# THE PREVALENCE OF BLANDM, BLAVIM GENES AMONG *ENTEROBACTER CLOACAE* BACTERIA

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**ABSTRACT**

**This study was aimed to detect the prevalence of *blaVIM and blaNDM* genes among *Enterobacter cloacae* isolates that were isolated from Iraqi patients. About 50 bacterial isolates were collected from different hospitals in Baghdad city and all these isolates were diagnosed using biochemical tests and CHROMagar culture media and conformed using the Vitek II system. The antibiotic susceptibility for *E.cloacae* isolates was determined using the disk diffusion (Kirby Bauer) method and the results showed that these bacteria were showed resistance to Cefepime and Meropenem antibiotics in percentage (50%), (40%) respectively. In addition it is resistant to Amikacin, ampicillin/sulbactam, Piperacillin, and aztreonam was (20%), (50%), (40%), and (55%) respectively. Also it has been noted that all isolates showed resistance to Cefixime in percentage (100%). EDTA combined disc test was used to detect the ability of *E. cloacae* isolates to produce carbapenemase and the results were showed that only 8 isolates were gave positive results , While the prevalence of *blaVIM and blaNDM* was determined using the conventional Polymerase chain reaction technique. And the result showed that only four isolates harbored blaNDM while blaVIM was present in 3 isolates**

**Keywords: Enterobacteriace, carbapenem resistance, metallo β lactmase, EDTA combined disc test**

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## انتشار جينات blaNDM و blaVIM بين بكتريا *Enterobacter cloacae*

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**المستخلص**

**هدفت هذه الدراسة الى تحديد انتشار جينات *blaVIM وbla NDM* بين عزلات بكتريا *Enterobacter cloacae* والتي عزلت من المرضى العراقيين .مايقارب خمسين عزلة بكتيرية تم جمعها من مختلف مستشفيات مدينة بغداد وتم تشخيص كل العزلات باستعمال الاختبارات الكيموحيوية ووسط CHROMagar ومن ثم تم تاكيد التشخيص باستعمال نظام Vitek II. تم تحديد الحساسية البكتيرية لعزلات *E.cloacae* باستعمال طريقه انتشار القرص (Kirby Bauer) واظهرت النتائج بان البكتريا اظهرت مقاومة لمضادات ,Cefepime Meropenem بنسبة مؤية (50%)و(40%) على توالي . بالاضافة ,كانت البكتريا مقاومة ل Amikacin, ampicillin/sulbactam, Piperacillin, and aztreonam ب (20%), (50%), (40%), (55%) بالتعاقب .كذلك,لوحظ بان كل العزلات مقاومة ل Cefixime بنسبة مؤية ( 100%). لقد استخدم اختبار EDTA combined disc لتحديد قدرة عزلات E. cloacae على انتاج انزيم carbapenemase واظهرت النتائج بان فقط 8 عزلات اظهرت نتائج ايجابية, بينما انتشار جينات *blaVIM* و *blaNDM* قد حددت باستعمال تقنية تفاعل البلمرة المتسلسل التقليدي .واظهرت النتائج بان فقط اربع عزلات تحمل *blaNDM* بينما ظهر *blaVIM* في ثلاث عزلات فقط.**

**كلمات مفتاحية: العائلة المعوية ,مقاومة الكاربنيم ,انزيمات البيتالاكتام المعدنية ,اختبار EDTA combined disc**

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## INTRODUCTION

Enterobacter is a rod-shaped, gram-negative bacteria belonging to the genus Enterobacteriaceae, which is facultative anaerobic and is not capable of spore production (6). The most clinically important species of this genus is Enterobacter cloacae, which is a bacterial strain usually present in the gut microbiota of healthy humans (12). As well as these bacteria is also recognized as the most common pathogen in nosocomial infections (13). This pathogen capable of producing a wide range of infections, such as urinary tract infections, pneumonia, and septicemia (1). This pathogen has recently emerged as a drug-resistant bacterial species; in both human and veterinary medicine, the production of antimicrobial resistance across Enterobacter spp., including resistance against -spectrum cephalosporins, is of serious importance (9). There is a rising understanding that E.cloacae behind *Escherichia coli and Klebsiella pneumonia* is also the third main genus of Enterobacteriaceae implicated in nosocomial infections. Carbapenems are commonly used to treat infections due to multidrug-resistant (MDR) Gram-negative rods containing cephalosporins or ESBL, such as Enterobacter species. However, carbapenemase-producing bacteria have prevented the use of carbapenems in medical practice (18, 11). CRE emergence, particularly with Enterobacter spp., is linked to the development of carbapenemases. A wide variety of carbapenemases was found in these bacteria which include ambler class A blaKPC, Metallo-β-lactamases (MBL) class B such as *blaVIM, blaIMP, blaNDM*, etc. (3, 20). CRE-caused nosocomial infections are serious challenges worldwide, and this is due to the quick spread of these infections across the globe. The mortality rate of CRE-induced infections is high, varying from 30-44 percent (3, 17). There are only a few studies regarding the abundance of carbapenem- resistant *Enterobacter cloacae* that were discovered in Baghdad This study was aimed to detect the prevalence of *blaVIM and blaNDM* genes among *Enterobacter cloacae* isolates that were isolated from Iraqi patients Hospital, Iraq, this research was performed.

## MATERIALS AND METHODS

**Collection and identification of the specimen:** About fifty bacterial specimens were collected from patients admitted to Baghdad hospitals as well as these bacteria were collected from wounds, UTI, and burned skin. All these 50 bacterial specimens were identified using biochemical tests and CHROMagar culture media, as well as all of them, were confirmed using ViteK II system.

**Antibiotic susceptibility**

The antibiotic susceptibility for *E.cloacae* was determined using Kirby Bauer method. This method was used to determine the susceptibility of E.cloacae to Meropenem, Cefepime, ampicillin/ sulbactam, Amikacin, Piperacillin, aztreonam, and Cefixime, and the result was interpreted according to the guideline of CLSI (2020).

**EDTA combined disc test**

This test was performed depends on the way provided by Galani *et al* (7). In which bacterial specimen was transferred from overnight growth of *E.cloacae* in brain heart infusion agar to 5 ml of normal saline and the inoculum was adjusted to 0.5 McFarland then the bacterial specimen was transferred from inoculum to the Muller Hinton agar. After the spreading of bacteria on the agar, Imipenem and Imipenem+ EDTA were placed on the agar surface and incubated for 18 hr at 37 C, and the results were interpreted according to the guideline of CLSI (2020).

**Molecular detection of carbapenemase**

DNA was extracted from E.cloacae isolates using WizPrep gDNA Mini Kit (Korea). The prevalence of carbapenem resistance genes (bla NDM and bla Vim) was detected using Conventional PCR technique. The primer used for detection of these genes was designed using (Primer 3 program https://primer3.ut.ee/) and was provided by alpha DNA company (Canada) and the sequence of the primer was illustrated in the table (1). The detection of these genes was performed by amplifying these genes in Conventional PCR technique with final concentration volume 25µl which was prepared by mixing 12.5µl of master mix with 2µl of primers, 3µl of DNA and the volume was completed to 25µl by the addition 7.5µ of nuclease free water. The mixture was place in the thermo-cycler (Bioneer Korea),

and the steps of amplification was as following: one cycle at 95°C for 5mins ,then 35 cycles as 30s at 95 °C ,annealing step at 58 °C for VIM and 52 for NDM for 30 S and extension for 10 mins at 72 °C and final extension at 72°C for 10 mins.

**Table 1. Sequence of the Primers (Vim and Imp)**

| *GENE* | | SEQUNCE 5 → 3 | Reference |
|---|---|---|---|
| *Vim* | F | GATGGTGTTTGGTCGCATA | 44 |
| | R | CGAATGCGCAGCACCAG | |
| *NDM* | F | ATGGAATTGCCCAATATTATGC | 45 |
| | R | CGAAAGTCAGGCTGTGTTG | |

## RESULTS AND DISCUSSION

From 50 bacterial specimen that are collected from different clinical source of Baghdad hospitals (UTI, wounds and burned skin) only 20 bacterial specimen was conformed using biochemical test and CHROMagar and the result of identification was reconfirmed using Vitek II system while the remaining of collected specimens were related to another pathogenic bacteria.

**Antibiotics susceptibility**

The antibiotic susceptibility test showed 10 isolates of *E.cloacae* were resistant to Cefepime (50%) and 8 isolates were resistant to Meropenem (40%) as well as only 4 isolates were resistant to Amikacin (20%).While another 10 isolates were resistant to ampicillin/sulbactam (50%), in addition 11 isolates were found to be responsible for providing resistance to aztreonam (55%), while resistance to Piperacillin was provided by only 8 isolates (40%) of *E.cloacae* and finally, all isolates were responsible for providing resistance to Cefixime (100%). The percentages of antibiotic susceptibility were showed in Figure (1).

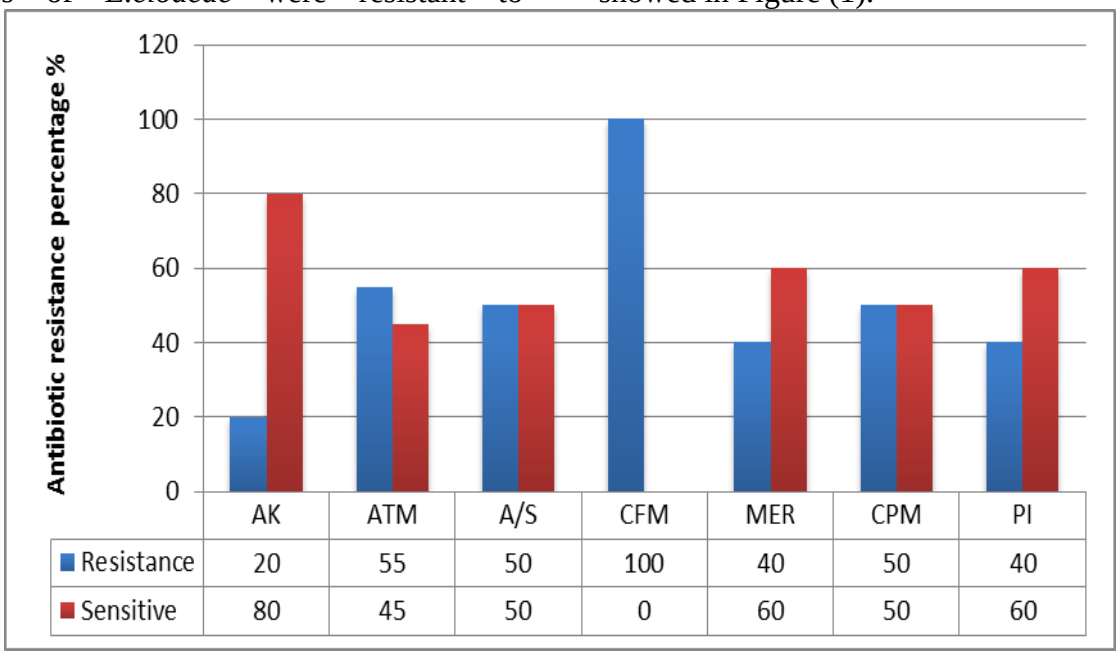


**Figure 1. The antibiotic susceptibility test of *E.cloacae*. In which AK (Amikacin), ATM (Aztreonam), A/S (Ampicillin/sulbactam), CFM (Cefixime), MER (Meropenem), CPM (Cefepime), and PI (Piperacillin)**

**Result of EDTA combined disc test**

This test was conducted only on 8 isolates because these isolates were resistant to all tested antibiotics and the result shows these 8 isolates were resistant to Imipenem and become sensitive after the addition of Imipenem + EDTA as shown in Figure (2), Which indicate these bacteria produce carbapenem-resistant genes.

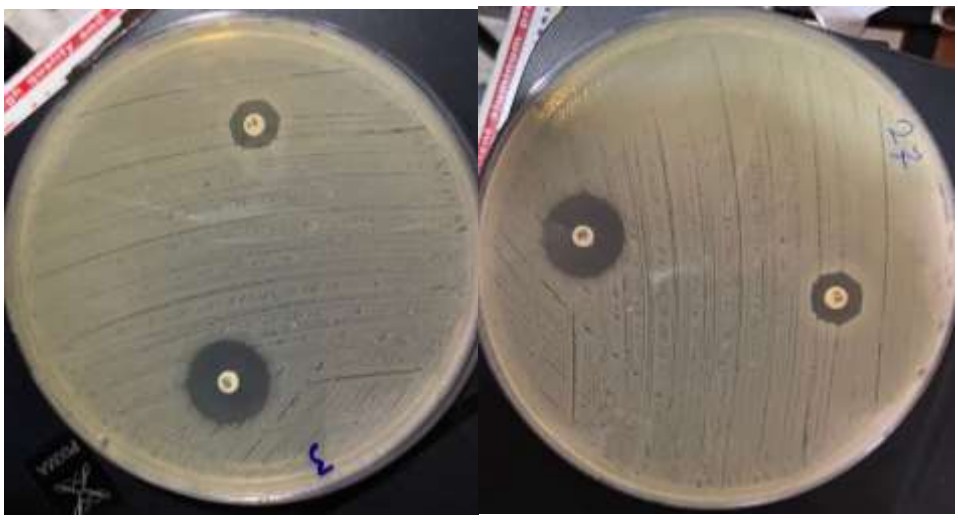

**Figure 2. EDTA combined disc test**

**Molecular detection of Carbapenem resistance genes:** This test was also conducted only on 8 isolates of *E. cloacae* and the result show out of 8 isolates only 4 isolates was carried *blaNDM* with molecular size of 490 bp while *blaVIM* was found only on three isolates with molecular size 390 bp as shown the following Figures (3A) and (3B).

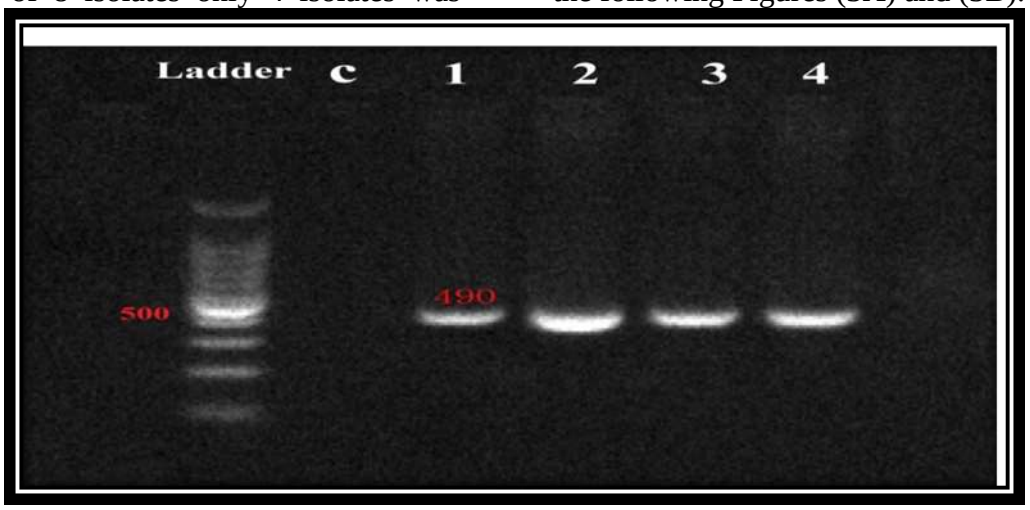


**Figure (3a) Detection the prevalence of *blaNDM gene in E.cloacae* using gel electrophoresis in which agarose concentration was 2% and DNA ladder from 100bp to 1500 bp**

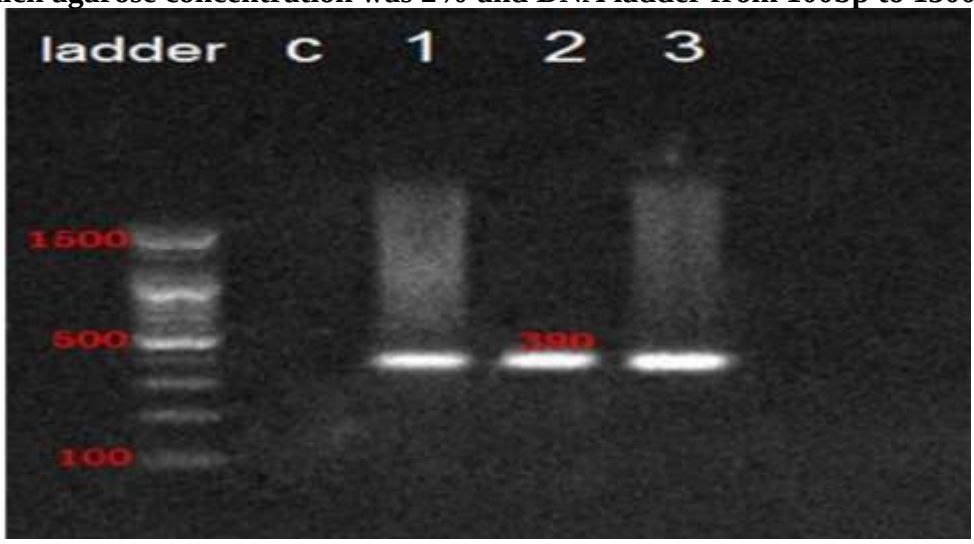


**Figure (3b) Detection the prevalence of *blaVIM* gene in *E. cloacae* using gel electrophoresis in which agarose concentration was 2% and DNA ladder from 100bp to 1500 bp**

There are few therapeutic possibilities with a rise in the production of MDR carbapenemases developing Enterobacter cloaca. Therefore, early detection is important to reduce the mortality of nosocomial infections caused by these species. (10, 18). *E. cloacae* are responsible for a wide range of nosocomial infections, especially wound infections, pneumonia, and bacteremia (15, 5). In the current study, most isolates (100%) were recovered from different clinical sources. In Consistent with our work, different researchers were documented isolation of these bacteria from different clinical sources included respiratory tract samples (17), skin (9). In contrast, in several studies from different countries like Brazil (18), China (22), a global surveillance program (14), and Korea (11). Furthermore, the most common sites for Enterobacter isolation were abdominal and blood samples. According to the results of susceptibility testing, 60% of our isolates showed MDR phenotype, this makes them a threat to public health in our area. This observation is not consistent with two research previously performed in Iran with a prevalence of 17.5% and 47.5% respectively (8, 16). Carbapenem resistance was characterized as resistance to one or more carbapenems in compliance with the CLSI guidelines (18). In the current analysis, 40%of isolates were not susceptible to Imipenem (carbapenem-resistant) and 8.7%, 29.2%, 25.7%, 35.1%, respectively, were recorded in many other reports from different regions (11, 5, 8, 2, 21).
In this study, the pattern of antimicrobial resistance and the existence of two carbapenemase genes were characterized among 8 *E. cloaca* clinical isolates. Which were Imipenem resistance and EDTA combined test positive that were recovered from an Iraqi population. However, Carbapenemase-producing *E. cloacae* has been reported in many countries and in current study, among evaluated carbapenemase genes only *bla*NDM (n=4/8, 50%) and *bla*vim (n=3/8, 37%) were detected. This study is the first reported presence of *bla*NDM among clinical isolates of *Enterobacter cloaca* in Iraq. While , studies published from other countries as Iran, China (5), Spain (4), Korea (11), and Mexico (3) with frequencies of 2.8%, 0%, 0%, and 100%, respectively. Indeed, an E. cloaca, the species which produces VIM-1, has been frequently recorded in Europe and has been a major nosocomial pathogen in southern Europe and Asia (21, 4). In this study , 3/8 of the isolates examined harbored the blaVIM-gene. An analysis in Spain showed that 52% and 100% of the E. cloacae isolates were found to be blaVIM producers. On the other hand, in Far Eastern studies, the rate of blaVIM (0.25%) was noted too close to our results. In addition, in an Iranian study, no carbapenemase gene was present in Enterobacter spp isolates Combined, the varying distributions between regions and strains in genetic diversity are more definitely attributable to the differences in results. Since this study not evaluate the existence of other carbapenemase genes from different groups of β-lactamases, we had to set the study's limitations to one being the comparatively limited sample size and the other being the other limitation of the work which is that to properly determine beta-lactam tolerance in our isolates, we could not evaluate the presence of different carbapenemase genes from different classes of β-lactamases.

**Conclusion*:*** In this study, we found for the first time the prevalence of *blaNDM* in *E. cloaca* from an Iraqi patients. Due to the rise in carbapenem-producing MDR *Enterobacter cloacae* in our country, hygiene regulations for hospitals and other healthcare facilities in developed countries will be required to monitor the fast spread of MDR Enterobacter isolates.

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