Detection of blaTEM, blaSHV, and blaCTX-M genes among the Extended-Spectrum $\beta$-Lactamases (ES$\beta$Ls) producing Enterobacteriaceae isolated from hospital-acquired infections and community in Egypt.

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Detection of \textit{bla}_{TEM}, \textit{bla}_{SHV}, and \textit{bla}_{CTX-M} genes among the Extended-Spectrum β-Lactamases (ESβLs) producing \textit{Enterobacteriaceae} isolated from hospital-acquired infections and community in Egypt.

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ABSTRACT

Background: Bacteria are resistant to an antibiotic such as \textit{Escherichia coli}, \textit{Klebsiella pneumonia}, and \textit{Enterobacter} \textit{sp.} responsible for morbidity in worldwide. ESβLs are a group of plasmids encoded enzymes that have the efficacy to hydrolyze β-lactams antibiotics. The spread of (ESβLs) representing a serious problem and threatening the ability to treat an infection.

Aim of the study: This study aimed to investigate ESβLs-producing \textit{Enterobacteriaceae} \textit{sp.} isolated from patients and healthy individuals and detect the resistant genes \textit{bla}_{TEM}, \textit{bla}_{SHV}, and \textit{bla}_{CTX-M}.

Patients and Methods: Two hundred bacterial isolates were recovered from patients and healthy individuals rectal swab samples. These isolates were screened for producing ESβLs and identified using both standard bacteriological methods and VITEK2 compact system). The antibiotics resistance of \textit{Enterobacteriaceae} was assessed by the disk diffusion method and detection of \textit{bla}_{TEM}, \textit{bla}_{SHV}, and \textit{bla}_{CTX-M} genes by multiplex PCR.

Results: Two hundred \textit{Enterobacteriaceae} screening for-producing ESβLs showed that 56% (112/200) produce ESβLs. One hundred and twelve ESβLs-\textit{Enterobacteriaceae} identified as following, \textit{Klebsiella pneumonia} 51.73% (58/112), \textit{Escherichia coli} 46.40% (52/112), and \textit{Enterobacter cloacae} 1.80% (2/112). The antibiotic resistance patterns of \textit{Enterobacteriaceae} showed high resistance to ciprofloxacin, levofloxacin, and amikacin with the ratio of (71.70%), (60.72%) and (60.72%), respectively. Furthermore, ESβLs \textit{Enterobacteriaceae} harbored genes \textit{bla}_{CTX-M} (78.6%), \textit{bla}_{TEM} (73.2%) and \textit{bla}_{SHV} (68.75%). The \textit{bla}_{TEM} was found the predominant gene in \textit{E. coli} isolates 80.8%, while \textit{bla}_{CTX-M} in \textit{Klebsiella pneumonia} 81%.

Conclusion: The present study showed a significant distribution of multidrug-resistant ESβLs-producing \textit{Enterobacteriaceae} in patients in the hospital- and community-acquired rectal infection. ESβLs-producing \textit{Enterobacteriaceae} species harboring co-existence resistant genes.

Keywords: \textit{Enterobacteriaceae}; ESβLs; resistance genes.

INTRODUCTION

Bacteria are resistant to an antibiotic such as \textit{Escherichia coli}, \textit{Klebsiella pneumonia}, and \textit{Enterobacter} \textit{sp.} responsible for morbidity in worldwide. Antibiotics resistance due to extended-spectrum β-lactamases (ESβLs) was firstly recorded in 1979 in Europe. ESβLs are a group of plasmids encoded enzymes that have the efficacy to hydrolyze β-lactams antibiotics. It was firstly reported that ESβLs producers are predominantly belonging to \textit{E. coli} and \textit{Klebsiella}.

Among several enzymes’ linkage with ESβLs activity, ESβLs class A include on cefotaximase (CTX-M), Temoneira (TEM), and SHV (to sulphydryl variable active site). These genes are commonly in \textit{Klebsiella pneumonia} and \textit{E. coli}. Investigation of these genes is important not only for their ability to hydrolyze β-lactam antibiotics but also because the plasmids responsible for ESβLs production regularly harboring genes encoding resistance to other antibiotic groups like aminoglycosides and fluoroquinolones. A previously
published study by Hassan et al., 2012 reported 98% of 65 Klebsiella pneumonia isolates obtained from Egyptian patient samples harbor SHV gene while 11% harbor CTX-M gene 6. In another study conducted in Egypt highlighted that the CTX-M gene is the predominant resistance gene in ESβLs Enterobacteriaceae 7. To date, few published studies were concerned with the assessment of ESβLs resistant genes in Enterobacteriaceae strains of the hospital and community setting. Therefore, this study aimed to isolate Enterobacteriaceae producing ESβLs species from rectal swab samples, antibacterial resistance pattern and determine the predominate ESβLs resistance gene in isolates of hospital settings versus the presence of those genes in rectal isolates from community settings.

PATIENTS AND METHODS

Samples collection

One hundred rectal swab samples were collected from 50 patients (two samples taken from each patient, one upon admission and second after 48h of admission) at Abu El-Reesh Pediatric Hospital, Cairo University Hospital, Egypt, and one hundred rectal swab samples from healthy individuals duration period extending from December 2016 to December 2019.

Cultivation and isolation of bacterial species

Rectal swab samples were cultivated on MacConkey media and incubated for 24 hours at 37°C aerobically. Colonies with positive lactose fermentation (Pink colonies) were collected. The pure cultures were identified based on morphological, physiological, and biochemical characteristics using microbiological methods 8th, 8 Bergey’s Manual of Systematic Bacteriology 9. Isolates identification was confirmed by the VITEK2 compact system (Biomerieux Inc., Marcy l’Etoile, France).

Screening of (ESβLs) production

The antibiotics synergy of ESβLs producing bacteria was detected by the double-disk synergy test (DDST) 10. Bacterial colonies from MacConkey agar equivalent to 0.5 McFarland are cultured on Mueller-Hinton agar media. The following antibiotic discs are used, cefotaxime 30μg/ml, ceftazidime 30μg/ml (third-generation cephalosporins), and amoxicillin-clavulanate 20/10μg/ml. Culturing plates were incubated at 35°C for 24h. ESβLs production activity is confirmed if there is an extension of the inhibition zone between any of the cephalosporins and amoxicillin-clavulanate disk (D-shape or keyhole shape) 11, 12.

Antibiotic resistance pattern

Antibacterial sensitivity testing was carried out by disc diffusion method and the results were expressed as resistant, intermediate, or susceptible according to CLSI guidelines 10. The antibiotics used in this study belonging to four groups antibiotics carbapenems include on (imipenem10μg and meropenem10μg), aminoglycosides (gentamicin 10μg/ml and amikacin 30μg/ml), fluoroquinolones (ciprofloxacin 5μg/ml and levofloxacin 5μg/ml), and polypeptides (colistin10μg/ml and polymyxin B 300U/ml).

Molecular detection of blaTEM, blaSHV, and blaCTX-M genes using PCR.

A total of 120 ESβLs producing Enterobacteriaceae were investigated for detecting three genes (blaTEM, blaSHV, and blaCTX-M) using multiplex PCR and specific primers (Table 1). The detection methods were designed according to the methods Randall et al., 2009 13.

<table>
<thead>
<tr>
<th>Gene type</th>
<th>Primer sequence (5’-3’)</th>
<th>Gene product length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM F</td>
<td>TCGTGTCGCCCTATTCCCTTTT</td>
<td>426</td>
<td>[19]</td>
</tr>
<tr>
<td>TEM R</td>
<td>GCGTGTAGCTCCTCCGGTCTC</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>SHV F</td>
<td>GTGGATGCCCGTGACGAACGC</td>
<td>551</td>
<td>[8]</td>
</tr>
<tr>
<td>SHV R</td>
<td>TGGCGCAAAAAAGGCGATCATTCT</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>CTX-M F</td>
<td>CGCTTTGCGATGAGCACAG</td>
<td>551</td>
<td>[8]</td>
</tr>
<tr>
<td>CTX-M R</td>
<td>ACCCGCATATCGTTGGT</td>
<td>212</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Primer sets used in PCR runs for tested isolates.

Statistical analysis:

The data were subjected to analysis of variance (ANOVA) by statistical package SPSS v17. The mean difference comparison between the treatments was analyzed by the Tukey HSD test at a significance level of P ≤ 0.05.

RESULTS

Screening of ESβLs producing Enterobacteriaceae

A total of 200 Enterobacteriaceae were screening for producing ESβLs, the results obtained showed that 112 (56%) bacterial isolates producing ESβLs. Thirty-two isolates (64%) from patients upon admission, 40 (80%) from the same patients after 48h of admission, and 40 (40%) from healthy individuals.

Identification of ESβLs Enterobacteriaceae

The results revealed that 112 bacterial isolates producing ESβLs (32 isolates from patients upon admission, 40 from same patients after 48h of admission, and 40 from healthy individuals) were
included to identify. The results obtained from morphological, physiological, and biochemical tests revealed that 100% of bacterial isolates are Gram-negative, rod shape, motile, ferment lactose sugar in MacConkey agar, and positive results in the triple sugar iron (TSI) test. Only 46.4% (52/112) from bacterial isolates have the ability to utilizing tryptophan and forming indole and produce decarboxylase enzyme. Moreover, 1.8% (2/112), 51.73% (58/112), and 53.5% (60/112) can produce H2S, urease, and citrate utilization respectively (Table 2). According to results obtained from the identification of 112 ESβLs - producing Enterobacteriaceae species, the most common species was found Klebsiella pneumonia followed by E. coli and Enterobacter cloacae with percent 51.8% (58/112), 46.4% (52/112), and 1.8% (2/112), respectively. This result was confirmed by VITEK-2 with an echelon ratio of 99%. In this study, The identification of bacterial species isolated from healthy individuals revealed that the most common species E. coli 22.3% (25/112) followed by Klebsiella pneumonia 13.38% (15/112), comparable isolates from patients the most common species were found Klebsiella pneumonia 38.35% (43/112) followed by E. coli 24% (27/112) and 1.8% (2/112) Enterobacter cloacae.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Health samples</th>
<th>1st samples</th>
<th>2nd samples</th>
<th>Total</th>
<th>%</th>
<th>Biochemical test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>motility TSI H2S Urease Citrate utilization Decarboxylase enzyme</td>
</tr>
<tr>
<td>E. coli</td>
<td>25</td>
<td>15</td>
<td>12</td>
<td>52</td>
<td>46.40%</td>
<td>+     +     -     -     -     +     +</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>15</td>
<td>16</td>
<td>27</td>
<td>58</td>
<td>51.73%</td>
<td>+     +     +     +     +     +     -</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1.80%</td>
<td>+     +     +     -     -     -     +</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>32</td>
<td>40</td>
<td>112</td>
<td>100%</td>
<td>--    --    --    --    --    --    --</td>
</tr>
</tbody>
</table>

Table 2: Identifications of 112 ESβLs producing Enterobacteriaceae species isolated from patients and healthy individuals.

Resistance patterns of Enterobacteriaceae

The antibiotic profile of Enterobacteriaceae isolated from patients showed the highest resistance to ciprofloxacin, levoflaxacin, and amikacin (71.76%), (60.72%) and (60.72%), while, they were sensitive to colistin, polymyxin, meropenem, and imipenem (95.22%), (95.22%) (44.16%), and (44.16%), respectively. Moreover, it was noted that bacterial species isolated from patients after 48h of admission highly resistant to bacterial species isolates from the same patients upon admission and healthy individuals (Table 3). E. coli isolated from patients after 48hours of admission showed the highest resistance to ciprofloxacin, levoflaxacin 91.63%, while, isolates from patients upon admission 66.6%. However, E. coli isolated from healthy individuals exhibit a low resistance level (Table 4). In K. pneumonia, from patients after 48hours of admission the highest resistance was observed against and ciprofloxacin, levoflaxacin 81.4% with a low resistance level to polymyxin B and colistin 3.70%. (Table 5). Enterobacter cloacae recorded the highest resistance to ciprofloxacin, levoflaxacin with 100%. K. pneumonia showed the resistance to meropenem and imipenem with 53.32%, 51.6%, followed by Enterobacter cloacae 50.0% and E. coli with 23.04%, respectively (table 4 and 5). Overall, K. pneumoniae showed the highest resistance level from E. coli isolates (Table 6).

Prevalence of blaTEM, blaSHV, and blaCTXM genes in Enterobacteriaceae species

In our study, 88/112 (78.5%) isolates it has blaCTXM resistant gene, 82/112 (73.2%) blaTEM gene and 77/112 (68.70%) blaSHV (table 7) Figure 1A, 1B, 2A and 2B.

The statistical analysis revealed that no significant statistical difference between the presence resistant genes blaTEM, blaSHV, and blaCTXM in healthy individuals isolates comparable with isolates collected from patients either upon admission or after 48h. of admissions. Also, the difference between the presences of the three genes in bacterial isolates collected from patients cases either upon admission or after 48h of admission was statistically insignificant (Table 8). Interestingly, the predominant resistant gene in bacterial species isolated from patients and healthy individuals is the blaCTXM gene followed by blaTEM and blaSHV (Table 8). blaCTXM was the predominant gene in Klebsiella pneumonia isolates (81.0%) while it was the second common in E. coli isolates (75.0%). The blaCTXM resistance gene was the predominant gene in E. coli isolates (80.8%), and the least gene in Klebsiella pneumonia isolates (67.2%), blaSHV, a second common gene in Klebsiella pneumonia (79.3%) (Table 9).
### Table 3: Antibiotic resistance pattern of 112 ESβLs producing Enterobacteriaceae species.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inpatients (72 isolates)</th>
<th>Healthy individual (40 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upon admission (32 isolates)</td>
<td>After 48h of admission (40 isolates)</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Amikacin</td>
<td>16 (50%)</td>
<td>0%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16 (50%)</td>
<td>0%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20 (62.5%)</td>
<td>0%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>20 (62.5%)</td>
<td>0%</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>2 (6.25%)</td>
<td>0%</td>
</tr>
<tr>
<td>Colistin</td>
<td>2 (6.25%)</td>
<td>0%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1 (4.375%)</td>
<td>0%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1 (4.375%)</td>
<td>0%</td>
</tr>
</tbody>
</table>

R= Resistant, I= Intermediate, S= Sensitive.

### Table 4: Antibiotics resistance pattern of E. coli

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Patients</th>
<th>Healthy individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upon admission</td>
<td>After 48h of admission</td>
</tr>
<tr>
<td>Amikacin</td>
<td>56.25%</td>
<td>0%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>56.25%</td>
<td>0%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>62.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>62.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>62.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Colistin</td>
<td>62.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>62.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>62.5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

R= Resistant, I= Intermediate, S= Sensitive.

### Table 5: Antibiotics resistance pattern of Klebsiella pneumonia

R= Resistant, I= Intermediate, S= Sensitive.
Antibiotics | E. coli 52 isolates | K. pneumonia 58 isolates | Enterobacter cloacae 2 isolates |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R (%)</td>
<td>I (%)</td>
<td>S (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>32.64</td>
<td>3.84%</td>
<td>63.36%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>36.48%</td>
<td>0.0%</td>
<td>63.66%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>44.16%</td>
<td>0.0%</td>
<td>55.65%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>44.16%</td>
<td>0.0%</td>
<td>55.65%</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>1.92%</td>
<td>0.0%</td>
<td>97.92%</td>
</tr>
<tr>
<td>Colistin</td>
<td>1.92%</td>
<td>0.0%</td>
<td>97.92%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>23.04%</td>
<td>0.0%</td>
<td>78.72%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>23.04%</td>
<td>0.0%</td>
<td>78.72%</td>
</tr>
</tbody>
</table>

Table 6: Antibiotics resistance pattern of total E. coli, Klebsiella pneumonia, and Enterobacter cloacae. R= Resistant, I= Intermediate, S= Sensitive

<table>
<thead>
<tr>
<th>Resistant gene</th>
<th>Number of isolates containing each gene</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla&lt;sub&gt;CTX-M&lt;/sub&gt;</td>
<td>88 bacterial isolates</td>
<td>78.6%</td>
</tr>
<tr>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>82 bacterial isolates</td>
<td>73.2%</td>
</tr>
<tr>
<td>bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>77 bacterial isolates</td>
<td>68.75%</td>
</tr>
</tbody>
</table>

Table 7: Summary of prevalence bla<sub>CTX-M</sub>, bla<sub>TEM</sub>, bla<sub>SHV</sub> genes in the 112 ESβLs producing Enterobacteriaceae species.

<table>
<thead>
<tr>
<th>Resistant genes</th>
<th>Patients</th>
<th>Healthy individuals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upon admission</td>
<td>After 48h of admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No gene</td>
<td>--------</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>One gene</td>
<td>bla&lt;sub&gt;CTX-M&lt;/sub&gt;</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Two genes</td>
<td>bla&lt;sub&gt;CTX-M&lt;/sub&gt; + bla&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>bla&lt;sub&gt;CTX-M&lt;/sub&gt; + bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt; + bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Three genes</td>
<td>bla&lt;sub&gt;CTX-M&lt;/sub&gt; + bla&lt;sub&gt;TEM&lt;/sub&gt; + bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 8: Distribution of bla<sub>CTX-M</sub>, bla<sub>TEM</sub>, bla<sub>SHV</sub> genes in ESβLs producing Enterobacteriaceae species.

<table>
<thead>
<tr>
<th>Resistant genes</th>
<th>E. coli (total 52 isolates)</th>
<th>Klebsiella pneumonia (total 58 isolates)</th>
<th>Enterobacter cloacae (total 2 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of isolates</td>
<td>Percentage %</td>
<td>Number of isolates</td>
<td>Percentage %</td>
</tr>
<tr>
<td>bla&lt;sub&gt;CTX-M&lt;/sub&gt;</td>
<td>39</td>
<td>75%</td>
<td>47</td>
</tr>
<tr>
<td>bla&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>42</td>
<td>80.8%</td>
<td>39</td>
</tr>
<tr>
<td>bla&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>30</td>
<td>57.7%</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 9: Distribution of bla<sub>CTX-M</sub>, bla<sub>TEM</sub>, bla<sub>SHV</sub> genes in ESβLs-producing Enterobacteriaceae.
In the last years, ESβLs producing Enterobacteriaceae have been increasingly recognized in hospitals in Egypt and, unfortunately, are linkage with multiple drug resistance. The prevalence of producing ESβLs-Enterobacteriaceae was found to be (56%) 12/200, comparable with a studies from Ghana (49.3%) 15, Ethiopia (57.6%) 16, India (57.5%) 17, Burkina Faso (58.0%) 18, and Uganda (62.0%) 19. Teklu et al., 2019 recorded 57.7% (246/426) from Enterobacteriaceae species isolated from clinical samples producing ESβLs 20. A current study from Turkey recorded a prevalence rate of ESβLs-Enterobacteriaceae carriage (34.3%) in the community. The predominant ESβLs production was observed in Klebsiella pneumonia, these results agree with Teklu et al., 2019 20. In this study, the antibiotics resistance patterns of ESβLs-producing Enterobacteriaceae species isolated from patients showed that highly resistant level to ciprofloxacin, levofloxacin, and amikacin (71.76%), (60.72%) and (60.72%), while, they were sensitive to colistin, polymyxin, meropenem and imipenem (95.22%), (95.22%) (44.16%) and (44.16%), respectively. Teklu et al., 2019 isolated Enterobacteriaceae producing ESβLs from clinical samples resistant to norfloxacin with ratio (58.8%), ciprofloxacin (46.3%), gentamycin (43.4%), but low resistance to meropenem (5.2%) and amikacin (13.8%) 20. The studies were conducted in Burkina which showed that 89% of ESβLs-producer isolates non-susceptible to gentamicin and 80% to ciprofloxacin 22. In Ghana, 91.2% of ESβLs-producer Enterobacteriaceae was found resistant to gentamicin and 41.1% to ciprofloxacin 22. In central India 50% from ESβLs-producer Enterobacteriaceae resistant to gentamicin and 87.5% to ciprofloxacin 22. While in Nepal 90.7% resistant to ciprofloxacin, 90.4 and, 63.12% to gentamicin 24. In this study, E. coli isolates from patients after 48hours of admission showed the highest resistance rate to ciprofloxacin, levofloxacin 91.63%, while isolates from patients upon admission showed a resistance rate of 66.6%. Several studies showed that E. coli isolates exhibit a resistance rate to ciprofloxacin and levofloxacin 86.6% 25. Zheng and Xiang-zhu, 2017 reported a resistance rate to ciprofloxacin and levofloxacin among E. coli isolates 85.08% and 80.42%, respectively 25. In the current study Klebsiella pneumoniae isolated from patients after 48hours of admission showed a resistance rate to ciprofloxacin, levofloxacin 62.5%. Zheng and Xiang-zhu, 2017 reported that Klebsiella pneumoniae resistant to ciprofloxacin, levofloxacin with a rate of 66.86%, and 50.0%, respectively 26. In our study K. pneumoniae showed the highest resistance to meropenem and imipenem 53.32%, 51.6%, followed by Enterobacter cloacae 50.0% and E. coli with 23.04%, and all producing ESβLs-Enterobacteriaceae showed a resistance rate to imipenem and meropenem 37.46% (42/112) and 36.57% (41/112), respectively. This finding disagrees with Teklu et al., 2019, which found producing ESβLs-Enterobacteriaceae resistant to meropenem 5.2%, E. coli (3.5%), and K. pneumoniae 10.7% 20.

**Prevalence of resistance genes blaTEM, blalSIGV, and blaCTX-M in ESβLs producing Enterobacteriaceae species**

In the current study, the predominant resistant gene was blaCTX-M78.6% followed by blalTEM 73.2%, and blalSIGV 68.75%. These results are in agreement with several studies 27, 28, 29 which indicate that dissemination of the blaCTX-M gene represents a...
global pandemic. The multinational survey performed by Ben-Ami et al., 2009 concluded that the blaCTX-M gene was predominant in E. coli while the blaTEM was predominant in Klebsiella pneumoniae. This result was not matched with our study, where the blaTEM was predominate in E. coli while blaTEM in Klebsiella. In our study, some ESβLs producing Enterobacteriaceae species harboring more than one resistant gene could be attributed to the participation of genetic elements in the mobilization of these genes. A low number of ESβLs producer isolates were not harboring one resistant gene at least from blaCTX-M, blaTEM, and blaSHV, a similar result was reported by Bajpai et al., 2017. In the same regard, the statistical analysis showed that the difference in the presence of the three ESβLs encoding genes in healthy individuals versus their presence in patient samples was not statistically significant. The presence of three resistance genes (blaTEM, blaCTX-M, and blaSHV) in ESβLs-producing Enterobacteriaceae species illustrate the genetic diversity among isolates due to horizontal gene transfer between different bacterial species.

In this study, the blaCTX-M gene was predominant in bacterial species isolated from patient samples upon admission and after 48h of admission with a percentage of 81.25% (26/32) and 82.5 (33/40), respectively. This result agreed with Hagel et al., 2019, who reported that blaCTX-M was represented in 81.1% of upon admission isolates and 84.1% of discharge isolates. Moreover, Pérez et al., 2019 showed that the blaCTX-M gene represents 83.17% of all isolates taken from patients upon admission. Data analysis of healthy individuals revealed that the blaCTX-M resistant gene was the most common gene detected in 72.5% of bacterial isolates. Also, the blaSHV gene was detected in 62.5% of the healthy individuals bacterial isolates. In the same regard, Valverde et al., 2004 reported that 70% of the non-hospitalized individual were colonized with ESβLs carrying the blaCTX-M gene. The predominance of a blaCTX-M gene in E. coli clinical patients isolates was also reported by Ahmed et al., 2014. As well as, blaCTX-M encoding ESβLs gene was detectable in 96.6% of community isolates.

CONCLUSION

From this study we can be concluded, ESβLs producing Enterobacteriaceae species are carried by patients and healthy individuals in the community. Fecal carriage of resistant Enterobacteriaceae species represents a high risk for spread multidrug resistance bacteria. ESβLs producing Enterobacteriaceae harboring co-existence resistant genes. The best choice for the treatment of ESβLs Enterobacteriaceae is polymyxin B and colistin. To prevent further spread ESβLs producing Enterobacteriaceae, it should be motivating the ideal use of antibiotics, and antibiotic resistance should keep under surveillance in Egypt.

REFERENCES