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Prevalence of Drug-Resistant *Klebsiella pneumoniae* Strains in Hospitalized Patients in Mashhad, Iran: A Cross-Sectional Study

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ABSTRACT

Background: *Klebsiella pneumoniae* is one of the important bacilli of the *Enterobacteriaceae* family and is considered as the primary pathogen in nosocomial infections, especially septicemia, pneumonia, etc. Widespread resistance to antibiotics is currently a major concern worldwide. The aim of this study is to determine the prevalence of antibiotic resistance and its association with variables such as age, gender, and department.

Methods: 90 strains of *Klebsiella pneumoniae* were identified by the phenotypic method and BD Phoenix M50, and the Phoenix system determined their antibiotic resistance according to the manufacturer's procedures. Then, a statistical relationship between antibiotic resistance and variables, including age, gender, and patient department, was obtained.

Results: The findings indicated that the Multidrug-resistant (MDR) and Extended-Spectrum Beta-Lactamase (ESBL) resistance in patients had a high prevalence (above 80%). The intensive care units (ICUs) and burn wards had the highest prevalence of these antibiotic resistance. Comparing drug resistance with gender showed that total drug resistance (TDR) was significantly higher in men. However, no statistically significant relationship was found between them and age.

Conclusion: Based on the findings, the high prevalence of antibiotic resistance in *Klebsiella pneumoniae* requires special attention to their control and prevention, especially in critical departments such as ICU and burn units, and therapeutic stewardships may need to be revised accordingly.

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Introduction

Klebsiella pneumoniae is a Gram-negative, opportunistic, encapsulated, and non-motile bacterium, ubiquitously present in various environmental niches, including water, soil, animals, and the surfaces of medical equipment (1-3). This bacterium can lead to various diseases, including septicemia, urinary tract infections, pneumonia, meningitis, surgical site and burn wound infections, liver abscesses, and soft tissue infections (4). Hypervirulent *Klebsiella pneumoniae* (hvKp) infections are notably high in Asia, and Iran is among the few countries where the disease is endemic. A predilection of the hvKp infection in Asians has been observed even among Asians residing in Western countries, although the reason for this remains unclear. However, there is a growing occurrence of infections caused by hvKp in other ethnicities and climes. As such, there is a need for heightened awareness of this condition, which may be further complicated by other infections (2). Owing to the escalating spread of *Klebsiella pneumoniae* resistance to carbapenems, the disease outcomes in Iran are often severe. Consequently, treatment has become increasingly challenging (2, 5).

Several classifications of antibiotic resistance have been proposed in the last 15 years (6). Multidrug-resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, Extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories) and totally drug-resistant (TDR) was defined as non-susceptibility to all agents in all antimicrobial categories (7).

The rising prevalence of MDR, XDR, and TDR *Klebsiella pneumoniae* infections presents a significant challenge and is associated with increased mortality rates (8).

The aim of this study was to investigate the prevalence of MDR, XDR, and TDR antibiotic resistance patterns in *Klebsiella pneumoniae* strains isolated from hospitalized patients in different hospital departments between March 20 and June 20, 2024.

Materials and Methods

Bacterial identification

The study began with the collection of clinical specimens from patients with suspected *Klebsiella pneumoniae* infections. A total of 90 strains were isolated using standard protocols. Initial identification was based on phenotypic characteristics, in which strains were evaluated for specific biochemical features: positive reactions to citrate utilization and urease production, along with negative motility, indole, and hydrogen sulfide (H_2S) tests.

To confirm the identity of the isolates as *K. pneumoniae*, the BD Phoenix M50 Compact automated system was used, which increases the accuracy of microbiological identification through advanced biochemical and profiling tests. The system uses a proprietary database that compares the metabolic activities of the isolates against known bacterial profiles.

Determination of antibiotic resistance by the Phoenix method

Antimicrobial susceptibility testing (AST) was conducted using the BD Phoenix M50 Compact automated system to ascertain the susceptibility patterns of the selected *K. pneumoniae* strains against a panel of antibiotics. The focus was particularly on identifying strains exhibiting resistance to carbapenems (CARB), AmpC beta-lactamases (AMPC), and extended-spectrum beta-lactamases (ESBL). Furthermore, resistance phenotypes were characterized into multidrug-resistant (MDR), extensively drug-resistant

(XDR), and totally drug-resistant (TDR) categories, as defined by established criteria in the literature.

Following the manufacturer's protocol, each sample was analyzed using two separate vials; one was specifically designed for gram-positive and the other for gram-negative bacteria. The AST broths were supplemented with indicators such as methylene blue and resazurin, which are crucial for visual assessment of metabolic activity. For gram-negative bacteria, a distinct color change (red) indicates bacterial growth and, by extension, antibiotic resistance.

The identification broth (ID broth) was used for bacterial identification, while the AST broth was specific for susceptibility testing, mirroring methodologies applied in previous studies, such as that by Hong et al.(9). Applying these advanced techniques can provide valuable information about emerging resistance patterns among clinical isolates of *Klebsiella pneumoniae*.

Statistical analysis

Various patient data, including age, gender, and department, were collected, and their statistical association with antibiotic resistance patterns was evaluated using SPSS software (version 27). ANOVA and t-tests were used to determine statistical relationships between variables, and a p-value of less than 0.05 was considered significant.

Results

Analyses showed that 83 out of 90 samples (92.2%) exhibited multidrug resistance (MDR). The lowest number of these cases was observed in the central operating room (two cases), while the highest was recorded in the Intensive care units (ICUs) (22 cases).

Additionally, 59 cases (65.6%) of XDR bacteria were identified, with the lowest number in the central operating room and trauma surgery (one case) and the highest in the ICU (20 cases). Finally, 10 cases (11.1%) of TDR bacteria were reported. The lowest occurrence of TDR was noted in the obstetrics, burn, central operating room, cardiology, and open-heart surgery wards (0 cases each), while the highest was in the infectious diseases and ICU wards (3 cases each). In this study, AMPC, CARB, and ESBL antibiotic resistance patterns were reported in 60 (66.7%), 62 (68.9%), and 73 (81.1%) patients, respectively.

The results showed a significant association between the type of hospital ward and the prevalence of infections caused by ESBL producing bacteria (p=0.033). However, no significant association was observed between the type of ward and other antibiotic resistance, including AMPC, CARB, MDR, XDR, and TDR (Table 1).

Specifically, the highest ESBL cases were recorded in the ICU (21 cases), while the lowest was observed in the Obstetrics ward (3 cases). Additionally, analyses revealed a significant association between gender and the incidence of TDR infections (p=0.037), with men showing the highest rates of TDR.

Furthermore, a significant relationship was identified between the variables XDR and TDR (p=0.015). However, the results indicated no significant association between gender and other drug resistances, including ESBL, AMPC, CARB, MDR, and XDR. The average age of participants in this study was 55 years, and analyses showed no significant association between age and the drug resistance examined (Table 1).

Table 1 provides a detailed summary of these findings, presenting the p-values associated with each variable. The variables MDR and XDR demonstrated significant associations with the production of ESBL, AmpC, and CARB.

Table 1. Antibiotic Sensitivity Pattern of Studied GAS Isolates to some commonly used Antibiotics.

Variable	*ESBL*	*AmpC*	*CARB*	*MDR*	*XDR*	*TDR*
Age	0.75	0.91	0.88	0.14	0.57	0.65
Gender	0.71	0.71	0.81	0.74	0.61	0.037
Department	0.033	0.37	0.77	0.24	0.78	0.408

Discussion

The findings of the present study indicate a high prevalence of antibiotic resistance among patients with infections caused by *Klebsiella pneumoniae* at a Hospital in Mashhad.

The prevalence of TDR, XDR, and MDR was reported to be 92.2%, 65.6%, and 11.1%, respectively. This was much higher than the values obtained in the studies of Farhadi et al. in 2021 and Mirzaei et al. in 2023 in Iran and more similar to the results of Davoudabadi in Tehran in 2023 (10-12).

The rates of antibiotic resistance patterns, including ESBL, AmpC, and CARB, were 81.1%, 66.7%, and 68.9%, respectively. This was relatively higher than the results obtained in the studies of Haghighifar in 2021 and Mirzaei et al. in 2023 in Iran, which reported ESBL as about 40%. However, the results of our study were similar to those of Davoudabadi et al., who reported its prevalence as 84.6% (11-13).

Kazemian et al. 2019 reported the prevalence of AmpC to be 20%, which differed from our results (14). Regarding CARB, our results were relatively similar to the study by Abbasi et al. in 2023 (15). This discrepancy in results may be due to differences in laboratory methods, patient samples, and geographic regions.

Statistical analyses revealed that various clinical and demographic factors, such as the hospitalization ward and gender, significantly impacted the incidence of antibiotic resistance.

Specifically, the Burn Unit was identified as the primary hotspot for ESBL cases, potentially due to the intensive care and extensive use of antibiotics in this ward. In contrast, the Obstetrics ward had the lowest number of cases, which may reflect differences in treatment protocols or the specific nature of patients in this ward. Additionally, the analyses revealed a significant association between gender and the incidence of TDR infections. Specifically, men had the highest rates of TDR, highlighting the need for further research to investigate the factors contributing to this gender difference. However, no significant association was found between female gender and the incidence of TDR. This finding suggests that other factors, aside from gender, may influence drug resistance in women, which should be explored in future studies.

The association between MDR and XDR resistances with the production of ESBL, CARB, and AMPC underscores the importance of closely monitoring and appropriately managing these resistances.

The average age of the participants in this study was 55, indicating a focus on middle-aged and elderly groups within the sample. This finding highlights the special importance of studying these age groups, as individuals in these categories are at greater risk of acquiring drug-resistant infections due to weakened immune systems or underlying health conditions. Identifying these high-risk groups can be crucial in designing preventive and therapeutic interventions.

Regarding sample distribution, the highest number of samples was collected from the hospital's Infectious Diseases department. This is likely due to the high prevalence of infections in this ward and the presence of patients with severe infectious diseases requiring intensive care and extensive antibiotic use. The overuse of antibiotics in this department can lead to increased drug

resistance, highlighting the importance of adhering to strict drug usage protocols.

In contrast, the lowest number of samples was collected from the Cardiology department. This may be due to the lower incidence of drug-resistant infections in patients in this ward. Additionally, differences in the types of diseases and the patient profiles in the Cardiology department may influence the number of samples collected. In this department, broad-spectrum antibiotics are likely used less frequently, which could be one of the factors contributing to the lower levels of drug resistance.

The distribution of samples and their demographic characteristics highlight the need for designing targeted strategies for different age groups and hospital wards. This approach could significantly reduce drug resistance and improve infection management in hospitals.

Despite the valuable findings of this study, several limitations should be considered. First, the study was conducted in a single hospital, and its results may not be generalizable to other healthcare facilities. Second, the data collected were limited to a short period (three months), which may not capture seasonal or long-term drug resistance patterns. Third, detailed information on patient antibiotic consumption was not recorded, which could have provided more profound insights into the factors influencing drug resistance. These limitations highlight the need for further, more comprehensive research with a broader geographic and temporal scope and consideration of additional factors.

This study has multiple implications for the management and control of drug resistance. In particular, our findings could help improve healthcare policies in hospitals and other healthcare settings. The data obtained can be used to design more precise and effective protocols for controlling and preventing the spread of drug-resistant infections.

Additionally, findings related to high-risk wards, such as the burn unit, can be valuable for training healthcare staff on properly using antibiotics and reducing drug resistance. The results of this study can also contribute to improving drug resistance monitoring systems and detecting outbreaks of resistant infections early. These results can help improve therapeutic strategies and infection prevention in hospitals. Ultimately, it is recommended that future research more closely examine the factors influencing the spread of these infections across different hospital wards. This study highlights the importance of closely monitoring antibiotic resistance and implementing effective strategies to control and prevent the spread of infections caused by resistant bacteria. These results can serve as a foundation for future planning in antibiotic resistance management and improving patient treatment outcomes.

Conclusion

This research indicates the prevalence of antibiotic resistance in *Klebsiella pneumoniae*-infected hospitalized patients in Mashhad with a very high rate of multidrug resistance (92.2%) and extensively drug-resistant organisms (65.6%). The significant variations in levels of antibiotic resistance among wards within hospitals, particularly elevated in intensive care and burn wards, underscore the need for targeted infection control strategies. The gender association with the presence of extensively drug-resistant infections also necessitates an investigation into their causes. In summary, these findings highlight the imperative need for ongoing surveillance, proper antibiotic stewardship, and investigation into effective treatment strategies to counter the growing menace of antibiotic resistance in healthcare settings.

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Ethics approval and consent to participate

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

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