Research Article

Extended-Spectrum β-Lactamases among Enterobacteriaceae Isolated from Urinary Tract Infections in Gaza Strip, Palestine

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Background. Extended-spectrum β-lactamase-producing organisms causing urinary tract infections are increasing in incidence and pose a major impendence to health-care facility, having limited therapeutic options. This study aimed to assess the prevalence of ESBLs in Enterobacteriaceae isolates causing urinary tract infections in Gaza strip, Palestine, and to characterize β-lactamase types and associated resistance genes.

Methods. Eighty-five Enterobacteriaceae isolates were recovered from urinary tract infections within three months in Gaza Strip hospitals. β-lactamase genes and the genetic environments of CTX-M, the identification of associated resistance genes, and the presence and characterization of integrons were tested by PCR and sequencing.

Results. The occurrence rate of ESBL among tested isolates was 30 (35.3%), and among ESBL-positive isolates, blaCTX-M was the highest followed by blaTEM. ESBL-CTX-M-1 group was confirmed in 93.3%, and the remaining carried CTX-M-9 group. CTX-M-15, CTX-M-3, CTX-M-1, CTX-M-27, and CTX-M-37 enzymes were demonstrated among the isolates with the majority (73%) being CTX-M-15. ISEcp-1 was demonstrated in 27 (90%, high incidence) of ESBL isolates. Class 1 integrons have been detected in higher rates (53.3%) in ESBL-positive isolates in comparison with non-ESBL isolates (6, 33.3%). Cassettes of integron-1 contain (aadA1, aadA2, aadA5, dfrA5, dfrA7, dfrA12, and dfrA17) genes. The aac(6′)-Ib-cr gene was demonstrated in 36.7% of ESBL-positive isolates. Conclusions. This study indicates that blaCTX-M-15 was the most prevalent β-lactamase in this region. Our study demonstrates for the first time in Palestine the identification of blaCTX-M-15 in P. rettgeri and S. liquefaciens, also blaCTX-M-37 in E. cloacae. The coexpression of multiple β-lactamase genes with aac(6′)-Ib-cr and qnr in the presence of ISEcp-1 and integrons in individual strains will increase the dissemination of highly resistant strains. ESBL producers were more resistant than non-ESBLs producers for almost all tested antibiotics.

1. Introduction

Urinary tract infection (UTI) is one of the most common widespread infections, mainly caused by Enterobacteriaceae, especially Escherichia coli, that are encountered by hospitalized and outpatients [1]. Normally, UTIs are treated with different classes of antibiotics such as β-lactams, β-lactam/β-lactamase inhibitors, carbapenems, and fluoroquinolones [2]. However, recent data worldwide reveal that these uropathogens have become resistant to most conventional drugs [3]. Extended-spectrum β-lactamases (ESBLs) are bacterial enzymes that hydrolyze oxyimino-cephalosporins and confer resistance to broad-spectrum cephalosporins and aztreonam [4]. Enterobacteriaceae harboring ESBLs is a global problem with limited available treatment options [5]. ESBL-producing bacteria are related with infections that are consequences of bad clinical facilities, inappropriate
antibacterial therapy, prolonged hospital stays, and greater hospital costs [6]. In the past years, there has been an increase in widespread dissemination of β-lactamase-mediated resistance with high significance in the prevalence of ESBL-producing Enterobacteriaceae [7].

ESBL-producing Enterobacteriaceae have become a major concern in clinical setting worldwide, causing outbreaks related to enzymes of CTX-M class being most common [8]. Recently, ESBLs of CTX-M type have been replaced by SHV- and TEM-ESBLs types worldwide among various members of Enterobacteriaceae [9]. Currently, CTX-M β-lactamasess are categorized into five classes according to their amino acid sequences and include the CTX-M-1, -2, -8, -9, and -25 clusters [10]. Presently, the dominants of CTX-M-1 and CTX-M-9 are most prevalent in geographical distributions [11–13], with many exhibiting CTX-M-15, which is the most widely distributed CTX-M enzyme worldwide [14–16].

Early investigation of patients infected with ESBL-producing urinary pathogens is necessary to prescribe the most efficient therapy and minimize the dissemination of infection by applying preventive measures. Therefore, the aim of this study was to detect the occurrence of ESBL-producing Enterobacteriaceae and investigate the molecular characteristics of β-lactamase-producing isolates obtained from three hospitals in Gaza Strip, Palestine.

2. Materials and Methods

2.1. Isolation and Identification. Eighty-five Enterobacteriaceae isolates were recovered from urinary tract infection samples obtained in various units of three hospitals in Gaza Strip, Palestine, during the period of three months in 2013. Only one bacterial isolate per patient was included in this study. All collected specimens were sent to the microbiology laboratory to be processed for bacterial isolation and identification. Standard methods for isolation and identification were used [17]. The isolates were confirmed to genus and species level by amplification and sequencing of 16S rRNA gene.

2.2. Antibiotic Susceptibility Testing. Susceptibility testing for all isolates to 15 antibiotic agents was performed by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [18] using commercial antibiotic disc panels comprising (μg/disk) ampicillin (10), cefoxitin (30), ceftazidime (30), cefotaxime (30), gentamicin (10), amikacin (30), tobramycin (10), amoxicillin-clavulanic acid (20/10), nalidixic acid (30), ciprofloxacin (5), imipenem (10), kanamycin (30), trimethoprim-sulfamethoxazole (1.25/23.75), tetracycline (30), and chloramphenicol (30).

2.3. Identification of β-Lactamase Genes and Genetic Environment of blaCTX-M Genes. PCR amplification and sequencing were used to determine the presence of the genes encoding TEM, SHV, OXA, and CTX-M type β-lactamasess. The genetic environment surrounding the blaCTX-M genes was studied by the amplification of ISEcp-1, orf477, and IS903 [19].

2.4. Detection of Non-β-Lactam Resistance Genes. Isolates were screened for the genes associated with resistance to tetracycline (tet(A) and tet(B)), sulfamethoxazole (sul1, sul2, and sul3), and gentamicin (aac(3)-I, aac(3)-II, and aac(3)-IV). The genes qnrA, qnrB, qnrS, qepA, and aac(6′)-Ib were studied by PCR and sequencing to identify the variants according to Jouini et al. [20].

2.5. Detection and Characterization of Integrons. The presence of intI1 and intI2 genes encoding for classes 1 and 2 integrase, respectively, and the presence of aacED1-sul1 genes within 3′-conserved region of class 1 integrons was identified by PCR. The variable regions of class 1 and 2 integrons were studied in intI1- or intI2-positive isolates by PCR and sequencing to identify the gene cassettes [19].

Positive and negative controls were kindly provided from the Universite’ Tunis-El Manar, Tunis, and were used in all PCR and sequencing experiments.

2.6. Data Analysis. The data of antimicrobial resistance of non-ESBL and ESBLs of Enterobacteriaceae isolates were analyzed by SPSS version 20 software (IBM Corporation, Somers, NY) by applying a Pearson’s chi-square test. Level of statistical significance was set at P < 0.05.

3. Results

3.1. Bacterial Strains. In this study, we screened 85 Enterobacteriaceae isolates from urine from three Palestinian hospitals in Gaza Strip for the determination of β-lactamase-encoding genes and to characterize their type, genetic environments of CTX-M, and associated resistance genes.

A total of 85 urinary tract infection samples were obtained from Al-Shifa Hospital (37; 43.5%), Balsam Hospital (27; 31.8%), and Al-Remal Center (21; 24.7%). The samples collected from Al-Shifa and Balsam hospitals were from inpatients, and the Al-Remal Polyclinic samples were from outpatients. Out of 85 bacterial isolates, Escherichia coli was the predominant isolate (60; 70.6%) followed by Klebsiella pneumoniae (15; 17.6%), Proteus mirabilis (3; 3.5%), Enterobacter cloacae (3; 3.5%), Serratia liquefaciens (2; 2.4%), Providencia rettgeri (1; 1.2%), and Morganella morgani (1; 1.2%).

3.2. Antimicrobial Susceptibility and ESBL-Positive Isolates. Among the 85 isolates collected, extended-spectrum β-lactamasess were confirmed in 30 isolates (35.3%); among these were E. coli (20 isolates), K. pneumoniae (5 isolates), E. cloacae (3 isolates), S. liquefaciens (1 isolates) and P. rettgeri (1 isolates).

The susceptibility to imipenem was 80.0% against ESBL-producing isolates. However, the resistance rate to cefotaxime and ampicillin was 100%, which was high among ESBL-producing isolates. Resistance to sulfamethoxazole/
trimethoprim, nalidixic acid, ciprofloxacin, kanamycin, and tetracycline among strains was 76.7, 66.7, 66.7, 60.0, and 60.0%, respectively. Low resistance in ESBL-producing isolates against gentamicin, chloramphenicol, cefoxitin, amikacin, and amoxicillin clavulanic acid was recorded, and the resistance rate was less than 40.0% (Table 1).

On comparing the antibiotic resistance of ESBL and non-ESBL Enterobacteriaceae, both showed low resistance to imipenem in comparison to other tested antibiotics. However, resistance to other antibiotics was higher among ESBL than non-ESBL isolates with statistical significance (Table 1). To the end, the resistant pattern of ESBL producers was significantly higher than that of non-ESBL producers for all 15 tested antibiotics except for imipenem (Table 1).

3.3. Identification of β-Lactamases. Molecular characterization of 30 ESBL-producing isolates revealed that all of them harbored blaCTX-M genes (100%), blaTEM, blaoxa, and blaSHV genes were detected in 10 (33.3%), 1 (3.3%), and 1 (3.3%) of the isolates, respectively.

In this study, 28 of the 30 strains were found to be positive for the ESBL-CTX-M-1 group, 23 were confirmed to carry blaCTX-M-15, 2 strains harbored blaCTX-M-3, 2 isolates carried blaCTX-M-1, and the remaining isolate carried blaCTX-M-37. Of the 2 remaining strains that are positive for the ESBL-CTX-M-9 group, 1 carried blaoxa and 1 harbored blaCTX-M-27 (Table 2).

β-lactamase genes identified by PCR and sequencing among the 20 ESBL-positive E. coli strains are as follows: blaCTX-M-15 (n = 11), blaCTX-M-15, blaTEM-1 (n = 4), blaCTX-M-1 (n = 2), blaCTX-M-14 (n = 1), blaCTX-M-27 (n = 1), and blaCTX-M-3 (n = 1). There were five ESBL-positive K. pneumoniae isolates; three isolates harbored (blaCTX-M-15, blaTEM-1, blaSHV-1), one carried (blaCTX-M-15, blaoxa, and blaTEM-1), and one carried (blaCTX-M-3). The three isolates of E. cloacae producing ESBL had the following β-lactamase genes: blaCTX-M-15, blaoxa-1, and blaoxa-1 (n = 1), blaCTX-M-15, blaoxa-1 (n = 1), and blaoxa-1 (n = 1). The blaCTX-M-15 was identified in ESBL-producing Serratia liquefaciens and Providencia rettgeri (Table 2).

Non-ESBL β-lactamases were identified in 18 isolates: E. coli (n = 10), K. pneumoniae (n = 6), P. mirabilis (n = 1), and Morganella spp. (n = 1). The β-lactamases that are not classified as ESBL include genes encoding TEM-1, SHV-1, and OXA-β-lactamase genes identified among the 18 non-ESBL-β-lactamase strains are as follows: blaoxa-1 was the most predominant gene, which was detected in 12 isolates (nine E. coli, one K. pneumoniae, one Morganella spp., and one P. mirabilis). The SHV-encoding genes were found among five isolates of K. pneumoniae in two variants; blaoxa-1, and blaoxa-1 with the frequency of 2 and 3, respectively. Finally, blaoxa-1 was detected in only one isolate of E. coli (Table 2).

3.4. Genetic Environments of blaCTX-M Genes. The genetic mechanisms that may be involved in the expression and mobilization of blaCTX-M genes and the genetic environments upstream and downstream of blaCTX-M genes were studied in ESBL-positive Enterobacteriaceae isolates by PCR and sequencing. The sequence of orf477 was found downstream of the ESBL-CTX-M-1 group members (blaCTX-M-15, blaCTX-M-3, blaCTX-M-1, and blaCTX-M-27) genes in twenty-seven isolates. The insertion sequence (IS903) was identified downstream of the blaCTX-M-14 gene in one isolate (NT134), whereas the downstream region of blaCTX-M-27 and blaCTX-M-37 in E. cloacae and E. coli, respectively, was unknown. The insertion sequence (ISEcp-1) was found upstream blaCTX-M genes in twenty-seven isolates; however, the upstream region of blaCTX-M-37, blaCTX-M-15, and blaCTX-M-14 in NT60, NT117, and NT134 isolates, respectively, was unknown.

3.5. Integrons and Arrangement of Gene Cassettes. Class 1 integrons have been demonstrated in ten ESBL-positive E. coli isolates with the following gene cassette arrangements: dhfr17 + aadA5 (6 isolates), dhfrA7 (2 isolates), and dfrA12 + aadA2 (one isolate); three of those integrons lacked the qacA1 and sul1 genes. In K. pneumoniae, class 1 integrons were present in 3 of the 5 ESBL-producing isolates with two different genetic arrangements (dfrA12 + aadA2) and dfrA5. Int1 was identified in two ESBL-positive E. cloaca, the gene cassette implicated in the resistance to streptomycin (aadA1) was detected in one isolate, and one of those integrons lacked the qacE1 and sul1 genes. The cassette that conferred resistance to trimethoprim (dfrA12) and streptomycin (aadA2) was found in integron-1 among ESBL-producing S. liquefaciens.

Five of non-ESBL E. coli contained class 1 integron with gene cassette arrangement (dhfr17 + aadA5) found in isolates. Class 1 integron has been demonstrated in non-ESBL-Morganella morganii Isolate (Table 2).

3.6. Non-β-Lactam Antimicrobial Agent-Coding Genes. A variety of genes which confer resistance to non-β-lactam antibiotics were observed among ESBL-producing Enterobacteriaceae: tetA and tetB (in 11 and 5, respectively, of tetracycline-resistant strains); eleven of the 30 ESBL isolates harbored sul genes (sul1: (n = 7); sul2: (n = 3); and sul3: (n = 1)). The aac(3)-II gene was found in 13 gentamicin-resistant isolates, and the aac(6′)-Ib-cr gene was detected in eleven isolates. The qnrB1 gene was identified in two isolates, and qnrA and qnrS1 genes were each identified in one isolate. Yet, five non-ESBL E. coli isolates harbored some non-β-lactam antibiotics coding genes such as sul-1 (n = 2), aac(3)II (n = 2), aac(6′)-Ib-cr (n = 1), tetA (n = 2), tetB (n = 1), and sul2 (n = 1). Non-ESBL-Morganella spp. carried sul1 gene (Table 2).

4. Discussion

A total of 85 Enterobacteriaceae isolates obtained from urinary tract infections from in- and outpatient populations during a three-month time period were evaluated for the production of ESBL, β-lactamase enzymes, and associated resistance genes.

The finding showed that among 85 urine isolates, E. coli was the most prevalent representing 60 (70.6%) of isolates followed by K. pneumoniae 15 (17.6%). This is in agreement
with other studies investigating Enterobacteriaceae causing urinary tract infections in Sri Lanka and Qatar [5, 21].

The prevalence of ESBL among our isolates was confirmed in 30 isolates (35.3%). This was similar to the findings of Liu et al., Giwa et al., and Caccamo et al. [22–24] where ESBL production among Enterobacteriaceae causing urinary tract infections was found to be 33.4%, 34.3%, and 36.0%, respectively.

Our findings demonstrated that imipenem was the most effective drug against ESBL isolates. Studies performed in Palestine from different clinical awards demonstrated the same as our findings about the effectiveness of imipenem [1, 25–27].

The results of our study showed that the frequency of antibiotic resistance among ESBL producers is higher than that of resistance in nonproducers; our findings correlate with other studies in Tanzania and India [28, 29]. The spread of ESBL producers in hospitals and their increased antibiotic resistance is alarming.

Resistance to imipenem was found to be low among ESBL producers (20.0%) and non-ESBL producers (12.7%) without statistical significance. The lower resistance to imipenem among ESBL and non-ESBL’s was reported in India [30]. The mechanism of resistance to carbapenem occurs by bacterial production of β-lactamas and decreases the permeability of the antibiotics by changes in porin channels in the cell wall or reduced susceptibility of bacterial cell toward meropenem through upregulation of efflux pumps [31]. The imipenem resistance in our study could be due to production of carbapenemase. This may be because patients in Palestinian hospitals are treated with carbapenems which may have a role in development of multidrug-resistant strains. Detection of carbapenemase genes are beyond the aims of this study and will be our planned future work.

Molecular genotyping of ESBL-containing isolates revealed the highest presence of CTX-M genes (100%) followed by TEM genes, which is in agreement with previous reports from the region and around the globe [1, 32]. CTX-M enzymes have become dominant extended-spectrum β-lactamases in Europe [33] and in many Middle Eastern countries [34].

In this report, 93.3% of Enterobacteriaceae isolates from urine harbored ESBLs-CTX-M-1 group, including bla_{CTX-M-15}, bla_{CTX-M-3}, bla_{CTX-M-1}, and bla_{CTX-M-37} genes, whereas 6.7% of the isolates carried the ESBL-CTX-M-9 group, including bla_{CTX-M-14} and bla_{CTX-M-27}. This finding is in agreement with study in Qatar that showed among the ESBL-producing Enterobacteriaceae infections in urine 90% of the CTX-M belonged to CTX-M-1 group and 8% belonged to the CTX-M-9 group [5]. In a recent study in USA, among the ESBL-producing Enterobacteriaceae, 80% and 20% were positive for CTX-M-1 group and CTX-M-9 group, respectively [32].

Genotypic characterization of all ESBL-positive isolates revealed that the majority (73%) of the CTX-M type was CTX-M-15. A lot of reports worldwide described that CTX-M-15 is the most prominent distributed ESBL-CTX-M enzyme [14–16]. Rapid dissemination of CTX-M-15 enzyme is reported in many countries. The easy transfer of this gene is associated with the epidemic plasmid [35].

One of the significant findings of this study is that this is the first report of bla_{CTX-M-15} in P. rettgeri and S. liquefaciens in the Middle East and also the first report of bla_{CTX-M-15} in E. cloacae in Palestine. In the previous reports, bla_{CTX-M-15} was identified in P. rettgeri causing urinary tract infections in Croatia [36], and bla_{CTX-M-15} was reported in E. cloacae in Southern China [22], Egypt [11], and Yemen [37].

In our study, CTX-M-15, CTX-M-3, CTX-M-1, CTX-M-14, CTX-M-27, and CTX-M-37 enzymes were demonstrated among the isolates and indicated a diversity of the CTX-M groups in clinical Enterobacteriaceae isolates recovered from Palestinian hospitals. Enterobacteriaceae harboring CTX-M-15 with CTX-M-14 have been reported as the predominant CTX-M-ESBL types in clinical isolates worldwide [11–13]. In a Canadian study that was conducted in 2007, among CTX-M-producing isolates from 11 Canadian medical centers, CTX-M-15 was the most common (86.5%), followed by CTX-M-14, CTX-M-3, and CTX-M-2 [38].

### Table 1: Comparison of antibiotics resistance in 30 ESBL and 55 non-ESBL Enterobacteriaceae.

| Antibiotics                        | Resistance in ESBL-producing isolates (%) | Resistance in non-ESBLs-producing isolates (%) | P value |
|------------------------------------|------------------------------------------|-----------------------------------------------|---------|
| Cefotaxime                         | 30 (100%)                                | 6 (11%)                                       | 0.000   |
| Ceftazidime                        | 15 (50%)                                 | 4 (7.3%)                                      | 0.000   |
| Gentamicin                         | 10 (33.3%)                               | 3 (5.5%)                                      | 0.001   |
| Ampicillin                         | 30 (100%)                                | 32 (58.2%)                                    | 0.000   |
| Imipenem                           | 6 (20.0%)                                | 7 (12.7%)                                     | 0.278   |
| Nalidixic acid                     | 20 (66.7%)                               | 7 (12.7%)                                     | 0.000   |
| Sulfamethoxazole/trimethoprim      | 23 (76.7%)                               | 18 (32.7%)                                    | 0.000   |
| Tobramycin                         | 15 (50.0%)                               | 5 (9.1%)                                      | 0.000   |
| Ciprofloxacin                      | 20 (66.7%)                               | 5 (9.1%)                                      | 0.000   |
| Kanamycin                          | 18 (60.0%)                               | 9 (16.4%)                                     | 0.000   |
| Tetracycline                       | 18 (60.0%)                               | 2 (3.6%)                                      | 0.000   |
| Cefoxitin                          | 10 (33.3%)                               | 1 (1.8%)                                      | 0.000   |
| Amikacin                           | 10 (33.3%)                               | 3 (5.5%)                                      | 0.001   |
| Amoxicillin-clavulanic acid        | 10 (33.3%)                               | 3 (5.5%)                                      | 0.001   |
| Chloramphenicol                    | 12 (40.0%)                               | 7 (12.7%)                                     | 0.012   |
| Class 1 integron | Non-β-lactamase genes | Genetic environment of blaCTX-M gene | β-lactamase genes | Antimicrobial resistance pattern | Hospital | Species | Bacterial code |
|----------------|------------------------|------------------------------------|-------------------|-------------------------------|----------|---------|--------------|
|                |                         | ESBLs                              |                   |                               |          |         |              |
|                |                         | CTX, GM, AMP, IMP, NAL, SXT, TOB, CIP |                   |                               | Al-Shifa | K. pneumoniae | NT13         |
| dhfr17 + aadA5 | + +                    | ISEcp-1                            | orf477            |                               |          | E. coli | NT14         |
|                |                         | ISEcp                              | orf477            |                               | Al-Shifa | E. coli | NT40         |
|                |                         | ISEcp                              | orf477            |                               | Balsam   | E. coli | NT55         |
| dfra12 + aadA2 | + +                    | ISEcp-1                            | orf477            |                               | Al-Shifa | K. pneumoniae | NT57         |
|                |                         | ISEcp                              | orf477            |                               |          | E. coli | NT58         |
|                |                         | ISEcp                              | orf477            |                               | Al-Shifa | E. coli | NT60         |
|                |                         | ISEcp                              | orf477            |                               |          | K. pneumoniae | NT66         |
| dfra12 + aadA2 | + +                    | ISEcp-1                            | orf477            |                               | Al-Shifa | E. coli | NT68         |
|                |                         | ISEcp                              | orf477            |                               |          | Enterobacter cloacae | NT69 |
| dhfr17 + aadA5 | - +                    | ISEcp-1                            | orf477            |                               | Al-Shifa | E. coli | NT71         |
| dfra12 + aadA2 | - +                    | ISEcp-1                            | orf477            |                               | Al-Shifa | Serratia liquefaciens | NT73 |
|                |                         | ISEcp-1                            | orf477            |                               |          | E. coli | NT75         |
|                |                         | ISEcp-1                            | orf477            |                               |          | E. coli | NT84         |
| dhfr17 + aadA5 | + +                    | ISEcp-1                            | orf477            |                               | Al-Shifa | E. coli | NT92         |
| dhfr17 + aadA5 | - +                    | ISEcp-1                            | orf477            |                               | Al-Shifa | E. coli | NT98         |
|                |                         | ISEcp-1                            | orf477            |                               |          | K. pneumoniae | NT106        |
| Variable region | qacE∆1 + sul1 | Int1 | Non-β-lactamase genes | Genetic environment of \( \text{bla}_{\text{CTX-M}} \) gene | \( \beta \)-lactamase genes | Antimicrobial resistance pattern | Hospital | Species | Bacterial code |
|-----------------|---------------|------|-----------------------|-----------------------------------------------|-----------------------------|--------------------------------|---------|---------|---------------|
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15}} \) | AMC, CAZ, CTX, AMP, NAL, SXT, FOX, TET | Al-Renal | E. coli | NT108         |
| dfxA5           | –             | +    | \( \text{aac}(3)\)II | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-3}} \) | AMC, CAZ, CTX, AMP, IMP, KAN, AMK, SXT, TOB, CHL | Al-Renal | K. pneumoniae | NT112 |
| –               | –             | –    | TetB                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CAZ, CTX, AMP, SXT, TET | Al-Renal | E. coli | NT116         |
| dfxA7           | –             | +    | TetA                  | Unknown orf477                        | \( \text{bla}_{\text{CTX-M-15-1s}} \) | AMC, CTX, AMP, NAL, SXT, FOX, TET | Balsam   | E. coli | NT117         |
| \( \text{dhfr}17 + \text{aadA5} \) | –             | +    | \( \text{aac}(3)\)II, \( \text{aac}(6')\)-1b-cr, sul1, \text{TetA} | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15}} \) | CAZ, CTX, AMP, KAN, NAL, AMK, SXT, TOB, CIP, TET | Balsam   | E. coli | NT121         |
| \( \text{dhfrA7} + \text{aadA5} \) | –             | +    | \( \text{aadA5} \)   | Unknown IS903                         | \( \text{bla}_{\text{CTX-M-14}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | E. coli | NT126         |
| \( \text{dhfrA7} \) | +             | +    | sul1                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | E. coli | NT129         |
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, AMK, SXT, TOB, CHL, CIP, TET | Balsam   | E. coli | NT134         |
| \( \text{aadA1} \) | +             | +    | \( \text{aadA5} \)   | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | E. coli | NT138         |
| \( \text{dhfr}17 + \text{aadA5} \) | –             | +    | \( \text{sul1} \)    | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | Providencia rettgeri | NT146 |
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, AMK, SXT, TOB, CIP, TET | Balsam   | E. coli | NT147         |
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, AMK, SXT, TOB, CIP, TET | Balsam   | E. coli | NT151         |
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | Enterobacter cloacae | NT157 |
| \( \text{aadA1} \) | +             | +    | \( \text{sul1} \)    | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | Enterobacter cloacae | NT157 |
| –               | –             | –    | \( \text{aadA5} \)   | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{CTX-M-15-1s}} \) | CTX, AMP, KAN, NAL, SXT, CIP | Balsam   | Enterobacter cloacae | NT157 |

Non-ESBLs

| Variable region | qacE∆1 + sul1 | Int1 | Non-β-lactamase genes | Genetic environment of \( \text{bla}_{\text{CTX-M}} \) gene | \( \beta \)-lactamase genes | Antimicrobial resistance pattern | Hospital | Species | Bacterial code |
|-----------------|---------------|------|-----------------------|-----------------------------------------------|-----------------------------|--------------------------------|---------|---------|---------------|
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{SHV-11}} \) | AMP | Al-Renal | E. coli | NT4 |
| –               | –             | +    | \( \text{aadA5} \)   | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{SHV-11}} \) | AMP | Al-Renal | K. pneumoniae | NT25 |
| –               | –             | +    | \( \text{sul2} \)    | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{SHV-11}} \) | AMP | Al-Renal | E. coli | NT28 |
| \( \text{dhfr}17 + \text{aadA5} \) | +             | +    | \( \text{sul1} \)    | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{SHV-11}} \) | AMP | Al-Renal | E. coli | NT29 |
| –               | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{SHV-11}} \) | AMP | Balsam   | K. pneumoniae | NT30 |
| \( \text{dhfr}17 + \text{aadA5} \) | –             | –    | TetA                  | IS\text{Ep}-1 orf477                  | \( \text{bla}_{\text{SHV-11}} \) | AMP | Balsam   | K. pneumoniae | NT36 |
| Variable region | qacEΔ1 + sul1 | IntI | Non-β-lactamase genes | Genetic environment of bla<sub>CTX-M</sub> gene | β-lactamase genes | Antimicrobial resistance pattern | Hospital | Species | Bacterial code |
|-----------------|---------------|------|------------------------|---------------------------------|-------------------|-------------------------------|----------|---------|----------------|
| −               | −             | +    | −                      | aac(3)I, aac(6′)-Ib-cr, TetA, TetB | >sul1             | −                             | NC3      | E. coli | NT64          |
| −               | +             | +    | −                      | sul1                            | −                 | −                             | NC89     | M. morganii | NT89          |
| −               | −             | −    | −                      | −                               | −                 | +                             | NC102    | K. pneumoniae | NT102         |
| −               | −             | +    | −                      | −                               | −                 | −                             | NC107    | E. coli | NT107         |
| −               | −             | −    | −                      | −                               | −                 | −                             | NC114    | K. pneumoniae | NT114         |
| −               | −             | +    | −                      | −                               | −                 | −                             | NC115    | E. coli | NT115         |
| −               | −             | −    | −                      | −                               | −                 | −                             | NC123    | E. coli | NT123         |
| −               | −             | −    | −                      | −                               | −                 | −                             | NC125    | P. mirabilis | NT125         |
| −               | −             | −    | −                      | −                               | −                 | −                             | NC130    | E. coli | NT130         |
| −               | −             | −    | −                      | −                               | −                 | −                             | NC131    | K. pneumoniae | NT131         |
| −               | −             | −    | −                      | −                               | −                 | −                             | NC149    | K. pneumoniae | NT149         |
this enzyme was described in clinical isolates of Enterobacteriaceae in Korea and France [40, 41]. Sequencing the amplification of PCR products from the two E. coli isolates detected the CTX-M-encoding gene as blaCTX-M-15; this gene has been demonstrated in clinical Enterobacteriaceae in France and Italy [41, 42]. CTX-M-27 enzyme has been reported in nosocomial outbreaks caused by Salmonella enterica in a neonatal unit in Tunisia [43].

Our study reported for the first time the presence of CTX-M-37 in clinical Enterobacteriaceae isolates in the Middle East. Its presence was recently reported in clinical strains of E. cloacae in Mongolia [44] and was also identified in S. enterica serotype Isangi from South Africa [45].

Our results showed that ten blaCTX-M-15-containing isolates carried more than one β-lactamase gene, and three K. pneumoniae isolates harbored blaCTX-M-15, blagSHV-1, and blaqTEM-1. One isolate coexpressed blaCTX-M-15 with other β-lactamase gene, and E. cloacae carried blaCTX-M-15, blaqTEM-1, and blaqOXA-1. In previous studies, Enterobacteriaceae isolates carrying multiple β-lactamase genes have been reported [34, 46, 47]. The occurrence of multiple β-lactamase genes in individual strains is a concern because it will enhance co-resistance and greater resistance to various classes of antibiotics.

Insertion sequences and integrons have played an important role in the dissemination of blaCTX-M. ISEcP-1 is the most common insertion element associated with blaCTX-M. Moreover, the role of ISEcP-1 is mobilization of the blaCTX-M gene, and it acts as a strong promoter which enhances the expression of blaCTX-M genes [48]. ISEcP-1 was demonstrated in high incidence (90%) among our isolates which is a growing problem of high resistance in our hospitals that can carry high risk through dissemination of blaCTX-M between patients. ISEcP-1 insertion sequence was found upstream of CTX-M enzyme in Enterobacteriaceae isolates from Tunisia and Croatia [19, 36].

In the present study, class 1 integrons were detected and included resistant genes in the variable region encoding resistance to streptomycin (aadA1, aadA2, and aadA5) and trimethoprim (dfrA5, dfrA7, dfrA12, and dfrA17) which increases the risk of the dissemination of antimicrobial resistance by horizontal transmission by integrons. The integrons lacking qacEΔ1 and sul1 genes have been previously reported in other countries [19, 49].

ESBL-positive isolates have been demonstrated to carry higher rates (16; 53.3%) of class 1 integrons compared to non-ESBL isolates (6; 33.3%). The integron contribution to transfer extended-spectrum cephalosporin resistance has been established [50]. The frequency of occurrence of integrons among our ESBL-positive isolates was 53% which is less than the rate of isolates from Europe [51]; in contrast to this, the integron frequency was 73% and 92% in isolates from Australia and India [50, 52]. In our study, the percentage of integron in non-ESBL-producing isolates was almost similar to the results obtained from studies performed in Spain and India [52, 53]. May be there are possibilities of transferring the integrons carrying ESBL genes and other drug-resistant determinants from ESBL to non-ESBL-producing isolates that make non-ESBL isolates more resistant and complicate antibiotic resistance problems in our hospitals.

The non-β-lactam antibiotic resistance pattern of the isolates showed that the sul and aac(3)-II genes were the most prominent identified in our isolates. This is in agreement with the findings of a study conducted in Spain [54]. The variant aac(6′)-Ib-cr gene has the ability to reduce susceptibility to aminoglycosides and ciprofloxacin [16]. This gene prevalence rate in our study was 36% among ESBL-producing Enterobacteriaceae isolates in comparison with 25% in a previous study in Palestine [55]. A significant association between resistance to broad-spectrum cephalosporins and resistance to quinolones was reported [56]. The association of qnrA1 gene with blaCTX-M identified in this study was similar to the findings of a study in Algeria [57]. We also observed the associations between qnrB1 and blaCTX-M which is in agreement with previously reported results in Tunisia [58]. Moreover, the correlation between qnrA and blaCTX-M demonstrated here was also reported in Yemen [37]. The presence of β-lactamases in association with other resistance genes, qnr gene and aac(6′)-Ib-cr, in the same strain could complicate the treatment of these pathogens. These results support previous reports that suggest larger dissemination of aac(6′)-Ib-cr with qnr determinants, in ESBL-producing isolates harboring blaCTX-M-15 gene [58–60].

Regarding the comparison of the occurrence of non-β-lactam genes within ESBL and non-ESBL isolates, the non-β-lactam genes were confirmed in 83% and 33.3% in ESBL and non-ESBL isolates, respectively. These findings could complicate the resistance problem and limit the antimicrobial drug therapy of infections caused by ESBL producers.

5. Conclusions

Our study highlights the high incidence of ESBL in Enterobacteriaceae recovered from urinary tract infections in Gaza hospitals, Palestine, with the increase in antibiotics resistance for most commonly used antibiotics in our hospitals except for imipenem that showed the highest activity against ESBL isolates. ESBL producers were found to be more resistant than non-ESBLs producers for almost all tested antibiotics. This study indicates that blaCTX-M-15 was the most prevalent β-lactamase in Gaza Strip hospitals. To our knowledge, this is the first report on identification of blaCTX-M-15 in P. rettgeri and S. liquefaciens and also blaCTX-M-35 in E. cloacae in the Middle East.

The associations between multiple β-lactamase genes in individual strains with other resistance genes such as aac(6′)-Ib-cr and qnr in the presence of ISEcP-1 and integrons increase the dissemination of highly resistant strains and complicate the problem of clinical conditions and treatment failure in our hospitals.

Data Availability

All data used to support the findings of the study are approved and included within the article.
Conflicts of Interest
The authors declare that they have no conflicts of interest.

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