

Molecular Identification of Carbapenem Resistance *Acinetobacter baumannii* causing Ventilator-Associated Pneumonia Isolated from Intensive Care Unit of Tertiary Care Hospital

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Abstract

Ventilator-associated pneumonia (VAP) is a serious complication in critically ill patients, significantly increasing morbidity and mortality. One concerning organism behind VAP is *Acinetobacter baumannii*, a multidrug-resistant bacterium has ability to evade treatment, particularly with carbapenems, the last-line antibiotics. This is especially worrisome within the confines of Intensive Care Units (ICUs) of tertiary care hospitals, hubs for high-risk patients and potential reservoirs of antimicrobial resistance. This study focused on identifying carbapenem-resistant *Acinetobacter baumannii* using both phenotypic and genotypic methods. In 132 isolates of *Acinetobacter baumannii*, we observed 96% resistance to the cephalosporins while least resistance found to colistin and tigecycline. However, a concerning 51.5% of isolates exhibited carbapenem resistance. Phenotypically confirmation of carbapenem resistance detected in 47% isolates by Combined Disc Test and 51.5% isolates by Modified Hodge Test and E-test. Genotypic analysis with RT-PCR revealed a diverse array of resistance genes: bla_{IMP} (33.82%), bla_{VIM} (25%), bla_{OXA}-Group (20.58%), and bla_{NDM} (8.82%). These findings highlight the alarming prevalence of carbapenem-resistant *Acinetobacter baumannii* in healthcare settings.

Keywords: Ventilator-Associated Pneumonia, *Acinetobacter baumannii*, Multidrug Resistance, Beta Lactamase

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INTRODUCTION

VAP is usually a bacterial nosocomial pneumonia that develops in patients with acute respiratory failure after 48 hrs of mechanical ventilation.¹ VAP is generally categorized on the basis of time of onset of pneumonia in two categories, early onset and late onset VAP. Early onset VAP is caused by antibiotic sensitive bacteria commonly carries a good prognosis, and occur within 4 days of hospitalization, whereas late onset VAP takes place after 5 or more days of mechanical ventilation, caused by multidrug resistance (MDR) organisms and has high patient mortality and morbidity.² Patients with early onset VAP, with a history of hospitalization within the past 90 days or administration of prior antibiotics are susceptible for MDR infection, and they should be treated as late onset patients.³

Acinetobacter spp. is one of the prime causes of Ventilator-associated pneumonia. It is gram-negative, non-lactose fermenter, non-saccharide fermenter, non-motile, oxidase negative coccobacilli showing intrinsic resistance to multiple antimicrobial drugs. *Acinetobacter* spp. is associated with opportunistic infections and nosocomial infections because it is widely spread in hospital environment and easily colonized on mucosal surface such as respiratory, urinary and intestinal tract. It has capability to hold on inanimate objects and survive on environment for up to five months even in the absence of nutrients.⁴ The infections, due to *Acinetobacter* spp. are mostly treated by cephalosporins, aminoglycosides, carbapenems, and tetracycline but now days, majority of *Acinetobacter* spp. isolates are susceptible to all currently available antibiotics. MDR *Acinetobacter* defined as resistance to minimum of three classes of antimicrobial regime such as β -lactamases, aminoglycosides, fluoroquinolones and carbapenem.⁵ The causative factor is the presence of mobile genetic elements such as plasmid and integrons which transfers the antimicrobial resistant gene to other organisms easily.^{6,7} Different mechanism of multidrug resistance have been identified in *Acinetobacter* spp. which involves production of β -lactamases, alterations in outer membrane protein, penicillin binding protein and increased activity of efflux pumps.⁸ One of the chief mechanisms of resistance

in *Acinetobacter* spp. is β -lactamase production which includes extended spectrum β -lactamase, metallo β -lactamase and oxacillinase.⁹ ESBLs belong to class A β -lactamases, which shows resistance to the third-generation cephalosporin (cefotaxime, ceftriaxone, and ceftazidime) and inhibited by clavulanic acid. Class B β -lactamases, also called as metallo- β -lactamases, act on penicillin's, cephalosporins and carbapenems but not on monobactam. Class C β -lactamases are ampC β -lactamases which confers resistance to cephalosporins in the oxyimino group, 7 α -methoxy cephalosporins which are not affected by β -lactamase inhibitor. Class D β -lactamase or OXA type β -lactamases have high hydrolytic activity against oxacillin and cloxacillin and they confer resistance to ampicillin and cephalothin, which are poorly inhibited by clavulanic acid.¹⁰ Carbapenem-resistant organisms are resistant to all those antibiotics to which AmpC β -lactamases producer are resistant. In addition, they are also resistant to carbapenems and resistance cannot overcome by β -lactamases/ β -lactamases inhibitors. The most commonly encountered β -lactamases in carbapenem resistance are carbapenem-hydrolysing class D β -lactamases (CHDLs), and class B metallo- β -lactamases (MBLs). So, this study was performed to determine the antibiotic resistance profiles, β -lactamase production by phenotypic method and existence of MBL (IMP, VIM, NDM), OXA (OXA23/40/58), and KPC-encoding gene in *Acinetobacter* spp. causing VAP.

MATERIALS AND METHODS

Bacterial Isolates

In the present study, we collected a total of 340 endotracheal aspirates using a mucous extractor under aseptic conditions.¹¹ Among these, patients who met the inclusion criteria, including patients on mechanical ventilation in ICU for >48 hours, Fever greater than 38.5°C, Leucocytosis ($\geq 10,000$ cells/mm³) or Leukocytopenia ($\leq 4,000$ cells/mm³), Radiograph infiltrates and all endotracheal aspirate samples were growing *Acinetobacter baumannii* isolates in significant >105cfu/ml in number were included in this study. Quantitative cultures were performed on ET aspirates and threshold was considered 105cfu/ml for diagnosis

of VAP. Primary inoculation of the samples was done on blood agar (BA), and MacConkey agar (MA) and all plates were incubated overnight at 37°C and observed for growth after 24 hr. Identification of bacteria was done by VITEK-2 compact automated system.

Antibiotic susceptibility testing (Kirby-Bauer's Disk Diffusion Method)¹²

Kirby-Bauer's disk diffusion method was used for antimicrobial susceptibility test. AST was done on Muller Hinton Agar (MHA) media according to clinical and laboratory standard institute (CLSI) guidelines.¹³ MDR strains were screened as per criteria illustrated by European Centre for Disease Control and Prevention and Centre for Disease Control and Prevention. Colistin susceptibility was determined by colistin broth disk elution method as per CLSI guidelines.¹³

Metallo Beta Lactamase (MBL) Detection by Phenotypic Methods: MBL production was screened by:-

Combined Disc iTest (CDT)¹⁴

The test culture was made on the Muller Hinton Agar (MHA) plate, as recommended by CLSI guidelines. Imipenem (10µg) and imipenem /iEDTA (10+750µg) discs were put on MHA at an interval of 20mm, distance from centre to centre. Plates were incubated for 16-18 hrs at 37°C. If the inhibition zone was ≥7mm around the IMP/EDTA disc, then IMP disc alone, indicated as MBL producers.

Modified Hodge Test¹⁴

The modified Hodge test was carried out to identify the activity of carbapenemase. *E. Coli* ATCC 25922 strain was inoculated on iMuller Hinton Agar (MHA) plate. Imipenem discs (10µg) were placed on the plate centre and test culture was streaked from the disc edge to the plate periphery. Plate was incubated for 16-18 hours at 37°C. Interpretation was made by presence of clover leaf zone or distortion of inhibition around the imipenem disc, indicated as presumptive carbapenems producer.

E-Test¹⁴

All imipenem resistance isolates were subjected to E-Test (Ezy MICTM Strip, HI media)

to detect minimum inhibitory concentration ratio and to confirm MBL production. The Imipenem (4-256 g/ml) and Imp-EDTA (1-64 g/ml) dilution ranges are double-sided on the E-Test MBL strip. On MHA agar, a lawn culture of 0.5 McFarland opacity test isolation was done. E-test strip was inoculated and incubated at 37°C overnight on agar surface. The plates were examined for imipenem and imipenem-EDTA minimum inhibitory concentration (MIC) values where the inhibition ellipses intersected the strip. When the MIC ratio of imipenem/imipenem plus EDTA was greater than eight, the test was considered MBL positive. The appearance of a phantom zone or a distortion of the imipenem ellipse was also considered positively.

Metallo Beta Lactamase (MBL) detection by Genotypic Method

PCR for Carbapenem resistance: Dynex laboratories manufactured kit, Praha 1, Prague was used for detection of carbapenemase activity of VIM, IMP, KPC, NDM & OXA-23/40/58 genes on Real Time PCR. ABI i7500 Fast Dx Real Time PCR (Peltier Block technology) & Type specific primer & probe were used to detect target genes. Bacterial DNA was isolated by using Qiagen DNeasy Mini Prep Kit. Detection of VIM/IMP/KPC/NDM/OXA-23/40/58 genes was done based on amplification of signals in the respective quencher dyes along with the detection of internal controls.

RESULTS

Bacterial Isolates

Among the 340 VAP cases, maximum isolates were found of *Acinetobacter baumannii* 132 (44.14%). 61 (46.21%) isolates of *Acinetobacter baumannii* were identified in early VAP patients and remaining 71 (53.79%) were identified from late VAP patients. 71 (53.78%) isolates were found as multidrug resistant (MDR).

Antimicrobial Sensitivity

Colistin & Tigecycline (Table) were found most sensitive drug of choice showed 100% and 96% sensitivity respectively. Imipenem was sensitive in 64 (48.48%) isolates of *Acinetobacter baumannii*.

Distribution of MBL by Phenotypic Method

62 (47%) isolates showed metallo-β-lactamase activity by Combined Disc Test and 68 (51.5%) by Modified Hodge Test. All Modified Hodge Test positive cases were also found resistant to Imipenem by E-Test.

Distribution of Carbapenem Resistance Gene by Genotypic Method

The results of RT-PCR are shown in Figure 1 and amplification process of gene in Figure 2. Resistance gene bla_{IMP} (33.82%), bla_{VIM} (25%), bla_{NDM} (8.82%), bla_{OXA} (20.58%), KPC (0%) was detected in 60 isolates.

DISCUSSION

Ventilator-Associated Pneumonia (VAP), is a frequent hospital-acquired infection that is acquired in patients admitted to ICU after 48 hrs of mechanical ventilation. It is associated with high morbidity and mortality, and become one of the most important public health issues with increased cost of treatment. High frequency of MDR pathogens in ICU is associated with increased incidence of VAP due to *Acinetobacter* spp. *Acinetobacter* spp. is one of the causes of severe

infections in patient on ventilator, resistant to most of the available antimicrobial agents. This poses a serious problem in choosing appropriate antimicrobial therapy for the treatment of critically ill patients as only few agents as

Table. Antibiotic sensitivity pattern of *Acinetobacter baumannii* (n=132) isolates, isolated from ET secretions

Antimicrobial Agent	Sensitive	Resistant
Amikacin	7 (5.3%)	125 (94.69%)
Gentamicin	8 (6.06%)	124 (93.93%)
Tobramycin	9 (6.8%)	123 (10.73%)
Ampicillin sulbactam	12 (9.09%)	120 (90.90%)
Piperacillin-Tazobactam	16 (12.12%)	116 (87.87%)
Ceftazidime	4 (3.03%)	128 (96.96%)
Cefotaxime	4 (3.03%)	128 (96.96%)
Ceftriaxone	5 (3.78%)	127 (96.21%)
Cefepime	5 (3.78%)	127 (96.21%)
Ciprofloxacin	6 (4.54%)	126 (95.45%)
Doxycycline	6 (4.54%)	126 (95.45%)
Trimethoprim+Sulfamethoxazole	4 (3.03%)	128 (96.96%)
Imipenem	64 (48.48%)	68 (51.51%)
Tigecycline	128 (96.96%)	4 (3.03%)
Colistin	132 (100%)	0 (0%)

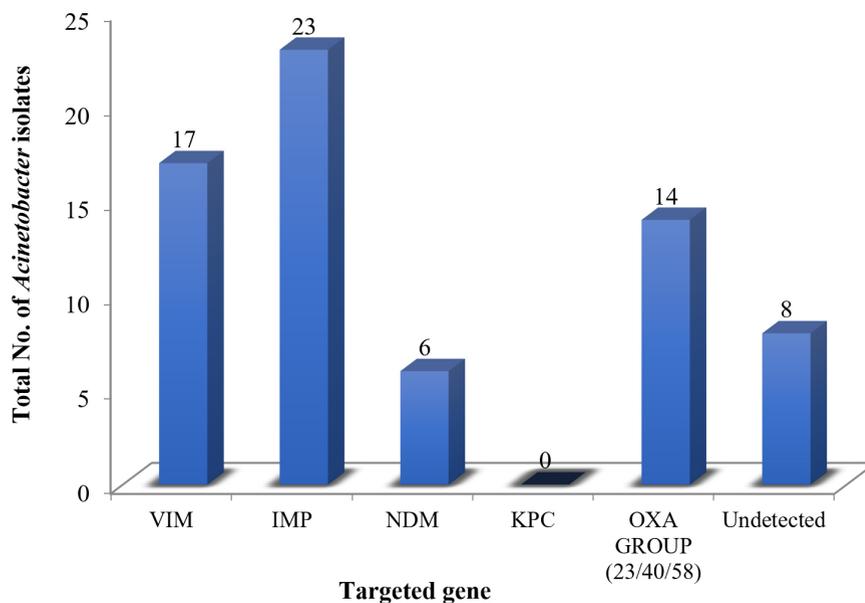


Figure 1. Distribution of *Acinetobacter* spp. based on Genotypic Resistance (VIM IMP NDM KPC & OXA-Group 23/40/58 gene)

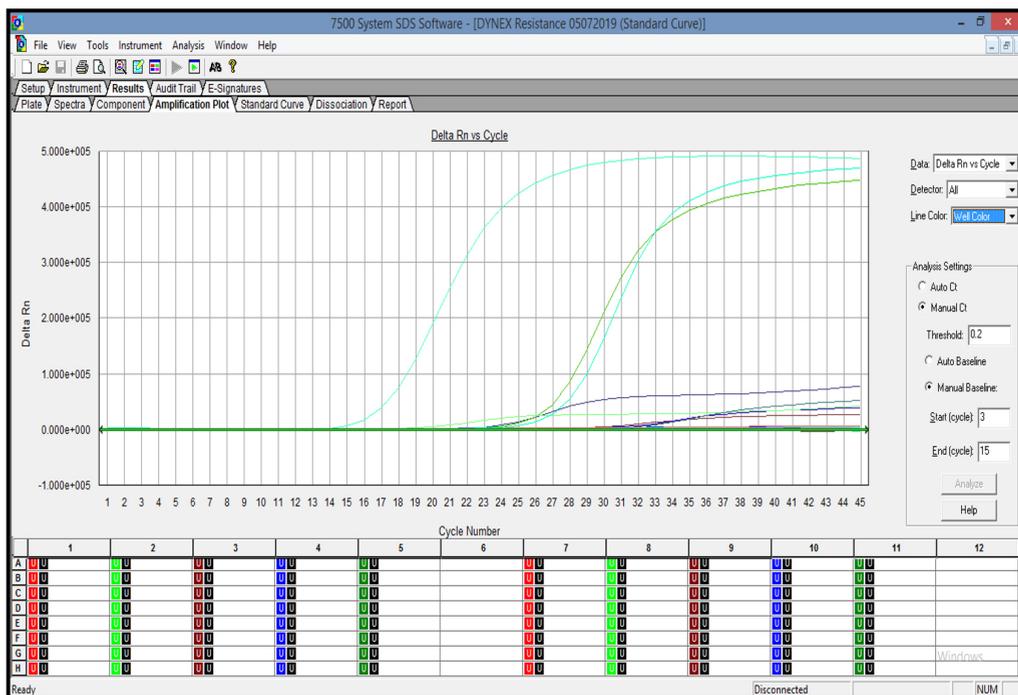


Figure 2. Amplification of genes during cycle running

Colistin and Tigecycline remains as therapeutic options. So, it is necessary to construct various strategies for infection control, Antimicrobial Resistance Surveillance Programme (AMSP), judicious prescription of antibiotics and cycling of antibiotics to reduce emergence of resistant strain. Distribution of *Acinetobacter* spp. in present setting was found high and similar findings were observed by other authors.^{15,16} This indicates high frequency of MDR pathogens in ICU is associated with increased incidence of VAP due to *Acinetobacter* spp. *Acinetobacter* spp. was found to be more common with late onset VAP. Late onset of VAP associated with higher rate of infection with MDR pathogen. Prior antimicrobial therapy before admission in ICU's can lead to alteration in normal flora. As *Acinetobacter* spp. has higher potential to stay in hospital environment for longer duration leading to nosocomial outbreak and other host factors such as underlying diseases also contribute in higher incidence of *Acinetobacter* infection. Imipenem resistance was found 51.5% in the present study. The results were in concordance with Goel *et al.*¹⁷ (44%). In other studies^{15,18} the resistance is more as compared to present study. Colistin and tigecycline were found to be the most

effective drugs against *Acinetobacter* spp. showing 100% sensitivity. Similar findings were found in the other author's studies.¹⁷⁻¹⁹ *Acinetobacter* spp. has become MDR, XDR and Pan drug resistant due to widespread usage of antimicrobials worldwide in ICU. Carbapenems have been considered the final resort to treat multi-drug resistant strain infections because of their capacity to treat ESBL and AmpC β -lactamase in a steady manner. The excessive usage of these antibiotics has now produced carbapenem-resistant strains. Production of Metallo β -lactamase (MBL) was found most common resistance mechanism in *Acinetobacter*. MBL gene are harboured as mobile elements and easily disseminate among the isolates. We found differences in the prevalence of MBL producing *Acinetobacter* spp. as compare to other studies.²⁰⁻²² It may be due to variations among different patient studied, different rates of antibiotic use in different hospitals and differences in geographical distribution which might have led to variations in the prevalence of the β -lactamases with varied resistance pattern.

Emergence of carbapenem mediated resistance in India is of serious concern. The detection of carbapenem mediated resistance

is of utmost importance in deciding the most appropriate therapeutic regimen for the treatment. In this study, carbapenem mediated resistance was mainly found due to class B and class D carbapenemase. Among class B bla_{IMP} and bla_{VIM} the results are in concordance with other studies,^{23,24} while in some studies²⁵⁻²⁸ lower percentage of isolates harbouring bla_{IMP} gene was observed. These variations were found due to change in pattern of resistance over different years and geographical difference within the countries over a period. Our study showed bla_{NDM} producer 8.82% while high percentage of bla_{NDM} was reported by many studies.²⁷ In present study we could not differentiate between the OXA genes as per our kit protocol. It could detect OXA-gene in total (23/40/58) rather than individual OXA gene as in other studies.^{15,26} Oxacillinase encoding gene can be intrinsic (bla_{OXA-51}) or acquired (bla_{OXA23'}, bla_{OXA40} and bla_{OXA58}). The oxacillin enzymes weakly hydrolyse carbapenems. In 8 isolates no gene was detected. Resistance in these strains can be attributed to presence of other mechanism such as defects of porins, decreased expression of outer membrane proteins and alteration in the binding site of penicillin binding protein. Early detection of these genes is useful in identification of carbapenem resistance *Acinetobacter* spp. as we observed that transfer of antimicrobial resistance gene to other bacterium and further spread of these in hospital environment is common. So, Epidemiological surveillance of resistance gene in *Acinetobacter* spp. is essential for outbreak detection. Carbapenem resistance gene are able to hydrolyse most of the β -lactams, including imipenem and meropenem especially in MDR strains. Therefore, detection of carbapenem producing *Acinetobacter* spp. is essential for treatment of patients and for controlling nosocomial spread of these genes. Our aim should be implementations of quality assurance management for infection control.

CONCLUSION

Acinetobacter spp. has been an opportunistic pathogen with high virulence factor in hospital-acquired infections. It is particularly multidrug resistant strain which causes severe infections in patients admitted in various hospitals.

Treatment of such infections is problematic due to their broad resistance to antimicrobial drugs. The early detection of carbapenem resistance gene producing strain, would be necessary to check dissemination of these strains in hospital which will help to reduce mortality rate. This study may help clinicians in prescribing antibiotics by knowing the predominant resistance mechanisms allows for targeted therapy by choosing antibiotics less susceptible to those mechanisms and predict the likelihood of encountering this multidrug-resistant organism in their ICU and adjust their empiric antibiotic choices accordingly. By knowing the molecular diversity of circulating *A. baumannii* isolates in ICU, can inform targeted environmental decontamination and surveillance strategies to prevent further spread within the hospital.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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