Antibiotic resistance profile & extended spectrum beta-lactamase (ESBL) production in *Acinetobacter* species

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Received June 22, 2006

**Background & objectives:** Members of the genus *Acinetobacter* are an important cause of nosocomial infections and with widespread resistance to various antibiotics. Extended spectrum beta lactamase (ESBL) associated resistance among *Acinetobacter* species is now known. The aim of this study was to speciate clinical isolates of *Acinetobacter*, analyze their resistance patterns, identify the production of ESBLs and compare the role of different cephalosporins in detecting ESBL production in the isolates.

**Methods:** One hundred and fifty clinical isolates of *Acinetobacter* were speciated by various phenotypic tests. Antibiotic susceptibility was determined by the standard disc diffusion method. ESBL production was detected by the double disk approximation test using clavulanate containing disk and four different cephalosporin disks. Results of the above test were confirmed using the NCCLS phenotypic confirmatory test for ESBLs on a limited number of isolates.

**Results:** Most of the isolates were of respiratory origin. *A. calcoaceticus A. baumannii* (Acb) complex was the predominant species isolated (75%). Most isolates were resistant to the antibiotics tested including the third generation cephalosporins. Most isolates were sensitive to carbapenems and cefoperazone-sulbactam. ESBL production was detected in 28 per cent of the isolates. In the double disc approximation test, cefepime and cefotaxime could detect most of the ESBLs in *Acinetobacter* isolates.

**Interpretation & conclusion:** A high level of antibiotic resistance was found in *Acinetobacter* in our study. Acb complex was the predominant and the more resistant species. Relatively high levels (28%) of ESBL have been detected in *Acinetobacter* and may reflect the scenario in India. ESBL production in *Acinetobacter* should be promptly detected and reported as it helps in treating individual cases and also in controlling the spread of these resistant phenotypes to other individuals.

**Key words** *Acinetobacter* - extended spectrum beta-lactamase (ESBL)

*Acinetobacter* is an important cause of nosocomial infections and has been associated with a wide variety of illnesses in hospitalized patients, especially patients in the intensive care units. *Acinetobacter* infections are often difficult to treat, because of the widespread antibiotic resistance. Further, these bacteria survive for a long time in the hospital environment, with enhanced opportunities for transmission between patients.

Extended spectrum beta lactamase (ESBL) associated resistance among *Acinetobacter* species is now a known phenomenon. ESBLs in *Acinetobacter*...
may be either chromosomal or plasmid mediated\textsuperscript{6,7}. PER-1 is an ESBL, which was detected in Acinetobacters in Turkey, France and Korea\textsuperscript{5,7,8}. In Turkey, 46 per cent and in Korea, 54.6 per cent of Acinetobacter species were found to produce PER-1. Increase in cefepime resistance is suggestive of the presence of PER-1 production, as cefepime is stable to AmpC but not to PER-1 $\alpha$ lactamase\textsuperscript{5}. In 2003, another ESBL, VEB-1, was identified in \textit{A. baumannii} in France\textsuperscript{3}. Routine detection of ESBL producing strains may be difficult because the synergy between cephalosporin and clavulanic acid, typically observed with ESBL, tends to be minimal with \textit{Acinetobacter} spp\textsuperscript{8}.

Previous Indian studies\textsuperscript{6} have described the successful transfer of plasmid bearing ESBL to \textit{Escherichia coli} DH5$\alpha$. However, the characterization and prevalence rates of ESBLs were not performed\textsuperscript{6}. The transferable nature of ESBL in \textit{Acinetobacter} may lead to increase in the basal level of multidrug resistance among other nosocomial pathogens by dissemination and integration of the R-plasmids\textsuperscript{6}. In India, increasing reports of infections caused by Acinetobacters, especially \textit{A. calcoaceticus}-\textit{A. baumannii} (Acb) complex, have been documented\textsuperscript{1}. It is therefore important to speciate and determine the antibiotic resistance profile and rate of ESBL production in \textit{Acinetobacter} spp. in the India.

We undertook this study to speciate \textit{Acinetobacter} isolates obtained from clinical samples by simple phenotypic methods and to determine their antibiotic susceptibility patterns, to determine the prevalence of ESBL in \textit{Acinetobacter} by the double disk synergy test and to compare the role of different cephalosporins in detecting ESBL production in the isolates.

**Material & Methods**

This prospective study was conducted at the Department of Microbiology at St. John’s Medical College and Hospital (SJMCH), Bangalore. A total of 150 isolates were obtained from clinical samples from March 2003 to March 2004. Isolates obtained either in pure culture or as predominant growth, were included in the study. Nonfermenting Gram-negative bacilli or coccobacilli that were oxidase negative and nonmotile were presumptively identified as belonging to Genus \textit{Acinetobacter}\textsuperscript{2}. Growth on MacConkey agar revealed non-lactose fermenting colonies.

The phenotypic identification scheme of the different genospecies of \textit{Acinetobacter} has been depicted in the Table. Carbon assimilation tests were performed on 112 random isolates using a simplified panel of carbon sources (histamine, histidine, citrate, malonate, trans-aconitate, phenylalanine, DL-4 aminobutyrate)\textsuperscript{2}.

Antimicrobial susceptibility of all 150 isolates was determined by the standard Kirby Bauer disk diffusion method\textsuperscript{6}. \textit{Escherichia coli} ATCC 25922 was used as control. Antibiotics included were amikacin (10 $\mu$g), cefotaxime (30 $\mu$g), ceftriaxone (30 $\mu$g), ceftazidine (30 $\mu$g), cefepime (30 $\mu$g) ciprofloxacin (5 $\mu$g), meropenem (10 $\mu$g), gentamicin (10 $\mu$g) and piperacillin (100 $\mu$g). Susceptibility to cefoperazone-sulbactam in 126 random isolates was also determined. Swenson \textit{et al}\textsuperscript{10} have validated the use of standard disk diffusion for all antimicrobials other than $\beta$-lactam antibiotics.

The double disk synergy test (DDST) was used to determine the ESBL production\textsuperscript{11}. Four different cephalosporin disks - ceftazidime, cefotaxime, ceftriaxone, cefepime (30 $\mu$g each) were placed around the amoxicillin-clavulanate disk (Oxoid, UK), at a center-to-center distance of 15 mm from the central disk. \textit{E. coli} ATCC 25922 was used as the negative control and an in-house ESBL producing \textit{Acinetobacter} strain was used as the positive control. An isolate was considered to be an ESBL producer if there was any enhancement between any of the four cephalosporins and the clavulanate-containing disks. An isolate was considered to be ESBL negative if there was no enhancement between any of the cephalosporins and the clavulanate-containing disks.

With the introduction of the phenotypic confirmatory test for ESBL production, the disk potentiation test was performed retrospectively on 30 isolates. National Committee for Clinical Laboratory Standards. Of these 30 isolates, 15 were ESBL-producing as per the DDST. In the disk potentiation test, four discs namely cefotaxime (30 $\mu$g), ceftriaxone/clavulanic acid (30 $\mu$g/10 $\mu$g), ceftazidime (30 $\mu$g) and ceftazidime / clavulanic acid (30 $\mu$g/10 $\mu$g), were used. A >5 mm increase in zone diameter for either antimicrobial tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBL production.

**Results**

About half of the \textit{Acinetobacter} isolates were obtained from respiratory samples (tracheal tap-29, sputum-22 and endotracheal tube tip-20). One-third samples were from pus (n=31) and wound swab (n=18).
Significant numbers of isolates (38%) were obtained from patients admitted in the intensive care units (ICUs). This was followed by patients admitted in surgical (22%), medical (18%) and burns ward (13%).

Based on the results of the biochemical and carbon assimilation tests, the species of Acinetobacter identified were A. calcoaceticus A. baumannii (Acb) complex (112, 75%), A. lwoffii (36, 24%) and A. junii (n=2). Acb complex comprises genospecies 1 (A. calcoaceticus), 3,13 and genospecies 2 (A. baumannii) which are grouped together as they are phenotypically indistinct. Majority of the isolates showed resistance to most of the antibiotics tested (Fig.1). Carbapenems and cefoperazone-sulbactam were the most effective amongst all the antimicrobials tested. About 14 per cent of the isolates were resistant to each of these drugs. Five isolates were resistant to both these drugs. The comparison between resistance patterns of Acb complex and A.lwoffii is shown in the Fig. 2. In general, Acb complex was found to be more resistant than A.lwoffii.

Of the 150 isolates of Acinetobacter species, 42 (28%) were found to be producing ESBL by the DDST with one or more of the cephalosporins used (Acb complex - 29 and A. lwoffii - 13). Of the 30 isolates chosen for the disk potentiation test, 15 isolates found ESBL-positive by the DDST were also ESBL-positive by the disk potentiation test and the remaining 15 isolates found ESBL-negative by the DDST were also ESBL-negative by the disk potentiation test. Thus, a 100 per cent concurrence was noted in the results obtained by the DDST and disk potentiation test for the 30 isolates tested retrospectively.

In DDST cefepime and cefotaxime together with the clavulanate containing disk detected most of the ESBL producing isolates. Of the 42 ESBL producing isolates, 32 (76%) were identified cefepime disk, 18 (43%) by cefotaxime, 15 (36%) by ceftazidime and 8 (19%) by using ceftriaxone disk.

**Discussion**

Acinetobacter, predominantly Acb complex, is increasingly found to be associated with nosocomial infections. Although the number of isolates was small, the frequency of ESBL producing isolates is high. The majority of the isolates were resistant to most of the antimicrobials tested. The comparison between resistance patterns of Acb complex and A.lwoffii is shown in the Fig. 2. In general, Acb complex was found to be more resistant than A.lwoffii.

**Table.** Phenotypic characteristics of 15 Acinetobacter genospecies (indicated in perentages) Ref.2

<table>
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<tr>
<th>Species</th>
<th>Genospecies</th>
<th>Of glucose</th>
<th>Gelatin hydrolysis</th>
<th>Haemolysis</th>
<th>Growth at 27°C</th>
<th>Growth at 44°C</th>
<th>Arginine hydrolysis</th>
<th>Histamine*</th>
<th>L-Histidine*</th>
<th>Citrate*</th>
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* carbon assimilation tests

**Fig. 1.** Antibiotic sensitivity patterns of 150 isolates (expressed as a percentage).
infections, especially in ICUs\textsuperscript{2,4}. They are most often associated with infections of the respiratory tract\textsuperscript{1,3}. Many Indian studies reported high levels of resistance in Acinetobacters\textsuperscript{3,4}. In our study, most of the Acinetobacter isolates belonged to the \textit{Acb} complex. Most isolates were from the critical care setting and the source was most often respiratory samples. There was a high level of resistance to majority of the antibiotics tested.

ESBL production in \textit{Acinetobacter} has been found to vary from 46 per cent in Turkey to 54.6 per cent in Korea\textsuperscript{5,8}. In our study, 28 per cent of the \textit{Acinetobacter} isolates (n=42) were ESBL-producing. The study by Joshi \textit{et al}\textsuperscript{6} showed the transferable nature of the ESBL plasmid in Acinetobacter. A study on multiresistant Gram-negative bacilli causing neonatal septicemia showed two of the six isolates of \textit{Acinetobacter} producing ESBL; but the number of isolates studied was too small to reach to any conclusion\textsuperscript{12}. The relatively high prevalence of ESBL in \textit{Acinetobacter} isolates in our study may be due to selection pressure due to extensive use of antibiotics.

Among the cephalosporins tested, cefepime was the most sensitive in detecting ESBLs. Yong \textit{et al}\textsuperscript{8} also concluded the same and suggested that ESBL detection in \textit{Acinetobacter} may be improved by decreasing the distance between the cefepime and amoxicillin-clavulinate disk to 5 mm edge-to-edge and by using a disk containing 20 μg clavulinate\textsuperscript{8}. In our study, the other effective cephalosporin in detecting ESBL was cefotaxime. Moreover, cefotaxime could detect 8 of the 10 ESBL-isolates not detected by cefepime. Therefore, cefepime and cefotaxime used together with amoxicillin-clavulanate in the DDST, increased ESBL detection to 95 per cent. On the other hand, ceftazidime was found to have a lower sensitivity of detecting ESBLs in Acinetobacters. Several Indian studies also used the with DDST multiple cephalosporins to detect ESBLs in \textit{Enterobacteriaceae} in order to increase the sensitivity of detection but not in \textit{Acinetobacter}\textsuperscript{13,14}.

Definitive identification and characterization of ESBL can only be confirmed by molecular techniques. However, these techniques are not available in all laboratories. Molecular detection of ESBLs could not be done in the present study. The other major limitation of this study was inability to perform the minimum inhibitory concentrations (MIC) of the various antibiotics and cephalosporins with and without clavulanic acid for ESBL detection. MICs for the former was not performed, as the study was mainly intended to analyze the ESBLs in \textit{Acinetobacter} and the latter due to the difficulty in procuring clavulanic acid.

To conclude, our study detected a high level of resistance in \textit{Acinetobacter} species to most antibiotics tested. \textit{Acb} complex was the predominant species of \textit{Acinetobacter} isolated and was also found to be more resistant than the other species. A relatively high level of ESBLs (28%) in \textit{Acinetobacter} has been found reflecting the Indian scenario. Cefepime and cefotaxime along with the clavulanate disk in the DDST detected most of the ESBLs in \textit{Acinetobacter}. Judicious use of antibiotics, especially in the ICUs, and appropriate infection control measures are necessary to control the spread of such infection in hospitals.

**References**


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