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Molecular Detection of AmpC gene families Encoding for Antibiotic Resistance among Escherichia coli isolated from Patients with Urinary Tract Infection (UTI) in Al-Najaf Hospitals

A thesis

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Abstract

our study was designed to detect and evaluation Ampicillin class C β-lactamases genes from *Escherichia coli* isolates in patients with UTI by using of PCR technique .

In the present study, 250 mid-stream urine samples were collected from patients suspected to had urinary tract infection (UTI). The urine samples collected from three hospitals in Najaf during the period from (january 2012 to April 2012) , and screened for the presence of *E. coli* . The growth of $\geq 10^5$ colony forming units/ml were considered significant bacteriuria.

A total of 130 (52%) samples with significant bacteriuria were detected. The study showed higher incidence of UTI in females (73.08%) than males (26.9%).The bacteria which were grown on culture of all the 130 urine samples with significant bacteriauria were as follows: 60 (46.2 %) isolates of *E. coli* and 70 (53.8%) were other isolates such as *P. aerogenosa* , *Proteus mirabilis* , *K. pneumonia* and *Staphylococcus aureus*.

The sixty isolates of *E. coli* isolates were initially screened for susceptibility to β-lactam antibiotics and the results demonstrated that there was 53(88.3%) of the tested isolates were resistant to both ampicillin and amoxicillin. All the 53 β-lactams resistant isolates were tested for their antibiotic resistance against 17 selected antimicrobial agents. All the isolates were found to be resistant to a minimum of 3 classes of antibiotics.

The majority of β-lactams resistant *E. coli* 30(56.6%) isolates were found to be able to produce β-lactamase enzyme with rapid iodometric method. All the 53 β-lactams resistant *E. coli* isolates were tested for cefoxitin susceptibility and ability to produce AmpC β-lactamase by two methods; modified three dimensional test and AmpC disk test , as well as further tested for their ability to produce chromosomal-mediated AmpC β-lactamases using ceftazidime-imipenem antagonism test. The results revealed that 28 (52.8%)

isolates were cefoxitin resistant and 23(43.4%) isolates were confirmed as AmpC producers by the two above methods , respectively. Conversely, none of the 53 isolates were identified as inducible of AmpC β-lactamase producers by the ceftazidime-imipenem antagonism test. Therefore, all of these *E.coli* isolates were considered plasmide mediated ampC β-lactamases.

The results showed that only 18 (34%) isolates gave positive results with bla_{AmpC} gene and all bla_{AmpC} positive isolates showed diversity presence of plasmid-mediated AmpC β-lactamases. The genes that encoded FOX, CIT, DHA, EBC, ACC and MOX were found in the percentage of 44.4, 38.9, 27.8, 50.0, 22.2 and 27.8, respectively. Multiplex PCR assay revealed that bla_{FOX} and bla_{CIT} as well as bla_{DHA} and bla_{EBC} *ampC* genes were detected in 3 (16.7%) and 2 (11.1%) isolates, respectively,While the remaining 13 (72.2%) isolates carried only one *ampC* gene. Such results showed that ampC β-lactamases producing by *E. coli* isolates were recognized in both phenotypic and molecular methods in local isolates recovered from patients suspected to have UTI in Najaf hospitals .