

Original Research Paper

# Genotypic Characterization of Antimicrobial Resistance-Associated Genes in *Citrobacter Freundii* Isolated from Patients with Urinary Tract Infection in Al-Najaf Governorate-Iraq

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**Abstract:** *Citrobacter freundii* is one of the most important pathogens that cause urinary tract infection in human because it harbors many types of antimicrobials resistance-associated genes. Therefore, this study aimed to evaluate the prevalence of antimicrobial resistance-associated genes *citrobacter freundii* isolated from patients with urinary tract infection in Al-Najaf governorate-Iraq. A total of 30 strains of *citrobacter freundii* isolated from urine samples of patients with urinary tract infection in Al-Najaf central hospital in Al-Najaf City, Iraq, during April to December 2018. Polymers chain reaction technique was used to detect the presence of antimicrobial resistance-associated genes. From the 30 strains of *citrobacter freundii*, 15 strains (50%) were positive for *bla-tem* gene, 16 strains (53.3%) were positive for *bla-shv* gene, 21 strains (70%) were positive for *bla-ctxm* gene, 3 strains (10%) were positive for *bla-ctxm-1* gene, 6 strains (20%) were positive for *bla-ctxm-2* gene. Interestingly, it should be noted that no any strain from the 30 we tested was carrying *pse1*, *bla-ctxm-8*, *bla-ctxm-9* or *bla-ctxm-25* genes. Our data showed that 19 strains (63.3%) were positive for *cat1* gene, 9 strains (30%) were positive for *cat2* gene and 4 strains (13.3%) were positive for *cat3* gene. On the other hand, 17 strains (56.6%) were carrying *qnrA* gene, 11 strains (36.6%) were carrying *qnrB* gene and *aac(6')-Ib-cr* gene. Also, the results proved that there were 7 strains (23.3%) being positive for *sul1* gene and 11 strains (36.6%) were positive for *sul2* gene. We also found 22 strains (73.3%) being positive for *strA-strB* gene and 4 isolates (13.3%) were positive for *aacC1* genes. No strain carrying *aacC2*, *tetA* and *tetB* genes was identified. *Citrobacter freundii* harbor large numbers of different antimicrobial resistance-associated genes that enable this pathogen to escape the effect of antibiotics which make this bacteria highly resistant causing urinary tract infection.

**Keywords:** *Citrobacter Freundii*, Urinary Tract Infection, Antimicrobials Resistance-Associated Genes, Iraq

## Introduction

*Citrobacter freundii* is consider as one of a commensal types of the intestinal tract of humans and different creatures but can also be found in water, sewage, food and hospital settings (Liu *et al.*, 2018). *Citrobacter freundii* is a Gram-negative, facultative anaerobic, encapsulated motile organism with long rod shape, a typical length of bacterium is 1-5  $\mu\text{m}$  (Hossain *et al.*, 2017; Aljanaby and Aljanaby, 2017).

*Citrobacter freundii* is often found in clinical specimens as an opportunistic pathogen was implicated with infections that are generally resstricted to infants and immuno-compromised people (Plakkal *et al.*, 2013; Bae *et al.*, 2018). *Citrobacter freundii* responsible for nosocomial infection, blood stream and wounds infections, gastroenteritis, endocarditis, pneumonia, septicemia, urinary tract infection meningitis and brain abscesses with high mortality and morbidity (Aljanaby and Gafil, 2013). *Citrobacter freundii* has many types of

antimicrobial resistance-associated genes such as *shv*, *tem*, *ctx-m* and others enable this pathogen to escape the effect of various antibiotics, thus became more virulent and cause different types of infection such as urinary tract infection (Liu *et al.*, 2017; 2018). In Iraq, there are limited studies focusing on the prevalence of antimicrobial resistance-associated genes in *Citrobacter freundii* isolated from patients with urinary tract infection. Therefore, the aim of this study was to investigate the prevalence of antimicrobial resistance-associated genes in *Citrobacter freundii* isolated from patients with urinary tract infection. This study will draw a good genotypic picture of this pathogen.

## Materials and Methods

### Study Design and Patients

This is a cross-sectional descriptive study, performed in Al-Najaf central hospital in Al-Najaf City, Iraq, during period from April to December 2018. A total of 461 urine samples were collected from patients infected with urinary tract infection, females (21) and males (9), age groups between 18 to 60 years old.

### Eligibility Criteria for Patients

Patients will be considered eligible for registration into this study if they fulfill all the inclusion criteria and none of the exclusion criteria as defined below:

1. Patients (female or male) at least equal or more than 18 years old
2. All infected patients have been diagnosis by physician and primary microscopic characters such as the presence of pus cells in urine samples
3. Patients should have sufficient capacity for informed consent
4. Patients should don't have any other infection
5. Patients should don't take any antibiotics for treatment

## Samples Collection, Culture and Bacterial Identification

Ten ml of mid-stream urine samples were collected in sterile disposable containers after cleaned the genitals. All urine samples were centrifuged at 2000 rpm for 5 min, immediately, the sediment incubated aerobically with brain hart infusion broth at 37°C for 24h then streaked with sterile loop on to blood agar and MacConkey agar (Oxoid™) plates (Mac Faddin, 2000). Colony Forming Units (CFUs) method was used for growing single and pure bacterial colony; all urine samples containing less than 10<sup>5</sup> CFUs/ml were excluded (Tan *et al.*, 2012). All emerged bacterial isolates were identified according to colony morphology and standard microbiological tests such as; colony morphology, Gram stain, oxidase test, catalase test, imvic test, motility test, coagulase test, growth on MacConkey agar (Oxoid™) (Mac Faddin, 2000).

**DNA extraction:** The previously published protocol (Yang *et al.*, 2008) was used for the extraction of total DNA as follows: Five pure and fresh colonies of *citrobacter freundii* strains were suspended in 200 µl of sterile deionized water and cells were placed in water bath (Memmert-Germany) at 100°C for 30 min, immediately the solution was placed in ice for 30 min and the other cellular components was removed by centrifugation at 9000 rpm for 15 min. Finally the supernatant was used as the DNA template.

**Polymerase Chain Reaction (PCR) detection of antimicrobials resistance-associated genes:** Polymerase chain reaction was used to detect 22 antimicrobials resistance-associated genes: All primers used in this study and all PCR thermo cycling conditions are listed in Tables 1 and 2, respectively. All PCR products were loaded on a 1.5% (w/v) agarose gel with 0.5 mg/mL safestain and were analyzed by gel electrophoresis. All primers used in this study and all PCR thermo cycling conditions are listed in Tables 1 and 2, respectively.

**Table 1:** Sequencing of primers used in PCR for 22 antimicrobials resistance-associated genes of *citrobacter freundii*

Name of gene	Oligo Sequence (3'→5')	Product Size (bp)	Reference
Ampicillin resistance-associated gene <i>Pse-1</i>	F: CGCTTCCCCTTAACAAGTAC R: CTGGTTCATTTTCAGATAGCG	419	Bacci <i>et al.</i> (2012)
β-lactamase resistance-associated genes <i>blatem</i>	F: CAGCGGTAAGATCCTTGAGA R: ACTCCCGTCGTGTAGATAA	643	Newier <i>et al.</i> (2013)
<i>blashv</i>	F: GGCCGCGTAGGCATGATAGA R: CCCGGCGATTGCTGATTTC	714	Newier <i>et al.</i> (2013)
<i>blactx-m</i>	F: AACCGTCACGCTGTTGTTAG R: TTGAGGCGTGGTGAAGTAAG	766	Newier <i>et al.</i> (2013)
<i>blactx-m-1</i>	F: GCGTGATACCACTTCACCTC R: TGAAGTAAGTGACCAGAATC	260	Iraz <i>et al.</i> (2015)
<i>blactx-m-2</i>	TGATACCACCACGCCGCTC TATTGCATCAGAAACCGTGGG	341	Iraz <i>et al.</i> (2015)
<i>blactx-m-8</i>	F: TTTGCCCGTGCATTGG R: CGACTTTCTGCCTTCTGCTCT	368	Du <i>et al.</i> (2014)
<i>blactx-m-9</i>	F: ATGGTGACAAAGAGAGTGCA R: CCCTTCGGCGATGATTCTC	870	Liu <i>et al.</i> (2017)

**Table 1:** Continue

<i>blactx-m-25</i>	F: TTGTTGAGTCAGCGG GTTGA R: GCGCGACCTTCCGGCCAAAT	490	Huang <i>et al.</i> (2017)
Chloramphenicol resistance-associated genes			
<i>Cat1</i>	F: CACCGTTGATATATCCCAATGGCATCGT R: CTGCCGACATGGAAGCCATCACAAAC	582	De Vito <i>et al.</i> (2015)
<i>Cat2</i>	F: CCTGCCACTCATCGCAGTAC R: AACGGCATGAACCTGAA	547	Adesiji <i>et al.</i> (2014)
<i>Cat3</i>	F: ATCGGCATCGGTTACCATGT R: ATCCCCTCTTGCTGATATT	310	Adesiji <i>et al.</i> (2014)
Quinolone resistance-associated genes			
<i>qnrA</i>	F: ATTTCTCAGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	Robicsek <i>et al.</i> (2006)
<i>qnrB</i>	F: GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469	Robicsek <i>et al.</i> (2006)
<i>aac(6)-Ib-cr</i>	F: TGACCAACAGCAACGATTCC R: TTAGGCATCACTGCGTGTTC	554	Shams <i>et al.</i> (2015)
Sulfonamides resistance-associated genes			
<i>Sul1</i>	F: GTGACGGTGTTCGGCATTCT R: TTTACAGGAAGGCCAACGGT	668	Levings <i>et al.</i> (2005)
<i>Sul2</i>	F: TCAACATAACCTCGACAGT R: GATGAAGTCAGCTCCACCT	707	Chu <i>et al.</i> (2001)
Streptomycin resistance-associated gene			
<i>strA-strB</i>	F: ATGGTGGACCCAAAACCTCT R: GTCTAGGATCGAGACAAAG	891	Tamang <i>et al.</i> (2013)
Aminoglycoside resistance-associated genes			
<i>aacC1</i>	F: ATGGGCATCATTGCGACATGTAGG R: TTAGGTGGCGGTACTTGGGTC	873	Hujer <i>et al.</i> (2006)
<i>aacC2</i>	F: ATGCATACGCGGAAGGCAATAAC R: CTAACCGGAAGGCTCGCAAG	861	Hujer <i>et al.</i> (2006)
Tetracycline resistance-associated genes			
<i>tetA</i>	F: GCTACATCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	Bacci <i>et al.</i> (2012)
<i>tetB</i>	F: TTGGTTAGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	659	Bacci <i>et al.</i> (2012)

**Table 2:** Thermo cycling conditions of PCR for 22 antimicrobials resistance-associated genes of *Citrobacter freundii*

Cycling condition °C/Time

Gene	Initial denaturation °C/Time	Denaturation	Annealing	Extension	N Cycles	Final extension °C/Time	Reference
<i>Pse-1</i>	94°C/12 min	94°C/1 min	57°C/30sec	72°C/5min	34	72°C/7 min	Bacci <i>et al.</i> (2012)
<i>bla-tem</i>	95°C/5 min	94°C/30 sec	52°C/45 sec	72°C/45sec	30	72°C/7 min	Newier <i>et al.</i> (2013)
<i>bla-shv</i>	95°C/5 min	94°C/30 sec	55°C/60 sec	72°C/45sec	30	72°C/7 min	Newier <i>et al.</i> (2013)
<i>blactx-m</i>	95°C/5 min	94°C/30 sec	57°C/45 sec	72°C/45sec	30	72°C/7 min	Newier <i>et al.</i> (2013)
<i>blactx-m-1</i>	94°C/4 min	94°C/1 min	54°C/60 sec	72°C/1min	30	72°C/10 min	Iraz <i>et al.</i> (2015)
<i>blactx-m-2</i>	95°C/5 min	95°C/50 sec	56°C/40 sec	72°C/60sec	35	72°C/5 min	Iraz <i>et al.</i> (2015)
<i>blactx-m-8</i>	95°C/5 min	95°C/50 sec	50°C/40 sec	72°C/60sec	35	72°C/5 min	Du <i>et al.</i> (2014)
<i>blactx-m-9</i>	95°C/5 min	95°C/50 sec	50°C/40 sec	72°C/60sec	35	72°C/5 min	Liu <i>et al.</i> (2017)
<i>blactx-m-25</i>	94°C/5 min	94°C/25 sec	53°C/40 sec	72°C/60sec	30	72°C/6 min	Huang <i>et al.</i> (2017)
<i>Cat1</i>	95°C/5 min	94°C/30 sec	62°C/45 sec	72°C/5min	30	72°C/7 min	De Vito <i>et al.</i> (2015)
<i>Cat2</i>	95°C/5 min	94°C/30 sec	55°C/30 sec	72°C/30sec	35	72°C/10 min	Adesiji <i>et al.</i> (2014)
<i>Cat3</i>	95°C/5 min	94°C/30 sec	55°C/30 sec	72°C/30sec	35	72°C/10 min	Adesiji <i>et al.</i> (2014)
<i>qnrA</i>	95°C/5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Robicsek <i>et al.</i> (2006)
<i>qnrB</i>	95°C/5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Robicsek <i>et al.</i> (2006)
<i>aac(6)-Ib-cr</i>	95°C/5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Shams <i>et al.</i> (2015)
<i>Sul1</i>	95°C/5 min	94°C/2 min	53°C/1 min	72°C/30sec	35	72°C/7 min	Levings <i>et al.</i> (2005)
<i>Sul2</i>	94°C/5 min	94°C/1 min	50°C/1 min	72°C/1 min	32	72°C/7 min	Chu <i>et al.</i> (2001)
<i>strA-strB</i>	94°C/2 min	94°C/60sec	55°C /60 sec	72°C/60sec	30	72°C/5 min	Tamang <i>et al.</i> (2013)
<i>aacC1</i>	95°C/5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Hujer <i>et al.</i> (2006)
<i>aacC2</i>	95°C/5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Hujer <i>et al.</i> (2006)
<i>tetA</i>	94°C/5 min	94°C/25sec	55°C /30 sec	72°C/50sec	34	72°C/5 min	Bacci <i>et al.</i> (2012)
<i>tetB</i>	94°C/5 min	94°C/25sec	55°C /30 sec	72°C/50sec	34	72°C/5 min	Bacci <i>et al.</i> (2012)

Statistical Analysis: Fisher's exact test was used in this study for the comparison between samples by using SPSS version 6 software. P values less than the 0.05 level of significance were considered statistically significant (Aljanaby and Gafil, 2013).

## Results

### Beta Lactamase Resistance Associated Genes

Out of 30 isolates, there were 15 isolates (50%) were positive for bla-tem gene (Fig. 1), 16 isolates (53.3%) were positive for bla-shv gene (Fig. 2), 21 isolates (70%) were positive for bla-ctxm gene (Fig. 3), 3 isolates (10%) were positive for bla-ctxm-1 gene (Fig. 4), 6 isolates (20%) were positive for bla-ctxm-2 gene (Fig. 5). We did not detect any isolate carrying pse1, bla-ctxm-8, bla-ctxm-9 and bla-ctxm-25 genes.

### Chloramphenicol Resistance Associated Genes

In this study, 3 genes were selected as a chloramphenicol resistance associated genes, the results proved that there were 19 isolates (63.3%) positive for cat1 gene (Fig. 6), 9 isolates (30%) were positive for cat2 gene (Fig. 7) and 4 isolates (13.3%) were positive for cat3 gene (Fig. 8).

### Quinolones Resistance Associated Genes

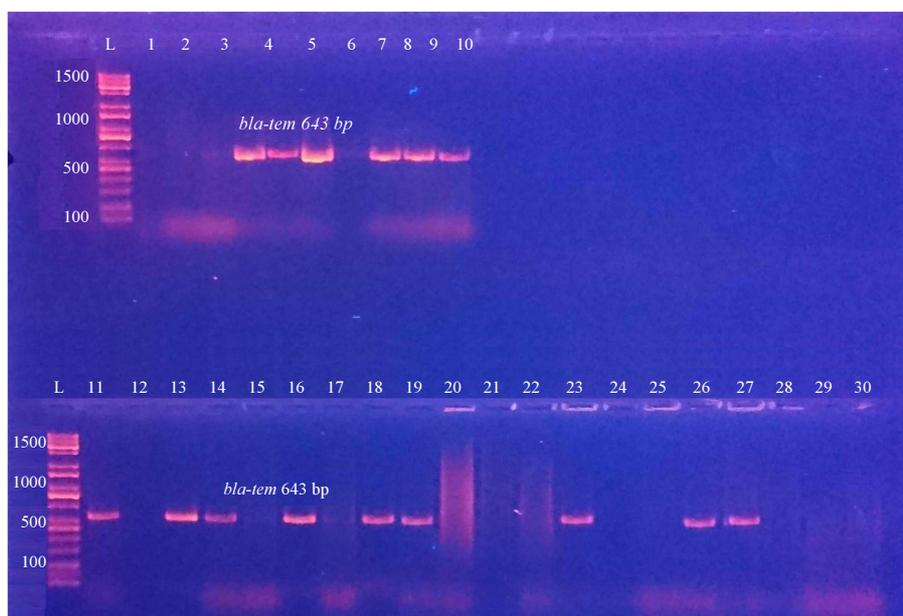
The results indicated that 17 isolates (56.6%) were carrying qnrA gene (Fig. 9), 11 isolates (36.6%) were carrying qnrB gene (Fig. 10) and aac(6)-Ib-cr gene (Fig. 11).

### Sulfonamide Resistance Associated Genes

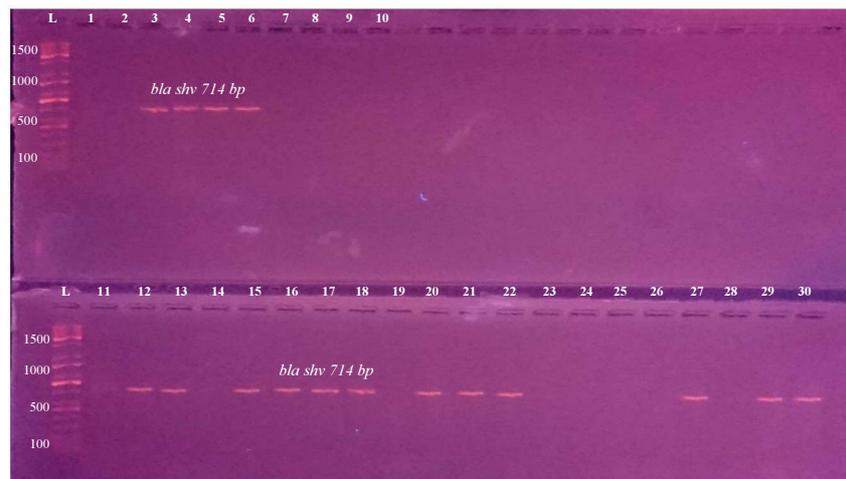
In the current study, genotypic detection of two genes that responsible for encoding sulfonamide resistance associated genes: sul1 and sul2 were detected by Polymerase chain reaction using specific primer sequences which yield products size 668 bp and 707 bp respectively. The results proved that there were 7 isolates (23.3%) positive for sul1 (Fig. 12) and 11 isolates (36.6%) were positive for sul2 gene (Fig. 13).

### Streptomycin, Aminoglycoside and Tetracycline Resistance Associated Genes

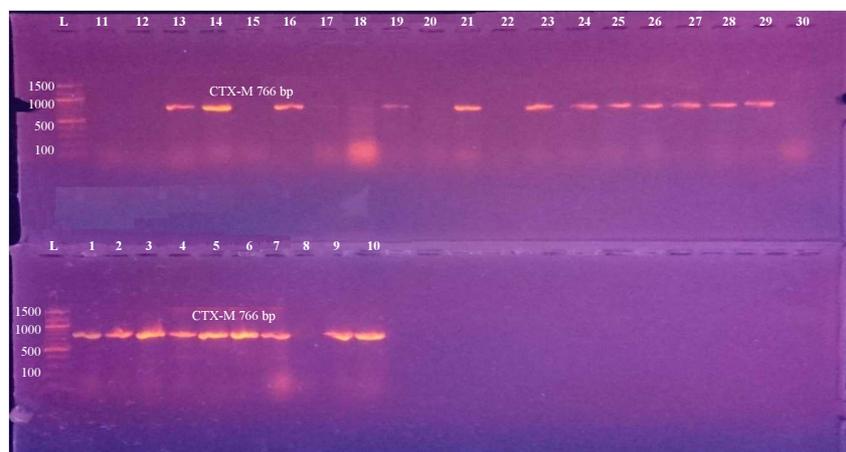
Different genes were used to detect the prevalence of Streptomycin, aminoglycoside and tetracycline resistance associated genes in 30 isolates of *Citrobacter freundii*. strA-strB gene was used as a streptomycin resistance associated genes, the results showed that there were 22 isolates (73.3%) were positive for strA-strB gene (Fig. 14). aacC1 and aacC2 genes were used as a aminoglycoside resistance associated genes, the results indicated that there were 4 isolates (13.3%) were positive for aacC1 gene (Fig. 15) while, there was no any isolates was carrying aacC2 gene. tetA and tetB genes were used as a tetracycline resistance associated genes, the results proved that there was no any isolate was carrying these genes. All numbers and percentages of antimicrobials resistance associated genes in *Citrobacter freundii* isolates are mentioned in Fig. 16 and Table 3.



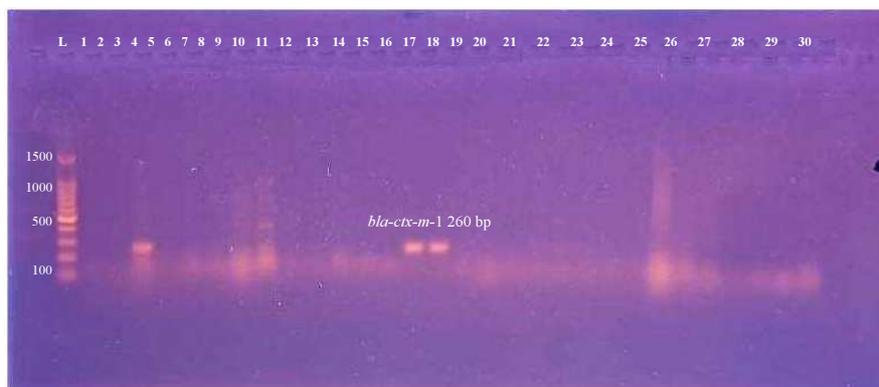
**Fig. 1:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with bla-tem gene show positive results at 643 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



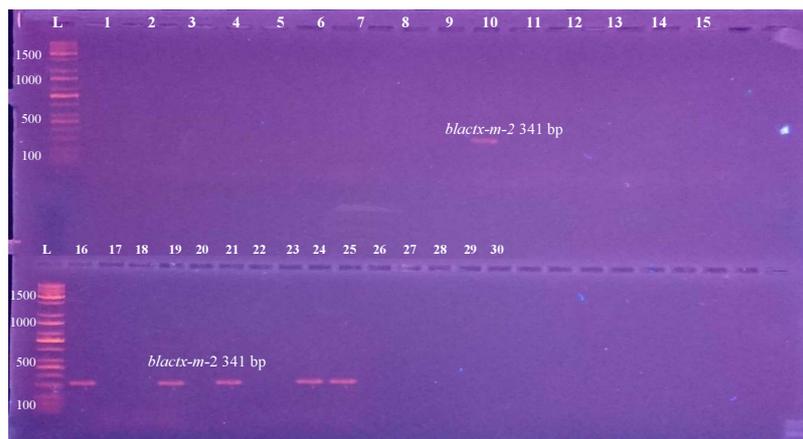
**Fig. 2:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *bla-shv* gene show positive results at 714 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



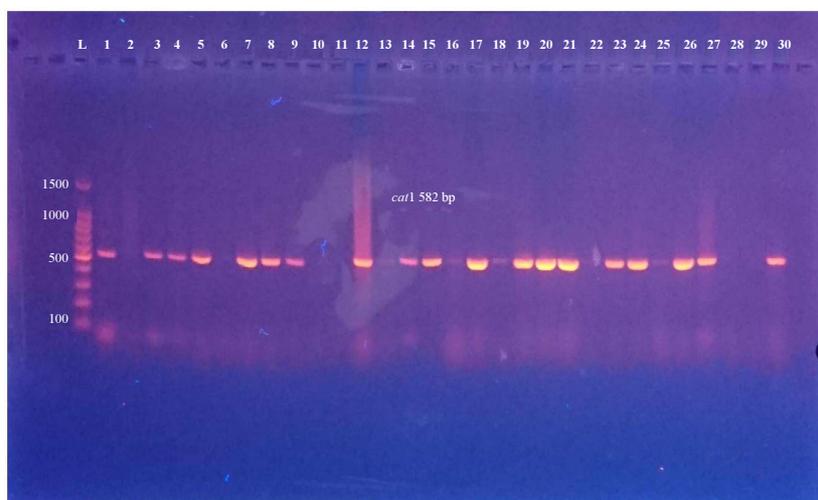
**Fig. 3:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *bla-ctx-m* gene show positive results at 766 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



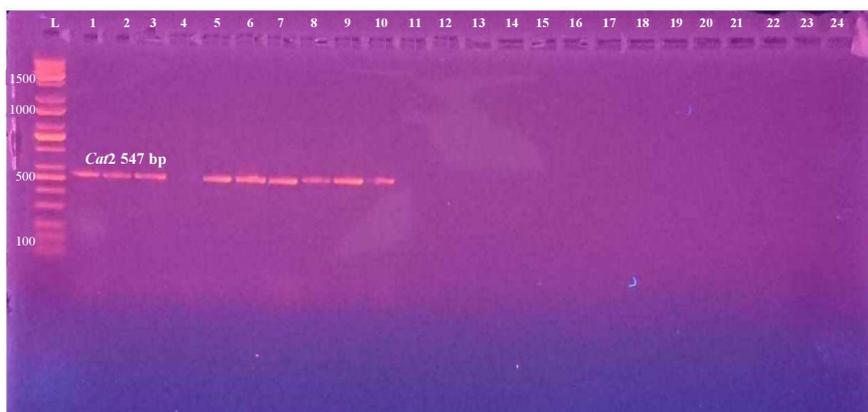
**Fig. 4:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *bla-ctx-m-1* gene show positive results at 260 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



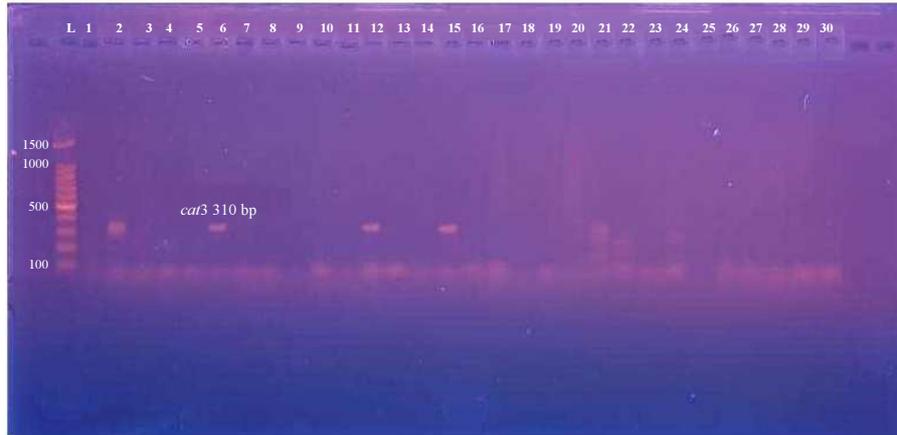
**Fig. 5:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *blaCTX-M-2* gene show positive results at 341 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



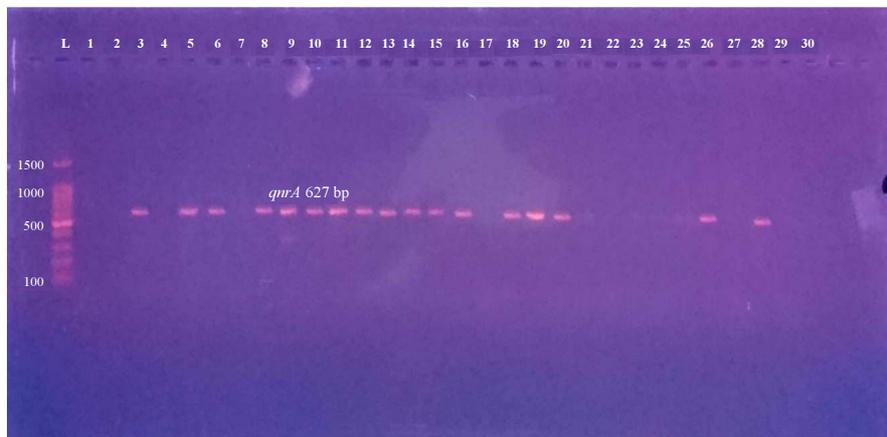
**Fig. 6:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *cat1* gene show positive results at 582 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



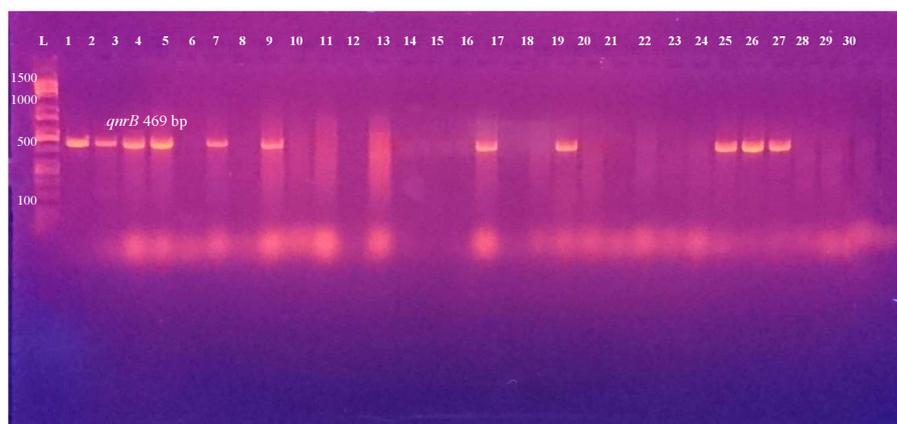
**Fig. 7:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *cat2* gene show positive results at 547 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



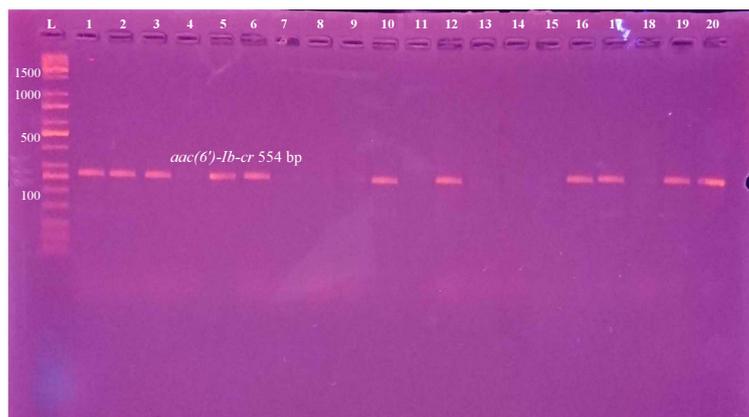
**Fig. 8:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *cat3* gene show positive results at 310 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



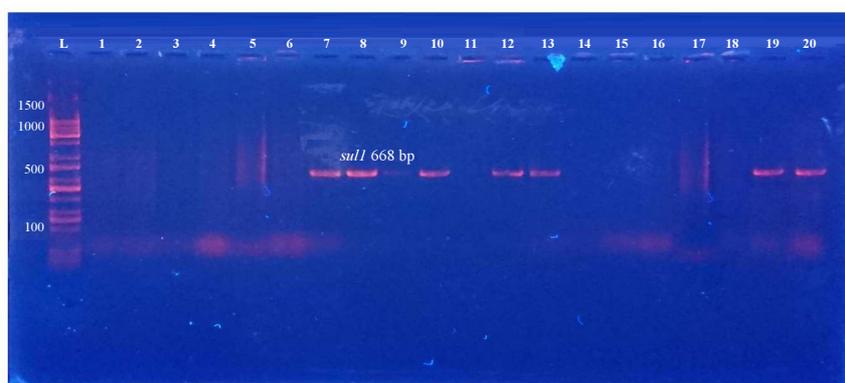
**Fig. 9:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *qnrA* gene show positive results at 627 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



**Fig. 10:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *qnrB* gene show positive results at 469 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



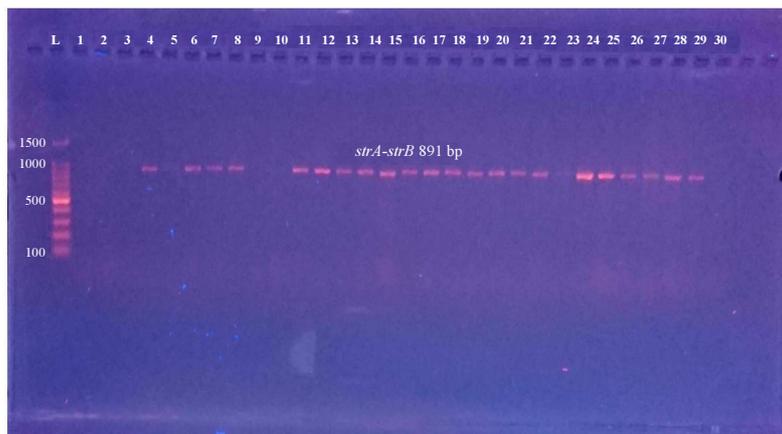
**Fig. 11:** Polymerase chain reaction amplified products from extracted DNA of *Citrobacter freundii* isolates. Amplified with *aac(6)-Ib-cr* gene show positive results at 554 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



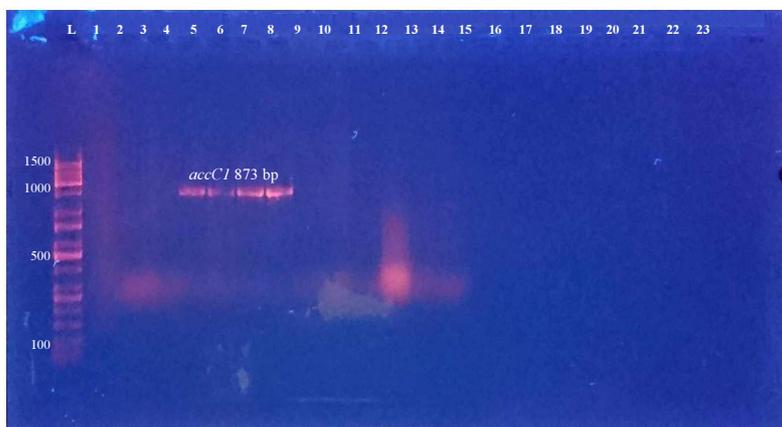
**Fig. 12:** Polymerase chain reaction amplified products from extracted DNA of *Citrobacter freundii* isolates. Amplified with *sul1* gene show positive results at 668 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



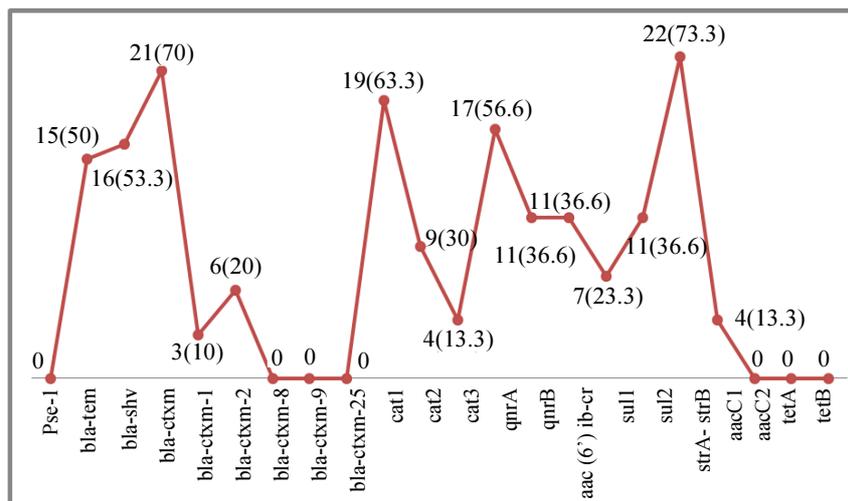
**Fig. 13:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *sul2* gene show positive results at 707 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



**Fig. 14:** Polymerase chain reaction amplified products from extracted DNA of 30 *Citrobacter freundii* isolates. Amplified with *strA-strB* gene show positive results at 891 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



**Fig. 15:** Polymerase chain reaction amplified products from extracted DNA of *Citrobacter freundii* isolates. Amplified with *accC1* gene show positive results at 873 bp. The electrophoresis was performed at 90 volt for 90 minutes. L: DNA molecular size marker 100 bp. (ladder: 100 to 1500 bp)



**Fig. 16:** Numbers and percentages (%) of antimicrobials resistance associated genes in *Citrobacter freundii* isolates from patients infected with urinary tract infection in Al-Najaf City-Iraq during period from April to December 2018. (N = 30)

**Table 3:** Genotypic profile of resistance associated genes in 30 *Citrobacter freundii* isolates from patients infected with urinary tract infection in Al-Najaf City-Iraq during period from April to December 2018

Isolate	Resistance associated genes	No. (%)	Gender	Age group
1	<i>cat1, cat2, qnrB, aac(6)-Ib-cr</i>	4(9.2)	F	51-60
2	<i>cat2, Cat3, qnrB, aac(6)-Ib-cr</i>	4(9.2)	F	18-30
3	<i>bla-shv, bla-ctxm, bla-ctxm-1, cat1, cat2, qnrA, qnrB, aac(6)-Ib-cr</i>	8(34.7)	F	41-50
4	<i>bla-tem, bla-shv, bla-ctxm, cat1, qnrB, sul2, strA-strB, aacC1</i>	8(34.7)	F	31-40
5	<i>bla-tem, bla-shv, cat1, cat2, qnrA, aac(6)-Ib-cr, sul2, aacC1</i>	8(34.7)	F	51-60
6	<i>bla-tem, bla-shv, bla-ctxm, cat2, cat3, qnrA, aac(6)-Ib-cr, strA-strB, aacC1</i>	9(39.1)	F	41-50
7	<i>cat1, cat2, qnrB, sul1, sul2, strA-strB, aacC1</i>	5(21.7)	M	51-60
8	<i>cat1, cat2, qnrA, sul1, strA-strB</i>	5(21.7)	M	41-50
9	<i>bla-ctxm, cat1, cat2, qnrA, qnrB, sul2</i>	6(26)	F	41-50
10	<i>cat2, qnrA, aac(6)-Ib-cr, sul1, sul2</i>	5(21.7)	F	41-50
11	<i>bla-tem, bla-ctxm, qnrA, sul2, strA-strB</i>	5(21.7)	M	31-40
12	<i>bla-shv, cat1, cat3, qnrA, aac(6)-Ib-cr, sul1, strA-strB</i>	5(21.7)	M	31-40
13	<i>bla-tem, bla-shv, bla-ctxm, bla-ctxm-2, qnrA, sul1, sul2, strA-strB</i>	8(34.7)	M	31-40
14	<i>bla-tem, bla-ctxm, bla-ctxm-1, cat1, qnrA, sul2, strA-strB</i>	7(30.4)	F	41-50
15	<i>bla-shv, bla-ctxm, bla-ctxm-1, cat1, cat3, qnrA, strA-strB</i>	7(30.4)	F	18-30
16	<i>bla-tem, bla-shv, bla-ctxm, bla-ctxm-2, qnrA, aac(6)-Ib-cr, strA-strB</i>	7(30.4)	F	18-30
17	<i>bla-shv, bla-ctxm, cat1, qnrB, aac(6)-Ib-cr, strA-strB</i>	6(26)	F	41-50
18	<i>bla-tem, bla-shv, bla-ctxm, qnrA, strA-strB</i>	5(21.7)	F	31-40
19	<i>bla-tem, bla-ctxm, bla-ctxm-2, cat1, qnrA, aac(6)-Ib-cr, sul1, strA-strB</i>	8(34.7)	F	41-50
20	<i>bla-shv, cat1, qnrA, qnrB, aac(6)-Ib-cr, sul1, strA-strB</i>	7(30.4)	F	31-40
21	<i>bla-shv, bla-ctxm, bla-ctxm-2, cat1, strA-strB</i>	5(21.7)	F	31-40
22	<i>bla-shv, bla-ctxm, strA-strB</i>	3(13)	F	31-40
23	<i>bla-tem, bla-ctxm, cat1</i>	3(13)	F	51-60
24	<i>bla-ctxm, bla-ctxm-2, cat1, sul2, strA-strB</i>	5(21.7)	F	51-60
25	<i>bla-ctxm, bla-ctxm-2, sul2, strA-strB</i>	4(9.2)	F	41-50
26	<i>bla-tem, bla-ctxm, cat1, qnrA, qnrB, sul2, strA-strB</i>	7(30.4)	F	41-50
27	<i>bla-tem, bla-shv, bla-ctxm, cat1, qnrB, strA-strB</i>	6(26)	M	41-50
28	<i>qnrA, qnrB, strA-strB</i>	3(13)	M	41-50
29	<i>bla-shv, bla-ctxm, strA-strB</i>	3(13)	M	51-60
30	<i>bla-shv, bla-ctxm, cat1</i>	3(13)	M	51-60

No(%): Numbers and percentages of *Citrobacter freundii* isolates that were carried to antimicrobials resistance associated genes, F: Female, M: Male

## Discussion

The TEM catalysts was one of the initially stretched out range beta lactamase to be distinguished, first found in 1965. The TEM sort  $\beta$ lactamase was named after the *E. coli* contaminated patient, Temoneira, in Athens, Greece (Aljanaby and Aljanaby, 2018a). What's more, the *bla-tem* quality (encoding the protein) is normally found on plasmids (Matsumoto *et al.*, 2014). In current study out of 30 isolates, there were 15 isolates with percentage rate (50%) were positive for *bla-tem* gene. This result is in agreement with the study by Praharaj *et al.* (2016) in India, who showed 50% of *C. freundii* isolated from urinary tract infections samples were positive to *bla-tem* gene. A similar study by Delgado-Valverde *et al.* (2016) reported that 65.8% of *C. freundii* isolates were positive to *bla-tem* gene. However, other study demonstrated that there was 10.4% of *C. freundii* isolates were positive to *bla-tem* gene (Akinduti *et al.*, 2011). Extended spectrum beta lactamase enzymes have been derived from TEM and SHV genes by mutations and have been well described in *Enterobacteriaceae* family (Liakopoulos *et al.*, 2016).

In the current study, Molecular detection of some antimicrobials resistance genes were detected by PCR

using specific primer sequences. In the present study 16 isolates with percentage rate (53.3%) were positive for *bla-shv* gene. The current study result is in agreement with those previously reported (Sharma *et al.*, 2016) showing 57.5% of *C. freundii* isolates from urinary tract infection samples were positive to *bla-shv* gene. Another study in Madagascar by Chereau *et al.* (2015) showed that there was 66.6% of *C. freundii* isolates from pregnant women urinary tract samples were positive to *bla-shv* gene. In the current years, another group of plasmid-interceded ESBLs (extended spectrum beta lactamase) called CTX-M which specially hydrolyze cefotaxime, has risen and the *bla-ctxm* quality is typically found on plasmids. CTX-M catalysts have for the most part been found in strains of *Salmonella enterica* serovar *Typhimurium* and *E. coli*, however have likewise been depicted in different types of *Enterobacteriaceae* (Brown *et al.*, 2018). Not at all like different ESBLs sorts, CTX-M family incorporates a complex and non-comparable gathering of catalysts. The primary investigation and arrangement of the amino corrosive groupings of the CTX-M variations ordered

these catalysts into five bunches (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25) (Aljanaby and Alhasnawi, 2017). CTX-M  $\beta$ -lactamases rose not by changes from prior plasmid intervened catalysts but rather by preparation of chromosomal *bla* qualities from microscopic organisms are firmly identified with *Escherichia coli* and discovered around the world, activation of *bla-ctxm* qualities happened by inclusion successions or by lesser degree bacteriophages. The inclusion succession assumes a part in the over-articulation of *bla-ctxm* qualities (Tamang *et al.*, 2013). The *bla* qualities were coordinated into portable hereditary structures and exchanged probably by conjugation into clinical microscopic organisms (Hardiman *et al.*, 2016). These prepared *bla-ctxm* qualities increment cefotaxime imperviousness to a significantly more prominent degree than imperviousness to ceftazidime (Aljanaby, 2018). The consequences of present study additionally showed that 21 isolates (70%) were positive for *bla-ctxm* gene, 3 isolates (10%) were positive for *bla-ctxm-1* gene, 6 isolates (20%) were positive for *bla-ctxm-2* gene, while, there was positive isolate carrying *pse1*, *bla-ctxm-8*, *bla-ctxm-9* and *bla-ctxm-25* genes. This is supportive to previously published study (Castanheira *et al.*, 2017) showing that there was 35.5% of *C. freundii* isolates were from samples revealed that ESBL (extended spectrum beta lactamase) producing isolates have *bla-ctxm* genes, these result is not in agreement with this study result. Chloramphenicol is one of the first broad-spectrum antibiotics used to treat gram-positive and gram-negative bacterial infections; the major resistance of mechanism to chloramphenicol is enzymatic inactivation by the plasmid transposon-mediated chloramphenicol acetyl transferases *cat1* gene and *cat2* gene (Huang *et al.*, 2017). Quinolones are expansive range antibacterial specialists, generally utilized for treatment of contaminations therefore, upgraded level of quinolone resistance has happened as of late for instance fluoroquinolones, for example, ciprofloxacin, beforehand appeared to have incredible action against clinical disengages of *C. freundii*, have turned out to be less successful because of their broad utilize (Aldred *et al.*, 2014). Quinolones are broad-spectrum antibacterial agents, commonly used for treatment of infections as a result, enhanced level of quinolone resistance has occurred in recent years (Aljanaby *et al.*, 2018). Mechanisms of Quinolone resistance is mediated by the mutation of chromosomal genes encoding DNA gyrase and/or topoisomerase IV or by the mutation of genes regulating the expression of efflux pumps (Correia *et al.*, 2017). *qnr* gene found on plasmid, quinolone resistance may also be related to plasmid-mediated quinolone

resistance genes, (*qnrA*, *qnrB* and *aac(6')Ib-cr*) (Aljanaby and Medhat, 2017). The *qnrA* and *qnrB* genes are coding for proteins belonging to the penta peptide repeat family interacting with DNA gyrase and topoisomerase IV enzymes to prevent quinolone inhibition (Aljanaby and Aljanaby, 2018b). Another plasmid mediated quinolone resistance gene is *aac(6')Ib-cr*, which encodes aminoglycoside acetyltransferase enzyme. This enzyme which can diminish a fluoroquinolone activity by adding an acetyl group to this antibiotic (Ramirez and Tolmasky, 2017) Sulfa drugs are an important class of synthetic bacteriostatic antibiotics still used today for the treatment of bacterial infections and those caused by other microorganisms, sulfa drugs achieve this bacteriostatic action by inhibiting the synthesis of folic acid in bacteria (Shama, 2015). They are also known as sulfa drugs and were the main source of therapy against bacterial infections before the introduction of penicillin in 1941. Although sulfonamides have for the most part been replaced by other agents, they still maintain considerable action in certain types of infection, for example in the urinary tract, eye and ear infections (Kapoor *et al.*, 2017). Aminoglycosides are highly potent, broad-spectrum antibiotics with many desirable properties for the treatment of life-threatening infections. Their history begins in 1944 with streptomycin and was thereafter marked by the successive introduction of a series of milestone compounds (kanamycin, gentamicin and tobramycin) which definitively established the usefulness of this class of antibiotics for the treatment of gram-negative bacillary infections (Krause *et al.*, 2016). Streptomycin is an aminoglycoside antibiotic produced by the soil actinomycetes *Streptomyces griseus*. It acts by binding to the 30S ribosomal subunit of susceptible organisms and disrupting the initiation and elongation steps in protein synthesis. It is bactericidal due to effects that are not fully understood (Aljanaby, 2013). The tetracycline which were discovered in the 1940s, are a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. Tetracycline are broad-spectrum agents, exhibiting activity against a wide range of gram-positive and gram-negative bacteria (Krause *et al.*, 2016). In USA 2008, the study by Srinivasan *et al.* (2008) who showed that the *strA-strB* gene was most prevalence gene in *C. freundii* isolates with 4.6% rate of, these result is not in agreement with present study. The study by Igbiosa *et al.* (2018) in Nigeria, showed that the prevalence of multidrug resistance isolates where it contains further antibiotic resistance genes, including those for aminoglycosides (*strA* and *strB*), beta-lactams (*bla-tem*), chloramphenicol (*cat2*), sulfonamide (*sul2*), tetracycline

(*tetA*) and trimethoprim (*dhfrA14*). In conclusion: *Citrobacter freundii* harbor large numbers of different antimicrobial resistance-associated genes that enable this pathogen to be more virulent and make it highly resistant against most antimicrobials and became more dangerous bacteria cause urinary tract infection.

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## Authors' Contributions

**Ahmed Abduljabbar Jaloob Aljanaby:** Designed the study, performed the statistical analyses and wrote the manuscript.

**Thualfakar Hayder:** Collected, cultured and identified of all bacterial isolates, DNA extracted, PCR technique and wrote the manuscript.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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