Prevalence of $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$ among $\beta$-lactamase producing Escherichia coli isolated from tertiary care hospitals in Taif, Saudi Arabia

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Abstract: Seventy clinical isolates of Escherichia coli were obtained from two tertiary care hospitals in Taif Governorate, Saudi Arabia and investigated for their susceptibility to 15 antibiotics of different classes using antibiotic disc diffusion method. Amikacin and imipenem were the most active antibiotics while, ampicillin was the least active. Multiplex drug resistance was detected in 52.9% of the isolates which were resistant to 3-6 antibiotics of three or more different classes. All isolates were tested for their ability to produce $\beta$-lactamases in general and extended spectrum $\beta$-lactamases in particular by iodometric overlay method using penicillin, cefotaxime, ceftriaxone and ceftazidime as substrates. It was found that 67%, 38.6%, 20% and 40% of isolates were found to degrade the previous substrates respectively. On the basis of CLSI criteria, 57.1% of the isolates were phenotypically categorised as ESBLs. On the other hand, molecular investigation of $\beta$-lactamases revealed that 54.3%, 7.1% and 10.0% of isolates were found to harbour $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$ genes respectively. More strict legislations for the use of antibiotics are suggested to avoid spread and emergence of antibiotic resistance.

Keywords: Escherichia coli, $\beta$-lactamases, ESBLs, $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$, $\text{bla}_{\text{CTX-M}}$.

I. Introduction

There has been a growing worldwide challenge of resistant clinical bacterial isolates to antimicrobials in general and $\beta$-lactams in particular. $^{[1]}$ The primary and the most prevalent cause of resistance to $\beta$-lactam antibiotics in Enterobacteriaceae is the enzymatic hydrolysis by $\beta$-lactamase. $^{[2,3]}$ The first reported $\beta$-lactamase is Temoniera $\beta$-lactamase or the so called TEM $\beta$-lactamase which was recovered from a clinical isolate of *E. coli* recovered from a the Greece patient named Temoniera. A sulphhydril variable (SHV) $\beta$-lactamase is the second reported $\beta$-lactamase which was recovered from *Klebsiella pneumonia*. $^{[4]}$ Both enzymes were narrow spectrum $\beta$-lactamases and are classified as class A $\beta$-lactamases according to Ambler classification of $\beta$-lactamases. $^{[4]}$

Due to substitution of one or more amino acids of the active sites of TEM and SHV-$\beta$-lactamases, the spectrum of these two enzymes was extended to include 3rd generation cephalosporins and monobactams $^{[5,6]}$, and thence, they are termed extended spectrum $\beta$-lactamases (ESBLs). ESBLs have the capacity to confer resistance to penicillins, monobactams and cephalosporins, with the exception of cephamycins and carbapenems. $^{[6]}$

Cefotaximase Munich or the so called CTX-M $\beta$-lactamases are another Ambler class A ESBLs. $^{[7,8]}$ These enzymes exhibit high hydrolytic activity on cefotaxime, ceftriaxone and aztreonam and their prevalence has dramatically increased worldwide. $^{[9,10]}$

Generally, TEM, SHV and CTX-M $\beta$-lactamases are inhibited by $\beta$-lactamase inhibitors like clavulanate, sulbactam and tazobactam. $^{[6,8]}$ It was found that $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$ are carried on plasmids in *E. coli*. $^{[11]}$ Presence of $\text{bla}_{\text{TEM}}$, $\text{bla}_{\text{SHV}}$ and $\text{bla}_{\text{CTX-M}}$ on plasmid enables them to spread horizontally through bacterial populations and between different bacterial species. $^{[8]}$

The extensive use of extended-spectrum $\beta$-lactams has been associated with an increase in the prevalence of ESBL producers. $^{[12]}$ However, bacterial isolates expressing the ESBLs phenotype varies globally across different geographical regions. A prevalence of lower rates of 3-8% has been reported in a few countries.
like Sweden, Japan and Singapore while, higher rates of prevalence of 30-60% have been reported in most countries like Portugal, Italy, New York, Latin American countries and Turkey. In the Kingdom of Saudi Arabia, the rates of reported ESBL varied between 8.5 and 38.5%. Rates of 31.7% and 41% have been reported in neighbour Gulf Countries like Kuwait and the United Arab Emirates respectively. In Saudi Arabia, blaTEM, blaSHV and blaCTX-M have been reported in clinical isolates of Enterobacteriaceae in Riyadh, Dammam and Dhahran. In the present study, the prevalence of blaTEM, blaSHV and blaCTX-M in E. coli clinical isolates is reported for the first time in Taif governorate.

II. Materials And Methods

2.1. Bacterial isolates:

Seventy clinical isolates of E. coli were recovered from different clinical specimens which collected from patients who admitted or hospitalized in two military hospitals in Taif governorate, Saudi Arabia from January and April 2016. The identity of the isolates was determined by API 20E (BioMérieux, France). The recovered isolates were stored at -80°C in tryptic soy broth containing 30% glycerol (v/v).

2.2. Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method using 15 different antibiotic discs (Oxoid, UK). The results were interpreted according to clinical laboratory standards institute. Both E. coli ATCC 25922 and K. pneumoniae ATCC 700603 were used as quality control standard strains.

2.3. Phenotypic detection of β-lactamase production

2.3.1. Initial ESBL production

Initial detection of potential ESBL production was determined according to guidelines of CLSI, 2014 i.e. resistance to either cefotaxime or ceftazidime or ceftriaxone or aztreonam.

2.3.2. Confirmation of ESBL production by double disk synergy (DDS) test

Briefly, a disc of amoxicillin/clavulanic acid (30µg) was placed at the center of Muller Hinton agar (MHA) plate and single disc of each of CAZ (30µg) and CTX (30µg) were placed 25-30 mm apart from each side of amoxicillin/clavulanic acid disc. The plates were incubated at 37°C for 18hrs. Isolate was considered to be ESBL producer when expanded zone of inhibition was observed around either ceftazidime and/or cefotaxime disc from side towards to amoxicillin/clavulanic disc.

2.3.3. Confirmation of β-lactamase and ESBL production by idometric-overlay method

Bacterial isolates were tooth-picked onto Muller-Hinton agar (Oxoid, UK) plates and allowed to grow for 18 hours at 37°C. The plates were overlaid with agarose containing toluene, soluble starch and the tested β-lactam substrate. The plates were incubated for 15 min at 37°C before addition of Lougal's iodine solution. After addition of Lougal's iodine solution the plates were then left at room temperature for a few minutes. The appearance of a well-defined halo zone indicated the production of β-lactamase.

2.4. Molecular detection of β-lactamases

All isolates of E. coli which were screened for blaTEM and blaSHV and blaCTX-M by conventional PCR technique using Mastercycler personal (Eppendorf, California, USA) and primers listed in (Table 1).

Table 1: Primers used for amplification of blaTEM, blaSHV and blaCTX-M genes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
</table>
| TEM    | F: GAGTATTCAACATTGCCGTTGTC  
R: TAAATCTGAGGCAACTTATCCT | 848bp | [58] |
| SHV    | F: ANGATCCCAATATCGCAGACGAC  
R: ATTCAGTTCGGTTCGCCACGCCGG | 231bp | [59] |
| CTX-M  | F: ATGTGCAAGYACCAGAARGT  
R: TGGGTRAARTARGTSACCAGA | 594bp | [60] |

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III. Results

3.1. Antibiotic susceptibility testing

All isolates were tested for their susceptibility to 15 antibiotics of different classes as shown in (Table 2). Out of the 70 investigated isolates, only 2 isolates were sensitive to all the tested antibiotics. As regard to the tested β-lactam antibiotics, ampicillin and cefazolin were the least effective β-lactams in which 91.5% and 87.2% of the isolates were resistant to these agents respectively. The resistance rates to 3rd and 4th generation cephalosporins were relatively lower than those of penicillins in which 37.1%, 50.2% and 22.9% of the isolates were resistant to ceftazidime, cefotaxime and cefepime respectively. Only one isolate was found to be resistant to imipenem.

Table 2: Antibiotic susceptibility pattern of E. coli clinical isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Susceptibility pattern</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>6 (8.6)</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>46 (65.7)</td>
<td>12 (17.1)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>9 (12.9)</td>
<td>10 (14.3)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>42 (60)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>44 (62.9)</td>
<td>12 (17.1)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>35 (50)</td>
<td>6 (8.8)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>54 (77.1)</td>
<td>13 (18.6)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>69 (98.6)</td>
<td>-</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>46 (65.7)</td>
<td>9 (12.9)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>40 (57.1)</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Sulphamethoxazole/trimethoprim</td>
<td>29 (41.4)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>62 (88.6)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>70 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>63 (90)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>33 (47.1)</td>
<td>1 (1.4)</td>
</tr>
</tbody>
</table>

As for the non-β-lactam antibiotics, all the isolates were sensitive to amikacin, while 10.2% and 11.4% of the isolates were resistant to gentamicin and chloramphenicol respectively. On the other hand, the resistance rates of ciprofloxacin, sulphamethoxazole/trimethoprim and tetracycline were 42.9%, 57.1% and 51.4%, respectively. Multiple drug resistance to 3-6 antibiotics was detected in 37 isolates (52.9%) as shown in (Table 3). Thirty-four of the multiple resistant strains (91.9%) were resistant to 3-4 antibiotics.

Table 3: Multiple drug resistance among E. coli clinical isolates

<table>
<thead>
<tr>
<th>No. of resistant antibiotic</th>
<th>No. of isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>20</td>
<td>28.6</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

3.2. Phenotypic detection of β-lactamase and ESBL production

On the basis of CLSI criteria, 57.1% (40/70) of the isolates were categorised as ESBLs (data not shown). The investigation of β-lactamases and ESBLs production using iodometric overlay method as shown in (Fig. 1) using penicillin G, cefotaxime, ceftazidime and cefepime revealed that 47 (67.1%), 27 (38.6%), 14 (20%) and 28 (40%) of the isolates were able to hydrolyse penicillin G, cefotaxime, ceftazidime and cefepime respectively. It was found that five isolates were resistant to β-lactams and yet they tested negative for β-lactamase production by the iodometric overlay method.

Figure 1: Detection of β-lactamase production by iodometric overlay method
3.3. Molecular detection of blaTEM, blaESHV and blaCTX-M

All isolates were screened for blaTEM, blaESHV and blaCTX-M as shown in (Fig. 2). Out of the investigated 70 E. coli isolates, 38 (54.3%), 5 (7.1%) and 7 (10%) isolates were found to harbour blaTEM, blaESHV and, blaCTX-M, respectively as shown in (Table 3).

Three isolates was found to harbour both blaTEM and blaESHV and 3 other isolates was found to harbour both blaTEM and blaCTX-M (Table 3). Twelve isolates (17.1%) were found to be producers for β-lactamases, as revealed by the iodometric overlay method, but did not harbour any of the three investigated β-lactamase genes. On the other hand, five isolates were resistant to different β-lactams and were negative for β-lactamase production by the iodometric overlay method (data not shown).

Table 3: Prevalence of blaTEM, blaESHV and blaCTX-M among clinical isolates of E. coli.

<table>
<thead>
<tr>
<th>Detected β-lactamase gene</th>
<th>No. of isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaTEM</td>
<td>38</td>
<td>54.3</td>
</tr>
<tr>
<td>blaESHV</td>
<td>5</td>
<td>7.1</td>
</tr>
<tr>
<td>blaCTX-M</td>
<td>7</td>
<td>10.0</td>
</tr>
<tr>
<td>blaTEM, blaESHV</td>
<td>3</td>
<td>0.08</td>
</tr>
<tr>
<td>blaTEM, blaCTX-M</td>
<td>3</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Fig. 2: PCR amplification of blaTEM (A), blaESHV (B), blaCTX-M (C) genes.

IV. Discussion

In the past decade, there has been a growing problem of antimicrobial resistance in clinical isolates of Enterobacteriaceae. [2] In this study, the susceptibility of E. coli clinical isolates to 15 antibiotics was investigated. E. coli is known to be associated with the production of diverse classes of β-lactamases. [28]

Ampicillin was the least active with a resistance rate of 88.6%. The previous rate is relatively higher than the rate which reported by a recent performed in Taif [29] in which 73.4% of the isolates were resistant to ampicillin. This suggests an increase of 15.2% in the incidence of resistance to ampicillin over the last eleven years. On the other hand, amikacin and imipenem were the most active antibiotics against the investigated E. coli isolates in which the resistance rates for these agents were 0% and 1.3% respectively. The previous finding was contradicted with a recent study carried out Taif, Saudi Arabia in which all E. coli clinical isolates were sensitive to amikacin and imipenem. [19]

In recent years, ESBLs production has become a progressively widespread problem due to the extensive and irrational use of the 3rd generation cephalosporins. [30] In this study, the total prevalence of ESBL production among investigated E. coli isolates was 57.1%. The previous finding is contradicted with the finding of Khanfar et al. in which a lower prevalence rate of 14.8% of ESBL production was detected among E. coli clinical isolates recovered from the Eastern Province of Saudi Arabia. [22]

Lower incidences of 20.4% and 35.8% for ESBLs were also reported in Riyadh [31] and Dammam [32] respectively. Also, Lower rate of ESBLs production was reported in countries neighbour to Saudi Arabia in which the rates of ESBLs production in other Gulf countries like Kuwait and the United Arab were 31.7% [33] and 41% [33] respectively.

On the other hand the surveillance studies in Egypt revealed that the prevalence of ESBLs production was 38.5% and 60.9% during the period of 2004 and 2006 respectively [34-35]. In contrast to the Middle East, lower rates of ESBLs production were reported in North America, Latin America and the far East-Western Pacific area where the rates of ESBLs production ranged between 3.3-23.6% [36].

β-lactamase production is the most common resistance mechanism of β-lactam antibiotics among Gram-negative bacilli. In this study, 67.1% (47/70), 38.6% (27/70), 20% (14/70) and 40% (28/70) were capable of hydrolyzing penicillin G, ceftaxime, cefazidime and cefepime, respectively, as inspected by iodine overlay method. The current study revealed that only five isolates were resistant to β-lactams and they were negative for β-lactamases production by the iodometric method. This suggests the involvement of other resistance mechanisms than β-lactamase production. The current study revealed that the prevalence of blaTEM among investigated E. coli isolates was 54.3% which is closely related to the incidence of a recent Egyptian study in
which 52.3% of *E. coli* isolates were found to harbour *blaTEM-1*. Regarding to *blaSHV* the current study revealed that *blaSHV* was found among 7.1% of *E. coli* isolates. In contrast to the previous result a higher rate was reported in *E. coli* recovered from Al-Alsha governorate, Saudi Arabia. The present study revealed that the incidence of *blaCTX-M* among *E. coli* isolates was 10%, the previous rate was lower than the rate which reported by a recent study that performed in Riyadh, Saudi Arabia in which 19.7% of investigated *E. coli* isolates was found to harbour *blaCTX-M*. On the other hand, the current study reported that the incidence of *blaCTX-M* among potential ESBLs producer *E. coli* isolates was 17.5%. The previous rate is lower than the rate which conducted by a recent study which performed in Dhahran, Saudi Arabia in which the incidence of *blaCTX-M* among potential ESBLs producer *E. coli* was 25%.

Twelve isolates (17.1%) were found to be of β-lactamases producers by iodometric-overlay method and did not harbour any of the three investigated β-lactamase genes. This suggests that they may produce other type(s) of β-lactamases. This study recommends the need for screening of *E. coli* and other Gram-negative bacteria for different types of β-lactamase genes to better understand the epidemiology of β-lactamases in Saudi Arabia.

### V. Conclusion

The current study demonstrated a high resistance rate to β-lactam antibiotics. Iodometric overlay method was able to detect β-lactamase production in isolates tested negative for *blaTEM*, *blaSHV* and *blaCTX-M* genes. The current is considered to be the first study in Saudi Arabia which addresses the prevalence of *blaTEM*, *blaSHV* and *blaCTX-M* genes in Taif area. Also, the current study is considered to be the second study which uses iodometric overlay method for detection of ESBL production. The present study high lightened the need for more strict legislations to control the use of β-lactam antibiotics to avoid the spread of resistance to these agents.

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### Reference


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