Journal of Medical Bacteriology

Carbapenem-Resistance in Clinical *Klebsiella pneumoniae* Isolates From Iran, Review Article

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ARTICLE INFO

Article type:
Review Article

Article history:
Received: 30 Nov 2021
Revised: 05 Dec 2021
Accepted 10 Dec 2021
Published: 27 Dec 2021

Keywords: *Carbapenem Resistance, Iran, Klebsiella pneumoniae, Oxacillinase.*

ABSTRACT

Background: Because of the current limitations of therapeutic methods, the worldwide appearance and spreading of carbapenem-resistant (CR)-*Klebsiella pneumoniae* has made it a key concern in the healthcare system. *K. pneumoniae*, the most frequent *Klebsiella* species is the cause of human infections, classified as top three pathogens of global concern which was established in the2014 WHO Global Report on Surveillance of Antimicrobial Resistance.

Methods: Embase, PubMed/Medline, Scopus, Google Scholar, Web of Sciences, and Iranian databases were searched to retrieve the potentially relevant studies. In this review, we explored the prevalence of *K. pneumoniae* generating three universal carbapenemases (*KPCs*, *NDMs*, and *OXA-48-like*) by following keywords: "carbapenem resistance" and"bla_{KPC}" and"Metallo lactamase-beta" and "bla_{NDM}" and "bla_{OXA}" and "*Klebsiella pneumoniae*" and Iran.

Results: After exploiting predefined inclusion and exclusion criteria, 37 articles were collected that reported prevalence of carbapenem-resistant *Klebsiella pneumoniae*. At finally, 20 studies reported bla_{NDM} , 17 studies reported bla_{KPC}, 14 studies reported bla_{OXA-48} and bla_{VIM} as gene cause resistance among *Klebsiella pneumoniae* strains. The described resistance to carbapenem varied across different studies, ranging from 4.4% to 100%.

Conclusion: Our findings demonstrated that the high prevalence of carbapenem-resistant *Klebsiella pneumoniae* expresses concern over most Iranian hospitals.

- ***Please cite this paper as***: Moghadam M, Ghavide lM, Kooti S, Meshkat Z, Ghazvini K, Arian E, Farsiani H. Carbapenem-Resistance in Clinical *Klebsiella pneumoniae* Isolates From Iran. *J Med Bacteriol.* 2021; **10** (3, 4): pp.51-65.

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Introduction

Klebsiella pneumoniae is a gram-negative bacterium, a clinically relevant pathogen that tends to acquire multidrug resistance (MDR). Currently, there are limited therapeutic methods for nosocomial and community-acquired infections such as septicemia, pneumonia, wound and urinary tract infections (UTIs) (1-3). Carbapenem-resistant *Klebsiella pneumoniae* is identified as a global superbug in the group of carbapenem-resistant *Enterobacteriaceae* (4).

Fast worldwide dissemination of MDR *K. pneumoniae*, along with other *Enterobacteriaceae*, gave rise to a significant threat to healthcare globally (5). Carbapenems resistance may result from carbapenemases generation and changes in permeability via losing porins as well as efflux system overexpression (6).

The most frequent carbapenemases characterized in *K. pneumoniae* until now are: class A β-lactamases, class B β-lactamases/Metallo- β-lactamases, and class D β-lactamases. During the time that these plasmid-encoded carbapenemases have been progressively reported over the world, their prevalence shows geographical variations (2, 7). Besides, they can also incorporate other resistance mechanisms such as the losing outer membrane porins that affect antibiotic uptake (8).

In class A carbapenemases, KPC enzymes dampen down all other major members, i.e. IMI, NMC-A, GES, SME, SFC-1, and SHV-38 to become the superior enzymes. Initially, KPC β-lactamases were detected in 27 states in the USA, but are nowadays widely spread worldwide, especially in Italy, Greece, South America, China, and Israel as well (9, 10). In 1996, the first KPC producer (a KPC-2-positive *K. pneumoniae*) was characterized on the eastern coast of the USA and after that, a series of variants have been detected. Until now KPC-2 stands as the most routinely identified variant (11, 12). Currently, there are 22 KPC variants, all of which are point-mutant derivatives of a repeated amino acid sequence (13).

The bla_{KPC} in *K. pneumoniae* has been shown on various plasmid types, such as IncA/C, IncF, IncI2, IncX, ColE1, and IncR, but the most prominent plasmid type is IncF with FII*K* replicons (13-15). Different bla_{KPC} genes are linked with a promiscuous transposon-related structure Tn*4401*. This type of transposon has skipped to various plasmids that are generally conjugative (16, 17).

The Metallo beta-lactamase (MBLs) are categorized as Class B of Ambler and vary from other carbapenemases due to having zinc at their active site, which promotes the hydrolysis of the antibiotic. A research group from India demonstrated a new variant of MBL that was first detected in a *K. pneumoniae* and *E. coli* isolate from a Swedish patient who had been treated in India in 2009 and has since spread over the world. The variant called New Delhi Metallo-lactamase (NDM) is encoded by the gene bla_{NDM}. To date, 15 NDM variants have been specified and most of them arise from Asia (18, 19). This gene was revealed as a significant global health challenge not only because of its ability to give resistance to nearly all beta-lactam antibiotics but also because of its fast propagation around the world. NDM spread via medical tourism, international trips, as well as possible exposures in the Balkans and the Indian subcontinent (20, 21). The *NDM-1* gene can be carried by a chromosome or a plasmid. Plasmids carry this gene can be transmitted to other bacteria, for example, the human intestinal flora (22). NDM has a global propagation and now

NDM producing gram-negative bacilli (predominantly in *Escherichia coli* and *K. pneumoniae)* has been demonstrated in more than 40 countries. India, Bangladesh, the United Kingdom, the Indian subcontinent, Pakistan, and the Middle East are regarded as the main pool for *bla*$_{NDM}$ producing bacteria (10, 23).

The first Ambler class D β-lactamase oxacillinase OXA-48, detected from a carbapenem-resistant *K. pneumoniae* isolated from a patient in Istanbul, Turkey, in 2001. OXA-48 hydrolyzes imipenem and penicillins, almost broad-spectrum cephalosporins (8, 10, 24). Another variant of OXA-48, namely OXA-181, has four amino acid substitutions (Glu168Gln, Ser171Ala, Thr104Ala, and Asn110Asp), was initially detected in 2007 in clinical isolates of *Enterobacter cloacae* and *K. pneumoniae* from India. Besides, OXA-232, another variant of OXA-48 has five amino acid substitutions (Glu168Gln, Ser171Ala, Thr104Ala, Asn110Asp, and Arg214Ser), was initially detected in clinical isolates of *K. pneumoniae* and *E. coli* in India (25). So far, 11 variants of *bla*$_{OXA-48-like}$ have been identified and have expanded to North Africa, the Middle East, and southern European countries with a fast spread in Turkey and France (18, 21, 26). These genes, identified by both phenotypic and genotypic methods. The most common phenotypic methods were carba NP- Test, E- test strips, DDST, and Modified Hodge test (MHT), in which the E- test strips are the recommended method to detect MBL producing bacteria. This method is relying on Metallo- β-lactamases inhibition by EDTA. Since the Hodge-test has lower specificity, it is not recommended for Metallo-β-lactamase identification (27, 28). Furthermore, the Real time-PCR method is a useful genotypic technique. Specificity and sensitivity, rapid identification of NDM-1 producers in less than 2 hours are some advantages of this method. Real time-PCR also can be used to detect OXA, VIM, KPC, and IMP-type. Loop-mediated isothermal amplification (LAMP) is another technique that was applied to detect NDM-1 producers in 2011 (24). The rapid multiplex- PCR is a reliable method to screen carbapenemases such as KPC and NDM enzymes, (less than 4 hours) (29-32). As one of the countries in the Middle East, Iran is considered a neighbor of countries in which NDM and OXA-48 producing-bacteria are endemic (33) objectives: Current systemic review aimed to investigate the prevalence of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA48}$, in Iranian hospitals from 2005 to 2020.

Materials and Methods

We carried out a systematic search on the prevalence of *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA48}$ genes in Iran using different electronic databases including Embase, Scopus, Web of Sciences, PubMed/Medline, and Iranian databases from 2005 to 2020. These databases were explored to find out potentially relevant pieces of literature. The search terms separately and along with the *Klebsiella pneumoniae* were applied: "carbapenem resistance" and "Metallo beta-lactamase" and "*bla*$_{KPC}$" and "*bla*$_{NDM}$" and "*bla.*$_{OXA}$" and "*Klebsiella pneumoniae*" and Iran. The same strategy applied for Iranian databases including Iranian Scientific Information Database (www.sid.ir) and Magiran (www.magiran.com). The search was limited to all original articles published in Persian or English that demonstrated the prevalence or incidence of these genes in *K. pneumoniae* by phenotypic and molecular (PCR) methods in Iranian cities.

The article search criteria were as follows: titles, abstracts and full texts. The literature was included

if 1) the study was in Persian or English; 2) the study collected clinical specimens; 3) the study was an original research or a brief report; 4) studies that comprised antibiotic susceptibility patterns by standard methods according to the Clinical Laboratory Standard Institute guidelines (CLSI). As a result of that, the exclusions criteria were as follows: 1) studies didn't involve the *bla*$_{OXA}$/ *bla*$_{KPC}$/ *bla*$_{NDM1}$, 2) studies focused on other mechanisms of carbapenem resistance, 3) review articles and studies in languages other than English or Persian, 4) systematic reviews or meta-analyses, congress abstracts, a duplicate publication for the same document, 5) articles available only in abstract form.

The variables extracted from included studies are as follows: author's name, publication year, study location, total samples or number of *K. pneumoniae*, carbapenem-resistant isolates (resistance against meropenem or imipenem), and diagnosis methods. All of the acquired data were organized in tables.

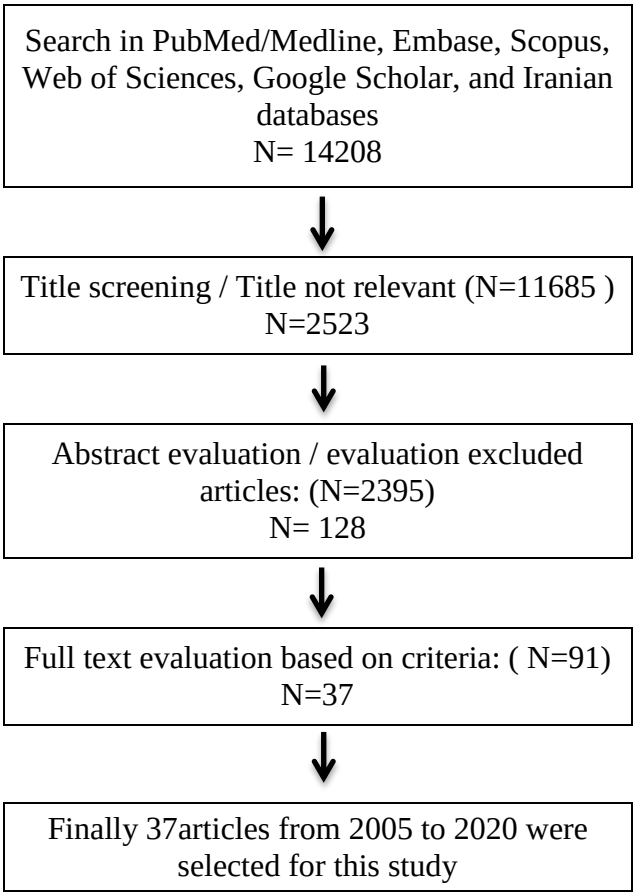

Figure 1: Study selection flowchart for this systematic review

Table 1: Characteristics of studies incorporated in the systematic review

Reference	Province	Total Sample	Specimen Type	Number of CRKP (imipenem)	Diagnosis Methodes	Genes of Responsible (number)	Time
(34)	Shiraz	211	U,B,S,Th,CPleural,	29(13.7%)	MHT DDST	*bla* $_{NDM-1}$ (27) *bla* $_{OXA-48}$(2)	2014-2015
(35)	Bandar Abbas	170	W,U,T,B,S aspirate, eye infection, BAL, As	12(7%)	PCR	*bla*$_{NDM-1}$ (4)	2018
(36)	Isfahan	100	U,T,C,W, Br,Af,A,S,B, CSF	68(68%)	MHT PCR	No detect	2017
(37)	Kashan	181	U,B,W,CSF, C, respiratory tract	36 (19.8%)	DDST PCR	*bla* $_{NDM-1}$ gene(20)	2013-2014
(38)	Tehran	270	U,B,St, W, CSF, S,A, T,C skin lesion, eye discharge,	41 (15.18%)	MHT PCR	*bla*$_{KPC}$ (33) by MHT No detect by PCR	2011-2013
(33)	Semnan	122	U, B, W, S, T	77 (63.11%)	PCR	*bla*$_{OXA-48}$ (21) *bla*$_{NDM-1}$(7) *bla*$_{IMP}$ (3)	2015-2016
(39)	Rasht	68	U	3(4.4%)	PCR	*bla*$_{NDM-1}$ (8) *bla*$_{OXA-1}$ (15)	2020
(40)	Isfahan	80	U, T, B, CSF, W,S,A, C, BAL	46 (57.5%)	MHT E-test PCR	*bla*$_{OXA-48}$ *(46)* *bla*$_{NDM-1}$(8)	2017
(41)	Kurdistan-Isfahan	183	T, B, U, C, W	129 (70.49%)	MHT PCR	*bla*$_{VIM}$(4) *bla*$_{IMP}$(1) *bla*$_{KPC}$(0)	2017
(42)	Tehran	45	U and F	11 (24.4%)	MHT PCR	*bla*$_{NDM-1}$, *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$. This is the first report on the detection of MBL NDM-1 in Iran.	2013
(43)	Kashan	181	U,CSF,C,W Respiratory	35 (19.3%)	PCR	*bla*$_{KPC}$ (21)	2013-2014
(44)	Shiraz	150	U,B ,W, S	24 (16%)	PCR	*bla*$_{IMP}$ (21) *bla*$_{VIM}$(2) *bla* $_{SPM}$(0)	2019

(45)	Tabriz and Mashhad	145	U,B ,W	12(8.27%)	PCR	*bla*$_{NDM-1}$(26)	2018
(46)	Tehran	180	U, B, S,W, T	14(7.77%)	MHT PCR	*bla*$_{NDM}$(3) *bla*$_{VIM}$(5) *bla*$_{KPC}$ (1)	2009-2012
(47)	Tehran	28	W	28 (100%)	MHT CarbaNP test PCR	*bla*$_{OXA-48}$(27) *bla*$_{VIM-4}$(1)	2011
(48)	Kerman	175	W, B, U, CSF, BAL	37(21.14%)	Carba NP test PCR	*bla*$_{OXA-1}$(38) *bla*$_{NDM-1}$(37)	2015-2017
(49)	Yazd	241	U,W,S	ND	PCR	No detect *bla*$_{OXA-48}$ *bla*$_{KPC,}$ *bla*$_{NDM}$	2015-2017
(50)	Tehran	181	U,B, S	100(55.2%)	MHT Real-Time PCR	*bla*$_{KPC}$(51) *bla*$_{OXA -23}$(51) *bla*$_{OXA -48}$(89)	2018
(51)	Isfahan	96	T, U,W , B,C, S, BAL,Th, CSF and pleural fluid	96(100%)	PCR	*bla*$_{OXA-48}$(56) *bla*$_{NDM-1(}$6)	2014-2016
(52)	Tabriz	50	U, B, W	50(100%)	Carba NP test MHT PCR	*bla*$_{OXA-48}$(39) *bla*$_{NDM}$ (24) *bla*$_{IMP}$ (11) *bla*$_{VIM}$ (6) *bla*$_{KPC}$ (4)	2018-2019
(53)	Isfahan	112	U, T, A, CSF, S, C, BAL and eye	49(43.75%)	MHT E-test PCR	*bla*$_{KPC}$ (32) *bla*$_{NDM-1}$(6)	2012-2013
(54)	Tehran	83	S, U, W, B, Intra-abdominal	20(24%)	CDDT MHT	MBL(3) KPC(5)	2011-2012
(55)	Shiraz	60	U, S, B, Th, W, A, nasal/c plural	33(55%)	DDST MHT	*bla*$_{NDM}$ (27) *bla*$_{OXA48}$ (6)	2019
(56)	Isfahan	29	B, U, S, W	29(100%)	MHT DDST	*bla*$_{VIM(}$3) *bla*$_{IMP}$(1) *bla*$_{OXA}$(1)	2011-2012
(57)	Zabol	110	S, U, B, W and other clinical specimens (trachea, synovial fluid and abscess)	8(7.2%)	DDST PCR	*bla*$_{NDM-1}$ (6)	2019
(58)	Babol	50		26(52%)	DDST	*bla*$_{VIM}$(15)	2015

(59)	Tehran	83	U, B, W, S, intra-abdominal, CSF	20(24%)	DDST PCR	MBL (3)	2011-2012
(60)	Urmia	182	U, S, tracheal discharges, B, W. St	45(24.7%)	DDST MHT RAP-DPCR.	*bla*$_{VIM}$(22) *bla*$_{NDM}$(7) *bla*$_{KPC}$(5) *bla*$_{IMP}$(3)	
(61)	Yazd and Karaj	130	U	61(46.9%)	MHT PCR	No detect *bla*$_{KPC}$	2013-2014
(62)	SEMNAN	120	U, B, mucus	ND	PCR	*bla*$_{NDM-1}$(9)	2016
(63)	Qom	79	urine, blood,bronchoalveolar-lavage, A,S, CSF, Peritoneum fluid, synovial fluid	79(100%)	DDST MHT PCR	*bla*$_{VIM}$(46) *bla*$_{OXA-48}$(36) *bla*$_{NDM}$(5) *bla*$_{IMP}$(6) *bla*$_{KPC}$(5)	2019
(64)	Kurdistan	114	urine , wound , blood , tracheal aspiration and sputum	28(24.5%)	DDST PCR	*bla*$_{VIM-1}$(4) *bla*$_{IMP-1}$(1)	2013-2014
(65)	Isfahan	98	U ,B, CSF, As,RS, Peritoneal fluid,Pericardial fluid	57(58.16%)	MHT	*bla*$_{KPC}$(70)	2016
(66)	Tehran	55	W, U, B, S, Th, , discharge, Pf	5(9.09%)	MHT	--	2011-2012
(67)	Abadan	114	ND	ND	DDST PCR	MBL(27) *bla*$_{KPC}$(14)	2014-2015
(68)	Kermanshah	60	U, B, sputum,W, RS	4(6.6%)	MHT PCR	*bla*$_{KPC}$(1) by MHT *bla*$_{VIM}$(3)	2015
(69)	Zanjan	149	U, B, S,Pus	12(8%)	DDST PCR	*bla*$_{VIM}$(5) *bla*$_{IMP}$(12)	2012-2013

: ND: not detected, MHT: Modified Hodge Test, DDST: *Double Disc Synergy Test*, PCR: Polymerase chain reaction, E test; Epsilometer test ,U; Urine, T; Tracheal, C; Catheter, W; Wound, Br; Bronchial, AF; Abdominal fluid, A; Abscess, S; Sputum,CSF; Cerebrospinal fluid, B; Blood, RS; Respiratory secretions, Th; throat , As;ascetic fluid, Pf; pleural fluid, St; stool, BAL; bronchoalveolar-lavage fluid.

Table 2: The frequent genes that were responsible for carbapenem resistance.

Gene responsible for CR	Number of studies	Number of genes responsible for carbapenem resistance
$bla_{\text{OXA-48}}$	14	338
bla_{KPC}	17	245
bla_{NDM}	20	230
bla_{VIM}	14	81
bla_{IMP}	12	47

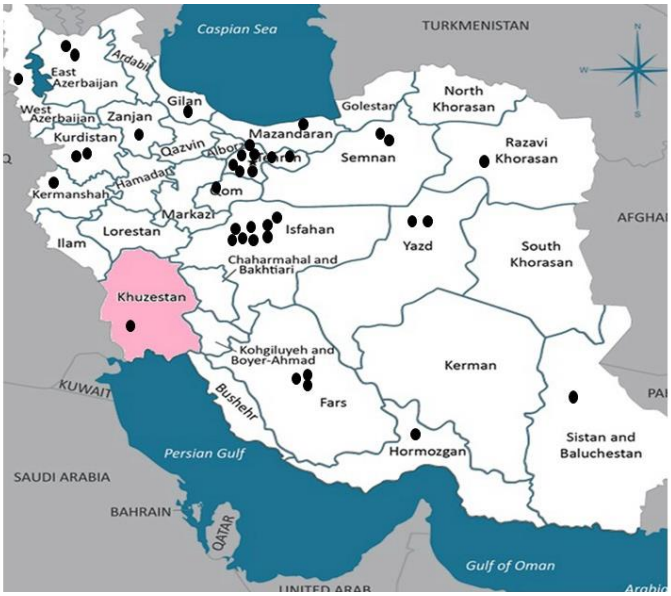

Figure 2: Distribution of studies in Iran.

Results

We retrieved 14208 studies by searching in the mentioned database. Initially, duplicate studies were excluded. Second, via title and abstract exploration, 2523 studies were subjected to a detailed full-text review. In the end, 37 articles were selected for this review. Fig. 1 shows exclusion criteria, via the investigation for title/abstract and full-text articles. The characteristics of the included articles are summarized in Table1.

Diagnosis methods in selected studies included: Carba NP test, Double disc synergy test (DDST), Modified Hodge test (MHT), E test, and PCR. Resistance was detected based on the Clinical and Laboratory Standards Institute (CLSI) guidelines in all selected studies. Finally-selected articles were cross-sectional studies, carried out in different cities of Iran (figure 1). The described resistance to

carbapenem varied across different studies, ranging from 4.4% to 100%.

Carbapenem resistance mechanisms in clinical isolates of K. pneumoniae

Of 37 selected studies, most of them involved the *bla*$_{NDM}$ gene. Besides, the most common genes which were responsible for carbapenem resistance among *K. pneumoniae* strains included: *bla*$_{OXA-48}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, respectively.

Discussion

Carbapenems are important therapeutic agents for the treatment of healthcare-associated infections. Indeed, Carbapenems are indicated in high levels of antibiotic resistance. *Klebsiella pneumoniae* has long been known to result in a severe community or hospital-acquired infections (69). Thus, the elevated prevalence of carbapenem resistance may result in higher mortality, make the hospital course longer, as well as spend more time and energy by the health system (9, 70). The patients' movement between countries may lead to the international distribution of carbapenemase-producing *K. pneumoniae* (71).

As mentioned earlier, a key mechanism of carbapenem resistance in *E. coli* and *K. pneumoniae* is carbapenemase production (i.e., KPC, VIM, NDM, IMP, and OXA) (2, 18). In 2011, NDM-1 and KPC enzymes changed to a worldwide problem (72). KPCs are usually found in *K. pneumoniae* are shown to be related to nosocomial infections (3). The most dominant multi-drug resistance clone in *K. pneumoniae* over the world included ST258, ST11, ST14, and ST15. These clones are also linked with higher mortality and morbidity (15). They are demonstrated by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), which trace strains at the genotype level and improve the knowledge of global epidemiology. *K. pneumoniae* ST258 has a key role in spreading KPC enzymes in the USA as well as the world (15, 73). Further studies are needed to characterize the role of every single intervention in controlling the KPC enzymes distribution. Besides, plasmid outbreaks (rather than clonal outbreaks) might respond more agreeably to antibiotic stewardship interventions rather than the infection control interventions. The epidemiology of *K. pneumoniae* producing KPCs shows geographical variations. It has been reported that these bacteria had endemic spread in Italy, Poland, the USA, China, Greece, Israel, Brazil, Colombia, Argentina, and Taiwan (74). Infections of NDM-producing *K. pneumoniae* are also related to high in-hospital mortality (75). NDM (New Delhi Metallo-b-lactamase) is one of the most clinically remarkable carbapenemases. Similar to KPC, the coexistence of NDMs and other carbapenemases (OXA-48) in *K. pneumoniae* has also been demonstrated globally (53, 55, 76, 77). Besides NDM-type carbapenemases, the IMP and VIM groups have also been identified over the world in *K. pneumoniae* (52, 56, 78). The *bla*$_{NDM}$ genes in *K. pneumoniae* have been illustrated on many broad-host-range plasmid types, including IncH, IncN, IncL/M, IncA/C, IncF, IncR, and IncX types (78-83). Many IncA/C plasmids with *bla*$_{NDM-1}$ also have different antibiotic resistance genes including 16S rRNA methylases (RmtC and RmtA), related to aminoglycoside resistance; CMY-type b-lactamases, linked with broad-spectrum cephalosporin resistance and QnrA, connected with quinolone resistance (15). Since the *bla*$_{NDM}$ genes are located in numerous broad-host-range plasmids, the expansion of NDM-1 is improved by horizontal gene transfer between bacteria (84). The OXA-48 is the most effective

class D carbapenemase for imipenem. It is also one of the most common class D carbapenemases (18). OXA-48 gene tendency has a propensity to be spread among enterobacterial species are higher than KPC and NDM genes (19, 85, 86). The current study showed that *bla*$_{\text{OXA-48}}$ gene, *bla*$_{\text{KPC}}$ gene, *bla*$_{\text{NDM-1}}$ gene control the carbapenem resistance in *K. pneumoniae* respectively. The prevalence of carbapenem resistance in *K. pneumoniae* showed variation in different cities of Iran. According to this survey, most of the studies were conducted in the central parts of the country. In these 21 studies, the most resistance-induced genes were related to OXA (class D), KPC (class A), NDM, VIM, IMP (class B). This variation may be due to geographical differences, inappropriate use of antibiotics, and lack of appropriate hospital supervision in antibiotics administration. Besides, it has been identified that important reservoirs of these carbapenemase producers act as significant origins for their global distribution. Therefore, healthcare workers, patients, or the hospital environment as reservoirs may have a role in nosocomial outbreaks. In this regard, restricted comprehensive surveillance programs addressing the prevalence of infection due to carbapenem resistance strains conducted in Iran. Typing the carbapenemase seems to be essential for managing the carbapenem resistance among Enterobacteriaceae.

Furthermore, fast identification of carbapenem-resistant strains is required for the efficient management of these infections (87). This review study involves 37 published reports from microbial resistance-inducing genes (i.e., *NDM, OXA, KPC, VIM, and IMP*) conducted on clinical specimens in different Iranian centers. The specimens include urine, blood culture, wound, sputum, intra-abdominal samples, cerebrospinal fluid, etc. In most of them, the gene expression data in different samples were not specified separately for each sample. According to the data presented in Table 2, OXA, KPC, NDM, VIM and IMP genes showed the highest antibiotic resistance frequency. Although primary Iranian studies demonstrated that the NDM gene was regarded as a rare gene in resistance development, recent studies show that this expression increases. Given that the studies were conducted in different geographical locations and different samples, the results are unique and not comparable.

Conclusion

In some hospitals in Iran, there is almost a high prevalence of carbapenem-resistant *K. pneumoniae* isolates. To hamper the further expansion of resistant isolates, appropriate and accurate detection of *bla*$_{\text{NDM}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{OXA}}$ genes in *K. pneuomoniae* by phenotypic and genotypic methods is essential. Fast and accurate characterization of the carbapenemase type found in *K. pneumoniae* is difficult by phenotypic antibiotic susceptibility tests. Therefore, novel molecular detection methods developed recently. Furthermore, hygiene conditions should be improved along with monitoring of drug-resistant isolates to better control this problem. In this study, we aimed to explore the frequency of carbapenem-resistant *Klebsiella pneumoniae* in Iran. Our results somehow can be helpful to reduce the mortality rate, cost of treatment as well as hospitalization time course.

Acknowledgment

Thanks to Mashhad University of Medical Sciences.

Funding information

There was no need for funding.

Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare no competing financial interest.

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