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The abundance of capsule (*wabG*) and fimbria (*fimH*) coding genes in multidrug-resistant (MDR) *Klebsiella pneumoniae* strains isolated from patients admitted to Isfahan hospitals

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Abstract

The high resistance of *K. pneumoniae* strains to various antibiotics is remarkable. The most important virulence factors for *K. pneumoniae* include fimbriae, capsule, lipopolysaccharide, outer membrane proteins, and iron transport molecules. The aim of this study was to investigate the prevalence of capsule (*wabG*) and fimbriae (*fimH*) coding genes in MDR *K. pneumoniae* strains isolated from patients admitted to Isfahan (Iran) hospitals. The antibiotic susceptibility pattern of the isolates was carried out by the disk diffusion method. Definitive confirmation of MDR isolates was done by tracing the *16S-23S ITS* gene, and the presence of capsule (*wabG*) and fimbriae (*fimH*) genes was investigated in the isolates. Data analysis was done using an independent parametric T-test and one-way analysis of variance (ANOVA). One hundred and two *K. pneumoniae* isolates were detected in the samples, including urine, respiratory tract, blood, throat, cerebrospinal fluid, direct discharge, wound secretions, pleural fluid, joint fluid, abscess discharge, stool, and sputum. Men were significantly more infected with *K. pneumoniae* than women. The highest frequency of the isolates was related to urine (40%), followed by the respiratory tract (27%). The largest number of isolates were found in the ICU (37%) and emergency (28%) departments. Out of the 102 isolates of *K. pneumoniae*, 50 isolates (49%) were MDR, and 50 (49%) were carbapenem-resistant. Of the 50 MDR isolates, 48 (96%) and 47 (94%) had *fimH* and *wabG* genes, respectively. High frequencies of MDR and carbapenem-resistant strains of *K. pneumoniae* with a high prevalence of *fimH* and *wabG* genes are significant and should be considered by healthcare management.

1. Introduction

K. *pneumoniae* is one of the most important pathogens associated with MDR infections with challenging treatment. Infections caused by the bacterium are becoming more severe because of the indiscriminate use of antibiotics (Molton et al., 2013; Tan et al., 2020), especially during the recent COVID-19 pandemic (Patil et al., 2023). This problem is more significant with regard to carbapenems (Reyes et al., 2019).

K. *pneumoniae* is an opportunistic pathogen. These pathogens, which are generally observed as commensals, have an increased advantage in

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causing infections in patients with weakened immune systems or with defective microbiomes, such as hospitalized patients (Magill et al., 2014; Boucher et al., 2009). The transmission rate of K. *pneumoniae* in hospitals is related to the length of hospitalization and the intensity of colonization. Except for contamination caused by faulty hygiene practices in medical equipment and blood products, the main reservoirs of K. *pneumoniae* transmission in the hospital are the digestive system of patients and the hands of hospital staff. The ability of this organism to spread rapidly often causes hospital-acquired infections. Epidemic nosocomial infections caused by MDR strains are extremely dangerous (Gorrie et al., 2022). For example, some studies after the Coronavirus 2 epidemic in Iran show that there was a large increase in the resistance of Klebsiella sp. isolates causing UTIS to three antibiotics, including ceftriaxone (CRO), imipenem (IPM), and gentamicin (GEN), after the coronavirus pandemic in 2021 (Hamidi Hesari et al., 2022).

Virulence factors, including fimbriae, capsule, outer membrane proteins (OMPs), lipopolysaccharide (LPS), and Siderophores, are important in the pathogenesis of K. pneumoniae (Zhu et al., 2021; Gan et al., 2022). The polysaccharide capsule is an important virulence factor in K. *pneumoniae* . Several capsule serotypes have been found in invasive infections caused by the bacterium. The capsule promotes the bacterium's resistance to phagocytosis, antimicrobial peptides, and complement lysis process (Pan et al., 2015; Xu et al., 2021). It has been shown that the capsule enhances the survival of K. *pneumoniae* in the bloodstream by protecting the bacterium from being captured by macrophages in the liver (Huang et al., 2022). Recent studies have found the *wabG* gene among the common genes in invasive and MDR K. *pneumoniae* strains (Hasani et al., 2020; Fursova et al., 2021). Fimbria is also known to play a role in the virulence of K. *pneumoniae* by contributing to colonization, irruption, and pathogenicity (Guo et al., 2017), especially in biofilm formation by MDR strains (Riwu et al., 2022). Recent studies have detected *fim* genes in MDR strains (Makhrmash et al., 2022; Jin et al., 2022).

Because of the increasing prevalence of MDR K. *pneumoniae* strains and the importance of fimbriae and capsules in the invasion of these strains, the present study aimed to investigate the multidrug resistance of K. *pneumoniae* strains isolated from patients admitted to hospitals in Isfahan (Iran) and to detect the fimbriae and capsule coding genes (*fimH* and *wabG*, respectively) in these strains.

2. Materials and methods

2.1. Clinical samples

Two hundred clinical samples of blood, urine, lung, and purulent secretions were collected in sterile containers from different departments of hospitals in Isfahan, Iran, from March 2022 to July 2022. The samples were rapidly transferred on ice to microbiology laboratory 9. The samples were inoculated on blood agar and McConkey agar. Of 200 isolates of K. *pneumoniae* , 102 were isolated on blood agar and McConkey agar and identified using microbiological and biochemical tests. Then, accurate identification was made by DNA extraction (by boiling) and PCR amplification of the 16S-23S internal transcribed spacer (16S-23S *ITS*) gene in K. pneumoniae . The obtained 130 bp band was visualized by agarose (1%) gel electrophoresis. The used primer pairs are listed in (Table 1) (Turton et al., 2010). The PCR protocol used is listed in (Table 2). K. *pneumoniae* ATCC 700603 was used as the positive control, and water was used as the negative control. The PCR products were visualized in 1% agarose gel electrophoresis.

Table 1: The sequences of primers used for accurate identification of *K. pneumoniae* .

Target gene	Sequences of primers (5′ 3′)	Amplicon size (bp)	Reference
16S-23S ITS	F:ATTTGAAGAGGTTGCAAACGAT R:TTCACTCTGAAGTTTTCTTGTGTTC	130	Turton et al. 2010

Table 2: The PCR protocol for the amplification of the *16S-23S ITS*) gene in *K. pneumoniae* (Turton et al., 2010).

Step	Number of cycles	Denaturation Temperature /Time	Primer annealing Temperature/Time	Extension Temperature/ Time
1	1	94 °C/5 min	-	-
2	35	95 °C/30 s	58 °C/90 s	72 °C/90 s
3	1	-	-	72 °C/10 min

2.2. Antibiotic sensitivity test

The sensitivity of K. *pneumoniae* strains to antibiotics was investigated using the disc diffusion method using antibiotic disks (Padtanteb, Iran), including meropenem (MEN, 10 µg), imipenem (IPM, 10 µg), ciprofloxacin (CP, 5 µg), gentamicin (GM, 10 µg), and cotrimoxazole (SXT, 10 µg). The results were recorded as the diameter of the growth inhibition zone around each antibiotic disk, and the data were interpreted by referring to the standard tables of Clinical and Laboratory Standard Institute-2021 (CLSI-2021). MDR isolates were used for the next experiments.

2.3. Detection of the virulence genes in MDR strains

The presence of *fimH* and *wabG* genes was investigated by PCR on the extracted DNA using the primers shown in (Table 3). For primer design, the gene sequence, along with 75 base pairs before it and 75 base pairs after it, were obtained from NCBI. Then, the specific forward and reverse primers were designed using Gene Runner software. The PCR protocol was achieved as detailed in (Table 4). The PCR products were visualized in 1% agarose gel electrophoresis. K. *pneumoniae* ATCC 700603 was used as the positive control, and water was used as the negative control.

Table 3: Primer sequences that were designed for genotypic identification of MDR strains.

Target gene	Sequences of primers (5 3′)	Amplicon size (bp)	**Ref**
fimH	F:TATGGCGGTGTGCTGTCGAG-3’ R:GGGAGGGTGACGGTGACATC-3’	329	This study
wabG	F:TCCCGGCTGCGATCTCTACC-3’ R:CGGAGCCGACGTAGATCAGG-3’	362	

Table 4: The PCR protocol for the amplification of *fimH* and *wabG* genes in *K. pneumoniae* **.**

Step	Number of cycles	Denaturation Temperature /Time	Primer annealing Temperature/ Time	Extension Temperature/Time
1	1	94 °C/5 min	-	-
2	35	94 °C/30 s	55 °C/30 s	72 °C/30 s
3	1	-	-	72 °C/5 min

2.2. Analysis of data

The data were analyzed using SPSS version 20 (Chicago SPSS, USA), and the graphs were plotted in Excel 2010 (Microsoft Corporation, USA). The differences between the averages and the relationships between the groups were determined using the parametric independent T-test and the analysis of variance (one-way ANOVA). The p-values lower than 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Molecular identification of the isolates

This study included 200 clinical samples from Isfahan (Iran) hospitals, from which 102 strains of K. *pneumoniae* were detected by phenotypic identification via biochemical tests, which were

then accurately identified by tracing a specific 130 bp fragment in *16S-23S ITS* gene of K. *pneumoniae* (Fig 1).

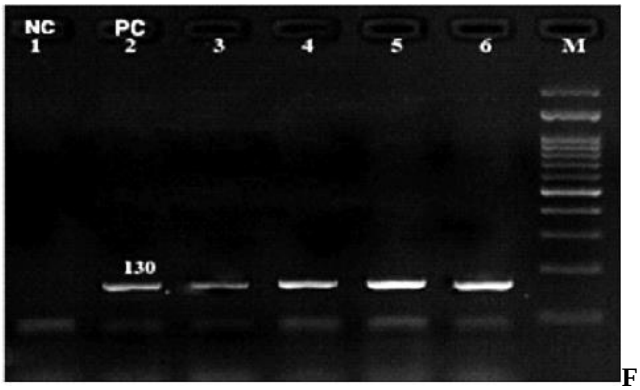

Figure 1. The 130 bp fragment, which was amplified by PCR in the *16S-23S ITS* gene of some *K. pneumoniae* strains on 1% agarose gel electrophoresis (M: 100 bp DNA marker, 1: negative control. 2: Positive control, *K. pneumoniae* ATCC 700603, 3-6: the amplified fragment in some detected clinical isolates. ATCC 700603

3.2. Distribution of the isolates

Of 102 isolates, 39 (38%) were from female patients, and 63 (62%) were from male patients. These results showed that the frequency of isolates in men was 1.61 times more than in women. Therefore, it seems that men were more infected by K. *pneumoniae* than women, with a significant difference ($P < 0.05$). According to the results presented in (Fig 2), out of 102 isolates, the highest frequencies were related to urine (40%) and respiratory tract (27%), respectively, and the lowest frequency was related to sputum, joint fluid, abscess, and feces (1%). There were significant differences between the distributions of the isolates in different clinical samples ($P < 0.05$). Ballén et al. (2021) detected 127 strains of K. *pnemoniae* in different clinical samples. They emphasized the differences between urine-achieved strains and the strains from other clinical sources. Urine strains showed the highest antibiotic resistance and the highest production of extended-spectrum beta-lactamases (ESBL). The urine strains also significantly presented higher levels of virulence genes, such as the uge gene, compared to the strains from other clinical sources. A high number of biofilm-producing strains were also found in urine. The rapid spread of these clinical strains, found by Ballén et al., as well as the high prevalence of K. pneumoniae in our study, has also been reported in other recent studies (Li et al. 2022, Niazadeh et al. 2022), is of concern. New therapeutic procedures are recommended to investigate the control of the adverse effects of these strains.

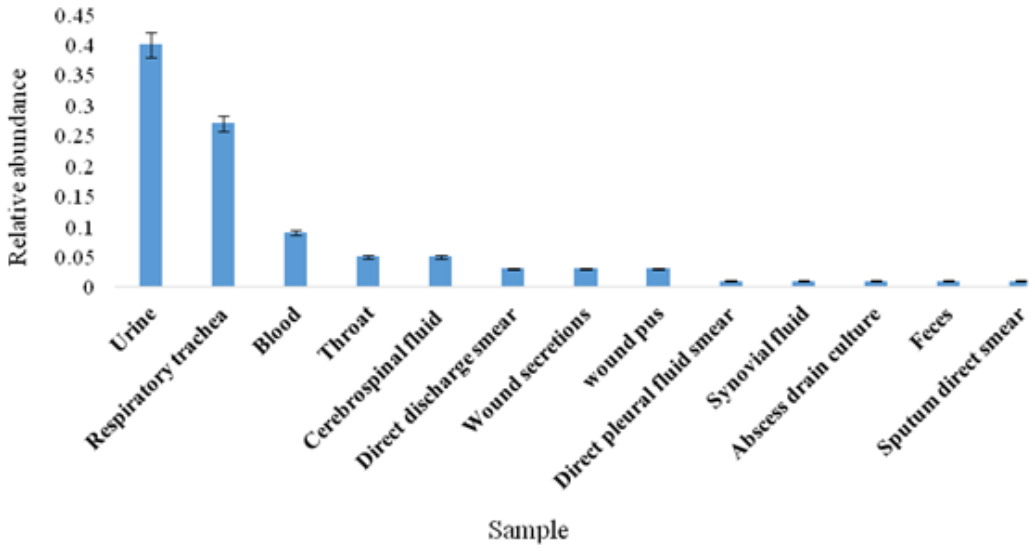

Figure 2. Frequency distribution of *K. pneumoniae* isolates according to the sample type.

According to the results presented in (Fig 3), out of 102 isolates, most were from ICU (37%) and emergency (28%) departments. There was a significant difference between the distributions of

the isolates in different departments of the hospitals ($P < 0.05$).

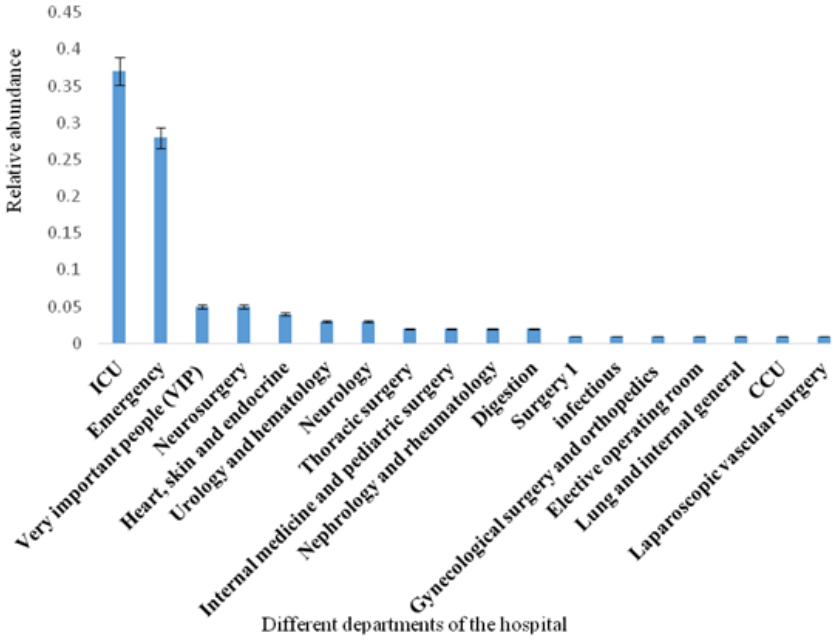

Figure 3. Frequency distribution of the isolates in different departments of the hospitals.

3.3. Antibiotic sensitivity

The results of antibiotic sensitivity pattern evaluation are shown in (Table 5). Out of 102 isolates of K. *pneumoniae*, 50 isolates (49%) had multiple drug resistance patterns, and 52 isolates (51%) had no multiple drug resistance pattern

Table 5: The antibiotic sensitivity pattern of the strains of *K. pneumoniae* according to the CLSI-2021 standard.

Antibiotic	MDR strains N=50			Non MDR strains N=52		
	Resistant (%)	Intermediate (%)	Sensitive (%)	Resistant (%)	Intermediate (%)	Sensitive (%)
Imipenem (IPM)	50 (49.01)	0	0	0	6 (5.88)	46 (45.09)
Meropenem (MEN)	50 (49.01)	0	0	19 (18.62)	7 (6.86)	26 (25.49)
Cotrimoxazole (SXT)	46 (45.09)	2 (1.96)	2 (1.96)	25 (24.5)	3 (2.94)	24 (23.52)
Ciprofloxacin (CP)	50 (49.01)	0	0	25 (24.5)	9 (8.8)	18 (17.46)
Gentamicin (GM)	50 (49.01)	0	0	3 (2.94)	9 (8.8)	3 (2.94)

Healthcare-associated infections (HAIs) by Gram-negative bacteria are a common problem worldwide (Cassini et al., 2016), particularly HAIs caused by MDR strains. The indiscriminate consumption of antibiotics has caused an increased incidence of MDR bacterial strains in recent years (Tacconelli et al., 2018). MDR K. *pneumoniae* strains commonly acquire ESBL or carbapenemase genes that cause this bacterium to become resistant to third-generation cephalosporins or carbapenems, leading to minimal options for antimicrobial therapy (Petrosillo et al., 2013). MDR K. *pneumoniae* is among the most prevalent causes of HAIs in hospitals and can cause urinary tract infections, pneumoniae, wound infections, and sepsis (Gorrie et al., 2022; Niazadeh et al., 2022).

3.3. Distribution of *fimH* and *wabG* genes in MDR isolates

Among 50 MDR isolates, 48 isolates (96%) had the *fimH* gene, of which 17 isolates were obtained from female patients and 31 from male patients. Among 50 MDR isolates, 47 isolates (94%) had the *wabG* gene, of which 18 isolates were

obtained from female patients and 29 isolates were obtained from male patients.

Fimbriae, capsule, and siderophores are the main virulence factors in K. *pneumoniae* that contribute to the pathogenicity of its clinical strains (Karampatakis et al., 2023). Hyper-virulence strains of K. pneumoniae are associated with the overproduction of capsules (Walker and Miller, 2020). Tan et al. (2020) investigated the K. *pneumoniae* strain SGH10 with several deletions that led to the production of mutants that interrupt different steps in the capsule production biosynthetic pathway. They showed the importance of the capsule in the pathogenesis of the bacterium, as the non-capsulated strains had less cellular survival in the gut and had increased susceptibility in contact with bile salts than the capsulated wild-type strain.

In the present study, of the 50 identified MDR strains of *K. pneumoniae*, 47 strains (94%) had the *wabG* gene. Choi et al. (2020) determined the diversity of lipopolysaccharide and capsular polysaccharide antigens in 645 K. pneumoniae isolates collected from blood samples in 13 countries. Among the isolates, 19.3% were resistant to carbapenems and 62.1% were MDR. A limited number of lipopolysaccharide antigen types and diverse capsular antigen types were seen in the isolates. Although the carbapenem resistance seen in all isolates in the present study is significant, the results showed that 49% of K. *pneumoniae* isolates were MDR, lower than that obtained by Choi et al. (2020). Given the high number of capsular antigen types in Choi's study and the high multidrug resistance and prevalence of capsule coding genes in both studies, investigating vaccination protocols based on less diverse antigens is advised to prevent invasive K. *pneumoniae* and MDR infections. By enhancing the resistance to phagocytosis, capsule-associated genes, including *wabG* (that encodes capsule *uge*, which encodes capsule lipoprotein) and *ycfM* (which promotes external membrane protein), are highly involved in K. *pneumoniae's* virulence. While other studies have been focused on the prevalence of capsule coding genes in clinical isolates of K. *pneumoniae* (Pan et al., 2015; Choi et al., 2020; Tan et al., 2020; Walker & Miller, 2020; Huang et al., 2022), only a few studies have investigated the wabG gene (Hasani et al., 2020; Ballén et al., 2021). We observed the presence of the *wabG* gene in 94% of K. *pneumoniae* strains, which was near its prevalence in the two previous studies (88.5% and 100%, respectively).

4. Conclusion

The high frequency of MDR strains of K. pneumoniae in different clinical sources in Isfahan, Iran, is significant. The highest frequency observed in urinary tract infections should be carefully considered by healthcare systems. The frequency of more than 90% of fimH and *wabG* genes in the MDR isolates should also be considered in the infection diagnostic, and future study on alternative treatment protocols that can overcome the virulence factors is proposed.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgment

This study involves human participants and was approved by the Ethics Committee of Falavarjan Branch, Islamic Azad University, Isfahan, Iran with the ethics code IR.IAU.FALA.REC.1401.004. We thank the research assistant of the University.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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