Prevalence of Extended-Spectrum Beta-Lactamase Producing Enterobacteriaeae from various Clinical Isolates in Al-ansar Hospital, Medina, Saudi Arabia

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Extended spectrum beta-lactamases (ESBLs) producing Enterobacteriaceae. The enzyme is an increasing problem in hospitals worldwide. Therefore, it is imperative to quantify the problem, and reinforce guidelines promoting appropriate antibiotic use. The purpose of this study was to evaluate the incidence of ESBL and to analyze their antibiotic susceptibility in Enterobacteriaceae isolate from different sources Al-ansar hospital, in Medina, Saudi Arabia. A total of 283 were examined by direct microscopy (Gram stain and methylene blue) and parallel cultured in selective and non-selective media, therefore obtained between January and June 2014. All isolates were identified and tested for susceptibility by the VITEK II system (bioMérieux, Marcy l'Etoile, France) using the card for Gram-negative strains (GN cards) and AST-N291. Antibiotic susceptibility testing to beta-lactam/beta-lactamase inhibitor, cephalosporins, aminoglycosides, and carbapenems were performed by disk diffusion method. As for the identity of recovered 101 Enterobacteriaceae ESBL produce isolates, including Escherichia coli (57%), Klebsiella pneumoniae (40%), and Proteus mirabilis (3%). ESBL phenotype was seen in 14 (40%) of tracheal secretion isolates, 43(39%) from urine, 3(37%) from pus, and 13(36%) from sputum. wound and blood specimens were also encountered at a frequency of 31% and 29%, respectively. E.coli was most resistance to cotrimaxazole (68%), nitrofurantoin (50%), amoxicillin/clavulanate (51%), piperacillin/tazobactam and ciprofloxacin (40% and 33%, respectively). K. pneumoniae was sensitive to imipenem (97%), meropenem (97%), amikacin (93%), and gentamicin (70%). P. mirabilis, E. cloacae, P. stuartii, and S. marcescens, strains were sensitive to amikacin, impenem, and meropenem antibiotics. Three isolates had reduced susceptibility to carbapenems. This study showed the existence of important ESBLs among the Enterobacteriaceae isolated from inpatients and outpatient. Continuous surveillance of ESBLs, in bacteria isolated from these patients have an important clinical impact, since, it can often provide valuable information for effective infection control measures and for the choice of appropriate antimicrobial therapy. The empiric use of third and fourth generation cephalosporins should be curtailed, as cephalosporin use was associated with an increased risk of ESBL production.

Key words: Extended spectrum beta-lactamases (ESBLs), Enterobacteriaceae, Escherichia coli, Klebsiella pneumoniae, antibiotic.

Resistance to β-lactam antibiotic agents, especially extended spectrum cephalosporins and other antibiotic agents among various clinical isolates of Gram-negative bacteria is on the rise worldwide. Beta-lactam antibiotics are among the most frequently used antibiotics for empirical therapy.

The first description of extended spectrum beta-lactamases (ESBLs) in 1983, production of beta-lactamase is one of the strategies adopted by bacteria to develop resistance to beta-lactam class of antibiotics. ESBLs are enzymes that mediated resistance to extended-spectrum cephalosporins (e.g. ceftriaxone, cefotaxime, and ceftazidime) by hydrolysis of these antibiotics and which are inhibited by beta-lactamase inhibitors such as
clavulanic acid. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated Gram-negative bacteria. These new beta-lactamases are derived from mutation in older beta-lactamase like (TEM-1, TEM-2, and SHV-1) which confer resistance to penicillins but not to expanded-spectrum cephalosporins. TEM-1 beta-lactamase spread worldwide in different species of bacteria.

Nosocomial infections caused by ESBL-producing Gram-negative bacteria have also been reported, which are mainly the result of extensive and inappropriate use of third-generation cephalosporins (ceftazidime, cefotaxime, and ceftriaxone). They can be found in a variety of Enterobacteriaceae species; however, majority of the ESBL producing strains are Escherichia coli, Klebsiella species. They have also been found in other genera of the Enterobacteriaceae including Citrobacter, Enterobacter, Proteus, Serratia, and Salmonella.

Confirmatory test susceptibilities to cefotaxime and ceftazidime alone those with clavulonate are compared using disk diffusion, increase significantly more than or equal to five mm increase in a zone diameter. The treatment of choice for ESBL-positive isolates includes carbapenem and beta-lactam/b-lactamase inhibitors.

Recently, high prevalence of antibiotic resistant bacteria and a trend of increase resistance under continued antibiotic. The major risk factors implicated are long-term exposure to antibiotics, nursing home residency, prolonged ICU stay, and catheterization.

This study was designed in Al-ansar hospital of Saudi Arabia to prevalence data on the occurrence of ESBL producing Enterobacteriaceae and analyse their antibiotic susceptibility pattern.

MATERIAL AND METHODS

Specimens and identification

During the study period from January to June 2014, two hundred eighty-seven specimens non-repeat culture were collected from inpatient and outpatient, Al-ansar, Medina. These were isolated from different sources including urine, sputum, tracheal aspiration, pus, and blood. Cultures were processed using standard microbiological methods. The specimens received were initially cultured on blood agar (Hi-Media, Mumbai, India) and MacConkey agar (Hi-Media, Mumbai, India). The blood culture bottles were incubated in the Bactec 9120 system as recommended by the manufacturer for seven days. When growth is detected in a blood culture bottle three drops of blood were sample with a sterile syringe and were inoculated onto blood agar, chocolate agar and Macconkey agar (Hi-Media, Mumbai, India). Urine samples were cultured on blood agar, and CLED agar (Hi-Media, Mumbai, India). The samples were incubated at 37°C under aerobic conditions for 24-48h. Identification of bacteria was carried out based on Gram staining, methylene blue, standard biochemical tests and VITEK II compact System (bioMérieux, Marcy l’Etoile, France) using GN-291 cards for Gram negative bacteria.

Antibiotic susceptibility testing

After identification, all isolates were subcultured on Muller Hinton agar (MHA) (Hi-Media, Mumbai, India). The organisms were suspended in saline to turbidity 0.5 McFarland standard prepared from the pure colonies was inoculated on Muller Hinton agar plates, and left for 15 min to allow moisture absorption at room temperature before applying the multi-disk on the agar. The agar plates were then incubated at 35°C for 18-24 h. E. coli (ATCC 25923) was used as controls. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and also by VITEK II (bioMérieux, Marcy l’Etoile, France)compact System using AST-GN291 card.

The antibiotics disks were as follows (µg/disc): Amoxicillin/clavulanate(20/10), piperacillin/tazobactam (100/10), ceftazidime (30), cefotaxime (30), ceftriaxone (30), amikacin (30), gentamicin (10) ciprofloxacin (5), trimethoprim-sulfamethoxazole (cotrimaxazole) (1.25/23.75), meropenem (10), imipenem (10), colistin (50), and nitrofurantoin for in vitro susceptibility of bacterial isolates to these antibiotics.

ESBL detection

CLSI guidelines to identify ESBL producing isolates using standard disk diffusion technique were followed, i.e., diameter zone of ≤ 22mm with ceftazidime, ≤ 27mm with aztreonam, ≤
27mm with cefotaxime, and ≤ 25mm with ceftriaxone were considered potential ESBL producers and further proceeded for confirmation. These tests were checked for quality using standard control ESBL negative strain of *E. coli* ATCC 25923.

**Double disc synergy assay**

Synergy between a disc of third generation cephalosporin such as cefotaxime, ceftriaxone or ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) disc was detected. MHA plates were prepared and inoculated with standardized inoculums of the bacteria (0.5 McFarland standard) to form a lawn culture. A 30 µg disc of each third generation cephalosporin antibiotic was placed on the agar at a distance of 15 mm (centre to centre) from a ceftazidime/clavulanic acid disc. *K. pneumonia* ATCC 700603 was used as the positive control. ESBL production was interpreted as positive if the inhibition zone around the test antibiotic disc increased toward the ceftazidime/clavulanic acid disc.

**RESULTS**

Of the 283 non-repetitive *Enterobacteriaceae* isolates that were included in the study. The subjects age varied from 19 years to 64 years. The majority of ESBL isolates were identified in 46-55 age group (29/101; 27%), followed by the 36-45 age group (24/101; 22%) whereas the age group 15-25 years (15/101; 14%) showed the lowest frequency of ESBL isolates (Fig. 1). There were 65 males (65/283; 24%) and 36 females (36/283; 13%) with a male to female ratio 1.8:1.

The maximum number of ESBL isolates were isolated from Male Medical Ward (MMW) 35/101 (34%), followed by Intensive Care Units (ICUs) 33/101 (33%), Female Medical Ward (FMW) 15/101 (15%), Outpatient 9/101 (9%), Male Surgical Ward (MSW) 5/101 (5%), Female Surgical Ward (FSW) 4/101 (4%) (Fig. 2).

ESBL production was observed in 101 (38%) isolates by double disk synergy and amongst these 58%, 40%, 3% isolates were *E. coli*, *K. pneumoniae*, and *P. mirabilis*, respectively (Table 1). The urinary tract infection was the major source of ESBL-producing isolates (43/101; 43%). Urine samples yielded *E. coli* (24/43; 49%); followed by *K. pneumoniae* (16/43; 26%). Also, wound swab samples revealed *E. coli* as a predominant isolates (18/24; 75%), while the predominant species isolated from the tracheal aspiration samples was *K. pneumoniae* (9/16; 56%) (Table 2 & 3).

Table 1 shows the antibiotic resistance of *Enterobacteriaceae*. For *P. mirabilis*, *E. cloacae*, *P. stuartii*, and *S. marcescens* strains were sensitive to amikacin, imipenem, and meropenem antibiotics. *E. cloacae* and *P. stuartii* were resistant to amoxicillin/clavulanate, piperacillin/tazobactam and ciprofloxacin. *E. coli*
was most resistance to cotrimaxazole (68%), nitrofurantoin (50%), amoxicillin/clavulanate (51%), piperacillin/tazobactam and ciprofloxacin (40% and 33%, respectively). *E. coli* was sensitive to imipenem (93%), amikacin (98%), and meropenem (92%). *K. pneumoniae* was sensitive to imipenem (97%), meropenem (97%), amikacin (93%), and gentamicin (70%). Three isolates had reduced susceptibility to carbapenems (Table 4).

### DISCUSSION

The major challenge to infection control is the prevention of the emergence and spread of ESBL-producing *Enterobacteriaceae*. Therapeutic options for treatment of infections involving ESBLs have also become increasingly limited. The efficacy of extended spectrum cephalosporins is compromised while co-resistance to cotrimaxazole, aminoglycosides and fluoroquinolones has been reported. It is necessary to know their prevalence in a hospital setting so as to enable the clinician to select early appropriate antibiotic regimens to reduce average length of stay in a hospital thereby reducing healthcare costs and to formulate an effective antibiotic policy.

Out of the overall 283 *Enterobacteriaceae* were assessed for ESBL phenotype, ESBL was detected in 101 (36%) of isolates. These

**Table 3.** ESBL pattern of *Enterobacteriaceae* in different source specimen.

<table>
<thead>
<tr>
<th>Species</th>
<th>Urine</th>
<th>Wound</th>
<th>Tracheal aspiration</th>
<th>Sputum</th>
<th>Blood culture</th>
<th>Pus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>24</td>
<td>18</td>
<td>5</td>
<td>9</td>
<td>2</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>16</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>24</td>
<td>14</td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>101</td>
</tr>
</tbody>
</table>

**Table 4.** Resistant Pattern of ESBL for clinical isolates of *Enterobacteriaceae*.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Number of isolates</th>
<th>AUG</th>
<th>P/T</th>
<th>CRO</th>
<th>CAZ</th>
<th>CXT</th>
<th>CPE</th>
<th>CUR</th>
<th>GM</th>
<th>AK</th>
<th>IMP</th>
<th>MER</th>
<th>CIP</th>
<th>FD</th>
<th>SXT</th>
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</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>140</td>
<td>51</td>
<td>40</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>47</td>
<td>33</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>35</td>
<td>50</td>
<td>68</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>105</td>
<td>63</td>
<td>47</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>50</td>
<td>30</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>63</td>
<td>46</td>
<td>57</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>8</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>-</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>13</td>
<td>100</td>
<td>100</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
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<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>33</td>
<td>67</td>
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<tr>
<td><em>P. stuartii</em></td>
<td>9</td>
<td>100</td>
<td>100</td>
<td>33</td>
<td>22</td>
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<td>0</td>
<td>100</td>
<td>-</td>
<td>86</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>8</td>
<td>90</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33</td>
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<td>0</td>
<td>0</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>


* SPICE organisms
findings are consistent with those previously reported from other countries as USA (10), Canada (11), China (12), Italy (13), India (14,15,16 &17), United Arab Emirates (18), Jordan (19), Egypt (20, 21), as well as Saudi Arabia (22,23).

ESBL isolates were from the intensive care unit are likely to have higher use of invasive devices such as urinary and vascular catheters. Nosocomial infections caused by ESBL producing pathogens are associated with risk factors such as old age (>45 years), previous antibiotic use, prolonged hospitalization, inadequate antibiotic therapy, and presence of invasive devices (5,10). Our result revealed that E. coli (39%) and followed by K. pneumoniae (27%) were the predominant isolates inpatient and outpatient of Al-ansar Hospital. Similar findings have been observed in Chain and United Arab Emirate (5& 12). The present study indicates that E. coli is still the most common cause of urinary tract infection. This finding is consistent with the other studies from Italy.6

In our study, we detected high rates of ESBL in E. coli (57%) and K. pneumoniae (40%) by screening test. This was in agreement with the results obtained by Saurina et al., 2000, in a study also in USA (10). On other hand, other study in Saudi Arabia (22) reported that E. coli are becoming more common than ESBL-producing Klebsiella spp. In United Arab Emirate and in the Italy, the rates of ESBL positivity in E. coli and K. pneumoniae isolates were lower than those of our study.18, 13.

Resistance to antibiotics poses a serious and growing problem, because such resistant bacteria are becoming more difficult to treat. These findings also suggest other possibilities for our high resistance rates, such as inappropriate, uncontrolled empiric therapy or cross acquisition of resistance rather than the development of natural resistance. So the empirical and the indiscriminate use of antibiotics should be avoided and prompt infection control strategies in hospitals with special consideration in critical patient should be established in order to decrease the emergence and the spread of drug resistance among bacterial pathogens25.

Treatment failures have been reported with the use of cephalosporins and beta-lactam/beta-lactamase inhibitor combinations (amoxicillin with clavulanate and piperacillin with tazobactam) for infections caused by ESBL-producing isolates25. The level of resistance reported here for amoxicillin/clavulanate is much higher than that reported in other studies22 and also much higher than the 25% resistance to piperacillin/tazobactam among ESBL isolates described in United Arab Emirates18, suggesting that this beta-lactam/ beta-lactamase inhibitor combination may not be useful in our setting. Susceptibility to other antibiotic was follows: 97% for meropenem, 95% for amikacin, 95% for imipenem, 67% for gentamicin, and 52% for of ciprofloxacin.

**CONCLUSION**

It is very important for the clinical microbiology laboratories to have the ability to detect and report ESBLs production in clinical isolates of Enterobacteriaceae. Besides, monitoring and judicious usage of ESBL, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBL. Amikacin and carbapenems remain the most effective drugs, but the presence of carbapenem-resistant ESBL-producing Enterobacteriaceae and occurrence of multidrug resistance are of concern. Infection control measures should be aggressively followed to prevent such infections among these high risk patients.

**REFERENCES**


