

Original Research Article

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Prevalence of ESBL and MBL producing *Acinetobacter* Isolates in Clinical Specimens in Tertiary Care Hospital, Assam, India

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ABSTRACT

Outbreaks of infection caused by strains of *A. baumannii* resistant to multiple antibiotic classes, including carbapenems, are a serious concern in many specialized hospital units, including intensive care units (ICUs). Clinical isolates of *Acinetobacter* species initially retained at least partial susceptibility against the 3rd and 4th generations viz cephalosporins, fluoroquinolones, semisynthetic aminoglycosides, carbapenems and 100% susceptibility to imipenem but the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance. The increase in the number of MBLs in *A. baumannii* is an ominous development in the global emergence of resistance to β -lactams. Keeping this things mind study was carried out to know the prevalence of ESBL & MBL producing *Acinetobacter* isolates in tertiary care hospital. A total of 31 no's *Acinetobacter* isolation was done from clinical specimens from different medical wards, surgical wards and ICU department of GMCH for a period of one year from August 2014--July 2015. Isolated *Acinetobacter* spp were tested for ESBL (Extended spectrum Beta Lactamase) by double disc synergy and MBL (Matelo Beta Lactamase) by imipenem and Imepenem+EDTA E-test method. Out of the total of 31 *Acinetobacter* isolates, MDR isolates found to be 13(41.94%) of which *A.baumannii* 12(92.30%) and *Acinetobacter lwoffii* 1(7.69%). MDR *Acinetobacter* were sensitive to Polymixin B (100%) followed by Tigecycline, colistin (92.85%). Out of the total of 31 *Acinetobacter* isolates, 32.25 % isolates are ESBL producer and 38.7% of isolates were MBL producer and all MBL producers were isolated from ICU. Maximum MDR, ESBL and MBL isolation rates were seen from tracheal aspirates. In our study 32.25 % isolates and 38.7% of isolates were ESBL and MBL producer respectively and all MBL producers were isolated from ICU. Therefore early detection is necessary to screen and confirm beta lactamase mediated resistant strains to avoid treatment failure and prevent the spread of MDR.

Keywords

MDR
A.baumannii,
ESBL producer,
MBL producer,
Matelo Beta
Lactamase

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Introduction

Acinetobacter spp play a significant role in the colonization and infection of patients admitted to hospitals (Bergogne-Berezin *et al.*, 1996). *Acinetobacter* is a Gram Negative

Coccobacillus (Munoz-Price *et al.*, 2008; Schreckenberger *et al.*, 2007). The organism is ubiquitous in nature; its ability to survive in varying temperatures, pH conditions, and

on dry, moist surfaces helps in the transmission and propagation of this organism in the hospital setting (Health care associated infection; Manchanda *et al.*, 2010).

It typically colonizes skin, the respiratory, urinary, gastrointestinal tract wounds and indwelling plastic devices of the hospitalized patients can cause infections in burn, trauma, mechanically ventilated and immunocompromised patients. It shows a special predilection for the ICU (Patwardhan *et al.*, 2008).

Clinical isolates of *Acinetobacter* species initially retained at least partial susceptibility against the 3rd and 4th generations viz cephalosporins, fluoroquinolones, semisynthetic aminoglycosides, carbapenems and 100% susceptibility to imipenem (Montefour *et al.*, 2008). In addition, intrinsic resistance and multi-drug resistance (MDR) pose a global medical challenge (Li *et al.*, 2007). Carbapenems which were once the mainstay of therapy are no longer effective in controlling the infections caused by this organism. Although these drugs are still active against the vast majority of *A. baumannii* strains worldwide, the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane-based mechanisms of resistance. The increase in the number of MBLs in *A. baumannii* is an ominous development in the global emergence of resistance to β -lactams. Rasmussen and Bush (Rasmussen *et al.*, 1997) reported, carbapenem resistance can be due to acquired carbapenemase production. Carbapenem resistance is mainly due to either reduced levels of drug accumulation or increased expression levels of the pump efflux. Other risk factors responsible for colonization and infection with MBL producers include age of patient,

duration of hospitalization, underlying diseases like diabetes, tumours or overcrowding in the hospital wards. Heritier *et al.*, (2005) reported that *Acinetobacters* may develop resistance to carbapenems through various combined mechanisms, including AmpC stable depression, decreased permeability, altered penicillin-binding proteins (PBPs) and, rarely, efflux pump over expression.

Outbreaks of infection caused by strains of *A. baumannii* resistant to multiple antibiotic classes, including carbapenems, are a serious concern in many specialized hospital units, including intensive care units (ICUs) (David *et al.*, 2012). The foremost implication of infection with carbapenem-resistant *A. baumannii* is the need to use "last-line" antibiotics such as colistin, polymyxin B, or Tigecycline.

The main objectives of this study prevalence of ESBL & MBL producing *Acinetobacter* isolates in tertiary care hospital.

Materials and Methods

A total of 31 no's *Acinetobacter* isolation was done from clinical specimens endo-tracheal aspirate and bronchoalveolar lavage, Blood /catheter tips, Pus / wound swab, Body fluids (Pleural fluid, ascetic fluid and cerebrospinal fluid etc) collected from different medical wards, surgical wards and ICU department of GMCH for a period of one year from August 2014--July 2015.

Samples were collected from patients of all age groups. The study was commenced with ethical approval and clearance certificate from the IEC, GMCH. Species identification was done by KBO14 and by Vitek2 compact. Antimicrobial susceptibility of isolated *Acinetobacter spp* were tested by modified Kirby-Bauer disc diffusion method

as per the recommendation of Clinical and Laboratory Standard Institute (CLSI) and VITEK 2 compact AST 281) as per guideline.

Acinetobacter baumannii ATCC19606 and *Acinetobacter lwoffii* ATCC 15309 were used as quality control strains. VITEK 2 compact –identification and sensitivity testing confirmation done by (GN card and AST 281) as per guideline.

The isolates were tested against antibiotics Table 1. Isolates showing resistance to at least three categories of drugs i.e. penicillins and cephalosporins, fluoroquinolones, and aminoglycosides, were considered multi-drug resistant (Abbo *et al.*, 2005). Extensive drug resistant (XDR) *Acinetobacter* were isolates displaying resistance to carbapenems in addition to resistance to penicillins and cephalosporins, fluoroquinolones, and aminoglycosides. Pan resistant *Acinetobacter spp.* was defined as *Acinetobacter* isolate that is resistant to the whole panel of antibiotics (Manchanda *et al.*, 2010).

Isolated *Acinetobacter spp.* were tested for ESBL (Extended spectrum Beta Lactamase) by double disc synergy and MBL (Metallo Beta Lactamase) by imipenem and Imipenem+EDTA E-test method.

Phenotypic detection of ESBL

Ceftazidime-resistant isolates were screened for producing ESBL. The double disk method was used for detection of this enzyme. Then the suspension was streaked onto Mueller-Hinton agar plates (Hi Media, Mumbai, India). A disc of either ceftazidime (30 µg) or cefotaxime alone (30µg) in combination with clavulanic acid (30µg/10 µg) was placed at the distance of 20 mm (centre to centre). After incubation overnight at 35°C, a positive test result was

considered as a 5 mm increase in inhibition halo compared with a disk without clavulanic acid.

Phenotypic detection of MBL

Phenotypic detection of MBL Imipenem-resistant isolates were screened for producing MBL. The double disk method was used to detect this enzyme.

Imipenem-resistant isolates were screened for producing MBL. Merpenem (MRP) with or without EDTA Ezy MIC™ strip (EM 092) method was used to detect this enzyme. Colonies from overnight cultures on blood agar plates were suspended in peptone water and adjusted 0.5 McFarland standard. Then the suspension was streaked onto Mueller-Hinton agar plates (HiMedia, Mumbai, India).

After incubation overnight at 35°C, a positive test result was considered as a MRP/MRP+EDTA ≥ 8 ; MBL positive strain.

Results and Discussion

Out of the total of 31 *Acinetobacter* isolates, *Acinetobacter baumannii* was the predominant species 28 (90.32%) isolated, followed by *Acinetobacter lwoffii* 2 (6.45%) and *Acinetobacter hemolyticus* 1 (3.23%). In the present study, the highest number of isolates 32.26 % (10) in age group 21-40 years and 51.61 % (16) *Acinetobacter spp.* isolated in male. *Acinetobacter* species isolated from patients in ICU was 80.65% (25) and other Non ICU hospitalized patients showed lower isolation rates 19.35%. Out of the total of 31 *Acinetobacter* isolates, MDR isolates found to be 13 (41.94%) of which *A.baumannii* 12 (92.30%) and *Acinetobacter lwoffii* 1 (7.69%). MDR *Acinetobacter* were sensitive to Polymixin B (100%) followed by

Tigecycline, colistin (92.85%) Minocycline and cefoperazone sulbactam (Table-1).

Out of the total of 28 *A.baumannii* 13 (46.43%) found to be ceftazidime and cefepime Resistant and Carbapenem resistance found in 42.85% *A.baumannii*. Out of the total of 31 *Acinetobacter* isolates, 32.25 % (10) isolates are ESBL producer and 38.7% (12) of isolates were MBL producer and all MBL producers were isolated from ICU.

In the present study, out of 31 *Acinetobacter* spp, the predominant species was *A. baumannii* (90.32%) followed by *A.lwoffii* (6.45%) and *A.hemolyticus* (3.23%). Similarly, Rahul Kamble *et al.*, (2012) isolated (87.2%) *A.baumannii* followed by *A. haemolyticus* (9.3%) and *A. lwoffii* (3.5%). Similarly by Sinha *et al.*, reported *Acinetobacter baumannii* as the predominant species (92.14%), while *Acinetobacter lwoffii* (6.42%) and

Acinetobacter haemolyticus (1.42%). Kalidas Rit *et al.*, 2010; Major infections found in different medical wards, surgical wards and ICU were due to *Acinetobacter baumannii* (74.02%), *A. lowfii* (14.2%), *A. haemolyticus* (7.79%), *A. junii* (3.8%) among *Acinetobacter* species. Predominance *A.calcoaceticus baumannii* complex is perhaps its non fastidious natures and higher prevalence in hospital environment. *Acinetobacter* spp. have emerged as particularly important organisms in intensive care units (ICUs), and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs in recent years.

In our study, *Acinetobacter* species isolated from patients in ICU most frequently (80.65%) and *A.baumannii* was the predominant species similar to study done by Sharma *et al.*, (2015) and Muntasir I. Omer *et al.*, (2014).

Table.1 Antimicrobial susceptibility of *A.baumannii* isolated from infected patients

Antimicrobial Agent	Breakpoint(micr/ml)MIC	Resistant % (<i>A.baumannii</i> n=28)
Amikacin	s<=16 R>=64	42.85
Gentamycin	s<=4 R>=16	42.85
Aztreonam	s<=8 R>=32	60.71
Ceftazidime	s<=8 R>=32	46.43
Cefepime	s<=8 R>=32	46.43
Cefoperazone+Sulbactum	s<=32 R>=64	14.29
Doripenem	s<= 2 R>=8	42.85
Imipenem	s<=4 R>=16	42.85
Meropenem	s<=4 R>=16	42.85
Ciprofloxacin	s<=1 R>=4	50
Levofloxacin	s<=2 R>=8	46.43
Minocycline	s<=8 R>=16	14.29
Tigecycline	s<=4 R>=8	3.57
Colistin	s<=4 R>=16	7.14
Cotrimoxazole	s<= 20 R>=320	42.85
Polymixin B	s<=2 R>=4	0

Table.2 Sample wise MDR *Acinetobacter* distribution

Origin of sample	<i>Acinetobacter</i> spp	
	Total n=31	MDR n=13
Respiratory	14	11(84.61%)
Wound swab	5	2(15.38%)
Blood	4	0
Urine	6	0
Body fluid	2	0

Fig.1 Distribution of MDR *Acinetobacter* in ICU and INDOOR

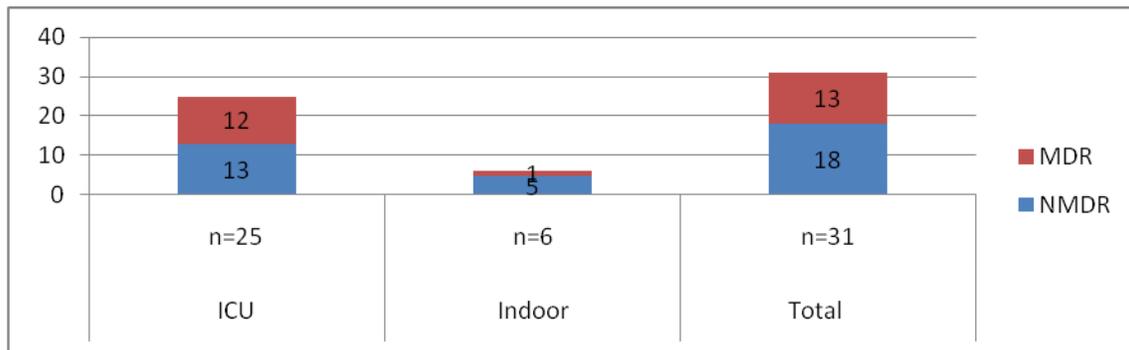


Fig.2 Prevalence of ESBL and MBL *Acinetobacter*

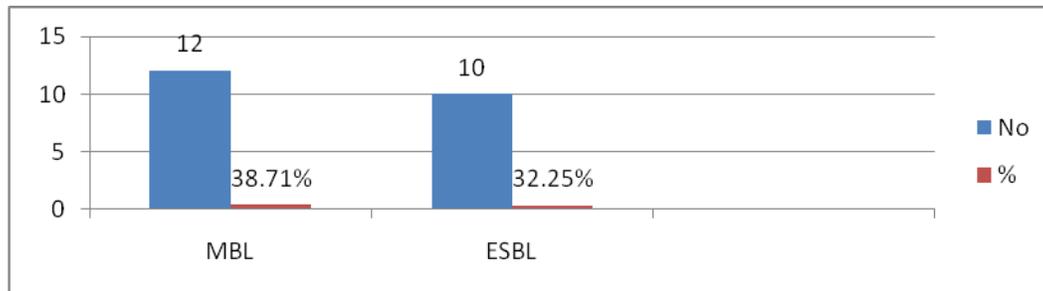
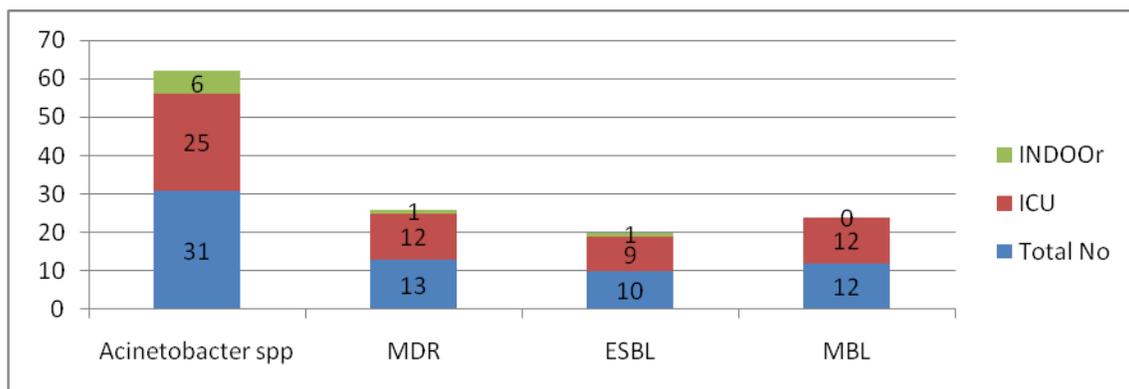


Fig.3 Distribution of ESBL and MBL *Acinetobacter* spp



This result corroborates the fact that a lot of risk factors associated with *Acinetobacter* infection exist in the ICU like potential environmental reservoirs for *A.baumannii*, opportunities for cross transmission, sick, immunocompromised patients who are colonized, patients having multiple wounds and indwelling devices, heavy use of broad spectrum antibiotics and frequent contamination of the hands of health care workers while patient care.

In the present study, *Acinetobacter* isolates, 32.25 % (10) isolates are ESBL producer and 38.7% (12) of isolates were MBL producer. Similarly ESBL production was detected in 28 per cent of the isolates in a study done by Sinha *et al.*, In a study done at AIIMS, New Delhi, 48.72% of *A.baumannii* strains were ascertained to be MBL- enzyme producers by the same method, thus implying rapid spread of resistance amongst this pathogen. In another study done by Neetu Gupta *et al.*, (2015), 31.5% of *Acinetobacter* species were ESBL producer by the DDST and 14.4% were MBL producers by the combined disc diffusion test.

Maximum MDR, ESBL and MBL isolation rates were seen from tracheal aspirates followed by wound swabs. High isolation of *Acinetobacters* in respiratory samples is perhaps due to its ability to survive in varying temperatures, pH conditions, and on dry, moist surfaces which helps in the transmission and propagation of this organism in the hospital setting.

In conclusion, *A.baumannii* is becoming a global medical challenge due to the emergence of multi-drug resistance. In our study 32.25 % isolates and 38.7% of isolates were ESBL and MBL producer respectively and all MBL producers were isolated from ICU. β -lactamase mediated resistance

mechanisms are accounting very high in the multidrug resistant isolates of *Acinetobacter* species. Therefore early detection is necessary to screen and confirm beta lactamase mediated resistant strains to avoid treatment failure and prevent the spread of MDR. Thus, due to such high prevalence of resistance, antibiotics must be used judiciously by the clinicians and appropriate infection control measures need to be implemented to control the spread of infections in hospitals.

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