NTU Journal of Pure Sciences
Double Blind Peer Review Journal
ISSN: 2789-1089 EISSN: 2789-1097
Available Online: https://journals.ntu.edu.iq/index.php/NTU-JPS
Volume 5, No. 1, 2026

Original Article

Screening *Klebsiella pneumoniae* in community-acquired infections and its relationship to antibiotic profile resistance in Kirkuk City

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Received: 21 July 2024
Accepted: 08 September 2024
Published: 12 April 2026
DOI: https://doi.org/10.56286/w4j6nv37
Article ID: 1084

Abstract

Community-acquired Klebsiella pneumoniae is a major cause of health problems due to the rising rates of antibiotic resistance, especially for the new generation of antimicrobial families. The objective of this study was to detect the prevalence of K. pneumoniae from clinical samples of community-acquired infections and its association with antimicrobial resistance. A total of 256 different clinical samples were obtained from outpatients at four main hospitals in Kirkuk city. In addition, 38 isolates were identified by culture and biochemical characteristics and then confirmed with the VITEK 2 system. The antibiotic resistance profile was determined by testing 30 antibiotics using both the disc diffusion method and the VITEK 2 AST-419 card. Results: This study involved 256 clinical samples; K. pneumoniae represented 14.8% of all isolates. The antibiotic sensitivity test showed 100% resistance of all isolates to the penicillin group while most isolates were sensitive (89.5%, 84.2%) to lipopeptide and Glycylcycline groups respectively. The data showed increased resistance of K. pneumoniae to the common antibiotics and MDR K. pneumoniae was the most prevalent among the isolates.

Keyword: *Klebsiella pneumoniae*, Community-acquired infections, MDR, Antibiotic resistance profile

Introduction

Klebsiella pneumoniae belongs to the family of Enterobacteriaceae classifies, a non-motile, Gram-negative encapsulated organism, under the genus Klebsiella. This bacterium is known for its ability to ferment lactose and its facultative anaerobic nature [1]. Opportunistic bacterium can cause community and nosocomial infections [2]. It has been associated with a broad range of clinical disorders such as pneumonia, infections within the abdomen, urinary tract infections (UTIs), wounds, bloodstream, and soft tissue infections [3, 4, 5]. The environment contains K. pneumoniae, particularly in soil, shallow waters, and non-living surfaces like medical

instruments. The microorganism establishes colonization on the mucosal surfaces of humans, specifically in the oropharynx and gastrointestinal system, and can potentially spread to other tissues [6]. An array of virulence factors contribute to the virulence and pathogenicity of K. pneumoniae isolates, enabling them to infect and invade the host's immune response, resulting in their ability to cause severe diseases. The capsule, lipopolysaccharide (LPS), fimbriae, and siderophores are the most extensively researched virulence components linked to K. pneumoniae [7, 8]. An increasing number of diseases are becoming more difficult to manage and prevent due to antimicrobial resistance. This poses a significant global threat to health and development. It has become a major public health issue in the 21st century [9, 10]. Multidrug-resistant (MDR) bacteria, including ESBL-producing K. pneumoniae and other Gram-negative bacteria, are widely distributed globally and exhibit a high incidence in both hospital and community settings [11]. K. pneumoniae has many resistance mechanisms to numerous antibiotics, including alteration of antibiotic target locations, modulation of metabolic pathways, efflux pump system activation, alteration of membrane permeability, and the production of drug-inactivating enzymes [12]. Regarding the production of enzymes that inactivate antibiotics, extended-spectrum beta-lactamase (ESBL) enzymes, which are encoded by plasmids, play an important role in dissolving bonds of beta-lactam rings derived from antibiotics such as Penicillins, Cephalosporins, and Aztreonam, as well as being inhibited by the effects of Clavulanate [13]. Furthermore, various modulating enzymes present on the same plasmid can confer resistance to different types of antibiotics, including aminoglycosides, fluoroquinolones, tetracyclines, and trimethoprim/sulfamethoxazole [14]. Antibiotic misuse has resulted in challenges in treating K. pneumoniae, limiting our options for effectively managing infections caused by this bacterium [15]. As a result, this study aimed to determine the frequency of K. pneumoniae isolated from various clinical sources, detect the incidence of drug resistance patterns (MDR, XDR, and PDR), and study the antibiotic susceptibility of K. pneumoniae isolates in community-acquired infections in Kirkuk City.

Materials and Method

Ethics statements

Before initiating the research, specific permission had been obtained from the Kirkuk health directorates (document number and date were 582 on 2023-9-17).

Sample collection

This research project carried out in Kirkuk City's four hospitals, AL-Naser Hospital Azadi Teaching Hospital, Kirkuk General Hospital, and General Paediatrics Hospital, during the period from September 2023 to December 2023, two hundred fifty-six (256) various clinical samples taken from outpatients (including females and males with different ages) were suffering from community-acquired infections. To collect sputum and urine samples, a sterile container was used, while to take other samples and transport them to the microbiology laboratory for processing, a sterile swab with transport media was utlised.

Isolation and bacterial identification of strains

All 256 clinical samples were plated on an ordinary medium (nutrient agar), enrichment medium (blood agar), selective and differential media (MacConkey agar and Eosin methylene blue agar), after an overnight incubation at 37 ˚C. Isolated colonies were identified by oxidase, catalase tests, Gram staining, and morphological characteristics, in addition to biochemical reactions. All 38 *K. pneumoniae* isolates were confirmed by utilising the VITEK 2 GN Identification card system according to the manufacturer's instructions.

Large creamy to white colonies of *K. pneumoniae* on the nutrient agar was produced; non- hemolytic colonies can be seen on blood agar whereas viscous, round, and lactose fermenter colonies were observed on the MacConkey agar then subcultured on Eosin methylene blue agar for distinguishing *Klebsiella* species from *E .coli*.

Antimicrobial resistance is evaluated

The antimicrobial susceptibility of *K. pneumoniae* was evaluated against 30 antibiotics belonging to 13 antibiotic classes was done utilising two methods (disc diffusion and Vitek 2 GN AST-N419 methods).

Kirby Bauer technique of disc diffusion on Muller Hinton agar (HIMEDIA, India) performed on all 38 isolates based on the Clinical and Laboratory Standards Institute (CLSI, 2023) [16]. In this method, we used 13 antibiotic disks including ampicillin 10 µg, cefazolin 30 µg, amoxicillin/clavulanic acid 30 µg, cefoxitin 30 µg, cefixime 5 µg, gemifloxacin 5 µg, aztreonam 30 µg, azithromycin 15 µg, norfloxacin 10 µg, doxycycline 30 µg, tetracycline 30 µg, trimethoprim 5 µg, and nitrofurantoin 300 µg. The other method was the Vitek 2 GN AST-N419 card. The antibiotics in this automated test were: ticarcillin, amoxicillin, ampicillin/sulbactam, ceftazidime/avibactam, ceftolozane/ tazobactam, piperacillin/tazobactam, ceftazidime/avibactam, ceftazidime, cefepime, cefotaxime,

imipenem, meropenem, colistin, tigecycline, amikacin, gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole, also performed on all *K. pneumoniae* isolates following manufacture instructions (bio-Merieux). Based on their antibiotic-resistant characteristics, we classified the isolates into the following groups:

If MDR is resistant to at least one agent in at least three antimicrobial categories and if (XDR) extensively drug-resistant)not susceptible to at least one agent in all but two or fewer antimicrobial categories) an isolate is classified as PDR which is apan-drug resistant if it demonstrates resistance to all listed antimicrobial agents [17].

Statistical analysis

The Statistical Analysis Systems-SAS (2018) programs were utilised to identify the impact of various factors on research parameters through statistical analysis. In this investigation, we employed the chi-square test to examine the significance between percentages at probabilities of 0.05 and 0.01.

Results and Discussion

The study involved the isolation and bacteriological identification of K pneumoniae strains. The current study demonstrated that out of 256 various samples, there were 148 (57.8%) samples.

This exhibited bacterial growth, of which 38 (14.8%) represent *K. pneumoniae*. The remaining rate 110 (43%) involved other bacteria, whereas 108 (42.2%) samples exhibited no bacterial growth as listed in table 1. The frequency of *K. pneumoniae* from total bacterial growth compared with negative growth was shown to be statistically highly significant (*p*-value = 0.0009).

Table 1. Various clinical samples cultural results

Bacterial growth		No growth	Total	p-value
k. pneumoniae	**Other bacteria**	108 (42.2%)	256 (100%)	** Chi-Square = 49.109 P-value = 0.0009
38 (14.8%)	110 (43%)			

** (p-value level of ≤ 0.01 considered highly significant)

Our results were consistent with the previous study's findings [18], which reported that bacterial growth represents 60/125 (48%) of community-acquired infections in Diyala City and the frequency of *K. pneumoniae* isolates was 12.0 % in different clinical sources. They contradicted the findings of the study [19], where the prevalence of *K. pneumoniae* isolate was 43.06%.

K. pneumoniae isolates prevalence rate among different clinical samples revealed the highest number and were observed in urine (47.4%), followed by wound swabs (21.1%), sputum (13.2%), burn swabs (10.5%), diabetic foot ulcer (2.6%), higher vaginal swab (2.6%), and bronchial wash (2.6%).

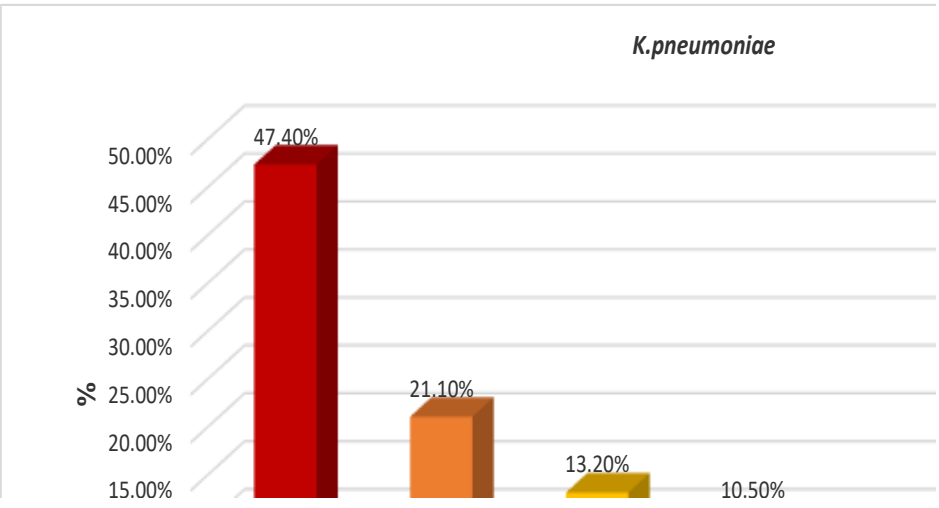


Figure 1. Illustrates the distribution of *K. pneumoniae* isolates in various clinical samples.

In a comparable study conducted by [20], the prevalence rate is higher in urine (41.94%), followed by wound swabs (19.35%), (12.90%) sputum, (4.83%) were obtained from ear swabs, (3.22%) high vaginal swabs, (1.61%) eye swabs and throat, (4.83%) blood, and (9.67%) represent other sources, respectively. Furthermore, another study [21] was carried out in Mexico which shows that the frequency of community-acquired *K. pneumoniae* strains was (46.1%) in urine, (25.6%) vaginal secretion, (15.3%) antral biopsy samples, (10.2%) sputum, and (2.5%) cerebrospinal fluid (CSF). These results were also similar to our results regarding the urine and sputum samples prevalence rate. The present study's findings showed that *K. pneumoniae* isolates were more prevalent in 21 male outpatients (55.3%) than in 17 females (44.7%), this finding agrees with [20], which found that the infection rate in males was 53.23 % more than females. On the other hand study [22], which obtained samples from different sites from both inpatients and outpatients, disagreed with our finding, and showed that the number of infected females (42) was higher than males (27). The infection rate of *K. pneumoniae* varies depending on the source of infection and the gender of the affected individuals. Various factors influence susceptibility to K. pneumoniae infection, including the pathogen's characteristics (such as its ability to cause disease and drug resistance), the host's intrinsic features (such as genetics, age, and immune response), and external factors (such as antibiotic use, exposure to the environment, diet, and alcohol consumption) [23].

Regarding the identification of *K. pneumoniae* isolates, they were initially identified by their morphology characteristics, culture, and biochemical assays, as shown in Figure 2.

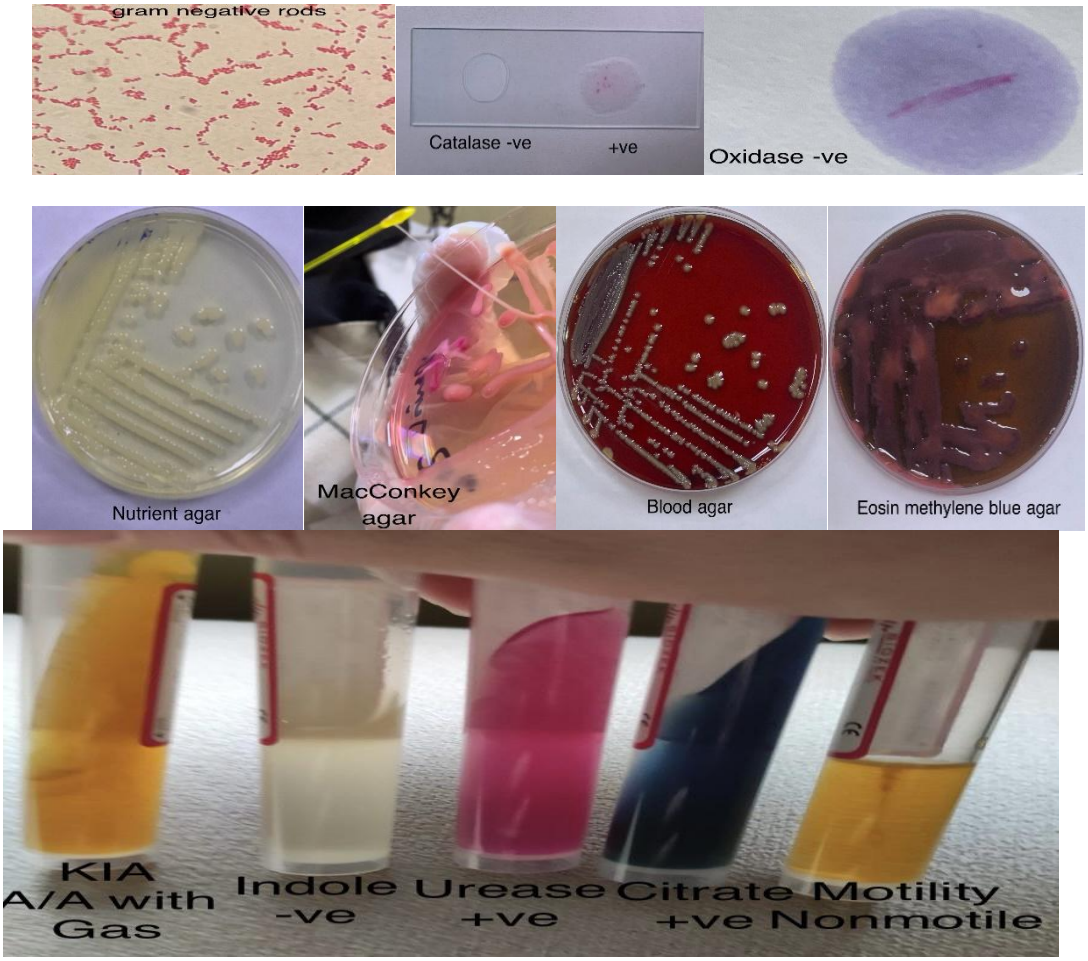
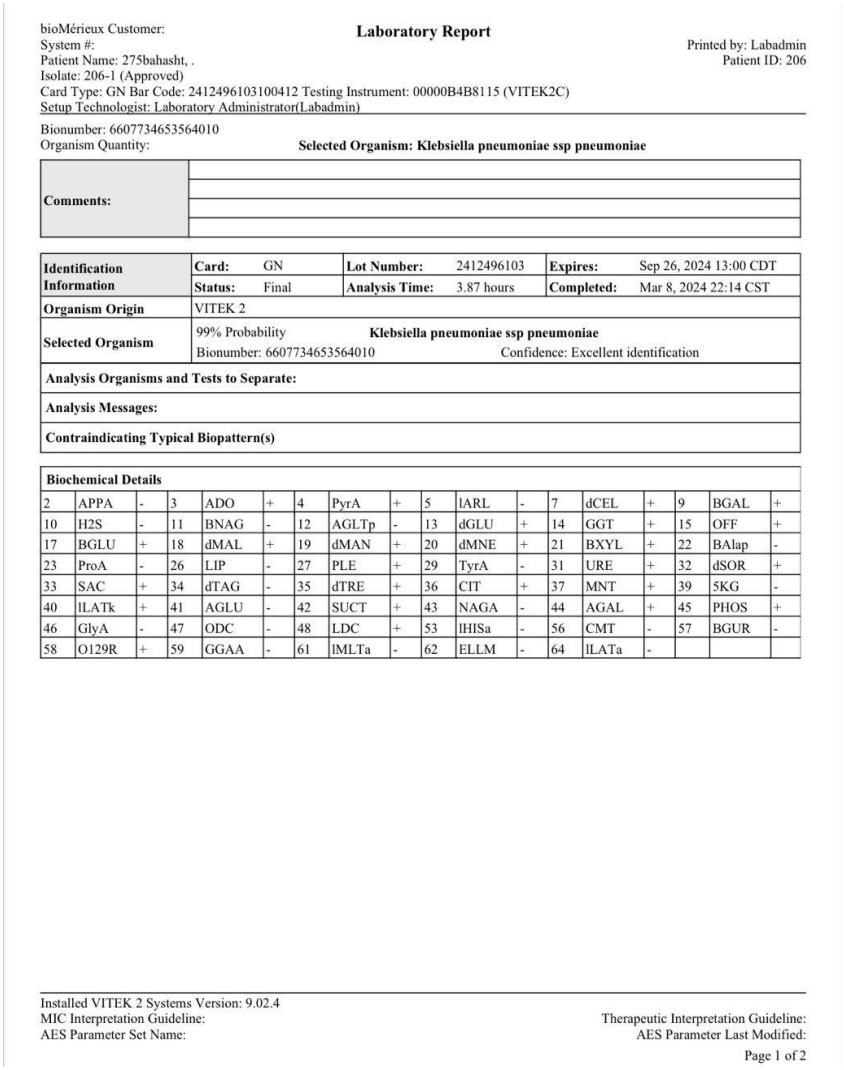


Figure 2. *K. pneumoniae's* morphological, cultural characteristics, and biochemical assays

Note: KIA (kligler iron agar), A/A (acid/acid), +ve (positive), -ve (negative).

Then all thirty-eight isolates were confirmed with the Vitek 2 compact [Figure 3].



bioMérieux Customer:
System #:
Patient Name: 275bahasht, .
Isolate: 206-1 (Approved)
Card Type: GN Bar Code: 2412496103100412 Testing Instrument: 00000B4B8115 (VITEK2C)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: Labadmin
Patient ID: 206

Bionumber: 6607734653564010
Organism Quantity:

Selected Organism: Klebsiella pneumoniae ssp pneumoniae

Comments:	

Identification Information	**Card:**	GN	**Lot Number:**	2412496103	**Expires:**	Sep 26, 2024 13:00 CDT
	Status:	Final	**Analysis Time:**	3.87 hours	**Completed:**	Mar 8, 2024 22:14 CST
Organism Origin	VITEK 2					
Selected Organism	99% Probability		**Klebsiella pneumoniae ssp pneumoniae**			
	Bionumber: 6607734653564010			Confidence: Excellent identification		
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Installed VITEK 2 Systems Version: 9.02.4
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 2

Figure 3. Conformation of *K. pneumoniae* isolates with Vitek 2 system

Antimicrobial resistance is evaluated

We conducted the antibiotic susceptibility test on all 38 isolates using both disc diffusion and Vitek 2 compact AST GN-419 methods, revealing varying levels of resistance to the 30 antibiotics used in this study. All 38 strains exhibited 100% resistance to Ticarcillin, Ampicillin, and Amoxicillin. Additionally, the strains showed resistance rates of 73.7% for Cefazolin, 71.1% for Cefixime, and 68.4% for (Cefotaxime, Aztreonam, and Trimethoprim). Furthermore, 55.3% of the strains were resistant to Ciprofloxacin, 52.6% to Tetracycline, and 50% to (Gemifloxacin and Trimethoprim/Sulfamethoxazole). The resistance rates towards amoxicillin/ clavulanate, ampicillin/ sulbactam, and nitrofurantoin were 47.4%, 44.7%, and 47.4%, respectively. Isolates were susceptible to other agents in beta-lactam combination drugs at rates of 65.8%, and 63.2% for Ceftolozane/ Tazobactam, Ceftazidime/ Avibactam, and Piperacillin/ Tazobactam. Sensitivity rates to Cefoxitin and Cefepime (Cephalosporin drugs) were 55.3% and 52.6%. Carbapenems class (Imipinime and Meropenem) showed sensitivity at 63.2% and 60.6%. In addition to aminoglycosides class (Gentamicin and Amikacin) at 73.7% and 65.8%, the antibiotics such as Doxycycline, Azithromycin, and Norfloxacin at sensitivity rates of 55.3% and 52.6. Colistin and Tigecycline were the most effective antibacterial agents for treating community-acquired *K. pneumoniae* infections, with sensitivity rates of 89.5% and 84.2% respectively as in Table (2).

Table 2. The table displays the antibiotic susceptibility tests of K. pneumoniae strains.

Antibacterial agents	*R*	*%*	*I*	*%*	*S*	*%*	*T*	*Class of antibiotics*
Ampicillin	38	100	0	0	0	0	38	
Amoxicillin	38	100	0	0	0	0	38	Penicillin
Ticarcillin	38	100	0	0	0	0	38	
Amoxicillin/ Clavulanate	18	47.4	3	7.9	17	44.7	38	B-lactam
Ampicillin/ Sulbactam	17	44.7	6	15.8	15	39.5	38	Combination agents

Ceftolozane/ Tazobactam	13	34.2	0	0	25	65.8	38	
Ceftazidime/ Avibactam	14	36.8	0	0	24	63.2	38	
Piperacillin/ Tazobactam	14	36.8	0	0	24	63.2	38	
Cefazolin	28	73.7	3	7.9	7	18.4	38	
Cefoxitin	17	44.7	0	0	21	55.3	38	
Ceftazidime	21	55.3	4	10.5	13	34.2	38	Cephalosporin
Cefixime	27	71.1	0	0	11	28.9	38	
Cefotaxime	26	68.4	0	0	12	31.6	38	
Cefepime	18	47.4	0	0	20	52.6	38	
Aztreonam	26	68.4	0	0	12	31.6	38	Monobactams
Imipenem	13	34.2	1	2.6	24	63.2	38	Carbapeneams
Meropenem	14	36.8	1	2.6	23	60.6	38	
Colistin	1	2.6	3	7.9	34	89.5	38	Lipopeptides
Tigecycline	1	2.6	5	13.2	32	84.2	38	Glycylcyclines
Gentamicin	10	26.3	0	0	28	73.7	38	Aminoglycoside
Amikacin	13	34.2	0	0	25	65.8	38	
Azithromycin	18	47.4	0	0	20	52.6	38	Macrolides
Doxycycline	16	42.1	1	2.6	21	55.3	38	Tetracycline
Tetracycline	20	52.6	2	5.3	16	42.1	38	
Ciprofloxacin	21	55.3	2	5.2	15	39.5	38	
Gemifloxacin	19	50	3	7.9	16	42.1	38	Fluoroquinolones
Norfloxacin	16	42.1	2	5.3	20	52.6	38	
Trimethoprim/ Sulfamethoxazole	19	50	0	0	19	50	38	Folate pathway antiagonists
Nitrofurantoin	18	47.4	7	18.4	13	34.2	38	Phenicols
Trimethoprim	23	68.4	0	0	15	39.5	38	

** (p-value level of ≤ 0.01 considered highly significant)

Notes: Resistance(R), Intermediate (I), Sensitivities (S), Percentage (%), Total (T).

The results of our investigation agree with the study conducted by [21], which documented a 100% resistance rate for ampicillin and ticarcillin. Furthermore, our findings agree with the results reported by [24], which indicated a 100% resistance rate for Amoxicillin in samples collected from outpatients. *K. pneumoniae* isolates have an inherent resistance to ampicillin and ticarcillin due to the synthesis of a chromosomal penicillinase known as SHV-1. Furthermore, it can acquire resistance mechanisms through mobile elements such as plasmids and transposons [25]. A study carried out by [20] found that the rates of resistance for Ceftazidime, and Cefepime were 54.84%, 46.77%, respectively. This finding is in agreement with the results in the current study. Regarding the cefotaxime resistance, our results are consistent with the results of the study [26], which reported that Cefotaxime resistance was 67.0%. In this study, Cefazolin resistance was 73.1% which disagrees with the study results of [27], which showed that Cefazoline resistance, was 56.64%. Concerning Beta-lactam combination agents (Amoxicillin/Clavulanate and Piperacillin/ Tazobactam), this study's results contradicted a study conducted [18], which reported increased resistance at rates of 86.6% for Amoxicillin/Clavulanate and 66.6 for Piperacillin/ Tazobactam. Our research results revealed a moderate to decreased resistance to Carbapenems, specifically Imipenem and Meropenem; these findings agree with the results reported in the study [28], which showed a resistance rate of 42.0% for Imipenem and 28.0% for Meropenem. Carbapenems remain to be the most efficient β-lactam antibiotics for the majority of species. Therefore, they must be used logically to treat severe cases including antibiotics for MDR bacteria, for regular β-lactams are ineffective [29]. The study [28] demonstrated that the resistance rates to Trimethoprim/Sulfamethoxazole, Nitrofurantoin, and Ciprofloxacin were 64.0%, 50.0%, 46.0%, respectively, which aligns with the findings of our results for these antibiotics. However, our isolates showed effective sensitivity to Amikacin and Gentamicin, which agrees with the results were based on a previous study [30]. In that study, the sensitivity rate for Amikacin was 77.2% and 70.4% for Gentamicin. The current study's results showed that out of 38 isolates, 23 (60.5%) were MDR, 8 (21.1%) were XDR, and 1 (2.6%) were PDR, while only 6 (15.8%) were non MDR, which was statistically highly significant (p value ≤ 0.01) as listed in Table (3).

Table 3. The *K. pneumoniae* strains exhibit a pattern of drug resistance.

No. of* K. pneumoniae *strains	**Resistance type**						**NON-MDR strains**		**P-value**
	MDR		**XDR**		**PDR**				
38	No.	%	No.	%	No.	%	No.	%	**

	23	60.5	8	21.1	1	2.6	6	15.8	Chi-Square= 37.754 P- value = 0.0008

Notes: Multi-drug resistant (MDR); extensively drug resistant (XDR); number (No.); and percentage (%).

The local study conducted by [31] showed that the prevalence of MDR *K. pneumoniae* isolates was 22 (73.333%), 6 (20.0%) XDR, and 2 (6.667%) PDR, which agrees with our findings. Furthermore, our results were different from the the results of the study [31] in that there were no susceptible isolates to one or two drugs among the 30 isolates. Due to overuse and abuse, *K. pneumoniae* has become very resistant to antibiotics. The appearance of PDR and MDR strains complicates prevention and treatment [32].

Conclusion

The current study data showed an increased resistance of *K. pneumoniae* to the common antibiotics, and MDR *K. pneumoniae* was the most prevalent among the isolates in community-acquired infections.

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