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Detection of Extended Spectrum β–Lactmase Gene CTX-M-1 in *Escherchia coli* and *Klebseilla pneumonia* Isolated from Urinary Tract Infection of Pregnant Women in Al-Nassyriah City

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Abstract:

This study was performed to detect the presence of CTX-M-1 gene in *Klebseilla pneumonia and Escherchia coli* are that causing urinary tract infections in pregnant women in Al- Nassyriah city , during the period from september (2016) to february (2017).

Three hundred and thirty (n= 330) urine samples were collected and cultured on appropriate media for initial isolation. Morphological, conventional biochemical tests and API 20 system were performed to identify the pathological bacteria agents.Ninety samples showed positive culture, the most common isolates were *E. coli* 57 (63.3%) followed by *K. pneumonia* 21(23.3%) and 12(13.3%) from other Gram negative bacteria.

ESBLs gene CTX-M-1(688 bp) was found in 22 isolates 17/57 (29.8%) in *E.coli* and 5/21 (23.8 %) in *K. pneumonia*.

KEYWORD: ESBLs, *E.coli*, *K.pneumoniae*,Pregnant Women ,UTI.

Introduction:

Urinary tract infection (UTI) is an infections caused by the existence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the tract of digestive which ,climbs to the opening of the urethra and begin to double to cause infection (Okonko *et al*., 2009). In dissimilarity to men, women are more susceptible to UTI, and this is mainly due to absence of prostatic secretion, shortened urethra, pregnancy and easiy contamination of the urinary tract with fecal flora (Haider *et al*., 2010).

Escherichia coli and *Klebsiella pneumonia* were of the common intestinal commensals normal flora , it's a members of enterobacteriaceae family, with importance role in diverse hospital infections and community acquired infections. These bacteria dominant in urinary tract infections. Frequently use of antibiotics to remedy *E. coli and K. pneumonia* infectious lead to increasing the multidrug resistance (MDR) and hence the problem in treating the infection specially UTIs (Trecarichi *et al.*, 2012*).*

β-lactamase production considered as an important mechanism between several model to resistance of antibiotic by *E.coli and K. pneumonia* (Nicolas-Chanoine *et al*., 2012).The ability to production ESBLs is contributors in the trouble of resistance to antimicrobial(Mirzaee *et al*., 2009). Cefotaxim hydrolyzed (CTX-M-1) ESBLs family has been increasing in frequency around the world (Woerther *etal*., 2013).

ESBLs originate mainly because of mutations in β-lactamases encoded by the TEM ,SHV and CTX-M-1 genes, the variants TEM and SHV are most widespread ESBLs, but strains expressing CTX-M-1 ESBLs have initiate to emerge in many countries (Ahmed *etal*.,2013).The β-lactamases CTX-M-1 are characterized by selective degradation of cefotaxime (Paterson and Bonomo 2005).This study aimed to detected CTX-M-1 ESBLs gene in *E.coli* and *K. pneumonia* isolated from pregnant women.

2. Materials and Methods:

Isolation of Bactaria:

We collected 330 urine samples from pregnant women from september (2016) to february (2017), who suspected to have urinary tract infection (UTI), consalted for AL- Nassyriah city hospital in Iraq. Urine samples transferred to laboratory and culture according to standard methods, after incubation for 24 h at 37°C, the positive culture were identified by conventional techniques and confirmed by using API 20 E system (Cowan and Steel,2014).

Detection of Extended Spectrum β-lactamase (ESBLs).

Double disk synergy test (DDST) method was used to detection of ESBLs production . *E.coli* and *K. pneumoniae* was identification by using tested by spreading on Mueller -Hinton agare plates,then (30µg) disk of cefotaxime,aztreonam (30µg) ,ceftazidime (30µg) and ceftriaxone (30µg) was dispensed around a disk of amoxicillin -clavulanate (20 µg amoxicillin / 10 µg clavulonic acid) positioned at a distance of (30mm) (centre to centre).Resulting by increasing the diameter of inhibition zone at the side of amoxicillin-clavunic acid disk (NCCLS,2007).

Detection of CTX-M-1 Gene by PCR Technique.

The DNA was extracted and purified according to the instructions of the manufacturing company (Geneaid / Korea). The DNA was extracted from (57) isolates of *E.coli and* (21) of *K.pneumonia*. The CTX-M-1 gene was amplified using primers CTX-M-1 F (5′-TTAGGAARTGTGCCGCTGYA-3′) and CTX-M-1R(5′-CGATATCGTTGGTGGTRCCAT-3′(Dallenne *et al*.,2010).

Total DNA (2 µl) was subjected to PCR in a 50µl reaction mixture containing 1X PCR buffer [10 Mm Tris–HCl (pH 8.3), 50Mm KCl], 1.5 Mm MgCl2 , 200 Mm of each deoxynucleotide triphosphate, 1.5 µl of each of the primers and 2.5 U of Taq polymrase.PCR Program was preformed with following: (1) cycles of (10 min.) initial denaturation at (94°C), followed by (35) cycles of denaturation at (90°C), (40 sec.) annealing at (60°C), (40 sec.) extension at (72°C), (60 sec.) and a final extension step of (7 min.) at (72°C). (Dallenne *et al*., 2010).

3. Results and Discussion:

Atotal of 330 urine samples were collected and cultured at different media ,only 90 samples given positive growth results. Gram-negative distributed after diagnosed using conventional techniques and conformed by using API 20 E system to 57(63.3%) *E.coli* and 21 (23.3%) *K.pneumoniae* and 12(13.3%) from other Gram negative bacteria as in Table (1). A high frequency of UTI commonly accepted during pregnancy .Among pregnant women the UTI bacterial infection is the predominant type , the physiological and hormonal changes in urinary tract include changes in ureteral dilatation, tone and bladder volume that may promote the infection in pregnant women.The isolation percentage of this study was in agreemeent with other studies such as Al- Muk and Hansony (2001) in Basrah (Iraq), Almushaita *et al*., (2013) in Saudi Arabia and Hamdan *et al* .(2011) in Sudan.

E.coli is the most implicated microorganisms and the second common bacterium isolated of Gram negative bacteria was *K.pneumoniae* that causing UTI in pregnant women in the present study. This study is correspond with results obtained in Tikrit city by Al-Jebouri and Mdish ,(2013), also in AL - Najaf city (Abdulla and Oleiwi,2016) and in Kirkuk (Ali *et al* .,2007) .

Results of detection of ESBLs production were showed that out of 90 isolates 26(28.9%) were ESBLs producing isolates, *E.coli* contain 17(29.8%) and *K.pneumoniae* contain 5(23.8%) ESBLs producing isolates (Table 1).

Table (1): Distribution of ESBLs Production and CTX-M-1 gene in *E.coli* and *K.pneumonia*.

Isolates	Number of isolates	ESBLs enzyme production	%	ESBLs producting isolates and have CTX-M-1 gene	%
Escherichia coli	57	17	29.8	13	76.5
Klebsiella pneumoniae	21	5	23.8	4	80.0
Klebsiella oxytoca	12	4	25.0	2	50.0
Total	90	26	28.9	19	73.1

The multidrug resistant isolates of *E. coli* has been increasing around the world and is associated

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with the acquisition of mobile element-encoded β-lactamases (Ramos *etal*.,2012). The CTX-M-1 β-lactamases production is a very important mechanism to resist for many antibiotic groups in *K.pneumoniae* and *E.coli* ,this family of plasmid-mediated ESBLs of ambler class A has been detected mainly in Asian countries , Europe and America (Castanheira *et al*.,2008).

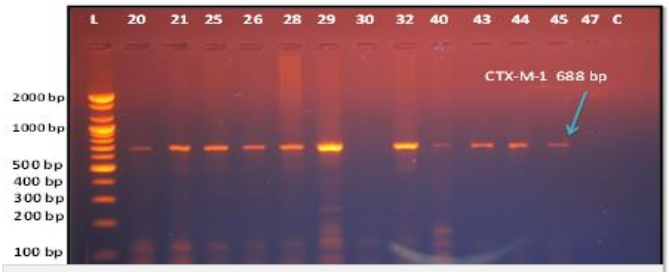
In this study, were the production detected of ESBLs in (28.9 %) of Gram negative isolated from the pregnant urine figure(1), this result were correspond with the study reported in Tikrit city 27.5% done by Alsamarai *et al*., (2016) and this study less than the study in Erbil province 61.6 % by Ahmad and Ali, (2014) .

The study revealed that *E.coli* was the most prevalent ESBL producing isolates 65.4%. This reuslt closed to Al-Nammi (2001) in Baghdad (Iraq), who reported that (72.6%) of *E. coli* isolates gave positive ESBLs, while ESBLs producing *K.pneumoniae* represent 19.2% in present study this near from local study showed 10.5 % done by Al-Charrakh , (2005). Such result was less than the study in Erbil province 40 % by Ahmad and Ali,(2014).

figure (1).Clearance the inhibition of producing of ESBLs due to clavunic acid through expansion of the zone of inhibition between AUG and each of CRO ,CAZ ,ATM and CTX.

The results showed individual band of CTX-M -1 gene were characterized in (688 bp) by comparison with the standard molecular DNA ladder (100-2000bp) figure (2),the CTX-M-1 was considered as a common type of ESBLs that detected at Asia, Europe, North and South America among multidrug-resistant in *E.coli* (Feizabadi *et al*.,2010).

The ESBLs gene CTX-M-1, predominates in this results which present four isolated of *K. pneumonia* out of 5 isolates producing ESBLs and in 13 isolates of *E.coli* out of 17 isolates producing ESBLs as in Table (1) ,this result corresponding with result of Al-Mayahie,(2013) in Al-kut city.The CTX-M-1gene predominates in Europe, while in other countries, the ESBL genes are more diverse (Livermore *et al*., 2012) .In Norway and Portugal,the CTX-M-1 is the ESBLs enzyme most frequently found in *E. coli* (Tofteland *et al*., 2007). Also,this results are in accordance with (Pitout *et al, 2005)* who reported that repeat UTIs were more likely to be caused by CTX-M-1producing strains than by non-CTX-M-producing strains.



Figure(2): Gel Electrophoresis [Ethidium bromide,Agarose (2%)] of PCR products from extracted Gram negative isolates DNA amplified with primers for ESBLs gene CTX-M-1 .lane(L),DNA Ladder size marker (2Kb ladder).Lanes:21,25,26,32,40,43 and 44 isolates of *E.coli*, lane 20,28, 29 and 45 isolates of *K.pneumoniae* that show positive results for CTX-M-1 of ESBLs genes .lane 30 and lane 47 isolates of *E.coli* show negative result for ESBLs genes .Lane (C) control.

CONCLUSION:

This study was conducted for detection of extended spectrum β-lactamases enzyme and CTX-M-1 gene among *E.coli* and *K. pneumonia* isolates that causes urinary tract infections for pregnant women at AL-Nassyriah city in Iraq.

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