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High Frequency of Multiantibiotic Resistance in *Klebsiella Pneumoniae* Isolated from Different Hospitals in Baghdad, Iraq

Farah Hadi Odaa[1*] and Harith Jabbar Fahad Al-Mathkhury[2]
[1,2] Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

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Abstract

The widespread use of antibiotics has contributed to a progressive rise of *Klebsiella pneumoniae* resistance to antibiotics, posing a challenge in the implementation of infection control. The current study aims to give an update on the antibiotic resistance status of local *K.pneumoniae* isolates. A total of one hundred and sixty specimens from different sources were collected. The VITEK 2 compact system was used to confirm the identification and test the antibiotic susceptibility. Multiple antibiotic resistance indices were estimated for each isolate. Out of the total samples, eighty (50%) *K. pneumoniae* isolates were identified by morphological and cultural characteristics and CHROM agar. Noticeably, the majority (28.75%) of the isolates in our investigation were obtained from cases of sputum. Moreover, piperacillin was the most resistant antibiotic (91.25%), whereas colistin (10%) was the least resistant antibiotic. 66 (82.5%) isolates were indicated as multidrug-resistant *K. pneumoniae,* and 13.6% of isolates were flagged as ESBL producers. What's more, 71 (53.75%) isolates had a MAR value > 0.2. *K. pneumoniae* developed an increased resistance to antibiotics, mostly prescribed by local physicians.

Keywords: *Klebsiella pneumoniae*, antibiotic resistance, MDR, VITEK, MRI.

1. Introduction

Klebsiella pneumoniae, as a member of the Enterobacteriaceae, is a Gram-negative, encapsulated, rod-shaped, non-motile, lactose-fermenting, gas-producing, facultative anaerobic bacterium (1, 2). The World Health Organization (WHO) lists *K. pneumoniae* as one of the top three bacteria of world concern. It is the second most common cause of community-acquired urinary tract infections (3).

Antibiotic resistance is a global issue causing significant public health problems and increased infections induced by antibiotic-resistant pathogens (4, 5). *K. pneumoniae* strains resistant to multiple antibiotics have emerged as a significant concern to the public's health during the last few decades; of note, antibiotic misuse is the primary cause of antibiotic resistance (6). *K.*

pneumoniae is intrinsically resistant to various antibiotics; it also possesses multiple mechanisms of antimicrobial resistance through selecting mutations in chromosomal genes and acquiring resistant determinants (7, 8). Moreover, *K. pneumoniae* is common in healthcare facilities, and its spread was aided by its ability to colonize the digestive tract and its high effectiveness of resistant variant selection (9). Throughout epidemics, the colonization of the hand and nasopharynx has led to the identification of a large number of carriers among patients and staff in clinical settings (10, 11).

Extended-spectrum beta-lactamases (ESBLs) are a class of enzymes produced by certain bacteria that can break down broad-spectrum antibiotics that belong to the penicillin and cephalosporin families and monobactam (12). Plasmids often harbor ESBLs, facilitating their transmission from one bacterial strain to another and even across different types of bacteria (13, 14). Unfortunately, *K.pneumoniae* has become progressively resistant to β-lactams, aminoglycosides, ESBL, and fluoroquinolones (15, 16). The current investigation seeks to provide an update on the antibiotic resistance status of local *K. pneumoniae* isolates.

2. Materials and Methods

2.1. Sample collection

A total of one hundred and sixty specimens from different clinical sources, including blood, mid-stream urine, sputum, wound fluids, pus, abscess, skin, tissue, swabs, nasal wash, bronchial wash, central venous line, expressed prostatic secretion, endotracheal tube, and Foley tips were collected in sterilized containers from hospitals including Baghdad Teaching Hospital, Ghazi Al-Hariri Hospital for Surgical Specialties, Nursing Home Hospital, and Teaching Laboratories at Baghdad Medical City, Baghdad, Iraq.

2.2. Isolation and Identification

All specimens were immediately streaked on MacConkey and blood agar (Accumix/India) and incubated at 37°C for 24 hours. Thereafter, the mucoid colonies with pink colour were subcultured onto CHROM agar. The VITEK 2 system was used for confirming the identification of *Klebsiella* spp. according to the manufacturer's instructions (Biomerieux, France).

2.3. Antimicrobial susceptibility testing

The test was performed with the automated VITEK® 2 compact instrument employing the sensitivity cards AST-N222, which included piperacillin, ceftazidime, cefepime, aztreonam, imipenem, meropenem Ticarcillin- Clavulanate, trimethoprim-sulfamethoxazole, Colistin, Gentamicin, Tobramycin, Amikacin, Minocycline, Ciprofloxacin, Sulfamethoxazole-Trimethoprim. The results were interpreted according to the Clinical and Laboratory Standards Institute (17) guidelines. *E. coli* ATCC 25922 was used as a quality control strain.

2.4. Determination of multiple antibiotic resistance indexes

The multiple antibiotic resistance (MAR) index was calculated using the approach outlined in (18) as follows:

$$\text{MAR index} = a/b \quad (1)$$

Where a is the number of antibiotics resisted by isolates, and b is the total number of antibiotics utilized in this research.

3. Results and Discussion

3.1. Isolation and Identification of *K. pneumoniae*

A total of 80 (50%) isolates were confirmed to be *K. pneumoniae.* Markedly, the present results (**Table 1**) demonstrated that the bulk (28.75%) of the isolates in our investigation were from sputum patients (P≤0.05), followed by mid-stream urine (26.25%), whereas blood specimens achieved the lowest level (1.25%). These results agreed with (19), as they documented that the highest isolation percentage of this bacteria was from sputum (53.77%), followed by urine (14.70%). In contrast to another local study achieved by (20), the sputum specimen represented the lowest isolation percentage (4.59%).

Table 1. The frequency of *K. pneumoniae* isolation from specimens.

Specimen	Number of specimens	Number of isolates	Percentage of isolation (out of 80 isolates)
Mid-stream urine	42	21	26.25
Sputum	40	23	28.75
Blood	4	1	1.25
Wound	8	3	3.75
Fluids	6	2	2.5
Pus	3	1	1.25
Abscess	2	0	0
Skin	2	0	0
Tissue	1	0	0
Swabs	4	2	2.5
Nasal wash	1	1	1.25
Bronchial wash	3	3	3.75
Expressed prostatic secretion	1	1	1.25
Endotracheal tube	22	13	16.25
Foley tips	15	6	7.5
Central venous line	6	3	3.75

Chi square = 21.14, degree of freedom = 12, P= 0.04

3.2. Antibiotic Susceptibility of *Klebsiella pneumoniae*

Antibiogram results demonstrated significant resistance to most antibiotics used in this study (**Figure 1**).

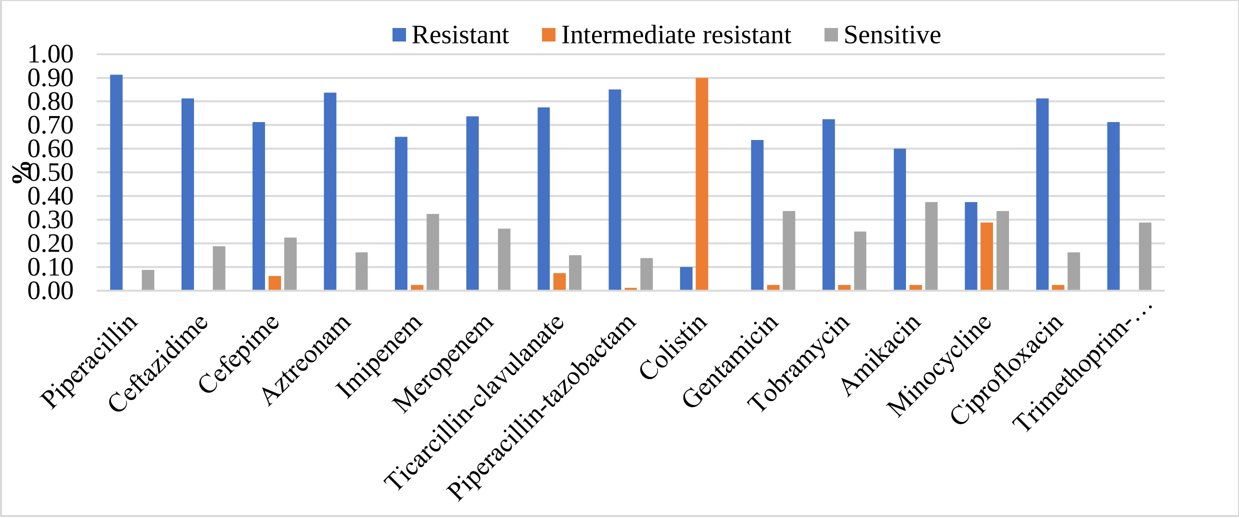

Figure 1. Antibiotic susceptibility of *Klebsiella pneumonaie* isolates (n= 80) of the current study.

Among the 80 isolates of *K. pneumoniae*, 66 (82.5%) were indicated as MDR *K. pneumoniae*. Moreover, of all MDR *K. pneumoniae*, 54 (81.8%) isolates were carbapenemase producers, and 9 (13.6%) isolates were ESBL producers.

According to the results of the antibiotic susceptibility test, as shown in **Figure 1**, the beta-lactam antibiotic piperacillin revealed the highest percentage of antibiotic resistance (91.25%), followed by piperacillin-tazobactam (85%), aztreonam (83.75%), ceftazidime and ciprofloxacin (81.25%), ticarcillin-clavulanate (77.5%), meropenem (73.75%), tobramycin (72.5%), cefepime and trimethoprim-sulfamethoxazole (71.5%), imipenem (65%), gentamicin (63.75%), amikacin (60%), and minocycline (37.5%). Additionally, the lowest resistance percentage belonged to colistin (10%).

These results matched with the results of a local study by (20), which indicated that the highest resistance towards beta-lactam antibiotics was against Piperacillin (95.9%) and aztreonam (82.8%). Also, the current findings are compatible with a previous study conducted in 2021 by (6), in which all *K. pneumoniae* isolates were resistant to piperacillin. Nonetheless, (21) reported that *K. pneumoniae* isolates developed a high resistance to penicillins and cephalosporins; yet, 66, 18, and 16% of *K. pneumoniae* isolates were sensitive, intermediate resistant, and resistant to piperacillin, respectively. Furthermore, the present findings are consistent with a local study carried out in Karbala City by (22), which stated that 27 (90%) of 30 *K. pneumoniae* were MDR.

When carbapenems were employed as the final choice to treat infections resulting from ESBL-producing bacteria, the present study revealed that 65% and 73.75% of isolates were resistant to imipenem and meropenem, respectively. Such results need more attention due to the risk of dissemination of resistance elements among nosocomial as well as community isolates (8). The current work found that *K. pneumoniae* resistance to aminoglycosides, especially tobramycin, gentamicin, and amikacin, was 72.5%, 63.75%, and 60%, respectively. Such results are in parallel with a local study done by (20), as they indicated that the resistance of *K. pneumoniae* to tobramycin, gentamicin, and amikacin was 62.1, 58.6, and 50.6%, respectively. Furthermore, the present findings were in disagreement with the results of a local study performed by (23) that highlighted that amikacin had the highest resistance level (97.5%), followed by gentamicin and tobramycin with 50% and 40%, respectively.

The vast majority of isolates (90%) had intermediate resistance to colistin, whereas 10% of them were resistant. These results agreed with those of (24), who reported that 89% of the isolates were intermediately resistant to colistin (MIC $\leq$ 2 μg/ml), whereas 11% were resistant (MIC $\geq$ 4 μg). Moreover, our results disagreed with a local study conducted by (25) on colistin resistance among *K. pneumoniae* isolated from different sources; they indicated that 42.7% of the bacterial isolates were colistin-resistant.

What's more, in the present study, ciprofloxacin-resistant *K. pneumoniae* was relatively high (81.25%). This result confirms the Iraqi study by (26), who found that ciprofloxacin had the highest resistance rate (52.5%) of all five fluoroquinolones used.

Regarding trimethoprim-sulfamethoxazole, the resistance percentage was 71.25%, which confirms the results scored by (27), as they reported that *K. pneumoniae* resistance to trimethoprim-sulfamethoxazole at a percentage of 71.1%. Nevertheless, the present findings disagreed with the outcomes of a local study done by (28), as they reported the percentage of *K. pneumoniae* resistance reached 28.57%. Same authors found that all their isolates were sensitive to minocycline.

There are various drawbacks to this study. We analyzed specimens from Baghdad, Iraq's capital, which may not be representative of the general situation in Iraq. To track trends in resistance patterns over time, larger research, including all cities and a larger sample size, is extremely desirable. From an epidemiological standpoint, determining antibiotic resistance genes through full genome sequencing might be a helpful complement to this work.

The results grouped in **Table 2** revealed that 71 (53.75%) isolates had a MAR value > 0.2. When the MAR index exceeded 0.2, it more likely means that the high-risk source of contamination is where antibiotics are frequently used (29, 30). Such a high MAR index recorded in this study, perhaps, relates to the high usage, misuse, or abuse of these antibiotics in our community.

Table 2. Frequency of MAR index**.**

MAR index	Number of isolates (%)
≤0.2	9 (11.25)
0.21-0.5	11 (13.75)
0.51-0.8	17 (21.25)
0.81-1	43 (53.75)

4. Conclusions

The studied *Klebsiella pneumoniae* isolates exhibited escalating antibiotic resistance patterns in addition to MDR, which necessitates shedding light on this problematic issue in treatment. Much work is needed to disclose the origin of such high resistance levels, for instance, MLST or ERIC genotyping**.**

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Conflict of Interest:

The authors declare that they have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

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Ethics Committee:

All participants agreed to provide the investigator with the specimens. The Ethics Committee of the College of Science, University of Baghdad, approved this work (Ref. CSEC/0922/0067). Informed consent according to the Declaration of Helsinki was obtained from all participants.

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