Short Communication

High Prevalence of Multidrug-Resistant in *Klebsiella pneumoniae* Isolated from Hospitalized Patients in Hilla City

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Abstract

Background: *Klebsiella pneumoniae* is a Gram-negative, rod-shaped bacterium that belongs to the family Enterobacteriaceae. People with impaired immune systems who are hospitalized and suffering from serious underlying diseases are the most common victims of *K. pneumoniae* infections. Patients with this condition are more likely to get urinary tract infections, bloodstream infections, wound infections, and respiratory tract infections during admission in hospital. **Objectives:** Determine the prevalence of *K. pneumoniae* isolates that were resistant to antibiotics in the main hospitals in Hilla City, Iraq. **Materials and Methods:** The 155 clinical samples from various clinical specimens underwent preliminary species identification by conventional and biochemical techniques. Antimicrobial susceptibilities were determined using disk diffusion test (Kirby-Bauer technique) findings. **Results:** Only 32 isolates (21%) of *K. pneumoniae* were identified out of 155 samples. The percentages of the resistant toward these antibiotics were as follows: Augmentin 81%, Aztreonam 88%, Ceftazidime 75%, Cefixime 75%, Cephalothin 84%, Ceftriaxone 72%, Cefotaxime 72%, Doripenem 50%, Ertapenem 56%, Imipenem 47%, Meropenem 56%, Piperacillin/Tazobactam 78%, and Penicillin 100%. All of the isolates (100%) in the existing study were found to be resistant to Penicillin, yet 28% of isolates were resistant to all antibiotics. Further, 9% of isolates were resistant to 12 antibiotics, 6% of isolates were resistant to 11 antibiotics. This study also showed that most of the isolates of *K. pneumoniae* were 100% multidrug-resistant. **Conclusions:** *Klebsiella pneumoniae* isolates had high levels of resistance to all antibiotics; the age group of 21–37 was more vulnerable to infection than other age groups; women are more susceptible to infection than men.

Keywords: Antibiotics resistance, Enterobacteriaceae, *Klebsiella pneumoniae*, sputum and MDR

Introduction

The genus *Klebsiella* is Gram-negative, non-motile, nonsporulating, lactose-fermenting, oxidase negative with a prominent polysaccharide capsule. *Klebsiella* bacteria are everywhere in nature, whereas in humans, they colonize the skin, pharynx, or gastrointestinal tract, sterile wound, and urine and may be regarded as normal flora in many parts of the colon, intestinal, and biliary tract. In the hospital setting, *Klebsiella pneumoniae* is largely an opportunistic infection that affects immunosuppressed patients with major underlying diseases. These patients get nosocomial infections of the urinary tract, blood, wounds, and lungs as a result of hospital care.[1] In addition, *K. pneumoniae* has been implicated in the development of serious community-acquired illnesses, including meningitis, pyogenic liver abscess, and community-acquired pneumonia.[2] It is the root of around one-third of all Gram-negative infections.[3] Modern healthcare faces a global problem with antimicrobial resistance (AMR). AMR is expected to cost the global economy $100 trillion by 2050 if global containment efforts are unsuccessful.[4] The number of deaths yearly related to AMR is expected to rise from the current 700,000 to 10 million. The number of individuals who contract multidrug-resistant (MDR) bacterial infections is rising globally.[5] Antibacterial medications are ineffective against the ESKAPE group of bacteria, which

Access this article online

Quick Response Code:

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

DOI: 10.4103/MJBL.MJBL_92_23

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Submission: 28-Jan-2023 **Accepted:** 26-Feb-2023 **Published:** 23-Jan-2026

How to cite this article: Ewad MM, Al-Khazraji SM, Al-Khafaji JKT. High prevalence of multidrug-resistant in *Klebsiella pneumoniae* isolated from hospitalized patients in Hilla City. Med J Babylon 2025;22:1533-8.

includes *Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococcus* spp.[6] *Klebsiella pneumoniae* is a bacterium that is often found nowadays and causes issues in the healthcare system.[7] Because of the advent of MDR strains linked to hospital outbreaks, *K. pneumoniae* strains are now acknowledged as a serious hazard to human health.[6] AMR in *K. pneumoniae* has been increasing, and the bacteria is now highly resistant to aminoglycosides, quinolones, and B-lactams.[8] In 2019, more than a third of *K. pneumoniae* isolates reported to the European Antimicrobial Resistance Surveillance Network were resistant to at least one of the antimicrobial classes under regular surveillance, including fluoroquinolones, third-generation cephalosporins, aminoglycosides, and carbapenems.[9]

Materials and Methods

Sample collection

A total of 155 clinical specimens (sputum, vaginal, wound, urine, and blood) were collected from various wellsprings of patients from the two genders and diverse age who joined in or conceded to the really four hospitals in Hilla City/Babylon region (General Teaching Hospital of Hilla, Merjan Medical Teaching Hospital/Babylon Hospital for Maternity and Pediatrics and Al-Sadiq Hospital). During a period from September 2021 to December 2021, all samples were collected in sterile containers and kept refrigerated until they were transported to the lab.

Isolation and identification of *Klebsiella pneumoniae*

Patients' 5 mL sputum samples were taken in the morning in sterile wide mouth containers. Sputum was cultivated into culture medium using swabs, and the mixture was incubated aerobically for 24 h at 37°C.[10] Urine samples were taken from the midstream, placed in sterile containers with screw caps, centrifuged for 15 min at 5000 rpm, inoculated onto culture medium, and incubated aerobically at 37°C.[11] Vaginal samples were obtained from patients with genital infection. Typically, a swab (high vaginal swab) is more convenient for aspirating the secretions. To keep the swab wet until it is taken to the laboratory, place it in tubes containing normal saline. The swab was put onto culture media and aerobically cultured for 24 h at 37°C.[11] In burns and wound, swabs were taken from the depth of burn or wound. A 2–5 mL of blood withdrawn aseptically using a disposable syringe. Blood samples were promptly transferred to a sterile blood culture bottle with Brain Heart Infusion Broth. Following that, the blood culture bottle was incubated for 24–72 h at 37°C.[10]

Using a sterile loop and an incubator set to 37°C for 24 h, all samples were cultured on blood agar and MacConkey agar (Himedia, India).[12] Strains maintained in glycerol-rich feeding broth at 4°C.[13] Bacteria cultured on MacConkey, blood, and nutrient agar were analyzed for their colony form, size, and texture. In order to examine a single colony in detail, we Gram-stained it and noticed it via an oil-immersion microscope at 100× magnification.[12] As cited in,[14] the isolates were classified based on their cellular and morphology as well as the results of biochemical tests.

Antibiotics resistance

The disk diffusion test was used for the detection of resistance to antibiotics. In terms of antibiotics, we used Augmentin, Aztreonam, Ceftazidim, Cefixime, Cephalotin, Ceftriaxone, Cefotaxime, Doripenem, Ertapenem, Imipinem, Meropenem, Pipercillin/Tazobactam, and Pencillin. *K pneumoniae* was classified as resistant or sensitive based on the zone of inhibition using CLSI criteria.[15]

Ethical approval

The Declaration of Helsinki was used as the ethical framework for the study. We got the patients' informed verbal permission and signed analytical consent before collecting any samples. After reviewing the study protocol, subject information, and consent form, a local ethics committee gave approval in document 9841 (dated April 11, 2021).

Results

Description of study population and specimen processing

A total of 155 samples taken from patients were tested in the lab, and 32 isolates of *K. pneumoniae* were discovered after taking into account the patients' cultural traits and the results of the biochemical tests.

The age of patients ranged from 4 to 69. There are significant differences between age groups ($P \leq 0.05$). The patients were divided into four groups according to their age, as in Table 1.

Nine (28%) were found to have urinary tract infections, seven (22%) were found in sputum samples from people with pneumonia, three (9%) were found in septicemia, four (12.5%) were found in vaginal infection, four (12.5%) were found in wound infection and five (16%) were found in people with diarrhea (stool). Women seemed to have suffered more

Table 1: Distribution of *Klebsiella pneumoniae* isolates according to age groups

Age group	Number of isolate	Percentage (%)	*P* value
4–20	4	12.5	0.000*
21–37	10	32	
38–53	11	33.5	
54–69	7	22	
Total	32	100	

**P* value is high significant

than men in general; in urinary tract infections (UTI) was (5), septicemia (3), pneumonia (5), only in diarrhea male was more than female (4) as shown in Table 2.

Antibiotic resistance pattern among *Klebsiella pneumoniae*

Thirty two isolates (KP1 to KP32) of *K. pneumoniae* were tested against 13 common antibiotics. According to the results in Figure 1, the results of the tests for antibiotic susceptibility revealed the following percentages of isolates who were resistant to certain antibiotics: Augmentin 81%, Aztreonam 88%, Ceftazidim 75%, Cefixime 75%, Cephalotin 84%, Ceftriaxone 72%, Cefotaxime 72%, Doripenem 50%, Ertapenem 56%, Imipinem 47%, Meropenem 56%, Piper./Tazo.78%, and Pencillin 100%.

In Table 3, we see the proportion of clinical samples that *K. pneumoniae* was able to survive after being separated. All clinical samples (100%) showed that *K. pneumoniae* was very resistant to penicillin, and all urine samples showed that *K. pneumoniae* was highly resistant to Augmentin, Aztreonam, Cephalotin, and Piper./Tazo (88%). Sputum-isolated *K. pneumoniae* was remarkably resistant to Augmentin, Aztreonam, Cephalotin (85%), and Piper./Tazobactam (100%), whereas blood-isolated *K. pneumoniae* was extremely effective against Ceftazidim, Cefixime, Ceftriaxone, Cefotaxime, and Ertapenem (100%). Isolated *K. pneumoniae* from wounds showed excellent resistant to the antibiotics ceftazidim, cefixime, ceftriaxone, cefotaxime, and ertapenem (100%). In spite of the fact that the *K. pneumoniae* found in the vagaina samples was shown to be highly resistant to aztreonam (100%).

Table 2: Distribution *of Klebsiella pneumoniae* isolates according to number, gender, and hospitals

Hospital	Gender F/M	Sample	Disease	Number of isolate	Percentage (%)
Merjan	3/0	Blood	Septicemia	3	9.4
Al-imam	4/0	Sputum	Pneumonia	4	22
Merjan	1/2	Sputum	Pneumonia	3	
Al-imam	1/3	Stool	Diarrhea	4	16
Babylon	0/1	Stool	Diarrhea	1	
Al-hilla	3/2	Urine	Uti	5	28
Al-imam	1/1	Urine	Uti	2	
Babylon	1/1	Urine	Uti	2	
Babylon	4/0	Vagina	Vagina infection	4	12.5
Al-hilla	2/2	Wound	Wound infection	4	12.5

Discussion

Description of study specimens

In addition to causing bacteremia septicemia, skin and soft tissue infections, pneumonia, UTI, and skin and lung infections.[16,17] *Klebsiella pneumoniae* is a major cause of a variety of other clinical disorders. The percent incidence distribution by patient specimens was consistent with the results revealed by Naqid *et al.*,[18] who discovered that the 130 *K. pneumoniae* isolates were obtained from a wide variety of clinical sample sources. Female patients were more likely to have *K. pneumoniae* isolated from clinical samples than male patients in recent research (76.2% vs. 0%) (23.8%). Female patients were shown to have a higher incidence of *K. pneumonia* in urine, blood, wound, and oral samples compared to male patients.[18] In women, the urethra is shorter than men's and is closer to the anus, where stool comes out. It's also close to the vagina, which can collect bacteria during sex. Because of this positioning bacteria from the anus or vagina have easy access to a woman's urinary tract making it easier for an infection to occur, whereas male patients had a higher frequency of isolates in sputum samples. *Klebsiella pneumoniae* was the most common bacteria isolated from the patients'

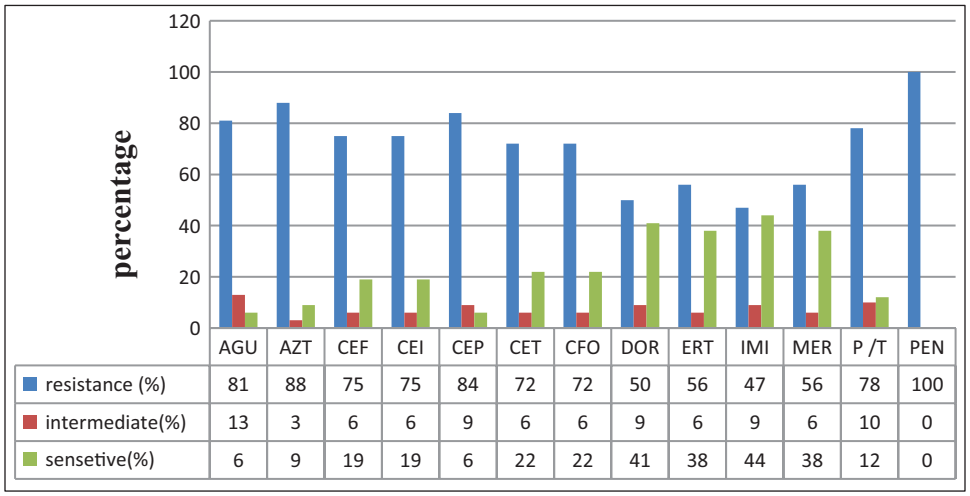

Figure 1: Susceptibility patterns of *Klebsiella pneumoniae* to different antibiotics used in this study

Abbreviations: AGU: Augmentin, AZT: Aztreonam, CEF: Ceftazidim, CEI: Cefixime, CEP: Cephalotin, CET: Ceftazidim, CFO: Cefotaxime, DOR: Doripenem, ERT: Ertapenem, IMI:Imipinem, MER: Meropenem, P/T: Pipercillin./Tazobactam., PEN: Pencillin

Table 3: Antibiotic resistance in *K. pneumoniae* isolated from clinical samples

Antibiotic	Blood (*N* = 3)		Sputum (*N* = 7)		Stool (*N* = 5)		Urine (*N* = 9)		Vagina (*N* = 4)		Wound (*N* = 4)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Augmentin	2	66	6	85	4	80	8	88	3	75	3	75
Aztreonam	2	66	6	85	4	80	8	88	4	100	4	100
Ceftazidim	3	100	6	85	2	40	5	55	3	75	3	75
Cefixime	3	100	6	85	2	40	6	66	3	75	4	100
Cephalotin	2	66	6	85	4	80	8	88	3	75	4	100
Ceftriaxone	3	100	6	85	2	40	6	66	2	50	4	100
Cefotaxime	3	100	6	85	2	40	6	66	3	75	3	75
Doripenem	1	33	4	57	1	20	7	77	2	50	1	25
Ertapenem	3	100	4	57	2	40	7	77	1	25	1	25
Imipinem	1	33	4	57	1	20	6	66	1	25	1	25
Meropenem	3	100	4	57	2	40	7	77	1	25	1	25
Piper./Tazo.	2	66	7	100	3	60	8	88	2	50	3	75
Pencillin	3	100	7	100	5	100	9	100	4	100	4	100

urine (66.2%), blood (12%), and wound swabs (10 %). According to several researches conducted in Iraq,[19,20] for instance, *Klebsiella* species are primarily found in urine, according to Akter *et al*.[21]

Antibiotic resistance pattern among *Klebsiella pneumonia*

AMR is a prominent cause of treatment failure for infectious diseases and a major source of worry for global health.[21,22] Today, it is believed that multidrug-resistant *K. pneumoniae* poses a serious threat to global public health.[23,24] As a result of widespread administration of broad-spectrum antibiotics to hospitalized patients, the prevalence of multidrug-resistant bacteria, such as *K. pneumoniae* carriage, has increased.[25] Sadly, *K. pneumoniae* has developed resistance to even cutting-edge treatments in certain regions, including third-generation cephalosporins.[26] Furthermore, *K. pneumoniae* has been identified as the most common pathogenic bacteria that develops resistance to broad-spectrum beta-lactam medications.[27] This is because *K. pneumoniae* produces extended-spectrum beta-lactamase. Beta-lactamase production is thought to be the most prevalent gram-negative pathogen mechanism,[28] and penicillin resistance is caused by *K. pneumoniae's* ability to transfer the plasmid generating beta-lactamase variations. These findings were in line with those of another study,[29] which discovered that all strains of *Klebsiella* spp. are penicillin-resistant. According to Gales *et al.*,[30] who found that Klebsiella's resistance to -lactams ranged from (63.3% to 70%), the high resistance is related to various mechanisms that mediate antibiotic resistance in *Klebsiella*. This may be because *Klebsiella* spp. were exposed to antibiotics as a result of sporadic antibiotic use during infectious diseases that caused by *Klebsiella*. In order to prevent antibiotics from entering the bacterial cell, Ghorashi *et al*.[31] show that *Klebsiella* may resist cephalosporin, penicillin, and other-lactams by varying the permeability of the plasma membrane. Seventy-four percent of *K. pneumoniae* isolates are resistant to cefotaxime, as was mentioned in the previous sentence. Infections caused by *K. pneumoniae* may be treated with imipenem and amikacin since these antibiotics are effective against the vast majority of *K. pneumoniae* isolates.[32] Recent research found that, among the antibiotics tested, *K. pneumoniae* demonstrated the greatest levels of resistance to Ampicillin (100%), Cefotaxime (100%), Piperacillin (100%), and Trimethoprim (80%). Both Gentamicin (54%) and Azithromycin (65%) have higher rates of resistance than the other antibiotics. Not only that, but Imipenem (4%), Chloramphenicol (12%), and Ofloxacin (35%) had the lowest rates of resistance, in that order.[33] Studies, Grillon *et al.*[34] conducted lately looked into whether or not *K. pneumoniae* isolated from blood was sensitive to levofloxacin (100%) and ciprofloxacin (90%) (100%). Cefoxitin, ceftriaxone, cefepime, and gentamicin were all ineffective against the *K. pneumoniae* isolates, and over 80% of them were resistant to all four. They also had the highest rates of ampicillin (93.8% resistance) and cefazolin (87.5% resistance).This outcome is analogous to research from less developed countries that discovered ampicillin resistance in all *Klebsiella* isolates.[27,35] This might be due to the affordability and ease of use of ampicillin, a widely used antibiotic. Doripenem, Ertapenem, Imipinem, Meropenem, and Piper./Tazo were shown to be 75% sensitive to the *K. pneumoniae* isolated from the wound swabs in the current study, whereas Ceftazidim and penicillin recorded 100% resistance. The *K. pneumoniae* bacterium was found to be primarily susceptible to amikacin and imipenem in an Iraqi investigation, but mostly resistant to ceftriaxone and tetracycline.[36] 100% of the isolated *K. pneumoniae* from the sputum samples, according to the findings of,[18] were ampicillin-resistant, whereas only 77.8% of them were gentamicin-sensitive. *K. pneumoniae* was shown to

be very susceptible to ertapenem and imipenem but highly resistant to ampicillin, ceftriaxone, and cefepime in the urine samples (96.5%). *Klebsiella pneumoniae* in urine samples showed a high degree of sensitivity to ertapenem (97.7%) and imipenem (97.7%), but a high degree of resistance to ampicillin (97.7%), ceftriaxone (60.5%), and cefepime (62.8%). There was an almost perfect agreement between the two groups (96.5%). To identify the ideal concentration, more researches are necessary.[37,38] This high prevalence may be due to the fact that the vast majority of *K. pneumoniae* isolates (90.91%) were collected from hospitalized patients, where many antimicrobials are administered simultaneously and therefore function as a selection pressure for drug resistance. According to the recommendations of Magiorakos *et al*.,[39] additional antimicrobial medicines were included in the assessment in this study, which improved the detection of MDR.

Conclusion

Klebsiella pneumoniae isolates had high levels of resistance to all antibiotics; the age group[19-33,35,39] was more vulnerable to infection; women are more sensitive to infection than men. Prediction of *K. pneumoniae* antibiotics resistance owing to misuse of antibiotics. The best way to detect this resistance accomplished by using samples of urine, because the most resistant isolates were found in urine samples.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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