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Evaluation of Virulence Factors and Antibiotic Resistance Patterns in Clinical Urine Isolates of *Klebsiella pneumoniae* in Semnan, Iran

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Abstract

Background: *Klebsiella pneumoniae* as an opportunistic pathogen can be the cause of a range of nosocomial and community - acquired infections. Many virulence factors help these bacteria overcome an immune system and cause various diseases. K1 and K2 capsular antigens, also *magA*, *wcaG*, and *rmpA* are well - known *K. pneumoniae* virulence factors. *Klebsiella pneumoniae* has been revealed to have the ability to acquire resistance to many antibiotics, which cause treatment failure.
Objectives: This study aimed at determining the prevalence of *magA*, *wcaG*, *rmpA*, Capsular type K1, Capsular type K2, TEM, and SHV in *K. pneumoniae* isolates.
Methods: A total of 173 non - duplicate *K. pneumoniae* isolates were collected from two different hospitals in Semnan, Iran, from urine specimens. *Klebsiella pneumoniae* was identified by conventional bacteriological tests. Disk diffusion test was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Detection of virulence factors, TEM, and SHV gene was performed by specific primers.
Results: Frequency of virulence factors was as follow: capsular type K2: 32.9%, *rmpA*: 20.2%, capsular type K1: 6.9%, and *wcaG*: 16.2%. Also, the SHV and TEM were observed in 46.8% and 33.5%, respectively. Antibiotics resistance rates were as follow, imipenem: 7.5%, ciprofloxacin: 16.1%, levofloxacin: 17.3%, amoxicillin - clavulanic acid: 30%, trimethoprim - sulfamethoxazole: 32.9%, cefepime: 34.1%, nitrofurantoin: 35.8%, amikacin: 36.4%, aztreonam: 39.3%, ceftazidime: 42.7%.
Conclusions: Frequency of some virulence factors including capsular type K2, *rmpA*, *wcaG*, and also resistant rate to imipenem, amikacin, and ceftazidime were significantly higher than similar studies. Presence of virulence factors accompanied by drug resistance should make bacteria an infectious agent and lead to treatment failure.

Keywords: *Klebsiella pneumoniae*, Virulence Factors, Antibiotic Resistance

1. Background

Klebsiella pneumoniae, as an opportunistic pathogen, can be the cause of a range of nosocomial and community - acquired infections (1-3). Pathogenic *K. pneumoniae* strains are responsible for urinary tract, respiratory, and blood infections and are associated with mortality and morbidity in patients (4-6). Pathogenicity of *K. pneumoniae* is the result of production of many virulence factors that help these bacteria overcome the immune system and cause various diseases. Different virulence factors, such as lipopolysaccharide (o - antigen), capsular polysaccharides (K antigen), fimbriae and siderophores contribute to the pathogenicity of *Klebsiella* (7, 8). Among these factors, the capsule is one of the most important virulence determinants, which helps achieve biofilm formation and leads to protection against phagocytosis, antimicrobial peptides, and serum bactericidal activity. There are more than 77 types of capsular antigens in *K. pneumoniae* strains, of which K1 and K2 serotypes are well known (9, 10).

Klebsiella pneumoniae also carries virulence - associated genes, which may encode capsules (*magA*, *K2A*, *WcaG*) and a capsular regulator gene (regulator of mucoid phenotype (*rmpA*). Mucoviscosity - associated gene A (*magA*) and WcGA genes located within the gene clusters are responsible for encoding capsular polymerase and capsular biosynthesis, respectively (11-13). Capsular biogenesis by *magA* is achieved through conversion of manose to fucose, which

may enhance the ability of the bacteria to evade phagocytosis by macrophage. The regulator of mucoid phenotype A (*rmpA*) gene located on the plasmid increases capsular polysaccharide production and is found in the hypermucoviscous phenotype. Moreover, two genes, including the *magA* and *rmpA*, were initially associated with invasive infections (14, 15).

Resistance of pathogenic bacteria to different antibiotics has become a serious worldwide problem because of the fatal outcome of defective treatment and the difficulty to find treatment options (16). *Klebsiella pneumoniae* has been revealed to have the ability to acquire resistance to many antibiotics, especially third generation cephalosporins. Multidrug resistance of *K. pneumoniae* against the antibiotics used for therapy intensifies the virulence potential of *Klebsiella*. Beta - lactam antibiotics are one of the most commonly used antibiotics in the treatment of bacterial infections and the production of B - lactamase enzymes are the most common bacterial resistance mechanisms (17, 18). In the recent years, Extended Spectrum Beta Lactamase (ESBL) producer *K. pneumoniae* have increased over the world. The ESBLs are divided to several groups; the main groups are TEM, CTX, and SHV derivatives (19, 20).

2. Objectives

The present study aimed at determining the prevalence of *magA*, *wcaG*, *rmpA*, Capsular type K1 and Capsular type K2 virulence genes along with SHV and TEM beta lactam resistance genes in *K. pneumoniae* isolates.

3. Methods

3.1. Ethics Statement

The ethics committee of the Semnan University of Medical Sciences, Semnan, Iran, approved this study (IR.SEMUMS.REC 1394.29).

3.2. Sampling

A cross sectional study was performed. A total of 173 non - duplicate *K. pneumoniae* isolates were collected during year 2015 at two different hospitals of Semnan, Iran, from urine specimens.

3.3. Bacterial Identification

The specimens were cultured on blood agar and Eosin Methylene Blue (EMB) agar (Merck, Darmstadt, Germany), and incubated at 37°C for 24 hours. *Klebsiella pneumoniae* was identified by conventional bacteriological tests. Gram stain was performed for suspected colonies. Biochemical tests, including lactose fermentation, gas production from glucose, FeS production, motility, indole production, sodium citrate utilization, and urea utilization were used for *K. pneumoniae* identification (21).

3.4. Antibiotic Susceptibility Testing

Disks diffusion test was performed according to the guidelines of the Clinical and Laboratory Standards Institute (22). Used disks (Rosco, Taastrup, Denmark) were amikacin (30 μg), ciprofloxacin (5 μg), amoxicillin - clavulanic acid (20/10 μg), ceftazidime (30 μg), imipenem (10 μg), cefepime (30 μg), aztreonam (30 μg), nitrofurantoin (300 μg), and trimethoprim - sulfamethoxazole (1.25/23.75 μg). *Escherichia coli* (ATCC 25922) strain was used for quality control.

3.5. DNA Extraction

DNA of each *K. pneumoniae* isolate was extracted from 1 mL of overnight bacterial culture. Extraction was performed as previously described by Kuske et al. (23). The supernatant was used as the template DNA in PCR reactions.

3.6. Detection of Capsular type K1, Capsular type K2, rmpA, wcaG, TEM, and SHV

Specific primers for detection of Capsular type K1, Capsular type K2, *rmpA*, *wcaG*, TEM, and SHV are shown in Table 1. For capsular type K1, capsular type K2, *rmpA*, and *wcaG* amplification were performed as follow: initial denaturation at 95°C for five minutes, followed by 35 cycles at 94°C for 30 seconds, 58°C for 90 seconds and 72°C for 90 seconds and a final extension at 72°C for 10 minutes (24). Polymerase Chain Reaction (PCR) conditions for TEM amplification were as follow: initial denaturation at 94°C for three minutes, 35 cycles at 94°C for 30 seconds, 45°C for one minute, 72°C for one minute, final extension at 72°C for 10 minutes (25). The SHV was amplified under the following conditions: 94°C for three minutes, 35 cycles at 94°C for 30 minutes, 60°C for one minute, 72°C for one minute, and a final extension at 72°C for 10 minutes (25). The PCR products were analyzed by electrophoresis with 1% agarose gel in 1X Tris - Acetate - EDTA buffer. The gels were stained with ethidium bromide and the PCR products were visualized under UV light (Figure 1).

3.7. Statistical Analysis

Confidence interval test was used to assess the statistical significance with confidence level of 95% ($\alpha = 0.05$).

Table 1. Specific Primers for Detection of Capsular Type K1, Capsular Type K2, *rmpA*, *wcaG*, TEM, and SHV

Gene	Primer	Sequence	Product Size (bp)	Reference
Capsular type K1	*MagA* - F	GGTGCTCTTTACATCATTGC	1283	Turton et al. (24)
	MagA - R	GCAATGGCCATTTGCGTTAG		
Capsular type K2	K2wzy - F	GACCCGATATTCATACTTGACAGAG	641	Turton et al. (24)
	K2wzy - R	CCTGAAGTAAAATCGTAAATAGATGGC		
rmpA	*rmpA* - F	ACTGGGCTACCTCTGCTTCA	516	Turton et al. (24)
	rmpA - R	CTTGCATGAGCCATCTTTCA		
wcaG	*wcaG* - F	GGTTGGKTCAGCAATCGTA	169	Turton et al. (24)
	wcaG - R	ACTATTCCGCCAACTTTTGC		
SHV	SHV - F	AAGATCCACTATCGCCCAGCAG	200	Shahcheraghi et al. (25)
	SHV - R	ATTCAGTTCCGTTTCCCAGCGG		
TEM	TEM - F	GAGTATTCAACATTTCCGTGTC	800	Shahcheraghi et al. (25)
	TEM - R	TAATCAGTGAGGCACCTATCTC		

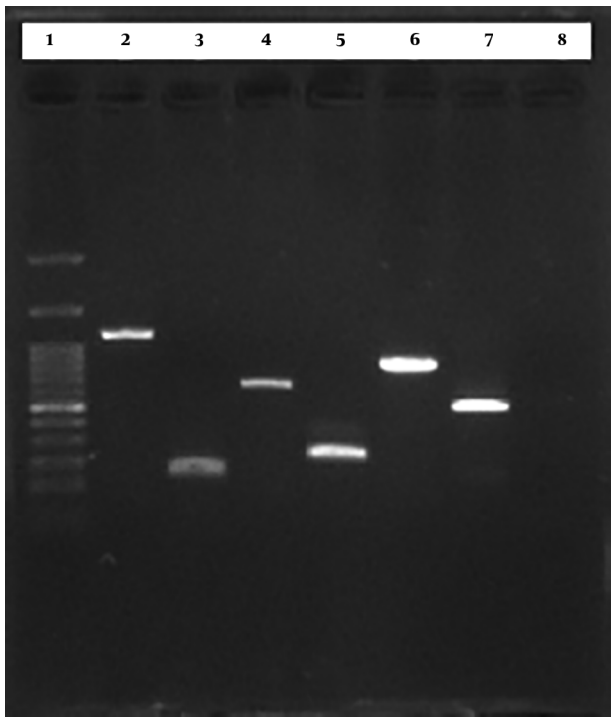

Figure 1. PCR products electrophoresis. 1: Marker (100 bp), 2: Capsular type K1 (1283 bp), 3: *wcaG* (169 bp), 4: Capsular type K2 (641 bp), 5: SHV (200 bp), 6: TEM (800bp), 7:rmp A (516 bp), 8: negative control.

4. Results

In this study, frequency of virulence factors was as follow: capsular type K2: 57 out of 173 (32.9% CI: 39.9%, 25.9%), *rmpA*: 35 out of 173 (20.2% CI: 26.2%, 14.2%), *wcaG*: 28 out of 173 (16.2% CI: 19.4%, 13%), capsular type K1: 12 out of 173 (6.9% CI: 10.6%, 3.2%). Also, the SHV was observed in 81 out of 173 (46.8% CI: 51.2%, 42.4%) and TEM was observed in 58 out of 173 (33.5% CI: 37.5%, 29.5%) cases. The resistant rates to antibiotics were as follow, imipenem: 13 out of 173 (7.5% CI: 9.8%, 5.2%), ciprofloxacin: 28 out of 173 (16.1% CI: 19.2%, 13%), amoxicillin - clavulanic acid: 52 out of 173 (30% CI: 33.9%, 26.1%), trimethoprim - sulfamethoxazole: 57 out of 173 (32.9% CI: 36.9%, 28.9%), cefepime: 59 out of 173 (34.1% CI: 38.2%, 30%), nitrfurantoin: 62 out of 173 (35.8% CI: 35.8%, 27.8%), amikacin: 63 out of 173 (36.4% CI: 40.5%, 32.3%), aztreonam: 68 out of 173 (39.3% CI: 43.5%, 35.1%).

5. Discussion

Klebsiella pneumoniae is the cause of nosocomial and community acquired infections, such as urinary tract, respiratory, and blood infections. It harbors many virulence-associated genes including *magA*, *rmpA*, *WcaG*, fimbriae, and siderophores, which help the bacteria overcome the immune system and cause infection. Drug resistant, especially ESBL - producing *K. pneumoniae* strains, contribute to treatment failure and increase morbidity and mortality in patients (26-28).

In this study, the frequency of SHV was significantly lower than 69.6% and 67.4% reported by Shahcheraghi et al. (25) and Feizabadi et al. (29), respectively. Frequency of TEM was significantly lower than 54%, reported by Feizabadi et al. (29), yet in accordance with 32.1% reported by Shahcheraghi et al. (25). These differences were the result of differences in infection control system and therapeutic regimens in different parts of Iran. Age of over 60, long hospitalization, diabetes and previous antibiotic

treatment were other factors associated with the ESBL phenotype (30).

Capsular type K1 frequency was significantly lower than 39.5% and 64.3% reported by Liu et al. (31) and Lee et al. (32), yet significantly higher than 1.4% reported by Maatallah et al. (33). Capsular type K2 frequency was significantly higher than 18.4% and 20% reported by Liu et al. (30) and Lee et al. (32), yet significantly lower than 5% reported by Maatallah et al. (33). Frequency of *rmpA* was significantly higher than 5.5%, 3.6%, and 7.0% reported by Derakhshan et al. (27), Maatallah et al. (33), and Derakhshan et al., respectively (34). Frequency of *wcaG* was significantly higher than 2.7% and 8.6% reported by Derakhshan et al. (27) and Maatallah et al. (33), yet significantly lower than 23.5% reported by Derakhshan et al. (34).

The resistance rate to imipenem was significantly higher than 1.4% and 0.05% reported by Liu et al. (31), and Derakhshan et al. (34), and also reports of Shahcheraghi et al. (25), Peerayeh et al. (26), Derakhshan et al. (27), Peerayeh et al. (28), and Ranjbar et al. (35), which reported no resistance to imipenem, yet significantly lower than 20.8% reported by Amraie et al. (36). The resistance rate to ciprofloxacin was significantly lower than 44.4%, 37.1%, 42.5%, and 52.5% reported by Derakhshan et al. (27), Liu et al. (31), Derakhshan et al. (34), and Ranjbar et al. (35), yet in accordance with 18%, 18%, and 15.6% reported by Shahcheraghi et al. (25), Peerayeh et al. (26), and Amraie et al. (36). The resistance rate to amoxicillin - clavulanic acid was significantly lower than 31.7% and 28.6% reported by Liu et al. (31) and Pereira et al. (37), yet in accordance with 100% and 60.5% reported by Derakhshan et al. (27) and Derakhshan et al. (34), respectively.

The resistance rate to nitrofurantoin was significantly lower than 40% and 71% reported by Ranjbar et al. (35) and Amraie et al. (36). The resistance rate to trimethoprim - sulfamethoxazole was significantly lower than 39%, 55.5%, 39.3%, 50%, 51.5%, and 54% reported by Peerayeh et al. (26), Derakhshan et al. (27), Peerayeh et al. (26), Derakhshan et al. (34), Ranjbar et al. (35), and Amraie et al. (36), respectively, yet in accordance with 32.9% reported by Liu et al. (31). The resistance rate to cefepime was significantly higher than 12.9% reported by Liu et al. (31), yet in accordance with 37.5% reported by Peerayeh et al. (26), also significantly lower than 80.5% and 57% reported by Peerayeh et al. (26) and Derakhshan et al. (34).

The resistance rate to amikacin was significantly higher than 14%, 27%, 10%, 22%, and 4.8% reported by Shahcheraghi et al. (25), Peerayeh et al. (26), Liu et al. (31), Amraie et al. (36), and Pereira et al. (37), yet in accordance with 31.5% reported by Derakhshan et al. (34), also significantly lower than 50% reported by Derakhshan et al. (27). The resistant rate to aztreonam was significantly lower than 100%, 59%, and 92.5% reported by Derakhshan et al. (27), Derakhshan et al. (34), and Ranjbar et al. (35), yet in accordance with 39% and 38.1% reported by Peerayeh et al. (26) and Pereira et al. (37), also, significantly higher than 30% reported by Liu et al. (31). The resistance rate to ceftazidime was significantly higher than 31.3%, 51.1%, 35.8%, and 4.8% reported by Shahcheraghi et al. (25), Peerayeh et al. (28), Liu et al. (31), and Pereira et al. (37), yet significantly lower than 100%, 57%, 100%, and 49.7% reported by Derakhshan et al. (27), Derakhshan et al. (34), and Ranjbar et al. (35).

6. Conclusions

Frequency of some virulence factors including Capsular type K2, *rmpA*, *wcaG*, and also resistance rates to imipenem, amikacin, and ceftazidime were significantly higher than similar studies. Presence of virulence factors accompanied by drug resistance make bacteria an infectious agent and lead to treatment failure.

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Footnotes

Authors' Contribution: All authors participated in the research design and contributed to different parts of the research.

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