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HIGH RATE OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* DETECTED FROM HOSPITAL EQUIPMENTS IN IRAN

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The objective of this study was to assess the prevalence, antibiogram, and related genes of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) among hospital environment samples. A total of 250 samples were taken from different surfaces and medical devices of three hospitals in Isfahan, Iran. All samples were cultured and *K. pneumoniae* strains were identified by conventional microbiological methods and polymerase chain reaction (PCR). Antibiogram of isolates was performed by disk diffusion method and production of carbapenemases and metallo-β-lactamases (MBLs) was confirmed using modified Hodge test and E-test, respectively. Molecular detection of the related genes was carried out by PCR. Overall, 37 (14.8%) *K. pneumoniae* strains were isolated, of which 34 (91.9%) strains were resistant to carbapenems. Twenty-eight (82.4%) isolates were positive for carbapenemases and seven (20.6%) isolates were phenotypically MBL producers. The results of PCR showed that the prevalence of $bla_{\text{OXA-48}}$, bla_{NDM}, bla_{IMP}, bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{TEM}, and class 1 integron among CRKP isolates was 70.6%, 52.9%, 2.9%, 100%, 82.4%, 55.9%, and 76.5%, respectively. However, bla_{KPC}, bla_{GES}, bla_{IMI}, bla_{VIM}, and class 2 integron were not detected in any of the isolates. This study showed that the environment of our hospitals is contaminated with CRKP and it emphasizes the importance of using standard methods for infection control.

Keywords: carbapenem-resistant *Klebsiella pneumoniae*, CRKP, OXA-48, NDM, hospital environment

Introduction

The emergence and dissemination of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) in hospitals is a great concern for healthcare systems worldwide [1]. CRKP is resistant to almost all antimicrobial agents, and

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outbreaks caused by this pathogen are associated with considerable morbidity and mortality [2]. Infected patients with CRKP can be a source of its spread in the hospital and can transfer it to other patients or even personnel [3]. Probably, the environment surrounding these patients is contaminated with these organisms and is responsible for disseminating directly or indirectly [4, 5]. Due to the vulnerability of patients in intensive care unit (ICU), the presence of CRKP strains in the environment of this ward is very important in terms of infection control [6, 7]. Undoubtedly, knowing the status of hospital environment contamination with CRKP and the use of effective methods for disinfecting the environment and medical devices from these organisms can help control infection in ICUs. Since there has not been a study on the prevalence of these organisms in hospital environments in Iran, this study aims to evaluate the prevalence, antibiotic resistance pattern, and related genes of CRKP among hospital environment samples in the ICUs of three hospitals of Isfahan, Iran.

Materials and Methods

Bacterial isolates

During a 3-month period, from May to July 2017, bacterial strains were isolated from 250 surface samples in the ICUs of three hospitals (Alzahra, Imam Musa Kazim, and Khorshid) of Isfahan, Iran. All samples were taken using swabs from different surfaces and medical devices including hospital beds, ventilators, aerators, suctions, monitors, stethoscopes, sphygmomanometer cuffs, pulse oximeter probes, chest leads, infusion pumps, oxygen bubblers, oxygen masks, cabinets, sinks, water taps, walls, phone handsets, and washing liquid. For sampling, sterile cotton-tipped applicators were moistened in 2 ml of sterile normal saline, rolled several times over a surface area (~20 cm^2), immersed in tryptic soy broth medium, vortexed gently, and aerobically incubated at 37 °C overnight. Then, tubes with turbidity were subcultured in blood agar and eosin methylene blue agar plates. After 24 h of incubation at 37 °C, all lactose-positive colonies were examined using conventional microbiological methods such as Gram-staining and biochemical (oxidase, sugar fermentation, IMViC, Kliger's iron agar, nitrate reduction, motility, etc.) tests [8]. To confirm the species, we carried out a polymerase chain reaction (PCR) detection based on the 16S–23S internal transcribed spacer sequence of *K. pneumoniae* [9]. This study was evaluated and approved by the ethics committee of Isfahan University of Medical Sciences (project no.: 395891).

Antibiotic susceptibility testing

Antibiotic susceptibility of isolates was determined by Kirby–Bauer's disk diffusion method, according to Clinical Laboratory Standard Institute (CLSI) recommendations [10]. For this end, we used 18 antibiotic disks (MAST, UK and Liofilchem, Italy), including imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), gentamicin (10 μg), ceftaroline (30 μg), piperacillin/tazobactam (100/10 μg), cefazolin (30 μg), cefuroxime (30 μg), ceftazidime (30 μg), cefepime (30 μg), cefoxitin (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), tigecycline (15 μg), aztreonam (30 μg), amoxicillin/clavulanic acid (20/10 μg), chloramphenicol (30 μg), and tetracycline (30 μg). *Escherichia coli* ATCC 25922 was used for quality control of disk diffusion method [10].

Carbapenemase and metallo-β-lactamase (MBL) screening assays

Carbapenemase activity was determined using the modified Hodge test (MHT) by ertapenem disk (MAST), according to CLSI guidelines [10]. To detect MBL activity, E-test method using strips containing meropenem/meropenem + EDTA (Liofilchem) was performed based on manufacturer's instructions.

PCR for the detection of antibiotic resistance genes

DNA was extracted using the boiling method and used as a template for PCR [11]. To detect antibiotic-resistant genetic elements, including bla_{KPC}, bla_{GES}, bla_{IMI}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, bla_{SHV}, bla_{TEM}, and bla_{CTX-M} as well as class 1 and 2 integrons, separate PCR reactions were performed. The list of all target genes and corresponding primers is presented in Table I [12–19]. PCR was performed using commercially available PCR Master Mix (Ampliqon, Denmark), according to the manufacturer's instructions. Briefly, 1 μl of template DNA (~100 ng/μl), 1 μl of each primer (10 pmoles/μl), and 9.5 μl of DNase-free distilled water were added to 12.5 μl of Master Mix in a final volume of 25 μl. The cycling conditions consisted of a 5-min initial denaturation step at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 45 s at primer-specific temperatures (Table I), extension at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. PCR products were resolved by standard electrophoresis on 1.5% agarose gel containing DNA safe stain.

Table I. List of primers, expected amplicon size, and annealing temperatures

Target gene	Primer sequence (5′–3′)	Annealing temperature (°C)	Amplicon size (bp)	References
bla_{KPC}	F: GATACCACGTTCCGTCTGG R: GCAGGTTCCGGTTTTGTCTC	58	246	[12]
bla_{GES}	F: GTTTTGCAATGTGCTCAACG R: TGCCATAGCAATAGGCGTAG	53	371	[13]
bla_{IMI}	F: ATGTCATTAGGTGATATGGC R: GCATAATCATTTGCCGTACC	50	879	[14]
bla_{VIM}	F: TTTGGTCGCATATCGCAACG R: CCATTCAGCCAGATCGGCAT	66	500	[15]
bla_{IMP}	F: GTTTATGTTCATACATCG R: GGTTTAACAAAACAACCAC	45	440	[15]
bla_{NDM}	F: GGGCAGTCGCTTCCAACGGT R: GTAGTGCTCAGTGTCGGCAT	52	475	[16]
bla_{OXA-48}	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	60	438	[17]
bla_{SHV}	F: ATGCGTTATATTCGCCTGTG R: TGCTTTGTTATTCGGGCCAA	60	753	[15]
bla_{TEM}	F: AAACGCTGGTGAAAGTA R: AGCGATCTGTCTAT	45	752	[15]
bla_{CTX-M}	F: TTTGCGATGTGCAGTACCAGTAA R: CGATATCGTTGGTGGTGCCATA	51	544	[18]
intI1	F: GGTCAAGGATCTGGATTTCG R: ACATGCGTGTAAATCATCGTC	52	484	[19]
intI2	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGAGTGACGAAATG	54	789	[19]

Results

Bacterial isolates

Table II demonstrates the relative frequency of CRKP strains isolated from hospital surfaces and medical devices. Overall, 250 samples were cultured, of which 28 (11.2%) samples were negative and 37 (14.8%) samples were contaminated by *K. pneumoniae* strains.

Antibiotic susceptibility testing

Table III demonstrates the results of antibiotic susceptibility testing in hospital environment isolates. The highest resistance rate was obtained for ertapenem, cefepime, ceftazidime, ceftaroline, cefuroxime, and cefazolin with 91.9%.

Table II. Relative frequency of CRKP strains isolated from hospital surfaces and medical devices

Sampling site	Sample size	Positive cultures [*n* (%)]	*K. pneumoniae* isolates [*n* (%)]	CRKP isolates [*n* (%)]
Hospital bed	61	60 (98.4)	10 (16.7)	10 (100.0)
Ventilator	47	42 (89.4)	6 (14.3)	5 (83.3)
Sphygmomanometer cuff	25	25 (100.0)	10 (40.0)	9 (90.0)
Sink	21	17 (80.9)	3 (17.6)	2 (66.7)
Oxygen bubbler	20	13 (65.0)	1 (7.7)	1 (100.0)
Wall	16	13 (81.3)	1 (7.7)	1 (100.0)
Suction	10	7 (70.0)	2 (28.6)	2 (100.0)
Pulse oximeter probe	9	9 (100.0)	2 (22.2)	2 (100.0)
Cabinet	8	6 (75.0)	0 (0.0)	0 (0.0)
Water tap	6	5 (83.3)	0 (0.0)	0 (0.0)
Monitor	5	5 (100.0)	0 (0.0)	0 (0.0)
Aerator	4	2 (50.0)	0 (0.0)	0 (0.0)
Infusion pump	4	4 (100.0)	0 (0.0)	0 (0.0)
Stethoscope	4	4 (100.0)	0 (0.0)	0 (0.0)
Chest lead	3	3 (100.0)	1 (33.3)	1 (100.0)
Oxygen mask	3	3 (100.0)	1 (33.3)	1 (100.0)
Phone handset	3	3 (100.0)	0 (0.0)	0 (0.0)
Washing liquid	1	0 (0.0)	0 (0.0)	0 (0.0)
Total	250	222 (88.8)	37 (16.7)	34 (91.9)

Note: CRKP: carbapenem-resistant *Klebsiella pneumonia*.

Table III. Antibiotic susceptibility of *K. pneumoniae* isolates ($n = 37$)

Antibiotic	Susceptible [*n* (%)]	Intermediate [*n* (%)]	Resistant [*n* (%)]
Imipenem	10 (27.0)	3 (8.1)	24 (64.9)
Meropenem	3 (8.1)	1 (2.7)	33 (89.2)
Ertapenem	3 (8.1)	0 (0.0)	34 (91.9)
Gentamicin	7 (18.9)	0 (0.0)	30 (81.1)
Ceftaroline	3 (8.1)	0 (0.0)	34 (91.9)
Piperacillin/tazobactam	4 (10.8)	0 (0.0)	33 (89.2)
Cefazolin	3 (8.1)	0 (0.0)	34 (91.9)
Cefuroxime	3 (8.1)	0 (0.0)	34 (91.9)
Ceftazidime	3 (8.1)	0 (0.0)	34 (91.9)
Cefepime	3 (8.1)	0 (0.0)	34 (91.9)
Cefoxitin	5 (13.5)	5 (13.5)	27 (73.0)
Ciprofloxacin	4 (10.8)	0 (0.0)	33 (89.2)
Trimethoprim/sulfamethoxazole	13 (35.1)	1 (2.7)	23 (62.2)
Tigecycline	19 (51.4)	7 (18.9)	11 (29.7)
Aztreonam	5 (13.5)	0 (0.0)	32 (86.5)
Amoxicillin/clavulanic acid	3 (8.1)	1 (2.7)	33 (89.2)
Chloramphenicol	14 (37.8)	8 (21.6)	15 (40.6)
Tetracycline	11 (29.7)	4 (10.8)	22 (59.5)

Tigecycline was the most effective antibiotic with 51.4% susceptibility rate followed by chloramphenicol (37.8%) and tetracycline (29.7%). Among the carbapenems, the highest and the lowest resistance rates were detected for ertapenem (91.9%) and imipenem (64.9%), respectively. Overall, 34 (91.9%) isolates were resistant to carbapenems.

Carbapenemase and MBL screening assays

The results of MHT on 34 isolates with resistance to carbapenems showed that 28 (82.4%) isolates were positive and 6 (17.6%) isolates were negative. In addition, phenotypic MBL production testing on these isolates identified 7 (20.6%) isolates as positive, 8 (23.5%) isolates as negative, and 19 (55.9%) isolates with non-determinable results.

PCR for the detection of antibiotic resistance genes

All CRKP isolates were examined by PCR for the presence of the special antibiotic resistance genetic elements. Twenty-four (70.6%) isolates harbored $bla_{\text{OXA-48}}$, 18 (52.9%) isolates were positive for the bla_{NDM}, and bla_{IMP} was detected in 1 (2.9%) isolate. Moreover, nine (26.5%) isolates had both bla_{NDM} and $bla_{\text{OXA-48}}$, simultaneously. Extended-spectrum β-lactamases (ESBLs)-encoding genes including bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{TEM} were identified in 100%, 82.4%, and 55.9% isolates, respectively. In addition, 16 (47.1%) isolates had bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{TEM} genes, concurrently. Three (8.8%) isolates were positive for the presence of $bla_{\text{OXA-48}}$, bla_{NDM}, bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{TEM}, together. Nevertheless, the results of PCR were negative for bla_{KPC}, bla_{GES}, bla_{IMI}, and bla_{VIM}. Furthermore, 26 (76.5%) isolates harbored class 1 integron, but class 2 integron was not detected in any of the isolates.

Discussion

In this study, we documented the existence of CRKP in the hospital environment and the surfaces of medical devices. As we know that CRKP can survive for more than 2 weeks on dry surfaces [20], this finding reveals the role of the hospital environment as a source of the transmission of CRKP to the hospitalized patients. In this study, the isolation rate of *K. pneumoniae* from hospital environment samples was 14.8%. In several studies conducted in Iran

that used the same method (wet sterile swab) for sampling, the isolation rate of *K. pneumoniae* from hospital environment samples was less than ours: Mehraban et al. (0.4%) [21], Ayatollahi et al. (4.5%) [22], Ekrami et al. (4.7%) [23], and Zazouli et al. (5%) [24]. This difference can be due to the difference in the level of environment contamination in the hospitals studied, the sampling ward, the sampling time, the sampling site, and cultivation method. Among the medical devices and surfaces, the highest level of relative contamination with *K. pneumoniae* was detected in sphygmomanometer cuffs (40%), followed by chest leads (33.3%), oxygen masks (33.3%), suctions (28.6%), pulse oximeter probes (22.2%), sinks (17.6%), hospital beds (16.7%), ventilators (14.3%), oxygen bubblers (7.7%), and walls (7.7%). CRKP strains were isolated from 10 of the 18 sites sampled: hospital beds, ventilators, sphygmomanometer cuffs, sinks, suctions, pulse oximeter probes, oxygen bubbler, wall, chest lead, and oxygen mask. In the study by Lerner et al. [1] in Israel, they have identified carbapenem-resistant *Enterobacteriaceae* strains in only 5 of the 14 sites sampled. About the antibiogram results of *K. pneumoniae* isolates, the highest resistance rate was observed for β-lactam antibiotics; however, the lowest resistance rate was identified for tigecycline. In this regard, the results of this study are consistent with the other two studies conducted on clinical isolates in the hospitals studied [25, 26]. In this study, $bla_{\text{OXA-48}}$ was the most prevalent carbapenemase detected, followed by bla_{NDM}. Similarly, our findings are in parallel with the studies of Solgi et al. [25, 26] in our region. We detected bla_{IMP} in one CRKP isolate. This isolate was obtained from a ventilator in the ICU of Alzahra hospital and was resistant to all the aforementioned antibiotics except gentamicin and chloramphenicol. Moreover, this isolate carried $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, and class 1 integron, simultaneously. To the best of our knowledge, this is the second report of bla_{IMP}-harbored *K. pneumoniae* from Iran after Khorvash et al. [27]. This study showed a high prevalence of ESBLs-encoding genes (bla_{SHV}, $bla_{\text{CTX-M}}$, and bla_{TEM}) among CRKP isolates, which is consistent with other studies conducted in Isfahan [25, 26] and Iran [28–30]. However, we have not detected bla_{KPC}, bla_{GES}, bla_{IMI}, and bla_{VIM} among CRKP isolates. In this regard, studies that were previously carried out in Isfahan have not been able to identify these genes [26, 31, 32]. On the contrary, Khorvash et al. [27] in their study identified bla_{VIM} in 10.3% of CRKP isolates from clinical isolates of Alzahra hospital. In conclusion, this study showed the existence of CRKP isolates in the patients' surroundings in the ICUs of our hospitals. It emphasizes the importance of using standard methods of infection control for disinfecting surfaces of medical devices and hospital environment.

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Conflict of Interest

The authors declare no conflict of interest.

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