Status of extended spectrum beta lactamase producing Escherichia Coli and Klebsiella species in urinary tract infection

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Background: Production of Extended Spectrum Beta Lactamases (ESBLs) is an important mechanism of beta-lactam resistance in Enterobacteriaceae. And the occurrence of ESBL producing isolates has increased worldwide.

Methods: The study was carried out in bacteriology laboratory of TUTH from March 2005 to May 2005 that included 371 midstream urine samples (msu). During these three months period, the MSU samples were investigated by conventional semi-quantitative culture technique, antibiotic susceptibility test and ESBL detection test.

Results: Out of 98 significant positive cases, 60 cases were due to E. coli, 5 were due to Klebsiella oxytoca and 4 were due to Klebsiella pneumoniae. Multidrug resistance (MDR, resistant to two or more than two classes of antibiotics) was observed in 61.7% (37/60) of Escherichia coli, 40% (2/5) of Klebsiella oxytoca and 75% (3/4) of Klebsiella pneumoniae. Out of total 69 cases of E. coli and Klebsiella species that were subjected to preliminary Ceftazidime ESBL screening test, 22 isolates were suspected of ESBL production. Finally 27.5% (19/69) were confirmed for production of ESBL by Double Disc Synergy Test (DDST). Out of 19 ESBL producing strains, 16 were found out to be E. coli, one was K. oxytoca and 2 were K. pneumoniae. All ESBL producing strains were found to be multidrug resistant.

Conclusion: There is a great need to conduct this type of study throughout the year to determine their seasonal variation and also for the study of increasing trends of MDR and ESBL. This type of study should also be conducted in other hospital as well.

Keywords: UTI, Extended Spectrum Beta Lactamase (ESBL), Escherichia coli, Klebsiella species, DDST

Introduction

ESBLs are group of enzymes occasionally present in Klebsiella species and Escherichia coli that confer upon the bacteria the additional ability to hydrolyze the beta-lactam rings of oxyimino- third and second generation cephalosporins (ceftaxime, ceftriaxone and ceftazidime) and/or aztreonam 1. These beta-lactamases generally belong to Ambler’s molecular class A 2 and Bush’s functional class 2be 3. They are susceptible to beta-lactamase inhibitors such as clavulanic acid 4 but do not effect cefamycins as cefoxitin, cefotetan and cefmetazole and carbapenems as imipenem and meropenem 5.

ESBLs include: Cephalosporin-hydrolysing mutants of TEM and SHV - the common plasmid-mediated penicillinases of Enterobacteriaceae (over 100 such variants are known) 6; CTX-M types that evolved separately, at least some of them...
via the escape and mutation of chromosomal ß-lactamases of Kluyvera spp. (over 30 variants are known) and Obscure types, e.g. VEB, PER and OXA (Class D) ESBLs from Pseudomonas aeruginosa.

ESBLs are not the sole ß-lactamases to confer resistance to 2nd and 3rd generation cephalosporins, but are the most important. They should be distinguished from other important modes of resistance to 2nd and 3rd generation cephalosporins as hyperproduction of chromosomal AmpC ß-lactamases, especially in Enterobacter spp. ß-plasmid-mediated AmpC ß-lactamases, in Klebsiella spp. and E. coli (rare); hyperproduction of K1 or KOXY chromosomal ß-lactamases in K. oxytoca (not K. pneumoniae); efflux-mediated resistance in P. aeruginosa and various ill-defined mechanisms in Acinetobacter spp.

ESBLs are clinically important because they destroy cephalosporins given as first-line agents to many severely ill patients, and delayed recognition and inappropriate treatment of severe infections caused by ESBL producers with cephalosporins has been associated with increased mortality. Many ESBL producers are multi-resistant to non-ß-lactam antibiotics such as quinolones, aminoglycosides and trimethoprim, narrowing treatment options.

The National Committee for Clinical Laboratory Standards (NCCLS) has developed broth microdilution and disk diffusion screening tests using selected antimicrobial agents. Each Klebsiella pneumoniae, K. oxytoca, or Escherichia coli isolate should be considered a potential ESBL-producer if the test results are as follows:

<table>
<thead>
<tr>
<th>Disc diffusion</th>
<th>MICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>cefpodoxime ≤ 22 mm</td>
<td>cefpodoxime ≥ 2 g/ml</td>
</tr>
<tr>
<td>cefotaxime ≤ 22 mm</td>
<td>cefotaxime ≥ 2 g/ml</td>
</tr>
<tr>
<td>aztreonam ≤ 27 mm</td>
<td>aztreonam ≥ 2 g/ml</td>
</tr>
<tr>
<td>cefotaxime ≤ 27 mm</td>
<td>cefotaxime ≥ 2 g/ml</td>
</tr>
<tr>
<td>ceftriaxone ≤ 25 mm</td>
<td>ceftriaxone ≥ 2 g/ml</td>
</tr>
</tbody>
</table>

Enterobacteriaceae isolates resistant to any indicator cephalosporin in the screening tests outlined above should be subjected to confirmatory tests. Confirmation of ESBL production depends on demonstrating synergy between clavulanate and those indicator cephalosporin(s) to which the isolate was initially found resistant. Methods that can be used for confirmation of ESBL production include: Double Disc Synergy Tests (DDST), Combination disc methods and Etest ESBL strips methods. K. pneumoniae ATCC 700603 (positive control) and E. coli ATCC 25922 (negative control) are supposed to be used for quality control of ESBL tests.

As the incidence of antimicrobial resistance rises, the costs associated with consequences also do and hence can be considered an economic burden to society more so in context of developing country like Nepal. Antibiotic susceptibility profile and reporting of drug resistant strain especially ESBL producing strains would enlighten the appropriate antibiotic therapy and would help in global awareness towards misuse and overuse of antibiotics. Therefore this study was conducted with the objective of studying the aetiological agents causing UTI and their antibiotic susceptibility profile with the special reference to Extended Spectrum Beta Lactamases producing strains.

Material and Methods

Study Population: The present research work was conducted in the well-equipped laboratory of Bacteriology section of Tribhuvan University Teaching Hospital, one of the major hospitals of Kathmandu valley, from March 2005 to May 2005. During the research period, 371 midstream urine specimens, collected from patients that were suspected of Urinary Tract Infection (UTI), was enrolled. The age of these patients ranged from 8 to 86 years.

Culture, Identification and Antibiotic susceptibility test: Semi quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria. An inoculating loop of standard dimension was used to take up approximately fixed (±10% error is accepted) and known volume of mixed uncentrifuged urine was inoculated on the surface of 5% Blood Agar (Oxoid, England) and MacConkey Agar (Oxoid, England). The inoculated MA and BA were incubated at 37°C for 24 hours. After incubation the number of colonies was counted on each plate and organisms per ml was estimated. The bacterial isolates in the plates were identified if significant growth occurs (≥10³CFU/ml). Blood agar was observed for haemolysis and MacConkey agar for lactose fermentation.

Identification of significant isolates was done by using standard microbiological techniques that included study of colony morphology, Gram staining and biochemical tests (catalase test, oxidase test, TSI test, SIM test, Citrate Utilization test, Urea hydrolysis test and others as required). Antibiotic susceptibility test of all the isolates was performed by Kirby Bauer disc diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS). In this method, the broth culture of test organism (comparable to McFarland tube no.0.5; inoculum density 1.5x10⁸ organisms per ml) was uniformly carpeted on the surface of Mueller Hinton agar (Oxoid). Then, antibiotic discs were seeded over the lawn culture of test organism. The inoculated and seeded MHA plate was then incubated at 37⁰C for 18 hours (or overnight). After incubation the zone diameter of each antibiotic was interpreted using the

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interpretative chart and the organism was reported “resistant”, “moderately/intermediate susceptible” or “susceptible. Control strains (ATCC) were used in parallel as a part of quality control test system.

Antibiotics used for gram negative bacteria were Ampicillin (10 µg per disc, Oxoid), Ciprofloxacin (5 µg per disc, Oxoid), Cephalexin (30 µg per disc, Oxoid), Norfloxacin (10 µg per disc, Oxoid), Nitrofurantoin (300 µg per disc, Oxoid) and Ceftazidime (30 µg per disc, Oxoid). Multi-drug Resistant isolates were defined as those, which showed resistance to two or more than two classes of antibiotics of the first line used for the sensitivity testing.

**Laboratory Detection of Extended Spectrum Beta Lacatamase Producing Strain:**

Screening test for ESBL detection: The indicator drug (Ceftazidime, 30 µg per disc, Oxoid) was included in primary susceptibility testing. As directed by NCCLS 10,11 the organisms that gave <22 mm diameter of zone of inhibition, were suspected of ESBL production i.e., regarded as ESBL screening test positive.

Confirmatory test for ESBL detection (Double Disc Synergy Test or DDST): E. coli and Klebsiella species that were suspected as ESBL producing strains in the screening test were subjected to the phenotypic confirmatory test. The Double Disc Synergy Test or DDST was performed by standard disc diffusion assay on Mueller Hinton Agar.

A MHA plate was inoculated with Nutrient broth culture of organism (comparable to McFarland nephometer tube no. 0.5) as for a routine susceptibility tests. Discs containing cefotaxime (30 µg per disc, Oxoid) and ceftazidime (30 µg per disc, Oxoid) were applied either side of one with Co-amoxyclov (20+10 µg per disc, Oxoid). The distance between Co-amoxyclov and either third generation cepaholsporin was adjusted 20 to 30 mm (center to center) or 15 mm (edge to edge) depending on the species 14, 15.

Enhancement of the inhibition zone towards the disc of Co-amoxyclov (clavulanic acid effect with either screening agent) was considered and confirmed suggestive of ESBL producing strain.

**Results**

Out of 371 MSU samples 98 (26.4%) samples showed monomicrobial significant growth (i.e. e\(^{10^{5}}\) CFU/ml, 218

**Table 1:** Pattern of bacterial Isolates and Multidrug Resistance among urinary isolates

<table>
<thead>
<tr>
<th>S.N</th>
<th>Organism</th>
<th>Number of cases</th>
<th>Total% MDR</th>
<th>Total MDR</th>
<th>Total MDR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Escherichia coli (N=37)</td>
<td>60</td>
<td>61.2</td>
<td>37</td>
<td>61.7</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella pneumoniae (N=3)</td>
<td>5</td>
<td>5.1</td>
<td>2</td>
<td>40.0</td>
</tr>
<tr>
<td>3</td>
<td>Klebsiella oxytoca (N=2)</td>
<td>4</td>
<td>4.1</td>
<td>3</td>
<td>75.0</td>
</tr>
</tbody>
</table>

**Table 2:** Antibiotic susceptibility profile of MDR strains in urinary tract infection

<table>
<thead>
<tr>
<th>S.N</th>
<th>Organism</th>
<th>Antibiotics used</th>
<th>Antibiotic Susceptibility Profile(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>R %</td>
</tr>
<tr>
<td>1</td>
<td>Escherichia coli (N=37)</td>
<td>Ampicillin</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalexin</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norfloxacin</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrofurantoin</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella pneumoniae (N=3)</td>
<td>Ampicillin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalexin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norfloxacin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrofurantoin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Klebsiella oxytoca (N=2)</td>
<td>Ampicillin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalexin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norfloxacin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nitrofurantoin</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\)Abbreviations: R, Resistant; I, Intermediate or Moderately Sensitive; S, Sensitive
(58.7%) samples were sterile, 34 (9.2%) samples showed insignificant growth (i.e.<10^3 CFU/ml) and 21 (5.7%) samples showed polymicrobial insignificant growth (i.e. more than two types of isolates).

Out of 98 significant culture positive cases, 83 (84.7%) were due to gram negative bacteria and 15 (15.3%) were due to gram positive bacteria. Eleven different species of bacteria were isolated among which *Escherichia coli* (61.2%) was found to be the most common one followed by *Klebsiella* species (9.2%), *Pseudomonas aeruginosa* (7.1%), *Enterococcus faecalis* (6.1%), *Staphylococcus aureus* (6.1%) and *Proteus mirabilis* (4.1%). The other organisms are *Citrobacter freundii* (2.0%), CONS (2.0%), *Morganella morganii* (1.0%) and *Staphylococcus saprophyticus* (1.0%).

Among the multidrug resistant *Escherichia coli*, 100% isolates were found to be resistant to Ampicillin, 81.08% resistant to Ciprofloxacin and 21.62% resistant to Nitrofurantoin.

The phenotypic profile for ESBL detection test result is expressed in figure 1. Out of 69 potential isolates (excluding non- *Escherichia* and non *Klebsiella* species), 22 isolates were suspected of ESBL production i.e. Ceftazidine screen positive. And out of 22 only 19 isolates were confirmed for ESBL production in DDST.

**Fig. 1:** Summary of phenotypic profiles of study isolates

- **Total isolates (total *E. coli* and *Klebsiella* species)**: 69 isolates
- **Negative ESBL Ceftazidine screen 47 isolates**
- **Positive ESBL Ceftazidine screen 22 isolates**
- **Confirmatory test (Double Disc Synergy Test)**
  - **ESBL confirmatory test negative (No CA effect)**: 3 isolates
  - **ESBL confirmatory test positive (CA effect)**: 19 isolates

**Discussion**

The increasing prevalence of antibiotic resistance is a cause of serious concern and multidrug resistance among some of the most important human pathogens is increasing. In our study Multidrug resistance was found out to be 61.7% (37/60) in *Escherichia coli* and that in *K. oxytoca* and *K. pneumoniae* was 40% (2/5) and 75% (3/4) respectively. Multidrug resistance (MDR) in our study is higher than in other studies that have same MDR criterion.

Outcome of prevalence of MDR depends on various factors, MDR criterion being the chief one followed by the types of antibiotics used in antibiogram, study isolates and study population. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used.

First described in 1983, ESBLs have contributed to dramatic increase in resistance to β-lactam agents among gram negative bacteria in recent years. However laboratory detection of ESBL can be problematic, because in many cases conventional breakpoints of resistance are not reached. All *E. coli* and *Klebsiella* species isolated were subjected to phenotypic laboratory detection of ESBL production. The NCCLS has issued guidelines for ESBL screening and confirmatory tests that apply only to *E. coli* and *Klebsiella* species. Hospital based study of in USA demonstrated that ESBLs are detected infrequently in non *Klebsiella* and non *E. coli* isolates of Enterobacteriaceae. Other isolates of *Enterobacteriaceae*, such as *Salmonella* spp., *Proteus mirabilis*, *Enterobacter* spp. and *Citrobacter freundii* and isolates of *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Stenotrophomonas maltophilia* also produce ESBLs. However, at this time, methods for screening and phenotypic confirmatory testing of these isolates have not been determined by NCCLS. NCCLS detection methods are based on a phenotypic profile that has potential to yield false positive and false negative results. In some of the isolates, additional mechanisms of resistance, such as AmpC- β-lactamases, porin changes, and inhibitor resistant TEMs (IRTs) and SHV β-lactamases with reduced affinities for β-lactamase inhibitors can mask CA inhibition. In addition hyperproduction of Class A ‘K1’ chromosomal protease by *K. oxytoca* can give positive clavulanate synergy test with ceftoxime and cefepime (never ceftazidime), so the producers are confused with ESBL producers.

Organisms inferred to have ESBLs should be reported resistant to ALL penicillins (except temocillin), cephalosporins (except cefoxitin), and to aztreonam, irrespective of routine susceptibility results. Treatment failures and death have occurred when cephalosporins were used against ESBL producers that appeared susceptible in vitro.
Despite the introduction of very promising molecular methods (e.g. DNA probes, PCR, nucleotide sequencing, isoelectric focusing and chip technology), the phenotypic detection tests are considered by many the simplest and most cost effective strategies for detection of ESBLs among gram negative bacteria.

**Conclusion**

Based on invitro susceptibility test and phenotypic Double Disc Synergy Test (DDST), it was concluded that a significant number of urinary isolates are MDR and often co-produce ESBL, which can result in unavoidable treatment failures. No doubt the reporting of MDR and ESBLs and other beta lactamases should continue to challenge treatment strategy for years to come.

**Recommendations**

All the hospitals and health institutes should have the provision for detection of ESBL producing strains as these are associated with treatment failure in many cases.

Even if ceftazidime and/or cefotaxime appear to be sensitive in vitro, but the zone of inhibition is ≤22 mm and ≤27 mm respectively, the organism should be suspected for ESBL production. If the organism is confirmed to do so, it should be reported to be resistant to all penicillins (except temocillin), cephalaxins (except cephalexin) and aztreonam. If the patient is not responding to a particular third generation cephalosporins, the clinicians should look for ESBL producing organisms.

Techniques for detection of enzymes with marginal ESBL activity, those expressed weakly, those produced alongside other enzymes (as AmpC, K1 protease) and techniques for detection of these AmpC, K1 protease should also be applied.

The study should be continued and the MDR and ESBL producing strains should be subjected for genetic study to acquire their detailed genetic makeup and to characterize the mechanism of drug resistance. The phenotypic methods outlined in our study will never be so precise as the best molecular methods.

**Acknowledgement**

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**References**

Escherichia Coli and Klebsiella species in urinary


