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Genetic Linkage of the Antibiotic Resistance Ability in the *Escherichia coli* UR4 Strain Isolated from Urine

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It is of interest to understand the antibiotic resistance ability in pathogenic bacteria at the molecular level. Characterization of bacteria by methods based on phenotypic, biochemical and molecular characters were followed herein; and plasmids were certain target in the present study. Seventeen bacterial isolates were isolated from urine, blood and stool samples of patients with urinary tract infections, bloody diarrhea and fever. They were characterized by phenotypic and biochemical criteria and were identified as belonging to *Escherichia coli* (*E. coli*). One of them, isolate number 4 from urine (UR4) was resistant to 14 types of antibiotics used. Hence this strain was subjected to molecular identification. DNA was isolated from *E. coli* UR4 and 16SrRNA gene was separated after agarose gel electrophoresis and then was sequenced. The sequence was subjected to gene bank and showed about 99.5% similarity to *E. coli* category. Growing of *E. coli* UR4 at elevated temperature (42°C) and treatment of colonies with sodium dodycyl sulphate, sodium azide and ethidium bromide revealed a mutants lacking antibiotic resistance ability with mutation percentage ranging from 0.6-14%. The mutation in *E. coli* UR4 was a stable character and no recovery of mutants was observed. Plasmid profile of the *E. coli* UR4 wild strain and its four mutants showed five plasmids in the wild strain and four only in its mutants. One plasmid of a molecular mass of about 600 bp is showed to be deficient or dissociated indicating on its role in antibiotic resistance ability in the *E. coli* UR4 strain.

Key words: Antibiotic resistance, genetic linkage, *E. coli* UR4 and mutation

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INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped, motile, facultatively anaerobic and non-sporulating bacterium. It belongs to family enterobacteriaceae in which most bacterial genera are human pathogens (Vogt and Dippold, 2005). It is part of the microflora of human colon and can benefit their hosts by producing vitamin K and by neutralization of pathogenic bacteria within the intestine and can be changed to pathogenic bacteria under certain conditions such as suppression of immune system (Reid *et al*., 2001).

Transmission of *E. coli* is commonly by fecal/oral route with contaminated foods. At least five types of intestinal infections that differ in pathogenic mechanisms have been identified for *E. coli* viz., enterotoxigenic, enteropathogenic, enterohaemorrhagic, enteroinvasive and enteroaggregative (Harvey and Champe, 2012). *E. coli* was isolated from many pathogenic cases such as gastroenteritis, neonatal meningitis, haemolytic-uremic syndrome, peritonitis, mastitis, cystitis, pyelonephrities, urethrities, vaginities, endometrities and prostatitis (Tauschek *et al*., 2002). Unfortunately, many authors described the existence of *E. coli* variants resistant to many antibiotics (Saffar *et al*., 2008). The antibiotic resistance ability in *E. coli* is due to secretion of enzymes by target strains, exopolyrsacharide production, modification of cell surface, drug efflux system and existence of genes associated to certain plasmids (Fange *et al*., 2009). This clearly showed that there is a need to continue research on *E. coli* strains to able to characterize the multidrug resistant strains and to understand the actual reasons of the antibiotic resistance phenomena which is many cases even at modification of cell membrane or secretion of enzymes and modifications of plasmid associated genes (Harvey and Champe, 2012).

The present study was undertaken to isolate and characterize the multidrug resistant strains of *E. coli* from Egyptian patients by possible biochemical and molecular methods. The study was also aimed to study the genetic determinants of the antibiotic resistance ability in *E. coli* UR4.

MATERIALS AND METHODS

Isolation of bacterial cultures and antibiotic sensitivity test: About 50 clinical samples of urine, blood and stool were taken from patients who did not respond to treatment by antibiotics Egyptian patients and were transferred asceptically to the Microbiology Lab. Bacterial cultures were isolated onto MacConkey agar media (Oxoid) and Hichrome *E. coli* selective media (HiCrome,™). Cultures grown on both media were predicted as *Escherichia* bacteria and were consequently, chosen for study. The antibiotic bioassay were studied using disc diffusion assay for 17 bacterial isolates grown on Hichrome media against 14 types of antibiotics listed in Table 1 (Ehinmidu, 2003). Results were taken according the manufacturer's instructions of antibiotic discs (Oxoid).

Identification of multidrug resistant bacteria: Gram staining, cell morphology, motility, catalase reaction, oxidase test, production of H_2S and growth of bacteria on both MacConkey agar and Hichrome agar confirmed that the 17 bacterial isolates belonged to genus *Escherichia*. The species was determined by tests listed in Table 2 (Wolfgang *et al*., 1998). Identification was carried out according to diagnostic key of Holt *et al*. (1994) and Garrity *et al*. (2005).

The *E. coli* UR4 strain which was resistant to all antibiotics used were subjected to molecular characterization by elucidation of 16 S rRNA cataloging

Table 1: Antibiotic sensitivity test of 17 bacterial isolates from many patients at two hospitals in Cairo

Isolate No. and code	Source of isolate	Antibiotics													
		CAZ	PRL	NOR	CTX	FOX	CRO	SAM	ATM	AK	CFP	AM	CIP	IMP	AM
UR1	Urine	R	R	R	I	R	R	S	R	R	R	R	R	I	R
UR3	Urine	I	R	R	R	R	R	I	R	I	R	R	R	S	R
UR4	Urine	R	R	R	R	R	R	R	R	R	R	R	R	R	R
UR7	Urine	R	R	R	R	I	R	R	R	R	S	R	R	S	R
UR8	Urine	R	R	R	R	R	S	R	I	I	R	R	R	R	I
UR10	Urine	R	R	R	R	R	R	R	R	R	R	R	I	R	R
UR12	Urine	R	R	R	R	R	R	R	R	R	I	R	R	R	R
UR13	Urine	R	R	R	R	R	R	R	R	R	R	R	R	R	S
UR15	Urine	R	R	R	R	R	R	R	R	S	R	R	R	I	R
UR18	Urine	I	R	R	R	R	R	R	R	R	I	R	R	R	R
UR22	Urine	R	R	R	R	I	I	R	R	R	R	R	R	R	R
UR23	Urine	R	I	I	R	R	R	R	R	R	R	R	R	R	I
BL6	Blood	R	R	I	R	R	R	R	S	R	R	R	R	R	R
BL7	Blood	R	R	R	R	I	R	R	R	R	S	R	R	S	R
BL10	Blood	R	R	R	R	R	R	R	R	R	R	R	I	R	R
ST1	Stool	R	R	R	I	R	R	S	R	R	R	R	R	I	R
ST2	Stool	R	R	R	R	R	I	R	R	R	R	R	R	R	R

R: Resistant bacteria, I: Intermediate, S: Sensitive

Table 2: Biochemical characteristics of the antibiotics resistant bacteria

	Biochemical characteristics								
Isolate No. and code	Source of isolate	Catalase	Coagulase	-Indole	Methylered	Oxidase	Vogus proskauer	Citrate utilization	H_2S production	Urease
UR1	Urine	+	-	+	+	-	-	-	-	-
UR3	Urine	+	-	+	+	-	-	-	-	-
UR4	Urine	+	-	+	+	-	-	-	-	-
UR7	Urine	+	-	+	+	-	-	-	-	-
UR8	Urine	+	-	+	+	-	-	-	-	-
UR10	Urine	+	-	+	+	-	-	-	-	-
UR12	Urine	+	-	+	+	-	-	-	-	-
UR13	Urine	+	-	+	+	-	-	-	-	-
UR15	Urine	+	-	+	+	-	-	-	-	-
UR18	Urine	+	-	+	+	-	-	-	-	-
UR22	Urine	+	-	+	+	-	-	-	-	-
UR23	Urine	+	-	+	+	-	-	-	-	-
BL6	Blood	+	-	+	+	-	-	-	-	-
BL7	Blood	+	-	+	+	-	-	-	-	-
BL10	Blood	+	-	+	+	-	-	-	-	-
ST1	Stool	+	-	+	+	-	-	-	-	-
ST2	Stool	+	-	+	+	-	-	-	-	-

	Biochemical characteristics											
Isolate No. and code	Motility	Lysine decarboxyl	Gelatin liquefaction test	Glucose	Lactose	Maltose	Mannitol	Sucrose	Salicin	l-arabinose	D-sorbitol	Blood hemolysis
UR 1	+	-	+	+	+	+	+	-	-	+	+	+
UR 3	+	-	+	+	+	+	+	-	-	+	+	+
UR 4	+	-	+	+	+	+	+	-	-	+	+	+
UR 4	+	-	+	+	+	+	+	-	-	+	+	+
UR 8	+	-	+	+	+	+	+	-	-	+	+	+
UR 10	+	-	+	+	+	+	+	-	-	+	+	+
UR 12	+	-	+	+	+	+	+	-	-	+	+	+
UR 13	+	-	+	+	+	+	+	-	-	+	+	+
UR 15	+	-	+	+	+	+	+	-	-	+	+	+
UR 18	+	-	+	+	+	+	+	-	-	+	+	+
UR 22	+	-	+	+	+	+	+	-	-	+	+	+
UR 23	+	-	+	+	+	+	+	-	-	+	+	+
BL6	+	-	+	+	+	+	+	-	-	+	+	+
BL7	+	-	+	+	+	+	+	-	-	+	+	+
BL10	+	-	+	+	+	+	+	-	-	+	+	+
ST1	+	-	+	+	+	+	+	-	-	+	+	+
ST2	+	-	+	+	+	+	+	-	-	+	+	+

analysis. DNA was extracted from UR4 strain according to protocol of Liu *et al.* (2002). PCR mediated amplification of the 16 S r RNA gene and its purification were done using PrepMan Ultra (Applied Biosystem), Microseq PCR and Miroseq Cycle Sequencing Applied Biosystem. The 16 S rRNA gene was amplified with primer mixture viz., 5'AGAGTTT GATCMTGGCTCAG '3 forward primer and 3' TACAG CATTGTTCCATTGGCAT '5 reverse primer.

The PCR mixture consisted of 30 picomoles of each primer, 10 ng of chromosomal DNA, 200 μM dNTPs and 2.5 Units of Taq polymerase in 50 μL of polymerase buffer. The PCR was carried out for 30 cycles in 94°C for 1 min., 55°C for 1 min and 72°C for 2 min. After completion, a fraction of the PCR mixture was examined using agarose gel electrophoresis (Ausubel *et al*., 1999) and the remnant was purified using QIA quick PCR purification reagents (Qiagen). DNA sequences were obtained using a 3130 X DNA sequencer (Genetic Analyzer, Applied Biosystems, Hitachi, Japan), BigDye Terminator Cycle Sequencing (see details below). The PCR product was sequenced using the same PCR primers. Blast program was used to assess the DNA similarities and multiple sequence alignment and molecular phylogeny were performed using Bio Edit software (Hall, 1999).

DNA sequencing: Automated DNA sequencing based on enzymatic chain terminator technique, developed by Sanger *et al.* (1977) was done using 3130 X DNA Sequencer (Genetic Analyzer, Applied Biosystems, Hitachi, Japan). The sequencing reaction was performed with four different fluorescent labels identifying the ddNTPs, instead of the radioactive labels. These flurophores were excited with two argon lasers at 488 and 514 nm, respectively when the respective bands passed the lasers during the electrophoresis. The specific emissions were detected and the data were collected for analysis (Prober *et al*., 1987 and Freeman *et al*., 1990). The thermal cycling mixture was as follows: 8 μL of BigDye terminator mix, 6 μL of the sequencing primer (10 pmol) and 6 μL of the PCR product, then the reaction was run in

the thermal cycler. The cyclic reaction composed of 1 min at 95°C, then 49 cycles of 30 sec at 95°C, 10 sec at 52°C and 4 min at 60°C. The products were purified using special column according to the instruction of the manufacturer. The eluates were taken and add high dye formamide with (1:1)/volume ratio, run at 95°C for 5 min for denaturation, shock on ice; then the sample become ready for sequencing in 3130 X DNA sequencer and analysis. The sequence of 16 S gene was sent to glue bank under accession number JQ388912.

Selection of *E. coli* UR4 mutants: Elimination of the antibiotic resistance ability was carried out according to Enan *et al.* (1996). A series of test tubes, each containing 9 mL nutrient broth with or without 20 mg mL^{-1} of each of the curing agents or antibiotics listed in Table 3, were inoculated with log phase cells of the *E. coli* UR4 wild strain to give 2×10^7 CFU mL^{-1}. Tubes supplemented with curing agents; tubes without curing agents were incubated at 30 and 42°C, respectively for 48 h. Tubes containing visible bacterial growth were transferred another time in another tubes like the first rounds of the experiment. After successive transfer, bacterial suspensions were plated onto nutrient agar plates and were counted. Number of colonies sensitive to the cold solution I [50 mM glucose, 25 mM Tris buffer (pH antibiotics listed in Table 4 were counted after their antibiotic bioassay onto separate nutrient agar plates (Enan *et al.*, 1996).

Plasmid isolation and agarose gel electrophoresis: Plasmids were extracted from UR4 wild strain and its mutants according to Sambrook *et al.* (1989):

- Multiple colonies were collected using a sterile loop and added to individual tubes containing 2 mL of nutrient broth, incubated overnight at 37°C
- One and half mililitter of the culture was poured into microcentrifuge tube and centrifuged at 12,000 rpm for 30 sec at 4°C
- The supernatant was removed, leaving the bacterial pellet as dry as possible
- The bacterial pellet was resuspnded in 100 μL of ice 8.0), 10 mM EDTA (pH 8.0)]. Vigorous vortexing was done to ensure that the pellet was completely dispersed in solution 1
- Two hundred microlitter of freshly prepared solution II [0.2 NaOH in 1% SDS) were added and the tube was closed tightly, inverted rapidly five times and stored on ice for 5 min. At this stage a viscous bacterial lysate was formed

Table 3: Effect of curing agents on the antibiotic resistance ability of *E. coli* UR4

Mutagen	Total colonies tested	No. of antibiotic resistant colonies	No. of antibiotic sensitive colonies	Mutation (%)
SDS	326	324	2	0.61
Elevated temperature	326	322	4	1.24
Sodium azide	405	402	3	0.74
Ethidium bromide	116	101	15	14.85
Penicillin	220	220	0	0
Ampicillin	200	200	0	0
Novobiocine	200	200	0	0
Tetracycline	250	250	0	0
Kanamycin	320	320	0	0
Vancomycin	310	310	0	0
Chloramphenicol	280	280	0	0

Table 4: Biochemical characteristics of the wild *E. coli* UR4 strain and its mutants

	Biochemical characteristics									
Mutant	Catalase	-Indole	M R	Oxidase	VP	Citrate utilization	H_2S production	Urease	Motility	Lysine decarboxyl
Wild strain	+	+	+	-	-	-	-	-	+	-
SDS	+	+	+	-	-	-	-	-	+	-
ET	+	+	+	-	-	-	-	-	+	-
SAZ	+	+	+	-	-	-	-	-	+	-
ETH	+	+	+	-	-	-	-	-	+	-

	Biochemical characteristics									
Mutant	Gelatin liquefaction test	Glucose	Lactose	Maltose	Mannitol	Sucrose	Salicin	L-arabinose	D-Sorbitol	Blood hemolysis
Wild strain	+	+	+	+	+	-	-	+	+	+
SDS	+	+	+	+	+	-	-	+	+	+
ET	+	+	+	+	+	-	-	+	+	+
SAZ	+	+	+	+	+	-	-	+	+	+
ETH	+	+	+	+	+	-	-	+	+	+

- One hundred and fifty μL of ice cold solution III [3M potassium acetate in 11.5% glacial acetic acid] were added to neutralize the reaction and the tubes were closed and vortexed for 10 sec to disperse solution III through the viscous bacterial lysate then stored on ice for 3-5 min
- The tubes were centrifuged at 12,000 rpm for 5 min at 4°C then the supernatant was transferred to a fresh tube
- Two volumes of ethanol (double the volume of the supernatant) were added (to precipitate the double stranded DNA) and mixed by votexing. The mixture was allowed to stand at room temperature for 2 min
- The tubes were centrifuged at 12,000 rpm for 5 min at 4°C
- The supernatant was removed and the tubes were inverted on a paper towel to drain away all fluids
- The pellets were rinsed with 1 mL of 70% ethanol and allowed to dry in the air for 10 min
- The pellets were redissolved in 50 μL of tris EDTA (TE) buffer (10 mM Tris HCl pH 8.0 in 1 mM EDTA), vortexed briefly and stored at 20°C

Agarose gel electrophoresis was carried out according to Davis *et al.* (1986). Twenty five microliter of tracking dye were added to 100 μL of plasmid DNA preparation. The sample was loaded on 0.7% agarose gel in Tris-EDTA buffer pH 8.2 and electrophoresis was conducted at 100 volt for 1-2 h. The gel was stained is EtBr (0.5 g mL^{-1}) for 15 min., distained in 1 mM $MgSO_4$ for 30 min, photographed by gel documentation system.

RESULTS

Isolation, characterization and identification of multidrug resistant bacteria: Urine, blood and stool samples were taken from patients who did not respond to medications and were analysed microbiologically onto both MacConkey agar and HiCrome™ agar media. About 17 culture grown well were chosen and were assayed against 14 types of antibiotics listed in Table 1. All the 17 bacterial cultures tested were showed to be multidrug resistant bacterial cultures.

All the 17 bacterial cultures were rod shaped, motile, Gram negative and catalase positive cells. Many biochemical tests used for bacterial identification were studied. Results are given in Table 2. All isolates showed positive results with regard to indole test, methyl red test, liquefaction of gelation, utilization of glucose, lactose, maltose, mannitol, L. arabinose, D-sorbitol and blood hemolysis; but showed negative results with other biochemical tests listed in Table 2. Following diagnostic key of Holt *et al.* (1994) and Garrity *et al.* (2005), all the 17 bacterial isolates were identified as belonging to *E. coli*.

Since, UR4 strain was resistant to all the 14 antibiotics used, it was important to characterize it by 16 S rRNA cataloging analysis for more description of this interesting multidrug resistant bacterium. DNA was isolated from this strain and the 16 S rRNA gene was amplified by PCR technique. Amplified 16 S gene was electrophoresed using agarose gel and the clear band of 16 S gene was taken and sequenced (Fig. 1). The sequence of 16 S gene was submitted to gene bank under accession number JQ388918. It was showed 99.5% similarity to *E. coli* category.

Selection of antibiotic resistance negative clones: To produce an antibiotic resistance negative clones, the UR4 strain was subjected to 20 mg mL^{-1} of antibiotics, SDS, sodium azide and ethidium bromide and was grown at elevated temperature (42°C). The results are presented in Table 3. In population treated with antibiotic, no negative clones (sensitive colonies to antibiotic used) were observed. There was no dependence of the number of either antibiotic resistance negative clones or antibiotic resistance positive clones on the applied dose (20 mg mL^{-1}) of the antibiotic used.

However, the occurrence of an antibiotic resistance negative colonies (sensitive colonies to the antibiotic) was found in populations either treated with curing agents or grown at elevated temperature. The mutation percentage was 0.61; 1.24; 0.74 and 14.85% in the experimental antibiotic resistant cells of *E. coli* UR4 after using the curing agents viz SDS; elevated temperature sodium azide; ethidium bromide, respectively. The produced mutants of the antibiotic resistance negative colones became sensitive to many antibiotics (Fig. 2). The mutants produced by SDS; elevated temperature; sodium azide; ethidium bromide were termed SDS; ET; SAZ; ETH and were used for further experiments.

CGGGGGGAGGCATAAAGATGTCTGTCGTACGGTCACAGG
AAGCAGCTTGCTGCTTTGCTGACGAGTGCCGGACGGGTG
AGTAATGTCTGGGAACTGCCTGATGGAGGGGGATAACTA
CTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACC
AAAGAGTGGGACCTTCGGCCCTCTTGCCGTCGGAGTGCC
CAGATGGAATTAGCAAGTAGGAGGCCATTGCCTCCCCTA
GGCCACGACCCTAGCTGGTAGGACTAATGCCCAGCCACA
TGAACCTTACCCCGTCCCGACCTTACGGGAGCACAGTGC
CGACCCCGTAAATCCCCCCCAGCCTGAGGGGCCATGAAG
GGGGGGGGGAGCCCTCCTTTCGTTTTTTTACTTTCAACC
CGCAAAATCGCAAGTTGAAAAAAAGCCAATAAC

Fig. 1: Sequence of 16S rDNA gene of the wild strain *E. coli* UR4

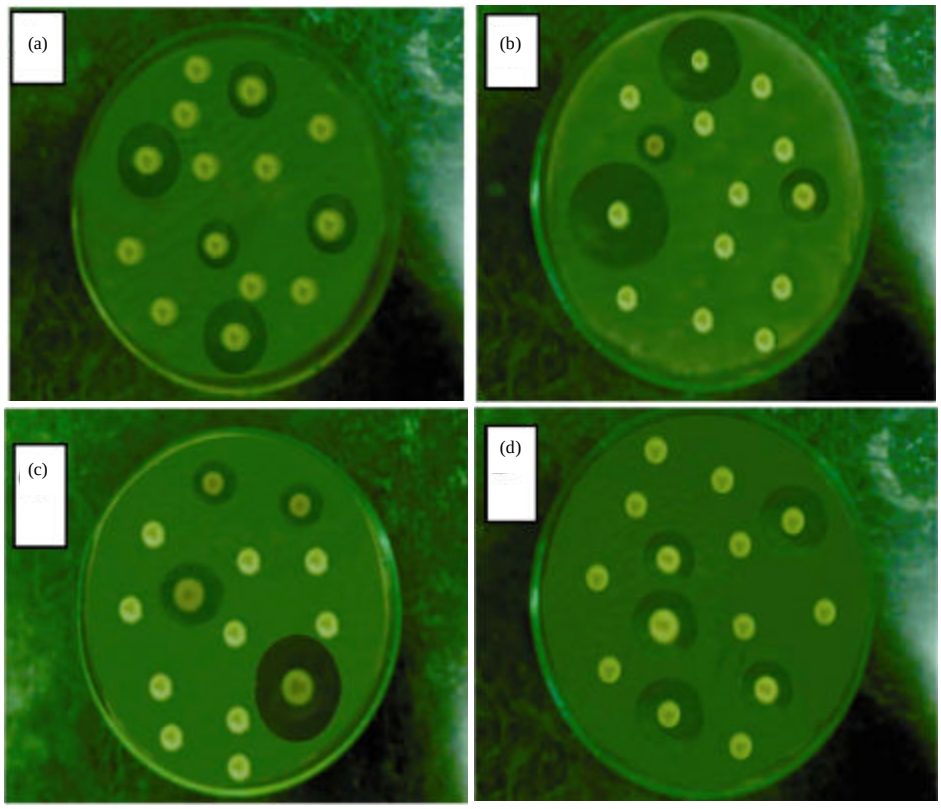

Fig. 2(a-d): Antibiotic sensitivity test of the *E. coli* UR4 mutants produced after curing with, (a) SDS, (b) Elevated temperature, (c) Sodium azide and (d) Ethidium bromide

Stability of mutation in the antibiotic resistance negative clones: SDS, ET, SAZ and ETH, were subcultured ten times in nutrient broth. After each subculture, antibiotic sensitively test was carried out. None of them was antibiotic resistant again but all colonies were sensitive to some antibiotic used (Fig. 2).

Comparison of the *E. coli* UR4 wild strain and its mutants: The antibiotic resistant wild strain *E. coli* UR4 and its mutants SDS, ET, SAZ and ETH which became sensitive to the antibiotic used were grown in nutrient broth. Growth values were compared. No differences in the rate of growth were observed.

Comparison of biochemical characteristics of the *E. coli* UR4 wild strain and its mutants: *E. coli* UR4 wild strain and its mutants SDS, ET, SAZ and ETH which became sensitive clones were subjected to many biochemical tests used for bacterial identification Table 4. Results were compared. There was a quite similar results with regard to biochemical tests listed in Table 4.

Plasmid profile: To study the genetic linkage of *E. coli* UR4, it mandatory to compare between the plasmid content of the wild strain of *E. coli* UR4 and its mutants SDS, ET, SAZ and ETH to elucidate whether antibiotic resistance ability is plasmid encoded or is linked to gene located on the chromosome. If the antibiotic resistance ability is plasmid encoded, one plasmid could be deficient in the antibiotic resistance negative variant. However, in case of the antibiotic resistance is chromosomal encoded, the plasmid profile of the wild strain and each mutant should be similar to each other.

Since, the *E. coli* UR4 wild strain and its mutants SDS, ET SAZ and ETH which became sensitive to antibiotics used had a quite similar physiological properties, plasmids were isolated of the wild strain of *E. coli* UR4 and its mutants SDS, ET, SAZ and ETH. A preparations of Lumda DNA (Omega) were used as standards. Plasmids and standard DNA were electrophoresed using agrose gel.; plasmid profile is given in (Fig. 3). The plasmid profile of the UR4 wild strain is shown in lane 1 and appears to contain five plasmids

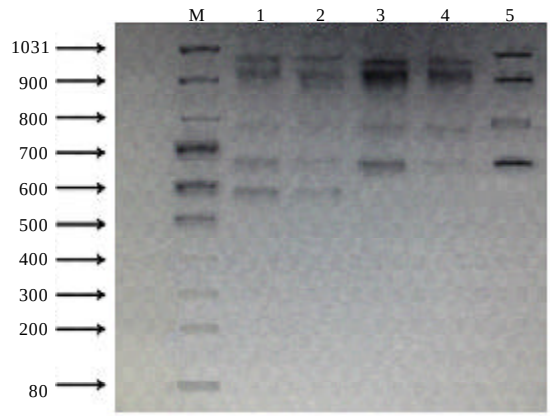

Fig. 3: Agrose gel electrophoresis of plasmids extracted from *E. coli* UR4, Lane 1: Wild strain, Lane 2: Mutant strains ET, Lane 3: SDS, Lane 4: Ethidium bromide, Lane 5: SAZ

with a molecular mass range from 80-1031 bp. The SDS, ET, SAZ and ETH mutants appeared to contain only four plasmids. One plasmid from each mutant of a molecular mass of about 600 bp was lost or dissociated in lane 2, 3, 4 and 5. This clearly indicated that this plasmid is the responsible one on the antibiotic resistance ability in the experimental *E. coli* UR4 strain and this antibiotic resistance ability appeared herein as plasmid encoded.

DISCUSSION

E. coli is a part of the normal flora of the human colon but can be pathogenic both within and outside of the gastrointestinal tract. The difference of the degree of virulence of different *E. coli* strains are caused by individual plasmid associated with each strain. Many strains of *E. coli* are pathogenic such as enteropathogenic; enterotoxigenic; enterohemorrhagic; enteroinvasive; enteroadherant which cause watery diarrhea; blood diarrhea; colitis; persistant watery diarrhea in children, respectively (Harvey and Champe, 2012. This clearly showed that there is a need to continue research on *E. coli* to control its growth for healthy reasons. Unfortunately, the existence of the antibiotic resistance ability in *E. coli* was previously shown by many authors (Whittam *et al*., 1993; Todar, 2007). Resistance of *E. coli* to antibiotic was in many reports due to genetic reasons and this clearly showed that there is a need to study this phenomena to able be find out new control strategies on the antibiotic resistant strains of *E. coli.*

In this study, 17 bacterial cultures were isolated on specific Hichrome medium from Egyptian patient suffering from urinogential infection symptoms even after antibiotic treatment. It was of interest to study such bacterial cultures. The 17 bacterial cultures appeared to be multidrug resistant bacteria. This supported latter work in this respect(Ehinmidu, 2003). Since this phenomena is very necessary clinically, isolates were identified as *E. coli* UR4 (Holt *et al*., 1994; Garrity *et al*., 2005). For further characterization of the most antibiotic resistant bacterium (UR4 strain), the molecular identification by 16 S r RNA gene sequence analysis was made and UR4 strain appeared to be similar to *E. coli* sequence in gene bank (Pot and Janssen, 1993). Later on, it was of interest to study the genetic determinants of the antibiotic resistance ability in the UR4. This to concur with previous study in this respect (Enan and Saad, 2000).

Growing of the experimental UR4 and treatment of its cells with some curing agents led to mutants lost the antibiotic resistance ability and similar work was published by Lichstein and Soule (1994). The mutation was stable character and the SDS, ET, SAZ and ETH mutants continued sensitive to most antibiotic used and no recovery was observed in any of the above mutants. This support latter findings in this respect. The wild *E. coli* strain contained five plasmids and this clearly indicated the genetic structure of this strain which expresses on high virulence and high ability of this strain to resistant all antibiotics used. This is in agreement with Schafer *et al.* (1999). The SDS, ET, SAZ and ETH mutants lacked one plasmid of molecular region of about 600 bp and this clearly indicated the role of this plasmid in the antibiotic resistance ability of *E. coli* UR4. Hence the antibiotic resistance ability in this strain was plasmid encoded.

Further study will be necessary to digest the plasmid containing the antibiotic resistance gene by restriction enzymes to understand its genetic map and to its modifications.

CONCLUSION

The 17 antibiotic bacterial isolates were isolated, characterized by phenotypic and biochemical criteria. They were identified as belonging to *E .coli.* One only of them was resistant to 14 antibiotics used; was further characterized by 16Sr RNA sequence analysis. It was identified and designated *E .coli* UR4. Mutants of such strain were obtained by some curing agents; and the plasmids of UR4 wild strain and its mutants were obtained. One plasmid of about 600 bp was showed to be the committed genetic factor for the antibiotic resistance ability in the *E .coli* UR4 strain.

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