ORIGINAL ARTICLE

Phenotypic and Genotypic Detection of Extended Spectrum Beta Lactamase Producing Escherichia Coli in Urinary Tract Infection of Pregnant Women in Benha

[1]Riham N. Ragab*, [1]Heba-Allah F. EL-Marakby, [2]Amira E. Khalil, [3]Nora M. Nassar, [4]Omnia Y. Habashy, [1]Doaa A. Shaker

[1]Microbiology and Immunology Department, Faculty of Medicine, Benha University, Benha, Egypt
[2]Obstetrics and Gynecology Department, Faculty of Medicine, Benha University
[3]Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University, Benha, Egypt
[4] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Benha University, Benha, Egypt

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Corresponding Author:
Riham N. Ragab, Ph.D
Address: Microbiology and Immunology Department, Faculty of Medicine, Benha University, Benha, Egypt.
Postal code: 13511
Tel.: 01002577195
rnragab@yahoo.com

ABSTRACT

Background*: Urinary Tract Infections (UTIs) caused by extended spectrum beta lactamase (ESBL) producing E.coli in pregnancy is a serious health problem increasing pregnancy–related complications and continue to be a challenge for choosing the suitable therapy since they may exhibit coresistance to many other classes of antibiotics.* ***Objectives****: to ascertain the genotypic and phenotypic characteristics of (ESBLs) producing E. coli from pregnant females attending the Obstetrics and Gynecology Department in Benha University hospitals.* ***Methodology****: 100 urine samples of pregnant females were presumptively identified using standard bacteriological methods. Positive cultures were tested for antibiotic sensitivity. Production of ESBL was confirmed by double disc synergy and MALDI-TOF MS assay. Loop mediated isothermal amplification (LAMP) was further applied for the ESBL gene genotypic detection.* ***Results:*** *34(82.93%) women out of 100 were colonized by ESBL producing E.coli. The rates of antibiotic susceptibility of E.coli isolates were 53.66% to Ampicillin, 43.9% to pipracillin, 30.9% to gentamicin, 43.9% to aztreonam, 21.9%, 39% and 17% to ceftriaxone, cefotaxime and cefpodoxim respectively. Concerning genotypic detection of ESBLs genes; CTX M-1 was (29.2%), followed by CTX M-2 (4.9%).* ***Conclusion*** *occurrence of ESBL synthesis among E. coli isolates is high. Periodical detection and checking of antimicrobial susceptibility of ESBL isolates in pregnant women, and the use of effective antimicrobial drugs are recommended to decrease the risk of high antibiotic resistance rate and reduce potential subsequent complications.*

INTRODUCTION

Urinary tract infections are defined by invasion of the urinary tract by microorganisms. UTIs are a common cause of severe infections in pregnant women. Pyelonephritis is the greatest common cause of septic shock in them[1].The physiological, anatomical and hormonal changes accompanying pregnancy lower the immune status of pregnant women and raise the chance of severe complications of UTI even in apparently healthy pregnant females [2]. Additionally, it has been connected to higher risks of mortality and morbidity in mothers and newborns[3].

E.coli is the furthermost frequently isolated organism from UTI in pregnancy. Other causative organisms include Gram-negative uropathogens like *Klebsiella species* and P*seudomonas species* and Gram Positive cocci such as *S. saprophyticus* and *Enterococcus spp*[4,5].

The resistance to different classes of antibiotics is an increasing danger; especially in UTI's that may confound the therapeutic choices. Inappropriate and unnecessary use of antibiotics has a major role in this issue. Beta-lactam antibiotics are the chief class used for the treatment of these infections but the rise and blowout of bacterial resistance to them cause treatment failure and recurrent infections [6].

The production of ESBLs is a crucial resistance mechanism to 3[rd]generation cephalosporins which are widely prescribed with the purpose of treating UTI's because of lesser nephrotoxicity effects[7]. (ESBLs) are transmitted by plasmids. They have the capacity to hydrolyze and render ineffective an extensive range of beta-lactam antibiotics, including penicillins, monobactams, and third generation cephalosporins [8].

The transfer of ESBLs enzymes is owing to high gene exchange within Enterobacteriaceae and a related group namely Pseumonadales. Thus, they are present in

almost all clinically significant Enterobacteriaceae family members chiefly *Klebsiella species* and *E. coli* [9].

ESBLs are genetically categorized into 9 families based on amino acid sequences, such as CTX-M, TEM, SHV, OXA, PER, VEB, GES and BES[6].

CTX-M family is one of frequently connected to antibiotic resistance. It comprises 130 distinct classes, categorized into five distinct groups, the CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25[10].

The aim of this study was to detect the ESBL-producing *E.coli* from UTI among pregnant women in Benha University hospitals.

METHODOLOGY

The study included 100 pregnant female patients complained with UTI symptoms and signs in The Obstetrics and Gynecology Department as out-patients and in patients after taking their consents.

Subjects:

Data and Specimen Gathering:

A standardized questionnaire was employed for clinical data collection. Study respondents provided a freshly discharged mid-stream urine sample of approximately 10mL by using a pre-labeled plastic container with wide mouthed, sterile, screw top. The containers were tagged with the date, time, and age of the participants.

Urine culture and bacterial identification of Isolates

The specimens were cultured in Cystine Lactose Electrolyte Deficient (CLED) medium. Identification of isolated organisms was conducted by colony morphology, Gram staining, oxidase testing, triple sugar iron agar examination, urease test, citrate utilization test, indol test, and methyl red test.

Antibiotic sensitivity testing of isolates by disk diffusion technique

24 hours freshly sub - cultured isolates were emulsified into 5mL saline water, turbidity was adjusted to 0.5 Macfarland turbidity and cultured on Muller Hinton agar (MHA) plates. Discs of cefotaxime (30 µg), cefpodoxime (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), Nitrofurantoin (300 µg), aztreonam (30 µg), pipracillin (30 µg), Gentamicin (10 µg), and Ampicillin (10 µg) were cautiously sited at equal distance on the inoculated MHA plates. Prepared plates were allowed to pre – diffused for 45 minutes at 37°C in the incubator. The inhibition Zones were carefully measured in millimeter using a meter rule. Bacteria isolates were classified according to standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2017).

Phenotypic Detection of ESBL Production by E.coli

Screening test

E. coli strains produced inhibition zones of < 17mm, 22mm, 27mm, 25mm and 27mm for cefpodoxime (10 µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30 µg) and aztreonam (30 µg) respectively by previous disk diffusion method, were regarded as ESBL positive and subjected to confirmatory tests.

Confirmatory tests

Double disk synergy test (DDST)

Suspension of screened isolates with ESBL positivity was compared with McFarland standard (0.5%) and plated on MHA plates. Amoxicillin/clavulanic acid (20/10µg) disc was centered in the plate. cefotaxime (30µg) and ceftazidime (30µg) discs were positioned 15 mm separately center to center to amoxicillin/clavulanic acid and aerobically incubated for 24 hours at 37°C.An expansion in the diameter of inhibition zone near the disc of amoxicillin/clavulanic acid was considered ESBL positive (Figure 1).

Fig. 1: Double disk synergy test method (DDST)

MALDI-TOF MS assay (matrix-assisted laser desorption ionization-time of flight mass spectrometry):

Antibiotic Hydrolysis Assay and Spectrum Acquisition[11]

Isolated *E.coli* strains were grown on MHA for 24 h at 37°C. Cefotaxime (0.5 mg/ml in H2O), was selected for the assay.

A 1 µL loopful of bacterial colonies was stirred in a ten µL of the antibiotic solution in antibiotic reaction buffer (10 mM NH_4CO_3/10 Ug/mL $ZnCl_2$/0.005% SDS), incubated for 3 h at 37 °C with continuous agitation, and then centrifuged at 14,000 rpm for 3 minutes. Equivalent portions of the supernatant were added to HCCA matrix (Thermofisher) and dissolved in 50 HCN/49.9% H_2O/0.1% TFA solution. 1 µL of this mix was placed on MALDI Tray and left to become dry. Spectra were acquired on a MALDI-TOF MS microflex.

For calibration, an external standard was prepared in the same way as the antibiotic samples.

Spectra were investigated using Flex Analysis v.3.4 software (Bruker).

The peak intensities of the non-hydrolyzed forms were compared to those of the hydro-lyzed forms. Strains were considered presumably sensitive if the intensity distributions of the non-hydrolyzed and hydrolyzed forms were resembled to those for the negative control. On the other hand, if the intensities of the hydrolyzed forms were accounted for 80% or greater of intensity of both non-hydrolyzed and the hydrolyzed forms, the strain is presumably resistant.

Detection of CTX-M groups 1, 2 genes of ESBL Producing *E.coli* by LAMP technique:

Preparation of DNA templates for bacterial DNA extraction:

In 200µl of distilled water, 3 or more E.coli colonies were suspended, then sited into a bath of boiling water for 10 minutes, after that conveyed over the ice for 5 minutes and lastly centrifuged at 12,000 rpm/ minutes for 5 minutes. Five µl of the supernatant were utilized as a template for the DNA.

LAMP primers

LAMP primers were designed by using the online Primer Explorer V4 software (table 1)

Table 1: LAMP primers

Primer name	Primer sequence (5´ to 3´)	Reference
CTX-M group 1		
F3	CACTGCGTCAGTTCACGC	Rivoarilala et al[12]
B3	CACGGCCATCACTTTACTGG	
FIP	TTGCTGTACGTCCGCCGTTTGTTTTCAACCGTCACGCTGTTGT	
BIP	CAGTCGGGAGGAAGACTGGGTTTTTGCGCTCATCAGCACGATA	
LF	TACAGCGGCACACTTCCTA	
CTX-M group 2		
F3	GGTGGTCCCGATAAAGTGAC	
B3	CTGTGCCCGCTGAGTTTC	
FIP	CGCCTGGAATGGCGGTATTGAGTTTTGGGTGATGAGACCTTCCGT	
BIP	GTGATACCACCACGCCGCTCGCTTTACCCAGCGTCAGAT	

The LAMP assay

In an Eppendorf tube, 25µl mixture of the following components: 0.8µM of every inner primers FIP and BIP, 0.2µM of every outer primers F3 and B3, and 0.4µM of each loop primers LF or/and LB when available, 10mM KCl, 10mM (NH4)2SO4, 20mM Tris-HCl, 1M betaine, 0.1% Tween 20, 4mM MgSO4, 0.4mM of each deoxynucleotide triphosphate, 5µl of DNA extract, and 8U Bst DNA polymerase. The mixture was then incubated at 65˚C in a bath of water. Distilled water (DW) was served as the negative control.

To identify LAMP products, 2 diverse methods were used:

1. Naked eyes observation and staining with Sybrgreen by adding 1µl of Sybrgreen diluted 1/10 to 25µl of the mix. Positive reactions were indicated by a change in color from orange to yellow, and negative reactions were shown by no color change
2. Five microliters of the LAMP product were loaded onto a 2% agarose gel for analysis using gel electrophoresis.

Statistical Analysis

The IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA) program was used for data analysis.

RESULTS

Demographic Characteristics of patients

100 pregnant females with symptoms and signs of UTI were investigated throughout the study period. The age of the participants was ranged from 25 to 44 years; the majority was between the age ranges of 25 to 34 years (47%). An equal percentage of participants had university education qualifications (50%). (49%) of the participants were in their third trimester of pregnancy .About (65%) of patients had a previous history of UTI, and multigravida patients were (72%) (table2).

Table 2: Sociodemographic and risk factor data of the participants

Variable	Frequency	Percentage
Age		
<25	24	24%
25-34	47	47%
35-44	22	22%
>44	7	7%
Education		
Primary	10	10%
Secondary	40	40%
Tertiary	50	50%
Duration of pregnancy		
First trimester	15	15%
Second trimester	36	36%
Third trimester	49	49%
History of UTI		
Yes	65	65%
No	35	35%
Use of catheter		
Yes	10	10%
No	90	90%
Gravidity		
Primigravida	28	28%
Multigravida	72	72%

Among 100 samples from participants, 79 samples were culture positive (79%). Five different bacteria were identified. *E. coli* considerd the predominant one (n=41(51.89%). Other isolated bacterial strains, include *Klebsiella, Proteus, Staphylococcus, and Pseudomonas*.The Antimicrobial susceptibility pattern of isolated strains: is illustrated in Table3.

Table3. Antimicrobial susceptibility testing (AST).

No and percentage of isolates	***E. Coli*** **n=41(**51.89%)		***S. Aureus*** **n=18(**22.78%)		***Klebsiella*** **n=12(**15.2%)		***Proteus*** **n= (6**7.6%)		***Pseudomonas*** **n= (2**2.53%)	
Antibiotic	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Ampicillin	22(53.66)	19(46.34)	15(83.33)	3(16.67)	7(58.33)	5(41.67)	4(66.67)	2(33.33)	0(0)	2(100)
Pipracillin	18(43.9)	23(56.1)	14(77.7)	4(22.3)	8(66.66)	4(33.33)	5(83.33)	1(16.66)	2(100)	0(0)
Nitrofurantoin	39(95.11)	2(4.89)	14(77.78)	4(22.22)	11(91.67)	1(8.33)	1(16.67)	5(83.33)	2(100)	0(0)
Gentamicin	25(30.98)	16(39.02)	17(94.44)	1(5.56)	11(91.67)	1(8.33)	6(100)	0(0)	2(100)	0(0)
Ceftriaxone	9(21.9)	32(78.1)	5(27.8)	13(72.2)	3(25)	9(75)	1(16.67)	5(83.33)	1(50)	1(50)
Cefotaxime	16(39.03)	25(60.97)	14(77.78)	4(22.22)	4(33.33)	^(66.67)	3(50)	3(50)	1(50)	1(50)
Cefpodoxim	7(17.07)	34(82.93)	15(83.3)	3(16.7)	3(25)	9(75)	2 (33.33)	4(66.67)	1(50)	1(50)
Aztreonam	18(43.9)	23(56.1)	-	-	10(83.3)	2(16.7)	5(83.33)	1(16,66)	2(100)	0(0)
Ceftazidime	10(25)	31(75.61)	4(22.2)	14(17.7)	5(41.6)	7(58.3)	4(66.6)	2(33.3)	1(50)	1(50)

Phenotypic detection of Extended Spectrum Beta – Lactamases by Disk diffusion and DDSD (Table4)

Table 4: Phenotypic detection of ESBL production by disk diffusion and DDSD

Bacterial isolates	ESBL screening by the disk diffusion method				ESBL confirmation by double disk diffusion testing
	Cefotaxime (30 µg) N (%)	Ceftazidime (30 µg) N (%)	Ceftriaxone (30 µg) N (%)	Aztreonam (30 µg) N (%)	Cefotaxime (30 µg) with clavulanic acid (10 µg) N (%)
E. coli (41)	25(60.97)	31(75.61)	32(78.1)	23(56.1)	22(53.65)

MALDI-TOF MS assay, Cefotaxime antibiotic hydrolysis by strains of *E. Coli* that produce ESBL was explored by detecting its molecular peak. A non-ESBL-producing strain showed peaks at 456 and 458 m/z, consistent to cefotaxime's non-hydrolyzed form. An ESBL positive strain created peaks at 370 and 414 m/z, whereas the peaks at 456 and 458 m/z shrank (figure2)

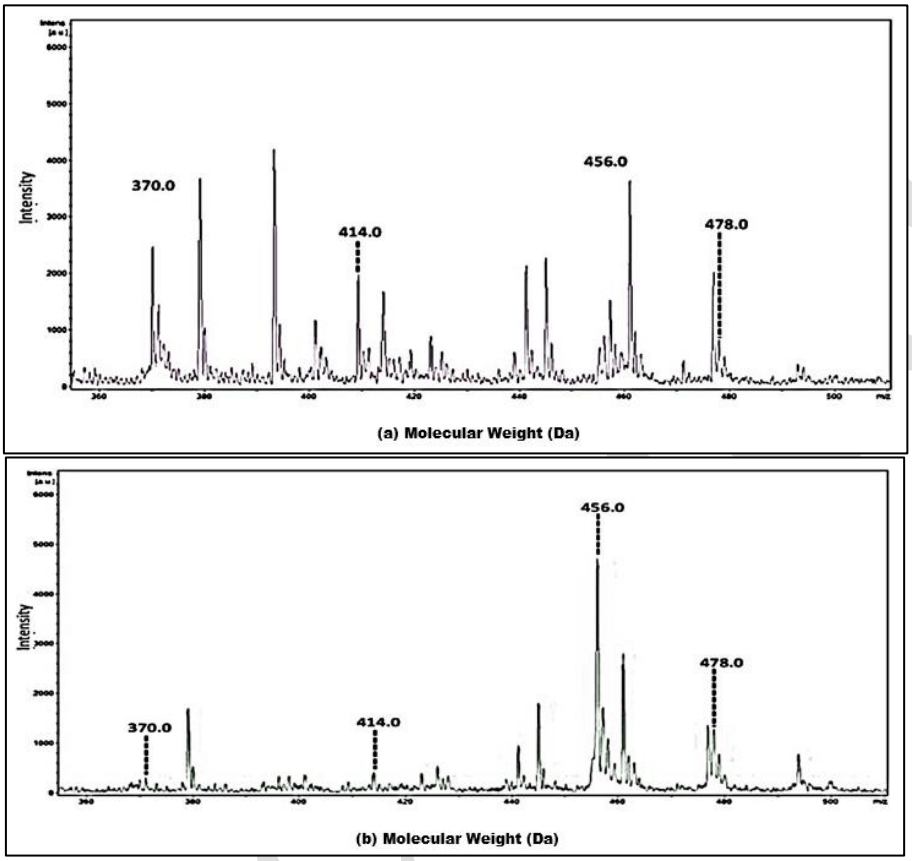

Fig. 2: MALDI-TOF spectra of the hydrolyzed form of cefotaxime (a) obtained from an ESBL producing strain and the corresponding non-hydrolyzed form (b) from a non-ESBL-producing strain

Genotypic Detection of ESBL Producing E.coli by LAMP

CTX-M group 1 was found in 12 of 41 *E. coli* that produces ESBLs while, only 2 strains proved to harbor CTX-M 2 on results of the gel electrophoresis (Figure3)

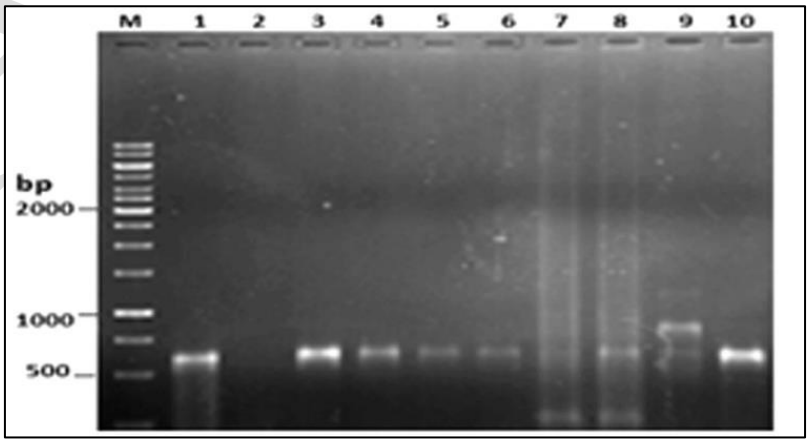

Fig. 3: LAMP assay: Migration on agarose gel

DISCUSSION

Many factors may predispose to UTI, for example age, sexual activity, pregnancy, instrumentation, urinary tract obstruction, and previous antibiotics [13].

We noticed that a significant proportion of the females were between the age ranges of 25 to 34 years (47%), these findings align with ***Maingi, et al***[6] who reported that the highest UTI prevalence was in pregnant females between the ages of 28 – 37(52.7%). This might be because of the fact that this age group is more sexually active and reproductive.

Vaginal microorganisms enter the urethra during sexual intercourse, making it easy for bacteria to be wiped into the urethra and subsequently migrate to the bladder, leading to infection. In the meantime, the natural close proximity of the of the urethra in women to the anus makes it disposed to to fecal flora contamination, particularly for expectant women who fail to exhibit personal hygiene throughout gestation[14].

The high UTIs prevalence in this age range increases serious worries about adverse birth outcomes which can be challenging to be managed in an era of prevalent antibiotic resistance.

Usually, pregnant females in their second and third trimesters showed higher UTI prevalence. The physiological and anatomical changes that take place during pregnancy could be the cause of this e.g., the expansion of the uterus during pregnancy plus increased hormonal effects on normal homeostatic balance making conditions favorable for bacterial invasion [16, 15].

Although the incidence of UTI in the first trimester was fairly low, it may serve as an incubation period for the microorganisms so UTI with significant bacterial counts was unable to be recognized in this period. Moreover the pregnant females may have competent immune systems to keep the bacteriological growth in check in this trimester, and so as the pregnancy grows, the pregnant females develop lower immune status and the UTI is clearly diagnosed in the second and third trimesters[17].

A significant association between parity and former history of UTI was reported in our research. These findings are comparable to Masinde, et al[18].

According to our culture results, 51% of patients have *E. coli* isolated from them; the remaining percentages were attributed to several strains of bacteria, including *Staphylococcus* (21%), *Klebsiella (*14%), *Proteus* (10%), and *Pseudomonas* (4%). Similar results by Simba, et al[19], revealed *E. coli* as the most repeatedly isolated organism causing UTI during pregnancy

The high ESBL production by bacterial isolates might result from the use of broad spectrum antibiotics in hospitals and the lack of laboratory screening of ESBLs production [6].

The test of double disk synergy detected only 53.6% of our screened ESBL isolates .This result correlates with an earlier study by Sahraoui, et al.[20] In contrast to Shaikh et al[21] who reported that DDST detected 77.78% of ESBL isolates which is greater than our results. The discrepancy in ESBLs producing strains might be due to differences in screening methods, study settings and study population[22].

The MALDI-TOF MS-based approach directly analyzes the enzymatic reaction of the -lactamase at the molecular level. This assay proceeds much more quickly, and results are obtained in just 4 hours.

On comparing the MS-derived data with the routine procedure data, it was found that the bacteria were classified identically based on their sensitivity and resistance [23].

The Enterobacteriaceae that produce ESBLs have CTX-M show resistance to 3rd-generation cephalosporins, contributing to a rapidly increasing global health crisis. E. coli is a primary host for the CTX-M 1 gene variants[24].

We detected CTX-M group 1 in 12 of 41 ESBL positive *E. coli* and CTX-M group 2 was identified in only 2 strains. According to Zeynudin [25], the most prevalent CTX-M group found was CTX-M group 1 also.

The study had a few limitations; the sample size was small which may affected the assessment of ESBLs producing bacteria strains among expectant women. Additionally, the study was done in a hospital facility; therefore, the results are not representative of other pregnant females at other health centers inside the same region.

CONCLUSION

The significant occurrence and resistance of ESBL strains towards frequently used antibiotics underscore the need of using suitable diagnostic techniques and targeted antibiotic treatment. The integration of phenotypic and genotypic testing offers significant insights for informing treatment strategies and mitigating potential consequences in pregnant individuals affected by UTI. Additional investigation is mandatory to examine the optimal lines for the management of ESBL-producing E. coli in this susceptible demographic. In its whole, this research enhances comprehension about UTIs in pregnant females and highlights the need of tackling antibiotic resistance within this particular setting.

Declarations:
Consent for publication: Not applicable
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REFERENCES

1. Habak PJ, Griggs, Jr RP. Urinary Tract Infection in Pregnancy. [Updated 2023 Jul 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-.
2. Storme O., Tiran Saucedo J., Garcia-Mora A., Dehesa-Dávila M., Naber K. G. Risk factors and predisposing conditions for urinary tract infection. Therapeutic Advances in Urology . 2019;11:p. 175628721881438.
3. Kalinderi K, Delkos D, Kalinderis M, Athanasiadis A, Kalogiannidis I. Urinary tract infection during pregnancy: current concepts on a common multifaceted problem. J Obstet Gynaecol. 2018 May;38(4):448-453. doi: 10.1080/01443615.2017.1370579. Epub 2018 Feb 6. PMID: 29402148.
4. Wing DA, Fassett MJ, Getahun D. Acute pyelonephritis in pregnancy: an 18-year retrospective analysis. Am J Obstet Gynecol. 2014 Mar;210(3):219.e1-6.
5. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections:epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13:269–284.
6. Maingi, E., Nyerere, A. and Makobe, C. (2023) Phenotypic and Genotypic Characterization of Extended Spectrum Beta-Lactamases Producing Bacteria Causing Urinary Tract Infections among Expectant Women Attending Antenatal Clinic at Ruiru Sub County Hospital, Kenya. Advances in Microbiology, 13, 571-587.
7. Ghonaim R.A., and H.A. Moaety. 2018. Comparison between multiplex PCR and phenotypic detection methods for identifying AmpC B-lactamases among clinical isolates of Enterobacteriaceae in Zagazig University Hospitals, Egypt. Clin. Microbiol. 7:3.
8. Castanheira M., Simner P. J., & Bradford P. A. (2021). Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-antimicrobial resistance, 3(3), dlab092.
9. Paulitsch-Fuchs AH, Melchior N, Haitzmann T, Fingerhut T, Feierl G, Baumert R, Kittinger C, Zarfel G. Analysis of Extended Spectrum Beta Lactamase (ESBL) Genes of Non-Invasive ESBL Enterobacterales in Southeast Austria in 2017. Antibiotics (Basel). 2022 Dec 20;12(1):1. doi: 10.3390/antibiotics12010001. PMID: 36671202; PMCID: PMC9854808.
10. Ghenea AE, Zlatian OM, Cristea OM, Ungureanu A, Mititelu RR, Balasoiu AT, Vasile CM, Salan AI, Iliuta D, Popescu M, Udriștoiu AL, Balasoiu M. TEM,CTX-M,SHV Genes in ESBL-Producing Escherichia coli and Klebsiella pneumoniae Isolated from Clinical Samples in a County Clinical Emergency Hospital Romania-Predominance of CTX-M-15. Antibiotics (Basel) . 2022 Apr 10;11(4):503.
11. kawamoto Y, Kosai K, Yamakawa H, Kaku N, Uno N, Morinaga Y, et al. Detection of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae using the MALDI Biotyper selective testing of antibiotic resistance-β-Lactamase (MBT STAR-BL) assay. J Microbiol Methods 2019;160:154-6.
12. Rivoarilala OL, Garin B, Andriamahery F, Collard JM (2018) Rapid in vitro detection of CTXM groups 1, 2, 8, 9 resistance genes by LAMP assays. PLoS ONE 13(7): e0200421.
13. Gururajan G, Kaliyaperumal KA, Ramasamy B. Prevalence of extended spectrum beta lactamases in uropathogenic Escherichia coli and Klebsiella species in a Chennai suburban tertiary care hospital and its antibiogram pattern. Research J Microbiol. 2011;6:796-804.
14. DCD . Jaypee Brothers Medical Publishers Ltd; New Delhi, India: 2013. DC Dutta's Texbook of Gynecology.
15. 15-Amiri M., Lavasani Z., Norouzirad R., Najibpour R., Mohamadpour M., Nikpoor A.R., et al. Prevalence of urinary tract infection among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful city, Iran, 2012-2013. Iran. Red Crescent Med. J. 2015;17(8)
16. Mittal P., Wing D.A. Urinary tract infections in pregnancy. Clin. Perinatol. 2005;32(3):749–764.
17. Adedze-Kpodo RK, Feglo PK, Agboli E, Asmah RH, Kwadzokpui PK. Genotypic characterization of extended-spectrum β-lactamase producing urinary isolates among pregnant women in Ho municipality, Ghana. Heliyon. 2022 Dec 22; 8(12):e12513. doi: 10.1016/j.heliyon.2022.e12513. PMID: 36593819; PMCID: PMC9803828.

18. Masinde A., Gumodoka B., Kilonzo A. and Mshana S.E. (2009) Prevalence of Urinary Tract Infection among Pregnant Women at Bugando Medical Centre, Mwanza, Tanzania. Tanzania Journal of Health Research, 11, 154-159.
19. Simba S.M., Omwenga E.O. and Musyoki, S.K. (2022) Prevalence of E. coli as a Causative Agent of Urinary Tract Infections and Its Drug Susceptibility Patterns among Pregnant Mothers Seeking Medicare at Kisii Teaching and Referral Hospital, Kenya. International Journal of Community Medicine and Public Health, 9, 1161
20. Sahraoui HL, Berny EH, Quasmaoui A, Charof R and Mennane Z. Detection methods of Enterobacteriaceae producing extended spectrum betalactamase. IJIAS (2016);15 (2):232-9.
21. Shaikh NK, Mundhada SG, Lalngaihzuali R and Ingole KV. Comparison of different phenotypic methods for the detection of extended spectrum blactmase (ESBL)] in bacterial isolates from tertiary care center. International Journal of Current Research and Review (2016);8 (11):10:14.
22. Thapa R., Lamichhane P., Banjara M.R. and Acharya G.P. (2015) Prevalence of Extended Spectrum β Lactamase Producing Uropathogens in Pregnant Women. Asian Journal of Pharmaceutical and Clinical Research, 8, 207-210.
23. Doern CD, Butler-Wu SM. Emerging and future applications of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry in the clinical microbiology laboratory: A report of the association for molecular pathology.J Mol Diagn 2016; 18(6):789-802.
24. Higgins O, Chueiri A, O'Connor L, Lahiff S, Burke L, Morris D, Pfeifer NM, Santamarina BG, Berens C, Menge C, Caniça M, Manageiro V, Kisand V, Hassan MM, Gardner B, van Vliet AHM, La Ragione RM, Gonzalez-Zorn B, Smith TJ. Portable Differential Detection of CTX-M ESBL Gene Variants, blaCTX-M 1 and blaCTX-M-15, from Escherichia coli Isolates and Animal Fecal Samples Using Loop-Primer Endonuclease Cleavage Loop-Mediated Isothermal Amplification. Microbiol Spectr. 2023 Feb 14; 11(1):e0331622.
25. Zeynudin A, Pritsch M, Schubert S. et al. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. BMC Infect Dis 18, 524.