International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Volume 16; Issue: 4; April 2026
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Pattern of Antibiotic Susceptibility Among *Klebsiella pneumoniae* Clinical Isolates in a Tertiary Care Hospital, Assam

Tonushyam Sonowal[1], Nayanjyoti Sarmah[2], Rohan Raj Kutum[3], Ranjita Khandait[4], Subhrendu S Sen[5], Migom Doley[6]

[1]Assistant Professor, Department of Microbiology, Pragjyotishpur Medical College and Hospital, Guwahati, Assam
[2]Assistant Professor, Dept. of Microbiology, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India
[3]Demonstrator, Dept. of Microbiology, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India
[4]Assistant Professor, Dept. of Microbiology, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India
[5]Professor & Head, Dept. of Microbiology, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India
[6]Associate Professor, Dept. of Community Medicine, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India

Corresponding Author: Dr Tonushyam Sonowal

DOI: https://doi.org/10.52403/ijhsr.20260403

ABSTRACT

Background: *K. pneumoniae* frequently offers a major health risk to patients since there are few available treatments, and it can develop multidrug-resistant (MDR). Therefore, identifying the frequency and patterns of antibiotic susceptibility of *K. pneumoniae* isolates from clinical specimens is crucial for the diagnosis and management of patients.
Aim: Finding the susceptibility pattern of *Klebsiella pneumoniae* isolated from clinical samples.
Materials and methods: From January 2024 to December 2025, a retrospective cross-sectional study was carried out at microbiology department, Lakhimpur Medical and Hospital, North Lakhimpur, Assam. Using a data collecting sheet, laboratory and socio demographic information was gathered from registered books and data record system. Using conventional protocols, all the clinical samples and processed. Gram stain, colony characterisation on culture media, and a number of biochemical tests were used to identify *K. pneumoniae*. The Kirby Bauer disc diffusion technique and VITEK system was used for antimicrobial susceptibility testing.
Results: A total of 1235 (24.9%) of the 4768 clinical specimens tested positive for bacteria, of which 131 (9.4%) were isolates of *K. pneumoniae*. Most of them were from adults, and they were mostly isolated from urine samples (64%). In our analysis, we found that *K. pneumoniae* was resistant to ampicillin (70%), cefuroxime (42%), amoxicillin/clavulanic acid (12%), piperacillin/tazobactam (15%), ceftazidime (7.6%), cefotaxime (38%), and cotrimoxazole (25%). Additionally, the rates of nitrofurantoin and ciprofloxacin resistance were 21%, and 26%, respectively. Multidrug resistance has been found in 58 (45%) *Klebsiella pneumoniae* isolates. Beta-lactamases were detected in 42 (72%) of the 58 Klebsiella pneumoniae isolates.

Using phenotypic techniques, 15 isolates of *Klebsiella pneumoniae* were positive for the synthesis of AmpC.

Conclusion: The severity of MDR *K. pneumoniae* was really concerning. Thus, it is highly advised that antimicrobial surveillance procedures and antimicrobial stewardship programs be strengthened in the study area.

Keywords: Antimicrobial susceptibility; Assam, *K. pneumoniae*; Tertiary care hospital,

INTRODUCTION

Antimicrobial resistance (AMR) is one of the biggest risks to public health in the world. Multidrug-resistant strains (MDRs) were responsible for more than a million deaths in 2019 [1]. Furthermore, it is predicted that over 5 million deaths would occur by 2030 because to the ease with which MDRs can spread, especially in healthcare settings [2, 3]. *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* are currently considered the main opportunistic pathogen species within the *Enterobacteriaceae* family. They cause a variety of infections, including pneumonia, bloodstream infections (BSIs), urinary tract infections (UTIs), and surgical site infections [4, 5]. Additionally, employing medical devices such as urinary catheters, venous catheters, and respiratory support equipment raises the risk of nosocomial infections caused by opportunistic pathogenic microorganisms [4]. Due in significant part to the widespread creation of extended-spectrum β-lactamases (ESBLs), both K. pneumoniae and E. coli have developed concerning levels of resistance to routinely used antibiotics over the past 20 years, especially third-generation cephalosporins. There are few treatment options available for ESBL-producing bacteria since they are resistant to aztreonam, cephalosporins, and penicillins [6]. The WHO has identified carbapenem-resistant Enterobacteriaceae, such as E. coli and K. pneumoniae, as a key priority group of pathogens for which new antibiotics are desperately needed in response to this expanding issue [7]. Resistance in these organisms is facilitated by various mechanisms. The synthesis of ESBLs and AmpC β-lactamases provides resistance to extended-spectrum cephalosporins, whereas the acquisition of carbapenemase genes such as KPC, NDM, OXA-48, and VIM leads to resistance against carbapenems, which are regarded as last-resort antibiotics. Furthermore, additional mechanisms such as porin mutations and the overexpression of efflux pumps contribute to treatment failures [8]. The swift dissemination of these resistance mechanisms has been enabled by horizontal gene transfer through plasmids and transposons, resulting in outbreaks in hospitals globally [9].

Global surveillance studies have indicated a rise in resistance rates within Enterobacteriaceae. In various parts of Asia and Latin America, the prevalence of ESBL in E. coli surpasses 50%, while the resistance to carbapenems in K. pneumoniae has shown a consistent increase over the past decade [4]. The situation in India is particularly alarming. Reports suggest that over 70% of K. pneumoniae isolates demonstrate resistance to third-generation cephalosporins, with carbapenem resistance rates exceeding 30% in numerous tertiary care hospitals [10]. These trends are indicative of both the excessive and inappropriate use of antibiotics in clinical settings, alongside the absence of effective antimicrobial stewardship programs.

The purpose of this study was to assess the antibiotic susceptibility profile in K. pneumoniae isolated from clinical specimens in a newly established tertiary care hospital Assam between Jan 2024 and Dec 2025 in order to enhance therapeutic treatment and clinical practice.

MATERIAL AND METHODS

Collection of Clinical Isolates:

Between January 2024 and December 2025, 131 *K. pneumoniae* strains were isolated

from patients who were attended and admitted to the Lakhimpur Medical College and Hospital, North Lakhimpur, Assam. For the enrolled patients, we gathered demographic information such as age and gender, department unit, and infection site. Blood, urine, sputum, bronchial aspirate, swabs (throat, and wound), and additional samples (Ascitic fluid, abscess, pleural fluid and synovial fluid) were among the samples. We took care to eliminate cultures that contained the same pathogen isolated from a single participant in the event of numerous infection events during the whole study period. Only non-duplicate clinical isolates that were obtained during the first infection episode throughout the study period were included in order to prevent data duplication. In order to avoid duplicate isolates, we defined them as isolates from the same patient that had an identical pattern of susceptibilities.

Culture:

All the clinical samples submitted for culture and sensitivity testing to the Microbiology laboratory were processed according to standard procedures. The samples were inoculated onto MacConkey agar and blood agar using a standard calibrated loop (0.001 ml). To verify notable growth, colonies were counted 24–48 hours after being incubated overnight at 37ºC. Following conventional microbiological methods, the organism was identified using colony morphology, gram staining, and biochemical assays. Phenotypic and Vitek 2 compact system (bioMerieux) confirmations were also conducted for further confirmation.

Antibiotic susceptibility testing:

To evaluate the susceptibility of isolates to various antibiotics, the Kirby Bauer's disk diffusion method was used. Figure 1 shows the Kirby Bauer's disk diffusion method.

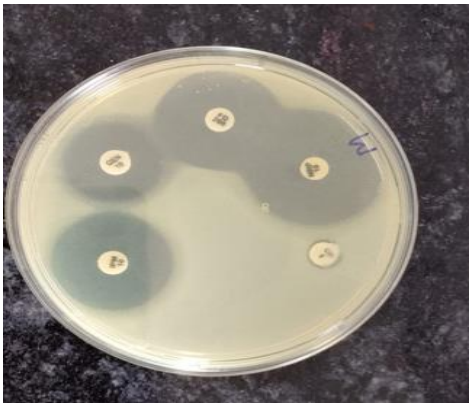

Fig 1: Antibiotic sensitivity of different antimicrobials by Kirby-Buer disc diffusion method

Using a panel including ceftazidime (30µg), ceftazidime/clavulanate (30/10µg), cefoxitin (30µg), cefepime (30µg), levofloxacin (5µg), aztreonam (30µg), gentamicin (10µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), nitrofurantoin (300 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), ampicillin (10 µg), and co-trimoxazole (25 µg),doxycycline (30 µg), minocycline (30 µg). The test organism was suspended in normal saline, adjusted to 0.5 McFarland standards, and streaked with a sterile swab stick on Mueller Hinton Agar (MHA). Antibiotic-impregnated disks were applied and incubated at 35°C ± 1°C overnight. Zone of inhibition diameters were recorded and interpreted as per updated CLSI guidelines.

Statistical analysis

The data collected was entered into Microsoft Excel and the raw data were screened for missing values and outliers. Data for this study were analyzed using descriptive statistics to summarize the variables and identify patterns within the sample. Categorical variables were analysed using frequencies and percentages. The results are shown using tables and figures as appropriate for visual representation.

RESULTS

Between January 2023 to December 2025, 131 strains of *K. pneumoniae* were isolated at microbiology laboratory, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam. Sixty-one (61%) percent

of the patients were female. According to the examination of incidence by age group, 41% of patients were between the ages of 31 and 45, followed by those between the ages of 16 and 30 and those more than 46 years ages of 22%. The incidence was lower for younger patients (Table 1). Furthermore, isolation rate of *Klebsiella pneumoniae* in outdoor patient (48%), adult ward (21%), intensive care unit (5%), and pediatric outpatient unit (3%) respectively (Table 1).

Table 1: Characteristics of patients from which *K. pneumoniae* were isolated

Variable	Category	Total
Sex	Male	51
	Female	80
Age	<28 days	0
	1month- 15 years	5
	16-30 years	42
	31-45 years	55
	> 46 years	29
Patient location	Adult OPD	63
	Pediatric OPD	4
	Adult ward	28
	Adult ICU	7

The majority of *K. pneumoniae* strains were identified from urine samples (64%), followed by pus samples (19%), sputum (6.1%), aural swabs (5%), and tracheal aspirate (2.5%) (Table 2).

Table 2: Distribution of *Klebsiella pneumoniae* isolates in different clinical samples

Clinical samples	No of *Klebsiella pneumoniae* isolates N (%)
Urine	84 (64%)
Aural swab	7 (5.3%)
Pus sample	25 (19.08%)
Throat swab	2 (1.52%)
Sputum	8 (6.1%)
Tracheal aspirate	3 (2.2 %)
Catheter tip	2 (1.52%)
Total	**131**

A significant percentage of ampicillin (70%), cefuroxime (42%), (amoxicillin/clavulanic acid (12%), piperacillin/tazobactam (15%), ceftazidime (7.6%), cefotaxime (38%), and cotrimoxazole (25%) resistance was observed in *K. pneumoniae* in our investigation (Table 3). Additionally, the rates of nitrofurantoin and ciprofoxacin resistance were 21%, and 26%, respectively. Additionally, imipenem had a low rate of resistance 9%, but meropenem shows 15% resistance rate. Fifty-eight (45%) isolates of *Klebsiella pneumoniae* have shown multidrug resistance.

Table 3: Resistance rates of the clinical isolates of *Klebsiella pneumoniae* to antimicrobial agents (Total number of isolates 131)

Antibiotics	Resistance rate N (%)
Ampicillin	93 (70%)
Cefotaxime	50 (38%)
Cefoxitin	30 (22.9%)
Cefuroxime	55 (41.9%)
Piperacillin-tazobactam	20 (15.2%)
Amoxiclav	16 (12.21%)
Ceftazidime	10 (7.6%)
Amikacin	19 (14.5%)
Tetracycline	13 (9.9%)

Meropenem	20 (15.26%)
Ciprofloxacin	34 (25.95%)
Cotrimoxazole	33 (25.19%)
Nitrofurantoin	28 (21.37%)
Imipenem	12 (9.16%)
Tobramycin	20 (15.26%)
Doxycycline	8 (6.1%)
Gentamicin	39 (30%)

Out of 58 isolates 42 (72%) *Klebsiella pneumoniae* isolates were positive for beta-lactamases [Fig 2].

Fig 2: Phenotypic confirmatory test for ESBL using Kirby-Bauer disk diffusion method. Positive test if the zone of inhibition around cefotaxime+ clavulanate and ceftazidime+clavulanate is increased by ≥ 5mm as compared to that of cefotaxime or ceftazidime on MHA plate

Fifteen (15) isolates of *Klebsiella pneumoniae* were screening positive for AmpC production by phenotypic methods.

DISCUSSION

Nosocomial infections have been a serious problem to healthcare systems around the world in recent decades, primarily in developing nations [11]. As one of the most prevalent pathogens, *K. pneumoniae* is a major contributor to serious infections. Being the primary nosocomial opportunistic pathogen, it is constantly exposed to different antibiotics, which causes resistance mechanisms to form and MDR strains to proliferate. However, geographic location, population, and antibiotic management may all have an impact on the rate of antibiotic resistance in *K. pneumoniae* [12]. The frequency and antibiotic resistance profiles of *K. pneumoniae* isolates from various specimen sources in inpatients and outpatients between January 2024 and December 2025 were examined in this investigation. Children aged 0–18 years had a low incidence rate, according to the analysis of incidence by age group. Adult patients aged 31 to 45 years had the highest incidence (41%) and those aged 16-30 years had the highest incidence (21%). These results are not consistent with those of Singh AK, Jain S et al., who found that Children had the highest infection rate [13]. Urine samples (64%), followed by pus samples (19%), sputum (6.1%), aural swabs (5%), and tracheal aspirate (2.5%) were the primary sources of *K. pneumoniae* isolation. Romanus et al.'s investigation, on the other hand, revealed the following distribution: 30.5% in lung samples, 23.6% in urinary tract samples, and 40% in blood cultures [14]. Compared to other samples, a significant percentage of bacteria were isolated from sputum and bronchoalveolar specimens (49.5%), according to another study. Urine isolates were the second most common group (16.3%), followed by blood sources (8.7%) [13]. According to a prior study conducted in Nigeria, where the incidence of this phenotype was 17%, we found 75% of ESBL *K. pneumoniae* isolates were present in our investigation [15].It is important to remember that the ESBL phenotype's prevalence may differ according on the geographic region; for example, it might range from 14% in France and 16% in England to 5% in the US [16]. Furthermore, according to the European Union's 2020 Annual Epidemiological Report, 33.9% of isolated *K. pneumoniae* were ESBL, and in Italy, this percentage was higher than 50%. However, research conducted in Iran by Kashefeh et al. and Kiaei et al. revealed that 65% and 41.4% of ESBL isolates, respectively, were present [17, 18]. Lastly,

Xu et al. found that 33.7% of *K. pneumoniae* isolates in China produced ESBL [18]. The widespread use of third-generation cephalosporins in society is probably the cause of the high occurrence of *K. pneumoniae* that produces ESBLs [19]. Our investigation found that ampicillin resistance has the highest incidence (70%). This result is consistent with research that found a 97% resistance rate in Russia and Iran [19]. The study found that tigecycline, imipenem, gentamicin, and fosfomycin were the most effective antibiotics against *K. pneumoniae*, with respective susceptibilities of 71%, 72%, 64%, and 61%. In recent years, fosfomycin, a broad-spectrum antibiotic that works against both Gram-positive and Gram-negative bacteria, has been crucial in the treatment of MDR *Enterobacteriaceae* [20]. Our findings on the use of fosfomycin to treat *K. pneumoniae* are consistent with those of other studies in which the resistance rate ranged from 16% to 10.9%. There are several ways to explain fosfomycin resistance, including decreased permeability, target site modification, and antimicrobial modifying enzymes [21]. Fosfomycin has been used more frequently in Europe and Asia in recent years to treat infections brought on by isolates of *Enterobacteriaceae* that are resistant to carbapenem [21].

Based on our findings, 78% of tested K. pneumoniae was sensitive to Nitrofurantoin, 89% to Imipenem, 85% to Meropenem, and 86% to Amikacin. This finding was consistent with a study conducted in Indonesia [22] and Iran [23].

CONCLUSION

The severity of MDR *K. pneumoniae* was really concerning. The results showed increased resistance to ampicillin and increased sensitivity to cefepime, imipenem, meropenem, and piperacillin/tazobactam. In summary, this study intends to emphasize the significance of tracking the development of antimicrobial susceptibility models in our area, putting in place surveillance systems for multidrug resistant microorganisms, and confirming the effectiveness of the empirical treatments used in order to reduce treatment failures and the spread of the antibiotic resistance phenomenon. To maintain the effectiveness of antibiotics and the health of patients, creative and ongoing surveillance techniques are a vital asset. Lastly, to decrease the transmission of infections and multi drug resistant bacteria obtained in nosocomial settings, sanitary conditions and preventative measures must be improved.

Declaration by Authors

Ethical Approval: Not required as it involved a retrospective study of anonymized patient data.

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest

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How to cite this article: Tonushyam Sonowal, Nayanjyoti Sarmah, Rohan Raj Kutum, Ranjita Khandait, Subhrendu S Sen, Migom Doley. Pattern of antibiotic susceptibility among *Klebsiella pneumoniae* clinical isolates in a tertiary care hospital, Assam. *Int J Health Sci Res.* 2026; 16(4):17-24. DOI: *https://doi.org/10.52403/ijhsr.20260403*

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