Resistant pattern of extended spectrum β-lactamase producing isolates from clinical specimen; an experience at a tertiary care hospital in Alkharj, Saudi Arabia

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1. Introduction

Indiscriminate use of broad spectrum antibiotics without proper investigation has resulted in dramatic increase in antibiotic resistance over the years1. The development of antibiotic resistance limits the choice of antibiotics to be used2. Extended spectrum β-lactamase producing enterobacteriaceae member are reported to be notorious for their ability to produce multidrug resistance by hydrolyzing β lactam antibiotics2,3. A single organism may harbor multiple ESBLs such as the serine cephalosporinases and Amp CA. Escherichia coli is the most frequently reported organism in the recent past as a common cause of urinary tract infection in community bases studies and is capable of producing β lactamases, such as CTX-M enzymes4.

For treatment of gram negative sepsis the extended spectrum β-lactam antibiotics2,3. A single organism may harbor multiple ESBLs such as the serine cephalosporinases and Amp CA. Escherichia coli is the most frequently reported organism in the recent past as a common cause of urinary tract infection in community bases studies and is capable of producing β lactamases, such as CTX-M enzymes4.

For treatment of gram negative sepsis the extended spectrum β-lactams are the most common drugs being used empirically. Threat of treatment failure is mainly due to the emergence of ESBL producing organisms5. The bacteria producing ESBL also imparts resistance against some other drugs like quinolones etc6. However, the incidence and resistance pattern of antimicrobial resistance of ESBL producing enterobacteriaceae varies from time to time and from place to place7. Even from one center to another the variation has been reported widely in literature. The frequency of ESBL producing organisms in Saudi Arabia is documented to vary from 4.8% to 15.8% in different regions8-11. However, the incidence and resistance pattern of antimicrobial resistance of ESBL producing enterobacteriaceae varies from time to time and from place to place7. Even from one center to another the variation has been reported widely in literature. The frequency of ESBL producing organisms in Saudi Arabia is documented to vary from 4.8% to 15.8% in different regions8-11.

Resistant pattern of ESBL producing organism has a lions’ share in constituting the antibiotic policy of an institute. An effective antibiotic policy is effective in combating against drug resistance7. Therefore, keeping in view the regional, time-based and intra-institutional variation regarding this subject it is advisable to collect such data with regular and frequent intervals of time.
Materials and Methods:

Bacterial isolates

A total of 131 non-repetitive isolates of enterobacteriaceae from 200 clinical samples of urine, blood, pus, wound swab, high vaginal swab, sputum were obtained from different clinics of the hospital (Medicine, Surgery, gynecology and obstetrics, pediatrics) over a period of Eight months (February to September, 2014). The study included patients of all age groups and both sexes. All the specimens were then inoculated on Blood agar, MacConkey agar and cysteine lactose electrolyte deficient medium (CLED) medium. All the media were incubated for 18-24 hours at 37C. Blood cultures were processed using the BACT/ALERT 3D system (bioMérieux, France).

Plates were observed for bacterial growth. Culture results were interpreted as significant and insignificant according to standard.

Identification and antimicrobial susceptibility testing using Vitek 2:

All isolates were identified and tested for susceptibility by the Vitek 2 system using the card for Gram-negative strains (GN cards) and AST-N291. The following antimicrobial agents were tested in the study: amikacin, gentamicin, ciprofloxacin, ceftazidime, cefotaxime, pipercillin/tazobactam, and trimethoprim/sulfamethoxazole. The cards were inoculated and incubated in the system according to the manufacturer’s instructions. All results were interpreted using the Advanced Expert System (AES) (software version VT2-R04.03). The isolates were initially screened positive if minimum inhibitory concentration (MICs) of ceftazidime and cefotaxime for these organisms were ≥ 2 mg/L using the Vitek 2 system AST-N0291 card12.

Objective:

To see the resistant pattern of ESBL producing enterobacteriaceae clinical isolates in our population

Results:

This was a cross sectional retrospective analysis of all the 200 different samples sent for microbiological examination in the diagnostic laboratory of Salman bin Abdulaziz university hospital over a period of eight months i.e. from February 2014 to September 2014.

Overall 200 samples were received during the study period. Most of the samples were urine (47.5%), followed by various swabs (23.5%) and pus (18.5%). Sputum and blood samples accounted for 5.5% and 5.0% respectively. (Table 1)

Out of 200 samples bacterial growth was noted in 168 sample of which members of enterobacteriaceae were isolated in 131 (77.9%) samples, and in the rest 37 (22.1%) samples other organisms like Staphylococcus aureus, Pseudomonas spp., etc. were isolated. (Table 2)

A total of 84 isolates of enterobacteriaceae showed ESBL production. This accounted for 42% (84/200 samples) of all the samples, 50% (84/168 samples) of all the isolates, and 64.1% (84/131 samples) of enterobacteriaceae isolates. Escherichia coli was the most frequent organism producing ESBL with a relative frequency of 42.85% (36/84). Klebsiella with a frequency of 27.38% (23/84) ranked second amongst ESBL producing organisms followed by 14.28% (12/84) isolates of Proteus and 9.52% (8/84) cases of Citrobacter. Sphingomonas were the least frequent accounted for 05.95% (5/84) cases (Table 3).

Susceptibility pattern of ESBL producing organisms is shown in table 4. It is apparent that meropenem, imipenem, pipercillin/tazobactam, gentamicin and amikacin are the most effective drugs against ESBL producing organisms in our study. The susceptibility to above said drugs ranged from 83% to 100% with a mean of 93.3% in our study. Amoxiclav, celepine, nitrofurantoin and ciprofloxacin showed intermediate susceptibility for ESBL producing enterobacteriaceae with a mean of 76.87%, 79%, 85% and 72.5% respectively. We found that ceftazidime, trimethoprim/sulfam and norfloxacin were found to be less effective with poor efficacy in the said study (Table 4).
TABLE 4: SUSCEPTIBILITY PATTERN OF ESBL PRODUCING ENTEROBACTERIACEAE

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli (36)</th>
<th>Klebsiella (23)</th>
<th>Proteus (12)</th>
<th>Citrobacter (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10µg)</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Amoxiclav (100/200µg)</td>
<td>72.3</td>
<td>27.3</td>
<td>74.6</td>
<td>25.4</td>
</tr>
<tr>
<td>Piperacillin/tazobactum (100/10 µg)</td>
<td>91.4</td>
<td>8.6</td>
<td>86.7</td>
<td>13.3</td>
</tr>
<tr>
<td>Amikacin (30µg)</td>
<td>91.1</td>
<td>9.1</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>Gentamicin (16µg)</td>
<td>89.9</td>
<td>10.1</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>Cefepine</td>
<td>83.3</td>
<td>16.7</td>
<td>81</td>
<td>19</td>
</tr>
<tr>
<td>Cefazidime (30µg)</td>
<td>84.5</td>
<td>15.5</td>
<td>52.7</td>
<td>47.3</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>35.1</td>
<td>64.9</td>
<td>82.6</td>
<td>17.4</td>
</tr>
<tr>
<td>Ciprofloxacin (5µg)</td>
<td>71.2</td>
<td>28.8</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>Imipenem (10µg)</td>
<td>97.1</td>
<td>2.9</td>
<td>98.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Trimetho/sulpham</td>
<td>53.3</td>
<td>45.6</td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>51.7</td>
<td>41.6</td>
<td>62</td>
<td>37</td>
</tr>
</tbody>
</table>

Discussion:

Changing pattern of antibiotic resistance has created many problems in the management of serious infections worldwide. Members of enterobacteriaceae are notorious for producing resistance against commonly and widely used antibiotics. Production of ESBL adds gravity to the situation. Since the first ever report of ESBL producing enterobacteriaceae in the mid 1980s, a number of studies have been published reporting such organisms in various clinical isolates. This also has affected greatly the antibiotic policy determination of any institute. This would be the first study of its kind in Salman bin Abdulaziz University hospital, Kharj Saudi Arabia.

In present study, majority samples were urine (47.5%) followed by swabs (23%) and sputum (15.5%). Sample from body fluids accounted for 21.5%. Roshan et al described ESBL production in 45.1% of urine samples, 30.5% of pus and 7.5% in body fluids. The selection of sample may be different in an inpatient setting versus OPD settings. Majority of our samples were referred from OPD. This may explain the slight disparity amongst the distribution of various clinical samples in our study from that of Roshan et al.

Enterobacteriaceae were found to be the most frequent (77.9%) organisms isolated in our study. This finding is in complete accordance with the findings of Qamar et al. and Seetha et al, who had described the frequencies of enterobacteriaceae to be 70.5% and 67.92%. However the frequency of samples collected and examined may vary from center to center.

Escherichia coli was found to be the most frequent ESBL producing organism in our study (42.85%) followed by Klebsiella (27.38%). The similar finding has been reported by Somily et al from the eastern region of the kingdom, however the percentage of E. coli (6.6%) described by them is much lower than our study. Researchers from other countries have reported 61.7% and 58% of E. coli in their studies. Our results are in accordance with the findings of international data available, however the percentage is much higher than reported from the central region of the kingdom. This is an established fact that the percentage of ESBL producing organisms reported from central region is much lesser than other regions of the kingdom, a fact that has been accepted by Somily et al. Relative frequency of ESBL producing species of Klebsiella was found to be 27.38% in present study. Roshan et al described the relative frequency of Klebsiella spp to be 21.1%. Another study from central region i.e. Riyadh has described 55% of ESBL Klebsiella spp in their study. This is quite apparent that the relative frequencies of Klebsiella species is very much variable not only from one region to another in the kingdom, amongst various countries worldwide.

Amikacin, imipenem, piperacillin/tazobactam, meropenem and gentamicin were associated with the least resistance against ESBL producing enterobacteriaceae in our study. Similar findings have been reported by Hassan et al from within the kingdom. Cefepime, ciprofloxacin and amoxiclav exhibited intermediate resistance against ESBL producing enterobacteriaceae in our study. Once considered drug of choice in the infections with enterobacteriaceae, ciprofloxacin is now becoming less effective with intermediate and highly variable resistant pattern. Although, lower resistant pattern has been described by Roshan et al and Hassan et al, 12 for ciprofloxacin, the drug efficacy in treating infections with ESBL producing enterobacteriaceae is now getting controversial. Some authors have considered ciprofloxacin may serve as an alternative choice for infections caused by ESBL producing enterobacteriaceae as poor efficacy has been shown by this drug. Nitrofurantoin is not commonly used drug in UTI in the kingdom, therefore the resistance is not that high for this drug. However, variable results have been described for nitrofurantoin resistant pattern in ESBL producing enterobacteriaceae. The least effective drugs in our study with highest resistant in ESBL producing enterobacteriaceae were cotrimoxazole and norfloxacin. Similar observations have been described by Roshan et al, Hassan et al 12 and Al-Zahran and Akhtar.

Conclusion and Recommendation:

The most difficult infections to be treated in a tertiary care hospital are the ones caused by ESBL producing enterobacteriaceae, as a continuously changing resistant pattern is being observed over a period of time. The institutes are recommended to carry out long term broad based studies before establishment of the antibiotic policy of the hospital so as to ensure quality treatment provided to patients.

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Conflict of Interest:

No conflict of interests is declared in the above said study.

References


