GENETIC TRANSFORMATION IN ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* O157:H7

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SUMMARY: Five hundred stool samples were collected from patients with diarrhea (infants and children under ten years of age) admitted to the Pediatric and Maternity Hospital in Erbil City from March 2007 to September 2007. The samples were cultured on different culture media and according to the colony morphology, biochemical reactions and by the use of API 20E system, 35 (7%) were diagnosed as E.coli I, 8 (1.6%) E.coli II, 17 (3.4%) E.coli III, 22 (4.4%) E.coli IV, 8 (1.6%) Shigella dysenteriae, 16 (3.2%) Salmonella arizonae, 12 (2.4%) Salmonella typhi and 6 (1.2%) Vibrio cholerae. In addition, cases of Entamoeba histolytica 175 (35%), Giardia lamblia 102 (20.4%) and Hymenolepis nana 2 (2.4%) were identified. No infectious agents were found in 75 (15%) of the samples. 22 (4.4%) of the samples had mixed infections. The sensitivity of E.coli O157:H7 isolate to different antibiotics was performed. There was a variation in the resistance ranging from 8.5-90%. The determination of the site of genes responsible for the antibiotic resistance in E.coli O157:H7 was performed using the genetic transformation method for E.coli DH5α laboratory strain with the DNA that is absent from the highly resistant strains, E.coli O157:H7 4 and E. coli O157:H7 6. The transformation process succeeded when using the plasmid DNA for strain 4 and failed when using strain 6. It was evident that the genes responsible for resistance to the following antibiotics were located on the plasmid DNA: amoxicillin, amoxiclav, ampicillin, cephalexine, cefixime, cefotaxime, doxycyclin, gentamycin, nalidixic acid, nitrofurantoin, rifampicin, streptomycin and tetracycline. Whereas the genes responsible for the following antibiotic resistance were located on the chromosome: amikacin, erythromycin, chloramphenicol, ciprofloxacin, tobramycin and trimethoprim.

Key words: Escherichia coli, genetic transformation, antibiotic resistance.

INTRODUCTION

Infectious diarrhea is one of world's causes of morbidity and mortality, resulting in about two million deaths per year. The majority of cases of serious diarrhea occur among children in developing countries. In contrast to third-world countries, pediatric infectious diarrhea is rarely fatal in industrialized countries (1).

Bacterial infections are very important causes of diarrhea in infants and young children worldwide (22). The principal microorganisms implicated are Salmo-

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nella (35), Shigella (34), Vibrio cholerae (37) and serotypes of Escherichia coli including EPEC, STEC, EAEC, EIEC and EHEC (26).

Antibiotic resistance has become a major clinical and public health problem during the lifetime of most people (17). There are many reasons for this problem, one of which is an over use of antibiotics (36) in addition to the chromosomal changes or the exchange of the genetic material via plasmid and transposons which help in transmission and spread of drug resistance among pathogenic bacteria (3).

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 (13). Genetic transformation is a process by which free DNA is incorporated into a recipient cell and brings about genetic change (18).

The current study was conducted to study the antimicrobial resistance of *E.coli* O157:H7 to different antibiotics and to determine the site of antimicrobial resistance genes by genetic transformation.

Table 1: Standard antisera used through the study.

	Standard antisera	Company
1	Anti *Escherichia coli* polyvalent I O1, O26, O86a, O111, O119, O127a, O128	DENKA SEIKEN / Japan
2	Anti *Escherichia coli* polyvalent II O44, O55, O125, O126, O146, O166	DENKA SEIKEN / Japan
3	Anti *Escherichia coli* polyvalent III (Anti *Escherichia coli* O157)	Serotest / England
4	Anti *Escherichia coli* polyvalent IV O6, O27, O78, O148, O159, O168	DENKA SEIKEN / Japan
5	Anti *Escherichia coli* H7	Serotest / England
6	Anti *Shigella flexneri* polyvalent (1-6)	Serotest / England
7	Anti *Shigella sonnei* polyvalent (1-6)	Serotest / England
8	Anti *Shigella dysenteriae* polyvalent (1-10)	Serotest / England
9	Anti *Shigella boydii* polyvalent (7-11)	Serotest / England
10	Anti *Salmonella* polyvalent O	Serotest / England
11	Anti *Salmonella* polyvalent H	Serotest / England
12	Anti *Vibrio cholerae* polyvalent O1 (Hikojima, Inaba, Ogawa)	RSHM / Turkey
13	Anti *Vibrio cholerae* O1 Ogawa	RSHM / Turkey
14	Anti *Vibrio cholerae* O1 Inaba	RSHM / Turkey
15	Anti *Vibrio cholera* polyvalent O139	RSHM / Turkey

MATERIALS AND METHODS

Specimen collection and diagnosis

A total of 500 stool samples were collected in clean disposable plastic containers from diarrheal patients (infants and children, below ten years of age) admitted to the Pediatric and Maternity Hospital in Erbil City from March 2007 to September 2007 and the relevant information were recorded from each patient including age, sex, geographical area, clinical symptoms and the type of feeding. Samples were then sent for direct macroscopical and microscopical examination in the laboratory of Maternity and Children Hospital to be checked for the presence of RBC's, WBC's and parasites and their stages. The sample was then cultivated on suitable culture media (MacConkey and blood agar {Oxoid, England}) and incubated at 37°C overnight. The growing colonies were cultured on further selective and differential media and diagnosed using the API 20E system. The diagnosed bacteria were selected and transferred to nutrient agar (Oxoid, England) slants and incubated at 37°C overnight. The slants were kept at 4°C until used. All bacteriological assays were performed according to Arora and Arora (6).

Diagnosis of bacterial isolates was confirmed using specific anti-sera, as shown in Table 1.

Table 2: Antibiotics used (5, 14).

Antibiotics' Names	Symbol	Stock Solution (mg / ml)	Final (Working) Concentration (µg / ml)	Solvent
Amikacin	Amk	20	15	D.W.
Amoxicillin	Amc	25	25	D.W.
Ampicillin	Amp	50	50	D.W.
Augmentin (Amoxicillin+Clavulanic acid)	Aug	10	30	Methanol
Cefixime	Cex	20	5	50% Methanol
Cefotaxime	Cef	10	30	D.W.
Cephalexine	Cep	45	30	D.W.
Chloramphenicol	Cm	34	30	Ethanol
Ciprofloxacin	Cip	10	5	D.W. and Ethanol
Doxycyclin	Do	10	30	D.W.
Erythromycin	Ery	10	10	Ethanol
Gentamycin	Gm	10	10	D.W.
Nalidixic acid	Nal	5	30	0.3M NaOH
Nitrfurantion	Nit	30	10	Dimethylformamide
Rifampicin	Rif	34	5	Methanol + 5 drops of 10N NaOH / ml
Streptomycin	Sm	10	25	D.W.
Tetracycline	Tc	5	15	50% Ethanol
Tobramycin	To	10	30	D.W.
Trimethoprim-sulfamethoxazole	Tm	20	20	Dimethylformamide

Antibiotic resistance test

To study the effect of different antimicrobials on *E.coli* O157:H7, both nutrient (Oxoid, England) and Mueller-Hinton agar (HHI MEDIA/ India) were used as growth media, after sterilization and cooling at 45°C, final concentration of antibiotics was added (Table 2) to media and poured into sterile Petri dishes. After solidification, the plates were inoculated by streaking method with the bacterial isolate then incubated at 37°C for 24 hours. The results were recorded the next day (4).

Genetic transformation

To determine the location of antibiotic resistance gene in *E.coli* O157:H7 the purified plasmid from *E.coli* O157:H7 (resistant to antibiotics) was transformed to another sensitive strain of *E. coli* DH5α (it is a bacterial strain that does not contain the plasmid used in this study and is a derivative of *E.coli* K-12, kindly provided by Dr. Farhad Abdulkareem from Erbil).

Transformation process includes

1. Preparation of competent cells

They were prepared by the method described by Mandle and Hige (19). Five ml of nutrient broth was inoculated with a single colony of *E.coli* DH5α (whose plasmid is manipulated genetically, plasmidless), incubated with shaking at 100 rpm for 24 hours at 37°C then 1ml of bacterial culture was added to 50 ml nutrient broth, incubated with shaking at 37°C, 100 rpm for 3-4 hours. The cells were harvested by centrifugation at 800 rpm, and then resuspended in 1 ml of cooled transforma-

Table 3: Enteropathogens detected in fecal samples from 500 children with diarrhea

Type of Enteropathogen	Number	Percentage (%)
E.coli polyvalent I	35	7
E.coli polyvalent II	8	1.6
E.coli polyvalent III	17	3.4
E.coli polyvalent IV	22	4.4
Shigella dysenteriae	8	1.6
Salmonella arizonae	16	3.2
Salmonella typhi	12	2.4
Vibrio cholerae	6	1.2
Entamoeba histolytica	175	35
Giardia lamblia	102	20.4
Hymenolepis nana	2	0.4
No pathogen identified	75	15
Mixed infection (E.coli polyvalent III + E.coli polyvalent I {12}, Salmonella typhi + E.coli polyvalent III {7}, Salmonella typhi + E.coli polyvalent III {3})	22	4.4
Total	**500**	**100**

tion buffer (TE buffer), then 39 ml of the same buffer added, the resuspended cell left on ice for one hour, centrifuged for 15 minutes at the same velocity, and resuspended in 1ml of cooled transformation buffer. To increase the efficiency of genetic transformation, the competent cells were kept at 4°C for 24 hours before adding the plasmid.

2. DNA uptake

The method of Lederberg and Cohen (16) was used for uptake of plasmid DNA for the process of transformation. Samples of 0.1ml of prepared plasmid DNA were plated on nutrient agar plates and incubated at 37°C for 24 hours to ensure that they are not contaminated with the bacterial cells. 1μg of prepared plasmid DNA (equivalent to 100 l) was added to the tube containing 0.2 ml of competent cells. The mixture was placed on ice for 30 minutes, and then exposed to heat shock at 42°C for 6 minutes (12). 1ml of fresh nutrient broth was then added to transformation mixture and incubated at 37°C for 60 minutes to allow the expression of antibiotic resistant genes. Five samples of (0.1ml) from transformation mixture were spread on nutrient agar plates containing the appropriate antibiotic, and 0.1 ml of competent cells was spread on nutrient agar containing the same antibiotics used as a control. All plates were incubated at 37°C for 48 hours. The number of transformation colonies were scored and purified several times on plates containing the different antibiotics used.

The genetic transformation frequency was calculated following the equation described by Puhler and Timmis (23):

Transformation Frequency = Number of transformation colonies per μg of plasmid DNA / Number of viable cells

Statistical analysis

The data were analyzed using the ANOVA test.

Table 4: Percent of resistance to antimicrobials of E.coli O157:H7 isolated from stool samples of patients with diarrhea.

Antibiotics at final concentration*	*E. coli* polyvalent III
Amk	S
Amc	89
Amp	90
Aug	88
Cex	35
Cef	35
Cep	80
Cm	S
Cip	28
Do	42
Ery	35
Gm	18
Nal	10
Nit	8.5
Rif	29
Sm	52
Tc	70
To	45
Tm	8.3

S: Sensitive *Abbreviations of antibiotics are in Table (2)

RESULTS

The causative agents of diarrhea are presented in Table 3. The most common isolated enteropathogens were Entamoeba histolytica, bacteria and Giardia lamblia. The difference between these groups was significant (P ≤ 0.05).

There was variation in the resistance to antibiotics of the isolate *E.coli* O157:H7 ranging from 8.5-90% whereas other isolates were sensitive (Table 4).

Table 5 shows that *E.coli* DH5α had the ability to receive purified *E.coli* O157:H7 plasmid DNA and transformed successfully. The number of the purified transformant colonies produced by the plasmid DNA was less than the original number. The number of transformant colonies obtained was 248 and then became 100 colonies when sub cultured on Amoxicillin, Amoxiclave, Nalidixic acid, Nitrofurantoin and Rifampicin at appropriate concentrations. Forty-three, 70, 56, 20, 13, 23, 53 and 76 colonies were obtained for Ampicillin, Cefalixine, Cefixime, Cefotaxime, Doxycylcin, Gentamycin, Streptomycin and Tetracycline, respectively. Transformation frequency for *E.coli* O157:H7 was 7.02×10^{-7}.

Table 5: Number of transformation colonies and transformation frequency of *E.coli* O157:H7 isolated from stool of patients with diarrhea.

Isolate	No. of transformant colonies	Number of colonies grown on nutrient agar containing antimicrobials in µg/ml																			Transformation frequency
		Amk*	Am	Aug	Amp	Cep	Cex	Cef	Cm	Cip	Do	Ery	Gm	Nal	Nit	Rif	Sm	Tc	To	Tm	
E.coli O157:H7 4	248	S	100	100	43	70	56	20	S	S	13	S	23	100	100	100	53	76	s	s	7.02×10^{-7}
E.coli O157:H7 6	Transformation did not succeed after repeating the process several times																				

DISCUSSION

The predominant causes of diarrhea in the stool of patients enrolled in this study were parasites and bacteria, while other etiological causes or organisms were of less significance which may be because of their need for special media and growth conditions for isolation including Yersinia and Campylobacter (13).

All 100 controls were investigated for *E.coli* (serotypes) and none were positive. 17 (3.4%) were *E.coli* Polyvalent III. These results were found to differ from the results of Sulaiman (33) who isolated polyvalent III at a rate of 37.5% of 40 isolates, in Erbil City. Our results, on the other hand, were similar to those reported by Shebib *et al.* (32) who identified the rate of STEC in 11.5%. The differences in the results may be due to the use of better methods for isolation and identification in our current study in addition to the fact that pathogenic *E.coli* is an important etiological agent in infants with diarrhea in Erbil City (24, 33).

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E.coli O157:H7 was found to be sensitive to Amk and Cip. On the other hand, it was found to be resistant to all other antibiotics, ranging from 8.5%-90%. However, ciprofloxacin and other quinolones are not approved for children because of the risk of damage to immature joints and most parental third-generation cephalosporins (e.g. cefotaxime) are administered only in a hospital setting. These results are also similar to those of Salah (29) who showed all isolates of *E.coli* to be resistant (97.5%) for Cm and less resistant for Amk (2%).

Sang *et al.* (31) study reported multidrug-resistance in enteroaggregative *E.coli* with persistent diar-

rhea in Kenyan children where the isolates were resistant to Tc and Aug. Another study (25) recorded the highest rates of resistance against Amc, Cm, Tc and Cip (100%) and the most common resistance pattern was for Amc, Gm, Cip, Tc, Cm (38.7%) and the least common resistance patterns was for Nit (3.2%).

The United States food and drug administration emphasizes the spread of drug resistance in the Enterobacteriaceae family from antibiotic-fed animals to human beings. Transmission of the R-plasmid from *E.coli* of poultry to humans occurs very commonly (25). Antibiotic resistant *E.coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as S.aureus and *E.coli*, often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. Indeed, *E.coli* is frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E.coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus *E.coli* and other enterobacteria are important reservoirs of transferable antibiotic resistance (38).

This variation in antibiotic resistance on *E.coli* is due to the genes that are located on conjugant plasmid and by conjugation, transformation or transduction processes, may be transferred to recipient *E.coli*; or the resistance genes may be located on bacterial chromosome and jump to resistance plasmid by transposition process (many composite transposons contain genes for antibiotic resistance and some bear more than one resistance gene). This is found in both Gram-negative and Gram-positive bacteria (39).

Genetic transformation experiment was performed to determine the site of genes that are responsible for antibiotic resistance in *E.coli* O157:H7 isolates. Table 5 showed that *E.coli* DH5α had received purified *E.coli* O157:H7 plasmid DNA from E4 isolate and transformed successfully, while the plasmid DNA sample for isolate E6 failed to enter the host DH5α by the transformation process after repeating the process several times. It is also evident that the number of the purified transformed colonies produced by the plasmid DNA were less than that started from, where the number of the transformant colonies obtained were 248 and became 100 colonies when sub-cultured on Amc, Aug, Nal and Rif at appropriate concentrations, and 76, 70, 56, 53, 43, 23, 20 and 12 colonies for Tc, Cep, Cex, Sm, Amp, Gm, Cef and Do, respectively.

Differences were observed in transformant colonies number for E4 isolates when cultured on different antibiotics separately indicating that the antibiotics resistance genes are located on different R-plasmid. This consists of two linking fragments, one called RTF-TC, which carries several numbers of genes which are specialized for plasmid replication process and copy number of plasmids and sometimes contain genes responsible for Tc resistance. The other fragment is called r-determinant, which contains genes responsible for other antibiotic resistance (7). These two DNA fragments of R-plasmid may enter the DH5α host in different efficiency because of their variation in size. In addition, it is reasonable to believe that there is more than one DNA species of plasmid DNA content on isolate E4 and transformed in different efficiencies. Irregular segregation of plasmid DNA species may result in differences in the number of transformant colonies for other antibiotics (11). Furthermore, irregular segregation of plasmid DNA species may result in differences in the transformant colonies for other antibiotics. On the other hand, the transformant colonies failed to grow on nutrient agar with Amk, Cm, Cip, Ery, To, Tm. This finding may be related to those genes which are responsible for resistance to these antibiotics, either located on chromosome or on large plasmid which cannot enter DH5α strain, acting as a host, or have transposition property (29).

Isolated plasmid DNA from E6 isolate was not successfully transferred even after repeating the transformation process several times. The large size of the plasmid may have been exposed to the breakage during its preparation. This could be considered as a reason for the failure of transformation of this isolate (11).

Al-Atraqchi (2) recorded the location of the antibi-

otics Amp, Amc, Cep, Cm, Cip, Ery, Gm, Nal, Rif and Tc genes on the plasmid DNA by the genetic transformation of the strain *E.coli*: K12JM83 with plasmid DNA purified from *E.coli*, while Tm gene, Sm and Neo were located on chromosomal DNA of *E.coli*. This is in agreement with our results. In addition, Salah (29) determined the location of genes responsible for antibiotic resistance in *E.coli* isolates Amk, Do and Tc located on plasmid DNA. This is also comparable to our results. Cm, To, and Tm genes were located on plasmid DNA. These data disagree with our results.

Since the original recognition of transposon Tn1 carrying an ampicillin resistant determinant on plasmid RP4, many other transposons have been reported among members of Enterobacteriaceae such as Tn2, Tn3 , Tn21, Tn401, Tn903, Tn1701, and Tn2601. All of these transposons carry the penicillinase (β-lactamase) genes (40).

Dale and Park (9) observed that the plasmid R100, which confers resistance to 4 different antibiotics Tc, Cm, Sm, and sulphonamides as well as to mercury salts, carries two copies of IS1 and two copies of IS10. They mentioned that the TEM β-lactamase is the most common type amongst plasmids in the Enterobacteriaceae and in many members of genus Pseudomonas. Al-Atraqchi (2) detected the transposition property among some antibiotic resistance genes and the results demonstrate that isolate 52 *E.coli*, 20 Morganella and 88 Salmonella contain transposons for neomycin and streptomycin resistance. These genetic transposable elements have the ability to jump from the chromosome of these isolates to plasmid DNA and integrate into genes encoding antibiotic resistance. This result agrees with To, Tm, Ery, and Cm results which were located on chromosomal DNA in this study. These plasmids responsible for antibiotic resistance may have jumped to chromosomal DNA.

Transformation frequency for the E4 isolate was 7.02×10^{-7}. The result of Al-Atraqchi (2) on *K.oxytoca* 31, *E.coli* 7, *E.coli* 97, *M.morgana* 20 and *Salmonella* 88, were 2.2×10^{-5}, 3.4×10^{-6}, 0.063×10^{-6}, 0.96×10^{-7} and 1.2×10^{-6}, respectively. Mohamed (21) recorded 0.8×10^{-4}, 1.9×10^{-4}, 1.6×10^{-4}, 1.3×10^{-4} and 2.8×10^{-4} for E1, E2, E3, E4 and E5, respectively. While Kheder (15) recorded 0.39×10^{-8}, 0.16×10^{-8}, 3.9×10^{-8}, 0.2×10^{-8}, 0.61×10^{-8}, 0.067×10^{-8} and 3.3×10^{-8} on *Pseudomonas aeruginosa* isolates P7, P14, P28, P31, P38, P40, and P51, respectively. Mawlud (20) recorded 0.3×10^{-5} and 0.5×10^{-7} for *Klebsiella pneumoniae* isolates K11 and K32, respectively. Hamasalih (10) recorded 6.94×10^{-7} for *P.aeruginosa* isolate P5.

The transformation frequency differs from one type of bacteria to another. These differences may be due to the size and shape of transferred plasmid; small circle plasmids are transferred much more efficiently than large circle plasmids in addition to the fact that the purity of plasmid affects transformation. We can conclude that the laboratory *E.coli* HD5α strains treated with $CaCl_2$ can represent an efficient host for accommodation of the plasmid DNA transfer of *E.coli* O157:H7. In addition, the preparation of plasmid DNA by Cesium chloride-ethidium bromide centrifugation can increase the transformation frequency because this procedure made plasmid DNA remains in (ccc) state (11).

Transformation frequency is influenced by several factors like size of plasmid DNA. Although the size of plasmid DNA is small, the transformation frequency becomes high or increased which means that the small size of plasmid DNA leads to genetic transformation to be successful. Another factor is the purity of plasmid DNA which has a high effect on genetic transformation. Whenever plasmid DNA is in a high degree of purity, the genetic transformation is increased. Moreover, energy which is needed for adherence of the plasmid DNA to the competent cell is considered as a factor on energy pathway of competent cells leading to prevention of the ability of plasmid DNA to enter (8, 30).

CONCLUSION

1. High prevalence of *Entamoeba histolytica* and *Giardia lamblia* that cause diarrhea followed by *E.coli* serotypes, *Salmonella spp.*, *Shigella* and *Vibrio cholerae*.

2. Enteropathogenic *E.coli* (EPEC) is considered as one of the major causative agents of children's diar-

rhea followed by ETEC, STEC and EAggEC.

3. The isolated *E.coli* O157:H7 revealed high resistance to most widely used antibiotics.

4. The genes responsible for Amc, Aug, Amp, Cep, Cex, Cef, Do, Gm, Nal, Nit, Rif, Sm and Tc resistance in *E.coli* O157:H7 are located on plasmid DNA, and others including Amk, Cm, Cip, To, Ery and TM are located on chromosomal DNA. These genes are successfully transferred to laboratory *E.coli* DH5α strains through transformation process.

REFERENCES

1. Afset JE, Bergh K, Bevanger L: High prevalence of a typical enteropathogenic Escherichia coli (EPEC) in Norwegian children with diarrhea. J Med Microbiol, 52:1015-1019, 2003.

2. Al-Atraqchi KIB: Molecular genetic studies of bacterial species isolated from patients with diarrhea. Ph D Thesis. College of Medicine Hawler, Medical University, Erbil, Iraq, 2006.

3. Al-Faisal, A: Genetic Engineering. Dar Al-Shrooq for Publishing Amman (Arabic), 1999.

4. Al-Najjar AR: Studies on the effect of surfactants on the antimicrobial activity of several antibiotics. Ph.D. Thesis, University of Manchester, UK, 1976.

5. Andrews JM: Determination of minimum inhibitory concentrations. J Antimicrob Chemother, 482: 5-16, 2001.

6. Arora B, Arora DR: Practical Microbiology. CBS Publishers and Distributors, New Delhi, 2007.

7. Clows R: Molecular structure of bacterial plasmids. Bact Rev, 36: 361, 1972.

8. Cosloy SD, Oishi M: Genetic transformation in E. coli: K-12. Practice of National Academics Science, USA. 70: 84-86, 1973.

9. Dale W, Park S: Molecular genetics of bacteria. 4th ed, John Wiley and Sons Ltd, England, 2004.

10. Hamasalih RM: The effect of both aqueous and alcoholic extracts of Quercus infectoria against antibiotic resistance of Pseudomonas aeruginosa In vitro and In vivo studies. M.Sc. Thesis, College of Education, University of Salahaddin, Erbil-Iraq, 2008.

11. Hardy K: Bacterial plasmid. 2nd ed, Am Soc Microbiol, USA, 1986.

12. Hoekstra WM, Bergman HN, Zuidweg EM: Transformation in E.coli, studies on the nature of donor DNA after uptake and interaction. Genetic Res Comb, 35:281-285, 1980.

13. Jawetz E, Melnick JL, Adelberg EA, Brooks GF, Butel JS, Morse SA: Medical Microbiology. 23rd ed, McGraw-Hill, USA, 2004.

14. Jean SS, Teny LJ, Hsueh PR, HO SW, Luh KT: Antimicrobial susceptibilities among clinical isolates of extended-spectrum cephalosporin, resistant Gram-negative bacteria in a Taiwanese university hospital. J Antimicrob Chemother, 49:69-76, 2002.

15. Kheder AK: Studies on antibiotic resistance by plasmids of Pseudomonas aeruginosa. Ph.D. Thesis, College of Education, University of Salahaddin, Erbil-Iraq, 2002.

16. Lederberg EM, Cohen SN: Transformation of S. typhimurium by plasmid Deoxyribonucleic acid. J Bacteriol, 119: 1072-1074, 1974.

17. Levy SB: Active efflux, a common mechanism for bicid and antibiotic resistance. J Appl Microbiol Symp, Suppl 92:65-71, 2002.

18. Madigan MT, Martinko JM, Parker J: Brock Biology of Microorganisms, 9th ed. Prentice-Hall, Inc. USA, 2000.

19. Mandle M, Hige A: Calcium-dependent bacteriophage DNA infection. J Mol Biol, 53:159-161, 1970.

20. Mawlud SQ: The effect of some medicinal plant extracts on curing plasmids of Klebsiella pneumoniae isolates from different environments. M.Sc. Thesis, College of Education University of Salahaddin, Erbil-Iraq, 2006.

21. Mohamed BG: Genetic study of Escherichia coli isolated from various human infections. M.Sc. Thesis, College of Education, University of Mosul (Arabic), 1999.

22. Nelson EW, Berhman ER, Kligman MR, Jenson: Nelson Textbook of Paediatrics. 15th ed, Vol1. WB Saunders, USA. pp 935-1123, 2004.

23. Puhler A, Timmis NK: Advances in Molecular Genetics. Springer-Verlag, Berlin, 1984.

24. Rasheed NE: Etiology of bloody diarrhoea among children admitted to maternity and children hospital. Diploma of Science in Paediatrics, College of Medicine, University of Salahaddin, 2003.

25. Roy R, Purushothaman V, Koteeswaram D, Dhillon AS: Isolation, characterization and antimicrobial drug resistance pattern of E.coli isolated from Japanese quail and their environment. Jappel poultres, 15: 442-446, 2006.

26. Ruohonen P: Other strains of E.coli outbreak. Pritzker Law Firm, 2008.

29. Salah HF: Effect of some medicinal plant extracts on antibiotic resistance by plasmid of Escherichia coli isolated from

different sources. M.Sc. Thesis, College of Education, University of Salahaddin, 2007.

30. Salle AJ: Fundamental Principle of Bacteriology. 7th ed. McGraw-Hill Book Company, USA, 1997.

31. Sang WK, Oundo JO, Mwituria JK, Waiyaki PG, Yoh M; Iida T, Honda T: Multidrug-resistant entero aggregative Escherichia coli associated with persistent diarrhea in Kenyan children. Emerging Infect Dis, 3(3), 1997.

32. Shebib Z, Abdulghani Z, Mehdi L: First report of E.coli O157 among Iraqi children. East Mediterr Hlth J, 9: 1-8, 2003.

33. Sulaiman SS: The identification, serological, classification, heamagglutination makers and antibiotic sensitivity patterns in Escherichia coli causing infantile gastroenteritis in Erbil. M.Sc. Thesis, College of Science, University of Salahaddin. (Arabic), 1994.

34. Sureshbabu J: Shigella infection. Honcode principles of the health of the net foundation. Web MD, 2006.

35. Todar K: Salmonella and Salmonellosis, University of Wisconsin-Madison, Dept Bacteriol Todar's online textbook of Bacteriology, 2005.

36. Webster M: Antibiotic resistance in E.coli. Hastings-on-Hudson. High School, Westchester, 2002.

37. Wikipedia: Cholera, Wikipedia foundation, Inc. http://en.wikipedia.org/org/cholera, 2008.

38. Wikipedia: Escherichia coli. Wikipedia foundation, Inc, 2008.

39. Woodford N, Johnson AP: Molecular bacteriology, protocols and clinical application. Humana Press Inc, 1998.

40. Yamamoto T, Tanako M, Nohra C: Transposition of the oxacillin hydrolyzing penicillinase gene. J Bacteriol 145: 808-813, 1981.

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