CURING THE DRUG RESISTANCE PLASMID IN *E. COLI* O157:H7

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Abstract. In this study, twenty five isolates of *E. coli* O157:H7 were identified among 200 samples taken from children under ten years old suffer from diarrhea. Isolates were identified from stool sample by using cultural, morphological, biochemical characteristics and serological test. The plasmid profile has been conducted by using gel electrophoresis. To control the antibiotic resistance of the tested *E. coli* O157:H7 isolates, curing of plasmid DNA was conducted using Ethidium bromide (EB) and elevated temperature (ET) at 46 °C. One of the most resistance isolate was chosen for this purpose E15 then treated with Ethidium bromide at 125 µg/ml. The results revealed that the genes encoded resistance to AK (Amikacin), CIP (Ciprofloxacin), CN (Gentamicin) and TMP + SXT (Trimethoprim + Sulphamethoxazole) (were cured from E15 and the percentage of curing was 28.57%. The results confirmed by conducting gel electrophoresis and showed that EB removed three plasmid of E15, while two plasmids remained. On the other hand, elevated temperature used also as curing agent for the same isolate and the results revealed that resistance to AK, CIP, CN and TMP + SXT genes were cured from E15 and the curing percent was 35.71% after incubating the isolate at 46 °C. The results confirmed by conducting gel electrophoresis and showed that tested isolate lost three plasmids after incubation at 46 °C.
Keywords: *E. coli O157:H7, antibiotic resistance, plasmid profile, plasmid curing*

Introduction

The human large intestine ordinarily harbors a huge microbial population, most bacterial, protozoan, and viral agent of diarrhea are not members of this normal gut flora but are acquired through contaminated food or water (Talaro and Talaro, 2002). *Escherichia coli* O157:H7 is an emerging public health concern in most countries of the world (Schlundt, 2001). It is an important cause of foodborne human disease. Complications related to infection include diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Nataro and Kaper, 1998). The constant increase in the antibiotic resistance of clinical bacterial strains has become an important clinical problem (Adamus-Bialek et al., 2013). This is a major problem in the world now "antibiotics resistance", so the medicine manufactures which yearly produced new generations of antibiotic to solute this problem, specially there are some bacteria considered a multiple antibiotic resistance as *Escherichia coli*, *Klebsiella* and others (Al-Faisal, 1999). Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera (Hamad, 2009). Antimicrobial resistance (AMR) is a global problem hindering treatment of bacterial infections, rendering many aspects of modern medicine less effective. AMR genes (ARGs) are frequently located on plasmids, which are self-replicating elements of DNA. They are often transmissible between bacteria, and some have spread globally. Novel strategies to combat AMR are needed, and plasmid curing and anti-plasmid approaches could reduce ARG prevalence, and sensitise bacteria to antibiotics (Michelle et al., 2018).

Antibiotic resistance increases the likelihood of death from infection by common pathogens such as Escherichia coli and Klebsiella pneumoniae in developed and developing countries alike. Most important modern antibiotic resistance genes spread

between such species on self-transmissible (conjugative) plasmids. These plasmids are traditionally grouped on the basis of replicon incompatibility (Inc), which prevents coexistence of related plasmids in the same cell. These plasmids also use post-segregational killing ('addiction') systems, which poison any bacterial cells that lose the addictive plasmid, to guarantee their own survival (Kamruzzaman et al., 2017).

Between 2000 and 2010, global human use of antibiotics increased by 36%, and the use of two last-resort antibiotics, carbapenems and polymyxins, increased by 45% and 13%, respectively (Van Boeckel et al., 2014). Antimicrobials have many non-human uses including in animals for growth promotion, veterinary treatment and aquaculture (Vyas and Piddock, 2015; Van Boeckel et al., 2015). In 2013, an estimated 131 109 tons of antimicrobials were used globally in food animals; by 2030 this is expected to increase to 200 235 tons (Van Boeckel et al., 2017). However, there is a growing trend to improve antimicrobial stewardship in many countries. For example, in Switzerland veterinary antimicrobial sales increased between 2006 and 2008, but then steadily decreased, reaching a 26.2% reduction in 2013 (Carmo et al.2017). In addition to human and animal use, many cleaning and personal hygiene products contain biocides, such as triclosan, which can select for mutants resistant to biocides, and in some cases to antibiotics used in medicine (Vyas and Piddock, 2015; Webber et al., 2015, 2017).

Plasmid curing is the process by which plasmids are removed from bacterial populations. This is an attractive strategy to combat AMR (antimicrobial-resistant) as it has the potential to remove ARGs (AMR genes) from a population while leaving the bacterial community intact. This means, for example, that the structure of the gastrointestinal microbiome of a chicken treated with a plasmid curing agent might remain largely unchanged, but potentially pathogenic bacteria which may unfortunately be transmitted into the food chain would be susceptible to antibiotics. Alternatively, a plasmid curing agent could be given to a patient prior to surgery, to reduce the likelihood of a resistant hospital acquired infection. Plasmid curing agents could also be taken by international travellers to reduce the global spread of AMR. Unfortunately, at the moment no such treatment options are in use. In fact, there are very few curing mechanisms that have been tested in vivo, even in experimental models. Therefore, research in this area is urgently needed. Recently, it was shown that 24% of non-antibacterial drugs impact growth of members of the human microbiome (Maier et al., 2018). Studies such as this would be important for determining any impact of anti-plasmid compounds on the microbiome.

The risk of *E. coli* O157:H7 occurs in its plasmid, because of its ability to transfer plasmids to other bacteria or other strains of *E. coli* by transformation, conjugation and transduction processes (Jawetz et al., 2004). Therefore, controlling and elimination of the resistance that conferred by R -plasmid by using biological mutagen, physical and chemical or other curing agents will be quite useful to eliminate such resistance (Jawetz et al., 2007). For this reasons this study concerned with the isolation and identification of *E. coli* O157:H7 from children suffering from diarrhea, study the antimicrobial resistance patterns of the isolated *E. coli* O157:H7 to different antibiotics, characterization of the plasmid DNA profile pattern of the isolates using gel electrophoresis technique, and elimination of the bacterial resistance to antibiotic for some isolates by curing using ethidium bromide and elevated temperature.

Materials and methods

Specimen collection

The samples were collected during the period 20st May 2013 to 1th November 2013. A total of two hundred (200) stool specimens were collected from infants and children (68 males and 132 females), aged one day- 10 years attending Rapareen Teaching Hospital for Children in Erbil City (Iraq) and the relevant information were recorded from each patient including age and sex. All stool samples were collected in clean disposable plastic containers from diarrheal patient. The specimens were transferred to the laboratory and processed within half an hour of collection.

Bacterial identification

Bacterial isolates were identified by performing morphological, cultural, biochemical, and serological test.

Antibiotic susceptibility test

To study the effect of different antibiotics on the isolates of *E. coli* 0157:H7, Mueller-Hinton agar was used as growth media (Wayne, 2005). Antibiotic resistance patterns of the isolates were determined using the Disc diffusion (Kirby Bauer) method; bacterium inoculate was adjusted to 0.5 McFarland standard of Clinical and Laboratory standards institute (Clinical and Laboratory Standard Institute, 2007). The test inoculums were spread onto Muller-Hinton agar using a sterile cotton swab. The tested antimicrobial agents were aseptically placed on the inoculated Muller Hinton agar and incubated overnight. The zones of inhibition were measured and interpreted according to (Clinical and Laboratory Standard Institute, 2007).

Plasmid DNA extraction (Gene Aid Company) laboratory protocol

Plasmid DNA was extracted and purified from 5 ml overnight culture of the selected isolates of the *P. mirabilis* grown in LB broth medium containing 100 μg/ml Ampicillin using a plasmid DNA purification kit, according to the manufacturer's instructions. A single bacterial colony was used to inoculate 5 ml LB, which was incubated overnight in a shaker incubator at 37 °C. A volume of 3 or 5 ml of each isolate in an eppendorf tube was centrifuged for 30 s at 13,000 rpm and the supernatant discarded. The bacterial pellet resuspended by vortexing in 250 μl of resuspension buffer (RNase A solution was added), until no clumps of the cell pellet remain. Two-hundred and fifty μl of lysis buffer added to resuspended cells and the tube closed and gently mixed by inverting the tube several times without vortexing. Three-hundred fifty μl of neutralization buffer was added and gently mixed by inverting the tube several times. Resuspended cells centrifuged at 13,000 rpm for 10 min at 4 °C, then a column inserted into collection tube. After centrifugation, supernatant transferred promptly into the column, and centrifuged at 13,000 rpm for 60 s. The column removed from the collection tube, the filtrate in collection tube discarded. The spin column placed back in the same collection tube. Five hundred μl of washing buffer A added and centrifuged at 13,000 rpm for 60 s. The column removed from collection tube, the filtrate discarded in collection tube, and then placed the spin column back in the same collection tube. Seven-hundred μl of washing buffer B was added, centrifuged at 13,000 rpm for 60 s. The filtrate discarded in collection tube and column placed back in the same collection tube, Centrifuged at

13,000 rpm for 60 s to dry the filter membrane. The column put in a clean and sterile eppendorf. Fifty µl of elution buffer added to the upper reservoir of the column, and let stand for 1 min. Then, the tube centrifuged at 13,000 rpm for 60 s. The purified DNA plasmid used immediately in downstream applications or store at -20 °C (Manufacturer protocol).

Plasmid profile

The extracted plasmids were electrophoresed in 0.7% agarose gel with Tris-borate ethylene diamine tetra-acetic acid (TBE), Agarose gel electrophoresis was used to separate DNA fragments according to size, 0.7% (w/v) agarose gel was made by adding 0.7 gm of agarose to 100 ml of 1X TBE buffer solubilized by heating at boiling temperature in microwave oven for 2 min, then the agarose was left to cool down at 55 °C before pouring in a tray to solidify. A comb was placed near one edge of gel, and gel was left to harden. After gel solidification, the comb was removed gently and the gel was soaked in a gel tank contain 1X TBE buffer and the placement of the gel in the tank should be in a way that the wells located on the negative (cathode) pole. The amount of TBE buffer had to be sufficient to cover about 2-3 mm of the gel, 1 kb DNA ladder (Fermentas) was used as molecular size marker of DNA fragment, 3µl of loading buffer was mixed with 10 µl plasmid DNA extract, and then samples were added carefully to individual wells. After loading the samples into wells, the power supply was set on 45 V for 15 min. Then the voltage changed to 75 V and the gel was run for 90 min. until the bromophenol blue dye migrated to the other end of the gel. The gel was immersed in ethidium bromide (0.5 µg/ml of D.W.) for 30-45 min. The gel was visualized by UV-trans illuminator and then photographed (Sambrook and Russella, 2001).

Ethidium bromide as a curing agent

Curing by ethidium bromide was done by the procedure mentioned by (Tomoeda et al., 1974). Five ml of LB broth containing appropriate antibiotic at final concentration inoculated with single colony of *E. coli* O157:H7 isolate, incubated at 37 °C for 24 h, after overnight 0.1 ml of the broth bacterial culture were inoculated into 5 ml LB broth containing 125 µg/ml of ethidium bromide agents and incubated with shaking at 37 °C for 48 h. Serial dilutions were prepared up to 10^{-6}, 0.1 ml from the last three dilutions were spread onto nutrient agar plates and incubated at 37 °C for 24 h. After incubation time, fifty colonies were transferred to nutrient agar plate and incubated over night at 37 °C representing the master plate. Five colonies were randomly chosen, picked up and transferred to Muller Hinton agar plates and tested for antibiotic sensitivity pattern, incubated at 37 °C for 24 h. After overnight incubation the percentages of curing colonies for antibiotic sensitivity pattern were calculated.

Curing of plasmid DNA by elevated temperature

This curing was carried out according to (Baldwin and Strickland, 1969). Ten ml of LB broth inoculated with single colony of *E. coli* isolate, after inoculation for 24 h at 37 °C, 10 ml LB broth culture inoculated with 0.2 ml of bacterial culture, incubated with shaking (100 rpm at 46 °C for 24 h), serial dilutions were prepared up to 10^{-7}, then 0.1 ml from last three dilutions spread on nutrient agar plates and incubated at 37 °C for 24 h. After incubation time, fifty colonies were transferred to nutrient agar plate and incubated over night at 37 °C representing the master plate. Five colonies were

randomly chosen, picked up and transferred to Muller Hinton agar plates and tested for antibiotic sensitivity pattern incubated at 37 °C for 24 h. After overnight incubation the percentages of curing colonies for antibiotic sensitivity pattern were calculated.

Antibiotic resistance pattern after plasmid curing

The colonies were screened for antibiotic resistance by the disk diffusion method after curing. Cured markers were determined by comparison between the pre- and post-curing resistance pattern of isolates. Loss of resistance markers gave an indication that those markers were probably located on plasmid and not on the chromosome.

Results and discussion

Identification of E. coli O157 isolates

They produce bright metallic green sheen colonies on Eosin Methylene Blue (EMB) agar and on Sorbitol MacConkey agar colonies of *E. coli* O157:H7 appear as colorless colony due to non sorbitol fermenting which consider as a selective media (Wistreich and Lechtman, 1980). Bacterial cells from smear preparation are gram negative short rods, motile, non-spore forming and presumptively are *E. coli* O157:H7 which in accordance with previous observations (Sharma et al., 2007). The biochemical tests for all bacterial isolates were negative for citrate, oxidase and urease production test, but they were positive for catalase and indole test. On Kligler Iron Agar (KIA) medium, all isolates of *E. coli* understudy produce a yellow slant and a yellow butt A/A reaction due to the fermentation of lactose and glucose and negative for H_2S production (Wistreich and Lechtman, 1980).

Serological test

Samples serotyped by application of solid agglutination test, using anti *Escherichia coli* O157:H7. Once the agglutination was detected, the reaction was considered positive according to the materials used. Three drops of physiological normal saline were added on a clean slide, and then with the aid of the loop, a small piece of fresh bacterial growth was mixed with the 3 drops in order to obtain a homogenous mixture. After that a drop of polyvalent antisera was added to the two drops (mixture) while the third drop was considered as control test. The appearance of clear agglutination within 1 min indicates a positive result.

Antibiotic sensitivity test

Antibiotic sensitivity testing was done for all isolates by using fourteen antibiotic types which include amikacin (AK), ampicillin (AMP), chloramphenicol (C), cephalothin (KF), ciprofloxacin (CIP), cefepime (FEP), ceftriaxone (CRO), cefotaxime (CTX), gentamicin (CN), imipenem (IPM), metronidazole (MET), tetracycline (TE), trimethoprim (TMP) and trimethoprim + sulphamethoxazole (SXT). Antibiotic discs of Amikacin (30 µg), Imipenem (10 µg), Ampicilin (25 µg), Gentamicin (30 µg), Chloramphenicol (30 µg), Cephalothin (30 µg), Ciprofloxacin (10 µg), Cefepime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Metronidazole (5 µg), Tetracycline (30 µg), Trimethoprim (5 µg) and Sulphamethoxazole (25 µg) were applied on the plates. The resistance rate of the isolates toward these antibiotics were 100% for AMP,

96% for MET, 76% for CTX, 72% for TE and KF, 68% for TMP, 56% for FEP, 52% for C and SXT, 48% for CRO, 28% for AK, 20% for CIP, while the lowest percent 16% was for CN, and all isolates were sensitive for IPM (*Table 1*).

Table 1. Percentages of resistance of bacterial isolates to different antibiotics

Antibiotics	Abbreviation	No. of Isolates	Resistant percentage of resistance %
Amikacin	AK	7	28
Ampicillin	AMP	25	100
Chloramphenicol	C	13	52
Cephalothin	KF	18	72
Ciprofloxacin	CIP	5	20
Cefepime	FEP	14	56
Cefotaxime	CTX	19	76
Ceftriaxone	CRO	12	48
Gentamicin	CN	4	16
Imipenem	**IMP**	**0**	**0**
Metronidazole	MET	24	96
Tetracycline	TE	18	72
Trimethoprim	TMP	17	68
Sulphamethoxazole	SXT	13	52

IPM was the most effective antibiotic against isolates of *E. coli O157* and 100% of these isolates were susceptible to IPM, this result was agree with Hadi (2008), Al-Hilali (2010), and Shamki et al. (2012). They reported that all *E. coli* isolated from different clinical source were sensitive for IPM. The high efficiency of these antibiotics may be the usage rarely in studied area and it is expensive drug. In the present work, 68% of the isolates were resistant to TMP, this result was in agreement with Nanakaly (2013) who stated that 52% of *E. coli* isolates were resistant for TMP, also Salah (2007) and Al-Hilali (2010) found that 59.03% and 72.2% of *E. coli* isolates were resistant to TMP. This may be result from mutational changes that lead to over production of the bacterial dihydrofolatereductase (Salah, 2007). Regarding to C resistance, 52% of isolates was resistant to it. Taye et al. (2013) mentioned that 100% of the *E. coli* O157:H7 isolates were resistant to C and Salah (2007) who found that 97.59% of isolates were resistant to C, while in a study conducted by Shamki et al. (2012) and Momtaz et al. (2012), it was found that 32.14% and 29.8% *E. coli* isolates were resistant to C. In the current study, it has been shown that the resistance raised to the third generation of cephalosporin 3GC such as CTX, and the results were 76% for CTX. These results were agreed with Al-Hilali (2010). Shamki et al. (2012) and Nanakaly (2013) they mentioned that (68.2, 96.4% and 87%) respectively of the isolates resistant for the CTX. High resistance pattern for AMP reported with a percentage of 100%, similar result obtained by Zinnah et al. (2008), Shamki et al. (2012) and Taye et al. (2013) whom found the resistance to AMP as 90%-100%, and 100% while Albert et al. (2009) reported 45.9%. This was explained by the overuse of antibiotics especially AMP in Erbil Hospitals, and also to the misuses of these antibiotic as they are prescribed without sensitivity test (Decre et al., 2002). On detecting the resistance of *E. coli* O157:H7 for KF, it was found that 72%

of the isolates was resistant to it, Shamki et al. (2012) and Dehkordi et al. (2014) reported that 100% and 48% respectively, of *E. coli* isolates showed resistance for KF, while Bekele et al. (2014) showed that the resistance of the isolates for KF was 12.8%. In our work, 56% of the isolates were resistant to FEP, this result agree with Kalantar et al. (2010) who found that 40.5% of the isolates resistance for FEP while Al-Hilali et al. (2010) and Shamki et al. (2012) mentioned that 77.3%, 92.85% of the isolates resistance for FEP. The resistance to CIP was 20%, this result in agreement with (Shamki et al., 2012; Bekele et al., 2014) recorded 25% and 17.9% respectively but the result that mentioned by Zinnah (2008) and Al-Hilali (2010) disagree who reported that all isolates were sensitive to CIP. This is due to that the ciprofloxacin is newly used in treatment in comparison with other antibiotics. Moreorever, Rice et al. (1992) showed that increasing uses of antibiotics was associated with development of resistance against them. The clinical use of fluoroquinolone in children should be restricted because of potential cartilage damage that occurred in research with immature animals, the safety and efficacy of oral ciprofloxacin in children is understudy (Elder, 2004). Jalal et al. (2010) showed that the resistance in bacterial population can be spread either by transfer of bacteria between people or transfer of resistance genes between bacteria (usually on plasmids) and by transfer of resistance genes between genetic elements within bacteria, on transposons or may chromosomally located. Susceptibility to antibiotics is changing in general and increase in antibiotic resistance has been shown worldwide. The main reason for this trend is the increase in antibiotic consumption, the abuse of board spectrum antibiotics or self medication (Younis et al., 2009).

Plasmid profile of E. coli O157:H7

Extracted plasmids analyzed by gel electrophoresis on 0.7% agarose gel as shown in *Figure 1*. The results revealed that most isolates have one band with molecular weight more than 10 kb while (E11, E13 and E21) have two bands with molecular weight more than 10 kbp, E16, E20 and E24 have three bands but E15 which is resistant for 13 antibiotics have 5 bands with molecular weight ranging between (2 kbp- more than 10 kbp) bp in size.

Analysis of plasmid DNA content by agarose gel electrophoresis showed variation in their size and more than one DNA bands appeared in many isolates. These results revealed that among 25 *E. coli* O157:H7 isolates, the size of the bands ranged from 2 Kbp to more than 10 Kbp. The presence of more than one DNA band in the agarose gel is a good indication for existence of more than one plasmid DNA species. The variation in the molecular weights of plasmids might be a result of these plasmids carrying different gene cassettes for resistance against different classes of antibiotics (Khan et al., 2007). The reported results indicate the dissemination of plasmids among *E. coli* O157:H7 isolates which may be carrying resistant genes against wide spectrum of clinically used antibiotics, which may explain the reason of evolution antibiotic resistant patterns in studied bacterial cultures. The study of Ehywarieme (2011) showed that some *E. coli* O157:H7 isolates had plasmid bands with sizes > 1.5 kbp. Aibinu et al. (2007) mentioned that all isolates of *E. coli* O157:H7 had harbored plasmids ranging in size between 21,563 and 27,444 kb. In a research conducted by Malkawi (1998) on *E. coli,* he demonstrated that the plasmid sizes were from (1.5–54) kbp. Tsen (1996) reported that a range of (2 – 22) kbp for plasmid sizes among *E. coli* isolates. Danbara et al. (1987) have also reported plasmid size variations between 3.9 kbp and 50 kbp in *E. coli* strains. As ampicillin showed the most resistance, therefore we have suggested

that the gene coding for ampicillin resistance could be located on the plasmid. Possibly, some antibiotic resistance genes may not be located on the plasmid but may be on the bacterial chromosome or on transposable elements (Transposon) (Farshad et al., 2012). This indicates that plasmids allow the movement of genetic materials, including antimicrobial resistance genes between bacterial species and strains.

Figure 1. Plasmid profile of E. coli O157:H7 isolates. (Lane 1: 10 000 bp DNA ladder, Lane 2: Plasmid content of E15, Lane 3: Plasmid content of E16, Lane 4: Plasmid content of E17, Lane 5: Plasmid content of E18, Lane 6: Plasmid content of E19, Lane 7: Plasmid content of E20, Lane 8: Plasmid content of E21)

Curing of plasmid in E. coli O157:H7 isolate E15 by ethidium bromide

Table 2 demonstrates the curing percent of plasmid DNA from *E. coli* O157:H7 isolates by ethidium bromide. For E15 isolate, ethidium bromide affect AK, CIP, CN and SXT genes with (28.57%). *Figure 2* shows the plasmid DNA profile for *E. coli* O157:H7 isolate (E15) and it is clear that two plasmid DNA were remain in the E15 isolate after treating with ethidium bromide.

Table 2. Curing of plasmid DNA from E. coli O157:H7 (E15) isolate by using ethidium bromide (EtBr)

Treatment	Antibiotic resistance pattern														
	AK*	AMP	C	KF	CIP	FEP	CTX	CRO	CN	IPM	MET	TE	TMP	SXT	
E15	R	R	R	R	R	R	R	R	R	S	R	R	R	R	28.57%
EtBr + E15	S	R	R	R	S	R	R	R	S	S	R	R	R	S	

Among various plasmids present in bacterial strains, R plasmids are very significant as they confer resistance to one or more antibiotics, thus possess a threat to chemotherapy. Treatment of cells with certain chemical and physical agents enhances the elimination of plasmids from host cells. This phenomenon is referred to as curing and has been used to ascertain the plasmid associated nature of genes. Susceptibility to curing agent also varies among plasmids (Madigan et al., 2000). Hamad (2009) study the effect of plasmid curing on the drug resistance determinants of MDR *E. coli*, the results revealed that some of the resistance markers (chloramphenicol and nalidixic

acid) were stably lost in all isolates, thereby confirming their location on plasmid that showed missing of 15 Kbp plasmid band after treating MDR *E. coli* with ethidium bromide, the results also showed losing of all resistant markers for (ampicillin, ceftazidime, cefotaxime, chloramphenicol, nalidixic acid and tetracycline) in some strains of MDR *E. coli,* no plasmid band obtained when these strain subjected to electrophoresis**.**

Ehywarieme (2011) study the impact of plasmid curing on the drug resistance determinant of *E. coli* O157:H7 by using acridine orange*,* the results showed that some of antibiotic resistance marker (Ciprofloxacin and Nalidix acid) were equally observed to be lost in all isolates except E4. The effect of ethidium bromide as curing agent might be due to their action as intercalating agent. This mutagen became inserted between two abnormal conformation can lead to insertion or deletion, thus can induce frameshift mutation (Nariman et al., 1998). On the other hand, the unsuccess of ethidium bromide in curing process for certain antibiotic genes may be attributed to their failure in inhibition of plasmid replication that carry resistance to these antibiotics due to incapability to create mutation in the original replication that specific for this plasmid, or because of their disability to prevent plasmid distribution mechanism on daughter cell as a result remaining of these plasmids in these isolates.

Curing of plasmid DNA by elevated temperature

Elevated temperature at 46 °C was used to cure the plasmid DNA that confers resistance to antibiotics in *E. coli* O157:H7 (E15) isolate and the main results are represented in *Table 3*. *Table 3* indicates that the E15 when treated with elevated temperature at 46 °C AK, CN, CIP, CRO and SXT genes were affected and the curing percent was (35.71%). *Figure 2* shows that E15 tested isolate missing three bands of plasmid DNA after incubation at 46 °C.

Table 3. Curing of plasmid DNA from E. coli O157:H7 (E15) isolates by using elevated temperature

Treatment	Antibiotic resistance pattern														
	AK*	AMP	C	KF	CIP	FEP	CTX	CRO	CN	IPM	MET	TE	TMP	SXT	
E15	R	R	R	R	R	R	R	R	R	S	R	R	R	R	35.71 %
Elevated temperature 46 °C + E15	S	R	R	R	S	R	R	S	S	S	R	R	R	S	

Nariman et al. (1998) studied the effect of elevated temperature on eliminating plasmid from *E. coli* isolates and the results indicated that CEP, CTX and CRO determinant were plasmid encoding and they concluded that this may due to the existence of two separate R-plasmids or one plasmid is carrying these antibiotic resistance genes. Nanakaly (2013) performed curing of plasmid DNA by elevated temperature (46 °C) for uropathogenic *E. coli*, she mentioned that E3 when treated with elevated temperature at 46 °C, the ciprofloxacin, ceftriaxone, nitrofurantoin, chloramphenicol, Cefotaxime sodium, gentamycin, sulfamethoxazole/trimethoprim and amikacin genes were affected and the curing percent was (66.6%), while when E25 isolate treated with elevated temperature at 46 °C, genes which responsible for ciprofloxacin, ceftriaxone, nitrofurantoin, cefotaxime, gentamycin,

sulfamethoxazole/trimethoprim and amikacin resistance were affected with the percentages of (58.3%). From the obtained results, a conclusion can be made that curing by elevated temperature is efficient method for plasmid curing, and this may be due to the fact that the enzymes which contribute to the DNA replication processes are more affected by this high temperature. The inactivation of these enzymes may be due to the change in the folding of polypeptide at this temperature, i.e. the enzymes are sensitive to elevated temperature (Khder, 2002). These results also agree with Radi and Rahman (2010) they concluded that curing by elevated temperature is the most efficient method among others. Furthermore, enzymatic activity declines above the optimum temperature that is characteristic of the heat stability of the particular enzyme (Hardy, 1986). However, plasmids appear to be dependent on host enzymes for their replication, therefore, most of the proteins synthesized during changing of temperature might be utilized for cell division, by that, chance of plasmid replication decreases then curing occurred (Radi and Rahman, 2010). Many researchers demonstrated the mechanism by which the elevated temperature create curing of plasmid DNA, of these, the effect of elevated temperature on plasmid curing may be due to decreasing the amount of synthesized DNA.
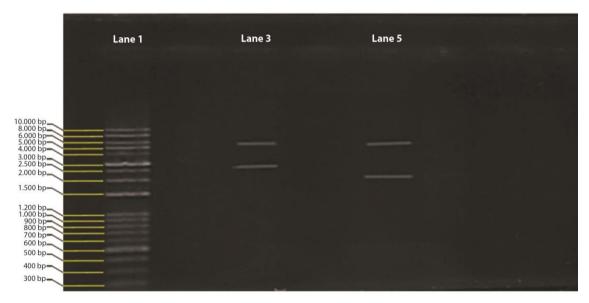

Figure 2. Plasmid profile of cured E15 by ethidium bromide and elevated temperature. (Lane 1: 10 000 bp DNA ladder, Lane 3: E15 after curing with ethidium bromide, Lane 5: E15 after curing with elevated temperature)

Conclusion

The article aimed to cure the drug resistance genes twenty-five isolates of E. coli O157:H7 obtained from 200 child stool samples, using using cultural, morphological, biochemical characteristics and serological test to identify the bacteria, and gel electrophoresis to register the plasmid profile, and utilizing Ethidium bromide and elevated temperature to cure the plasmid DNA.

For this purpose, two different types of curing methods were used and the results obtained was a preliminary indication of association of drug resistance of the clinical isolate of *E. coli* O157:H7 (E15*)* with plasmids. Among the two curing method used, elevated temperature displayed greater success rate than the ethidium bromide.

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