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Multidrug Resistance and Genetic Profiling of Clinical *Klebsiella pneumoniae* Isolates: A Threat Iraqi Hospitals

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ABSTRACT

Research objective: *The main objective of this work was to assess the prevalence, antimicrobial resistance, virulence genes profiles, and genetic determinants in aminoglycoside-resistant K. pneumoniae clinical isolates in different medical institutions of Najaf, Iraq.* ***Method****: A total of 162 K. pneumoniae isolates were characterized that included no duplicate. Resistance patterns, and carriage of genes were determined by the use of phenotypic methods, automated VITEK 2 system and the use of PCR based genotyping.* ***Result:*** *The resistance to aminoglycoside among the isolates was 30.3% or 49 isolates. Twenty-six (16.1%) isolates were found positive using a string test to indicate hypermucoviscous phenotype. The resistance genes that were predominant included aac(6')-Ib (100 percent), followed by rmtB (43.8 %) and ant(3'') I (50 %). Among the hypervirulent isolates, capsular serotype K57 and K20 dominated. Concerning, half (50 %) of aminoglycoside resistant isolates were extremely drug resistant (XDR), and 8.3 % were pan-drug resistant (PDR).* ***Conclusions:*** *These results reinstate the necessity of improving infection control and antimicrobial stewardship programs in hospitals of Iraq.*

Keywords*:* ***Klebsiella pneumoniae*****, multidrug resistance, Aminoglycoside resistance, Hypervirulent, Capsular serotype.**

Article Information

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1.INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, opportunistic pathogen and a major cause of healthcare-associated infections worldwide, particularly among immunocompromised individuals and patients undergoing invasive medical procedures. It is frequently implicated in urinary tract infections, pneumonia, bloodstream infections, surgical site infections, and device-related complications [1]. This is because over the last 20 years K. pneumoniae has emerged as a serious clinical problem because of its extraordinary capability to obtain resistance determinants and virulence factors in a horizontal manner via the transfer of plasmids, integrons, and transposons [2]. One of the factors attributed to the global crisis of antimicrobial resistance is the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains of *K. pneumoniae*. Such isolates show an exceptional resistance to almost all classes of commonly used antibiotics such as aminoglycosides, beta-lactams, and carbapenems, commonly referred to as last-line agents [3]. Carbapenem-resistant *K. pneumoniae* has been declared a critical priority pathogen whose infection needs new treatment methods that should be developed

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through research [4].Special attention is recovery of resistance to aminoglycosides, most often co-occurring with other mechanisms, with aminoglycoside-modifying enzymes (AMEs) (AAC*(6): Ib*, *ANT(3): I*, *16 S rRNA* methylases (*RmtB*)) being the main agents [5] . At the same time, clinically, there has also arisen the problem of hypervirulent K. pneumoniae (hvKp) strains. Although initially reported in community-acquired liver abscesses in East Asia, these strains are becoming more frequently detected in both the community and the hospitals throughout the entire world. The key characteristics of hypervirulent strains are the increased capsule synthesis, their tissue invasiveness, and the virulence plasmid harbouring the genes, including *rmpA*, *rmpA2*, capsular serotypes K1, K2, K20 and K57 [6]. Worryingly, recent research has reported isolates with hypervirulence and extensive antibiotic resistance, a double threat that does not only complicate the diagnosis but also treatment [7]. In developing countries such as Iraq the issues are compounded by poor infrastructure to diagnose, mass prescriptions of antibiotics, and lack of infection control practices. Limited surveillance data on molecular epidemiology of K. pneumoniae exist in Iraq although there is rising morbidity and mortality with hard to treat infection. Specifically, research that attempts to examine aminoglycoside resistance gene, capsular serotypes and hypervirulence markers in clinical isolates is absent. This paper attempts to fill the above gap by offering a thorough molecular and phenotype description of clinical *K. pneumoniae* isolates obtained in hospitals within Najaf in Iraq. The study aims at examining aminoglycoside resistance mechanisms, profile of virulence genes, capsule serotypes, and carbapenemase genes. The findings aim to inform empirical treatment decisions, support the development of national antimicrobial stewardship policies, and enhance infection control efforts within Iraqi healthcare institutions.

2.MATERIALS AND METHODS

2.1 Ethical and Scientific Approval: Ethical clearance for this study was obtained from the Ethics Committee of the College of Medicine, University of Kufa, and the Ethics Committee of the Health Directorate of Al-Najaf Province. All necessary health precautions and safety protocols were strictly observed during the sampling process.

2.2 Study Design and Bacterial Isolates: This cross-sectional study was conducted from December 2024 to May 2025 in Najaf Governorate, Iraq. A total of 162 non-duplicate Klebsiella pneumoniae isolates were collected from various clinical specimens (urine, wounds, diabetic foot ulcers, etc.) from five hospitals and diagnostic laboratories. Identification of isolates was initially performed using standard biochemical tests and confirmed with the VITEK 2 compact system (bioMérieux, France).

2.3 Antimicrobial Susceptibility Testing: Antibiotic susceptibility testing was carried out using the Kirby-Bauer disk diffusion method and the VITEK 2 system according to CLSI 2025 guidelines. Minimum inhibitory concentrations (MICs). The isolates were classified as multidrug-resistant (MDR), extensively drug-resistant (XDR), or pan-drug resistant (PDR) according to standardized international definitions.

2.4 Detection of Hypermucoviscous Phenotype: The string test was used to detect the hypermucoviscous phenotype. A single bacterial colony was stretched with a loop, and the formation of a viscous string >5 mm was considered a positive result, indicating hypermucoviscosity.

2.5 Genomic DNA Extraction: Genomic DNA was extracted from overnight bacterial cultures using a boiling method. Briefly, bacterial suspensions were centrifuged,

resuspended in sterile distilled water, heated at 95°C for 10 minutes, and centrifuged again. The supernatant containing DNA was used for PCR.

2.6 Detection of Resistance and Virulence Genes:PCR was used to detect genes encoding aminoglycoside-modifying enzymes (AMEs) *aac(6')-Ib*, *ant(3")-I*, and *aph(3')-I* the *16S rRNA* methyltransferases rmtB, carbapenemases (*bla_NDM*, bla_OXA-51, OXA-23), and virulence factors (*rmpA*, *uge*, *fimH*, *wabG*, *acrAB*). Capsular serotype-specific genes (K1, K2, K5, K20, K54, and K57) were also targeted.

2.7 PCR Protocol: PCR reactions were carried out in a total volume of 25 µL containing 12.5 µL of master mix (Promega), 1 µL each of forward and reverse primers listed in table (2-1) (10 µM), 2 µL of template DNA, and 8.5 µL of nuclease-free water. Thermocycling conditions varied by gene target and followed previously published protocols. The thermo cycling program was to denaturate the DNA at 94-95 C. Anneal at temperatures which were specific to each gene (50-60 C as given in table.1) and extend the resulting primer at 72 C. This would normally be repeated 30 or 35 times to get enough amplification. These amplification products could then be visualized in an 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination using a gel documentation system.

Table 1: The specific primer sequence used in study.

Target gene	**Primer sequence (5′–3′)**	**size (bp)**	**Annealing tem (°C)**	**References**
aac(6')-Ib	F:TTCGGATGCTCTCTCGTGGCCTA R: CTGCAGTGCCTCTGCCTGTTT	482	55°	Park *et al.*, (2006)
ant(3")-I	F:CATCATGAGGGAAGGCGTG R: GACTACCTGGTGATCTTCG	787	55°	Hu *et al.*, 2013
aph(3')-I	F:ATGGGCTCGCGATAATGTC R:CTCACCGAGGCACATTTCAT	600	55°	Zou *et al.*, 2014
rmtB	F:ATG AAC ATC AAC GAT GCC CTC R: CCT TCT GAT TGG CTT ATC CA	769	60°	Hu *et al.*, 2013
acrAB	F:ATCAGCGGCCGGATTGGTAAA R:CGGGTTCGGGAAAATAGCGCG	312	53°	Wasfi *et al.*, 2016
fimH	F:TGCTGCTGGGCTGGTCGATG R:GGGAGGGTGACGGTGACATC	550	55°	Ferreira *et al.*, 2019
uge	F:TCTTCACGCCTTCCTTCACT R:GATCATCCGGTCTCCCTGTA	534	54°	Brisse *et al.*, 2009
webG	F:ACCATCGGCCATTTGATAGA R:CGGACTGGCAGATCCATATC	683	55°	Turton *et al.*, 2010
rmpA	F:ACTGGGCTACCTCTGCTTCA R:CTTGCATGAGCCATCTTTCA	535	50°	Siu *et al.*, 2011
blaNDM	F**:**GGTTTGGCGATCTGGTTTTC R**:**CGGAATGGCTCATCACGATC	813	52°	Gondal *et al.*, 2020
blaOXA-23	F**:** GAT CGG ATT GGA GAA CCA GA R**:** ATT TCT GAC CGC ATT TCC AT	501	52°	Poirel *et al.*, 2004
blaOXA-51	F**:** TAATGCTTTGATCGGCCTTG R**:**TGGATTGCACTTCATCTTGG	353	52°	Poirel *et al.*, 2004

Target gene	Primer sequence (5′–3′)	size (bp)	Annealing tem (°C)	References
K20	F: CGG TGC TAC AGT GCA TCA TT R: GTT ATA CGA TGC TCA GTC GC	881	58°	Fang *et al.*, 2007
K57	F: CTC AGG GCT AGA AGT GTC AT R: CAC TAA CCC AGA AAG TCG AG	1037	50°	Fang *et al.*, 2007

Data Analysis: Frequency distribution and correlation with resistance types.

3. RESULTS

An epidemiologically significant trend is divulged by the fact that Klebsiella pneumoniae isolates were distributed in various hospitals and clinical samples. On the whole, most of the isolates were obtained on urine samples (56.2%) The second-most frequent source was diabetic foot (DF), 17.9 percent, followed by wound swabs, 13.6 percent and burn wounds 6.8 percent, blood, 3.1 percent, and high vaginal swabs (HVS) and sputum, 1.2 percent each. The largest number of isolates (40.1%) was contributed by al-Sadir Hospital and their main source was DF and urine samples. Al-Hakeem Hospital came next with 22.9 percent of isolates which were mostly urine samples, pointing to a high prevalence of UTIs. Isolates at the Burn Center comprised 7.4 percent of the total number of isolates; almost all isolates were burn wounds, which is in line with the specific population served by that center. Isolates collected at Public Health laboratories accounted 10.5%; mostly urine. Other hospitals (Al-Forat, Al-Zahra, Al-Najaf, and others) as well as private laboratories had isolates of 6.2 to 3.1.

Table 2: Epidemiological Characteristics.

Sample origin	No (%) of *K. pneumoniae* isolated from							Total (%)
	Urine	DF†	Wound	Burn	Blood	HVS†	Sputum	
Al-Sadir	21	28	14	-		-	2	65 (40.1)
Al-Forat	6	-	3	-	1	-	-	10 (6.2)
Al-Hakeem	34	-	1	-	1	1	-	37(22.9)
Al-Zahra	3	-	-	-	2	-	-	5 (3.1)
Burn center	-	-	-	11	-	1	-	12 (7.4)
Al-Najaf	7	-	-	-	1	-	-	8 (4.9)
Public Health	16	1		-	-	-	-	17 (10.5)
Private	4		4	-	-	-	-	8 (4.9)
Total (%)	91 (56.2)	29 (17.9)	22 (13.6)	11 (6.8)	5 (3.1)	2 (1.2)	2 (1.2)	162 (100)

The table: 3 includes the difference between hypermucoviscous and classical strain of Klebsiella pneumoniae in terms of the clinical sources in which they are identified. We found a total of 162 K. pneumoniae isolates, 26 (16%) of them were hypermucoviscous and 136 (84%) classical strains. Both phenotypes were most frequent in urine, with percentages of 56.2 of the total number of isolates. Nonetheless, classical strains predominated this type as 81 out of the 91 isolates (59.6%) presented being this subtype in contrast to 10 (38.4%) that had hypermucoviscous strains. In

comparison, there was a different pattern with diabetic foot infection whereby the hypermucoviscous strain was more common (13 isolates, 50%) as opposed to the classical strain (16 isolates, 11.8%). This implies that hypermucoviscous phenotype may be related to the soft tissue infection of diabetics.Wound, burn and blood samples were more likely to be linked to classical strains with 15.4, 7.3 and 2.9 percent of the classical isolates vs 3.9 percent of each evaluated in the hypermucoviscous group. It is noteworthy that, no hypermucoviscous isolates were recovered in sputum or high vaginal swabs (HVS) and classical strain comprised 1.5 % of isolates in each of the sample types.

Table3: Hypermucoviscous Phenotype.

Sample source	No. (%) of patients infected with:		Total (%)
	Hypermucoviscous	Classical	
Urine	10 (38.4)	81 (59.6)	91 (56.2)
Diabetic foot	13 (50)	16 (11.8)	29 (17.9)
Wound	1 (3.9)	21 (15.4)	22 (13.6)
Burn	1 (3.9)	10 (7.3)	11 (6.8)
Blood	1 (3.9)	4 (2.9)	5 (3.1)
Sputum	0 (0)	2 (1.5)	2 (1.2)
HVS†	0 (0)	2 (1.5)	2 (1.2)
Total (%)	26 (100)	136 (100)	162 (100)

Of 48 studied isolates of aminoglycoside-resistant Klebsiella pneumoniae, 41.7 percent (20 isolates) were (MDR). The most common strains were (XDR) strains representing 50 percent (24 isolates) of cases and were resistant to one or two categories of antibiotics. In particular, 8.3% (4 isolates) were detected to be (PDR)as shown in Table 4.

Table 4: Resistance profile.

Resistance Type	No. of Isolates (%)	Description
MDR	20 (41.7%)	Resistant to ≥1 agent in ≥3 categories
XDR	24 (50%)	Resistant to all but 1–2 categories
PDR	4 (8.3%)	Resistant to all tested antimicrobial agents

Of the screened virulence genes in the AMEs gene isolatesin table:5, *acrAB* was the most common with 93.8 percent of isolates exhibiting it. Some of which are the *fimH* gene which is related to adhesion and biofilms in 64.6 percent of isolates and *uge* and *webG* of capsules biosynthesis and lipopolysaccharide structures with prevalence of 52.1 percent and 43.4 respectively. It is noteworthy that no isolated strain contained the *rmpA* hypermucoviscosity and virulence associated gene. In relation to resistance gene, AMEs gene *aac(6)Ib* was of universal occurrence (100 percent) mentioning its central role in aminoglycoside resistance. Other found AME genes included *ant (3")-I* and *aph(3')-I* found in 50 percent and 33. 3 percent of isolates respectively. A high-level aminoglycoside resistance gene 16S polymerase methyl transferase gene- *rmtB* was detected in 43.8%.

A capsular serotype analysis showed that K20 was predominant (29.2%), followed by K57 (16.7%), implying that some serotypes dominated and are perhaps associated with higher pathogenicity or hospital transmission. With regard to carbapenemases genes, 83.3 percent of the isolates were found to possess blaNDM. A further 58.3 and 37.5 percent of samples were carrying *blaOXA-51* and *blaOXA-23*,respectively.

Table 5: Virulence and capsular serotype.

Virulence gene	**Prevalence (%)**	**AMEs and 16S rRNA Methylases gene**	**Prevalence (%)**
acrAB	93.8	aac(6')-Ib	100
fimH	64.6	ant(3")-I	50
uge	52.1	aph(3')-I	33.3
webG	43.4	rmtB	43.8
rmpA	0		
Capsular serotype	**Prevalence (%)**	**Carbapenems gene**	**(%)**
K20	(14)29.2	blaNDM	40 (83.3%)
K57	(8)16.7	blaOXA-23	18 (37.5%)
		blaOXA-51	28 (58.3%)

The graph (**Figure 1**) shows that the resistance isolates of Klebsiella pneumoniae are too high against most of the antibiotics. Various antibiotics such IMP (Imipenem), AK (Amikacin), TOB (Tobramycin) and CP (Ciprofloxacin) were found to be 100 percent resistant, which shows that these antibiotics are sum-totally ineffective in relation to these isolates. The resistance of another subdivision of beta-lactam antibiotics (CTX (Cefotaxime, 95.1%), TCC (Ticarcillin-clavulanate, 87.5%), FOX (Cefoxitin, 81.3%), and CAZ (Ceftazidime, 75%)) was also very high. Intermediate resistance percentages with FEP (Cefepime, 66.7%), CRO (Ceftriaxone, 57.5%), CST (Colistin, 72.9-83.3%, DO (Doxycycline, 79.2-83.3%), and F (Fosfomycin, 75%). In the meantime, ATM (Aztreonam) demonstrated the resistance rate of 72.9%, and NET (Netilmicin) was a bit lower but still extremely high 89.4 %, which significantly restricts treatment options with aminoglycoside and monobactam antibiotics. The only medication with insignificantly high resistance of 10 percent is AMC (Amoxicillin-clavulanic acid).

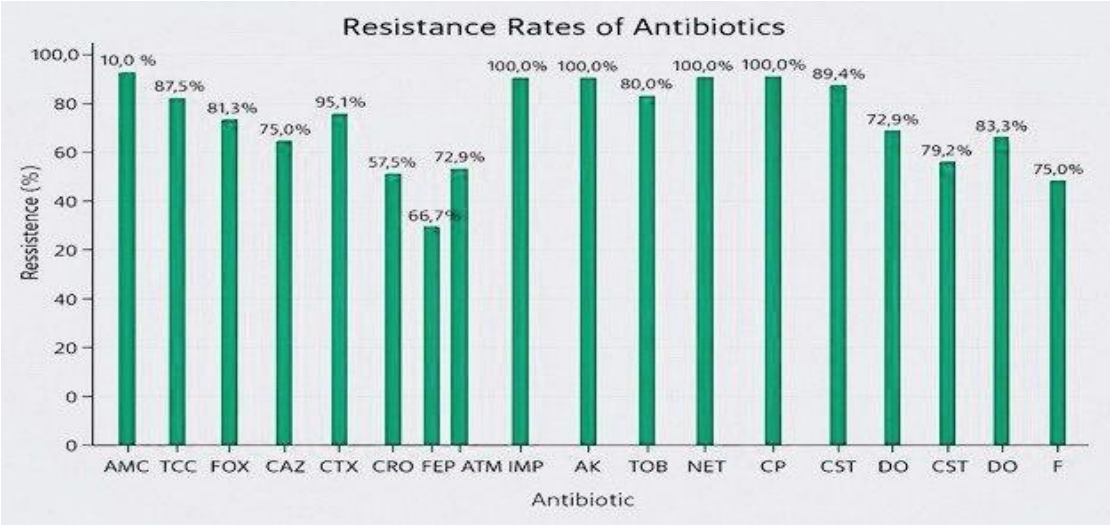

Figure 1: Antibiotic percentage.

4. DISCUSSION

The distribution of *K. pneumoniae* isolates according to sample origin and hospital source indicate that urinary tract infections (UTIs) are the predominant clinical source of *K. pneumoniae* in this setting, consistent with previous studies in Iraq and other regions that identify UTIs as the most common site for *K. pneumoniae* infections, especially in catheterized or immunocompromised patients. However, the relatively high proportion of isolates from diabetic foot wounds (17.9%) underscores the particular vulnerability of diabetic patients to MDR, which may be higher than in some earlier local reports [8]. That showed lower rates of *K. pneumoniae* in diabetic wounds, possibly reflecting an increase in uncontrolled diabetes prevalence or inadequate wound care practices. Compared with studies outside Iraq, such as [9]in Asia, the pattern of urine being the primary source of *K. pneumoniae* is similar, although those studies often reported a higher proportion of respiratory isolates, which are infrequent here (1.2% from sputum). The scientific significance of these findings lies in highlighting the need for enhanced infection control, particularly in urinary and diabetic foot care, to reduce the burden of resistant *K. pneumoniae*. Future research should focus on identifying risk factors for infection in these key patient groups, implementing antimicrobial stewardship programs targeting urinary and wound infections, and conducting molecular epidemiological studies to track the clonal spread of resistant strains within and between these hospitals. Regarding the analysis of *K. pneumoniae* isolates by phenotypic type and clinical source reveals that classical strains were predominant, accounting for 136 out of 162 cases (84%), while hypermucoviscous (HMV) strains represented 26 cases (16%). Urine was the most frequent source for both types, with 81 classical (59.6%) and 10 hypermucoviscous (38.4%) isolates, reflecting the high burden of urinary tract infections (UTIs) in hospitalized and catheterized patients. Interestingly, diabetic foot infections had the highest proportion of HMV strains (13 out of 26 HMV cases; 50%), suggesting a stronger association between hypermucoviscosity and chronic wound infections, likely due to biofilm formation and evasion of immune response. In contrast, wounds and burns were predominantly associated with classical strains (15.4% and 7.3%, respectively), while HMV strains were rare in these sources (3.9% each). Bloodstream infections and respiratory samples (sputum, HVS) accounted for very few cases overall, with no HMV isolates detected from these sites. These findings partly align with prior studies such as [10], which reported the emergence of hypervirulent *K. pneumoniae* (often hypermucoviscous) in community-acquired infections, particularly liver abscesses, though those studies found higher prevalence of respiratory and invasive HMV isolates than observed here. The relatively limited distribution of HMV strains in the study, especially their absence in blood and sputum, may indicate that classical MDR strains still dominate nosocomial settings in Iraq, while hypermucoviscous strains may be more common in community-acquired or outpatient cases not captured in this study.

The scientific significance lies in the distinct clinical profiles of HMV vs. classical *K. pneumoniae*, where HMV strains tend to be more virulent but less resistant, while classical strains are more drug-resistant but less virulent. The high prevalence of classical strains in our setting underscores the ongoing threat of treatment-resistant infections, while the presence of HMV strains in diabetic foot infections raises concern for difficult-to-treat chronic infections with increased morbidity.

The classification of antibiotic resistance among aminoglycoside-resistant *K. pneumoniae* isolates revealed a concerning distribution of MDR phenotypes. Specifically, 20 isolates (41.7%) were identified as MDR, showing resistance to at least one agent in three or more antimicrobial categories. XDR strains represented the majority, accounting for 24 isolates (50%), and were resistant to all but one or two antibiotic categories. More alarmingly, 4 isolates (8.3%) met the criteria **(**PDR), exhibiting resistance to all tested antibiotics, thus leaving no effective therapeutic options. These findings align with global reports of rising resistance in *K. pneumoniae*, such as those by Hogea *et al* [11]. And Gholami *et al.* [12], and mirror the regional emergence of XDR and PDR phenotypes in Iraq and neighboring countries. Compared to earlier local studies that reported lower PDR rates, the current data indicate a worsening resistance scenario. This trend may be attributed to antibiotic misuse, lack of stewardship programs, and poor infection control practices. Scientifically, the dominance of XDR strains highlights the urgent need for alternative therapeutic strategies and strict containment measures, while the presence of PDR strains signals a potential post-antibiotic era where standard treatments are no longer viable. Future studies should aim to explore novel treatment approaches (e.g., bacteriophage therapy or antimicrobial peptides), evaluate the efficacy of last-resort antibiotics like ceftazidime-avibactam, and implement nationwide genomic surveillance to monitor resistance gene dissemination and clonal spread of high-risk strains.

The genetic analysis of *Klebsiella pneumoniae* isolates revealed a high prevalence of multiple virulence and resistance determinants. Among the virulence genes, ***acrAB*** was the most frequently detected (93.8%), highlighting its crucial role in antibiotic efflux and multidrug resistance. The ***fimH*** adhesin gene (64.6%) and ***uge*** gene (52.1%), both associated with colonization and immune evasion, were also common, while ***webG*** (43.4%) involved in lipopolysaccharide biosynthesis, was moderately prevalent. Notably, ***rmpA,*** a key marker of hypermucoviscosity and hypervirulence, was entirely absent, indicating a dominance of classical rather than hypervirulent strains in this population.

Regarding resistance genes, the aminoglycoside-modifying enzyme ***aac(6')-Ib*** was universally present (100%), confirming its central role in conferring high-level resistance to aminoglycosides. The other AME genes, ***ant(3")-I*** (50%) and ***aph(3')-I*** (33.3%), also contributed significantly to the resistance phenotype. The 16S rRNA methylase gene ***rmtB,*** which leads to high-level resistance to all aminoglycosides, was found in 43.8% of isolates, reflecting a growing trend of plasmid-mediated resistance mechanisms.

Capsular serotyping showed a predominance of ***K20*** **(29.2%)** and ***K57*** **(16.7%),** both of which are commonly associated with invasive and persistent infections, though not necessarily with hypervirulence. Among carbapenemase genes, ***blaNDM*** was the most prevalent (83.3%), confirming its widespread dissemination and significant role in carbapenem resistance in Iraqi hospitals. ***blaOXA-51*** (58.3%) and ***blaOXA-23*** (37.5%) were also frequently detected, suggesting co-occurrence of multiple carbapenemases, which further complicates treatment options.

These findings are in partial agreement with previous studies such as [13,14], which reported a high prevalence of AME and carbapenemase genes in *K. pneumoniae*, particularly ***aac(6')-Ib*** and ***blaNDM*****.** However, the complete absence of ***rmpA*** and relatively low prevalence of hypervirulence markers differ from studies conducted in East Asia [15,16], where hypermucoviscous and

hypervirulent strains are more common. This suggests that, in the Iraqi clinical context, resistance is driven primarily by classical strains harboring high-risk resistance genes rather than hypervirulent clon. These results indicate a severe public health threat posed by highly resistant but not necessarily hypervirulent *K. pneumoniae* strains. The high rates of *blaNDM* and AME genes indicate widespread horizontal gene transfer and the presence of mobile genetic elements, which facilitate rapid spread of resistance within hospitals and the community. The dominance of *acrAB* further implies that efflux pump-mediated resistance plays a vital role in the multidrug-resistant phenotype.

The results of this study demonstrate alarmingly high resistance rates among aminoglycoside-resistant *Klebsiella pneumoniae* isolates, with 100% resistance observed to key antibiotics including imipenem, amikacin, tobramycin, and ciprofloxacin, indicating the presence of extensively or even pandrug-resistant strains. High resistance levels were also seen against β-lactam antibiotics such as cefotaxime (95.1%) and ticarcillin-clavulanate (87.5%), while the lowest resistance was noted with amoxicillin-clavulanic acid (10%), though its clinical utility remains limited in severe infections. These findings are consistent with previous regional and international studies, including those by [17]. Ahmed *et al.*, and [18]. Gizatullina *et al*., which reported widespread distribution of ESBL and carbapenemase-producing *K. pneumoniae*. However, compared to earlier local studies in Iraq, such as Mohammed *et al.*,[19]. which documented lower carbapenem resistance rates (60–70%), this study indicates a worrying increase in resistance levels, potentially reflecting increased antibiotic misuse or inadequate infection control. Interestingly, the absence of the *rmpA* hypervirulence gene contrasts with East Asian studies like Yang *et al.*, [20], which found a high prevalence of hypervirulent strains, suggesting that while these isolates are extensively resistant, they predominantly represent classical rather than hypervirulent *K. pneumoniae*. Scientifically, the high prevalence of the *blaNDM* gene (83.3%) underscores the risk of horizontal gene transfer of carbapenemase genes to other pathogens, exacerbating the spread of resistance. Despite the lack of hypervirulence markers, the extreme resistance alone poses a critical public health concern. Therefore, future research should include multicenter epidemiological studies across Iraq to map resistance patterns comprehensively, molecular typing to explore clonal relationships and possible outbreaks, susceptibility testing of newer combination antibiotics like ceftazidime-avibactam, and screening for additional hypervirulence genes to monitor the potential emergence of highly resistant and hypervirulent strains. ,

CONCLUSIONS

this study highlights the alarming spread of multidrug-resistant *K. pneumoniae* in Najaf hospitals, with high rates of aminoglycoside resistance and carbapenemase genes, particularly *aac(6')-Ib* and *blaNDM*. Also, the dominance of the classics over the hypervirulent phenotype, along with the full absence of *rmpA*, suggests that it is classics, rather than hypervirulent clones that will dominate resistance in such an environment of clinical settings. The coexistence of resistance and virulence genes, such as *acrAB* and *fimH*, raises serious concerns about treatment difficulty and infection severity. Epidemiologically, these findings highlight the need to adopt effective infection control strategies, intense antimicrobial stewardship interventions, and the adoption of rapid diagnostic assays to identify the resistance and virulence determinants in early stages. Molecular surveillance should be done on a continuous basis to detect evolution and spread

of high-risk clones whereas development of new means of treatment therapies- including bacteriophage therapy, antimicrobial peptides, and newer combinations of β-lactam/β-lactamases should be encouraged. In the end, unless we act together now at an institutional, national and regional scale, the further development and distribution of these high-risk *K. pneumoniae* strains may culminate in a post-antibiotic world where common infections become resistant to treatment and morbidity and mortality may be much higher, and health costtronomically higher.

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