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Research Paper

Integron profiles and sulfonamide resistance genes in *Klebsiella pneumoniae* isolated from diabetic patients with urinary tract infection

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ABSTRACT

Klebsiella pneumoniae is an opportunistic pathogen and an important cause of pneumonia, bacteremia and urinary tract infection (UTI). *K. pneumoniae* infection is historically associated with diabetes. Therefore, the aim of the present study was to determine the Integron types and sulfonamide resistance genes in *K. pneumoniae* isolated from diabetic patients with urinary tract infection. In this descriptive-cross-sectional study, 90 *K. pneumoniae* isolates were collected from urine samples of diabetic patients. Antibiotic susceptibility testing was performed using disk diffusion methods. Detection of the sulfonamide resistance determinants and integron types were carried out through PCR. In this study, 90 *K. pneumoniae* isolates were obtained from diabetic patients with UTI, with a mean age of 69.8 ± 9.2 years; 58.9% were female. Antibiotic resistance was highest to gentamicin (70%) and ceftriaxone (65.5%), and lowest to imipenem (32.2%), with significant resistance also observed to trimethoprim-sulfamethoxazole (50%). Among the isolates, *sul1* (91.8%) and *intI* (67%) were the most prevalent resistance genes followed by *dfrA1* (63%) and *sul2* (52%). The *dfrA5* gene was not detected in any of the isolates. The predominance of *sul1* and class I integrons among *K. pneumoniae* isolates from diabetic patients with UTI underscores their key role in the spread of multidrug resistance. These findings emphasize the importance of targeted antimicrobial stewardship, infection control, and monitoring strategies to prevent treatment failure in this high-risk population.

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1. Introduction

Klebsiella pneumoniae is a prominent Gram-negative opportunistic pathogen frequently associated with urinary tract infections (UTIs), particularly in immunocompromised populations such as diabetic patients [1]. Diabetes mellitus increases susceptibility to UTIs through multiple mechanisms, including impaired neutrophil function, altered bladder physiology, and elevated glucose levels in urine, which promote bacterial growth and colonization [2]. Among the antimicrobial agents historically used to treat UTIs, sulfonamides have played a central role; however, their clinical efficacy has been increasingly compromised by the emergence of resistant *K. pneumoniae* strains [3]. Sulfonamide resistance in *K. pneumoniae* arises through two major genetic mechanisms, namely the acquisition of *sul* genes, which encode modified dihydropteroate synthase (DHPS) enzymes with reduced affinity for the drug, and *dfr* genes, which produce altered dihydrofolate reductase (DHFR) variants that enable sustained tetrahydrofolate synthesis despite exposure to sulfonamides and trimethoprim and these resistance determinants are commonly carried on mobile genetic elements, including plasmids and integrons, that promote their rapid dissemination within bacterial populations [4].

Integrons, particularly class I integrons, are genetic platforms capable of capturing and expressing multiple gene cassettes, many of which confer resistance to a wide range of antibiotics [5,6]. The presence of integrons and associated gene cassettes in *K. pneumoniae* not only facilitates multidrug resistance but also complicates empirical therapy and infection control [7]. Several studies have highlighted the global spread of sulfonamide-resistant *K. pneumoniae*, with integron-mediated gene cassettes playing a critical role in the persistence and propagation of resistance [8]. However, data specifically focusing on diabetic patients with UTIs remain limited, despite the high prevalence of infections and the increased risk of treatment failure in this population. This study aims to determine the prevalence of sulfonamide resistance genes and to identify the types of integrons present in *K. pneumoniae* isolates from diabetic patients with UTI.

2. Materials and Methods

2.1 Study design and sampling

In this descriptive cross-sectional study conducted over a one-year period in 2025, a total of 90 *K. pneumoniae* isolates previously obtained from diabetic patients with UTI were analyzed. These isolates had been collected as part of a research project approved by Babol University of Medical Sciences (tracking code: 724133814) and were preserved in the microbial bank of the Department of Microbiology, from which they were retrieved for the present investigation. Bacterial identification had initially been performed using standard microbiological and biochemical procedures, and the reference strain *K. pneumoniae* ATCC 13883 was employed as a positive quality control throughout the study [9]. The project protocol was reviewed and approved by the Ethics Committee of Babol University of Medical Sciences under the ethical approval code IR.MUBABOL.HRI.REC.1403.180.

2.2 Antimicrobial susceptibility testing

In agree with the clinical and laboratory standards institute guideline (CLSI; M100-S35) [10], disk diffusion test was done on the Mueller-Hinton agar (MHA) petri (Merck, Darmstadt, Germany) for ciprofoxacin (CIP; 5µg), ceftazidime (CAZ; 30µg), cefotaxime (CTX; 30µg), tetracycline (TET; 30µg), imipenem (IPM; 10µg), ampicillin (AMP; 10µg), cefepime (FEP; 30µg), aztreonam (ATM; 30µg), amikacin (AN; 30µg), gentamicin (GM; 10µg), and trimethoprim-sulfamethoxazole (SXT; 5µg) (Padtan Teb Co, Iran). Isolates resistant to at least three or more different antimicrobial classes considered as multi-drug resistant (MDR) [11].

2.3 Genomic DNA extraction

Genome extraction was performed according to the boiling method described by Mostaghemi et al. in 2024 [12]. The quantity of extracted DNA was checked by electrophoresis on the 1.0% agarose gel, while the purity and concentration of template DNA was assessed at 260/280 nm (Thermo Scientific Nanodrop 2000 Spectrophotometer). Template DNA stored at -20°C for further analysis.

2.4 Molecular detection

A set of specific primers was used for polymerase chain reaction (PCR) was shown in Table 1 [13,14]. PCR reaction was done in a Techne TC-512 thermal cycler (Eppendorf, Hamburg, Germany) at the total volume of 25 µl comprises of 1.3 µl of genomic DNA, 12.5 µl of Taq DNA Polymerase Master Mix RED (Ampliqon, Stenhuggervej, Odense M, Denmark), 0.8 µl of each primer, and 9.6 µl of aquapure water. PCR products were subjected to the ultraviolet transilluminator (Bio-Rad, Hercules, USA) after running at 100 V for one hour on a 1.0% agarose/Tris/Borate/EDTA (TBE) 0.5X (45 mM-Tris-borate, 1 mM-EDTA, pH= 8.0) gel stained with DNA safe stain (SinaClon, Tehran, Iran).

2.5 Statistical analysis

After collecting the data, statistical analyses were performed using SPSS software, version 22.0 (IBM, Armonk, NY, USA). Descriptive statistics were calculated to summarize the demographic and clinical characteristics of the participants. The Chi-square test was applied to compare categorical variables, and a P-value < 0.05 was considered statistically significant.

3. Results

In this study, 90 *K. pneumoniae* samples were isolated from diabetic patients with UTI. The age range of the patients was from 5 to 89 years with a mean age of 69.8 ± 9.2 years. Overall, 53 isolates were obtained from female patients (58.9%) and 37 from males (41.1%). The results showed that the highest resistance rates were to gentamicin, and ceftriaxone, 70%, and 65.5%, respectively (Table 2). While, the lowest resistance rates was against imipenem with 32.2%. Moreover, 58.9% of isolates were considered as MDR.

The distribution of resistance genes showed that *sul1* was the most prevalent, detected in 71 isolates (78.9%), followed by *sul2* in 47 isolates (52.2%) and *sul3* in 12 isolates (13.3%), while no isolate carried the *dfrA5* gene; in contrast, *dfrA1* was identified in 57 isolates (63.3%). Also, the *intI* gene was present in 61 isolates (67.7%), and *intII* was detected in 32 isolates (35.5%).

4. Discussion

In this study, we characterized 90 *K. pneumoniae* isolates from diabetic patients with urinary tract infections, a patient population inherently at higher risk of complicated infections due to immunological dysfunction and altered urinary tract physiology. The high mean age and predominance of female patients align with the well-recognized demographic pattern of urinary tract infections among individuals with diabetes. Our antibiotic susceptibility data reveal a worrying level of resistance. These findings align with previous reports in *K. pneumoniae*, where β-lactam resistance, particularly to third-generation cephalosporins, is common due to extended-spectrum β-lactamase (ESBL) production, whereas carbapenems often remain more effective [15].

At the molecular level, our absence of the *dfrA5* gene in all isolates suggests that this particular trimethoprim-resistance cassette may be uncommon in our geographic or clinical context, or perhaps that other *dfr* variants predominate. In contrast, the high prevalence of ***sul*** genes point to widespread sulfonamide resistance. The dominance of *sul1* is consistent with many integron studies which indicated *sul1* is frequently associated with the 3′-conserved segment of class I integrons. For example, environmental and clinical *Klebsiella* isolates showed very high rates of *intI1* and that most integrons carried *sul1* in the 3′ CS [12]. Also, a study in China reported 57.5% of *K. pneumoniae* isolates harboring class I integrons, with diverse resistance gene cassettes [16]. Similarly, research in Iran also documented high prevalence of class I integrons in urinary *K. pneumoniae* isolates [17]. The high ratio of class I integrons in our isolates likely reflects strong selective pressure in the hospital environment, especially under frequent antibiotic use, including sulfonamides and cephalosporins.

Class I integrons, embedded in mobile genetic elements (e.g., plasmids, transposons), facilitate rapid horizontal gene transfer, which amplifies resistance dissemination [18]. Comparing with other geographical contexts, there are some differences. For example, Firoozeh et al. (2019) reported 45.8% of *K. pneumoniae* isolates with class I integrons, but only 0.7% with class II, and none with class III, which is lower than our class I prevalence [15].

Table 1. Primer sequences used in this study

Target genes	Primer sequences (3→'5')	Amplicon size (bp)
Sul1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	432
Sul2	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	293
Sul3	F: CAGATAAGGCAATTGAGCATGCTCTGC R: GATTTCCGTGACACTGCAATCATT	563
dfrA1	F: TGGAGTTATCGGGAATGGC R: AACATCACCTTCCGGCTCG	343
dfrA5	F: ACGGAGTGATTGGTTGCGG R: CTCTGTAAATCTCCCCGCC	279
intI	F: GCCTGTTCGGTTCGTAAGCT R: CGGATGTTGCGATTACTTCG	585
intII	F: GGGCAGTCGCTTCCAACGGT R: GTAGTGCTCAGTGTCGGCAT	475

Table 2. The full results of antibiotic resistance profile

Antimicrobial agents	Resistance No (%)	Intermediate No (%)	Susceptible No (%)
Trimethoprim-sulfamethoxazole	45 (50.0)	3 (3.3)	42 (46.7)
Cefoxime	46 (51.1)	2 (2.2)	42 (46.7)
Cefepime	49 (54.4)	0 (0)	41 (45.5)
Ceftriaxone	59 (65.5)	2 (2.2)	29 (32.2)
Ciprofloxacin	53 (58.8)	0 (0)	37 (41.1)
Imipenem	29 (32.2)	3 (3.3)	55 (61.1)
Ampicillin/Sulbactam	55 (61.1)	0 (0)	27 (30.0)
Gentamicin	63 (70.0)	5 (5.5)	17 (18.8)
Amikacin	36 (40.0)	2 (2.2)	52 (57.7)

Meanwhile, an Iraq study found 100% of sampled *K. pneumoniae* isolates carried class I integrons, highlighting variation across regions and hospital policies [19]. The presence of *sul3* in our isolates, though not as common as *sul1*, is clinically relevant. Some studies reported unusual *sul3*-associated integrons in *K. pneumoniae*, indicating that non-classic integron structures may circulate in certain settings [20]. This may reflect local plasmid dynamics or selective pressures specific to our patient cohort. The absence of *dfrA5* and the detection of integron class II (around 35%) and class III (18%) in smaller proportions suggest a complex genetic architecture of resistance in our isolates.

Although class II and III integrons are generally less common, they may still contribute to diversity in resistance gene cassettes in different settings [21]. One possible explanation for the discrepancies in integron prevalence between our study and others may lie in differences in local antibiotic prescribing practices, particularly in treatment of UTIs in diabetic patients. Selection for integron-bearing strains is likely stronger in environments with heavy sulfonamide and cephalosporin use. Another factor may be genetic diversity for the genomic plasticity of certain strains in acquiring foreign genetic material. Moreover, diabetic patients may harbor *K. pneumoniae* strains with different genetic backgrounds compared to non-diabetic populations, which could influence the distribution of mobile genetic elements.

Our data underscore the critical role of class I integrons in mediating sulfonamide resistance in *K. pneumoniae* from diabetic UTIs. The very high frequency of *sul1* and class 1 integron-integrase gene suggests that interventions targeting integron-mediated gene cassettes could be vital to control resistance spread. Continuous surveillance of integron classes, associated gene cassettes, and antibiotic susceptibility in this high-risk patient group is warranted. Overall, these findings underscore the importance of implementing local antimicrobial stewardship programs, as reducing the unnecessary use of sulfonamides and third-generation cephalosporins may help control the selection and spread of integron-bearing, multidrug-resistant *K. pneumoniae*. Future investigations should incorporate sequencing of integron variable regions to characterize specific gene cassettes and determine their contribution to antimicrobial resistance and associated clinical outcomes.

This study has several limitations, including a relatively small number of isolates, which may limit the generalizability and statistical strength of the conclusions, and the fact that all isolates were collected from a single geographic region, potentially restricting the applicability of the findings to broader resistance trends.

This study reveals a high prevalence of sulfonamide resistance and class I integrons in *K. pneumoniae* isolates from diabetic patients with UTIs. The dominance of *sul1* and *intI1* highlights their role in multidrug resistance. These findings underscore the need for ongoing surveillance and rational antibiotic use to control the spread of resistant strains.

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Authors' contributions

AP, MS: contributed substantially to the conception and design of the study. MR, MB: data collection, and initial drafting of the manuscript. MR, AP, MB: performed data analysis, contributed to interpretation of the results, and critically revised the manuscript for important intellectual content. MS: assisted with methodology development, supervised the project, and contributed to manuscript editing. All authors read and approved the final version of the manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical declarations

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Babol University of Medical Sciences under the ethical approval code IR.MUBABOL.HRI.REC.1403.180. Written informed consent was obtained from all participants prior to enrollment. For participants unable to sign, consent was obtained from legal guardians as required.

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