

Research article

Mechanisms of resistance to carbapenems among *Acinetobacter* isolates

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Abstract

Objectives: To identify carbapenem resistance mechanism in *Acinetobacter* isolates from clinical samples.

Methods: Eighty six isolates of *Acinetobacter* spp obtained from various samples were included in the study. Antibiotic susceptibility was determined by Kirby-Bauer disc diffusion method. Isolates showing resistance to Meropenem were further tested for Amp C beta lactamase production by Amp C disc test and Metallobetalactamase (MBL) production by disc potentiation test.

Results: Among eighty six isolates of *Acinetobacter* sp, forty eight strains (55%) were found to be resistant to Meropenem by disc diffusion method. Out of forty eight resistant strains, twenty nine strains (60%) were isolated from intensive care units, 10 strains (20%) from medical wards and 9 (19%) from surgical wards. Among these resistant strains, six (12%) were found to be Amp C betalactamase producers, 39 strains (81%) produced MBL, 3 strains (6%) were negative for both Amp C and MBL.

Conclusion: In our study we found that meropenem resistance in *Acinetobacter* was more in Intensive care units and the major resistance mechanism to carbapenem among *Acinetobacter* isolates was due to production of metallo beta lactamase.

Keywords: *Acinetobacter*, Metallo beta lactamase, Amp C, Carbapenem resistance

1. Introduction

Acinetobacter sp is a leading cause of nosocomial infections and is intrinsically resistant to many antibiotics. The characteristic feature of *Acinetobacter* sp. is their ability to survive in the hospital environment for prolonged periods. Treatment of *Acinetobacter* infections is a challenge to the clinicians making the treatment and infection control complicated because of their resistance to multiple antibiotics.^{1,2} Since *Acinetobacter* sp are resistant to aminoglycosides, fluoroquinolones, ureidopenicillins and third-generation cephalosporins, carbapenems have emerged as an important agent for managing these infections. But increasing usage of these antibiotics has resulted in the development of carbapenem resistant strains.^{3,4} Production of metallo beta lactamase has been implicated as the major cause for the development of resistance to *Acinetobacter*.⁵ This is a matter of great concern because MBL producers most often exhibit resistant phenotype to additional classes of drugs since they originate nosocomially. Carbapenem resistance in *Acinetobacter* is attributed to various causes such as reduced expression of outer membrane proteins (29kDa,33-36 kDa) and production of carbapenamases.⁶ A recent study done by Dheepa *et al.*, showed 10% of *Acinetobacter* isolated from clinical samples were metallo beta lactamase producers and 73% were Amp c producers.⁷ The objective of the study was to know the prevalence and mechanisms of resistance to carbapenems in meropenem-resistant *Acinetobacter* spp.

2. Materials and Methods

This study was a prospective study done in a tertiary care hospital. Eighty six isolates of *Acinetobacter* sp obtained from various samples were included in the study. Standard guidelines were followed for sample collection. Samples were processed according to standard Microbiological procedures.⁸ Non fermenting gram negative bacilli, oxidase negative and non motile were considered as *Acinetobacter* sp. Samples showing growth were subjected to antibiotic sensitivity testing as per CLSI guidelines.⁹ Antibiotic susceptibility was determined by Kirby-Bauer disc diffusion method. Antibiotics included were ampicillin (10 mcg), amikacin (30 mcg), ceftriaxone(30mcg), ciprofloxacin (5mcg), gentamicin(10 mcg), meropenem (10 mcg), piperacillin + tazobactam (100/10mcg). Cefoxitin (30 mcg) was included to screen for Amp c beta lactamase production. Isolates showing resistance to meropenem were tested for MBL production.

i) Amp C disc test¹⁰:

The strains that were resistant to cefoxitin were further subjected to Amp C disc test to confirm Amp C production. A lawn culture of E.coli ATCC 25922 is prepared on MHA plate. A cefoxitin disc (30mcg) is placed in the center of the plate. A sterile plain disc moistened with 10 – 20 µl of sterile saline is placed besides the cefoxitin disc (almost touching it). The plain disc is inoculated with several colonies (5 -10) of test organism. The plates are incubated overnight at 37°C. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc is a positive test (Fig 1)

ii) Disc potentiation test¹¹:

This test was done for the detection of metallo β lactamase production. The test strain was inoculated onto MHA plate. Two imipenem discs were placed on the inoculated plates approximately 30 mm apart and 10µl of 0.5M EDTA was added to one imipenem disc the plates are incubated at 37°C. An increase in the zone size of at least 7mm around imipenem + EDTA disc in comparison to the imipenem disc alone indicates production of MBL. (Fig 2)

Fig -1: A positive Amp C disc test - A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc



Fig -2: A positive metallobeta lactamase test - An increase of more than 7mm is noted with imipenem+EDTA disc when compared with imipenem disc alone. (Disc potentiation test for Metallobetalactamase detection)



3. Results

Among eighty six isolates of *Acinetobacter* sp, forty eight strains (55%) were found to be resistant to meropenem by disc diffusion method. The distribution of *Acinetobacter* sp in different wards is given in Table 1. The distribution of *Acinetobacter* amongst different samples is given in table II. Among these 48 resistant strains, six (12%) were found to be Amp C beta lactamase producers by Amp C disc test, 39 strains (81%) produced MBL by disc potentiation test using meropenem and EDTA discs. 3 strains (6%) were negative for both Amp C and MBL. All these strains that were resistant to meropenem were also resistant to all the other classes of antimicrobials.

Table I – Distribution of carbapenem resistant *Acinetobacter* among ICU and Wards

Wards	Isolation rate
Intensive care units	29 (60%)
Medical wards	10 (20%)
Surgical wards	9 (19%)

Table II – Distribution of carbapenem resistant *Acinetobacter* among various samples

Wound swab	33 (68%)
ET secretions	11 (22%)
Urine	4 (8%)

4. Discussion

Acinetobacter sp, a gram negative bacteria has become one of the most dangerous pathogen to treat. It has become a predominant hospital pathogen along with *Pseudomonas aeruginosa*. It is resistant to almost all the antibiotics that are frequently used. It can cause infections like UTI, wound infections, pneumonia, endocarditis and life threatening septicemia.¹² In our study we found that meropenem resistance amongst *Acinetobacter* isolates is about 55%. Another Indian study by Taneja *et al*¹³ reported 20% resistance to carbapenems among *Acinetobacter*. Another similar study conducted by Sinha *et al.*,¹⁴ which involved 150 clinical isolates of *Acinetobacter*, 14% of the isolates were found to be resistant to meropenem.

Our study also showed that majority of the isolates (56%) were from Intensive care units. A study conducted by Sinha *et al*¹⁴ showed 42.8% of the isolates from medical intensive care units. This shows that *Acinetobacter* can survive well in the hospital environment especially in the intensive care units and can cause frequent hospital acquired infections.

Our study revealed that strains that were resistant to meropenem were also resistant to other classes of antimicrobials like III generation cephalosporin and β lactam – β lactamase inhibitor combinations like piperacillin-tazobactam. In such situations treatment options are very limited leaving the physicians to choose either colistin or polymyxin B. The capacity of *Acinetobacter* species for extensive antimicrobial resistance may be due in part to the organism's relatively impermeable outer membrane and its environmental exposure to a large reservoir of resistance genes¹⁵.

Our study showed that 81% of *Acinetobacter* isolates produced MBL. An Indian study on the *Acinetobacter baumannii* stated that 70.9% isolates produced metallo beta lactamase.¹⁶ The present study revealed 12% of *Acinetobacter* produce Amp C. A similar study conducted by Dheepa *et al.*, 73% isolates were found to be AmpC β -lactamase producers.⁷

To conclude, multidrug resistant *Acinetobacter* sp are common in hospital especially in intensive care units and treating the infections caused by this organism is very difficult as they are resistant even to carbapenems which are considered to be the life saving drugs. There should be a surveillance mechanism in the laboratory to detect metallo beta lactamases. This menace of multidrug resistant organism can be controlled only by following standard precautions and a good antibiotic policy.

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