

First Report Of New Delhi Metallo-Beta-Lactamase (NDM) Producing *Citrobacter Braakii* Isolated From Diabetic Foot Infection In Iraq

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ABSTRACT

Background: Diabetic foot infection (DFI) is an infection in soft tissue or bone in patient with diabetes mellitus, *Citrobacter* species are infrequent nosocomial pathogens and cause a range of infections. the aim of the current study we report the isolation of carbapenem genes among *Citrobacter* species isolated from DFI. **Methods:** about 111 swabs specimen were collection from patient suspected with DFI during the period of study, determine the antibiotic susceptibility of *Citrobacter* spp isolates by using Kirby-Bauer disk diffusion method to 23 antimicrobial agents according to the CLSI (2021). Molecular detection for MBL genes by multiplex PCR. **Results:** from 111 swabs specimen *Citrobacter* spp isolates had represented in 3 (4.34%) among Gram-negative bacteria. Antibiotic susceptibility test revealed that one isolate of *Citrobacter braakii* was MDR and the other isolates was PDR. The PCR data of MBL genes revealed that the frequency of MBL genes among *Citrobacter braakii* as following *blaVIM*, *blaNDM*, and *blaSIM* were 100%. **Conclusion:** the study show the appear MDR and PDR among *Citrobacter* species and *Citrobacter braakii* isolate carry the MBL genes (*blaVIM*, *blaNDM*, and *blaSIM*) where the dissemination of NDM producing *Citrobacter braakii* considered first reported in Iraq.

Keywords Diabetic foot infection (DFI), Metallo-Beta-Lactamase (NDM), *Citrobacter Braakii*.

Article Information

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INTRODUCTION

Diabetic foot infection (DFI) is one of the common consequences for peoples with diabetes, and has grown to be a major reason for non-traumatic amputation (Shabhay *et al.*, 2021). According to some studies about 15-34% of diabetic patients are at risk for developing foot ulcers during their lives (Armstrong *et al.*, 2020). *Citrobacter braakii* and *Citrobacter freundii*, two of the 15 species in the genus *Citrobacter*, are Gram-negative rods in the *Enterobacteriales* family they can be found in water, soil, sewage, and the intestinal tracts of humans and animals (Ducepe *et al.*, 2019). It has been linked with

many hospitals and community acquired infections in humans, based on articles it has been illustrated that 0.8 percent of Gram-negative diseases due to *Citrobacter* species, where in nosocomial settings, about 3-6 percent *Citrobacter* species causes hospital infections among all *Enterobacteriales* family (Kumar and Branton, 2015). Yong *et al.*, (2009) demonstrated that the *blaNDM* gene was identified in Indian patient in Sweden who had previously been treated in New Delhi hospitals (2008) Worldwide attention is now focused on NDM-1 positive *Enterobacteriaceae*, as this gene can be

acquired from the environment by bacteria and can be distributed from India into another country during travel (Walsh, 2005). Since, it has been discovered in many parts of India, Egypt and Europe (Leylabadlo *et al.*, 2015). In Iraq, the initial report to the isolation of NDM-producing *K. pneumoniae* was reported from France in 2010 from an Iraqi trauma patient referred to France for therapy (Poirel *et al.*, 2011). Then, in 2014, published the first article that reported the presence of NDM-producing *P. aeruginosa* isolated from Najaf, Iraq (Alshara *et al.*, 2014). The emergence of NDM among *Citrobacter freundii* was reported in Brazil (Barberino *et al.*, 2018), but the dissemination of NDM producing *Citrobacter braakii* considered first reported in Iraq. Therefore, the aim of the current study we report the isolation of carbapenem genes among *Citrobacter* species isolated from DFI.

MATERIALS AND METHODS:

Specimen collection and bacterial isolates:

This cross section study was conducted in Al-Sader Medical City in the Najaf province during the period from December 2021 to March 2022. The study population consisted of 111 swabs specimen from both gender males and females patients clinically suspected by the physician to have DFI, and the specimens immediately transported to the microbiology laboratory, all Gram-negative isolates from swab in this study had been identified by depending on Morphological characteristic, Microscopically examination (gram-stain), and Biochemical tests according to standard method described by MacFaddin and Hart (MacFaddin, 2000) (Hart, 2006). *Citrobacter* species isolates were also identified by the VITEK-2 compact system.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing of *Citrobacter* spp isolates was performed by using disk diffusion methods (Kirby-Baure method), The resistance profile to 23 antibiotics disks including: ampicillin (10 μ g), piperacillin (100 μ g), piperacillin-tazobactam (110 μ g), amoxicillin-clavulanate acid (30 μ g), ceftazidime (30 μ g), cefotaxime (30 μ g), ceftriaxone (30 μ g), Aztreonam (30 μ g), cefixime (5 μ g),

cefepime (30 μ g), meropenem (10 μ g), imipenem (10 μ g), gentamicin (10 μ g), amikacin (30 μ g), tobramycin (10 μ g), ciprofloxacin (5 μ g), levofloxacin (30 μ g), norfloxacin (30 μ g), chloramphenicol (30 μ g), nitrofurantoin (300 μ g), Trimethoprim-Sulphamethazole (25 μ g), tetracycline (30 μ g), colistin (25 μ g). All susceptibility results were interpreted according to the standard values performed by CLSI (2021).

Molecular detection of Carbapenem gene:

DNA was extracted from the isolates by using the protocol kit of the manufacturing company (Favorgen, Taiwan), *Citrobacter* spp were screening for detection the presence MBLs genes by multiplex PCR, the primers sequence was published in previous article (Poirel *et al.*, 2011). Amplicons were separated by agarose gel electrophoresis in 1.5 % (w/v) agarose gel, stained with ethidium bromide. The positive results were detection when the DNA band base pairs of sample equal to the target product size. The PCR were prepared in total volume 50 μ l PCR mixture including 25 μ l Promega Master mix, 1.5 μ l forward primer (10 μ M), 1.5 μ l reverse primer (10 μ M), 2 μ l DNA template (10-250 ng), and 8 μ l nuclease free water. PCR conditions had performed in T3000 thermocycler (Biometra).

RESULTS:

The results indicated that out of 111 swabs were cultured, there 93 (83.7%) of specimens were positive for the presence of growth bacterial, while 18 (16.2%) had non growth bacterial. Out of 93 of bacteria isolates were bacterial growth and the rate of Gram-negative bacteria was 69 (74.1%) and 34 (36.5%) was Gram positive bacteria. Where only three isolates of *Citrobacter* species 3 (4.34%) were identified as 2 isolates (*Citrobacter braakii*) and 1 isolate (*Citrobacter freundii*) based upon colonial characteristics and conventional biochemical tests and VITEK 2 compact system test.

Antibiotic susceptibility test of *Citrobacter* species isolates indicated that one isolate of *Citrobacter braakii* was MDR (isolate resistant to three or more categories of antibiotics) where was sensitive to amikacin, tetracycline,

and nitrofurantoin. While the other isolates was PDR (isolates resistant to all class of antibiotics), PDR to *Citrobacter* spp considered first reported in these isolates in Iraq among patient with DFI.

Molecular Detection and Distribution of MBLs genes: *Citrobacter* spp isolates were examined by multiplex PCR for the occurrence gene determinants encoding MBL genes (*blaIMP*,

blaVIM, *blaNDM*, *blaSPM*, and *blaSIM*). The results indicated that the presence only one *Citrobacter braakii* isolate carry the MBL genes (*blaVIM*, *blaNDM*, and *blaSIM*) as shown in figure (1). Where the appearance of the NDM producing *Citrobacter braakii* considered first reported for those isolate in Iraq.

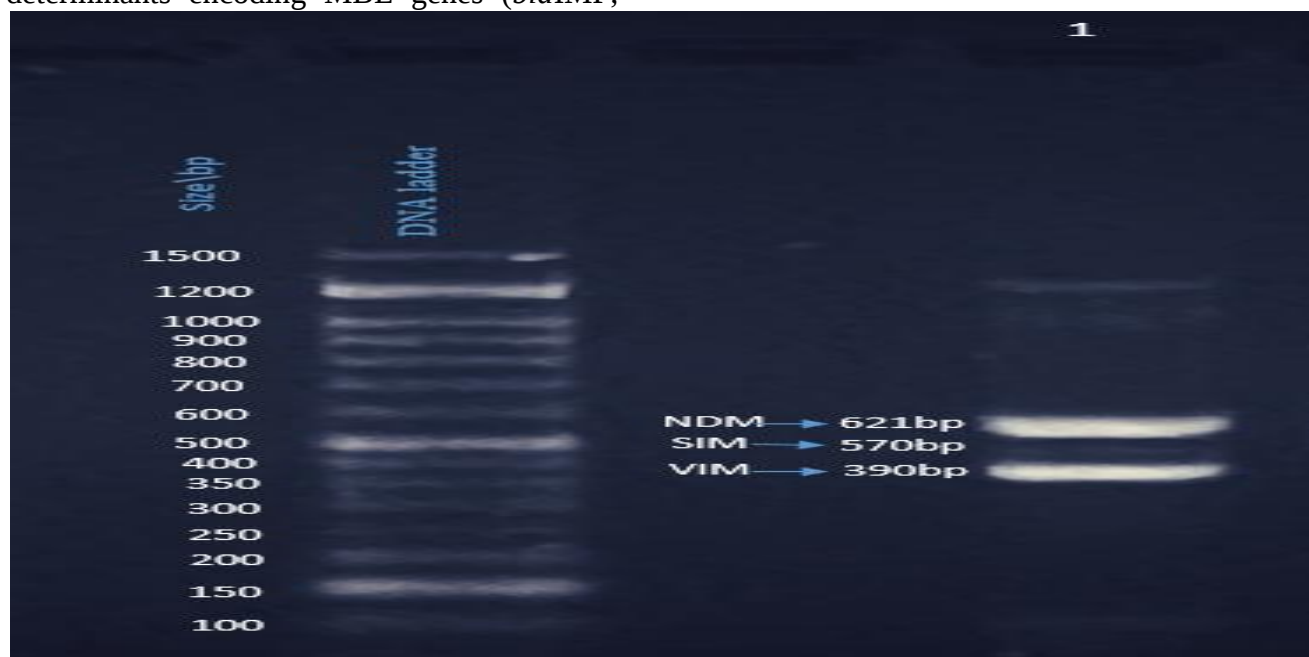


Figure 1: Agarose gel electrophoresis of image that show multiplex PCR amplified product from extract DNA of *C.braakii* isolate with *blaNDM*,*blaVIM*,*blaIMP*,*blaSIM*,*blaSPM* genes primers

Lane (1) show positive results with *blaNDM* gene (621 bp),*blaSIM* gene (570 bp),and *blaVIM* gene (390 bp). DNA ladder with size\bp (100-1500bp).

DISCUSSION:

Diabetic foot (DF) is refers to the alterations and abnormalities that occur either separately or together in the feet and legs of diabetic patients(Braun and Silva, 2021).These reasons are less likely to penetrate antimicrobial therapies in DFI, making it more challenging to deliver effective antimicrobial activity to the target location and accelerating the emergence of bacterial resistance(Li *et al.*, 2022). In the current study the prevalence of *Citrobacter* spp was 3 (4.34%) among isolates from DFI, similar results have been found previous studies done in Malaysia and Turkey with frequency 2.1% and 2.8% respectively (Kow *et al.*, 2022;Sen and Demirdal, 2020). The other findings the prevalence of *Citrobacter* spp was

Citrobacter braakii was MDR , where was sensitive to only amikacin, tetracycline, and nitrofurantoin, this results similar to study performed by Pepperell *et al.*, (2002) Who found during that study, the biggest group of multi-resistant *Enterobacteriales* discovered belonged to the genus *Citrobacter*. Most efficacy antimicrobial agents against *Citrobacter* spp were imipenem and amikacin (Sami *et al.*, 2017). While the other isolates was PDR, PDR to *Citrobacter* spp considered first reported in these isolates in Iraq among patient with DFI. The high death rate associated with *Citrobacter* infections may attributed to ineffective empirical drug treatment. *Citrobacter* spp are majority

resistant to Extended Spectrum Cephalosporins (Pepperell *et al.*, 2002).

In the present study the prevalence of MBL genes (*blaVIM*, *blaNDM*, and *blaSIM*) among *C. braakii* isolate was 100%, this results correspond to study conducted in China revealed the appearance of NDM -producing *C. braakii* among specimens of urinary tract infection (Han *et al.*, 2022). While first isolation for NDM -producing *C. braakii* in Iraq among specimens diabetic foot infection. Among genus *citrobacter* , the most commonly species that harbored the MBL genes was *Citrobacter freundii* like the study in Germany done by Yao *et al.*, (2021) who reported the *C. freundii* harbored the MBL genes were *blaVIM* (n=8; 17%) and *blaNDM* (n=3; 7%).

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Conclusion:

This study show the emergence of MDR and PDR among *Citrobacter* species and molecular detection revealed that the presence only one *C. braakii* isolate carry the MBL genes (*blaVIM*, *blaNDM*, and *blaSIM*) and NDM producing *C. braakii* considered first reported for those isolate in Iraq.

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