hi Media Mumbai, India) was placed at the center of the plate and the test strain was streaked from the edge of the disk to the periphery of the plate in four different direction. The culture plate was incubated at 37°c for 24 hours. In MHA plate, the test isolates shows clover leaf indentation due to the presence of carbapenemase enzyme production. The test isolate was examined as positive (7).

EDTA disk synergy test (DDST)

Done for the ability of the detection of MBL possessing isolates EDTA disk synergy test an full night liquid culture of the test isolate was accommodate to a turbidity of 0.5 Farland Mc criterion and spread on surface of MHA plate. A meropenem (MRP) disk in10 µg concentration or 30 µg ceftazidime (CAZ) disks was placed on MHA agar plate. A solution of 0.5 M EDTA was gets ready by dissolving 186.1 g of EDTA disodium salt (Brand Reachem) in 1000 ml of distilled water. The pH was adjusted to 8.0 using NaOH (Himedia) and sterilized by autoclaving.

A 10mcg meropenem disk or 30 mcg ceftazidime disks kept on MHA plate and 6 mm diameter blank disk (Himedia) was kept on the inner surface of the lid of the MH Agar plate and 10 µL of freshly prepared 0.5 M EDTA solution was put on it and transferred to the surface of the MHA agar plate. The distance of meropenem disk and EDTA disk kept between 10 mm and incubate at 37°c for 24 hours. After 24 hours of incubation the zone of enhancement in between the meropenem and EDTA disk in comparison with the zone of inhibition on the far side of the drug was interpreted as positive for MBL production (2).

Results and observations

Out of 210 Acinetobacter species isolated from the various clinical samples were 92.8% A. baumanii followed by A. lwoffi (5.8%), A Haemolyticus (1.4%) showing in table 1 and figure 1.

Table1. Showing the distribution of *Acinetobacter species* isolated from various clinical samples.

S.NO.	Species	No. of isolates in percentage (Out of 210)
1	*Acinetobacter baumanii*	92.8%
2	*A.lwoffi*	5.8%
3	*A.haemolyticus*	1.4%

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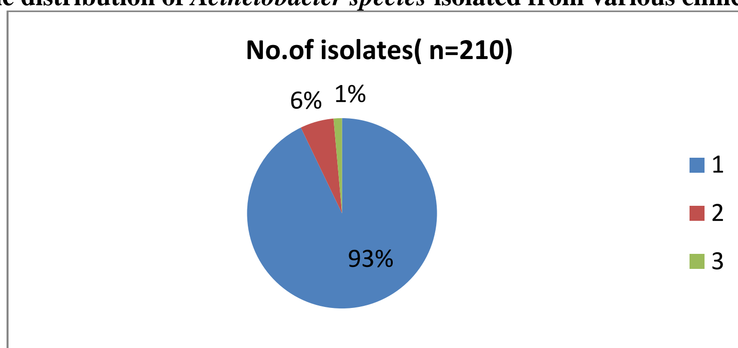
Figure 1: Showing the distribution of *Acinetobacter species* isolated from various clinical samples.



In our study we found isolation rate of *A. baumanii* species was highest from pus samples (35.7%) followed by urine (32.3%), 13.8% samples were isolated from sputum,7.6%, isolated from ET Tip, (6.1%), biopsy sample(2.5%) and Body fluid (2.0%)samples were isolated from blood showing in table 2.

Table 2:Showing the number of *Acinetobacter species* from various clinical samples in percentage.

Clinical Samples	No. of Acinetobacter species
Blood	13 (6.1%)
Pus	75 (35.7%)
Urine	68 (32.3%)
Sputum	29 (13.8%)
E T Tip	16 (7.6%)
Body fluid	04 (2.0%)
Biopsy sample	05 (2.5%)
Total	210

In this study we observed *Acinetobacter* isolate were resistance to ceftriaxone followed by cefepime, gentamicin, ceftazidime, ciprofloxacin, meropenem, imipenemand amoxi-clav. Acinetobacter spp. was more susceptible for Colistin Polymixin Band Tigicycline showing in table 3.

Table3: Showing antimicrobial resistance pattern of *Acinetobacter species.*

Antibiotics	Sensitive %	Resistance %(n=210)
Piperacillin Tazobactum	22(10.4%)	188(89.5%)
Amoxicillin-clavulanate	32(15.2%)	178(84.7%)
Ceftazidime	16(7.6%)	194(92.3%)
Cefepime	14(6.6%)	196(93%)
Ceftriaxone	5(2.3%)	205(97.6%)
Imipenem	36(17.1%)	174(82.8%)
Meropenem	32(15.2%)	178(84.7%)
Gentamicin	14(6.6%)	196(93.3%)
Amikacin	38(18.0%)	172(81.9%)
Ciprofloxacin	30(14.2%)	180(85%)
Levofloxacin	46(21.9%)	164(78%)
Tigycycline	175(83.3%)	35(16.6%)
Colistin	210(100%)	0
PolymixinB	207(98.5%)	3(1.4%)
Cotrimaxazole	35(16.6%)	175(83.3%)

We observe 84% *Acinetobacter spp*. were carbapenem resistance. Out of 84% carbapenem resistance 58 were Carbapenem producing strain were positive by modified hodge test, and 49 test isolates were MBL positive by IMP-IMP EDTA Double disk synergy test showing in table 4.

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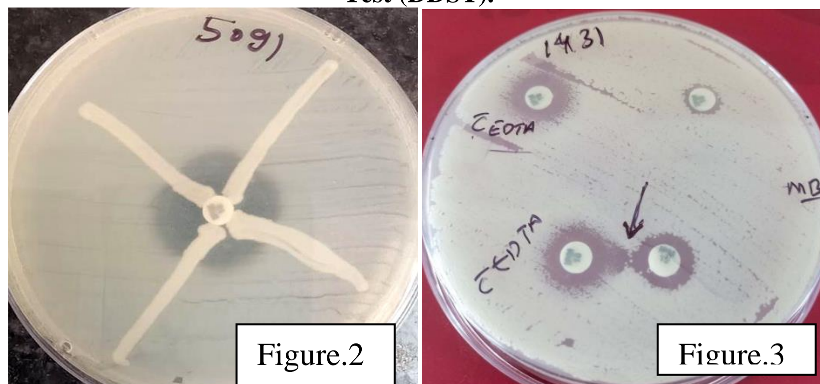
Table4: Showing the distribution of metellobetalactamase enzyme producing strain by phenotypic method.

S.NO.	Phenotypic Test	No. of Positive Tests
1	IMP-IMP EDTA Double disk synergy test	49%
2	Modified Hodge Test	58%

Figure 2: **Showing Modified Hodge Test (MHT) and figure3Showing Imipenem EDTA Double Disc Synergy Test (DDST).**



Discussion

Acinetobacter spp. has emerged as significant pathogens causing nosocomial infections. Treatment of these pathogens has become a major challenge to clinicians worldwide, due to their increasing prevalence to antibiotic resistance. To mark this matter, we have assemble a panel of *Acinetobacter spp.* strains expressing different antimicrobial resistance determinants such as small spectrum β-lactamases, extended-spectrum β-lactamases, OXA-type-carbapenemase, metallo-beta-lactamases, and over-expressed AmpC β-lactamases. The bacterial strains display different resistance phenotypes were collated in the years of 2008 and 2013 from Severance Hospital, Seoul. *Acinetobacter baumannii* has become a life threatening pathogen (8). Moreover, carbapenem resistance among *A. baumannii* isolates limited therapeutic choice for the treatment of A. baumannii infections which might lead to increasing morbidity and mortality rates (9, 10). Fattouh M. et al. (2014) studies mention on the prevalence of the carbapenemases among Egyptian A. baumannii clinical isolates (11–12).The phenotypic identification of carbapenemases has the lead of low cost, methods with the absence of costly equipment however, it undergo from low specificity and sensitivity. Consequently, the screening by PCR for few genes responsible for the carbapenem resistance, too some insertion sequences were taken as the gold standard procures to estimate the sensitivity of the separate phenotypic. The different device can give to carbapenem resistance, however, the production of MBL and CHDLs remain the most frequent mechanisms among A. baumannii isolate (13).

Infections caused by multidrug resistant gram negative bacterial where Carbapenem antibiotic proved most potent agents for treatment. MBL production is a most important mechanism to hydrolyze the Carbapenem antibiotics which emerged as the Carbapenem resistance. As per the therapeutic significance these bacterial isolates in study were also showing resistance for many other antibiotic groups like beta-lactams, aminoglycosides, fluoroquinolones and out of these, options left for therapy are use of Polymixin B and Colistin antimicrobial agent which carry potential toxicity (14) MBL producing strains may share in horizontal MBL gene transfer to different pathogens in the hospital areas due to intrinsic capability of MBL producing strains. As early detection of MBL producing bacteria in infections is need to treat appropriate with in time limit which might reduce the mortality when patient stay in hospital (7) Joshi et al. examined that 9% of bacteriologically positive *Acinetobacter baumannii* isolates collected from a hospital in India (15).

In our study Acinetobacter spp. were isolated from various clinical sample 92.8% was A.baumanii followed by A. lwoffi (5.7%), A Haemolyticus (1.4%). Isolation rate of A.baumanii species was highest from blood sample (35.7%) followed by pus (32.3%),13.8%sample were isolated from sputum sample, 7.6% was isolated from ET Tip sample.85% Acinetobacter spp. were carbapenem resistance. This type of study conducted by Siau et al. who exhibited a relative high prevalence of *A. baumannii* in South-East Asian countries, and they observed that the hot and humid climatic changes contributed to this high incidence of infection (16). Similar study was done by manisha kumara et.al in Nepal who observed that among 324 samples of Acinetobacter spp, 167 isolates were *A. calcoaceticus baumannii* Acb complex followed by 83 A. lwofi, 38 A. haemolyticus, 30 A. radioresistens (17). In the recent years the other study done at government medical college Amritsar in2021 91.6% isolates were Acinetobacter Baumanii & 5.6% Acinetobacter lwoffi, 2.8% Acinetobacter hemolyticus were observed in various clinical samples (18). In this study we observed In our study we found isolation rate of *A. baumanii* species was highest from pus samples (35.7%) followed by urine (32.3%), 13.8% samples were isolated from sputum,7.6%,

isolated from ET Tip, (6.1%) and samples were isolated from blood samples. In our current study A. baumannii 92.8% was observed to be the frequent cause of infections. Like our study, W. Nageeb et al., also proved that A. baumannii was the only Acinetobacter spp. come across in clinical specimens and this supported the finding that infections by other Acinetobacter spp. are infrequent (19). Lone R, Shah A. et. al., (2009) and Basustaoglu AC et. al., (2001) studies which also identified that among others *Acinetobacterspp,A. baumannii* was the most prevalent in clinical specimens and the most often responsible for nosocomial infections (20, 21).In our study out of 210 Acinetobacter spp. 84% were carbapenem resistance. Out of 84 carbapenem resistance 58 were positive by modified hodge test and 49 were MBL positive by DDST. The similar study was done in Greece in (2007) to evaluate different laboratory test for detection of MBL 98% was positive by MHT (22).

Conclusion

In the light of current study it can be concluded that, that MHT and Double disk synergy test were equally efficient to detect MBL production, Simultaneous existence of different carbapenemases is a complication to compute with and should be seriously considered for different and newer therapeutic strategies. Emergence of *Acinetobacter spp.* alarming threat and excessive use of carbapenem drug therefore early detection and prompt infection control measures is important to prevent spread of MBL to other gram negative bacteria.

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