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ORIGINAL ARTICLE

Molecular characterization of intestinal carriage of carbapenem-resistant Enterobacteriaceae among inpatients at two Iranian university hospitals: first report of co-production of bla_{NDM-7} and bla_{OXA-48}

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Abstract Gastrointestinal colonization of carbapenem-resistant Enterobacteriaceae (CRE) could serve as a reservoir for the transmission of these pathogens in the clinical setting. The aim of this study was to investigate the intestinal carriage of CRE and to analyze risk factors for CRE carriage. Rectal swabs were collected from 95 patients at two Iranian university hospitals. CRE screening was performed using selective media (CHROMagar and MacConkey agar). Polymerase chain reaction (PCR) was used to detect carbapenemase-encoding genes. Clonal relatedness was investigated by pulsed-field gel electrophoresis (PFGE). The rate of carriage of CRE in hospitalized patients was 37.9%. Overall, 54 CRE isolates were identified, of which 47 were carbapenemase-producers. All of the 54 CRE were detected using CHROMagar compared with 52 CRE detected using MacConkey agar. Fifteen patients were colonized by multiple CRE isolates. Three significant risk factors for CRE carriage were detected: intensive care unit (ICU) hospitalization, antibiotic exposure, and mechanical ventilation. bla_{OXA-48} was the most frequent carbapenemase detected, followed by bla_{NDM-1} and bla_{NDM-7}. Eleven carbapenemase-producing Enterobacteriaceae (CPE) isolates co-harbored bla_{NDM-1} and bla_{OXA-48}. Also, six CPE isolates co-harbored bla_{NDM-7} and bla_{OXA-48}. We did not detect bla_{KPC}, bla_{GES}, bla_{IMP}, or bla_{VIM}. PFGE analysis showed that *Escherichia coli* clones were diverse, while *Klebsiella pneumoniae* isolates were divided into four clusters. Cluster I was the major clone carrying bla_{OXA-48} and $bla_{CTX-M-15}$ genes. In our study, the carriage rate of CRE was high and the emergence of CPE isolates among patients is alarming. The implementation of adequate preventive measures such as active surveillance is urgently needed to control the spread of CPE in the healthcare setting.

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Introduction

Enterobacteriaceae are common human pathogens that are associated with both nosocomial and community-acquired infections [1]. Carbapenems comprise the most effective treatment of choice for severe infections due to extended-spectrum beta-lactamase (ESBL) producers [2]. Carbapenem-resistant Enterobacteriaceae (CRE) have been increasingly reported worldwide. The emergence of CRE is a serious threat to public health because they are resistant to almost all beta-lactam antibiotics [3]. Resistance to carbapenems in CRE is mainly due to the production of carbapenemases [1, 2].

Intestinal colonization of CRE and their dissemination from the intestinal tract may facilitate the transmission of these pathogens among patients in healthcare settings. The rate of carriage of CRE varies in different parts of the world. The most important reservoir of NDM-1-producing bacteria is

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the Indian subcontinent, whereas it seems that a secondary reservoir of these bacteria has been established in the Balkans regions and the Middle East [4, 5]. In recent years, the emergence of OXA-48 producers has been reported in many countries. The Middle East, Turkey, and North Africa can be considered as the main reservoirs of OXA-48 producers; however, the occurrence of OXA-48 producers in European countries has been reported in recent years [6–9].

Recent studies in Iran have focused on the detection of CRE in clinical samples [10]; thus, no data are available about the fecal carriage of CRE. As intestinal colonization of CRE among inpatients is considered one of the most important reservoirs of hospital-acquired infections, the detection of fecal carriers of carbapenemase-producing Enterobacteriaceae (CPE) is becoming an important issue, and it is an important health problem [11].

The aim of the present study was to investigate the intestinal carriage of CRE and comparisons of risk factors among CRE carriers and non-CRE carriers at two university hospitals in Iran. Herein, we report, for the first time in the Iran, isolates of *Klebsiella pneumoniae* and *Escherichia coli* co-harboring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$.

Methods

Patient samples and data collection

This hospital-based cross-sectional study was conducted from July to November 2015 in two university hospitals in Iran. Rectal swab specimens were randomly collected from 95 inpatients, as previously informed consents were obtained from all participants. The swab was inserted 2 to 3 cm into the rectum, rotated several times, and rectal swab specimens were inoculated immediately in trypticase soy broth (5 mL) containing a 10-μg ertapenem (ETP) disk (Mast Group Ltd., Merseyside, UK) [12]. For each patient, the following data were recorded: age, sex, unit of hospitalization, invasive medical device utilization, history of surgery, presence of wounds, transfer from another hospital, transfer between hospital units, and exposure to antibiotics. Comparison of risk factors was considered between CRE carriers and non-CRE carriers.

Identification of CRE colonies

We used two different phenotypic methods for the detection of CRE in rectal swab specimens. Method 1: following an overnight incubation at 37 °C, all tubes were vortexed and 100 μL of suspension was subcultured onto MacConkey agar (Difco, Detroit, MI) and then a 10-μg ETP disk was placed on the plate [12]. Likely, CRE colonies were considered those with an inhibition zone of ≤27 mm around the ETP disk on MacConkey agar plates [13]. Method 2: all specimens subcultured directly onto CHROMagar KPC medium (CHROMagar Company, Paris, France). CRE colonies on CHROMagar KPC plates were identified according to the manufacturer's instructions [14, 15]. Isolates were confirmed by using standard biochemical tests and API 20E (bioMérieux, Marcy-l'Étoile, France).

Susceptibility testing and MIC determination

The susceptibility of isolates was determined by the disk diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. The following antibiotics were tested: imipenem (IMP: 10 μg), meropenem (MEM: 10 μg), ETP (10 μg), ceftazidime (CAZ: 30 μg), cefotaxime (CTX: 30 μg), cefepime (CPM: 30 μg), ciprofloxacin (CIP: 5 μg), amikacin (AK: 30 μg), gentamicin (K: 30 μg), aztreonam (ATM: 30 μg), and tigecycline (TGC: 15 μg) (Mast Group Ltd., Merseyside, United Kingdom). Minimal inhibitory concentrations (MICs) of IMP, MEM, ETP, and colistin were determined by gradient test strips (Liofilchem, Roseto degli Abruzzi, Italy). *Escherichia coli* ATCC 25922 was used as the control strain in susceptibility testing. All CRE isolates were screened for carbapenemase production by the modified Hodge test (MHT) according to the CLSI guidelines [16].

Detection of carbapenemase and CTX-M genes

Polymerase chain reaction (PCR) experiments were carried out using primers specific for the genes encoding bla_{KPC}, bla_{GES}, bla_{VIM} bla_{IMP}, bla_{NDM}, $bla_{\text{OXA-48}}$, and $bla_{\text{CTX-M}}$, as previously described [17–19]. Selected amplicons were sequenced using an ABI Capillary System (Macrogen Research, Seoul, Korea).

PFGE

Genomic DNA was prepared in agarose blocks and digested with the restriction enzyme *Xba*I. The DNA fragments were separated by use of the CHEF-DR III System (Bio-Rad Laboratories, Hercules, CA) at 6 V/cm for 20 h, 14 °C, 120 °C included angle, with the pulse time increasing from 5 to 30 s and 2.2 to 45 s for *E. coli* and *K. pneumoniae*, respectively. DNA fragments patterns were analyzed with GelCompar II software (Applied Maths, Kortrijk, Belgium). Dendrograms were constructed by the unweighted pair group method with arithmetic mean (UPGMA) and analyzed based on the criteria of Tenover et al. [20].

Statistical analysis

Statistical analyses were performed by Fisher's exact test with SPSS v21 software to compare variables of different groups. A *t*-test was used to compare the age of CRE carriers and non-

CRE carriers. p-Values <0.05 in all experiments were considered statistically significant.

Results

Prevalence of CRE in patients

Two university hospitals, located in different provinces of Iran, were involved in this study. In total, 95 rectal samples were collected from patients (50 patients from hospital A and 45 patients from hospital B). Overall, 36/95 (37.9%) patients were colonized with CRE isolates, and, in total, 54 CRE isolates were collected from rectal swabs of these patients. A higher proportion (28/36; 77.8%) of colonization with CRE isolates was identified among admitted patients in hospital A compared to hospital B (8 carriers).

This study showed that 21 (58.33%) out of 36 patients were colonized with one CRE isolate, whereas 12 (33.33%) and 3 (8.33%) patients were colonized with two and three different species, respectively (refer to Table 3).

Comparison of risk factors between CRE carriers and non-CRE carriers

There was a significant association between CRE carriers and antibiotics exposures, except in the case of ciprofloxacin and piperacillin/tazobactam therapy. Exposure to the antibiotics third-generation cephalosporins (p = 0.00001), colistin (p = 0.004), vancomycin (p = 0.03), and meropenem (p = 0.04) were associated with CRE colonization. The other significant risk factors found were as follows: hospitalization in intensive care unit (ICUs) [ICU-2 (p = 0.00004) and general ICU (p = 0.007)], mechanical ventilation (p = 0.0004), urinary catheter (p = 0.04), recent surgery (p = 0.03), patients transferred from another hospital (p = 0.004), transfer between hospital units (p = 0.008), and male patients (0.02) (Table 1).

Prevalence of CRE isolates

Of the 95 rectal swabs, 36 and 35 CRE-colonized patients were detected using CHROMagar KPC and MacConkey agar plus ETP disk, respectively. The prevalences of CRE isolates using the two methods are shown in Table 2.

Susceptibility testing and MIC determination

The rates of resistance of CRE to IMP, MEM, and ETP by disk diffusion were 88.9% (48/54), 94.4% (51/54), and 100% (54/54), respectively. Moreover, the resistance rates to cephalosporins were high. Fifty isolates (92.6%), 54 isolates (100%), and 53 isolates (98.1%) were resistant to ceftazidime, cefotaxime, and cefepime, respectively. The percentages of resistance to other antimicrobial agents were as follows: amikacin, 24%; gentamicin, 35%; aztreonam, 77.7%; and ciprofloxacin, 74%. All CRE isolates were susceptible to tigecycline and colistin. The MICs of carbapenem agents tested against CPE isolates are shown in Table 3.

Phenotypic and genotypic detection of carbapenemase production

The results of the phenotypic and genotypic carbapenemase detection tests are shown in Table 3. Seven out of 54 CRE isolates displayed a negative MHT result (12.9%), while 47 isolates were positive (85.1%).

Thirty isolates carried a single gene, including $bla_{\text{NDM-1}}$ in four *K. pneumoniae*, one *E. cloacae*, and one *E. coli* isolates, $bla_{\text{NDM-7}}$ in one *K. pneumoniae* isolate, $bla_{\text{OXA-48}}$ in 12 *K. pneumoniae*, ten *E. coli*, and one *Proteus mirabilis* isolates. The combination of $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ was found in eight *K. pneumoniae* and three *E. coli* isolates. Furthermore, the co-harboring of two genes, $bla_{\text{NDM-7}}$ and $bla_{\text{OXA-48}}$, was found in four *K. pneumoniae* and two *E. coli* isolates. We did not detect bla_{KPC}, bla_{GES}, bla_{IMP}, or bla_{VIM} among the CRE isolates. Thirty-four out of the 54 (63%) CRE isolates also produced $bla_{\text{CTX-M}}$. The nucleotide sequences of the $bla_{\text{NDM-1}}$, $bla_{\text{NDM-7}}$, and $bla_{\text{OXA-48}}$ genes were assigned to the GenBank accession numbers KX467530, KX467529, and KX671151, respectively.

The clonal relatedness among CPE

The PFGE results showed that 20 isolates of the 28 carbapenemase-producing *K. pneumoniae* were divided into four clusters (clusters I–IV), and eight isolates were singletons (Fig. 1). Cluster I with 12 isolates was the major common clone. The PFGE analysis showed that there was a great clonal diversity among the 15 *E. coli* isolates. The data analysis revealed that there were only two minor clusters (clusters I and II). The first cluster consisted of two isolates and the second cluster consisted of three isolates (Fig. 2).

Discussion

The prevalence of CRE fecal carriers is increasing worldwide and has become a serious problem in the healthcare setting [11, 21, 22]. In the study described herein, mainly hospitalized patients in the ICUs were screened for intestinal carriage, and the rate of carriage was high (37.9%). The carrier rate found was higher than in a 2012 report from Greece (12.8), as well as one from Korea (0.3) [23, 24].

Gastrointestinal colonization with multiple CRE isolates in the same patient has been observed in several studies [25–27]. In this study, we detected 15 patients with intestinal

Table 1 Summary of risk factors associated with carbapenem-resistant Enterobacteriaceae (CRE) colonization

Variable	CRE carriers, no. (%); $n = 36$	CRE non–carriers, no. (%); $n = 59$	p-Values <0.05
Age, years (mean ± SD)	50.55 ± 20.62	49.52 ± 23.43	0.82
Sex, male, *n* (%)	22 (61.1)	22 (37.3)	0.02
Unit of hospitalization, *n* (%)			
Infectious	3 (8.3)	9 (15.2)	0.32
Emergency ICU	2 (5.6)	7 (11.9)	0.30
General ICU	3 (8.3)	19 (32.2)	0.007
ICU-1	5 (13.9)	9 (15.2)	0.85
ICU-2	18 (50)	7 (11.9)	0.00004
ICU-3	5 (13.9)	8 (13.6)	0.96
Invasive medical device utilization, *n* (%)			
Mechanical ventilation	29 (80.6)	26 (44)	0.0004
Urinary catheter	29 (80.6)	36 (61)	0.04
Surgery	28 (77.8)	33 (55.9)	0.03
Presence of wounds	23 (63.9)	26 (44)	0.06
Transfer from another hospital, *n* (%)	14 (38.9)	8 (13.5)	0.004
Transfer between hospital units	32 (88.9)	38 (64.4)	0.008
Antibiotic exposures, *n* (%)			
Carbapenem	29 (80.6)	36 (61)	0.04
Third-generation cephalosporin	13 (36.1)	56 (94.9)	0.00001
Ciprofloxacin	13 (36.1)	23 (38.9)	0.77
Colistin	15 (41.7)	9 (15.2)	0.004
Vancomycin	29 (80.6)	35 (59.3)	0.03
Piperacillin/tazobactam	9 (25)	18 (30.5)	0.56

colonization of multiple CRE isolates. These results are most likely due to the intra- and inter-species transmission of bla_{NDM} and bla_{OXA-48} genes within the gut microbiome. The prevalence of CRE colonization among inpatients in hospital A (56%) was much higher than that in hospital B (17.7%), which could be due to factors such as hand hygiene non-compliance, breaches in environmental sanitation in all hospital areas (especially in the ICU), increased duration of hospitalization, and extensive use of broad-spectrum antimicrobial agents. The rapid identification of inpatients colonized with CRE could be an important strategy to control the transmission of these organisms in healthcare facilities [11, 28].

The significant risk factors for increased rates of gut colonization with CRE in the present study were as follows: antibiotic exposure (mainly third-generation cephalosporins), ICU admission (mainly ICU-2 and general ICU), mechanical ventilation, indwelling urinary catheter, transfer being refereed from other hospitals, previous surgery, and transfer between hospital units. Our results are similar to previous reports in other parts of the world [11, 23, 29]. In contrast to our study, previous studies have reported that the presence of wounds and ciprofloxacin use were associated with CRE colonization [23, 30].

We also detected carbapenemases bla_{NDM-1}, bla_{NDM-7}, and bla_{OXA-48} in different multidrug-resistant Enterobacteriaceae species that co-produce $bla_{CTX-M-15}$. The predominant species found in our study was *K. pneumoniae*, followed by *E. coli*, while other species have been isolated sporadically from

Table 2 The isolates recovered from 95 rectal samples using the two different methods

CRE isolates	CHROMagar KPC		MacConkey agar	
	Total	Carbapenemase-producing	Total	Carbapenemase-producing
K. pneumoniae	33	29	33	29
E. coli	19	16	18	15
E. cloacae	1	1	1	1
P. mirabilis	1	1	–	–
Total isolates	54	47	52	45

Table 3 Clinical, phenotypic, and genotypic characteristics of the 54 CRE isolated from intestinal carriage in Iran

Patient	Species	Unit/hospital	Data of isolation (day/month)	MHT	MICs (μg/mL)			Carbapenemases	bla_{CTX-M}	PFGE clusters
					ETP	MEM	IMP			
P1	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	8	8	12	NDM-7, OXA-48	CTX-M-15	Cluster I
P2	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	8	8	12	NDM-1	−	Cluster III
P3	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	8	8	16	NDM-7, OXA-48	CTX-M-15	Cluster I
	E. coli	ICU-2/HA	12/10/2015	+	8	8	8	NDM-7, OXA-48	−	Singleton
P4	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	8	8	12	NDM-7, OXA-48	−	−
P5	*K. pneumoniae*	ICU-2/HA	12/10/2015	−	8	8	64	NDM-7	CTX-M-15	Singleton
	E. coli	ICU-2/HA	12/10/2015	+	1	0.5	<4	OXA-48	CTX-M-15	Singleton
P6	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	8	1.5	<4	OXA-48	CTX-M-15	Cluster I
P7	*E. coli*	ICU-2/HA	12/10/2015	+	ND	ND	ND	−	CTX-M-15	−
P8	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	ND	ND	ND	−	CTX-M-15	−
P9	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	ND	ND	ND	−	CTX-M-15	−
	E. coli	ICU-2/HA	12/10/2015	+	8	8	12	NDM-1, OXA-48	CTX-M-15	Singleton
P10	*K. pneumoniae*	ICU-2/HA	15/10/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
P11	*E. coli*	ICU-2/HA	15/10/2015	+	8	8	12	NDM-7, OXA-48	CTX-M-15	Cluster I
P12	*K. pneumoniae*	ICU-2/HA	15/10/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
	K. pneumoniae	ICU-2/HA	15/10/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
P13	*K. pneumoniae*	ICU-2/HA	12/10/2015	+	8	8	256	NDM-1, OXA-48	CTX-M-15	Cluster IV
	E. coli	ICU-2/HA	15/10/2015	+	1	0.25	<4	OXA-48	CTX-M-15	Singleton
P14	*K. pneumoniae*	ICU-2/HA	15/10/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
	E. coli	ICU-2/HA	15/10/2015	+	8	8	32	OXA-48	CTX-M-15	Cluster II
P15	*K. pneumoniae*	ICU-1/HA	17/11/2015	+	8	8	16	NDM-1	−	Cluster III
P16	*K. pneumoniae*	ICU-1/HA	17/11/2015	+	8	8	12	NDM-7, OXA-48	CTX-M-15	Singleton
P17	*K. pneumoniae*	ICU-1/HA	17/11/2015	+	8	8	256	NDM-1, OXA-48	CTX-M-15	Cluster I
P18	*K. pneumoniae*	ICU-1/HA	17/11/2015	+	8	8	12	NDM-1, OXA-48	−	Singleton
	E. coli	ICU-1/HA	17/11/2015	+	1	0.25	<4	OXA-48	CTX-M-15	Singleton
P19	*E. coli*	ICU-1/HA	17/11/2015	+	8	8	12	NDM-1, OXA-48	−	−
P20	*K. pneumoniae*	ICU-1/HA	17/11/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
P21	*K. pneumoniae*	ICU-3/HA	17/11/2015	+	8	8	12	NDM-1, OXA-48	−	Cluster III
	E. coli	ICU-3/HA	17/11/2015	+	8	8	16	NDM-1, OXA-48	−	Singleton
P22	*K. pneumoniae*	ICU-2/HA	17/11/2015	+	8	8	256	NDM-1, OXA-48	CTX-M-15	Cluster IV
P23	*E. coli*	ICU-3/HA	17/11/2015	+	8	8	16	OXA-48	CTX-M-15	Cluster II
P24	*K. pneumoniae*	ICU-3/HA	17/11/2015	+	8	8	8	NDM-1, OXA-48	−	Cluster II
P25	*K. pneumoniae*	ICU-3/HA	17/11/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
P26	*K. pneumoniae*	ICU-3/HA	17/11/2015	+	8	8	8	OXA-48	CTX-M-15	Cluster I
	E. coli	ICU-3/HA	17/11/2015	+	0.25	0.25	<4	OXA-48	CTX-M-15	Singleton
	P. mirabilis	ICU-3/HA	17/11/2015	+	8	8	48	OXA-48	CTX-M-15	−
P27	*K. pneumoniae*	ICU-2/HA	17/11/2015	+	8	2	<4	OXA-48	CTX-M-15	Singleton
	K. pneumoniae	ICU-2/HA	17/11/2015	+	8	8	6	OXA-48	CTX-M-15	−
	E. coli	ICU-2/HA	17/11/2015	+	8	1.5	<4	OXA-48	−	Singleton
P28	*E. coli*	ICU-2/HA	17/11/2015	+	8	8	6	OXA-48	CTX-M-15	Cluster II
	E. coli	ICU-2/HA	17/11/2015	+	8	8	6	OXA-48	−	Singleton
P29	*K. pneumoniae*	ID/HB	1/7/2015	+	8	8	16	NDM-1, OXA-48	−	Singleton
P30	*E. coli*	ID/HB	1/7/2015	−	ND	ND	ND	−	−	−
P31	*K. pneumoniae*	G-ICU/HB	18/6/2015	−	ND	ND	ND	−	−	−
	K. pneumoniae	G-ICU/HB	18/6/2015	−	ND	ND	ND	−	−	−
P32	*E. coli*	E-ICU/HB	31/7/2015	−	ND	ND	ND	−	CTX-M-15	−
P33	*K. pneumoniae*	G-ICU/HB	1/8/2015	−	8	8	8	NDM-1	−	Singleton

Table 3 (continued)

Patient	Species	Unit/hospital	Data of isolation (day/month)	MHT	MICs (μg/mL)			Carbapenemases	bla_{CTX-M}	PFGE clusters
					ETP	MEM	IMP			
	E. coli	G-ICU/HB	1/8/2015	+	8	8	8	NDM-1	−	Singleton
	E. cloacae	G-ICU/HB	1/8/2015	+	8	3	4	NDM-1	−	−
P34	*K. pneumoniae*	G-ICU/HB	20/9/2015	+	8	8	16	NDM-1, OXA-48	CTX-M-15	Cluster II
	E. coli	G-ICU/HB	20/9/2015	+	8	1.5	<4	OXA-48	−	Singleton
P35	*K. pneumoniae*	ID/HB	18/9/2015	+	8	8	6	OXA-48	CTX-M-15	Singleton
P36	*K. pneumoniae*	E-ICU/HB	19/10/2015	+	8	8	<4	OXA-48	−	Cluster IV
	K. pneumoniae	E-ICU/HB	19/10/2015	−	8	8	256	NDM-1	CTX-M-15	Singleton

ICU, intensive care unit; ID, infectious diseases; G-ICU, general intensive care unit; E-ICU, emergency intensive care unit; HA, hospital A; HB, hospital B; MHT, modified Hodge test; ETP, ertapenem; MEM, meropenem; IMP, imipenem; MICs, minimal inhibitory concentrations; PFGE, pulsed-field gel electrophoresis

fecal carriage. Interestingly, bla_{OXA-48} was the most frequently detected carbapenemase and bla_{NDM-1} was second, which is similar to other reports [21, 31]. The bla_{OXA-48} gene was detected in 40 isolates of three species of Enterobacteriaceae (*K. pneumoniae*, *E. coli*, and *P. mirabilis*). Also, the bla_{NDM-1} gene was detected in 17 isolates of three species of Enterobacteriaceae (*K. pneumoniae*, *E. coli*, and *E. cloacae*). The potential dissemination of bla_{OXA-48}-producing *P. mirabilis* isolates is a major problem, because this organism is intrinsically resistant to colistin, which is the agent of last resort against CPE isolates [16].

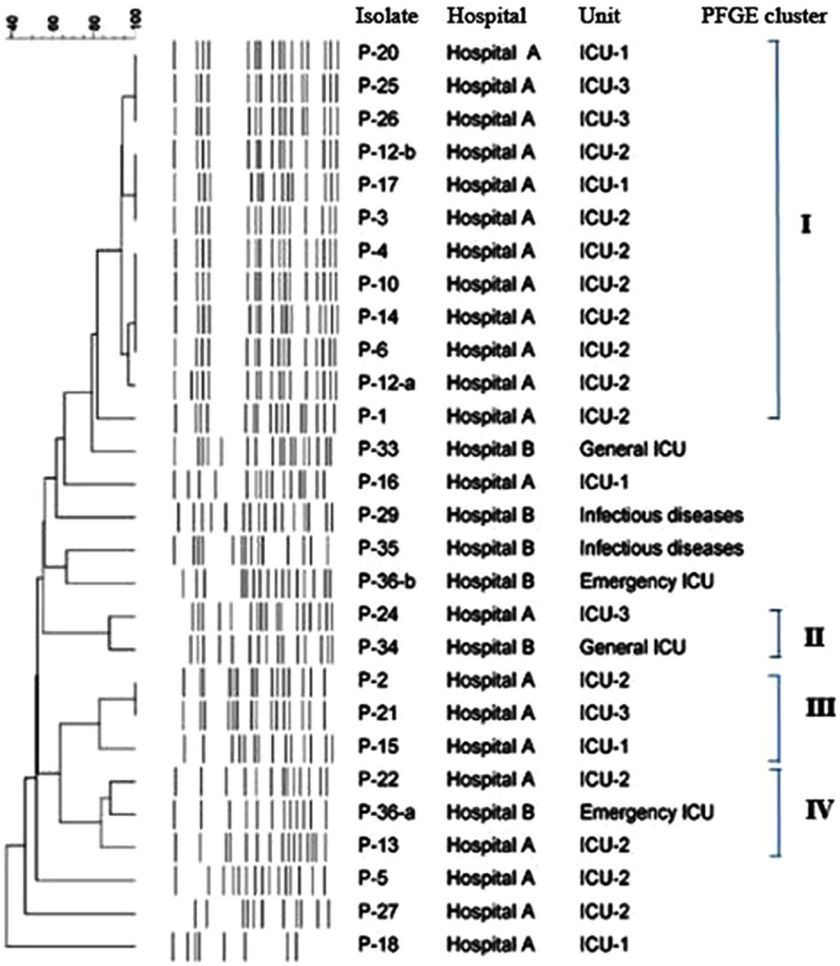

Fig. 1 Results of the pulsed-field gel electrophoresis (PFGE) analysis and the unweighted pair group method with arithmetic mean (UPGMA) dendrogram in 28 carbapenemase-producing *Klebsiella pneumoniae* isolates. Based on ≥80% profile similarity, four clusters (I–IV) were defined and seven isolates were considered singletons. Cluster I was the major clone, carrying bla_{OXA-48} and $bla_{CTX\ M-15}$ genes, and was recovered mainly from ICU-2 in university hospital A

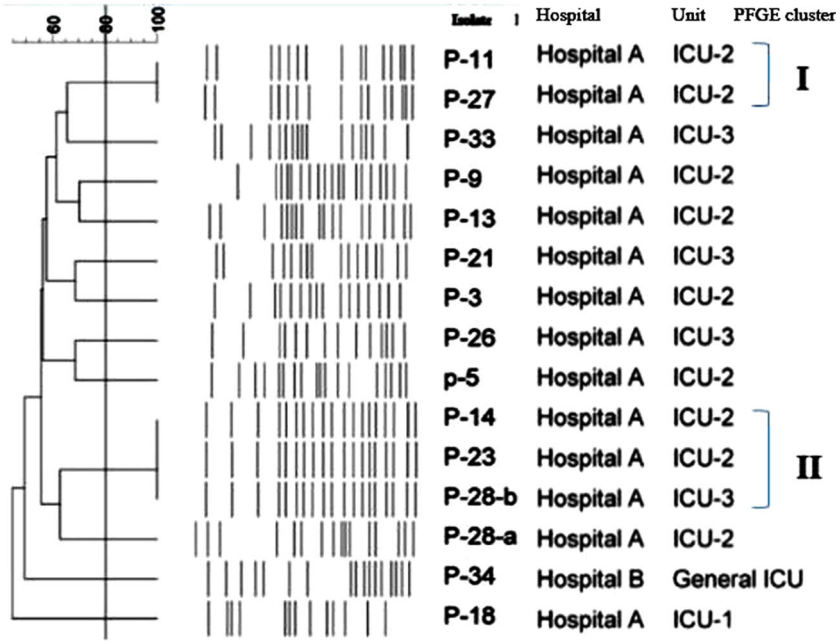

Fig. 2 Results of the PFGE analysis and the UPGMA dendrogram in 15 carbapenemase-producing *Escherichia coli* isolates. Based on ≥80% profile similarity, two minor clusters (I and II) were defined and ten isolates were singletons

The co-harboring of $bla_{\text{NDM-1}}$ with the $bla_{\text{OXA-48}}$ carbapenemase in *E. coli* and *K. pneumoniae* isolates has been detected in many studies [9, 32, 33]. We detected seven *K. pneumoniae* and five *E. coli* isolates co-harboring the $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$ genes. The co-existence of these two carbapenemase-encoding genes poses a therapeutic challenge to clinicians, due to limited treatment choices and the possibility of global spread by means of cross-border transfer [34].

A comparison of MacConkey agar plus ETP disk with CHROMagar KPC for the isolation of CRE isolates showed that both media are appropriate for the detection of CRE carriage, but CHROMagar KPC performed better than MacConkey agar plus ETP disk. The detection of OXA-48 producers by the Centers for Disease Control and Prevention (CDC) method is difficult due to their usually low MICs for carbapenems, low inoculum, and presence of other CRE isolates. These results are in agreement with previous data [4, 35, 36].

Among the 47 CRE isolates with a positive MHT result, 44 isolates harbored the bla_{NDM} and $bla_{\text{OXA-48}}$ or both types of gene. False-positive results could be due to the production of ESBL and AmpC beta-lactamase combined with porin loss [37]. We have shown that MHT is highly sensitive and suitable for the screening of class D carbapenemase (OXA-48), which is similar to previous findings by Woodford et al. [38].

In our study, based on PFGE analysis, *K. pneumoniae* isolates had been more clonal compared to *E. coli* isolates. The prevalent cluster among carbapenemase=producing *K. pneumoniae* was cluster I in hospital A. All of the *K. pneumoniae* isolates in cluster I were positive for $bla_{\text{CTX-M-15}}$, which was probably similar to the successful international clone described by Nematzadeh et al. [39]. In addition, these isolates in cluster I were not only harboring $bla_{\text{CTX-M-15}}$, but were also carrying the $bla_{\text{OXA-48}}$ gene. However, dissemination of cluster I has been observed in ICU-1 and ICU-3. Therefore, intra-ward transmission of this cluster between ICU-1 and ICU-3 could be suspected. The PFGE clonal analysis of ten carbapenem-resistant *E. coli* has shown that these isolates were clonally unrelated. Our results showed that both carbapenemase-producing *K. pneumoniae* and *E. coli* cluster I strains were isolated among inpatients who shared a room; hence, the PFGE profile of the strains was identical. Therefore, it is possible that a spread of CPE from patient to patient occurred.

The present study had several limitations. First, fecal sampling was carried out in only two hospitals, from two different Iranian cities; thus the results may not be representative of the whole country. Second, the lack of some medical details (such as underlying disease and previous admissions in the last year), lack of history data on international travel, and contact with animals, is also a limitation. Third, a multivariable risk factor analysis was not carried out with the risk factors identified in the univariate analysis.

In conclusion, the present study shows a high rate of CRE intestinal colonization among inpatients. These results are a warning for hidden fecal carriage in patients with CRE isolates. The high rate of fecal carriage of NDM and OXA-48-producing Enterobacteriaceae among inpatients in Iran alert on the large dissemination of these genes to other hospitals and the community [29]. These results suggest that the implementation of adequate preventive measures such as active surveillance and antibiotic stewardship are urgently needed

to control the spread of CRE isolates in the healthcare setting in our country.

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Compliance with ethical standards

Ethical statement This project was done based on ethical guidelines as previously approved by the Pasteur institute of Iran (project no: IR.PII.REC.1395.51).

Conflict of interest No conflicts of interest declared.

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