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 (*blaNDM*, *blaKPC*, *blaCTX-M*, *blaVIM*, *blakIM*, *blaGPM*, *blaOXAX*, and *blaOXAS*) and ESBL (*blaCTX-M*, *blaSHV* and *blaTEM*) 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 *blaSHV* gene in hvKp and cKp was 94% (15/16) and 87.5% (336/384), respectively. The cKp isolates carried *blaNDM* (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 *blaSHV* gene were strongly associated with hvKp-*rmpA*+.

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

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. pneu-
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), lipo-polysaccharide (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 blaKDM and blaOXA 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 (Payam-bare-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 (Merk, 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 (Merk, Germany) with 30% sterile glycerol at -70°C.

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 μg), meropenem (10 μg), piperacillin (30 μg), piperacillin-tazobactam (100-10 μg), trimethoprim/sul-famethoxazole (1.25/23.75 μg), ceftazidime (30 μg), cefepime (30 μg), ampicillin-sulbactam (10-10 μg), aztreonam (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), and tetracycline (30 μg) (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 |
|-------------|---------------------|-----|
| blaIMP      | GGAATAGTAGTGGCTTAAYTCTC | 21  |
|             | GTTTGAYAAAACAAACACCC  |
|             | GATGTGGTTGATGCACATA   |
|             | CGAATGGCGAGCACCAG     |
|             | AAAATCTGGGATACCAAGAC  |
|             | AACATTACGCCTGGAACAGG  |
|             | CGCGTATGATGGATGTTCGCG |
|             | GCCCATATATATGCACCCCG  |
|             | TATATCCTAAGGCAAGG     |
|             | CACACAAATATGCGCTAAAC  |
|             | ATITTCAGACCTTTACGCC   |
|             | TATGCGTGCACTGTAGC     |
|             | AGCGCCCTAGGCAAAATTAAC |
|             | ATCCCGAGATTTTAAAC      |
|             | CATTCCGCTGTCGTCCCTTATC|
|             | TTAGGAATGTGCGCCCTGTA  |
| K1          | GTAGTTATCGAAGCAATGC   | 18  |
|             | GCCCAAGTTAATAGATCGGT  |
| K2          | GACCGCAATTTCACTACTGAGG|
|             | CTCTGAAGTTAATGCAAGCC  |
| K5          | TGTATGATGGTGCTAGCGGA |
|             | CTTGAAACCACCCAAATTC   |
| K20         | CGTGTCGCAATATGCATATT  | 18  |
|             | GTTATACGATGCATGCCTGAC|
| K54         | GATGTAGCTGATGGTGCT    |
|             | GCTTGACAACAAACCATAGCAG|
| K57         | CTGGCGGCCGATAGTCTGAG  |
|             | CACTAACCAGAAAATCGGAG  |
| rmpA        | ACTGCGCTACTCTGCTTCA   | 16  |
|             | CTTGCAATGACCCATFTTCA  |
| iucA        | CCAATCCGGCGTACCGCTGTC |
|             | CGAGGGCTGACGATGTTGCT  |
| imB         | AGAGGCCTGATTTGCGGTGTTG|
|             | CGATCTCGTGAACATCCGGCGTGTAG |
| pagO        | TGTCCTTGAACATTACCTTCC |
| LuxR        | CGTGGCGCGATGGAAACATA  |
|             | TGAGCCAAATGTAATGCGCAAAGGA |

ESBL identification genes. The presence of genes associated with ESBLs such as blaCTX-M, blaSHV, and blaTEM, 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 blaNDM, blaIMP, blavIM, blaKPC, blasm, blaOXAX, and blaOXAX181 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%)    | 26 (8.3%)     | 31 (8.3%)    |
|                  | Meropenem           | 5 (31.3%)   | 168 (33.6%)   | 173 (33.5%)  |
| Cephalosporin    | Ceftazidime         | 4 (25%)     | 58 (26.6%)    | 62 (33%)     |
|                  | Ceferpine           | 3 (18.8%)   | 57 (29.3%)    | 59 (29.7%)   |
| Beta-lactam inhibitor | Piperacillin-tazobactam | 1 (6.3%)   | 18 (9.3%)     | 19 (9.5%)    |
|                  | Ampicillin-sulbactam| 0 (0%)      | 56 (29.1%)    | 56 (29.4%)   |
| Beta-lactam      | Piperacillin        | 11 (68.8%)  | 261 (52.1%)   | 271 (54.4%)  |
| monobactam       | Aztreonam           | 2 (12.5%)   | 62 (29.3%)    | 66 (16.5%)   |
| Sulfonamide      | Trimethoprim/sulfamethoxazole | 8 (50%) | 64 (22.9%)   | 72 (23.5%) |
| Fluoroquinolone  | Ciprofloxacin       | 4 (25%)     | 103 (26.9%)   | 107 (26.8%)  |
| 30s              | Gentamicin          | 1 (6.3%)    | 76 (19.7%)    | 77 (19.5%)   |
|                  | Tetracycline        | 0 (0%)      | 128 (33.3%)   | 128 (32.8%)  |

**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*4, 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*1, *K*2, *K*3 and *K*4 types. The *K*4 positive strains were cultured from sputum (30%; *n* = 3), trachea (20%; *n* = 2), urine and blood (10%; *n* = 1 for each of them) specimens, while *K*4 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 *blaCTX-M* gene, and 116 (30%) carried *bla*TEM genes. These genes occurred in different combinations, *blaCTX-M+blaSHV* and *blaCTX-M+blaSHV+blaTEM* most frequently occurred together in 79 (20.5%) and 77 (20%) isolates, respectively. Twenty (5.2%) of the isolates possessed *blaSHV+blaTEM* genes, whereas two (0.5%) of isolates carried *blaCTX-M+blaTEM* 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 *blaSHV* gene and one (6%) strain had *blaCTX-M* gene. Therefore, only one strain of hvKP-rmpA+ strains was positive for both *blaCTX-M* and *blaSHV* genes. None of hvKP-rmpA+ strains were positive for *blaTEM* gene (Table 3).

**Frequency of carbapenemase genes.** All *K. pneumoniae* isolates were negative for *blaIMP, blaKPC, blaSHV, blaOXA-48*, and *blaOXA-181*. But the results revealed that 30 (7.8%) cKP isolates possessed *blaNDM* gene. The *blaNDM* 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 *blaNDM* 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...
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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 |
|----|-----------|-----------------|----------------|-------------|---------|
|    | blaCTX-M | blaSV | iucA | iroB | luxR | pagO | K1 | K2 | K3 | K4 |     |
| 1  | +        | +     | +    | +    | +    | +   | +  |     |     |     | Urine |
| 2  | +        | +     | +    | +    | +    |     | +  |     |     |     | Urine |
| 3  | +        | +     | +    | +    | +    |     | +  |     |     |     | Blood |
| 4  | +        | +     | +    | +    | +    |     |     |     |     |     | Wound |
| 5  | +        | +     | +    | +    | +    |     |     |     |     |     | Trachea |
| 6  | +        | +     | +    | +    | +    |     |     |     |     |     | Sputum |
| 7  | +        | +     | +    | +    | +    |     |     |     |     |     | Urine |
| 8  | +        | +     | +    | +    | +    |     |     |     |     |     | Sputum |
| 9  | +        | +     | +    | +    | +    |     |     |     |     |     | Sputum |
| 10 | +        | +     | +    | +    | +    |     |     |     |     |     | Trachea |
| 11 | +        | +     | +    | +    | +    |     |     |     |     |     | Trachea |
| 12 | +        | +     | +    | +    | +    |     |     |     |     |     | Wound |
| 13 | +        | +     | +    | +    | +    |     |     |     |     |     | Trachea |
| 14 | +        | +     | +    | +    | +    |     |     |     |     |     | Sputum |
| 15 | +        | +     | +    | +    | +    |     |     |     |     |     | Trachea |
| 16 | +        | +     | +    | +    | +    |     |     |     |     |     | Sputum |
| Total | 1   | 15   | 13   | 16   | 16   | 10  | 3  | 1  | 2  | 15 |

*Only the results of positive genes have been shown (All were negative for blaTEM, blaNDM, blaIMP, blaVIM, blaAPM, blaKPC, blaCTX-M, blaSV, K1 and K2*).

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 K1 (62%) and K2 (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 blaNDM gene. Also, 15 hvKp-rmpA+ strains carried blaSV gene and only one strain had blaCTX-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

| Total N (%) | ESBL genes profile | Samples (N, %) |
|-------------|--------------------|----------------|
| 79 (20.5%)  | cKp isolates       | Urine (26, 6.7%) |
|             | bla<sub>CTX-M</sub>, bla<sub>HIV</sub> | 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%) |
| 77 (20%)    | bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>HIV</sub> | 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<sub>TEM</sub>, bla<sub>HIV</sub> | Urine (15, 3.9%) |
|             |                    | Wound (3, 0.7%) |
|             |                    | Trachea (1, 0.26%) |
|             |                    | Sputum (1, 0.26%) |
| 2 (0.5%)    | bla<sub>TEM</sub>, bla<sub>CTX-M</sub> | Wound (2, 0.5%) |
| 16 (53.3%)  | bla<sub>CTX-M</sub>, bla<sub>HIV</sub> | Trachea (8, 26.6%) |
|             |                    | Urine (4, 13.3%) |
|             |                    | Wound (4, 13.3%) |
| 12 (40%)    | bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>HIV</sub> | Urine (9, 30%) |
|             |                    | Wound (1, 3.3%) |
|             |                    | Discharge (1, 3.3%) |
|             |                    | Blood (1, 3.3%) |
| 1 (3.3%)    | bla<sub>TEM</sub>, bla<sub>HIV</sub> | Urine (1, 3.3%) |
| 1 (3.3%)    | bla<sub>TEM</sub>, bla<sub>CTX-M</sub> | Urine (1, 3.3%) |

*One bla<sub>ndaM</sub> + 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-<i>rmpA</i>+ 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-<i>rmpA</i>+ were less resistant than cKp to imipenem, piperacillin-tazobactam,
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Cefepime, ampicillin-sulbactam, aztreonam, ciprofloxacin, gentamicin, and tetracycline. In this study, the number of hvKp-rmpA+ possessed ESBL genes, especially blaSHV gene (94%) is significantly higher compared to carbapenemase genes. None of hvKp-rmpA+ strains possessed carbapenemase genes. But, 7.8% of cKp isolates carried blaNDM 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 blaSHV and blaCTX-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, blaVIM-2 and dhfrI cassette arrangement in a class 1 integron (29). A study in Spain revealed the first description of a blaCTX-M-15 blaOXA-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/huca/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 blaSHV 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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