Journal of Applied Genetics
https://doi.org/10.1007/s13353-024-00893-w

MICROBIAL GENETICS • ORIGINAL PAPER

Antibiotic resistance and its correlation with biofilm formation and virulence genes in *Klebsiella pneumoniae* isolated from wounds

Mohammed Allami[1,2,3] · Eman Jassim Mohammed[4] · Zainab Alnaji[5] · Salsabil A.jassim[6]

Received: 16 December 2023 / Revised: 29 May 2024 / Accepted: 12 July 2024
© The Author(s), under exclusive licence to Institute of Plant Genetics Polish Academy of Sciences 2024

Abstract

Klebsiella pneumoniae is the most important species of the *Klebsiella* genus and often causes hospital infections. These bacteria have a high resistance to most of the available drugs, which has caused concern all over the world. In this study, we investigated the antibiotic resistance profile and the ability to produce extended-spectrum beta-lactamase (ESBL) among *K. pneumoniae* isolates, and then we investigated the relationship between these two factors with biofilm formation and the prevalence of different virulence genes. In this study, 130 isolates of *K. pneumoniae* isolated from wounds were investigated. The antibiotic resistance of the isolates was evaluated by the disk diffusion method. The microtiter plate method was used to measure biofilm formation. The prevalence of virulence genes was detected by multiplex PCR. Among the examined isolates, 85.3% showed multidrug resistance. 87.6% of the isolates were ESBL-positive. Imipenem, meropenem, and fosfomycin were the most effective drugs. The ability of the isolates to produce biofilm was strong (80%), moderate (12.3%), and weak (7.6%), respectively. *fim*H, *mrK*D, *ent*B, and *tol*C virulence genes were observed in all isolates. High prevalence of antibiotic resistance (especially multidrug resistance), high prevalence of ESBL-producing isolates, the ability of all isolates to biofilm formation, and the presence of *fim*H, *mrK*D, *ent*B, and *tol*C virulence genes in all isolates show the importance of these factors in the pathogenesis of *K. pneumoniae* isolates in Iraq.

Keywords *Klebsiella pneumoniae* · Antibiotic resistance · Virulence genes · Biofilm · Multidrug resistance · Extended-spectrum beta-lactamase

Communicated by Agnieszka Szalewska-Palasz.

✉ Mohammed Allami
alhamdany012@gmail.com

Eman Jassim Mohammed
emanjassim@uomustansiriyah.edu.iq

Zainab Alnaji
zainab.mohsin@uomisan.edu.iq

Salsabil A.jassim
salsabelahmad18@gmail.com

1 Department of Dentistry, Al-Manara College for Medical Sciences, Misan, Iraq

2 Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

3 Microbiology Laboratory, Qal'at Saleh General Hospital, Misan Health Department, Misan, Iraq

4 Department of Microbiology, College of Science, Mustansiriyah University, Baghdad, Iraq

5 College of Pharmacy, University of Misan, Misan, Iraq

6 Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Introduction

Klebsiella pneumoniae is a gram-negative bacillus, a member of the Enterobacteriaceae family, this bacterium exists as a saprophyte in the pharynx, oral cavity, and digestive system of some people and can be an opportunistic cause of infections such as meningitis, septicemia, bacteremia, wound infection, pneumonia, urinary tract infection, and hospital infections should be considered (Manandhar et al. 2020; Yoon et al. 2022). The high prevalence of MDR isolates among hospital infections has made it difficult to choose the right drug to treat this bacterium (Mbelle et al. 2020). *K. pneumoniae* is increasing in causing disease due to the reduction of the host's defense as a result of complex and long surgeries and the use of different drugs. Since *K. pneumoniae* causes disease in people with weak immune systems, increasing the resistance to antibiotics in these bacteria can be considered a serious threat. Therefore, it is very important to determine the pattern of antibiotic resistance in common pathogenic

Published online: 20 July 2024

bacteria in order to guide experimental and specific treatments against a specific pathogen. On the other hand, the global spread of *K. pneumoniae* isolates with MDR has become a serious risk (especially since these MDR isolates are also reported from hospital infections) (B. F. Araújo et al. 2018; F. Araújo et al. 2012).

Extended-spectrum β-lactam (ESBL) are enzymes that can hydrolyze oxyimino-cephalosporins and are inhibited by beta-lactamase inhibitors (Mohammed et al. 2022a, b). In many countries, due to excessive use of antibiotics, *K. pneumoniae* isolates have become resistant to many antibiotics and can produce different enzymes such as broad-spectrum beta-lactamases to resist antibiotics (Jensen et al. 2020). During the last 5 decades, the increase of resistance in *K. pneumoniae* has been very high, previously first resistance to certain antibiotics such as ampicillin, trimethoprim, and tetracycline was reported, but now this range of antibiotic resistance has become very wide (Brisse et al. 2014). Aminoglycosides, Fluoroquinolones, Trimethoprim-sulfamethoxazole, and Cephalosporins are among the antibiotics to which *K. pneumoniae* have become resistant due to the acquisition of plasmids that encode broad-spectrum β-lactam and aminoglycoside-converting enzymes, therefore, the possibility of treating infections *Klebsiella* is weakened by these antibiotics (Holt et al. 2015). Furthermore, *K. pneumoniae* has been reported to be the most common pathogenic bacteria resistant to extended-spectrum β-lactam antibiotics (Naqid et al. 2020).

Also, one of the mechanisms of antibiotic resistance in this bacterium is the production of biofilm, and in fact, biofilm is a population of bacteria that are trapped in an extracellular matrix (Jensen et al. 2020). This matrix consists of proteins, exopolysaccharides, DNA, and lipopeptides. By forming a biofilm, *K. pneumoniae* isolates can stay away from the immune system and antibiotics and lead to antibiotic resistance. In addition, the pathogenicity of *K. pneumoniae* is due to various pathogenic factors that enable the bacteria to overcome the host's innate immunity and cause infection in the host. *K. pneumoniae* virulence factors include lipopolysaccharides (*htr*A), capsule, biofilm formation ability (mrKD), excessive mucosal viscosity (*mag*A, *rmp*A), iron absorption systems (*kfu*, *entB*, *ybtS*, *iucC*, *iut*A), serum resistance (*tra*T), efflux pumps (*acr*AB, *tol*C, *mdt*K), and adhesins (*fim*H) (Dalir et al. 2021; Zhu et al. 2021).

Despite the high prevalence of infections caused by *K. pneumoniae* in Iraq, a comprehensive study in which the characteristics of this bacterium in terms of drug resistance, beta-lactamase enzyme production, biofilm formation, and coding of virulence genes has not been investigated. This study aimed to investigate the factors involved in increasing the resistance and pathogenesis of *K. pneumoniae*.

Material and methods

Identification of *bacteria*

This study analyzed 130 K. *pneumoniae* isolated from wound swab samples collected from Ibn Al Katteeb hospitals, Al-Kindi Teaching Hospital, and private medical clinics in Baghdad between February 2019 and April 2021. Among the patients, 81 were women between 6 and 64 years old (median, 53 years) and 49 were male patients between the ages of 8 and 71 years (median, 49 years). The samples were cultured on MacConkey agar medium. After 24–18 incubation at 37 °C, the grown *K. pneumoniae* isolates were confirmed by microbiological methods, including gram staining and microscopic observation of bacilli, as well as biochemical tests (API-20E test strip) (bioMerieux, France). To definitively confirm *K. pneumoniae* in the studied isolates, a PCR test was performed to trace the 16S rRNA gene.

Antibiotic resistance profile

The pattern of antibiotic resistance in the isolates was done using the disk diffusion method according to CLSI (2020) criteria. The isolates were cultured in Brain Heart Infusion (BHI) medium and incubated at 37 °C for 18–24 h. After the growth of bacteria and creating turbidity equal to a 0.5 McFarland standard, they were cultured on Mueller Hinton agar culture medium using sterile swabs. Antibiotic-resistant test was performed in the presence of amikacin (AK: 30 µg), amoxicillin/clavulanic acid (AMC: 20/10 µg), ampicilin (AM: 25 µg), aztreonam (ATM: 30 µg), cefepime (CPM: 30 µg), cefoxitin (FOX: 30 µg), ceftazidime (CAZ: 30 µg), ceftriaxone (CRO: 30 µg), ciprofloxacin (CIP: 5 µg), ertapenem (ETP: 10 µg), fosfomycin (FOS: 200 µg), gentamicin (GEN: 10 µg), imipenem (IPM: 10 µg), meropenem (MEM: 10 µg), nitrofurantoin (NIT: 300 µg), norfloxacin (NOR: 10 µg), piperacillin (PI: 100 µg), piperacillin/tazobactam (P/T 100/10 µg), and trimethoprim/sulfamethoxazole (STX: 1.25/23.75 µg) discs made by Bioanalyse, Turkey. Isolates that were resistant to at least three classes of antibiotics were recorded as MDR isolates. *Escherichia coli* ATCC 25922 strains were used as negative control and *K. pneumoniae* ATCC 700603 as positive control.

β-Lactamase characterization

To confirm the production of broad-spectrum beta-lactamases, a combined disc method was performed based on the inhibitory property of clavulanic acid on beta-lactamases and using ceftazidime, ceftazidime-clavulanic acid, cefotaxime, and cefotaxime-clavulanic acid discs (Bioanalyse, Turkey). For this purpose, a bacterial suspension adjusted

to a turbidity equal to a 0.5 McFarland standard was spread on Mueller–Hinton agar and disks containing cephalosporin were placed against disks containing cephalosporin-clavulanic acid. ESBL production was evaluated by comparing the diameter of the growth zone around the disc without clavulanic acid with the disc with clavulanic acid. In ESBL-positive isolates, the diameter of the non-growth zone around the disk with clavulanic acid is at least 5 mm larger than the disk without clavulanic acid.

Genotypic diagnosis of virulence factors

DNA extraction of *K. pneumoniae* isolates was performed using a DNA extraction kit manufactured by Fermentas-Germany according to the manufacturer's instructions. The quality and quantity of extracted DNA were checked by electrophoresis on agarose and using a Nanodrop device. After extracting the DNA of each sample, they were kept at minus 20 °C until PCR was done. The primers were divided into groups (A, B, C, D, E) based on their suitable annealing temperature. Since the annealing temperature of some primers was similar, the Multiplex-PCR method was used to detect virulence genes. The sequence of primers used in this study and the annealing temperature of each primer are shown in Table 1.

Measurement of biofilm production

The microtiter plate method and staining with 0.1% crystal violet were used to measure biofilm formation. First, each strain was cultured in tubes containing 5 ml of TSB medium and incubated for 24 h at 37 °C. In the next step, to equalize the turbidity of the microbial suspensions, dilution was done in a fresh TSB medium (preparation of a suspension with a turbidity equal to a 0.5 McFarland standard). Then, 150 µl of microbial suspension was poured into each of the wells of 96-well microplates and placed in an incubator at 37 °C for 24 h. Then, the supernatant solution was removed and the wells were gently washed 3 times with 200 µl of physiological saline. The microplate was placed upside down at

Table 1 The sequence and annealing temperature of the primers of the virulence genes used

Group	Virulence gene	Primer sequence (5′ to 3′)	Annealing temp. (°C)	Product size (bp)	References
A	*kfu*	F: GGCCTTTGTCCAGAGCTACG R: GGGTCTGGCGCAGAGTATGC	61	638	(Albasha et al. 2020)
	fimH	F: GCCAACGTCTACGTTAACCTG R: ATATTTCACGGTGCCTGAAAA	61	180	(Mirzaie and Ranjbar 2021)
	mrKD	F: CCACCAACTATTCCCTCGAA R: ATGGAACCCACATCGACATT	61	**226**	(Mirzaie and Ranjbar 2021)
B	*ent*B	F: CTGCTGGGAAAAGCGATTGTC R: AAGGCGACTCAGGAGTGGCTT	61	385	(Mirzaie and Ranjbar 2021)
	*mag*A	F: GGTGCTCTTTACATCATTGC R: GCAATGGCCATTTGCGTTAG	59	1282	(Mirzaie and Ranjbar 2021)
	*rmp*A	F: ACTGGGCTACCTCTGCTTCA R: CTTGCATGAGCCATCTTTCA	59	516	(Mirzaie and Ranjbar 2021)
	*iut*A	F: GGGAAAGGCTTCTCTGCCAT R: TTATTCGCCACCACGCTCTT	59	920	(Anis et al. 2021)
C	*acr*AB	F: ATCAGCGGCCGGATTGGTAAA R: CGGGTTCGGGAAAATAGCGCG	60	312	(Mirzaie and Ranjbar 2021)
	*tol*C	F: ATCAGCAACCCCGATCTGCGT R: CCGGTGACTTGACGCAGTCCT	60	527	(Mirzaie and Ranjbar 2021)
	*iuc*C	F: GTGCTGTCGATGAGCGATGC R: GTGAGCCAGGTTTCAGCGTC	60	944	(Caneiras et al. 2019)
D	*tra*T	F: GGTGTGGTGCGATGAGCACAG R: CACGGTTCAGCCATCCCTGAG	56	288	(Mirzaie and Ranjbar 2021)
	mdtK	F: GCGCTTAACTTCAGCTCA R: GAT GATAAATCCACACCAGA	*56*	453	(Elmanakhly et al. 2022)
	*ybt*S	F: GACGGAAACAGCACGGTAAA R: GAGCATAATAAGGCGAAAGA	56	242	(Mirzaie and Ranjbar 2021)
	*htr*A	F: CGTTCTGCCAGGATGGTTCT R: CCCCAATGATGACATCGCCT	56	1071	(Mirzaie and Ranjbar 2021)
E	16 S rRNA	F: ATTTGAAGAGGTTGCAAACGAT R:TTCACTCTGAATTTTCTTGTGTTC	57	130	(Albasha et al. 2020)

a temperature of 65° to dry. To fix the biofilm better, 96% ethanol was used in the amount of 100 µl. After 15 min, the alcohol was removed and the microplate was air-dried. One hundred microliters of 1.5% crystal violet dye were added to all the wells, and after 20 min, the plate was washed with water to remove the excess dye from the wells. One hundred fifty microliters of 33% acetic acid were added to the wells to release the dyes bound to the biofilm to the surface of the microplate. To measure the amount of biofilm production, the absorbance of the wells was measured at a wavelength of 550 nm using an ELISA reader. If optical density (OD) is less than 0.1, it indicates weak biofilm formation, if OD is between 0.1 and 1, it indicates moderate biofilm formation, and if OD is greater than 1, it indicates strong biofilm formation. *Pseudomonas aeruginosa* PAO1 strain was used as a positive control for biofilm assay.

Statistical analysis

SPSS24 statistical analysis program (chi-square test) was used to analyze the percentage of presence of different factors examined among the isolates, and a p-value < 0.5 was considered significant.

Results

Study population

In this study, 760 wound swabs were collected and cultured, and 130 isolates of *K. pneumoniae* were detected. Of all isolates, 81 (62.3%) were related to women and 49 (37.6%) were to men. A significant relationship (p-value < 0.05) was observed between the distribution of *K. pneumoniae* isolates and the gender of the patient. In this study, we divided the examined patients into two groups: greater than/equal or less than 30 years. Of all isolates, 68 (52.3%) were related to ≤ 30 years age group and 62 (47.6%) were related to > 30 years age group. There was a significant relationship (p-value < 0.05) between the distribution of *K. pneumoniae* isolates and the patient's age.

Biofilm

All the investigated isolates in this study could form biofilm. The ability of the isolates to biofilm formation was strong at 104 (80%) isolates, moderate at 16 (12.3%) isolates, and weak at 10 (7.6%) isolates, respectively. The relationship between the ability to form biofilm and the gender of the patient showed that the ability to strong biofilm formation was higher in women 70 (67.3%) than in men 34 (32.6%). This ability in moderate biofilm formation isolates was equal to 8 (50%). The ability of isolates to weaken biofilm formation was higher in males 7 (70%) than in females 3 (30%).

Virulence genes

Based on the PCR results, *fim*H, *mrK*D, *ent*B, and *tol*C genes were observed in all 130 (100%) isolates. *mag*A and *iut*A genes were the least prevalent among the isolates. The prevalence of the remaining virulence genes was respectively, *mdt*K 122 (93.8%), *ybt*S 109 (83.8%), *tra*T 93 (71.5%), *acr*AB 86 (66.1%), *htr*A 82 (63%), *kfu* 81 (62.3%), *rmp*A 70 (53.8%), *iut*A 43 (33%), and *mag*A 22 (16.9%).

Antibiotic resistance profile and its relationship with biofilm formation and virulence genes

The results of the Kirby-Bayer antibiotic resistance showed that imipenem, meropenem, and fosfomycin were the most effective against the isolates, while piperacillin and ampicillin showed the least effectiveness. The relationship between antibiotic resistance and biofilm formation showed that among the 19 antibiotics examined, the isolates that had resistance to aztreonam, ceftriaxone, fosfomycin, piperacillin, imipenem, nitrofurantoin, ertapenem, trimethoprim/sulfamethoxazole, and amoxicillin/clavulanic acid antibiotics had a high ability of $\geq 80\%$ in strong biofilm formation. The moderate and weak ability of biofilm formation in isolates resistant to aztreonam, meropenem, nitrofurantoin, and ertapenem antibiotics was equal. The ability to form moderate biofilm was not observed in imipenem-resistant isolates. Figure 1A shows the relationship between antibiotic resistance profile and biofilm formation ability.

The distribution of virulence genes among antibiotic-resistant isolates showed that *fim*H, *mrK*D, *ent*B, and *tol*C virulence genes were present in all antibiotic-resistant isolates. The virulence gene mdtK had a high presence among antibiotic-resistant isolates and was observed in all isolates resistant to fosfomycin, imipenem, and amoxicillin/clavulanic acid. *ybt*S virulence gene was present in all isolates resistant to fosfomycin and imipenem (Table 2).

Relationship between virulence genes and biofilm formation

Among the examined virulence genes, the isolates containing all genes (except *tra*T and *htr*A) had the ability of 80% to form strong biofilm. Isolates containing the *mag*A gene were not able to form moderate biofilm. Isolates containing *kfu* and *acr*AB genes were not able to form weak biofilm. Figure 1B shows the relationship between virulence genes and biofilm formation ability.

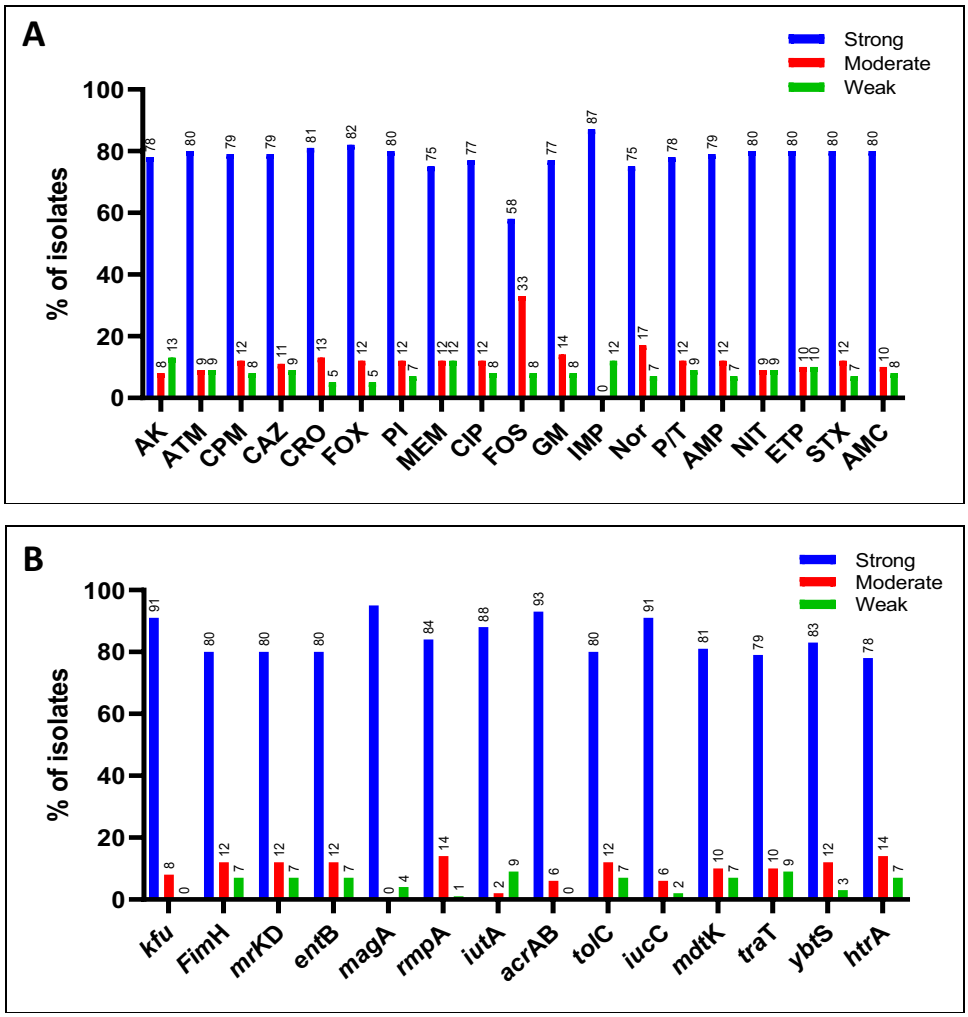

Fig. 1 Shows the relationship between **A** antibiotic resistance profile and **B** virulence genes with biofilm formation

MDR isolates and its relationship with biofilm formation and virulence genes

Investigation of antibiotic sensitivity showed that 111 (85.3%) isolates were MDR. Most MDR isolates were resistant to fosfomycin, meropenem, and ceftriaxone. Figure 2A shows the relationship between MDR isolates and antibiotic resistance profile. MDR in isolates that showed resistance to nitrofurantoin, piperacillin/tazobactam, norfloxacin, ceftriaxone, amikacin, meropenem, ciprofloxacin, fosfomycin, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, and ertapenem were more in patients over 30 years old. The relationship between multidrug resistance isolates and the gender of the patient showed that the average antibiotic resistance in female patients (52.8%) was higher compared to male patients (51%) (Table 3). The ability to biofilm formation among MDR isolates was weak and moderate biofilm (100%) and strong biofilm (81.7%), respectively (Data not shown). More than 76% of all isolates encoding virulence genes (except *mag*A and *iut*A) had MDR. Figure 2B shows the relationship between MDR isolates and virulence genes.

ESBL isolates and their relationship with antibiotic resistance, biofilm, and virulence genes

The results of broad-spectrum beta-lactamase enzyme identification showed that 114 (87.6%) of the examined isolates were producing this enzyme. Most ESBL-positive isolates were resistant to meropenem, nitrofurantoin, and cefoxitin. Figure 3A shows the relationship between ESBL-positive isolates and antibiotic resistance profile. The ability to form biofilm among ESBL-positive isolates was weak biofilm (100%), moderate biofilm (93.7%), and strong biofilm (86.5%). At least 82% of the isolates encoding virulence genes (except for *iut*A 76%) were ESBL-positive. Figure 3B shows the relationship between ESBL-positive isolates and virulence genes.

Discussion

The emergence of antibiotic resistance organisms has recently been recognized as a major global concern, and infections caused by MDR or ESBL-producing organisms

Table 2 Relationship between the overall pattern of antimicrobial resistance in *K. pneumoniae* isolated from wound swabs and virulence genes. The statistically significant values ($p < 0.05$) are shown in bold numbers

Antibiotics/Total resistant	*kfu*	*fim*H	*mrK*D	*ent*B	*mag*A	*rmp*A	*iut*A	*acr*AB	*tol*C	*iuc*C	*mdt*K	*tra*T	*ybt*S	*htr*A
Amikacin 46 (35.3%)	25 (54)	46 (100)	46 (100)	46 (100)	**6 (13)**	27 (58)	17 (36)	25 (54)	46 (100)	17 (36)	45 (97)	37 (80)	36 (78)	27 (58)
Aztreonam 92 (70.7%)	54 (58)	92 (100)	92 (100)	92 (100)	**13 (14)**	44 (47)	30 (32)	59 (64)	92 (100)	**28 (30)**	88 (95)	90 (97)	75 (81)	60 (65)
Cefepime 110 (84.6%)	68 (61)	110 (100)	110 (100)	110 (100)	**16 (14)**	57 (51)	**37 (33)**	74 (67)	110 (100)	40 (36)	103 (93)	75 (68)	92 (83)	69 (69)
Ceftazidime 97 (74.6%)	**58 (59)**	97 (100)	97 (100)	97 (100)	18 (18)	50 (51)	31 (31)	55 (56)	97 (100)	34 (35)	89 (91)	65 (67)	77 (79)	**59 (60)**
Ceftriaxone 101 (77.6%)	64 (63)	101 (100)	101 (100)	101 (100)	17 (16)	52 (51)	**26 (25)**	68 (67)	101 (100)	40 (39)	98 (97)	**71 (70)**	89 (88)	67 (66)
Cefoxitin 89 (68.4%)	52 (58)	89 (100)	89 (100)	89 (100)	13 (14)	**52 (58)**	28 (31)	**57 (64)**	89 (100)	**31 (34)**	88 (98)	63 (70)	75 (84)	59 (66)
Piperacillin 130 (100%)	81 (62)	130 (100)	130 (100)	130 (100)	**22 (16)**	70 (53)	43 (33)	86 (66)	130 (100)	47 (36)	122 (93)	93 (71)	109 (83)	82 (63)
Meropenem 16 (12.3%)	8 (50)	16 (100)	16 (100)	16 (100)	2 (12)	10 (62)	3 (18)	8 (50)	16 (100)	**5 (31)**	14 (87)	15 (93)	12 (75)	9 (56)
Ciprofloxacin 89 (68.4%)	58 (65)	89 (100)	89 (100)	89 (100)	13 (14)	44 (49)	**29 (32)**	62 (69)	89 (100)	28 (31)	85 (85)	67 (75)	77 (86)	**53 (59)**
Fosfomycin 12 (9.2%)	7 (58)	12 (100)	12 (100)	12 (100)	1 (8)	**6 (50)**	2 (16)	**7 (58)**	12 (100)	4 (33)	12 (100)	9 (75)	12 (100)	10 (83)
Gentamicin 107 (82.3%)	71 (66)	107 (100)	107 (100)	107 (100)	16 (14)	54 (50)	**33 (30)**	75 (70)	107 (100)	35 (32)	102 (95)	79 (73)	90 (84)	71 (66)
Imipenem 8 (6.1%)	5 (62)	8 (100)	8 (100)	8 (100)	1 (12)	4 (50)	2 (25)	6 (75)	8 (100)	4 (50)	8 (100)	5 (62)	8 (100)	(75)
Norfloxacin 40 (40%)	24 (60)	40 (100)	40 (100)	40 (100)	**7 (17)**	22 (55)	15 (37)	24 (60)	40 (100)	**15 (37)**	38 (95)	26 (65)	33 (82)	24 (60)
Piperacillin/Tazobactam 98 (75.3%)	66 (67)	98 (100)	98 (100)	98 (100)	15 (15)	**49 (50)**	32 (32)	69 (70)	98 (100)	32 (32)	93 (94)	77 (78)	83 (84)	62 (63)
Ampicillin 128 (98.4%)	79 (61)	128 (100)	128 (100)	128 (100)	22 (17)	69 (53)	41 (32)	84 (65)	128 (100)	46 (35)	120 (93)	92 (71)	107 (83)	80 (62)
Nitrofurantoin 73 (56.1%)	46 (63)	73 (100)	73 (100)	73 (100)	15 (20)	38 (52)	28 (38)	48 (65)	73 (100)	28 (38)	69 (94)	**57 (78)**	59 (80)	42 (57)
Ertapenem 40 (30.7%)	26 (65)	40 (100)	40 (100)	40 (100)	6 (15)	23 (57)	**9 (22)**	**26 (65)**	40 (100)	**17 (42)**	39 (97)	26 (65)	34 (85)	19 (47)
Trimethoprim/Sulfamethoxazole 98 (75.3%)	54 (55)	98 (100)	98 (100)	98 (100)	**12 (12)**	47 (47)	28 (28)	57 (58)	98 (100)	27 (27)	84 (85)	64 (65)	77 (78)	60 (61)
Amoxicillin/Clavulanic acid) 114 (87.6%)	**70 (61)**	114 (100)	114 (100)	114 (100)	22 (19)	**62 (54)**	40 (35)	75 (65)	114 (100)	41 (35)	114 (100)	**83 (72)**	92 (80)	68 (59)

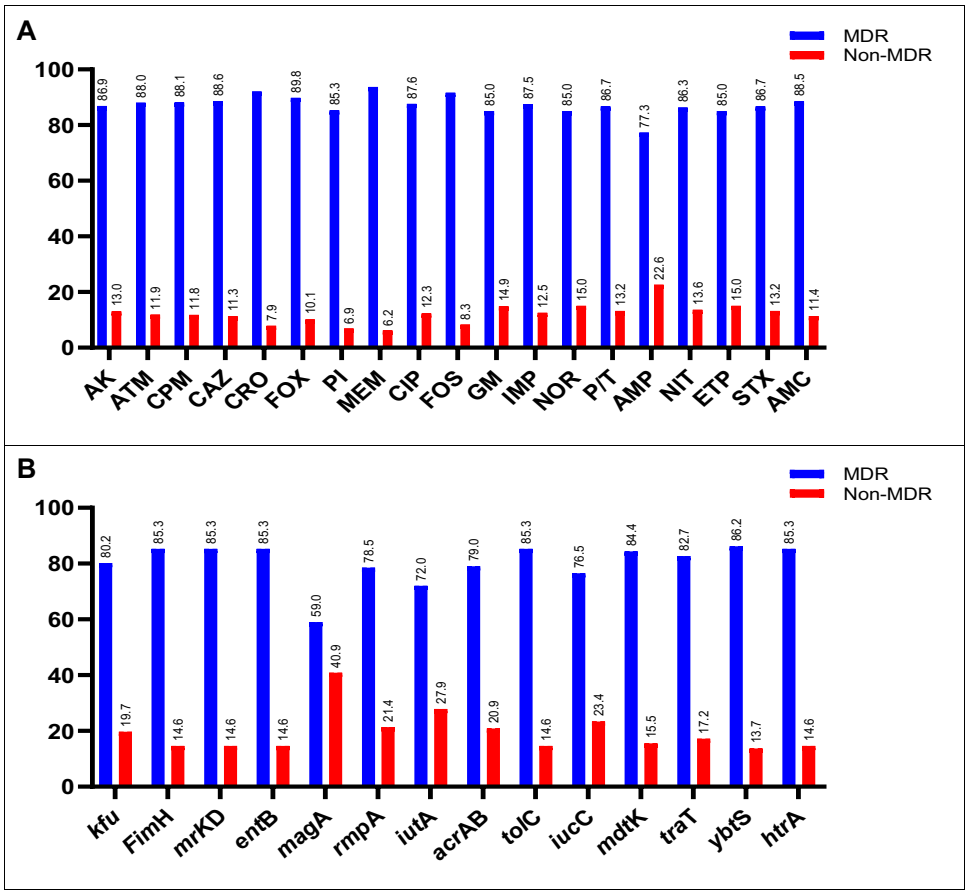

Fig. 2 Relationship between **A** antibiotic resistance sand **B** virulence factors in MDR isolate of *K. pneumoniae* isolates

Table 3 Distribution of multidrug resistance isolates among different age groups and gender of patients. Only statistically significant values ($p < 0.05$) are shown

Antimicrobials	MDR	Age groups (%)		*p*-value	Gender (%)		*p*-value
		≤ 30 YO	**> 30 YO**		**Female (*n* = 81)**	**Male (*n* = 49)**	
Amikacin 46 (35.3%)	40 (86.9)	9 (22.5)	31 (77.5)		28 (70)	12 (30)	
Aztreonam 92 (70.7%)	81 (88)	46 (56.7)	35 (43.2)		54 (66.6)	27 (33.3)	
Cefepime 110 (84.6%)	97 (88.1)	56 (57.7)	41 (42.2)		61 (62.8)	36 (37.1)	0.033
Ceftazidime 97 (74.6%)	86 (88.6)	62 (72)	24 (27.9)	0.011	51 (59.3)	35 (40.6)	
Ceftriaxone 101 (77.6%)	93 (92)	38 (40.8)	55 (59.1)		61 (65.5)	32 (34.4)	
Cefoxitin 89 (68.4%)	80 (89.8)	46 (57.5)	34 (42.5)		48 (60)	32 (40)	
Piperacillin 130 (100%)	111 (85.3)	62 (55.8)	49 (44.1)	0.042	67 (60.3)	44 (39.6)	
Meropenem 16 (12.3%)	15 (93.7)	7 (46.6)	8 (53.3)		10 (66.6)	5 (33.3)	
Ciprofloxacin 89 (68.4%)	78 (87.6)	33 (42.3)	45 (57.6)		53 (67.9)	25 (32)	0.041
Fosfomycin 12 (9.2%)	11 (91.6)	4 (36.3)	7 (63.6)		8 (72.7)	3 (27.2)	
Gentamicin 107 (82.3%)	91 (85)	47 (51.4)	44 (48.3)		59 (64.8)	32 (35.1)	0.027
Imipenem 8 (6.1%)	7 (87.5)	4 (57.1)	3 (42.8)		5 (71.4)	2 (28.5)	
Norfloxacin 40 (40%)	34 (85)	14 (41.4)	20 (58.8)		22 (64.7)	12 (35.2)	
Piperacillin/Tazobactam 98 (75.3%)	85 (86.7)	40 (47)	45 (52.9)		51 (60)	34 (40)	
Ampicillin 128 (98.4%)	99 (77.3)	50 (50.5)	49 (49.4)		54 (54.5)	45 (45.4)	
Nitrofurantoin 73 (56.1%)	63 (86.3)	28 (44.4)	35 (55.5)		40 (63.4)	23 (36.5)	
Ertapenem 40 (30.7%)	34 (85)	15 (44.1)	19 (55.8)		23 (67.6)	11 (32.3)	
Trimethoprim/Sulfamethoxazole 98 (75.3%)	85 (86.7)	36 (42.3)	49 (57.6)		54 (63.5)	31 (36.4)	0.039
Amoxicillin/Clavulanic acid) 114 (87.6%)	101 (88.5)	40 (39.6)	61 (60.3)	0.021	66 (65.3)	35 (34.6)	0.03

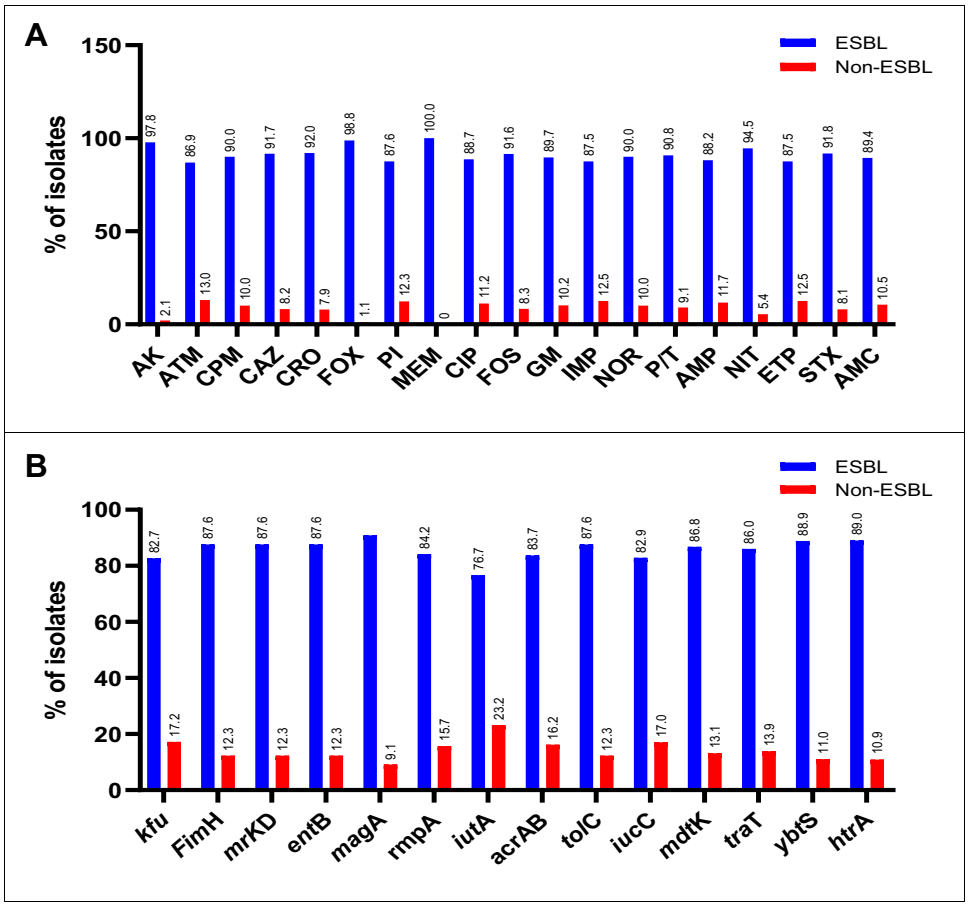

Fig. 3 Relationship between **A** antibiotic resistance and **B** virulence factors with ESBL-positive *K. pneumoniae* isolates

in particular are considered a major threat to clinicians and patients (Allami et al.2022; Sokhn et al. 2020). In this study, 130 isolates of K. pneumonia were collected from wound swabs. The prevalence and correlation between antibiotic resistance, biofilm formation, and virulence genes were investigated. Regarding gender, the findings of the current research indicated that the prevalence of the *K. pneumoniae* isolates was higher in female patients (62.3%) compared to males (37.6%). Similar results have been reported in different countries, such as Iraq (Naqid et al. 2020) and Lebanon (Sokhn et al. 2020).

In this study, imipenem, meropenem, and fosfomycin were most effective against the isolates, while piperacillin and ampicillin showed the least effectiveness. Other studies have reported similar results in Iran (Peerayeh et al. 2014; Rastegar et al. 2019), Pakistan (Fatima et al. 2021), and Portugal (Caneiras et al. 2019). One of the reasons that causes 100% ampicillin resistance in *K. pneumonia* isolates in developing countries is due to the low cost and easy administration of this antibiotic (Fatima et al. 2021; Naqid et al. 2020; Susethira and Uma 2016). In this study, the prevalence of *K. pneumoniae* with MDR was 111 (85.3%) isolates. Similar results were reported in other studies, Egypt (82.5%) (El-Domany et al. 2021), Iran (92%) (Mirzaie and Ranjbar 2021), Nepal (91%) (Manandhar et al. 2020) and less Spain (40%) (Ballén et al. 2021). Most of the MDR isolates in this study were resistant to fosfomycin, meropenem, and ceftriaxone. In the study of Mirzaie et al., most of the MDR isolates were resistant to imipenem, meropenem, and beta-lactam antibiotics (Mirzaie and Ranjbar 2021). In other studies, most MDR strains were resistant to trimethoprim/sulfamethoxazole and ciprofloxacin (Shadkam et al. 2021). In this study, multidrug resistance isolates was observed in women (52.8%) more than men (51%). Gender inequalities can influence the level of access to various health facilities. Where patriarchal values are prominent, boys and men are often prioritized for treatment over female family members. In 2018, the WHO published a working paper that promotes an enhanced focus of gender and equity in relation to antimicrobial resistance. The guidance document describes how better understanding risk factors and drivers, as well as who might "fall through the net" is essential in reducing antimicrobial resistance (Jones et al. 2022). The prevalence of ESBL-positive isolates in our study was 114 (87.6%). This rate of ESBL production in our isolates is similar to that observed in Kurdistan/Iran (88%) (Shakib et al. 2018), but higher than that reported in other reports from Nepal (60%) (Manandhar et al. 2020), Egypt (40%) (El-Domany et al.

2021), Pakistan (33%) (Fatima et al. 2021), Lebanon (27%) (Sokhn et al. 2020), and Spain (43%) (Ballén et al. 2021). Since the first description of ESBL enzymes in the literature in 1983, they have become increasingly common worldwide (Peerayeh et al. 2014). In our study, most ESBL-positive isolates were resistant to meropenem, nitrofurantoin, and cefoxitin. In the study of El-Domany et al., all ESBL-producing isolates (100%) were resistant to cefotaxime, ceftazidime, ceftriaxone, and cefoperazone (El-Domany et al. 2021). Of course, it should be noted that in this study, resistance among ESBL isolates to ceftriaxone and ceftazidime was 91% and 92%, respectively. In Peerayeh et al.'s study, ESBL-positive isolates were most resistant to ceftazidime and aztreonam (Peerayeh et al. 2014). This is of great concern because some antibiotic resistance genes are carried by mobile genetic elements that facilitate horizontal genetic exchange and the spread of antimicrobial resistance genes within and between species. On the other hand, MDR is associated with increased healthcare costs, longer hospital stays, and high mortality rates. In addition, today there are few alternatives to effective treatments (Ballén et al. 2021).

Biofilm is one of the most important factors that cause bacteria to become partially or completely resistant to a wide range of antibiotics. This provides the basis for extensive and MDR and creates conditions for the organism to increase its ability to survive in the environment. The results of the present study showed that all isolates (100%) could form biofilm. This result is consistent with Ali et al.'s study in Iraq (Ali and Hussein 2022). In other studies, isolates unable to form biofilm have been reported (Mirzaie and Ranjbar 2021; Shadkam et al. 2021; El-Domany et al. 2021). Comparing the results of our study with other studies showed that our isolates had a high ability to form biofilm. The ability to form biofilm in our study was 80% strong, 12.3% moderate, and 7.6% weak, respectively. The results of biofilm formation in Mirzaie et al.'s study were 71% strong biofilm, 20% moderate biofilm, and 14% weak biofilm (Mirzaie and Ranjbar 2021). The isolates that had the characteristic of strong biofilm formation showed higher resistance to antibiotics compared to other isolates. *K. pneumoniae* has many pathogenic factors such as capsular polysaccharide, adhesion, and siderophores, which play a role in the pathogenicity of these bacteria. The presence of virulence factors in *K. pneumoniae* is important because it is the most important cause of death in patients before the start of antibiotic treatment (El-Domany et al. 2021). The distribution of virulence genes among isolates showed that *fim*H (type 1 fimbriae gene), *mrK*D (type 3 fimbriae adhesion gene), *ent*B (enterobactin synthase component B), and *tol*C (efflux pump) genes were present in all isolates. *mag*A and *iut*A genes were the least prevalent among the isolates. In the study of Shakib et al. in Kurdistan-Iran, *ent*B (81%) and *mrk*D (65%) were the most prevalent (Shakib et al. 2018). In the study of Ballén et al. in Spain, all the isolates carried the *ent*B gene and the *fim*H and *mrk*D genes were present in most of the isolates (98%) (Ballén et al. 2021). In the study of Fatima et al. in Pakistan, *fim*H and *ent*B genes were reported in all the examined isolates (Fatima et al. 2021). Type 1 fmbriae (*fm*H-1) and Type 3 fmbrial adhesion (*mrk*D) are the most common adhesive factors of bacterial cells that cause *K. pneumoniae* to adhere to the epithelial and endothelial cells of the urinary tract and cause urinary infection. Studies have shown that fimbrial adhesion type 3 plays an important role in biofilm formation in *K. pneumoniae*, but its exact mechanism has not yet been identified (Ballén et al. 2021; Khalil et al. 2019). Enterobactin synthase component B production helps the pathogens in biofilm formation and infection development. Studies revealed that enterobactin biosynthesis is an iron-uptake protein produced by *Klebsiella* because of the presence of *ent*B gene in its genome (Fatima et al. 2021). The most effective drug efflux pump *E. coli*'s AcrAB works with TolC to extrude a wide variety of antimicrobial compounds from the cell, including antibiotics, detergents, dyes, and organic solvents (Ali and Hussein 2022). The results of the present study showed that a significant number of common antibiotics that are used in hospitals are not effective in treating a high percentage of infections caused by *K. pneumoniae*, and their use in treatment, in addition to increasing treatment costs, causes there is more resistance. As a result, the treatment regimen prescribed by doctors must be based on the results of the antibiogram of the clinical microbiology laboratory. This study showed that in Baghdad, the antibiotics imipenem, miropenim and fosfomycin can still be used as effective drugs to treat *K. pneumoniae* infections if necessary. According to the results shown in this research and other previous studies in this field, it can be concluded that antibiotic resistance is increasing in this bacterium. One of the reasons for this increasing resistance can be the formation of biofilm. The formation of biofilm in the isolates makes the antibiotic unavailable to the bacteria. As a result, the increase in antibiotic resistance makes the treatment of infection longer and more difficult, and in addition imposes a high economic cost on the patient and the society. It also seems that the increase in biofilm production is related to the emergence of resistance to several drugs, which shows the importance of investigating the presence of biofilm in resistant isolates.

Conclusions

In our study, we reported the high prevalence of MDR *K. pneumoniae* strains with resistance to multiple antimicrobial agents, the ability to form biofilm, and the presence of virulence-associated genes which can be considered a major challenge for the treatment of *K. pneumoniae*-related

infections and further spread of resistance genes to other regions. However, further studies are needed to show other epidemiological aspects of the *K. pneumoniae* strains in different regions in our country.

Acknowledgements We are grateful to all those who participated in our research and those who helped in collecting the samples. We are also grateful to Professor Kazem Al-Hamdani from the College of Medicine University of Babylon for his scientific and practical help in completing this research.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval The study protocol was approved by the Ethics committee of the Iraqi Ministry of Health (2018,7133). Informed consent was obtained from all the patients before participation.

Conflict of interest The authors declare no competing interests.

References

Albasha AM, Osman EH, Abd-Alhalim S (2020) Detection of several carbapenems resistant and virulence genes in classical and hypervirulent strains of *Klebsiella pneumoniae* isolated from hospitalized neonates and adults in Khartoum. BMC Res Notes 13:1–7. https://doi.org/10.1186/s13104-020-05157-4

Ali SA, Hussein OM (2022) Molecular study on Efflux pumps of *Klebsiella pneumonia* isolated from patients with Cystitis. RJPT 15(10):4559–4564. https://doi.org/10.52711/0974-360X.2022.00765

Anis RH, Ahmed SM, Esmaeel NE (2021) Virulence determinants associated with biofilm formation by *Klebsiella pneumoniae* causing hospital-acquired bloodstream infection. Microbes Infect Dis 2(2):317–325. https://doi.org/10.21608/mid.2021.62223.1117

Araújo F, Ribeiro C, Silva I (2012) *Klebsiella pneumoniae* spinal epidural abscess treated conservatively. Acta Reum Port 37(3):260–3

Araújo BF, Ferreira ML, Campos PAD (2018) Hypervirulence and biofilm production in KPC-2-producing *Klebsiella pneumoniae* CG258 isolated in Brazil. J Med Microbiol 67(4):523–528. https://doi.org/10.1099/jmm.0.000711

Ballén V, Gabasa Y, Ratia C (2021) Antibiotic resistance and virulence profiles of *Klebsiella pneumoniae* strains isolated from different clinical sources. Front Cell Infect Microbiol 11:738223. https://doi.org/10.3389/fcimb.2021.738223

Brisse S, Passet V, Grimont PA (2014) Description of Klebsiella quasipneumoniae sp. nov., isolated from human infections, with two subspecies, Klebsiella quasipneumoniae subsp. quasipneumoniae subsp. nov. and Klebsiella quasipneumoniae subsp. similipneumoniae subsp. nov., and demonstration that *Klebsiella singaporensis* is a junior heterotypic synonym of *Klebsiella variicola*. Int J Syst Evol Microbiol 64(Pt_9):3146–3152. https://doi.org/10.1099/ijs.0.062737-0

Caneiras C, Lito L, Melo-Cristino J (2019) Community-and hospital-acquired *Klebsiella pneumoniae* urinary tract infections in Portugal: virulence and antibiotic resistance. Microorganisms 7(5):138. https://doi.org/10.3390/microorganisms7050138

Dalir A, Razavi S, Talebi M (2021) Antibiotic susceptibility pattern and distribution of virulence factors among *Klebsiella pneumoniae* isolated from healthy volunteers. Iran J Med Microbiol 15(6):676–683. https://doi.org/10.30699/ijmm.15.6.676

El-Domany RA, Awadalla OA, Shabana SA (2021) Analysis of the correlation between antibiotic resistance patterns and virulence determinants in pathogenic *Klebsiella pneumoniae* isolates from Egypt. Microb Drug Resist 27(6):727–739. https://doi.org/10.1089/mdr.2020.0236

Elmanakhly AR, Bendary MM, Safwat NA (2022) Carbapenem-resistant *Klebsiella pneumoniae*: diversity, virulence, and antimicrobial resistance. Infect Drug Resist 6177–6187. https://doi.org/10.2147/IDR.S387742

Fatima S, Liaqat F, Akbar A (2021) Virulent and multidrug-resistant *Klebsiella pneumoniae* from clinical samples in Balochistan. Int Wound J 18(4):510–518. https://doi.org/10.1111/iwj.13550

Holt KE, Wertheim H, Zadoks RN (2015) Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc Natl Acad Sci U S A 112(27):E3574–E3581. https://doi.org/10.1073/pnas.1501049112

Jensen TS, Opstrup KV, Christiansen G (2020) Complement mediated *Klebsiella pneumoniae* capsule changes. Microbes Infect 22(1):19–30. https://doi.org/10.1016/j.micinf.2019.08.003

Jones N, Mitchell J, Cooke P (2022) Gender and antimicrobial resistance: what can we learn from applying a gendered lens to data analysis using a participatory arts case study? Front Glob Womens Health 3:745862. https://doi.org/10.3389/fgwh.2022.745862

Khalil MA, Hager R, Abd-El Reheem F (2019) A study of the virulence traits of carbapenem-resistant *Klebsiella pneumoniae* isolates in a Galleria mellonella model. Microb Drug Resist 25(7):1063–1071. https://doi.org/10.1089/mdr.2018.0270

Manandhar S, Zellweger RM, Maharjan N (2020) A high prevalence of multi-drug resistant Gram-negative bacilli in a Nepali tertiary care hospital and associated widespread distribution of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-encoding genes. Ann Clin Microbiol Antimicrob 19(1):1–13. https://doi.org/10.1186/s12941-020-00390-y

Mbelle NM, Feldman C, Sekyere JO (2020) Pathogenomics and evolutionary epidemiology of multi-drug resistant clinical *Klebsiella pneumoniae* isolated from Pretoria. South Africa Sci Rep 10(1):1232. https://doi.org/10.1038/s41598-020-58012-8

Mirzaie A, Ranjbar R (2021) Antibiotic resistance, virulence-associated genes analysis and molecular typing of *Klebsiella pneumoniae* strains recovered from clinical samples. AMB Express 11:1–11. https://doi.org/10.1186/s13568-021-01282-w

Mohammed EJ, Hasan KC, Allami M (2022a) Phylogenetic groups, serogroups and virulence factors of uropathogenic *Escherichia coli* isolated from patients with urinary tract infection in Baghdad. Iraq Iran J Microbiol 14:445. https://doi.org/10.18502/ijm.v14i4.10230

Mohammed EJ, Hasan KC, Allami M (2022b) Phylogenetic groups, serogroups and virulence factors of uropathogenic *Escherichia coli* isolated from patients with urinary tract infection in Baghdad. Iraq Iran J Microbiol 14(4):445–457. https://doi.org/10.18502/ijm.v14i4.10230

Naqid IA, Hussein NR, Balatay AA (2020) The antimicrobial resistance pattern of *Klebsiella pneumonia* isolated from the clinical specimens in Duhok City in Kurdistan Region of Iraq. J Kermanshah Univ Med Sci 24(2):e106135. https://doi.org/10.5812/jkums.106135

Peerayeh SN, Rostami E, Siadat SD (2014) High rate of aminoglycoside resistance in CTX-M-15 producing *Klebsiella pneumoniae* isolates in Tehran. Iran Lab Med 45(3):231–237. https://doi.org/10.1309/LMDQQW246NYAHHAD

Rastegar S, Moradi M, Kalantar-Neyestanaki D (2019) Virulence factors, capsular serotypes and antimicrobial resistance of hypervirulent *Klebsiella pneumoniae* and classical *Klebsiella pneumoniae*

in Southeast Iran. Infect Chemother 51(4):e39. https://doi.org/10.3947/ic.2019.51.e39

Shadkam S, Goli HR, Mirzaei B (2021) Correlation between antimicrobial resistance and biofilm formation capability among *Klebsiella pneumoniae* strains isolated from hospitalized patients in Iran. Ann Clin Microbiol Antimicrob 20:1–7. https://doi.org/10.1186/s12941-021-00418-x

Shakib P, Kalani MT, Ramazanzadeh R (2018) Molecular detection of virulence genes in *Klebsiella pneumoniae* clinical isolates from Kurdistan Province. Iran Biomed Res Ther 5(8):2581–2589. https://doi.org/10.15419/bmrat.v5i8.467

Sokhn ES, Salami A, El Roz A (2020) Antimicrobial susceptibilities and laboratory profiles of *Escherichia coli, Klebsiella pneumoniae*, *and Proteus mirabilis* isolates as agents of urinary tract infection in Lebanon: paving the way for better diagnostics. Med Sci 8(3):32. https://doi.org/10.3390/medsci8030032

Yoon H, Song B, Yoon SS (2022) A case of *Klebsiella pneumoniae* meningitis associated with brain abscess and endophthalmitis. J Neurosonol Neuroimag 14(2):95–98. https://doi.org/10.31728/jnn.2022.00129

Zhu J, Wang T, Chen L (2021) Virulence factors in hypervirulent *Klebsiella pneumoniae*. Front Microbiol 12:642484. https://doi.org/10.3389/fmicb.2021.642484

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.