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RESEARCH ARTICLE

Molecular Detection of Extended Spectrum-Beta Lactamase Producing *Escherichia coli* Isolated from Pregnant Women Infected with Urinary Tract Infection

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Abstract

During pregnancy, the urine becomes low concentration and increase the volume of the bladder as a result of an increase of the plasma volume. These factors combined and leading to stasis of urine and ureterovesical reflux [3].A uropathogenic strain of *E. coli* (UPEC) was the most predominant cause of infection in the urinary tract system in the world [4; 5]. The main bacterial mechanism of resistance to the ß-Lactame antibiotic was the ß-Lactamase production [8]. Generally, ESBLs genes were SHV type, TEM type, CTX-M type, and OXA. The SHV type of extended-spectrum beta-lactamases was the more commonly present in clinical isolates than any type of extended-spectrum beta-lactamases [25]. A total of two hundred and seventy nine midstream urine samples were collected randomly from the pregnant women suspected to be infected with urinary tract infection admitted to Al-Sader Medical City, Najaf-Iraq, from Oct. 2017 to Dec. 2018. Cultural and Biochemical properties were studied carefully. Vitec 2 system was used to confirm the identification. Extended spectrum beta lactamase genes (*bla TEM, bla SHV, bla CTX-M,* and *bla OXA*) were detected by using polymerase chain reaction technique. The highest percent of infected pregnant women was 42.8% in the age group 20-25 years, most of them (76.5%) in the third trimester of pregnancy. According to the educational level (38.13%) of pregnant women had completed the primary school. The recovery of UPEC was 62.60%. From the analysis samples the percentage of *E. coli* isolates in pure culture were 10.71% (6/56), 39.29% (22/56) and 50% (28/56), in the first, second and third trimesters, respectively. On the other hand, the recovered percentage of *E. coli* as a mix growth were occupied different ratios there were 18.75%(3/16), 37.5%(6/16) and 43.75%(7/16) in the first, second and third trimesters, respectively. Also, the results revealed that the commonest gene was *CTX-M* 90.9% (as a single, double and more than two genes). The majority of the *E. coli* strains carried two or more ESBL genes, of these isolates, 1/22 (4.55%) had two types of ESBL genes were *TEM* and *CTX-M*-positive; tow (2/22: 9.09%) were *OXA*- and *CTX-M*-positive. Three of these isolate had three types of ESBL genes 3/22 (13.64%) and only five strain 5/22 (22.72%) carried all 4 gene types. From the previous results we conclude the presence of the ESBL bearing strains of UPEC at high level, and this finding may be affected the pregnant women and her fetus.

Keywords: *UTI, UPEC, Pregnancy trimesters, ESBL.*

Introduction

The infection of structures participating in the excretion and elimination of urine i.e. urethra, urinary bladder, ureters and the kidneys which defined as urinary tract infections by the international classification of diseases (ICD).In pregnancy, serious adverse mothers and fetus outcomes can result from the urinary tract infection [1]. Twenty percent of the pregnant have been reported for urinary tract infections, and it was the common reason of obstetrical ward [2]. When the asymptomatic bacteriuria was

untreated, that was considered as a risk factor for acute cystitis and pyelonephritis in pregnant women. The danger of UTIs was increased during pregnancy. The physiological change in pregnant women can lead to the ureters dilation around of the sixth week. This was medically called "pregnancy hydronephrosis", which summits at twenty-two to twenty-six weeks and remains until delivery. The progesterone level becomes high during the pregnancy and these increase it related to decreasing the bladder and ureteral tone. During pregnancy, the urine becomes low concentration and increase the volume of the bladder as a result of an increase of the plasma volume. These factors combined and leading to stasis of urine and ureterovesical reflux [3].

A uropathogenic strain of *E. coli* (UPEC) was the most predominant cause of infection in the urinary tract system in the world [4; 5]. According to the hypothesis, uropathogenic *E. coli* strain can transmission from the urinary system of pregnant women who suffer from decrease level of immune response to the reproductive system [6].Based on the latest epidemiological studies, a high prevalence of uropathogenic *E. coli* resistance (50-100 %) to the predominant antibiotic used for the patient [4, 7]. The main bacterial mechanism of resistance to the ß-Lactame antibiotic was the ß-Lactamase production [8].

The ß-Lactam antibiotic widely used in the treatment of infection by Gram-negative bacilli bacteria. The resistances of them in Enteriobacteriace family become to recognize the worldwide problem [9]. This study was designed to investigate the prevalence of ESBL producing *E. coli* among pregnant women infected with UTI. Extended-spectrum ß-Lactamases can be classified into four major groups: TEM, SHV, CTX-M, and OXA types. The majority of extended-spectrum ß-lactamases determined in clinical isolates during the 1980s–1990s were the SHV or TEM kinds, while, since the middle of 2000s CTX-M has been determined in diverse members of the *Enterobacteriaceae*, but specifically in *E. coli*, and developed to major type of extended spectrum ß-Lactamases in the antimicrobial resistance around the world (Pitout *et al*., 2009).

Materials and Methods

Patients and Study Design

This cross-sectional study included 279 urine samples, which were collected randomly from pregnant women suspected to have urinary tract infection, those patients admitted to the AL-Sadder Medical City in AL-Najaf AL-Ashraf Provence-Iraq, from Oct. 2017 to Dec. 2018.

Ethical Approval

Patient's agreements were taken before their inclusion in the study.

Identification of *E. coli* Strains

Microscopic, cultural, and biochemical properties were carefully studied to identify the bacterial isolates. Further identification was achieved by using Vitek$_2$- Compact system. Gram-negative (GN) card was used for this purpose for *E. coli* identification. All the following steps were done according to [10] and the manufactures instructions (Biomerieux, France).

Extraction of Total DNA

Three to five pure and fresh colonies of *E. coli* were inoculated from MacConkey agar plate into 300 µl of distilled water. Then cells were lysed by heating at 100^{o}C for 20 minutes in water bath, then the cells were immediately placed in ice for 30 minutes, and the other cellular components were removed by centrifugation at 8500 rpm for 10 minutes. Finally, the supernatant was used as the DNA template [11].

Master Mix and DNA Marker: Table one demonstrated the master mix and DNA marker type and components that used in this study.

Table 1: Master Mix and Deoxyribonucleic Acid Marker

Master Mix		
Type	**Description**	**Company**
Vial	Top DNA Polymerase (1 U) Each: dNTP (dATP, dCTP, dGTP, dTTP) (250mM) Tris-HCl (pH 9.0) (10 mM) KCl (30mM), $MgCl_2$ (1.5mM) Stabilizer and tracking dye.	Sigma/ USA
DNA Marker		
DNA	**Description**	**Company**

Marker		
100 bp Ladder with loading dye	100-3000 base pairs (bp). The ladder consists of 13 double-strand DNA fragment ladder with Size of (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000 and 3000 bp). All other fragments appear with equal intensity on the gel.	Bioneer Corporation (Korea)

Primers used in this Study: Table two demonstrated the oligo-sequence primers of beta-lactamase genes that used in this study.

Table 2: Oligo-sequence primers of β-lactamase

β - lactamase genes	Oligo-Sequence (3′→5′)	Product Size (bp)	Reference
bla TEM	F:CAGCGGTAAGATCCTTGAGA R: ACTCCCCGTCGTGTAGATAA	643	[12]
blaSHV	F:GGCCGCGTAGGCATGATAGA R:CCCGGCGATTTGCTGATTTC	714	
blaCTX-M	F:AACCGTCACGCTGTTGTTAG R:TTGAGGCGTGGTGAAGTAAG	766	
blaOXA	F:ATATCTCACTGTTGCATCTCC R:AAACCCTTCAAACCATCC	618	[13]

Polymerase Chain Reaction Mixture

Twenty micro liters were used as the total volume of Mixture of PCR in the current study as follows:

- Master Mix 5 µl.
- Primer forward and primer reverse (each alone) 2.5 µl.
- DNA template 5 µl.
- Deionizing water 5 µl.

Thermo-cycling Conditions of PCR

The PCR cycling program parameters conditions used in the current study were shown in table three.

Table 3: Programs of PCR thermo-cycling conditions used in the current study

Gene	Initial Denaturation °C/ Time	Cycling condition °C/ Time				Final extension°C /Time	Reference
		Denaturation	Annealing	Extension	Number of Cycles		
bla TEM	95°C 5 min	94°C 30 sec	52°C 45 sec	72°C 45sec	30	72°C 7 min	[12]
bla SHV	95°C 5 min	94°C 30 sec	55°C 60 sec	72°C 45sec	30	72°C 7 min	

bla CTX-M	95°C 5 min	94°C 30 sec	57°C 45 sec	72°C 45sec	30	72°C 7 min	
bla-OXA	94°C 4 min	94°C 1 min	55°C 60 sec	72°C 1min	30	72°C 10 min	**[13]**

Agarose Gel Preparation and DNA Loading

This method was carried out according to **[14]**: as follows:

- One gram of agarose was added to 100 ml of Tris-borate-EDTA (TBE) with final concentration 10x (90 ml distilled water + 10 ml TBE).
- The prepared Tris-borate-EDTA was boiled, then allowed to cool to 45°C, and 0.5 mg/ml of safe stain was added.
- The agarose poured in equilibrated gel tray and left until cooled and became more hardened.
- Five microliters of PCR product were loaded to the agarose gel wells followed by DNA marker to one of the wells. The gel tray was fixed in electrophoresis chamber and TBE buffer was added to the chamber. The electric current was performed at 70 volts for 80 minutes. The PCR products were detected in a 1.5% agarose gel.
- Finally, the electrophoresis result was detected by using a gel documentation system; the positive results were proved when the DNA band base pairs of sample equal to the target product size.

Results and Discussion

The present study results revealed that the ratios of the infected pregnant women were differed according to the age group. Figure one demonstrated that the highest percent was 42.8% (119/278"One patient reported to be missed value") in the age group 20-25 years, while the lowest percent was 3.2% in the age group more than 38 years (P>0.01). Other age groups occupied different ratios there were 22.3% (62/278), 23.4%(65/278), and 8.3% (23/278) for the age groups 14-19 years, 26-31 years and 32-37 years, respectively.

These results were similar to those results published by [15], who reported that the prevalence of UTIs in the age group 21-25 years was 44.6% followed by 27.69%, 16.92% and 16.15% for the age groups 26-30, 31-35 and 16-20 years, respectively. On the other hand, the lowest ratio was 4.61% for the age group 36-40 years. In contrast to the results published by [16], who reported that the highest percent depending on the age group of the infected pregnant women was 33.6% for the age group 27-32 years, while the lowest ratio was 3.5% for the age group 39-44 years.

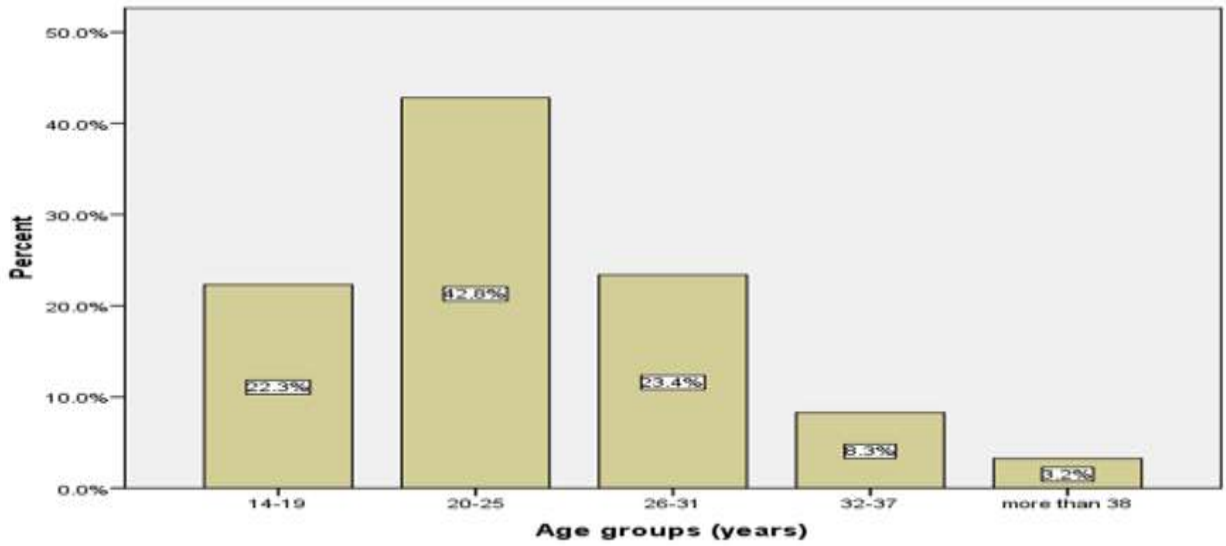

Figure 1: Distribution of the pregnant women according to the age groups (Chi-square =132.791)

According to the educational levels the results in figure two illustrated that the highest ratio (38.13%) of pregnant women had completed the primary school (P>0.01), while the lowest percent (2.52%) was found to be completed the diploma. Other ratios were 20.14%, 16.55%, 12.23% and 10.43% for pregnant women never been to school, intermediate level, secondary level and bachelor level, respectively.

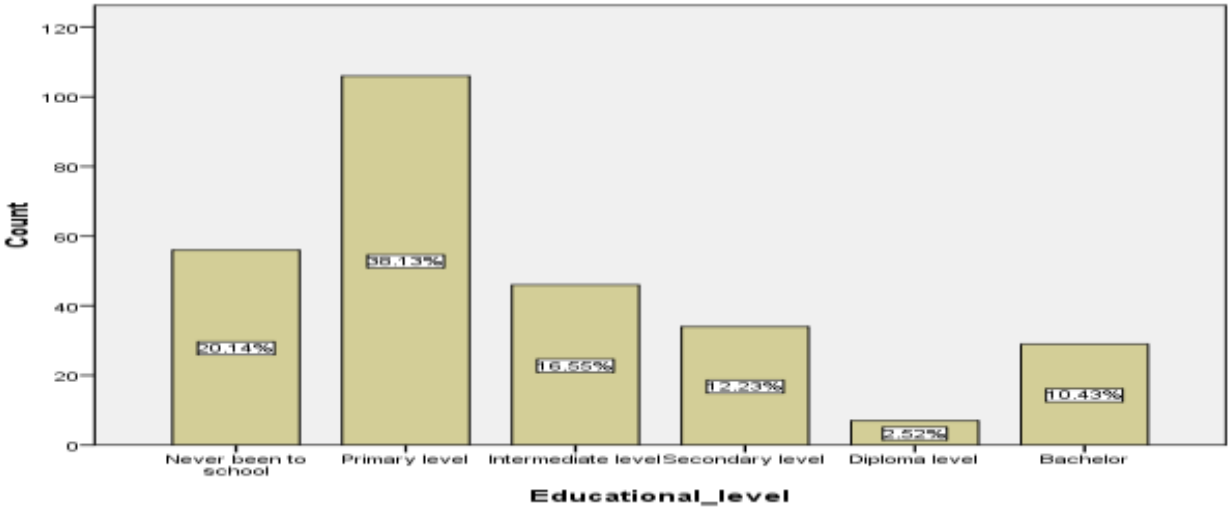

Figure 2: Distribution of pregnant women according to the educational level (Chi-square= 122.014)

Figure 3 demonstrated significantly elevation (P>0.01) in the ratio of infected women by UTIs (76.5%) (81/120) in the third trimester of pregnancy. Also, the percent of infected pregnant women in the second trimester were significantly high (62.1%) (72/116) in comparison with non-infected women in the same trimester. While there was no significant difference between these two groups in the first trimester (48.8%) (21/43).

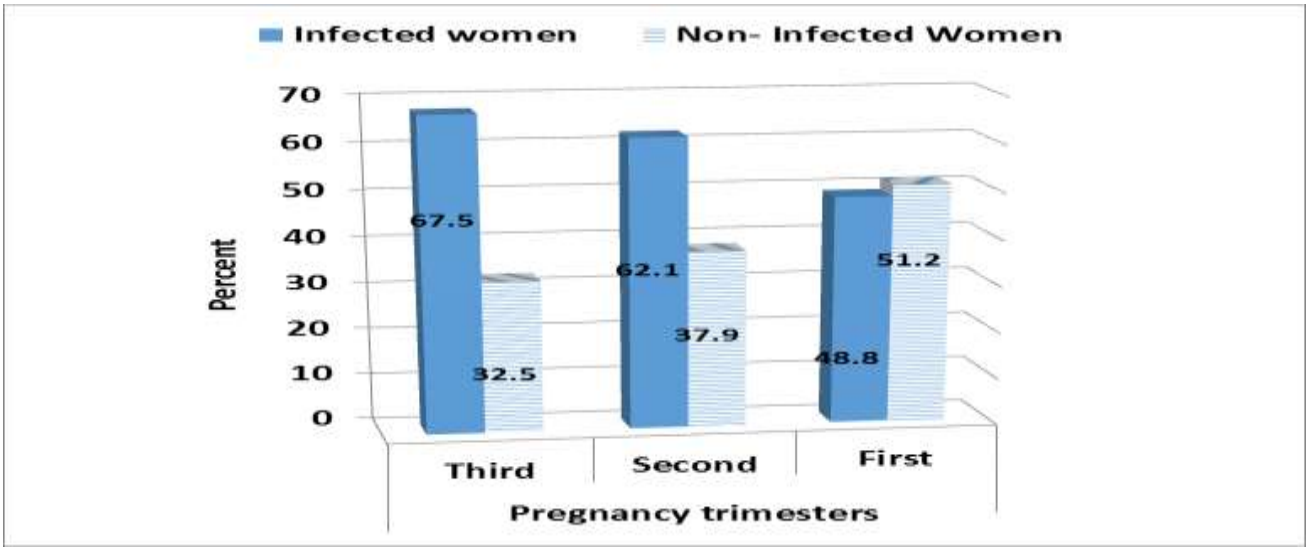

Figure 3: The prevalence of Urinary tract infections among pregnant women distributed according to the pregnancy trimester. (Chi-square =13.893 for 3rd trimester, 6.759 for 2nd trimester, 0.023 for 1st trimester)

The results in figure three were found to be similar to the results reported by [15], mention that the infection of UTIs were high in third trimester (78.46%), compared to the first and second trimesters which recorded 9.23% and 12.30%, respectively. On the other hand, the results disagree to those results previously described by [16], which verified high frequency of UTIs in the second trimester 50.4% compared to the first trimester 23.0% and third trimester 26.5%. The housewives group of pregnant women was occupied the highest ratio (92.8%) (P> 0.01) among infected women, in comparison to employee and student groups, which possess 6.1% and 1.1%, respectively. These

results were clearly illustrated in figure two. These results were agreed with results published previously by [17], who reported that the housewives ratio was 96% in comparison to employee group (4%). While the results in figure two disagreed with those results reported by [18], which showed that the housewives were occupied less percent (10%) than employee group (52.2%).

Isolation and Identification of Bacteria

The recovery of UPEC was 62.60%. In the pregnant sampled in present study this similar to the findings of [19] which reported 56.79% in the India, but disagree with study reported by [20] who reported 84% occurrence of *E. coli* in UTIs episode in Iran. From the analysis samples the percentage of *E. coli* isolates in pure culture were 10.71% (6/56), 39.29% (22/56) and 50% (28/56), in the first, second and third trimesters, respectively. On the other hand, the recovered percentage of *E. coli* as a mix growth were occupied different ratios there were 18.75% (3/16), 37.5%(6/16) and 43.75%(7/16) in the first, second and third trimesters, respectively. Also the result in figure four demonstrate that, the ratios of urine sample which gave other types of bacteria results were 9.3% (4/43), 39.53% (17/43), and 51.17% (22/43) in the first trimester, second trimester and third trimester respectively. As the same time the results which appear negative growth results were 13.56% (8/59), 45.76% (27/59) and 40.68% (24/59) in the first, second and third trimesters respectively. Past research which has shown different observations compare with the present study, isolation of *E. coli* in pure culture was 56.5%, mixed-growth with other type of bacteria was 9.5%, infection by other type of bacteria only was 12.5% and no growth of any type of bacteria 21.5% [21].

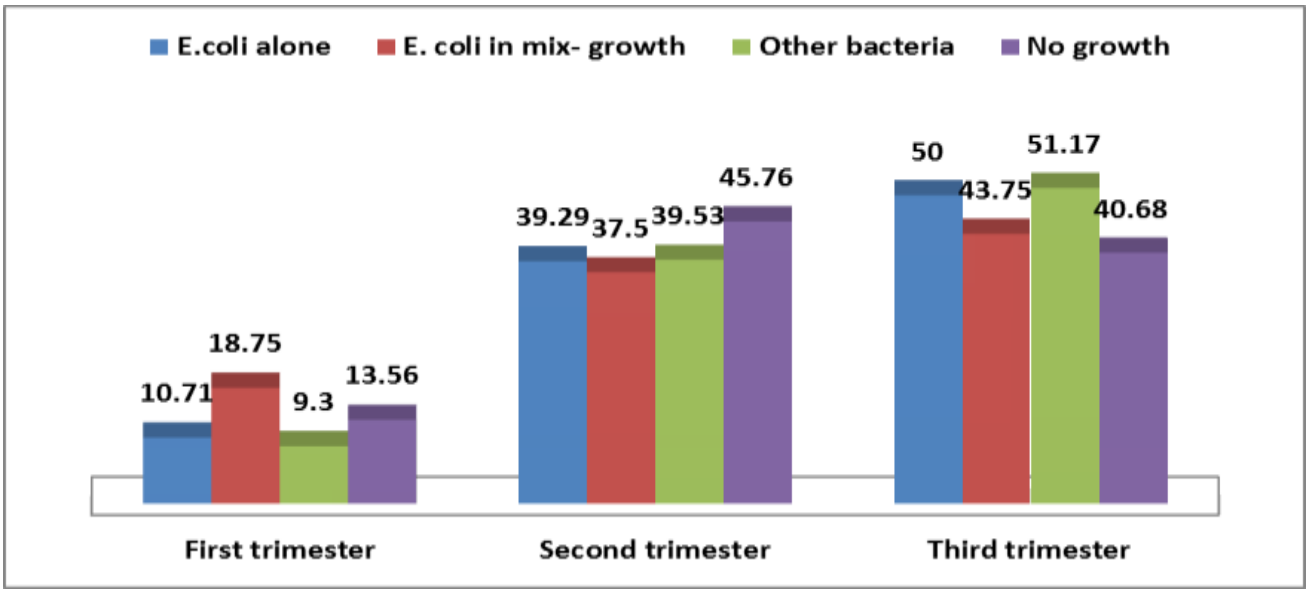

Figure 4: The percentage of the causative agents in three trimesters of pregnancy

The present study results showed the commonest gene was CTX-M 90.9% (as a single, double and more than two genes) this result which similar to [22] who show prevalence of CTX-M gene in these isolates 98.6%, but disagree with [23] which show TEM and CTX-M genes 82.9% and 0.0% respectively among *E. coli* that carried ESBL genes. The majority of the *E. coli* strains carried two or more ESBL genes, of these isolates, 1/22 (4.55%) had two types of ESBL genes were TEM and CTX-M-positive; tow (2/22: 9.09%) were OXA- and CTX-M-positive. Three of these isolate had three types of ESBL genes 3/22 (13.64%) and only five strain 5/22 (22.72%) carried all 4 gene types figure. The distribution of the four genes in the present study slightly near to [22] which show the gene distribution CTX-M (43.84%) (32/73), OXA (1.37%) (1/73), both CTX-M and TEM (8.2%) (6/73), both CTX-M and OXA (30.14%) (22/73), both CTX-M and SHV (2.74%), (CTX-M, OXA and TEM genes) was (12.33%) (9/73) and finally the isolates which have all the four genes (1.37%) (1/73). But, the clear difference was in the [24] study which find TEM (91.0%) (31/34), SHV (3.0%) (1/34), CTX-M (0.0%) (0/34), both TEM and SHV (6.0%) (12/34).

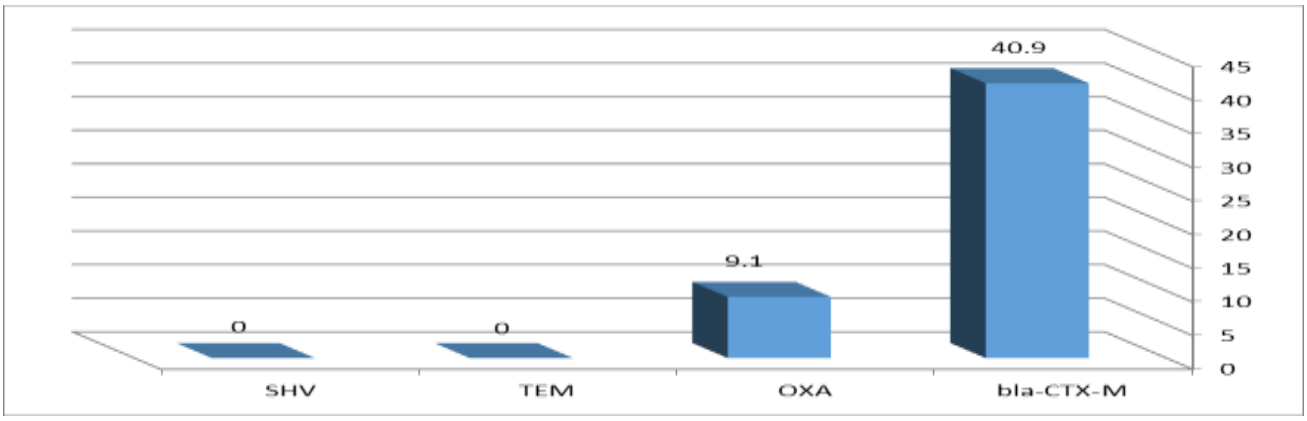

Figure 5: Prevalence of single gene of extended spectrum β-lactamases among *E. coli* by using PCR Technique

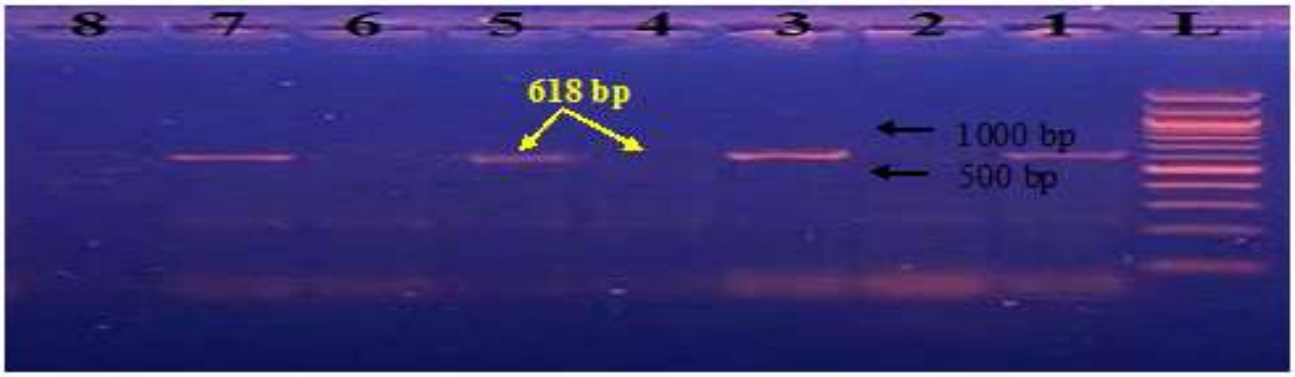

Figure 6: Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of *blaOXA* gene (618 bp) from extracted total DNA of *E. coli*. Lane: L: 100 bp ladder marker. Lane: 1, 3, 5 and 7 positive results. Lane: 2, 4, 6 and 8 negative results

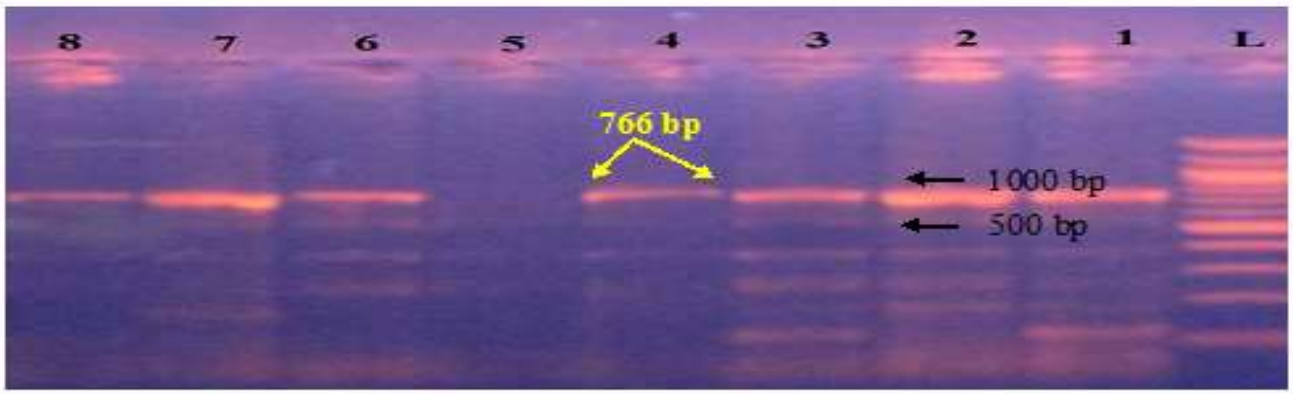

Figure 7: Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of *bla-CTX-M* gene (766 bp) from extracted total DNA of *E. coli*. Lane: L: 100 bp ladder marker. Lane: 1, 2, 3, 4, 6, 7 and 8 positive results. Lane: 5 negative results

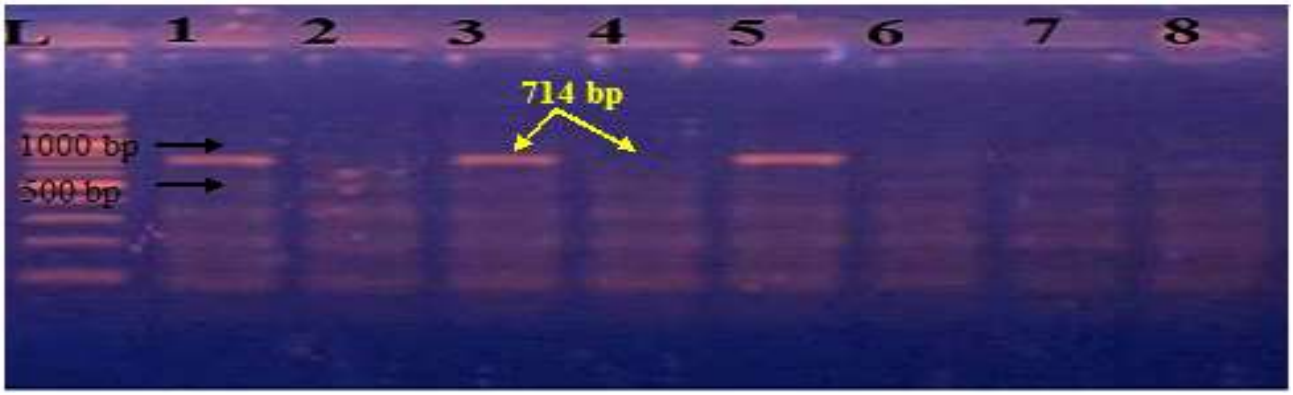

Figure 8: Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of *bla-SHV* gene (714 bp) from extracted total DNA of *E. coli*. Lane: L: 100 bp ladder marker. Lane: 1, 3 and 5 positive results. Lane: 2, 4, 6, 7 and 8 negative results

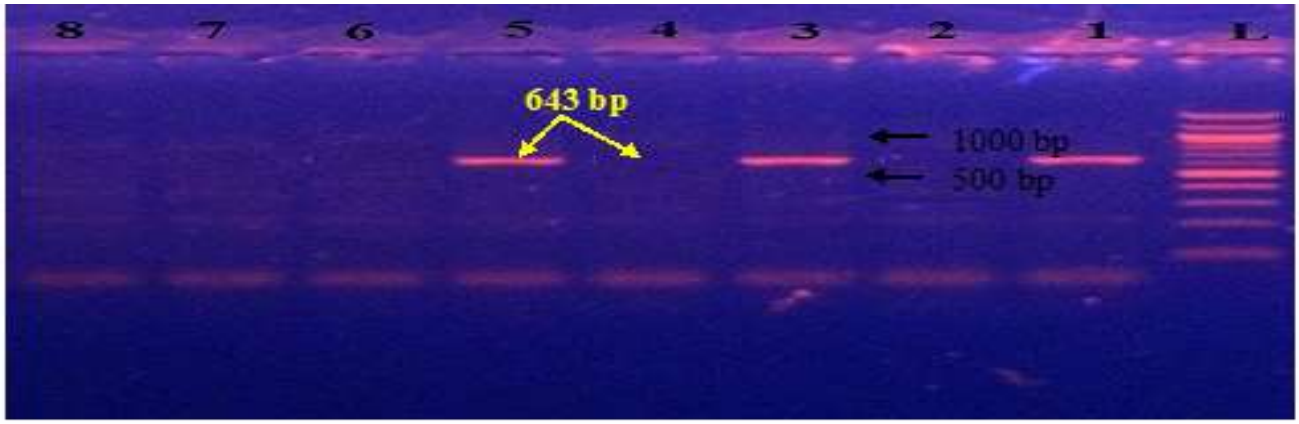

Figure 9: Ethidium bromide-stained agarose gel electrophoresis of conventional PCR amplified products of *bla-TEM* gene (643 bp) from extracted total DNA of *E. coli*. Lane: L: 100 bp ladder marker. Lane: 1, 3 and 5 positive results. Lane: 2,4,6,7 and 8 negative results

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