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Research Article

Molecular Detection of *wzx1* and *wzy* Genes in Multi Drugs Resistance *E. coli* Isolates

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ABSTRACT

E*scherichia coli* have been genetically changes in Iraqi environments, the present study was carried out to detection *E coli* isolates sero-group using o-antigens gene cluster, tow genes were amplified used multiplex PCR, the results of present study show that present isolates have more than one plasmid in different size (500-700 bp). PCR results show about 100% of isolates were had a positive results of PCR products of O45 wzx1 gene (451 bp) while 25% of isolates were a positive PCR products of O45 wzy1 gene (255 bp) and about 25% of isolates have tow genes. A new finding in present study that 50% of isolates have other copy of O45 wzx1 gene, these results concluded that it may be important in Iraqi isolates identification and classification however we need more information about genes sequencing.

Keywords: o-antigens, O45 wzx1 gene, O45 wzy1. Multiplex PCR.

INTRODUCTION

E*scherichia coli* sero-groups have been developed using the *wzx* (O-antigen flippase) and the *wzy* (O-antigen polymerase) genes which found in the O-antigen gene cluster of each organism (DebRoy *et al*., 2005). Lipopolysaccharide is endotoxin which components of the outer membrane of some Gram-negative bacteria. It is consist of two layers a hydrophilic polysaccharide and a hydrophobic as lipid A that responsible of endotoxin bioactivity, many studies have been identified some enzymes and genes coding for proteins which responsible for the biosynthesis and export of lipopolysaccharide in most Gram-negative bacteria (Bastin *et al*., 1993; Paton *et al*., 1999). There are some differences in structure of lipopolysaccharide in bacterium to another, this because of discover additional enzymes and gene products which responsible of basic structure modifications of lipopolysaccharide especially in pathogenic bacteria (Batisson *et al*., 2003) .

These modifications related to the virulence of bacteria and not important of bacterial survival. the enzymes genes which involved in O-antigen synthesis clustered in a chromosomal region called O-antigen gene cluster (rfb cluster), that found between the galF gene and the and other gene (Wang *et al*.., 1991;Wang *et al*.., 2001). The number of genes in these clusters depending on the complexity of the polysaccharide, and strains of different sero-groups can show completely different gene sets (D'Souza *et al.*, 2002).

MATERIALS AND METHODS

Pathogenic bacteria; *E.coli* was obtained from advance microbiology lab in biology department\ college of science \Babylon university in suitable media , then antibiotic sensitivity was detected using disc methods to cefixime, erythromycin, gentamicin, amikacin, meropenem, Nitrofurantoin, ceftazidime, norfloxacin, tetracycline, cefotaxime, aztronam.

DNA extraction and PCR experiment

DNA was extracted and PCR performed according to PCR colony methods then the following primers were used to amplify O45 wzx1 (F) CCG GGT TTC GAT TTG TGA AGG TTG ,(R) CAC AAC AGC CAC TAC TAG GCA GAA, and O45 wzy1 (F) GAA ATT ATG CCA TCT TGG CGA GCG , (R) CAT GTG AAG CCT GAA GGC AAA CTC (DebRoy et al.., 2004).

PCR conditions and size products

PCR experiments performed as a following; pre-denaturation for 5 min at 94˚C, then 35 cycles (30 s at 94˚C, 30 s at 59˚C, 30 s at 72˚C, and finally 10 min at 72˚C). Electrophoresis was implemented using 1% agarose 0.5 X for 60 min , 70 V and 20 mA all testes had control represented by *E coli* HB101 [pomega which have the following genetic description F–, *thi*-1, *hsd*S20 (rB–,mB–), *sup*E44, *rec*A13,*ara*-14, *leu*B6, *pro*A2, *lac*Y1, *gal*K2, *rps*L20(strr), *xyl*-5, *mtl*-1.].

RESULTS AND DISCUSSION

The results of present study show there were 8 isolates labeled as E1, E2, E3, E4, E5, E6, E7, V5, which resistance to some antibiotic which show in table (1). All isolates were sensitive to Erythromycin (15mcg), Nitrofurantoin (300 mcg) and Norfloxacin (10 mcg).

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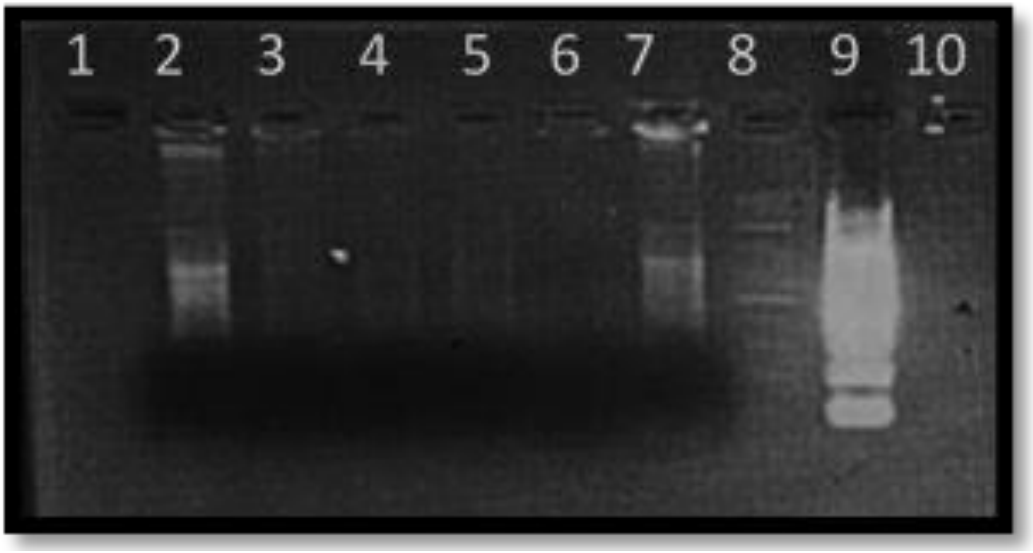

Figure 1: Plasmids profile of *E coli* isolates, the lanes 1-8 represent *E coli* isolates, lane 9 DNA ladder , lane 10 *Ecoli* HB101.

Table 1: Inhibition zone diameters (mm) of antibiotic sensitivity of *Ecoli* isolates and E coli HB 101.

Bacteria isolates	Erythro-mycin 15mcg	Ceftazidime 30mcg	Nitro-furantoin 300mcg	Cefo-taxime 30mcg	Cefixime 5mcg	Norfloxacin 10mcg	Aztronam 30mcg	Amikacin 30mcg	Gentamicin 10mcg
S.S	17	-	35	-	-	23	-	-	16
E2	29	-	30	14	-	25	29	30	20
E4	31	-	23	14	9	21	-	34	25
E3	31	-	25	-	-	30	-	35	31
V5	25	-	15	14	13	25	-	32	26
E5	40	15	20	41	32	23	21	37	32
E1	30	-	10	-	23	25	-	-	-
E6	20	10	14	13	-	30	-	34	-
E7	15	-	25	20	10	21	-	-	-

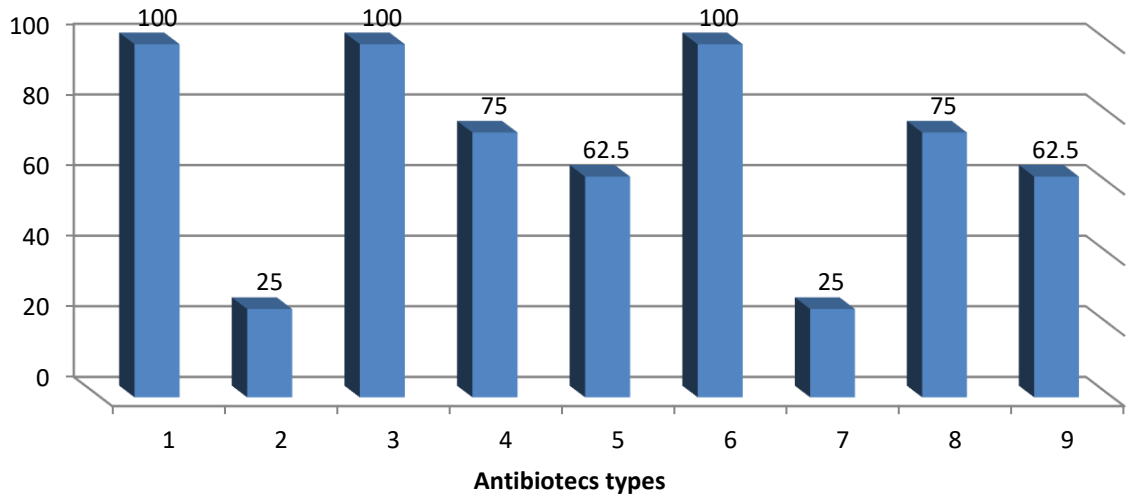

Fig 2: Antibiotic sensitivity percentage in Ecoli isolates lane1 Erythromycin, 15 mcgm; lane 2 Ceftazidime 30 mcg, lane 3 Nitrofurantoin 300 mcg; lane4 Cefotaxime 30 mcg; lane 5 Cefixime 5 mcg; lane 6 Norfloxacin 10 mcg; lane 7 Aztronam 30 mcg; lane 8 Amikacin 30 mcg; lane 9 Gentamicin10mcg

about 75% of isolates were sensitive to Cefotaxime (30 mcg) and Amikacin (30 mcg). While Cefixime (5 mcg) and Gentamicin (10 mcg) were effected in 62.5% of isolates, the least effected of antibiotics were Ceftazidime (30 mcg) and Aztronam (30 mcg) it were 25% of isolates

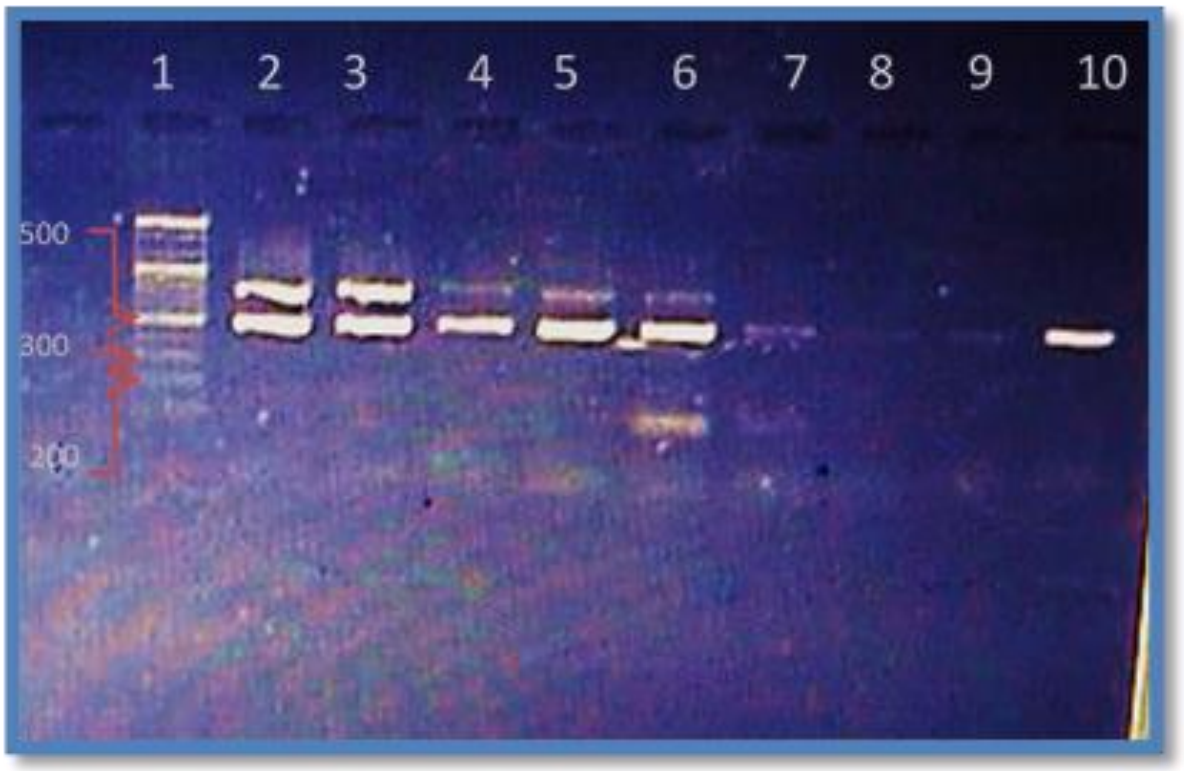

Figure (3) Multiplex PCRs targeting the *E. coli* O*wzx* and O*wzy* genes Lane 1 M, molecular weight, lane2-9 bacterial isolates lane 10 *E coli* HB101. multiplex PCR showing the amplified *wzx* 255 bp and *wzy* 451 bp.

(figure 2 and table 1). Antibiotic resistance in these isolate may be because resistance gene in plasmids, also, it may be acquired by adaptation to environments factors or by genetic ways like transformation, transduction or conjugation. In Iraq there were many isolates of *E coli* have been recorded that it were resistance to antibiotic (Al-Jebouri and Mdish 2005; Ibrahim *et al*., 2014; Mohammed *et al*., 2014).

The DNA profile of these isolates show that some of them have mega-plasmid in tow isolates and about 500 bp plasmid was appeared in 5 isolates and about 700 bp plasmid appeared in tow isolates. The present finding clarified how these isolates resistance to some antibiotics which tested in present study, the Iraqi environment helped bacteria to be resistance because of absence lack of health awareness in some rural population and absent of sewage processing; all these helped bacteria to be more pathogenesis and more resistance to antibiotic and to be had a new genetic materials. This results deal with other studies in Iraq like Al-Hamdani and shadan (2015) whom found that *E coli* isolates have more than one plasmid in each isolates from UTI patients in Basra and Baghdad (Zianbe et al.,2011).

About 100% of isolates show positive results of PCR products of O45 wzx1 gene (451 bp) while 25% of isolates show positive PCR products of O45 wzy1 gene 255 bp (figure 4), about 25% of isolates have tow genes. A new finding in present study that 50% of isolates have other copy of O45 wzx1 gene it may be a new virulence properties or a new gene like O45 wzx1 gene. It is need more investigation to detection its loci and its sequencing. All previous studies in Iraq about E coli didn't record any information about this a new products, DebRoy *et al*.., (2004) used these gene clusters to identified E coli subgroups by PCR , who found that these primers improved high efficiency in bacterial identification. In Iraq this identification may be useful in pathogenesis treatments and drug synthesis (Al-Terehi *et al*.., 2015) We conclude that our finding may be important in Iraqi isolates identification and classification however we need more information about genes sequencing.

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