Molecular characterization of antimicrobial drug resistance in *Escherichia coli* isolated from clinical samples

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Abstract: A total of 49 different clinical samples (urine n=30, stool n=10, and blood n=8) were collected from patient admitted to the Al-Sadder medical City in Al-Najaf Governorate-Iraq. The results demonstrated that 49 specimens (100%) were diagnosed as *E. coli* by cultural, biochemical characteristics and Vitek2® system. Polymerase Chain Reaction has been used to detect of some genes which coding antimicrobial resistance in *E. coli* isolates. Regarding genes that responsible for ESBL enzymes ($bla_{\text{CTX-M}}$, bla_{OXA} and bla_{TEM}), the current results proved that bla_{TEM} genes have highest rate (97.95%) followed by bla_{TEM} and bla_{OXA} (93.75%) for each.

Keywords: *Escherichia coli*, $bla_{\text{CTX-M}}$, bla_{OXA}, bla_{TEM}, by product, Iraq.

Introduction

Antimicrobial resistance (AMR) is when a microbe evolves to become more or fully resistant to antimicrobials which previously could treat it[1]. When a bacterial strain resistant to three or more different antimicrobial classes defined as MDR bacteria[2].

Since meat and its byproducts are important sources of human deals, it should be free of contamination and hazard[3]. Thus *E. coli* and the other member of *Enterobacteriaceae* are important reservoirs of transferable antibiotic resistance[4]. *E.*

coli may use various biochemical pathways to escape the lethal action of drugs: (i) decreased intracellular accumulation of the antibiotic by an alteration of outer membrane permeability, diminished transport across the inner membrane, or active efflux; (ii) alteration of the target by mutation or enzymatic modification; (iii) enzymatic detoxification of the drug; and (iv) by passing of the drug target. The coexistence of several of these mechanisms in the same host can lead to multidrug resistance (MDR)[5].

Antibiotic resistance in MRSA is determined by the *mec*A gene, whichencodes an altered penicillin binding protein (PBP-2a), that reduces the binding affinity for methicillin andother β-lactam antibiotics[6].

Materials and Methods

DNA extraction: Three to five pure and fresh colonies of *E. coli* were inoculated from MacConkey agar plate into 300 µl of distilled water. Then cells was lysed by heating at 100 ^{o}C for 20 minutes (in water bath), and then immediately the cells were placed in ice for 30 minutes, and the other cellular components was removed by centrifugation at 8500 rpm for 10 min. Finally the supernatant was used as the DNA template[7].

Extended spectrum beta lactamases Primers: Primers used were supplied from Bioneer and were listed in Table (1).

PCR amplification: The reaction mixture contain Go Taq® Green Master Mix, X2 which is premixed ready-to-use solution containing bacteriology derived *Taq*DNA polymerase dNTP, $MgCl_2$, and reaction buffers at optimal concentrations and its recommended for any amplification reaction that to visualized by agarose gel electrophoreses and ethidium bromide staining.

Agarose gel electrophoresis: Agarose gel was prepared by dissolving 1.5 gm of agarose powder in 100 ml of TBE buffer (pH 8) in boiling water bath, allowed to cool to 50˚C, then ethidium bromide (at concentration of 0.5 mg/ml) was added. A tape was placed across the end of the gel tray, the comb was fixed at one end of the tray for making wells used for loading DNA samples[8].

Table (1): target genes, amplification sizes and cycling conditions.

Gene name	Initial denaturation	Cycles	Denaturation	Primer annealing	Elongation	Final elongation	References
***bla*$_{TEM}$**	94ºC / 30 sec	35	94ºC / 30 sec	45ºC / 1 min	72ºC / 1 min	72ºC / 10 min (then 4°C→ ∞	9
***bla*$_{CTX-M}$**	94ºC / 30 sec	35	94ºC / 30 sec	60ºC / 1 min	72ºC / 1 min	72ºC / 10 min (then 4°C→ ∞	10
***bla*$_{OXA}$**	94ºC / 5 min	30	94ºC / 50 sec	55ºC / 50 sec	72ºC / 1min	72ºC / 10 min (then 4°C→ ∞	11

Results and Discussion

CTX-M-type enzymes were the most common type of ESBL in *E. coli* isolates compared with SHV and TEM enzyms[12]. Since then, an increase in the CTX-M β-lactamases has been seen in many countries in Europe and Asia[13]. The *bla*$_{CTX-M}$ was reported the most prevalent *bla*-gene in Korea[14].

Results showed that *erm*C was detected in 6 (50%), another high detection show moderate prevalence 35.9%[15], while another study found that 3.9% of staphylococci isolatescarried *erm*C[16].

On the other hand, TEM-1, which is responsible for most of the ampicillin resistance in; 94% of *E. coli* strains isolated in Spain, 89% of *E. coli* strains isolated in Hong Kong, and in 78% of *E. coli* strains isolated in London[17].

The OXA enzymes are regarded as OXA-type ESBLs and have been discovered mainly in *P. aeruginosa* in specimens from Turkey and France[18].

Pyridoxine (V B6) significantly increased growth of *Cymbopogon citrates* L. plants, especially in plants treated with 200 mg/l[19]. Similresults were recorded by [20] on *Antirrhinum majus* plant, and on corn plant[21,22].

Table (2): Prevalence of extended spectrum beta lactamases genes of *E. coli* according to infections site (n=48).

Genes	Urine Total Isolates (n=30)	Stool Total Isolates (n=10)	Blood Total Isolates (n=8)	Total (48)
***bla*$_{CTX-M}$**	29 (96.66%)	9 (90%)	7 (87.5%)	45 (93.75%)
***bla*$_{OXA}$**	29 (96.66%)	9 (90%)	7 (87.5%)	45 (93.75%)
***bla*$_{TEM}$**	30 (61.22%)	10 (20.40%)	8 (16.32%)	48 (97.95%)

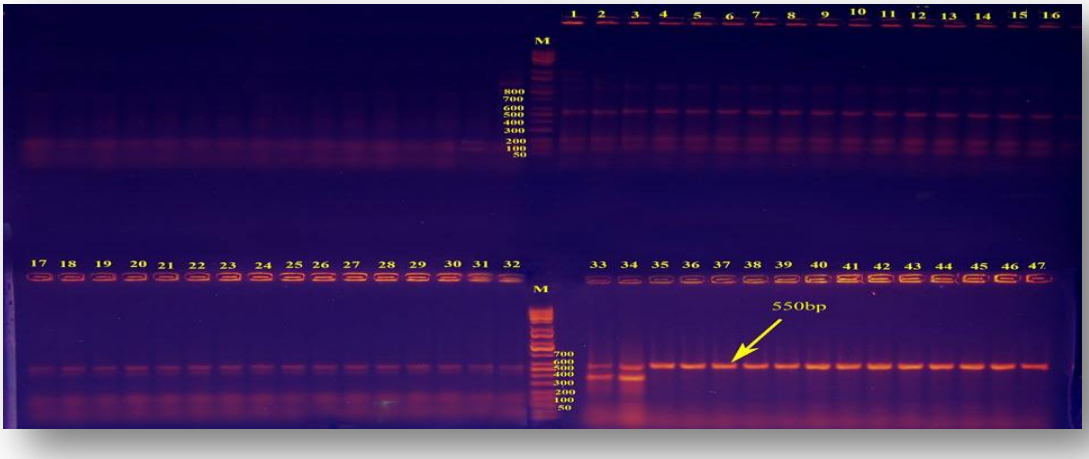

Photo (1): Ethidium bromide-stained agarose gel electrophoresis of monoplex PCR amplified products from extracted total DNA of 49 *E. coli* isolates isolated from different clinical specimens. Lane: (1 to 47 isolates) amplified with diagnostic *bla*$_{CTX-M}$ gene, show positive results at 550 bp . The electrophoresis was performed at 80 volt for 95 minutes. (L): DNA molecular size marker (50 bp ladder).

Conclusion

Our findings showed that there was relationship between phenotypic and genotypic detection of antimicrobial resistance in *E. coli*.

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