Journal of Medical and oral biosciences
ISSN (Online): 3007-9551
ISSN (Print): 3007-9543

JMOB
Open Access DOAJ

Type: Research article
Published: 08/ 11/2024

Molecular detection of multidrug resistance Klebsiella species isolates from urinary tract patients in Thi-Qar province

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OPEN ACCESS

ARTICLE INFO
Received: 21/ 09/2024
Revised: 29/ 09/ 2024
Accepted: 01/ 10/2024
Publish online: 08/11/2024

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CITATION

Liath Lateef Salih , Haydar Khamis Al-maliky. (2024). Molecular detection of multidrug resistance Klebsiella species isolates from urinary tract patients in Thi-Qar province. JMOB. 1;(4): 01-10.
DOI: https://doi.org/ 10.58564/jmob.61

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Abstract

The bacterium causing urinary tract infections (UTIs), *Klebsiella pneumoniae* is a major globally. It is a gram-negative bacterial genus, a member of the Enterobacteriaceae family. It is a rod-shaped, non-motile, non-spore-forming with a prominent capsule made of polysaccharides. This study designed to isolate and identify *Klebsiella species* from urinary tract patients in Thi-Qar province. One hundred specimens were collected from UTIs-infected patients, ageing (11) to (76) years, .in three hospitals (Al-Hussein Teaching Hospital, Al-Nasiriya Teaching Hospital, and Bint Al-Huda Hospital) in Thi-Qar province between November 2023 and March 2024.The isolates were identified and their features concerning antibiotic resistance were investigated using the VITEK II system.Then examining the isolated bacterial proteins started with the polymerase chain reaction (PCR) assay identifying 16S rRNA . Results showed that rmpA, AcrAB, and fimH1 were the virulence genes. On the culture medium, the most commonly observed gram-positive bacteria were 25 (30.49%), E. coli 19 (23.17%), and *K. pneumoniae* 33 (40.24%).Using the VITEK II system revealed 33 samples including *Klebsiella spp.* bacterial presence. Men had the highest rate (77.97%) while the women rate (59.02%) regarding bacterial infections. Examining 70 drugs against K. pneumoniae, the study found that 45.67% of isolated bacteria were resistant to antibiotics, 9.91% were intermediate, and 44.42% of the bacteria were sensitive to antibiotics. Comprising 100% Ampicillin, 93.94% Amoxicillin, and 63.64% Ceftriaxone. The study revealed that successful treatments for *K. pneumoniae* are Cefpirome and Faropenem. The study finds isolates of 22 Klebsiella pneumoniae. PCR results of Gram-negative bacterial amplification helped to identify the isolates by means of the 16S rRNA amplification. According to the research, isolates of *Klebsiella pneumoniae* show great potential as targets for antibiotics and other medications. Therefore, the authors request a future study to determine the epidemiology of *Klebsiella pneumoniae* in all Iraqi provinces.

Keywords: urinary tract infection, Klebsiella ,multidrug resistance, PCR.

Introduction

Urinary tract infections (UTIs) are well known in medicine as relatively common diseases(1). Multidrug-resistant *Enterobacteriaceae* (including the principal causative agents; *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus* spp) generate an extended-spectrum beta-lactamases (ESBL) and carbapenemases UTIs (2). In most regions of Iraq, urinary tract infection is widespread and remains the main health concern in many

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developing countries (3) . Among the most typical bacterial illnesses, including nosocomial diseases and short-term infections, are the UTI. *Enterobacteriaceae* are generally prone to urine contamination (4) .About 75% to 90% of all cases of urinary tract disorders are brought about by *E. coli* (3) .Uropathogens occur in a varied array of structures, although *E. coli* is still the most prevalent. *Klebsiella pneumoniae*, *Enterobacter*, *Proteus spp.*, and *Enterococcus spp.* are some additional typical uropathogens (5). The UTIs can damage the kidneys, bladder, ureters, and urethra. The three primary forms of UTIs are urethritis, pyelonephritis, and cystitis referring to the inflammation of the urethra, kidney, and bladder, respectively. Members of the *Enterobacteriaceae* family, *Klebsiella* species are facultative anaerobes that are encapsulated, non-motile, Gram-negative, and capable of digesting lactose. The natural flora of the gastrointestinal tracts of animals and humans contains *Klebsiella* spp. as a significant opportunistic pathogen. But given the perfect conditions, these bacteria can cause a number of illnesses in both disorders that affect both humans and animals for example mastitis, pneumonia, and urethral infections(6).Among the numerous are different lipopolysaccharides (LPS), fimbrial and non-fimbrial adhesins, and iron-scavenging systems multidrug-resistant bacteria produce significant Infections acquired in hospitals and in the community that are challenging to cure by using existing medications. β-lactam antibiotics limit peptide bond formation by acting as competitive inhibitors of these Penicillin-binding proteins. These result in the creation of irreversible covalently bonded penicilloyl-enzyme complexes with weakly cross-linked peptidoglycans, which therefore ease bacterial lyses (7).

Methodology

Study design and participants

Between (November 2023 to March 2024), a total of 120 samples were obtained from patients affected with UTIs at Al-Hussein Teaching Hospital, Al-Nasiriya Teaching Hospital, and Bint Al-Huda Hospital. The patients' age ranged from (11) to (76) years.

Study tools and data collection

The bacterial isolates were identified by culturing on bacteriological media, colony morphology, biochemical reactions and final diagnosis by VITEK-II system. Urine culture on plates MacConkey and blood agar. A minor amount of urine samples was grown on the media using sterile swabs by streaking technique then incubated for 24 h. The pure colonies from the growing bacteria were isolated and submit these samples to VITEK-II. According to instruction of the primer synthesizer business, the primers (originally lyophilized), were dissolved in the free ddH2O to reach a final concentration of 100 µM/µl which functioned as a stock solution that stored at -20 ^{o}C. The stock primers were used to create a work primer with a concentration of 10 µM/µl. The data for the primers were provided (Table 1).

Statistical analysis

The data of the current study was statistically analysis by using SPSS (Statistical Package for the Social Sciences), version 26, based in using both Descriptive and non-parametric chi-square, at p. value < 0.05.

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Table. 1: Primer details used in the current experiment

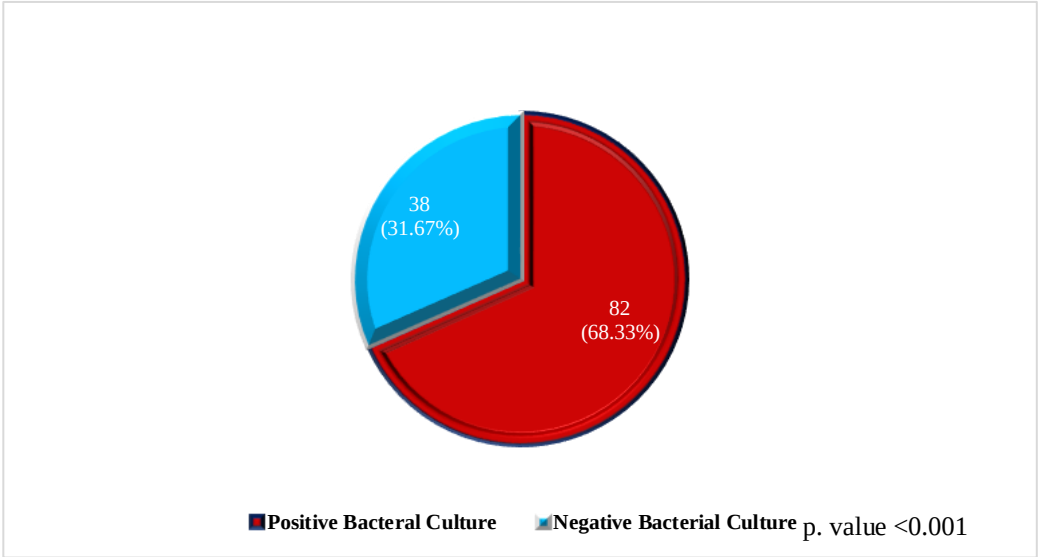
Gene	Primer name	Sequence (5'-3')	PCR size	Accession number
16S *Rrna*	F	ATGACGTCAAGTCATCATGG	353 bp	CP147165.1
	R	AGGAGGTGATCCAGCCGCA		
RmpA	F	ACTGGGCTACCTCTGCTTCA	535 bp	KY403906.1
	R	CTTGCATGAGCCATCTTTCA		
fimH-1	F	TGCTGCTGGGCTGGTCGATG	550 bp	CP152422.1
	R	GGGAGGGTGACGGTGACATC		
AcrAB	F	ATCAGCGGCCGGATTGGTAAA	312 bp	CP147165.1
	R	CGGGTTCGGGAAAATAGCGCG		

Results

Urinary tract infection

The study covered 120 patients with UTIs , with 82 patients (68.33%) having bacterial infections, while 38 (31.67%) had no infections (Figure.1). The study indicated a substantial variation in the frequency of bacterial infections between male and female groups. The highest positive bacterial infection was found in males (77.17%), then in females (59.02%). The investigation found the top isolated bacteria as *K. pneumonia* (40.24%), followed by gram-positive bacteria (30.49%) and E. coli (23.17%). The lowest bacterial isolates were *P. mirabilis* and *E. aeruginosa* (1.22%).

Figure.1: The prevalence of bacterial infection in UTI patients



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Identification of *Klebsiella pneumoniae* isolates

The study identified 33 *Klebsiella pneumoniae* isolates from 40 samples using a variety of morphological, physiological, and biochemical tests. The VITEK II system was used to authenticate the isolates' bacterial identification .Colony morphology exhibited huge, pink, regular-edged, spherical colonies with a mucoid texture generated by *Klebsiella* when they fermented lactose. The percentage of isolated *K. pneumonia* was 27.5%, with the highest infection in males (63.64%) and females (36.36%). The VITEK II system revealed that all 40 isolates previously confirmed to be *Klebsiella* species. The study also identified a non-significant difference between *K. pneumonia* infection and sex of patients, with the highest infection in males (63.64%) and in the females (36.36%) . *K. pneumonia* infection and age group of patients, with the highest infection in the 50th age group (33.33%) and the lowest rate of in the 60th age group (21.21%).

Table 2 : Prevalence of *K. pneumonia* infection according to sex

K. pneumonia		
Sex	No.	%
Male	21	63.64
Female	12	36.36
Total	33	100
CalX^2= 2.45 TabX^2= 3.84 DF= 1 P. value 0.117		

Antibiotics Susceptibility

The study examined 70 antimicrobials against *K. pneumoniae*, indicating that 45.67% of the isolates were resistant, 9.91% intermediate, and 44.42% sensitive. Of the 33 isolates examined, 100% were resistant to ampicillin, followed by 93.94% to amoxicillin, and finally 63.64% to ceftriaxone. The research also showed that 33 of the isolates tested responded effectively to cefpirome 100%. The most sensitive antibiotics were Latamoxef (100%), Ceftazidime / Avibactam and Ceftolozane / Tazobactam (100%), and Isepamicin (93.94). The most successful therapy for *K. pneumoniae* was imipenem, with the most sensitive antibiotics being ceftazidime, ceftazidime / Avibactam, ceftolozane / Tazobactam, ceftriaxone, cefuroxime, cenoxacin, ceprofloxacin, doripenem, enrofloxacin, and faropenem. The study underscores the relevance of understanding antibiotic resistance and sensitivity in treating *K. pneumoniae* in Table 3.

Table 3. Antibiotics Susceptibility of k. pneumonia

Antibiotics	**Resistance**		**Intermediate**		**Sensitive**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amikacin	4	12.12	2	6.06	27	81.82
Amoxicillin	31	93.94	2	6.06	0	0.00
Amoxicillin / Clavulanic Acid	22	66.67	9	27.27	2	6.06

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Antibiotics	Resistance		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Ampicillin	33	100.00	0	0.00	0	0.00
Ampicillin/Sulbactam	24	72.73	8	24.24	1	3.03
Azlocillin	31	93.94	2	6.06	0	0.00
Aztreonam	4	12.12	6	18.18	23	69.70
Carbenicillin	30	90.91	2	6.06	1	3.03
Cefaclor	25	75.76	7	21.21	1	3.03
Cefadroxil	23	69.70	8	24.24	2	6.06
Cefalexin	25	75.76	5	15.15	3	9.09
Cefalotin	28	84.85	5	15.15	0	0.00
Cefamandole	30	90.91	2	6.06	1	3.03
Cefazolin	30	90.91	1	3.03	2	6.06
Cefcapene	25	75.76	4	12.12	4	12.12
Cefditoren	8	24.24	4	12.12	21	63.64
Cefepime	2	6.06	2	6.06	29	87.88
Cefixime	6	18.18	6	18.18	21	63.64
Cefmetazole	12	36.36	6	18.18	15	45.45
Cefonicid	27	81.82	5	15.15	1	3.03
Cefotetan	7	21.21	8	24.24	18	54.55
Cefotiam	20	60.61	4	12.12	9	27.27
Cefoxitin	4	12.12	6	18.18	23	69.70
Cefpirome	0	0.00	0	0.00	33	100.00
Cefpodoxime	14	42.42	6	18.18	13	39.39
Cefprozil	28	84.85	4	12.12	1	3.03
Cefradine	30	90.91	2	6.06	1	3.03
Cefsulodin	29	87.88	4	12.12	0	0.00
Ceftazidime	5	15.15	9	27.27	19	57.58
Ceftazidime / Avibactam	0	0.00	0	0.00	33	100.00
Ceftibuten	26	78.79	3	9.09	4	12.12
Ceftizoxime	3	9.09	3	9.09	27	81.82
Ceftolozane/Tazobactam	0	0.00	0	0.00	33	100.00
Ceftriaxone	21	63.64	2	6.06	10	30.30
Cefuroxime	33	100.00	0	0.00	0	0.00
Cinoxacin	15	45.45	2	6.06	16	48.48
Ciprofloxacin	4	12.12	1	3.03	28	84.85
Doripenem	0	0.00	0	0.00	33	100.00
Enrofloxacin	14	42.42	6	18.18	13	39.39
Faropenem	0	0.00	0	0.00	33	100.00
Fleroxacin	20	60.61	2	6.06	11	33.33
Gatifloxacin	5	15.15	3	9.09	25	75.76

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Antibiotics	Resistance		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Gentamicin	2	6.06	1	3.03	30	90.91
Imipenem	6	18.18	3	9.09	24	72.73
Isepamicin	2	6.06	0	0.00	31	93.94
Latamoxef	0	0.00	0	0.00	33	100.00
Levofloxacin	5	15.15	2	6.06	26	78.79
Lomefloxacin	6	18.18	2	6.06	25	75.76
Loracarbef	28	84.85	2	6.06	3	9.09
Marbofloxacin	24	72.73	2	6.06	7	21.21
Meropenem	2	6.06	3	9.09	28	84.85
Mezlocillin	30	90.91	3	9.09	0	0.00
Moxifloxacin	4	12.12	1	3.03	28	84.85
Nalidixic Acid	18	54.55	2	6.06	13	39.39
Netilmicin	3	9.09	1	3.03	29	87.88
Nitrofurantoin	3	9.09	15	45.45	15	45.45
Norfloxacin	15	45.45	2	6.06	16	48.48
Ofloxacin	4	12.12	2	6.06	27	81.82
Pipemidic Acid	18	54.55	0	0.00	15	45.45
Piperacillin	33	100.00	0	0.00	0	0.00
Piperacillin/Sulbactam	17	51.52	10	30.30	6	18.18
Piperacillin/Tazobactam	11	33.33	18	54.55	4	12.12
Prulifloxacin	10	30.30	2	6.06	21	63.64
Ticarcillin	31	93.94	2	6.06	0	0.00
Ticarcillin/Clavulanic Acid	33	100.00	0	0.00	0	0.00
Tigecycline	0	0.00	0	0.00	33	100.00
Tobramycin	4	12.12	1	3.03	28	84.85
Tosufloxacin	4	12.12	0	0.00	29	87.88
Trimethoprim	23	69.70	2	6.06	8	24.24
Trimethoprim/ Sulfamethoxazole	21	63.64	2	6.06	10	30.30
Susceptibility %	45.67		9.91		44.42	
$CalX^2$= 1562.1 $TabX^2$= 183.5 DF= 138 p. value <0.001						

Molecular detection of *16S rRNA* of *Klebsiella pneumonia*

The study attempted to identify the isolates of *Klebsiella pneumoniae* utilizing biochemical tests and molecular approaches. Out of the 22 isolates, the bacteria were identified using biochemical testing and molecular techniques. The PCR results of the amplification of the partial region of the gene *16S rRNA* of Gram negative bacteria were 353 bp in size. The PCR products of the amplification of the *AcrAB* gene were 312 bp in size. All 22 isolates underwent molecular identification by PCR amplification of the *K.*

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pneumonia AcrAB gene using specific primers. The results showed that the amplified fragments were 312 bp in size. All of the isolates (95.45%) produced good findings (550 bp). The PCR results of the amplification of the partial region of the gene fimH gene were 550 bp.(Fig 2.A and B)

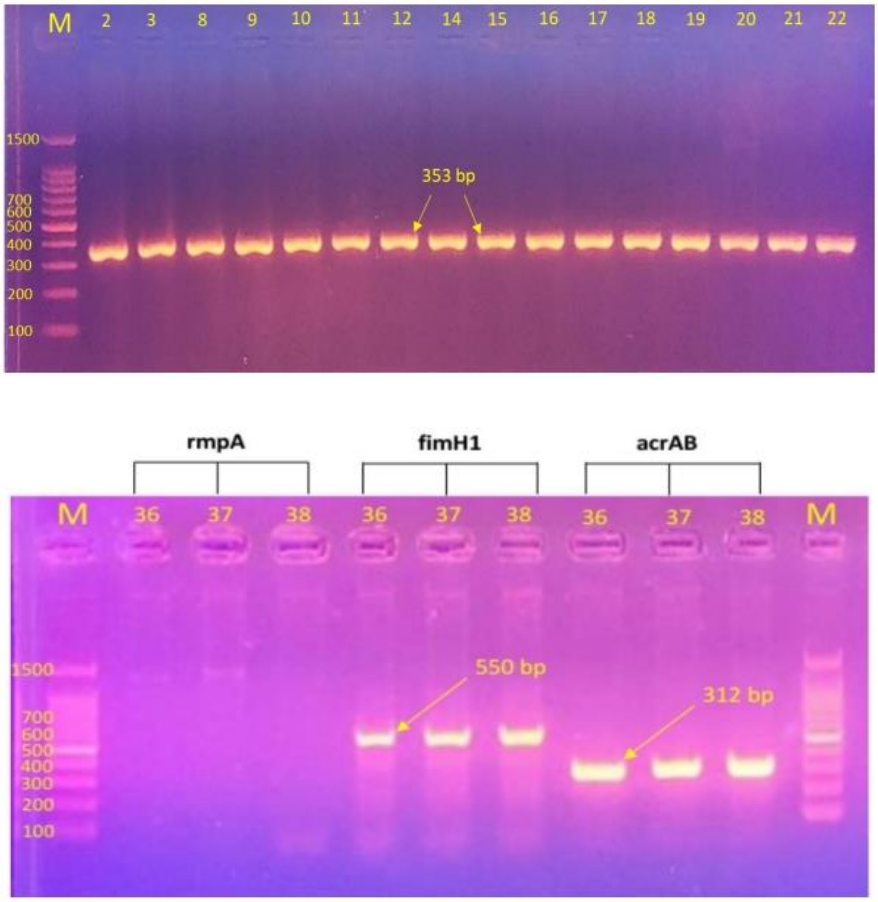

Figure.2: (A) PCR products of the amplification of partial region of gene 16S rRNA of Gram-negative bacteria. **(B)** PCR products of the amplification of partial region of genes rmpA and fimH1 and acrAB in the isolates of *Klebsiella pneumoniae*

Discussion

The need for medical attention in the community is often associated with UTIs. When it comes to treating bacterial UTIs, the key is to identify the specific organism responsible for the illness and then choose the right antibiotics to kill it. In Iraq, as for most developing countries, UTIs are a major public health concern (8). Only 82 out of 120 patients (68.33%) had a positive bacterial culture when they were clinically diagnosed with a UTI. This finding was consistent with that of other Iraqi researchers. A research done by Alsamarai and Ali, (2016) (9) in Tikrit indicated that 234 out of 563 patients (41.6%) had positive cultures (10). In addition, they also stated that out of 135 urine samples taken in Baquba, only 110 samples indicated bacterial growth, representing for 81.4% of the total. Furthermore, out of 774 specimens, 278 (or 35.9%) tested positive for bacterial cultures in the Wasit (11). Of the 120 UTI isolates evaluated, 33 (27.5%) were found to be *K. pneumoniae*. The obtained results were lower than the findings in Romania (12). These bacteria are common in the resident flora and have several pathogenic factors that affect

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their ability to cause illness, which may explain why they are so common in UTIs. The variance in results linked to other research might be ascribed to changes in sample size and the time of the investigation. The present investigation demonstrates that *K. pneumoniae* was frequent among 63.64% of males, and it was 36.36% inside women. These results agrees with a research in India, who demonstrated that uropathogenic *K. pneumoniae* in males (31.6%) was more prevalent than females (7.3%)(13). Also, in Pakistan, that men were more vulnerable to uropathogenic *K. pneumonia* (14). Also, Korea, reported the rate of *K. pneumoniae* in UTIs was present in 7.7% of males and 5.3 % in females (15). The isolated strain of *K. pneumoniae* was submitted to testing against 70 antibiotics with various mechanisms of action. The findings of this analysis indicated that 45.67% of the recovered bacteria displayed resistance to antibiotics, 9.91% shown intermediate sensitivity, and 44.42% were resistant to antibiotics among the 33 *K. pneumoniae* isolates investigated. The resistance for ampicillin rate was 100% and the piperacillin sensitivity rate was 100%; both are penicillins (16). One of the most utilized medications for treating infections of the urinary tract is ampicillin, the subsequent prevalent resistance in Iraqi isolates presents a substantial hurdle to antibiotic treatment (17). Based on high antibiotic resistance, Thomson and Amyes, (1993) found that 88.2 % of *Klebsiella* isolates from illnesses in Scotland were ampicillin-resistant (18). For molecular identification, all 33 isolates were submitted to PCR amplification of 16S rRNA with K 16S-F and K 16S-R primers, which are appropriate for the PCR amplification of *16S rRNA* of *K. pneumonia*. The amplified fragment were roughly 353 bp ,also obtained the same size when they used the same primer (19). The results for all 40 isolates were positive (353 bp bands), identifying them as *K. pneumonia* . *AcrAB* gene presence in *Klebsiella pneumoniae* isolates from a Brazilian intensive care unit was studied by *Ferreira et al.,* (2018). Their study indicated that *AcrAB* was 100% of isolate , (41%) of the strains possessed efflux pump genes, including *AcrAB* (20). They also remark out that comparing to other efflux pump genes, *AcrAB* was more frequent in *K. pneumoniae* strains. Additionally, comparing to other clinical specimens, the urine samples contained a larger quantity of the *AcrAB* efflux pump gene (21). Moreover, they found that 90% of *K. pneumoniae* strains from diverse environmental and clinical sources possess the *fimH* gene (22).

Conclusion

The study approved that most patients attributed with UTI o *klebsiella* were men ageing from 51to 60. Further, *Klebsiella pneumoniae* has been shown to be particularly resistant to multiple classes of antimicrobials in clinical isolates (MDR). The ampicillin and cefuroxime groups showed the highest rates of resistance, while the cefprozil and doripenem were the most effective against *klebsiella pneumonia*. The virulence factors linked to *Klebsiella pneumonia* isolates are mainly attributed to *AcrAB* gene (100%) and *fimH* (95.45%), with no effect revealed by *rmpA* gene (0 %).

DECLARATIONS

Acknowledgments

We would like to thank the College of Veterinary Medicine and Surgery at Al-Shatra University for their aid and support. This paper is part of Liath master thesis.

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Funding
The author did not receive any source of fund.

Competing interests statement No conflict of interest related with publishing of this article.

Ethics statement

The author approved that this research follows the journal's Attach Ethic Approval guidelines as appeared on the journal's author guidelines page.

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