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A Microbiological Study of *Acinetobacter baumannii* with Special Reference to Multi-Drug Resistance

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A B S T R A C T

Acinetobacter calcoaceticus baumannii (ACB) is a significant pathogen in hospitals with a growing incidence of multi-drug resistant (MDR) strains. This was a prospective study carried out on ACB isolates from clinical samples between 2013 and 2015. Anti-microbial susceptibility testing was done by Kirby-Bauer Disk Diffusion method (KBBD) method. CRAB strains were tested by Modified Hodge test (MHT) to detect MBL producers. Of the 190 ACB isolates, 142 (75%) were MDR strains. 93% were hospital acquired and 7% community acquired. MDR was seen only in hospital acquired isolates. *Acinetobacter* was most commonly isolated from ICU (51.41%); followed by medicine ward and surgical wards. 34% of the isolates were from endotracheal tubes which were followed by sputum (14%), pus (13%), blood (10%), suction catheter (7%) and urine (5%). The maximum number of isolates was seen in patients above 60 years of age (28.94%). Among all samples, 120 (63%) were CRAB strains of which 63 out of 120 (53%) were found to be MBL producers by Modified Hodge test (MHT). A high incidence of MDR was seen in ACB from samples of hospitalized patients. MHT is an economical test which can be included in routine testing for detection of MBL phenotypes.

Introduction

Acinetobacter baumannii is an important nosocomial pathogen ranked second after *Pseudomonas aeruginosa* among aerobic non-fermentative gram-negative bacilli (Schreckenberger *et al.*, 1999; Simor *et al.*, 2002). It causes respiratory and urinary tract infections, meningitis, endocarditis, burn infections, and wound sepsis, especially in intensive care units (ICUs) (Chastre *et al.*, 2000). *A. baumannii* infections are often difficult to eradicate due to high-level resistance to many antibiotics as a result of intrinsic and acquired mechanisms (Yong *et al.*, 2003) Carbapenems (e.g., imipenem and meropenem) have become the drugs of choice against *Acinetobacter* infections in many centers but are being compromised by the emergence of carbapenem-hydrolyzing β-lactamase (carbapenemase) of molecular classes B and D (Livermore *et al.*, 2002). Therefore studies of epidemiology and antibiotic resistance are necessary to help in treatment of infections.

The detection of carbapenemase production is complicated because some carbapenemase-producing isolates demonstrate elevated yet susceptible Carbapenem minimum inhibitory concentration (MIC). CLSI has published guidelines for detection of isolates producing carbapenemase (CLSI document M100).

Various methods like, EDTA disk synergy (EDS) test, MBL E-test, EDTA-based microbiological assay are used for detection of MBLs (Noyal *et al.,* 2009). For isolates that test susceptible to a Carbapenem but demonstrate reduced susceptibility either by disk diffusion or MIC testing, performing a phenotypic test for carbapenemase activity, the Modified Hodge Test (MHT), is recommended (Calfee *et al.,* 2008; Wayne *et al.,* 2009; Deshpande *et al.,* 2006; Siegel *et al.,* 2007).

This study was taken up to isolate and identify the *A. baumannii* from various clinical samples and to determine their Carbapenemase activity by MHT.

The present study aims at:

To detect the prevalence rate of *A. calcoaceticus baumannii* (ACB) infections from various clinical samples.

To find out the antimicrobial resistance pattern of *A. baumannii*.

To find the incidence of MBL production amongst CRAB (Carbapenem-resistant *Acinetobacter baumannii*).

Materials and Methods

The study was carried out in the Department of Microbiology and MGM Central Research Laboratory at MGM Medical College and Hospital, Kamothe, Navi Mumbai, for 2 years duration (December 2013 to December 2015).

190 *Acinetobacter baumannii* isolates obtained from various clinical samples including endotracheal tube, sputum, pus, blood, suction catheter tip, tracheostomy tube, urine, tissue and Foley's catheter tip etc. were included in the study. *Acinetobacter baumannii* were identified using standard microbiological procedures. The Kirby-Bauer Disk Diffusion method (KBBD) was carried out for antimicrobial susceptibility testing as per CLSI guidelines 2014. All Carbapenem-resistant *Acinetobacter baumannii* (CRAB) strains were tested by Modified Hodge test (MHT) for detection of MBL producers.

All the isolates were tested for Carbapenemase production by MHT.

Modified Hodge test

Procedure

0.5 McFarland dilution of the *E. coli* ATCC 25922 in 5 ml of Broth or saline was prepared. A lawn of the 1:10 dilution of *E. coli* ATCC 25922 was streaked on a Mueller Hinton agar plate and allowed to dry for 3–5 minutes. A 10 µg Imipenem, disc was placed in the centre of the plate. In a straight line, test organism from the edge of the disc to the edge of the plate was streaked.

Plates were incubated overnight at 35°C ±2°C for 16–24 hours. After 16–24 hours of incubation, the plates were examined for a clover leaf-type indentation at the intersection of the test organism and the E. coli 25922, within the zone of inhibition of the Carbapenem susceptibility disc. MHT Positive test has a clover leaf-like indentation of the *E. coli* 25922 growing along the test organism growth streak within the disc diffusion zone. MHT Negative test has no growth of the *E. coli* 25922 along the test organism growth streak within the disc diffusion (CDC-Center of disease control).

Results and Discussion

In our study, out of the 190 ACB isolates, 142(75%) were multidrug resistant. 93% were hospital acquired and only 7% were community acquired. Among the in-patients, *Acinetobacter* was most commonly isolated from ICU (51.41%) which include medicine ICU (32%), surgical ICU (9.60%), neonatal ICU (9.60%), followed by medicine ward (13%), neurosurgery ward (12%), general surgery ward (11%), orthopaedics ward (5.64%) and cardiothoracic ward (3.38%). 34% of the isolates were isolated from endotracheal secretion which were followed by sputum (14%), pus (13%), blood (10%), suction catheter (7%) and urine (5%) samples.

MDR was seen only in hospital acquired isolates. Among all samples, 120 (63%) were CRAB strains of which 63 out of 120 (53%) were found to be MBL producers. The Modified Hodge test (MHT) was used by us to detect carbapenemase activity. It is easily available in clinical microbiology routine settings and recommended by the CLSI for phenotypic detection of carbapenemase. Maximum ACB isolates showed resistance to cephalosporins whereas least resistance was seen to fluoroquinolones.

Mahajan *et al.,* (2012), showed highest isolation being from the ICU (51.61%), surgery (21.5%), paediatrics (10.2%), orthopaedics (7.5%) and medicine (5.7%). In the study done by Prashanth and Badrinath (2006), the highest (42%) isolates were from ICU, medicine (37%), paediatrics (23%), surgery (21%) and orthopaedics (2.3%) wards. Also in a study by Jaggi *et al.,* (2012), the proportion of *A. baumannii* isolates was higher in the ICU (76.7%) as compared to the in-patient wards (18.7%) and OPD (4.5%) pointing towards the fact that *A. baumannii* is a predominantly ICU bug. Our 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, immune compromised 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.

However some other studies by Lahiri *et al.,* (2004) and Mindolli *et al.,* (2010) showed different results from our study, as they found highest isolation from surgical ward (Table 1 and 2).

In our study the highest number the isolates (34%) were from endotracheal secretions, followed by sputum (14%), pus (13%), blood (10%), suction catheter (7%), tracheostomy tube (6%), urine (5%), tissues (4%), Foley's catheter tip (2%) etc (Table 3). Peymani *et al.,* (2011) also showed similar findings, where maximum isolates were from ET (37%). Mindolli *et al.,* (2010) in contrast, found only 3.5% isolates from ET and a maximum of 29% from pus samples.

In our study, the maximum no. of isolates were from patients above 60 years of age (28.94%), followed by 21-30 years (17.36%), 31-40 years (14.21%) and least were from the paediatric age group of 2-10 years of age (2.10%). Among (0-1) years age group, maximum isolates (12.10%) were seen in new born babies with endotracheal tubes who were all preterm. This showed that presence of endotracheal tubes and preterm birth was predisposing factors for developing ACB infection in neonates (Table 4). Mahajan *et al.,* (2012), found different results where, 10.75% isolates were isolated in age < 17 years, 22.58% in age between 18-35 years, 41.93% in 36-60 years of age groups and 24.72% of growth in age > 60 years.

Table.1 Location wise isolation of *Acinetobacter* species (n=190)

Location	Isolation	Percentage
Out patient	13	6.8%
In patient	177	93.2%
Total	190	100

Table.2 Inpatient Distribution of Isolation of *Acinetobacter* species (N= 177)

Ward	Isolation	Percentage
ICU	91	51.41
Medicine	23	12.99
Neuro Surgery	21	11.86
Surgery	19	10.73
Orthopedics	10	5.64
CVTS	6	3.38
Others	7	3.95
Total	177	100

Table.3 Clinical specimens showing isolation rates from different clinical samples

Types of samples	Isolates of *Acinetobacter*	Percentage
Endrotracheal tube	64	33.68
Sputum	26	13.68
Pus	25	13.15
Blood	20	10.52
Suction Catheter tip	13	6.84
Tracheostomy tube	11	5.78
Urine	7	3.68
Tissue	8	4.21
Foley's catheter tip	4	2.10
Swab from suture site	4	2.10
Others	8	4.21
Total	190	100

Table.4 Age wise isolation of *Acinetobacter baumannii*

Age group	Isolation	Percentage
0-1	23	12.10
2-10	4	2.10
11-20	10	5.26
21-30	33	17.36
31-40	27	14.21
41-50	20	10.52
51-60	18	9.47
>60	55	28.94

Table.5 Antibiotic resistance pattern

Name of Antibiotics	Conc (mcg)	Resistance in Hospitalized (n=177)	Resistance in non-Hospitalized (n=13)	Resistance in Hospitalized (%)	Resistance in non-Hospitalized (%)
Amikacin (AK)	30	136	02	76.83	15.38
Ciprofloxacin (CIP)	05	125	04	70.62	30.76
Ceftotaxime (CTX)	30	148	06	83.61	46.15
Cefuroxime (CXM)	30	164	07	92.65	53.84
Augmentin (AMC)	30	168	07	94.91	53.84
Lomefloxacin (LOM)	30	123	03	69.49	33.33
Ceftazidime (CAZ)	30	157	04	88.70	30.76
Cefeperazone (CPZ)	75	145	03	81.92	23.07
Gentamicin (GEN)	10	138	03	78.37	23.07
Neticillin (NET)	30	76	01	42.93	7.69
Perfloxacin (PF)	5	95	00	53.67	00
Ofloxacin (OF)	5	35	00	19.77	00
Imipenem (I)	10	107	00	60.45	00
Meropenem (MRP)	10	117	01	66.10	7.69
Cefepime (CPM)	30	158	02	89.26	15.38
Ticarcillin + Clavulanic acid (TCC)	75/10	145	01	81.92	7.69
Piperacillin + Tazobactam (PIT)	100 /10	156	02	88.13	15.38
Ceftriaxone (CTR)	30	167	02	94.35	15.38
Cefoperazone + Sulbactum (CFS)	50/ 50	161	01	90.96	7.69

Table.6 Percentage of MDR in Hospitalized and Non- Hospitalized patients

Total no of ACB	MDR ACB in Hospitalized (n=177)	MDR ACB in non-Hospitalized (n=13)	Percentage (%)
190	**142**	**0**	**75%**

Table.7 Incidence of Carbapenem-resistant *Acinetobacter baumannii among Acinetobacter baumannii* isolates

Total ACB	Carbapenem –resistant *Acinetobacter baumannii* (CRAB)	Percentage (%)
190	**120**	**63%**

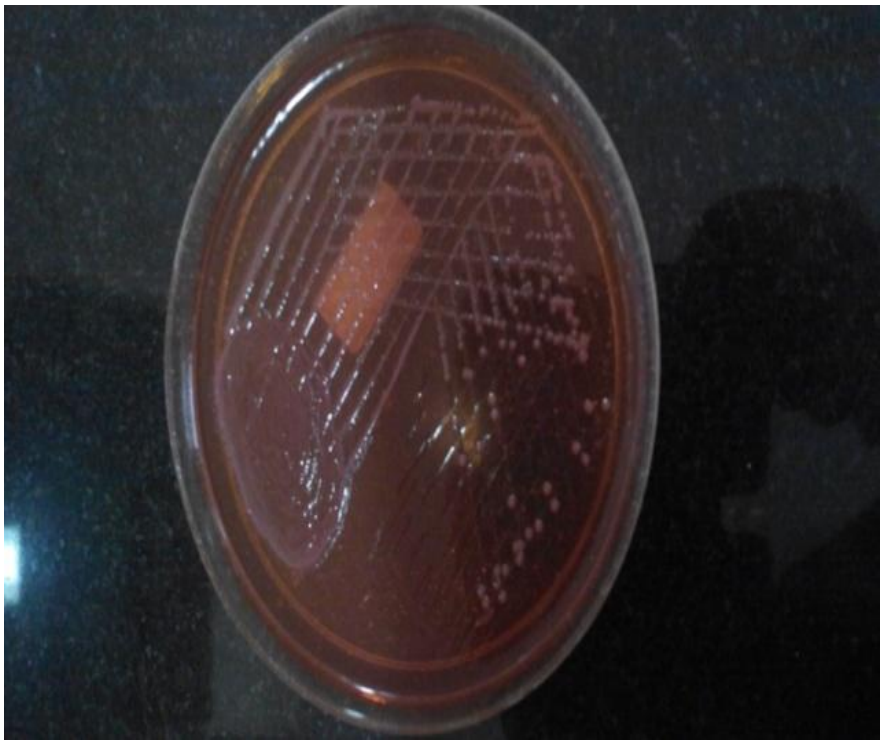
Table.8 Incidence of MBL production amongst CRAB (Carbapenem-resistant *Acinetobacter baumannii)* by Modified Hodge Test (MHT)

Carbapenem –resistant *Acinetobacter baumannii* (CRAB)	MBL Producers By MHT	Percentage (%)
120	63	53%

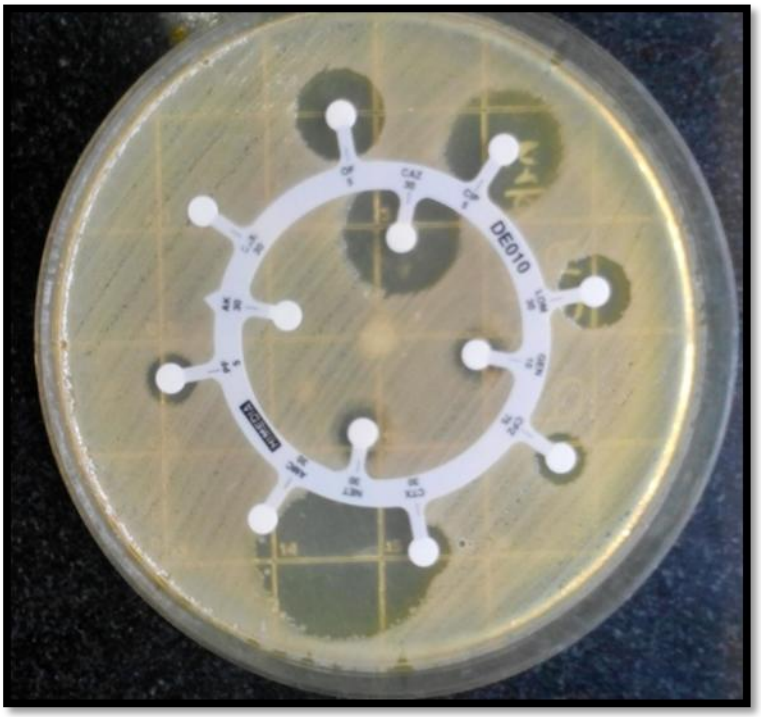
Table.9 Comparison of the clinical specimens yielding ACB isolates among various series

Sample	Present study	Peymani *et al.*, (2011)	Mindolli *et al.*, (2010)	Dent *et al.*, (2010)
ET tube	34%	37%	3.5%	-
Urine	5%	21%	28%	16%
Sputum	14%	9%	25%	31%
Blood	10%	7%	14%	10%
Pus	13%	8%	29%	13%
Body fluids	4%	6%	0.5%	-
Catheter tips	2%	6%	-	9%

Photograph.1 Growth of ACB on MacConkey agar



Photograph.2 Kirby Bauer Disc Diffusion Test



Photograph.3 Modified Hodge Test

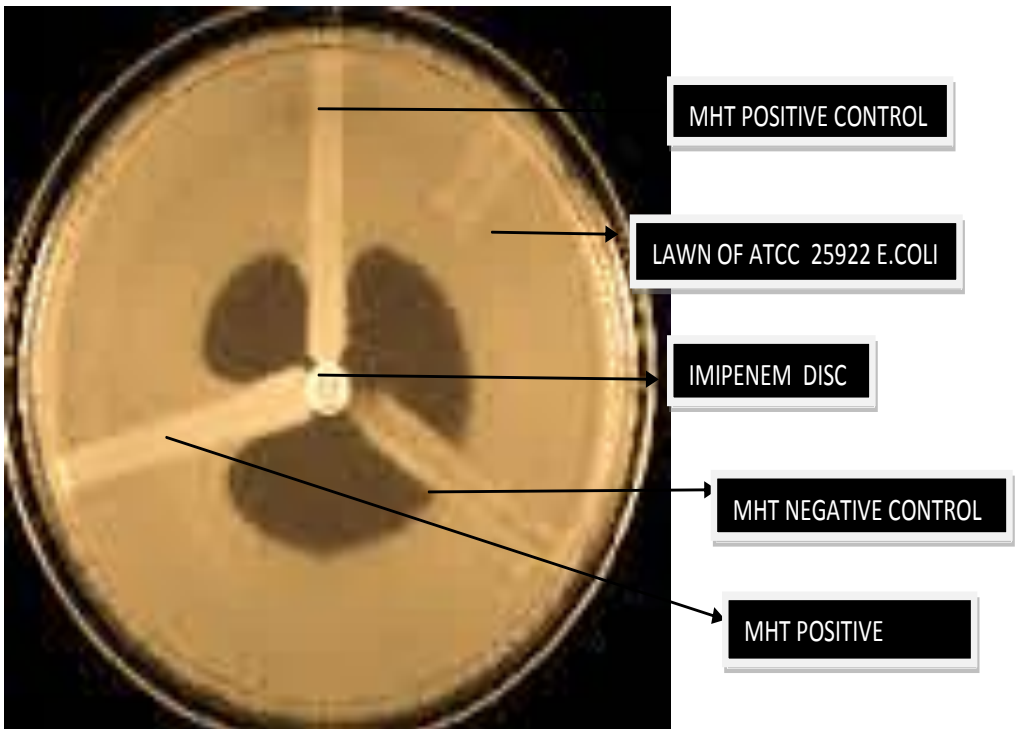


Fig.1 Pie chart showing the distribution of ACB isolates between in-patients and out-patients

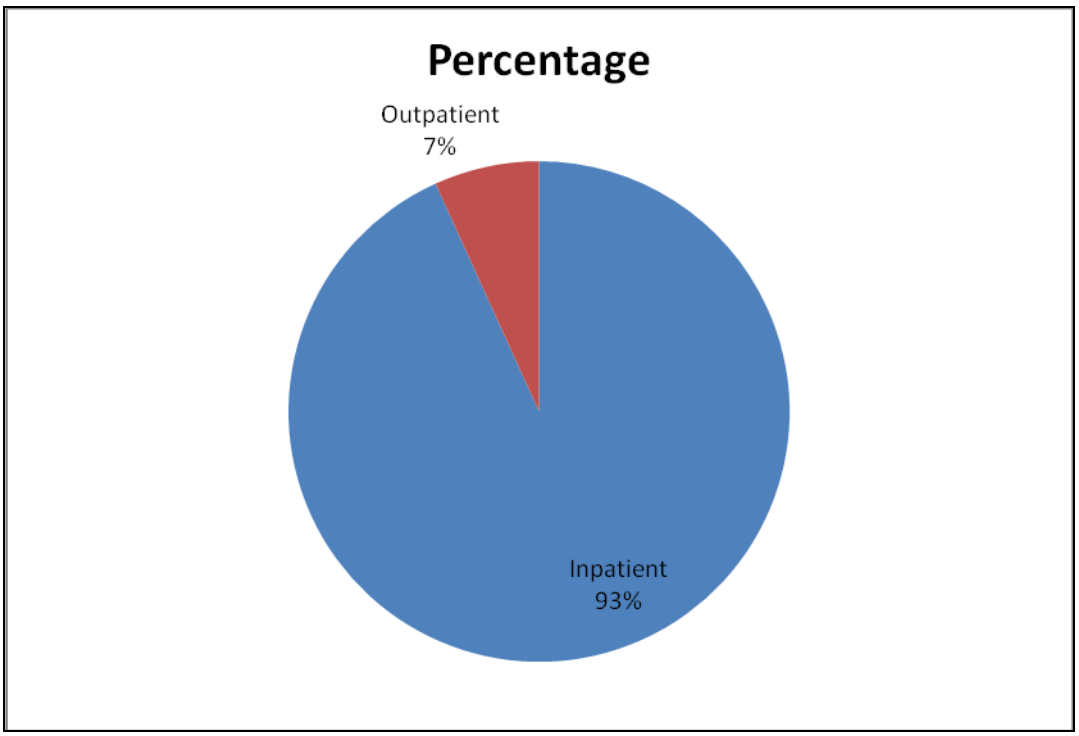


Fig.2 Pie chart showing the distribution of ACB isolates in various wards and ICU

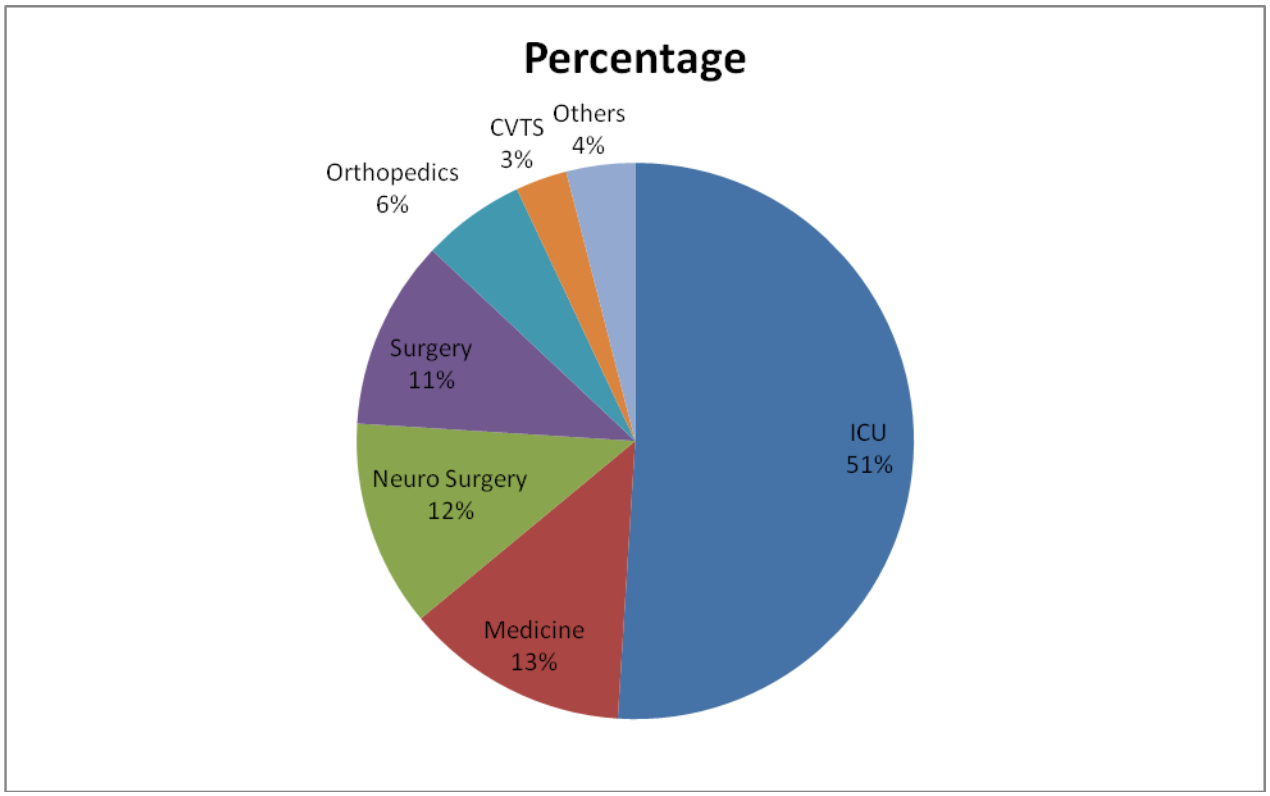
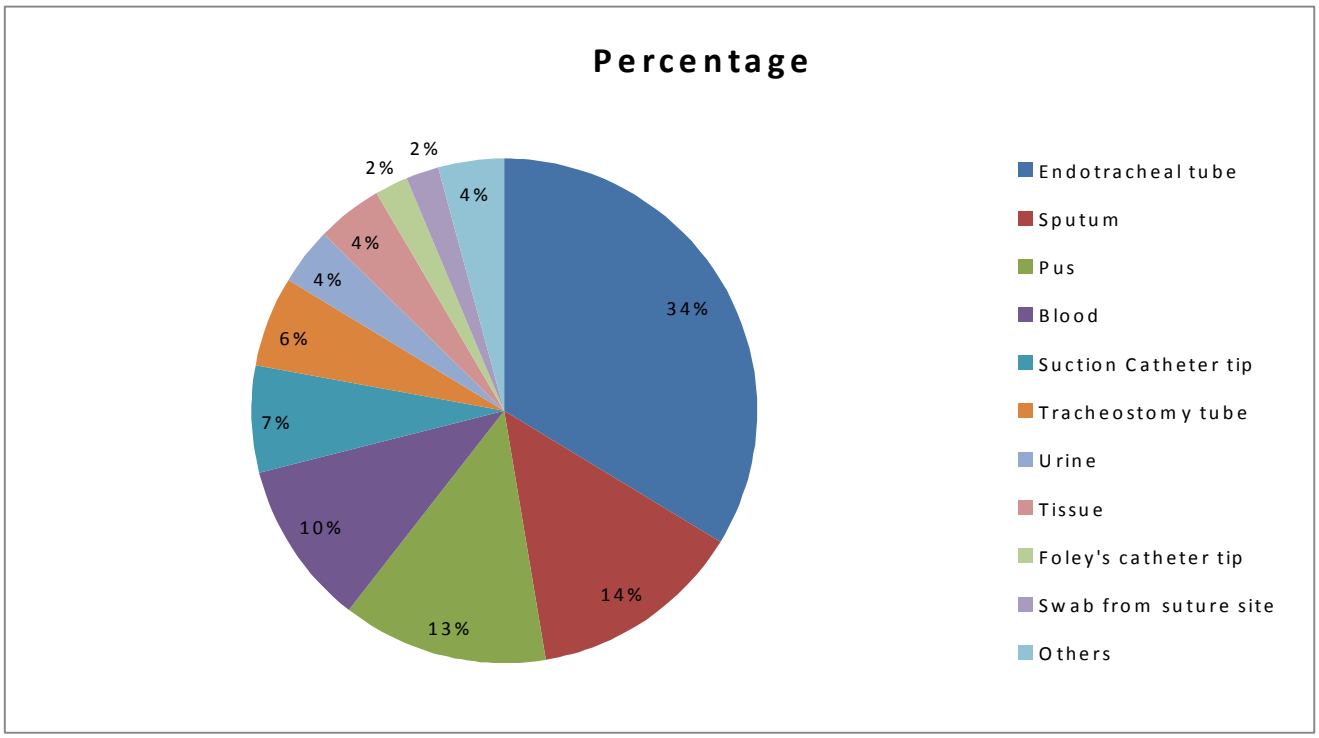


Fig.3 Pie chart showing the ACB isolates from various clinical specimens



In contrast, the study done by Prashanth and Badrinath (2006), showed 60.47% isolates of *Acinetobacter* in age > 40 years and 39.53% in age < 40 years, which is comparable with findings of our study.

In our study, *Acinetobacter* showed high degree of resistance (>80%) to Cephalosporins (90% to cefuroxime, 84.73% to ceftazidime, 81% to cefotaxime, 77.89% to cefaperazone), to piperacillin + tazobactum (83%), to gentamicin (74.21%), and to netillin (40.52%) and least resistance to fluroquinolones, which included 18.42% to ofloxacin and 50% to perfloxacin. The organism was found to be highly resistant to Carbapenems (56.31% resistant to imipenem and 62.10 % resistant to meropenem) (Table 5). This is different from findings of Mindolli, PB *et al.*, (2010) which showed lowest resistance to Carbapenems (9.5%) and piperacillin + tazobactum (9.5%) and high resistance to Ofloxacin (73.5%). Jaggi *et al.*, (2012), showed findings similar to our study. More than 85% isolates were resistant to cephalosporins, 90% resistant to piperacillin + tazobactum and 89% resistant to carbapenems. Muthusamy and Boppe (2012) found 100% resistance to carbapenems and 99% resistance to cephalosporins. Peymani *et al.*, (2011) found that the resistance to Piperacillin+tazobactum was 89%, Ticarcillin+clavulanic acid 83%, Augmentin 89%, Ceftazidime 92%, Cefepime 88%, Ceftriaxone 94%, Meropenem 56%, Imipenem 54%, Gentamicin 86%, Amikacin 81% and Ciprofloxacin 86%. These findings were similar to the results of our study. This shows that the extensive use of carbapenems has created a selective antibiotics pressure which in turn has resulted in increased prevalence of carbapenems resistant *Acinetobacter baumannii* (CRAB).

Our study found that 63% of ACB isolates were CRAB strains out of which 53% were

MBL producers. Amudhan *et al.*, (2011) have found that out of a total of 116 consecutive, non-duplicate carbapenems resistant *A. baumannii* isolates from various clinical specimens 113 (97%) were MBL producers. Niranjan, DK. *et al.*, (2013), reported that out of a total of 30 imipenem resistant, consecutive non-repeat clinical isolates of *A. baumannii* at a tertiary hospital in Delhi, all but one were MBL producer, as identified by the modified Hodge test (MHT). Khajuria *et al.*, (2014), have found MBL producers to be 60% (using the MHT) in their 368 isolates, which included 155 (42.11%) with reduced susceptibility to imipenem (Fig. 1–3; Table 6–9).

A. baumannii accounts for a substantial proportion of endemic nosocomial infections. Multidrug resistance is being increasingly reported in these pathogens and posing a threat to hospitalized patients due to the limitation of therapeutic options. The acquisition of multidrug resistance is related to environmental contamination and contact with transiently colonized health care providers. Carbapenems have been the drug of choice for treatment of infections caused by *A. baumannii*. However, in recent years, the number of isolates showing resistance to carbapenems has increased worldwide. This is mediated by the lack of drug penetration (i.e. porin mutations and efflux pumps) and/or carbapenem-hydrolyzing beta-lactamase enzymes such as OXA carbapenamases and metallo-beta-lactamases.

There is high incidence of multi-drug resistance seen in *Acinetobacter calcoaceticus baumannii* (ACB) isolates acquired from samples of hospitalized patients. Modified Hodge test, which is easy to carry out and not a very expensive one, can be included in routine testing for detection of MBL phenotypes. This will go a long way in early detection and help control infections due to this highly resistant organism.

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