Research Article

Resistance Profiles In Klebsiella Pneumoniae From Clinical Samples And Phenotypic Detection Of Carbapenemase Producing Klebsiella Pneumoniae By Carba NP Test In Tertiary Care Hospital U.P.

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Abstract:

Background: Klebsiella pneumoniae is a major opportunistic pathogen responsible for a wide range of severe nosocomial and community-acquired infections. The rapid global dissemination of multidrug-resistant (MDR) and carbapenem-resistant K. pneumoniae (CRKP) strains severely restricts therapeutic alternatives and significantly raises patient mortality rates.
AIM: This study aimed to evaluate the antibiotic susceptibility pattern of K. pneumoniae from clinical isolates and evaluate the carbapenemase-producing strains using the rapid Carba NP test within a tertiary care hospital.
Materials & Methods: An observational cross-sectional study was conducted from May 2024 to July 2025 at the Department of Medical Microbiology, Santosh Medical College and Hospital (Ghaziabad) and Rama Medical College (Harpur). A total of 335 non-duplicated K. pneumoniae isolates collected from various clinical specimens (including urine, sputum, pus, and blood) across all age groups and genders were included. Bacterial identification was achieved through standard cultural characteristics and biochemical profiling. Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar using the Kirby-Bauer disc diffusion method, and phenotypic validation of carbapenemase production was executed using the Carba NP test in strict accordance with CLSI guidelines.
Results: Out of 335 isolates, the highest rate was retrieved from urine samples (40.3%), followed by sputum (26.6%) and pus (14.0%). The General Medicine ward presented the heaviest burden (54.43%), followed by Surgery (20.30%). The highest incidence occurred within the 51–60 age group (73 cases), demonstrating an overall male predominance in older populations, while a female predominance was isolated among young adults aged 21–30 years. In sensitivity pattern. Alarming rates of resistance were observed for ampicillin (>80% across sputum, pus, and blood) and early-to-late-generation cephalosporins. Carbapenems (meropenem and imipenem) sustained solid sensitivity in blood (84.4%) and urine (73.3% meropenem, 79.3% imipenem), but critical reduction in pus samples, where resistance rates were between 36.2% and 40.4%.
The Carba NP test successfully identified 45 phenotypic carbapenemase-producing isolates (yielding an overall positivity rate of 13.43%). Proportional carbapenemase positivity was most concentrated in endotracheal (ET) tube specimens (36.36%) and pus samples (31.91%).
Conclusions: The study shows an alarming level of multidrug resistance and a significant rise in carbapenemase producing K. pneumoniae isolates, particularly within device-associated (ET tube) and wound (pus) environments.

Keywords: Klebsiella pneumoniae, Multidrug Resistance, Carbapenemase, Carba NP Test, Antibiogram.

Introduction

Klebsiella pneumoniae, belongs to the Enterobacteriaceae family, it is naturally present in the human and animal gastrointestinal tract [1]. However, it is the most common cause of nosocomial and community-acquired infections. The Pathogenic K. pneumoniae strains can cause a wide variety of infectious diseases, like urinary tract, respiratory tract and blood stream infections [2]. Klebsiella species causes about 70% of the infections caused by this genus. Klebsiella pneumoniae is present in many parts of the body and thus can cause multiple infections. Though it is a part of our healthy microflora, but it has been known to cause serious infections in all vulnerable patients. These infections were earlier managed by antibiotics like carbapenems but due to the emergence of resistance because of ESBL production, these antibiotics are not very useful in multidrug resistant organisms [3].
K. pneumoniae isolates that belong to the carbapenem-resistant Enterobacteriaceae (CRE) family are characterized by their resistance to carbapenems that is a class of commonly used wide-spectrum antibiotics. The World Health organisation identified them as a “critical concern”, meaning that there is a serious need for the research and development of new antibiotics to target this significant threat to human health [4]. In fact, K. pneumoniae is the major cause of nosocomial pneumonia and one of the few Gram-negative rods that can cause primary pneumonia. Classical K. pneumonia (cKP) is characterized by nosocomial infections, which are most common in elderly or immunodeficient patients [5].

Furthermore, the emergence and dissemination of carbapenem-resistant K. pneumoniae (CRKP) strains has posed an urgent threat to public health on a global scale. Carbapenem resistance in K. pneumoniae is mainly owing to the acquisition of carbapenemase genes associated with mobile elements such as plasmids. In fact, CRKP strains are characterized by multiantibiotic-resistance profiles that involve most β-lactams, including carbapenems, along with other non β-lactam antibiotics, limiting therapeutic options and affecting the clinical outcome of infections, with high mortality rates reported in some categories of patients [6].

According to the World Health Organization's assessment of the global status of antibiotic resistance, K. pneumoniae is one of the top three bacteria of global concern as many antibiotics used to treat bacterial diseases are showing multi drug resistance [7]. Furthermore, among the top ten MDR bacteria in intensive care units, K. pneumoniae strains are now the second most prevalent MDR bacteria [8].

Material Method:

It is an Observational-Cross sectional study that was done in the Department of Medical Microbiology, Santosh Medical College and Hospital, Ghaziabad and Rama Medical College, Harpur, in the time duration of May 2024 to July 2025. The sample size was calculated by the formula:

Sample size= n = [DEFF*Np(1-p)]/ [(d^2/Z$^2_{1-\alpha/2}$*(N-1) + p*(1-p)]

Where z= Confidence level at 95% (standard value of 1.96)

p= prevalence

q= 1-p

e= margin of error (0.05)

In this study the Patient from all Age and gender, Patient from OPD/IPD and Klebsiella pneumoniae from all clinical samples were included.

The Unlabelled, Contaminated and duplicate sample will not be included and isolates other than Klebsiella pneumoniae will be excluded from the study.

All clinical sample were collected under sterile technique and were transported immediately to the laboratory for further processing. All sample were processed by performing Gram's staining, cultured on Blood agar, MacConkey's agar, CLED agar. The identification of Klebsiella pneumoniae was done by Gram's staining, colony characteristics, type of haemolysis produces on blood agar and lactose fermentation. Biochemical reactions such as Indole, Urease, Citrate, Methyl red, Voges praoskauer, TSI, were done for the identification of Klebsiella pneumoniae. Antibiotic susceptibility testing was done on Muller Hinton Agar by Disc Diffusion Method their interpretation was done according to CLSI guidelines.

The carbapenamase producing Klebsiella pneumoniae were identified by Carba NP as described by CLSI guidelines.

Result: Out of the sample received in the Microbiology Department from May 2024 to July 2025, 335 isolates were of Klebsiella pneumoniae. The distribution pattern was similar from the previous study.

Table 1. Sample wise distribution of Klebsiella pneumoniae:

Sample	Number of isolates	Percentage
Urine	135	40.3%
Sputum	89	26.6%
Pus	47	14.0%
Blood	32	9.6%
BAL Fluid	17	5.1%
ET Tube	11	3.3%
Ascitic Fluid	4	1.1%

Table 1. Represents the sample wise distribution of Klebsiella pneumoniae, out of 335 non-duplicated Klebsiella pneumoniae isolates the urine sample shows the highest number of isolates (135, 40.3%) followed by sputum sample (89, 26.6%), pus sample (47, 14.0%), blood sample (32, 9.6%), BAL Fluid (17, 5.1%), ET Tube shows 11isolate of 3.3%, Ascitic Fluid account for 1.1% (4 isolates).

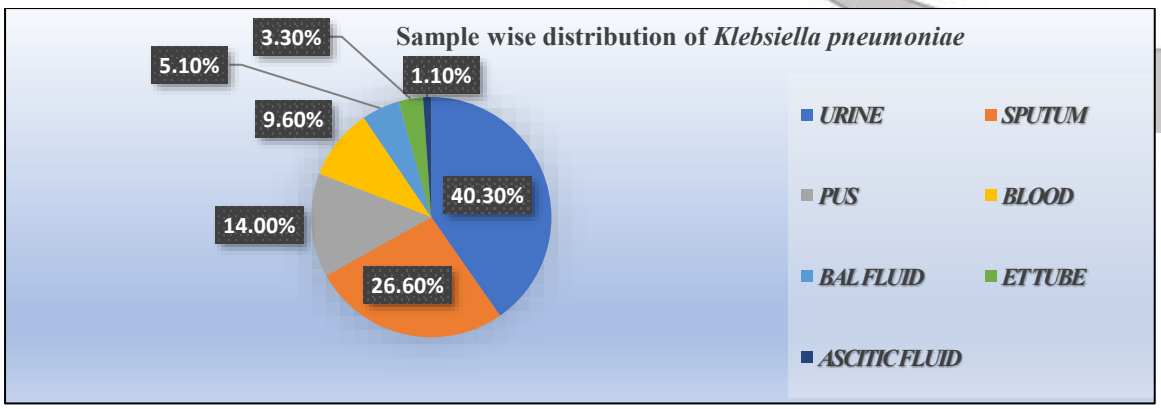

Fig 1. Sample wise distribution of Klebsiella pneumoniae.

Table 2. Ward Wise distribution of Klebsiella Pneumoniae:

Ward Name	Number of isolates	Percentage
General Medicine	179	54.43%
Surgery	68	20.30%
Urology	32	9.55%
Respiratory Medicine	21	6.27%
Gynecology	16	4.78%
Paedia	9	2.69%
Neurology	6	1.79%
ENT	3	0.90%
Gastrology	1	0.30%

Table 2, shows the ward wise distribution of klebsiella pneumoniae isolates, General Medicine shows the highest 179 cases (54.43%), followed by surgery 68 cases (20.30%), and urology 32 cases (9.55%). The remaining 16.72% cases were reported from respiratory medicine, gynecology, paediatrics, neurology, ENT and Gastrology.

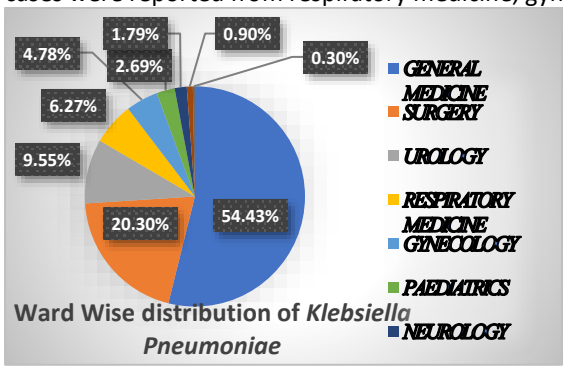

Fig. 2: Ward Wise distribution of Klebsiella Pneumoniae:

Table 3. Age wise distribution of Klebsiella pneumoniae:

Age	Male	Female	Total
1-10 Years	5	4	9
11-20 Years	10	8	18
21-30 Years	17	19	36
31-40 Years	14	25	39
41-50 Years	37	28	65
51-60 Years	42	31	73
61-70 Years	38	14	52
>70 Year	28	15	43

The highest rate of cases occurred among the patient of age group 51-60 Years (73 cases), followed by 41-50 years (65 cases), 61-70 years (52 cases), >70 years (43 cases). The male shows the majority of cases among these

age group while in young adults of age group 21-30 years the female predominance exists. Age group 1-10 years shows the lowest number of cases (9 cases).

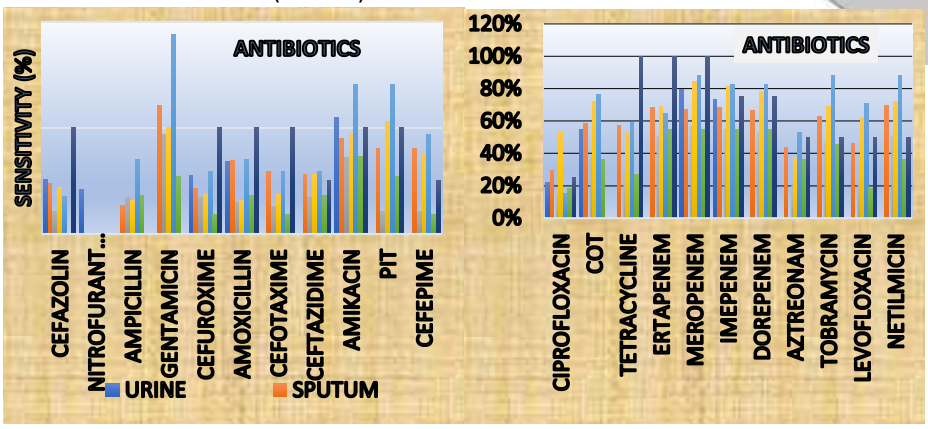

Fig 3 Antibiogram of Klebsiella isolates

Fig 3 Shows the antibiogram of Klebsiella pneumoniae, out of 135 urine sample, Meropenem shows the highest efficacy rate of 79.3% susceptibility followed by imipenem (73.3%), amikacin and cotrimoxazole shows the same susceptibility rate (54.8%), but amikacin shows intermediate rate of 22.2%. Nitrofurantoin shows the highest resistance rate (>57%).

In blood meropenem shows 84.4% and imipenem 81.3%, however in sputum sample imipenem had a susceptibility rate of 68.5% and resistance rate was 12.4%. In pus samples the sensitivity rate drops slightly imipenem showing 55.3% & meropenem 57.4%; Netilmicin shows the high efficacy particularly in sputum with 69.7% and 29.2% resistance rate.

Aminoglycosides like gentamicin shows high sensitivity in sputum isolates (60.7%) and resistance rate of 27.0%; in blood it shows 50% sensitivity and 40.6% resistance rate but works poorly in pus isolates with 48.9%. Amikacin is 44.9% sensitive and 28.1% resistant.

In fluroquinolones; levofloxacin is 62.5% sensitive and 9.4% resistance for blood sample but in pus sample the sensitivity rate decreased (23.4%) and resistance rate was 68.1%. Ciprofloxacin in sputum was 29.2% sensitive and 52.8% resistance and in pus it shows 14.9% sensitivity rate while 80.9% resistant rate. In blood 53.1% sensitive; pipracillin tazobactam was highly sensitive in blood (53.1%) and 40.4% in sputum. Ampicillin was 86.5% resistant in sputum, 80.9% resistant in pus and 84.4% resistant in blood.

In first and second generation cephalosporins cefazolin shows 74.2% resistance rate in sputum and 89.4% in pus isolates of K. pneumoniae; the resistant rate of cefuroxime is 69.7% in sputum and 83.0% in pus.

Third and fourth generation cephalosporins/ monobactams like cefotaxime, ceftazidime, cefepime and aztreonam shows the high resistance rate. Cefotaxime shows 60.7% resistance rate in sputum, 78.7 % resistance in pus; ceftazidime also shows the high resistance rate of 62.9% in sputum and 83.0 % resistance in pus. Aztreonam show 50.6% resistance rate in sputum and 74.5% resistance rate in pus isolates of Klebsiella pneumoniae.

Table 5: Sample wise distribution of Carbapenamase producing Klebsiella pneumoniae by Carba Np Test:

Sample	Carba Np test Positive	Percentage%
Urine (n=135)	14	10.37
Sputum (n=89)	08	8.99
Pus (n=47)	15	31.91
Blood (n=32)	03	9.37
BAL Fluid (n=17)	1	5.88
ET Tube (n=11)	4	36.36

From 68 isolates that were resistant to carbapenem, only 45 isolates were confirmed as carbapenemase producers. The Table 5 shows the sample wise distribution of Carbapenamase producing Klebsiella pneumoniae by phenotypic detection, that was done by the Carba NP test. The percentage of carbapenemase positivity was

highest in ET Tube isolates (4/11; 36.36%) and Pus samples (15/47; 31.91%). It remained lower but clinically impactful in Urine (14/135; 10.37%), Blood (3/32; 9.37%), Sputum (8/89; 8.99%), and BAL Fluid (1/17; 5.88%).

Discussion: The prevalence of multidrug-resistant (MDR) and carbapenem-resistant Klebsiella pneumoniae (CRKP) presents an urgent threat in clinical environments, notably restricting empirical therapeutic options and elevating patient mortality rates (Pattolath et al., 2023). This cross-sectional study mapped the phenotypic susceptibility and Carbapenamase production profiles of 335 non-duplicate K. pneumoniae isolates recovered from tertiary care facilities in Uttar Pradesh, providing baseline epidemiological insights into regional resistance traits.

The highest volume of isolates were from urine samples (40.3%), followed by sputum (26.6%) and pus (14.0%). This aligns closely with similar multi-center evaluations in India where urinary tract infections (UTIs) dominate Klebsiella clinical burdens (Patidar, 2021) [9].

The study shows that the General Medicine ward accounted for the vast majority of cases (54.43%), with the Surgery ward representing the second largest cases (20.30%). This underscores the dual hospital threat of K. pneumoniae across both prolonged medical stays and post-operative therapeutic environments [9, 10].

This study shows that the infection rates showed a definitive peak among older patients, specifically within the 51–60 years group (73 cases) and the 41–50 years (65 cases). Interestingly, while an overall male predominance was recorded in advanced age groups, a female predominance emerged exclusively within young adults aged 21–30 years (19 females vs. 17 males). This demographic shift is highly indicative of the biological susceptibility of young adult females to community-acquired and nosocomial UTIs driven by Enterobacteriaceae colonization (Patidar, 2021) [9].

The antibiogram profiles generated across the 335 isolates shows critical multi-drug resistance in Klebsiella pneumoniae isolates that invalidate traditional beta-lactam therapies:

In this study carbapenems retained reasonable overall susceptibility in urine (Meropenem 79.3%, Imipenem 73.3%) and blood (84.4% sensitivity, 15.6% resistance), pus samples demonstrated a precarious drop in susceptibility (55.3% to 57.4%) paired with high resistance levels (36.2% to 40.4%). This focal drop implies localized selection pressure or compartmentalized transmission of Carbapenamase-encoding plasmids within surgical and wound infection environments (Kumar et al., 2018; Patidar, 2021) [9] [11].

In present study out of the 335 Klebsiella pneumoniae isolates total 68 isolates were resistant to carbapenem (imipenem), only 45 isolates were confirmed as carbapenemase producers, establishing an overall phenotypic carbapenemase positivity rate of 13.43%. The absolute volume of positive tests was highest in pus (15/47) and urine (14/135). However, the highest proportional rates of carbapenemase-positivity were discovered in invasive device-associated settings: Endotracheal (ET) Tube isolates yielded 36.36% positivity (4/11), closely followed by Pus samples at 31.91% (15/47). This hyper-prevalence in ET tube and pus configurations is clinically significant. Patients requiring mechanical ventilation or carrying indwelling devices face a disproportionately high risk of acquiring highly virulent, carbapenemase-producing strains—a finding closely aligned with regional observations that identify device dependency as a major accelerator for metallo-beta-lactamase (MBL) and Klebsiella pneumoniae carbapenemase (KPC) propagation (Kumar et al., 2018; Pasha et al., 2023) [11, 12].

The prompt biochemical response of the Carba NP test—relying on the acidification of phenol red driven by imipenem hydrolysis (Shinde, 2017)—proves invaluable in this context. It saves 12 to 18 hours off standard diagnostic turnaround times, providing clinicians with the rapid data needed to implement strict contact isolation and optimize life-saving therapeutic selections (Pasha et al., 2023; Shinde, 2017) [12, 13].

Conclusion

This study establishes a baseline of high multidrug resistance among clinical Klebsiella pneumoniae isolates in Uttar Pradesh, marked by extensive resistance to first-, second-, and third-line antibiotics. While Carbapenems (meropenem and imipenem) remain the most potent defenses, showing their highest efficacy in blood (84.4%) and urine (73.3%–79.3%) isolates. However, a highly critical drop in carbapenem susceptibility occurs in pus samples (< 55%), (the notable emergence of carbapenem resistance—particularly localized within ET Tube (36.36%) and Pus (31.91%) environments—signals a severe institutional threat, alongside near-total failure of fluoroquinolones in deep tissue infections.

The Carba NP test functions as a highly effective, rapid, and economical phenotypic screening tool for routine clinical diagnostic workflows. Deploying this assay allows tertiary care facilities to drastically reduce diagnostic turnaround times, enabling early target therapy adjustments, reinforcing institutional antibiotic stewardship, and halting the clonal dissemination of superbugs within critical care units.

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