

Research Article

Molecular typing and integron detection of multidrug-resistant *Klebsiella pneumoniae* clinical isolates recovered from Baquba Teaching Hospital in Iraq

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Abstract

Klebsiella pneumoniae is a gram-negative bacterium that causes severe illnesses and is antibiotic-resistant. This study aimed to determine the antibiotic resistance profile and prevalence of class II and III integrons and ERIC-PCR among clinical isolates of *K. pneumoniae*. The study was conducted between September 2022 and January 2023. Fifty isolates were obtained from 230 specimens (wounds, burns, blood, fluid, ears, urine and sputum). Macroscopic, microscopic, and biochemical assays were used to identify all *K. pneumoniae* isolates, which were confirmed with the genetically by 16S rRNA. All isolates were examined for various types of clinically significant antibiotic drugs. The results of resistance to antibiotics indicated resistance to Amoxicillin-clavulanic acid (98%), Meropenem (38%), Ceftazidime (96%), Amikacin (48%), Trimethoprim-sulfamethoxazole (58.62%) and Levofloxacin (46%). The testing for antibiotic susceptibility of the *K. pneumoniae* isolate showed that 24 (48%) of the isolates were multi-drug resistant (MDR). *K. pneumoniae* β -lactamase producers (ESBL) appeared 33 (66%). Enterobacterial repetitive intergenic consensus (ERIC) amplification of 16 clinical *K. pneumoniae* isolates showed 14 (87.5%) of each of them revealed at least one amplification band. ERIC-PCR typing found two groups, A and B, with identical antimicrobial resistance patterns within the same group. While Integron II showed that 1 (6.25%) of *K. pneumoniae* isolates was integrase gene positive. Class III integrons were seen in all isolates at a rate of 16 (100%). Continuous monitoring and characterization of integrons and their associated gene cassettes could be helpful in controlling the rate of antibiotic resistance by planning to take preventive measures to hinder the spread of resistant strains.

Keywords: Antibiotic resistance, ERIC-PCR, Integrons., *Klebsiella pneumoniae*, Multidrug-resistance stains

INTRODUCTION

A Gram-negative encapsulated bacteria called *Klebsiella pneumoniae* possesses some virulence components, including a capsule, an endotoxin, a siderophore, an iron-scavenging mechanism, and adhesins, which are essential to its pathogenesis (Ahmadi *et al.*, 2022). Antibiotic resistance is a major global health concern, and resulting in significant financial losses due to the inappropriate use of antibiotics (Cancica *et al.*, 2019). Most *K. pneumoniae* isolates are multi- and extensively drug-resistant. (MDR, XDR) thus limiting therapeutic options for treating infections caused by *K. pneumoniae* (Li *et al.*, 2020). The production of antibiotic-inactivating

enzymes causes resistance; also, resistance is caused by modifications to efflux pumps, permeability of membranes, or molecule targets (Ahmadi *et al.*, 2022). Beta-lactamase enzymes, such as extended-spectrum lactamase (ESBL), produce resistance to lactam antibiotics such as penicillins and cephamycin (Kuinkel *et al.*, 2021; Najj and Abdal Kareem, 2021). *Klebsiella pneumoniae* can also produce Biofilms, which are bacterial populations placed in an extracellular matrix. Proteins, exopolysaccharides, DNA, and lipopeptides make up this matrix (Mohammed, 2021). ERIC-PCR is a successful method for genotyping *K. pneumoniae* isolates from various origins, and it is regarded as one of the most efficient, simple, and cost-effective procedures

(Mahmud *et al.*, 2022). Integrons are important genetic factors in Multi-drug resistance transmission of genes in gram-negative microbes. It recombines mobile gene cassettes at specified sites (Jahanbin *et al.*, 2020). The study aimed to determine molecular diversity using ERIC-PCR and integron detection of multidrug-resistant *K. pneumoniae* clinical isolates recovered from Baquba hospitals in Iraq.

MATERIALS AND METHODS

Isolation and identification of bacterial isolates

Two hundred and thirty clinical specimens (wounds, burns, blood, fluid, ears, and sputum) were conducted between September 2022 and January 2023. They were collected from many Baqubah facilities, including Al-Batool Teaching Hospital and Baqubah Teaching Hospital in Iraq. The isolates were identified using 16S rRNA and their colony characteristics and gram stain. The purified isolates were kept in Brain heart broth with 20% glycerol at -20°C to detect antibacterial agent susceptibility.

The Kirby-Bauer method was used to test the susceptibility of 6 antibiotics Amoxicillin-clavulanic acid (AMC) (20/10 µg), Ceftazidime (CAZ) (30 µg), Meropenem (MEM) (10 µg), Amikacin (AK) (30 µg), Levofloxacin (LEV) (5 µg) and Trimethopri-sulfamethoxazole (TS) (1.25/23.75 µg) for the detection of *K. pneumoniae* phenotypes based on the drug resistance patterns. Multidrug-resistant (MDR) patients make phenotyping classifications depending on sensitivity tests.

Phenotypic detection of Extended-spectrum β -lactamase (ESBL) production

Production of ESBL *K. pneumoniae* was identified using the double-disk synergy test (DDST) (Humphries *et al.*, 2021).

Detection of biofilm

The micro-titer plate described by Ghellai *et al.*, (2014) An ELISA reader was used to measure the concentration at 630 nm. The control well's OD value was subtracted from all of the test OD values (OD_c). The results were divided into three categories based on absorbance: strong "2 x OD_c < OD", moderate "OD_c < OD ≤ 2 x OD_c", and non-biofilm "OD" ≤ "OD_c".

Extraction of DNA from genomes

The genomic DNA was extracted from isolates employing Genomics DNA Purification Kits as per the instructions provided by the manufacturer (Promega USA). The PCR reaction tubes were placed in a thermal cycler that was set up as follows: The conditions for each cycle were 30 sec. At 94 °C, 30 sec. of repeated changing annealing temperatures (from 48 °C to 60 °C ac-

ording to primers) and 30 sec. of last extension at 72° C for 5 minutes. An agarose gel electrophoresis procedure was used to detect amplified PCR products. The primers used are shown in Table 1.

Standard sequencing

The resolved amplicons of PCR were commercially read in the forward direction, according to the sequencing company's instructions manual (Macrogen Inc., Geumcheon, Seoul, South Korea). A Universal primer 16S rRNA27F (5'-AGAGTTTGATCC TGGCTCAG-3'), 1492R5'-TACGGTTACCTTGTTACGACTT-3' Annealing temp 60 °C, Product size 1500bp was used (Sharma *et al.*, 2010).

Statistical analysis

For analysis of statistics, SPSS software (version 23) was used. The Chi-square statistic was used to compare the capability of biofilm formation to resistance to antibiotics (Allison, 2012). An anti-biogram was utilized to detect the bacterial groupings that were generally homogenous. The correlation matrix between susceptibility agents and phylogenetic tree was determined using the Fisher Exact Test.

Ethical approval

Ethical approval for samples collected was obtained from the Iraqi Ministry of Health – Diyala Health Department by 41462 on 6.9/2022/

RESULTS AND DISCUSSION

Fifty primary clinical isolates of gram-negative bacteria were identified as *Klebsiella pneumoniae* using Macroscopic, Microscopic, and Biochemical assays, which were confirmed genetically by 16S rRNA. The source of these specimens were as follows: 6(12%) isolates from Wounds, 9(18%) isolates from Burns, 23 (46 %) isolates from urine, 2(4%) isolates from ears, 3 (6 %) isolates from sputum, 5(10%) isolates from blood and the last 2(4%) from fluid. The difference in incidence rates could be attributable to many contributing factors, such as socio-demographic data, hospitalization, health status, and seasonal variation (Assafi *et al.*, 2022). In addition to the sample size, the collection method and loss of environmental and personal care were taken (Gebremariam *et al.*, 2019).

Molecular investigation of *Klebsiella pneumoniae* DNA

Total extracted DNA from *Klebsiella pneumoniae*

A genomic DNA purification kit (Promega, USA) extracts total genomic DNA from *K. pneumoniae* isolates. Extraction DNA from 16 clinical isolates PDR transferred by gel electrophoresis depending on band size. The Quantus Fluorometer was used to determine the con-

Table 1. Primers used for Eric,IntI,and IntII genes detection

Primer name	Primer sequence 5` 3`	Annealing temp (C)	Producte size (bp)	Reference
ERIC-F	ATGTAAGCTCCTGGGGATTAC	48	800	(Parsaie <i>et al.</i> , 2017)
ERIC-R	AAGTAAGTGACTGGGGTGAGCG			
intII-F	TTATTGCTGGGATTAGGC	51	300	
IntII-R	ACGGCTACCCTCTGTTATC			
Int III-F	AGTGGGTGGCGAATGAGTG	36	300	(Jahanbin <i>et al.</i> , 2020)
IntIII-R	TGTTCTTGATCGGCAGGTG			

Table 2. Antibioqram susceptibility of *Klebsiella pneumoniae* isolates

Antibiotic	Resistant isolates No. %	Sensitive isolates No. %
Amoxicillin-clavulanic acid	49(98)	1(2%)
Ceftazidime	48(96%)	2(4%)
Meropenem	19(38%)	28(56%)
Amikacin	24(48%)	21(42%)
Levofloxacin	23(46%)	25(50%)
Trimethopri-sulfamethoxazole	40(80%)	9(18%)

centration and purity of DNA. The DNA contents in the extracts ranged from 13 ng/ l to 25 ng/ l. The cultivation methods, bacteria group, pellet volume, and extraction kit type had an impact on the quality and characteristics of nucleic acid. The genotyping of 16 isolates showed that they were all *K. pneumoniae* with a 1500-bp 16S *rRNA* gene. Gene cleared up any uncertainties regarding the diagnosis (Fig.1).

Antimicrobial Sensitivity Test

The results of fifty *K. pneumoniae* isolates tested for resistance to 6 different antibiotics are mentioned in Table 2, indicating that the isolates varied in their capacity to resist antibiotics.

Antibiotics ceftazidime showed resistance in 96% of the isolates (Table 2). These findings support the results obtained by Muslim (2022), who observed that ceftazidime had a resistance rate of roughly 95 % and also corresponded to a study in Egypt that revealed a high resistance rate to third-generation cephalosporins (Al-Baz *et al.*, 2022).

Susceptibility testing revealed that 19 (38%) of *K.pneumoniae* isolates were resistant to imipenem. This antibiotic showed higher activity (62%) against these isolates. The findings correspond with previous studies conducted in Erbil, Iraq (Ali and Esmeal, 2017), which indicated that meropenem was the most efficient treatment. This finding is likely because carbapenems are used much less frequently for treating illness in this country than other antibiotics. However, the rise of carbapenem resistance needs close monitoring, particularly in *K. pneumoniae* immunocompromised individuals and in hospitalized patients. The antimicrobial capacity of isolates to the aminoglycosides a group, including amikacin, was 48% agreed with Mo-

hamad (2022) but disagreed with Nirwati *et al.* (2019) showed that *K. pneumoniae* isolates were 100% sensitive to amikacin. There are three methods by which *K. pneumoniae* resists amikacin: Firstly, antibiotics are modified using an alteration enzyme. Second, chromosomal mutations in target protein-encoded genes lower bacterial permeability to the antibiotic. (Lia *et al.*, 2022).

Phenotypic screening for Extended-Spectrum Lactamase (ESBL) production

The percentage of ESBLs generating isolates determined by using the disk diffusion method showed that 33(66%) isolates produced ESBLs enzyme, while 17(34%) of the isolates were non-ESBLs enzyme producers in *K. pneumoniae* isolates. The same opinion is shared by Ayatollahi *et al.*(2020). Which reported that 64% of *K. pneumoniae* strains produced ESBLs.

ESBL enzyme could be found in Enterobacteriaceae, primarily *K. pneumoniae* and other bacteria, especially in Gram-negative bacteria, because of the ability of genetic elements, especially plasmids with different sizes that carry ESBLs genes to transfer, clone and conjugation (Karamptakis *et al.*,2023).

Detection of biofilm formation

The fifty isolates had biofilm formation properties and an absorbency value of 0.032 (Table 3). It shows that 24% of isolates created strong biofilms, in agreement with the study by Mohammed in 2022, in which strong biofilm formation was 24.14, while 38% formed moderate biofilms and 38% did not form biofilms. These agreed with Husham, 2022, in which moderate biofilm and non-biofilm were 32%.

isolate's ability to make biofilms differed because, generally, various factors influence the ability to produce

Table 3. Absorbency values and biofilm pattern by MTP method (n=50)

NO. isolates	percentage%	Biofilm formation
19	38%	Non- biofilm
12	24%	Strong
19	38%	Moderate

biofilms, like the detecting method and the media, the incubation condition and the type of process's surface, the kind of surface employed for that operation because the materials on which the biofilm is generated differ in composition. The layer of membrane established on the microtiter plate's polystyrene surface is far better than the silicon surface of the catheters (Slettengren *et al.*, 2020).

Relation of biofilm formation and antibiotic resistance for *Klebsiella pneumoniae*

The total biofilm formation in *K. pneumoniae* is statistically significant (p . value ≤ 0.02) with antibiotic resistance, as shown in Table 4. Ceftazidime, Amoxicillin-clavulanic acid, and Trimethopri-sulfamethoxazole showed the highest correlation in biofilm formation of 96.77%, 90%, and 83.33%, respectively.

Resistance to antibiotics was stronger in *K. pneumoniae*, which formed biofilms than in *K. pneumoniae*, which did not produce biofilms. The conclusion formed by these findings is the closest to the study of Shadkam *et al.* (2021). This contrasts with our result (Hasan *et al.*, 2022; Hassan and Khider, 2020). It was discovered that antibiotic-susceptible isolates generate stronger biofilms than resistant ones. Other possible reasons for the link between biofilm production and antibiotics include quicker conjunctive Transfer of plasmid or the multipurpose effect of certain gene regulators, which confer antibiotic resistance and increased biofilm production ability.

The present study supports the involvement of biofilm formation in the resistance of *K. pneumoniae* clinical isolates acquired from hospitals. In clinical specimens,

the strength of biofilm development varied (strong, Moderate and weak). As a result, the characterization of nosocomial microbes is extremely beneficial in controlling and treating infections caused by these infections (Karimi *et al.*, 2021).

Biofilm formation is substantially more prevalent in MDR and PDR isolates than in XDR isolates (p . value ≤ 0.04), which are (45.16% and 38.70%) MDR and PDR, respectively, compared to 16.12 % XDR (Table 5). The result disagreed with the study performed by Mohamed (2022), who reported that biofilm formation was more prevalent in PDR (38%) more than MDR (26%). The multi-drug-*Klebsiella pneumoniae* bacteria develop a stronger biofilm than non-multidrug strains Seifi *et al.*, (2016). Research into the processes of biofilm production in *K. pneumoniae* will eventually help in the treatment of biofilm-mediated diseases and reduce mortality and morbidity among people with potentially deadly nosocomial diseases (Shadkam *et al.*, 2021).

This result appeared to be the most statistically significant (p . value ≤ 0.04) for biofilm production across beta-lactamase-producing enzyme (ESBL (70.9) in *K. pneumoniae* isolates.

Besides expressing pumps for efflux in the living cell membrane, the biofilm blanket can block enzyme excretion, nutrients, or Even minor substances that aggregate within biofilms to create a more favorable environment by stabilizing the biofilm while altering pH and levels of ions. Finally, some bacterial cells are crucial as a mechanical protective mechanism in biofilm preservation (Raouf and Fayidh, 2022).

In the present study, the presence of beta-lactamase enzymes and biofilm formation among isolates *K. pneumoniae* demonstrated the significance of these factors in transferring resistance.

Molecular typing by Integron Class II genes and Integron class genes among *Klebsiella pneumoniae* Isolates

Integron class II is defined in this study by a small per-

Table 4. Relationship between biofilm formation and antibiotic resistance *K.pneumoniae*

Antibiotics	Strong (n=13)%	Moderate (n=18) %	Total	Non- biofilm(n=19)%
Meropenem	46.15	50	48.38	21.05
Ceftazidme	100	94.44	96.77	94.73
Amikacin	53.84	44.44	48.38	47.36
Amoxicillin-culvanic acid	92.30	100	96.77	100
Trimethopri-sulfamethoxazole	84.61	88.88	83.87	68.42
Levofloxacin	46.15	44.44	45.16	47.36

Table 5. Relationship between biofilm formation and (MDR, XDR and PDR) *K. pneumoniae*

Clinical isolates	Biofilm formation			
	Strong(n=12%)	Moderate(n=19)%	Total%	Non- biofilm(n=19)%
MDR	30.76	55.55	45.16	52.63
XDR	15.38	16.66	16.12	15.78
PDR	38.46	38.88	38.70	21.05

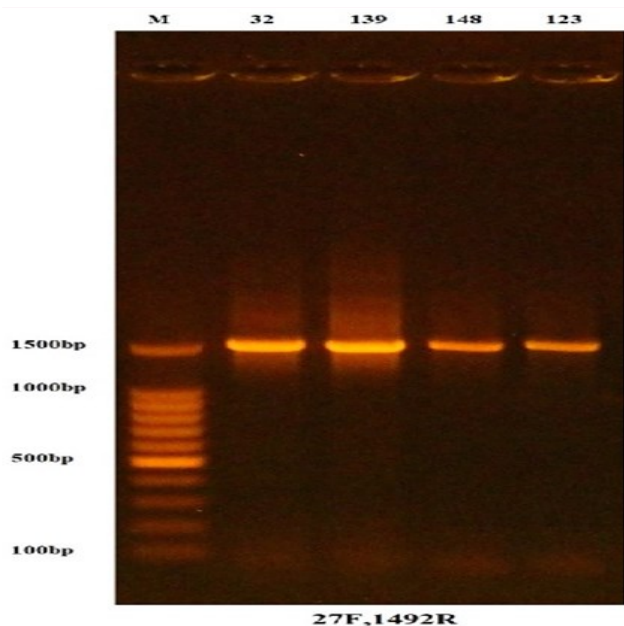


Fig. 1. Showing 16S rRNA gene of primer amplification in *K.pneumoniae* samples fractionated on 1% electrophoresis of agarose gel stained with Eth.Br. M: 100bp ladder marker, NC: negative control 60 minutes of electric current at 100 volts/amp

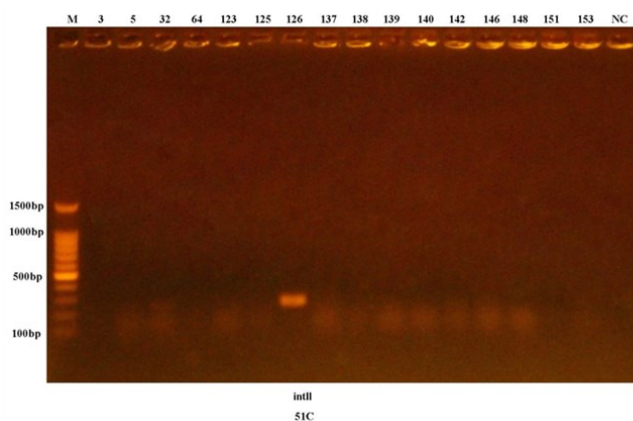


Fig.2. Showing amplification of IntI1 gene of bacterial species fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker (Lanes 3-153 resemble PCR products)

centage in isolate 1 (6.25%), as shown in Fig. 2, which is resistance to all antibiotics (PDR). It is in agreement with the results of Delarampour *et al.*(2020). There is only one isolate with Integron class II. While it differed from the results of a previous study by Laibi *et al.* (2021), it contained the Integron Class II at 100%.

The present analysis discovered class III integrons in all strains at a rate of 16 (100%) (Fig. 3). That does not agree with what several studies have shown. for the sequences of The Presence of Integrons and Their Relation Integron transfer and resistance to microbes. However, there is insufficient information on the incidence of class III and its relationship to antibiotic re-

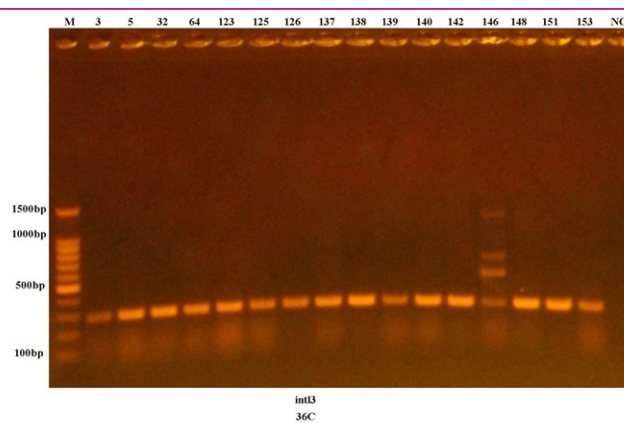


Fig.3. Showing the results of the amplification of IntI3 gene of bacterial species fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker (Lanes 3-153 resemble PCR products).

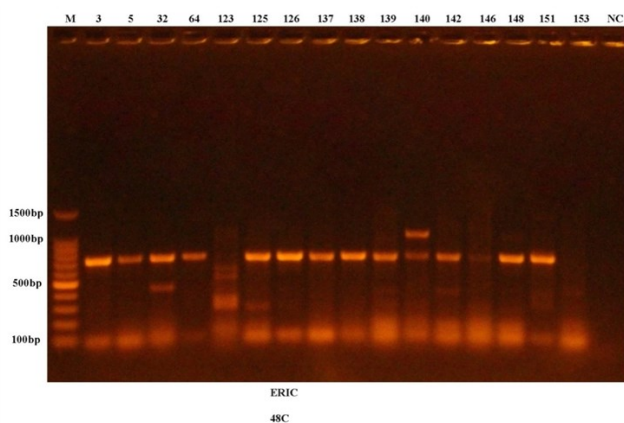


Fig. 4. Showing the results of the amplification of ERIC gene of bacterial species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker (Lanes 3-153 resemble PCR products)

sistance (Mohammadipour *et al.*, 2017).

Other studies showed the presence of a small percentage of this class, ranging from 0 to 10. Rowe-Magnus *et al.*, (2001) indicated the presence of high resistance in gram-negative bacterial isolates that contain integron III (Dayih, 2018). Samir *et al.* (2023) showed isolated *K.pneumoniae* has Integron class III 20 (35%). Continuous surveillance and characterization of the integrons and their related gene cassettes could aid in the control of antibiotic resistance by designing preventive steps to stop the emergence of resistant strains (Jahanbin *et al.*, 2020).

Molecular typing by ERIC-PCR

The ERIC-PCR produced the banding pattern of 16 *K.pneumoniae* isolates (from different specimens and locations) indicated the sizes ranging from (300-8000) bp were frequent among those isolated (Fig. 4). The ERIC type of the isolates was submitted to computerized analysis to evaluate genetic relationships based on the Diced coefficient. *K. pneumoniae* genotype iden-

Table 7. Detected mutation pattern in *K. pneumoniae* PCR products of 16S rRNA amplicons in comparison to its matching NCBI reference sequences (GenBank accession no. KP244269.1). The letter "g" stands for "genomic"

Sample No.	Native	Allele	Position of nucleic acid in PCR fragment	Variant summary in PCR fragment	Position of nucleic acid in the reference genome	Type of point mutation
S1	C	T	134	g.134C>T	134	Transition
S1	T	G	408	g.408T>G	408	Transversion
S1	A	G	410	g.410A>G	410	Transition
S1,S3	G	T	426	g.426G>T	426	Transversion
S2	A	G	826	g.826A>G	826	Transition
S2,S3	G	-	782	g.782Gdel	782	Deletion
S2,S3	G	-	841	g.841Gdel	841	Deletion
S2,S3	A	G	900	g.900A>G	900	Transition
S2,S3	G	-	905	g.905Gdel	905	Deletion
S2,S3	A	G	969	g.969A>G	969	Transition
S2,S3	A	-	971	g.971Adel	971	Deletion
S3	T	A	408	g.408T>A	408	Transversion

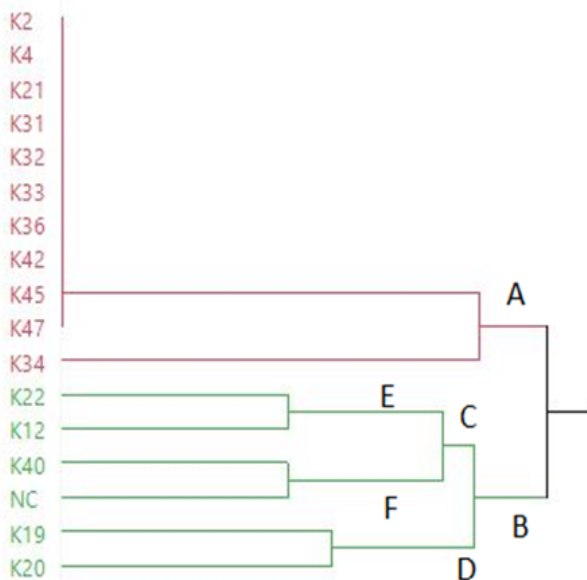


Fig. 5. Dendrogram of 16 *K. pneumoniae* isolates based on the results of ERIC-PCR method

tified 14 distinct ERIC types, yet two strains were un-typeable due to no banding patterns following PCR reaction and electrophoresis.

The ERIC dendrogram of *K. pneumoniae* isolates showed two major clusters, A and B. Cluster A had the largest 11 isolates, whereas clustered B was smaller than cluster A, as shown in Fig. 5. It is made up of five isolates, which were also split into two groups sub-clusters, C and D. C was also divided into two sub-clusters, E and F.

Relationship between antibiotic susceptibility test and phylogeny among isolates

Phylogenics B, D and E had higher resistance patterns to Meropenem than other phylogenetic were 100%. While phylogenetic A, C and F were sensitive to Meropenem. The result is not statistically significant among phylo-

genics ($p. value \leq 0.05$) (as shown in Fig.6). All phylogenics were resistant to Ceftazidame 100%.The results appear statistically significant among phylogenics($p. value \leq 0.05$) (as shown in Fig. 7.

Phylogenics B, D and E had higher resistance patterns to amikacin than other phylogenetic were 100%, while phylogenics A, C and F were sensitive to amikacin. The result appears not statistically significant among phylogenics ($p. value \leq 0.05$), (as shown in Fig. 8.

All phylogenetic resistance to Amoxicillin –clulvanic acid was 100%. The result appears statistically significant among phylogenics($p. value \leq 0.05$) (as shown in Fig.9. Phylogenetic F, D, and B had higher resistance pattern to Trimethopri-sulfamethoxazole than other phylogenics. The result appears to be no statistically significant among phylogenics. ($p. value \leq 0.05$) (as shown in Fig. 10.

Phylogenics E, D and B had higher resistance patterns to Levofloxain than other phylogenics The result appears not statistically significant among phylogenics ($p. value \leq 0.05$) (as shown in Fig. 11.

Genetic identification *Klebsiella pneumoniae* on 16S rRNA gene sequences

Three samples (assigned S1, S2, and S3) were included in the current examination. These specimens were examined for their ability to amplify the 16S rRNA sequences partly. Thus, the variation of these ribosomal sequences can be used for the description of these bacterial species due to the possible ability of rRNA sequences to adapt to variable genetic diversity. Sequenced reactions showed their accurate identification With the amplicons of 1312 bp. After performing NCBI blastn on these PCR amplicons (Zhang *et al.*, 2000).The NCBI BLASTn engine reported over 99% sequence similarity among the sequenced specimens and the *K. pneumoniae* referenced target sequenc-

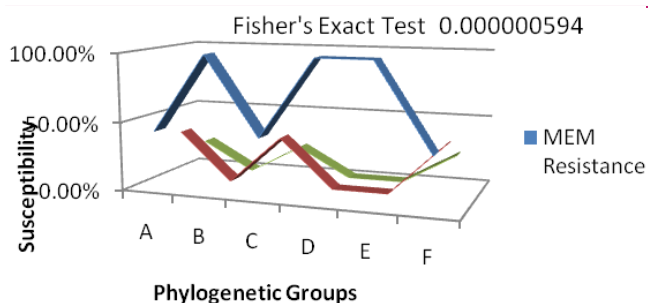


Fig. 6. Comparison of Meropenem susceptibility among phylogeny of *K. pneumoniae*

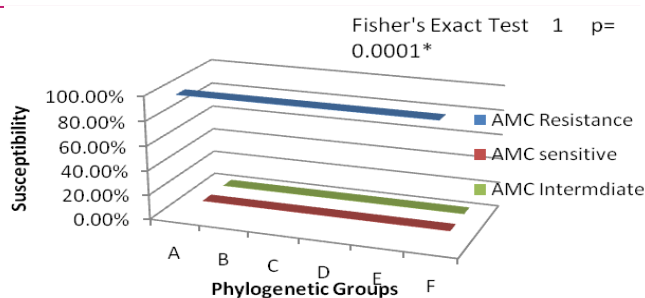


Fig. 9. Comparison of Amoxicillin-clavulanic acid susceptibility among phylogeny of *K.pneumoniae*

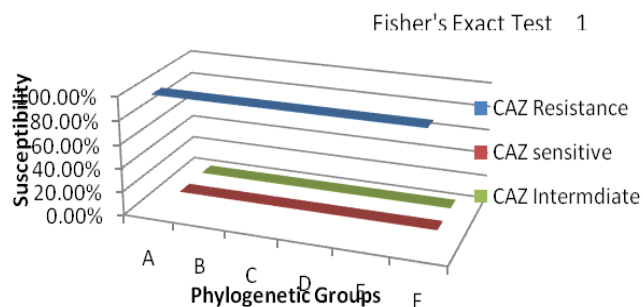


Fig. 7. Comparison of Ceftazidame susceptibility among phylogeny of *K.pneumoniae*

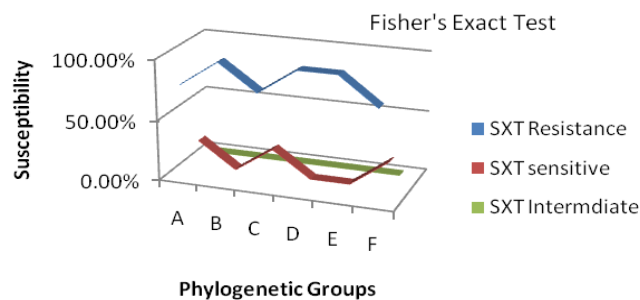


Fig. 10. Comparison of Trimethopri-sulfamethoxazole susceptibility among phylogeny of *K.pneumoniae*

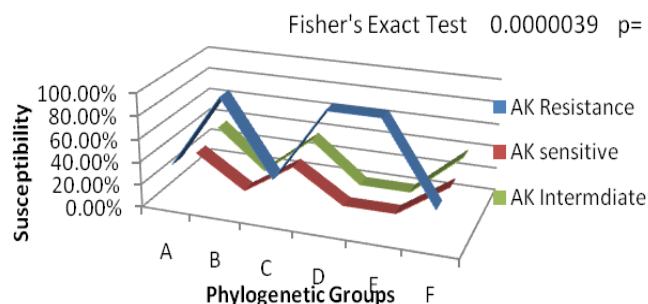


Fig. 8. Comparison of Amikacin susceptibility among phylogeny of *K.pneumoniae*

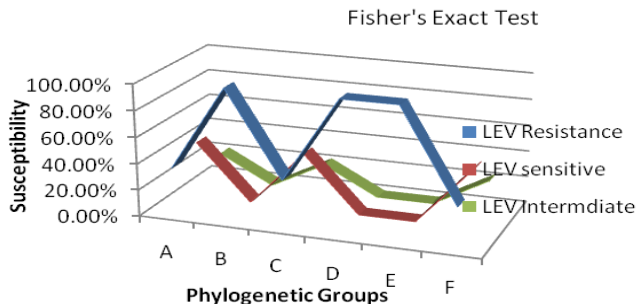


Fig. 11. Comparison of Levofloxacin susceptibility among phylogeny of *K.pneumoniae*

es. The recovered PCR fragments' exact locations and other information were determined by comparing the obtained nucleic acid sequence of these specimens to the returned nucleic acid sequences (GenBank acc. KP244269.1). The complete length of the target loci was determined using the NCBI server, and the start and end sites of the targeting loci have been confirmed with the most similar bacterial targets.

The findings of the sequenced demonstrated the presence of twelve nucleic acid variations (134C>T, 408T>G, 408T>A, 410A>G, 426G>T, 782Gdel, 826A>G, 841Gdel, 900A>G, 905Gdel, 969A>G, and 971Adel), that were variably distributed in S1 – S3 samples compared with the reference sequences of *Klebsiella pneumoniae* (GenBank acc. no. KP244269.1) as shown in Table 7.

The identified variants showed three different patterns of biological diversity in the investigated S1, S2, and S3, as some observed variants showed exclusive existence in each of these isolates. In contrast, other identified variants showed a common existence in more than

one sample. These distributions were observed in S1, S2, and S3 samples isolated from blood, urine, and burn sources.

Conclusion

The present study concluded that *K. pneumoniae* has become common in Baquba Teaching Hospital in Iraq and is one of the leading causes of infection outbreaks. There was a significant occurrence of *K. pneumoniae*, which was resistant to various antibiotics and a prolific ESBL generator. Resistance to antibiotics may enhance the ability of some bacterial species to form biofilms. Biofilm-forming *K. pneumoniae* isolates were resistant to many antibiotics in this investigation. The findings indicated a probable connection between medication resistance and biofilm formation among clinical isolates of *K. pneumoniae*. This may be a significant problem in treating *K. pneumoniae* associated infections. Additional studies could expand on new possibilities for avoiding nosocomial *K. pneumoniae* infections.

Eric, Integron I, and Integron II impotence virulence factors for antibiotic resistance were more resistant to nine different classes of antibiotics in I infection, especially beta-lactamase inhibitor combinations, cephalosporin GIII, tetracycline, sulfonamides and floral pathway antagonists and contained fewer foraminoglycosides, fluoroquinolones and carbamates.

Conflict of interest

The authors declare that they have no conflict of interest.

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