Iraqi Journal of Biotechnology, 2026, Vol. 25, No. 1, 326-338

Detection of *Acrab, Tolc, Mdtk* Genes and Biofilm Forming in *Klebsiella pneumoniae* Isolated from Different Cases

Douaa Rashed Hamady*, Rana Mujahid Abdullah

*Department of Biology, College of Education For pure Sciences (Ibn Al–Haitham), University of Baghdad, Baghdad, Iraq.

Received: October 29, 2024 Accepted: November 28, 2024 Published March 30, 2026

Abstract

Background: *Klebsiella pneumoniae* is a clinically important opportunistic pathogen associated with a wide range of human infections. Its ability to develop multidrug resistance and form biofilms is largely mediated by efflux pump systems, which significantly limit the effectiveness of antimicrobial therapy. **Objective:** This study aimed to investigate the prevalence of the efflux pump genes *acrAB*, *TolC*, and *mdtK* and to evaluate their association with biofilm formation in clinical isolates of *K. pneumoniae*. Materials and **Methods:** A total of 130 clinical samples were collected and cultured on selective media, followed by identification using standard biochemical tests and confirmation by 16S rRNA gene detection. Antibiotic susceptibility was assessed using standard methods. Biofilm formation was evaluated phenotypically, and the presence of efflux pump genes (*acrAB*, *TolC*, *mdtK*) was determined by molecular techniques. **Results:** Out of 130 clinical samples, 50 (38.4%) were identified as *K. pneumoniae*, with the highest isolation rate from urine samples (50%), followed by sputum (28%), wounds (12%), blood (6%), and burns (4%). The isolates exhibited high resistance rates to multiple antibiotics, including trimethoprim/sulfamethoxazole (78%), ciprofloxacin (68%), tobramycin (66%), and imipenem (64%). All isolates demonstrated biofilm-forming ability, with 62% classified as strong, 26% as moderate, and 12% as weak biofilm producers. Molecular analysis revealed that *acrAB* and *TolC* genes were present in 100% of isolates, while *mdtK* was detected in 70%. **Conclusion:** The widespread presence of efflux pump genes and strong biofilm-forming capacity among *K. pneumoniae* isolates likely plays a crucial role in their multidrug resistance and reduced susceptibility to commonly used antibiotics.

Keywords: *Klebsiella pneumoniae*, *acrAB*, *TolC*, *mdtk*, Biofilm.

Corresponding author E-mail: (douaarashed@yahoo.com , dr.ranamujahid@gmail.com).

Introduction

K.pneumoniae is Gram-negative (1, 2). It belongs to the family Enterobacteriaceae (3). Its length ranges from (0.6-6) micrometers, while its width ranges from (0.3-1) micrometers, arranged singly, in pairs, or in non-motile chains (4). *Klebsiella* is a lactose fermentation that contains a prominent polysaccharide capsule with a wide thickness, which gives a mucoid appearance to the colonies on agar plates. *K. pneumoniae* is divided into three different groups based on the small number of pneumoniae gene sequences. These ethnic groups have been reclassified into three species including *K. pneumoniae* /KpI; *K.quasipneumoniae* /KpII and *K. variicola* /KpIII (KpI, KpII, KpIII) which can infect humans (5).

On MacConkey agar, its colonies appear large, pink, round, and mucous, indicating lactose fermentation and acid production. It also grows rapidly on normal media. It also grows rapidly on CHROMO agar, nutrient agar, blood agar, eosin methylene blue agar (EMB). It is commonly found in water, plants, soil and from the mucosal surfaces of mammals (6).

While blood agar, grey-white, non-hemolytic, mucous colonies. However, some researchers have reported that *K. pneumoniae* isolates have been shown to be able to produce hemolysin on blood agar and appear as dark pink, non-mineralized mucous colonies on EMB. On the other hand, *K. pneumoniae* *K. pneumoniae* is ubiquitous in nature and can be found in soil, sewage, water, plants, and on the mucosal surfaces of mammals (9). The virulence factors of *K. pneumoniae* isolates are mainly mediated by four major groups of virulence factors including: capsular polysaccharide (CPS), lipopolysaccharide (LPS), fimbriae (types 1 and 3), and siderophores. A number of additional factors such as urease production, cytotoxins, enterotoxins, hemolysin, protein-tyrosine kinase, heat labile and heat stable endotoxins, and phosphotyrosine-protein phosphatase are also involved. *K. pneumoniae* forms biofilms as a key step in pathogenesis. Biofilms are organized groups of bacterial cells embedded within a matrix that self-produces extracellular polymeric substance (EPS), adhering to abiotic or biological surfaces (10). One of the most important features of the biofilm state is that antibiotic-resistant biofilms can be up to 1000 times more resistant to antibiotics than planktonic cells (11). *AcrAB* and *OqxAB* genes are the most investigated efflux pumps in *K. pneumoniae* with respect to antibiotic resistance. Efflux pumps have been reported as one of the mechanisms responsible for antibiotic resistance in biofilm structures (12). The study aimed to investigate the prevalence of *acrAB*, *TolC*, and *mdtk* and their ability to form biofilms of *K. pneumoniae* isolates.

Materials and Methods

Isolation and identification of *K. pneumoniae*

One hundred thirty clinical samples from (Al-Karkh General Hospital, Al-Imamain Al-Kadhimiya Hospital, Al-Horook Hospital, Ghazi Al-Hariri Hospital) hospitals from different clinical sources including (urine, sputum, blood, wounds, burns) were collected from 15/10/2023 to 25/12/2023. Then the sample was placed on MacConkey agar, blood agar, methylene eosin blue agar, and chromium agar, and then incubated for 24 hours at 37°C. Then the samples were examined in terms of color, size, shape, hemolytic activity, and staining. Then the pure colony was transferred to nutrient agar for preservation and other biochemical tests including indole, methyl red, Voges Proskauer, citrate use, catalase, oxidase, and urease, in addition to examining their movement. The confirmed cultures were preserved in brain and heart broth including 20% glycerol and stored at -20°C for further study.

The ethical approval was obtained from the College of Education for Pure Science (Ibn Al-Haitham) at the University of Baghdad and the Iraqi Ministry of Health and Environment no. (39891) on 19/10/2023.

Antibiotic sensitivity test

The susceptibility testing of bacterial isolates to some antibiotics from different groups was performed using Vitek 2 Compact system using AST-GN cards (Antibiotic Susceptibility Test Card) containing 16 antibiotics at different concentrations distributed in 64 wells. All bacterial isolates were tested for their susceptibility to Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Minocycline, Trimethoprim/ Sulfamethoxazole and the results were taken according to Mustafa and Abdullah (13).

Quantification of biofilm formation using microtiter plate method

The ability of (50) bacterial isolates to form biofilm was investigated using a 96-well microtiter plate as reported by Gomaa (14) with some modifications as follows:

1. The tubes containing the brain heart infusion broth medium with 3% sucrose

K. pneumoniae appears as metallic blue colonies on CHROM agar. *K. pneumoniae* is responsible for urinary tract infections (UTIs), septicaemia, pneumonia (7), wound infections, nosocomial infections in intensive care units and septicaemia in new-borns (8).

2. were inoculated with pure bacterial
3. medium for a period of (18-24) hours (modified).
4. Comparing the turbidity of the bacterial suspension with the standard McFarland turbidity which gives approximately 5×10^8 cells/ml.
5. Place in the holes of the first column of the microtiter plate (200) microliters of brain heart infusion broth only without the bacterial suspension (negative control), and place in the other three holes of the first column (200) microliters of brain heart infusion broth inoculated with the bacterial suspension (at a rate of three replicates for each isolate) and in the same way for all bacterial isolates until the last column.
6. The microtiter plate was incubated for 24 hours at a temperature of 37 °C, after which the contents of the holes were disposed of and washed with Phosphate Buffer Saline solution at a rate of three times, then emptied and left to dry at room temperature.
7. Place 200 microliters of 1% crystal violet dye into each well of the microtiter plate for 15 minutes to stain the formed biofilms, then wash with distilled water and leave to dry at room temperature.
8. The crystal violet dye attached to the biofilms is removed by adding 200 microliters of 95% ethanol to each well. Then, the absorption of the crystal violet color (Optical Density (OD)) is measured in each well using an ELISA device at a wavelength of 570 nm.
9. The ability of the bacterial isolates to form biofilms is determined into four groups: non-biofilm-forming, weakly forming, medium-forming, and strong-forming, colonies growing on MacConkey's depending on the OD of the bacterial biofilms

- OD ≤ ODc non-film forming
- ODc < OD ≤ 2xODc Weakly film forming
- 2 xODc < OD ≤ 4xODc Moderately film forming
- xODc < OD 4 Strongly film forming.

DNA extraction

DNA was extracted from (50) bacterial isolates grown on MacConkey agar using a DNA purification kit (Promega, USA) according to the manufacturer's instructions. DNA was measured using a Quantus Fluorometer, and DNA was screened for *16S rRNA* genes *acrAB*, *TolC* and *mdtk* using primers (Macrogen, Korea) listed in Table (1). The dried primer was dissolved in sterile distilled deionized water to a concentration of 100 pmol/µl and then diluted to 10 pmol/µl according to the manufacturer's instructions.

Amplification of *16SrRNA, acrAB, TolC* and *mdtk* genes by PCR

A Thermal Cycler (USA/Thermo Fishe Scientific) was used to perform the PCR reaction. The final PCR reaction mixture of 24 µl contained 12.5 of Go Taq Green Master Mix, 1 µl of each of the forward and reverse primers, 7.5 µl of sterile distilled deionized water, 2 µl of template DNA as mentioned in Table (2). The PCR amplification of the four genes *16SrRNA, acrAB, TolC* and *mdtk* was verified under the conditions mentioned in Tables (3) and (4) by electrophoresis on a 2% agarose gel at 100 V for 60 min using a ladder (100-1500 base pairs) as a molecular weight marker, and monitoring the PCR product using a UV-Transilluminator under UV light with a wavelength of 320 nm (15).

Table 1. The primer sets used for PCR assay in this study.

Genes	Primer Sequence (5´- 3´)	Product size	Reference
16SrRNA	F: GCAAGTCGAGCGGTAGCACAG	260	(16)
	R: CAGTGTGGCTGGTCATCCTCTC		
acrAB	F: ATCAGCGGCCGGATTGGTAAA	312	(17)
	R: CGGGTTCGGGAAAATAGCGCG		
toIC	F: ATCAGCAACCCCGATCTGCGT	527	(17)
	R: CCGGTGACTTGACGCAGTCCT		
mdtk	F: GCGCTTAACTTCAGCTCA	453	(17)
	R: GATGATAAATCCACACCAGAA		

Table 2. Polymerase chain reaction mixture (PCR Products).

Master mix components	Volume(µl)
Master Mix	12.5
Forward primer	1
Reverse primer	1
Nuclease Free Water	7.5
DNA	2
Total volume	24

Table 3. The optimal conditions for amplifying 16S rRNA by PCR.

PCR Steps	Temperature (°C)	Time	Cycles' Number
Initial Denaturation	95	3min	1
Denaturation	95	45sec	28
Annealing	58	sec45	
Extension	72	1 min	
Final Extension	72	min5	1

Table 4. The optimal conditions for amplifying acrAB, TolC and mdtk by PCR.

PCR Steps	Temperature (°C)	Time	Cycles' Number
Initial Denaturation	94	1min	1
Denaturation	94	30sec	35
Annealing	52	sec30	
acrAB			
TolC	52		
mdtk	43		
Extension	72	sec90	
Final Extension	72	Min10	1

Statistical analysis

The Pearson's correlation coefficient was utilized to determine the correlation between Biofilm formation and MIC of antibiotics.

Results

Distribution of *K. pneumoniae* Isolates

Fifty bacterial isolates of *Klebsiella pneumoniae* were obtained from different clinical sources. The highest proportion was isolated from urine samples (25/50, 50%), followed by sputum (14/50, 28%), wounds (6/50, 12%), blood (3/50, 6%), and burns (2/50, 4%), as shown in Table (5).

Table 5. The source, number and percentages of K. pneumoniae bacteria isolates.

Isolates source	Isolates number	%
Urine	25	50
sputum	14	28
wound	6	12
blood	3	6
Burns	2	4
Total number	50	100

Antibiotic sensitivity of *K. pneumoniae*

Antibiotic susceptibility testing revealed varying resistance rates among the isolates. Resistance to imipenem was observed in 64% of isolates, meropenem in 60%, amikacin in 40%, gentamicin in 56%, tobramycin in 66%, ciprofloxacin in 68%, minocycline in 34%, and trimethoprim/sulfamethoxazole in 78%.

Detection of biofilm production by *K. pneumoniae*

All *K. pneumoniae* isolates (100%) demonstrated the ability to form biofilms using the microtiter plate method. Strong biofilm formation was detected in 31 isolates (62%), moderate biofilm formation in 13 isolates (26%), and weak biofilm formation in 6 isolates (12%).

Correlations between Biofilm formation and MIC of antibiotics

Statistical analysis indicated a strong positive correlation between biofilm formation and the minimum inhibitory concentration (MIC) of antibiotics (r = 0.816), as illustrated in Figure (6).

Figure 6. The relationship between Biofilm formation and MIC of antibiotics.

		Biofilm	MIC
Biofilm	Pearson Correlation	1	0.816
MIC	Pearson Correlation	0.816	1

.

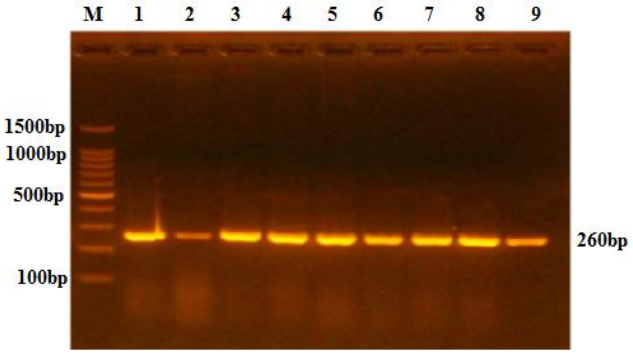
Detection of *16SrRNA* in *K. pneumoniae*

The results of the polymerase chain reaction were confirmed by analyzing the bands using gel electrophoresis and by comparing with the multiplexed bands and the DNA ladder size indicator. The results showed that all *K. pneumoniae* isolates (50 isolates) at a rate of 100% possess the *16SrRNA* gene as shown in Figure (1).

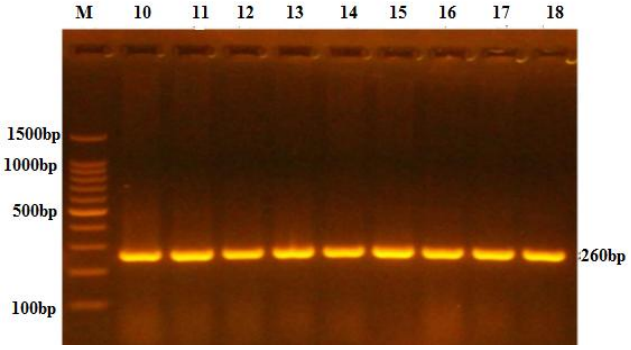
Genetic detection of some efflux system genes of *K. pneumoniae*

The results of the current study displayed that 50 bacterial isolates (100%) belong to *K. pneumoniae* that possess the *acrAB* and *TolC* genes. After comparing the multiple bands with the ladder size indicator bands, it was found that all of them have a molecular weight of 312 and 527 base pairs respectively as shown in Figure (2, 3).

The results of the current study displayed that 35 bacterial isolates (70%) belong to *K. pneumoniae* that possess the *mdtk* gene. After comparing the multiple bands with the ladder size indicator bands, it was found that all of them have a molecular weight of 453 base pairs, as shown in Figure (4).



A



B

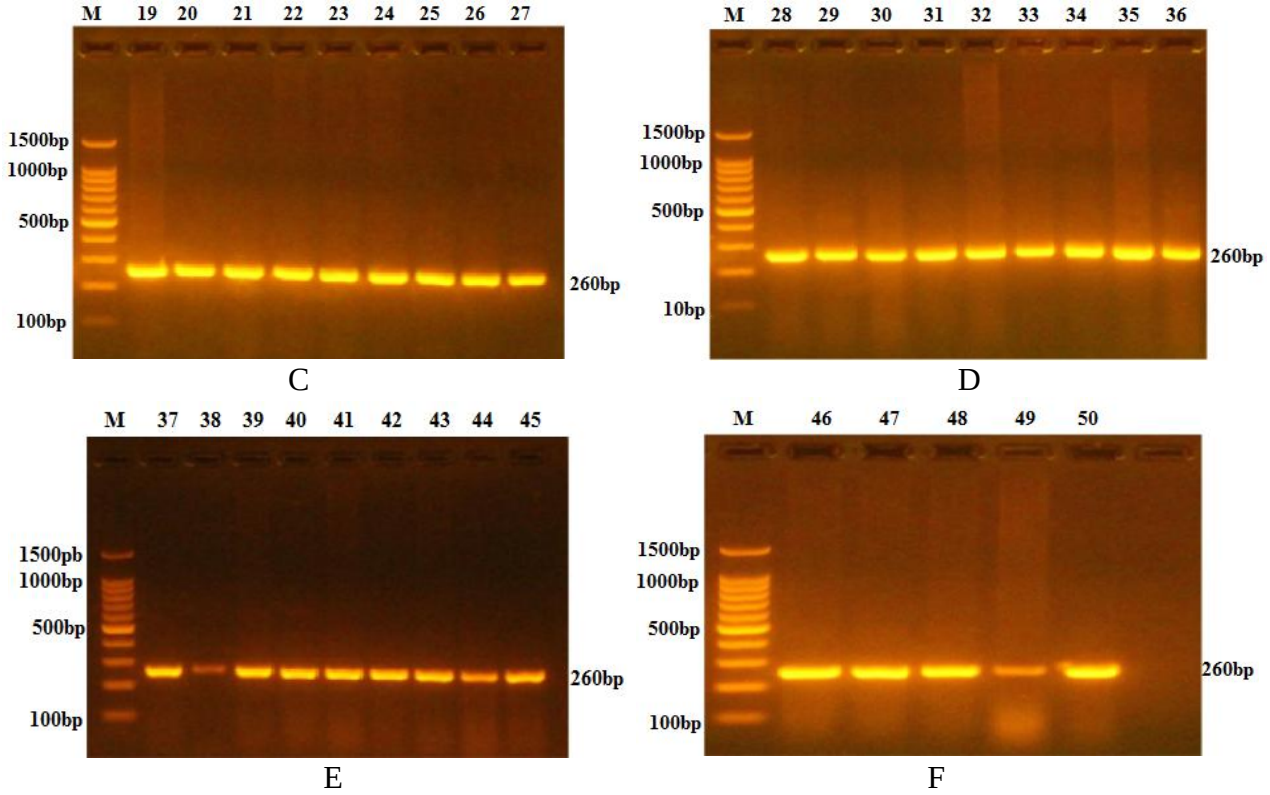
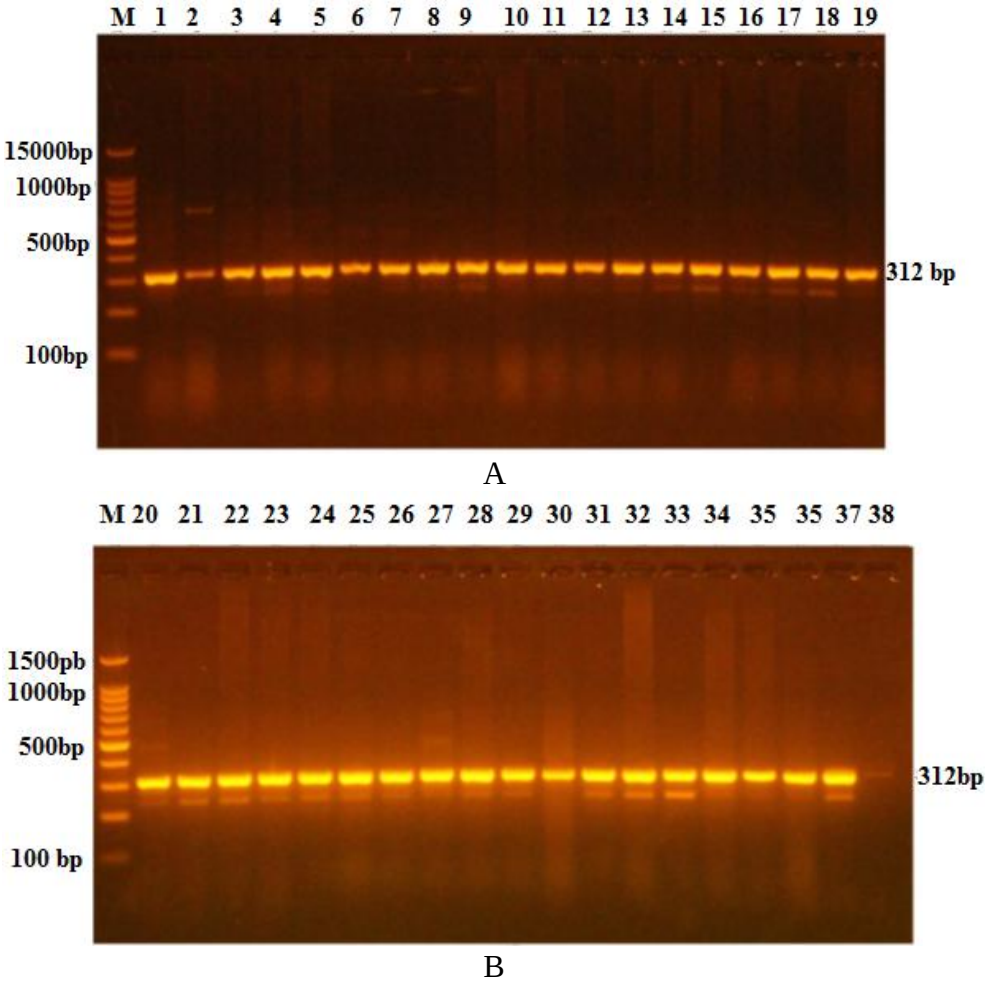
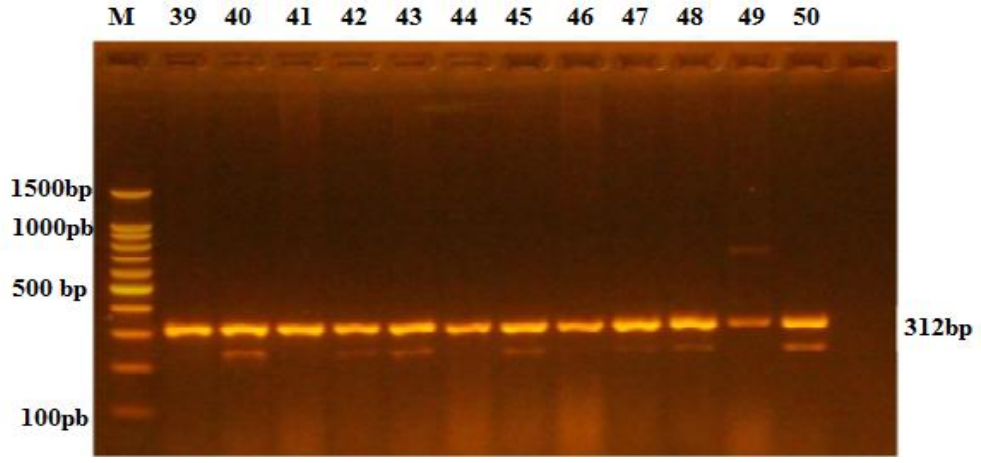


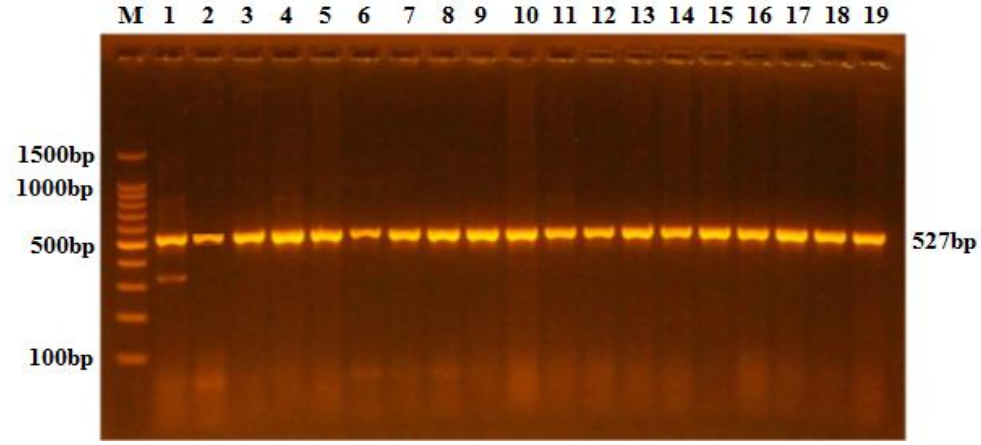
Figure 1. Results of the amplification of 16 S genes (260bp) of bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lines (1-9) in Figure (A1), (10-18) in Figure (B1), (19-27) in Figure (C1), (28-36) in Figure (D1), (37-45) in Figure (E1), (46-50) in Figure (F1).



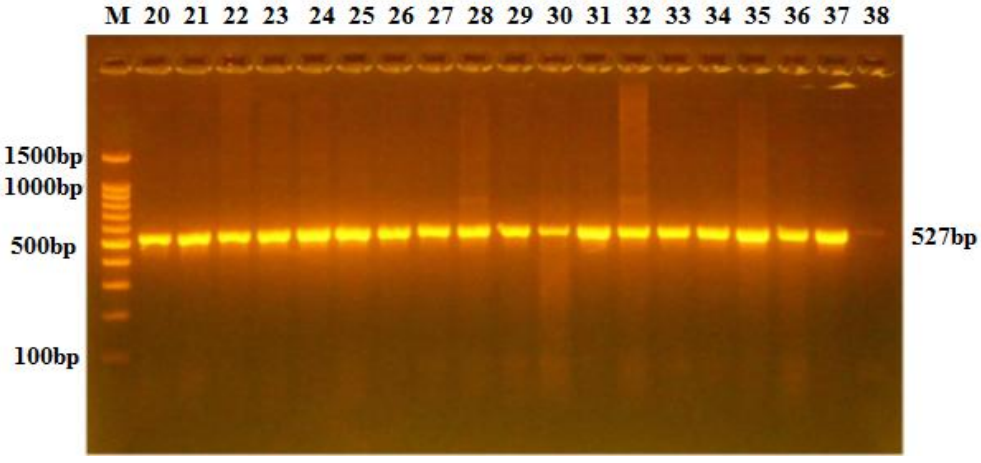


C

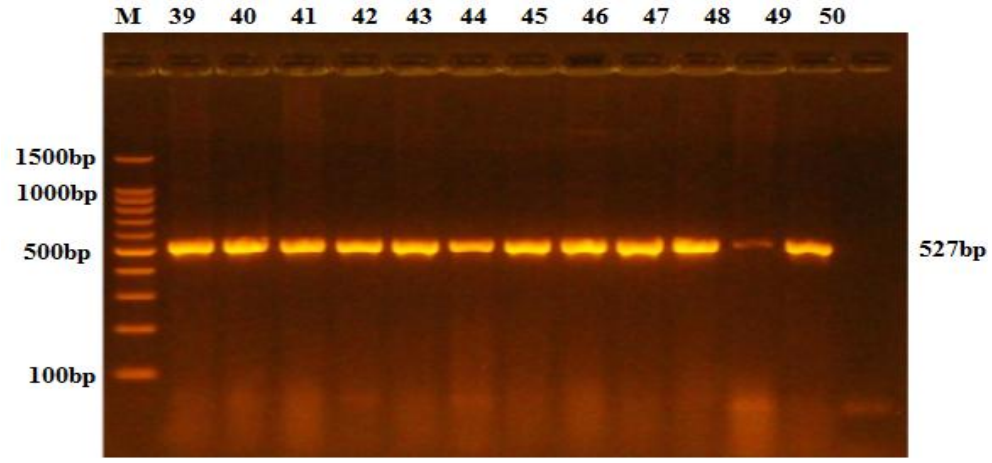
Figure 2. Electrophoresis of the PCR product of the *acrAB* gene (312 base pairs) of *K. pneumoniae* isolates on agarose gel at a concentration of (0.2%) and a potential difference of 100 volts for 60 minutes. Line M (volume index) 100–1500 base pairs. Lines (1-19) in Figure (A2), (20-38) in Figure (B2), (39-50) in Figure (C2).



A

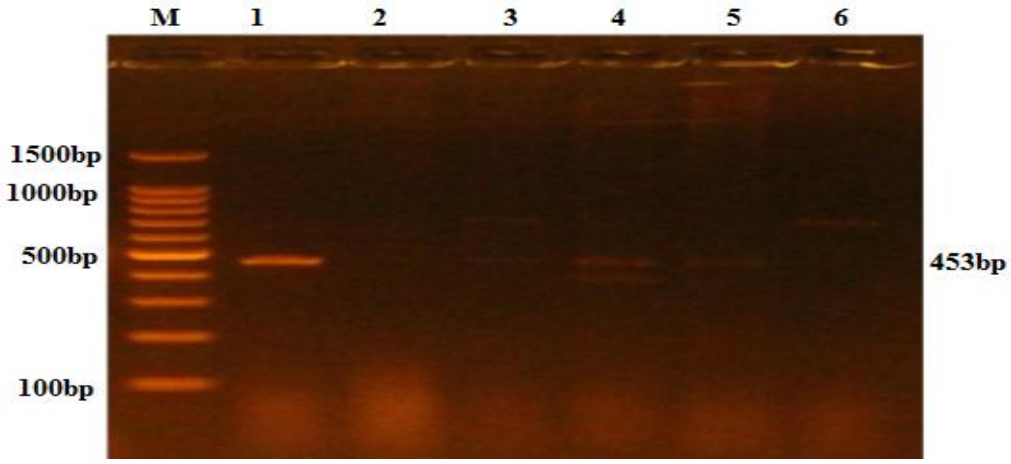


B

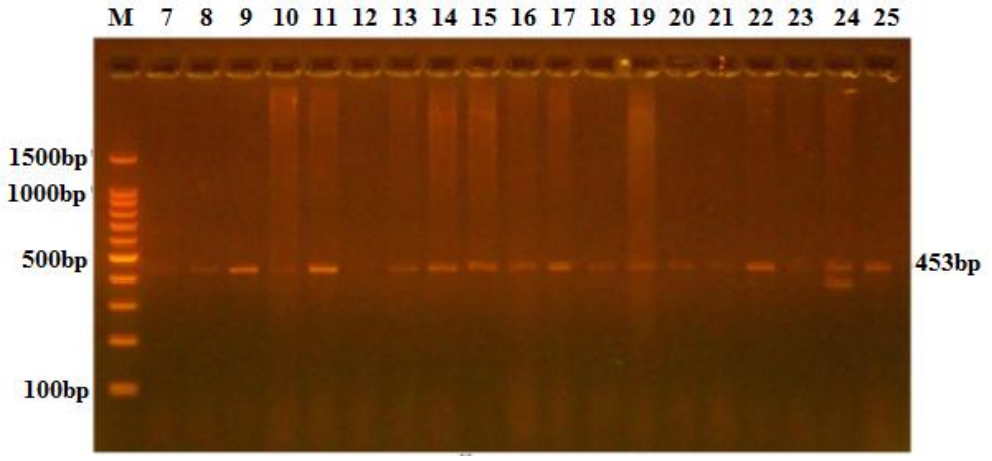


C

Figure 3. Electrophoresis of the PCR product of the *TolC* gene (527 base pairs) of *K. pneumoniae* isolates on agarose gel at a concentration of (0.2%) and a potential difference of 100 volts for 60 minutes. Line M (volume index) 100–1500 base pairs. Lines (1-19) in Figure (A3), (20-38) in Figure (B3), (39-50) in Figure (C3).



A



B

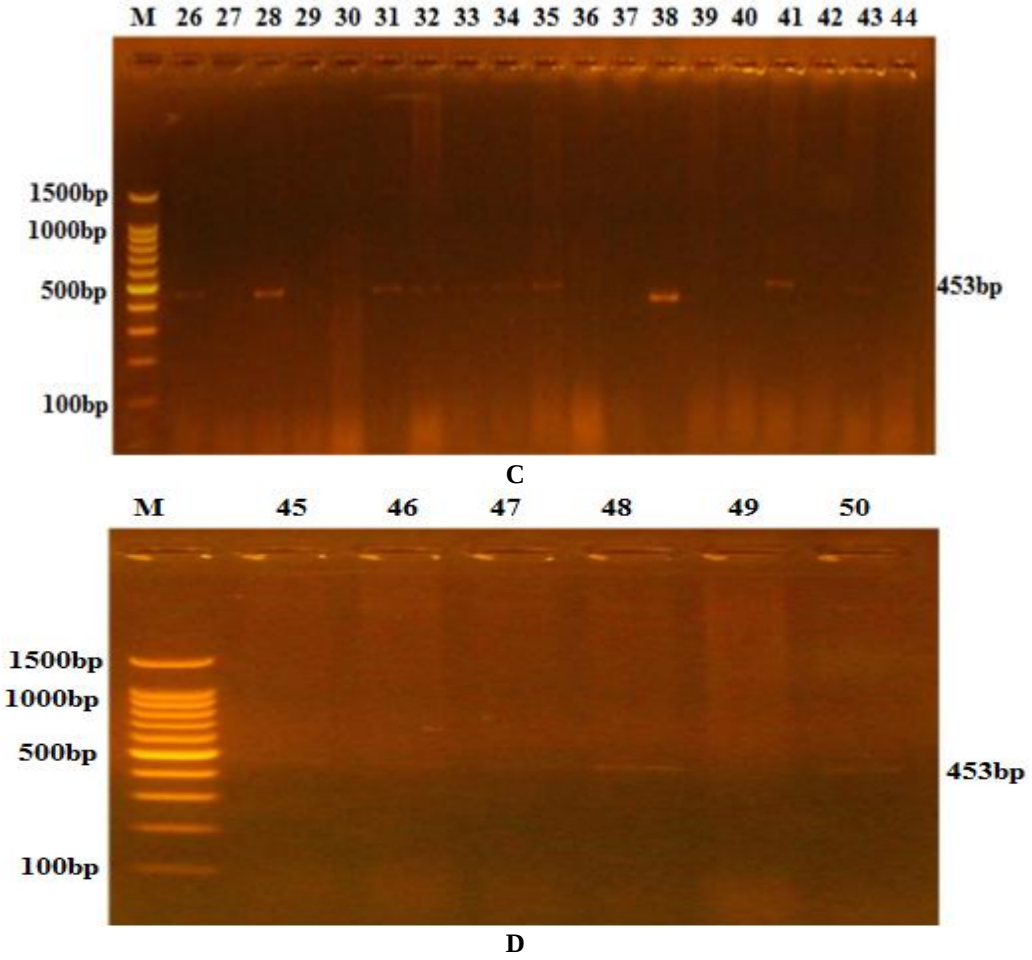


Figure 4. Electrophoresis of the PCR product of the *mdtk* gene (453 base pairs) of *K. pneumoniae* isolates on agarose gel at a concentration of (0.2%) and a potential difference of 100 volts for 60 minutes. Line M (volume index) 100 – 1500 base pairs. Lines (1-6) in Figure (A4), (7-25) in Figure (B4), (26-44) in Figure (C4), (45-50) in Figure (D4).

Discussion

The findings of the present study show that *Klebsiella pneumoniae* strains sampled from various clinical sources show the pathogen's strong association with the clinical setting of urinary tract infections. As shown in previous investigations, the prevalence of urine samples from patients with *K. pneumoniae* indicates that this organism has adapted to become a foremost uropathogen due to its capacity for attachment to the uroepithelia, and for creating biofilms that can survive within the urinary system (18-20). The differences in isolation frequency for sputum or wound sources could be attributed to a variety of demographic factors related to each patient, the departments of the respective hospitals, and local epidemiological conditions (21-23).

In this study, it was found that *K. pneumoniae* is highly resistant to many different classes of antibiotics, and this demonstrates the alarming increase of multi-drug resistance in *K. pneumoniae*. Of great concern is the resistance to carbapenems such as imipenem and meropenem as they are usually the last option available for treating the most severe infections caused by Gram-negative organisms (24, 25). In addition, there have been several other studies describing similar resistance patterns indicating that carbapenem-resistant *K.pneumoniae* is widespread in hospitals (26-28). The resistance of *K.pneumoniae* to aminoglycosides and fluoroquinolones is likely related to a number of mechanisms including aminoglycoside-modifying enzymes, mutations in the target sites, plasmid-mediated resistance determinants, and decreased permeability of outer membranes (29-31).

The ability of bacteria to produce biofilm, which is an important virulence factor, plays a major role in the ability of these organisms to persist and withstand being treated with antimicrobials. In this study, the majority of *K. pneumoniae* isolates also produced biofilm, while most of the isolates were classified as strong biofilm producers. The results of this study are consistent with prior research that identified a high prevalence of biofilm-producing *K. pneumoniae*, especially among isolates from patients with urinary tract infection (UTI) or that were obtained from medical devices (32-34). In addition to being a physical and physiological barrier, biofilms are barriers to the penetration of antibiotics and protect bacterial cells against host immune defences, contributing to higher rates of treatment failure and chronic infections (35-37).

The fact that there is a good correlation between how well an organism forms its biofilm and how high a dose of antimicrobial is necessary to kill it supports the concept that biofilms provide a mechanism for the organism to develop resistance to an antimicrobial agent. Compared to planktonic cells, biofilm-associated cells show different gene expression patterns from planktonic (non-biofilm forming) cells, have decreased metabolic rates, and can tolerate much higher concentrations of antibiotics (38-40). In addition to this, multiple studies have shown correlations between biofilm production and multi-drug resistance in clinical isolates of *K. pneumoniae*, supporting the clinical significance of the biofilm-mediated mechanism for developing resistance (33-35).

The *acrAB* and *TolC* efflux pump genes were shown to be present in all strains, along with the high occurrence of the *mdtK* gene. The AcrAB-TolC system is one of the most well-characterized mechanisms used by *Klebsiella pneumoniae* to evince antibiotic resistance. This system functions to confer resistance to a variety of different classes of antibiotics (β-lactams, fluoroquinolones, tetracyclines, and chloramphenicol) (41-43). The extensive distribution of these genes amongst multidrug-resistant isolates has been previously reported in regional and international studies, demonstrating their important participation in antimicrobial resistance (44-47).

MdtK, while detected at a significantly lower frequency than acrAB and TolC, is still present in a large percentage of strains, suggesting it plays a role in quinolone and related antimicrobial resistance. It is believed that the MdtK efflux pump, which is a member of the MATE family, results in less accumulation of antibiotics inside the cell and allows bacteria to survive better when exposed to antimicrobials (48, 49). The overall differences in the frequency of *mdtK* across multiple studies may be attributed to geographic differences, differing patterns of antibiotic use, and the selective pressure applied to pathogens in the hospital environment (50).

The combination of multidrug resistance, biofilm forming ability and multiple efflux pump genes, as exhibited by *K. pneumoniae* isolates in our study account for very high resistance rates found in this study. Our report matches findings reported previously that the antimicrobial resistance of *K. pneumoniae* is a complex process resulting from a combination of phenotypes and genetic determinants (51, 52). Understanding how these mechanisms interact will help in improving infection control practices, developing guidance to antibiotic stewardship programmes, creating alternative therapeutic approaches for treating multidrug resistant *K. pneumoniae* infections.

Conclusion

The high occurrence of *acrAB*, *TolC*, and *mdtk* among *K. pneumoniae* isolates and the capacity to produce biofilm may contribute to their low susceptibility to many antimicrobial agents.

References

1. Abd Al-Rhman,R.M. and Al-Aubydi M.A. (2016). Investigating the Adjuvanticity of *K. pneumoniae* Capsular Polysaccharide with Formalin-Killed S. aureus Against Live S. aureus Infection in Mice. Iraqi Journal of Science, 57(2A): 893-900.
2. Ali, Z.S. and Shami, A.M. (2023). Molecular Study of Siderophore Genesin CarbapenemsResistant *Klebsiella pneumoniae* Isolated from Urinary Tract Infection Patients. Iraqi Journal of Biotechnology, 22(1): 183-192.

3. Khalid, T.M. and Ghaima, K.K. (2022). Molecular Detection of acrAB and oqxAB Genes in *Klebsiella pneumoniae* and Evaluation the Effect of Berberine on their Gene Expression. Iraqi Journal of Biotechnology, 21(2): 124-135.
4. Mustafa, M.S. and Abdullah, R.M. (2020). Role of oqxA and oqxB Genes in the Development of Multidrug Resistant Phenotype among Clinical *Klebsiella pneumoniae* Isolates from Various Cases. Iraqi Journal of Science, 61(8): 1902-1912.
5. Maatallah, M.; Vading, M.; Kabir, M.H.; Bakhrouf, A.; Kalin, M.; Naucler, P., *et al*. (2014). *Klebsiella variicola* is a frequent cause of bloodstream infection in the Stockholm area, and associated with higher mortality compared to *K. pneumoniae.* PloS one, 9(11): e113539.
6. Al-Musawi, A. (2018). Genotypic and phenotypic typing of clinical *Klebsiella pneumoniae* local isolates: M.Sc. thesis. College of Science. Mustansiriyah University.
7. Abdulhasan, G.A. (2015). The biological effect of Rosmarinus officinelis L. essential oil on biofilm formation and some fimbrial genes (fimH-1 and mrkD) of *Klebseilla pneumoniae*. Iraqi Journal of Science, 56(3C): 2553-60.
8. Fodah, R.A.; Scott, J.B.; Tam, H.H., Yan, P.; Pfeffer, T.L.; Bundschuh, R. *et al*. (2014). Correlation of *Klebsiella pneumoniae* comparative genetic analyses with virulence profiles in a murine respiratory disease model. PloS one, 9(9): e107394.
9. Piperaki, E.T.; Syrogiannopoulos, G.A.; Tzouvelekis, L.S.; Daikos, G.L. (2017). *Klebsiella pneumoniae*: virulence, biofilm and antimicrobial resistance. The Pediatric Infectious Disease Journal, 36(10): 1002-5.
10. Zubair, M.; Ashraf, M.; Arshad, M.; Raza, A.; Mustafa, B.; Ahsan, A. (2014). Formation and significance of bacterial biofilms. International Journal of Current Microbiology and Applied Sciences, 3(12): 917-23.
11. Hamady, D.R.; Ibrahim, S.K. (2020). The study on ability of Escherichia coli isolated from different clinical cases to biofilm formation and detection of csgD gene responsible for produce Curli (Fimbriae). Biochemical & Cellular Archives, 20(2).
12. Al-Saadi Z.H.A. and Abdullah, R.M. (2019). Phenotypic and molecular detection of Escherichia coli efflux pumps from UTI patients. Biochemical & Cellular Archives, 19.
13. Mustafa, M.S. and Abdullah, R.M. (2024). Measurement of Some Inflammatory Biomarkers and Genotyping of GramNegative Bacteria Isolated from Acute Leukemia Patients. Iraqi Journal of Science, 65(6): 3057-74.
14. Gomaa, N.A. (2021). Prevalence, antimicrobial resistance, and biofilm formation of *Klebsiella pneumoniae* isolated from human and cows. Zagazig Veterinary Journal, 49(1): 27-41.
15. Mustafa, M. (2020). Prevalence of Quinolones Resistance Proteins Encoding Genes (*qnr* genes) and Co-Resistance with β-lactams among *Klebsiella pneumoniae* Isolates from Iraqi Patients. Baghdad Science Journal, 17(2): 0406.
16. Tayebeh, F.; Amani, J.; Nazarian, S.; Moradyar, M. and Mirhosseini, S.A. (2016). Molecular diagnosis of clinically isolated *Klebsiella pneumoniae* strains by PCR-ELISA. Journal of Applied Biotechnology Reports, 3(4): 501-505.
17. Abbas, H.; Shaker, G.; Khattab, R.; Askoura, M. (2021). A new role of metformin as an efflux pump inhibitor in Klebsiella pneumonia. Journal of Microbiology, Biotechnology and Food Sciences, 11(1):e4232-e.
18. Mustafa, M.S. and Abdullah, R.M. (2020). Investigation for some Aminoglycosides Modifying Enzymes-Encoding Genes and Co-Resistance to Fluoroquinolones among *Klebsiella pneumoniae* Isolates from Different Clinical Cases. Iraqi Journal of Science,61(11): 2866-2878.
19. Razavi, S.; Mirnejad, R. and Babapour, E. (2020). Involvement of *AcrAB* and *OqxAB* efflux pumps in antimicrobial resistance of clinical isolates of Klebsiella pneumonia. Journal of Applied Biotechnology Reports, 7(4): 251-257.
20. Mohammed, A.N.; Al-Rawi, D.F. and Buniya, H.K. (2023). Evaluation of Antibiotic Resistance of *Klebsiella Pneumoniae* Isolated from Patients in Hospitals in Iraq. Acta Microbiologica Bulgarica 39(4): 411-417.
21. Abd Al-Rhman, R.M. and Al-Aubydi M.A. (2015). Determination the relationship between some genetic aspects with the capsule formation for pathogenic *Klebsiella pneumoniae* serotypes K1 &K2. Iraqi Journal of Science, 56(8B): 1385-93.
22. Omar, F. and Ibrahim, A. (2023). The Prevalence of Integron Class I and II Among Multi-Drug Resistance Producing Klebsiella Pneumonia. Iraqi Journal of Agricultural Sciences, 54(3):619-29.
23. Mohammed, B.J.; Saleh, Z.F.; Esmaeel, J.R.; Klaif, S.F,; Jawad, M.S. and Fahad K.H. (2020). Identification and phylogenetic study of *Klebsiella pneumoniae* via molecular-based techniques targeting *16S rRNA*, *magA*, and *rmpA* in camels from Al-Diwaniyah city, Iraq. Annals of Tropical Medicine and Health, 23(5): 218-25.
24. Effah, C.Y.; Sun, T.; Liu, S. and Wu, Y. (2020). Klebsiella pneumoniae: an increasing threat to public health. Annals of Clinical Microbiology and Antimicrobials, 19: 1-9.
25. Al Bshabshe, A.; Al-Hakami, A.; Alshehri, B.; Al-Shahrani, K.A.; Alshehri, A.A.; Al Shahrani, M.B., *et al*. (2020). Rising *Klebsiella pneumoniae* infections and its expanding drug resistance in the intensive care unit of a tertiary Healthcare Hospital, Saudi Arabia. Cureus, 12(8).
26. Krause, K.M.; Serio, A.W.; Kane, T.R. and Connolly, L.E. (2016). Aminoglycosides: an overview. Cold Spring Harbor Perspectives in Medicine. 6(6): a027029.
27. Ajeel, E.A. and Mohammed, R.K. (2022). Genotype and Phenotype Investigation of CTX-M Gene among Multidrug Resistant *Klebsiella*

pneumoniae Isolates. Iraqi Journal of Biotechnology, 21(2): 400-408.
28. Hasan, T.H.; Alasedi, K.K.; Aljanaby, A.A.J. (2021). A comparative study of prevalence antimicrobials resistance *Klebsiella pneumoniae* among different pathogenic bacteria isolated from patients with urinary tract infection in Al-Najaf City, Iraq. Latin American Journal of Pharmacy. 40: 174-178.
29. Hasan, T.; Alshammari, M. and Yousif, H. (2020). Extended Spectrum Beta-Lactamase Producing Klebsiella Pneumonia Isolated from Patients with Urinary Tract Infection in Al-Najaf Governorate–Iraq. International Journal of Advances in Science, Engineering and Technology (IJASEAT), 8(1): 13-16.
30. Albaayit, S.; Al-Khafaji, A. and Radif, H. (2018). Investigation of plasmid-associated fluoroquinolone resistance in nosocomial *Pseudomonas aeruginosa* isolated from infected burn wounds. Journal of Biological Sciences, 18: 514-519.
31. Al-Ruobayiee, M.R. and Ibrahim, A.H. (2023). The Relationship Between OqxAB Efflux Pump and Drug Resistance in *Klebsiella pneumoniae* Isolated from Clinical Sources. Al-Rafidain Journal of Medical Sciences, 1(5).
32. Khoshnood, S.; Akrami, S.; Saki, M.; Motahar, M.; Masihzadeh, S. andDaneshfar, S., *et al*. (2023). Molecular evaluation of aminoglycosides resistance and biofilm formation in *Klebsiella pneumoniae* clinical isolates: A cross-sectional study. Health Science Reports, 6(5): e1266.
33. Vuotto, C.; Longo, F.; Pascolini, C.; Donelli, G.; Balice, M.; Libori, M. *et al*. (2017). Biofilm formation and antibiotic resistance in *Klebsiella pneumoniae* urinary strains. Journal of Applied Microbiology, 123(4): 1003-1018.
34. Ostria-Hernandez, M.L.; Juárez-de la Rosa, K.C.; Arzate-Barbosa, P.; Lara-Hernández, A.; Sakai, F.; Ibarra, J.A., *et al*. (2018). Nosocomial, multidrug-resistant *Klebsiella pneumoniae* strains isolated from mexico city produce robust biofilms on abiotic surfaces but not on human lung cells. Microbial Drug Resistance, 24(4): 422-433.
35. Maharjan, G.; Khadka, P.; Siddhi Shilpakar, G.; Chapagain, G. and Dhungana, G.R. (2018). Catheter-Associated Urinary Tract Infection and Obstinate Biofilm Producers. Canadian Journal of Infectious Diseases and Medical Microbiology. 2018(1): 7624857.
36. Razaq, F.J.A.; Kareem, A.A. and Maklef, E.A. (2023). Molecular Detection of Some Genes Responsible for Biofilm Formation in *Acinetobacter Baumannii* Isolated from Different Sites of Infection. HIV Nursing, 23(2): 510–513.
37. Aubaid, S.H.; Falih, E.S. and Ibrahim, K.S. (2022). Biofilm Formation of Staphylococcus Aureus in Multiple Sclerosis Patients and its Essential Role in the Pathogenicity of the Disease. Journal of Techniques, 4(3): 14-18.
38. Riegman, P.; Becker, K.; Zatloukal, K.; Pazzagli M.; Schröder, U. and Oelmuller, U. (2019). How standardization of the pre-analytical phase of both research and diagnostic biomaterials can increase reproducibility of biomedical research and diagnostics. New Biotechnology, 53: 35-40.
39. Budiarso, T.Y.; Amarantini, C. and Pakpahan, S. (2021). Biochemical identification and molecular characterization of *Klebsiella pneumoniae* isolated from street foods and drinks in Yogyakarta, Indonesia using 16S rRNA gene. *Biodiversitas* Journal of Biological Diversity, 22(12).
40. Mohamed, I.Q. and Al-Taai, H.R.R. (2023). Phylogenetic Analysis of *Klebsiella pneumoniae* Isolated from Nosocomial and Community Infection in Diyala, Iraq. Iraqi Journal of Science. 64(6): 2726-2740.
41. Ahmed, Z.S. and Mawlood, A.H. (2023). Molecular Characterization of Efflux Pump and Porin Related Genes in Multidrug Resistance *Klebsiella pneumoniae* Isolates Recovered from Erbil Hospitals. Journal of University of Babylon for Pure and Applied Sciences, 31(2): 115-127.
42. Ranjbar, R.; Tabatabaee, A.; Behzadi, P. and Kheiri, R. (2017). Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping of Escherichia coli strains isolated from different animal stool specimens. Iranian Journal of Pathology, 12(1): 25.
43. Sun, J.; Deng, Z. and Yan, A. (2014). Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. Biochemical and Biophysical Research Communications, 453(2): 254-267.
44. Mathur, T.; Singhal, S.; Khan, S.; Upadhyay, D.; Fatma, T. and Rattan, A. (2006). Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian Journal of Medical Microbiology, 24(1): 25-29.
45. Wasfi, R.; Elkhatib, W.F. and Ashour, H.M. (2016). Molecular typing and virulence analysis of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from Egyptian hospitals. Scientific Reports, 6(1): 38929.
46. Suresh, K. and Pillai, D. (2024). Prevalence of antimicrobial resistance, biofilm formation, efflux pump activity, and virulence capabilities in multi-drug-resistant *Klebsiella pneumoniae* isolated from freshwater fish farms. Journal of Water and Health, 22(4): 721-734.
47. Al-Dahmoshi, H.; Ali, S.A. and Al-Khafaji, N (2022). Efflux pumps among urinary E. coli and *K. pneumoniae* local isolates in Hilla City, Iraq. *The Global Antimicrobial Resistance Epidemic: Innovative Approaches and Cutting-Edge Solutions*, 239: 264.
48. Alsanie, W.F. (2020). Molecular diversity and profile analysis of virulence-associated genes in some *Klebsiella pneumoniae* isolates. Practical Laboratory Medicine, 19: e00152.
49. Yuhan, Y.; Ziyun, Y.; Yongbo, Z.; Fuqiang, L. and Qinghua, Z. (2016). Over expression of AdeABC and AcrAB-TolC efflux systems confers tigecycline resistance in clinical isolates of

Acinetobacter baumannii and *Klebsiella pneumoniae*. Revista da Sociedade Brasileira de Medicina Tropical, 49(02): 165-1671.

50. Li, X.-Z.; Plésiat, P. and Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clinical Microbiology Reviews, 28(2): 337-418.
51. Ni, R.T.; Onishi, M.; Mizusawa, M.; Kitagawa, R.; Kishino, T.; Matsubara, F.; *et al*. (2020). The role of RND-type efflux pumps in multidrug-resistant mutants of *Klebsiella pneumoniae*. Scientific Reports, 10(1): 10876.
52. Padilla, E.; Llobet, E.; Doménech-Sánchez, A.; Martínez-Martínez, L.; Bengoechea, J.A. and Albertí, S. (2010). *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrobial Agents and Chemotherapy, 54(1): 177-83.