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Molecular Detection of Antibiotics Profile of *Citrobacter freundii* Isolated from Burn Infection

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fifty samples were taken for this study from burn victims receiving care at the Al-Hussien medical city between February and October 2021. In order to isolate *Citrobacter freundii*, two swabs were taken: one for culturing and the other for direct examination.
Only12 (4.1%) of the 50 samples were isolated from burn and wound infections at the molecular level and 12 (4.1%) from culture. The results isolation and laboratory diagnosis as well as biochemical test approved that there is only 12 isolates belong to *C.freundii* confirmed by molecular detection by specific primers.
Burn and wound is one of the rare causes of infection. Associated with sub sequent urinary tract infection and burn and wound infection.

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Three antibiotics were employed at the molecular level , the results showed that 10 samples were resistant to the (Tet b) gene, all samples were 100% sensitive to the PIP gene, and all samples (100% resistant to the erna gene).

Keywords: Citrobacter freundii; antibiotics pattern; qrnb; Erna; Tetb; pip.

1. INTRODUCTION

Numerous nosocomial infections affecting the respiratory tract [1], urinary tract [2], and bloodstream are typically caused by *Citrobacter freundii*, a gram-negative bacterium that is a member of the *Enterobacteriaceae* family [3] . Additionally, prior studies have connected it to high-mortality brain abscesses and newborn meningitis [4].

Opportunistic infections that are resistant to numerous medicines Considering the infection's severity, The most prevalent virulence factors identified in *C. freundii* linked with diarrhea are toxins, which include Shiga-like toxins and heat stable toxins [5,9]. Kauffmann and Moller first hypothesized the carcinogenicity of *C. freundii* in the 1940s [10]. Certain strains of *C. freundii* exhibit a noticeable presence of both Shiga-like and heat-stable toxins [9, 11]

Cervical carcinoma has been reported to be effectively treated by *C. freundii*, making it a useful tool in the treatment of some tumors [2]. Certain isolated strains of *C. freundii* are fatal to HeLa cells, an immortal human cell line derived from cervical cancer cells [2]. Aggressive adherence of *C. freundii* in goat cells has been seen; this has been proven to contribute to the pathogenicity of *t*he organism, but it does not ensure infection. It has been discovered that the virulence genes of *C. freundii* are homologous or identical to those of *Salmonella* and *E. coli* pathotypes [6] .

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of fifty burn and wound swabs samples of burn infections were admitted to the Al-Hussien medical city in Karbala province / Iraq.

2.2 DNA Extraction

According instructed of the kit (Genaid U.S.A.), DNA was extracted from the bacterial isolate
PCR detection of particular gene markers :This folding protein was amplified using Qnrb primer, a detection primer described in Table (1).

2.3 Identification of *Citrobacter freundii* Antibiotic Resistance by PCR

Specific PCR was performed to identify the antibiotic resistance genes mentioned in Table (1)& (2). using a template of nucleic acid (DNA) isolated from bacterial cells. Five liters of extracted DNA, one and a half liters of master mix, two and a half liters of upstream and downstream primers, and two and a half liters of nuclease-free water made constituted a single reaction combination. The PCR products were filtered using a 1.5% concentration agarose gel.

Table 1. Sequences of detection primers in addition to their amplicon size base pair (bp) and condition

Gene	Primer sequence ($5' - 3'$)	Size (bp)	PCR condition	Reference
*Qnr*b	F-5' CTCTGGCRYTMGTYGGCGAA3' R-3"TTYGCBGYYCGCCAGTCGAA5'	590	$94°C$10 min1x $94°C$ 1min $62 - 66C$1 min 35x $72°C$ 1 min $72°C$10 min1x	In current study procedure designed by Optimise Protocol Writer online

Table 2. Primer sequences for antibiotic resistance and their amplicon size base pair (bp) and condition

Genes	Primer sequence $(5' - 3')$	Size (bp)	PCR condition	Reference
Erna	F-5'AACACCCTGAACCCAAGGGACG 3' R-5'CTTCACATCCGGATTCGCTCGA3'	405	$94°C10$ min1x $94°C2$ min $55°C$ 1 min 40x $72°O10$ min1x	[3]
PIP	F-5' GCAGGAGCATCAGATAGTTCT 3' R-5' GGGATTTATTGTATGCTACAA 3'	169	$94°C10$ min1x $94°C - 1$ min $62°C$ 1min35x $72°C$ 1 min $72°C$ 10min1x	[9]
Tet b	F-5'AAAACTTATTATATTATAGTG3' R-5' TGGAGTATCAATAATATTCAC3'	315	$94°C10$min1x $94°C$ 1 min $62°C$ 1 min 35x $72°C10$ min1x	[7]

The Table (2) contains a list of primers and PCR conditions used to identify the antibiotic resistance gene of *C. freundii*. However, each 25 l PCR container comes with 12.5 l of master mix, 2.5 l of free nuclease water, 5 l of 0.1 g/ml DNA extraction, and 2.5 l of each upstream and downstream primer. The polymerase chain reaction amplicon was determined using gel electrophoresis on 1.5% agarose gels for 40 minutes at 70 V[8].

3. RESULTS AND DISCUSSION

3.1 Isolation of *Citrobacter freundii*

From February to October 2021, a total of fifty swabs were taken from burn and wound infections at Al-Hussien medical city. As indicated in Table (3) out of 150 clinical samples, only 12 had molecularly positive results based on onrb. Due to the deficiency of knowledge regarding *C. freundii*, this study concentrated on it.

We collected clinical isolates from the *Citrobacter freundii* complex from Al-Hussien medical city since preliminary testing revealed that onrb was uncommon in isolates of *Citrobacter koseri* or *Citrobacter amalonaticus*. Molecular results accounted for 10% of the total. 11% of the isolates exhibited resistance or intermediate susceptibility to ciprofloxacin. 12 samples demonstrated no susceptibility to further antibiotics.

Several specialized primers, including species- and genus-specific qnrb adhesion gene primers, were utilized to validate the earlier findings. In contrast to the allelic ladder, this resulted in a distinct 615 bp DNA fragment, as demonstrated in Fig (1). The percentage of identification was reduced to 12 (20%).

Prokaryotic and eukaryotic evolution studies have made extensive use of ESBL sequences, which are helpful for phylogenetic analysis. Since a 549–700 bp section of the cpn60 coding region, known as the "universal target" can be amplified using universal PCR primers, chaperonin sequences have also been used as targets for the detection and identification of organisms [14,12].

3.2 Molecular Antibiotics Profile

C. freundii is usually treated with clindamycin and pipracillin combined, yet there is little information known regarding its resistance [15]. According to a recent study, tetracycline varies in both resistance and sensitivity; in comparison to allelic

Table 3. Number and percentage of bacteria isolated from samples.

No. of swab samples	Diagnosis percentage			
	On culture		Qnrb on molecular	
	Positive results	Negative results	Positive result	Negative result
50 samples	$12(4.1\%)$	38	$12(4.1\%)$	38

ladder, the percentage of resistance was 10 (66.7 %).

This result was consistent with the results obtained by [14]. However, these findings are consistent with those obtained by, who discovered that all isolates possessed Tetracycline resistance due to the antibiotic's inability to penetrate the cell wall. Tetracycline antibiotics work by stopping aminoacyl-tRNA from binding to the mRNA-ribosome complex, therefore suppressing the production of proteins. They primarily bind to the 30S ribosomal subunit of the mRNA translation complex to accomplish this. The tetM gene, which was found on the bacterial chromosome of tetracycline-resistant strains of C*. freundii*, was previously believed to be the source of tetracycline resistance. This outcome was similar to that attained by Jacoby [10,13].

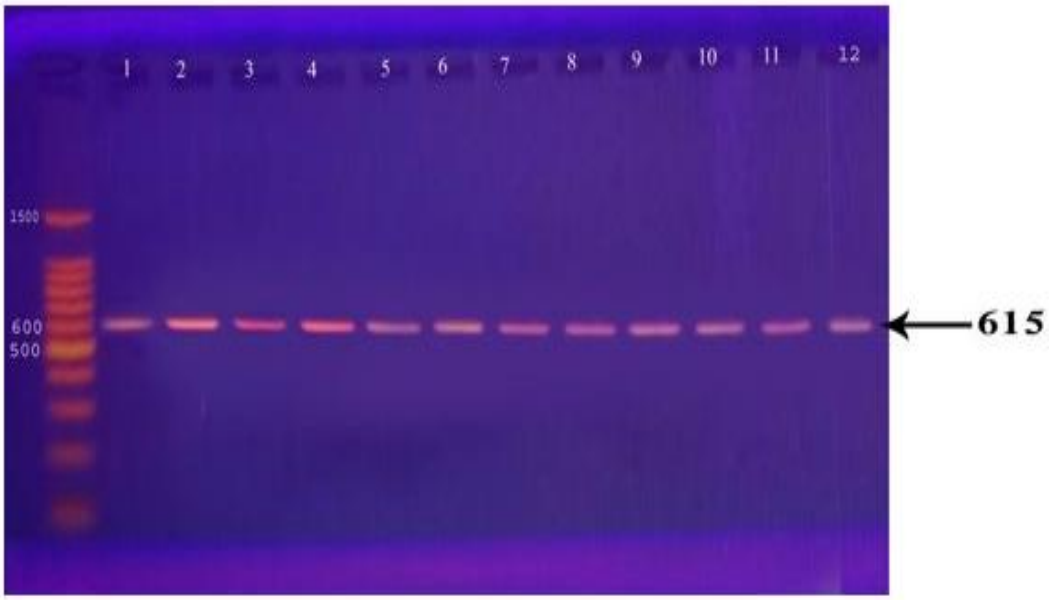

Fig. 1. Agarose gel electrophoresis at 70 volt for 50 min for onrb PCR products visualized under U.V light at 301 nm after staining with with ethidium bromide *L: 1500bp ladder; lane (1-12) were positive for this gene,the size of product is 620bp*

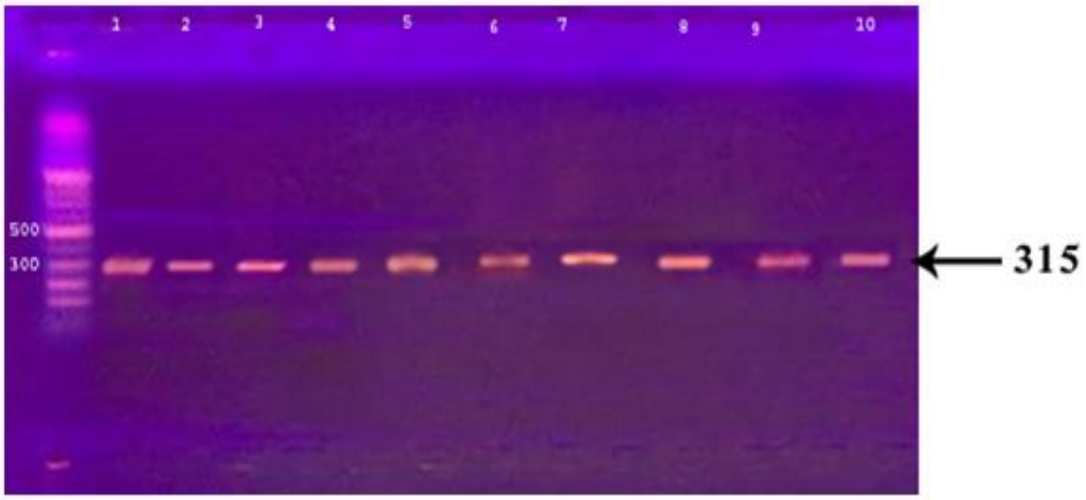

Fig. 2. 1% Tet B PCR products were separated by agarose gel electrophoresis at 70 volts for 50 minutes and visualized under UV light at 301 nm after staining with ethidium bromide L: 1500bp ladder; lanes (1-10) were positive for this gene;product size is 315bp

Resistance to tetracycline was caused by the drug's inability to reach targeted sites due to the cell wall's role as a limiting factor in preventing antibiotic entry into the cells, as described by Sanchez-Cespedes [20].

When compared to allelic ladder, clindamycin, the second antibiotic to which was 100% resistance, gave 405 base pairs.

The results that were obtained corresponded with the results that Poirel et al[18] provided, which revealed that all isolates were completely resistant to these antibiotics. Clindamycin's main effect is bacteriostatic. It works similarly to macrolides in inhibiting bacterial protein synthesis by inhibiting ribosomal translocation. It has been confirmed that metronidazole binds to the large bacterial ribosome subunit's 50S rRNA in certain *C freundii* strains [18,20]. Pipracin, the last choice, is highly sensitive; as fig indicates, there was no band. The qnrB alleles present in two *Citrobacter* strains are similar to those reported on transmissible plasmids and in other *Enterobacteriaceae*. *E. coli* was able to readily acquire the qnrB genes from these strains that carried either qnrB2 or qnrB4 on multiresistant plasmids [19,23].

Even though the remaining qnrb-positive Citrobacter strains did not exhibit any symptoms of qnrB transfer, 7 out of 24 of them were able to produce putative transconjugants after being selected with ampicillin, sulfonamide, or trimethoprim, indicating that the majority of the qnrB alleles are located on a chromosome [22,16].

Petrella *et al* sequenced and cloned the gene from C. koseri isolate CK4, cko, encoding a low-level constitutively expressed -lactamase. The sequence was then added to the EMBL database (accession number AF477396) [17]. We amplified a homologue using PCR primers that we developed.

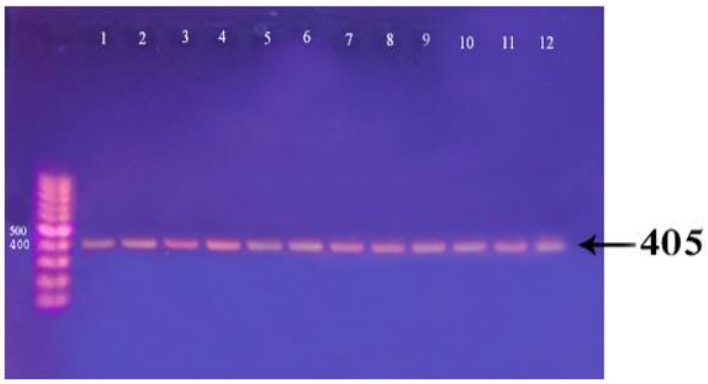

Fig. 3. 1% after staining with ethidium bromide, erna PCR products were electrophoretically separated on an agarose gel at 70 volts for 50 minutes and visualized under UV light at 301 nm. L: 1500 bp ladder; lane (1-12) was positive for this gene; the product size is 405 bp

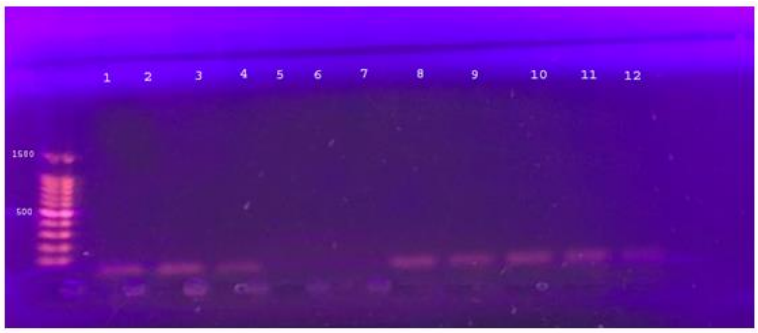

Fig. 4. 1% PIP PCR products were separated by agarose gel electrophoresis at 70 volts for 50 minutes and visualized under UV light at 301 nm after staining with ethidium bromide L: 1500 bpladder; lane (1-12) were negative for this gene; product size is 196 bp

The findings agreed with those of Wang et al [24, 25] who found that decreasing the rate of drug activation inside the cell reduced the rate of drug activation. Active efflux, increased activity of oxygen-consuming enzymes (including catalase, peroxidase, and superoxide reductase), and increased activity of DNA repair mechanisms all contributed to the medication being removed from the cell more quickly

In addition to being a well-known source of diarrheal infections, *Citrobacter freundii* is frequently responsible for nosocomial infections and is developing an increasing resistance to drugs (MDR). The deletion or inactivation of genes encoding nitro reductase activity, as elucidated by Wang et al [24, 25], is the well-established mechanism of pipracin resistance.

4. CONCLUSION

Citrobacter freundii is a commonly recognized cause of diarrheal diseases and a prevalent cause of nosocomial infections. It is developing a more antibiotic-resistant (MDR) variations. This study aimed to evaluate the genetic diversity, antibiotic resistance profiles, and in vitro pathogenicity aspects of *C. freundii* from diarrhea patients and healthy individuals..

5. RECOMMENDATIONS

Since this bacteria is a common cause of wound infections and has practical significance because it is resistant to several antibiotics, we suggest giving attention on it.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brenner DJ. Classification of citrobacteria by DNA hybridization: designation of Citrobacter *farmeri s*p. nov., Citrobacter *youngae sp*. nov., Citrobacter braakii sp. nov., Citrobacter werkmanii sp. nov., Citrobacter *sedlakii sp*. nov., and threeunnamed Citrobacter genomospecies . Int. J. Syst. Bacteriol. 1993;43:645–658.
2. Cattoir V, Poirel L, Mazel D, Soussy CJ, Nordmann P. Vibrio splendidus as the source of plasmid-mediated QnrS-like quinolone resistance determinants. *Antimicrob.* Agents Chemother. 2007;51:2650–2651.
3. Cavaco LM, Hasman H, Xia S, Aarestrup FM *qnrD*, a novel gene conferring transferable quinolone resistance in Salmonella enterica serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob.* Agents Chemother. 2009;53:603–608.
4. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing; 19th informational supplement. CLSI document M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA; 2009.
5. Da Re S. The SOS response promotes *qnrB* quinolone-resistance determinant expression. EMBO Rep. 2009; 10:929–933.
6. Espedido BA, Partridge SR, Iredell JR.. *bla*$_{IMP-4}$ in different genetic contexts in Enterobacteriaceae isolates from Australia. *Antimicrob*. Agents Chemother. 2008;52:2984–2987.
7. Jacoby GA, Gacharna N, Black TA. Miller GH, Hooper DC. Temporal appearance of plasmid-mediated quinolone resistance genes. *Antimicrob.* Agents Chemother. 2009;53:1665–1666.
8. Jacoby GA, Han P. Detection of extended-spectrum β-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli. J. Clin. Microbiol. 1996;34:908–911.
9. Jacoby GA. *qnrB*, another plasmid-mediated gene for quinolone resistance. *Antimicrob. Agents Chemother.* 2006;50:1178–1182.
10. Janda JM, Abbott SL, Cheung WK, Hanson DF. Biochemical identification of citrobacteria in the clinical laboratory. J. Clin. Microbiol. 1994;32:1850–1854. .
11. Jiang Y. Complete nucleotide sequence of Klebsiella pneumoniae multidrug resistance plasmid pKP048, carrying *bla*$_{KPC-2}$, *bla*$_{DHA-1}$, *qnrB4*, and *armA*. Antimicrob. Agents Chemother. 2010;54:3967–3969.
12. Kehrenberg C, Friederichs S, de Jong A, Schwarz S. Novel variant of the *qnrB* gene, *qnrB12*, in Citrobacter werkmanii. Antimicrob. Agents Chemother. 2008;52:1206–1207.
13. Kim HB. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob. Agents Chemother. 2009;53:639–645.

14. JJ, Links MG, Hill JE, Dumonceaux TJ, Kimani J, Jaoko W, Wachihi C, Mungai JN, Peters G A, Tyler S, Graham M, Severini A, Fowke KR, Ball TB, Plummer FA. Molecular definition of vaginal microbiota in east african commercial sex workers. Appl Environ Microbiol. 2011;77(12):4066–4074.
DOI: 10.1128/AEM.02943-10
15. O'Hara CM, Roman SB, Miller JM. Ability of commercial identification systems to identify newly recognized species of Citrobacter. J. Clin. Microbiol. 1995; 33:242–245.
16. Park YJ, Yu JK, Lee S, Oh EJ, Woo GJ. Prevalence and diversity of *qnr* alleles in AmpC-producing Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii and Serratia marcescens: a multicentre study from Korea. J. Antimicrob. Chemother. 2007;60: 868–871.
17. Petrella S, Renard M, Ziental-Gelus N, Clermont D, Jarlier V, Sougakoff W. Characterization of the chromosomal class A β-lactamase CKO from *Citrobacter koseri*. FEMS Microbiology Letters. 2006; 254(2):285–292,
Available:https://doi.org/10.1111/j.1574-6968.2005.00028.
18. Poirel L, Rodriguez-Martinez JM, Mammeri H, Liard A, Nordmann P. Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob.* Agents Chemother. 2005;49:3523–3525.
19. Saga T, Sabtcheva S, Ishii Y, Kaku M, Yamaguchi K. Characterization of *qnrB*-like gene in Citrobacter species of American Type Culture Collection (ATCC) strains, abstr. C2-713. *Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother*. American Society for Microbiology, Washington, DC; 2009.
20. Sanchez-Cespedes J. Two chromosomally located *qnrB* variants, *qnrB6* and the new *qnrB16*, in Citrobacter spp. isolates causing bacteraemia. Clin. Microbiol. Infect. 2009;15:1132–1138.
21. She RC, Simmon KE, Petti CA. Identification of bacteria by DNA target sequencing in a clinical microbiology laboratory, p..*In*Persing D. H.. (ed.), Molecular microbiology: Diagnostic principles and practice, 2nd ed. ASM Press, Washington, DC. 2011;479–489.
22. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin. Microbiol. Rev. 2009;22:664–689.
23. Verdet C. Emergence of DHA-1-producing Klebsiella spp. in the Parisian region: genetic organization of the *ampC* and *ampR* genes originating from *Morganella morganii*. *Antimicrob.* Agents Chemother. 2006;50: 607–617.
24. Wang M, Jacoby GA, Mills DM, Hooper DC. SOS regulation of *qnrB* expression. Antimicrob. Agents Chemother. 2009;53:821–823.
25. White PA, McIver CJ, Deng Y, Rawlinson WD. Characterisation of two new gene cassettes, *aadA5* and *dfrA17*. FEMS Microbiol. Lett. 2000;182:265–269.

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