Introduction

Nosocomial infections caused by multidrug resistant (MDR) Gram negative bacteria expressing Extended Spectrum beta lactamases (ESBL) pose serious therapeutic challenge to clinicians due to limited therapeutic options. ESBLs are plasmid mediated enzymes capable of hydrolysing and inactivating a wide variety of \( \beta \) lactams, including third generation cephalosporins, penicillins, and aztreonam, but are susceptible to \( \beta \) lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. They are mainly found in Escherichia coli, Klebsiella species, Proteus species but can also occur in other members of Enterobacteriaceae family and in some nonenteric organisms such as Acinetobacter species.\(^2\)

Amp C enzymes are chromosomally encoded \( \beta \)-lactamases which confer resistance to the oxyimino group containing cephalosporins (Cefotaxime,ceftriaxone and ceftazidime) and the 7 methoxy cephalosporins (cefoxitin and ceftotetan) and are not affected by inhibitors (clavulanic acid, tazobactam and sulbactam). They are commonly found in Enterobacter, Serratia, Providencia, Aeromonas, Morganella morganii, Citrobacter freundii, Haflma and Pseudomonas aeruginosa.\(^3\)

Since the ESBL genes are usually found in large plasmids, they also contain other antimicrobial resistant genes. Therefore most ESBL producing organisms are also resistant to aminoglycosides, fluoroquinolones, tetracyclines, Chloramphenicol, sulfonamides. Carbapenems are the mainstay of therapy for infections caused by ESBL producing organisms.\(^4\) Nevertheless their resistance to a wide variety of common antimicrobials has made the proliferation of ESBL producing strains a serious global health concern that has complicated treatment strategies. In this context, routine screening for ESBL producers in the laboratory is of great importance for their early identification and management.\(^5\)

Several factors have been reported to increase the risk of colonisation and infection with ESBL producing bacteria which include prolonged hospital stay, prior antibiotic usage, presence of invasive device and severe underlying disease. It is also imperative that risk factors for infection with ESBL producing organisms be clearly identified so that effective strategies can be developed to curtail the emergence and spread of these strains.\(^6,7,8\)

This study was undertaken to determine the prevalence of ESBL production among nosocomial isolates of E.coli and Klebsiella pneumoniae and their impact on the clinical outcome.

Materials and Methods

The study was conducted for a period of two months (July 2006 and August 2006) A total number of 101 consecutive, non-repetitive, clinical isolates of E.coli (72) and Klebsiella pneumoniae

Abstract

Background: Extended spectrum \( \beta \) Lactamases (ESBL) producing Escherichia coli (EC) and Klebsiella pneumoniae (KP) has increased in recent years leading to limitations of treatment options. The present study was undertaken to determine the prevalence of ESBL production among nosocomial isolates of EC and KP and their impact on clinical outcome.

Methods: One hundred and one isolates of EC and KP obtained from patients hospitalized for \( \geq 48 \) hours were included in the study. They were tested for ESBL production by double disc synergy test (DDST) and E test. Co-resistance to fluoroquinolones, aminoglycosides, trimethoprim - sulphamethoxazole, aztreonam and \( \beta \) lactamase inhibitor combinations and susceptibility to carbapenems were determined by disc diffusion method. Production of AmpC was screened using Cefoxitin discs. They were designated as colonizers or pathogens using clinical data and laboratory parameters. Risk factors assessed were variables related to hospital stay and antibiotics used. The outcome was followed up.

Results: Of 101 isolates, 68 (49 EC, 19 KP) were ESBL producers, 14 being colonizers and 54 pathogens. They were obtained from Blood (4), Respiratory secretions (4), exudates (12) and Urine (48). A high degree of co-resistance was observed to other antibiotics. Susceptibility to carbapenem was universal. Two isolates were AmpC producers. Increased duration of hospital stay of \( \geq 7 \) days was a significant risk factor (\( P < 0.0005 \)). Prior exposure to antibiotics was also an important contributing factor.

Conclusion: The high prevalence of ESBL in EC and KP is associated with a multitude of infections in hospitalized patients with a significantly longer duration of hospital stay, increased morbidity and greater hospital charges. This necessitates emphasis on interventional strategies to control and prevent their spread in health care centers.
subjected to antibiotics susceptibilty testing by disc diffusion

Microbiological procedures

exudates(15), and respiratory secretions(4). The source of these isolates were Urine(78), blood(4), from the cultures of specimens from patients hospitalised for >29 were obtained from the clinical Microbiology laboratory of controls,

Mean age 40.29,

Clinical data collected included the demographic characteristics , provisional diagnosis, duration of hospital stay , presence of indwelling devices, period of stay in the Intensive care units (ICU),classes of antibiotics used, presence of underlying diseases such as Diabetes mellitus, malignancy, etc. The therapy instituted was recorded and outcome of treatment was followed up.

Statistical Analysis

Proportions were compared using Chi -Square test to determine the significance of factors influencing acquisition of ESBL producing strains. Difference were considered significant if p was < 0.05, Analysis were performed using SPSS software.

Results

Of 101 isolates, 68 (67.3%) were ESBL producers and 33 (32.7%) were non ESBL producers. Among the ESBL producers 49 were Escherichia coli and 19 were Klebsiella pneumoniae. Two isolates were AmpC producers. The source of ESBL producing strains were urine(n=48), exudates (n=12), blood (n=4) and respiratory secretions(n=4). Among the 68 ESBL isolates 54 were pathogens and 14 were considered colonisers after careful evaluation with clinical parameters. Majority of the ESBL producers (72%) were obtained from the patients in non ICU wards of the health care facility. These strains were co-resistant to several antibiotics including ciprofloxacin and ofloxacin (86.8%), amikacin (25%), piperacillin- tazobactam (13.2%) and ceftipime(30.8 %). All ESBL producer were susceptible to imipenem.

The demographic and clinical characteristics are shown in table 1.

Mean age of patients with ESBL producing isolates was 40.29 (SD 22.29) and there were equal number of male and female patients. Mean duration of hospital stay was 15.4(6SD13.6) days. The difference in duration of stay in the hospital was a significant risk factor for acquiring infection with ESBL producing strains (P=0.005). Exposure to multiple antibiotics, stay in ICU, presence of invasive device and underlying illness were not significant risk factors in this study. However prior exposure to beta lactam antibiotics was recorded in 41 patients with ESBL producing organisms. This is an important contributing factor . Six patients in the study population died, all the expired patient had been infected with ESBL producing organisms and were in th ICU.

Discussion

ESBLs most commonly produced by the Enterobacteriaceae ,confers resistance to β lactam and monobactam antibiotics. These enzymes, often expressed by genes carried on large transferable plasmids constitute an important mechanism of resistance in nosocomial Gram negative pathogens.

The introduction of the third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β lactamase-mediated bacterial resistance to antibiotics. The first report of plasmid encoded β lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983.Most of these ESBL have evolved by genetic mutation from native β lactamases TEM-1,TEM-2 and SHV-1. These parent enzymes are commonly found in Gram negative bacteria, particularly Enterobacteriaceae and are highly active against penicillins and early generation of cephalosporins. Other ESBLs that do not belong to the TEM or SHV families have also been reported, and these are termed “CTX-M” (highlighting their greater activity against cephalotaxime

Table 1 : Demographic and Clinical Characteristics of the Isolates

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ESBL positive [68]</th>
<th>ESBL negative [33]</th>
<th>Total [101]</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>40.29(5D22.29)</td>
<td>41.33(5D26.51)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sex - Males</td>
<td>34(73.9%)</td>
<td>12(26.1%)</td>
<td>46</td>
<td>0.197</td>
</tr>
<tr>
<td>Females</td>
<td>34(61.8%)</td>
<td>21(38.2%)</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Duration of hospital stay &gt;7 days</td>
<td>50</td>
<td>3</td>
<td>53</td>
<td>0.0005</td>
</tr>
<tr>
<td>&lt;= 7 days</td>
<td>18</td>
<td>30</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Mean duration of hospital stay (days)</td>
<td>15.4(5D13.6)</td>
<td>4.8(5D2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underlying illness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>20(66.7%)</td>
<td>10(33.3%)</td>
<td>30</td>
<td>0.888</td>
</tr>
<tr>
<td>Others</td>
<td>14(63.6%)</td>
<td>5(36.4%)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>34(69.4%)</td>
<td>15(30.6%)</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Stay in ICU</td>
<td>20(80%)</td>
<td>5(20%)</td>
<td>25</td>
<td>0.119</td>
</tr>
<tr>
<td>Non ICU</td>
<td>48(63.2%)</td>
<td>28(36.8%)</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Ventilated</td>
<td>11(91.7%)</td>
<td>1(8.3%)</td>
<td>12</td>
<td>0.055</td>
</tr>
<tr>
<td>Not ventilated</td>
<td>57(83.8%)</td>
<td>12(16.2%)</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Exposure to antibiotics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B lactams</td>
<td>41(71.9%)</td>
<td>16(28.1%)</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Carbapenems</td>
<td>0</td>
<td>100(100%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides / fluoroquinolones</td>
<td>9(56.3%)</td>
<td>7(43.8%)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Others(doxo, tetra, macrolides, Cotrimoxazole)</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0.266</td>
</tr>
<tr>
<td>No antibiotics</td>
<td>15(62.5%)</td>
<td>9(37.5%)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovered</td>
<td>62(65.3%)</td>
<td>33(34.7%)</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Expired</td>
<td>6(100%)</td>
<td>0</td>
<td>6</td>
<td>0.079</td>
</tr>
</tbody>
</table>

* P value < 0.05 is significant

(29)were obtained from the clinical Microbiology laboratory of a 1200 bedded tertiary care hospital.The isolates were obtained from the cultures of specimens from patients hospitalised for > 48 hours. The source of these isolates were Urine(78), Blood(4), exudates(15), and respiratory secretions(4).

Microbiological procedures

Bacterial isolates of E.coli and Klebsiella pneumoniae were subjected to antibiotics susceptibility testing by disc diffusion technique according to CLSI guidelines with suitable quality controls, E.coli ATCC 25922 , Klebsiella pneumoniae 700603. The drugs tested were ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefepime(30µg), ciprofloxacin (5µg), gentamycin (5µg) and amikacin (30µg) [Himedia laboratories , Mumbai], piperacillin – tazobactam (100/10µg) and imipenem(10µg) [ BD diagnostics, Bawam, Haryana]

ESBL screening: Isolates were tested for ESBL production by phenotypic method of inhibitor potentiated disc diffusion. Minimal inhibitory concentration (MIC) was determined by E test ESBL strips with cefotaxime/clavulanic acid and Cefazidime/clavulanic acid. AmpC beta lactamase production was screened by using cefoxitin (30µg) discs.

Clinical data

The isolates were designated as colonisers or pathogens using a combination of clinical data and laboratory parameters. The clinical data included the demographic characteristics , provisional diagnosis, duration of hospital stay , presence of
than ceftazidime). Several ESBLs that are not closely related to any of the three well-established families have been reported. Examples include SFO, BES, BEL, TLA, GES, PER and VEB types. These ESBLs are remarkable for their geographical diversity. ESBLs now number >500 distinct enzymes and convey varying degree of resistance to penicillins, cephalosporins, β-lactam inhibitors and monobactams.

They are of greatest concern because the infections caused by these are often multidrug resistant. Patients with infections due to ESBL-producing organisms have significantly longer hospital stay and incur greater hospital charges than do patients without these infections. The associated mortality rate is also higher. Detecting the presence of ESBL producing pathogens in specimens obtained from patients has important implications for clinical decision making, chiefly by influencing the choice of appropriate therapy. In addition to its clinical value, their detection can also aid in infection-control measures by helping to guide patient isolation strategies.

We detected ESBL production by using E test ESBL strips. The E test ESBL strip is a commercially available agar based test that has excellent sensitivity and specificity for the confirmation of ESBL producing strains. The MIC to cefotaxime and Ceftazidime were determined and compared with their MIC in the presence of clavulanic acid. The prevalence of ESBL production among 101 isolates was 67.3%. These strains were co-resistant to several antibiotics including ciprofloxacin and ofloxacin (86.8%), amikacin (25%), piperillin-tazobactam (13.2%) and cefipime (30.8%). All ESBL producer were susceptible to imipenem. These finding suggest that carbapenems are the best choice for treatment of ESBL related infections.

The isolates were designated as colonizers or pathogens using a combination of clinical data and laboratory parameters. A detailed clinical history was sought to rule out colonization and to assess risk factors for acquiring infection with ESBL producing strains. In this study increased duration of hospital stay (≥ 7 days) was the most significant risk factor (P=0.0005). Mean duration of hospital stay was 15.4 days among patients with ESBL producing organisms and 4.8 days among patients with non-ESBL producing organisms. Though mechanical ventilation, urinary and vascular catheterization were not significantly associated with ESBL, they are surrogate markers of nursing care manipulations which increases with length of hospital stay.

Underlying severe and prolonged disease were not significant risk factors for acquiring ESBL. 20.5% of the ESBL isolates were deemed as colonizers. Empirical antibiotic therapy promotes such colonization in hospitalized patients with resistant strains by eradicating susceptible flora. They are the reservoirs of infection and facilitate the persistence of multidrug resistant bacteria in the hospital.

Increased mortality due to ESBL was not encountered in this study. Yet they contributed to prolonged hospital stay and increased antibiotic usage leading ultimately to economic burden in the hospitalized patients. Though exposure to multiple classes of antibiotics including beta lactams was observed in 80% of patients infected with ESBL producers, this factor was not significantly associated with development of infection with ESBLs.

Numerous studies have assessed risk factors for colonization and infection with ESBL-producing organisms. Analysis of the results of these studies yields a plethora of conflicting results, likely due to the differences in study populations, selection of cases, selection of controls, and sample size. Nevertheless, some generalizations do exist. Patients at high risk for developing colonization or infection with ESBL-producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices are present (urinary catheters, endotracheal tubes, central venous lines) for a prolonged duration. The median length of hospital stay prior to isolation of an ESBL producer has ranged from 11 to 67 days. Yet they contributed to prolonged hospital stay and may be higher in patients infected with ESBL-producing organisms than with non-ESBL-producing organisms of the same species.

**Conclusion**

The high prevalence of ESBL in *E. coli* and *Klebsiella pneumoniae* is associated with a multitude of infections in hospitalized patients. Emphasis must be placed on the rational and judicious use of all antimicrobial agents. Accurate detection of ESBL producers, their treatment strategies and infection control policies are of paramount importance in curtailing this growing epidemic. A knowledge of ESBL properties, the risk factors for acquisition and differentiation between ESBL and other resistance mechanisms will help physicians opt for the best treatment modalities.

**References**


