Original Research

BIOTECHINTELECT, 2024, 1 (1): e5
https://jbiotechintel.com/index.php/biotechintel

pISSN: SOON
eISSN: SOON
OPEN ACCESS

Investigation of Antibiotic Resistance Patterns of Klebsiella pneumoniae Isolates from Educational and Medical Centers of Mazandaran University of Medical Sciences, 2020-21

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Article history:
Received 17 July 2024
Revised 19 August 2024
Accepted 29 August 2024
Published online 01 October 2024

Keywords: *Klebsiella pneumoniae, antibiotic resistance, Mazandaran*
How to cite this article: Hazrati Damavandi, V., Ahanjan, S., Gholami, M., Divsalar, S., Nemati Hoolaee, E., Ahanjan, M., & Ayu Gusti, M. (2024). Investigation of Antibiotic Resistance Patterns of Klebsiella pneumoniae Isolates from Educational and Medical Centers of Mazandaran University of Medical Sciences, 2020-21. *BiotechIntellect, 1*(1), 1-10. https://doi.org/10.61838/biotechintellect.1.1.5

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ABSTRACT

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated bacilli, *Klebsiella pneumoniae* acts as an opportunistic pathogen in the occurrence of hospital-acquired infections. This bacterium is often resistant to several classes of antibiotics, including beta-lactams. The aim of this study was to investigate the frequency and antibiotic resistance of *Klebsiella pneumoniae* strains isolated from hospitalized patients in the educational and medical centers of Mazandaran University of Medical Sciences from 2020-2021. In this descriptive-cross-sectional study, 50 *Klebsiella pneumoniae* strains were collected from various clinical samples of hospitalized patients in educational and medical centers affiliated with Mazandaran University of Medical Sciences. Sample cultures were performed on Eosin methylene blue and Blood agar media, and after 24 hours of incubation, Gram staining and oxidase tests were conducted on the grown colonies. Gram-negative and oxidase-negative bacilli were identified, and the presence of *Klebsiella pneumoniae* was confirmed by culturing bacteria on TSI, SIM, MR-VP, citrate, and urea media. Then, antibiotic susceptibility testing was performed using the disk diffusion method according to CLSI guidelines. The antibiotic disks included ciprofloxacin, imipenem, meropenem, cefepime, ceftazidime, gentamicin, amikacin, and ceftriaxone. In this study, among the 50 isolates examined, 61% were resistant to ciprofloxacin, 60% to imipenem, 49% to meropenem, 56% to cefepime, 58% to ceftazidime, 35% to gentamicin, 13% to amikacin, and 55% to ceftriaxone. Amikacin was identified as the most sensitive and effective antibiotic in this study. The results of the current study indicate an increase in the resistance of *Klebsiella pneumoniae* isolates to various antibiotics compared to previous studies. Further studies in this area can better guide us in confronting the antibiotic resistance of these infectious bacteria. Therefore, for infection control and to prevent the spread of drug-resistant bacteria, precise management in drug prescription and identification of resistant isolates is essential.

What is "already known":	• Klebsiella pneumoniae is an opportunistic pathogen in hospital infections. • It often shows antibiotic resistancy to beta-lactams, etc. • 50 Klebsiella pneumoniae were collected from hospitalized patients in Mazandaran. • The bacterium was determined by cultur on TSI, SIM, MR-VP, citrate, and urea media.
What this article adds:	• Antibiotic resistance was checked by the disk diffusion method according to CLSI guidelines. • Order of antibiotic resistancy were ciprofloxacin, imipenem, meropenem, cefepime, ceftazidime, gentamicin, amikacin, and ceftriaxone. • Amikacin was identified as the most sensitive and effective antibiotic in this study.

1. Introduction

Antibiotic resistance is a serious problem for human health, affecting patients in hospitals worldwide [1]. The world health organization has made many recommendations to governments for controlling and preventing antibiotic resistance, the most important of which include assessing antibiotic resistance, proper use of antibiotics, selling antibiotics only with a doctor's prescription, and controlling and preventing infections [2, 3]. Various studies have shown that the indiscriminate and irregular use of antibiotics leads to antibiotic resistance [4]. The WHO has identified bacterial resistance to antibiotics as one of the most important threats to global health, accounting for a high number of annual deaths [5]. Unfortunately, in recent years, in addition to the general issue of antibiotic resistance, cases of bacterial drug resistance to more than two different classes of antibiotics, commonly referred to as multidrug resistance, have increased, especially among hospital isolates, such that infections caused by multidrug-resistant Gram-negative bacilli are recognized as one of the important causes of death in hospitalized patients [6]. *Klebsiella pneumoniae* is a Gram-negative bacilli belonging to the large family Enterobacteriaceae. This microorganism is part of the normal human microflora, and about one-third of individuals are intestinal carriers of this microbe. *Klebsiella pneumoniae* plays a role as one of the important causative agents of hospital-acquired infections in causing important diseases such as urinary tract infections, pneumonia, septicemia, and soft tissue infections [7].

Over the past two decades, reports have emerged of community-acquired invasive *Klebsiella pneumoniae* isolates causing emerging diseases. The high resistance of Klebsiella to antibiotics and their rapid spread in various hospital departments have created major treatment problems, leading to septicemia and patient deaths [8]. Antibiotic resistance is considered a fundamental problem in the treatment and control of infections [9]. The continuous use of beta-lactam antibiotics and the selective pressure caused by these agents have led to the emergence of various types of drug resistance to this class of antibiotics in Gram-negative bacteria, especially *Klebsiella pneumoniae* [10]. One of the most important mechanisms of resistance to beta-lactam drugs is the production of enzymes called extended-spectrum beta-lactamases, which are mainly located on plasmids, facilitating their spread [11]. Beta-lactam antibiotics are commonly prescribed worldwide and include penicillins, cephalosporins, monobactams, and carbapenems [12]. Recently, *Klebsiella pneumoniae* has shown significant resistance to broad-spectrum drugs, including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides. This resistance has led to a growing global problem in selecting effective antibiotic treatments for hospital-acquired infections [13].

The aim of this study is to investigate the frequency and antibiotic resistance of *Klebsiella pneumoniae* strains isolated from hospitalized patients in the departments of educational and medical centers of Mazandaran University of Medical Sciences.

2. Materials and Methods

This descriptive-cross-sectional study was conducted to investigate the frequency and evaluate the drug resistance of *Klebsiella pneumoniae* isolates obtained from clinical samples of hospitalized patients in the wards of Mazandaran University of Medical Sciences teaching hospitals. For this purpose, 50 *Klebsiella pneumoniae* isolates were collected from various wards of Mazandaran University of Medical Sciences hospitals from February 2020 to August 2021 from different clinical samples. First, clinical samples were cultured in the laboratories of the studied hospitals on two basic media, Blood Agar and Eosin methylene blue (EMB) and after growth, they were subculture again and transferred to the microbiology group. After 24 hours of incubation at 37°C, they were examined by microbiological and biochemical tests related to the identification of *Klebsiella pneumoniae* species. Sample cultures were performed on EMB and Blood agar media. After 24 hours of incubation at 37°C, Gram staining and oxidase tests were performed on the grown colonies. Gram-negative and oxidase-negative bacilli were identified, and the presence of *Klebsiella pneumoniae* was confirmed using bacterial cultures on TSI, SIM, MR-VP, Citrate, Urea. Agar media, and the Indole test. Then, antimicrobial susceptibility testing was performed by the disk diffusion method on

Mueller Hinton Agar medium [Merck, Germany] according to CLSI also API (Figures 1 a-d).

3. Results and Discussion

This cross-sectional study, 50 non-duplicate *Klebsiella pneumoniae* positive samples were collected from various clinical specimens, including urine, blood, sputum, wounds, and tracheal tubes, from patients hospitalized in hospitals affiliated with Mazandaran University of Medical Sciences. Of the 50 samples examined, 39 were from women and 11 from men, and the average age of the individuals was 44.7 years. The prevalence of isolates varied across different hospital departments. The highest number of *Klebsiella pneumoniae* isolates was obtained from the intensive care unit, and the lowest from the neonatology department [2]. Also, based on the source [type] of the isolated sample, urine accounted for 58% (29 samples) and wounds for 6% [3 samples], respectively, representing the highest and lowest positive samples out of 50 isolates. Other Klebsiella isolates were obtained from blood samples at 26% (13 samples) and tracheal samples at 10% [5 samples].Of the 50 *Klebsiella pneumoniae* strains examined, the highest antibiotic resistance was observed against ceftazidime (58%), and the lowest antibiotic resistance was observed against amikacin at 13% (Figure 1). Table 1 shows the resistance, sensitivity, and intermediate sensitivity to the antibiotics used in the isolates.

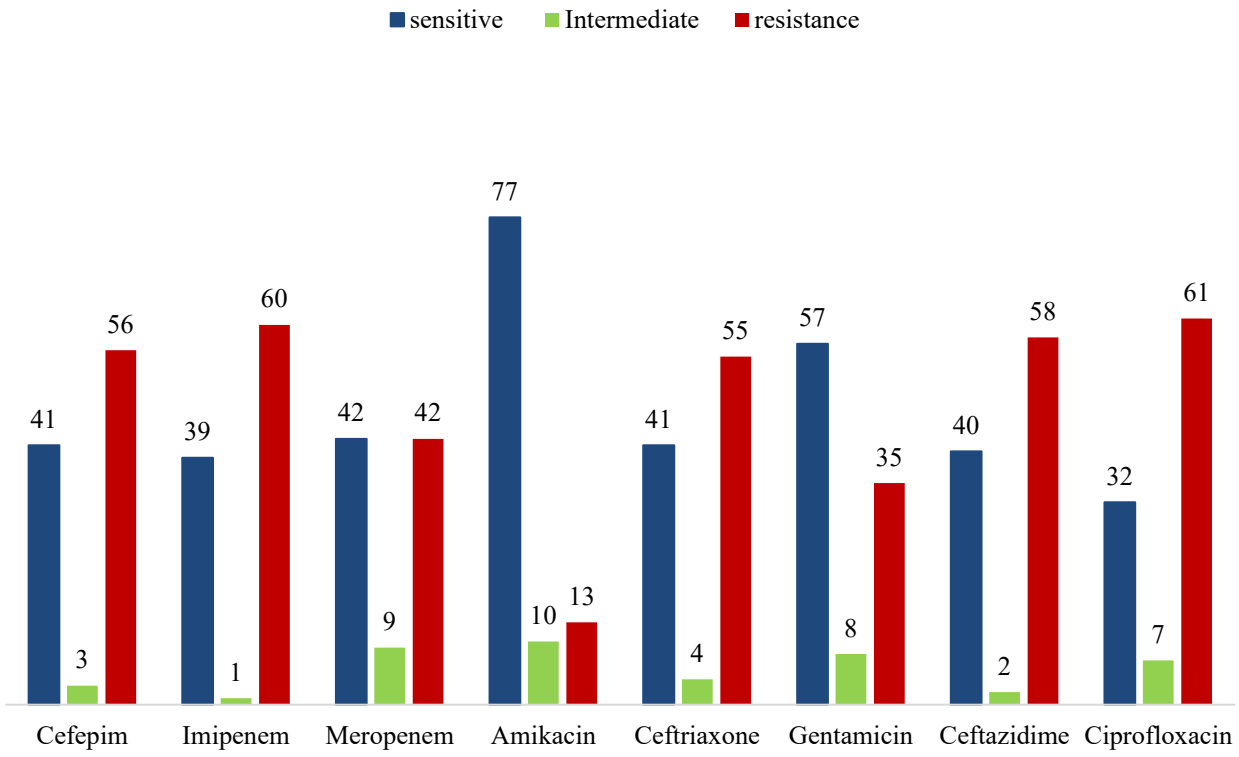

Figure 1: Percentage of Resistance (R), Intermediate (I), and Sensitive (S) drug susceptibility of samples

Table 1. Percentage of Sensitivity and Resistance of 50 *Klebsiella pneumoniae* Isolates

Antibiotic	resistant	Intermediate	Susceptible
Cefepim	56	3	41
Imipenem	60	1	39
Meropenem	49	9	42
Amikacin	13	10	77
Ceftriaxone	55	4	41
Gentamicin	35	8	57
Ceftazidime	58	2	40
Ciprofloxacin	61	7	32

Multidrug-resistant strains of *Klebsiella pneumoniae* have become a serious problem in hospitals due to their increased resistance to antibiotics, easy dissemination, and biofilm production [14]. The increasing number of carbapenem-resistant strains also emphasizes the importance of an appropriate antimicrobial regimen based on the antibiotic susceptibility pattern. The level of antibiotic resistance among isolates acquired from the

community and hospitals is increasing, which is a major global problem [15, 16]. The present study was conducted on *Klebsiella pneumoniae* causing nosocomial infections, and the isolated strains showed the highest antibiotic resistance to cefepime. In this study, among the 50 isolates examined, 61% were resistant to ciprofloxacin, 60% to imipenem, 49% to meropenem, 56% to cefepime, 58% to ceftazidime, 35% to gentamicin, 13% to amikacin, and 55% to ceftriaxone. Amikacin was identified as the most sensitive and effective antibiotic in this study.In a study conducted in China in 2014, the results showed that imipenem and gentamicin were the most effective antibiotics, which was somewhat consistent with the results obtained in this study. The results also showed that cefotaxime was the least effective antibiotic with 96.8% resistance. In our study, ciprofloxacin was reported as the least effective antibiotic with 61% resistance [17].

Haidari et al. in a systematic review and meta-analysis article showed that there is a relatively high prevalence of drug resistance in *Klebsiella pneumoniae* isolates in Iran. The highest resistance rates for *Klebsiella pneumoniae* isolates were observed against ampicillin [82%], nitrofurantoin [54%], and aztreonam [55%], while in their study, 52% of samples were resistant to co-trimoxazole, 51% resistant to ceftazidime, 43% resistant to cefepime, and 43% resistant to ceftriaxone [18].

Khamsipour et al. [19] showed that widespread resistance to the mentioned antibiotics has emerged for *Klebsiella pneumoniae* isolates: 41.1% ceftriaxone, 36.7% trimethoprim-sulfamethoxazole, 32.2% amikacin, 34.4% cefepime, 26.7% gentamicin. The resistance rate to amikacin in our study was lower than in the mentioned study, which could be attributed to changes in drug prescription patterns.

In a study entitled "Determining the Antibiotic Resistance Pattern of Klebsiella Species Isolated from Samples of Hospitalized Patients in Imam Khomeini [RA] Hospital “of Tehran , Sultan Dalal and colleagues investigated 300 Klebsiella isolates from 1200 hospitalized patients. The results showed that the different Klebsiella species, in order of frequency, were: pneumoniae [94%], oxytoca [4%], ozaenae [1%], and rhinoscleromatis [1%]. Also, in terms of infection source, the collected samples, in order of frequency, were: urine, sputum, vaginal, wound, stool, and blood. Furthermore, the antibiotic resistance of Klebsiella species was reported as follows: amoxicillin 97%, cephalothin 39%, gentamicin 30%, colistin 55%, nalidixic acid 2%, chloramphenicol 26%, kanamycin 17%, and tetracycline 28% [20].

In another study, Moghaddas et al. showed that antibiotic resistance rates were as follows: 7.5% imipenem, 16.1% ciprofloxacin, 32.9% trimethoprim-sulfamethoxazole, 32.2% amikacin, 34.4% cefepime, and 26.7% gentamicin [21]. Mohammadi et al. reported in 2016 that among of 67 clinical samples examined, the highest number of *Klebsiella pneumoniae* strains were isolated from urine samples and the lowest from wound samples, which was largely consistent with our results. Furthermore, the highest antibiotic sensitivity was related to imipenem and gentamicin, and the highest antibiotic resistance was related to trimethoprim-sulfamethoxazole and ceftriaxone. In our study, the highest sensitivity was related to amikacin, and a significant increase in resistance to imipenem was observed, which could be related to the higher prevalence of antibiotic resistance genes and the indiscriminate prescription of this antibiotic [22]. In 2020, Dalbar et al. conducted a study to investigate the antibiotic susceptibility pattern and virulence factors of *Klebsiella pneumoniae* isolated from healthy volunteers. Three hundred and fifty stool samples were collected from healthy individuals, sales representatives visiting healthcare centers in northwest Tehran to obtain health cards. Among the stool samples examined, 60 [17.1%] *Klebsiella pneumoniae* were isolated. Their results showed that the highest resistance rates were for piperacillin-tazobactam [41.6%], meropenem [28.8%], and co-trimoxazole [18.3%]. Additionally, all strains were susceptible to amikacin, gentamicin, and imipenem. They concluded that antibiotic resistance was common among *Klebsiella pneumoniae* isolated from the stool of healthy participating volunteers. The transmission of resistant bacteria and plasmids through oral and fecal sources is a threat to the general public, which can complicate treatment options for community-acquired infections caused by *Klebsiella pneumoniae* [23].Ahanjan and colleagues also evaluated the

antibiotic resistance and prevalence of beta-lactamase genes in *Klebsiella pneumoniae* in teaching hospitals of Mazandaran University of Medical Sciences in 2014. Their results showed that the highest resistance was against cefotaxime [100%] and ceftazidime [100%], while gentamicin [63%] showed the highest sensitivity [24].In reviewing the results of this study and comparing them with other reports, some discrepancies are observed, which can be attributed to the possibility of error during the experiment. This is because, in the accurate effect of antibiotics on bacteria and the determination of sensitive and resistant states of bacteria in vitro, factors such as the type of antibiotic disc used, the depth, and the composition of the culture medium play a major role and can lead to false changes in resistance statistics. Furthermore, the distribution of resistant strains in geographical areas can be due to climatic conditions and excessive antibiotic use in that region.

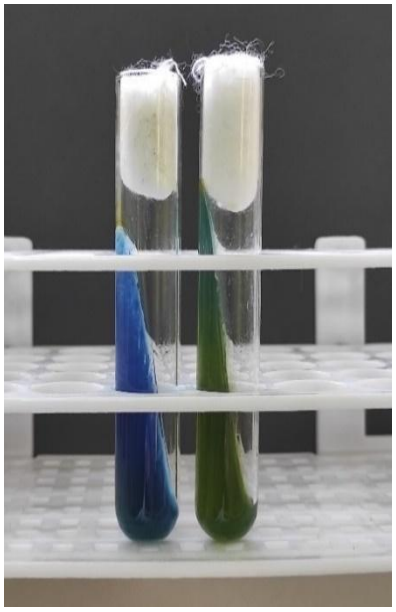

Figure 2a. Simon Citrate Agar Medium. Right: *Escherichia coli* [citrate negative]

Left: *Klebsiella pneumoniae* [citrate positive]

Figure 2b. Urea agar medium. Right side *Escherichia coli* [urease negative]

Left: *Klebsiella pneumoniae*(Uurease positive)

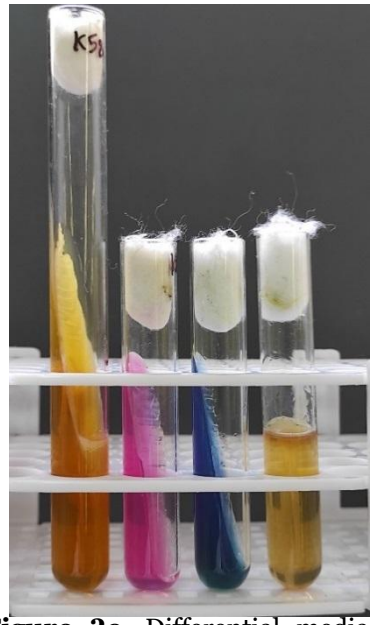

Figure 2c. Differential media after cultivation of *Klebsiella pneumonia*. Media from right to left: SIM, Simon citrate, urea agar and TSI

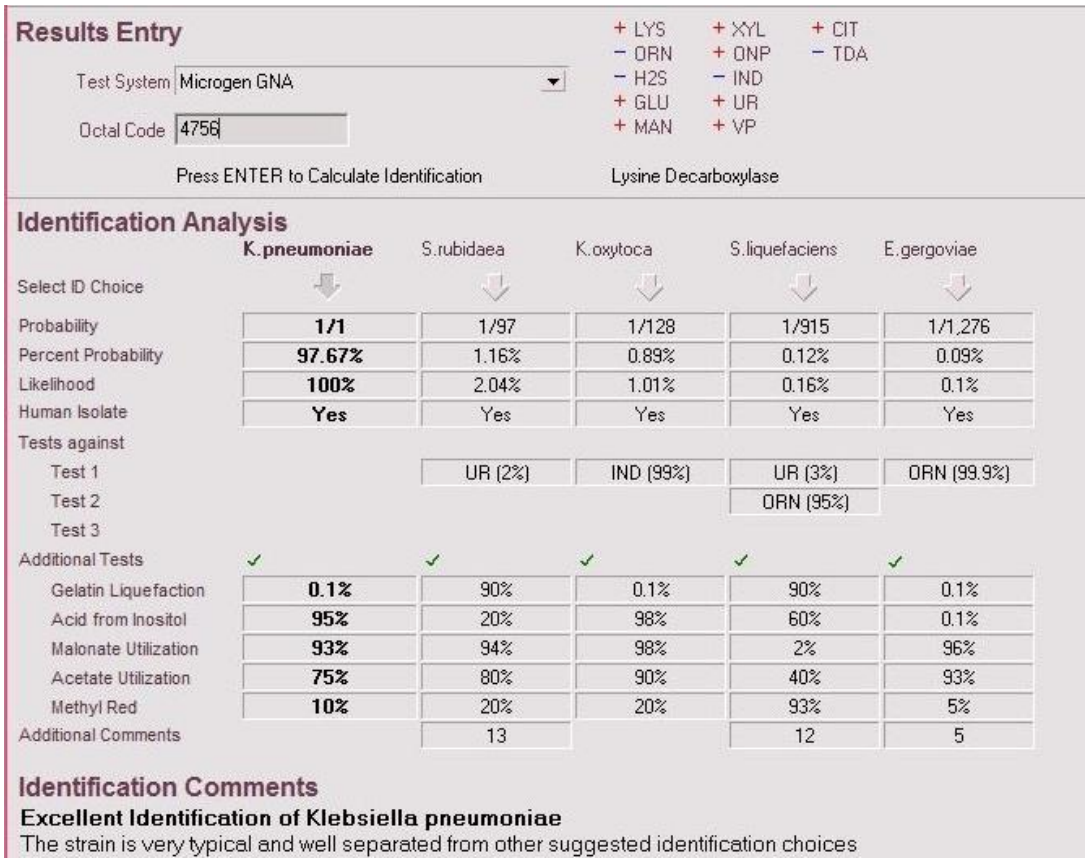

Figure 2d. API test result for *Klebsiella pneumoniae* isolate

4. Conclusion

The excessive use of antibiotics in recent years has led to the spread of resistant bacteria, especially multidrug-resistant strains. Under these circumstances, treating many infections caused by important pathogens such as *Klebsiella pneumoniae* has been fraught with many difficulties, increasing the risk of death for affected patients. The results of this study indicate an increasing trend of resistance to existing and common treatments against *Klebsiella pneumoniae* isolates from patients hospitalized in Mazandaran teaching hospitals, which, if not given sufficient attention, will lead to irreparable health and treatment consequences in the not-too-distant future.

5. Declarations

5.1. Acknowledgments

The author thanks the Editor-in-Chief for the invitation to prepare this editorial manuscript.

5.2. Authors' Contributions

NRS was the sole author.

5.3. Declaration of Interest

The authors of this article declared no conflict of interest.

5.4. Ethical Considerations

All ethical principles were adheried in conducting and writing this article.

5.5. Transparency of Data

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

5.6. Funding

This research was carried out independently with personal funding and without the financial support of any governmental or private institution or organization.

5.7. Using Artificial Intelligent chatbots

No AI chatbot has been used in this study.

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