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).  

**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 (Duceppe *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 μg), piperacillin(100 μg), piperacillin-tazobactam(110 μg), amoxicillin-clavulanate acid(30 μg), ceftazidime(30 μg), cefotaxime(30 μg), ceftriaxone(30 μg), Aztreonam(30 μg), cefixime(5 μg), cefepime(30 μg), meropenem(10 μg), imipenem(10 μg), gentamicin(30 μg), tobramycin(10 μg), ciprofloxacin(5 μg), levofloxacin(30 μg), norfloxacin(30 μg), chloramphenicol(30 μg), nitrofurantoin(300 μg), Trimethoprim-Sulphamethazole(25 μg), tetracycline(30 μg), colistin(25 μg). 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 base pairs of sample equal to the target product size. The PCR were prepared in total volume 50 μl PCR mixture including 25 μl Promega Master mix, 1.5 μl forward primer (10 μM), 1.5 μl reverse primer (10 μM), 2 μl DNA template (10-250 ng), and 8 μl 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](image)

Lane (1) show positive results with blaNDM gene (621 bp), blasSIM 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
resist 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%).

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.

REFERENCES


