Detection of ESBL (extended spectrum β lactamases) from urinary isolates of multi drug resistant enterobacteriaceae in a tertiary care hospital in Mangalore

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Abstract

Introduction: The incidence of Extended Spectrum β Lactamase (ESBL) producing strains is increasing over the years. ESBL producing organisms pose problem for treatment. ESBL producers are also higher in uropathogens and baseline knowledge of ESBL organisms is mandatory for formulating control measures hence an attempt was therefore made to study ESBL production from urinary samples at a tertiary care hospital in Mangalore. Aim: To detect the number of ESBL producers among uropathogens in our centre and to compare two methods of ESBL detection. Materials and Methods: 1121 urinary samples were processed, of this 319 were Multi-drug resistant and were tested for ESBL production by Double Disc approximation and CLSI Confirmatory Test. Results: Out of 319 MDR samples, 153 were Enterobacteriaceae 93 were positive for ESBL i.e., 60.78%. Inpatients were more than outpatients and the two methods of ESBL detection were comparable. In this study ESBL was 60.78% among MDR enterobacteria and enterobacteriaceae being the commonest organism in UTI, an attempt to contain ESBL organism may have some impact on decreasing the load of MDR organism. Conclusion: The burden of ESBL's among Enterobacteriacea uropathogens continues to pose a challenge in treatment. Carbapenems were a mainstay in treating these cases and either methods of ESBL detection may be conviently used. However attempts to minimize ESBL spread through hygiene,contact precautions,suitable antibiotic policy to include cheaper and more effective antimicrobials are the need of the hour.

Key words: CLSI confirmatory test, double-disc diffusion, ESBL, multidrug resistance.

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INTRODUCTION

Unlike the developed countries, India like other developing countries has a heavy burden of MDR (Multi drug resistance) Organism, and many of the MDR are caused by, ESBL (extended spectrum β lactamases) producers.1,2,3 In some studies in India, ESBL percentage is 62-100% among E-Coli and Klebsiella.1 ESBL enzymes hydrolyse β Lactam rings and hence resistant to cephalosporins, aztreonam but are inhibited by Clavulanic acid.2 UTI is one of the most common infections4,5 for which antimicrobial agent is usually prescribed.6 Enterobacteriaceae are leading cause in Urinary tract infection (UTI).6,7,8 ESBL producers are also higher in uropathogens.8 Hence detection of ESBL from enterobacteriaceae was attempted. This part of India particularly coastal areas is geographically different from main land India and still very little data exists.2 Studies suggest the need for area or institution based approach for screening or intervention if needed, for this baseline knowledge of ESBL organisms is mandatory, without which control measures cannot be formulated.3 ESBL producers from community are also on the rise making it a public health concern8 studies suggest, that ESBL positive patient in UTI had longer hospital stay and more symptoms9, hence in this studies the true burden of ESBL...
enterobacteriaceae in urine culture positive sample and MDR organism was attempted.

**MATERIALS AND METHODS**

The study was conducted at the Department of Microbiology. After ethical clearance, 1121 urinary samples from Sep 2013 to Nov 2013 that were clinically suspected for UTI were processed. Out of this 472 were reported with sensitivity. From this 319, samples that were MDR (Multi Drug Resistance) i.e. resistant to 1st, 2nd, 3rd generation cephalosporin and 153 were Enterobacteriaceae, these 153 samples were further processed for ESBL detection.

**Antibiotic Susceptibility testing**

The isolates were tested as per CLSI guidelines. The drug discs used for screening were Amoxicillin/Clavulanic acid (Amoxclv) (20/10µg), Cefazolin (30µg), Cefuroxime (30µg) and Cefotaxim (30µg), for ESBL production, after standardization to 0.5 Mc Farland.

1. **Double Disc approximation**

   The organism was swabbed on Mueller Hilton agar. Amoxclv (20/10µg) and Cefotaxim (30µg) discs were placed 15mm apart and incubated (18-24 hrs). Organisms showing extension towards Amoxclv disc were considered as ESBL producers. [fig :1]

2. **CLSI Confirmatory Test**

   Cefazidine (30µg) and Cefazidineclavulanate (30/10µg) discs were placed and organisms that showed more than 5mm increase in zone on the Cefazidine – clavularate were considered ESBL producers. [fig:1]

**RESULT**

Out of the 1121 urine samples processed 472 were culture positive, among this 319 were MDR; 153 of MDR organism were enterobacteriaceae and were further tested for ESBL production; of which 93 Enterobacteriaceae were positive ESBL production. [Table 1]

Only 2 samples out of 93 showed negative by Double disc but positive by CLSI confirmation test.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Age</th>
<th>MDR +ve</th>
<th>ESBL +ve</th>
</tr>
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<tbody>
<tr>
<td>Birth -18</td>
<td>11</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>19-40</td>
<td>43</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>41-60</td>
<td>48</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>61-90</td>
<td>51</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>153</td>
<td>93</td>
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<th>Table 2</th>
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<tbody>
<tr>
<td>IP</td>
</tr>
<tr>
<td>MDR - 120</td>
</tr>
<tr>
<td>ESBL - 73</td>
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</table>

Among the total MDR enterobacteriaceae urine isolates, IP (In patient) sample were more than OP (outpatient) sample. [Table 2] The age group ranged from 37 days to 86 years, and maximum no of samples were from the older age (above 61 years) and least from less than 18 years [Table 1]. Females were more than males for both MDR and ESBL.[Table 3]

**DISCUSSION**

Our findings of ESBL enterobacteriacea is 60.78% which is similar to previous studies in this area. Among the Enterobacteriacea, the most predominant ESBL producers was E.coli followed by Klebsiella pneumonia. This was in contrast to a study by T.Menon et al where enterobacter was predominant. Carbapenems were100% sensitive and the mainstay in treatment of these cases by T.Menon et al. (6.6 –68%) (11, 12, 13). MDR positive samples were 67.58% (319 out of 472). There was no significant difference between the two methods of ESBL detection. Double Disc diffusion as compared to CLSI confirmatory test statistically showed a sensitivity of 97.84%; hence may be concluded that both methods are equally effective for detecting ESBL. Some studies find MDR more in older age group. In this study though MDR was more in older age group; p value was 0.95 i.e. not significant MDR and ESBL were more in IP’s as compared to OP’s which is similar to other studies. In this study ESBL was 60.78% among MDR enterobacteriacea and enterobacteriacea being the commonest organism in UTI an attempt to contain ESBL organism may have some impact on decreasing the load of MDR organism. ESBL producers are increasing throughout the world and studies associate it with increase use of antibiotic. UTI is a common bacterial infection and antibiotics are usually prescribed prior to culture result, also UTI is frequently misdiagnosed.

**CONCLUSION**

In this study both the method of ESBL detection were comparable hence any method may be conveniently used. The ESBL load was comparable to other studies done in this area hence some policy to contain ESBL in this area if effective may be adopted here also. An effective antibiotic stewardship will go a long way to contain this menace of ESBL’s among uropathogens.
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