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Research Article

Detection of Class 1 Integron among *Klebsiella pneumoniae* Clinical Isolates in Baghdad Hospitals

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Abstract

Background: Extensively drug-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) is a significant problem currently due to the increasing prevalence of this pathogen.

Objectives: To underscore the growing threat of antibiotic-resistant *K. pneumoniae* by investigating the prevalence of class 1 integrons among selected multidrug-resistant (MDR) clinical isolates, with the goal of emphasizing the need for strengthened surveillance and targeted antimicrobial strategies.

Methods: Seventy-four *K. pneumoniae* isolates have been identified out of 200 clinical samples from different clinical sources (urine, burns, blood, sputum, wounds). Isolates were obtained from November 2024 to March 2025, in Al-Kadhimiya Teaching Hospital and the Laboratory at College of Science Microbiology Department, University of Baghdad. The isolates were first detected by biochemical testing, chromogenic agar and later confirmed using the VITEK 2 method. Antimicrobial susceptibility was evaluated utilizing the disc diffusion technique (Kirby-Bauer method). The presence of the class 1 integron gene was confirmed via conventional PCR.

Results: A total of 200 clinical samples were collected from the 74 *K. pneumoniae* isolates; 51 (68.9%) exhibited multidrug resistance. Resistance to cefotaxime was observed in 86%, followed by tetracycline (80%), while imipenem exhibited the highest sensitivity at 84%. Class 1 integron gene was identified in 80% of the 10 selected multidrug-resistant isolates.

Conclusion: The high incidence of class 1 integrons in multidrug-resistant *K. pneumoniae* clinical isolates highlights the essential role of integrons in the spread of antibiotic resistance. This indicates the worldwide emergence of *K. pneumoniae* pathogenic strains resistant to colistin and carbapenems, highlighting the imperative for ongoing molecular surveillance and efficient antibiotic stewardship.

Keywords: Clinical isolates; Class 1 integron; *Klebsiella pneumoniae*; MDR; Nosocomial infections; PCR.

Introduction

The pathogenicity of *Klebsiella pneumoniae* (*K. pneumoniae*) is intricately associated with the increase in the level of antibiotic resistance among this bacteria and multiple virulence features, notably the capsule, which impedes neutrophil phagocytosis and serum complement-mediated eradication (1). *K. pneumoniae* is a clinically important species in the Enterobacteriaceae family and a gram-negative bacillus, and it has emerged as one of the leading nosocomial pathogens. It is associated with an array of community-acquired and nosocomial infections, including septicemia, respiratory infections, urinary tract infection (UTI), bacteremia and surgical site infection, all of which contribute significantly to the global burden of morbidity and mortality, particularly among hospitalized patients (2). Due to widespread dissemination and rapid spread of multidrug-resistant Enterobacteriaceae, especially in *K. pneumoniae*, dependence on carbapenems, especially imipenem (IMP) and meropenem (MEM), becomes more important as empirical therapy for severe infections due to MDR isolates. Because of their high potency and broad-spectrum antimicrobial activity, carbapenems are often considered as "antibiotics of last resort" to treat resistant Gram-negative pathogens (3, 4). *K. pneumoniae* is a primary culprit in severe hospital-acquired infections, and its ability to form biofilm makes it worse to treat. Both of these three-dimensional structures cause biofilms to become less sensitive towards antibiotics (reduced antibiotic susceptibility) since antibiotics experience impaired diffusion into the extracellular matrix material and encounter bacteria with altered metabolism. Several antibiotic groups, such as cephalosporins, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole have been used to treat *K. pneumoniae* infections. Nevertheless, carbapenems are

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still the first line of treatment for severe infections, especially those with MDR backgrounds (5). The spread of antibiotic resistance in *K. pneumoniae* is mainly due to mobile gene elements (MGEs), including insertion sequences, transposons and integrons (6), that promote uptake and horizontal transmission of resistance genes. These MGEs mediate the transfer of resistance genes between species, which facilitates the rapid transmission and increase of antimicrobial resistance in bacterial populations (7). Integrons are one of the major groups among MGEs, which consist of *intI* encoding an integrase belonging to the tyrosine recombinase family, an *attI* recombination site, and a promoter (8). There have been reported five distinct groups of integrons including one associated with antibiotic resistance Type I integrase ORF9 (9). The class 1 integrons are the most abundant and are found commonly in clinical isolates of Gram-negative bacteria such as *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Yersinia* due to their genetic association with transposons and their frequent integration into conjugative plasmids (10). The combination of MDR, powerful virulence factors and extensive transmissibility makes *K. pneumoniae* one of the most challenging clinical threats and a difficult-to-treat bacterium (11).

This study aimed to underscore the growing threat posed by antibiotic-resistant *Klebsiella pneumoniae* strains by investigating the prevalence of class 1 integron among selected MDR clinical isolates. The findings are intended to highlight the need for enhanced surveillance and targeted antimicrobial strategies.

Materials and Methods

Sample collection: The ethics committee of Baghdad College of Science (CSEC/0522/0062) approved the study. In the current study, clinical samples were obtained from Al-Kadhimiya Teaching Hospital and Al-Yarmouk Teaching Hospital in Baghdad from November 2024 to March 2025. Seventy-four samples were selected out of the 200samples obtained from various clinical sources: Urine, wound swabs, sputum, blood, and burn swabs. After being transported by a well-closed tank with ice bags, the collected specimens were cultured on blood agar, MacConkey agar, and HiCrome™ UTI agar media, then incubated at 37°C for 24 hours to allow for bacterial growth and preliminary identification based on colony morphology. On MacConkey agar, the colonies displayed significant mucoid pink pigmentation, signifying their biochemical properties as lactose-fermenting organisms and the mucoid morphology attributed to the polysaccharide capsule. Two hundred clinical samples were collected from several clinical sources, including burns, sputum, wounds, and urine. *K. pneumoniae* was characterized using HiCrome UTI Agar, MacConkey Agar, and Blood Agar for sample culture. The bacterial isolates were identified based on Gram staining, colony cultural characteristics, pigment production on CHROM agar, and biochemical assays, including catalase, oxidase, urease, Simmon's citrate, indole, Methyl Red (MR), and Voges-Proskauer tests. with growth assessed at 37°C for 24 hours. Eventually, the confirmation of identification tests encompassed the analysis using the VITEK-2 compact system (12).

The String Test: The string test serves as a diagnostic tool for the phenotypic detection of hypermucoviscous *K. pneumoniae* (HvKP) strains. In this procedure, freshly cultured bacterial isolates are streaked onto MacConkey agar and incubated under aerobic conditions at 37°C for 24 hours. The formation of a viscous string, typically greater than 5 mm in length, when a bacterial colony is stretched with a loop, is indicative of the hypermucoviscous phenotype. A sterile inoculation loop was gently used to touch and pick up the unit colony perpendicular to the surface after incubation. A positive string test was considered when the length of the mucous thread exceeded 5 mm (hypermucoviscous phenotype). The test was conducted in triplicate for repeatability, and the results were confirmed by using molecular markers like *rmpA*. Results Positivity to the string test was frequently found linked to genotypically characterized, hypervirulent strains, although it was not in all cases (13).

Antibiotic susceptibility assay: The antibiotic susceptibility testing was carried out with the following antibiotics: cefotaxime (CTX 30 µg), amoxicillin-clavulanate (AMC 30 µg), amikacin (AK 10 µg), chloramphenicol (CHL 30 µg), ciprofloxacin (CIP 5 µg), tetracycline (TET 30 µg), nitrofurantoin (NIT 100 µg), levofloxacin (LE 5 µg), imipenem (IMP 10 µg), and gentamicin (GEN 30 µg), all obtained from Himedia, India. The CLSI (2024) (14) was used as the standard guideline for interpreting the results. Optimal growth was achieved by inoculating a single well-isolated bacterial colony in 5 mL of Luria-Bertani broth (Himedia, India) incubated with shaking at 37°C for 24 h. On the following day, 15–25 µL of the overnight culture was inoculated into 3 mL of sterile Phosphate-Buffered Saline (Himedia, India) after the incubation. Turbidity of the bacterial suspension was adjusted to an optical density that produced a 0.5 McFarland standard when visually inspected against a commercial reference tube to standardize bacterial concentration. The standardized suspension was spread uniformly onto Mueller-Hinton Agar (Condalab, Spain) plates with a sterile cotton swab using three-dimensional streaking to cover the entire surface of the agar. This procedure was performed in order to obtain a uniform lawn of bacterial growth for the antibiotic susceptibility tests. Antibiotic disks borne on two separate groups were distributed

spatially to prevent overlapping zones of inhibition. The two disks were transferred to the agar surface at a distance of at least 24 mm apart from each other according to the protocol as in the Clinical and Laboratory Standards Institute (CLSI) method in order to make zone measurements as accurate and reliable as possible. The prepared plates were inoculated with the standardized bacterial suspension and incubated at 37°C in an upright position for 24 hours. Following incubation, the diameters of the inhibition zones surrounding each antibiotic disk were carefully measured in millimeters with a ruler (expressed in mm). These measurements were accurately documented for all tested antibiotics. The test isolate was subsequently evaluated by the generated data to establish its antimicrobial susceptibility pattern. Results interpretation was performed according to CLSI breakpoint values, which classify the isolates into susceptible, intermediate, or resistant. This approach facilitated the production of comparable and intelligible susceptibility profiles, providing knowledge of the resistance profile of the isolates and helping to promote a rational therapy (15, 16).

DNA extraction: *K. pneumoniae* Genomic DNA picking Genomic DNA of K. pneumoniae isolates was extracted using the Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions with some modifications for higher DNA yield and purity. An overnight culture of *K. pneumoniae* was incubated in 1.5 mL Luria-Bertani broth at 37°C, and the 1.5 mL bacterial suspension was centrifuged and collected at 14,000 rpm for two minutes. The bacterial pellet was subsequently resuspended in 200 µL of GB buffer containing lysozyme (20 mg/mL) to facilitate the breakdown of Gram-negative cell walls following the removal of the supernatant. The suspension was incubated at 37°C for 30 minutes, after which 20 µL of Proteinase K and 200 µL of GD buffer were introduced. Samples were then homogenized and incubated for 10 minutes at 60°C to improve protein digestion and to ensure complete cell lysis. Then 200 µL absolute ethanol was added, and the lysate was loaded onto a DNA spin column. The flow-through was discarded after centrifugation. The bound DNA was washed twice with W1 buffer and twice with wash buffer following the manufacturer's protocol, including a centrifugation step at every washing in order to efficiently eliminate contaminants, in particular remaining salt and proteins. Finally, purified genomic DNA was eluted with 100 µL of pre-warmed (60°C) Elution Buffer (TE buffer) to get the maximal DNA recovery.

Conventional PCR-based molecular diagnosis of *intI* gene: The presence of the *intI* gene was determined by PCR with specific primers as previously described. These primers were synthesized by Macrogen, Inc (Korea) and provided in a lyophilized state. On reaching laboratory, pellets were resuspended in nuclease-free water to obtain workable stock solutions. Oligonucleotide primers that amplified 266 bp of the *intI* gene were used as described (Table 1). The PCR mixture for the extracted genomic DNA contained 2 µL of DNA, 1 µL each of the forward and reverse primers (10 µM), 12.5 µL of a commercially available master mix (CWbio, South Korea), and 8.5 µL of deionized water (Bioneer/United States) added to a total reaction volume of 25 µL; the final volume was made up with nuclease-free water (Bioneer, USA). The PCR thermal cycler program included initial denaturation at 95°C for 5 min, 30–35 cycles of denaturation at 94°C for 30 sec, primer annealing at 58°C for 30 sec (optimal temperature depending on primer melting temperature), and extension at 72°C for 30 sec. A final extension at 72°C for 10 min was carried out. After amplification, the products were kept at 4°C until analyzed. PCR products were examined by agarose gel electrophoresis. The PCR products (5–10 µL) were analyzed by electrophoresis (1.5% agarose gel in 1× TBE buffer) and stained with 0.5 µg/mL ethidium bromide. Electrophoresis was performed at 150 V for 30 min, and fragment sizes were estimated by running a 1500 bp double-step DNA ladder. The DNA bands were visualized with a nucleic acid imaging system using UV light. A single intense band at 266 bp corresponding to the expected size confirmed the amplification of *intI* gene. It is to be mentioned here that Gel electrophoresis in this protocol was used only to validate the presence and size of the PCR products, and not for checking the quality or integrity of the genomic DNA before amplification.

Table 1: The specific primer sequences used for amplification of *intI* gene

Primer Name	Sequence	Product Size	Tm (°C)	Reference
intI-F	TCTCGGGTAACATCAAGG	266 bp	58°C	(17)
intI-R	AGGAGATCCGAAGACCTC			

Statistical Analysis: The Statistical Package for the Social Sciences (SPSS, 2019) was used to analyze the influence of different variables or groups on the distribution of study parameters. To determine the significance of the association of variables, the Chi-square (χ^2) test was applied at probability levels of 0.05 and 0.01.

Results

Isolation and Identification of *K. pneumoniae*: Among the 200 samples, only 74 isolates (37%) were classified as *K. pneumoniae*. Blood agar served as an enrichment medium to promote bacterial proliferation (Figure 1: A). On MacConkey Agar, the isolates exhibited a pink, mucoid morphology, signifying lactose fermentation (Figure 1: B), but on HiCrome™ UTI Agar, the colonies manifested a prominent blue-green hue with a mucoid texture (Figure 1: C). Alongside the 74 *K. pneumoniae* isolates (37%), 102 samples (50.1%) demonstrated growth of several bacterial species, while the remaining samples (12.9%) displayed no bacterial growth. The distribution of *K. pneumoniae* isolates from clinical sources included 33 isolates (44.6%) from urine, 19 isolates (25.7%) from burn samples, 10 isolates (13.5%) from wound swabs, and 12 isolates (16.2%) from sputum, as presented in Table 2. To validate the diagnosis of *K. pneumoniae*, all isolates displaying characteristic morphology received additional biochemical testing, and the VITEK-2 Compact System was utilized for the acquired isolates, as illustrated in Table (3).

Table 2: The distribution and percentages of *K. pneumoniae* isolates from clinical samples

Type of clinical sample	Number of Isolates/ Number of samples	Percentage of isolates
Urine	33/95	34.7%
Burns	19/51	37.3%
Wounds	10/28	35.7%
Sputum	12/26	45.2%
Total	74/200	37.0%
Chi-Square: χ^2 (P-value)	--	1.165** (0.761)
** (P>0.01).		

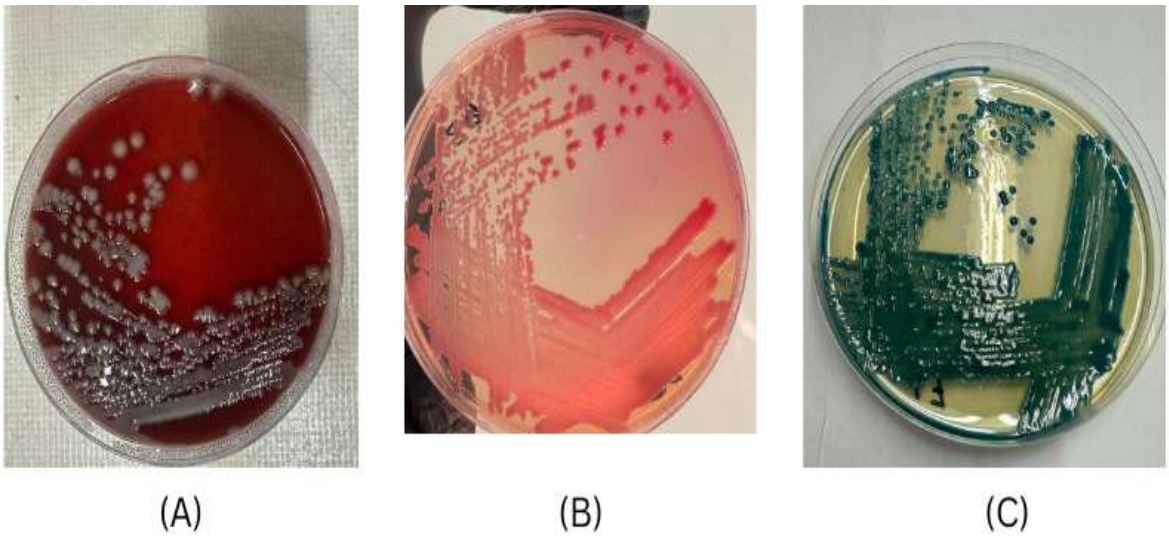

Figure 1: Growth characteristics of *K. pneumoniae* on selective and differential media. (A) Smooth, moist, and mucoid colonies on blood agar without hemolysis; (B) Lactose-positive colonies appearing pink on MacConkey agar; (C) Distinctive blue-green colonies on HiCrome™ UTI agar, facilitating presumptive identification of *K. pneumoniae*

Table 3: The VITEK-2 compact system result of *K. pneumoniae*

Identification Information					Analysis Time:		3.88 hours		Status:		Final				
Selected Organism					99% Probability		*Klebsiella pneumoniae* ssp *pneumoniae*								
					Bionumber:		2601734673164010								
ID Analysis Messages															
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	-			

Phenotypically identifying hypermucoviscous *K. pneumoniae* (Hvkp): Among the 74 analyzed isolates, 30 (40.5%) were positive for specific potentially hypervirulent strains as shown in (Table 4) and (Figure 2).

Table 4: Results of String test in sample (positive and negative)

String test	Positive result	Negative result
74	30	44
% Percentage	40.5%	59.5%
Chi-Square: χ^2 (P-value)	3.648 * (0.0498)	

* (P≤0.05).

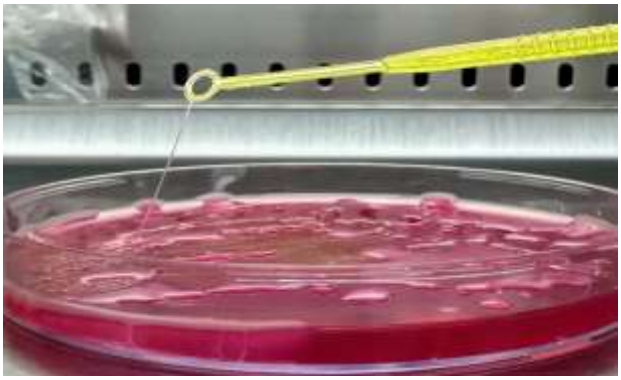

Figure 2: String Test of Hvkp isolated from burns Antibiotic resistance profile of local *K. pneumoniae* isolates

Table 5 shows that *K. pneumoniae* samples displayed significant antibiotic resistance. The isolates exhibited resistance rates of 86% to cefotaxime and 66% to ciprofloxacin. Amikacin and nitrofurantoin exhibited significant resistance rates of 58% and 56%, respectively. Furthermore, tetracycline resistance was identified in 80% of isolates, whereas 52% of isolates exhibited resistance to amoxicillin-clavulanic acid. Gentamicin and levofloxacin had moderate resistance rates of 38% each. Imipenem was the most efficacious antibiotic, exhibiting resistance in (14%) of isolates, although chloramphenicol demonstrated a comparatively low resistance rate of (16%) (Figure 3). Seventy-four clinical isolates of *K. pneumoniae* were examined for their antibiotic susceptibility profiles. Of them, 51 isolates (68.9%) were identified as multidrug-resistant (MDR), characterized by resistance to three or more classes of antibiotics (Figure 4a). Among the MDR isolates, 29 (57%) were designated as extensively drug-resistant (XDR), demonstrating resistance to all or nearly all antimicrobial agents, except one or two. Furthermore, 8 isolates (16%) from the MDR cohort were classified as pan-drug-resistant (PDR), exhibiting resistance to all tested antibiotic classes (Figure 4b). The remaining 23 isolates (31.1%) were not categorized as MDR (sensitive isolates).

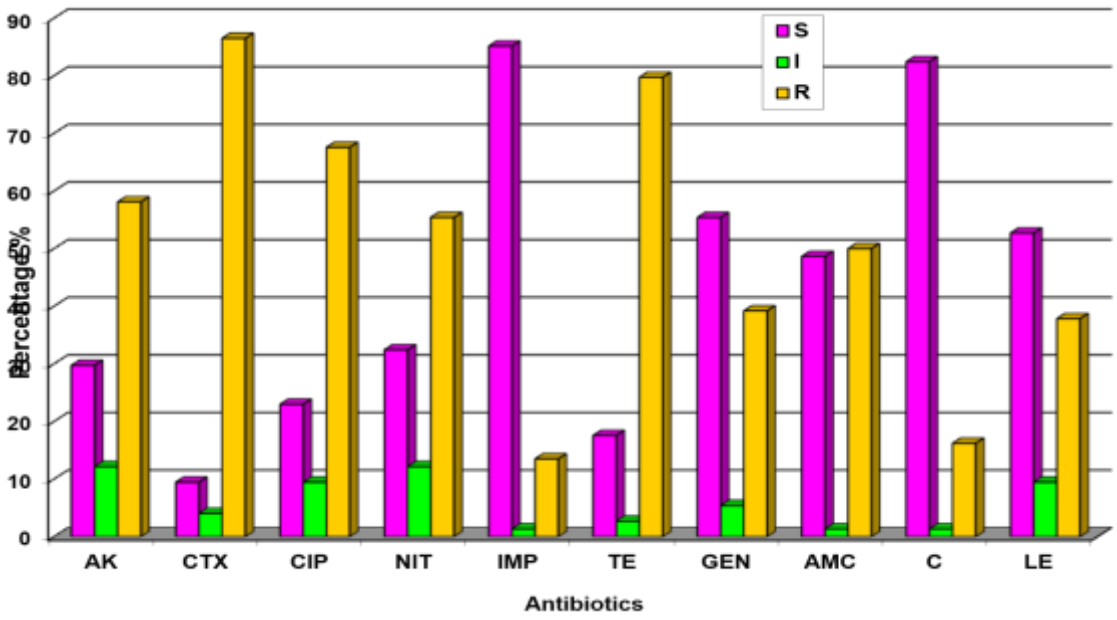

Figure 3: Antibiotic resistance pattern of *K. pneumoniae*. Amoxicillin-clavulanate (AMC), cefotaxime (CTX), amikacin (AK), chloramphenicol (C), tetracycline (TE), ciprofloxacin (CIP), Nitrofurantoin (NIT) Gentamicin (GEN), levofloxacin (LE), and imipenem (IMP), I = Intermediate, R = Resistant, S = Sensitive

Table 5: Distribution of the K. pneumoniae cases according to Antibiotics sensitivity results

Antibiotics	S	I	R	P-value
Amikacin	22 (29.7%)	9 (12.2%)	43 (58.1%)	0.0001 **
Cefotaxime	7 (9.5%)	3 (4.1%)	64 (86.5%)	0.0001 **
Ciprofloxacin	17 (23.0%)	7 (9.46%)	50 (67.5%)	0.0001 **
Nitrofurantoi	24 (32.4%)	9 (12.2%)	41 (55.4%)	0.0001 **
Imipenem	63 (85.1%)	1 (1.4%)	10 (13.5%)	0.0001 **
Tetracycline	13 (17.6%)	2 (2.7%)	59 (79.7%)	0.0001 **
Gentamicin	41 (55.4%)	4 (5.4%)	29 (39.2%)	0.0001 **
Amoxicillin-clavulanate	36 (48.7%)	1 (1.4%)	37 (50.0%)	0.0001 **
Chloramphenicol	61 (82.4%)	1 (1.4%)	12 (16.2%)	0.0001 **
Levofloxacin	39 (52.7%)	7 (9.5%)	28 (37.8%)	0.0001 **
P-value	0.0001 **	0.0087 **	0.0001 **	---

** (P≤0.01).

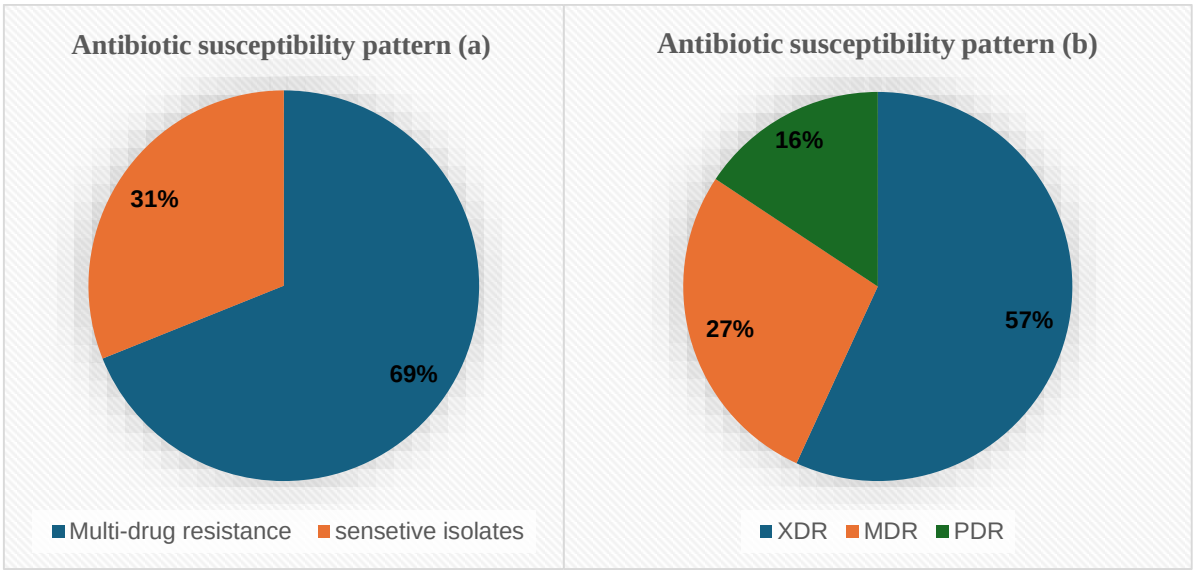

Figure **4**: Proportional Distribution of Antimicrobial Resistance Patterns Among Clinical *K. pneumoniae* Isolates Including MDR Multidrug-Resistant, XDR Extensively Drug-Resistant, and PDR Pan-Drug Resistant Phenotypes

Molecular detection of *intI* gene in *K. pneumoniae* isolates: The detection of *intI* gene was performed on ten selected multidrug-resistant *K. pneumoniae* isolates from the total clinical isolates. The selected isolates had been screened from 51 MDR strains for their different resistance patterns, their phenotypic properties (positive result to the string test) and the source of isolation. As indicated in Table 6, PCR detection showed that 8 (80%) of the 10 examined isolates were *intI* positive. This was confirmed by a specific amplicon band of 266 bp, directly visualized in the agarose gel electrophoresis image (Figure 5), and the successful amplification of the target integrase gene. **(**Table 7) shows the resistance patterns of MDR *K. pneumoniae* isolates tested for the *intI* gene.

Table 6: Antimicrobial Resistance Patterns and Presence of *intI* Gene in *K. pneumoniae* Isolates from Various Clinical Sources

Isolate	Antibiotic resistance	Presence of *intI*	source
34	MDR	+	Wounds
30	MDR	-	Burns
46	MDR	+	Burns
19	XDR	+	Burns
i7	MDR	-	UTI
55	PDR	+	Burns
i8	MDR	+	UTI
17	PDR	+	Burns
21	MDR	+	Sputum
25	MDR	+	Wounds
Positive: No. (%) Negative: No. (%)		8 (80.0%) 2 (20.0%)	--
Chi-Square: χ^2 (P-value)		3.610 * (0.04859)	--

* (P≤0.05).

Table 7: Resistance profiles of MDR *K. pneumoniae* isolates tested for class 1 integron

No. of isolate	AK	CTX	CIP	NIT	IMP	TE	GEN	AMC	C	LE
34	I	R	R	R	S	R	R	S	S	R
30	S	R	I	I	I	R	R	S	S	R
46	R	R	I	R	S	R	S	R	S	R
19	R	R	R	R	R	R	R	R	R	R
55	I	R	R	R	R	R	I	R	R	R
17	R	R	R	R	R	R	R	R	R	R
25	I	R	R	R	S	S	R	S	S	S
21	S	R	R	R	I	R	R	I	I	R
i7	S	R	R	R	S	R	R	I	I	R
i8	I	R	R	S	I	R	R	I	R	R

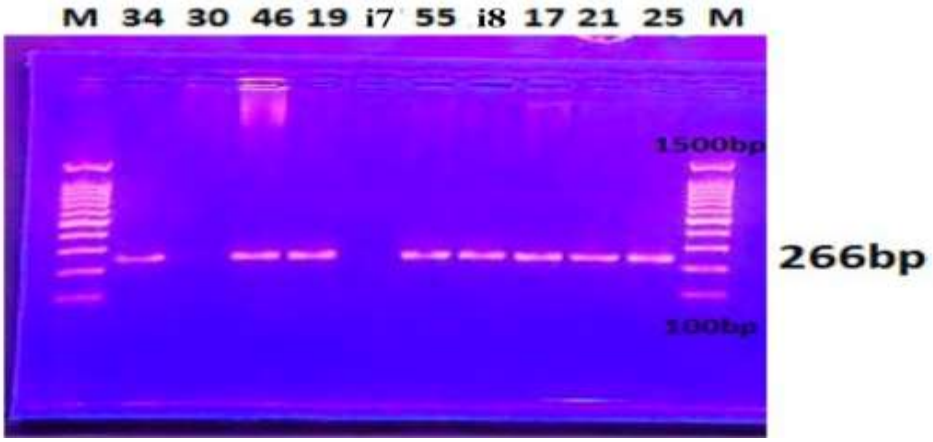

Figure **5**: Agarose gel 1.5% electrophoresis of PCR amplified products for *intI* gene stained with Ethedium Bromide. Lane M: l500bp Ladder marker. Isolate no. with positive bands of 266bp of *int* 1 gene as follow:34,46,19,55, i8,17,21 and 25.

Discussion

The characteristically mucoid and pink appearance of *Klebsiella* colonies on MacConkey agar medium overlaid with red broth (due to lactose fermentation and capsular polysaccharide) is an important phenotypic trait of the organism. This finding is in accordance with previously reported data from Iraq showing a similar morphology on both MacConkey and HiCrome™ UTI when isolating *K. pneumoniae* from clinical isolates (18). The overall isolation rate in the present work (37%), is close to the 40% reported by Abd Al-Hamed et al. for urinary and wound assessments in Diwaniya hospitals (19). Urine specimens being the major source of isolates is consistent with the findings of Alara et al., where *K. pneumoniae* accounted for 42% of Gram-negative urinary pathogen infections in hospitalized patients (20). A close rate was reported in Dohuk, where 46% of strains were isolated from urine samples (21). With HiCrome UTI agar used in the present study, *K. pneumoniae* could be presumptively identified by its typical blue-green colony appearance, further highlighting the previous work that had been done for chromogenic media in uropathogen detection, particularly in cases of polymicrobial infections (22). Culture-based techniques combined with an automated identification system like VITEK 2 improved the accuracy of the diagnosis in this series. This is consistent with routine procedures in microbial diagnostics and has been reported in numerous regional studies.

Concerning hypermucoviscous features, the positive string test reported in the current study was slightly higher than that reported by another study from Baghdad (36%) but slightly lower than that reported from Basrah (45%) (22, 23, 24). These close results from different regions of Iraq may reflect the widespread distribution of hvKP. These variants are clinically important because of their aggressive behavior, frequently being linked to invasive disease, including liver abscess, bacteremia, and metastatic disease. International studies have found the prevalence of hvKP as 42% in China and 40% among bloodstream infections in South Korea, both associating the hvKP phenotype with higher rates of complications and longer hospitalization. The positive association between class 1 integron carriage and string test results may be explained by the genetic linkage of integrons with virulence genes such as regulation of mucoid phenotype A (*rmpA*. The *rmpA* gene enhances capsule production and leads to the hypermucoviscous phenotype, suggesting that integron-positive isolates not only accumulate resistance determinants but may also harbor factors contributing to increased virulence (25, 26, 27).

Two antibiotics showed worrisome resistance rates in the antimicrobial susceptibility results in this study:

Cefotaxime (86%) and ciprofloxacin (66%). These results may be the reason for fluoroquinolone overuse as well as the larger prevalence of ESBL producers. Moreover, resistance levels of both amikacin (58%) and nitrofurantoin (56%) were consistent with the previous reports of Baghdad, without significant increase over the period of the collection of the isolates (28). Moreover, resistance to tetracycline (58.8%) and to amoxicillin-clavulanic acid (52%) goes hand in hand with other global and regional reports and has been associated with the spread of tetA/B genes and the overexpression of β-lactamases (29, 30).

A moderate resistance level was detected against gentamicin and levofloxacin (38% for both) compared to the other drugs, suggesting a possible benefit of the use of these agents in some particular clinical settings and/or in combination therapy or in cases of infections with low bacterial burden. Imipenem was the most sensitive (84%) among the tested antibiotics, but the emergence of carbapenemase-producing *K. pneumoniae* (CPKP) has limited its clinical utility. This is of particular concern in the immunocompromised and geriatric populations, in which CPKP infections have been associated with few therapeutic options, treatment failure, and higher mortality rates (31). Chloramphenicol had the lowest resistance rate (16%), which can be attributed to its reduced use and less selective pressure. But its application is still limited in clinical practice owing to the toxicity.

Multidrug resistance is a serious problem with 68.9% of isolates in the current study found to be MDR, 57% of which were XDR, and 16% were PDR. These results are similar to previous studies documenting the rising burden of resistant *K. pneumoniae* phenotypes in hospitals in Iraq (32, 33). Local differences indicated by the XDR and PDR rates could represent dissimilar prescribing habits, local quality control measures, and resistance determinant dissemination, highlighting the need for local application of antimicrobial stewardship and continuous surveillance (34).

The presence of the class 1 integron gene (*intI*) in 80% of these 10 MDR isolates indicates a possible role in mediating resistance gene exchange. This detection rate is, however, noteworthy and might indeed be a small concerted sample for a generalization. These results are consistent with a study from Iran in 2017 in which the majority (90%) of *K. pneumoniae* isolates carried *intI* (35). Another study involving MDR *K. pneumoniae* strains from hospitalized patients (10) reported a detection rate of 83.3%, and another recent work showed a prevalence of 72.7% among the strains obtained from different infection sources (36). The presence of an integron (especially class 1) in a hypermucoviscous *K. pneumoniae* isolate suggests that the strain has an enhanced capacity to accumulate and spread antibiotic resistance. This has two major implications. First, clinicians might face an infection that is unusually severe (due to hvKp virulence factors like rmpA-mediated capsule production) yet unresponsive to standard antibiotics (due to integron-borne resistance genes). Second, such strains can act as reservoirs of resistance and virulence genes, disseminating them to other bacteria. Academic and clinical experts now stress the importance of surveillance for integrons in hvKp. Routine string testing of *K. pneumoniae* isolates (to flag hvKp) supplemented by molecular screening for integron genes can help early identification of these high-risk strains. By monitoring the emergence of hvKp, with emphasis on mechanisms of antimicrobial resistance, infection control teams can implement timely interventions to contain outbreaks (37, 38). In summary, the integron mechanism – by inserting resistance cassettes into plasmids – significantly bridges the realms of virulence and drug resistance in *K. pneumoniae*. Appreciating this connection is vital for developing strategies to curb the spread of hyper-virulent, integron-fueled resistant *K. pneumoniae*, and reinforces the need for judicious antibiotic use to avoid fueling this dangerous convergence of traits.

Limitations

The study was limited by the relatively small number of isolates tested for the presence of class 1 integrons and by its restriction to a few hospitals in Baghdad. In addition, only conventional PCR was used without sequencing, which may not fully reveal the diversity of gene cassette arrays. These factors may limit the generalizability of the findings and highlight the need for broader, multicenter studies using advanced molecular tools

Conclusion

The notably high prevalence of class 1 integrons among (*MDR*) *Klebsiella pneumoniae* isolates recovered from various clinical sources underscores its pivotal role in facilitating the acquisition and dissemination of antimicrobial resistance determinants within clinical settings. This indicates the worldwide emergence of *K. pneumoniae* pathogenic strains resistant to colistin and carbapenems, highlighting the imperative for ongoing molecular surveillance and efficient antibiotic stewardship. Further investigations are warranted to elucidate the structural composition of gene cassette arrays within these integrons and to clarify their functional contribution to resistance phenotypes.

Authors' declaration:
We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in the College of Science, University of Baghdad, under reference number CSEC/0522/0062.
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Data availability:
Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' contributions:
Study conception & design: (Alyaa Razooqy Hussein). Literature search: (Jasim Salam Jasim). Data acquisition: (Jasim Salam Jasim). Data analysis & interpretation: (Alyaa Razooqy Hussein). Manuscript preparation: (Jasim Salam Jasim). Manuscript editing & review: (Alyaa Razooqy Hussein).

References
1. Bai R, Guo J. Interactions and implications of Klebsiella pneumoniae with human immune responses and metabolic pathways: a comprehensive review. Infect Drug Resist. 2024;17:449–62. https://doi.org/10.2147/IDR.S451013
2. Chakraverty R, Kundu AK. Hospital-Acquired Infections in Intensive Care Unit and their Management: The Indian Perspective. Springer; 2025. https://link.springer.com/book/10.1007/978-981-96-0018-2
3. Razzaq T, Ali Y, Khan S, Nadeem Z, Batool A, Ibraheem M, et al. Antibacterial drug exposure and risk of carbapenem-resistant Klebsiella pneumoniae: a review. Zenodo. 2025;3(2):3007–1593. https://doi.org/10.5281/zenodo.15279758
4. Albermani M, Salman WA, Hamad GK, Hadi EA. Infection control measures to reduce hospital infection rates in the medical city burn center. J Fac Med Baghdad. 2019;60(4):191–4. https://doi.org/10.32007/med.1936/jfacmedbagdad.v60i4.3
5. Russo A, Fusco P, Morrone HL, Trecarichi EM, Torti C. New advances in management and treatment of multidrug-resistant Klebsiella pneumoniae. Expert Rev Anti Infect Ther. 2023;21(1):41–55. https://doi.org/10.1080/14787210.2023.2151435
6. Gidla VVS, Anjaneyulu K, Bhanu RV, Dinesh G. Integrons: a mobile genetic element of concern in antimicrobial resistance gene transfer: an overview. Indian J Vet Sci Biotechnol. 2023;19(3):1–5. https://doi.org/10.48165/ijvsbt.19.3.01
7. Tao S, Chen H, Li N, Liang W. The application of the CRISPR-Cas system in antibiotic resistance. Infect Drug Resist. 2022;15:4155–68. https://doi.org/10.2147/IDR.S370869
8. Wang L, Zhu M, Yan C, Zhang Y, He X, Wu L, et al. Class 1 integrons and multiple mobile genetic elements in clinical isolates of the Klebsiella pneumoniae complex from a tertiary hospital in eastern China. Front Microbiol. 2023;14:985102. https://doi.org/10.3389/fmicb.2023.985102
9. Taha RQ, Mansoor RJ, Kareem SA, Mohsein OA. Unraveling the genomic landscape of multidrug-resistant Klebsiella pneumoniae: a focus on resistance islands. Caspian J Med Nat Sci. 2025;6(2). https://doi.org/10.17605/cajmns.v6i2.2754
10. Omar FH, Ibrahim AH. The prevalence of integron class I and II among multidrug-resistant Klebsiella pneumoniae. Iraqi J Agric Sci. 2023;54(3):619–29. https://doi.org/10.36103/ijas.v54i3.1775
11. Alara JA, Alara OR. An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis. Infect Disord Drug Targets. 2024;24(3). https://doi.org/10.2174/1871526523666230725103902
12. Al-Ruobayiee MR, Ibrahim AH. The relationship between OqxAB efflux pump and drug resistance in Klebsiella pneumoniae isolated from clinical sources. Al-Rafidain J Med Sci. 2023;5(1):S106–12. https://doi.org/10.54133/ajms.v5i1S.309
13. Alansary IMM, Al-Saryi NA. Emergence of hypervirulent Klebsiella pneumoniae isolates from some Iraqi hospitals. Rev Res Med Microbiol. 2024;35(2):88–96. https://doi.org/10.1097/MRM.0000000000000354
14. CLSI. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2024. https://clsi.org/shop/standards/m100
15. Abdel-Hamid RM, El-Mahallawy HA, Allam RM, Zafer MM, Elswify M. Changing patterns of bacterial profile and antimicrobial resistance in high-risk patients during the COVID-19 pandemic at a tertiary oncology hospital. Arch Microbiol. 2024;206(6). https://doi.org/10.1007/S00203-024-03965-X
16. Firoozeh F, Mahluji Z, Khorshidi A, Zibaei M. Molecular characterization of class 1, 2 and 3 integrons in clinical multidrug-resistant Klebsiella pneumoniae isolates. Antimicrob Resist Infect Control. 2019;8(1). https://doi.org/10.1186/s13756-019-0509-3
17. Mustafa SS, Batool R, Kamran M, Javed H, Jamil N. Evaluating the role of wastewaters as reservoirs of antibiotic-resistant ESKAPEE bacteria using

phenotypic and molecular methods. Infect Drug Resist. 2022;15:5715–28. https://doi.org/10.2147/IDR.S368886
18. Al-Rubaye DS, Hamza HM, Abdulrahman TR. Genotyping of Klebsiella spp. isolated from different clinical sources. Iraqi J Sci. 2016;57(3B):1937–51. https://doi.org/10.24996/ijs.2016.57.3B.21
19. Abd Al-Hamed ZA, Abd Al-Mayahi FS. Antibiogram of Klebsiella pneumoniae isolated from clinical and environmental samples in Al-Diwaniyah hospitals. Al-Qadisiyah J Pure Sci. 2021;26(2):44–54. https://doi.org/10.29350/qjps.2021.26.2.1296
20. Mahmood MK, Farhan SD, Ahmed BA, Nori W. Risk factors analysis of urosepsis following retrograde intrarenal surgery: a retrospective study. Al-Rafidain J Med Sci. 2025;8(1):48–55. https://doi.org/10.54133/ajms.v8i1.1643
21. Turki Monawer A. Molecular analysis of Klebsiella pneumoniae isolates collected from sputum samples in Duhok, Iraq. Al-Nahrain J Sci. 2025;28(1):91–5. https://doi.org/10.22401/ANJS.28.1.10
22. Jain C, Nikita N. Evaluation of chromogenic agar media for isolation, identification and direct antibiotic susceptibility testing of uropathogens. Int J Pharm Res Allied Sci. 2023;12(2):7–12. https://doi.org/10.51847/Kd4vp42v9B
23. Kherd A, Saaban A, Adyee, Ibrahim Fadhel IE. Wang-ball polynomials for the numerical solution of singular ordinary differential equations. Iraqi J Sci. 2021;62(3):941–9. https://doi.org/10.24996/ijs.2021.62.3.24
24. Ibraheim HK, Madhi KS, Jasim AS, Gharban HAJ. Molecular identification of Klebsiella species from pneumonic goats, Iraq. Open Vet J. 2024;14(11):2980–8. https://doi.org/10.5455/OVJ.2024.v14.i11.26
25. Hmood AH, Alammar MHM. Genotypic and phenotypic surveillance of multidrug resistance of hypermucoid K. pneumoniae among clinical isolates in Al-Najaf City, Iraq. Al-Kufa Univ J Biol. 2023;15(3):122–8. https://doi.org/10.36320/ajb/v15.i3.14615
26. Chhabra P, Rouhani S, Browne H, Peñataro Yori P, Siguas Salas M, Paredes Olortegui M, et al. Homotypic and heterotypic protection and risk of reinfection following natural norovirus infection in a highly endemic setting. Clin Infect Dis. 2021;72(2):222–9. https://doi.org/10.1093/cid/ciaa019
27. García-Fernández S, Simner PJ, Thomson G, Faron M, Bosboom R, van Griethuijsen A, et al. Rapid identification from rectal swabs of the clinically most relevant carbapenemase genes from gram-negative bacteria using the BD MAX Check-Points CPO assay. Diagn Microbiol Infect Dis. 2022;102(1):115554. https://doi.org/10.1016/j.diagmicrobio.2021.115554
28. Jabar ZA, Auhim HS, Hussein AR. Molecular detection of fimH and mrkD genes of strong biofilm producers and MDR Klebsiella pneumoniae. Int J Health Sci. 2022;6(S4):9225–35. https://doi.org/10.53730/ijhs.v6ns4.11954
29. Chowdhury MSR, Hossain H, Rahman MN, Hossain MA, Saha O, Zinnah MA, et al. Emergence of highly virulent multidrug- and extensively drug-resistant Escherichia coli and Klebsiella pneumoniae in buffalo subclinical mastitis cases. Sci Rep. 2025;15:11704. https://doi.org/10.1038/s41598-025-95914-x
30. Al-Marzooq F, Mohd Yusof MY, Tay ST. Molecular analysis of antibiotic resistance determinants and plasmids in Malaysian isolates of multidrug-resistant Klebsiella pneumoniae. PLoS One. 2015;10(7):e0133654. https://doi.org/10.1371/journal.pone.0133654
31. Almidhatee HRM, Alkufaishi ZHJ, Al-Saffar AKH, Alhamawandi JAA. Detection of molecular resistance mechanisms in Klebsiella pneumoniae through carbapenem enzymatic genes. J Angiother. 2024;8(4). https://doi.org/10.25163/angiotherapy.849602
32. Fouad NA, Totl KJ. Antibiotic resistance in Klebsiella pneumoniae and its impact in mixed type isolates. Iraqi Journal of Medical Sciences. 2024;22(2):369–376. doi:10.22578/IJMS.22.2.22 https://iraqijms.net/index.php/jms/article/view/1059
33. Al-Qaysi AMK, Ahmed MM, Habeeb WH, Al-Meani SAL, Janaby MS AL, Alalwani AK, et al. Genetic variants of multidrug-resistant Klebsiella pneumoniae isolated from Al-Ramadi Teaching Hospital, Iraq. Open Microbiol J. 2024;18(1). https://doi.org/10.2174/0118742858298979240628070603
34. Mohammed MJ, Mahmood SS. The prevalence of pks genotoxin among Klebsiella pneumoniae isolated from different clinical samples in Baghdad, Iraq. Iraqi J Sci. 2024;65(7):3716–24. https://doi.org/10.24996/ijs.2024.65.7.13
35. Jazayeri Moghadas A, Kalantari F, Sarfi M, Shahhoseini S, Mirkalantari S. Evaluation of virulence factors and antibiotic resistance patterns in clinical urine isolates of Klebsiella pneumoniae in Semnan, Iran. Jundishapur J Microbiol. 2018;11(7). https://doi.org/10.5812/jjm.63637
36. Fadare FT, Fadare TO, Okoh AI. Prevalence and molecular characterization of integrons and associated gene cassettes in Klebsiella pneumoniae and K. oxytoca recovered from diverse environmental matrices. Sci Rep. 2023;13(1). https://doi.org/10.1038/s41598-023-41591-7
37. Sheikh AF, Abdi M, Farshadzadeh Z. Molecular detection of Class 1, 2, and 3 integrons in hypervirulent and classic Klebsiella pneumoniae isolates: a cross-sectional study. Health Sci Rep. 2024;7(5):e1962. https://doi.org/10.1002/hsr2.1962

38. Hetta HF, Alanazi FE, Sayed Ali MA, Alatawi AD, Aljohani HM, Ahmed R, et al. Hypervirulent Klebsiella pneumoniae: Insights into Virulence, Antibiotic Resistance, and Fight Strategies Against aSuperbug. Pharmaceuticals (Basel). 2025;18(5):724. https://doi.org/10.3390/ph18050724

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الكشف عن الإنتغرون من الصنف الأول بين عزلات الكلبسيلا الرئوية السريرية في مستشفيات بغداد

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الخلاصة:
الخلفية: تعد بكتريا الكليبسيلا الرئوية المقاومة للأدوية بشكل واسع مشكلة صحية ملحة في الوقت الحالي، نظرا لازدياد معدل انتشار هذا الممرض. هدفت هذه الدراسة إلى تحديد مدى انتشار انتكرونات الفئة في السلالات المقاومة لعدة مضادات حيوية من كليبسيلا الرئوية.
الأهداف: تسليط الضوء على التهديد المتزايد الذي تشكله بكتيريا كليبسيلا الرئوية المقاومة للمضادات الحيوية من خلال التحقيق في انتشار إنتغرونات الفئة الأولى بين العزلات السريرية المختارة المقاومة للأدوية المتعددة، بهدف التأكيد على الحاجة إلى تعزيز المراقبة واستراتيجيات مكافحة الميكروبات المستهدفة.
المنهجية: من أصل 200 عينة سريرية، تم عزل 74 عزلة (37%) تعود لبكتيريا الكليبسيلا الرئوية .جرى تحديد العزلات أوليا باستخدام الاختبارات الكيميائية الحيوية والأجار الكروموجيني، ثم تم التأكيد بواسطة نظام VITEK 2.تم تقييم الحساسية للمضادات الحيوية باستخدام تقنية الانتشار بالأقراص (طريقة Kirby-Bauer) كما تم الكشف عن وجود جين الإنترون من الفئة الأولى بواسطة تقنية PCR التقليدية.
النتائج: من بين 74 عزلة، أظهرت 51 عزلة (68.9%) مقاومة متعددة للمضادات الحيوية. سجلت أعلى مقاومة ضد السيفوتاكسيم بنسبة 86%، تلتها التتراسيكلين بنسبة 80%، بينما أظهر الإيميبينيم أعلى نسبة حساسية بلغت 84%. تم الكشف عن جينات الإنترون من الفئة الأولى في 80% من أصل 10 عزلات مختارة متعددة المقاومة.
الاستنتاج: يبرز ارتفاع معدل انتشار إنتغرونات الفئة الأولى في العزلات السريرية لبكتيريا الكليبسيلا الرئوية متعددة المقاومة للأدوية المتعددة الدور الأساسي للإنتغرونات في انتشار مقاومة المضادات الحيوية. ويشير هذا إلى ظهور سلالات ممرضة من الكليبسيلا الرئوية مقاومة للكوليستين والكاربابينيمات على مستوى العالم، مما يبرز ضرورة المراقبة الجزيئية المستمرة والإدارة الفعالة للمضادات الحيوية.
الكلمات المفتاحية: الكليبسيلا الرئوية، المقاومة المتعددة للمضادات، العدوى المكتسبة في المستشفيات، اختبار الانتشار بالأقراص، إنترونات الفئة الأولى، تفاعل البوليميراز المتسلسل.