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HIGH PREVALENCE OF EXTENDED-SPECTRUM ß-LACTAMASE (BLACTX-M-15) AND NEW DELHI METALLO-B-LACTAMASE-1 (NDM-1) GENES AMONG HIGH-LEVEL CARBAPENEM RESISTANCE KLEBSIELLA PNEUMONIA: AN ALARM FOR OUR HEALTH SYSTEM

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ABSTRACT

Background: The **e**xtended-spectrum β-lactamase (ESBL) carbapenems-resistant *Klebsiella* isolates are considered one of the most significant challenging in the treatment of patients in hospitals. The aim of this study was to determine the prevalence of important carbapenem resistance genes ESBL subtypes and between *K. pneumoniae* from patients at hospital in Tehran, Iran.

Methods: Fifty-four isolates of K. *pneumoniae* were isolated from Shariatee Hospital in Tehran from February 2013 to July 2016. Antibiotic testing was done by using the standard disk diffusion method and E-test MIC. The confirmation of carbapenemase activity was performed using an MHT and a new method called the carbapenem inactivation method test (CIM). Finally, a polymerase chain reaction (PCR) and sequencing of related genes was performed.

Results: Our PCR data demonstrate that blaCTX-M group's 40 (81.4%) genes were the most prevalent in our hospital followed by group genes $bla_{CTX\text{-}M\text{-}3}$ (18.51%) and $bla_{CTX\text{-}M\text{-}2}$ (20.38%). The distribution of the CTX-M group revealed that $bla_{CTX\text{-}M\text{-}15}$ 23 (42.6%) was the dominant subtype. The coexistence of multiple genes included blaTEM, CTX-M and blaSHV, and CTX-M The presence of blaNDM1, blaOXA-48, and blaKPC were identified in the carbapenem-resistant isolates, 22 (40.7%), 10 (18.5%), and 7 (12.9%) respectively.

Conclusion: Our research showed that a CIM test for the first time in Iran is possible and has a high facility for the fast identification of carbapenem-resistant *Klebsiella* (CRK). We are encountered with the emergence of CTX-M, OXA-48, KPC, and NDM1 harboring CRK strains in our hospitals. Therefore, the treatment of patients infected with these isolates will be an important future concern in our clinical settings.

Running Head: Resistance genes among carbapenem-resistant *Klebsiella pneumonia*

Keywords: New Delhi metallo-beta-lactamase-1, Klebsiella pneumoniae, Carbapenem, Extended-spectrum ß-lactamase

HAUTE PREVALENCE DES GENES DE LA β-LACTAMASE A SPECTRE ETENDU (BLACTX-M-15) ET DU NOUVEAU DELHI METALLO-B-LACTAMASE-1 (NDM-1) PARMI LA RESISTANCE AU CARBAPENEM DE HAUT NIVEAU KLEBSIELLA PNEUMONIA: UN ALARME POUR NOTRE SYSTEME DE SANTE

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ABSTRAIT

Contexte: Les isolats de Klebsiella résistants aux carbapénèmes à β-lactamase à spectre étendu (BLSE) sont considérés comme l'un des défis les plus importants dans le traitement des patients en milieu hospitalier. Le but de cette étude était de déterminer la prévalence d'importants sous-types de BLSE de gènes de résistance au carbapénème et entre K. pneumoniae chez des patients hospitalisés à Téhéran, en Iran

Méthodes: Cinquante-quatre isolats de K. pneumoniae ont été isolés de l'hôpital Shariatee de Téhéran de février 2013 à juillet 2016. Le test des antibiotiques a été réalisé à l'aide de la méthode de diffusion sur disque standard et du test EIC MIC. La confirmation de l'activité de la carbapénémase a été réalisée à l'aide d'un MHT et d'une nouvelle méthode appelée test de la méthode d'inactivation du carbapénème (CIM). Enfin, une réaction en chaîne de la polymérase (PCR) et le séquençage de gènes apparentés ont été réalisés.

Résultats: Nos données de PCR démontrent que les 40 gènes du groupe blaCTX-M (81,4%) étaient les plus prévalents dans notre hôpital, suivis des gènes du groupe blaCTX-M-3 (18,51%) et blaCTX-M-2 (20,38%). La distribution du groupe CTX-M a révélé que blaCTX-M-15 23 (42,6%) était le sous-type dominant. La coexistence de plusieurs gènes comprenait blaTEM, CTX-M 14 et blaSHV, et CTX-M). La présence de blaNDM1, de blaOXA-48 et de blaKPC a été identifiée dans les isolats résistants au carbapénème, 22 (40,7%), 10 (18,5%) et 7 (12,9%) respectivement.

Conclusion: nos recherches ont montré qu'un test CIM pour la première fois en Iran est possible et dispose d'une grande facilité d'identification rapide de Klebsiella (CRK) résistant au carbapénème. Nous sommes confrontés à l'émergence de souches CTK-M, OXA-48, KPC et NDM1 hébergeant des souches CRK dans nos hôpitaux. Par conséquent, le traitement des patients infectés par ces isolats constituera une préoccupation future importante dans nos environnements cliniques.

Running Head: Gènes de résistance parmi la pneumonie à Klebsiella résistante au carbapénème

Mots-clés: métallo-bêta-lactamase-1 de New Delhi, Klebsiella pneumoniae, carbapénème, ß-lactamase à spectre étendu

INTRODUCTION

In recent years, the use of third-generation cephalosporin (TGC) has led to the promotion of a selection of β-lactamase producer bacteria that performs a hydrolysis of TGC, especially the oxyimino-cephalosporins [1,31] .These enzymes, capable of hydrolyzing the newer β-lactams, are referred to as extended-spectrum β-lactamases (ESBLs) [1,2]. At hospital sites, ESBL K. pneumoniae-resistant isolates are becoming an emerging public health concern. The ESBL-producing isolates of Klebsiella. spp. (ESBL-K) have been involved in several outbreaks of nosocomial infections throughout Asia [3], including Iran [4,32], the United States of America [5], the Far East, and Europe [6]. Recently, bla_{CTX-M}-related genes have appeared as the leading type of ESBLs in many parts of the world, including Asia, Europe [7], the United States [8], and South America [9]. On the basis of amino acid sequence, more than 60 variants that include CTX-M-45, CTX-M-25, CTX-M-9, CTXM-8, CTX-M-2, and CTX-M-1 [10]. These Klebsiella ESBL-producing isolates may be have. TEM, SHV, and CTX-M-β-lactamases [10]. ESBL-K strains are prevalent in Tehran hospitals, but relatively little data are available about β-lactamase genes. Moreover, carbapenem antibiotics are useful for treating infections caused by ESBL-producing gram-negative bacteria. In recent years, carbapenem resistance is considered one of the most significant challenges in the treatment of patients in hospitals. The rapid spread of the carbapenem-hydrolyzing β-lactamase KPC between Klebsiella isolates CR in the Tehran region has turned alarming. Treatment of infection caused by this pathogen has become a matter of significant challenge in hospitals in Iran due to its high levels of resistance to virtually all classes of antibiotics. Therefore, the identification of KPC resistance is important for the appropriate choice of antibiotic treatment in addition to infection control measures to thwart the distribution of resistant Klebsiella strains in hospital settings. The aim of this study was to determine the prevalence of ESBL subtypes and important carbapenem resistance genes (KPC/OXA-48/NDM1) among Klebsiella cultured from patients at a hospital in Tehran, Iran.

MATERIALS AND METHODS

Bacterial strains

The present work is a descriptive cross-sectional study carried out from August 2013 to July 2016. Fifty-four high-level resistant isolates of *K. pneumoniae* were obtained from Shariatee Hospital in Tehran between February 2013 and July 2016. The *K.*

pneumoniae 7881 strain containing the blaSHV and blaTEM genes was used as a control. The samples yielding the isolates were obtained from different wards, including the ICU and ICU General, Neurology, Internal ICU, Post HSCT, Post-hematopoietic Stem Cell Transplantation, BAL (Bonchoalveolar lavage), Blood, Hematology, Oncology, Emergency, and Gland wards. Antibiotic susceptibility was checked as recommended by the Clinical and Laboratory Standards Institute (CLSI) procedure [11]: Ceftriaxone (CRO: 30 µg), Ceftazidime (CAZ: 30 µg), Cefotaxime (CTX: 30 µg), Imipenem (IMP: 10µg), Meropenem, Ampicillin-sulbactam, (ZOX: 30 µg), Gentamicin (GM: 10 µg), Amikacin (AN: 30 µg), Ciprofloxacin (CIP: 5 µg) (BBL), and K. pneumoniae ATCC 700603 were applied as the control in all tests. The Minimum Inhibitory Concentrations (MICs) of Imipenem (IMP: 10 µg) (MAST, Merseyside, UK) against isolates showing reduced susceptibility to this carbapenem was determined using the E-test MIC assay. Antibiotic susceptibility testing was performed as recommended by the Clinical Laboratory Standards Institute (CLSI) guideline and, using disks, Escherichia coli 25922 was applied as a control. The MICs for Cp, IMP, MP CAZ, and CRO were determined by the E-test method according to CLSI guidelines. Isolates exhibiting MIC ≥4 µg were screened for the production of ESBL.

Modified Hodge Testing (MHT)

The confirmation of carbapenemase activity was done using an MHT as previously stated [11]. The K. pneumonia, a positive control MHT Positive Klebsiella pneumoniae ATCC1705, and MHT Negative Klebsiella pneumoniae ATCC1706 were used as a positive control. In plates, the presence of a distorted or clover leaf-shaped inhibition zone was considered as positive for carbapenemase-producing isolates, as recommended by the document M100-S24 of the Clinical and Laboratory Standards Institute methods [11,12].

Carbapenem Inactivation Method Test (CIM)

The CIM was performed as previously stated with brief changes [3]. The isolates were cultured on MHA (Mueller-Hinton agar) plates and a full 12-µl inoculation loop of each strain was immersed in 420 µl of sterile distilled water, and an active susceptibility meropenem disc (MEM) was homogenized in the solution. After two hours incubation at 35°C, the disk was detached and placed on an MHA plate inoculated with a suspension of OD595 1.25 (correlates with a McFarland value of 0.5) standard of susceptible E. coli indicator strain ATCC 25922 with a sterile cotton swab. Finally, incubation of the plate was done at 35°C for 24 h. The results of the test were surveyed after overnight incubation: The inhibition zone in plates around each disk was measured. Plates with inhibition circles <10 mm in diameter were considered to indicate CIM positivity [13-15].

Polymerase chain reaction amplification (PCR) and DNA sequence analysis

The total genomic DNA from the clinical isolates was extracted by the boiling method: four colonies of each isolate in 600 ml of distilled water for 12 min. and centrifuged at 11,000 rpm for 12 min. The supernatant was used for the PCR test. PCR amplification was performed using the specific primers for the blaKPC family, Uni-KPC-F (5′-ATGTCACTGTATCGCCGTCT-3′) and Uni-KPC-R (5′-TTACTGCCCGTTGACGCCC-3′) genes as previously described(16). PCR amplification and primers for the detection of blaCTX-M, blaTEM, blaSHV, blaKPC, blaOXA-48-like, blaNDM, blaVIM, and blaIMP genes was carried out as previously described by Hosseinzadeh et al. and Fursova et al [17,18,33,34]. The sequences were aligned and compared using the online BLAST software, (BLAST http: //www.ncbi.nlm. nih.gov).

RESULTS

Of the eight available antibiotics, the most effective was colisitin (100% of the strains were susceptible). The results of the antimicrobial susceptibility test are shown in Table 1. Distribution of collected samples included BAL 22 (40.7%), Wound 5 (9.2%), Urine 9 (16.6%), Blood 9 (16.6%), Plural 3 (5.5%), Sputum 4 (7.4%), Abscess 1 (1.8%), and Abdominal 1 (1.8%) (Fig.4). Klebsiella spp. was detected in 25.9% (n=14) isolates in the <50 years age group and 70.0% (40) isolates in the ≥ 50 years age group, but this difference was not statistically significant (P > 0.05). Thirty-one (57.4%) males and 23 (42.5%) females were infected with Klebsiella (Fig.3). Isolates with positive results of the carbapenem inactivation method test (CIM) and modified Hodge testing (MHT) were considered carbapenem-resistant. MIC results for five antibiotics Cp, IMP, MP, CAZ, and CRO showed a high level of resistance (MIC≥4). The phenotypic confirmatory test showed that among the 54 isolates, 54 (100%) were ESBL producers (14). The PCR test of the ESBL-producing isolates showed that TEM 24 (44.4%) isolates were positive for the bla_{TEM} gene, 40 (80.1%) positive for the bla_{CTX-M} groups, and 30 (55.6%) isolates had the bla_{SHV} gene. Moreover, the SHV types were characterized as SHV-27, SHV-11, and SHV-1.

TABLE 1: THE DETAILED RESULTS OF CARBAPENEM-RESISTANT ISOLATES

Phenotypic Tests			MIC value (µg/ml)					Hospital unit	Isolation date Date	Age	Sex	Site of Isolation	Strain	Gene		N
MHT	CIM	DDST	CRO	CAZ	MP	IMP	Cp							ESBL	CP Genes	
Positive	Positive	Positive	12	192	24	32	8	Internal ICU	December /2016	26	M	BAL	klebpne	blaCTX-M15	bla NDM-1	1
Positive	Positive	Positive	16	192	32	32	24	ICU General	November/ 2016	71	F	BAL	Kleb spp.	blaCTX-M15	blaNDM-1	2
Positive	Positive	Positive	32	192	32	32	12	Neurology	November/ 2016	14	M	BAL	klebpne	blaCTX-M15	blaNDM-1	3
Positive	Positive	Positive	32	256	32	32	32	Neurology	November/ 2016	51	F	Wound	Kleb spp.	blaCTX-M15	blaNDM-1	4
Positive	Positive	Positive	24	256	32	24	24	Urology	December /2016	59	M	Urine	klebpne	blaCTX-M15	blakpc	5
Positive	Positive	Positive	32	256	24	32	32	Oncology	December /2016	62	M	BAL	Kleb spp.	blaCTX-M15	blakpc	6
Positive	Positive	Positive	16	256	32	32	12	Internal ICU	December /2016	90	M	Wound	klebpne	blaCTX-M15	-	7
Positive	Positive	Positive	32	192	32	32	12	Internal ICU	December /2016	69	F	BAL	klebpne	blaCTX-M15	-	8
Positive	Positive	Positive	24	128	32	32	24	CCU (Heart)	December /2016	75	M	BAL	Kleb spp.	blaCTX-M15	-	9
Positive	Positive	Positive	32	192	32	32	24	Urology	November/ 2016	86	M	BAL	klebpne	blaCTX-M2	blaNDM-1	10
Positive	Positive	Positive	24	256	32	24	32	Glands	November/ 2016	75	F	BAL	Kleb spp.	blaCTX-M15	blakpc	11
Positive	Positive	Positive	32	256	32	32	32	General Internal	December /2016	90	F	Blood	Kleb oza	blaCTX-M15	blakpc	12
Positive	Positive	Positive	32	256	32	32	32	ICU General	December /2016	72	M	BAL	Kleb spp.	blaCTX-M15	blaNDM-1	13
Positive	Positive	Positive	32	256	24	32	32	Oncology	December /2016	53	M	Wound	Kleb spp.	blaCTX-M15	blakpc	14
Positive	Positive	Positive	24	192	32	32	24	General Internal	December /2016	76	M	Sputume	Kleb spp.	blaCTX-M15	blaOXA-48	15
Positive	Positive	Positive	32	256	24	32	32	Oncology	December /2016	65	M	Wound	Kleb spp.	TEM,SHV	-	16
Positive	Positive	Positive	32	192	32	24	12	Neurology	December /2016	68	M	BAL	Kleb spp.	blaCTX-M3	blaNDM-1	17
Positive	Positive	Positive	24	256	32	32	12	General Internal	December /2016	86	M	Sputume/trach	klebpne	blaCTX-M15	blaOXA-48	18
Positive	Positive	Positive	32	256	32	32	24	ICU General	December /2016	64	M	Blood	Kleb spp.	blaCTX-M3	blaNDM-1	19
Positive	Positive	Positive	32	256	32	32	24	ICU General	August /2015	59	F	BAL	klebpne	blaCTX-M15	-	20 G
Positive	Positive	Positive	24	256	32	24	32	Oncology	October/ 2015	55	F	Urine	Kleb spp.	TEM,SHV	-	21
Positive	Positive	Positive	24	192	32	32	32	Neurology surgery	September/ 2015	37	F	Blood	Kleb spp.	blaCTX-M2	blaOXA-48	22
Positive	Positive	Positive	32	256	32	24	32	Oncology	September/ 2015	16	F	Abscess	klebpne	blaCTX-M15	-	23
Positiv	Positiv	Positi	24	256	32	32	32	General	October/	64	F	Urine	kleb	blaCTX	bla	24

e	e	ve						Internal	2015				pne	-M3	ND M-1	
Positive	Positive	Positive	24	192	24	24	24	General Internal	October/ 2015	70	F	BAL	klebpne	blaCTX-M15	blaND M-1	25
Positive	Positive	Positive	24	256	32	32	32	ICU nerves	October/ 2015	32	F	Urine	klebpne	blaCTX-M3	blaOXA-48	26
Positive	Positive	Positive	32	256	32	32	12	ICU General	October/ 2015	70	F	BAL	Kleb spp.	TEM,SHV	blaND M-1	27
Positive	Positive	Positive	32	256	32	32	12	ICU General	October/ 2015	88	F	BALV	klebpne	blaCTX-M15	blaOXA-48	28
Positive	Positive	Positive	24	192	32	24	12	Emergency Blood	September/ 2015	64	M	Blood	klebpne	blaCTX-M2	-	29
Positive	Positive	Positive	12	256	24	32	24	ICU General	December/ 2015	65	M	BAL	klebpne	TEM,SHV	blaND M-1	30
Positive	Positive	Positive	24	192	32	24	32	ICU General	August /2015	50	M	BAL	Kleb spp.	blaCTX-M3	blaOXA-48	31
Negative	Negative	Positive	12	192	24	32	24	ICU General	December /2015	72	F	BAL	Kleb spp.	blaCTX-M3	-	32
Positive	Positive	Positive	24	256	24	12	32	surgery room	September/ 2015	46	M	Abdominal F	klebpne	TEM,SHV	blaND M-1	33
Positive	Positive	Positive	24	192	24	12	32	Lung	December /2015	78	F	BAL	Kleb spp.	TEM,SHV	blaOXA-48	34
Positive	Positive	Positive	24	256	32	24	32	ICU General	August /2015	49	M	BAL	klebpne	blaCTX-M2	-	35
Positive	Positive	Positive	24	256	32	32	32	Blood	October/ 2015	59	M	Blood	klebpne	blaCTX-M15	blaND M-1	36
Positive	Positive	Positive	24	192	24	32	32	General Internal	September/ 2015	64	F	Wound	Kleb spp.	blaCTX-M15	blaND M-1	37
Positive	Positive	Positive	32	256	24	32	24	ICU General	September/ 2015	39	M	Urine	Kleb spp.	blaCTX-M3	blakpc	38
Positive	Positive	Positive	32	256	24	32	32	ICU General	May /2015	56	M	Plural	klebpne	blaCTX-M3	blaOXA-48	39
Positive	Positive	Positive	32	256	24	32	32	General Internal	May /2015	80	M	Sputum	Kleb spp.	blaCTX-M2	blaND M-1	40
Positive	Positive	Positive	32	192	24	32	32	General Internal	May/ 2015	58	F	Sputum	Kleb spp.	blaCTX-M3	blaND M-1	41
Positive	Positive	Positive	32	256	32	32	32	Hematology	May /2015	69	M	Blood	klebpne	blaCTX-M2	blaND M-1	42
Positive	Positive	Positive	16	256	32	32	24	ICU General	April /2016	59	M	Blood	klebpne	blaCTX-M2	-	43
Positive	Positive	Positive	32	256	32	24	32	Oncology	March /2016	77	M	Urine	klebpne	blaCTX-M3	blakpc	44
Positive	Positive	Positive	32	256	32	32	12	ICU (Heart)	April /2016	30	M	BAL	Kleb spp.	blaCTX-M2	blaND M-1	45
Positive	Positive	Positive	32	256	32	32	24	Oncology	April /2016	30	F	Urine	klebpne	TEM,SHV	blaOXA-48	46
Positive	Positive	Positive	32	192	32	32	32	Emergency (Blood)	April /2016	43	M	Plural	klebpne	TEM, SHV	blaND M-1	47
Positive	Positive	Positive	32	256	32	32	32	ICU General	April /2016	46	F	BAL	klebpne	blaCTX-M15	-	48
Positive	Positive	Positive	32	256	32	32	32	Emergency (Blood)	April /2016	43	M	Plural	klebpne	TEM,SHV	-	49
Positive	Positive	Positive	32	256	24	32	32	Emergency	March /2016	43	M	Blood	klebpne	blaCTX-M2	-	50

								(Blood)								
Positive	Positive	Positive	16	256	32	32	32	POST HSCT	April /2016	50	F	BAL	Kleb spp.	TEM,SHV	-	51
Positive	Positive	Positive	32	256	24	32	32	POST HSCT	April /2016	33	F	Urine	kleb pne	blaCTX-M2	blaNDM-1	52
Positive	Positive	Positive	32	192	32	24	24	ICU (Heart)	April /2016	30	M	Urine	kleb pne	blaCTX-M2	blaOXA-48	53
Positive	Positive	Positive	32	256	32	32	32	ICU	March /2016	72	F	Blood	kleb pne	blaCTX-M15	blaNDM-1	54

Abbreviations: POST HSCT; Post-hematopoietic stem cell transplantation, BAL; Broncho alveolar lavage, MIC = Minimum inhibitory concentration; IMP = Imipenem; MHT = Modified hodge test; DDST = Double disk synergy test; ICU = Intensive care unit; Cp= Ciprofloxacin;IMP= Imipenem; MP= Meropenem; CAZ= Ceftazidime; CRO=Ceftriaxone ; CIM= Carbapenem Inactivation method; N= Isolates number; F=Female;M=Male; Cp Genes= Carbapenem Genes;

Coexistence of multiple gene-encoding ESBLs identified among the isolates included bla_{TEM}, CTX-M bla_{SHV}, and bla_{TEM}, SHV (Fig. 1). Our PCR data demonstrated that among the *K. pneumoniae* clinical strains, $bla_{CTX\text{-}M15}$ 23 (42.6%) gene were the most prevalent in our hospital followed by group genes $bla_{CTX\text{-}M2}$ 11 (20.38%) and $bla_{CTX\text{-}M3}$ 10 (18.51). The distribution of CTX-M groups revealed that $bla_{CTX\text{-}M\text{-}15}$ was the dominant subtype. In the present study, all 44 CTX-M–harboring Klebsiella isolates studied were found to be resistant to cefotaxime and ceftazidime by using the standard disk diffusion method and E-test MIC, according to the CLSI guidelines. Of the 55 high level resistance Klebsiella included in the study, 40 (74.0%) were found, by the PCR reaction test, to carry a carbapenemase-encoding gene, and all PCR-positives (100%) were shown to produce carbapenemase by the CIM except one isolate (Table 2). The results of MHT in all ESBL isolates were positive. BlaNDM1, blaOXA-48 and blaKPC were identified in carbapenem-resistant isolates, 22 (%40.7), 10 (%18.5), and 7 (%12.9) respectively.

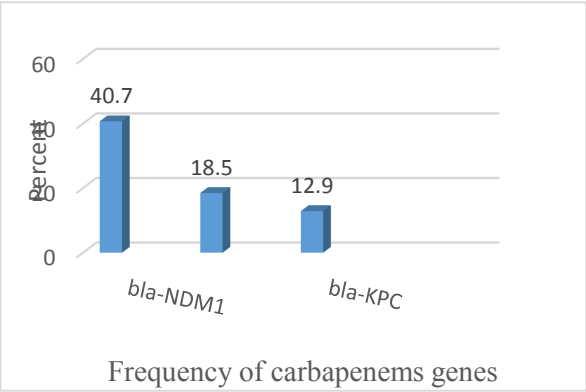

Fig1: Distribution of important Carbapenems resistance genes (KPC, OXA-48, and NDM1) among *Klebsilla* cultured from patients

Fig2: The MHT performed on a Muller Hinton Agar plate. (1) MH positive result

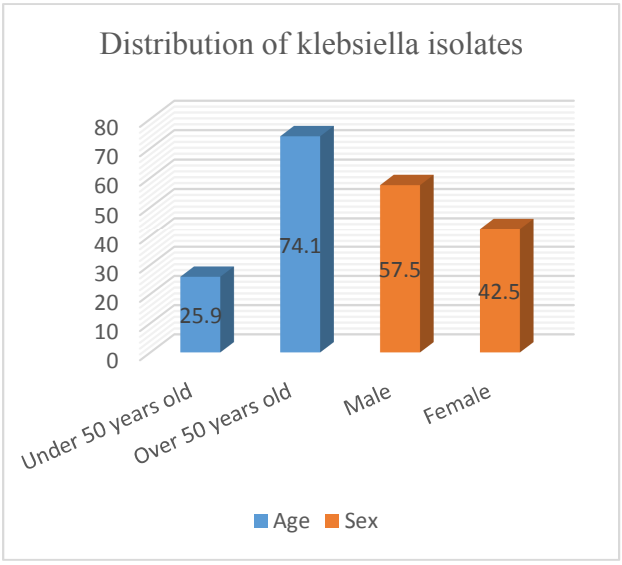

Fig3: Distribution of Klebsiella isolates based on Sex and Age

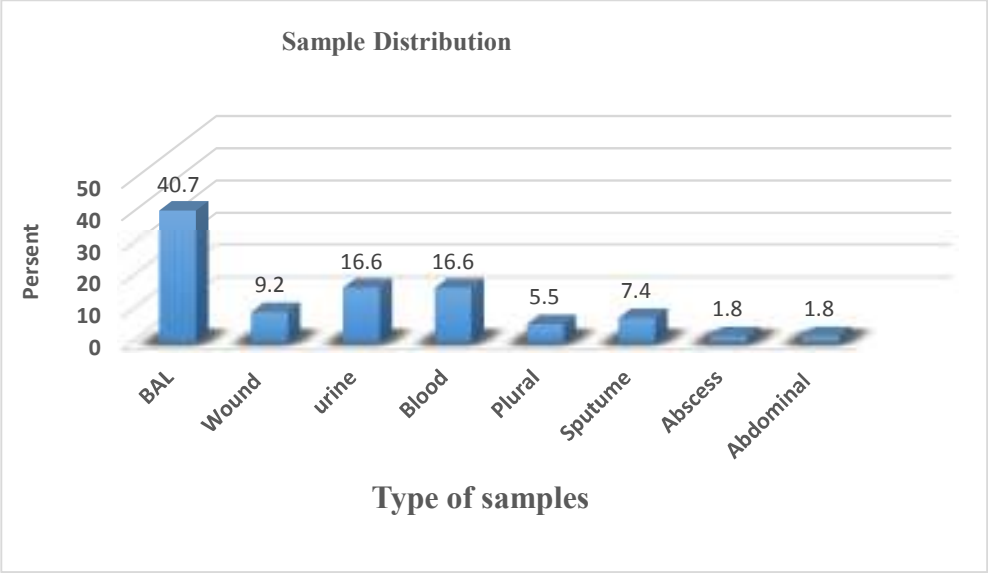

Fig4: Distribution of Klebsiella positive samples

DISCUSSION

ESBL carbapenem-resistant Klebsiella (CRK) strains have become globally disseminated in recent years, causing concern over the control of hospital infections [14]. In this situation, with increasing high-level resistant isolates, early detection of CPK strains is critical. A study by van der Zwaluw et al. [15] showed that CIM is a new method having high specificity and sensitivity for detecting carbapenemase producers. The modified Hodge test and CIM methods were used in our research. Results demonstrated that, of the 54 isolates, only one was CIM-negative (2.0%), while all the other isolates emerged positive in the modified Hodge test. The application of the CIM test for the detection of carbapenem-resistant isolates in our study showed it is capable of detecting carbapenemase production. Using PCR, 40 (74%) of these isolates were found to contain a carbapenemase-encoding gene. These results of CIM are consistent with other findings stated by some other researches [14,15-19]. With respect to our research, CIM for the first time in Iran was found to be very efficient and low-cost in the recognition of carbapenemase-producing K. *pneumoniae* isolates. In the clinical setting, the distribution of ESBL-producing *K. pneumoniae* was considered an important therapeutic and epidemiological concern. During the past decade, CTX-M.15, CTX-M-14, and CTX-M-2 have been the most prevalent CTX-M enzyme in different European countries and Iran [20,21]. The present study further showed that the CTX-M_1 group had a high (92.7%) prevalence in clinical ESBL-producing isolates, and the most common subtype was CTX-M_{15}. In a study by Agamy et al. in Saudi Arabia, a high number of isolates (97.3%) carried the bla_{SHV} gene [22]. Moreover, Ghafourian et al. [23], in contrast to our results in Iran, reported that 94% of K. pneumoniae strains harbored the bla_{SHV} genotypes. In our study, TEM-1 and SHV-1 subtypes were the majority of subtypes of TEM and SHV in K. spp. strains. Further, it characterized all the K. pneumoniae isolates from the Labbafi Nejad and Zahedan hospitals that carried the SHV-11, TEM-1 subtypes, SHV-5, and SHV-12 were reported to be the dominant ESBLs in Iran [24,25]. In agreement with our study, Dedeic-Ljubovic et al. confirmed the presence of CTX-M-15 in KPC isolates, which belongs to the CTX-M-1 group [20], in one of the most important hospitals in Bosnia-Herzegovina. Researchers in South America introduced CTX-M-2 and CTX-M-8 enzymes as the predominant ESBL types [26]. Similar to our finding, in United States, CTX-M-15 was most common genotype but the CTX-M-4 and CTX-M-2 groups were rarely detected [26]. In contrast, with a low frequency of the CTX-M genotype among KP in the United States, our region showed a wide range of strains (high prevalence of carbapenem-resistant strains harbored bla_{CTX-M}). Of 55 K. pneumoniae isolates, 22 (40.7) were bla_{NDM-1} positive. Our findings and previous studies indicate that the frequency of bla_{NDM-1} harboring K. pneumoniae strains in Iranian hospitals show an increasing trend. A research by Zowawi et al. showed that the most common carbapenemase were 16 isolates of NDM and 35 isolates of OXA-48 types [27]. In our finding, the blaOXA-48-like gene was identified in 10 (18.5) isolates; while two isolates were positive in MHT and

CIM. Phenotype OXA-48-positive in KPC strains has been stated in several regions, including France, Russia, Turkey, Saudi Arabia, Taiwan, and China, and can be a serious concern in hospital settings [17]. In our study, a small number of carbapenem-resistant isolates lacked the targeted genes. The reasons for this may be the reduced permeability of the outer membrane, presence of other genes, AmpC betalactamases, and an extended-spectrum beta-lactamases (ESBLs) [17,28,29]. In the present study, we detected the bla_{KPC} gene in just seven (12.9) CR isolates that were susceptible to tigecycline and polymyxin B. Similar to our findings, other KPC-harboring isolates revealed a resistance to the majority of antimicrobial agents [30]. In summary, we have described for the first time the high coexistence of blaKPC/NDM1/OXA-48 and bla_{CTX-M} -producing Klebsiella. spp. in KP isolates in Tehran, and characterized the CIM method as being efficient for the detection of CRK strains in hospital settings in Iran.

Conclusion: Our research confirmed that the CIM test is practicable in our laboratory and has a high capability of fast detection of CRK. We are faced with the emergence of CTX-M, OXA-48, KPC, and NDM_1 harboring CRK strains in our hospitals. All isolates showed a high level of resistance (ESBL-positive/carbapenem-positive), indicating a situation that was considered to pose a future threat and highlighting the necessity of further surveillance in a hospital setting. So, with the rapid emergence of multi-drug resistance isolates over time, the prevention of the spread of the CPK and the initiation of appropriate antimicrobial therapy will be a matter of serious concern in our clinical settings.

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