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 metallobetalactamase.

**Keywords:** *Acinetobacter*, Metallo betalactamase, 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, 33kDa, 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 betalactamase 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.\(^4\) Non fermenting gram negative bacilli, oxidative negative and non motile were considered as *Acinetobacter* sp. Samples showing growth were subjected to antibiotic sensitivity testing as per CLSI guidelines.\(^8\) Antibiotic susceptibility was determined by Kirby-Bauer disc diffusion method. Antibiotics included were ampicillin (10 mcg), amikacin (30 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg), gentamicin (10 mcg), meropenem (10 mcg), piperacillin + tazobactam (100/10mcg). Cefoxitin (30 mcg) was included to screen for Amp C betalactamase production. Isolates showing resistance to meropenem were tested for MBL production.

i) Amp C disc test\(^9\):

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 beside 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 test\(^10\):

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)
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 betalactamase 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

<table>
<thead>
<tr>
<th>Wards</th>
<th>Isolation rate</th>
</tr>
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<tbody>
<tr>
<td>Intensive care units</td>
<td>29 (60%)</td>
</tr>
<tr>
<td>Medical wards</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Surgical wards</td>
<td>9 (19%)</td>
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</tbody>
</table>

Table II – Distribution of carbapenem resistant Acinetobacter among various samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Isolation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swab</td>
<td>33 (68%)</td>
</tr>
<tr>
<td>ET secretions</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Urine</td>
<td>4 (8%)</td>
</tr>
</tbody>
</table>

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. In our study we found that meropenem resistance amongst Acinetobacter isolates is about 55%. Another Indian study by Taneja et al. reported 20% resistance to carbapenems among Acinetobacter. Another similar study conducted by Sinha et al., which involved 150 clinical isolates of Acinetobacter, showed 42.8% resistance to carbapenems among Acinetobacter. Our study also showed that majority of the isolates(56%) were from Intensive care units. A study conducted by Sinha et al. showed 14% 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 genes.

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

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 betalactamas. This menace of multidrug resistant organism can be controlled only by following standard precautions and a good antibiotic policy.
References

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