

## Original article

# Detection of various resistance mechanisms associated with *Acinetobacter* infections in hospitalised patients

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### Abstract:

**Background:** Infections by multidrug resistant *Acinetobacter* is posing a major threat to hospitalised patients. This study aimed to detect the phenotypic drug resistance mechanisms which are of diagnostic relevance and OXA51 gene among clinical isolates of *Acinetobacter*.

**Materials & Methods:** *Acinetobacter* isolates from invasive infection sites, numbering 120 were analysed. Significant organisms were phenotypically characterised for Amp C Beta lactamase (Amp C) and Metallo Beta Lactamase enzyme production (MBL). OXA 51 carbapenemase gene was detected among the clinical isolates of *Acinetobacter* by PCR.

**Results:** Multidrug resistant (MDR) strains were 87 percent among all the isolates. Resistance to meropenem and imipenem were detected in 75 and 70 percent of the isolates respectively. Amp C producers were 73 percent (88 isolates) while MBL producers were at a level of 24.1 percent (29 isolates). OXA-51 gene was detected in all *Acinetobacter baumannii* isolates.

**Conclusion:** Multidrug resistance associated with multiple resistance mechanisms was a key finding. In addition to AmpC and MBL production substantial proportion of strains showed an intrinsic presence of the OXA 51 gene in *A.baumannii*.

**Key Words :** *Acinetobacter*, resistance mechanisms, MBL, AmpC, OXA 51 gene.

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### Introduction

*Acinetobacter* is increasingly being recognised as a major pathogen in nosocomial infections, particularly in patients admitted to intensive care units.<sup>1</sup> Members of the genus *Acinetobacter* continue to attract interest because of their intrinsic and acquired resistance mechanisms limiting treatment options.<sup>2</sup> There is mounting evidence that *A.baumannii* has a naturally occurring carbapenemase gene intrinsic to it.<sup>3,5</sup>

Presence of chromosomally encoded resistance gene OXA 51 has been identified in most of the *A.baumannii* strains.<sup>3</sup> Thus making OXA 51 a genus marker.<sup>6</sup>

In view of the increasing prevalence of *Acinetobacter* infection and emergence of resistant strains, this study was undertaken to detect resistance mechanisms of diagnostic relevance by phenotypic

methods and the gene OXA51 among clinically significant isolates of Acinetobacter.

### **Aims**

This study aimed to detect the phenotypic drug resistance mechanisms which are of diagnostic relevance and OXA51 gene among clinical isolates of Acinetobacter.

### **Method**

All critically ill patients requiring admission to the intensive care unit of the hospital during October 2010 to March 2012 were included in the study following clearance of the study protocol by the institute ethics committee. Hundred and twenty patient's yielded significant growth of Acinetobacter, as single isolate, from various clinical specimens. They were identified by standard methods to species level and tested against panel of antibiotics recommended for various clinical entities according to CLSI guidelines.<sup>7</sup> In view of the rising trend of usage and to confirm the absolute resistance to Meropenem and Polymyxin B, their Minimum Inhibitory Concentrations (MIC) were determined. Isolates were designated as multidrug resistant if they were non-susceptible to at least  $\geq 1$  agent in  $\geq 3$  categories of antibiotics<sup>8</sup>

Detection of AmpC  $\beta$ -lactamase was done by Amp C disc test method<sup>9</sup>. Briefly a lawn culture of E. coli ATCC 25922 was made on 90mm, Mueller Hinton Agar (Cation adjusted MHA) plate. Sterile disks (six mm) were moistened with sterile saline (20  $\mu$ l) and inoculated with colonies of test organism. These were placed beside a cefoxitin disk (almost touching) on the MHA plate. After overnight incubation at 37<sup>0</sup>C a positive test appeared as a flattening or indentation of the cefoxitin zone of inhibition while a negative test had an undistorted zone. (Figure: 1)

Detection of Metallo- $\beta$ -lactamase (MBL) was done by Combined Disc Diffusion Test (CDDT), a method described by Clare Franklin et al.<sup>10</sup> Briefly, lawn culture was made with the test strain on MHA plate. Two discs of imipenem (10  $\mu$ g) one with 0.1 M EDTA and the other without EDTA were placed on the surface of the agar plate 25mm apart (centre to centre). An increase in the zone diameter of more than 4mm around imipenem-EDTA as compared to that of plain imipenem disc was considered positive for MBL.(Figure 2)

OXA51 gene was detected from the clinical isolates of Acinetobacter by PCR, DNA. Amplification of the OXA 51 gene was carried out according to the method of Turton JF.<sup>11</sup> The sequence of primers used for polymerisation were

Forward – 5' CCAATCACAGCGCTTCAA 3'and

Reverse –5'CCCATCCAGTTAACCAGCCTAC 3'with amplicon size of 625 base pairs. The PCR products were documented using a GelDoc system Bio-Rad (USA)

### **Results**

During the study period a total of 120 critically ill patients yielded significant growth of Acinetobacter, which were established as cause of infection and not colonizers, based on presence of inflammatory cells and abundant gram negative coccobacilli in the direct smear of specimen from wound swabs. Quantitative culture of endotracheal aspirate (ET) was done to establish its role in respiratory tract infection. Urine isolates were correlated with symptoms and presence of polymorphs by microscopy. Blood isolates were clinically correlated with elevated sepsis markers such as procalcitonin or CRP, and repeat isolations from multiple cultures. The isolates included 115 A. baumannii and five A. Iwoffii. Wound swabs accounted for 67(58.3 percent) of the isolates,

followed by ET aspirates 15(13.0 percent), blood 13(11.3 percent) and urine 10(8.7 percent). Majority of *A.lwoffii* were isolated from wound swabs. Antibiotic resistance pattern of 120 *Acinetobacter* isolates is shown in Figure 3.

Minimum Inhibitory Concentration (MIC) of Meropenem, ranged from 0.5µg/ml to >512 µg/ml. Among the isolates 91 (75.3 percent) out of 120 were found to be resistant to Meropenem by agar dilution method.(Figure 4) The only antibiotic with invitrosuceptibility(100 percent sensitive) against the isolates was Polymyxin B by agar dilution technique. Multidrug resistance was noted in 87% of the isolates. Resistance to carbapenem was noted in 30.8% of the isolates from blood stream infection, while it was about 74.8% from other samples. This difference was found to be significant ( $p = 0.0024$  by chi-square test).

Amp C production was the commonest mechanism of resistance seen in 73.3%(88/120) of the isolates , followed by production of MBL in 29 isolates(24.1%) while dual mechanism of resistance both Amp C and MBL production was seen in 18.3%(22/120) of the isolates. Fourteen isolates did not demonstrate any of the mechanisms of resistance that were tested. (Figure: 5)

OXA 51 gene was detected in 115 of the *Acinetobacter baumannii* by PCR. None of the five *A.lwoffii* isolates had OXA 51 gene.

### Discussion

*Acinetobacter baumannii* has become established as one of the common opportunistic bacterial pathogens in the health care setups and is primarily associated with hospital-acquired infections. It has been entitled as a “red alert” human pathogen, creating an alarm among the medical fraternity. This has resulted due to its extensive antibiotic resistance spectrum.<sup>12</sup>Among

the species *A. baumannii* is responsible for majority of infections almost to the exclusion of other species and this is also the species where multidrug resistance has been observed most commonly.<sup>13</sup> The present study also reflected a similar pattern.<sup>14, 15</sup> Distribution of the different types of infection varies from one hospital to another and is probably related to the hospital population and the type of procedures and interventions performed.<sup>16</sup> In the present study wound infections in the post trauma patients (road traffic accidents accounting for a large percentage of admissions in the critical care unit of this hospital) accounted for the majority of isolates. Bloodstream infections (10.8percent) had protracted course with fatal outcome in a major proportion of the cases.

Reports of *A. baumannii* becoming resistant to almost all antimicrobial agents are increasingly being reported.<sup>17, 18</sup> In this study eighty-seven percent of isolates were multidrug resistant similar to that reported in a study from this region.<sup>19</sup>

Carbapenems are the drug of choice for multidrug resistant *Acinetobacter* spp, and are increasingly being used in tertiary care hospitals due to infections by ESBL and Amp C producing Gram negative bacteria. Studies have demonstrated concomitant increase in multi resistant strains during the course of treatment with carbapenems.<sup>20,21</sup> High percentage of resistance to Imipenem and Meropenem among our isolates of *A. baumannii* is similar to several studies in recent years.<sup>19,22,23,24</sup> Reports from India and elsewhere have shown carbapenem resistance ranging from 14 percent to 50 percent.<sup>25,26</sup>

Phenotypic detection of resistance mechanisms as a part of diagnostic algorithm in a clinical microbiology laboratory provides added information for guiding appropriate therapy. Amp C production by *A.baumannii* was the commonest mechanism of

resistance noted in this study. This could be due to intrinsic Amp C cephalosporinase enzyme in this organism designated as *Acinetobacter*- derived cephalosporinase.<sup>27</sup> Whereas MBL was seen only in 18.3% of the isolates. Resistance to carbapenems in the absence of class B metallo  $\beta$  lactamase enzyme (MBL) production can be attributed to the production of other enzymes like Carbapenamase Oxacillinase or to nonenzymatic mechanisms, including changes in outer membrane proteins (OMPs), multidrug efflux pumps and alterations in the affinity or expression of penicillin-binding proteins.<sup>28, 29</sup> Among the MBL negative isolates in this group 67 percent were resistant to Meropenem and 65.9 percent to Imipenem. A finding which was statistically significant. (Table: 1). This indicates that other mechanisms as mentioned above, may be responsible for the resistance. *A. baumannii* codes for a naturally occurring carbapenemase gene, blaOXA-51-like, which is intrinsic to the species, which have a higher rate of multi drug resistance than those that do not carry this gene. All our isolates carried the OXA-

51gene, increasing the potential for developing resistance

### Conclusion

Multidrug resistant *A.baumannii* infection in hospitalised patients has shown multiple resistance mechanisms. Ability to survive for prolonged periods in hospital environment makes these organisms liable to pose a risk to hospitalised patients with protracted stay (including stay in the ICU) and for those on mechanical ventilation. Treatment options appear to be limited, with large proportion of strains exhibiting reduced susceptibility to carbapenems. Several mechanism including AmpC, MBL production and intrinsic presence of the OXA 51 gene could be responsible for this. Further studies need to be carried out to detect the plasmids carrying the ISAbal-blaOXA-51-like gene which may play a role in enhancing the expression of the intrinsic OXA51 gene. Other mechanisms like loss of outer membrane protein and efflux pumps in MDR *Acinetobacter* need to be explored.

Figure:1 AmpC enzyme detection by Amp C disc test method.

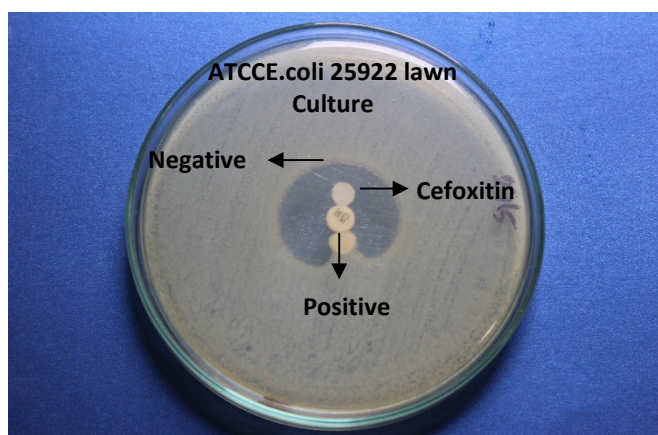


Figure:2 Detection of Metallo-β-lactamase(MBL) by combined disc diffusion test

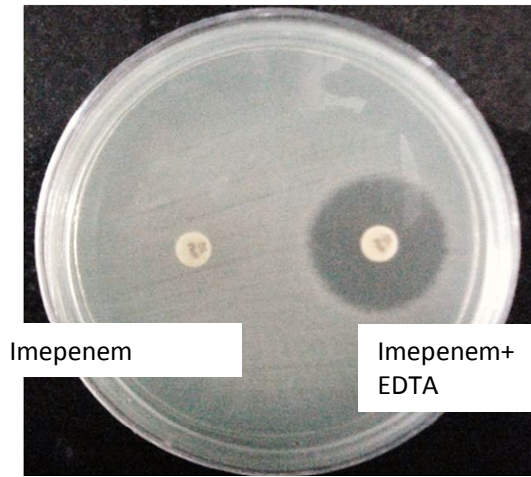


Figure: 3. Antibiotic resistance pattern among the clinically significant Acinetobacterspecies isolated from hospitalised patients.

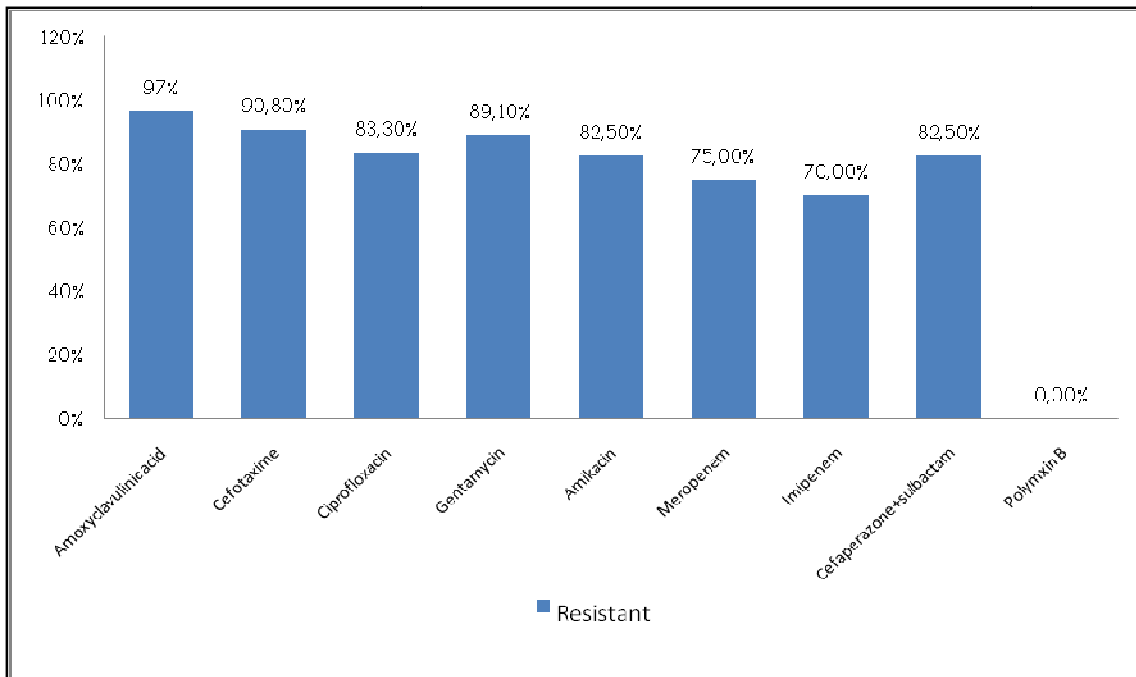


Figure: 4 Meropenem susceptibility by Minimum Inhibitory Concentration

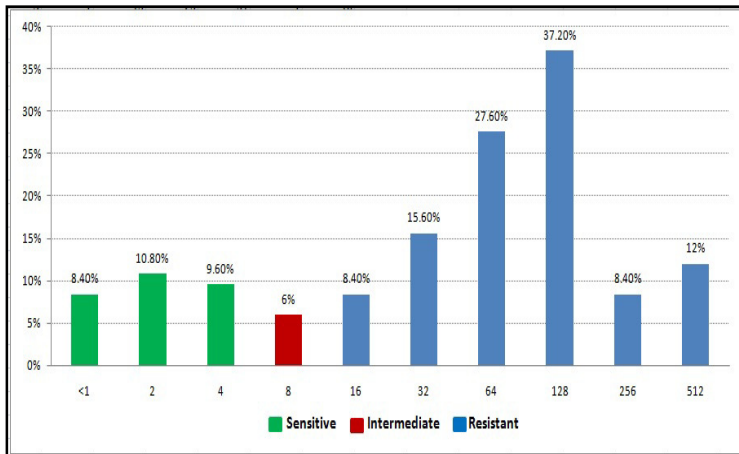


Figure: 5 Phenotypically detected, resistance mechanisms among multidrug resistant Acinetobacterbaumannii from hospitalised patients

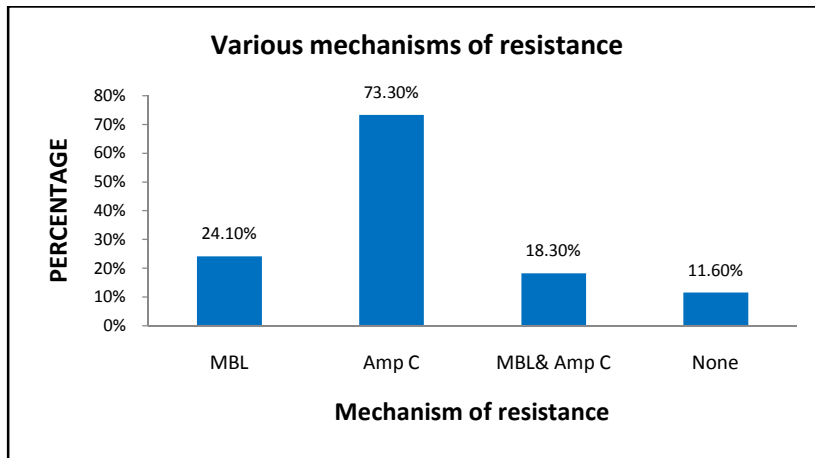


Table: 1 Resistance to carbapenem in phenotypically detected MBL positive and negative strains of Acinetobacter

Antibiotic	No. of resistant isolates (%)		p value*
	MBL positive (n = 29)	MBL negative (n = 91)	
Meropenem	29 (100)	61 (67.0)	0.0009
Imipenem	24 (82.8)	60 (65.9)	0.1365

Statistical test: Chi-square with significant p value at <0.05

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