Research Article

Rapid Detection of Beta-Lactamases Genes among Enterobacterales in Urine Samples by Using Real-Time PCR

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The objective of this study was to develop and evaluate newly improved, rapid, and reliable strategies based on real-time PCR to detect the most frequent beta-lactamase genes recorded in clinical Enterobacterales strains, particularly in Tunisia (blaSHV-12, blaTEM, blaCTX-M-15, blaCTX-M-9, blaCMY-2, blaoXA-48, blONDM-1, and blIMP) directly from the urine. Following the design of primers for a specific gene pool and their validation, a series of real-time PCR reactions were performed to detect these genes in 78 urine samples showing high antibiotic resistance after culture and susceptibility testing. Assays were applied to DNA extracted from cultured bacteria and collected urine. qPCR results were compared for phenotypic sensitivity. qPCR results were similar regardless of whether cultures or urine were collected, with 100% sensitivity and specificity. Out of 78 multiresistant uropathogenic, strains of Enterobacterales (44 E. coli and 34 K. pneumoniae strains) show the presence of the bla group. In all, 44% E. coli and 36 of K. pneumoniae clinical strains harbored the bla group genes with 36.4%, 52.3%, 70.5%, 68.2%, 18.2%, and 4.5% of E. coli having blaSHV-12, blaTEM, blaCTX-M-15, blaCTX-M-9, blaCMY-2, blaoXA-48 group genes, respectively, whereas 52.9%, 67.6%, 76.5%, 35.5%, 61.8, 14.7, and 1.28% of K. pneumoniae had blaSHV-12, blaTEM, blaCTX-M-15, blaCTX-M-9, blaCMY-2, blaoXA-48, and blONDM-1 group genes, respectively. The time required to have a result was 3 hours by real-time PCR and 2 to 3 days by the conventional method. Resistance genes of Gram-negative bacteria in urine, as well as cultured bacteria, were rapidly detected using qPCR techniques. These techniques will be used as rapid and cost-effective methods in the laboratory. Therefore, this test could be a good candidate to create real-time PCR kits for the detection of resistance genes directly from urine in clinical or epidemiological settings.

1. Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases in humans and represent a real public health problem in terms of both their frequency and their difficulty of treatment. Enterobacterales is primarily responsible for UTIs. Escherichia coli is the main pathogen responsible for cystitis and pyelonephritis as well as other species of Enterobacterales, such as Proteus mirabilis and especially Klebsiella pneumoniae [1, 2].

Beta-lactams are the antibiotics preferentially used against these Enterobacterales. The emergence and spread of these bacteria producing extended-spectrum β-lactamases (ESBLs) or carbapenemases, have become a concern. In fact,
the increase in the number of strains expressing β-lacta-
mases (E. coli, K. pneumoniae) might be explained by the
massive use of broad-spectrum cephalosporins (third-gener-
ation cephalosporins: C3G and fourth-generation cephalo-
sporins: C4G). Moreover, this selection pressure adhered to
the broadening of the TEM and SHV spectrum (by mutation
of the blaTEM-1 and blaSHV-1 genes) and to the emergence
of the CTX-M family, capable of hydrolyzing the penicillins,
broad-spectrum cephalosporins, and aztreonam [3].

The dissemination of CTX-Ms enzymes has resulted in a
generalization of their distribution throughout the world [4].
They are now the most extensively ESBLs in the world where
the most common mutants are CTX-M-15 and CTX-M-14
belonging, respectively, to the CTX-M-1 and CTX-M-9
groups [5]. In Africa and Europe, recent studies have found
a substantial increase in ESBL-producing Gram-negative
bacteria causing community urinary tract infections, par-
ticularly harboring the blaCTX-M-15 allele [6, 7].

Furthermore, over the last two decades, extended-
spectrum β-lactamase (ESBL) and plasmid-mediated
AmpC- (pAmpC-) producing Enterobacteriales exhibiting
resistance to the 3GCs has been increasingly isolated in
humans [8]. Among the AmpC β-lactamase genes, particu-
larly blaCMY-2 and blaOXA-48 are the most common in E. coli
and K. pneumoniae strains, respectively, of human and com-
panion animal [9, 10].

On the other hand, additional enzymes called carbape-
nemases have been detected in E. coli and K. pneumoniae
strains. The OXA-1 type enzymes are sometimes hosted
alongside the CTX-M group exhibiting ESBL activity, and
class D β-lactamases hydrolyzing carbapenems (for example
OXA-23, OXA-24/40, OXA-48, OXA-51, and OXA-58) are
commonly found in Pseudomonas aeruginosa and Acinetob-
acter baumannii [11, 12].

As elsewhere in the world, the spread of multidrug-
resistant (MDR) E. coli, often ST131, complicates treatment
and attributes to massive use of previously reserved antibi-
otics, such as carbapenems and colistin [13]. One poten-
tial way to address these issues is to switch from empirical ther-
apy to early-targeted therapy, detecting antibiotic resistance
genes directly from clinical samples, with no culture that can
take 2 to 3 days to become available. This identification can
be achieved by metagenomic sequencing [14] although there
is skepticism about the implementation, depending on costs
and workflows [15]. The polymerase chain reaction (PCR)
system was more immediately deployable, was less expensive,
and has been frequently studied for proliferate mecA or carba-
penemases genes, making it easier to treat infections [16, 17].

For this reason, our work is aimed at developing a qPCR
system for the detection of blaTEM, blaSHV-12, blaCTX-M-15,
blaCMY-2, blaOXA-48, blaIMP-I, and blaNDM-1 group
genes directly from urines and applying it in clinical E. coli
and K. pneumoniae strains. An alternative to its methods
through PCR can reduce the wait time to just a few hours.

2. Materials and Methods

2.1. Microbial Study. During the study period from Decem-
ber 2018 to December 2020, 2000 urine samples were col-
lected from patients aged 20 to 65 years. Five hundred of
them were culturally positive. Urine samples were collected
from patients in health facilities as well as community
patients in the towns of Sfax, south of Tunisia. All the strains
collected were identified using the API 20E system (bioMé-
rieux SA, Marcy l’Etoile, France).

2.1.1. Phenotypic Characterization. Urine analysis and strain
identification were performed by conventional methods.
Antimicrobial susceptibility study was determined using the
standard disk diffusion method on Mueller Agar-Hinton
(Oxoid) according to Clinical Laboratory Guidelines and
Institute Standards [18]. Tested antibiotics (Bio-Rad) were as
follows: amoxicillin-clavulanate, (10 μg) (AMX); amoxicillin,
(20 μg/10 μg) (AMC); cefotaxime, (30 μg) (CTX); ceftazidime,
(30 μg) (CAZ); cefoxitin (30 μg) (FOX); amikacin (30 μg)
(AN); ciprofloxacin (10 μg) (CIP); nalidixic acid (10 μg)
(NA); gentamicin (10 μg) (GEN); netilmicin (30 μg) (NET);
tobramycin (10 μg) (NN); fosfomycin (10 μg) (FFL); trimeth-
oprime + sulfamid (10 μg) (SXT); imipenem (10 μg) (IPM);
and colistin (10 μg) (CL). The diameters of the zones of inhi-
bition were interpreted according to the recommendations of
the CLSI [18]. All strains isolated were screened for
extended-spectrum β-lactamase (ESBL) production by the
double-disk synergy test [19].

2.2. Molecular Methods

2.2.1. DNA Extraction. The DNA template from urine sam-
ple was conducted as follows: the urine (4–10 ml) was cen-
trifuged at 15000 rpm for 5 min, the supernatant contain-
ing the harvested DNA was collected and stored at -20°C
until its use in the PCR experiments.

The preparation of the DNA template from urine sam-
ple was conducted as follows: the urine (4–10 ml) was cen-
trifuged at 12,000 rpm for 10 min, with the resulting
bacterial pellet resuspended in 100 μl sterile
distilled water and heated at 100°C for 10 minutes. After
centrifugation at 15000 rpm for 5 min, the supernatant con-
taining the harvested DNA was collected and stored at -20°C
until its use in the PCR experiments.

2.2.2. Primer Design. Based on the literature, primer design
was performed using the BLAST (English Basic Local Align-
ment Search Tool) program to detect
β-lactamase genes
encoding extended-spectrum β-lactamases (blaSHV-12,
blaTEM and blaCTX-M-15) [23], Plasmid-mediated AmpC-lactamases
(blaCMY-2) [24], and classes B (blaIMP-I, blaNDM-1) and D
(blaOXA-48) carbapenemases [25]. These primers were verified
by Primer 3 (Table 1).

2.2.3. Real-Time PCR Amplification Program. The qPCR
assay was performed on a CFX96™ real-time PCR thermo-
cycler (BioRad, France).
Each reaction was carried out in a 20 μl reaction mixture containing 1 μl of template AND extracted directly from urine or from strains (50 ng/μl), 100 nM of each primer, and 10 μl of the SYBR green. The optimal program of the qPCR includes an initial denaturation at 95°C for 3 min, followed by 40 cycles of: 95°C for 10 s; a hybridization temperature of 56°C for the genes \( \text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV-12}}, \text{bla}_{\text{CTX-M-15}}, \text{bla}_{\text{CTX-M-9}}, \text{bla}_{\text{CMY-2}} \) and 60°C for the genes of group \( \text{bla}_{\text{OXA-48}}, \text{bla}_{\text{IMP-1}}, \text{bla}_{\text{NDM-1}} \) for 10 s and 72°C for 30 s. A melting step was performed at the end of the amplification; it was performed using the following cycling parameters: 60°C for 30 s and 5°C temperature changes to the end temperature of 95°C. The amount of amplified product was monitored by detecting the fluorescence energy emitted by SYBR green. Each PCR run included a negative control (no template control).

### Table 1: List of primers used for qPCR amplification of ESBLs and carbapenemase genes.

| Genes | Primer sequence (5’ → 3’) FW: Forward RV: Reverse | T’m (°C) | Product size (bp) GenBank |
|-------|-----------------------------------------------|---------|----------------------------|
| \text{bla}_{\text{SHV-12}} | FW: AGCCGCTTGAGCAAATTTA \text{RV: } GCTGGCAGATCCATTTCTA | 59.99 | 77 \text{ LC229232.1} |
| \text{bla}_{\text{TEM}} | FW: GATAAACGGACGCGGTA \text{RV: } GATACGGGAGGGCTTACCAT | 60.04 | 78 \text{ MG860488.1} |
| \text{bla}_{\text{CTX-M-15}} | FW: CACCAATGATATTGCGGTA \text{RV: } GTTCGCGTGTTGTAATAG | 60.34 | 77 \text{ MG288771.1} |
| \text{bla}_{\text{CTX-M-9}} | FW: TACCTCACCGCTCAACC \text{RV: } ACCGTGGTGACGATTTAG | 60.11 | 78 \text{ CP028990.1} |
| \text{bla}_{\text{CMY-2}} | FW: CCAGAAGTCCGAAACA \text{RV: } CCGTGCTTAGGGTCA | 59.87 | 65 \text{ LC229227} |
| \text{bla}_{\text{OXA-48}} | FW: GTAGTCAGGCATCGTTA \text{RV: } CCGTICTGATGCCTCA | 60.02 | 73 \text{ MN54469.1} |
| \text{bla}_{\text{IMP-1}} | FW: GCCAAAGTCCGCCAAATTAT \text{RV: } TCAAGAGTGATGCGTCA | 60.17 | 92 \text{ MK088089.1} |
| \text{bla}_{\text{NDM-1}} | FW: ATGGAGACTGGCGACCAAC \text{RV: } GCCATGTGAGATAGGA \text{AAG} | 61.10 | 87 \text{ LC413788.2} |

### Table 2: Specificity and sensitivity of the qPCR system for \( \text{bla} \) group gene detection.

| Resistance gene target | Clinical urines (n = 78) | Strains (n = 78) |
|------------------------|--------------------------|-----------------|
|                        | True (+) = A True (-) = D | Sensitivity; specificity, % | True (+) = A True (-) = D | Sensitivity; specificity, % |
| \text{bla}_{\text{SHV-12}} | 34 0 0 44 | 100; 100 | 34 0 0 44 | 100; 100 |
| \text{bla}_{\text{TEM}} | 46 0 0 32 | 100; 100 | 46 0 0 32 | 100; 100 |
| \text{bla}_{\text{CTX-M-15}} | 56 0 0 22 | 100; 100 | 56 0 0 22 | 100; 100 |
| \text{bla}_{\text{CTX-M-9}} | 42 0 0 36 | 100; 100 | 42 0 0 36 | 100; 100 |
| \text{bla}_{\text{CMY-2}} | 29 0 0 49 | 100; 100 | 29 0 0 49 | 100; 100 |
| \text{bla}_{\text{OXA-48}} | 7 0 0 71 | 100; 100 | 7 0 0 71 | 100; 100 |
| \text{bla}_{\text{IMP-1}} | 0 0 0 78 | 100; 100 | 0 0 0 78 | 100; 100 |
| \text{bla}_{\text{NDM-1}} | 1 0 0 77 | 100; 100 | 1 0 0 77 | 100; 100 |

Each reaction was carried out in a 20 μl reaction mixture containing 1 μl of template AND extracted directly from urine or from strains (50 ng/μl), 100 nM of each primer, and 10 μl of the SYBR green. The optimal program of the qPCR includes an initial denaturation at 95°C for 3 min, followed by 40 cycles of: 95°C for 10 s; a hybridization temperature of 56°C for the genes \( \text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV-12}}, \text{bla}_{\text{CTX-M-15}}, \text{bla}_{\text{CTX-M-9}}, \text{bla}_{\text{CMY-2}} \) and 60°C for the genes of group \( \text{bla}_{\text{OXA-48}}, \text{bla}_{\text{IMP-1}}, \text{bla}_{\text{NDM-1}} \) for 10 s and 72°C for 30 s. A melting step was performed at the end of the amplification; it was performed using the following cycling parameters: 60°C for 30 s and 5°C temperature changes to the end temperature of 95°C. The amount of amplified product was monitored by detecting the fluorescence energy emitted by SYBR green. Each PCR run included a negative control (no template control).

2.3. Determination of Specificity and Sensitivity of the qPCR Using Cultured Bacteria or Bacteria Harvested from Urine. Specificity and sensitivity were determined using the following formula: specificity = \((D/C + D) \times 100\) and sensitivity = \((A/A + B) \times 100\), where A is true positive, B is false negative, C is false positive, and D is true negative (Table 2). The values of the correlation coefficients \( R^2 \) were calculated by the standard curve method. These values \( R^2 \) were 0.98 for all genes.

2.4. Statistical Analysis. Statistical tests including the \( \chi^2 \) test, multivariate logistic regression analysis to interpret the
associations between the genes of the bla group and the different levels of antibiotic resistance, OR, and 95% CI were calculated as well as the analysis of Spearman’s rank correlation. A *p* value of 0.05 was counted as statistically significant in this study. All data was done using IBM SPSS version 21.0.

### 3. Results

Of the 500 positive urine cultures, only the most predominant isolates were isolated, and among them, 90 isolates were considered multidrug-resistant strains, as these isolates were found to be resistant to at least two classes of antibiotics. Forty-four strains were identified as *E. coli*, which was predominant, followed by 34 strains of *K. pneumoniae*, 3 strains of *Enterobacter cloacae*, 2 strains of *Pseudomonas aeruginosa*, 2 strains of *Enterococcus faecalis*, 1 strain of *Morganella morgani*, 1 strain of *Citrobacter koseri*, 1 strain of *Aeromonas hydrophila*, 1 strain of *Acinetobacter*, and 1 strain of *Staphylococcus aureus*.

The qPCR system was used for the detection of antimicrobial resistance genes (Tables 2 and 3) in 78 uropathogenic *Enterobacterales* strains (44 *E. coli* and 34 *K. pneumoniae* included 40 strains of *E. coli* and 20 strains of *K. pneumoniae* ESBL producers), regardless of whether the DNA was extracted from bacteria in culture, or directly from urine.

qPCR reactions were initially performed at different annealing temperatures designated for each primer pair (Table 1).

#### 3.1. Evaluation of Specificity and Sensitivity of the qPCR

**Using Cultured Bacteria or Bacteria Harvested from Urine.**

The newly developed qPCR system was effective in detecting genes of the bla group. The use of this system has shown that all the *Enterobacterales* isolates tested have at least one gene of the bla group. Of these, 31 contained more than three genes from the bla group. qPCR amplification of DNA extracted directly from urine was also performed. A concordance of 100% was found between the results of resistance genes detected directly from urine to those using purely isolated colonies (Table 2).

For the targeted bla group genes, the specificity and sensitivity of qPCR were both determined to be 100%.

#### 3.2. Distribution of the Types blaSHV, blaTEM, blaCTX-M-15, blaCTX-M-9, blaCMY-2, blaOXA-48, blaNDM-1, and blaIMP-1 Genes in Clinical Enterobacterales Strains.

qPCR data show that among the *E. coli* clinical strains, group genes *blaCTX-M-15* (70.5%, 31 strains), *blaCTX-M-9* (68.2%, 30 strains), *blaTEM* (52.3%, 23 strains), and *blaSHV-12* (34%, 16 strains) were the most prevalent followed by group genes *blaCMY-2* (18.2%, 8 strains) and *blaOXA-48* (4.54%, 2 strains). None of these stains show the presence of the genes of the group *blaNDM* and *blaIMP-1*.

In *K. pneumoniae*, *blaCTX-M-15* (76.5%, 26 strains) was the most detected followed by *blaSHV-12* (52.9%, 18 strains), *blaTEM* (67.6%, 23 strains), *blaCMY-2* (61.8%, 21 strains), *blaCTX-M-9* (35.3%, 12 strains), *blaOXA-48* (14.7%, 5 strains), and *blaNDM* (2.9%, 1 strain) group genes (Figure 1).

In addition, we found that 98.7% (*n* = 77) of the clinical *E. coli* and *K. pneumoniae* strains tested had at least one gene from the bla group with up to 34 different bla genotypes.

Otherwise, 25 (56.8%) of *E. coli* and 23 (61.5%) of *K. pneumoniae* had more than 2 genes. The most frequent combinations of 3 or more genes from the bla group of isolates tested have been summarized in Table 3.

#### 3.3. Antibiotic Resistance Rates of the Clinical Enterobacterales Strains.

The antimicrobial susceptibility testing performed for 78 Enterobacterales isolates (44 *E. coli* and 34 *K. pneumoniae*) showed that 60 (90.9%) of isolates were ESBL producers (40 *E. coli* and 20 *K. pneumoniae*).

Multidrug-resistant *E. coli* and *K. pneumoniae* strains show strong resistance to penicillin-family antibiotics such as amoxicillin, amoxicillin-clavulanate, ticarcillin, and 3rd-generation cephalosporin antibiotics such as cefotaxime and ceftazidime.

According to the test of the sensitivity of multiresistant *E. coli*, a high level of resistance was also recorded to ofloxacin, ciprofloxacin and nalidixic acid with a percentage of 95.5%, 93.2% (% CI [81.34% - 98.57%]), and 88.6% (% CI [75.44% - 96.20%]), respectively. Strains of *K. pneumoniae* also showed a high level of resistance to ofloxacin 85.3% (% CI [68.94% - 95.04%]), ciprofloxacin 82.4% (% CI [65.46% - 93.23%]), and nalidixic acid 85.3% (% CI [68.94% - 95.04%]). A relative low resistance was recorded for imipenem with a percentage of 2.3% for *E. coli* and 29.4% (% CI [15.09% - 47.47%]) for *K. pneumoniae*.

Otherwise, resistance to cefotaxime can also be considered to be low in strains of *E. coli* and *K. pneumoniae* with a percentage of 9.1% (% CI [2.53% - 21.66%]) and 41.2 (% CI [24.64% - 59.30%]), respectively. Clinical strains of *E. coli* were also resistant to other non-β-lactam antibiotics such as netilmicin 59.1% (% CI [43.24% - 73.66%]), gentamicin 45.5% (% CI [30.39% - 61.15%]), tobramycin 63.6% (% CI [47.77% - 77.59%]), amikacin 25% (% CI [13.19% - 40.33%]), bactrim 68.2% (% CI [52.42% - 81.39%]), and fosfomycin 9.1% (% CI [2.53% - 21.66%]). In addition, the 34 strains of *K. pneumoniae* showed resistance to netilmicin, gentamicin, tobramycin, amikacin, bactrim, and fosfomycin with a percentage of 52.9% (% CI [35.12% - 70.22%]), 61.8% (% CI [43.56% - 77.83%]), 61.8% (% CI [43.56% - 77.83%]), 17.6% (% CI [6.76% - 34.53%]), 67.6% (% CI [49.47% -...
None of these strains shows resistance to colistin (Table 4).

3.4. Relationship between Genotypic and Phenotypic Results of Resistance of Strains to Antibiotics.

qPCR was carried out for the 78 uropathogenic strains of *Enterobacteriaceae* (44 E. coli and 34 *K. pneumoniae*) to analyze the ESBL genes as well as the determinants of resistance to drugs conferring resistance to \(\beta\)-lactam. The detailed associations of drug resistance with bla group detected in *K. pneumoniae* and *E. coli* isolates have been summarized in supplementary Tables 5–7).

Resistance to four or more \(\beta\)-lactam antibiotics was associated with the presence of four genes from the bla group. Indeed, the analysis of the present data using the chi-square test showed a highly significant correlation between the resistance to four or more \(\beta\)-lactams and blaTEM \((P \text{ value} = 0.006)\), blaCTX-M9 \((P \text{ value} = 0.008)\), and blaOXA-48 \((P \text{ value} = 0.006)\) (Table 5). Many strains harboring the blaTEM genes have also cohosted the genes of
| Antibiotics | blaTEM | blaSHV-12 | blaCTX-M9 | blaCTX-M15 | blaCMY-2 | blaOXA-48 |
|-------------|--------|-----------|-----------|-----------|----------|----------|
|             | +      | - P value | +         | - P value | +        | - P value | +        | - P value | +        | - P value |
| Resistance to ≥4 β-lactam antibiotics | 32.60% (15/46) | 6.25% (2/32) | 0.006 | 23.52% (8/34) | 20.45% (9/44) | 0.744 | 33.33% (14/42) | 8.33% (3/36) | 0.008 | 17.85% (10/56) | 31.81% (7/22) | 0.179 | 66.66% (4/6) | 18.05% (13/72) | 0.006 |
Table 6: Relationship between bla genes and antibiotic resistance in the 78 clinical strains (by multiple logistic regression analysis).

| Antibiotics                  | Resistance to ≥4 β-lactam antibiotics | blaTEM OR (95% CI) | blaSHV-12 OR (95% CI) | blaCTX-M9 OR (95% CI) | blaCTX-M15 OR (95% CI) | blaCMY-2 OR (95% CI) | blaOXA-48 OR (95% CI) |
|------------------------------|---------------------------------------|---------------------|-----------------------|-----------------------|-----------------------|----------------------|----------------------|
|                              | χ²  P value                            | χ²  P value         | χ²  P value           | χ²  P value           | χ²  P value           | χ²  P value           | χ²  P value           |
| Resistance to ≥4 β-lactam antibiotics | 5.921 0.015                           | —                   | —                     | 3.764 0.05            | —                     | —                     | 9.835 0.002           |
| FOX                          | —                                     | —                   | —                     | —                     | —                     | —                     | 11.136 0.002          |

χ²: Chi-square; OR: Odds ratio; 95% CI: 95% Confidence Interval.
the blaCTX-M-9 groups. This positive association between the gene(s) of the blaTEM and/or blaCTX-M-9 group and resistance to four or more antibiotics was confirmed by multiple logistic regression analysis (Table 6) and by Spearman’s rank correlation analysis (Table 7). In addition, a positive correlation was also recorded between the blaCMY-2 gene and the agent FOX. This association was proved by the chi-square test ($P < 0.001$; Table 5), multiple logistic regression ($P = 0.002$; Table 6), and Spearman’s rank correlation analysis ($P < 0.001$; Table 7).

4. Discussion

qPCR system is a faster and more efficient technology for detection sensitivity to antibiotics compared to classical phenotypic and conventional methods [26]. It has been widely used for the research of resistance genes in clinical samples, but principally to support infection controlling rather than guiding therapy [21]. We explored its potential to detect important genes for antibiotic resistance to Enterobacterales in the clinic urine without culture.

Unlike most tests currently available (conventional methods), our qPCR system offers the advantage of detecting the target group of 7 bla genes (blaSHV, blaTEM, blaCTX-M-9, blaCTX-M-15, blaCMY-2, blaOXA-48, and blaNDM) after 2 hours with similar sensitivity and specificity which was obtained for both urine and cultured bacteria. For this reason, the detection of antibiotic resistance genes directly from biological samples could be a useful tool in Tunisia and other countries where the blaCTX-M gene clusters were predominant. TheCTX-M, TEM, and SHV enzymes were among the most common variants in Tunisia [27], in Palestine, in Egypt [28], and in European region [29] with varying prevalence rates. These results are similar to our qPCR data. Among the clinical strains of E. coli, blaCTX-M-15 (70.5%) and blaCTX-M-9 (68.2%) group genes were the most prevalent followed by blaTEM (52.3%), blaSHV (36.4%), blaCMY-2 (18.2%), and blaOXA-48 (4.5%) group genes. In K. pneumoniae strains, blaCTX-M-15 (85.3%) was the most detected followed by blaSHV-12 group (52.9%), blaTEM (67.6%), blaCMY-2 (61.2%), blaCTX-M-9 (35.3%), blaOXA-48 (14.7%), and blaNDM-1 (2.9%) group genes.

The qPCR test achieves a sensitivity of 100% and 100% specificity of the β-lactamase genes in clinical urine and cultured strains. Our results are in agreement with those of Schmidt et al. [14] who developed a multiplex tandem PCR (MT-PCR) for the detection of 16 genes of the ESBLs family in clinical urine and isolates cultured with a sensitivity of 100% and a specificity of 95-100% [30].

Uniformly, these results using urine directly are comparable to that proven by others using analogous methodology on cultivated isolates. Chavada and Maley [31] evaluated the MT-PCR to look for 12 β-lactamases genes in cultured Gram-negative isolates, achieving 95% sensitivity and 96.7% specificity [31].

Singh et al. [32] have developed a real-time multiplex PCR test to detect 10 β-lactamases, such as ESBLs, AmpCs, and carbapenemases genes. The diversity of genes sought was higher than in our study, although the blaCTX-M group was neglected [32].

Moreover, Willemsen et al. [33] used qPCR to detect ESBL-encoding genes (blaCTX-M-like, blaTEM and blaSHV) accessing 98.9% sensitivity and 100% specificity compared to a reference chip [33].

In this study, we also investigated the relationship between phenotypic and genotypic results of ESBL-producing clinical isolates of E. coli and K. pneumoniae. We detected a relatively high percentage of genes previously shown to be associated with resistance to antibiotics belonging to the β-lactams [34].

The strains which carry the SHV and TEM genes show only relatively low resistance to third-generation cephalosporins such as cefixime, cefotaxime, ceftazidime, and monobactam; when coexisting with the genes of the blaCTX-M group, the rate resistance of these bacteria to these antibiotics will be significantly increased [35]. Apart from the many variants of CTX-M that have been reported in recent years, CTX-M-15 belongs to a specific group of these genes, which is defined by increased hydrolysis activity of ceftazidime [36].

Strains with the blaCTX-M-9 gene group only are characterized by a relatively low level of resistance to CAZ, but in combination with blaCTX-M-15, this resistance was markedly increased. The resistance of strains to CTX and CAZ may be explained by the presence of blaCTX-M15 [3].

The blaTEM and blaCTX-M-9 group genes were positively related with resistance to more than four β-lactams, according to statistical analysis of our data.

The CMY-2 gene encoded by the plasmid was detected by qPCR in 51.7% of cefoxitin resistant isolates and this result was statistically significant ($P < 0.05$). These results were consistent with two studies conducted in Egypt by Rensing et al. [8] and Fam et al. [37] in which CMY-2 was detected in 86.9% and 76.5%, respectively.

Three of K. pneumoniae and one E. coli strains carrying blaOXA-48 are resistant to imipenem. β-Lactamase OXA-48 hydrolyze significantly carbapenems such as imipenem and penicillins, but not extended-spectrum cephalosporins [38].
These findings support the idea that the qPCR technique can reveal the link between certain bla group genes and resistance to multiple β-lactam drugs. Multiple resistance genes coexisting in a single strain increase the risk of them spreading to new strains, and the diversity of their resistance complicates molecular detection and treatment.

However, despite the sensitivity of 100% was achieved for resistance tested in clinical urine and culture isolates, the qPCR system could not detect new or currently rare determinants if their number increases over time, which limits the number of targets that can be screened. In addition, this system could not distinguish the genes which code for ESBLs and non-ESBLs from blaTEM and blaSHV, as long as they are 10 times rarer than blaCTX-M among E. coli causing urinary tract infections [39].

Thus, our system does not interfere with the collapse antherapy, indeed during our work we have used qPCR to detect, only, the variants of ESBL frequently found in our region.

Finally, qPCR cannot predict resistance to cephalosporins and carbapenems in Enterobacterales isolates which is caused only by β-lactamases, but by other resistance mechanisms such as changes in membrane permeability and efflux pump [40]. These limitations could be linked with those of the conventional method, which only gives results for at least 2 days. If resistance prevails, this shows that most patients are undertreated either by a random treatment, by an agent with limitations but little resistance, or by an antibiotic which would usually be reserved (such as ertapenem) becomes the standard of empirical care.

5. Conclusion

In conclusion, the real-time PCR system accurately detected β-lactamase-producing Enterobacterales directly from biological samples or using purely isolated colonies.

Our results showed a concordance between the results found by the classical method and those by the molecular method. Here, we showed the high prevalence of ESBLs in Tunisia. In summary, an efficient detection system could be put in place to have a diagnosis, a rapid, specific, and reliable antibiotic therapy, and the management of infection control programs. All the tests validated during our work will be soon be marketed as a rapid and cost-effective methods in the laboratory.

Data Availability

All data and additional information regarding this study are available to third parties under reasonable request.

Ethical Approval

Approval and consent were not required.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors’ Contributions

Mariem Yengui was responsible for the conception and design of the work and writing of the manuscript. Rahma Trabelsi and Nourelhoua Mathlouthi were responsible for the interpretation of data for the work and writing and reviewing of the manuscript. Radhouane Gdoura, Mejdi, and Lamia Khannous were responsible for the supervision of the project, important intellectual contributions, and final approval of the version to be published. All authors read and approved the final manuscript.

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