Afghanistan Journal of Infectious Diseases
AJID
https://ajid.ghalib.edu.af/index.php/ajid

2025 Jan 3(1): 23-29

Phenotypic Diversity and Antibiotic Resistance Pattern of Extended-Spectrum Beta-Lactamase (ESBLS) Producing *Klebsiella pneumonia* Isolated From Patients in Three Selected Centers in Isfahan, Iran

Tahereh Motallebirad [1], Mohammad Reza Mohammadi [2], *Davood Azadi [3]

1. *Department of Research and Development, Satras Biotechnology Company, Islamic Azad University of Khomein, Khomein, Iran*
2. *Department of Bacteriology, Faculty of Medical sciences, Tarbiat Modares University, Tehran, Iran*
3. *Department of Biology, Faculty of Basic Sciences, Lorestan University, Khorramabad, Iran*

ARTICLE INFO

Type: Original Article
Received: 2024/08/17
Accepted: 2024/11/30

*Corresponding Author:
E-mail:
Davood.azadi@gmail.com

To cite this article: Motallebirad T, Mohammadi MR, Azadi D. Phenotypic Diversity and Antibiotic Resistance Pattern of Extended-Spectrum Beta-Lactamase (ESBLS) Producing *Klebsiella pneumonia* Isolated From Patients in Three Selected Centers in Isfahan, Iran. Afghanistan Journal of Infectious Diseases. 2025 Jan 3(1): 23-29.
https://doi.org/10.60141/ajid.73

ABSTRACT

Background: *Klebsiella pneumoniae* is a significant pathogen responsible for a wide range of infections, particularly urinary tract infections (UTIs). We aimed to determine the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* strains and analyze their antibiotic resistance patterns in isolates collected from patients in three hospitals in Isfahan, Iran.

Methods: This study was conducted over a 13-month period (2022–2023) in three educational hospitals in Isfahan, Iran. Out of 350 clinical specimens obtained from patients with UTIs, wounds, blood, and sputum, 142 *Klebsiella* isolates (40.5%) were identified and isolated according to standard methods. Antibiotic susceptibility was tested using the disk diffusion method according to CLSI standards, and ESBL production was confirmed using the combined disk method.

Results: Among the 142 isolates, 113 (31%) were derived from urine, while 29 (8.2%) were from wounds, sputum, and blood samples. Antibiotic resistance was highest against nitrofurantoin (45.5%), sulfamethoxazole (44.8%), and nalidixic acid (41.3%). In contrast, the most effective antibiotic was amikacin, with an 85.7% susceptibility rate. Additionally, 125 isolates (88%) of were identified as ESBL positive showed resistant to at least 6 antibiotics used in this study.

Conclusion: Amikacin remains the most effective antibiotic against *Klebsiella* species. However, the increasing prevalence of ESBL-producing strains poses a critical challenge for treating hospital-acquired infections. These findings highlight the need for rigorous antibiotic stewardship and infection control measures to mitigate the spread of resistant *Klebsiella* strains.

Keywords: *Klebsiella*, Urinary tract infections, Antibiotic resistance, Extended-spectrum beta-lactamase

Introduction

Klebsiella pneumoniae, a member of the Enterobacteriaceae family, is a gram-negative bacillus commonly found as part of the intestinal microbiota (1). While it typically exists as a commensal organism, *K. pneumoniae* is also a prominent opportunistic pathogen responsible for various infections, including urinary tract

infections (UTIs), pneumonia, septicemia, and wound infections. In women, especially younger individuals, the anatomical proximity of the urinary and genital tracts to the anal region increases susceptibility to UTIs caused by this pathogen. After *Escherichia coli*, *K. pneumoniae* is the second most common cause of UTIs, emphasizing its clinical importance (2-4).

Antibiotic resistance in *Klebsiella* is a significant concern, particularly in healthcare settings, where it is a leading cause of nosocomial infections. Resistance arises from intrinsic mechanisms and acquired factors, such as the horizontal transfer of resistance genes via plasmids, transposons, and integrons (5). Predominant resistance mechanisms in *K. pneumoniae* include the production of extended-spectrum beta-lactamases (ESBLs), alterations in membrane permeability, biofilm formation, and efflux pump overexpression (6, 7). ESBLs, in particular, hydrolyze a wide range of beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams, rendering these treatments ineffective (8).

The widespread presence of ESBL-producing *K. pneumoniae* strains complicates the management of hospital-acquired infections, as these strains are often multidrug-resistant (MDR). This resistance contributes to higher morbidity, mortality, prolonged hospital stays, and increased healthcare costs. Factors such as overuse of antibiotics, inadequate antibiotic susceptibility testing, and insufficient infection control measures have exacerbated the issue (9). Therefore, detection of ESBL production is critical for effective treatment and infection control.

Given the rising prevalence of antibiotic-resistant *K. pneumoniae* in Isfahan, we aimed to investigate the phenotypic diversity, ESBL production, and antibiotic resistance patterns of *Klebsiella* isolates from clinical specimens obtained in three hospitals. The findings aim to inform strategies for mitigating the spread of resistant strains and optimizing therapeutic approaches.

Materials and Methods

Study design and sample collection

This study was conducted over a 13-month period (2022–2023) in three educational hospitals in Isfahan, Iran. A total of 350 patients, comprising 185 hospitalized patients and 165 outpatients, were enrolled. The sample population consisted of 265 females and 85 males. Clinical specimens included 280 urine samples (80%), 42 wound swabs (12%), 15 sputum samples (4.3%), and 13 blood samples (3.7%). Samples were collected using sterile containers and transported to the microbiology laboratory within 2 hours under refrigerated conditions (4°C) to maintain sample integrity.

Sample Size Rationale

The sample size of 350 patients was determined based on a preliminary analysis to ensure sufficient power for detecting significant differences in antibiotic resistance patterns. This sample size was also guided by hospital admission rates and prevalence estimates of *K. pneumoniae* in similar studies.

Isolation and identification of Klebsiella isolates

Specimens were cultured on Blood Agar, MacConkey Agar, and Xylose Lysine Deoxycholate (XLD) agar and incubated aerobically at 37 °C for 24 hours. After incubation, mucoid colonies suggestive of *Klebsiella* were subjected to Gram staining to confirm gram-negative bacilli morphology. Afterward, to accurate identification of *Kelbsiella* all isolates were subjected to differential biochemical tests such as MR/VP, urease, lysine iron agar, TSI (Triple Sugar Iron Agar), SIM (Sulfide, Indole, Motility), simon citrate, arginine, and ornithine decarboxylase to accurate identification. The results of these

biochemical tests were compared with standard reference tables for species identification (10).

Determination of antibiotic susceptibility of isolates

Antibiotic susceptibility testing was performed using the disk diffusion (Kirby-Bauer) method in accordance with CLSI 2022 guidelines (11). Isolates were first grown in Tryptic Soy Broth (TSB) at 37 °C for 18–24 hours. The bacterial suspension was standardized to a turbidity of 0.5 McFarland (approximately 1.5×10^8 CFU/mL). A sterile swab was used to inoculate Mueller-Hinton agar (Merck, Germany) plates evenly. Antibiotic disks (Cefotaxime (30 µg), Ceftizoxime (30 µg), Amikacin (30 µg), Nalidixic acid (30 µg), Ciprofloxacin (30 µg), Nitrofurantoin (30 µg), and Sulfametoxazole (30 µg) (Padtan Teb Iran) were placed on the agar, and plates were incubated at 37 °C for 24 hours. *K. pneumoniae* ATCC 700603 was used as a control strain. Zone diameters were measured and interpreted as susceptible (S), intermediate (I), or resistant (R) based on CLSI standards. Beta-lactamase production was determined using the combined disk method. Data were analyzed using WHONET version 5.6 software.

Statistical Analysis

Statistical analysis was performed to validate the results. Antibiotic resistance percentages were calculated, and chi-square tests were used to compare resistance patterns between different sample types. A *P*-value of <0.05 was considered statistically significant.

Results

Out of 350 clinical samples, 142 *K. pneumoniae* isolates (40.5%) were identified. These comprised 113 isolates (80%) from urine samples and 29 isolates (20%) from wound, sputum, and blood specimens. A chi-square test revealed a statistically significant association between the type of clinical sample and the frequency of *K. pneumoniae* isolates ($\chi^2 = 58.92$, $P < 0.001$), indicating a higher prevalence in urine samples compared to other sample types.

Antibiotic susceptibility testing was performed on all 142 isolates using seven antibiotics (Table 1). The highest resistance rates were observed for nitrofurantoin (45.5%, 65/142 isolates), sulfamethoxazole (44.8%, 64/142), and nalidixic acid (41.3%, 59/142). Statistical analysis (chi-square test) showed significant differences in resistance rates among the tested antibiotics ($\chi^2 = 74.31$, $P < 0.001$). The most effective antibiotics were amikacin, with 85.7% (122/142 isolates) susceptibility, ciprofloxacin (79.3%, 113/142), and ceftizoxime (66.7%, 95/142). Pairwise comparisons using Fisher's exact test indicated significantly higher susceptibility rates for amikacin compared to nitrofurantoin and sulfamethoxazole ($P < 0.01$).

Table 1: Results of antibiotic susceptibility of *Klebsiella* isolated from patients samples.

Antibiotic	*Susceptible*	*Resistant (%)*	*Intermediate*	*Breakpoints*
Cefotaxime	53	20.8	26	23-25
Ceftizoxime	66.7	29.6	3.7	22-24
Amikacin	85.7	12.5	1.8	15-16
Nalidixic acid	54	41.3	4.7	14-18
Ciprofloxacin	79.3	20.7	5.2	16-20
Nitrofurantoin	36.8	45.5	15.9	15-16
Sulfamethoxazole	50	44.8	5.2	11-15

Among the 142 isolates, 88% (125/142) were positive for ESBL which resistant to at least three antibiotics, demonstrating a high prevalence of multidrug resistance (MDR). The association between ESBL and sample type was statistically significant ($\chi^2 = 25.67$, $P < 0.01$), with higher ESBL rates in urine isolates compared to other samples.

The results highlight a concerning trend of multidrug resistance among *K. pneumoniae* isolates, with nitrofurantoin and sulfamethoxazole showing particularly high resistance rates. The high efficacy of amikacin suggests its potential as a first-line treatment for infections caused by this bacterium. However, the increasing resistance to commonly used antibiotics underscores the need for regular susceptibility monitoring and tailored antibiotic stewardship programs (Figure 1).

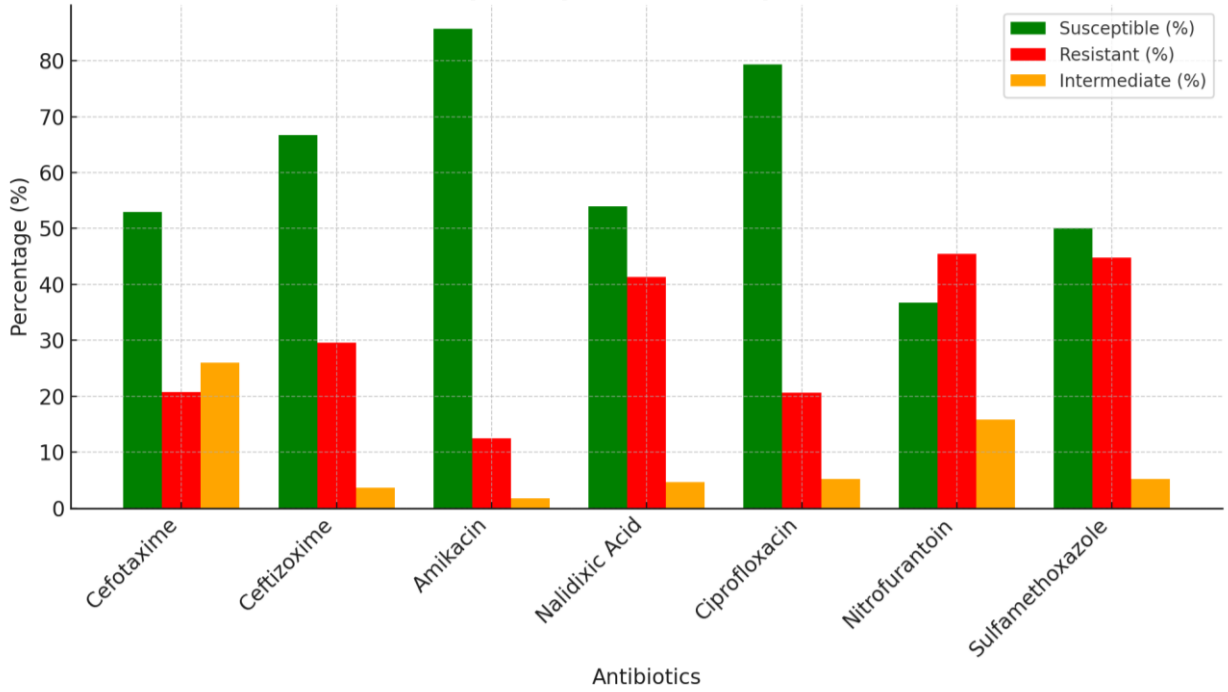

Figure 1: The chart illustrates the percentages of susceptible, resistant, and intermediate isolates of *K. pneumoniae* for each tested antibiotic. The green susceptible isolates, red resistant isolates, and orange intermediate isolates.

Discussion

Antibiotic resistance among *K. pneumoniae* isolates represents a critical challenge in healthcare settings, particularly in hospital environments where the prevalence of multidrug-resistant (MDR) strains is rising. In this study, resistance rates were highest for nitrofurantoin (45.5%), sulfamethoxazole (44.8%), and nalidixic acid (41.3%), while amikacin demonstrated the highest efficacy, with 85.7% susceptibility. The findings align with global reports of *K. pneumoniae* as a leading cause of nosocomial infections, highlighting the urgent need for strategies to combat resistance (12).

The prevalence of ESBL-producing *K. pneumoniae* isolates in this study (88%) reflects the significant role of ESBL enzymes in mediating resistance. These enzymes, which degrade a broad range of beta-lactam antibiotics, often co-occur with resistance to other drug classes, further limiting therapeutic options. The high levels of resistance to nitrofurantoin observed here are concerning and differ

from lower rates reported in earlier studies, who found resistance rates of 31.3% *(*13*)*.. This discrepancy may be attributed to regional variations in prescribing practices and the selection pressures unique to the study area. Similarly, while amikacin remains highly effective, consistent with a study (14), variations in resistance rates reported across regions underscore the need for local susceptibility testing to guide empirical treatment.

The clinical and public health implications of these findings are profound. The overuse and misuse of antibiotics have exacerbated resistance, emphasizing the need for antimicrobial stewardship programs tailored to local resistance patterns (15). Such programs can help optimize antibiotic use, reducing the emergence of resistant strains. Additionally, the high prevalence of MDR *K. pneumoniae* necessitates stringent infection control measures, including active surveillance of hospitalized patients and robust environmental decontamination protocols. Amikacin's continued efficacy supports its use as a first-line treatment in many regions, but its effectiveness must be monitored closely through regular susceptibility testing (16).

The broader public health impact of antibiotic resistance in *K. pneumoniae* extends beyond clinical management. The rapid dissemination of resistant strains in healthcare settings poses a significant threat to global health, requiring policymakers to invest in enhanced surveillance systems and research into alternative treatments. Potential solutions, such as bacteriophage therapy and antimicrobial peptides, represent promising avenues for addressing this growing crisis (17, 18).

While this study provides valuable insights into the resistance patterns of *K. pneumoniae* isolates in Isfahan, certain limitations must be acknowledged. The single-center design may limit the generalizability of the findings, and molecular analyses to identify specific resistance genes were not conducted. Future research should focus on genomic characterization of resistant strains and explore novel therapeutic approaches to combat MDR *K. pneumoniae*. Addressing these challenges will require a coordinated effort among clinicians, researchers, and policymakers to mitigate the impact of antibiotic resistance on public health (19, 20).

Conclusion

This study highlights the urgent need to address antibiotic resistance in *K. pneumoniae*, particularly the high prevalence of MDR and ESBL producing strains. While amikacin remains highly effective, rising resistance to commonly used antibiotics like nitrofurantoin and sulfamethoxazole underscores the importance of antimicrobial stewardship programs, routine susceptibility testing, and strict infection control measures in healthcare settings. To combat this growing threat, it is essential to explore alternative therapies such as bacteriophage therapy and antimicrobial peptides, invest in surveillance systems, and promote rational antibiotic use. Future research should focus on understanding the molecular mechanisms of resistance and developing strategies to mitigate the impact of MDR *Klebsiella* infections on public health.

Acknowledgements

The authors are grateful to office of Vice-Chancellor for Research of Isfahan University of Medical Sciences (960003) for the support of the current study.

Conflicts of interest

There are no conflicts of interest.

Reference

1. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global

expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis. 2013;13(9):785-96.
2. Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase–producing K. pneumoniae: importance of combination therapy. Clin Infect Dis. 2012;55(7):943-50.
3. Stahlhut SG, Struve C, Krogfelt KA, Reisner A. Biofilm formation of *Klebsiella pneumoniae* on urethral catheters requires either type 1 or type 3 fimbriae. FEMS Immunol Med Microbiol. 2012;65(2):350-9.
4. Puerta-Fernandez S, Miralles-Linares F, Sanchez-Simonet M, Bernal-Lopez M, Gomez-Huelgas R. R aoultella planticola bacteraemia secondary to gastroenteritis. Clin Microbiol Infec. 2013;19(5):E236-E7.
5. Heidary M, Nasiri MJ, Dabiri H, Tarashi S. Prevalence of drug-resistant *Klebsiella pneumoniae* in Iran: a review article. Iran J Public Health. 2018 Mar;47(3):317-26.
6. Tripathi PC, Gajbhiye SR, Agrawal GN. Clinical and antimicrobial profile of Acinetobacter spp.: An emerging nosocomial superbug. Adv Biomed Res. 2014;3.
7. Ahanjan M, Naderi F, Solimanii A. Prevalence of beta-lactamases genes and antibiotic resistance pattern of *Klebsiella pneumoniae* isolated from teaching hospitals, Sari, Iran, 2014. Journal of Mazandaran University of Medical Sciences. 2017;27(149):79-87.
8. Wiener J, Quinn JP, Bradford PA, Goering RV, Nathan C, Bush K, Weinstein RA. Multiple antibiotic–resistant Klebsiella and Escherichia coli in nursing homes. JAMA. 1999;281(6):517-23.
9. Mobasherizadeh S, Shojaei H, Azadi D, Havaei SA, Rostami S. Molecular characterization and genotyping of methicillin-resistant Staphylococcus aureus in nasal carriage of healthy Iranian children. J Med Microbiol. 2019 68(3):374-8.
10. Ghaffari K, Falahati V, Motallebirad T, Safarabadi M, Tashakor AH, Azadi D. Microbiological and Molecular Study of Paranasal Sinus Infections of Children with Malignancy and Unknown Origin Fever in Markazi Province, Iran. Current Ther Res. 2024;100:100745.
11. Rai S, Dash D, Agarwal N. Introducing the new face of CLSI M100 in 2023: An explanatory review. Indian J Med Microbiol. 2023;46:100432.
12. Motallebirad T, Tashakor A, Abniki R, D. A. 15 years of phenotypic and genotypic surveillance and antibiotic susceptibility pattern of actinomycetes (*Mycobacterium*, *Nocardia*, *Rhodococcus*, etc.) in clinical and environmental samples of Iran. Diag Microbiol Infect Dis. 2023 9:116080.
13. Safarabadi M, Motallebirad T, Azadi DAJ. Healthcare-associated infections in Iranian pediatric and adult intensive care units: A comprehensive review of risk factors, etiology, molecular epidemiology, antimicrobial sensitivity, and prevention strategies during the COVID-19 pandemic. J Intens Care Med. 2024 6:08850666241249162.
14. Yousefi Mashouf R, Alijani P, Saidijam M, Alikhani M Y, Rashidi H. Study of Antibiotic Resistance Pattern and Phenotypic Detection of ESBLs in *Klebsiella pneumoniae* Strains Isolated from Clinical Samples and Determination of Minimum Inhibitory Concentrations of Imipenem and Ceftazidim Antibiotics. Avicenna J Clin Med 2014; 20 (4) :295-302
15. Manchanda V, Singh N, Goyal R, Kumar A, Thukral S. Phenotypic characteristics of clinical isolates of Klebsiellea pneumoniae & evaluation of available phenotypic techniques for detection of extended spectrum beta-lactamases. Indian J Med Res. 2005;122(4):330.
16. Chaisaeng S, Phetburom N, Kasemsiri P, Putthanachote N, Wangnadee N, Boueroy P, Kerdsin A, Chopjitt P. Phenotypic and Genotypic Profiles of Extended-Spectrum Beta-Lactamase-Producing Multidrug-Resistant *Klebsiella pneumoniae* in Northeastern Thailand. Antibiotics. 2024 Sep 25;13(10):917.
17. Behzadian Nejad Q, Abdollahi A, Najar Peerayeh S, Forouhesh Tehrani H. Evaluation of bla-ctx-m-type gene in multi drug resistance Klebsiella pneumonia species isolated from clinical samples. Razi Journal of Medical Sciences. 2009;15(60):37-45.

18. Agha-Seyed Hosseini M, Firoozeh F, Piroozmand A, Gilasi HR. Carbapenemase-producing *Klebsiella pneumoniae* strains among clinical specimens in Kashan (2014-2015). KAUMS Journal (FEYZ). 2016;20(3):267-73.
19. Irajian G, Jazayeri Moghadas A. Frequency of extended-spectrum beta lactamase positive and multidrug resistance pattern in Gram-negative urinary isolates, Semnan, Iran. Jundishapur Journal of Microbiology. 2010:107-13.
20. Mohammadi S, Mohammadi B, Zandi S, Ramazanzadeh R, Rouhi S. Antibiotic sensitivity in strains of *Klebsiella pneumonia* isolated from clinical samples Besat hospitals of Sanandaj (2013-2014). Zanko J Med Sci 2016; 17 (52) :1-9.