ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 10, pp.9283--9286, October, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CLASS 1 INTEGRONS GENE IN DRUG RESISTANT *E. COLI* AND ISOLATED FROM DIFFERENT CLINICAL SPECIMENS IN JAZAN AREA K.S.A

[1*]Abdelhaleem, A. A., [1]Homeda, H.E., [1]Hershan, A. A., [1]Makeen, A. M. and [2]Alsanosy, R.M.

[1]Medical Research Center University of Jazan, Jazan, K.S.A.
[2]Deanship of Scientific Research University of Jazan, Jazan, K.S.A.

ARTICLE INFO

Article History:
Received 09th July, 2014
Received in revised form
16th August, 2014
Accepted 04th September, 2014
Published online 25th October, 2014

Key words:
Class 1 Integrons,
MDR,
E. coli

ABSTRACT

Background: in the recent years, there is emergence and dissemination of *E. coli* strains resistance to broad-spectrum of antimicrobial agents. Resistance in bacteria is largely due to the genetic exchange of resistance genes, and this exchange are due to genes called integrons.
Objectives: the aim of this study were to assess the presence of class 1 integrons in drug resistance *E. coli* in the study area, and to analyze the association between the gene and MDR in bacteria.
Methods: One hundred and eighty of *E. coli* isolates from different clinical samples , 72 from wounds, 62 from urine, 35 from stool, and 11 from blood, were included in this study, isolates were collected and selected from Jazan general hospital laboratories, Jazan K.S.A. The susceptibility testing was performed by disk diffusion method. Class 1 integrons was detected by PCR.
Results: Class 1 integrons was detected in 98 out of 180 of isolates with frequency 54.4%, while the frequency of negative class 1 integrons was 45.6%. MDR was found in 77%, and the association between the presence of an integrons and multidrug resistance was significant ($p<0.05$), with frequency 98.9%. Integron 1 gene was present among different isolates sample sources, with majority in isolates from urine sample.
Conclusion: most of resistant *E. coli* are carrying class 1 integrons, which were of crucial importance for the occurrence and transmission of multidrug resistance, and the proportions of drug resistance in class 1 intigron positive strains were higher than in those not carrying integrons.

INTRODUCTION

E. coli are often used in antimicrobial resistance studies because they are found in high numbers in warm-blooded mammals including humans, they are a common human pathogen, resistance is found in all strains (pathogenic and non pathogenic), they have the ability to transfer resistance between different strains and species of bacteria within the gastrointestinal tract and between *E. coli* strain itself (Österblad *et al*., 2000 and Oppegaard *et al*., 2001). Also, *E. coli* reside primarily in mammalian hosts, thus being subjected to the pressures of antimicrobial use and other environmental factors. This makes them an ideal agent for surveillance and research into factors that may contribute to the selection and spread of resistant bacteria (Houndt and Ochman, 2000 and Bartoloni *et al*., 2006). Further, these bacteria are abundant in the environment making them a predominant vehicle for the transmission of resistance genes (O'Brien, 2002; Cohen, 1992 and Erb *et al*., 2007).

Corresponding author: Abdelhaleem, A. A.,
Medical Research Center University of Jazan, Jazan, K.S.A.

In the recent years, there is emergence and dissemination of *E. coli* strains resistance to broad-spectrum of antimicrobial agents (Kang *et al*., 2005 and Martinez-Freijo *et al*., 1998). Resistance in bacteria is largely due to the genetic exchange of resistance genes (O'Brien, 2002), and this exchange are due to genes called integrons (Phongpaichit *et al*., 2008). Integrons are genetic elements, found in the bacterial chromosome, posses genetic determinants, and it is responsible for antimicrobial resistance. Many gene cassettes of integrons contain antimicrobial resistance genes in gram-negative bacteria. Class 1 integrons are the most commonly studied and also responsible for resistant found in *E. coli* isolates. Three classes of integrons, with clinical and epidemiological relevance for antibiotic resistance, have been described. Class 1 is the best characterized integrons, have been reported in clinical and environmental isolates of several Gram-negative bacilli (Fluit and Schmitz, 1999 and Jones *et al*., 1997). Multi drug resistance become problem worldwide (Oppegaard *et al*., 2001; Houndt and Ochman, 2000 and Bartoloni *et al*., 2006). Mutation in chromosomal DNA or acquisition of resistant gene (integron) are one of the most tools that lead to multi drug resistance in *E.coli*. *E. coli* cause intestinal and extra-intestinal diseases (O'Brien, 2002 and Cohen, 1992). Isolated *E. coli* from different specimen may resist to many antimicrobial

agent and there may be proportional of MDR and integron (Kang *et al*., 2005). Polymerase chain reaction technique (PCR): Is one of the valid methods used to detect antibiotics resistance genes in bacteriology and virology. Recently several polymerase chain reaction assays have been developed for detection of class 1 integrons (Japoni *et al*., 2008).

MATERIALS AND METHODS

One hundred and eighty clinical isolates of well identified *E. coli* were isolated from different clinical samples , 72 from wounds, 62 from urine, 35 from stool, and 11 from blood, were included in this study. Isolates were collected from Jazan general hospital laboratories, Jazan KSA.

Antimicrobial susceptibility testing

The susceptibility testing was performed with disk-diffusion method as published before (Jorgensen *et al*., 1999). Antimicrobial testing included amino glycosides, beta-lactams +/- beta-lactamase inhibitors, carbapenems, quinolones, carboxamide and cephamycin. Multiple drug resistance (MDR) was defined as resistance to one or more agents in three or more classes of tested drugs. Strains with intermediate susceptibility were classified as resistant.

Detection of integrons by Polymerase Chain Reaction (PCR)

Template preparation & DNA extraction

Template was prepared by mixing 300 µL of an overnight bacterial culture and 700 µL of DW. DNA was isolated by the Promega DNA Kit (Catalog NO 0000089642), following manufacturer's instructions.

PCR Reaction

To prepare a 20 µL reaction PCR was performed using 1 µL of 10× dNTPs mix (1.25 mM each of dATP, dCTP, dGTP and dTTP), 1 µL of each primer (0.5 µM), 4 µL of 10× PCR buffer, 2 µL of $MgCl_2$ (50 mM), 8.8 µL of sterile distilled water (SDW), 0.2 µL of *Taq* DNA polymerase (5 U/µL) and 2 µL of the DNA template. DNA was amplified by PCR using the following cycle conditions: 94°C for 5 min, followed 94°C for 30s, 59°C for 30 s, 72°C for 30s (35 cycles) and a final extension of 72°C for 7 min. The primers used to amplify the *intI1* gene (class 1) were those described previously (Rosser and Young 1999) (F, 5′-ATCATCGTCGTAGAGACGTCGG-3′; R, 5′GTCAAGGTTCTGGACCAGTTGC-3′). The expected sizes of PCR products were 892 bp.

Electrophoresis of amplification products

PCR products were concentrated by electrophoresis in 1% agarose gels at 100 V in 0.5× TAE buffer (0.4 M Tris–HCl, 0.02 M $Na_2EDTA{\cdot}2H_2O$, 0.2 M sodium acetate, 1.02 M acetic acid), and visualized by ultraviolet illumination after staining the gels with flouro-safe dye (0.5 mg/L).

Data analysis

The association between the presence of an integrons and multidrug resistance was analyzed by using (SPSS; Version10.0). The P Value was calculated by Z test for two population proportions.

RESULTS

Presence of integrons 1 genes

Among the study isolates of *E. coli* class 1 integrons was detected on 98 isolates out of 180 with frequency 54.4%, while the frequency of negative class 1 integrons was 45.6% as shawn in table (1). Positive integrons I gene was detected by presence of 892 pb band were as no band detected in the negative isolates figure (1).

Table 1. Distribution and frequency of Integron 1 gene among tested *E. coli* isolates

E. coli isolates	Number	percentage
Integron 1 positive isolates	98	54.4%
Integron 1 negative isolates	82	45.6%
Total	180	100%

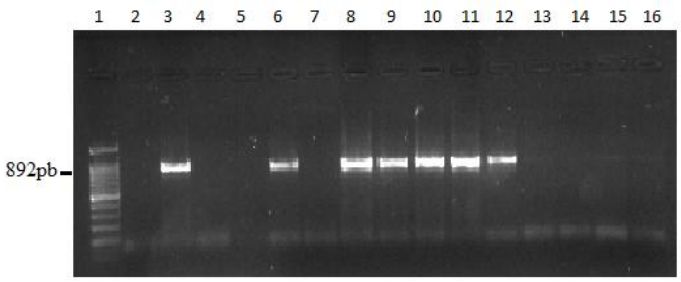

Figure 1. Agarose gel electrophoresis of Integrons 1 gene PCR amplification products: Lane 1 a 100 base-pair DNA ladder; lanes 2-16 *E. coli* isolates; Isolates from lanes 3, 6, 8, 9, 10, 11, and 12 were positive class1 integrons gene, and lanes 2,4,5,7,13,14,15, and 16 were negative

MDR among isolates

MDR was found in 77% of the isolates. Isolates with positive integrons 1 showed more antibiotic resistance than that with negative integrons gene. 97 out of 98 isolates had the gene were antibiotics resistance The P-Value was 0.006. The association between the presence of an integrons and multidrug resistance was significant ($p<0.05$), with frequency 98.9%. Whereas 42 out of 82 isolates had no gene were antibiotics resistance with frequency 51% table (2), Figures (1,2).

Table 2. MDR among tested *E. coli* isolates

E. coli isolates	R	S	Percentage of R
Integron 1 positive isolates (98)	97	1	98.9%
Integron 1 negative isolates (82)	42	40	51%
Total	139(77%)	41(23%)	

S: sensitive, R: resistance

Relation between presence of intgron1 with isolates sample sources

Integron 1 gene was present among different isolates sample sources, and it was more detected in isolates from urine sample with 40/ 62, followed by wound isolate 32/72, stool isolate 20/35, and isolates from blood was 6/11 as shown in table (4).

Table 3. Example of Antibiotic susceptibility test for 15 *E. coli* isolates

EC: *E. coli* isolate, S: sensitive, R: resistance.

E.coli isolates	Isolate source	Integron 1 gene	Antibiotic susceptibility test									
			E	Tet	Amc	Ofx	NA	Gen	Imp	Cfx	AK	Cip
EC1	urine	POSITIVE	S	R	S	R	I	R	R	S	S	R
EC2	urine	POSITIVE	R	R	R	R	R	I	R	R	R	R
EC3	urine	POSITIVE	R	R	R	I	R	S	R	R	R	R
EC4	urine	NEGATIVE	R	R	R	R	R	S	R	R	R	R
EC5	wound	NEGATIVE	R	R	S	R	I	S	R	S	S	R
EC6	wound	POSITIVE	R	R	R	R	R	S	R	R	R	R
EC7	urine	POSITIVE	R	R	R	S	R	S	S	S	S	R
EC8	urine	POSITIVE	R	R	R	R	R	S	R	R	R	R
EC9	stool	POSITIVE	S	R	R	R	R	S	R	R	R	R
EC10	urine	POSITIVE	R	R	R	R	R	S	R	R	R	R
EC11	urine	POSITIVE	R	R	R	R	R	S	R	R	R	R
EC12	wound	POSITIVE	R	R	S	R	I	S	R	S	S	R
EC13	stool	NEGATIVE	R	R	S	R	R	R	R	S	R	R
EC14	stool	NEGATIVE	R	R	R	S	R	S	S	S	S	R
EC15	stool	NEGATIVE	S	R	R	R	R	S	R	S	S	R

E: Erythromycin, Tet: Tetracycline, AMC: Ampicillin, OFX: Ofloxacin, NA: Nalidixic acid, Gen: Gentamycin, Imp: Impenim, Cfx: Cefoxacim, AK: Amikacin, Cip: Ciprofloxacin.

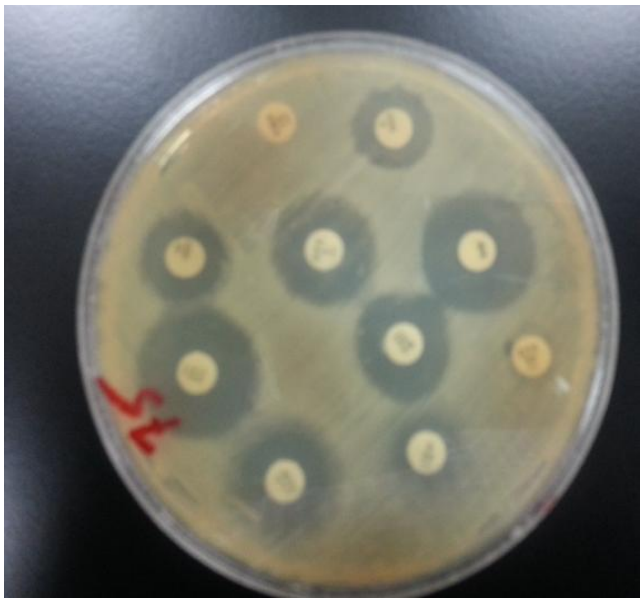

Figure 2. Antimicrobial susceptibility test result, Left example of resistant *E. coli* isolate, and Right sensitive isolate

Table 4. The presence of class 1 integrons according to the sample source of isolates

Sample source (N)	Class 1 integron positive isolates N (%)	Class 1 integron negative isolates N (%)
Urine (62)	40(40)	22(22)
Wound (72)	32(32)	40(40)
Stool (35)	20(20)	15(15)
Blood (11)	6(6)	5 (5)
Total (180)	98(54)	82(46)

DISCUSSION

Multi drug resistance (MDR) become problem worldwide. Mutation in chromosomal DNA or acquisition of resistant gene (integron) are one of the most tools that lead to multi drug resistance in *E.coli.* The class1 integron gene are most detected among isolates of clinical importance, and most of the known antimicrobial resistance gene were belong to this class. Hall and Stokes, (1993) initially described integrons as genetic elements that express resistance to different antibiotics, a general description later confirmed by Rechia and Hall, (1995). Our results provide additional evidence to support this view. In this study class 1 integron was detected on 98 isolates of *E. coli* out of 180 with frequency 54.4%, while the frequency of negative class 1 integron was 45.6%. The prevalence of class 1 integrons among isolates of *E. coli* investigated in this work agrees with that reported by Chang *et al*, (2000). MDR was found in 77% of the total *E. coli* isolates, which indicates increasing of MDR among the isolates circulating in the study area. On the other hand positive integron 1 isolates showed more antibiotic resistance than that with negative integron gene. 97 out of 98 isolates with gene positive were antibiotics resistance the P-Value was 0.006. The association between the presence of an integron and multidrug resistance was significant ($p<0.05$), with frequency 98.9%, same finding was observed before in study done in Greece by Dakić *et al*. (2011) although there is a deference on the isolates source that they used isolates from chickens, and in our study we used isolates from human sources. 42 out of 82 isolates with negative integrons 1 gene were antibiotics resistance with frequency 51%, this results indicated that there was other

factors that responsible of MDR rather than the presence of the gene, one of this factor could be the random use of antibiotics among the population lived at the study area without a doctor request, no published data support this observation. In this study Integron 1 gene was present among different isolates sample sources, isolated *E. coli* from different specimens could increase the resistance to many antimicrobial agents and there may be proportional of MDR and integron (Kang *et al*., 2005). *E. coli* isolated from urine sample showed the highest presence of integron 1 gene, same finding detected by Poly *et al*, (2000), they mentioned that integrons are frequently found among uropathogenic *E. coli*. This study showed that most of resistant *E. coli* are carrying class 1 integrons, which are of crucial importance for the occurrence and transmission of multidrug resistance.

Acknowledgements

We gratefully acknowledge the Saudi Basic Industries Corporation (SABIC) KSA for supporting this work (Grant No 46/4/33), Deanship of scientific research at Jazan university KSA, and also the laboratories of Microbiology of Jazan general hospital for their kind collaboration in the selection of clinical isolates.

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