International Journal of Sciences

Research Article Volume 8 – March 2019 (03)

Detection of E.Coli Strains Isolated from Water Sources and Diarrhea Cases by Random Amplified Polymorphic DNA in Basrah Governorate

Zeena Hashim Abd Al-Wahid[1], Munaff Jawdat Abd Al-Abbas[1]

[1]Department of Biology, College of Science, University of Basrah, Basrah, Iraq

Abstract: One hundred samples of water and thirty of diarrheal cases were collected . Coliform bacteria was detected in most water samples by MPN to comparison with clinical coliform , tap water showed the higher rates of coliform than other sources . 23 different bacterial species from water and 3 from diarrhea were identified by *16SrDNA* . *E.coli* 26 (35%) as the prodomient species from water and 27 (79%) from diarrhea followed by *K. pneumonia* 11 (14.1%) and 4 (11.8%) respectively. Antibiotic susceptibility showed all *E.coli* isolates (100%) were MDR and the Ciprofloxacin was the more active antibiotic against isolates of water (59%) and clinics (38%) . In addition , the antibiotic susceptibility profiles showed the identical relatedness of 22 (53.65%) *E.coli* strains from water and diarrhea . Moreover , RAPD analysis showed 14 (34.14%) of *E.coli* strains from water and diarrhea were identical indicating to the fecal contamination of water.

Keywords: *E.coli*, RAPD, Fecal Contamination, Diarrhea, *16S rDNA*, Strains

Introduction

Water is the foundation of life's permanence , while water becomes a vehicle to transport water borne disease that caused dying to people worldwide (Fawell and Nieuwenhuijsen , 2003) . Water borne disease is actual health problem infected many people in a very short time by drinking , washing and bathing in a contaminated water with pathogenic bacteria from human excreta such as cholera , salmonellosis , shigeliosis , gastroenteritis , typhoid fever and diarrhea that caused death for 3.4 million people worldwide and to 4000 child (Cabral , 2010 ; WHO , 2011 ; UNICEF, 2014). Diarrheal infection is one of the most common water borne disease as a result to consume water contaminated with bacteria causing damage the balance of intestinal tract and losing large quantity of water and electrolyte out of the body (Slutsker *et al.*, 1997) . *E. coli* is a fecal coliform bacteria found in the intestines of human and warm blooded animals as a flora , however , *E. coli* can refer to the fecal contamination and failure of sanitation if detected in the water by Most Probable Number method , since there is some strains cause intestinal and extra – intestinal infections (Chao *et al*. 2003). On the other hand , the misuse and overuse of antibiotics facilitate Multi drug resistance (MDR) bacteria diffusion producing serious health problem (Reller *et al.*, 2009). Furthermore , antibiotics can also used to show the biotype profile of bacteria isolated from two sources such as stool and water (Muringani *et al.*, 2016) . Random Amplified Polymorphic DNA is genomic DNA finger print used to determine the genetic relatedness among bacterial strains from one or more sources , in addition to the type of microorganism for epidemiological purposes (Maiti *et al*.,2009) . There is a paucity in studies around the ability to determine the source of human infection (Maiti *et al*.,2009).The aim of this study was to determination the frequency of coliform bacteria in the water sources , and if there was a relatedness between *E.coli* strains from water and diarrhea , in addition to the antibiotic susceptibility profile of these bacteria .

Materials and methods

Samples collection

Most probable number (MPN) was performed according to Girdoniya (2011) . One hundred samples of house-hold drinking , tap , storage tanks of hospital , storage tanks of houses and filter water (from Basrah governorate from 8 October of 2017 to 15 march of 2018) . In addition , thirty samples of diarrhea collected from patients were tested to isolate coliform , the positive result of MPN and diarrheal stool were cultured on MacConkey agar (LAB , U.K.) to gain a pure colonies .

Published at: http://www.ijsciences.com/pub/issue/2019-03/
DOI: 10.18483/ijSci.1943; Online ISSN: 2305-3925; Print ISSN: 2410-4477

Zeena Hashim Abd Al-Wahid (Correspondence)
+

Identification the bacteria by *16SrDNA* amplification .

The DNA of 107 isolates including 75 from water and 32 from diarrhea extracted according to the procedure of Presto™ Mini g DNA bacteria kit (Geneaid , Taiwan) after refresh the pure colony in Brain heart infusion broth (LAB , U.K.) for 18 h. at 37 °C . *16SrDNA* was amplified according to Miyoshi *et al*.(2005) by using Alpha primers (Promega , USA) including 27F 5-AGAGTTTGATCCTGGCTCAG-3 and 1492 5-GGTTACCTTGTTACGACTT-3 of 1500bp . PCR reaction mixture (50µl) contains 25 µl of Go Taq Green master mix (Promega , USA) , 19 µl of Nuclease Free water (Bioneer , Korea) , 2 µl of DNA template and 2 µl from each primers . The thermo cycler (Applied Biosystem , USA) condition for amplification 95°C for 5min. followed by 35 cycles at 95 °C for 30 sec , 55° C for 30 sec and 72°C for 1min , the final extension was done at 72°C for 5 min . Agarose gel electrophoresis was performed (2 % of agarose powder, 25 ml of TBE buffer and 0.2 of Ethidium bromide) with 100bp DNA ladder (Promega , USA) to detect *16SrDNA* bands under UV transilluminator (Wisd , Korea) .

***16S rDNA* sequencing**

Twenty µl of PCR product for *16 S rDNA* gene were sending to Macrogen company for purifying and sequencing .All bacteria identified by BLAST related to National Center for Biotechnology Information.

Phylogenetic tree

The Phylogenetic tree were drawn by MAFFT (Multiple alignment program for nucleotides sequences) " http://mafft.cbrc.jp/alignment/server/ and viewed by forester 1064 after concatenated by comparison the result through Clustal Omega .

Antibiotic susceptibility test

Antibiotic susceptibility test was performed to 41 *E. coli* isolates from water and diarrhea to bio- type *E. coli* from both sources and to determine the suitable antibiotic for treatment by using fifteen antibiotic (Mast diagnostics , U.K.) according to Bauer *et al*.(1996) .

RAPD analysis

Random Amplified Polymorphic DNA (RAPD) was performed according to Nielsen *et al*., (2014) with 22 *E.coli* strains (detected by antibiotic profile) including 7 from water and 15 from diarrhea by using 5-AAGAGCCCGT-3 (Promega , USA) . Total volume 25 µl contains 12 µl of Go Taq Green master mix (Promega , USA) , 7 µl of Nuclease Free water (Bioneer , Korea) , 4 µl of DNA template and 2 µl from each primers . Thermo cycler (Bioneer , Korea) condition for amplification 94°C for 4min. followed by 45 cycles at 94 °C for 30 sec , 37° C for 40 sec and 72°C for 40 sec , the final extension was done at 72°C for 7 min . The PCR product detected by agarose gel electrophoresis identical to of *16s r DNA with* 25/100 bp Mixed DNA ladder (Bioneer , Korea) . The distance between RAPD bands of all isolates were calculated according to ladder's bands by Microsoft word then transferred to the program " Unweighted pair group method with Arithmetic mean" (UPGMA) to show the result as a dendogram (Garcia-Vallve and Puigbo ,2009) .

Results

MPN Most of water samples had been contaminated with coliform bacteria . Since , MPN test of the house drinking water , filtered water of houses with hospital water, daily storage tank of houses and tap water of houses were showed 29 of 34 (85.3%) , 3 of 4 (75%) , 25 of 29 (86.2%) , and 28 of 29 (96.6 %) respectively (Table 1) .

Bacterial identification

16 S r DNA for 112 isolates were obtained on agarose gel (2%) at a suitable size 1500bp as (Figure 1) . The sequencing of these isolates showed identification of 107 isolates including 23 bacterial species from water sources including *E. coli* 26 (35%) , *Klebsiella pneumonia* 11(14.1%), *Enterobacter cloacae* 8(10.3%), *Enterobacter ludwigii* 4 (5.13%) , *Aeromonas veronii* 4(5.13%) , *pseudomonas otitidis* 3(3.8%) , *Enterobacter xiangfangensis* 2(2.6%) , *Kluyvera cryocrescens* 2(2.6%) and 1 (1.3%) for all of *Enterobacter mori* , *pseudomonas mosselii* , *kluyvera georgiana* , *Aeromonas jandaei, Enterobacter cancerogenus* , *Pseudomonas aeroginosa* , *Klebsiella oxytoca* , *Acinetobacter calcoaceticus* , *Acinetobacter pittii* , *Pseudomonas putida* , *Enterobacter hormaechei* , *Aeromonas hydrophyla* , *Acinetobacter junii* , *Shigella sonnei* and *Leclercia adecarboxylata* and 3 bacterial species from diarrhea cases including *E.coli* 27(79.4%) , *Klebsiella pneumonia* 4(11.8%) and *Enterobacter cloacae* 1(2.9%) as Table (1) .

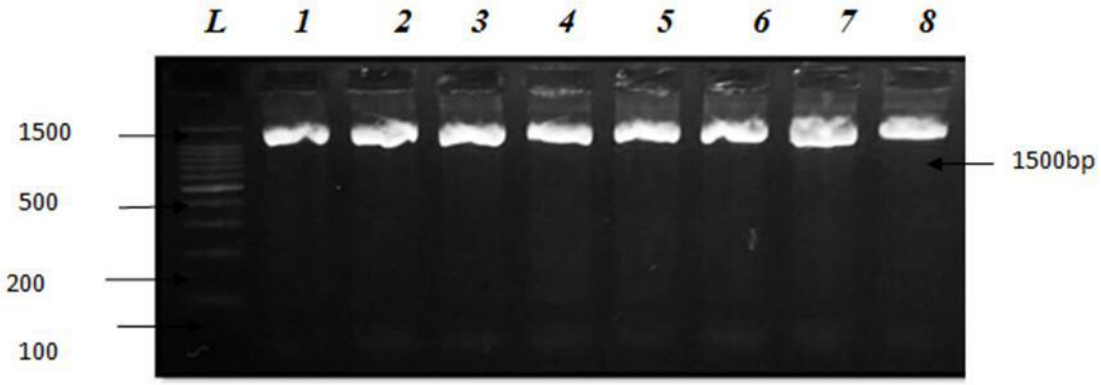


Figure 1: Agarose gel electrophoresis of PCR product for *16 S r DNA* (1500bp). Lane L : 100 bp DNA ladder , lanes 1-8 : *16 S r DNA* for bacterial isolates .

Phylogenetic tree

The Phylogenetic tree of water isolates included 21 bacterial species with their ATCC (Figure 2) . While , Figure (3) included 3 bacterial species of diarrhea cases with their ATCC .

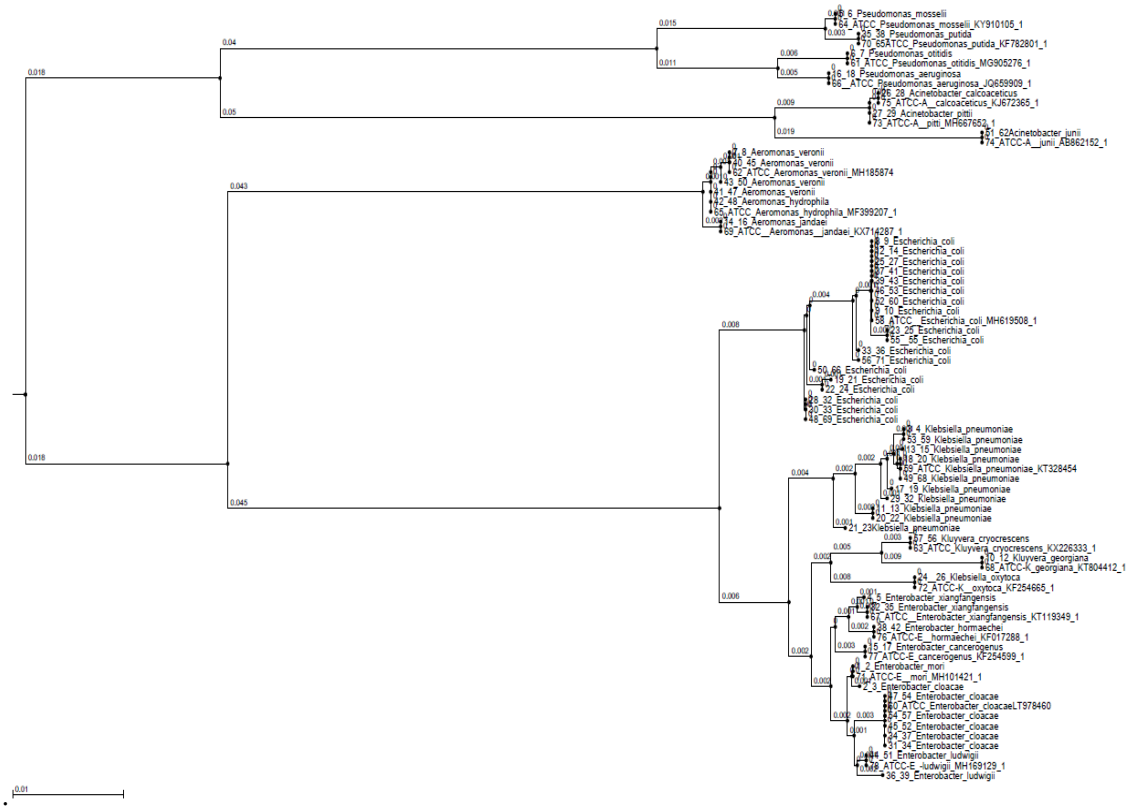


Figure 2: Rooted Neighbor Joining Phylogenetic tree constructed from concatenated sequences of 1310 bp for each strain (derived from an alignment of 16SrDNA sequences) then produced from a MAFFT alignment and visualized using forester version1046. This N-J tree showing the distribution and Phylogenetic relationships of 21 species isolated from water with their reference strains (ATCC). All horizontal branch lengths were drawn to scale. Bootstrap values after 1000 repetitions are indicated

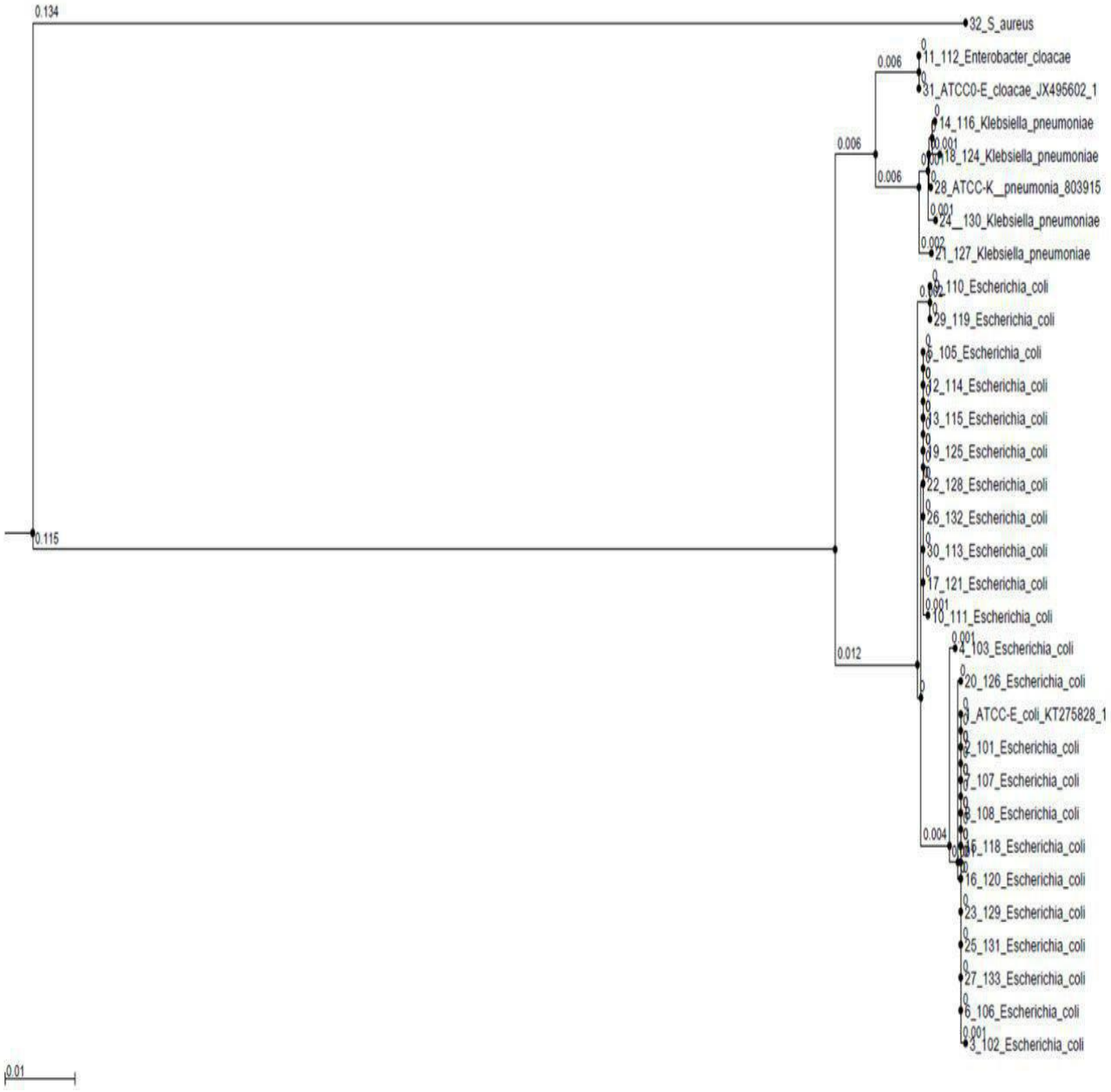


Figure 3: Rooted Neighbor Joining Phylogenetic tree constructed from concatenated sequences of 1318 bp for each strain (derived from an alignment of 16SrDNA sequences) then produced from a MAFFT alignment and visualized using forester version1046. This N-J tree showing the distribution and Phylogenetic relationships of 3 species were isolated from diarrhea with their reference strains (ATCC). All horizontal branch lengths were drawn to scale. Bootstrap values after 1000 repetitions are indicated S. aureus : out group .

Frequencies of bacterial species in sources

The frequency of bacterial species in drinking water showed high percentage of *E. coli* 7 (28%) then *Enterobacter cloacae* 4(16%) , *Enterobacter ludwigii* 2 (8%) and 1 (4%) of other bacterial species (Table 1) . *E.coli* represent 1 (33%) of filtered water and 67% of gram negative isolates (not identified) . 1 (25%) of storage tank of hospital was *E.coli* and 3(75%) are not identified . Tap water showed 9 (37.5 %) of *E.coli* **,** 4 (16.6%) for each *Klebsiella pneumonia* and *Enterobacter cloacae***,** 2 (8.3 %) of *Enterobacter xiangfangensis* and1 (4.2 %) for each

different species . Daily storage tank of houses showed 7 (31.8 %) of *Escherchia coli* , 5 (22.7%) of *Klebsiella pneumonia* ,2(9.09) of *Aeromonas veronii* and 1(4.5 %) for each different species . On the other hand , the frequency of clinical isolates showed 26 (81.3 %) of *E. coli* ,5 (15.6 %) of *K . pneumonia* and 1 (3.1 %) of *E. cloacae* . The frequency of bacterial species in clinical source was higher than in water sources with significant differences ($P \leq 0.05$) .

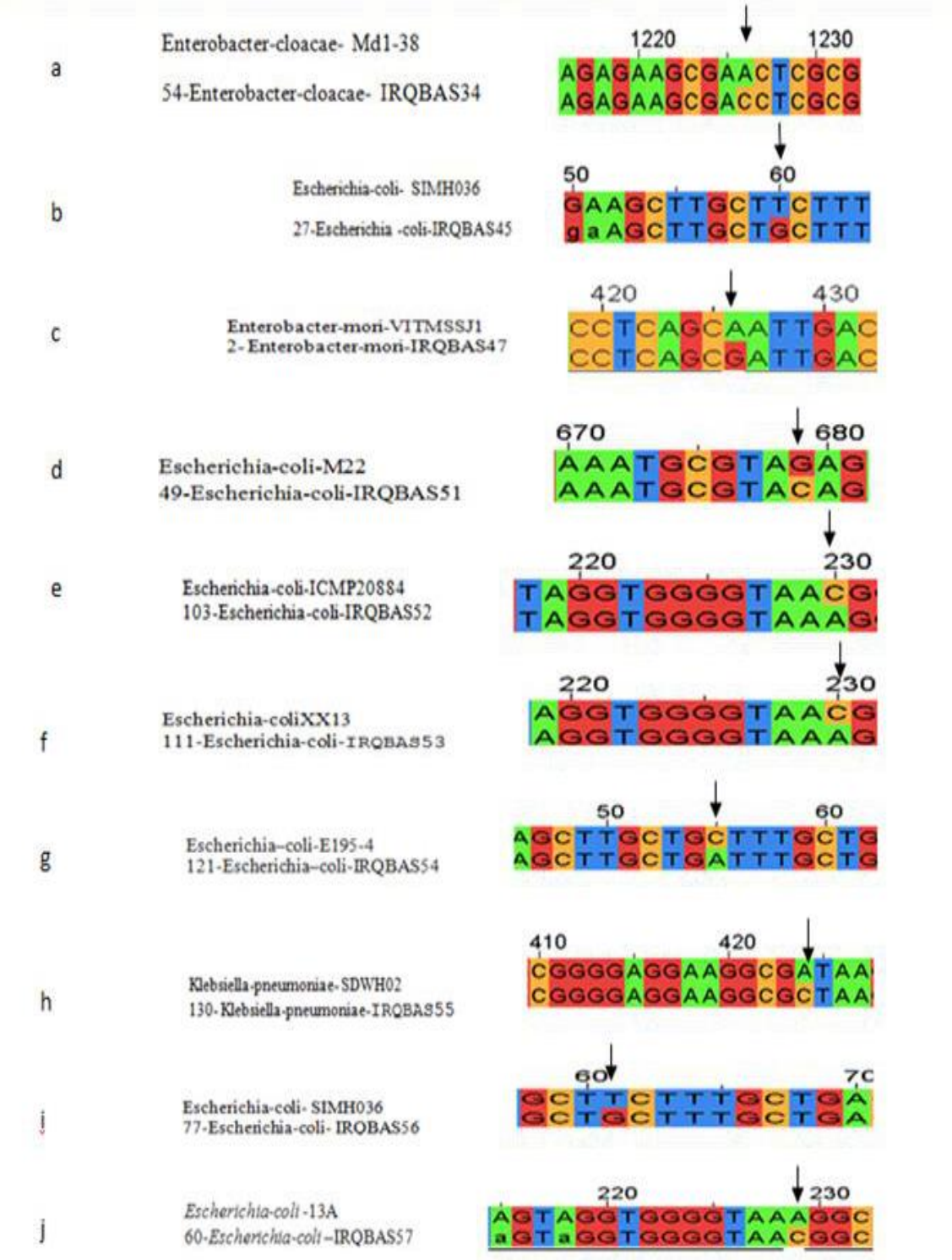
Table 1: Frequency of bacterial species in the sources and MPN index .

No.	Source	MPN Index %	n	Bacterial species	N	%
1	Drinking water (R.O) of houses	85.3	25	*Escherchia coli*	7	28
2				*Enterobacter cloacae*	4	16
3				*Enterobacter ludwigii*	2	8
4				*Enterobacter mori*	1	4
5				*Aeromonas veronii*	1	4
6				*Klebsiella pneumonia*	1	4
7				*Aeromonas jandaei*	1	4
8				*Enterobacter cancerogenus*	1	4
9				*Pseudomonas aeruginosa*	1	4
10				*Klebsiella oxytoca*	1	4
11				*Acinetobacter pittii*	1	4
12				*Pseudomonas otitidis*	1	4
13				*Kluyvera cryocrescens*	1	4
14				*Leclercia adecarboxylata*	1	4
15				*Acinetobacter junii*	1	4
16	Filter water of houses	75	1	*Escherchia coli* *Gr-ve	1 2	33% 67%
17	Storage tank of hospital water	75	1	*Escherchia coli* *Gr-ve	1	25% 75%
18	Tap water	96.6	24	*Escherchia coli*	9	37.5
19				*Klebsiella pneumonia*	4	16.6
20				*Enterobacter cloacae*	4	16.6
21				*Enterobacter xiangfangensis*	2	8.3
22				*Pseudomonas otitidis*	1	4.2
23				*Aeromonas veronii*	1	4.2
24				*Aeromonas hydrophila*	1	4.2
25				*Enterobacter ludwigii*	1	4.2
26				*Shigella sonnei*	1	4.2
27	Daily storage tank of houses water	86.2	22	*Escherchia coli*	7	31.8
28				*Klebsiella pneumonia*	5	22.7
29				*Aeromonas veronii*	2	9.09
30				*Pseudomonas otitidis*	1	4.5
31				*Pseudomonas mosselii*	1	4.5
32				*Kluyvera georgiana*	1	4.5
33				*Acinetobacter pittii*	1	4.5
34				*Pseudomonas putida*	1	4.5
35				*Enterobacter ludwigii*	1	4.5
36				*Kluyvera cryocrescens*	1	4.5
37				*Enterobacter hormaechei*	1	4.5
38	Diarrhea		32	*Escherchia coli*	26	*81.3*
39				*Klebsiella pneumonia*	5	*15.6*
40				*Enterobacter cloacae*	1	*3.1*

$P \leq 0.05$

4- Invention of new strains

Seventeenth isolates 12 from water and 5 from diarrhea were recorded in DDBJ and NCBI as new strains differed from their type strains in some nucleotide position (Figure 4) , as transversion mutation showed in strains No. 54-*Enterobacter cloacae* IRQBAS34 versus *Enterobacter cloacae* Md1-38 (C instead A) at 1226 bp , No. 27-*Escherichia coli* IRQBAS45 versus *Escherichia coli* SIMH036 (G instead T) at the position 60 bp , No. 49-*Escherichia coli* IRQBAS51 versus *Escherichia coli* M22 (C instead G) at the position 679 bp , No. 103- *Escherichia coli* IRQBAS52 and No.111-*Escherichia coli* IRQBAS53 were closely related to *Escherichia coli* ICMP20884 and XX13(respectively) (A instead C) at the position 230 bp, No. 121-*Escherichia coli* IRQBAS54 versus *Escherichia coli* E195-4 (A instead C) at the position 55 bp , No.130- *Klebsiella pneumoniae* IRQBAS55 versus *Klebsiella pneumoniae* SDWH02 (C instead of A) at the position 424 bp , No.77 - *Escherichia coli* IRQBAS56 versus *Escherichia coli* SIMH036 (G instead T) at the position 61 bp , No.60- *Escherichia coli* IRQBAS57 versus *Escherichia coli* 13A (C instead A) at the position 229 bp , No. 75-*Enterobacter ludwigii* IRQBAS59 was identical to *Enterobacter ludwigii* strainYPB10-1 but with four mutations ,three were transversion C instead T at the position 420 bp , T instead C at the position 1101bp and A instead C at the position 1102 bp and transition mutation A instead G at the position 437 bp . In addition ,there were six isolates as transition were showed in No. 2- *Enterobacter mori* IRQBAS47 versus *Enterobacter mori* VITMSSJ1 (G instead A) at the position 426 bp , No. 116-*Klebsiella pneumoniae* IRQBAS46 versus *Klebsiella pneumoniae* CCFM8358 (T instead C) at the position 132 bp, No.30-*Escherichia coli* IRQBAS49 versus *Escherichia coli* B70 (T instead C) at the position 174 bp , No. 32- *Klebsiella pneumoniae* IRQBAS50 versus *Klebsiella pneumoniae* YYM25 (T instead C) at the position 380 bp , No.74-*Klebsiella pneumoniae* IRQBAS58 versus *Klebsiella pneumoniae* NJ8 (C instead T) at the position 146 bp , No. 76- *Leclercia adecarboxylata* IRQBAS60 versus *Leclercia adecarboxylata* EGTM31 (C instead T) at the position 147 bp Figure (19) . Finally , there was a frame shift mutation in isolate No. 5- *Enterobacter xiangfangensis* IRQBAS48 versus *Enterobacter xiangfangensis* SitB501 (deletion T) at the position 418 bp .


a
Enterobacter-cloacae- Md1-38
54-Enterobacter-cloacae- IRQBAS34
1220
1230
b
Escherichia-coli- SIMH036
27-Escherichia -coli-IRQBAS45
50
60
c
Enterobacter-mori-VITMSSJ1
2- Enterobacter-mori-IRQBAS47
420
430
d
Escherichia-coli-M22
49-Escherichia-coli-IRQBAS51
670
680
e
Escherichia-coli-ICMP20884
103-Escherichia-coli-IRQBAS52
220
230
f
Escherichia-coliXX13
111-Escherichia-coli-IRQBAS53
220
230
g
Escherichia–coli-E195-4
121-Escherichia–coli-IRQBAS54
50
60
h
Klebsiella-pneumoniae-SDWH02
130-Klebsiella-pneumoniae-IRQBAS55
410
420
j
Escherichia-coli- SIMH036
77-Escherichia-coli- IRQBAS56
60
7C
j
Escherichia-coli -13A
60-Escherichia-coli –IRQBAS57
220
230

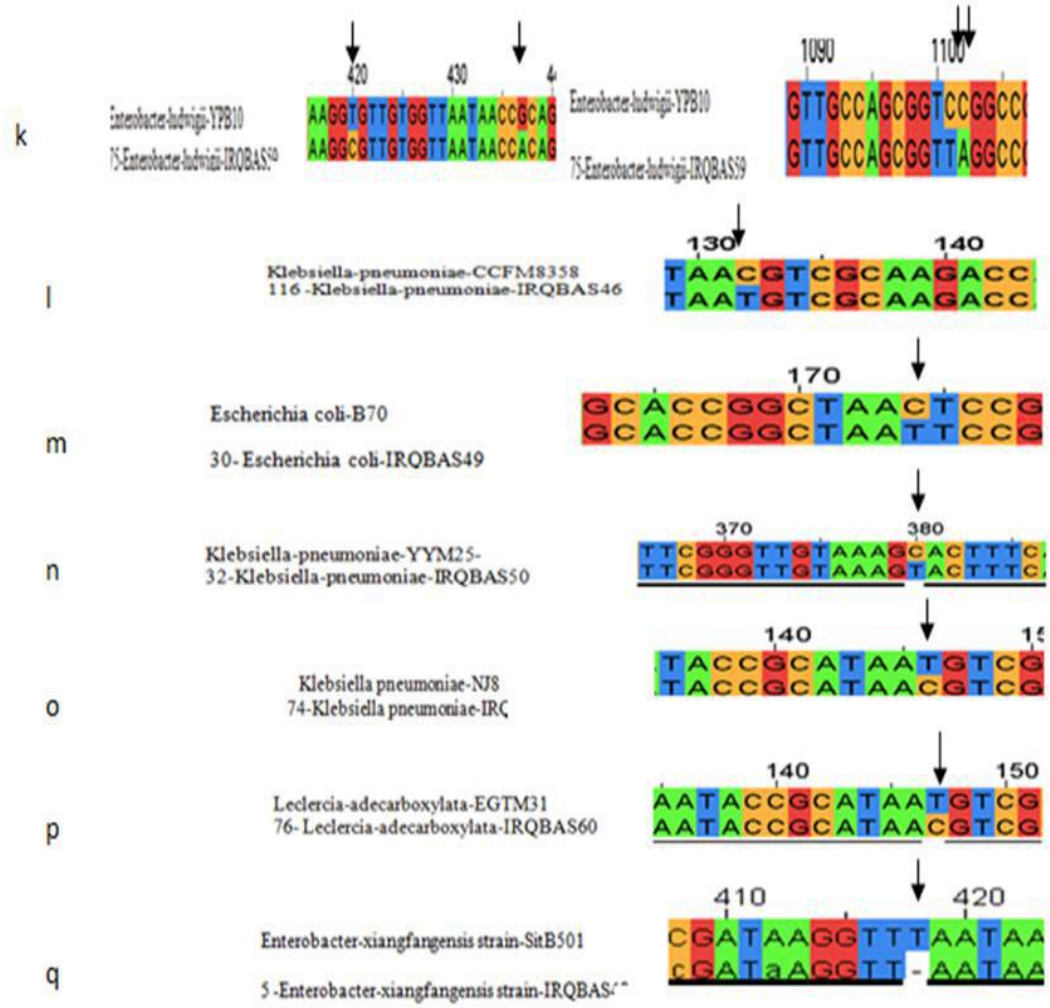


Figure (4) : a) Transversion mutation (C instead A).(b) Transversion mutation (G instead T) .(c) Transition mutation (G instead A) (d) Transversion mutation (C instead G) (e) Transversion mutation (A instead C) (f) Transversion mutation (A instead of C) (g) Transversion mutation (A instead C) (h) Transversion mutation (C instead A) (i) Transversion mutation (G instead T) (j) Transversion mutation (C instead A) (k) Three transversion mutation (C instead T) , T instead C and A instead C and transition mutation A instead G (l) Transition mutation (C instead T) (m) Transition mutation (T instead C) (n) Transition mutation (T instead C) (o) Transition mutation (C instead T) (p) Transition mutation (C instead T) (q) Frame shift mutation .

Antibiotic susceptibility

The antibiotic susceptibility to 41 *E. coli* isolates showed 5 groups of identical *E. coli* strains from water and diarrhea , each having the same antimicrobial drugs , represented 53.66% as (Table 2) . GroupA (No.21,24 from water and No. 108 ,109, 110,111,114, 115 ,119 and 132 from diarrhea) was resistant to all the 15 antibiotics. Group B (No.49 ,67 from water ,No. 103 ,129 from diarrhea) was sensitive to ciprofloxacin (CIP) only . Group C (No. 42 from water and No. 101 ,105 from diarrhea) was sensitive to cefoxitin (FOX) only . Group D (No.60 from water and No. 102 ,112 from diarrhea) was sensitive to only ciprofloxacin (CIP) and ampicillin sulbactum (SAM) . Group E (No. 30 from water and No. 118 from Diarrhea)was sensitive ampicillin sulbactum (SAM) only .

Table (2): Biotypes of *E. coli* isolates from water and diarrhea .

Groups	No. of isolate	CIP	SAM	FOX	IMI	MEM	AK	TS	PTZ	TM	CRO	CTX	SAZ	PRL	T	GM
A	21W 24W 108C 109C 110C 111C 114C 115C 119C 132C	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B	49W 67W 103C 129C	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C	42W 101C 105C	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R
D	60W 102C 112C	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R
E	30W 118C	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R

R: Resistance S:Sensitive

Ciprofloxacin (CIP) , Ampicillin sulbactum (SAM) , Cefoxitin (FOX) , Impenem (IMI) , Meropenem (MEM) , Amikacin (AK) , Trimthoprim sulfamethoxazole (TS) , Piperacillin tazobactam (PTZ) , Tobramycin (TM) , Cotrimoxazole (CRO) , Cefotaxime (CTX) , Ceftazidime (CAZ) , Piperacillin (PRL) , Tetracycline (T) , Gentamicin (GM) .

On the other hand ,all *E. coli* isolates from both sources (n=41) showed high resistance rates to antibiotic (Table 3) . In detail ,the water isolates showed 100% of isolates were resistant to Cefotaxime (CTX) , Ceftazidime (CAZ) , Piperacillin (PRL) , Tetracycline (T) and Gentamicin (GM) , 94% resistant to Amikacin (AK) , Trimthoprim sulfamethoxazole (TS) , Piperacillin tazobactam (PTZ) , Tobramycin (TM) and Cotrimoxazole (CRO) , 82% to Cefoxitin (FOX) and Impenem (IMI) , 64% to Ampicillin sulbactum (SAM) , 58 to Meropenem (MEM) and 41% to Ciprofloxacin (CIP) . While , 100% of clinical isolates were resistant to Cotrimoxazole (CRO) , Cefotaxime (CTX) , Ceftazidime (CAZ) , Piperacillin (PRL) , Tetracycline (T) , Gentamicin (GM) and Meropenem (MEM) , 95.8% to Piperacillin tazobactam (PTZ) and Tobramycin (TM) , 87% to Trimthoprim sulfamethoxazole (TS) , 79 % to Ampicillin sulbactum (SAM) , Impenem (IMI) and Amikacin (AK) , 75% to (FOX) and 62 % to Ciprofloxacin (CIP) with significant differences among them when ANOVA showed CIP and FOX the best antibiotic against clinical *E.coli* while CIP and MEM the best antibiotic against water isolates .

Table (3) : The antibiotic resistance profile of identical and non identical *E.coli* isolated from water and diarrhea cases .

Source	no. of isolate	CIP	SAM	FOX	IMI	MEM	AK	TS	PTZ	TM	CRO	CTX	CAZ	PRL	T	GM
water	17	7	11	14	14	10	16	16	16	16	16	17	17	17	17	17
%		41	64	82	82	58	94	94	94	94	94	100	100	100	100	100
clinical	24	15	19	18	19	24	19	21	23	23	24	24	24	24	24	24
%		62	79	75	79	100	79	87	95.8	95.8	100	100	100	100	100	100

* $P \leq 0.05$

Ciprofloxacin (CIP) , Ampicillin sulbactum (SAM) , Cefoxitin (FOX) , Impenem (IMI) , Meropenem (MEM) , Amikacin (AK) , Trimthoprim sulfamethoxazole (TS) , Piperacillin tazobactam (PTZ) , Tobramycin (TM) , Cotrimoxazole (CRO) , Cefotaxime (CTX) , Ceftazidime (CAZ) , Piperacillin (PRL) , Tetracycline (T) , Gentamicin (GM) .

1- Random Amplified Polymorphic DNA (RAPD) - PCR

According to RAPD performed with 22 (53.66%) *Escherichia coli* isolates that had identical patterns of antibiotic susceptibility(Figure 5to9) , there were 14 (34.14%) identical *E. coli* strains from water and diarrhea divided into 4 groups including the strain No.21 from house storage tank and No.24 from house tap water were identical to six clinical strains No. 108 ,109, 111, 115 ,119 and 132 (Figure 10 and Table 4) ,the strain No. 67 of drinking water (R.O) was identical to clinical strain No. 129, strain No. 42 of storage tank water was identical to clinical strain No.105, strain No. 30 of drinking water was identical to clinical strain No. 118, while strain No. 60 and 102 were closely related, but the other isolates of the two sources were consider as not related .

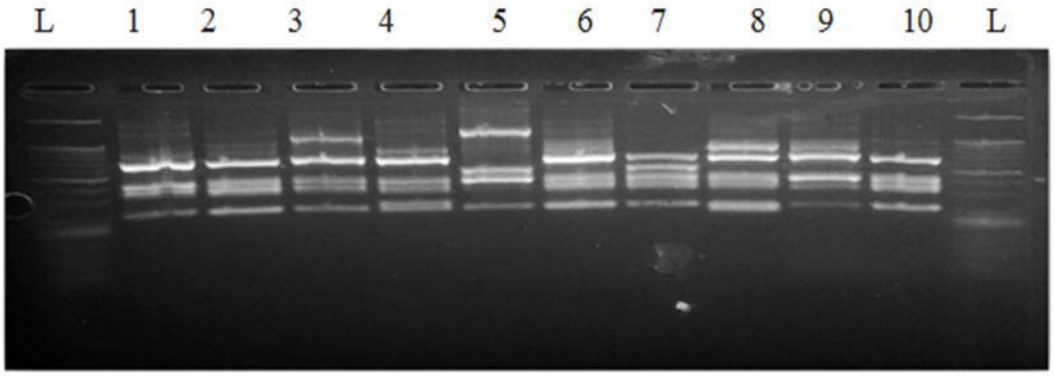


Figure5: Agarose gel electrophoresis (2%) showed RAPD patterns of *Escherichia coli* . Lane L : 25/100 bp Mixed DNA ladder. Lines1,2: Strains No.21,24 (water isolates) identical to line 3, 4, 6, 8, 9 and10 No. 108,109,111,115,119 and 132 (clinical isolates) .

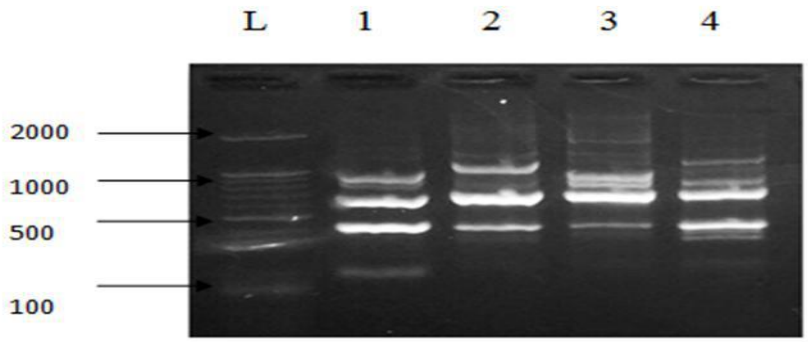


Figure6:Agarose gel electrophoresis (2%) showed RAPD patterns of *Escherichia coli* . Lane L : 25/100 bp Mixed DNA ladder ,line 2 strain No.67(water isolates) identical to line 4 No. 129(clinical isolates).

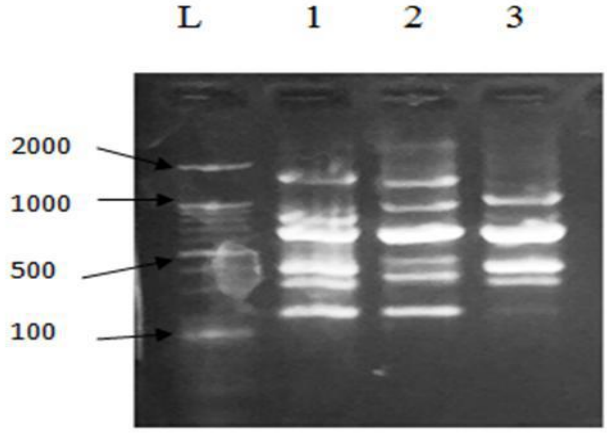


Figure 7 :Agarose gel electrophoresis (2%) showed RAPD patterns of *Escherichia coli* . Lane L : 25/100 bp Mixed DNA ladder , line1 Strain 42 (water isolate) identical to line 3 No. 105 (clinical isolates).

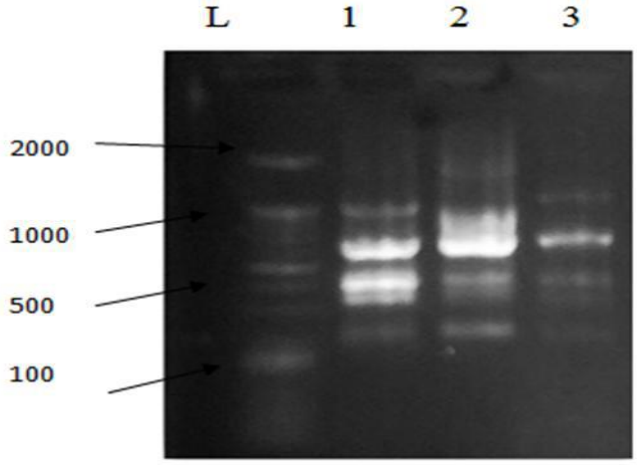


Figure 8 :Agarose gel electrophoresis (2%) showed RAPD patterns of *Escherichia coli* . Lane L : 25/100 bp Mixed DNA ladder , lines 1: strain No. 60(water isolate) closely related to line 2 strain No.102 (clinical isolates).

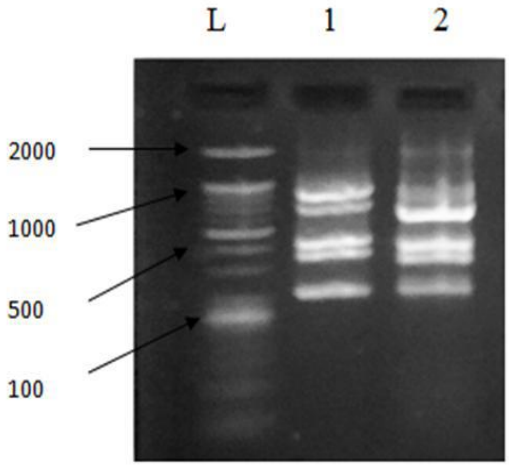


Figure 9 :Agarose gel electrophoresis (2%) showed RAPD patterns of *Escherichia coli* . Lane L : 25/100 bp Mixed DNA ladder , line 1: strain No.30(water isolate) identical to line 2 No.118 (clinical isolate) .

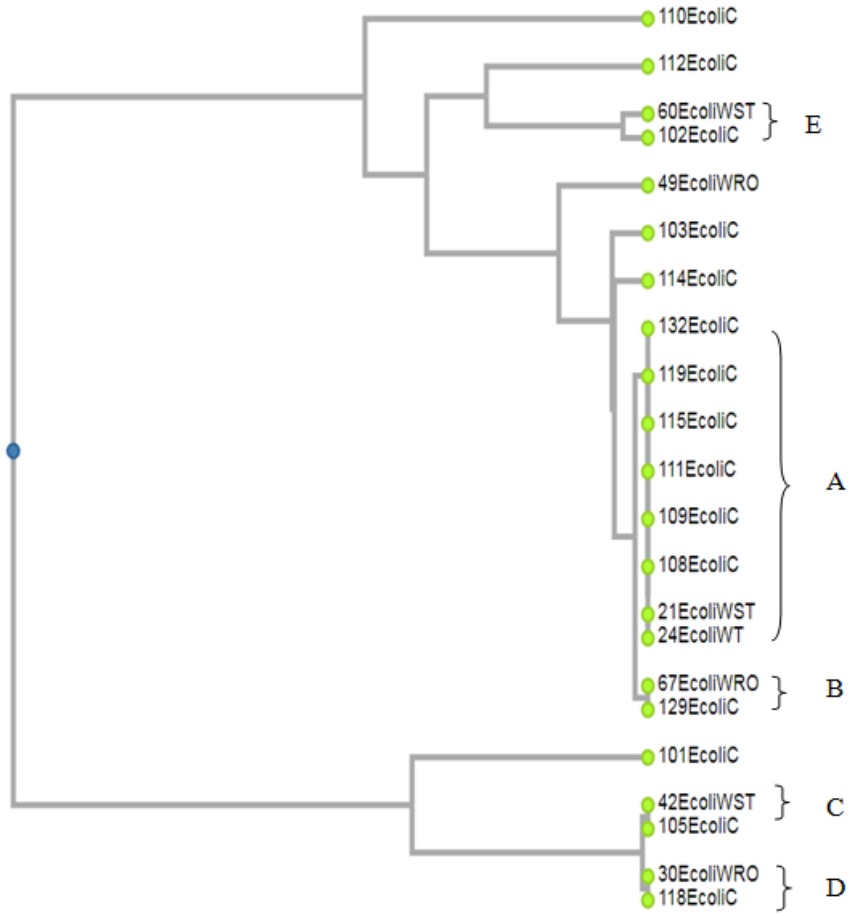


Figure 10 : Dendogram of *Escherichia coli* strains from water (21,24,30,42,49,60 and 67) and Diarrhea (101,102,103,105,108, 109, 110, 111, 112, 114, 115, 118, 119 , 129 and 132) performed by variables related to RAPD band using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm . Strains No.21 , 24 identical to No.108,109,111,115,119,132 ,strain No. 67 identical to strain No. 129, strain No.42 identical to 105 , strain No. 60 closely related to No. 102 and strain No.30 identical to 118 .

Table 4: The distance matrix among *E.coli* strains .

Strain	132	119	115	114	111	110	109	108	24	21	112	102	60	129	103	67	49	105	101	42	118	30
132	0																					
119	0.000	0																				
115	0.000	0.000	0																			
114	6.272	6.272	6.272	0																		
111	0.000	0.000	0.000	6.272	0																	
110	46.620	46.620	46.620	46.350	46.620	0																
109	0.000	0.000	0.000	6.272	0.000	46.620	0															
108	0.000	0.000	0.000	6.272	0.000	46.620	0.000	0														
24	0.000	0.000	0.000	6.272	0.000	46.620	0.000	0.000	0													
21	0.000	0.000	0.000	6.272	0.000	46.620	0.000	0.000	0.000	0												
112	55.447	55.447	55.447	52.497	55.447	68.392	55.447	55.447	55.447	55.447	0											
102	37.047	37.047	37.047	31.991	37.047	44.352	37.047	37.047	37.047	37.047	23.688	0										
60	20.284	20.284	20.284	21.515	20.284	39.859	20.284	20.284	20.284	20.284	30.010	4.150	0									
129	2.131	2.131	2.131	2.774	2.131	43.631	2.131	2.131	2.131	2.131	43.997	28.762	16.769	0								
103	5.514	5.514	5.514	7.407	5.514	31.186	5.514	5.514	5.514	5.514	69.164	34.075	19.865	6.930	0							
67	2.131	2.131	2.131	2.774	2.131	43.631	2.131	2.131	2.131	2.131	43.997	28.762	16.769	0.000	6.930	0						
49	15.276	15.276	15.276	13.495	15.276	62.768	15.276	15.276	15.276	15.276	47.506	42.245	34.868	10.640	20.832	10.640	0					
105	109.572	109.572	109.572	116.215	109.572	167.475	109.572	109.572	109.572	109.572	99.468	133.686	125.122	116.232	130.892	116.232	104.369	0				
101	72.044	72.044	72.044	76.760	72.044	131.741	72.044	72.044	72.044	72.044	127.381	99.826	85.731	83.165	72.598	83.165	80.788	42.088	0			
42	109.572	109.572	109.572	116.215	109.572	167.475	109.572	109.572	109.572	109.572	99.468	133.686	125.122	116.232	130.892	116.232	104.369	0.000	42.088	0		
118	96.490	96.490	96.490	104.298	96.490	160.530	96.490	96.490	96.490	96.490	95.028	125.502	114.473	103.818	118.091	103.818	93.779	0.898	36.344	0.898	0	
30	96.490	96.490	96.490	104.298	96.490	160.530	96.490	96.490	96.490	96.490	95.028	125.502	114.473	103.818	118.091	103.818	93.779	0.898	36.344	0.898	0.000	0

2- The association between diarrhea and fecal contamination water

As the result to RAPD test there was 14 (34.15%) identical *E.coli* strains including 5 from water and 9 from diarrhea with significant differences between the identical and non identical strains (Table 5) .

Table 5 : The disruption of *E.coli* strains groups between from diarrhea cases and water sources .

Total no. of water strains	Water sources	No. of *E. coli* strains in water	Total no. of clinical strains	Groups	No. of *E . coli* strains in diarrhea
5	S.T.H T	21 24	9	A	132 119 115 111 109 108
	R.O	67		B	129
	S.T.H	42		C	105
	R.O	30		D	118
34.15%					

$P \leq 0.05$ (S.T.H = Daily storage tanks of houses , R.O = Drinking water of houses , T = Tap water)

Discussion

Most Probable Number (MPN) is used because it is simple , fast , qualitative method to estimate and isolate coliform bacteria from water and to compare it with coliform isolated from diarrhea (Cappuccino and Sherman , 1996 ; Ahmed *et al.* , 2013) , MPN result showed the five sources of water were not suitable to use after comparing the result with WHO guidelines , as a result to high level of coliform bacteria per 100 ml , that is indicating to the poor sanitation of water , failure of chlorine pumping and possibility to the presence of pathogenic bacteria from fecal origin (Tharannum *et al.*, 2009) . *16 S r DNA* is a gold standard to identify bacterial isolates , it is fast and reliability method in a comparison with biochemical method , furthermore , providing a formation about the evolutionary relationships (Bosshard *et al*., 2006) . On the other hand , the Phylogenetic tree of water isolates didn't have *Shigella* and *Leclercia* because they haven't enough size like other isolates .

All bacteria isolated from water were a possible to cause infection , since it is a coliform and can become opportunistic especially when consumed with water , coliform should be not detected in 100 ml of water specified for human , since the intestinal tract was the natural inhabitant for these bacteria and the presence of coliform in water source indicated to fecal contamination (Gavriel *et al*., 1998 ; Heiman and Bowen , 2013) *E. coli* was the prodominant genus represented 26 (35%) of the present study in agreement with Kumar *et al.* ,(2013) . Coliform indicated to fecal contamination and caused gastrointrites espicially *E. coli* serotype O157:H7 and O111 associated with water borne disease , mortality , bloody diarrhea , watery diarrhea , abdomen crumps and hemolytic uramic syndrom due to failure of kidney (Ahmed *et al*., 2005 ; Garba *et al.* 2009) , *Klebsiella* represented 11(14.1%) in a water specified for human , however , *Klebsiella* causes multi-types of infections and its environmental strains has the same virulence factor of clinical strains. (Podschun *et al*., 2001) .On the other hand , the clinical isolates showed *E.coli* (79.4%) . However , there are six pathogenic strains having virulence factor responsible for severe diarrhea (Kaper *et al.*, 2004 ; Schmidt , 2010) . *K. pneumonia* (11.8%) is oppturstic pathogen causing multi infection in human including diarrhea that is the caustive agent to 22 diarrheal infection in China (Gassama-Sow *et al.*, 2010 ; Lu *et al.*, 2017) . *E. cloacae* is a specific strain

having shiga like toxin and usually related to health care infection (Probert *et al.*, 2014) .

Antibiotic susceptibility test Table (1) showed there was a five groups of identical strains from different water sources and diarrhea indicated to the fecal contamination of the tap , storage tanks and drinking water since the similarity of antibiotic profile was found between the *E.coli* isolated from both sources that can give a possible cause to diarrhea cases especially when 65.63% of clinical *E.coli* were as an axenic culture . On the other hand , the RAPD analysis showed four groups of identical strains including 5 strains from water and nine from diarrhea (Table 5) indicating to actual health problem and there is a route between sewage water and human using water . Nevertheless , *E.coli* isolated from both sources may be have the same virulence factor (Obi *et al.*, 2007 ; Ramalivhana *et al*., 2010) . Antibiotic susceptibility test show not only biotype but also high resistance rate when 100% of *E.coli* isolates were MDR because it is resistance to more than three antibiotics related to different classes (Magiorakos *et al*., 2012) . One hundred percent of clinical and water isolate resistant to Cefotaxime (CTX) , Ceftazidime (CAZ) , Piperacillin (PRL) , Tetracycline (T) and Gentamicin (GM) since bacteria have and developed many mechanism to resist antibiotic such as producing beta lactam enzyme , modification the target sites , point mutation , reduce cell permeability , in addition to transfer the resistance gene by trans-genetic gene such as plasmid (Livermore *et al.*, 2001 ; Pitout *et al*. , 2008) while ciprofloxacin was the best through this study in agreement with Muringani *et al.* (2016) .

Conclusions

Water was unsafe **,** many species of bacteria were found in the water from the five sources **,** *E.coli* and *K. pneumonia* were the most common bacteria in water and diarrhea samples , the five sources of water were contaminated with coliform bacteria referring to the stool as a contamination source .Since , the most *E.coli* strains isolated from water were identical to strains isolated from diarrhea specimens .

References

1. Ahmed, T. A. S. N. I. A., Acharjee, M., Rahman, M. S., Meghla, M., Jamal, J., Munshi, S. K., & Noor, R. A. S. H. E. D. (2013). Microbiological study of drinking water: qualitative and quantitative approach. *Asian Jr. of Microbiol. Biotech. Env. Sc*, *15*(4), 23-458.
2. Ahmed, W., R. Neller and M. Katouli, (2005). Host species-specific metabolic fingerprint Database for Enterococci and *Escherichia coli* and its application to identify sources of fecal contamination in surface waters. Applied Environ. Microbiol., 71: 4461-4468
3. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, *45*(4_ts), 493-496.
4. Bosshard, P. P., Zbinden, R., Abels, S., Böddinghaus, B., Altwegg, M., & Böttger, E. C. (2006). *16S rRNA* gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of non fermenting Gram-negative bacteria in the clinical laboratory. *Journal of clinical microbiology*, *44*(4), 1359-1366.
5. Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International journal of environmental research and public health*, *7*(10), 3657-3703.
6. Campana, R., Scesa, C., Patrone, V., Vittoria, E. and Baffone,W. (2006) Microbiological study of cosmetic products during their use by consumers: health risk efficacy of preservative systems. Lett Appl Microbiol 43, 301–306
7. Cappuccino, J. G., & Sherman, N. (1996). Microbiology: a laboratory manual. Publishing Co., Inc., Menlo Park, California
8. Chao, K. K., Chao, C. C., & Chao, W. L. (2003). Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical freshwater environments. *Journal of Microbiology, Immunology and Infection*, *36*(4), 288-293.
9. Farris, J. S. (1982). "Out groups and Parsimony". Systematic Biology. 31 (3): 328–334. doi:10.1093/sysbio/31.3.328. ISSN 1063-5157.
10. Fawell , J. and Nieuwenhuijsen , M.J. (2003).Contaminants in drinking water. Br. Med. Bull.68:199-208.
11. Garba, I., M.B. Tijjani, M.S. Aliyu, S.E. Yakubu, A. Wada-Kura and O.S. Olonitola,(2009).Prevalence of *Escherichia coli* some public water sources in Gusau Municipal, North Western Nigeria. Bayero J. Pure Applied Sci., 2: 134-137.
12. Garcia-Vallve S. and Puigbo P. (1999-2009). "Dendo UPGMA: A dendrogram construction utility", Universal Rovira i Virgili (URV).Tarragona. Spain.
13. Gassama-Sow, A., Diallo, M. H., Wane, A. A., Seck, A., Samb-Ba, B., Sow, P. S., & Aïdara-Kane, A. (2010). Genetic determinants of antibiotic resistance in diarrheagenic Klebsiella pneumoniae subspecies ozaenae: an emerging enteropathogen in Senegal. *Clinical infectious diseases*, *50*(3), 453-454.
14. Gavriel, A. A., Landre, J. P. B., & Lamb, A. J. (1998). Incidence of mesophilic Aeromonas within a public drinking water supply in north-east Scotland. *Journal of Applied Microbiology*, *84*(3), 383-392.
15. Girdoniya, M. V. (2011) MPN Test to Detect the Presence of Coliforms in Pond Water and Potable Water.
16. Heiman KE & Bowen A (2013). Shigellosis. In: Travelers' Health (Ed.). Infectious Diseases Related to Travel. Centers for Disease Control and Prevention, 1600 Clifton Rd. Atlanta, GA, USA. 30: 333.
17. Hidron, A. I., Edwards, J. R., Patel, J., Horan, T. C., Sievert, D. M., Pollock, D. A., & Fridkin, S. K. (2008). Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infection Control & Hospital Epidemiology*, *29*(11), 996-1011
18. Janda, J. M., & Abbott, S. L. (2006). The Genera Klebsiella and Raoultella.*The Enterobacteria* (2nd ed., pp. 115-129). Washington, USA: ASM Press.
19. Kaper, J.B., Nataro, J.P., Mobley, H.L.(2004) Pathogenic *Escherichia coli*. Nat Rev Microbial , 2:123-40 .
20. Kumar, D., Malik, S., Madan, M., Pandey, A., & Asthana, A. K. (2013). Bacteriological analysis of drinking water by MPN method in a tertiary care hospital and adjoining area Western UP, India. *J Environ Sci*, *4*, 17-22.

21. Lee, M. F., Peng, C. F., Lin, Y. H., Lin, S. R., & Chen, Y. H. (2008). Molecular diversity of class 1 integrons in human isolates of Aeromonas spp. from southern Taiwan. *Jpn J Infect Dis*, *61*(5), 343-349.
22. Livermore, D. M., Winstanley, T. G., & Shannon, K. P. (2001). Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *Journal of Antimicrobial Chemotherapy*, *48*(suppl_1), 87-102.
23. Lu, M. C., Chen, Y. T., Chiang, M. K., Wang, Y. C., Hsiao, P. Y., Huang, Y. J., ... & Lai, Y. C. (2017). Colibactin contributes to the hypervirulence of pks+ K1 CC23 Klebsiella pneumoniae in mouse meningitis infections. *Frontiers in cellular and infection microbiology*, *7*, 103.
24. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Paterson, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.
25. Maiti, B., Shekar, M., Khushiramani, R., Karunasagar, I., & Karunasagar, I. (2009). Evaluation of RAPD-PCR and protein profile analysis to differentiate Vibrio harveyi strains prevalent along the southwest coast of India. *Journal of genetics*, *88*(3), 273-279.
26. Miyoshi, T., Iwatsuki, T., & Naganuma, T. (2005). Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-size filters. *Applied and environmental microbiology*, *71*(2), 1084-1088.
27. Muringani, B. N., Obi, C. L., Apalata, T., & Vasaikar, S. D.(2016) . Bacterial Etiology Of Diarrheal Syndrome And Susceptibility Profile Of Enteropathogens Among Patient Attending Mthata General Hospital. 3 (3) .
28. Nielsen, K. L., Godfrey, P. A., Stegger, M., Andersen, P. S., Feldgarden, M., & Frimodt-Møller, N. (2014). Selection of unique Escherichia coli clones by random amplified polymorphic DNA (RAPD): Evaluation by whole genome sequencing. *Journal of microbiological methods*, *103*, 101-103.
29. Obi , C.L., Ramalivhana , J., Samie ,A., Igumbor , E.O. (2007) Prevalence pathogenesis, and antibiotic susceptibility profiles of *Aeromonas* isolatesfrom stool samples of patients in the Venda region of South Africa. *J Health Popul Nutr*;25:428-35.
30. Pitout, J. D. D., Revathi, G., L Chow, B., Kabera, B., Kariuki, S., Nordmann, P., & Poirel, L. (2008). Metallo-β-lactamase-producing Pseudomonas aeruginosa isolated from a large tertiary centre in Kenya. *Clinical Microbiology and Infection*, *14*(8), 755-759.
31. Probert, W. S., McQuaid, C., & Schrader, K. (2014). Isolation and identification of an Enterobacter cloacae strain producing a novel subtype of Shiga toxin type 1. *Journal of clinical microbiology*, JCM-00338.
32. Probert, W. S., McQuaid, C., & Schrader, K. (2014). Isolation and identification of an Enterobacter cloacae strain producing a novel subtype of Shiga toxin type 1. *Journal of clinical microbiology*, JCM-00338.
33. Ramalivhana, J. N., Obi, C. L., Samie, A., Labuschagne, C., & Weldhagen, G. F. (2010). Random amplified polymorphic DNA typing of clinical and environmental Aeromonas hydrophila strains from Limpopo province, South Africa. *Journal of health, population, and nutrition*, *28*(1), 1.
34. Reller, L. B., Weinstein, M., Jorgensen, J. H., & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical infectious diseases*, *49*(11), 1749-1755.
35. Schmidt, M. A. (2010). LEE ways: tales of EPEC, ATEC and EHEC. *Cellular microbiology*, *12*(11), 1544-1552
36. Slutsker, L., Ries, A. A., Greene, K. D., Wells, J. G., Hutwagner, L., & Griffin, P. M. (1997). Escherichia coli O157: H7 diarrhea in the United States: clinical and epidemiologic features. *Annals of internal medicine*, *126*(7), 505-513.
37. Tharannum, S., Sunitha, S., Nithya, J., Chandini, M., Vanitha, J., Manjula, T. S., & Shyam Sundar, C. (2009). Molecular confirmation of the presence of coliforms in drinking water using polymerase chain reaction. *Kathmandu Univ J Sci Eng Technol*, *5*(2), 130-136.
38. United Nation Children's Fund (UNICEF) (2014) World Water Day 2025: 4,000 children die each day from a lack of safe water., http://www.unicef.org/wash/ index_25637.htm
39. WHO (2010) Millennium Development Goals: progress towards the health-related Millennium Development Goals.,http://www.who.int/mediacentre/factsheets/fs290/en/index.html.