# Detection of Extended Spectrum β-Lactamase and AmpC gene in *Escherichia coli* Isolated from Pregnant Women in Misurata City

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الملخص

عدوى المسالك البولية (UTI) هي أكثر أنواع العدوى البكتيرية شيوعًا أثناء الحمل. الإشريكية القولونية هي أكثر الكائنات الحية شيوعًا التي تسبب عدوى المسالك البولية المكتسبة من المجتمع والمستشفى. قد تفسر الأنماط المختلفة لاستهلاك المضادات الحيوية في المستشفى تنوع وانتشار إنزيمات B-lactamase المقاومة المصاحبة للأمراض. لذلك ، تم فحص انتشار ESBLsالمنتجة للإشريكية القولونية في التهاب المسالك البولية بين النساء الحوامل في 300 عزلة بكتيرية. 160 عزلة كانت موجبة الجرام بينما كانت سالبة الجرام 140. كانت الإشريكية القولونية أكثر مسببات الأمراض عزلة (57٪). فيما يتعلق بمقاومة المضادات الحيوية ، كانت جميع عزلات الإشريكية القولونية (100٪) للأمبيسيلين والأوكساسيلين والجيل الأول من السيفالوسبورين والسيفاليكسين والسيفرادين. هم أكثر حساسية للجيل الثالث والرابع من السيفالوسبورين ، والتي أوصت بعلاج التهاب المسالك البولية بين النساء الحوامل. تم فحص جميع عزلات الإشريكية القولونية لإنتاج ESBLs عن طريق اختبار تآزر القرص المزدوج واختبار AmpC لإنزيم .AmpC أظهرت النتائج أن (36٪) من العزلات المختبرة كانت موجبة لـ ESBLsو (20٪) موجبة لـ AmpC و (10٪) تحتوي على إنزيمات ESBLs و .AmpC تم اكتشاف الجين CTX-Mباستخدام طريقة PCR ، والتي كشفت أن 53 ٪ من العينات التي تم فحصها كانت إيجابية.CTX-M

***Abstract***

Urinary tract infections (UTI) are the most common bacterial infections during pregnancy. *E.coli* is the most common organism causing both community as well as hospital acquired UTI. The various patterns of antibiotic consumption in hospital may account for the diversity and spread of β -lactamase resistant enzymes that associated with diseases. Therefore, prevalence of ESBLs producing *E.coli* in UTI among pregnant women investigated in 300 bacterial isolates. 160 of the isolates were gram positive, while 140 were gram negative. *E.coli* was the most frequently isolated pathogen (57%). Regarding to antibiotic resistance, all *E. coli* isolates were (100%) to Ampicillin and Oxacillin, and first-generation Cephalosporin, Cephalexine and Cephradine. They more sensitive to the third and fourth generation of Cephalosporin, which recommended treating UTI among pregnant women. All *E.coli* isolates screened for ESBLs production by double-disc synergy test and AmpC test for AmpC enzyme. The result revealed (36%) of tested isolates were ESBLs positive, (20%) AmpC positive and (10%) have both ESBLs and AmpC enzymes. CTX-M gene detected using the PCR method, which revealed that 53% of investigated samples were CTX-M positive.

**Keywords:** *Escherichia coli*; UTI; pregnancy; ESBLs; CTX-m; PCR

### *Introduction*

Bacterial producing extended spectrum β-lactamases have been increased in the word associated with many diseases (Elgrabulli, *et al*.,2015; Iqbal, *et al.,* 2017; Lhwak & Abbus, 2018). Various national pattern of anti-biotic consumption in hospitals probably account for the difference in distribution of their enzymes (Oliveira *et al*; 2011). B-lactamases resistant expressed chromosomal and plasmid mediated born. ESBLs production *E.coli* where detected in hospital as well as community acquired infection. It well documented in patients with urinary tract infection (Hussain, *et. al* ;2011; Tayh, *et.al*, 2019; Elgrabulli, *et al.,* 2015; Pandit, *et al.,* 2020, Hassuna *et al*; 2020). It also the main pathogen among pregnant women (Rowińska *et. al*., 2015; Youssef *et al*; 2019; Lhwak & Yahya; 2018). The AmpC and B-lactam test is use to conform resistance to methoxy B-lactams such as Cefoxithin, narrow extend and broad-spectrum Cephalosporin, Tazobactam and not inhibited by Clvalouic acid or another β-lactamases inhibitor (Coudron, *et al*; 2000). Detection of ESBL producer bacteria from UTI patient is important to select appropriate therapy (Monstein *et al*; 2007). The aim of current study is to determine the phenotype and the prevalence of antibacterial resistance *E.coli* to currently used antibiotic to treat UTI among pregnant women in Misurata city.

## Materials and Methods

### *Bacteria sample*

A total of 300 bacterial samples were isolates from urinary tract infection (UTI) among pregnant women from four different hospitals in Misurata city-Libya in period between April 2018 to August 2019. Patient's medical history recorded from their medical files. Pregnant women under antibiotic treatment not included. All bacteria isolates identified by microscopic and chemical standard method and confirmed by (Analytic Profile Index 20E) API 20 E.

### *Bacterial susceptibility testes*

Antibiotic sensitivity of the isolated bacteria was determined by standard Disc Diffusion method (Bauer and Kirby,1966) on Hinton Agar using different antibiotic (30µg) include Clavulanic acid, Tazobactam, Oxacillin, Ampicillin, Imipenem, Cefotaxime, Ceftazidime, Ceftriaxone, Cephradine, Cefuroxime, Cefoxitin, Cefixime, Cefepime, Meropenem. Bacterial that resist to 4-10 antibiotic classified as multi drug resistant bacteria (MDR).

### *Double Disc Synergy test (DDST)*

Isolates showing inhibition zone size below the (Clinical and Laboratory Standards Institute) CLSI stated break points were considered a potential ESBL-producer when the inhibition zone size for ceftriaxone >25 mm.

Double disk synergy test (DDST) method was used to detection of ESBLs production. *E.coli* was identification by using tested by spreading on Mueller-Hinton agar plates, then disk of Cefotaxime, Ceftazidime Ceftriaxone and Cefepime was dispensed around a disk of amoxicillin- Clavulanic acid positioned at a distance of (20mm) (centre to

centre). Resulting by increasing the diameter of inhibition zone at the side of amoxicillin-Clavulanic acid disk (NCCLS, 2007).

The presence of AmpC β-lactamases was detected by initial screening with Cephoxitin disc. Isolates which showed Cefoxitin zone diameter >14 mm was considered screen positive for AmpC β-lactamase production ،and secondly by the synergy between Cefepime and Tazobactam.

***Detection of CTX-M Gene by PCR Technique:***

The DNA extraction from obtained *E.coli* colony carried out using QIAGEN , according to the instructions of the manufacturing company at Misurata central laboratory. The DNA concentration and purity assessed by using Nanodrop. PCR was used to detect CTX-M gene (593 Bp) amplification using two primers, CTX-M- F (ATG TGC AGY ACC AGT AAR GTK ATG GC) and CTX-M- R (TGG GTR ARR TAR GTS ACC AGA AYC AGC GG) (Carthagenomics, France) (Monstein *et al*, 2007). PCR mixture consisted of 6.25 µL (1x) master mix, 0.5 µL (1 pm) of each primer, 0.5 ml (250 ng) DNA and 4.75 µL distill water in sterile 0.2 ml micro centrifuge tubes. PCR Program was performed with following: Initial denaturation at (95°C), (3min), followed by (35) cycles of denaturation at (95°C), (15 sec) annealing at (67°C), (30 sec.) extension at (72°C), (1 sec.) and a final extension step of (5 min) at (72°C) (Dallenne *et al*., 2010). Reaction products were detected using 1% agarose gel with ethidium bromide.

***Results and Discussion***

The most common treatment strategy is use antimicrobial drugs; however, the wide use of antibiotics plays a significant role in increasing resistance to them. Consequently, the treatment of diseases caused by bacterial pathogens has become very difficult. Therefore, determining the susceptibility of a microorganism to antibiotic is essential to good treatment. In this study, a total of 300 clean-catch mid-stream urine samples collected from UTI pregnant women screened for ESBL production. Bacterial identification methods (Gram stain and API 20E) revealed that Gram positive bacteria was the dominant, 54% (160) while Gram negative represented by 47% (140) samples. This is in argument with Hussein and Muhsin (2018) and disagree with Al. Haidari and Shehab (2017); Hamza and Altaky (2007).

In this study, the *E. coli* was the main cause of UTI in pregnant women and represented by 57% (80) of isolated Gram-negative bacteria (Table 1). This result is in agreement with other studies (Tayh *et al*; 2019, Al-Salamy, 2012). Other gram negative was *Klebsiellosis* spp. *Enterobacter* spp. *Pseudomonas* spp. and *Proteus* spp. represented by 18.57%, 12.14%, 7.15%, and 5% respectively Table (1). While Gram positive represented by *Staph*. Spp. 29%, *Strepto*. Spp. 29.55%, *Bacillus* 20% and *Enterococcus* spp. 17.2%.

Table (1): The number and percentage of Gram-negative bacteria isolates .

| Gram negative Bacteria | Total | % |
|---|---|---|
| ***Escherichia coli*** | 80 | 57.14% |
| Klebsiella spp | 26 | 18.57% |
| Enterobacter spp | 17 | 12.14% |

| | | |
|---|---|---|
| Pseudomonas spp | 10 | 7.15% |
| Proteus spp | 7 | 5% |
| Total | 140 | 100% |

Phenotypical susceptibility testes methods revealed that *E.coli* isolates (80) were highly resistance to 10 of tested antibiotics in this study (Table 2). They more sensitive to third and fourth generation of Cephalosporin such as Ceftazidime that recommended to be using in treatment UTI. This finding is in agreement with other studies ( Moore *et al*, 2016 & Reed *et al*, 2019).

Table (2): Antibiotic susceptibility pattern of *E.coli* isolates

| The antibiotics used | Symbol | Percentage of resistance | Sensitivity percentage |
|---|---|---|---|
| Ampicillin | AM | 100% | 0% |
| Oxacillin | OX | 100% | 0% |
| Cephalexin | CL | 100% | 0% |
| Cephradine | CE | 100% | 0% |
| Cefuroxime | CXM | 62% | 37% |
| Cefoxitin | FOX | 67% | 31% |
| Cefotaxe | CTX | 41% | 59% |
| Ceftriaxone | CRO | 42% | 57% |
| Ceftazidime | CAZ | 24% | 76% |
| Cefixime | CFM | 36% | 64% |
| Cefepime | FEP | 39% | 61% |
| Meropenem | MEM | 70% | 30% |
| Imipenem | IPM | 66% | 34% |
| Tazobactam | TPZ | 62% | 37% |

Some research studies found low sensitive to third-generation (Hassan *et al*, 2013, Igbal, *et al*; 2017). Double disc synergy test applied on isolates that showed resistance to more than 11 antibiotics. The result showed that 36% of *E.coli* isolates are significantly β -lactamase producer **(**Table 3).This agrees with other studies (Tayh *et al*., 2019, Adwan & Abu Jaber; 2015). AmpC β-lactamase type observed in 20% of isolates. However, 10% of investigated *E.coli* produce the two type of β -lactamase, ESBLs and AmpC. The coexistence of the two types of β-lactamases also observed by other investigators (Buzayan & El-grabulli, 2015 ; Grover *et al*, 2013).

Table (3): Determination of ESBL +ve & AmpC –ve and ESBL+AmpC +ve

| Samples Numbers | ESBL +ve | AmpC -ve | ESBL+AmpC +ve |
|---|---|---|---|
| 80 | 29 (36%) | 16 (20%) | 8 (10%) |

DNA Extraction showed that all isolates contain one double, single, high sharpness and clearly from extracted DNA, which indicates the concentration and purity. (Figure 1)

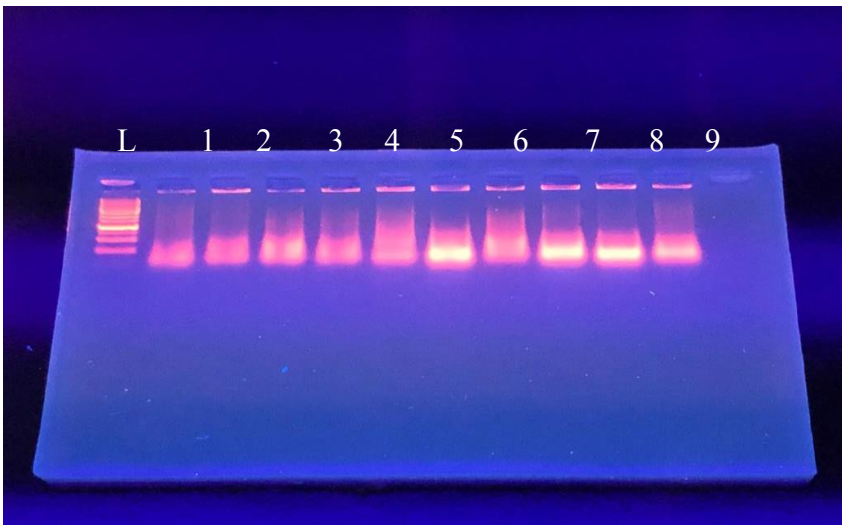


Figure (1). 1% agarose TBE gel run for 30 minutes at 50v. Lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 showing bacterial genomic DNA.

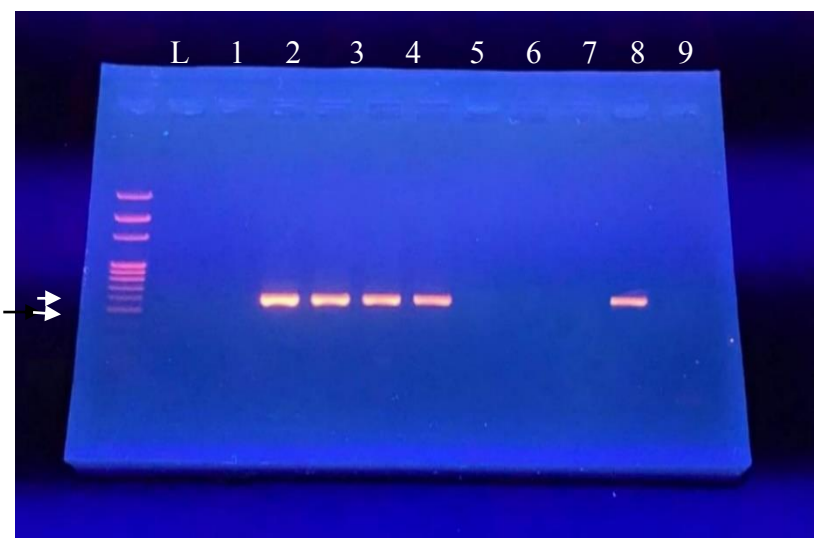


Figure (2). PCR product of bla-CTX-M gene amplification on 1% agarose gel TBE gel run for 50 minutes at 50v.

Lane L=100 bp, lane 1, 2, 7, 8, 9=negative results, lane3,4,5,6=positive result, PC = Positive Control, NC= Negative Control.

The PCR assay has the advantage of identifying genotypic resistance to antibiotic more rapidly and reliably. In this study PCR product on 1% agarose showed the presence of 593bp DNA fragments in 53% of investigated *E.coli* samples which evidence emergence of CTX-m gene (Figure 2). Similar results were obtained by Hussain, *et al.,* 2011 (57%), Zorgani *et al*., 2016 (51%). on other hand, this result contrasts with Tayh, *et al.*; 2019 who found 100% of *E.coli* isolates carries CTX-m gene. The miss use of antibiotics and frequent use of

cephalosporin may lead to success spread of CTX-m gene (Gelbanol, *et al*.;2015, Elgrabulli, *et al.*,2015; Rossolini, *et al.,*2008).

***Reference***

-AlHaidari S & Shehab R. 2017. Using Chromagar Orientation Medium in Identification of Pathogenic Bacteria of Renal Failure Patients. Al-Mustansiriya Science Journal. 28(1): 96-107

- Alsalamy A. 2012. Identification of broad-spectrum beta-lactam isolates of Escherichia coli isolated from urine. Kufa Journal of Medical and Veterinary Sciences. 55-65

-Adwan G & Abu Jaber A. 2017. Frequency and Molecular Characterization of β-lactamases Producing *Escherichia coli* Isolated from North of Palestine. Microbiology Research Journal International. 11(5): 1-13

- Bauer A, Kirby W, Sherris J & Turk M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of clinical pathology.45 (4):493-6

- Buzayan M & El-Grabulli F. 2014. Detection of ESBL and AmpC-lactamases producing in uropathogen *Escherichia coli* isolates at Benghazi Center of Infectious disease and immunity. J.Curr.Microbiol.App.Sci.3 (2):145-153

- Clinical and Laboratory Standards Institute (CLSI). 2010. Performance- standards for antimicrobial susceptibility testing. 20th informational supplement. M 100-S20.Wayne. Pennsylvania .30 (1)

- Coudron P, Moland E & Thomsonks K. 2000. Occurrence and detection ofAmpC Beta - lactamases among *Escherichia coli*, *Klebsiella pneumonia* and *Proteus* microbial isolates at veteran's medical center. J.clin. microbiology.38: 1791-1796

- Dallenne C, Costa A, Decre D, Favier C & Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important b-lactamases in *Enterobacteriaceae*. Journal of Antimicrobial Chemotherapy. 65(3): 490-495

- El-Grabulli F, Buzayan M & Mathlouthi N. 2015.Emergence of CTX-M gene Among Positive *E.coli* isolates from UTI out patients at Benghazi. The second symposium on theories and application of basic and biosciences. 5 September 2015.

- Gelband H, Miller-Petrie M, Pant S, Gandra S, Levinson J & Barter D, White A, Laxminarayan R. 2015. The state of the World' s Antibiotics. Center for Disease Dynamics, Economic & Policy. 79 Pages.

- Grover N, Sahni A & Bhattacharya S. 2013. Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. Med J Armed Forces India. 69(1): 4–10

- Hussain M, Hasan F, Shah A., Jung, A, Rayamajhi N, Cha S & Yoo H. 2011. Prevalence of class A and b-lactamases in clinical *Escherichia coli* isolates from Pakistan institute of medical science, Islamabad, Pakistan. Japanese Journal of Infectious Diseases. 64: 249-52

- Hussein F & Muhsin A. 2018. Isolation and Identification of some Microorganisms Causing Vaginitis and Cervicitis and Relationship of Risk Factors with these Infections. Rafidain Journal of Science. 27(3):77-94

- Hassan M, Alkharsah K, Alzahrani A, Obeid O, Khamis A, Diab A. 2013. Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. The Journal of Infection in Developing Countries. 7(8):618-29

- Hamza H & Al take E. 2007. Isolation and Identification of Pathogenic Bacteria from Women with Disturbance in Urinary System in Hilla. Technical Journal. 20(1): 7-12

- Hassuna N, Khairalla A, Farahat E, Hammad A & Abdel-Fattah M. 2020. Molecular Characterization of Extended-spectrum β-Lactamase Producing *E.coli* Recovered from Community-Acquired Urinary Tract Infections in Upper Egypt. Scientific Reports Journal. 10 (1): 1-8

- Iqbal R, Ikram N, Shoaib M, Asad J, Mehmood R, Niazi A, Asghar A, Ashfaq B, Naseer F. 2017. Phenotypic confirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ESBL) producing Uropathogens. Journal of Applied Biotechnology & Bioengineering. 3(3):344-349

- Lhwake N & Abbas Y. 2018. Detection of Extended Spectrum β-Lactamase Gene CTX-M-1 in *Escherichia coli* and *Klebseilla pneumonia* Isolated from Urinary Tract Infection of Pregnant Women in Al-Nassyriah City. Journal of thi-Qar Science. 92-96

- Moore C, Sona S, Poda S, Putchhat K & Sopheary S. 2016. Antimicrobial susceptibility of uropathogens isolated from Cambodian children. Paediatr Int Child Health. 36: 113-117

- Misurata Central Laboratory: Misurata- Libya. Fax: 051-2620121. Email: Labcent2003@yahoo.com

- Monstein H, Ostholm-Balkhed A, Nilsson M, Nilsson M, Dornbusch K & Nilsson L. 2007. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in *Enterobacteriaceae*. APMIS. 115(12):1400-8.

- National Committee for Clinical Laboratory Standards (NCCLS). 2003. Performance standards for disk susceptibility testing. approved standard M 100-S13

-Oliveira F, Paludo K, Arend L, Farah S, Pedrosa F & Souza E.2011. Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. Genet Mol Res Journal. 10(4):4114-25

- Pandit R, Awal B, Shrestha S, Joshi G, Rijal B & Parajuli N. 2020. Extended-Spectrum β-Lactamase (ESBL) Genotypes among Multidrug-Resistant Uropathogenic *Escherichia coli* Clinical Isolates from a Teaching Hospital of Nepal. Interdisciplinary Perspectives on Infectious Diseases. 1-8

-Rossolini G, D'Andrea M & Mugnaioli C. 2008. The spread of CTX-M-type extended-spectrum beta-lactamases. Clinical Microbiology and Infection Journal. 14 (s1):33-41

- Rowińska J, Małyszko J & Wieliczko M. 2015. Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems. Archives of Medical Science. 11(1): 67-77

- Reed T, Krang S, Miliya T, Ly S & Turner P. 2019. Antimicrobial resistance in Cambodia. International Journal of Infectious Diseases. 98-107

- Tayh G، Al Laham N, Ben Yahia H, Ben Sallem R, Elottol A & Ben Slama, K. 2019. Extended-Spectrum β-Lactamases among *Enterobacteriaceae* Isolated from Urinary Tract Infections in Gaza Strip, Palestine. BioMed Research International. 11 pages.

- Youssef M, Rizk H & Hassuna N. 2019. Phenotypic and Genotypic Characterization of Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* in Asymptomatic Bacteriuria in Pregnancy. Microbial Drug Resistance Journal. 25(5):731-738

- Zorgani A, Mathlouthi N, El Salabi A, Bakour S, Jridi Y, Slama K, Rolain J & Chouchani C. 2016. Carbapenemases and extended-spectrum β-lactamases producing *Enterobacteriaceae* isolated from Tunisian and Libyan hospitals. 10(7): 718-727