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Original Article

Genotypic Detection of *qnrA* and *qnrC* Genes in *Citrobacter koseri* Isolated from Patients with Urinary Tract Infection

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Abstract

The urinary tract infection (UTI) is a prevalent infection that affects people of all ages. Bacterial agents are the most common causes of UTIs. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and other *staphylococcal* species, *Citrobacter freundii* and *Citrobacter koseri* (*C. koseri*) account for a smaller number of infections. These pathogens are transported into the urinary tract from the colonic biotope into dysbacteriosis. Urine samples were randomly collected from 249 outpatients who were suspected of having UTIs. After genital cleaning, 10 mL of urine specimens were collected in a sterilized bowel. Then, the specimens were centrifuged at 2,000 rpm for 5 min and the residue was aerobically incubated with the broth infusion of brain flasks at 37°C for 24 h and then applied with a sterile ring onto blood agar plates and MacConkey agar (OxoidTM). Out of 249 urine samples, the results proved that there were 176 (70.7%) and 51(20.5%) gram-negative and gram-positive bacteria isolates, respectively. However, the results demonstrated that there were 22 (8.8%) urine samples with no growth. In addition, the results showed that eight various antimicrobials are used to treat *C. koseri*. In the current study, *C. koseri* was treated with eight different antimicrobial agents. The antimicrobial resistance rate for 7 isolates against Cefotaxime, Ceftriaxone, Ciprofloxacin, and Levofloxacin was high for 6 (85.71%) isolates. The results indicated that 6 and 5 isolates had 85.71% and 71.42% antimicrobial resistance against Ceftazidime and Levofloxacin, respectively. Whereas Gentamicin showed a moderate rate of resistance (4 isolates, 57.14%), and Amikacin resistance was found in 5 isolates, accounting for 28.57%. The bacterial isolates had a high susceptibility rate to Imipenem. The *qnrA* gene was found in 6 (85.71%) isolates. However, the recorded data demonstrated that there is no isolate carrying the *qnrC* gene. Among all pathogenic bacteria, *C. koseri* was the lowest causative agent of UTI in this study and was highly resistant to most antimicrobials except Imipenem, which was a good antibiotic with 100% sensitivity.

Keywords: Antimicrobials, Bacterial agents, *Citrobacter koseri*, UTI

1. Introduction

The urinary tract infection (UTI) is a widespread infection that occurs at all ages (1). The bacterial agents are the most probable causes responsible for UTIs (2). Urinary tract infections are bacterial infections caused by normal microflora, such as *Escherichia coli* and *Staphylococcus epidermidis* which are the most common agents isolated from UTI patients. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and other *staphylococcal* species, *Citrobacter freundii* and *Citrobacter koseri* (*C. koseri*) account for a smaller number of infections. These pathogens are transported into the urinary tract from the colonic biotope into dysbacteriosis (3, 4).

Citrobacter koseri is a gram-negative organism, negative to oxidase, able to motility, and has no

capsule (5, 6). *Citrobacter koseri* recently became more resistant to various antibiotics, such as beta-lactamases, cephalosporins 3rd generation, aminoglycosides, and quinolones, (inhibited DNA replication by targeting important bacterial enzymes DNA gyrase and topoisomerase IV, which form topoisomerase IV/DNA gyrase–DNA complexes) (7, 8).

The newer quinolone antibiotics (especially Ciprofloxacin and Levofloxacin) are the most used antibiotic to treat gram-negative bacteria causing UTIs. Bacterial agents resist the quinolones by many mechanisms (such as mutations that alter the sites of drug targets and mutations that reduce drug accumulation). Furthermore, plasmid-mediated quinolone resistance was discovered late in the game to protect cells from the lethal effects of quinolones. In Enterobacteriaceae *qnr* (A, B, C, D, and S), *aac (62) Ib-c*, and *qep*A are mediating the quinolones resistance (9-12).

The number of studies focusing on *C. koseri*, which causes resistance to various antibiotics, is limited in Iraq. Therefore, the main objective of this study is to examine the *qnrA* and *qnrC* genes of *C. koseri* isolated from outpatients infected with UTIs in Najaf, Iraq (13).

2. Materials and Methods

2.1. *Citrobacter koseri* Isolates

Urine samples were randomly collected from 249 outpatients with suspected UTIs. After genital washing, 10 mL of urine were collected in sterile disposable containers. All urine samples were centrifuged for 5 min at 2,000 rpm, then aerobically incubated with broth infusion of brain flasks at 37°C for 24 h before being put on blood agar and MacConkey agar plates with a sterile ring.

A single, pure bacterial colony was grown using the colony-forming units (CFUs) method. All urine samples with fewer than 105 CFU/ml were discarded. The VITEK 2 system was used to diagnose *C. koseri* suspected colonies.

2.2. Antimicrobial Sensitivity Test

Using the conventional disc diffusion method, the isolates were tested for resistance to up to eight antibiotics. All isolates were inoculated on Moller Hinton Agar, then the antimicrobial discs were added. The incubation period was 24 h at 37ºC and the inhibition zone diameter of each disc was measured and compared with the control measure. Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Gentamicin (15 µg), Amikacin (30 µg), Ciprofloxacin (5 µg) and Levofloxacin (5 µg) (Bioanalyse.Turkey) were used. The clarification of the results was as recommended by National Committee for Clinical Laboratory Standards (NCCLS).

2.3. DNA Extraction

Total DNA extraction was accomplished by the following steps: 5 pure and fresh colonies of *C. koseri* strains were suspended in 200 µl of sterile deionized waters and cells were put in water at 100°C for 30 min. The solution was then immediately placed on ice for 30 min and the remaining cellular components were centrifuged for 15 min at 9,000 rpm. Finally, the DNA template was made from the supernatant.

2.4. Polymerase Chain Reaction to Detect Genes Associated with the Resistance to Antimicrobials

The used primers and the PCR thermocycling conditions are listed in table 1, and the identification of two quinolone resistance genes in table 2.

Table 1. Primer sequence used in a polymerase chain reaction for *Citrobacter koseri* resistance-associated genes

Gene	Sequence	bp
qnrA	F-ATTTCTCACGCCAGGATTTG R- GATCGGCAAAGGTTAGGTCA	516
qnrC	F-GGGTTGTACATTTATTGAATC R-TCCACTTTACGAGGTTCT	447

Table 2. Polymerase chain reaction thermal cycling conditions for *Citrobacter koseri* quinolone resistance genes

Genes	Temperature (°C) / Time					Number Of Cycles
	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	
qnrA	95°C/5min	94°C/60sec	55°C/60sec	72°C/2min	72°C/5min	30
qnrC	95°C/5min	95°C/60sec	55°C/60sec	72°C/60sec	72°C/5min	30

Both PCR products were loaded onto a 1.5% agarose gel (w/v) with a safety stain of 0.5 mg/mL and analyzed using gel electrophoresis.

2.5. Statistical Analysis

In the present study, Fisher's method was used to compare samples using the SPSS software (version 6). A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Numbers and Percentages of Total Bacterial Isolates

Out of 249 urine samples, the results proved that 176 (70.7%) and 51 (20.5%) isolates were gram-negative and gram-positive, respectively. However, the results demonstrated that 22 (8.8%) of urine samples were not showed any growth (Figure 1).

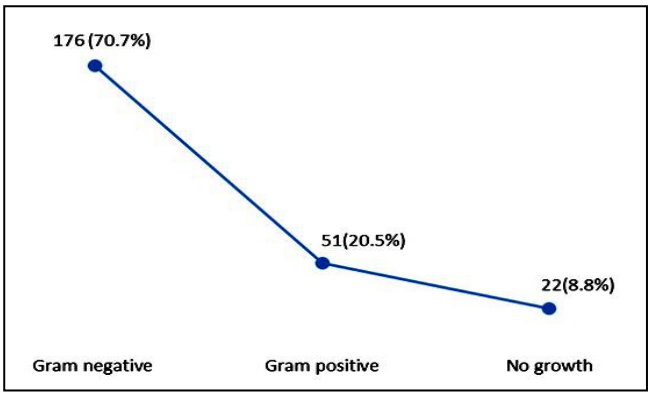

Figure 1. Total sample isolates (numbers and percentages) from patients infected with urinary tract infection

Based on the microscopic evaluation, culture characteristics, and biochemical test, the results revealed that 91 (36.54%), 59 (23.69%), 10 (4.01%), and 9 (3.61%) isolates were diagnosed as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, respectively. Moreover, 7 (2.81%), 29 (11.64%), 15 (6.02%), and 7 (2.81%) isolates were diagnosed as *Citrobacter koseri*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*, respectively, as shown in (Figure 2). Seven *C. koseri* isolates were confirmed by the VITEK 2 system.

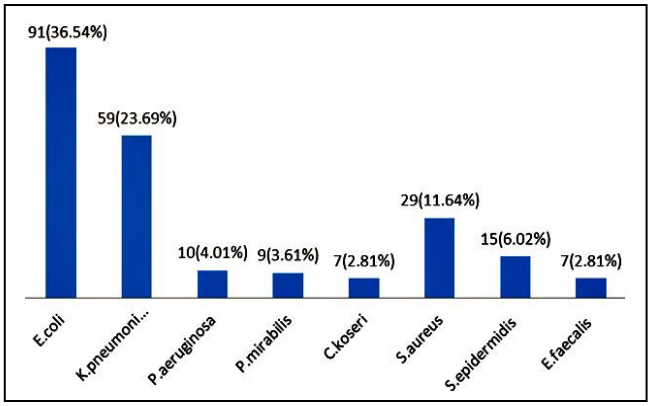

Figure 2. Total bacterial isolates from patients with urinary tract infection (numbers and percentages)

3.2. Antimicrobial Sensitivity Test

In the current study, *C. koseri* was treated with eight different antimicrobial agents. The antimicrobial resistance rate for 7 isolates against Cefotaxime, Ceftriaxone, Ciprofloxacin, and Levofloxacin was high for 6 (85.71%) isolates. The results showed that 6 (85.71%) and 5 (71.42%) isolates had antimicrobial resistance against Ceftazidime and Levofloxacin, respectively. Whereas, Gentamicin showed a moderate rate of resistance (457.14%) isolates. In contrast, Amikacin resistance was found in 5 isolates, accounting for 28.57%. As shown in table 3, the bacterial isolates had a high susceptibility rate to Imipenem (100%).

Table 3. Total *Citrobacter koseri* isolates were resistant to 7 antimicrobials identified from patients with urinary tract infection (numbers and percentages)

Antibiotics	Sensitive	Resistant	Percentage of resistance
Cefotaxime	1	6	85.71%
Ceftriaxone	1	6	85.71%
Ceftazidime	2	5	71.42%
Imipenem	7	0	0
Gentamicin	3	4	57.14%
Amikacin	5	2	28.57%
Ciprofloxacin	1	6	85.71%
Levofloxacin	1	6	85.71%

3.3. The genes Contributing to Quinolone Resistance

Based on the obtained results, 6 (85.71%) isolates carried the *qnrA* gene, as shown in figure 3. Whereas, the recorded data demonstrated that there was no isolate carrying *the qnrC* gene.

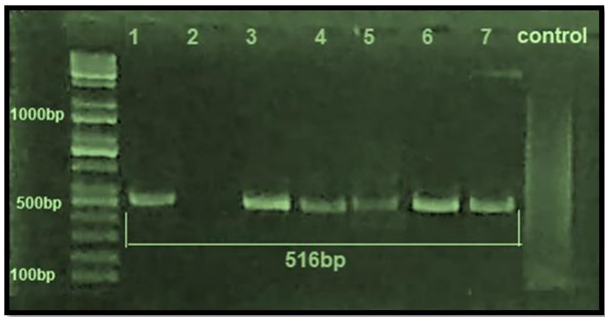

Figure 3. Extracted DNA from *Citrobacter koseri* isolates, gives positive results at 516 bp when amplified with the *qnrA* gene

4. Discussion

Bacterial urinary tract infections are among the most prevalent reasons for people to seek medical care (14). The active treatment of patients with bacterial UTIs typically means forming organizations through which the disease can be detected and selecting appropriate antibiotic agents for the organism in question (10). Urinary tract infection is common in many of Iraq's regions and remains a major medical problem (9).

Citrobacter koseri is an extremely adaptable, opportunistic bacterium with remarkable mechanisms for surviving and transmission within the host (15). In children and adults with a compromised immune system, this pathogen causes many pathogenic cases with a high morbidity and mortality rate, such as hospital-acquired infections, pneumonia, septicemia, wound infection, gastrointestinal infection, endocarditis, sepsis, and meningitis, (16, 17). Resistance to antibiotics affects infection incidence and the spread of pathogens, and increases death rates and treatment costs (18). The treatment for patients with UTIs is focused on antimicrobials (19). Although overuse of primary antibiotics, such as Ampicillin and Chloramphenicol has aided in the emergence and spread of many drug-resistant major strains around the world, different types of antibiotics, including those belonging to the quinolone family (e.g., Ciprofloxacin and Levofloxacin), have been used in the treatment of UTI due to their little resistance. These antibiotics attack the enzymes DNA gyrase and topoisomerase IV, which are required for the replication of genetic material (20, 21).

After the use of anti-quinolones, strains of resistance to these antibiotics appeared, as the bacteria resisted the quinolones by several mechanisms, such as a mutation in genes that encodes gyrase enzymes on their own or with a mutation in genes that encode to topoisomerase IV. On the other hand, the plasmids *qnr* (A, B, C, D, and S), *aac(62)Ib-c*, and *qep*A are mediating the quinolones resistance (22, 23).

Based on the findings of the present study, *C. koseri* was the lowest causative agent of UTI among all pathogenic bacteria. In addition, it was highly resistant to most antimicrobials, except for Imipenem, which was a good antibiotic with 100% sensitivity. Most *C. koseri* isolates harbored the *qnrA* gene at an 85.71% rate, whereas no isolate had the *qnrC* gene.

Authors' Contribution

Study concept and design: A. S. A. A.
Acquisition of data: T. H. H.
Analysis and interpretation of data: K. A. T.
Drafting of the manuscript: G. S. B.
Critical revision of the manuscript for important intellectual content: A. S. A. A.
Statistical analysis: T. H. H.
Administrative, technical, and material support: T. H. H.

Ethics

The study design was approved by the ethics committee of University of Alkafeel, Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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