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ORIGINAL ARTICLE

Bacteriological characteristics of hypervirulent *Klebsiella pneumoniae rmpA* gene (hvKp-*rmpA*)-harboring strains in the south of Iran

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ABSTRACT

Background and Objectives: To provide data on the occurrence of classical *K. pneumoniae* (cKp) and hypervirulent *Klebsiella pneumoniae* (hvKp) strains harboring the gene encoding regulator of mucoid phenotype A (*rmpA*) and evaluated characteristics of virulence biomarkers, carbapenemase, extended-spectrum-β-lactamase (ESBL)-producing, and capsule serotypes among *K. pneumoniae* clinical isolates collected in the south of Iran.

Materials and Methods: A total of 400 *K. pneumoniae* isolates were collected. First, the *K. pneumoniae* isolates were screened for *rmpA* gene by PCR, and then they were characterized for the presence of the virulence genes (*pagO, iucA, iroB, luxR*), capsular serotype genes (K1, K2, K5, K20, K54, and K57), carbapenemase (bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SPM}, bla_{OXA-48}, and $bla_{OXA-181}$) and ESBL (bla_{CTX-M}, bla_{SHV} and bla_{TEM}) genes. For all *K. pneumoniae* isolates phenotypic tests include of string test and disk diffusion test were performed.

Results: In total, 16 (4%) hvKp-*rmpA*+ and 384 (96%) cKp were observed. Of hvKp-*rmpA*+ strains, 16 (100%) were carried *pagO, iroB*, and *luxR* genes, and 13 (81.3%) strains harbored *iucA* gene. The most prevalent capsular type genes were K1 (62%) and K2 (19%) in hvKp-*rmpA*+ strains. The incidence of bla_{SHV} gene in hvKp and cKp was 94% (15/16) and 87.5% (336/384), respectively. The cKp isolates carried bla_{NDM} (30/384; 7.8%) gene.

Conclusion: Our data suggest that the incidence of hvKp was low. Also, hvKp-*rmpA*+ strains have less antibiotic resistance than cKp isolates. Serotypes K1 and K2, and bla_{SHV} gene were strongly associated with hvKp-*rmpA*+.

Keywords: Beta-lactamases; Carbapenem-resistant Enterobacteriaceae; *Klebsiella pneumonia*

INTRODUCTION

Klebsiella pneumoniae is one of the most common causes of infections, such as pneumonia, pyogenic liver abscesses, soft tissue infection, urinary tract infections, and bacteremia. There are mainly two pathotypes of *K. pneumoniae* that include: classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumoniae* (hvKp). Most cKp infections occur in immunocompromised patients (1). The hvKp strains cause severe infections in healthy and immunocompromised individuals such as liver abscesses, meningitis, and endophthalmitis. These hvKp strains frequently create infections at multiple sites and subsequently spread as metastasis. The hypermucoviscosity in these strains is result of overproduc-

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tion of capsular polysaccharide and such strains can cause serious community-acquired infections (2, 3). Several virulence genes have been described related to being pathogenicity of hvKp, including the regulator of mucoid phenotype A gene (*rmpA/A2*), lipopolysaccharide (*waaL/E*), iron acquisition systems aerobactin (*iucABCDiutA*)/salmochelin (*iroBCDN*), transcriptional regulator (*LuxR*), PhoPQ-activated integral membrane protein (*pagO*), Fimbrial synthesis (*FimA/B/C*) (4). A study showed that iron acquisition systems (*iroBCDN, iucABCDiutA*) along with the presence of *rmpA/A2*, the enhancer of capsule production, can significantly cause invasive infection (5).

Among *K. pneumoniae* strains, cKp known are notorious for its resistance to common antimicrobial agents. Previous studies reported that the majority of hvKp were sensitive to commonly used antibiotics (except for the inherent resistance to ampicillin), but in the past few years increasing the incidence of multidrug-resistant hvKp, especially carbapenem-resistant hvKp, extended-spectrum-β-lactamase (ESBL) producing hvKp, and polymyxin resistant hvKp, is emerging (6-8). The *K. pneumoniae* resistance to β-lactams antibiotics is frequently caused by ESBLs. Infections caused by these bacteria are complicated issues, due to resistance to other antibiotic classes and limited choices of available antibiotics (9, 10). Some *K. pneumoniae* carbapenem-resistant strains became hyper-virulent via acquiring virulence plasmid and this combination represents a major challenge for treatment and control of infections (11). The incidence of hvKp resistant strains due to the presence of some carbapenemase genes such as bla_{NDM}, and bla_{OXA} is a worrisome threat (12).

We investigated the frequency of hvKp-*rmpA* harboring strains and evaluated various virulence biomarkers, antibiotic resistance such as carbapenem-resistant, and ESBL-producing, and capsule serotypes amongst *K. pneumoniae* clinical isolates collected in a hospital in the south of Iran.

MATERIALS AND METHODS

Clinical bacterial isolation. Four hundred *K. pneumoniae* isolates were obtained from culture-positive patients at the main tertiary teaching hospital of Bandar Abbas, located in the south of Iran (Payambare-Azam-therapeutic center) from 2018-2020. Clinical *K. pneumoniae* isolates were collected from urine, trachea, wound, blood, sputum, discharge, broncoalveolar lavage (BAL), eye, pleural fluid, bile, and ascites samples of patients who were admitted to the hospital. The samples were culture within 24 h on MacConkey agar (Merck, Germany). Two presumptive *K. pneumoniae* colonies were picked from MacConkey agar plate and confirmed as *K. pneumoniae* by standard biochemical tests (13). From each sample one confirmed *K. pneumoniae* isolate was selected and stored in nutrient broth (Merck, Germany) with 30% sterile glycerol at -70ºC.

Ethics statement. Ethical approval was obtained from the Hormozgan University of Medical Sciences ethical committee, compliant with the Declaration of Helsinki (approval no. IR.HUMS.REC.1397.012).

Antibiotic susceptibility testing. The antimicrobial susceptibility testing was carried out for all *K. pneumoniae* isolates for 12 antibiotics using standard disk diffusion test according to Clinical and Laboratory Standards Institute guidelines (CLSI) (14). The antibiotic disks used in the study were imipenem (10 ug), meropenem (10 ug), piperacillin (30 ug), piperacillin-tazobactam (100-10 ug), trimethoprim/sulfamethoxazole (1.25/23.75 ug), ceftazidime (30 ug), cefepime (30 ug), ampicillin-sulbactam (10-10 ug), aztreonam (30 ug), ciprofloxacin (5 ug), gentamicin (10 ug), and tetracycline (30 ug) (MAST Group Ltd, Merseyside, UK). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls strains for antimicrobial susceptibility testing.

String test. The presence of hypermucoviscosity phenotype of all isolates was assessed using the string test. Positive string test was defined as the formation of a viscous capsular string >5 mm long (15).

DNA extraction and screening PCR for hvKp-*rmpA*. Crude DNA of all confirmed *K. pneumoniae* isolates were extracted by boiling method. The *K. pneumoniae* isolates were screened by a PCR assay for detected of hvKp by *rmpA* gene as described before (16) (Table 1).

PCR assay for *pagO, iucA, iroB, luxR*. The hvKp-*rmpA*+ isolates were subjected to a simplex-PCR assay detecting the major hvKp genes as

described by Ye et al. (17) (Table 1).

PCR detection of capsular types associated genes. For hvKp-*rmpA*+ isolates determined capsular serotypes K1, K2, K5, K20, K54, and K57 by using a simplex-PCR, as study previously (18, 19) (Table 1).

Table 1. Primers used for identification of antibiotic resistance, virulence biomarker and serotypes in this study.

Target gene	Sequence (5′ to 3′)	Ref
bla_{IMP}	GGAATAGAGTGGCTTAAYTCTC	21
	GGTTTAAYAAAACAACCACC	
bla_{VIM}	GATGGTGTTTGGTCGCATA	
	CGAATGCGCAGCACCAG	
bla_{SPM}	AAAATCTGGGTACGCAAACG	
	ACATTATCCGCTGGAACAGG	
bla_{NDM}	CCGTATGAGTGATTGCGGCG	22
	GCCCAATATTATGCACCCGG	
bla_{OXA-48}	TATATTGCATTAAGCAAGGG	23
& $bla_{OXA-181}$	CACACAAATACGCGCTAACC	
bla_{KPC}	ATTTTCAGAGCCTTACTGCCC	24
	TATCGTTGATGTCACTGTATCG	
bla_{SHV}	AGCCGCTTGAGCAAATTAAAC	20
	ATCCCGCAGATAAATCACCAC	
bla_{TEM}	CATTTCCGTGTCGCCCTTATTC	
	CGTTCATCCATAGTTGCCTGAC	
bla_{CTX-M}	TTAGGAARTGTGCCGCTGYA	
	CGATATCGTTGGTGGTRCCAT	
K1	GTAGGTATTGCAAGCCATGC	18
	GCCCAGGTTAATGAATCCGT	
K2	GACCCGATATTCATACTTGACAGAG	19
	CCTGAAGTAAAATCGTAAATAGATGGC	
K5	TGGTAGTGATGCTCGCGA	
	CCTGAACCCACCCCAATC	
K20	CGGTGCTACAGTGCATCATT	18
	GTTATACGATGCTCAGTCGC	
K54	CATTAGCTCAGTGGTTGGCT	
	GCTTGACAAACACCATAGCAG	
K57	CTCAGGGCTAGAAGTGTCAT	
	CACTAACCCAGAAAGTCGAG	
rmpA	ACTGGGCTACCTCTGCTTCA	16
	CTTGCATGAGCCATCTTTCA	
iucA	CCAACTCCGTCCGTACCCTGTCA	17
	CGAGGGATCGACGATGGTGTTCT	
iroB	AGAGGCTGGATTGGTGGCGTTTG	
	CGATCTGTGGAATACCGGCGTGTAG	
pagO	TGCTCTTGAAACTATCCCTCC	
	GGCAATAACTCCCGTCCA	
LuxR	CTTTGCCGGCATGGAACATA	
	TGAGCCAAATGTATGCCAAGGA	

ESBL identification genes. The presence of genes associated with ESBLs such as bla_{CTX-M}, bla_{SHV} and bla_{TEM} for all isolates was surveyed by different PCR assays as described previously (20) (Table 1).

Detection of carbapenemase genes. The all of isolates were examined to determine the presence of selected carbapenemase genes including bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SPM}, bla_{OXA-48}, and $bla_{OXA-181}$ by simplex-PCR method (21-24) (Table 1).

RESULTS

Samples and *K. pneumoniae* isolates. In this study, a total of 400 *K. pneumoniae* isolates were recovered from clinical samples including urine (49.3%; n = 197), trachea (16.3%; n = 65), wound (13.3%; n = 53), sputum (7%; n = 28), blood (6.5%; n = 26), discharge (2.5%; n = 10), BAL (2.3%; n = 9), eye (1%; n = 4), pleural fluid (0.5%; n = 2), catheter (0.5%; n = 2), bile (0.5%; n = 2), and ascites (0.25%; n = 1).

Antibiotic susceptibility patterns. The disk diffusion results showed that 283 (70.8%) of the all *K. pneumoniae* isolates were resistant to piperacillin, 270 (67.5%) to trimethoprim / sulfamethoxazole, 235 (58.8%) to ceftazidime, 225 (56.3%) to cefepime, 217 (54.3%) to ampicillin-sulbactam, 217 (54.3%) to aztreonam, 207 (51.7%) to ciprofloxacin, 165 (41.3%) to piperacillin-tazobactam, 155 (38.8%) to gentamicin, 134 (33.5%) to meropenem, 128 (32%) to tetracycline, and 104 (26%) to imipenem. Among hvKp-*rmpA*+ strains, the highest resistance rate was obtained against piperacillin (11, 68.8%), followed by trimethoprim / sulfamethoxazole (8, 50%), meropenem (5, 31.3%), ceftazidime, ampicillin-sulbactam, ciprofloxacin (4, 25% for each of them), cefepime (3, 18.8%), aztreonam (2, 12.5%), gentamicin, piperacillin-tazobactam and imipenem (1, 6.3% for each of them). None of hvKp-*rmpA*+ strains showed resistance to tetracycline. Antibiotic resistance of hvKp-*rmpA*+ and cKp isolates are presented in Table 2.

String test. Of all the *K. pneumoniae* isolates only 22 (5.5%) isolates were positive for the string test phenotype. Of 22 isolates that were positive for the string test, eight isolates lacked *rmpA* gene.

Table 2. Antibiotic resistance of hypervirulent *K. pneumoniae* (hvKP) and classical *K. pneumoniae* (cKP)

Antibiotic agent		hvKp-*rmpA*+ (n=16)	cKP (n=384)	Total (n=400)
Carbapenem	Imipenem	1 (6.3%)	103 (26.8%)	104 (26%)
	Meropenem	5 (31.3%)	129 (33.6%)	134 (33.5%)
Cephalosporin	Ceftazidime	4 (25%)	231 (60.2%)	235 (58.8%)
	Cefepime	3 (18.8%)	222 (57.8%)	225 (56.3%)
Beta-lactam inhibitor	Piperacillin-tazobactam	1 (6.3%)	164 (42.7%)	165 (41.3%)
	Ampicillin-sulbactam	0 (0%)	217 (56.5%)	217 (54.3%)
Beta-lactam	Piperacillin	11 (68.8%)	272 (70.8%)	283 (70.8%)
monobactam	Aztreonam	2 (12.5%)	215 (56%)	217 (54.3%)
Sulfonamide	trimethoprim / sulfamethoxazole	8 (50%)	262 (68.2%)	270 (67.5%)
Fluroquinolone	Ciprofloxacin	4 (25%)	203 (52.9%)	207 (51.7%)
30s	Gentamicin	1 (6.3%)	154 (40.1%)	155 (38.8%)
	Tetracycline	0 (0%)	128 (33.3%)	128 (32%)

Samples and screening of hvKp-*rmpA*+ strains. Of 400 *K. pneumoniae* isolates tested, 16 (4%) carried the *rmpA* gene and were identified as hvKp, and 384 (96%) isolates that did not harbor *rmpA* gene were detected as cKp. hvKp-*rmpA*+ strains were isolated from trachea (31%; n = 5), urine (25%; n = 4), sputum (25%; n = 4), wound (12.5%; n = 2), and blood (6%; n = 1) specimens (Table 3).

PCR assay for *pagO, iucA, iroB, luxR*. After determining the hvKp-*rmpA*+ strains by PCR, we investigated the possible presence of other cardinal virulence and capsular type genes related to the pathogenicity of hvKp. Of 16 hvKp-*rmpA*+ strains, 16 (100%) carried *pagO, iroB,* and *luxR* genes. Thirteen of the hvKp-*rmpA*+ strains (81.3%) harbored *iucA* gene (Table 3).

Capsular types. The most prevalent capsular type gene was K_1 that occurred in 10 (62%) hvKp-*rmpA*+ strains. Three (19%) strains harbored the K_2 gene, and two (12.5%) strains and one (6%) strain carried K_5 and K_{20} genes, respectively. None of the hvKp-*rmpA*+ strains were positive for K_{54} and K_{57} types. The K_1 positive strains were cultured from sputum (30%; n = 3), trachea (30%; n = 3), wound (20%; n = 2), urine and blood (10%; n = 1 for each of them) specimens, while K_2 strains were recovered from trachea, urine and sputum cultures (Table 3).

ESBL genes. The most prevalent ESBL gene was bla_{SHV} that occurred in 336 (87.5%) cKp isolates. One hundred ninety-three (50%) isolates harbored the bla_{CTX-M} gene, and 116 (30%) carried bla_{TEM}. These genes occurred in different combinations, bla_{CTX-M}+bla_{SHV} and bla_{CTX-M}+bla_{SHV}+bla_{TEM} most frequently occurred together in 79 (20.5%) and 77 (20%) isolates, respectively. Twenty (5.2%) of the isolates possessed bla_{SHV}+bla_{TEM} genes, whereas two (0.5%) of isolates carried bla_{CTX-M}+bla_{TEM} genes. The isolates that possessed ESBL genes were mostly cultured from urine and trachea specimens (Table 4).

Among 16 hvKp-*rmpA*+ strains, 15 (94%) strains carried bla_{SHV} gene and one (6%) strain had bla_{CTX-M} gene. Therefore, only one strain of hvKp-*rmpA*+ strains was positive for both bla_{CTX-M} and bla_{SHV} genes. None of hvKp-*rmpA*+ strains were positive for bla_{TEM} gene (Table 3).

Frequency of carbapenemase genes. All *K. pneumoniae* isolates were negative for bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SPM}, bla_{OXA-48}, and $bla_{OXA-181}$ genes. But the results revealed that 30 (7.8%) cKp isolates possessed bla_{NDM} gene. The bla_{NDM} producers were mostly cultured from urine (47%; n = 14), trachea (20%; n=6) followed by wound (17%; n = 5), sputum, discharge and blood (3%; n = 5 for each of them). However, none of the hvKp-*rmpA*+ strains were positive to selective carbapenemase genes. Table 4 shows the distribution of the bla_{NDM}+ gene in relation to the ESBL genes, and clinical samples.

DISCUSSION

Over the recent years, epidemiological data have shown often highly prevalent hvKp especially in

Table 3. Distribution of ESBL, carbapenemase, virulence, capsular genes, string test, and specimens in 16 hvKp-*rmpA*+ strains

No	ESBL gens		Virulence genes				Capsular genes				String test	Samples
	bla_{CTX-M}	bla_{SHV}	*iucA*	*iroB*	*luxR*	*pagO*	K1	K_2	K_5	K_{20}		
1	+	+	+	+	+	+	+				+	Urine
2		+	+	+	+	+				+	+	Urine
3		+		+	+	+			+		+	Urine
4				+	+	+	+				+	Blood
5		+	+	+	+	+	+				+	Wound
6		+	+	+	+	+	+				+	Trachea
7		+	+	+	+	+	+				+	Sputum
8		+	+	+	+	+		+			+	Urine
9		+	+	+	+	+		+			+	Sputum
10		+		+	+	+				+		Trachea
11		+	+	+	+	+		+			+	Trachea
12		+	+	+	+	+	+				+	Wound
13		+	+	+	+	+	+				+	Trachea
14		+	+	+	+	+	+				+	Sputum
15		+	+	+	+	+	+				+	Trachea
16		+	+	+	+	+	+				+	Sputum
Total	1	15	13	16	16	16	10	3	1	2	15	

∗Only the results of positive genes have been shown (All were negative for bla_{TEM}, bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{KPC}, bla_{OXA48}, bla_{OXA181}, K_{54} and K_{57}).

China (6, 7, 25, 26). In the current study using the presence of the *rmpA* gene, only 4% of *K. pneumoniae* were detected as hypervirulent. hvKp-*rmpA*+ strains were mostly derived from trachea, followed by urine and sputum of patients referred to the hospital. Further, other several important results were identified from our study. First, all the 16 hvKp-*rmpA*+ strains, were positive for *pagO*, *iroB* and *luxR* genes, biomarkers that were shown to be accurate for differentiating hvKp from cKp strains (7). Second, the most prevalent K_1 (62%) and K_2 (19%) capsular types were detected in hvKp-*rmpA*+ strains. Third, and most importantly, among hvKp-*rmpA*+ strains, none possessed carbapenemase genes, and only 7.8% of cKp isolates harbored the bla_{NDM} gene. Also, 15 hvKp-*rmpA*+ strains carried bla_{SHV} gene and only one strain had bla_{CTX-M} gene. These results show that hvKp-*rmpA*+ strains have less antibiotic resistance than cKp isolates.

At present, an accurate test of differential hvKp and cKp strains is needed for epidemiologic studies. Russo et al. demonstrated that certain virulence biomarkers have high specificity and sensitivity for the detection of hvKp strains. These biomarkers include *rmpA, rmpA2*, *peg*, *iroB*, and *iucA* which are associated with severe illness or death. Siderophore production strongly predicted hvKp strains (27). Our study utilized five virulence biomarkers (*rmpA, pagO, iucA, iroB, luxR*) and identified 16 hvKp strains from 400 patients. A previous study reported the epidemic spread of hvKp infections in Asian populations, especially in China, South Korea, Taiwan, and Iran (8). The results of our study were in agreement with another study in Iran that showing a low prevalence hvKp among *K. pneumoniae* were isolated from community-acquired urinary tract infections (11 out of 105) (28). Also, other studies in Iran have shown a lower frequency of hvKp strains (1, 29, 30). Therefore, based on the results of studies can conclude that the epidemic spread of hvKp strains in Iran is not consistent with results reported by Lee et al. (8).

K. pneumoniae based on capsular polysaccharides are divided into at least 78 serotypes. Among the various serotypes of *K. pneumoniae*, serotypes K1 and K2 are most associated with hvKp strains (31). Furthermore, other capsular polysaccharides serotypes of hvKp strains, including K5, K16, K20, K28, K54, K57, K63, and KN1, have been reported (8). In the current study, K1 and K2 serotypes are the most

Table 4. Frequency of ESBL genes profile and specimens in 384 cKp isolates and 30 bla_{NDM}+ strains

Total N (%)	ESBL genes profile	Samples (N, %)
	cKp isolates	
79 (20.5%)	bla_{CTX-M}, bla_{SHV}	Urine (26, 6.7%)
		Trachea (18, 4.6%)
		Wound (14, 3.6%)
		Sputum (6, 1.5%)
		Blood (6, 1.5%)
		BAL (3, 0.7%)
		Discharge (1, 0.26%)
		Ascites (1, 0.26%)
		Pleural fluid (1, 0.26%)
		Catheter (1, 0.26%)
77 (20%)	bla_{TEM}, bla_{CTX-M}, bla_{SHV}	Urine (30, 7.8%)
		Trachea (17, 4.4%)
		Wound (12, 3.1%)
		Sputum (7, 1.8%)
		Discharge (5, 1.3%)
		Blood (3, 0.7%)
		BAL (3, 0.7%)
20 (5.2%)	bla_{TEM}, bla_{SHV}	Urine (15, 3.9%)
		Wound (3, 0.7%)
		Trachea (1, 0.26%)
		Sputum (1, 0.26%)
2 (0.5%)	bla_{TEM}, bla_{CTX-M}	Wound (2, 0.5%)
	bla_{NDM}+ strains*	
16 (53.3%)	bla_{CTX-M}, bla_{SHV}	Trachea (8, 26.6%)
		Urine (4, 13.3%)
		Wound (4, 13.3%)
12 (40%)	bla_{TEM}, bla_{CTX-M}, bla_{SHV}	Urine (9, 30%)
		Wound (1, 3.3%)
		Discharge (1, 3.3%)
		Blood (1, 3.3%)
1 (3.3%)	bla_{TEM}, bla_{SHV}	Urine (1, 3.3%)
1 (3.3%)	bla_{TEM}, bla_{CTX-M}	Urine (1, 3.3%)

*One bla_{NDM}+ strain none positive for each ESBL genes.

predominant among hvKp strains, which is consistent with the results of the other studies (1, 32-34). In contrast to previous reports revealed that K2 capsular serotype among hypermucoviscous *K. pneumoniae* isolates was associated with more types of invasive infections than K1 isolates. K5, K20, K54, and K57 serotypes have been reported in China with different identification rates, such as K5 2.4% (2/84), K20 4.8% (4/84), and K57 9.6% (8/84) (35), K5 3.1% (3/96), K20 6.3% (6/96), K54 3.8% (4/96) and K57 10.4% (10/96) (36) and K5 40.5% (15/37), K20 8.1% (3/37), K54 21.6% (8/37), and K57 18.9% (7/37) (37). In the current study, we found K5 (12.5%) and K20 (6%), also none of the hvKp-*rmpA*+ strains possessed K54 and K57 genes, can be suggested that the serotype frequency of hvKp strains varied in different regions of Asia.

In similar to previous studies that hvKp strains were highly sensitive to routinely used antibiotics compare to cKp (6-8), the results of the current study showed that hvKp-*rmpA*+ were less resistant than cKp to imipenem, piperacillin-tazobactam,

cefepime, ampicillin-sulbactam, aztreonam, ciprofloxacin, gentamicin, and tetracycline. In this study, the number of hvKp-*rmpA*+ possessed ESBL genes, especially bla_{SHV} gene (94%) is significantly higher compared to carbapenemase genes. None of hvKp-*rmpA*+ strains possessed carbapenemase genes. But, 7.8% of cKp isolates carried bla_{NDM} gene. Like the report in China, the incidence of ESBLs was found to be significantly greater in cKp isolates than in hvKp strains (25). Similarly, a report in Iran showed that 90.0% and 63.6% of hvKp strains from urinary tract infections harbored bla_{SHV} and bla_{CTX-M}, respectively (28). Lam et al. suggested that the higher antimicrobial susceptibility of hvKp may be due to hyper-expression of K1 capsule, which may provide a physical barrier against penetrating foreign DNA to bacteria in conjugation, transformation, and also CRISPR/Cas systems. However, in recent years, antibiotic resistance in hvKp strains increased over time, such as ESBLs and carbapenemase-producing (38). Another report in Iran showed that hvKp strains were resistant to imipenem and carried an aacA7, bla_{VIM-2}, and dhfrI cassette arrangement in a class 1 integron (29). A study in Spain revealed the first description of a $bla_{CTX-M-13}$, bla_{OXA-48} and *armA*-harbouring hvKp of clone ST23 and capsular serotype K1 (39).

The string test was shown to be significantly associated with the hvKp strains (7), as we observed that one hvKp-*rmpA*+ strain was not hypermucoviscosity phenotype. The new data has suggested that the terms hypervirulent and hypermucoviscous are two different phenotypes that should not be used synonymously. Hypervirulent associated with genes which must be detected such as yersiniabactin, aerobactin, and the *rmpA/rmpA2* genes, but the absence of hypermucoviscosity phenotype is not an appropriate way to exclude hypervirulence (31).

Parrott et al. concluded that the string test was positive in only two-thirds of *rmpA/iucA/peg344* gene-positive isolates and had a specificity of 95.2% and a sensitivity of 66.7%. Also, showed that perhaps this test would best serve as a negative predictive value test in regions of low prevalence (40). However, another recent study reported that some cKp strains possess hypermucoviscous phenotype (27). In this study, it was stated that the hvKP-*rmpA*+ strains were found most frequently in samples of trachea origin. However, it is generally known that hvKP is highly associated with intraperitoneal infections such as liver abscesses. The distribution of bacterial origins in this study is expected to have a significant impact. One of the limitations of the present study was the lack of evaluation of more virulence and antibiotic resistance genes which could have made a more accurate assessment of the molecular status of the bacteria.

CONCLUSION

The prevalence of hvKp as defined by validated virulence biomarkers was low in the south of Iran (Bandar Abbas city). Given that some studies have shown the frequency of hvKp strains epidemic in Iran (8), discrepancies in the distribution of these strains may be due to differences in various geographical areas. We also showed that hvKp-*rmpA*+ strains have less antibiotic resistance than cKp isolates. The frequency of ESBL genes were strongly associated with cKp isolates, although bla_{SHV} gene detected was high in hvKp-*rmpA*+ strains. Therefore, more molecular epidemiologic researches are needed on cKp and hvKp strains in other regions of Iran.

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REFERENCES

1. Rastegar S, Moradi M, Kalantar-Neyestanaki D, Golabi dehdasht A, Hosseini-Nave H. Virulence factors, capsular serotypes and antimicrobial resistance of Hypervirulent *Klebsiella pneumoniae* and classical *Klebsiella pneumoniae* in southeast Iran. *Infect Chemother* 2019; 10.3947/ic.2019.0027.
2. Pajand O, Darabi N, Arab M, Ghorbani R, Bameri Z, Ebrahimi A, et al. The emergence of the hypervirulent *Klebsiella pneumoniae* (hvKp) strains among circulating clonal complex 147 (CC147) harbouring $bla_{NDM/OXA-48}$ carbapenemases in a tertiary care center of Iran. *Ann Clin Microbiol Antimicrob* 2020; 19: 12.
3. Russo TA, Marr CM. Hypervirulent *Klebsiella pneumoniae*. *Clin Microbiol Rev* 2019; 32(3): e00001-19.
4. Paczosa MK, Mecsas J. *Klebsiella pneumoniae:* going on the offense with a strong defense. *Microbiol Mol*

Biol Rev 2016; 80: 629-661.
5. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae,* an urgent threat to public health. *Proc Natl Acad Sci U S A* 2015; 112(27): E3574- E3581.
6. Lin ZW, Zheng JX, Bai B, Xu GJ, Lin FJ, Chen Z, et al. Characteristics of Hypervirulent *Klebsiella pneumoniae:* does low expression of *rmpA* contribute to the absence of Hypervirulence? *Front Microbiol* 2020; 11: 436.
7. Liu C, Du P, Xiao N, Ji F, Russo TA , Guo J. Hypervirulent *Klebsiella pneumoniae* is emerging as an increasingly prevalent *K. pneumoniae* pathotype responsible for nosocomial and healthcare-associated infections in Beijing, China. *Virulence* 2020; 11: 1215-1224.
8. Lee CR, Lee JH, Park KS, Jeon JH, Kim YB, Cha CJ, et al. Antimicrobial resistance of hypervirulent *Klebsiella pneumoniae:* epidemiology, hypervirulence-associated determinants, and resistance mechanisms. *Front Cell Infect Microbiol* 2017; 7: 483.
9. Kazemian H, Heidari H, Ghanavati R, Ghafourian S, Yazdani F, Sadeghifard N, et al. Phenotypic and genotypic characterization of ESBL-, AmpC-, and Carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates. *Med Princ Pract* 2019; 28: 547-551.
10. Lee CH, Su LH, Tang YF, Liu JW. Treatment of ESBL-producing *Klebsiella pneumoniae* bacteraemia with carbapenems or flomoxef: a retrospective study and laboratory analysis of the isolates. *J Antimicrob Chemother* 2006; 58: 1074-1077.
11. Feng Y, Lu Y, Yao Z, Zong Z. Carbapenem-resistant hypervirulent *Klebsiella pneumoniae* of sequence type 36. *Antimicrob Agents Chemother* 2018; 62(7): e02644-17.
12. Mataseje LF, Boyd DA, Mulvey MR, Longtin Y. Two hypervirulent *Klebsiella pneumoniae* isolates producing a bla_{KPC-2} carbapenemase from a Canadian patient. *Antimicrob Agents Chemother* 2019; 63(7): e00517-19.
13. Winn Washington C, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiolgy. Lippincott, Williams & Wilkins, Philadelphia. 2006.
14. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 30th informational supplement, CLSI document M100-S30. 2020. Clinical and Laboratory Standards Institute, Wayne, PA.
15. Shon AS, Bajwa RP, Russo TA. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae:* a new and dangerous breed. *Virulence* 2013; 4: 107-118.
16. Nadasy KA, Domiati-Saad R, Tribble MA. Invasive *Klebsiella pneumoniae* syndrome in North America. *Clin Infect Dis* 2007; 45(3): e25-28.
17. Ye M, Tu J, Jiang J, Bi Y, You W, Zhang Y, et al. Clinical and genomic analysis of liver abscess-causing *Klebsiella pneumoniae* identifies new liver abscess-associated virulence genes. *Front Cell Infect Microbiol* 2016; 6: 165.
18. Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC. *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. *Clin Infect Dis* 2007; 45: 284-293.
19. Turton JF, Baklan H, Siu LK, Kaufmann ME, Pitt TL. Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella* sp. and comparison of isolates within these serotypes. *FEMS Microbiol Lett* 2008; 284: 247-252.
20. Dallenne C, Da Costa A, Decre D, Favier C, Arle G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae. J Antimicrob Chemother* 2010; 65: 490-495.
21. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011; 70: 119-123.
22. Lascols C, Hackel M, Marshall SH, Hujer AM, Bouchillon S, Badal R, et al. Increasing prevalence and dissemination of NDM-1 metallo-β-lactamase in India: data from the SMART study (2009). *J Antimicrob Chemother* 2011; 66: 1992-1997.
23. Potron A, Nordmann P, Lafeuille E, Maskari ZA, Rashdi FA, Poirel L. Characterization of OXA-181, a carbapenem-hydrolyzing class D beta-lactamase from *Klebsiella pneumoniae. Antimicrob Agents Chemother* 2011; 55: 4896-4899.
24. Corbellini S, Caccuri F, Gelmi M, De Francesco MA, Fiorentini S, Caruso A, et al. Emergence of carbapenem-resistant *Klebsiella Pneumoniae* strains producing KPC-3 in Brescia Hospital, Italy. *New Microbiol* 2014; 37: 177-183.
25. Li L, Yuan Z, Chen D, Xie X, Zhang B. Clinical and microbiological characteristics of invasive and hypervirulent *Klebsiella pneumoniae* infections in a teaching hospital in China. *Infect Drug Resist* 2020; 13: 4395-4403.
26. Liu C, Guo J. Hypervirulent *Klebsiella pneumoniae* (hypermucoviscous and aerobactin positive) infection over 6 years in the elderly in China: antimicrobial resistance patterns, molecular epidemiology and risk factor. *Ann Clin Microbiol Antimicrob* 2019; 18: 4.
27. Russo TA, Olson R, Fang CT, Stoesser N, Miller M, MacDonald U, et al. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical K. *J Clin Microbiol* 2018; 56(9): e00776-18.

28. Taraghian A, Nasr Esfahani B, Moghim S, Fazeli H. Characterization of hypervirulent extended-spectrum β-Lactamase-producing *Klebsiella pneumoniae* among urinary tract infections: the first report from Iran. *Infect Drug Resist* 2020; 13: 3103-3111.
29. Tabrizi AMA, Badmasti F, Shahcheraghi F, Azizi O. Outbreak of hypervirulent *Klebsiella pneumoniae* harbouring bla_{VIM-2} among mechanically-ventilated drug-poisoning patients with high mortality rate in Iran. *J Glob Antimicrob Resist* 2018; 15: 93-98.
30. Alizade H, Jajarmi M, Aflatoonian MR, Kalantar-Neyestanaki D, Shoja S, Ghanbarpour R. Comparative Prevalence of $bla_{CTX-M-15}$ Gene with Virulence Genes and Serotypes in *Klebsiella pneumoniae*. *Jundishapur J Microbiol* 2018; 11(4): e61285.
31. Catalán-Nájera JC, Garza-Ramos U, Barrios-Camacho H. Hypervirulence and hypermucoviscosity: Two different but complementary *Klebsiella* spp. phenotypes? *Virulence* 2017; 8: 1111-1123.
32. El-Mahdy R, El-Kannishy G, Salama H. Hypervirulent *Klebsiella pneumoniae* as a hospital-acquired pathogen in the intensive care unit in Mansoura, Egypt. *Germs* 2018; 8: 140-146.
33. Yan Q, Zhou M, Zou M, Liu WE. Hypervirulent *Klebsiella pneumoniae* induced ventilator-associated pneumonia in mechanically ventilated patients in China. *Eur J Clin Microbiol Infect Dis* 2016; 35: 387-396.
34. Zhang Y, Zhao C, Wang Q, Wang X, Chen H, Li H, et al. High prevalence of hypervirulent *Klebsiella pneumoniae* infection in China: geographic distribution, clinical characteristics and antimicrobial resistance. *Antimicrob Agents Chemother* 2016; 60: 6115-6120.
35. Guo Y, Wang S, Zhan L, Jin Y, Duan J, Hao Z, et al. Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. *Front Cell Infect Microbiol* 2017; 7: 24.
36. Liu C, Shi J, Guo J. High prevalence of hypervirulent *Klebsiella pneumoniae* infection in the genetic background of elderly patients in two teaching hospitals in China. *Infect Drug Resist* 2018; 11: 1031-1041.
37. Sun Y, Wu H, Shen D. Clinical and molecular analysis of *Klebsiella pneumoniae* causing liver abscess in China. *J Mol Microbiol Biotechnol* 2016; 26: 245-251.
38. Lam MMC, Wyres KL, Duchêne S, Wick RR, Judd LM, Gan YH, et al. Population genomics of hypervirulent *Klebsiella pneumoniae* clonal-group 23 reveals early emergence and rapid global dissemination. *Nat Commun* 2018; 9: 2703.
39. Hernández M, López-Urrutia L, Abad D, De Frutos Serna M, Ocampo-Sosa AA, Eiros JM. First report of an extensively drug-resistant ST23 *Klebsiella pneumoniae* of capsular serotype k1 co-producing *CTX-M-15*, *OXA-48* and *ArmA* in Spain. *Antibiotics (Basel)* 2021; 10: 157.
40. Parrott AM, Shi J, Aaron J, Green DA, Whittier S, Wu F. Detection of multiple hypervirulent *Klebsiella pneumoniae* strains in a New York City hospital through screening of virulence genes. *Clin Microbiol Infect* 2021; 27: 583-589.