Original Article

Antibiotic Susceptibility Profile of Carbapenem-Resistant *Klebsiella pneumoniae* Isolated from Clinical Specimens in Iraq

Ali A. Al-Shammari[1,2], Alaa H. Al-Charrakh[2]

[1]Department of Dentistry, Al-Zahrawi University College, Karbala, Iraq, [2]Department of Microbiology, College of Medicine, University of Babylon, Hilla, Iraq

Abstract

Background: Infections caused by carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) isolates are a major threat to public health. Antibiotic misuse and overuse lead to the emergence of CR-Kp. **Objectives:** This study investigated the distribution and phenotypic virulence factors of CR-Kp isolates. **Materials and Methods:** The specimens were collected from 1029 patients from major hospitals in Babylon City. *K. pneumoniae* was diagnosed based on biochemical characteristics and the Vitek 2 compact system. Antibiotic susceptibility patterns and biofilm formation by CR-Kp were also investigated. **Results:** This study found that 66 isolates belonged to *K. pneumoniae*, 14 of which belonged to (CR-Kp). It was found that 5/14 (35.72%) of urine samples were CR-Kp, 4/14 (28.57%) from burns were CR-Kp, and 1/14 (7.14%) were from diabetic foot. Results also showed that all CR-Kp isolates were highly resistant to most of the antibiotics tested, while they showed a lesser degree of resistance to nitrofurantoin, tigecycline, colistin, gentamicin, and trimethoprim-sulfamethoxazole. The results showed that 54 (81.82%) of all 66 *K. pneumoniae* isolates were multidrug-resistant, nine (13.63%) were extensively drug resistant, and three (4.55%) were pan-drug resistant. The results of biofilm formation showed that 11 (78.57%) isolates had a strong biofilm formation capacity, moderate biofilm was shown in two (14.29%) isolates, and one (7.14%) isolate showed no biofilm formation. The results also showed that 11 (78.57%) of the isolates were hypermucoviscous phenotypes. **Conclusion:** A high percentage of CR-Kp isolates were from wounds. They were resistant to most antibiotics. Most of the CR-Kp isolates have moderate to high degrees of viscosity, and a high percentage of CR-Kp isolates have a strong capacity for biofilm formation. Colistin and tigecycline antibiotics are the most effective against CR-Kp isolates.

Keywords: Antibiotic susceptibility, carbapenem resistance, hypervirulent, *Klebsiella pneumoniae*

Introduction

Klebsiella pneumoniae is an important pathogen responsible for various infections, the most frequent of which are pneumonia, sepsis, bloodstream infections, meningitis, pyogenic liver abscesses, infections of urinary tract infections (UTIs), and wounds.[1] Infections caused by carbapenem-resistant *K. pneumoniae* (CR-Kp) isolates are a major threat to public health. Such infections can increase the mortality rates of critically ill and debilitated patients hospitalized in intensive care units and can have a negative impact on the financial costs of their hospitalization all over the world.[2]

In addition to the clinical environment, *K. pneumoniae* is frequently found in foods, including raw vegetables, powdered infant formula, meat, fish, and street foods, and it has been considered an important foodborne pathogen.[3] In powdered infant formula, *K. pneumoniae* is included in the hazard identification category "B" according to the FAO and WHO guidelines on microorganisms.[4] In the commonly recorded epidemics of gastrointestinal sickness (mostly in association with the Enterobacteriaceae family), fresh fruits were shown to be the source of bacterial contamination, and *K.*

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1596_23

Address for correspondence: Dr. Alaa H. Al-Charrakh, Department of Microbiology, College of Medicine, University of Babylon, Hilla, Iraq.
E-mail: ahani67@gmail.com

Submission: 23-Oct-2023 **Accepted:** 10-Aug-2025 **Published:** 30-Sep-2025

How to cite this article: Al-Shammari AA, Al-Charrakh AH. Antibiotic susceptibility profile of carbapenem-resistant *Klebsiella pneumoniae* isolated from clinical specimens in Iraq. Med J Babylon 2025;22:803-9.

pneumoniae was identified as a prominent foodborne pathogen.[5]

K. pneumoniae has many virulence factors, including capsules, exopolysaccharides associated with mucoviscosity, lipopolysaccharides, adhesins, and iron uptake systems, that have been shown to play important roles in its pathogenesis. The capsule is an important virulence factor that is involved in at least two pathogenic mechanisms: protection of the bacteria from phagocytosis and direct inhibition of the host immune response.[6]

The mechanism of antimicrobial resistance in gram-negative bacteria arises from the expression of antibiotic-inactivating enzymes and non-enzymatic pathways, which may result from increased intrinsic resistance due to mutations in chromosomal genes or be acquired through the transfer of mobile genetic elements carrying resistance genes or non-enzymatic mechanisms like Qnr for fluoroquinolone resistance in Enterobacteriaceae.[7,8]

Multidrug-resistant (MDR) *K. pneumoniae* strains have been isolated from different samples.[9] Dietary intake is one of the primary routes for the introduction of antibiotic-resistant bacteria and their genes into the human digestive tract. Consumption of specific food categories might influence gut antibiotic resistance gene diversity.[10]

Hypervirulence and carbapenem resistance have emerged as two distinct evolutionary directions leading high-risk *K. pneumoniae* lineages to epidemic success. CR-Kp has become a looming threat in clinical settings owing to extended antibiotic-resistant phenotypes and global dissemination via mobile genetic elements, causing much higher mortality than carbapenem-susceptible *K. pneumoniae*.[11]

Materials and Methods

Clinical specimens

The specimens were collected from 1029 patients (aged from 19 to 70 years) who attended four main hospitals in Babylon City: Al-Hilla General Teaching Hospital, Marjan Medical City, Al-Kifl General Hospital, and Al-Imam Al-Sadiq General Teaching Hospital during a period of six months, lasting from December 2022 to May 2023. The specimens were obtained from different sites of infection (urine, wounds, burns, diabetic foot, ear, sputum, and high vaginal swab). Each swab was taken carefully from the sites of infection and transferred to the Laboratory of Microbiology/College of Medicine. Urine (mid-stream urine) was collected from patients suffering from UTIs in sterile screw-cap containers. Swabs from burns, wounds, and ears were collected from patients before they took any antibiotics or cleaning, and swabs from diabetic foot patients, whose diagnosis depended on the physician, were collected before cleaning.

Bacterial identification and antibiotic susceptibility testing

K. pneumoniae isolates were initially identified by their morphological characteristics on blood agar, MacConkey agar, and biochemical tests based on Forbes *et al*.[12,13] Identification of *K. pneumoniae* was then confirmed using the Vitek 2 system (BioMerieux, France).

Antibiotic susceptibility testing (AST) was performed using the Vitek 2 system (bioMerieux), AST GN76 kit, and Kirby–Bauer disc diffusion methods (Becton Dickinson, Germany), and interpretation was done according to Clinical and Laboratory Standards Institute guidelines.[14]

Susceptibility testing was performed on Mueller–Hinton agar (bioMerieux, France), using overnight cultures at a 0.5 McFarland standard followed by incubation at 35°C for 16–18 h. Antibiotic resistance patterns (ARPs) (MDR, extensively drug resistant [XDR], and pan-drug resistant [PDR]) of all isolates were detected using the definitions of Magiorakos *et al*.[15]

Detection of biofilm formation and string test of carbapenem-resistant *Klebsiella pneumoniae* isolates

The biofilm formation by CR-Kp was conducted using the tissue culture plate method according to the procedure of Sultan and Nabiel.[16] The string test was performed for the detection of the hypervirulent *K. pneumoniae* (hvKp) phenotype,[17] and the score of the degree of viscosity of the isolates was interpreted as follows:

1–3 mm = + (low).

4–6 mm = ++ (moderate).

7–9 mm = +++ (high).

From 10 mm to more than 10 mm = ++++ (very high).

Ethical approval

All subjects involved in this work were informed, and consent was obtained verbally from each one before the collection of the samples. This study was approved by a local committee on publication ethics at the College of Medicine, University of Babylon, according to document No. 223 on November 23, 2022.

Phenotypic detection of carbapenemase in carbapenem-resistant *Klebsiella pneumoniae* isolates

Several methods were performed to detect carbapenemase in CR-Kp isolates, such as the modified Hodge test, cultivation of CR-Kp on CHROMagar, and susceptibility to cefoxitin disc using DDT. Negative control *K. pneumoniae* ATCC BAA-1706 was used as a carbapenem-susceptible strain obtained from the Central Public Health Laboratory in Baghdad.

Results

Isolation of *Klebsiella pneumoniae* from clinical samples

In this study, out of 251 clinical isolates, only 66 (26%) belonged to *K. pneumonie*, of which 45/66 (68.1%) were obtained from female patients and 21/66 (31.8%) recovered from male patients. Among patients with *K. pneumoniae*-positive cultures, 27/66 (40.1%) were community-acquired (outpatients), while 39/66 (59.9%) were hospitalized (inpatients).

It was found that most of the *K. pneumoniae* isolates, 28 (42.4%), were recovered from urine samples, whereas the lowest isolation rate was from samples of sputum and ear swabs. However, no isolates were recovered from samples of blood and vaginal swabs. The absence of *K. pneumoniae* isolates from samples of blood and vaginal swabs may be due to the low numbers of samples collected during the study, as shown in Table 1.

Antibiotic susceptibility test

The results also found that out of 66 *K. pneumoniae* isolates, 14 (21.2%) were phenotypically resistant to carbapenem antibiotics (CR-Kp) using the disc diffusion method and Vitek 2-compact system. However, all CR-Kp were resistant to all members of the carbapenems (IMP, MEM, and ETP) [Figures 1 and 2]. The distribution of CR-Kp among specimens is illustrated in Table 2.

Distribution of carbapenem-resistant *Klebsiella pneumoniae* isolates according to the site of infection

The results of DDT found that 5/14 (35.7%) of CR-Kp isolates were recovered from wound specimens, 4/14 (28.5%) from urine, 4/14 (28.5%) from burn specimens, and 1/14 (7.1%) from diabetic foot specimens, as shown in Table 2.

Figure 3 shows the multiple ARPs of all (No. 66) *K. pneumoniae* isolates. As shown in the results of AST and ARPs [Figure 3], two (14.2%) of the CR-Kp isolates (No. 14) were MDR, nine (64.2%) were XDR, and three (21.4) were PDR.

Table 1: Distribution of *Klebsiella pneumoniae* isolates from different clinical specimens

Type of specimen	Total no.	Positive growth bacteria	No. of *Klebsiella pneumoniae* isolates	%
Urine	296	70	28	42.42
Burns	128	42	13	19.69
Wound	197	61	12	18.19
Diabetic foot	189	47	8	12.12
Sputum	86	14	3	4.55
Ear	83	11	2	3.03
High vaginal swab	50	6	0	0
Total	1029	251	66	100

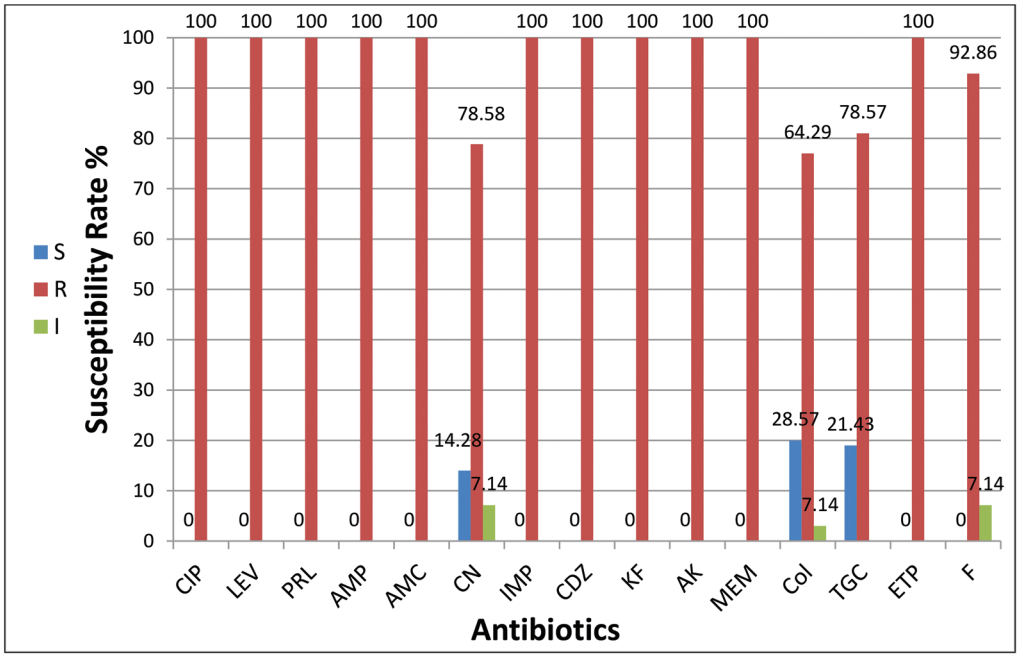

Figure 1: Antibiotic susceptibility profile of carbapenem-resistant *Klebsiella pneumoniae* isolates by DDT

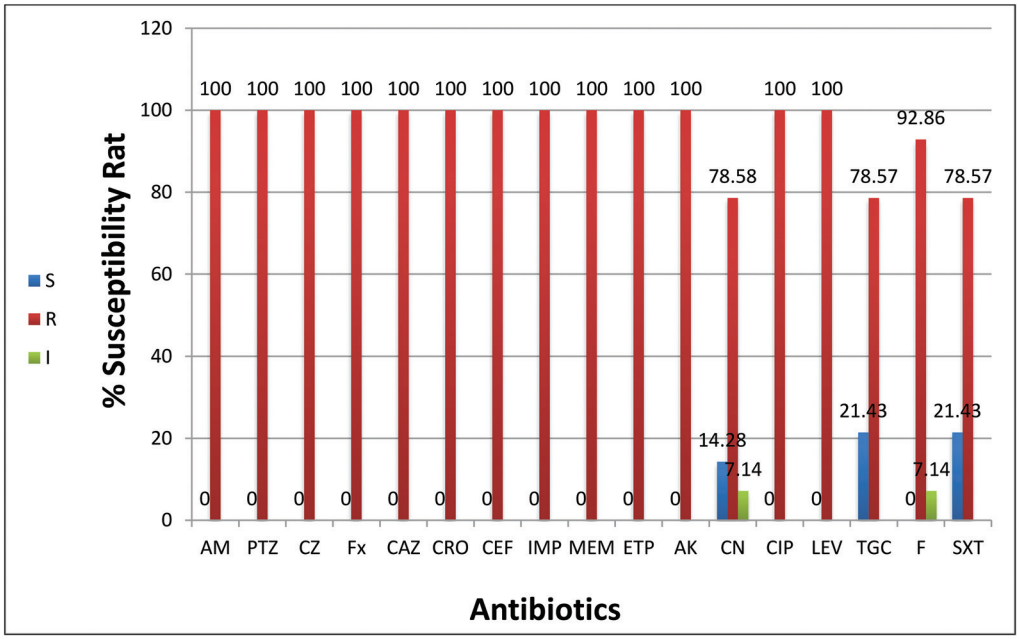

Figure 2: Antibiotic susceptibility profile of carbapenem-resistant *Klebsiella pneumoniae* isolates by the Vitek 2 system

Table 2: Distribution of carbapenem-resistant *Klebsiella pneumoniae* according to the site of infection

Site of infection	No. of carbapenem-resistant *Klebsiella pneumoniae* isolates	%
Wound	5	35.72
Urine	4	28.57
Burns	4	28.57
Diabetic foot	1	7.14
Total	14	100

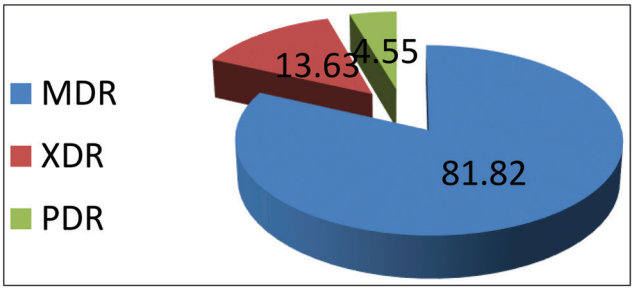

Figure 3: Multiple antibiotic-resistant phenotypes of *Klebsiella pneumoniae* isolates (No. 66)

Table 3: String test string of (n = 14) carbapenem-resistant *Klebsiella pneumoniae* isolates

No. of isolate	String test by mm	Degree of viscosity	No. of isolates	String test by mm	Degree of viscosity
1	5 mm	++	8	8 mm	+++
2	3 mm	+	9	4 mm	++
3	6 mm	++	10	2 mm	+
4	2 mm	+	11	0	–
5	0	–	12	10 mm	++++
6	7 mm	+++	13	7 mm	+++
7	0	–	14	9 mm	+++

String test

The results of the string test showed that 11/14 (78.57%) of CR-Kp isolates were positive for the hypermucoviscous phenotype test (string test), as shown in Table 3.

Detection of biofilm formation using tissue culture plate method

A total of 14 isolates of CR-Kp were tested for their ability to produce biofilm. From these isolates, 11/14 (78.57%) isolates had a strong biofilm formation capacity, moderate biofilm was shown in 2/14 (14.29%) isolates, and 1/14 (7.14%) isolates were weak or had no biofilm formation,

Table 4: Result of tissue culture plate method

Isolates (no.)	Biofilm formation		
	Strong	Moderate	None/weak
Carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) (14)	11/14 (78.57%)	2/14 (14.29%)	1/14 (7.14%)

Table 5: Results of biofilm formation of carbapenem-resistant *Klebsiella pneumoniae* isolates by ELISA

Isolate no.	Optical denisty of biofilm
CR-Kp4	0.728
CR-Kp9	0.319
CR-Kp12	0.267
CR-Kp18	0.801
CR-Kp23	0.235
CR-Kp29	0.644
CR-Kp36	0.118
CR-Kp39	0.557
CR-Kp42	0.749
CR-Kp45	0.812
CR-Kp50	0.471
CR-Kp54	0.196
CR-Kp60	0.753
CR-Kp63	0.399

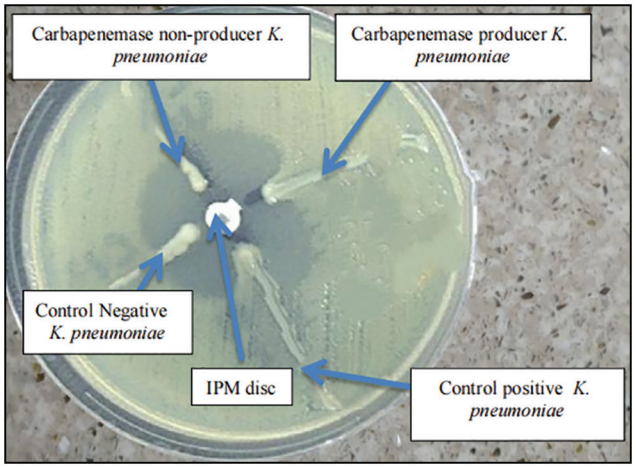

Figure 4: Modified Hodge test

as shown in Table 4. The results of biofilm formation, which were read at 570 nm by an ELISA reader, are listed in Table 5.

Phenotypic screening of carbapenemase in carbapenem-resistant *Klebsiella pneumoniae* by modified Hodge test

The results showed that 13 (92.8%) of CR-Kp isolates (N = 14) were carbapenemase producers [Figure 4].

Phenotypic detection of extended-spectrum betal-lactamase and AmpC β-lactamase by CHROMagar

Extended-spectrum betal-lactamase (ESBL) production was phenotypically confirmed in all CR-Kp isolates based on growth on CHROMagar-ESBL plates; all these isolates showed after overnight growth with blue colonies, as shown in Figure 5A and B.

CR-Kp isolates exhibited resistance to cefoxitin by the disc diffusion test (≤18 mm), considered as putative AmpC β-lactamase producers. In this study, all isolates

Table 6: Phenotypic-putative positive extended-spectrum betal-lactamase (ESBL) and AmpC β-lactamase producers among carbapenem-resistant *Klebsiella pneumoniae* isolates (no = 14)

No. (%) of ESBL producers by:				No. (%) of AmpC producers
Ceftriaxone ≤19 mm	Cefotaxime ≤22 mm	Ceftazidime ≤19 mm	CHROMagar technique	Cefoxitin ≤14 mm
14 (100)	14 (100)	14 (100)	14 (100)	14 (100)

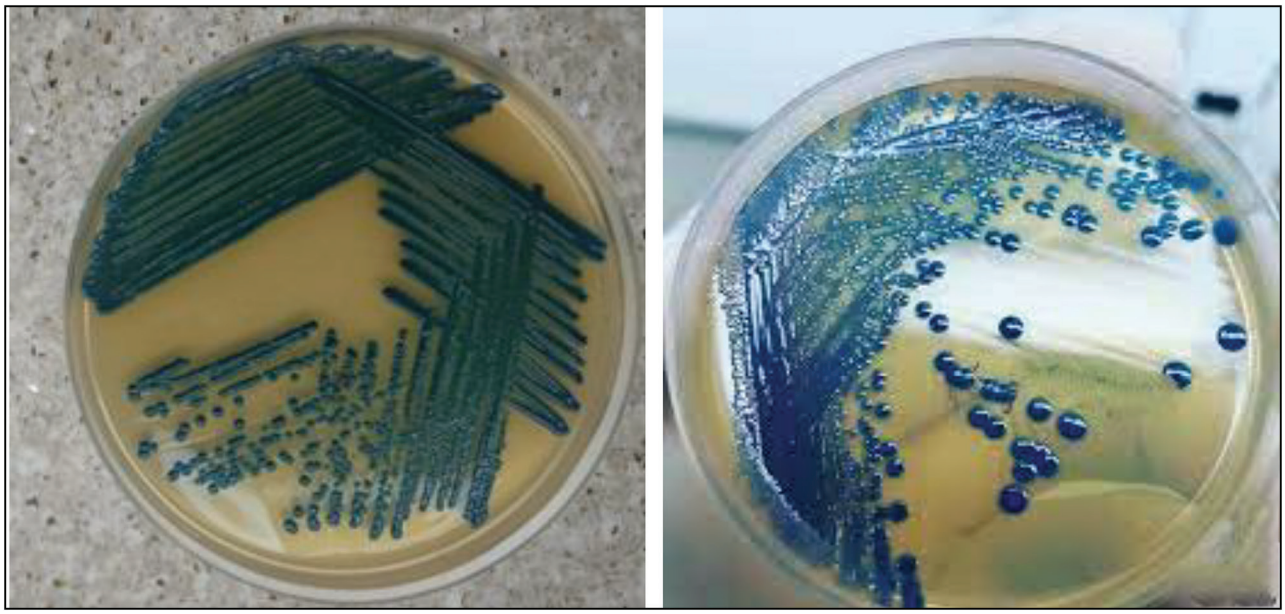

Figure 5: CHROMagar showing extended-spectrum betal-lactamase production in carbapenem-resistant *Klebsiella pneumoniae* isolates (a and b)

demonstrated resistance to cefoxitin and were considered potential AmpC β-lactamase producers [Table 6].

Discussion

The results of this study revealed that *K. pneumoniae* isolates were highly prevalent in UTIs (42.4%) compared with other types of specimens. This result was compatible with the studies conducted by Naqid *et al*.[18] and Abdal *et al*.[19] at isolation rates of 66.2% and 71.5%, respectively. However, other studies conducted by several authors reported a low isolation rate of *K. pneumoniae* from the urine specimens, at an isolation rate of 26.1%.[20]

The result of the diabetic foot infection was 12.1%. This result was compatible with several authors who revealed that the diabetic foot infection was caused by *K. pneumoniae* and other anaerobic bacteria.[21,22]

The results showed that 78.5% of the isolates were positive for the hypermucoviscous phenotype test (string test). This result was compatible with the studies conducted by Saki *et al*.[23] and Hussein *et al*.[24] who revealed that 35.3% and 53% of *K. pneumoniae* isolates were positive for the string test, respectively.

The results also found that out of 66 *K. pneumoniae* isolates, 14 (21.2%) were phenotypically resistant to carbapenem antibiotics (CR-Kp). The result was compatible with the studies conducted by Mohammed *et al*.[25] and Al-Hasnawi[26] who found that the percentages of CR-Kp from all *K. pneumoniae* isolates were 23.5% and 15%, respectively.

The highest percentage of CR-Kp was isolated from wound and urine specimens. CR-Kp isolates were resistant to most classes of antibiotics, but they were slightly susceptible to colistin, tigecycline, gentamicin, and trimethoprim-sulfamethoxazole. It was found that most CR-Kp isolates possess a hypermucoviscosity phenotype. Colistin and tigecycline antibiotics are the most effective against CR-Kp isolates.

The results showed that the resistance rates of CR-Kp against imipenem, meropenem, and ertapenem were 100%. Other studies conducted by several authors[27-29] also found that the resistance rate against these antibiotics was 100%.

This study found that two (14.2%) of CR-Kp isolates (No. 14) were MDR, nine (64.2%) were XDR, and three (21.4) were PDR. Another study conducted by[24] found that 5% of CR-Kp isolates were MDR, 76.2% were XDR, and 18.7% were PDR.

Taha *et al*.[30] found that 5% of CR-Kp isolates were MDR and 76.2% were XDR, and 18.7% were PDR. Another study by Haji *et al*.[31] found that 28%, 38%, and 33% of CR-Kp isolates were MDR, XDR, and PDR, respectively. The study conducted by Ahmed and Mawlood[32] documented that MDR, XDR, and PDR were 38%, 37% and 25%, respectively. Another study conducted by Abdelula *et al*.[33] found that all isolates of *K. pneumoniae* (100%) were XDR. However, these findings are not compatible with the study conducted by Shukla *et al*.[34] who revealed that MDR isolates were 4/22 (18.1%), XDR isolates were 15/22 (68.1%), and PDR isolates were 3/22 (13.6%). Another study by Al-Hasnawi[26] showed that 85% of isolates were MDR, 3% were XDR, and 2% were PDR.

Regarding the biofilm formation by isolates of CR-Kp, the results revealed that out of 14 isolates, 11 (78.57%) had a strong biofilm formation capacity. A study conducted by Mohammed *et al*.[35] found that 80% of *K. pneumoniae* isolates were strongly biofilm producers, 5% of isolates were moderately biofilm producers, 5% of isolates were weakly biofilm producers, and 10% of isolates were unproductive for biofilm. The existence of non-biofilm producing isolates may be due to heterogeneity in the origins of bacteria, such as genetic characterization, sources of isolates, environmental conditions, the absence of quorum sensing (which represents the first steps in biofilm formation) or the absence of the gene responsible for biofilm formation.

Conclusion

A high percentage of CR-Kp isolates were from wounds, and CR-Kp were resistant to most antibiotics. Most of the CR-Kp isolates have moderate to high degrees of viscosity, high percentage of CR-Kp isolates have a strong capacity for biofilm formation. Colistin and tigecycline antibiotics are the most effective against CR-Kp isolates.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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