Molecular Study of Genetic Diversity in *Escherichia coli* Isolated from Tap Water in Baquba

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Abstract

One hundred and ten samples of the tap water in Baquba city were collection .Ten isolates were obtained. The results of the sensitivity test showed that 10 isolates were multidrug, 10 isolates exhibited moderate resistance ranging from 5-8 antagonists. Through the use of the RAPD-PCR profiling system, the vast majority of *Escherichia coli* strains under study have large genomes and high virulence factors by belonging to group A2. Results of genetic profiling using the ERIC- PCR and BOX-PCR system showed several groups of isolates were identified as having a genetic variation and B2 group was given the highest proportion of ERIC- PCR system,70% in ERIC While A2 group was given the highest proportion of BOX- PCR system,40% in the BOX indicating that there is a negative spread The present study indicates that there is a correlation between taxonomic systems was evident by the distribution of isolates in each pattern and group.

Keywords: *E. coli, ERIC-PCR, BOX-PCR, RAPD–PCR.*

Introduction

Escherichia coli is a gram-negative bacteria belonging to the Enterobacteriaceae family, short bacilli, facultative anaerobic(1). *E. coli* is a major component of the human normal intestinal flora. Among the intestinal pathogens(2). There are several virulence factors that contribute to *E. coli* pathogenicity, such as enterotoxins, endotoxin and biofilm formation(3). These bacterial strains are a potential reservoir for antimicrobial resistance genes(4). The widespread development of resistance to several different antibiotics is generally as a result of lateral or horizontal gene transfer. Many studies have demonstrated that plasmid transfer between bacteria occurs in diverse environments(5). *E coli* bacteria are used as biomarkers such as their use as a source of faecal contamination in environmental samples. Where the presence of these strains (whose habitat is the intestines of humans and animals) in the water indicates fecal pollution resulting from humans or animals, and thus environmental pollution.(6;7). PCR fingerprinting method like enterobacterial repetitive intergenic consensus (ERIC- PCR) and BOX-PCR has been extensively used to study genetic relationship as they have discriminatory capability in differentiating different genera of bacteria(8).

The aim of this study is study virulence factors and genetic diversity for *E. coli* bactera isolates from environmental sources (water).

Materials and Method

E. coli **sample collection and identification:** In this study, ten isolates of *E. coli* were isolated from 110 tap water sample at the Baquba city,during October-December 2019.

Take known volume (30 mL) of water samples collected from tap water in Baquba city, cultured on Maconkey broth medium to ensure that the samples are contaminated with Gram-negative bacteria, and then cultured on contaminated samples on Maconkey agar plates, and then cultured on Eosin methylene blue agar plates for isolation of enteric bacteria. Gram stain and other biochemical tests were used such as IMViC Tests,Urea and Kligler Iron Agar.(9).

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Susceptibility test of antibacterial agents

Susceptibility of all the isolates to different antibiotics was determined by the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (10). The antibiotic discs used in this study were (Tetracycline (30µg), Imipenem (10µg), Doxycycline (30µg), Aztreonam (30µg), Levofloxacin (5µg), Cefuroxime (30µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Azithromycin (15µg), Cefoxitin (30µg), Ticarcillin- clavulanate (75/10µg), Cefpodoxime (10µg). Ampicillin-sulbactam (10/10µg)) Each antibiotic concentration was applied on the surface of Muller Hinton agar plates inoculated with E. coli isolates and incubated at 37°C for 24 h.

Biofilm formation detection using Micro Titer Plate method (MTP): The bacterial isolates were cultured on nutrient broth for 18- 24 hr at 37°C aerobically. About 200 µL of the bacterial suspensions were transferred into polystyrene plates containing 96 wells and a broth without bacterial inoculum was used as a negative control. The plate was incubated for 24 hr. The bacterial suspensions then removed and each well was washed three times with sterile saline solution (0.9% NaCl). The cells stuck on the walls were fixed with 200 µL of methanol for 10-15 minutes. The methanol was removed, the plates were left at room temperature to dry and they were stained with 200 µL of crystal violet 0.5% for 10-15 minutes. The plates then washed with distilled water 2-3 times and dried at room temperature. The absorbency was taken in an ELISA reader at wavelength of 630 nm, [11]. The value of the optical densities for each isolate (ODi) was obtained by averaging the three wells, and this value was compared to the optical density of the negative control (ODc). The isolates were classified into four categories, according to the mean optical densities (ODi) in relation to the ODc results. If ODi ≤ ODc; considered non-adherent, ODc≤ODi≤2*ODc; considered moderately adherent and if 2*ODc≤ODi which considered strongly adherent[12].

Detection of Acyl-homoserine lactone(AHL) by colorimetric method: The isolates were incubated in 5 mL Mueller Hinton broth overnight at 37°C. 1.5 mL of the suspension was centrifuged at 10,000 rpm for 15 min, the supernatant was transferred and this step was repeated twice Subsequently liquid-liquid extraction using ethyl acetate for 10 min and the organic phase (top) was removed. Next, the samples were dried at 40°C, they transferred 40 µL to a microplate and added 50 µL of 1:1 solution of hydroxylamine 2 M: NaOH 3.5 M and 50 µL of1:1 solution of FeCl3 10% in HCl 4 M: Ethanol 95%. Finally, the optical density at 520 nm was measured in a plate reader.[13].

Rep-PCR profile and dendrogram construction(ERIC, RAPD, BOX)

E. coli DNA was prepared for PCR according to the method described [14].

Rep-PCR was performed using The ERIC forward primer (5'-ATGTAAGCTCCTGGGGATTCAC-3') and reverse primer (5'AAGTAAGTGACTGGGGTGAGCG-3')[15] were used to amplify repetitive equences present in the chromosomal DNA of E. coli isolates. ERIC-PCR was carried in 25µl, volume comprising of 100ng of E. coli DNA, 1.5µl (10 pmol) of each primer and 12.5µl Mastermixe. Filtered water was added to the mixture to make a final volume of 25µl. Reactions were carried out using a programmable thermocycler according to the following thermocycling conditions: initial denaturation at 94 °C for 1 minute, with the next 30 cycles consisting of a denaturation step at 94 °C for 30 seconds, annealing at 52 °C for 35 seconds, extension at 72 °C for 4 minutes, and a final extension for 5 minutes at 72 °C.

RABD(1247) (5 - AAGAGCCCGT-3) [16]. PCR was carried out in a 25µl reaction mixture, comprising of 100 ng of E. coli DNA, 1.5µl (10 pmol) each primer and 12.5µl Mastermixe. Filtered water was added to the mixture to make a final volume of 25µl. conditions were as follows 95°C for 15 min, 35 cycles of 94°C for 1 min, 38°C for 1 min and 72°C for 2 min, with a final 10 min elongation step at 72°C.

BOX (5 - CTACGGCAAGGCGAC-GCTGACG-3) (17). PCR was carried out in a 25µl reaction mixture, comprising of 100 ng of E. coli DNA, 1.5µl (10 pmol) each primer and 12.5µl Mastermixe. Filtered water was added to the mixture to make a final volume of 25µl.

Conditions were as follows: after an initial denaturation at 94_C for 5 min, 35 cycles of denaturation (94_C, 1 min), annealing (40_C, 2 min) and extension (72_C, 2 min) were performed, followed by a final extension (72_C, 10 min). Amplicons (15µl) were analyzed PCR products were detected in 1.5 % agarose gel, stained with ethidium bromide and visualized by transilluminator. Dendrograms were constructed using unweighted pair-group method of arithmetic average (UPGMA).

Results and Discussion

Ten *E. coli* isolates were obtained from 110 samples represented 9.09 % from the total samples collected from tap water the Baquba City between October tothe December 2019. *E. coli* identified known through the classical diagnostic and genetic diagnostic tests. All results shown in figure 1,2,3 and 4 also in Table 1.

All isolates of E. coli were tested for antibiotic sensitivity test according disck diffusion test against 12 different antibiotics Previously mentioned. The percentage of resistance to the antibiotics used as follows: Aztreonam was 80%, Cefpodoxime 70%, Trimethoprim- sulfamethoxazole 55 %, Cefoxitin 45%, Cefotaxime 45%, Doxycycline 70%, Azithromycin 60%, Ticarcillin-clavulanate 25%, Ampicillin-sulbactam 15%, Tetracycline 65%, Levofloxacin 50 % and Imipenem 30% as shown in the chart 1.

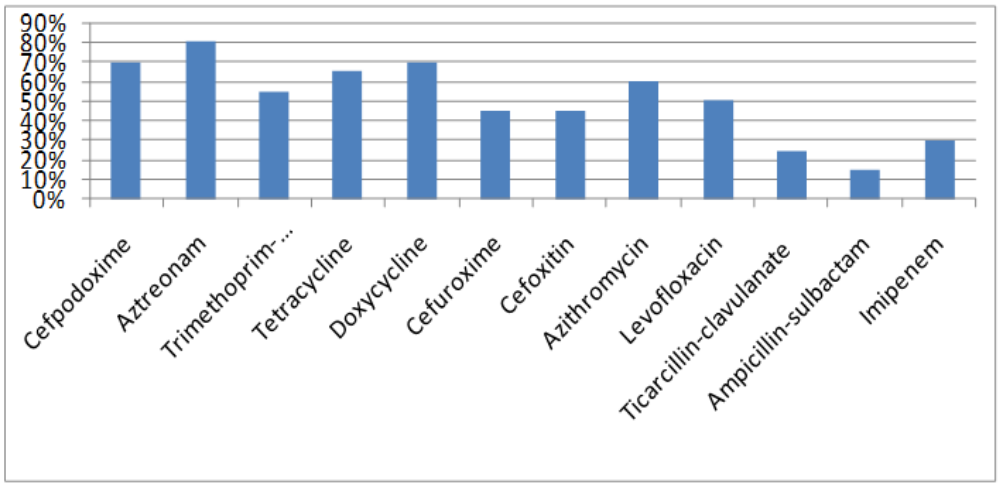

Fig. 1 : A diagram showing the resistance level of isolates for each Antibiotic

Transmission of the resistance between the intestinal family strains and the transition may be through spaying or bacterial coupling. Break the betalactam ring, or add proximity between the antibody and target sites (PBPs) or alter the permeability barrier [18,19].

Biofilm formation (Micro-titer plate method (MTP)): All *E coli* isolates from water were 10 (100%) isolates forming biofilms, 80% of isolates were Moderate biofilm where 20% of isolates were strongly biofilm

In other studies on bacterial resistance and biofilm formation in *E. coli* . in study for a total of 88 isolates the percentages of non-forming strains and weak, moderate and strong production capacity biofilms were 6.8, 36, 39.7 and 17.4%, respectively[20]

Detection of Acyl-homoserine lactone (AHL) by colorimetric method: Nine E coli isolates from water (90%) isolates Producer of Acyl-homoserine lactone.

Several studies have reported that *E. coli* is not able to produce AHL, yet has the ability to alter their pattern of gene expression and phenotypic properties in response to AHL[21].

Genotyping systems:

ERIC-PCR Profiling System: The isolates are classified according to the ERIC typing system and on the basis of the number of bands they own and according to the Dic program, the two main groups are apaered Group A and Group B:

The A group was characterized by having only 2 isolates . While group B was characterized by 8 isolates out of a total of 10 isolates under study. This group was divided into 2 subgroups under Group B, B1, B2 as shown in Diagram 2. The B1 group has one isolate only. The B2 group has 7 isolates.

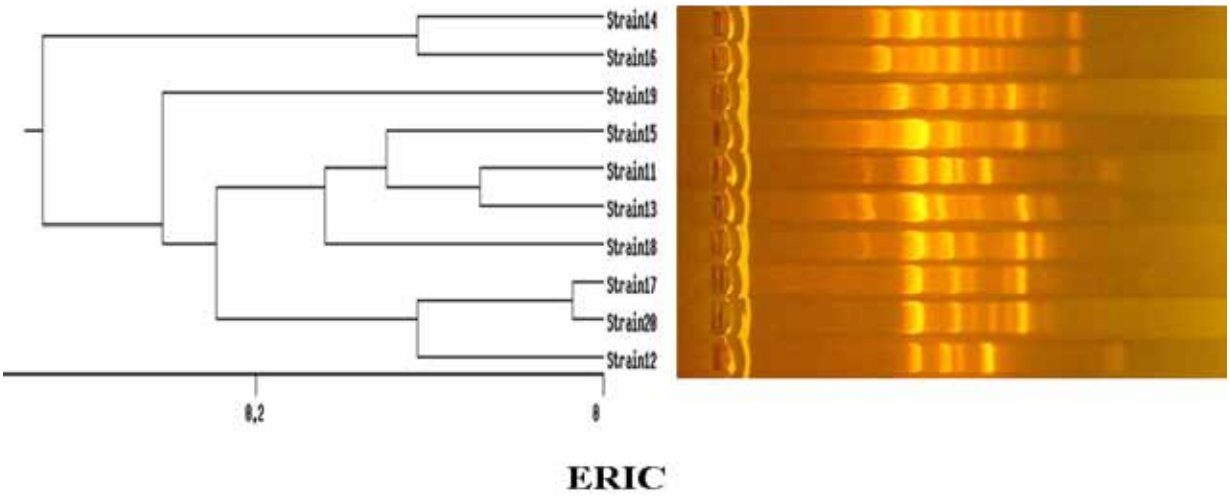

Fig. 2 :Dendritic analysis,and The resulting packets of ERIC - PCR reaction to some isolates of E.coli bacteria. (using electric relay technology for 1.5 hours and a voltage of 50 volts. The letter M denotes the volumetric guide 1kb

Many of the studies that used the ERIC-PCR system . Some of them differed in the size of the bands, their number or the number of patterns that emerged. Part of this difference may be due sometimes to the numerical variation of isolates used . The study explained that eighteen profoles each contained 2 to 5 isolates showing a similar genetic pattern. Similar genotype [22], The study showed that genetically modified isolates of the ERIC system of 27 different types can be grouped into 4 groups with similarities from 86 percent. Its bands are from 3 to 15 and the size is from 0.1 to 5.0 kb. [23]

BOX-PCR Profiling System: The isolates are classified according to the BOX typing system and on the basis of the number of bands they own and according to the Dic program, the two main groups are apaered Group A and Group B:

The A group was characterized by having 6 isolates. This group was divided into 2 subgroups under Group A, A1, A2 . The A1 group has two isolates. The B2 group has 4 isolates. While group B was characterized by 4 isolates. This group was divided into 2 subgroups under Group B, B1, B2 as shown in Diagram 3. The B1 group has one isolate only. The B2 group has 3 isolates.

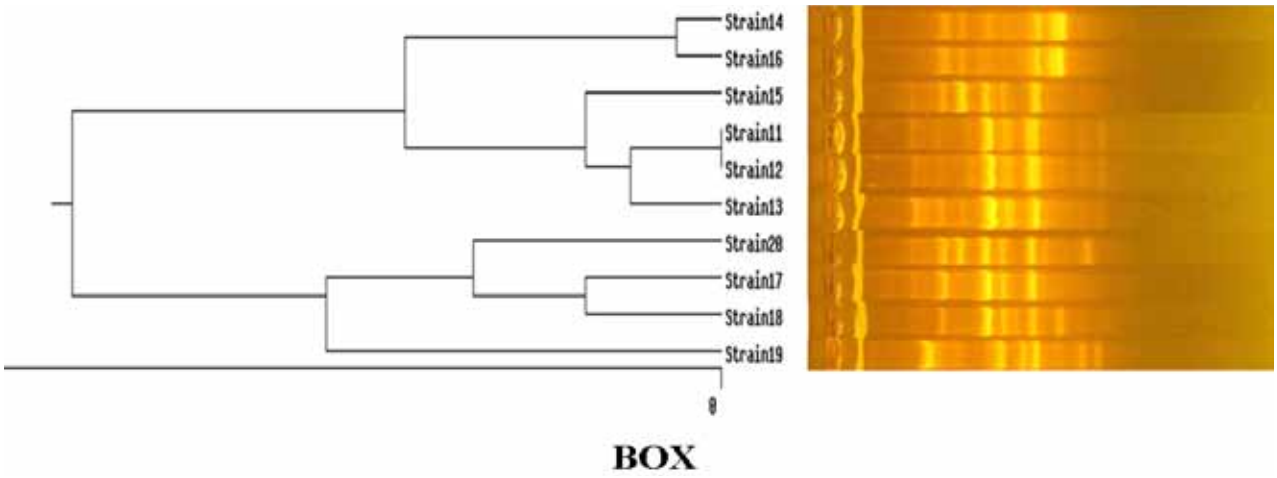

Fig. 3 :Dendritic analysis and the resulting packets of BOX- PCR reaction to some isolates of *E.coli* bacteria. (using electric relay technology for 1.5 hours and a voltage of 50 volts. The letter M denotes the volumetric guide 1kb)

BOX-PCR was second only to the PCR-5 GTG method. The researchers noted that the method of BOX-PCR showed high discriminative strength by generating 127 clear packets ranging from 0.16 to 3.9 kB whereas ERIC-PCR generated 61 packets ranging from 0.56 to[24]. The study also showed that stereotyping method that exploit the repeated elements distributed to the genome are more useful for assessing genetic diversity[25].

RAPD -PCR Profiling System: The isolates are classified according to the RAPD typing system and on the basis of the number of bands they own and according to the Dic program, the two main groups are apaered Group A and Group B:

The A group was characterized by having 6 isolates. This group was divided into 2 subgroups under Group A, A1, A2 . The A1 group has two isolates. The A2 group has 4 isolates. While group B was characterized by 3 isolates . This group was divided into 2 subgroups under Group B, B1, B2 as shown in Diagram 6. The B1 group has two isolated. The B2 group has one isolate. While one isolation was out group.

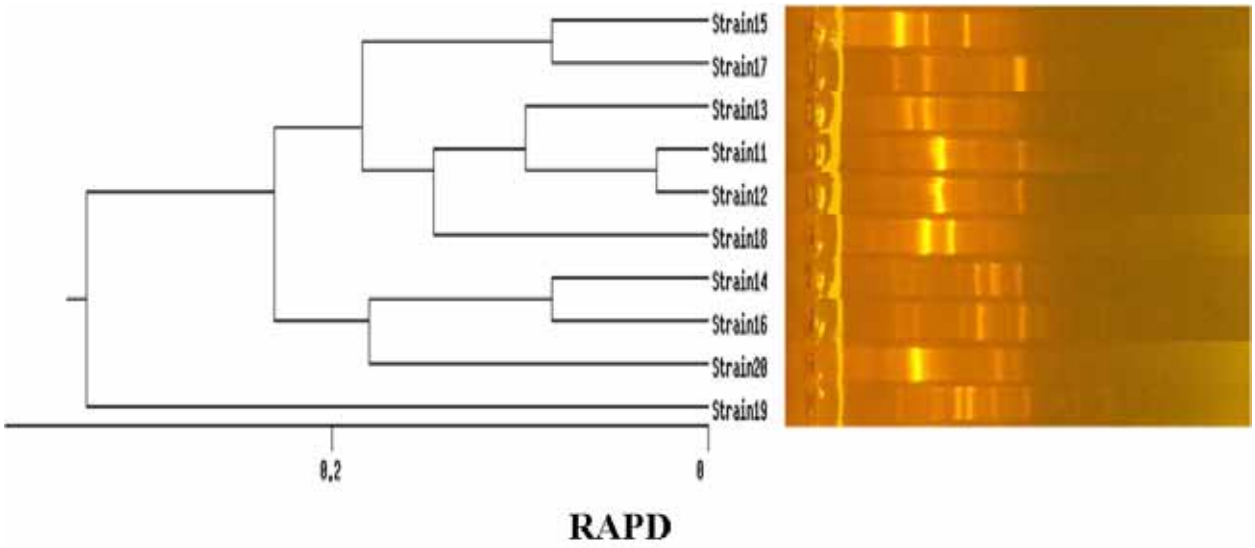

Fig. 4: Dendritic analysis and The resulting packets of RAPD- PCR reaction to some isolates of *E.coli* bacteria. (using electric relay technology for 1.5 hours and a voltage of 50 volts. The letter M denotes the volumetric guide 1kb)

The presented dual RAPD-PCR method has demonstrated a high level of resolution that allows for identification of distinct E. coli clones. Historically, RAPD assays have exhibited low reproducibility[26].

Table 1: Patterns of total isolates of *E. coli* according to the ERIC, BOX, RAPD system, number and percentage according to each category.

ERIC	**A**		**B1**	**B2**	
No. isolated	2		1	7	
Percentage %	20%		10%	70%	
BOX	A1	A2	B1	B2	
No. isolated	2	4	3	1	
Percentage %	20%	40%	30%	10%	
RAPD	A1	A2	B1	B2	Out group
No. isolated	2	4	2	1	1
Percentage %	20%	40%	20%	10%	10%
Total	10 (100%)				

Conclusion

The present study indicates that there is a correlation between taxonomic systems was evident by the distribution of isolates in each pattern and group.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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References

1. KaperJB, NataroJP, Mobley LT. Pathology Escherichia coli. Nat Rev Microbiol. 2004;2: 123–140.
2. Doughari HJ, Ndakidemi PA, Human IS, Benade S . Virulence factors and antibiotic susceptibility among verotoxic non O157: H7 Escherichia coli isolates obtained from water and wastewater samples in Cape Town, South Africa. Afri. J. Biotechnol. 2011; 10(6): 60-68.
3. Farrokh C, Jordan K, Auvray F, Glass K,Oppegaard H, Raynaud S. Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. Int .J. .Food. Microbiol. 2013; 162: 190-212.
4. Le Page S, Dubourg G,Baron SA, Rolain JM., Raoult D. Noglobal increase in resistance to antibiotics: a snapshot of resistance from 2001 to 2016 in Marseille, France. Eur J Clin. Microbiol. Infect. Dis. 2019; 38: 395-407.
5. Jarocki P, Podleśny M, Komoń-Janczara E, Piotr J, Kucharska J, Glibowska A, Targoński Z. Comparison of various molecular method for rapid differentiation of intestinal bifidobacteria at the species, subspecies and strain level. BMC. Microbiol. 2016; 22(16): 159.
6. Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Martinez JL. Tackling antibiotic resistance: The environmental framework. Nat. Revi. Microbiol. 2015; 13: 310–317.
7. Montealegre MC, Roy S, Böni F, Hossain MI, Caduff L. Risk Factors for Detection, Survival, and Growth of Antibiotic-Resistant and Pathogenic Escherichia coli in Household Soils in Rural Bangladesh . Applied .Environmental. Microbiol. 2018; 84 (24): 8–18.
8. Momtaz H, Karimian A, Madani M, Safarpoor DF, Ranjbar R, Sarshar M. Uropathogenic Escherichia coli in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob. 2013; 12(8).
9. Chess T. Microb. 18th edition. McGrow Hill. United State. 2012
10. CLSI (Clinical & Laboratory Standards institute). Performance standard for antimicrobial susceptibility testing Seventeenth informational supple -ment.2018; 27(1): 100-170.
11. Tang J, Kang M, Chen H, Shi X, Zhou R., Chen J. The Staphylococcal nuclease prevents biofilm formation in Staphylococcus aureus and other biofilm-forming bacteria. Sci. China. Life. 2011;54(9):863-9.
12. Almeida C, Azevedo NF, Santos S, Keevil CW. Correction: Discriminating Multi-Species Populations in Biofilms with Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA FISH). PLoS ONE.2013; 8 (6): 101-371.
13. Dietrich J, Mckee A, Keasling J. High-Throughput Metabolic Engineering: Advances in Small-Molecule Screening and Selection. Rev. Bioche. 2010;79:563-590.
14. Adwan K, Jarrar N, Abu-Hijleh A, Adwan G, Awwad E .Molecular characterization of Escherichia coli isolates from patients with urinary tract infections in Palestine. Journal of Medical Microbiology 2014;.63: 229–234.
15. Ardhami MA, Ranjbar R. Molecular typing of uropathogenic E coli strains by ERIC –PCR method. Medi. Bacteriol. 2016;8(4):2291-2296.
16. Nielsen KL,Godfrey PA, Stegger M, Andersen PS. Selection of Unique Escherichia coli Clones by Random Amplified Polymorphic DNA (RAPD): Evaluation by Whole Genome Sequencing .J. Microbiol. Method.2014;5:101-103.
17. Gambero ML, Blarasin M, Bettera S, AlboJG. Genetic diversity of Escherichia coli isolates from surface water and groundwater in a rural environment .J.water.Health.2017;15(5):757-765.
18. Turner PJ.. Extended-spectrum, B-lactamase. Clin. Infect. Dis.2005; 41(suppl. 4), s273-5.
19. Prats G, Miretis B, Miro E. Cephalosporin Risistant Escherichia coli among summer camp attendees

with salmonellosis . Emerg. Infect. Dis. 2003; 9(10), 1273-1278.

20. Shikh H, Shakibaie MR. Proteus Strains Isolated from Hospitalized Patients; Matter of Hospital Hygiene and Antimicrobial Stewardship. Pak. J. Biol. Sci. 2013; 16(22):1496-1502.
21. Ahmer BM. Cell-to-cell signalling in Escherichia coli and Salmonella enterica. Mol. Microbiol. 2004; 52(4):933-945.
22. Xia Y, Liang Z, Su X, XiongY. Characterization of carbapenemase genes in enterobacteriaceae species exhibiting decreased susceptibility to carbapenem in a university hospital in Chongqing, China. Ann Lab Med.2012; 32, 270-275.
23. Alitheen Z Y, Raha NS, Samuel A, Yeap L. Random amplifed polymorphic DNA-PCR and ERIC PCR analysis on Vibrio parahaemolyticus isolated from cockles in Padang, Indonesia. International Food Research Journal. 2009;16: 141-150.

24- Mohapatra BR, Broersma K, Mazumder A.Comparison of five rep-PCR genomic fingerprinting method for differentiation of fecal Escherichia coli from humans, poultry and wild birds Authors. Microbiology Letters 2007;98–106.

25. Van belkum A, Hermans PW.BOX PCR Fingerprinting for Molecular Typing of Streptococcus pneumoniae. Method Mol Med. 2001;48, 159-168.

26- Meunier JR, Grimont PA. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res Microbiol. 1993;144:373–379.