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Molecular study of MDR *Klebsiella pneumonia*genes isolates from urinary tract infections

Wissam Hadi and Baydaa A. Hassan
University of Kufa-Faculty of Science-Department of Biology
Iwnajm@gmail.com

Abstract: This study was completed in laboratories of Biology Department in Faculty of Science **.** It explainsgenes that responsible for MDR in *Klebsiella pneumonia* that isolated from UTI patients in the province of AL-Najaf. Total of specimens 220 (100%) , 153(69.5%) samples were female and 67 (30.5%) were male , so from total specimens of 205 (93.2%) showed significant bacterial growth included 143(69.8 %) of female specimens and 62(30.2%) from male specimens the results showed that they have been elected 6 bacterial species ,the most common pathogen was *Escherichia. coli*115(56.1%) , *Klepsiella . pneumoniae* 60 (29.3 %), *Proteus. mirabilis* 12 (5.9%) *S.aureus* 9 (4.4%), ,*enterobacter. cloacae* 7(3.4%) , and *P.aeruginosa* 2 (0.9%).Antibacterial susceptibility test was conducted for 60 *K. pneumoniae* isolates against 22 commonly used antibacterial agents by using the disk diffusion method , the highest rate of resistance was seen with Amoxicillin- clavulanic Acid 60/60 (100%)followed Trimethoprime - sulfamethaxazol 44/60(73.3%)and the low rate resistance was seen withimpenem 4/60 (6.7%)and Nitrofurantoin was 3/60 (5 %) .The capacity of some *K. pneumoniae*isolates to biofilm formation by phenotypic method which included Congo Red Agar Method (CRA) , from the 60 (100%) isolates of *K. pneumoniae* 48 (80%) were biofilm producers when that appearance of black dry crystalline colonies on the CRA plates and 12 (20 %) were non - biofilm producers when the colonies of *K. pneumoniae*remained pink or red colored , Molecular study of antibiotic resistance genes (*gyrA,, Ant(2")-1a,,blaSHV-1*) were detected in MDR *K. pneumoniae*isolates(20/20 (100%) , 11/20 (55%) , and 20/20 (100%) respectively.

Keywords:*Klepsiella pneumonia*, MDR, Antibiotic resistance genes,

1.Introduction : Infections of the Urinary Tract are regarded as one of the most serious illnesses, ranking second among bacterial infections in the medical community, and they are prevalent, according to estimates of the number of individuals afflicted. Around 150 million individuals every year" The cost-estimation technique for identifying and treating urinary tract infections is critical since the expenses are substantial, and laboratory testing is required to achieve a state of recovery **(1) .** Urinary tract infections are dependent on the virulence of the causative bacteria and the host's sensitivity, because the infection occurs in a person whose urinary tract is anatomically and naturally normal, as well as the possibility of bacteria ascending automatically from the urethra to the bladder and, in some cases, the kidney **(2)**. *Klebsiella pneumoniae* is a member of the *Enterobacteriaceae* family and is one of the most significant opportunistic pathogens causing nosocomial and community acquired infections **(3).**

2. Materials and Methods

2.1:Samples collection and bacterial identification

A total number of specimens (220) were collected and worked on it from patients withl UTI casesadmitted to Al-Hakam Hospital , Al- Zahraa Hospital and AL-Aman center for reaserch in AL-Najaf Governorate , during the period from (October , 2022 to January , 2023), all specimens were collected in a way to avoid any potential contamination , specimens were taken and close it until transported to advanced Microbiology laboratory/ College of Science / University of Kufa and culturing on different media for 24 h at cultivate 37 °C for bacterial diagnosis .

2.2. : Antibiotic susceptibility test for *K. pneumoniae*isolates

Muller Hinton agar was prepared , it is sterilized in the autoclave and poured in petri dishes , then antibiotic resistance *K. pneumoniae*isolates were streaked by sterile swab on petri dish and

placed antibodies disc and incubated the dishes at 37 ° C for 24 h , the diameter of inhibition zones was measured using a meter ruler **(4).**

2.3: Detection of biofilm formation for *K. pneumoniae*isolates

Biofilm production by isolated from Urinary tract infection pathogens in our study was detected by phenotypic method which included Congo Red Agar Method (CRA) , *K. pneumoniae*isolates inoculated aerobically on the CRA plates and incubated at 37°C for 48 h , Appearance of black dry crystalline colonies on the CRA plates indicated positive biofilm production while the colonies of biofilm no producer remained pink or red colored negative **(5).**

2.4: Molecular Techniques

2.4. 1: Extraction of Genomic DNA

Genomic DNA was extracted by using a methodof**(6).**

2.4.2: Molecular Identification : Gel electrophoresis was used to determine of DNA via UV trans illuminator , the primer was planned by Alpha DNA company, Canada as in table (1)

Table(1): Primers used in this study

Primer Type	Primer sequence (5'-3')	Amplicon size (pb)	Reference
blaSHV-1	F:5- GGGTTATTCTTATTTGTCGC-3 R:5-TTAGCGTTGCCATTCCTC-3	927	**(7)**
Ant(2")-1a	F:5- ACGCCGTGGGTCGATGTTTGAGTG-3 R:5-ACGCCGTGGGTCGATGTTTGATGT-3	572	**(8)**
gyrA	F:5- GGTATACCGTCGCGTACTTT-3 R:5-CAACGAAATCGACCGTCTCT-3	311	**(9)**

2.4.3 : PCR Thermo - cycling conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table (2).

Table (2) : Amplification conditions of genes were used by PCR reactions

Gene Name	Temperature (◦c) / Time					Cycles Number
	Initial Denaturation	Cycling conditions			Final Extension	
		Denaturation	Annealing	Extention		
blaSHV-1	95/5 min	95/45 sec	53/45 sec	72/1min	72/7 min	35
Ant(2)"-1a	94/5 min	94/30 sec	55/30 sec	72/1min	72/10 min	30
gyrA	95/2 min	95/10 sec	57/30 sec	72/1 min	72/7 min	40

3.Results and Discussion

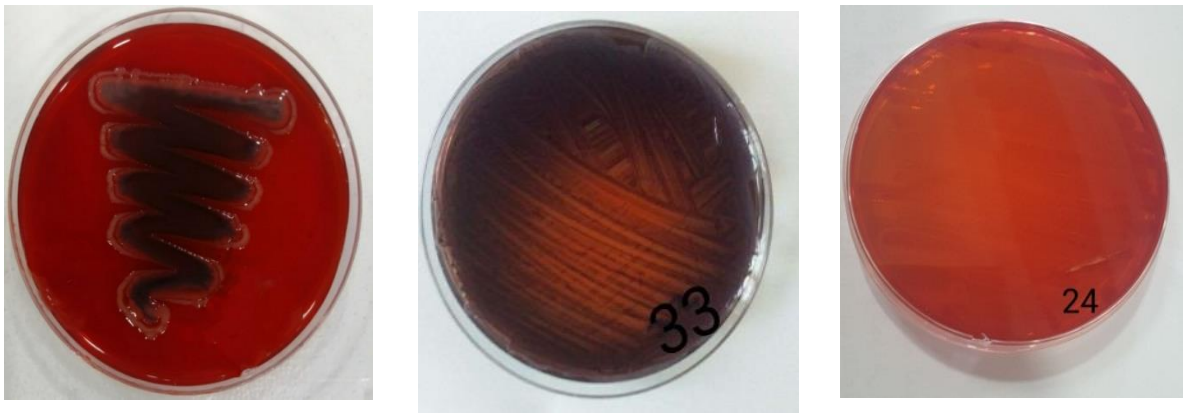
3.1: Antibiotics susceptibility of *K. Pneumonaeii*solates:Based on the following Table(3)

Type of antibiotic_class (sub_class)	Antibiotic disk	No. (%) antibiotic Resistance	
		Resistance	Susceptible
Fluoroquinolones	Levofloxacin	7 (11.7 %)	53(88.3%)
	Ciprofloxacin	20 (33.3 %)	40(66.7%)
	Norfloxacin	15 (25%)	45(75%)
quinolones	Nalidixic Acid	19 (31.7%)	41(68.3%)
β-lactamscephalosporines	Ceftazidime	30 (50%)	30(50%)
	Ceftriaxone	39 (65%)	21(35%)
	Cefepime	32 (53.3%)	28(46.7%)
	Cefoxitin	19 (31.7%)	41(68.3%)
	Cefotoxime	32 (53.3%)	28(46.7%)
	Cefixime	15 (25%)	45(75%)
β-lactams carbapenems	Meropenem	7 (11.7%)	53(88.3%)
	Impenem	4 (6.7%)	56(93.3%)
β-lactamspenicillns	Amoxicillin_ clavulanic acid	60 (100%)	0(0%)
Aminoglycosides	Tobramycin	23 (38.3%)	37(61.7%)
	Gentamicin	35 (58.3%)	25(41.7%)
	Amikacin	6 (10%)	54(90%)
Sulfonamides (antifolate)	Trimethoprime-sulfamethaxazol	44 (73.3%)	16(26.7%)
	Trimethprim	42 (70%)	18(30%)

Macrolides	Erythromycin	20 (33.3%)	40(66.7%)
Nitrofurans	Nitrofurantoin	3 (5%)	57 (95%)
β-lactamsmonobactams	Azitronem	11 (18.3%)	49(81.7%)
Tetracyclins	doxycyclin	12 (20)	48(80%)

3.2: Detection of Biofilm Formation

All of *K. pneumoniae* isolates were detected for biofilm formation by phenotypic method which included Congo Red Agar Method (CRA) , from the 60 (100%) isolates of *K. pneumoniae* 48 (80%) were biofilm producers when that appearance of black dry crystalline colonies on the CRA plates and 12 (20 %) were non - biofilm producers when the colonies of *K. pneumoniae*remained pink or red colored figure (3-1) .



(A) (B) (C)

Figure (3-1) : Biofilm formation by some *Klebsiella pneumonia*isolates
A & B : Biofilm formation C :without Biofilm formation .

3.3: Detection of genes that responsible for multidrug-resistant (MDR) in *Klebsiella pneumoniae*

Molecular study of antibiotic resistance genes (*gyrA*,, *Ant(2'')-1a*,and,*blaSHV*_1) were detected in (MDR)*K. pneumoniae* isolates, 20/20 (100%),11/20 (55%) , and 20/20 (100%) respectively. Figure (3-1), Figure (3-2), and figure (3-3) respectively .Fluoroquinolones are a class of synthetic broad spectrum antibiotics The resistance to Fluoroquinolones involves structural alterations in its targets (DNA gyrase and topoisomerase IV) which are essential for all bacterial species **(10)** . These enzymes provide a balancing DNA uncoiling /coiling function critical for sustaining a sufficient level of chromosomal relaxation necessary for replication **(11)** . FQ resistance mechanisms are similar in *K. pneumoniae* and many different pathogenic species, and involve acquisition of mutations in quinolone resistance-determining regions (QRDRs) of *gyrAgene*, mutations led to the emergence of the pandemic FQ- and multidrug-resistant *K. Pneumoniae***(12).**Mutations of GyrA within the quinolone resistance-determining regions have been found to be the main mechanism for quinolone resistance in Enterobacteriaceae. It has been shown that only some of the mutations in the gyrA gene identified from clinical sources were involved in fluoroquinolone resistance. Whether different patterns of gyrA mutation are related to antimicrobial resistance against ciprofloxacin and levofloxacin is unclear**(13)**

Aminoglycoside antibiotics block protein synthesis by targeting the A site or recognition site located in the 16S rRNA of the bacterial 30S ribosomal subunit where codon— anticodon accuracy is assessed

(14) . Even though clinical applications of aminoglycosides have not completely halted, the ever-increasing resistance to all major antimicrobial drugs has once again led to an interest in these compounds , particularly their application in the treatment of Severe infections by Gram-negative bacteria **(15) .**

Beta-lactam antibiotics are it one of the greatest usually prescribed treatment classes with many therapeutic indications , the mechanism of action for this antibiotics comprise , the peptidoglycan or murine is a vital basic of the bacterial cell wall this gives mechanical constancy for it , that is an very conserved constituent of within the Gram-positive and Gram-negative covers , the beta-lactam antibiotics prevent the latter stage in peptidoglycan creation via acyl ting the trans peptidase involved in cross-linking peptides to make peptidoglycan**(16) .**

The goals for activities of beta-lactam antibiotics it called as penicillin-binding proteins (PBPs) , the binding, in turn, interrupts the terminal transpeptidation method and gives failure of viability and lysis , and by autolytic methods with the bacterial cell , β-Lactamases are by far the greatest significant resistant process in Gram-negative bacilli , with the popularization of genetic techniques , an increasing number of this enzymes have been categorized different in amino acid series and hydrolytic activity for β-lactam antibiotics **(17)** .

Gram-negative bacteria, inducible appearance of β-lactamases is ordinarily originate in chromosomal β-lactamases whereas plasmid-mediated enzymes are usually constitutively expressed improvement appearance for this hydrolytic action is frequently controlled whitin promoters appear in upstream genes **(18)**

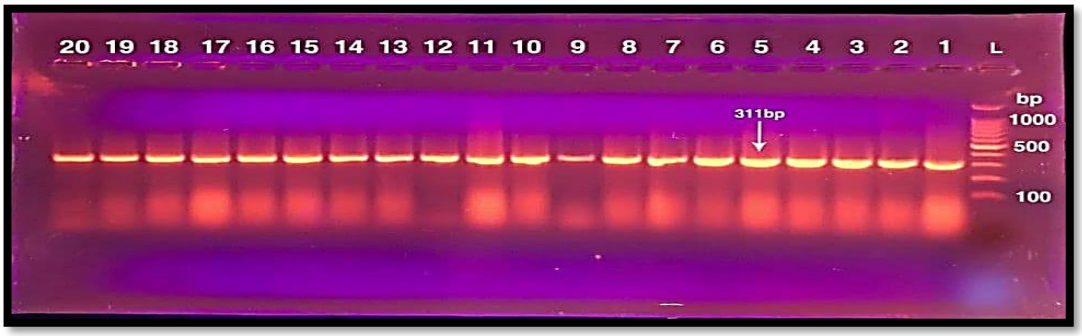


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Figure (3-2): Agarose gel (1.5)g with ethidium bromide stained of mono-plex PCR amplified with product(311bp) from extract DNA of *K. Pneumoniae*. isolates with *gyrA* gene primers , performed at(70 V, 1.5 h.) Lane (L) DNA molecular size marker (100-bp ladder) , all *K. pneumoniae* isolatesshowed positive results for *gyrA* gene .

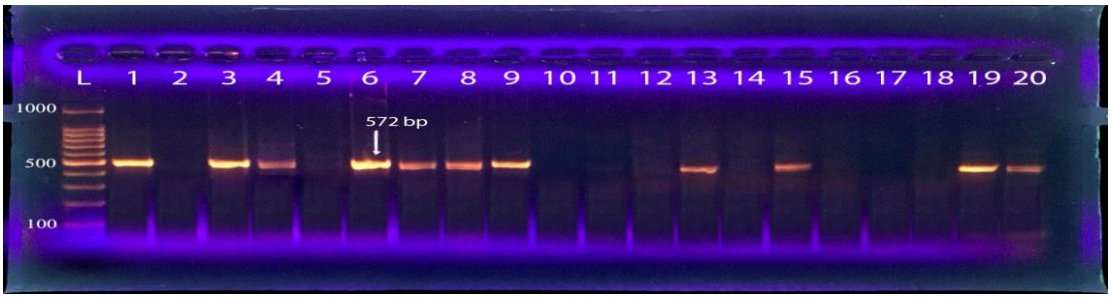

Figure (3-3): Agarose gel(1)g with ethidium bromide stained of mono-plex PCR amplified with product (572bp) from extract DNA of *K. pneumoniae*isolates with *Ant(2")-1a* gene primers , performed at (70 V, 1.5 h.) Lane (L) DNA molecular size marker (100-bpladder) , Lane (1, 3, 4,6,7,8.9.13.15,19,20) showed positive results for *Ant(2")-1a* gene .

Figure (3-4) Agarose gel(0.8)g with ethidium bromide stained of of mono-plex PCR amplified with product(927bp) from extract DNA of *K. Pneumoniae*. isolates with *SHV_1* gene primers , performed at(70 V, 1.5 h.) Lane (L) DNA molecular size marker (100-bp ladder) , all *K. pneumoniae* isolates showed positive results for *SHV_1* gene.

References

1. Medina,M. and Castillo-Pino, E. (2019). An introduction to the epidemiology and burden of urinary tract infections. Therapeutic advances in urology, 11, 1756287219832172

2. **Klein,R. D**. and Hultgren, S. J. (2020). Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies. Nature reviews. Microbiology, 18(4), 211–226.

3. Togawa, A.; Toh, H. and Onozawa, K. (2015). Influence of the bacterial phenotypes on the clinical manifestations in *Klebsiella pneumoniae* bacteremia patients: a retrospectivecohort study. J. Infection Chemother., 21:531-537.

4. Clinical and Laboratory Standards Institute (CLSI) .(2022).Performance Standards for Antimicrobial Susceptibility Testing; 29thed. supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

5. Ruchi, T.; Sujata, B. and Anuradha, D. (2015). Comparison of phenotypic methods for the detection of biofilm production in uro-pathogens in a tertiary care hospital in India. Int J Curr Microbiol App Sci, 4(9), 840–849.

6. Yang,J.L.; Wang, M.S. ; Cheng, A.C.; Pan, K.C. ; Li, C.F. and Deng, S.X. (2008). A simple and rapid method for extracting bacterial DNA from intestinal microflora for ERIC-PCR detection. World J. Gastroenterol., 14: 2872-2876.

7. Hadis, A.; Iraj, P.; Ayat, N.; Hasan, H.; Farid, A. J.; Morovvat, T.; Naser, S. and Allireza, M. S. (2015). Sulfonamide Resistance Genes (*sul*) M in Extended Spectrum Beta Lactamase (ESBL) and Non-ESBL Producing *Escherichia coli* Isolated From Iranian Hospitals. Jundishapur J Microbiol; 8(7): e19961.

8. Aggen, J.B.; Armstrong, E.S. and Goldblum, A.A. (2010).Synthesis and spectrum of the neoglycoside ACHN-490. Antimicrob Agents Chemother;54:4636–42.

9. Johnning, A.; Kristiansson, E.; Fick, J.; Weijdegård, B. and Larsson, D.G.J. (2015) .Resistance Mutations in gyrA and parC are Common in *Escherichia* Communities of both Fluoroquinolone-Polluted and Uncontaminated Aquatic Environments. Front. Microbiol. 6:1355.

10. Munita, J. M. and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiol. Spectr*. 4.

11. Baranello, **L. ;** Levens, D.; Gupta, A. and Kouzine, F. (2012). The importance of being supercoiled: How DNAmechanics regulate dynamic processes. Biochim. Biophys. Acta 1819, 632–638 .

12. Johnson, **J. R.** et al., (2015). Greater ciprofloxacin tolerance as a possible selectable phenotype underlying the pandemic spread of the H30 subclone of *Escherichia coli* sequence type 131. *Antimicrob. Agents Chemother*. 59, 7132–7135.

13. Yingmei, F.U.; Wenli,Z.; Hong, W.; Song, Z.; Yang, C.; Fanfei, M. and Fengmin, Z. (2013) . Specific patterns of *gyr A* mutations determine the resistance difference to ciprofloxacin and levofloxacin in *K. Pneumoniae* and *Escherichia coli*. BMC Infectious Diseaes8, 13, 1–6.

14. Davis, M.A.; Baker, K.N. and Orfe, L.H. (2010). Discovery of a gene conferring multiple-aminoglycoside resistance in *Escherichia coli.* Antimicrob Agents Chemother;54:2666-9.

15. Livermore, D.; Mushtaq, S. and Warner, M. (2011). Activity of aminoglycosides, including ACHN-490, against carbapenem resistant *Enterobacteriaceae* isolates. J. Antimicrob Chemother,66:48–53.

16. Eckburg, P.B.; Lister, T.; Walpole, S.; Keutzer, T.; Utley, L.; Tomayko, J.; Kopp, E.; Farinola, N. and Coleman, S. (2019) . Tolerability, Pharmacokinetics, and Drug Interaction Potential of SPR741, an Intravenous Potentiator, after Single and Multiple Ascending Doses and When Combined with β-Lactam Antibiotics in Healthy Subjects. Antimicrob Agents Chemother. ;63(9) .

17. Bush, K. (2010). Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr. Opin. Microbiol. 13, 558–564.

18. Bush, K. and Fisher, J. F. (2011). Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram- negative bacteria. Annu. Rev. *Microbiol.* 65, 455–478.