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Genetic relationship, antibiotic resistance pattern and virulence factors of *Klebsiella pneunoniae* strains isolated from meningitis patients

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ABSTRACT

Backgroud: Meningitis due to *Klebsiella pneunoniae* is increasingly reported from several Asian countries. Microbiological characteristic of *K. pneumoniae* strains causing meningitis is not yet explored in Iran. The aim of this study was to demonstrate antibiotic resistance pattern and virulence factors, as well as the genetic relationship of *K. pneumoniae* strains isolated from meningitis patients.

Materials and Mehtods: Eight *K. pneumoniae* isolates were collected from hospitalized patients at Imam Khomeini hospital, Tehran-Iran from 14 May 2018 to 15 Mar 2020. The antibiotic resistance pattern was determined by disc diffusion method. Antibiotic resistance genes and virulence-associated genes were traced by Polymerase Chain Reaction. Repetitive Extragenic Palindromic Polymerase Chain Reaction was used for evaluation of the genetic relationships among isolates.

Findings: Six out of eight isolates were resistant to almost all the 15 antibiotics tested. These six isolates harbored triple antibiotic resistance genes: $bla_{\text{OXA-48}}$, $bla_{\text{SHV,}}$ and *aac (6')-Ib*. Five of these isolates co-harbored *aac (3)-IIa* gene. Besides, bla_{TEM} and $bla_{\text{CTXM-1}}$ were detected in five and three isolates respectively. $bla_{\text{NDM-1}}$ was identified in one isolate. All isolates harbored *acrAB*, *ompK36*, and *tolC* resistance genes. None of the isolates were related to *K1* or *K2* capsular serotypes. The most commonly detected virulence genes were *entB* (100%), *mrkD* (100%), *ybtS* (62.5%) and *kfu* (25%). rep-PCR fingerprinting discriminated seven isolates into three clusters.

Conclusion: Simulations presence of the genes coding for Extended Spectrum Beta Lactamases, Carbapenemases, and Aminoglycoside Modifying Enzymes narrows therapeutic alternatives and imposes a heavy load on the public health system. Antibiotic susceptibility test is recommended however, Carbapenems can still be considered as first-line medications for preliminary empirical treatment of *K. pneumoniae* meningitis before obtaining susceptibility test results. Low genetic diversity suggests the circulation of certain *K. pneumoniae* clones in hospital which highlights the establishing of effective infection observation and prevention program.

Keywords: *Klebsiella pneumoniae,* antibiotic resistance, virulence genes, cerebrospinal fluid

INTRODUCTION

Despite the introduction of new antibiotics, the mortality rate of acute bacterial meningitis (ABM) is still high globally and the comparative proportion of organisms causing the disease has changed recently (Lu et al., 2002). Even though meningitis caused by *K. pneumoniae* was rare in the past, after implementation of vaccination against Gram positive organisms that more frequently caused bacterial meningitis an increase has been observed in the incidence of the meningitis caused by Gram-negative pathogens such as *Escherichia coli* and *K. pneumoniae* (Ellis et al., 2019; Scheld et al., 2002). Currently two evolutionary divisions exist for *K. penumoniae*: classical *K. pneumoniae* (cKP) which most commonly infects people with immune defect, especially diabetics and patients struggling with malignancies, and hyper virulent *K. pneumoniae* (hvKP) that can infect immune deficient as well as immunocompetent hosts (Paczosa et al., 2016).

The capability of *K. pneumoniae* for establishing a successful infection stems from possession of numerous virulence factors most of which fall in the following classes: Capsule, lipopolysaccharide (LPS), siderophores, pili, outer membrane proteins (OMPs), iron transport systems, and genes that contribute in allantoin metabolism (Li et al., 2014; Paczosa et al., 2016). *K. pneumoniae* capsular polysaccharide (K antigen) is a crucial virulence factor that can be used for typing of *K. pneumoniae* isolates to different serotypes (Choi et al., 2020). Amongst 78 capsular serotypes that are currently identified in *K. pneumoniae*, a few serotypes (chiefly K1 and K2) have hyper-mucoid phenotype and the measure of mucoidy seems to be associated with the invasiveness of infections they produce (Li et al., 2014).

Infections caused by multidrug-resistant (MDR) *K. pneumoniae* frequently result in failure of treatment and longstanding hospitalization (Paterson et al., 2005). *K. pneumoniae* utilizes two efflux pump systems: The *AcrAB-TolC* and *mdtK*, to expel antimicrobial drugs from its cell that can lead to emergence of MDR organisms (Wasfi et al., 2016). Two outer-membrane proteins: *ompK35* and *ompK36* are in charge of creating pores in the outer membrane of Gram-negative bacteria thorough which cephalosporin and carbapenem antibiotics enter the cell. Loss of or mutation in these porins can bring about organisms that might have reduced susceptibility to the mentioned antibiotics (Shi et al., 2013).

Treatment of infections caused *K. pneumoniae* has become more complex since it acquired plasmid-encoded extended-spectrum beta-lactamase (ESBL) genes (El Fertas-Aissani et al., 2013). ESBLs are enzymes produced by certain bacteria that hydrolyze penicillins, the first three generations of cephalosporins, and aztreonam (Paterson et al., 2005). Hence, carbapenems are extensively used as the last line agents for the treatment of infections caused by ESBL producing *K. pneumoniae* strains as their β-lactam ring is more resistant to hydrolysis by these enzymes (Shi et al., 2013). However, resistance to carbapenems can be created by several mechanisms mainly combination of porin loss with the expression of ESBLs, and the production of carbapenemases (Paterson et al., 2005). Carbapenemases are recently emerged β-lactamase enzymes that are able to hydrolysis carbapenems (Queenan et al., 2007).

Therapeutic options for treatment of carbapenemase-producing *K. pneumoniae* strains are limited and mainly relies on use of tigecycline, colistin, fosfomycin, and aminoglycosides (Falagas et al., 2007). Aminoglycosides are most commonly used for this purpose because of their wide availability and cost effectiveness (Forge et al., 2000). Nevertheless, aminoglycoside resistance is a growing challenge that occurs principally due to two

phenomena: enzymatic modification of drugs and target site modification in bacteria that are mediated by aminoglycoside modifying enzymes (AMEs) and 16S rRNA methylase genes, respectively (Krause et al., 2016). Molecular genetic techniques are valuable tools for the assessment of genetic relationship between bacterial isolates, especially if there is a suspected outbreak. Repetitive Extragenic Palindromic Polymerase Chain Reaction (rep-PCR) offers speedy typing results that are cheap and reproducible with a fairly high differentiating power (Nielsen et al., 2011). Studies have already shown the applicability of rep-PCR method for the study of *K. pneumoniae* epidemiology (Hou et al., 2015; Nielsen et al., 2011).

In recent decades increasing reports of *K. pneumoniae* meningitis has been published from several Southeast Asian countries (Ku et al., 2017; Xu et al., 2019). This high prevalence has been linked with the emergence of hvKP strains in that region and the mortality rate is reported from 48.5% to 66% (Ku et al., 2017). Yet there is no data regarding antibiotic resistance pattern and virulence profile of *K. pneumoniae* strains causing meningitis in Iran. In the current study, we investigated antibiotic resistance pattern, most common virulence genes, and genetic relationships among *K. pneumoniae* strains isolated from CSF of meningitis patients admitted to a large hospital in Tehran.

MATERIALS AND METHODS

Bacterial isolates and identification

In this descriptive cross-sectional study conducted during 14 May 2018 to 15 March 2020, a total of eight *K. pneumoniae* CSF isolates were obtained from laboratory staff of Imam Khomeini hospital (a 1400 bed teaching hospital in Tehran). *K. pneumoniae* isolates were identified by conventional biochemical tests and were further confirmed by PCR amplification of *khe* gene which is specific for *K. pneumoniae* (Wong et al., 2014). Table 1 shows characteristic of primers used for amplification of *Khe* gene.

Table 1. characteristic of primers used for genotypic detection of *K. pneumoniae* isolates

Gene	Primer sequence (5’ to 3’)	Product size (bp)	Tm (C)
khe	F: GGCGAGGTTTACGTCTCAAC	271	57
	R: GTACTTCTTGTTGGCCTCGC		57

Antimicrobial susceptibility testing

Antibiotic susceptibility of *K. pneumoniae* isolates was determined by the disc diffusion method (Kirby Bauer) according to Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2019). The following antibiotic discs were used for this purpose: ciprofloxacin (5µg), augmentin (10 µg), cefoxitin (10 µg), cefotaxime (10µg), ceftriaxone (10µg), azterionam (30 µg), trimetoprim/sulfamethoxazole (1.25/23.75 µg), ampicillin (10 µg), piperacillin tazobactam (100/10µg), tetracycline (30µg), cefipime (30 µg), gentamicin (10 µg), ceftazidime (30 µg), imipenem (10µg) and meropenem (10µg). *Escherichia coli* ATCC 25922 was used as the positive control.

PCR amplification of antibiotic resistance genes

Extraction of DNA was performed by boiling method. PCR was conducted for amplification of ESBL genes (*blaSHV, blaTEM, blaCTXM-1*), carbapenemase encoding genes (*blaKPC, blaNDM-1, blaIMP, blaOXA-48*), and genes encoding aminoglycoside modifying enzymes (AMEs) and 16 SrRNA methylases (*aac (6')-Ib, aac (3)-IIa, aac (3)-Ia, aac (3)-IVa, ant (2'')-Ia, ant (4')-IIa, aph (3')-Ia, armA, rmtB* and *rmtC*) using previously

described primers. Table 2 demonstrates sequences and characteristics of primers used for amplification of most of these genes.

Table 2. Characteristics of primers used for amplification of antibiotic resistance genes.

Gene	Primer sequence (5' to 3')	Product size (bp)	Tm (C)	Reference
aac(6') -Ib	F: TTGCGATGCTCTATGAGTGGCTA	482	59	(Fernández et al., 2015)
	R: CTCGAATGCCTGGCGTGTTT			
aac(3') -IIa	F: GGCAATAACGGAGGCGCTTCAAAA	536	63	(Fernández et al., 2015)
	R: TTCCAGGCATCGGCATCTCATACG			
aac(3)- Ia	F: GCAGTCGCCCCTAAAACTAA	464	62	(Fernández et al., 2015)
	R: CACTTCTTCCCGTATGCCCAACTT			
aac(3)-IVa	F: TCGGTCAGCTTCTCAACCTT	314	56	(Fernández et al., 2015)
	R: GATGATCTGCTCTGCCTGTG			
ant(2'')-Ia	F: ACGCCGTGGGTCGATGTTTGATGT	572	65	(Fernández et al., 2015)
	R: CTTTTCCGCCCCGAGTGAGGTG			
ant(4')- IIa	F: CCGGGGCGAGGCGAGTGC	423	66	(Fernández et al., 2015)
	R: TACGTGGGCGGATTGATGGGAACC			
aph(3')- Ia	F: CGAGCATCAAATGAAACTGC	624	55	(Fernández et al., 2015)
	R: GCGTTGCCAATGATGTTACAG			
armA	F: CCGAAATGACAGTTCCTATC	846	55	(El-Badawy et al., 2017)
	R: GAAAATGAGTGCCTTGGAGG			
bla_{IMP}	F: GGAATAGAGTGGCTTAAYTCTC	232	58	
	R: GGTTTAAYAAAACAACCACC			
bla_{NDM}	F: GGTTTGGCGATCTGGTTTTC	621	58	
	R: CGGAATGGCTCATCACGATC			
rmtB	F: ATGAACATCAACGATGCCCTC	769	60	(El-Badawy et al., 2017)
	R: CCTTCTGATTGGCTTATCCA			
Bal_{OXA-48}	F: GCGTGGTTAAGGATGAACAC	438	58	(Nordmann et al., 2011)
	R: CATCAAGTTCAACCCAACCG			
rmtC	F: CAGGGGTTCCAACAAGT	246	55	(Guven et al. 2016)
	R: GAAGAGTATATAGCTTGAACATAAGTA			
bla_{KPC}	F: CGTCTAGTTCTGCTGTCTT	796	58	
	R: CTTGTCATCCTTGTTAGGC			

Detection of capsular serotypes and virulence-associated genes

Genes specific for two invasive capsular serotypes (K1 and K2) were investigated by PCR as described previously (Compain et al., 2014). Virulence-associated genes (*entB, iutA, ybtS, magA, rmpA, mrkD, allS, kfu*, and *traT*), and genes encoding efflux pump systems and outer-membrane porins (*acrAB, mdtK, tolC, ompK35*, and *ompK35*) were traced by PCR using primers previously described. Table-3 presents characteristics of primers used for amplification of K1 and K2 specific genes and some important virulence associated genes.

Table 3. Primers used for amplification of K1 and K2 specific genes and some important virulence genes

Gene	Primer sequence (5' to 3')	Product size (bp)	Tm (C)	Reference
ybtS	F: GACGGAAACAGCACGGTAAA	242	53	(Compain et al., 2014)
	R: GAGCATAATAAGGCGAAAGA			
entB	F: GTCAACTGGGCCTTTGAGCCGTC	400	63	(Compain et al., 2014)
	R: TATGGGCGTAAACGCCGGTGAT			
K2	F; CAACCATGGTGGTCGATTAG	532	55	(Compain et al., 2014)
	R: GGGTCTGGCGCAGAGTATGC			
Kfu	F: GGCCTTTGTCCAGAGCTACG	637	61	(Compain et al., 2014)
	R: GGGTCTGGCGCAGAGTATGC			
iutA	F: GGGAAAGGCTTCTCTGCCAT	920	56	(Compain et al., 2014)
	R: TTATTCGCCACCACGCTCTT			
magA/K1	F: GGTGCTCTTTACATCATTGC	1283	54	(Compain et al., 2014)
	R: GCAATGGCCATTTGCGTTAG			

Analysis of genetic relationships between isolates

Genetic similarities between isolates were explored by rep-PCR method. Two inosine-containing primers: REP 1R-I "IIIICGICGICATCIGGC" and REP2-I "ICGICTTATCIGGCCTAC" were used for amplification of regions between noncoding repetitive DNA sequences in bacterial genome. PCR products were run on 1.5% agarose gel with a voltage of 80 volts for 120 minutes, using 1kb DNA ladder. The DNA fingerprints obtained were analyzed in http://insilico.ehu.eus/dice_upgma/ using Dice similarity coefficient. Isolates with DNA similarity of ≥ 90% were assigned to the same cluster.

RESULTS

Antibiotic Susceptibility Pattern

All isolates were resistant to ampicillin. Six out of eight isolates showed resistance to at least one agent of more than three antimicrobial classes and hence were MDR. All of these isolates were resistant to quinolones, cephalosporins, amoxicillin-clavulanate, and aztreonam. Two isolates were susceptible to all of these antibiotics, except for resistance to Augmentin in one isolate. The lowest resistance rate was detected to tetracycline (25%) followed by imipenem (50%). The antibiotic susceptibility patterns of all isolates are presented in Table 4.

Table 4. antibiotic susceptibility of *K. pneumoniae* strains isolated from meningitis patients

Antibiotic	No. (%)		
	Sensitive	Intermediate	Resistant
Imipenem	3(37.5)	1(12.5)	4 (50)
Meropenem	2 (25)	2 (25)	4 (50)
Gentamicin	2 (25)	0 (0)	6 (75)
Ciprofloxacin	1 (12.5)	1 (12.5)	6 (75)
Cefoxitin	2 (25)	0	6 (75)
Cefotaxime	0 (0)	2 (25)	6 (75)
Ceftriaxone	2 (25)	0 (0)	6 (75)
Augmentin	1 (12.5)	0 (0)	7 (87.5)
Aztrionam	2 (25)	0 (0)	6 (75)
Ampicillin	0 (0)	0 (0)	8 (100)
Trimethoprim- sulfamethoxazole	3 (37.5)	0 (0)	5 (62.5)
Piperacilin- tazobactam	2 (25)	1 (12.5)	5 (62.5)
Tetracycline	6 (75)	0 (0)	2 (25)
Cefipime	2 (25)	0 (0)	6 (75)
Ceftazidime	2 (25)	0 (0)	6 (75)

Detection of Antibiotic Resistance Genes

The six MDR isolates harbored at least one gene of three different classes of antibiotic resistance genes (ARGs): ESBLs (bla_{SHV}), carbapenemases (bla_{OXA-48}), and AMEs (*aac(6')-Ib*). The most frequent ESBL gene was bla_{SHV} which was detected in 7/8 (87.5%) of isolates followed by bla_{TEM} (62.5%) and bla_{CTXM-1} (37.5%). Simultaneous presence of bla_{SHV} and bla_{TEM} was detected in 62.5% of isolates and co-occurrence of three genes was found in 25% of isolates. The most frequent carbapenemase gene was bla_{OXA-48} with a prevalence rate of 75% among isolates, while bla_{NDM-1} was detected only in one isolate (12.5%). No PCR product was found for bla_{IMP} and bla_{KPC}. Amongst the AMEs and 16 SrRNA methylases genes that were traced, *aac (6')-Ib* and *aac (3)-IIa* were found in 75% and 62.5% of isolates respectively, while *aac (3)-Ia*, *aac (3)-IVa*, *ant (2'')-Ia*, *ant (4')-IIa*, *aph (3')-Ia*, *armA*, *rmtB*, and *rmtC*) were not detected. The two isolates that were susceptible to most of the tested antibiotics were negative for all three classes of ARGs except for the presence of bla_{SHV} in one of these isolates. Table 5 presents the frequency of ARGs detected in eight isolates. Figure 1 presents pictures of gel electrophoresis of the most commonly detected ARGs.

Table 5. Frequency of antibiotic resistance genes (ARG) detected in *K. pneumoniae* strains isolates from meningitis

isolates	bla_{SHV}	bla_{TEM}	bla_{CTXM}	bla_{OXA-48}	bla_{NDM-1}	*aac(6')-Ib*	*aac(3)-IIa*
1	+	+	-	+	-	+	+
2	+	+	-	+	-	+	-
3	+	+	-	+	-	+	+
4	+	+	+	+	+	+	+
5	-	-	-	-	-	-	-
6	+	-	+	+	-	+	+
7	+	+	+	+	-	+	+
8	+	-	-	-	-	-	-
No. (%) positive	7 (87.5)	5 (62.5)	3 (37.5)	6 (75)	1 (12.5)	6 (75)	5 (62.5)

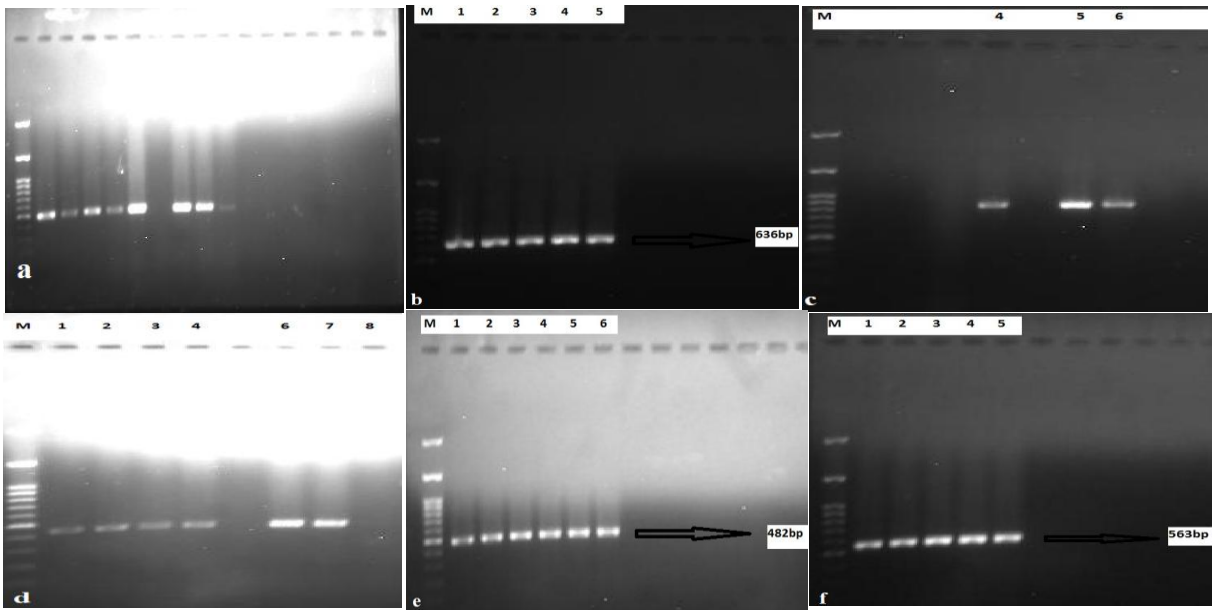

Figure 1. Pictures of gel electrophoresis of antibiotic resistance genes; a: bla_{SHV}, b: bla_{TEM}, c: bla_{CTXM}, d: bla_{OXA-48}, e: *aac(6')-Ib*, f: *aac(3)-IIa*

Detection of Genes Coding for Outer Membrane Porins, MDR Efflux Pumps, and Virulence Factors

All eight isolates harbored *acrAB-tolC* efflux pump system but were negative for *mdtK*. Of the two genes coding for outer membrane porins, *ompK36* was observed in all isolates while *ompK35* was not detected. PCR assay of wzi genes specific for K1 and K2 capsular serotype demonstrated that none of the isolates were related to K1 or K2 capsular serotypes. Genes encoding for type 3 fimbrial adhesin (*mrkD*) and entrobactin (*entB*) were present in 100% (8/8) isolates. Additionally, genes coding for yersiniabactin (*ybtS*), ABC iron transport system (*kfu*), and serum resistance (*traT*) were detected in 62.5%, 25%, and 12.5% of isolates respectively. Genes in charge of overproduction of capsule: mucoviscosity associated gene A (*magA*) and regulator of mucoid phenotype (*rmpA*), as well as gene coding for aerobactin sidrophore (*iutA*) and a gene involved in allantoin metabolism (*allS*), were not detected in this study. The frequency of virulence-associated genes detected in all isolates is shown in Table 6. In addition, Figure 2 shows pictures of gel electrophoresis of virulence associated genes.

Table 6. frequency of virulence-associated genes detected in *K. pneumoniae* strains isolates from meningitis

isolates	*entB*	*ybtS*	*kfu*	*mrkD*	*traT*	*acrAB*	*TolC*	*ompK36*
1	+	+	+	+	-	+	+	+
2	+	-	-	+	-	+	+	+
3	+	+	+	+	-	+	+	+
4	+	-	-	+	+	+	+	+
5	+	-	-	+	-	+	+	+
6	+	+	-	+	-	+	+	+
7	+	+	-	+	-	+	+	+
8	+	+	-	+	-	+	+	+
No. (%) positive	8 (100)	5 (62.5)	2 (25)	8 (100)	1 (12.5)	8 (100)	8 (100)	8 (100)

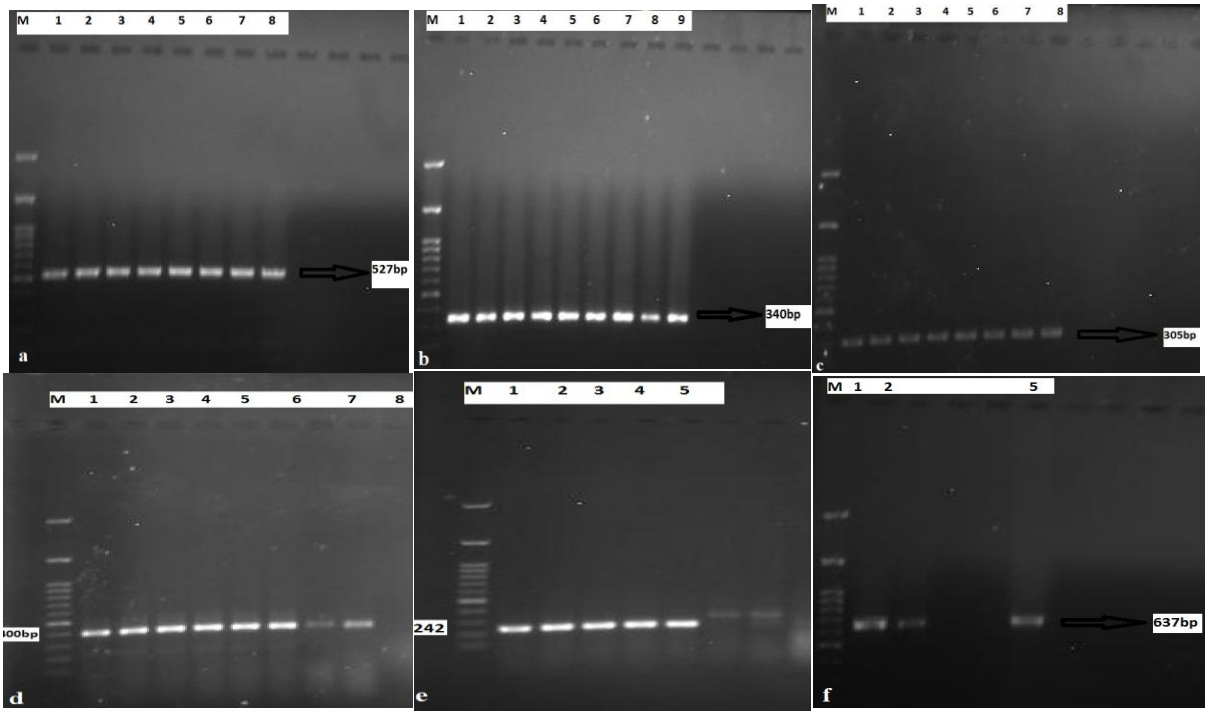

Figure 2. Pictures of gel electrophoresis of most commonly detected virulence associated genes; a: *tolC*, b: *mrkD*, c: *ompK36*, d: *entB*, e: *ybtS*, f: *kfu*

Determination of genetic relationships between isolates

rep-PCR analysis using a similarity cut-off of ≥90%, discriminated eight isolates into three clusters (three strains in cluster A and two strains in each of clusters B and C) and one sporadic strain. Isolates 1, 2, 3 that were assigned to cluster A, harbored *bla*$_{OXA-48}$ and either one or two of AME genes. Isolates 5 and 8 were assigned to cluster B, both were negative for *bla*$_{OXA-48}$ and AME genes and were phenotypically susceptible to imipenem and gentamicin. Isolates 6 and 7 which were assigned to cluster C also harbored *bla*$_{OXA-48}$ and AME genes. Isolate 4 which represented the sporadic strain was the only *bla*$_{NDM1}$ positive isolate. Figure 3 shows dendogram and virtual gel images obtained by rep-PCR.

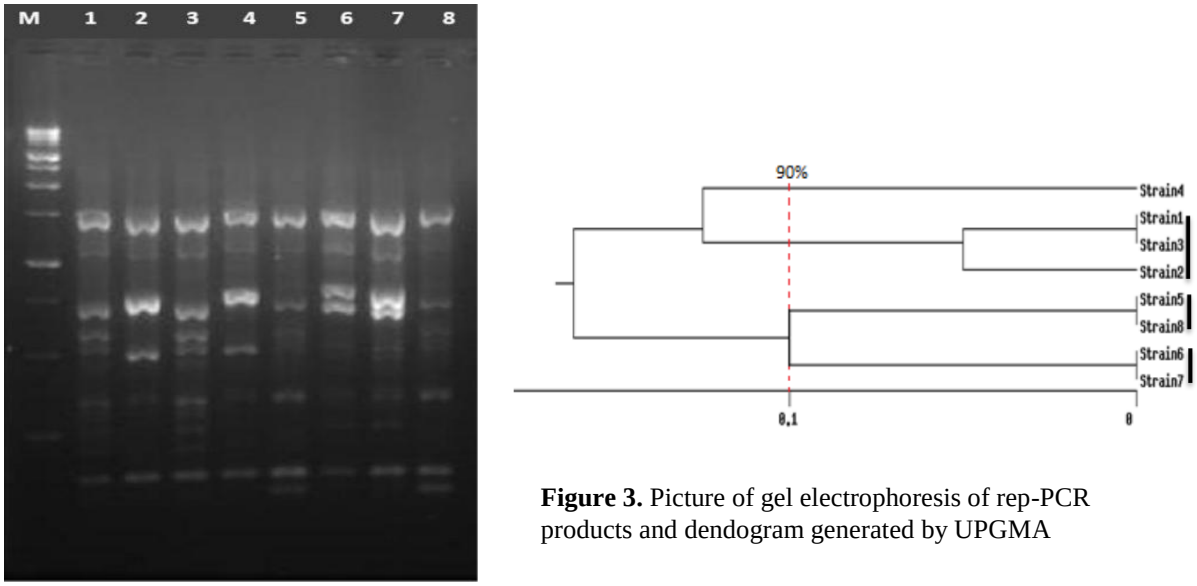

Figure 3. Picture of gel electrophoresis of rep-PCR products and dendogram generated by UPGMA

DISCUSSION

In this study, we collected 8 *K. pneumoniae* isolates during almost two years' period. Meningitis caused by *K. pneumoniae* had been relatively uncommon in the past but its frequency seems to be growing over time (Tang et al., 1994). A retrospective study in Qatar found only ten cases of adult *K. pneumoniae* meningitis from 2007 to 2012 (Khan et al., 2014). Although *K. pneumoniae* meningitis is much more common in Taiwan, Hong Kong, and China, these high reports are primarily due to the emergence of hvKP strains in that geographical region (Xu et al., 2019).

Carbapenems are the most widely used antibiotics for treatment of MDR *K. pneumonia* globally (Grundmann et al., 2017). In our study 75% of isolates were MDR and 50% of isolates showed resistance to carbapenems. This rate is higher than previous reports from Iran which found that resistance to imipenem was 25.7% (Kiaei et al., 2019) and 37.9% (Rastegar et al., 2019) but lower than reports from turkey 68% (Aksöz et al., 2015) and congruent with a report from Saudi Arabia (Al Bshabshe et al., 2020). These data suggest an annual increase in the frequency of carbapenem-resistant *K. pneumoniae* (CRKP) in Iran. Our results showed that tetracycline with a resistance rate of 25% was the most effective antibiotic. Efficacy of tetracycline against CRKP is previously reported from Iran (Jafari et al., 2018).

Limited numbers of antibiotics can be used as alternatives to carbapenems for treatment of CRKP (Grundmann et al., 2017), amongst which aminoglycosides are the most commonly accessible and cheap drugs (Forge et al., 2000). In our study resistance to gentamicin was detected in 75% of isolates. Resistance to gentamicin is

reported between 34.6% to 52.4% from different parts of Iran (Nasiri et al., 2018; Mokhtari et al., 2018; Rastegar et al., 2019), 60% from Egypt (El-Badawy et al., 2017), and 57.1% from Saudi Arabia (Al Bshabshe et al., 2020). Jafari et al. (2018) reported that 93.3% of CRKP isolates were resistant to gentamicin. Similarly, in our study, 100% (4/4) of CRKP isolates were resistant to gentamicin. These data show that resistance to aminoglycosides is higher among CRKP strains compared to carbapenem-susceptible strains.

We explored two general ESBL genes: bla_{SHV} and bla_{TEM}, and one common variant of bla_{CTXM} gene: bla_{CTXM-1}. The redundancy rates were 87.5% for bla_{SHV}, 62.5% for bla_{TEM}, and 37.5% for bla_{CTXM-1}. The prevalence rate of these genes was reported 67.4%, 54%, and 46.51% respectively in a study published from Iran in 2010 (Parvin et al., 2010). Previous studies have shown a higher frequency of ESBL genes among CRKP isolates (Moghadampour et al., 2018). As per our study, 100% (4/4) of CRKP isolates harbored bla_{SHV} and bla_{TEM} genes and 50% (2/4) carried bla_{CTXM-1} gene.

Amongst the genes coding for aminoglycoside- resistance *aac (6') Ib* and *aac (3) IIa* were detected in 75% (6/8) and 62.5% (5/8) of isolates respectively and 62.5% were positive for both genes. The prevalence of *aac (6') Ib* and *aac (3) IIa* was reported 44.6% and 43% respectively in a study from Spain (Fernández et al., 2018) and 84% and 25% respectively in a study from Greece (Galani et al., 2019). In accordance with our study, several studies from Iran found that *aac (6') Ib* and *aac (3) IIa* were the most common AME genes in aminoglycoside resistant isolates (Nasiri et al., 2018; Harir et al., 2018).

We explored four carbapenemase encoding genes in the current study, the most prevalent of which was bla_{OXA-48} (75%) followed by bla_{NDM-1} (12.5%). The presence rates of these genes were reported 58% and 2% respectively in a study from Turkey (Aksöz et al., 2015), and 29% and 75% respectively in a study from Egypt (Ragheb et al., 2020). In line with our study, several studies from Iran found that bla_{OXA-48} was the most frequent carbapenemase gene followed by bla_{NDM-1} (Moghadampour et al., 2018; Jafari et al., 2018). However, studies from south of Iran mostly reported bla_{NDM-1} as the main carbapenemase gene (Kiaei et al., 2019; Shoja et al., 2018). This discrepancy may be explained by traffic with neighboring countries. As previously suggested high prevalence of bla_{OXA-48} gene among *K. pneumoniae* strains in Tehran might be due dissemination of clones harboring this gene from Turkey (Jafari et al., 2018). Travel of passengers from south of Iran to India, Pakistan, and Bangladesh and vice versa might have caused dissemination of NDM1 positive strains in that part of the country.

In this study, rep-PCR fingerprints distinguished seven isolates into three clusters and one strain was assigned to be sporadic. This pattern of genetic similarity suggests that three clones of *K. pneumoniae* might have been transmitted from patient to patient in hospital, while one patient might have acquired the infection from a distinct clone. Comprehension of mode of transmission is vital for planning effective control schemes for prevention of *K. pneumoniae* dissemination (Granov et al., 2020). Unfortunately, our study suffers from the lack of patients' demographical data. For this reason, we were not able to correlate the result of genotypic epidemiology of strains with the situation in which patients acquired the infection.

CONCLUSION

Our work revealed high resistance to aminoglycoside and medium resistance to carbapenems among *K. pneumoniae* strains isolated from meningitis patients, with the existence of *aac (6')-Ib* and *aac (3)-IIa*, and bla_{OXA-48} genes being primarily responsible for this resistance. This suggests that carbapenems can be considered as first-line drugs for initial empirical treatment of *K. pneumoniae* meningitis before obtaining

susceptibility test results. Low diversity in genetic relationships among isolates could be due to circulation of some clones in hospital which emphasizes on the establishment of effective infection surveillance and prevention program.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest.

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