

GENOTYPES OF SEVERAL *ESCHERICHIA COLI* ISOLATES FOR POLLUTION EVALUATION

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ABSTRACT : The present study was carried out to using PCR-RAPD technique to evaluation pollution in Hilla city using *E. coli* isolates as contamination indicators, samples were collected from different source (river water, sewage water, waste water, tabs, lab tables, wound, patients with UTI and high vaginal swabs) then antibiotics sensitivity implemented using disc method and high resistance isolated were choosing to genotyping detection for evaluation the source of contamination, the results show that *E. coli* isolates were growth in UTI, wound and one isolate in HVS samples, antibiotics sensitivity show variety of resistance. Genotyping performed using five primers of RAPD. The results show that primer 1 and 5 more efficient in PCR amplification it had 138 and 94 band, respectively. The similarity index conducted using jaccard index and its divers among isolates, isolate 11 and 6 were had high differences index and appeared in a major groups in tow primers, while other isolates appeared in related groups, the mean of all similarity index of five primers show that isolated 11 was the more differences than others with low percentage of similarity. From these results, we can conclude that the source of contamination was from UTI patients that can be transfer to other habitats with high antibiotics resistance. This indicated that the health awareness must be spread in society to avoid genetic developments and habitat adaptation in bacteria.

Key words : *E. coli*, pollution dictators, PCR-RAPD, genotype.

INTRODUCTION

E. coli strains have been integrated in to different environmental microbial communities from divers sources, it has various phenotypes and genotypes, several classifications of *E. coli* were performed according to different characterization, somatic (O), capsular (K) and flagella (H) antigens used to classify *E. coli* to traditional serotype (Kaper and Nataro, 2004). Phylogenic features used to classified *E. coli* to A, B1, B2 and D, multi-locus sequence typing (MLST) used in a new groups (Clermont *et al*, 2013).

E. coli is commensal and pathogen strains caused a different human diseases led to death more than two million individuals in every year (Kaper *et al*, 2004). It has been used as pollution indicator because it's mainly inhabits in the lower intestinal tract humans and other warm blood mammals, it is always discharged to environment through faces or wastewater effluent (Jank *et al*, 2017).

The first sequencing of Genome of *E. coli* analysis recorded at 1997, to date more than 4800 genome has

been analyzed. *E. coli* considered the suitable evidence to study micro-organism evolution, environment pollution, and in several biotechnology application like genetic engineering and protein production (Tenailon *et al*, 2016; Jang *et al*, 2017).

The RAPD method has been the important techniques used in molecular labs for different investigations such as genetic polymorphism, taxonomy and phylogenetic studies. Recently, RAPD used to detected genotoxicity and DNA damage like adducts and breakage (Atienzar *et al*, 2002; Hans *et al*, 2004; Atienzar and Jha, 2006).

Here, we aimed to genotyping of *E. coli* isolates from different source in Hilla city to evaluation pollution during January-April, 2018.

MATERIALS AND METHODS

Samples collection

About 50 samples were collected from different sources ; water (river water, wastewater and sewage water) swabs (tab bath, lab tables) clinical samples (urine, wound, high vaginal swabs and feces), samples collected according to ethical approval of ministry of environment

and health in Iraq and according to lab lows ISO for pathogenic bacteria isolated all samples collected in sterile countenair and according to standard methodology to avoid contamination during work. Samples transfer to microbiology lab. All samples were culture in suitable media to estimate micro-organism communities, then macroscopic and microscopic examination implemented to diagnosis, here we studied *E. coli* genotyping, thus it re-culture in EMB for more purified colonies.

Morphological identification of *E. coli*

All isolates were cultured at 37°C on nutrient agar, bacterial colonies were large, thick, as henwhite, moist, smooth, opaque or translucent discs. EMB media assisted to visual discriminated of *Escherichia coli* among other lactose-fermenting enteric species and the *Salmonella* and *Shigella* genera.

Antibiotic sensitivity

It was detected using disc methods totobramycin (TOB), chloramphenicol (C), Clindamycin (DA), ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamycin (GN), Norfloxacin (NOR), Imipene (IMP), Amikacin (AK), tetracyclin (TE), Azothromycin (AZM).

DNA extraction

Bacterial DNA extraction according to Dafa *et al* (2000) in briefly a colony of bacteria isolates were re-culture on luria agar for 18 hours. Then pick colonies were transfer to PCR- tube contain 30 µl of dH₂O with mixing, the mixtures incubated at 95°C for 7 minutes in water bath, after incubation Mixtures centrifuged at 10,000 rpm for 15 min, then transferred the supernatant to a new tubes and stored at -20°C till it used for PCR.

PCR amplifications

RAPD amplification were by five different primers (Table 1), which were performed using clever scientific thermal cycler. RAPD amplification reaction mixtures consisted of 1 µl of bacterial DNA, 10 pm primers (Table 1) and 12.5 µl master mix (Promega) then it completed to 25 µl by dH₂O. PCR condition was according to the following : 95°C for 5 min, (95°C for 45 sec, 44-52 c for

45, 72 for 1 min) 40 cycle, and the final extension 72°C for 10 min. bands were visualized by the electrophoresis 1.5% agaros for 60 min at 70V, 20mA.

Phylogenetic analysis

The phylogentic tree created using the dendrogram program unweighted pair group method with arithmetic mean (UPGMA) algorithm by Dendro UPGMA: Adendrogram construction utility, similarity index were calculated according to Jaccard index.

RESULTS AND DISCUSSION

The results of present study shows diverse microbial community (bacterial, molds and fungi) it purified for diagnosis then *E. coli* was transfer to EMB media, *E. coli* didn't appeared in all water samples and water sources, feces and all environmental swabs, its appeared in clinical samples like urine, high vaginal swabs and wounds the previous studies in Iraqi samples show variety of results, some of their recoded present *E. coli* in clinical samples (Abdul-Razzaq and Abdul-Lateef, 2011) and others in hospital wastewater (Aziz *et al*, 2014). The presence of *E. coli* in other its habitat depending on several factors like management of biosafety in labs and hospital, also contamination processing in addition of health awareness.

Antibiotic sensitivity was implemented for all isolated bacteria using disc diffusion method. Table 2 shows antibiotics resistance percentage in present bacteria isolates, it show more resistance to Clindamycin while it more sensitive to Amikacin high vaginal swab was completely sensitive (100%) to 6 types of antibiotics TOB, C, CRO, AK, CN and AZM. Isolates from wound shows completely sensitive (100%) to AK. Urine isolates shows more sensitive to AK (7.69) while it more resistance to IMP and AZM (76.92%).

The diversity of Antibiotic sensitivity in *E. coli* isolates in present study can be explained by effective of genetic factors like gene transfer by plasmids or a new DNA recombination, transposable elements and bacterial conjugation, studies have been found genes encoded to antibiotics resistance in *E. coli* genome, chromosomal and extra-chromosomal elements in Iraq (Ibrahim *et al*, 2014; Zahid *et al*, 2017, Al-Terehi *et al*, 2018).

The genotyping study are shown in Fig. 1 (A, B, C, D and E) and Tables 1-6, the isolates were choosing to RAPD according to its resistance to antibiotics, genotyping implemented using RAPD-PCR technique for 5 primers, then mean of similarity for all primers was calculated to estimate the source of pollution in Hilla city, primer 1 has (138) bands for 11 isolates, primer 2 has (67) bands, primer 3 has 37 bands, primer 4 has 41 bands

Table 1 : Primers used in RAPD-PCR.

Primer	Sequences (5 to 3)	Annealing TM °C	References
1	GAGGCCCTTC	44.3	Fritsch <i>et al</i> (1993)
2	CGCAGACCTC	44.2	Roberts and Crawford (2000)
3	AACGCGCAAC	42	Akopyanz <i>et al</i> (1992)
4	GTTTCCGCCC	52.1	
5	GCGATCCCCA	42.5	

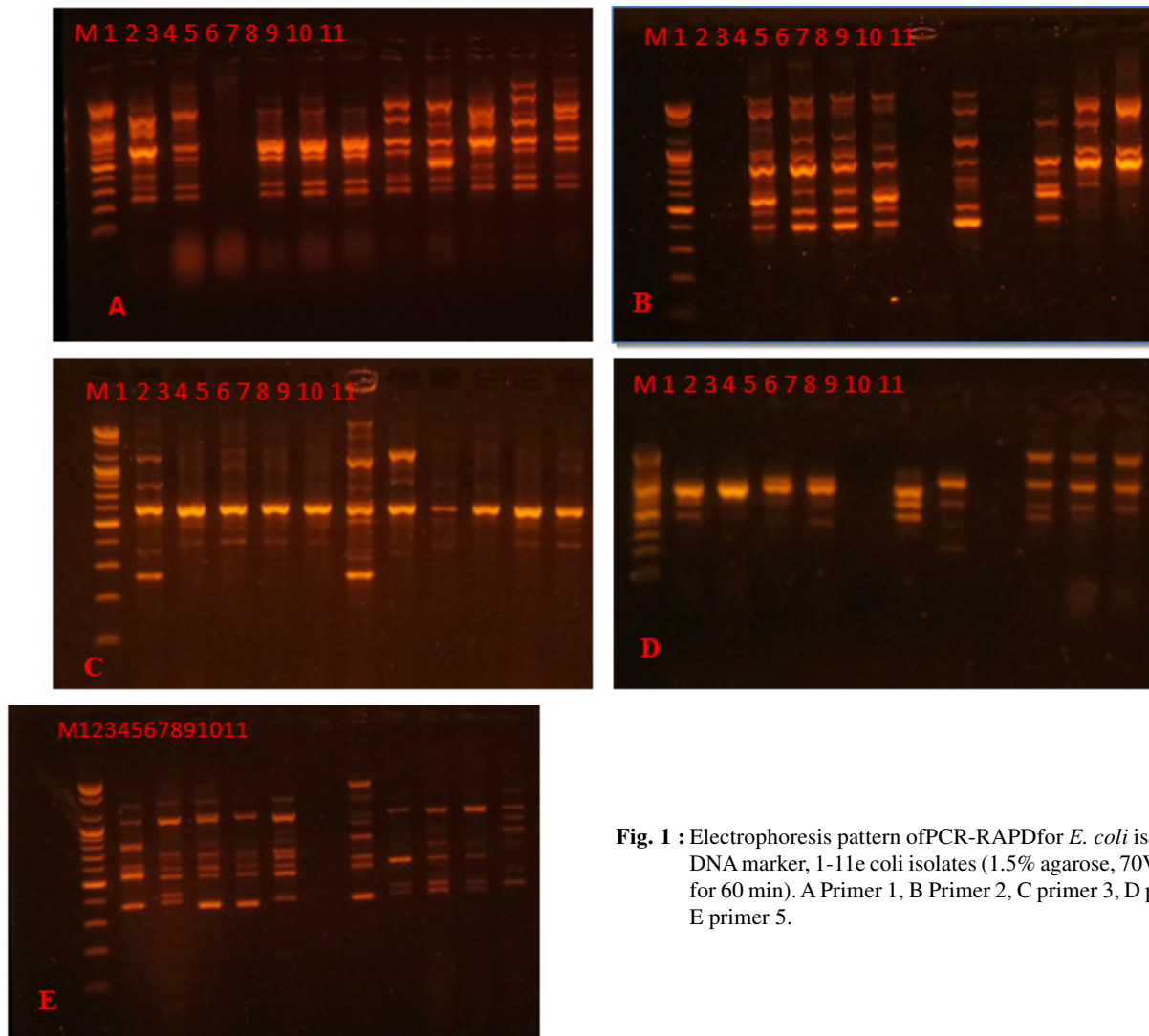


Fig. 1 : Electrophoresis pattern of PCR-RAPD for *E. coli* isolates M DNA marker, 1-11 *E. coli* isolates (1.5% agarose, 70V, 20mA, for 60 min). A Primer 1, B Primer 2, C primer 3, D primer 4, E primer 5.

Table 2 : Antibiotic sensitivity percentage of *E. coli* isolates.

Antibiotics	Urine	Wound	High vaginal swap
TOB	46.15	50.00	0
C	30.76	100	0
IMP	76.92	100	100
CRO	46.15	50	0
AK	7.69	0	0
CIP	38.46	100	100
CN	30.76	50	0
NRO	38.46	100	100
TE	61.53	100	100
AZM	76.92	100	0
DA	84.61	50	100

Tobramycin (TOB), chloramphenicol (C), Clindamycin (DA), ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamycin (GN), Norfloxacin (NOR) Imipene (IMP), Amikacin (AK), tetracyclin (TE), Azothromycin (AZM)

and primer 5 has 94 bands as in Figure 1(A, B, C, D and E, respectively).

The similarity index was depended in present study

according to Jaccard index that implemented using UPGM dendrogram software, the similarity index was divers according to primer sequence and its sites, primer 1 the most similar isolates was (1 vs 2), which isolated from urine and (9 vs 5) isolated from urine and wound respectively (Table 3) while the most differences was seen between (5 vs 3) which also isolated from urine with correlation coefficient (CP) = 0.84745691133762.

According to primer 2 the most similar was seen in (4 vs 3), (4 vs 2), (5 vs 2), (3 vs 2) (5 vs 3) and (5 vs 4) while the most differences were (11 vs 1) and (10 vs 1), correlation coefficient (CP) = 0.984680781222743. All these diversity was in isolates from urine (Table 4).

The diversities according to primer 3 were between (10 vs 7), (4 vs 2) and (9 vs 8), it were appeared as identical isolates, the most different was between 11 and all other isolates it was extremely different with CP = 0.920758666012682 (Table 5).

Isolate 8 and 5 were show extremely difference with

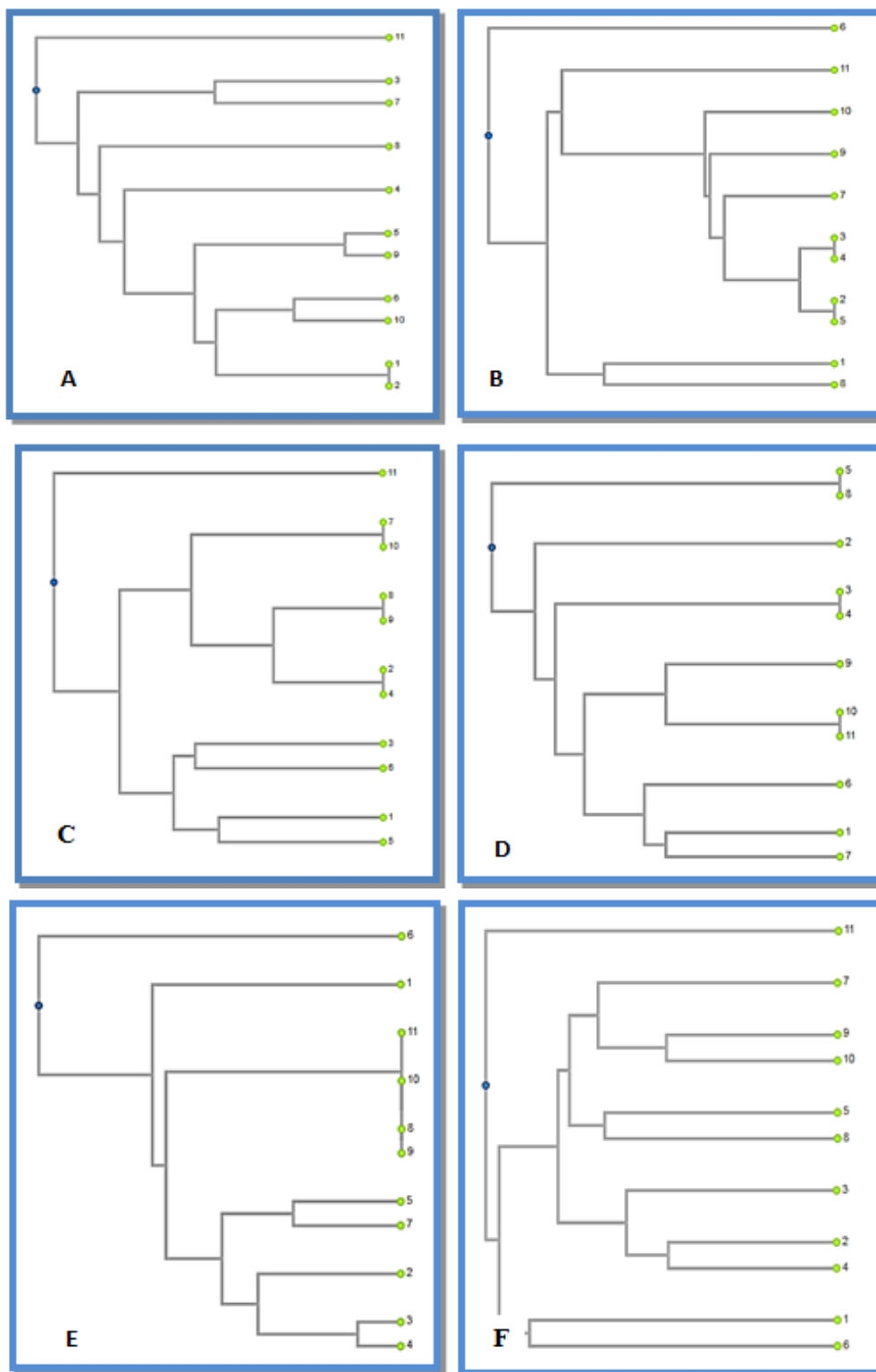


Fig. 2 : Phylogenetic trees of PCR-RAPD for *E. coli* isolates 1-11 *E. coli* isolates. A Primer 1, B Primer 2, C primer 3, D primer 4, E primer 5, F mean of similarity index.

other isolates while other isolates show low similarity between its according to primer 4 with CP = 0.96496991379824 (Table 6).

Primer 5 results show that identical between (11 vs 8, 9 and 10), (10 vs 8 and 9) and (9 vs 8), the low similarity

was seen between (6 vs 11, 10, 9 and 8) with CP = 0.969825331941494 (Table 7).

Means of the five primer similarity matrix were shows in Table 9, the similarity values between (0.1644-0.6406), as we saw isolate 11 was more differ with other

[illegible][illegible][illegible][illegible]

Table 7 : Similarity matrix of *E. coli* isolates according to primer 5.

Isolates	1	2	3	4	5	6	7	8	9	10	11
1	1	0.333	0.545	0.500	0.462	0.111	0.286	0.417	0.417	0.417	0.417
2		1	0.700	0.636	0.583	0.250	0.500	0.417	0.417	0.417	0.417
3			1	0.900	0.667	0.222	0.462	0.385	0.385	0.385	0.385
4				1	0.750	0.200	0.538	0.462	0.462	0.462	0.462
5					1	0.182	0.750	0.538	0.538	0.538	0.538
6						1	0.200	0.100	0.100	0.100	0.100
7							1	0.462	0.462	0.462	0.462
8								1	1.000	1.000	1.000
9									1	1.000	1.000
10										1	1.000
11											1

Table 8 : Cophenetic correlation coefficient (CP), of *E. coli* isolates for primers.

Primer	Cophenetic correlation coefficient (CP)
1	0.84745691133762
2	0.984680781222743
3	0.920758666012682
4	0.96496991379824
5	0.969825331941494
Similarity mean of primers	0.975825391339971

Table 9 : Mean of similarity matrix of *E. coli* isolates.

Isolates	1	2	3	4	5	6	7	8	9	10	11
1	1	0.3744	0.3492	0.3360	0.3574	0.3528	0.2714	0.2710	0.3044	0.2722	0.2206
2		1	0.5476	0.6464	0.4778	0.3428	0.4396	0.3444	0.4944	0.4788	0.1940
3			1	0.5674	0.4600	0.3190	0.4156	0.2864	0.3524	0.3612	0.2130
4				1	0.4488	0.3200	0.4322	0.3172	0.4622	0.4216	0.2418
5					1	0.2244	0.3710	0.5104	0.4722	0.4342	0.2100
6						1	0.2622	0.1600	0.2710	0.1702	0.1644
7							1	0.3226	0.4290	0.5640	0.2800
8								1	0.5634	0.4544	0.3238
9									1	0.6406	0.3838
10										1	0.3572
11											1

isolates.

According to jaccard index phylogenetic trees were created using UPGM, Fig. 2 shows all trees according to RAPD-PCR results, isolate 11 appeared as a main group in to tow primer; one and three (A and C), also isolate 6 appeared as a main group in to two primer tow and five (B and E), isolates 5 and 8 appeared as a main group in one primer 4 (Fig. D). All these isolates were isolated from urine of patients suffered from urinary tract infection. The mean of similarity index show that isolate 11 was a main group while other isolates made of two minor groups the first consist of two branches and the second consist of two isolates (Fig. 2 E).

The results of present study show that there were ten isolates which collected from urine and wound were had a similarin genotypes, thus its improved that *E. coli* may be polluted hospitals environment and transferred from patients to other by different ways. The present results shows diversity in genotyping, in general this led to show different phenotypic also its provides important information about its physiological and ecological information's (Meric *et al*, 2013), the phenotypic characteristics are forming biofilms and carbon source utilized (Cooper and Lenski, 2000). The genotyping

happened for environment adaptation, or by genetic transfer ways, transformation conjugation and transduction, which can acquisition of new genes in *E. coli* (van Elsas *et al*, 2011; Doi *et al*, 2012). Mutation in genome play important role in genetic diversity. Touchon *et al* (2009) recorded increasing *E. coli* genome to 18 000 genes, about 2000 genes had been appeared in genomes.

RAPD-PCR used in present study to evaluation the source of pollution, two of primers show good amplification results and mean of similarity index confirm these results. Studies used RAPD –PCR to other purposes like flora analyzing. Nielsen *et al* (2014)

proposed RAPD method as a fast, reproducible, high-resolution and low cost method to identification *E. coli* isolates prior to WGS or other high-resolution typing schemes. Early, Kärkkäinen *et al* (1996) used RAPD-PCR to identified intra species variation in *E. coli* the results show that it be reproducible and sensitive technique which less demanding, more rapid and more economical than either serotyping or ribotyping. Vogel *et al* (2000) used RAPD typing for Epidemiologic typing of *E. coli*, they found that this technique had high discriminatory capacity. From this we concluded that the one sources of pollution in Hilla city was patients with UTI as well as, to absence health awareness about bacterial infections and treatment.

CONCLUSION

The source of contamination was from UTI patients that can be transfer to other habitats with high antibiotics resistance. This indicated that the health awareness must be spread in society to avoid genetic developments and habitat adaptation in bacteria.

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