

Molecular Study of Tetracycline and Quinolone-Resistant *Klebsiella Pneumoniae* Urinary Tract Infections in Pregnant Women in Diwaniyah (Shamiya City) - Iraq

Oday Mitib Hadi¹, Majed H. Hadi Al-husseini²

^{1,2}Department of, Pathological Analysis, College of Health and Medical Techniques /Kufa, Al-Furat Al-Awsat Technical University, Iraq

Abstract

Hospital infections caused by *Klebsiella pneumoniae* are associated with substantial morbidity and mortality because of limited treatment options. The use of fluoroquinolone antibiotics has spread widely in the past decades leading to the emergence of resistant bacterial strains. The plasmid-mediated quinolone resistance (PMQR) is considered as a common mechanism contributed to resistance among Gram-negative. While Mechanisms of resistance to tetracycline through the acquisition of tet genes mainly include efflux pumps, ribosomal protection, and enzymatic deactivation. Mutations also contribute to the antibiotic resistance. The tet genes found at the highest frequency in gram-negative bacteria are related to efflux pumps, which are coded by the tetA, tetB, gene. Method: The current study included two hundred twenty-five Pregnant women as if They had an infection of the urinary tract. and were treated in the Shamiya General Hospital and the Pregnant Care Center in the Shamiya sector from October 2021 to March 2022. One hundred forty-nine different types of bacteria were isolated and using diagnostic methods nineteen isolates of *Klebsiella pneumoniae* were obtained. Results The results revealed that 225 pregnant women were the results were positive for Urinary tract infections were found in 149 patients, and the bacteria that grew was diverse. The age group of 21–25 had the highest risk of infection. The findings revealed that women with an elementary education had the highest likelihood of injuries, with roughly 86 pregnant women infected at a rate of 43% (64/149). The probability value is smaller than 0.05 by 0.000. As a result, the disparity in educational levels significantly impacts the injuries. According to this research, the most common bacterial diseases include Then 19 positive samples of *Klebsiella pneumoniae* were detected using chemical and morphological methods. *Klebsiella* showed great resistance to tetracycline (89.47%), while the percentage of resistance to Doxycycline was (73.70%), and the resistance was much lower to quinolones, where there is a high percentage of opposition Ciprofloxacin was (31.58%) and Levofloxacin (26.32). After that, all samples were submitted to a genetic PCR analysis utilizing tetracycline and quinolone genes primers (tetA, tetB, qnrA, qnrB) **Keywords:** antibiotic, *Klebsiella pneumoniae*, UTI, tetA, tetB, qnrA, qnrB ,quinolone, tetracycline, pregnant women

1. Introduction

The upper or lower urinary tract might get infected when there an infection that has spread to the urinary tract. This illness is also known as cystitis, which refers to an infection of the bladder, and pyelonephritis (an infection of the kidneys). Bacteria that enter the urinary tract through the urethra and proliferate in the bladder are the most common culprits in the development of urinary tract infections (UTIs). The urinary tract is home to some of the most common types of infections. This line of defense can be breached even though the urinary system is designed to keep out such tiny invaders. There are no germs in urine, despite the fact that it contains a wide range of substances like water, salt, and waste products. A full-blown infection of the urinary system can result if bacteria multiply in this situation. Infections of the urinary tract are caused by bacteria entering the bladder or the kidney and causing them to multiply in the bloodstream. Most women will experience a urinary tract infection (UTI) at least once

in their adulthood [1].

Klebsiella pneumoniae is the most prevalent cause of urinary tract infections., which is second only to *Escherichia coli* in frequency. The most common cause of human sickness is *Escherichia coli*. *Klebsiella pneumoniae* was initially described by Karl Friedlander in 1882. Following his investigation into It was found that pneumonia was caused by an encapsulated bacillus, which was found in the lungs of people who died of it. When *Klebsiella* was first found in 1886, the bacteria was referred to as *Bacillus Friedlander*. *Klebsiella pneumoniae*, a Gram-negative, encapsulated, and non-moving bacterium, can cause pneumonia in patients with diabetes or alcoholism. Since *K. pneumoniae* has a high prevalence of antibiotic resistance, it has been identified as one of the most frequent infectious disease pathogens and as such, a significant threat to public health [2, 3].

There are a lot of moving parts when it comes to the emergence of antibiotic resistance. However, it demonstrates the practical effects of long-term use

of antibiotics. Because of natural selection, many different genetic pathways have been created. Over time, acquired resistance has led to the creation of bacteria that are resistant to multiple drugs (MDR) and/or are extremely drug-resistant (XDR), are resistant to the majority of antibiotics and have no opportunity for therapeutic innovation. Enzymes like carbapenemases, metallo-lactamases and oxacillinases are among the many that *K. pneumoniae* is capable of making [4].

Conjugation in plasmids and transposons is used to transmit tetracycline resistance genes. There are, however, a few chromosomal genes that are present in some isolates. It is possible to acquire resistance mechanisms such as efflux pumps and enzyme inactivation by acquiring tet genes. There has been an upsurge in clinical *K. pneumoniae* quinolone resistance in developed and developing nations alike because quinolones are widely used to treat urinary tract infections that produce ESBLs [5, 6]. DNA gyrase and DNA topoisomerase IV mutations were previously cited for Enterobacteriaceae resistance to quinolones, but a new study has found that PMQR (plasmid-mediated Quinolone Resistance) is responsible. The PMQR gene is responsible for the low-level quinolone resistance [7].

3. Patients and Methods 3.1 Data Collection

A total of 225 samples of the pregnant women's urine were collected for testing. Isolates were collected from the Al-Shamiya Hospital and Maternity Care Centers in the Al-Shamiya region of the Al-Diwaniyah Governorate in Iraq between October 2021 and March 2022. The Medical Technical College/Kufa of Al Furat Al Awsat Technical University's Department of Pathological Analysis conducted this examination. *K. pneumoniae* bacteria were isolated from patients who had contracted their illnesses either in a hospital or out in the community. Of these, 19 unique strains were chosen for further study. During the course of the study, clinical urine samples were collected from people who were experiencing urinary tract infections. The researchers then isolated the germs from those samples.

Bacterial Isolation and Identification

Isolates of *K. pneumoniae* were recognized as such through the use of Microscopy, culture, Gram stain,

and other traditional biochemical tests are all options. Cultivation was carried out on blood agar, eosin methylene blue media, and McConkey agar. They were bought from Neogen in the UK and Hi-media in India. Microbes that pose a threat to human health were identified by means of culture-specific genetic tests, typical characteristics, and chromium-agar cultures. Following Gram staining and routine biochemical assays, bacterial isolates were investigated using catalase, indole formation, methyl red, voges proskauer, simon citrate, urase, and triple sugar iron assays to identify whether the bacteria were gram-positive or gram-negative.

Assay for Susceptibility to Antimicrobial Drugs

Antibiotic susceptibility testing was performed on Mueller-Hinton agar plates using the disk diffusion method (Kirby Bauer) in accordance with the clinical laboratory standards institute guidelines (Hi-media India). Amikacin (10 g), Rifampin (5 g), Ciprofloxacin (10 g), Gentamicin (30 g), Chloramphenicol (10 g), and Rifampin Clindamycin (15 g) were found in the discs of antibacterial drugs utilized in this investigation. Eethromycin (15 g), Doxycycline (10 g), Levofloxacin (5 g), and Tetracycline (10 g) were also found. Mast laboratories provided the antibiotics used in this study (Bioanalyse Company Turkey). The CLSI criteria were used to convert the diameters of the zones of inhibition for particular antibacterial drugs into susceptible, intermediate, and resistant groups. More than one class of antibiotic-resistant microbes were discovered.

Multiplex Polymerase Chain Reaction the genes involved in the (Tet A, Tet B, Qnr A, and Qnr B)

In order to test for the presence of (Tet A, Tet B, Qnr A, and Qnr B,) PCR was used. Table 2 lists the primer sequences used in gene PCR. To optimize the PCR for this experiment, the following conditions were used: an initial denaturation period of five minutes at 94 and 95 degrees Celsius, 30,35 cycles of denaturation at 55, 57, 72 and 72 degrees Celsius for 30 seconds or 1 minute, followed by a one-time cycle that lasted five minutes at a temperature of four degrees Celsius, followed by a final extension period that lasted ten minutes at 72 degrees Celsius.

Table (1). Primers used in gene PCR and their sequences

Reference	Product Size (bp)	Oligo-Sequence (3'→5')	Name of genes
(Boroumand et al., 2021)	576	GGTTCACCTCGAACGACGTCA :F R: CTGTCCGACAAGTTGCATGA	tet A
Sobur et al., 2019)	634	F: CCTCAGCTTCTCAACGCGTG R: GCACCTTGCTGATGACTCTT	tet B
Shahin et al. 2020)	519	F ATTTCTCACGCCAGGATTTG R G ATCGGCAAAGGTTAGGTCA	qnrA
(Herrera et al., 2021)	469	F: G ATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	qnrB

2. Statistical Analysis

Statistics were used to examine the data and explain

the distribution of variables based on their absolute and/or relative (percent) frequency of appearance. In order to find significant relationships between several factors of Isolated urinary tract infection

associated *Klebsiella pneumoniae* strains. The chi-square (2) tests were carried out with the use of IBM SPSS statistics software (Version 20. SPSS Inc, United States). If the P values for both tests were less than 0.01, they were considered very significant.

3. Result

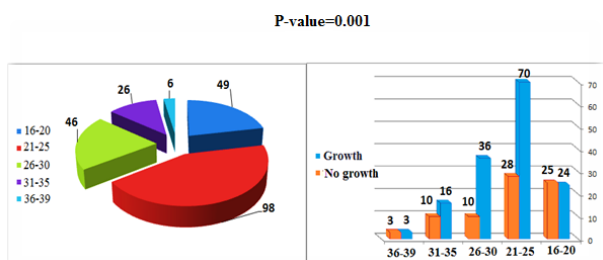


Figure 1: Pregnancy distribution by age group (chi-square=11.642).

In the current study, there were 225 pregnant women (149 with UTI and the rest without) (76 not infected with UTI). The recent study's findings revealed that the number of infected pregnant women was distributed throughout five age groups. Figure (1) shows that the largest age group included 70 infected pregnant women aged 21–25 years, with a percentage of 31.11 percent (70/225). However, the lowest age group was 36–39 years old, with 1.33 percent (3/225) of pregnant women infected. The remaining age categories were allocated as follows: 10.67 percent (24/225), 16 % (36 /225), and 7.11 percent (16/225) for the age groups 16-20, 26-30, and 31-35. Because the p-value is greater than 0.05, the age-related differences in infection rates are significant.

The outcomes were very similar to published by Rudri Bai et al. [8]. It also shows that UTI infections are prevalent in people aged 21--25. In comparison to other groups, However, the findings contradict those previously reported by Kolo et al. [9]. who found that the third age group, 26–30 years, had the greatest bacterial infections compared to the other groups. The discrepancies in outcomes could be related to variances in environmental factors, community social practices, personal hygiene standards, and health-care-seeking behaviors.

Figure (2) the p-value is 0.275 greater than 0.05, Consequently, the difference in blood types has no bearing on the likelihood of being infected.

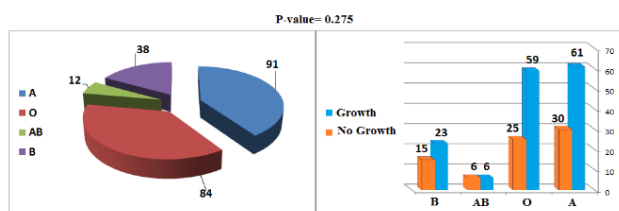


Figure (2) Distribution of pregnant women based on their blood types. (chi – square =0.365).

Infection in pregnant women is distributed according to four blood groups. Figure 2-4 demonstrated the highest blood group was a group, about 61 of infection in pregnant women by percentage, 27.11%

(61/225). But the lowest blood group was AB group, about 6 infections in pregnant women by percentage 2.66% (6/225). The rest of the blood groups were distributed according to 26.22% (59/225) and 10.22% (23/225) for blood groups O and BAs an example, there is no difference in infection risk between the two blood groups, as shown in Figure 2, with a p-value of 0.275 greater, than 0.05. Figure 2 shows comparable results to those found in Benli et al. [10].

When it came to urinary tract infections, type a blood had the highest rate, followed closely by type O. Blood types B and AB had the lowest rates, and no correlation was found between blood type and the risk of infection. However, these findings differed from those published by Mahmoud et al. [11]. The most infected were women with blood type O, followed by those with blood type B, with blood types A and AB having the lowest rates of infection. This association between blood type and the occurrence of urinary tract infections in women was particularly strong. The type and characteristics of patients studied may be a factor in determining the results of different studies in this area.

Figure (3) the p-value is 0.603 greater than 0.05, therefor the difference between Pregnancy trimesters is not significant to infection

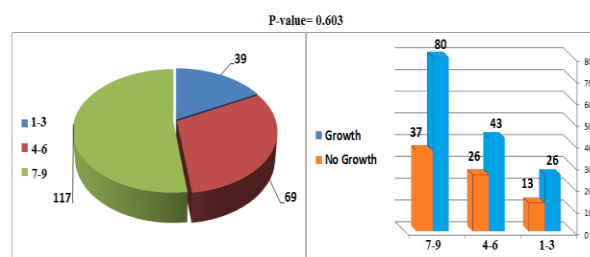


Figure (3) Distribution of the pregnant women according to the Pregnancy trimesters (chi – square =0.161).

Figure 3 has three Pregnancy trimesters of infected pregnant women the highest number of infections during Pregnancy third trimesters were (7-9) months about 80 infected pregnant women with a percentage of 68.37%(80/117). But the lowest number of infections during Pregnancy first trimesters was (1-3) months of infection about 26 infections in pregnant women with a percentage of 66.67% (26/39). But the number of infections during Pregnancy second trimesters at (4-6) months was 43 by a percentage of 62. 32% (43/69)

According to Kolo et al. [9] and Dinc [12], the results shown in figure 3 were expected to be identical. In the third trimester, the rate of UTI infection was significantly higher than in the first and second trimesters. It's possible that this is related to an uptick in activity. Obstructed mechanically by uterine pressure during pregnancy in the third trimester of pregnancy, the enlarged uterus has an impact on the ureter. Increase the relaxation impact on smooth muscle as well. Stress on the bladder as a result of pregnancy hormones and the descending section may cause the urine to get stagnant, which can lead

to an increase in the growth of bacteria. On the other hand, Prathibha's 2019 results are in odds with these findings. In the second trimester of pregnancy, urinary tract infection was observed, during the third trimester, the most instances of urinary tract infection were reported. It was slightly higher in the first trimester than in the third.

Figure (4) the p-value is 0.000 less than 0.05, therefore the difference between educational level is significant to infection

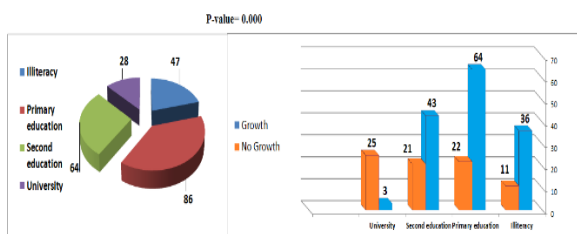


Figure (4) Distribution of the pregnant women according to level of educational ($\chi^2 = 29.987$)

Figure 6 shows the educational level indicative of pregnant women. The highest level of education was primary education, with about 86 pregnant women, with an infection rate of 43% (64/149). As for the lowest level of university education, it was about 28 pregnant women, with an infection rate of 2.01% (3/149). The remaining educational level was distributed as 24.16% (36/149) and 3.36% (43/149) for literacy and secondary education, respectively.

Figure 6 shows results that are similar to those published by Almkhtar [13], as shown in the figure. The percentage of bacterial growth in illiterate pregnant women was the highest, and the percentages were the lowest among those who obtained primary and secondary education, and women at the university level had the lowest bacterial growth. This may be owing to low or moderate education attributed to a lack of information sources about UTIs, which may increase the incidence of UTIs. There are some differences between this study and those previously mentioned by Ezugwu et al. [14], which indicated that the emergence of bacterial growth in women at the university level is greater than at secondary and primary levels, and illiterate women have less bacterial growth.

Figure (5) the p-value is 0.001 less than 0.05, therefore the difference between type of birth is significant to infection

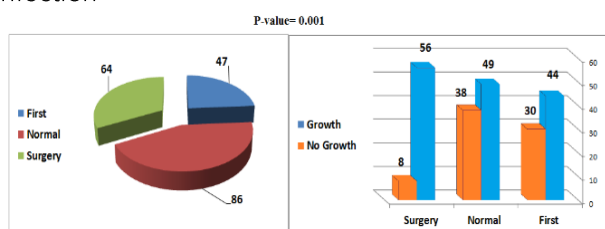


Figure (5) Distribution of the pregnant women according to type of birth ($\chi^2 = 18.279$).

In Figure 7, there are three cases from the type of delivery until the infection of the pregnant woman.

The highest incidence of pregnant women by caesarean section was about 59 pregnant women infected by 37.58% (56/149). As for the least number of cases of infection with pregnant women, the first pregnancy was about 44 cases of infection with pregnant women, or 29.53% (44/149). As for the infection of a pregnant woman with vagus delivery, about 49 cases of pregnant women are infected with a percentage of 32.89% (49/149). Gundersen et al. [15]. reported consistent results in Figure 7. An increased risk of postpartum urinary tract infection was seen in women who delivered via caesarean section as opposed to vaginally. incisions and the insertion of urine catheters during the procedure Al-Mamoryi et al. [16] published similar results, however these new ones differ significantly.. The study found a higher risk of UTI among women given via normal vaginal birth than those delivered via cesarean section.

antibiotic sensitivity

Antibiotic	Resistant/%	Intermediate	Sensitive	Sum
TE	17 (89.47)	2 (10.53)	0 (0.0)	19
RA	19 (100)	0 (0.0)	0 (0.0)	19
DO	14 (73.70)	0 (0.0)	5 (26.30)	19
CN	3 (15.79)	1 (5.26)	15 (78.95)	19
CIP	6 (31.58)	1 (5.26)	12 (63.16)	19
DA	18 (94.74)	1 (5.26)	0 (0.0)	19
E	17 (89.47)	0 (0.0)	2 (10.53)	19
C	9 (47.37)	0 (0.0)	10 (52.63)	19
AK	3 (15.79)	1 (5.26)	15 (78.95)	19
Lev	5 (26.32)	1 (5.26)	13 (68.42)	19

Abbreviations: CN =Gentamycin, Amikacin= AK, RA= Rifampin, C= Chloramphenicol, E=Eethromycin ,TE= Tetracycline, A, IPM= Impanel, Mem =Meropenem, CIP = Ciprofloxacin, Lev= Levofloxacin, DO= Doxycycline , DA=Clindamycin , R=Resistance I=Intermediate

Rifampin was able to kill all of the isolated Klebsiella pneumoniae in this study. These findings are similar to those published by Singh in 2019. The results, on the other hand, differ from those of Mhawesh et al 2020. The treatment resistance of Klebsiella was 50%. Clindamycin was shown to be ineffective in the trial (94.74 percent). Mardan et al. [17]? published results that were similar to ours. Mhawesh et al. [18] disagrees with the outcomes of this study, though. Klebsiella had a 30% resistance rate to this drug. Despite the fact that the study found 90% erythromycin and 90% tetracycline resistance This findings are comparable to those that were reported in the past by Mardaneh et al. [19] as well as [20].

The results, on the other hand, are in conflict with the findings of Bokaeian et al. [21]. Chloramphenicol (47.36 %) results from this study are identical to those from Hooper et al. [22] The results, on the other hand, diverge from those of Simon-Oke et al. [23]. The results showed that 18% of the Klebsiella samples tested had chlorophenol resistance. The Ciprofloxacin (31.58 percent) outcomes of this study are agree to those reported by Kou et al. [24]. On the other hand, the results don't match with those

from Mirzaie et al. [25], who found that 75% of the Klebsiella samples tested had Ciprofloxacin resistance. Levofloxacin (26.31 %) was shown to be effective in this investigation, which is in line with the findings of Rizwan et al. [26]. the other hand, are at odds with those of Aljanaby [27]. Which showed that 95% of the Klebsiella samples tested had resistance to Levofloxacin. Similarly, Jafari-Sales et al. [28]. And Fatima et al. [29]. published comparable results for Gentamycin and Amikacin (15.79 percent), respectively. The results, on the other hand, conflict with those previously reported by Alsumairy et al. [30] which demonstrated that Klebsiella was resistant to Gentamycin and Amikacin with a 95% prevalence rate.

Molecular detection

Table (2) shows the results of genes for Klebsiella pneumoniae

qnr B	qnrA	Tet B	tetA	No
- ve	- ve	- ve	+ ve	K:1
- ve	- ve	- ve	+ ve	K:2
- ve	- ve	- ve	+ ve	K:3
- ve	- ve	- ve	+ ve	K:4
- ve	- ve	+ ve	+ ve	K:5
- ve	- ve	- ve	+ ve	K:6
- ve	- ve	- ve	+ ve	K:7
+ ve	- ve	+ ve	+ ve	K:8
- ve	- ve	- ve	+ ve	K:9
- ve	- ve	- ve	+ ve	K:10
+ ve	- ve	+ ve	+ ve	K:11
- ve	- ve	- ve	+ ve	K:12
- ve	- ve	- ve	- ve	K:13
- ve	- ve	- ve	+ ve	K:14
- ve	+ ve	+ ve	+ ve	K:15
- ve	- ve	- ve	+ ve	K:16
-ve	- ve	- ve	+ ve	K:17
- ve	- ve	+ ve	+ ve	K:18
- ve	- ve	- ve	- ve	K:19
2	1	5	17	Nu. +ve
17	18	14	2	Nu. -ve
19	19	19	19	Total
10 %	5 %	26 %	90 %	%

The tetA gene (tetracyclin group) was shown to be According to the findings of Liu et al. [31], this gene is the most common in this study. According to Kashefieh et al., however, the results were different (2021). Tet B was found in 26 percent (5/19) of the klebsiella isolated, according to the findings. The findings were consistent with those of Jafari-Sales et al. [28]. On the other side, Khamesipour et al. [32]. found that the percentage of tetB was higher than previously reported (64.1 percent). qnrB, on the other hand, accounted for 10% of the sample (2/19), which was in line with recent findings by Das et al. [33] Despite this, the study's findings contradict those previously reported by Shams et al. [34]. The expression rate of genes was 46%.. qnrA, the gene with the lowest frequency, was found in the current investigation. One Klebsiella isolate was found to have a 5% (1/19) prevalence. According to Samer et al. [35], the findings were in line with those previously reported by Mardaneh and his colleagues. Contrary

to expectations, the findings are at odds with those of Taraghian et al. [36] The development of the resistance gene qnr A accounted for 22.2% of the total.

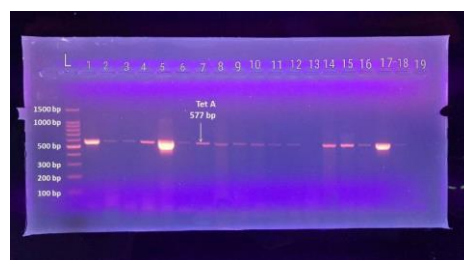


Figure (1) Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of TetA (577 bp) from extracted total DNA of Klebsiella pneumonia. Lane: L: 100 bp ladder marker. Lane 1,2,3,4,5,6,7,8,9,10,11,12,14,15,16,17and 18 positive results. Lane: 13 and 19 negative results



Figure (2) Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of TetB (634 bp) from extracted total DNA of Klebsiella pneumoniae. Lane: L: 100 bp ladder marker. Lane 5,8,11.15and 18 positive results. Lane: 1,2,3,4,6,7,9,10,12,13,14,16,17 and 19 negative results

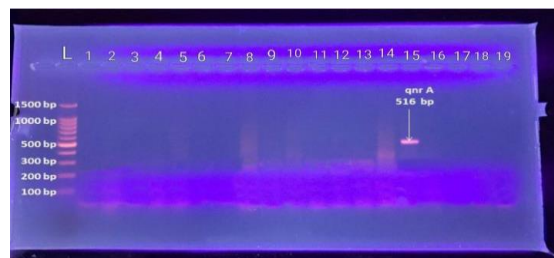


Figure (3) Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of qnrA (516 bp) from extracted total DNA of Klebsiella pneumonia. Lane: L: 100 bp ladder marker. Lane 15 positive results only. Lane: 1,2,3,4,5,6,7,8,9,10,11,12,13,14,16,17,18 and 19 negative results

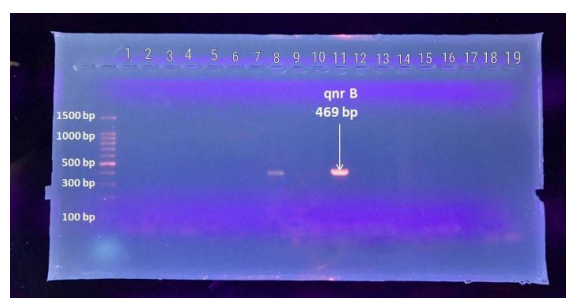


Figure (4) Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of qnrB :516 bp from extracted total DNA of Klebsiella pneumonia. Lane: L: 100 bp ladder marker. Lane 8 and 11 positive results. Lane 1,2,3,4,5,6,7 ,9,10, ,12,13,14,15,16,17,18 and 19 negative results

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