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Molecular study for antibiotic-resistance patterns of extended-spectrum B- Lactamase producing Klebsiella Pneumoniae from a UTI of pregnant women in Diwaniyah (Shamiya city) - Iraq

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Abstract---Background: Klebsiella pneumoniae is a member of the Enterobacteriaceae family. K. pneumoniae isolates produce enzymes that cause the hydrolysis of penicillins and cephalosporins.These enzymes are known as broad-spectrum beta-lactamases (ESBL). The resistance of K. pneumoniae to beta-lactamase antibiotics is usually mediated by beta-lactamase genes, and the present study aims to determine the prevalence of ESβL producing K. pneumoniae isolates with their antibiotic susceptibility pattern in urine samples of pregnant women with UTI. Method: The current study included two hundred twenty-five pregnant women as if they were suffering from urinary tract infection 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 149 patients with urinary tract infections, and the bacterial growth was of different types the highest rate of infection was in the age group of 21–25 years. Then, 19 positive samples of *Klebsiella pneumoniae* were detected using chemical and morphological methods. Penicillin resistance was 100%. Ceftazidime, Azithromycin, Ceftriaxone, Amoxicillin/clavicle acid, and Ceftriaxone were the consequences of the resistance (68.54 %). Gentamycin, Ciprofloxacin, Levofloxacin, and Amikacin, on the other hand, showed resistance at 31.58 %, 26.32 percent, and 15.79 %, respectively. On the one hand, all isolates were

responsive to Impanel and Meropene, indicating that the resistance rate was zero percent. After that, all samples were submitted to a genetic PCR analysis utilizing beta-lactam gene primers (*ampC*, *blaCtxm*, *blaSHV* and *bla*TEM). Conclusion: The percentage of cephalosporin-resistant isolates was found in *K. pneumoniae*. 68.43% of which has the ability to produce ESBL. The current study found that some isolates of *Klebsiella pneumoniae* contain more than one resistance gene in a phenomenon called multi-replication resistance plasmids. carbapenems treatments showed high efficacy against *Klebsiella pneumoniae*.

***Keywords*---**antibiotic, ESBL, *Klebsiella pneumoniae*, UTIs, *ampC. blaCTX-M, blaSHV*, *blaTEM*, B-lactam, pregnant women.

Introduction

A urinary tract infection (UTI) is defined as the presence of more than 100,000 microscopic cells in 1 mL of urine along with cystitis, pyelonephritis, and asymptomatic bacteria. UTIs can affect people of all ages and genders, but because of the female urethra's closeness to the anus and hormonal activity, it is more common in women than in men. UTIs are a prevalent concern among women, particularly during pregnancy. UTIs affect 10–20% of women, according to estimates. Bacteria, particularly E. coli, cause the majority of UTIs (Flores-Mireles,*et al* 2015).

Gastrointestinal bacterium that contaminates the region around the rectum and spreads to the bladder, infecting the urethra. Due to changes in the urinary system, UTIs are more likely during pregnancy. Because the uterus rests directly on top of the bladder as it expands, the increasing weight of the uterus might prevent urine from draining from the bladder, resulting in infection. In addition to pregnancy, there are additional host-related variables that enhance the risk of urinary tract infection, such as sexual factors, urine factors, urine osmolality, and so on. Introital variables, vaginal pH, and secretory state are all important considerations (Almukhtar, 2018).

The importance of this research is that UTIs are more harmful in pregnant women, with up to 50% of those with asymptomatic bacteriuria (ABU) developing pyelonephritis. Furthermore, intrauterine growth restriction and low-birth-weight newborns are more common in pregnant women. Preterm labor has been linked to having a UTI. They also spend more time in hospitals, have larger medical expenditures, and are more likely to suffer septic shock and die as a result (Wagenlehner *et al.*, 2019). Bacteria include *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Ezugwu *et al.,* 2021). *K. pneumoniae* accounts for only 10-15% of the total UTIs throughout the world, it occupies second place after *E. coli* among uropathogens. .

Klebsiella pnemoniae virulence factor Capsular polysaccharide (CPS), lipopolysaccharide (LPS), siderophores, and fimbriae are well-studied virulence

factors of *K. pneumoniae* that aid in host survival (also known as pili). These are necessary for adherence, colonization, invasion, and infection development. Outer membrane proteins, porins, efflux pumps, iron transport systems, and genes involved in allantoin metabolism are among the more recently discovered virulence factors (Paczosa, 2016).

Antibiotic resistance is a global problem within the Enterobacteriaceae family, which is the most prevalent cause of complicated UTIs. Those with multidrug-resistant urinary tract infections are three times more likely to get inappropriate empirical antibiotic treatment than patients without multidrug-resistant diseases. Infections caused by *Klebsiella* are treated with broad spectrum of antibiotics such as, cephalosporins, fluoroquinolones, aminoglycosides and carbapenems and it has been found that resistance has developed to these antibiotic.

The development of beta-lactamase is mostly linked to the attainment of resistance determinant genes carried on giant plasmids, whose horizontal gene transfer between species has become a severe worldwide health issue. Beta-lactam (B-lactam) medicines are one of the most commonly used antibiotics for treating *K. pneumoniae* infections. However, bacteria that produce extended-spectrum beta-lactamases (ESBLs) have recently emerged as a serious health concern. The ability of ESBLs to hydrolyze penicillins, broad-spectrum cephalosporins, and monobactams, resulting in their inactivation. *SHV*, *TEM*, and *CTX*-M are enzymes that hydrolyze the b-lactam ring to inactivate b-lactams (Jamal *et al.*, 2020).

3. Patients and Methods

3.1 Data Collection

A total of 225 urine samples were taken from pregnant women. Isolates were collected from Al-Shamiya Hospital and Maternity Care Centers in the Al-Shamiya area of Iraq's Al-Diwaniyah Governorate between October 2021 and march 2022. The Department of Pathological Analysis at Al Furat Al Awsat Technical University's Medical Technical College/Kufa did this research. From hospital and community-acquired illnesses, 19 isolates of *K. pneumoniae* bacteria were chosen. The germs were isolated from clinical urine samples of individuals with urinary tract infections for the investigation.

Isolation and Identification of Bacteria

Klebsiella pneumoniae isolates were identified by culture, Gram stain, microscopy, and standard biochemical tests. For cultivation, blood agar, eosin methylene blue media, and McConkey agar (Neogen-United Kingdom and Hi-media India) were used for cultivation. Pathogenic microorganisms were identified by culture-specific phenotypic tests, typical phenotypic traits, and by culture on chromium agar. Bacterial isolates were then identified by Gram staining (identification of gram-positive or gram-negative organisms) and a series of standard biochemical assays, Catalase, Oxidase, Indole production, Methyl red, Voges Proskauer, Simon citrate, Urase, Triple sugar Iron .

Antimicrobial Drugs Susceptibility Assay

An antibiotic susceptibility assay was performed via the disk diffusion method (Kirby Bauer), according to the clinical laboratory standards institute criteria on Mueller-Hinton agar plates (Hi-media India).The discs of antibacterial agents used in this study contained amikacin (10µg), ,imipenem (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), Cefixime (30 µg), Amoxicillin/clavicle acid (20/10 µg), ciprofloxacin (10 µg), gentamicin (30 µg), Azithromycin(15µg) ,Penicillin G(10 µg), Levofloxacin(5 µg), Meropenem (10 µg),. All the antibiotics were obtained from Mast laboratories (Bioanalyse Company Turkey). Diameters of the zones of inhibition for individual antibacterial agents were translated into susceptible, intermediate, and resistant categories, according to the clinical and laboratory standards institute (CLSI 2021) criteria. Multiple drug resistant microorganisms were identified as resistant to three or more antibacterial classes. Isolates showing inhibition zones of ≤ 27 mm for cefotaxime and ≤ 22 mm for ceftazidime were screened as potential ESBL producers.

Multiplex Polymerase Chain Reaction for Beta-Lactamase-Encoding Genes

PCR was performed for the *bla*TEM, *blaSHV ,bla CTX-m* and *bla ampC* beta-lactamase genes. The sequences of primers used for gene PCR are listed in Table 2. The PCR was optimized according to the following experimental conditions: initial denaturation at 94 °C for five minutes 30,35, 40 cycles, respectively from 94 °C for 30 seconds, 56 °C for 30 sec, and 72 °C for 1 min, followed by a final extension step at 72 °C for 10 and 7 min with a one-time cycle held at 4 °C for five min.

Name of genes	Oligo-Sequence (3′→5′)	Product Size (bp)	Reference
*bla*TEM	F: TCAACATTTTCGTGTCGCCC R: AACTACGATACGGGAGGGCT	766	(Yang, *et al.*, 2020)
blaSHV	F: CTTTACTCGCCTTTATCG R: TCCCGCAGATAAATCACCA	827	(Kolar *et al* .,2020)
ampC bla	F:TTCTATCAAMACTGGCARCC R: CCYTTTTATGTACCCAYGA	550	Shahein, *et al.*, (2021)
blaCTX-M	F: ATGTGCAGYACCAGTAA R: CCGCTGCCGGTYTTATC	536	(Tabi , 2018)

AmpC beta-lactamases =*ampC bla*

blaCTX-M =beta-lactamases refers to his potent in hydrolytic activity against cefotaxime antibiotic beta-lactamases named after first patient isolated from Temarian = *bla*TEM
blaSHV= SHV beta-lactamases related to sulfhydryl variable

Statistical analysis

The obtained data have been statistically analyzed; the distribution of variables was explained on the basis of their absolute and/or relative (%)frequencies of

appearance. In order to detect significant correlations between different variables of *Klebsiella pneumoniae* strains isolated from patients with urinary tract infection. The chi-square (x2) tests were used by using IBM SPSS statistics software (Version 20.SPSS Inc, United States). For both tests, P values of ≤ 0.01 were considered highly statistically significant.

Result & Discussion

Anthropometric Results

The current study have 225 pregnant women (149 infected with UTI and (76 non infected with UTI). The current study results demonstrated that number of the infection pregnant women, they distributed according to five age groups. Figure (1)revealed the highest age group was in 21-25 years about 70 infection pregnant women by percentage 31.11%(70/225). But lowest age group was in 36-39 years about 3 infection pregnant women by percentage 1.33% (3/225). The rest age groups distributed according to 10.67% (24/225), 16% (36/225) and 7.11% (16/225) for age group 16-20,26-30 and 31-35 respectively. Figure 1 the p-value is 0.001 greater than 0.05, therefor the difference between age groups is significant to infection

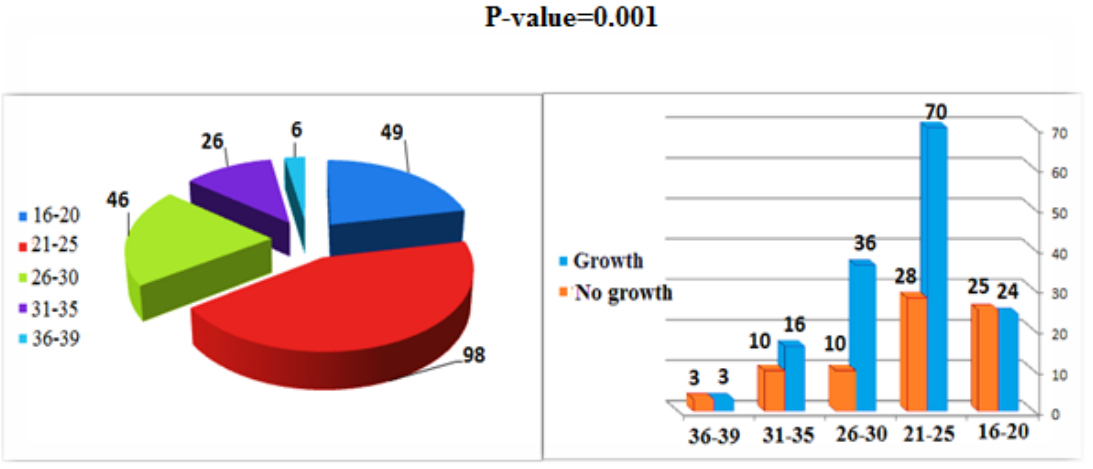

Figure-1- Distribution of the pregnant women according to the age groups ($chi - square$ =11.642)

The results in figure 1 were found to be similar with results reported by Rudri *at el* 2018. mention that the infection of UTIs were high in age 21-25 years Compared to other categories. On the other hand, the results disagree to those results previously described by Kolo *et al* 2021. which the third category 26-30 years had the most bacterial infections compared to the rest of the groups. the variation results may have been due to differences in the environmental conditions, social habits in the community, the standard of personal hygiene, and health care-seeking habits.

An antibiotic sensitivity assay was performed for nineteen *Klebsiella pneumoniae* isolates using disc antibacterial agents. The results are shown in Table 3.

Antibiotic	Resistant/%	Intermediate	Sensitive	Sum
CN	3 (15.79)	1 (5.26)	15(78.95	19
P	19 (100)	0 (0.0)	0 (0.0)	19
CIP	6 (31.58)	1 (5.26)	12(63.16)	19
AMC	14(73.70)	4(21.04)	11 (5.26)	19
CRD	14(73.70)	1 (5.26)	4(21.04)	19
AK	3 (15.79)	1 (5.26)	15(78.95)	19
AZM	14(73.70)	0 (0.0)	5 (26.30)	19
CTX	13 (68.42)	0 (0.0)	6 (31.58)	19
Lev	5 (26.32)	1 (5.26)	13 (68.42)	19
IPM	0 (0.0)	0 (0.0)	19 (100)	19
Mem	0 (0.0)	0 (0.0)	19 (100)	19
CAZ	14(73.70)	1 (5.26)	4(21.04)	19

Abbreviations: CN =Gentamycin, Amikacin= AK, AZM Azithromycin, P=Penicillin G, AMC Amoxicillin/clavicle acid, IPM= imipenem , Mem =Meropenem, CIP = Ciprofloxacin, Lev= Levofloxacin, CAZ= Ceftazidime, CRD =Ceftriaxone, CTX= Cefixime, R=Resistance I=Intermediat S=Sensitive

The majority of the isolated *Klebsiella pneumoniae* found in the current study showed resistance to Penicillin G (100), which is consistent with the findings of Ahmed, 2021. On the other hand, the results disagree with those previously published by Etukudo *et al.*, 2020. whereas Klebsiella's resistance to Pencilin was 50%. The results of this study demonstrated antibiotic resistance to ceftriaxone (73.68%). These results are similar to those posted earlier by Ali et al. 2022. On the other hand, the results disagree with those results previously published by Johnson *et al.*, 2021. *Klebsiella pneumoniae* was sensitive to ceftriaxone with a percentage of 84.6%. The results of this study for the antibiotic Azithromycin (73.68%) are similar to those published by Tawfeeq et al.2016.? On the other hand, the results disagree with those previously described by Orole, 2020, and Azithromycin resistance was 30%. The results of this study demonstrated antibiotic resistance to cefixime (68.42%). These results are similar to those posted earlier by Ahmed *et al.*, 2021. On the other hand, the results disagree with those results previously described by Zubair et al. 2019. The results of this study for the antibiotic Ciprofloxacin (31.58%) are similar to those published by Yandai et al. in 2019 On the other hand, the results disagree with those results previously published by Mirzai & Ranjbar, 2021. which indicated the presence of Ciprofloxcin resistance to *Klebsiella* and a percentage of 75%. The results of this study for the antibiotic Levofloxacin (26.31%) are similar to those published by Riziet al.202On the other hand, the results disagree with those results previously. Aljanaby, 2018.which indicated the presence of levofloxacin resistance to Klebsiella and a percentage of 95%. The results of this study for the antibiotic Gentomycin and Amikacin (15.79%) are similar to those published by Jafari-Sales *et al.*,2020. ,and Fatima & Mussaed,2018, respectively. On the other hand, the results disagree with those results. Alsumairy *et al.*,

2018. indicated the presence of Gentomycin and Amikacin resistance to Klebsiella and a percentage of 95%. The study showed that 100% of the isolates were sensitive to Imipenem and Meropenem and the results were similar to those reported by Romyasamit, *et al* 2021. Other researchers reported the isolation of Klebsiella pneumoniae resistant to carbapenem with elevated proportions. Hosny, *et al* 2021.

Molecular detection

The present study results showed the commonest gene was bla tem 90%(17/19) (bate-lactame group) this result which similar to Aljanaby & Alhasani,2016 .On the other hand, the results differed with those previously described by Zaniani *et al.,* 2012.The results of the current study showed that the shv gene came in second place with 80%(14/19) of the total isolates that were subjected to the study. The results were similar to those that were previously reported by Kashefieh *etal.,* 2021. On the other hand, the results disagree to those results previously described by Al-Ouqaili, 2018. The SHV gene rate was 48%. and about blaCtxm 74 %(13/19)the results similar to results reported by Koirala *et al.*, 2021. On the other hand, the results disagree to those results previously described by Finjan,& Mahmood, 2022 blaCTX-M gene was found in (3.1%) .While the proportion of ampC gene was 21% (4/19)The results were similar to those that were previously reported by Kazemian *et al.*, 2019 .On the other hand, the results disagree to those results previously described by Cheung *et al.*, 2016 The incidence of this gene was 77%

No	blaTEM	blaSHV	blaCtxm	ampC
K1	+ve	-ve	-ve	-ve
K2	+ve	+ve	+ve	-ve
K3	+ve	+ve	+ve	-ve
K4	+ve	+ve	+ve	-ve
K5	+ve	+ve	+ve	-ve
K6	+ve	+ve	+ve	-ve
K7	+ve	+ve	-ve	-ve
K8	+ve	-ve	-ve	-ve
K9	+ve	+ve	+ve	-ve
K10	+ve	-ve	+ve	+ve
K11	+ve	+ve	+ve	-ve
K12	+ve	+ve	-ve	-ve
K13	-ve	+ve	+ve	-ve
K14	-ve	-ve	+ve	+ve
K15	+ve	+ve	+ve	+ve
K16	+ve	+ve	-ve	-ve
K17	+ve	-ve	+ve	-ve
K18	+ve	+ve	-ve	+ve
K19	+ve	+ve	+ve	-ve
Nu. +ve	17	14	13	4
Nu. -ve	2	5	6	15
%	90%	80%	74 %	21%

AmpC beta-lactamases
=*ampC* *bla*
blaCTX-M =beta-lactamases refers to his potent in hydrolytic activity against cefotaxime antibiotic
beta-lactamases named after first patient isolated from Temarian = *blaTEM*
blaSHV= SHV beta-lactamases related to sulfhydryl variable
K=isolate symbol
Nu. +ve= positive result
Nu. –ve =negative result
Figure(2) shows results bla CTX-M gene

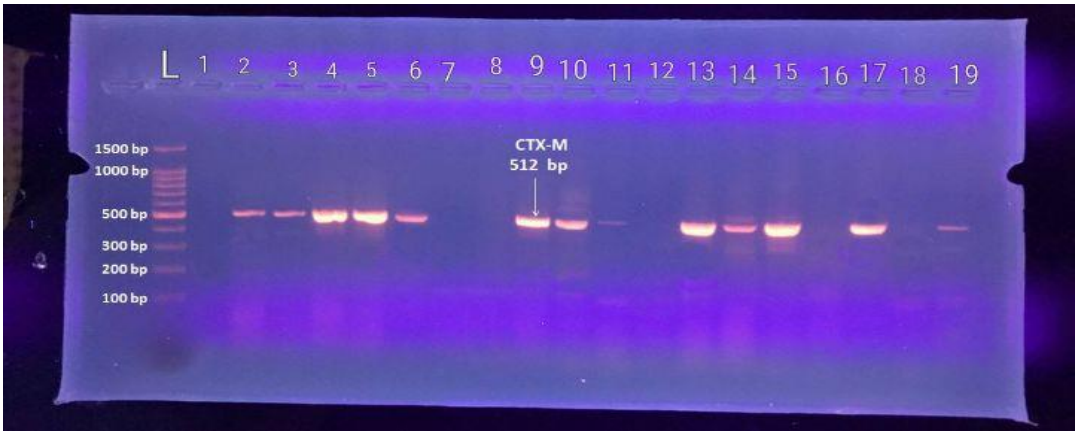

Figure (2)Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of bla CTX-M gene (512 bp) from extracted total DNA of Klebsiella pneumonia. Lane: L: 100 bp ladder marker. Lane 2,3,4,5,6,9,10,11,13.14,15,17and 19 positive results. Lane: 1,7,8,12,16, and 18 negative results

Figure(3) shows results bla *SHV* gene

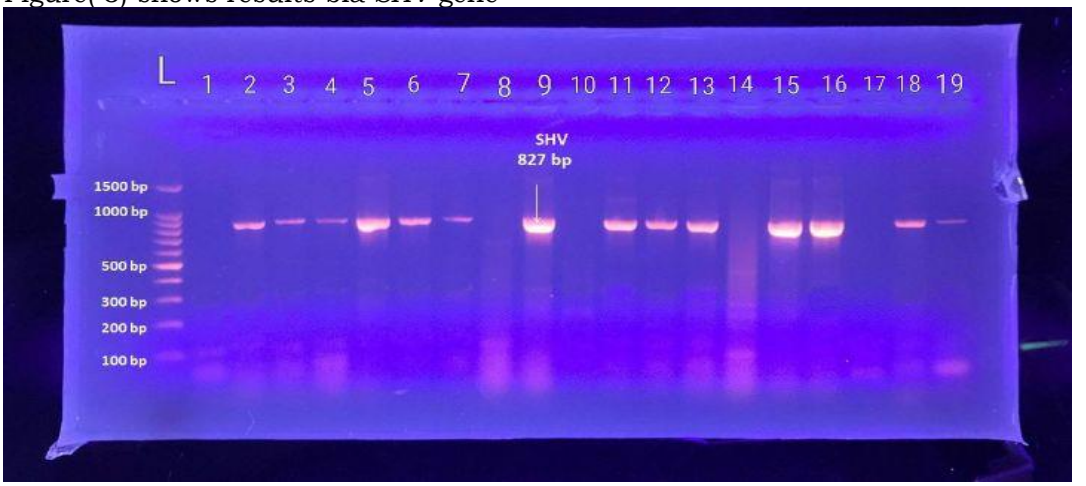

Figure (3)Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of bla SHV (877 bp) from extracted total DNA of Klebsiella pneumonia. Lane: L: 100 bp ladder marker. Lane

2,3,4,5,6,7,9,11,13,15,16,18and 19 positive results. Lane: 1,8,10,14and 17 negative results

Figure(4) shows results bla bla blaTEM gene

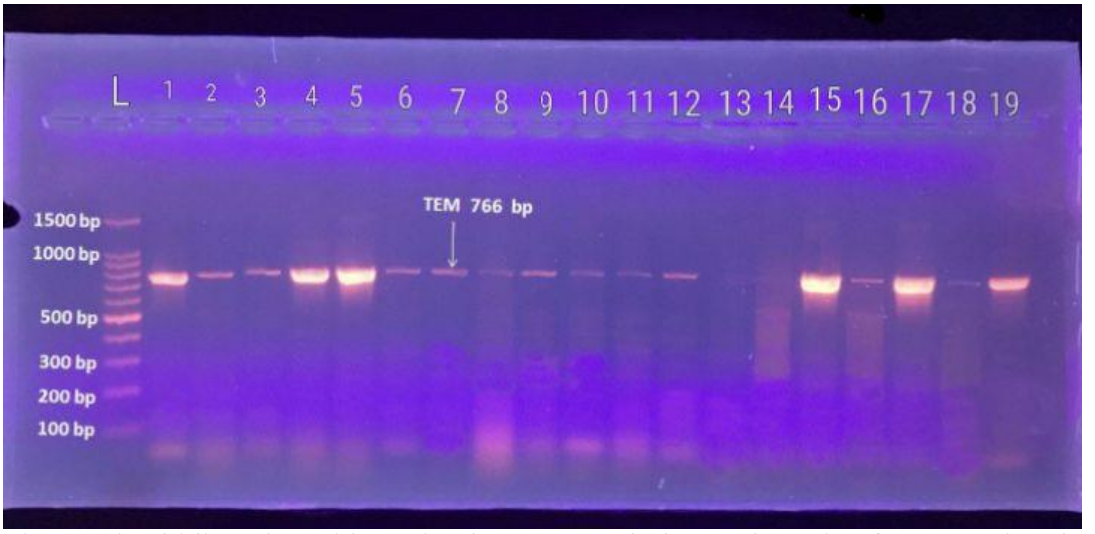

Figure (5)Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of blaTEM (766 bp) from extracted total DNA of Klebsiella pneumonia. Lane: L: 100 bp ladder marker. Lane 1,2,3,4,5,6,7,8,9,10,11,12,15,16,17,18and 19 positive results. Lane: 13 and 14 negative results.

Figure(5) shows results bla bla ampC gene

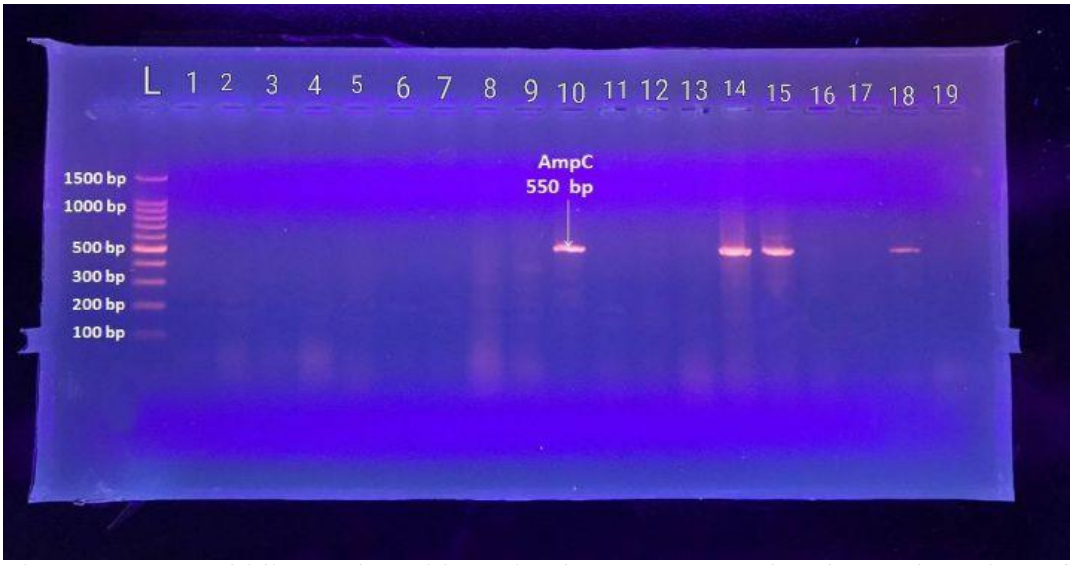

Figure (30-4)Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of ampC (550 bp) from extracted total DNA of Klebsiella pneumoniae. Lane: L: 100 bp ladder marker. Lane

10,14,15and 18 positive results. Lane: 1,2,3,4,5,6,7,8,9,11.12,13,16,17 and 19 negative results

Conclusion

The study showed that Klebsiella had high levels of resistance to penicillin as well as to sporins, while it was quite sensitive to two types of carapenems, using imipenem and meropenem on the other hand. When the genetic test was done, it was found that the beta-lactam inhibitor genes were dominant and that the bacteria had more than one resistance gene.

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