Prevalence of Multidrug Resistance, ESBL and MBL production in Acinetobacter spp.

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Abstract: Background: Acinetobacter are important cause of nosocomial infections with widespread resistance to various antibiotics. Extended spectrum beta lactamase (ESBL) and metallobeta-lactamase (MBL) associated resistance among Acinetobacter is now known. This study aims to determine antibiotic susceptibility patterns of Acinetobacter isolates, prevalence of multidrug resistance, ESBL production and MBL production. Material and methods: 107 Acinetobacter isolates were identified by standard microbiological testing. Antimicrobial susceptibility testing was performed by modified Kirby Bauer method as per the CLSI guidelines. Multidrug resistance was determined. ESBL production was detected by double disc method and CLSI phenotypic confirmatory test. MBL production was detected by combination disc test using imipenem and imipenem/EDTA disc. Results: The maximum sensitivity of Acinetobacter was seen to imipenem (57.00%) and amikacin (55.14%). Maximum resistance was observed to ceftazidime (100%), cefotaxime (100%) and piperacillin (100%). A. baumannii was more resistant to majority of drugs used and A. junii was more susceptible to majority of the drugs used. 32 (29.90%) Acinetobacter strains were extended spectrum beta lactamase (ESBL) producers. Out of the total 46 imipenem resistant Acinetobacter isolates, 40 (86.95%) Acinetobacter were MBL producer and all of them were A. baumannii strains. Multiple drug resistance was common among Acinetobacter isolates. Significantly higher percentage of multidrug resistance was found in A. baumannii strains compared to other Acinetobacter spp (P<0.05). Conclusion: Multidrug resistance in A. baumannii was more common compared to other spp. ESBL and MBL production should be promptly detected and reported to control the spread of resistant phenotypes to other individuals. Keywords: Acinetobacter, multidrug resistance, ESBL, MBL production.

Introduction

Acinetobacter has emerged as an important nosocomial pathogen associated with a wide variety of illnesses in hospitalized patients, especially in the intensive care units imposing greater challenge to clinical management and infection control. The Infectious Diseases Society of America reported this microorganism as one of the "red alert" pathogens. Acinetobacter are highly resistant to various antimicrobial agents. Extensive use of broad-spectrum antibiotics has increased multidrug resistance. These multidrug-resistant isolates are resistant to extended-spectrum cephalosporins and carbapenems. Carbapenem-hydrolyzing β-lactamases of Ambler class B (metalloenzymes), Ambler class D (oxacillinases) and extended-spectrum β-lactamases (ESBLs) of Ambler class A are sources of multidrug resistance in A. baumannii. Transferrable metallo-β-lactamases (MBLs) are the most feared because of their ability to hydrolyze virtually all drugs in that class, including the carbapenems. Carbapenem-resistant A. baumannii strains are increasingly recovered from hospitalized patients worldwide. Mechanisms for carbapenem resistance include mutation in porins, loss of outer membrane proteins and efflux mechanisms. MBL producing strains are frequently resistant to aminoglycosides and fluoroquinolones but remain susceptible to polymyxins. Carbapenem resistance due to MBL and other carbapenemase production has a potential for rapid dissemination, as it is often plasmid mediated. Consequently, the rapid detection of carbapenemase production is necessary to initiate effective infection control measures to prevent their dissemination. This study aims to determine antibiotic susceptibility patterns of Acinetobacter isolates and the prevalence of multidrug resistance, ESBL production and MBL production.

Material and methods

This study was carried out in the department of Microbiology from August 2008 to September 2010. 107 Acinetobacter isolates were obtained from clinical specimens and were identified by standard microbiological techniques. Antimicrobial susceptibility testing of all 107 isolates was performed by modified Kirby Bauer method as per the CLSI guidelines. Antibiotics tested were Ceftazidime (CAZ), Ciprofloxacin (CIP), Imipenem (IPM), Gentamicin (GM), Tobramycin (TOB), Amikacin (AK), Piperacillin-tazobactam (P/T), Cefepime (CPM), Cefotaxime (CTX), Tetracycline (TC), Piperacillin (PIP), Trimethoprim-Sulfamethoxazole (COT), Gatifloxacin (GAT). All the isolates were tested for ESBL.

Double disc method: For testing ESBL, a lawn culture of test strain was exposed to discs of amoxycillin (20 µg +10 µg) and cefotaxime (30 µg) placed at a distance of 2 cm from center to center. After overnight incubation, there was extension of zone of inhibition of cefotaxime.
A colony of the suspected isolate was suspended in Mueller Hinton broth and turbidity was adjusted to 0.5 McFarland opacity standards. Lawn culture was prepared on Mueller Hinton agar and combination disc test was put. The combinations used were imipenem (I) and imipenem-EDTA (I-EDTA). Imipenem (10 µg) and combined imipenem/EDTA (750 mg) discs (Hi-media laboratories Pvt. Ltd., Mumbai) were placed on the agar plates. After overnight incubation at 35°C, inhibition zones of the imipenem with and without EDTA were compared. The test was considered MBL positive if a >6 mm increase in the zone diameter for imipenem/EDTA was observed.\(^{[8],[9]}\)

### Statistical analysis

\( P \) value was reported and a value of <0.05 was considered significant. The statistical analysis was performed using Chi square test, Chi square with Yate’s correction and Fisher exact test.

### Results

A total of 107 \textit{Acinetobacter} strains were isolated from the processed clinical specimens. The maximum sensitivity of \textit{Acinetobacter} was seen to imipenem (57.00%), amikacin (55.14%), followed by gatifloxacin (44.87%) and tobramycin (41.12%). Maximum resistance was observed to ceftazidime (100%), cefotaxime (100%), piperacillin (100%) and piperacillin-tazobactam (86.92%). Imipenem resistance was seen in 46 (43.00%) \textit{Acinetobacter} strains (Fig 1).

In general wards and in ICU, \textit{A. baumannii} was more resistant to majority of drugs used. \textit{A. junii} was more susceptible to majority of the drugs used (Fig 2 & 3).

Out of total 107 \textit{Acinetobacter} strains, 32 (29.90%) \textit{Acinetobacter} strains were extended spectrum beta lactamase (ESBL) producers. Of the 32 ESBL producers, as many as 23 (71.87%) were \textit{A. baumannii}, followed by 9 (28.13%) \textit{A. lwoffii}. Similar results were obtained by both the double disc method and CLSI phenotypic confirmatory method for ESBL production. Only imipenem resistant \textit{Acinetobacter} isolates (46) were tested for MBL production. Out of the total 46 imipenem resistant \textit{Acinetobacter} isolates, 40 (86.95%) \textit{Acinetobacter} were MBL producer and all of them were \textit{A. baumannii} strains. Multiple drug resistance was common among \textit{Acinetobacter} isolates. There were total 96 (89.71%) \textit{Acinetobacter} isolates that showed resistance to 6 or > 6 drugs, of which 84 (98.82%) were \textit{A. baumannii} and 8 (61.53%) were \textit{A. calcoaceticus}. Total 88 (82.24%) \textit{Acinetobacter} isolates (96.47% \textit{A. baumannii} and 46.15% \textit{A. calcoaceticus}) showed resistance to 7 or more than 7 drugs. Eighty one (75.70%) isolates of \textit{Acinetobacter} showed resistance to 8 or more than 8 drugs of which 91.76% were \textit{A. baumannii} and 23.07% were \textit{A. calcoaceticus}. All the
Acinetobacter isolates showing resistance for 9 or more than 9, 10 or more than 10 and 11 or more than 11, 12 and more than 12 drugs were A. baumannii. There were 10 (9.34%) isolates which showed resistance to thirteen drugs. Significantly higher percentage of multidrug resistance was found in A. baumannii strains compared to other Acinetobacter spp (P<0.05) (Table 1).

**Table 1: Multidrug resistance in Acinetobacter isolates (n=107)**

<table>
<thead>
<tr>
<th>Multi drug resistant (No. of drugs)</th>
<th>A. baumannii</th>
<th>A. calcoaceticus</th>
<th>A. lwoffii</th>
<th>A. junii</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=85 (%)</td>
<td>84 (98.82)</td>
<td>8 (61.53)</td>
<td>2 (50.00)</td>
<td>1 (33.33)</td>
<td>96 (89.71)</td>
</tr>
<tr>
<td>n=13 (%)</td>
<td>82 (96.47)</td>
<td>6 (46.15)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>88 (82.24)</td>
</tr>
<tr>
<td>n=4 (%)</td>
<td>78 (91.76)</td>
<td>3 (23.07)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>81 (75.70)</td>
</tr>
<tr>
<td>n=3 (%)</td>
<td>76 (89.41)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>76 (71.02)</td>
</tr>
<tr>
<td>n=2 (%)</td>
<td>71 (83.52)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>71 (66.35)</td>
</tr>
<tr>
<td>n=107 (%)</td>
<td>54 (63.52)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>54 (50.46)</td>
</tr>
<tr>
<td>6 &amp; &gt;6</td>
<td>35 (41.17)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>35 (32.71)</td>
</tr>
<tr>
<td>9 &amp; &gt;9</td>
<td>10 (11.76)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (9.34)</td>
</tr>
</tbody>
</table>

(*Chi square test, Chi square with Yate’s correction and Fisher exact test, P<0.05)

**Discussion**

Acinetobacter is an important nosocomial pathogen with high mortality rates. It is "a prime example of mismatch between unmet medical need and the current antimicrobial research and development pipeline". Acinetobacter spp. are notorious for their ability to acquire antibiotic resistance. Antimicrobial resistance among Acinetobacter spp. has increased substantially in the past decade creating a major public health dilemma. Carbapenem is the most potent antibiotic currently available, but resistant strains have emerged.

We have studied the antimicrobial resistance pattern among 107 Acinetobacter isolates by Kirby-Bauer disc diffusion method. In our study, Acinetobacter isolates showed resistance to most of the antibiotics available. Maximum sensitivity was observed to imipenem (57.00%), amikacin (55.14%), followed by gentamicin (44.87%) and tobramycin (41.12%). Maximum resistance was observed to cefazidime (100%), cefotaxime (100%), piperacillin (100%), cefepime (98.13%) and piperacillin-tazobactam (86.92%). Imipenem resistance was seen in 46 (43.00%) Acinetobacter strains (Fig 1). Sinha et al[11] reported maximum sensitivity to meropenem (86.00%), ciprofloxacin (36.00%), amikacin (33.00%), cefepime (26.00%), ceftazidime (26.00%) and maximum resistance was reported to piperacillin (90.00%) and cefotaxime (87.00%). Acinetobacter spp. are universally resistant to penicillin, ampicillin and cephalothin. Various susceptibility to second and third generation cephalosporins have been reported.[12] Acinetobacter species possess a wide array of β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins and carbapenems. AmpC cephalosporinases are chromosomally encoded and confer resistance to broad-spectrum cephalosporins. Class D OXA-type enzymes, Class B metallo-β-lactamases (MBLs), such as VIM and IMP, hydrolyse a broad array of antimicrobial agents, including carbapenems. Increasing antimicrobial resistance leaves few therapeutic options for multidrug-resistant (MDR) Acinetobacter infection.[10]

In the present study, 43.00% of Acinetobacter were imipenem resistant. Out of these, 60.71% were imipenem resistant A. baumannii (IRAB) compared to 16.66% A. calcoaceticus. Sinha et al[11] reported 35.00% imipenem resistant Acinetobacter. Lee et al[13] reported 21.18% IRAB. Acinetobacter has intrinsic resistance to extended spectrum cephalosporins, have an outer membrane with selective permeability to β-lactams, and by modification of outer membrane porins, diminish permeability to other antibiotics. Also, they have chromosomal β-lactamases. All of these intrinsic mechanisms cause resistance to the extended spectrum β-lactam antibiotics.[14] Extended spectrum β-lactamases (ESBL) continue to be a major problem in clinical setups the world over and knowledge about their prevalence is essential guide towards appropriate antibiotic treatment. Significant high levels of Acinetobacter spp. produce ESBL and these ESBL producers are MDR. Routine antimicrobial susceptibility tests may fail to detect such ESBL producers. But a simple, rapid and approximately inexpensive method like double disc approximation method may help to screen all the clinical Acinetobacter isolates for ESBL production.[15] In our study we have tested all the Acinetobacter strains for ESBL production by both double disc method and CLSI phenotypic confirmatory method for ESBL production. In our study, out of the 107 Acinetobacter isolates, 32 (29.90%) Acinetobacter isolates were ESBL producers. Of these 32 isolates, as many as 71.87% A. baumannii were ESBL producer, followed by A. lwoffii (28.13%). Similar results were obtained by both the double disc method and CLSI phenotypic confirmatory method for

ESBL production. Vahaboglu et al\textsuperscript{[20]} reported ESBL production in 46.00% Acinetobacter strains. In a study by Yong et al\textsuperscript{[16]} 54.63% Acinetobacter were ESBL producers. Sinha et al\textsuperscript{[11]} isolated 28.00% Acinetobacter which were ESBL producers. In our study, out of the total ESBL producers, 71.87% were A. baumannii compared to 28.13% of A. Iwofii. Sinha et al\textsuperscript{[11]} reported that 69.04% of ACB complex were ESBL producer compared to 30.96% A. Iwofii strains. Inherent to Acinetobacter, mainly all A. baumannii strains are chromosomally encoded AmpC cephalosporinases, also known as Acinetobacter-derived cephalosporinases (ADCs). Unlike that of AmpC enzymes found in other gram negative organisms, inducible AmpC expression does not occur in Acinetobacter spp.\textsuperscript{[17]} So, we have not tested the strains for AmpC class of β-lactamase production. Carbapenem-resistant Acinetobacter spp. are increasingly recovered from hospitalised patients worldwide and in some cases are associated with high morbidity and mortality rates. Mechanisms of resistance in such strains have been associated with decreased permeability, efflux pump overexpression, and, more lately, production of carbapenemases. Metallo-β-lactamases (MBLs), mainly of types IMP and VIM, are increasingly associated with the reduced susceptibility to carbapenems seen in several gram-negative species. However, despite the worldwide occurrence of epidemic carbapenem-resistant strains, MBL-producing Acinetobacter isolates have been found to be disseminated only in specific geographic areas. Therefore, the detection of these enzymes is of major importance in the control of Acinetobacter hospital infections. Several schemes have been proposed for the phenotypic detection of MBL-producing gram-negative species, including Acinetobacter. These tests take advantage of the zinc dependence of MBLs by using chelating agents, such as EDTA, to inhibit enzyme activity. However, the phenotypic appearance of MBL-carrying organisms seems to depend on the nature of the bacterial host, since carbapenem-susceptible Enterobacteriaceae organisms may carry MBL genes not readily detectable by conventional assays. A recent study introduced a more sensitive procedure for MBL detection in a broad range of host organisms, including carbapenem-susceptible isolates.\textsuperscript{[18]} In our study, of the 46 imipenem resistant Acinetobacter strains, 86.95% Acinetobacter were MBL producers, all of which were A. baumannii. In the study by Yong et al\textsuperscript{[8]} MBL production rate in imipenem resistant Acinetobacter ranged from very occasional to as high as 50.00%. Lee et al\textsuperscript{[13]} reported MBL production in imipenem resistant Acinetobacter to be 15.10% (range 0-34%). Yong et al\textsuperscript{[8]} reported 6.95% MBL producing A. baumannii strains. Of the 46 imipenem resistant Acinetobacter strains, 40 strains were MBL producers. The remaining isolates may possess other enzymes mediating carbapenem resistance, such as OXA-type β-lactamases (class D) or AmpC β-lactamases and/or other mechanisms such as outer-membrane permeability and efflux mechanisms that were not checked.\textsuperscript{[19]} Wide adaptability to the environment and the emergence of multidrug-resistant strains has led Acinetobacter as one of the "superbugs" in the hospital which has been elevated to the highest degree of importance.\textsuperscript{[20]} MDR Acinetobacter spp. are defined as the isolate resistant to at least three classes of antimicrobial agents-all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones, and aminoglycosides.\textsuperscript{[21]}

In the present study, there were total 96 (89.71%) Acinetobacter isolates that showed resistance to 6 or more than 6 drugs of which 84 (98.82%) were A. baumannii, 8 (61.53%) were A. calcoaceticus, 2 (50.00%) were A. haemolyticus and A. lwoffii and a single strain each of A. Iwofii (33.33%) and 50.00% of A. junii (Table 1). There were total 88 (82.24%) Acinetobacter isolates (96.47% A. baumannii and 46.15% A. calcoaceticus) which showed resistance to 7 or more than 7 drugs. Eighty one (75.70%) isolates of Acinetobacter showed resistance to 8 or more than 8 drugs of which 91.76% were A. baumannii and 23.07% were A. calcoaceticus. All the Acinetobacter isolates showing resistance for 9 or more than 9, 10 or more than 10, 11 or more than 11 drugs and 12 or more than 12 drugs were A. baumannii. There were only 10 (11.76%) isolates which showed resistance to twelve drugs and all of them were A. baumannii (Table 1). Table 1 also shows that a significantly higher percentage of multidrug resistance was found in A. baumannii strains compared to other Acinetobacter spp (P<0.05). MDR Acinetobacter infections are independently associated with increased hospital and ICU lengths of stay compared with the outcomes for uninfected patients and those infected with drug-susceptible Acinetobacter. Acinetobacter spp. (A. baumannii in particular) have become resistant to many classes of antibiotics. MDR A. baumannii (MDR AB) infections tend to occur in immunosuppressed patients, in patients with serious underlying diseases, and in those subjected to invasive procedures and treated with broad-spectrum antibiotics.\textsuperscript{[22]} A. baumannii exhibits a remarkable ability to rapidly develop antibiotic resistance, which led from fully susceptible to multidrug-resistant strains within three decades.\textsuperscript{[24]} Seifert et al\textsuperscript{[23]} reported that A. baumannii strains are generally more resistant than other spp. and A. junii and A. Iwofii strains are more susceptible. Prashanth et al\textsuperscript{[24]} also reported that MDR isolates are mostly A. baumannii. Different terms like extensive drug resistant (XDR), and pandrug resistant (PDR) have been used with varied definitions to describe the extent of antimicrobial resistance among Acinetobacter spp. However, to date, unlike Mycobacterium tuberculosis, internationally, there are
no accepted definitions for the extent of resistance in the bacteria. ‘XDR Acinetobacter spp.’ shall be the Acinetobacter spp. isolate that is resistant to the three classes of antimicrobials described above (MDR) and shall also be resistant to carbapenems. Finally, ‘PDR Acinetobacter spp.’ shall be the XDR Acinetobacter spp. that is resistant to polymyxins and tigecycline. The above definitions have been described keeping in view the different mechanisms of resistance known till date and the antimicrobials being used to treat various Acinetobacter spp. infections. These definitions further help to clearly define the extent of resistance and rational antimicrobial therapy.[21]

References

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