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Enterobacter Cloacae: The association of antibiotic resistance, integron class I and carbapenemase genes

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Abstract

The opportunistic pathogen, E. *Cloacae* has been reported to carry carbapenemas genes worldwide. Our objective was assessing the association of antibiotic resistance, integron class I and carbapenemase genes among E. *Cloacae*. Herein, 200 E. *Cloacae* were collected and identified. The antibiotic resistance of them was evaluated using Kirby Bauer method. The existence of class I integron, carbapenemase genes was investigated using polymerase chain reaction (PCR). Of the 200 E. *Cloacae* isolates collected, 120 isolates (60%) were from male and 80 isolates (40%) were from females. Of them, 110 isolates (55%) showed a pattern of MDR phenotype. Of these, 18 isolates (9%) showed resistance to imipenem. Based on PCR test, 134 isolates (67%) had class I integrons. Also, out of 110 MDR isolates, 52 isolates (72%) were positive in terms of the presence of class I integrons. Isolates with integrons were mostly from urinary (61%) and blood (44%) and from ICU settings (46%) and inpatients (38%). A significant relationship was observed between the presence of integron and resistance to ciprofloxacin, imipenem, meropenem, and norfloxacin antibiotics. The prevalence of blaIMP, blaOXA-48 were 18% and 4%, respectively, but none of other carbapenemase genes were detected. The existence of class I integron was high among E. *Cloacae* from Baghdad city. The carriage of genes resistance to carbapenems were significantly associated to the class I integrin.

Keywords: *Enterobacter Cloacae*; Antibiotic resistance; Integron class I; Carbapenemase genes

1. Introduction

Enterobacter species from members of the *Enterobacteriaceae* family involved in the development of various clinical infections, especially in hospital settings, and are currently one of the major causative agents of nosocomial infections. *Enterobacter Cloacae* (*E. Cloacae*) are involved in the development of diseases such as pneumonia and skin, lower respiratory tract, soft tissue, blood and urinary tract infections [1-3]. In recent years, the emergence and spread of highly drug-resistant strains of these species are of great concern.

There have been many reports of the presence of *Enterobacter* species with multiple drug resistance (MDR) patterns in the clinical wards of hospitals around the world [4-6]. This bacterium is resistant to antimicrobial drugs through a variety of mechanisms, including changes in the cell permeability to drugs, the efflux pump, the receptor modification for drugs, and the drug destroying enzymes [7,8]. The genes encoding these enzymes are either chromosomal in origin or released by other transferable genetic elements (TGEs) such as plasmids, transposons, and integrons. Integrons are elements that can be located in plasmids, chromosomes, or transposons [9,10]. These elements are among the factors involved in the development of MDR and, like plasmids and transposons, are part of the TGEs in the acquisition and dissemination of resistance factors [11]. In total, integrons absorb gene cassettes that contain one or more genes (often drug resistance genes) with a conserved locus.

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More than 60 different gene cassettes have been identified in the integration group that make them resistant to important antibiotics such as aminoglycosides, penicillins, cephalosporins, carbapenemas, trimethoprim, rifampine, erythromycin, chloramphenicol and quaternary ammonium compounds [3,4,6].
Some studies have reported integrons containing more than one gene cassette that facilitate bacterial isolates to develop to MDR phenotype. The class I integration is one of the most important in the transfer of drug resistance genes. Due to the rapid spread of these genes among other species, identifying the presence of these integrons can provide advantageous information about the prevalence of resistant strains of *Enterobacter* spp, and how to develop it [7-10]. In the last two decades, *Enterobacter* species with a pattern of MDR have increased significantly in hospital settings and have caused many problems for physicians and infection control specialists. The aim of this study was to determine the relationship between the presence of class I integrons and different drug resistance patterns and carbapenemase genes in *E. Cloacae*.

2. Material and methods

In this analytical study, a total of 200 clinical isolates of *E. Cloacae* were collected from different wards of Baghdad hospitals from May 2018 to October 2020. Bacterial isolates were from various clinical specimens including blood, urine and urinary catheters, trachea, wound, sputum, bronchoalveolar lavage and cerebrospinal fluid and from patients admitted to intensive care units, intrauterine and orthopedic wards. The collected samples were cultured on blood agar medium (Merk, Germany) and eosin methylene blue and sent to the laboratory for identification. Then, using standard laboratory tests (microbiology and biochemistry) related to the identification of *Enterobacter* species were identified as follows: Gram staining, oxidase tests, production of indole, urea hydrolysis, motility, Citrate (culture on Simon Citrate medium), culture on Kligler Iron Agar media (KIA, (11) Methyl Red and Voges Prosquare (VP-MR) tests. Moreover the *hsp60* species specific gene was amplified using Forward: 5'- AGAAGAGAGGCGTGGTTGC-3' and Reverese: 5'-ATGCATTCGGTGGTGATCATCAG-3' primers. Bacteria isolated after diagnosis on the surface of the test. % Glycerol was stored at -80 ° C until further testing.

2.1. Antibiotic susceptibility

Antibiotic susceptibility patterns of all isolates was performed according to CLSI 2020 and use the following discs: gentamicin, tobramycin, ticarcillin- clavulanic acid, carbenicillin, ofloxacin, cefotaxime, ceftazidime, imipenem, meropenem, aztreonam, levofloxacin, trimethoprim, ciprofloxacin and piperacillin-tazobactam. For this purpose, first the Mueller Hinton Agar medium (pH 7.2 to 7.4, Merk, Germany) was used. The standard microbial suspension was then prepared on a half-McFarlane turbidity. They were incubated for 24 hours at 35 °C and then the results were recorded according to the instructions. Antibiotic discs were purchased from MAST UK. Escherichia coli strain ATCC 25922 was used to control the test.

2.2. Polymerase chain reaction

To determine the prevalence of class I integron, a polymerase chain reaction (PCR) test was used. In this method, a primer for integron class I gene, IntF, 5′-ATCATCGTCGTAGAGACGTCGG IntR, 5′-GTCGACGT, 5′-GTCGACGT was used. Finally, electrophoresis of the products on 1% agarose gel was determined. The DNA extraction of all samples was performed using boiling method. To ensure the existence of DNA, the nanodrop device was used at two wavelengths of 260 and 280 nm.The PCR reaction was performed in a total volume of 25 µl and consisted of the following materials: 200 µmol dNTP, 10 picomoles per 1 ml of primer, MgCl2, 0.5 U of Taq polymerase enzyme and 50 ng of template DNA.

Amplification of the class I integron gene was performed under the following conditions using a thermocycling device (biosystems, US). Primary denaturation temperature (94 ° C for for 5 minutes), 35 cycles of 94 ° C for 3min, the annealing temperature (55 ° C for 30 sec) and the amplification temperature (72 ° C for 1 min) and at the end the final amplification temperature (72 ° C for 10 min).

The existence of *blaIMP, blaOXA-48*, *blaVIM, blaNDM* and *blaKPC* genes was investigated by PCR (table1).

Table 1 Sequence of primers of carbapenemase genes

Primers	Sequence 5'----3'	Annealing Temp.	Amplicon size (bp)	Reference
bla_{KPC-2}	F: TTGCCGGTCGTGTTTCCCTTTAGC R: GGCCGCCGTGCAATACAGTGATA	64	282	[12,13]
bla_{VIM}	F: CATTGTCCGTGATGGTGATGAGT R: GCGTGTCGACGGTGATGC	61	205	
bla_{OXA-48}	F: CGCCCGCGTCGACGTTCAAGAT R: TCGGCCAGCAGCGGATAGGACAC	65	484	
bla_{NDM1}	F: CGCACCTCATGTTTGAATTCGCC R: GTCGCAAAGCCCAGCTTCGC	61	1015	
blaIMP$_1$	F: GGGTGGGGCGTTGTTCCTA R: TCTATTCCGCCCGTGCTGTC	62	198	

Data were analyzed using SPSS 20 software and Chi-square and Fisher's exact tests. In the analysis of the results, groups with moderate and complete resistance were integrated and $P < 0.05$ was considered statistically significant.

3. Results

Of the 200 *E. Cloacae* isolates collected, 120 isolates (60%) were from male and 80 isolates (40%) were from females. The mean age of patients was 51±5 years (age range 14 to 83 years). Most isolates were collected from urine (82, 41%) and blood (50, 25%) and from patients admitted to ICU settings (88 patients, 44%) and internal ward (66 patients, 33%).

Table 2 The antibiotic resistance pattern of *E. Cloacae* isolates

Disk/Resistance (N=200)	Susceptibility %	Intermediate %	Resistance %
CAZ	34	0.0	66
CTX	36	0.0	64
CN	31	5	61
AMC	37	8	55
IPM	52	8	40
MEM	53	9	38
PITZ	68	2	30
TI-CLA	63	0.0	37
OFX	46	3	51
GN	58	4	38
AZ	26	4	70
TOB	44	0.0	56
CP	29	2	69
TMP	47	3	50
LFX	31	4	65

Based on the results of antibiotic susceptibility test, 110 isolates (55%) showed a pattern of MDR (in the classes of beta-lactam, aminoglycoside and quinolone antibiotic drugs) as shown in table 2. Of these, 18 isolates (9%) showed resistance to imipenem, 14 isolates (7%) showed moderate resistance and 168 isolates (84%) were susceptible. It was also found that among MDR-*E. Cloacae*, 110 isolates (55%) were sensitive to meropenem and 6 isolates (3%) showed intermediate resistance.

Based on PCR test, 134 isolates (67%) had class I integrons. Also, out of 110 MDR isolates, 52 isolates (72%) were positive in terms of the presence of class I integrons. Isolates with integrons were mostly from urinary (61%) and blood (44%) and from ICU settings (46%) and inpatients (38%). No significant correlation between integrons class I and MDR pattern and between class I integrons and resistance to antibiotics gentamicin, Amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime, aztreonam, piperacillin, trimethoprim was observed. But a significant relationship was observed between the presence of integron and resistance to ciprofloxacin, imipenem, meropenem, and norfloxacin antibiotics.

The prevalence of bla_{IMP}, bla_{OXA-48} were 18% and 4%, respectively, but none of other carbapenemase genes were detected. The relation of class I integron and carbapenemase genes has been shown in table 3 and figure 1.

Table 3 The relation of class I integron and carbapenemase genes

Integron	bla_{IMP} (n=38)	bla_{OXA48} (n=8)	Resistance profile
Class I (n=134)	34%	6%	CP, IMP, MER, NOR

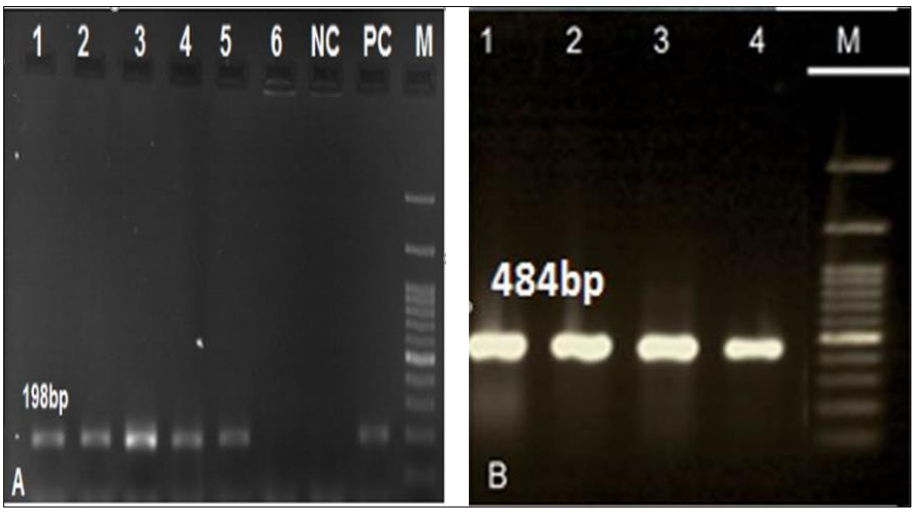

Figure 1 A blaIMP1 with 198bp size, B: blaOXA-48-like with 484bp size, M: 100bp DNA marker, PC: positive control, NC: negative control

4. Discussion

The opportunistic pathogen, *E. Cloacae* has been reported to carry carbapenemas genes worldwide [14, 15]. This study showed that 134 *E. Cloacae* isolates (67%) had class I integron. In general, there is a lot of information about the prevalence of different classes of integrons in a species of *Klebsiella* and *E. coli*, but little is known about the species of *Enterobacter*. Studies on the prevalence of different classes of integrons and their content have different results in terms of the existence of different factors of drug resistance; In different geographical areas, the prevalence of class I integrons in gram-negative pathogens has been reported from 28.5% (8% to 89.2%). They reported that 34% of the *Enterobacteriaceae* family members were positive in terms of the presence of class I integrons [16, 17]. In a study by Ibrahim *et al*., in Malaysia on *Enterobacteriaceae* isolates, it was found that 61.4% of the isolates had class I integrons. In China, 76.3% of isolates were positive in terms of the presence of class I integrons [18]. Antibiotics are another factor involved in different statistics on the prevalence of class I integrons in different geographical areas. In this study, most isolates containing class I integron (46%) were isolated from the ICU. The main reasons for the prevalence of resistant organisms in this ward can be the following: long-term hospitalization of patients in this ward, the severity of the

patient's illness and the use of invasive therapeutic tools such as chips and catheters. In the present study, there was a significant relationship between resistance to carbapenems and the presence of class I integrons. Moreover, the existence of *blaIMP* gene was significantly higher among isolates containing class I integron. In this study, for the first time in our area, the class I integron and carbapenemases were detected in *E. Cloacae.* The limitations of this study included lack of gene expression and narraw resistance genes assessed.

5. Conclusion

The existence of class I integron was high among *E. Cloacae* from Baghdad city. The carriage of genes resistance to carbapenems were significantly associated to the class I integrin.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interests.

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