Prevalence of ESBL, MBL and Amp C Producing XDR *Acinetobacter* Isolates from Lower Respiratory Tract Specimens

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**ABSTRACT**

**Introduction:** *Acinetobacter baumannii* has become a red alert pathogen in ICU as a result of their profundity in developing multidrug (MDR) and extreme drug resistance (XDR). The problem is further worsened by the emergence of resistant enzymes like ESBL, MBL and Amp C which are not detected by routinely used laboratory susceptibility testing. As there is lack of therapeutic options against such strains, knowledge of prevalence of such strains and resistance mechanism and antimicrobial susceptibility pattern will help in framing institutional antibiotic policy and better management of patients. Study aimed to know the prevalence of XDR *A. baumannii* and the rate of ESBL, MBL and Amp C enzymes production in them.

**Method and Materials:** The study was prospective observational study. Transtracheal or bronchoscopic aspirates collected aseptically from 335 patients were processed by semi-quantitative method on the blood agar and Mac-Conkey agar. Isolates were identified up to the species level with BD Phoenix automated system. The susceptibility testing of all the *A. baumannii* isolates was done against 22 drugs belonging to nine different categories by automated system as well as by Kirby bauer’s method. All the strains were further tested for ESBL, MBL and Amp C production.

**Results:** *A. baumannii* was the commonest isolate 88 (36.82%) with high MDR (100%) and XDR 76(86.33%) frequency. All the XDR were 100% resistant to cephalosporins, tetracycline, doxycycline, gentamycin, netilmicin and ticarcillin/clavulenic acid. About 25 (32.8%) XDR strains were resistant to all the carbapenem and eight (10.52%) were susceptible only to colistin and polymyxin B. ESBL, MBL and Amp C production in XDR strains was seen in 25 (32.9%), 28 (36.8%) and 45 (59.21%) strains respectively. The 8 isolates which were sensitive only to colistin and polymyxin B produced ESBL, MBL and Amp C in 100% strains.

**Conclusion:** there is high prevalence of XDR *Acinetobacter* isolates from ICU patients. The production of resistant enzymes like ESBL, MBL and Amp C is also on a rise. Our data will help in framing Institutional antibiotic policy.

**Key-words:** *Acinetobacter Baumannii*, ICU, LRTI, XDR, Resistant Enzymes

**INTRODUCTION**

During the last three decades, outbreaks of infections caused by *Acinetobacter* spp. have become a growing concern in hospitals. This organism commonly targets the critically ill and immunocompromised patients leading to various hospital acquired infections in them, such as ventilator associated pneumonia, urinary tract infections, blood stream infections and skin and soft tissue infections. Apart from its ability to chronically colonize these vulnerable set of patients, infections due to *Acinetobacter* spp are difficult to treat because of their profundity to develop multidrug resistance and extreme drug resistance rendering it a red alert pathogen. Beta lactams are the most widely used group of antibiotics against these bacteria but the excessive and injudicious use of these antibiotics has led to the development of resistance in this pathogenic bacteria. *Acinetobacter* spp. exhibits various mechanisms of drug resistance including mutations in the outer membrane proteins, development of efflux proteins and most importantly the production of beta lactamase enzymes (extended spectrum beta lactamas, metallo beta lactamas and Amp C). The problem with resistant strains is further worsened by the fact that they are not recognized in the routine laboratory testing as they may appear falsely susceptible leading to patients receiving ineffective antibiotics. The strains exhibiting beta lactamases may not only lead to significant therapeutic failure but also to the spread of these resistant enzymes to other organisms. The prevalence of MDR, XDR strains and the strains producing resistant enzymes is variable throughout the world and is rapidly changing over time. The various studies conducted worldwide have reported that *Acinetobacter* spp. is rapidly developing resistance to almost all the drugs that are routinely used in clinical practice and currently there are no new antimicrobial drugs active against these organisms in clinical trials.

We have noticed an increased isolation rate of XDR *A. baumannii* from lower respiratory tract samples from ICU patients over the last few months. There is paucity of data in India, regarding prevalence, susceptibility pattern and the presence of resistant enzymes in XDR strains, so we present our findings that were observed during the study. Knowledge of prevalence of such strains will help in framing institutional antibiotic policy and better management of patients.

**MATERIAL AND METHODS**

The present prospective observational study was conducted in the Department of Microbiology at teaching tertiary...
care hospital during Nov 2012- April 2013. Transtracheal or bronchoscopic aspirates were collected aseptically from 335 patients of all age and sex groups admitted to the ICU and requiring mechanical ventilation for atleast 3 days were included in the study. Samples of only new patients who were admitted for the first time were enrolled in the study. Samples were plated on the blood agar and Mac-Conkey agar plates using semi-quantitative method and incubated aerobically overnight at 37°C. Tracheal aspirates showing ≥ 10^5 colony forming units (CFU/ml) and bronchial secretions with ≤10^4 CFU/ml were regarded as significant counts. Single or mixed growth isolated from all samples were identified by standard microbiological techniques. All the consecutive, non duplicate, non fermenting. Gram negative cocobacilli, non motile, oxidase negative, catalase positive organisms were further identified using conventional techniques as well as automated system, B.D Phoenix. (Becton Dickinson, U.S.A).

The susceptibility testing of all isolates was done against 22 drugs belonging to nine different classes of antibiotics by disc diffusion technique using CLSI guidelines. (aminoglycosides, antipseudomonal carbapenens, antipseudomonal fluoroquinolones, antipseudomonal penicillins with beta lactamase inhibitor, extended spectrum cephalosporins, folate pathway inhibitors, penicillin with beta lacamase inhibitor, polymyxin and tetracycline). The MDR isolates were defined as resistance to at least one agent in three or more antimicrobial category and the XDR Acinetobacter was defined as an organism that was non susceptible to ≥1 agent in all but ≤2 categories. Strains were tested for gentamicin, amikacin, imipenem, meropenem, cefotaxime, ceftazidime, cefepime, pipercillin-tazobactam, colistin, trimethoprim-sulphamethaxazole, ciprofloxacin, levofloxacin, tetracycline drugs by BD phoenix automated system and against tobramycin (10µg), netilimcin (30µg), doripenem (10µg), ticarcillin-clavulanic acid (75/10µg), ceftriaxone (30µg), ampicillin-sulbactam (10/10µg), polymyxin B (300µg), doxycycline (30µg), minocycline (30µg) (Hi-media, concentration in µg) by Kirby Bauer’s disk diffusion method as per CLSI guidelines. Further, resistant enzymes like extended spectrum beta lactamases (ESBL), metallo beta lactamases (MBL) and Amp C were detected in all the isolates.

ESBL detection
Screening of all isolates was done using cefotaxime, cefepime and ceftazidime. Further confirmation was done by double disc approximation test in which a disc containing ceftazidime-clavulanic acid is placed in the center of the plate and another disc containing ceftazidime alone was placed 15 mm from the first disc. Enhancement of the zone of inhibition of ceftazidime clavulanic acid 5mm more than ceftazidime disc alone was considered a positive result. E.coli ATCC 25922 was used as negative quality control.

MBL-Detection
It was done using double disc synergy test. A disc containing imipenem was placed in the center of the plate and another disc containing imipenem and EDTA was placed 30 mm from the first disc. A zone enhancement of ≥ 7mm for imipenem disc as compared to imipenem alone was considered as positive.

Amp C Detection
An overnight culture suspension of E.coli ATCC 25922 was used to make a lawn culture on Mueller-Hinton agar plate. A 30µg cefoxitin disc along with a blank disc moistened with sterile normal saline and inoculated with few colonies of test organism were placed on the plate almost touching each other. The plate was incubated at 37°C for overnight. A flattening or indentation of zone of inhibition of cefoxitin was interpreted as positive.

STATISTICAL ANALYSIS
Microsoft office 2007 was used for the analysis. Descriptive statistics like mean and percentages were used for the analysis.

RESULTS
During the study period, a total of 335 samples were received from the patients admitted to ICU. Male to female ration was 2:1. Out of 335 samples 239 (71.34%) were culture positive and 96 (28.65%) were culture negative. Out of 239 culture positive samples, pure isolate was present in 229(95.81%) samples and poly-microbial (two isolates) were present in 16 (6.6%) specimens. Acinetobacter baumannii was the commonest organism isolated in 88 (36.82%) samples, followed by P. aeruginosa 78 (32.63%), Klebsiella spp. 33 (13.80%), E. coli 19 (7.94%), Citrobacter spp. 25 (10.46%), Proteus spp 1(0.41%) and S.aureus 7(2.9%).

All the A.baumannii isolates were 100% resistant to cefotaxime, ceftriaxone, ceftazidime, cefepime and trimethoprim/sulfamethaxazole. Out of 88 isolates of A. baumannii, 76 (86.36%) were XDR. All the XDR isolates were 100% resistant to cephalosporins, tetracycline, doxycycline, gentamicin, netilmicin and ticarcillin/clavulanic acid. About 25 (32.84%) XDR strains were resistant to all the carbapenem tested and eight (10.52%) XDR were resistant to all drugs tested except colistin and polymyxin B. Polymyxin B and colistin were the most effective drugs (100%) against XDR strains followed by minocycline (62.92%), imipenem (55.56%), doripenem (37.04%), meropenem (52.24%) and Ampicillin/ sulbactam (3.71%). Table - 1 shows the susceptibility pattern of all and XDR A. baumannii strains. Out of 76 XDR isolates of Acinetobacter spp, 25 (32.9%) were ESBL producers. 37 strains were found to be meropenem resistant which were further tested for MBL production. Out of 37 meropenem resistant strains, MBL production was seen in 28 (36.8%) strains. Amp C production was seen in 45 (59.21%) strains (Table 2). The 8 isolates which were sensitive only to colistin and polymyxin B produced ESBL, MBL and AmpC in 100% strains. (Table 3)

DISCUSSION
A.baumannii infection has become a critical challenge to health care system particularly in ICU patients. In the present study A. baumannii was the most common isolate from the
LRTI where as in our previous reports *Acinetobacter* spp. was found to be second most common bacteria after *Pseudomonas aeruginosa* in ICU patients.\(^1\)\(^,\)\(^2\)\(^,\)\(^3\) The emergence of MDR and XDR isolates is a serious problem that has made it difficult to select an empirical antimicrobial for the treatment of *A. baumannii* infections. Various studies conducted worldwide have reported the prevalence of multidrug resistant *A. baumannii* clinical isolates to be 50–70%.\(^4\) In a study conducted by Kuo Chen *et al* in Taiwan, the prevalence of XDR *Acinetobacter* spp. was shown to increase from 1.3% in 2002 to 41.0% in 2010.\(^5\) In the present study also an alarmingly high rate of MDR and XDR (87%) isolates were observed. All *Acinetobacter* isolates in our study were 100% resistant to all generations of cephalosporins, trimethoprim/sulfamethoxazole and 80-90% resistant to aminoglycosides and beta lactam/ beta lactamase inhibitor combination. This high level of resistance to these routinely prescribed drugs could be attributable to the injudicious use of these drugs, thus resulting in selection of the resistant strains.

The problem of drug resistance in *Acinetobacter* isolates is further accentuated by the presence in them of resistant enzymes which are not easily detected in routine laboratory. Hence the resistant strains are detected as falsely susceptible leading to patients receiving ineffective drugs. The prevalence of ESBL enzymes in our study was observed in 32.9% isolates which is almost similar to another study done in India who observed ESBL production in 28% of the isolates.\(^6\) But a few studies done internationally have shown slightly higher ESBL production ranging from 46% - 54.6%.\(^7\)\(^,\)\(^8\)\(^,\)\(^9\) Amp C production was observed to be 51.13% in our study which is in concordance with other studies done by Gupta *et al* and Bhattacharjee *et al*.\(^1\)\(^0\)\(^,\)\(^1\)\(^1\) The ESBL and Amp C producing isolates hydrolyze 3\(^{rd}\) and 4\(^{th}\) generation cephalosporins as well as monobactams. Carbapenems are one of the most clinically important classes of antibiotics as well as last resort of treatment used against life-threatening *A. baumannii* infections. They are generally very stable against hydrolysis by most β lactamases including ESBL and AmpC producing strains. However reports on carbapenem resistant *A. baumannii* are on rise globally.\(^1\)\(^1\)\(^,\)\(^1\)\(^2\) We also observed a high rate of resistance to cabapenems against *A. baumannii* but worrisome finding of the study was that about 33% XDR strains were resistant to all three carbapenems i.e meropenem, imipenem and doripenem suggesting the possibility of treatment failures. In our study, the MBL production was observed in 31.8% strains. Similar studies done in different parts of India have also observed high rate of MBL production ranging from 48.72%-56.82%.\(^1\)\(^3\)\(^,\)\(^1\)\(^4\) This differences in prevalence of various resistant enzymes could be due to variations in characteristics of patients studied and the rates at which different antibiotics are used in different hospitals as has been proved earlier also.\(^1\)\(^5\) But all these studies highlight increased prevalence of MBL production leaving us with further limited therapeutic options as MBL producing isolates hydrolyze all beta lactams except aztreonam. In our study, Amp C was the most common beta lactamase produced simulating the findings of Sharan *et al* but contrary to few other studies.\(^1\)\(^6\)\(^,\)\(^1\)\(^7\) Various beta lactamase enzymes in the bacteria are encoded either by chromosomal genes or by the plasmids or the transposones, enabling further spread of these enzymes in the hospital settings.\(^1\)\(^8\) Hence this high rate of ESBL, MBL and Amp C production by the clinical isolates of *Acinetobacter* spp. warrants routine detection of beta lactamase enzymes which will enable the clinicians not only to prescribe appropriate treatment to patients but also to prevent the spread of these enzymes. ICU patients in particular are prone to infections by these MDR, XDR organisms producing resistant enzymes owing to their weak immune system as well as lack of effective antibiotic policy.

The emergence of *A. baumannii* strains resistant to all routinely tested antimicrobials has led to necessary revival of the old antibiotics like minocycline and polymyxins (colistin or polymyxin E and polymyxin B. Minocycline is a second-line antibiotic for a number of common bacterial infections in the clinical setting. Recent studies have indicated that minocycline is a promising drug for the treatment of *A. baumannii* infections.\(^1\)\(^9\)\(^,\)\(^2\)\(^0\) In our study also minocycline was found to be quite effective (63%) against XDR strains. Overall efficacy of polymyxins has been highly encouraging in both adults and paediatric populations. In

### Table-1: Sensitivity profile of XDR strains

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Percentage sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>0%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1.4%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>55.56%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>52.24%</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>50%</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>2.5%</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>0%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2.4%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>4.2%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0%</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>0%</td>
</tr>
<tr>
<td>Ticarcillin-Clavulanic acid</td>
<td>0%</td>
</tr>
<tr>
<td>Ampicillin-Sulbactam</td>
<td>3.7%</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0%</td>
</tr>
<tr>
<td>Minocycline</td>
<td>62.92%</td>
</tr>
<tr>
<td>Colistin</td>
<td>100%</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Table-2: Percentage of Beta Lactamase producing strains in XDR Acinetobacter isolates

<table>
<thead>
<tr>
<th>Total strains</th>
<th>XDR isolates (76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL producing strains</td>
<td>25 (32.9%)</td>
</tr>
<tr>
<td>Amp C producing strains</td>
<td>45 (59.21%)</td>
</tr>
<tr>
<td>MBL producing strains</td>
<td>28 (36.8%)</td>
</tr>
</tbody>
</table>

### Table-3: Percentage of Beta Lactamase producing strains sensitive only to polymyxins

<table>
<thead>
<tr>
<th>Resistant Enzyme</th>
<th>XDR isolates (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL</td>
<td>100%</td>
</tr>
<tr>
<td>Amp C</td>
<td>100%</td>
</tr>
<tr>
<td>MBL</td>
<td>100%</td>
</tr>
</tbody>
</table>
our study all (100%) isolates were sensitive to polymyxins. Another warning signal of our study was the isolation of three strains that were resistant to all the classes of drugs except polymyxins (colistin and polymyxin B). Polymyxins, which are potentially toxic, have been ultimately considered as the last-resort treatment of XDR strains. Unfortunately, resistance to polymyxins have also been reported all over the world. Rate of resistance to polymyxins have recently been reported to be as high as 3.2% for MDR A. baumannii. Even higher rate of resistance to polymyxins are reported from Korea.28 Moreover treatment of patients with polymyxins has been associated with the emergence of pandrug resistance, poor clinical outcomes and breakthrough infections.29 Isolation of three polymyxins only susceptible strains from our institute raises a concern, since these patients can act as reservoir and may disseminate the infection to other patients. Though we have not yet isolated any strain that is polymyxin resistant but there is an impending risk of emergence of polymyxin resistance in our institute. Threats posed by the global increase in antibiotic resistant bacterial strains continue to cause alarm, and some observers suggest that this problem, caused by the use and misuse of antibiotics, is now threatening to take societies back to a pre-antibiotic era.

CONCLUSION

We conclude that there is high frequency of isolation of XDR Acinetobacter isolates from ICU patients. XDR isolates demonstrate increased production of ESBL, MBL and Amp C enzymes. Acquisition of these multiple resistance mechanisms by XDR Acinetobacter spp. leaves us with limited therapeutic options like minocycline and polymyxins. This report lents an urgent need to take stringent actions for implementing infection control practices, rational use of antibiotics as well as for framing an institutional antibiotic policy. Collaboration between clinicians and clinical microbiologist may be the most useful measure to optimize the use of antimicrobials.

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