# Extent of antimicrobial resistance in Acinetobacter species in a Tertiary Care Teaching Hospital

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## Abstract
*Acinetobacter*, gram negative coccobacillus, has become a frequent pathogen in hospitals and other health care settings. *Acinetobacter* species cause a wide variety of illness in debilitated and hospitalized patients especially in intensive care units (ICU). Carbapenems constitute the backbone of treatment of complicated *Acinetobacter* infections. However, resistance to Carbapenem is established and observed globally, leading to limited therapeutic options. The study was designed to understand the extent of resistance in *Acinetobacter* species in our hospital which is located in an island separated from mainland India. Isolates showing resistance to either Imipenem (IMP) and/or Meropenem by disc diffusion method was considered as Carbapenamase producing and further subjected to identification by phenotypic methods. Of the 160 isolates, 111 were resistant to either Imipenem and/or Meropenem. The susceptibility patterns of antibiotics tested suggests high resistance to 3rd/4th generation Cephalosporins (CTR 93.7%, CAZ 88.29%, CPM 85.59%) and least resistance to Colistin and Polymyxin B. MHT alone was positive in 66.98% isolates suggesting production of OXA type class D β-lactamase and DDST alone was positive in 7.55% isolates suggesting production of Metallo-β-lactamase /Group B β-lactamase. The present study adds to the literature available in respect to increasing instances of Carbapenem resistance and their presumptively mechanism of resistance.

**Keywords:** Antimicrobial, Resistance, Carbapenem, Acinetobacter.

## Introduction
*Acinetobacter*, gram negative coccobacillus, has become a frequent pathogen in hospitals and other health care settings.[1] The genus *Acinetobacter* was created in 1957 to include all non- motile Achromobacter species. It was not until 1971, that genus *Acinetobacter* was officially recognised following comparative biochemical studies by Baumann, leading to the establishment of type species *Acinetobacter calcoaceticus*. There are 34 species in the genus *Acinetobacter* recognised after DNA- DNA Hybridization technique and DNA sequencing analysis. The often quoted *A. calcoaceticus-A. baumannii* complex refers to the 4 species, *A. baumannii, A. calcoaceticus, A. pittii* (previously genomic species 3) *and A. nosocomialis* (previously genomic species 13TU), which can be distinguished only by using molecular techniques.[2] *Acinetobacter baumannii* has been extensively studied and is an established nosocomial pathogen. However *A. pittii* and *A. nosocomialis* are increasingly identified as causative agents of nosocomial infections, accounting for 29% bacteremia in the USA, 24-25% in Taiwan, 50% in Korea and 66% in Norway.[3] *Acinetobacter* species cause a wide variety of illness in debilitated and hospitalized patients especially in intensive care units (ICU). These bacteria survive for long time in the hospital environment, and there by the opportunity of cross infection are enhanced[4]. More recently, the pathogens have spread to other locations in the hospital and to non hospital populations and health care settings.[1]

Carbapenems constitute the backbone of treatment of complicated *Acinetobacter* infections. However, resistance to Carbapenem is established and observed globally, leading to limited therapeutic options.[5] The incidence of multi drug resistant(MDR) *Acinetobacter* species, defined as those strains that are resistant to three or more classes of antibiotics, has increased.[1,6] Several mechanism are responsible for resistance to Carbapenems in *Acinetobacter* species. These are changes in penicillin binding proteins, reduced outer membrane permeability and predominantly, production of Carbapenem hydrolysing enzymes like oxacillinases or Carbapenem hydrolysing class D Beta lactamases(CHDL) and Metallo-β lactamases.[5]

The study was designed to understand the extent of resistance in *Acinetobacter* species in our hospital which is located in an island separated from mainland India.

## Materials and Methods
The study was conducted in the Clinical Microbiology laboratory of Medical college hospital during the period April 2015-March 2016. One hundred and sixty isolates of *Acinetobacte*r species, obtained from equal number of patients, were considered for the study. These isolates were obtained from different clinical samples like respiratory secretion, sputum, blood, body fluid and urine. *Acinetobacter* was presumptively identified based on Grams stain morphology, motility and oxidase reaction. A non-fermenting, non-motile and oxidase negative isolate is presumptively identified as Acinetobacter species.

Further identificationto the species level is based on glucose oxidation test, growth at 37° C and 44° C, haemolysis on sheep blood agar, gelatin hydrolysis, arginine dihydrolase activity test.[7]

Antimicrobial susceptibility of the isolates were screened by Kirby-Bauer disc diffusion method using Amikacin(AK 30µg), Gentamicin(GEN 10 µg), Ceftriaxone(CTR, 30µg), Ceftazidime(CAZ, 30µg), Cefipime(CPM, 30µg), Levofloxacin (LE, 5µg), Ciprofloxacin(CIP,5µg), Cotrimoxazole(COT, 1.25/23.75µg), Imipenem(IMP, 10 µg), Meropenem(MRP,10µg), Piperacillin-Tazobactum(PIT, 100/10µg), Polymyxin B(PB, 300U) and Colistin(CL, µg) and interpreted as per CLSI guidelines.[8] Susceptibility to Cefoxitin (30 µg) disc was used for presumptively identification of Amp C beta lactamase production.

Isolates showing resistance to either Imipenem (IMP) and/or Meropenem by disc diffusion method was considered as Carbapenamase producing and further subjected to identification by phenotypic methods.

**1. Modified Hodge Test[9]**

Lawn culture of overnight broth culture of Escherichia coli, ATCC 25922, standardized to 0.5 Mc Farland turbidity standard is made on a Mueller-Hinton agar (MHA) plate. After drying the plate for 10 minutes, a disc containing 10µg of Imipenem is placed at the center of the plate. An overnight broth culture of the test strain is streaked heavily from the center to periphery of the plate. After overnight incubation at 37° C in ambient air, the presence of distortion in the zone of inhibition is interpreted as positive for Carbapenamase production. A known carbapenamase producing standard strain, Klebsiella pneumoniae ATCC BAA 1705, was used a s positive control for MHT.

**2. Detection of Metallo-β-lactamase- DDST[10]**

A lawn culture of test strain is made on Mueller Hinton Agar (MHA) plate. An Imipenem (10µg) disc is placed at the center of the plate. Another disc containing IMP (10 µg) and EDTA (750µg) is placed at a distance of 15mm, center to center, away from the Imipenem disc. After overnight incubation, at37° C in ambient air, an extension of zone around Imipenem disc on the side nearest to EDTA disc and/or difference of 7mm or more in the zone of combined IMP-EDTA disc is considered as positive for Metallo-β-lactamase production.

**3. Tests to determine MIC**

Minimum Inhibitory Concentration for Imipenem, Meropenem, Tigecycline and Colistin was determined by automated system, BD Phoenix(BD, USA).

## Results

During the period, 160 Acinetobacter species were isolated from various clinical samples. The source of the clinical isolates is given in Fig. 1. Of the 160 isolates, 111 were resistant to either Imipenem and/or Meropenem. The antimicrobial susceptibility pattern of Carbapenem resistant isolates is given in Fig. 2. The MHT and DDST was performed on 106 isolates. The comparison of DDST and MHT is given in Table 1. The MIC of Colistin and Tigecycline is given in table 2 and 3 respectively.

**Table 1: Comparison of MHT with DDST (n=106)**

| | | MHT | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| DDST | Positive | 04 | 08 | 12 |
| | Negative | 71 | 23 | 94 |
| | Total | 75 | 31 | 106 |

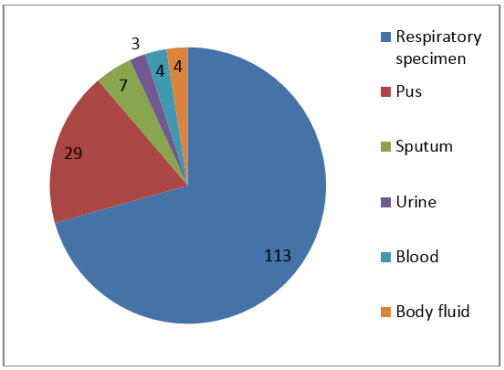


**Fig. 1: Distribution of specimen (n=160)**

**Table 2: MIC of Colistin (n= 111)**

| MIC | 0.5ugm/ml | 1ugm/ml | 1.5ugm/ml |
|---|---|---|---|
| No. of isolates | 99 | 04 | 08 |

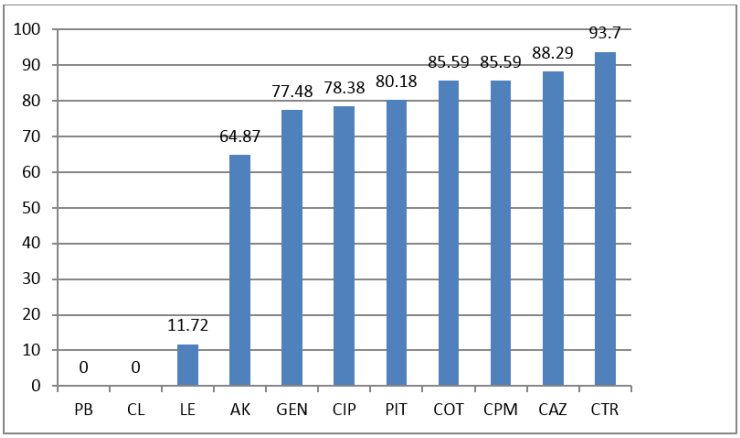


**Fig. 2: Antimicrobial resistance pattern of Carbapenem resistant Acinetobacter species (n=111)**

**Table 3: MIC of Tigecycline (n= 111)**

| MIC | 0.5ugm/ml | 1ugm/ml | 2ugm/ml | 4ugm/ml |
|---|---|---|---|---|
| No. of isolates | 12 | 43 | 19 | 37 |

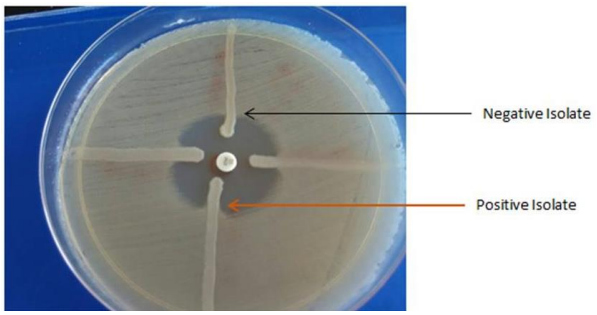


**Fig. 3: Modified Hodge Test**

## Discussion

Over the past many years there has been a rapid increase in infections caused by *Acinetobacter* species, especially in hospital settings. The alarming rise in infections is accompanied by an even more disturbing emergence of multidrug resistant organism.

The present study evaluated 160 non repetitive clinical isolates of *Acinetobacter* species obtained during the period April 2015 to March 2016. During the same period, 8597 clinical specimen were received for culture and sensitivity, thus *Acinetobacter* representing 1.86% of organisms isolated. Of the 8597 specimens processed, a significant growth was seen in 1666(19.37%) which included 1122 (67.35%) gram negative bacteria, 474(28.45%) gram positive bacteria and 70(4.2%) yeasts. *Acinetobacter* accounted for 9.6% of all isolates and 14.26% of gram negative bacteria isolated. There is wide variation in isolation rates of *Acinetobacter* species in literature. Global data provide prevalence rates ranging from 1.02% - 9.5%.[11-13] The prevalence rates shows wide variation in relation to the geographical area, the hospital setting and socio-economic status of the patients[14]. In comparison, the occurrence rates in ICU are higher. The reported data world over provide ICU prevalence rate of 3.7% to 19.2%[15]. The ICU prevalence rates are higher in resource poor nations of Asia and Africa[14]. The data from India suggest a prevalence in ICU of 10%-22.6%.[11,14,16-18] In the present study, 61 of 160 isolates of Acinetobacter were obtained during patients stay in the ICU. During the same period, 479 patients were admitted to the ICU, suggesting an ICU prevalence of 12.73%. Overall, the infection rates is well above acceptable levels but yet is better compared to hospitals with similar working conditions.

### Antimicrobial resistance

In the last decade, the Carbapenems were increasingly and widely used to treat complicated infection by *Acinetobacter* species. The acquisition and spread of resistance determinants on mobile genetic elements, presence of chromosomally coded β lactamases and effective permeability barriers have rendered many groups of antibiotics non efficacious. Multi drug resistant *Acinetobacter* is becoming a global threat with a therapeutic impasse increasingly described in Literature.[2,14]

In our study, 111(69.37%) isolates of *Acinetobacter* species were resistant to Imipenem and Meropenem by Kirby Bauer disc diffusion method. The same was confirmed by obtaining MIC of Imipenem and Meropenem by automated system, BD Phoenix(BD,USA). The MIC was >16 µg/ml for both Imipenem and Meropenem, thus there was concordance between disc diffusion and MIC.

The susceptibility patterns of other antibiotics tested, given in figure 2, suggests high resistance to 3rd/4th generation Cephalosporins (CTR 93.7%, CAZ 88.29%, CPM 85.59%) and least resistance to Colistin and Polymyxin B. These findings are consistent with earlier reports by same author but in different geographical location.[4]

**Colistin and Tigecycline resistance**

In the last few years Colistin therapy has gained significant role in treatment of MDR gram negative bacteria. They act by disrupting outer cell membrane by binding to lipopolysaccharide and phospholipids of gram negative bacteria. They can also prevent the pathophysiological effects of circulating endotoxin.[19] Hence, there is an increased interest in using the drug. In our study, we report 100% sensitivity to Colistin as tested by MIC. The MIC of Colistin, table 2, is within our comfort levels and may be concluded that creeping resistance is yet to find a place in our hospital setting. However, we cannot be complacent as Resistance to Colistin in *Acinetobacter* species is being reported frequently in literature now, with rates ranging from 1.2%-4.16%.[6,14,19-20] It should be noted that, while reporting and deciding Colistin resistance in *Acinetobacter* species, great care should be taken to distinguish between colonisation and true infection. Studies have reported colonization with Colistin resistant organism in as many as 33.34% of subjects.[20]

Similarly, Tigecycline-a glycylcycline group of antibiotic, has been increasingly used in complicated Acinetobacter infections. In the present study, we report 100% sensitivity to Tigecycline. It may be interesting to note that there is trend of increasing MIC, as little more than one third (37/111) of Carbapenem resistant isolates had MIC of 4 µg /ml. This could well be warning for the judicious use of precious antibiotics. Reports of resistance to Tigecycline is being reported and it ranges from 14.2%-66%. The wide variation in resistance pattern appears when Carbapenem resistant strains are compared with non Carbapenem resistant strains.[6]

**MHT and DDST**

Modified Hodge Test (MHT) is a phenotypic method used to detect Class D β Lactamases like OXA type carbapenamases, which are the predominant cause of Carbapenem resistance in *Acinetobacter* species. Double Disc Synergy Test (DDST), using Imipenem and Imipenem-EDTA, is used for detection of Class B/ Metallo-β-lactamase.[9-10,21-22]

Of the 111 Carbapenem resistant isolates of *Acinetobacter* species, MHT and DDST could be performed on 106 isolates. In brief MHT alone was positive in 71(66.98%) isolates suggesting production of OXA type class D β-lactamase and DDST alone was positive in 8(7.55%) isolates suggesting production of Metallo-β-lactamase /Group B β-lactamase. However, in 4 isolates both MHT and DDST was positive, thereby suggesting co-existence of both mechanism of resistance. In as many as 23/106(21.7%) isolates both MHT and DDST were negative suggesting mechanism other than Carbapenem hydrolysing enzymes, such as porin channel loss, efflux pump mechanism.[23] However, this cannot be considered as conclusive evidence for production of hydrolysing enzymes, as confirmation for the presence of resistance genes is conclusive.

**Drawbacks**

1. Species identification, though attempted, could not be completed. Phenotypic identification by automated system, BD Phoenix system (BD, USA) was not satisfactory and hence discontinued. Molecular characterization could not be undertaken for operational difficulties.[24,25]
2. Identification of molecular mechanism of resistance in the isolates would have conclusive and more informative. However, with reagents being made available, the same will be undertaken as extension of the present study.

## Conclusion

The present study adds to the literature available in respect to increasing instances of Carbapenem resistance and their presumptively mechanism of resistance. The resistance to Carbapenems though very high, is not complemented with a corresponding increased resistance to Colistin and Tigecycline. As highlighted by the shortcomings of the study, species identification of *Acinetobacter* is imperative. With non *Acinetobacter baumannii* complex species emerging as important pathogens, species identification is essential as they have different clinical outcomes.[26]

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