



Evaluation of Multidrug Resistance of *Klebsiella pneumoniae* Isolated From Different Clinical Samples

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Abstract

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Background: *Klebsiella pneumoniae* is a globally recognized opportunistic pathogen associated with multidrug resistance (MDR).

Objective: This study evaluated the phenotypic distribution of MDR and carbapenem resistance among *K. pneumoniae* isolates.

Materials and Methods: A total 200 specimens collected from four teaching hospitals in Nasiriya, Iraq(Nasiriya General Hospital, Al-Musawiy, Al-Haboubi, Bint Al-Huda), between October and December 2025, from which 88 isolates were identified as *K. pneumoniae* using biochemical methods and the VITEK®2 system. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method, and carbapenemase production was detected using the Modified Hodge Test (MHT).

Results: Blood samples constituted the majority of isolates (35.2%). MDR was observed in 88 isolates, with the highest resistance rates to ampicillin (68.18%) and ceftazidime (62.5%). Carbapenemase production was confirmed in 32.95% of isolates.

Conclusion: These findings indicate a high burden of MDR *K. pneumoniae*, underscoring the urgent need for strengthened infection control measures and rational antibiotic management in local hospitals.

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1.Introduction

Klebsiella pneumoniae is one of the most important pathogenic bacteria and it is a prominent cause of both hospital- acquired and community-acquired infections . It is a Gram-negative rod-shaped bacterium belonging to the family Enterobacteriaceae and is associated with high morbidity and mortality. *K. pneumoniae* is responsible for a wide range of clinical infections, including burns, surgical site infections, urinary tract infection (UTI), meningitis, pneumonia, and bloodstream infection(1) The problem of antimicrobial resistance is a serious challenge globally, which makes the treatment plan even harder and also causes higher morbidity and mortality. The classifications under which the antibiotics fall include aminoglycosides, carbapenems, fluoroquinolones, and third-generation cephalosporins, which have consistently failed in their effectiveness against *K. pneumoniae* (2).Carbapenem antibiotics are

perceived to be the first-line treatment options. Enterobacteriaceae strains with resistance to at least one of the four available antibiotics of carbapenem, imipenem, ertapenem, meropenem, and doripenem, fall under Carbapenem Resistant Enterobacteriaceae (CRE) (3) There are several mechanisms of multidrug resistance, including extended-spectrum β -lactamases (ESBLs) and carbapenemases (e.g., KPC, OXA-48, NDM), modification of antibiotic target sites, biofilm formation, activation of efflux pumps, and decreased outer membrane permeability because porin loss (4). According to Ambler classification, several classes of β -lactamases including, classes A, B, D, and C. Class A includes (KPC, SHV, CTX-M-15, TEM₁), class B(VIM, NDM, IMP), class D (OXAs), and class C(AmpC, CYM-2) (5). Therefore, the aim of the study was to investigation of multidrug-resistant of *Klebsiella pneumoniae* isolates.

2. Materials and Methods

2.1. Sample collection

All subjects participating in this study were informed, and the consent form was verbally obtained from each one before sample collection. All procedures performed in the studies involving human participants were in accordance with the ethical standards of province Thi-Qar University, Iraq. In this study, 200 specimens were collected isolated from clinical samples, including blood, wound swabs, urine, sputum, and abscesses. These were obtained from Nasiriya General Hospital in province Thi-Qar, Iraq between October 2025 and December 2025.

2.2. Isolation and Identification of *K. pneumoniae*

The samples were cultured on Blood agar and MacConkey agar for 24 hours at 37°C, and in negative sampling, extending to 48h. The isolates were first identified by biochemical test (IMVIC test, urease test), followed by confirmation using VITEK®2 (bioMérieux, Marcy l'Etoile, France).

2.3. Antimicrobial Sensitivity Test

The antibiotic susceptibility of *K. pneumoniae* was assessed using the Kirby- Bauer disk diffusion method. Substantially, MacConkey agar was used to prepare *K. pneumoniae* for overnight growth and Resuspended in Mueller-Hinton broth (0.5 McFarland). Then, use Mueller-Hinton agar to inoculate. Ampicillin; AM(10 μ g), Piperacilin; PI(5 μ g), Gentamicin; GEN(10 μ g), Levofloxacin; LEV (5 μ g), Amikacin AK(30 μ g), Cefoxitin; FOX(30 μ g), Ceftriaxone; CRO(30 μ g), Cefepime; CEF(30 μ g), Meropenem; MEM(10 μ g), Imipenem; IPM(10 μ g), Doripenem; DOR(10 μ g), Ertapenem; ETP(10 μ g), Trimethoprim-Sulfamethoxazole; SXT(25 μ g), Ciprofloxacin; CIP(5 μ g). The inhibitory zone was evaluated after the agar plate was infected at 37°C for 18 to 24 hours. The 2018 CLSI Taxonomy classified the outcomes as resistant, intermediate, and sensitive.

2.4. Evaluation of carbapenemase production

A typical *Escherichia coli* 0.5 McFarland standard was prepared as a 1:10 dilution in saline, and this was spotted on an MHA plate, and an Oxoid Imipenem ten microgram disk was placed on the plate. The colonies of the testing species were selected and spotted in a line along the edge of the disk. The plate was incubated at 35°C \pm 2°C for 24h. The result was considered positive for a carbapenemase-producing bacterium if there was escalated bacterial growth with a clover-leaf indent. (6).

2.5. Statistical Analysis

The present data was statistically analyzed by using SPSS version 26, based on using Chi-square at a p-value less than 0.05

3. Results

3.1. Isolation and identification of *K. pneumoniae*

200 samples collected ,75(37.5%) are Gram positive and 125(62.5%) are Gram negative. Eighty-eight of 125(70.4%) are *K. pneumoniae*. When cultured on MacConkey agar plates, *K. pneumoniae* appeared large, pink, and mucoid. While, in blood agar it appears mucoid, white. This bacterium was identified by biochemical test (as in Table 1 below). Eighty-eight isolates were collected from clinical specimens including (27) isolates from sputum, (31) from blood, (16) from urine, (11) from swabs, (3) from other types of specimens, as shown in table 2.

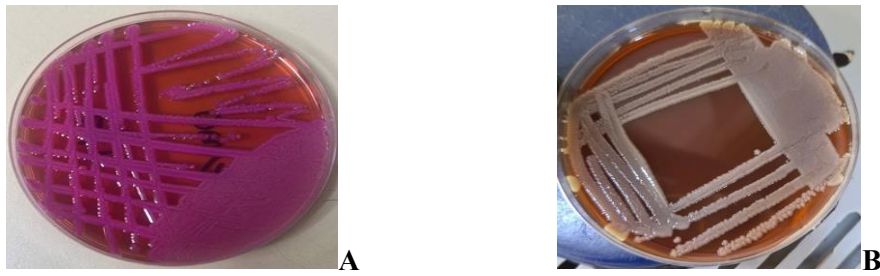


Figure 1: *K.pneumoniae* growth on A) MacConkey agar and B) Blood agar

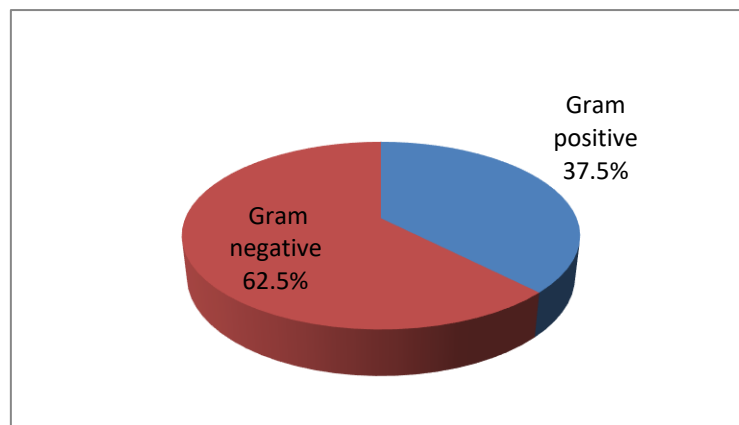


Figure 2: Frequency of Gram positive and Gram negative isolation in current study

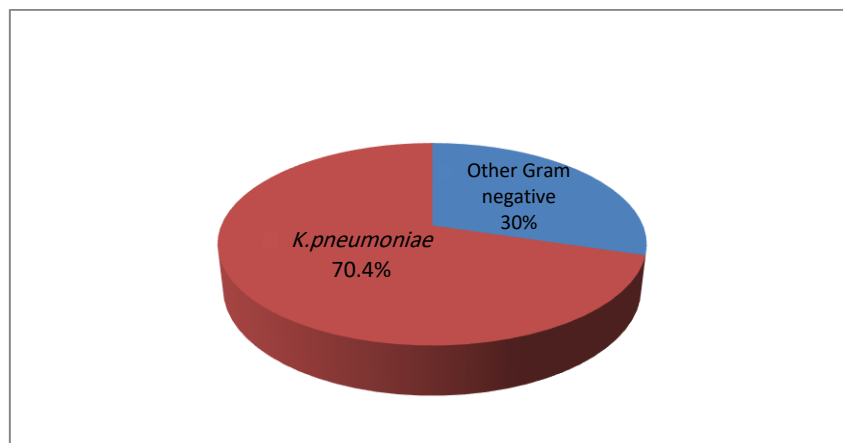


Figure 3: Percentage of *K.pneumoniae* among Gram negative bacteria

As shown in Table 1, the isolates were negative for the indole test and methyl red(MR) test, while positive reaction were observed for Voges proskauer (VP) test, citrate utilization test, and urease tests.

Table 1: Biochemical tests

Biochemical test	result
Indol test	-
Methyl red (MR) test	-
Voges proskauer (VP) test	+
Citrate utilization test	+
Urease test	+

* (-): negative results , (+): positive results

Table 2 : Distribution of Sources of *K. pneumoniae* isolates

Source	No.	Percentage%
Sputum	27	30.68
Blood	31	35.23
Urine	16	18.18
Swabs(wound, ear,skin)	11	12.50
Leg abscess	1	1.14
Stool	1	1.14
H.V.S	1	1.14
Total	88	100
$\text{CalX}^2= 87.7$ $\text{TabX}^2= 12.59$ $\text{DF}= 6$ $\text{p. value} < 0.01$		

3.2. Antibiotic sensitivity test

As table3 below showed the most isolates were multidrug resistance, where the highest rate of resistance was observed 60(68.18%) to ampicillin, followed by Ceftazidim55(62.5%), Cefepime52 (59%), Trimethoprim- sulfamethoxazole 51(57.9%), Ceftriaxone 44 (50%), Ciprofloxacin43(48.86%), Levofloxacin40(45.45%), Piperacillin37(42.05%), Amikacin35(39.77%), Gentamicin34(38.6%), Ertapenem29(32.95%), Imipenem28(31.8%), Meropenem27(30.68%), Doripenem20 (22.7%). As shown in the table below, sensitive to antibiotics showed Gentamicin high rate sensitivity to *K. pneumoniae* followed by Amikacin, and the least is ampicillin.



Figure 4: Antibiotic sensitivity test.

Table 3: Antibiotic sensitivity test

Antibiotic	Resistance		Intermediate		Sensitive	
	n	%	n	%	n	%
Ampicillin	60	68.18	4	4.55	24	27.27
Piperacillin	37	42.05	5	5.68	46	52.27
Ceftazidime	55	62.50	3	3.41	30	34.09
Cefepime	52	59.09	2	2.27	34	38.64
Ceftriaxone	44	50.00	1	1.14	43	48.86
Amikacin	35	39.77	0	0.00	53	60.23
Gentamicin	34	38.64	0	0.00	54	61.36
Imipenem	28	31.82	4	4.55	56	63.64
Ertapenem	29	32.95	1	1.14	58	65.91
Meropenem	27	30.68	1	1.14	60	68.18
Doripenem	20	22.73	0	0.00	68	77.27
Ciprofloxacin	43	48.86	1	1.14	44	50.00
Levofloxacin	40	45.45	0	0.00	48	54.55
Trimethoprim-sulfamethoxazole	51	57.95	0	0.00	37	42.05
Total Susceptibility	39.64		1.57		53.17	
CalX ² = 132.7 TabX ² = 38.89 DF= 26 p. value <0.01						

*R: Resistance, I: Intermediate, S: Sensitive

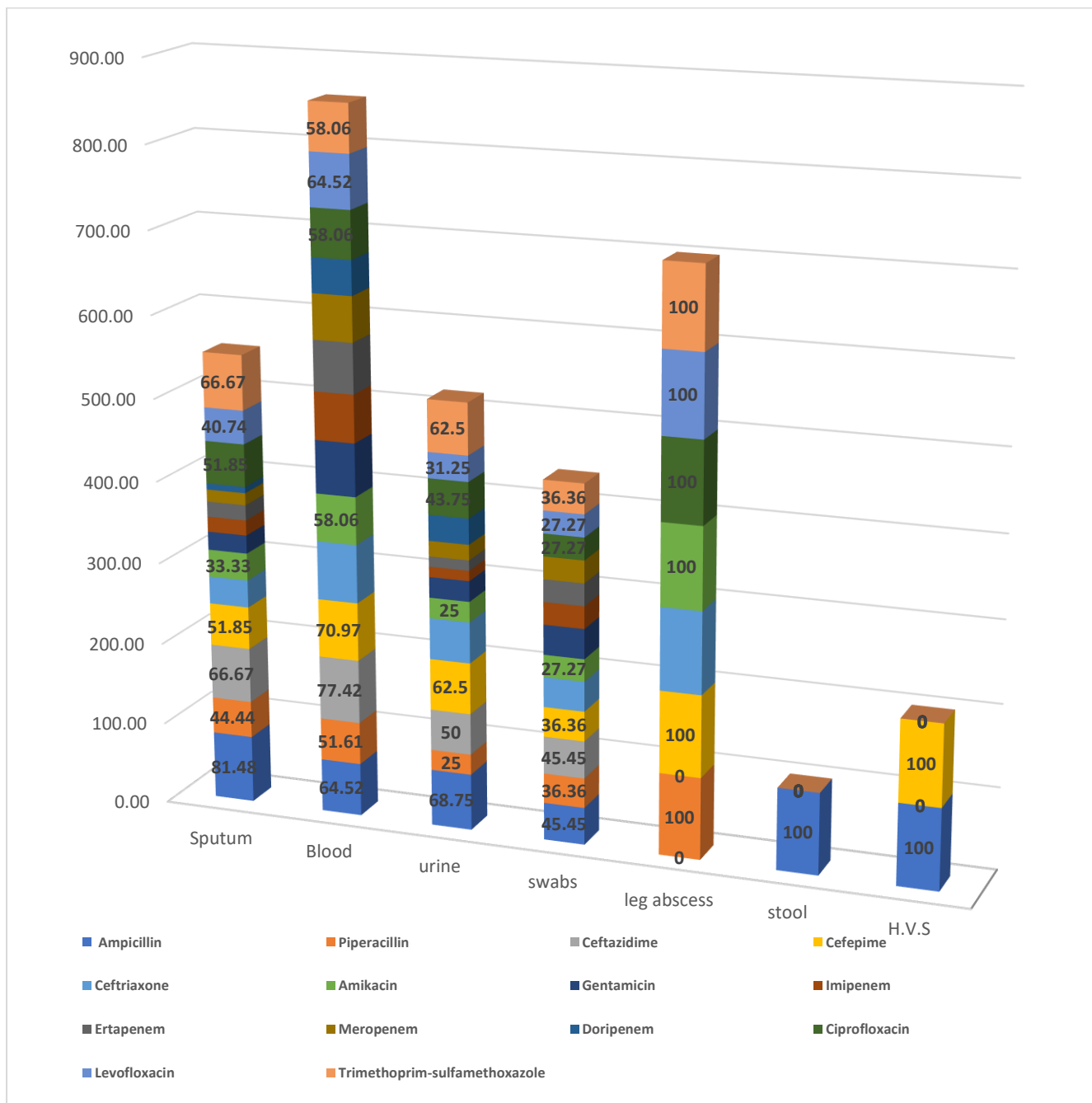


Figure 5: Antibiotic Resistance Percentage of *Klebsiella pneumoniae*.

3.3.Modified Hodge test (MHT)

In this test, it was found that out of 88 isolates, 29/88 isolates have resistance to carbapenem (32.95%), which have cloverleaf formations in the MHT test, and (59/88) isolates(67.05%) have been found to have sensitivity to carbapenem.

4.Discussion

Klebsiella pneumoniae has been identified as a serious causal agent of a broad spectrum of infections in humans. In the present study, *K. pneumoniae* was mostly isolated from blood. These findings contradict those presented in the work of (7), where the higher isolation rate of *K. pneumoniae* was observed in urine cultures, 45.3%, followed by vaginal and wound swabs, 19.9%, sputum and

bronchial aspirates, 15.4%, blood cultures, 12.5%, and samples taken from medical devices, 6.9%. In another study, which agreed with the current study, reported that *K. pneumoniae* was most isolated from blood (32.6%) (8).

High resistance was found for ampicillin (96.9%). On the other hand, high susceptibility values of 93.8% and 82.3% were established for Ertapenem and Imipenem, respectively(9).The present study are agreed with a study conducted in Iran, which reported that *K.pneumoniae* isolates exhibited extensive resistance to third-generation Cephalosporins, with approximately 50% of the isolates also demonstrating resistance to gentamicin (10). In contrast, local study have indicated that clinical isolates of *K. pneumoniae* exhibited relatively low resistance rates to Meropenem and Imipenem (11, 20).

In current study, *K.pneumoniae* resistance to Imipenem (31.82%)and Meropenem (30.68%). Another study showed most isolates of *K.pneumoniae* resistant to Imipenem and Meropenem 15 (51.7%), but to Ertapenem 29(100%)(12).In previous study from southern Rajathan reported that 40% of *K.pneumoniae* isolates were resistant to Imipenem (13).

In present study , *K. pneumoniae* resistance to ciprofloxacin(48.86%) and susceptible to Amikacin (60.23%). that consistent with a previous study (14), showed that *K. pneumoniae* isolates were 100% susceptible to Amikacin but showed resistance of 38.75% to ciprofloxacin. The variability in antimicrobial resistance profiles can be attributed to many reasons, such as differences in sample size, specimen type, geographic location, site of infection, susceptibility of patients to infections, among many others (15).

In current study, approximately 88 of the isolates of *K.pneumoniae* exhibited multidrug-resistant patterns, depicting a profoundly alarming scenario prevailing in Iraq. The results revealed marked disparities among the isolates regarding resistance patterns against each and every antibiotic used. Development and dissemination of antimicrobial resistance are mostly due to improper and overuse of antimicrobials. Other major causes are insufficient access and provision regarding Water, Sanitation, and Hygiene for humans as well as livestock, improper Infection Control Practices prevailing in healthcare facilities, unavailability of easily affordable treatment and diagnostic tools, and insufficient implementation of antimicrobial resistance regulations (16).

The factors that may have contributed to the increasing prevalence of the drug-resistant isolates include surveillance programs for infection, which are not well advanced, the scarcity of laboratory infrastructures, deficiencies in communication between microbiologists and physicians, and deficiency of standardized isolate identification and antimicrobial testing criteria, coupled with poor sanitation conditions (17).

In current study,29/29(100%), resistance to carbapenems by using MHT ,which agreed with another study showed 93.1% resistance to carbapenems (18). The Modified Hodge Test (MHT) can be employed to detect carbapenemase-producing bacteria, but its sensitivities are very low for Metallo- β -lactamases (MBLs), and its specificity cannot be targeted exclusively for serine carbapenemases. Other drawbacks are the variability of performance and the possibility of producing false-positive results. Additionally, MHT cannot be utilized for detecting carbapenemases in non-fermenting Gram-negative bacilli. Later, more reliable phenotypic techniques using the the modified carbapenem inactivation method (mCIM) and Carba NP test had been employed for detecting carbapenemase-producing bacteria (19).

5.Conclusion

It confirms that *K. pneumoniae* isolates in the Nasiriya General Hospital have developed significant resistance to standard treatments, including carbapenems. The prevalence of MDR and CRE strains calls for strict antibiotic stewardship and enhanced infection control measures with a view to impeding further dissemination of these "superbugs" within the healthcare facility.

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