South Asian Journal of Research in Microbiology

5(1): 1-13, 2019; Article no.SAJRM.52559
ISSN: 2582-1989

Isolation and Culture Dependent Characterization of *Escherichia coli* from the Sewage Waste Water of Lahore, Pakistan

Abida Bano[1] and Basharat Ali[1*]

[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore - 54590, Pakistan.

Authors' contributions

This work was carried out in collaboration between both authors. Author AB conducted the sampling, experimental work and wrote the first draft of the manuscript. Author BA designed the study, wrote the protocol and managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2019/v5i130122
Editor(s):
(1) Dr. Obafemi Yemisi Dorcas, Assistant Lecturer, Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria.
Reviewers:
(1) Justyna Mazurek-Popczyk, University of Zielona Góra, Poland.
(2) Arnaldo Sarti, Institute of Chemistry - IQ - Unesp - Araraquara, Brazil.
(3) Byron Baron, University of Malta, Malta.
Complete Peer review History: https://sdiarticle4.com/review-history/52559

Original Research Article

Received 06 September 2019
Accepted 11 November 2019
Published 15 November 2019

ABSTRACT

Aims: Infectious disease haemorrhagic uremic syndrome (HUS), present in sewage water, its cross contamination with drinking water may affect the community. This study was conducted to isolate the pathogenic strain of *E. coli* O157 from the sewage waste water of Lahore, Pakistan. The aim of this study was to evaluate biofilm formation and antibiotic susceptibility pattern for the isolated strains. Selective Hichrome EC O157 agar media was used for the isolation of *E. coli*.

Methodology: Taxonomic status of strain was confirmed by 16S rRNA gene sequencing. Serotyping of *E. coli* O157 strains was accomplished by Prolex^{TM} *E. coli* O157 Latex Test Reagent Kit results for O157. Bacterial strains were also evaluated for biofilm formation, toxin related genes (*stx1*, *stx2*, *stx2c*, *stx2d*) and antibiotic sensitivity.

Results: Bacterial strains showed resistance against amoxicillin, tobramycin, tetracycline and nitrofurantoin antibiotics. Maximum biofilm formation was shown by strain E124 when used as

**Corresponding author: Email: basharat.ali.mmg@pu.edu.pk;*

monoculture. In cocultures, strains E35 and E101 were the most efficient biofilm formers. PCR amplification recorded negative results for shiga toxin genes.
Conclusion: Shiga toxin genes were not present in any of the *E. coli* strain, from which we can suggest that our environment is free of shiga toxin genes. *E. coli* was present in sewage water, its cross contamination with drinking water may affect the community. Therefore, waste water should be treated properly before discarding it into the common water bodies.

Keywords: Shiga toxin; haemorrhagic uremic syndrome; biofilm formation; sewage water; biofilm production.

1. INTRODUCTION

Water plays a very vital role in the socioeconomic development of human population. Pure water is colorless, free of germs, smell, turbidity and abnormal taste. Whereas, polluted water contains microbial load, poisonous chemical substances and organic and inorganic wastes [1]. After oxygen, water is second most important ingredient for the sustenance of life [2]. In China, according to their standards 75 percent of people have no access to fresh drinking water [3]. Compounds of mercury emanating from mining process and nitrogen used in agriculture contaminate natural water bodies after discharge [4].

Hospital or medical waste is defined as any waste generated during diagnosis or treatment of the disease. Globally many problems arises due to inappropriate medical waste management techniques. Incineration of solid waste is accompanied all around the world to reduce medical waste. Solidification with cement and recycling are the other methods to reduce medical waste [5]. To control infections and to maintain hygiene levels, hospital wastes should be effectively disposed according to established policies. Hospital waste can contaminate the environment that can cause the spread of diseases. Poor management of waste can lead to land pollution that can contaminate ground water resources. Environment and public health is at great risk due to occurrence of large number of pathogenic organisms. The major source of potable water in developing countries is ground water reservoirs due to long retention time and natural retention capacity of aquifers, but they can be contaminated due to poor sanitation [6].

Escherichia coli is a gram negative facultative anaerobe which ranges from harmless commensals living in the gastrointestinal tract of humans to pathogenic strains causing mortality rate in humans and animals. Based on the presence of virulence factors, *E. coli* is toxin-producing sub- divided into different pathotypes such as enterotoxigenic (ETEC), enteroaggregative (EAggEC), shiga (STEC), enteropathogenic (EPEC), diffusely adherent (DAEC) and enteroinvasive (EIEC) in humans [7].

In gene transfer has played a key role in the emergence of genetic diversity of *E. coli* [8]. Use of different antibiotics such as cephalosporins, penicillins, tetracycline, and sulfa drugs has increased risk of antimicrobial resistance of *E. coli* [9]. Multiple antimicrobial resistance in STEC and non-STEC is due to spread of genetic element i.e transposons, integrons and plasmids [10].

In 1977 *E. coli* (STEC) O157:H7 strains producing shiga toxin were first isolated from cattle in Argentina that was identified as STEC O157:H7. The primary reservoir for strains O157:H7 and non-O157 STEC is cattle [11]. Cattle is asymptomatic carrier for *E. coli*, this organism reside in its hindgut and is shed through feces [12]. It can be transmitted through consumption of un-pasteurized dairy products, contaminated fruits and vegetables, undercooked meat or water contaminated by faeces from carriers [13]. Shiga toxin producing *E. coli* O157:H7 is responsible for many outbreaks and sporadic cases (Hemorheagic Uremic Syndrome). However STEC non O157 also cause severe illness in humans [14]. Many outbreaks of bloody diarrhea due to *E. coli* O157:H7 has been reported in United States, Canada, and Japan [15]. In 2011, Germany and other European countries faced an extraordinary outbreak caused by extremely virulent *E. coli* strain, due to presence of shiga toxin (Stx) acquired through gene transfer. Countries faced serious economic loss due to export bans and withdrawl of food from the market [16]. Around 100 serotypes of *E. coli* which produce shiga toxins have been reported [17]. Two types of protein toxins stx1 and stx2 present in *E. coli* O157 are major virulence factors for the cause of bloody diarrhea [18]. Stx1 and stx2 or their variants causes cell death by inhibiting protein synthesis of host cells [14].

E. coli can be detected by different methods. Polyphasic taxonomy technique is used to detect *E. coli* strains. Different types of differential, selective and chromogenic media are used for its detection like coliform agar, Chromocult® coliform agar, and Rainbow agar [19]. *E. coli* O157:H7 can be detected by agglutination test. Polymerase chain reaction (PCR) is useful tool to detect the presence of pathogens. For genetic diversity, toxin production genes can be isolated through PCR assays like real time PCR, multiplex PCR etc. 4-methylumbelliferyl-β-D-glucuronide (MUG) is also an important test for *E. coli* O157 strains identification. If an organism produces the enzyme glucuronidase, substrate orthonitrophenyl-beta-glucuronide will be brokendown to liberate ortho-nitrophenyl producing yellow color.

The main aim of this study was to evaluate the diversity of *E. coli* strains from community waste water of Lahore, Pakistan. Strains were isolated on *E. coli* O157 selective medium. Then, strains were identified by 16S rRNA sequencing. Afterwards, agglutination test was performed to identify *E. coli* O157 strains. Strains were further screened for the presence of shiga-toxin related genes that included *stx1a*, *stx2a*, *stxc* and *stx2d*. Finally, biofilm forming potential of isolated *E. coli* strains was evaluated. The rationale of study was to evaluate cross contamination of sewage water with agricultural soil or drinking water in the local environment. Potential pathogenic hazards are targeted in this study.

2. MATERIALS AND METHODS

2.1 Sampling and Isolation of Bacterial Strains

Sixteen samples of waste water were collected from different towns of Lahore, Pakistan. Samples were immediately brought to the laboratory for further processing. For the selective isolation of *E. coli*, Hichrome EC O157 agar medium (Himedia Laboratories, USA) was used. One ml of the sample was added into pre-autoclaved 9 ml sorbitol mackonkey broth. About 25 µl of each sample was aseptically spreaded on the EC O157 agar medium and incubated at 37ºC for 24 h. On the basis of pigment production, different shades of purple colored colonies were selected. EC O157 agar medium is a selective medium for the isolation of *E. coli* O157:H7 described by Rappaport and Henigh from environmental samples [20]. It contains sorbitol and chromogenic mixture which after cleavage by *E. coli* O157: H7 produces dark purple to magenta colored colony, while other strains of *E. coli* produces pink colored colonies. The other ingredients include casein enzymic hydrolysate which provides nitrogenous, growth nutrients and carbonaceous medium. Sodium chloride maintains osmotic balance. Sodium lauryl sulphate and bile salts prevents gram-positive organisms to grow. Addition of 0.25 ml potassium tellurite specifically picks the serogroups and prevent *Providencia* and *Aeromonas* species.

2.2 16S rRNA Gene Sequencing

Bacterial DNA was extracted from overnight grown cultures by using FavorPrep Tissue Genomic DNA Extraction kit (Taiwan, Pingtung). DNA was amplified by using a pair of forward 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse 1522r (5'-AAGGAGGTGATCCA (AG) CCGCA-3') primers. Dream Taq™ Green PCR Master Mix (Fermentas) was used for PCR amplification. PCR reaction mixture was incubated at specific conditions i.e., denaturation at 94ºC for 5 min, annealing at 55ºC for 1 min, extension at 72ºC for 2 min. Final extension was carried at 72ºC for 5 min. The samples were stored at -20ºC after completion of 35 PCR cycles. PCR amplified fragments were visualized by agarose gel electrophoresis. Purified DNA fragments (1.5 kb) were sent to First Base Labotatories, Singapore for sequencing. The sequences were analyzed and trimmed using bioinformatics tool CHROMAS lite version 2.4.1.0 and searched for homology in NCBI BLAST (Basic Local Alignment Search Tool). Phylogenetic tree was constructed by using neighbor joining method with the help of mega 6 software.

2.3 PCR Detection of Shiga Toxin Gene

E. coli O157 produces shiga toxin which cause hemorrhagic uremic syndrome (HUS). Shiga toxin genes *stx1* and *stx2* including its variants that differ in the sequence of nucleotides were amplified through PCR. For PCR amplification, 4 set of primers were used that included Stx 1-a-F (5'-TCTCAGTGGGCGTTCTTATG-3'), Stx1-b-R (5'-TACCCCCTCAACTGCTAATA-3'), Stx2-a-F (5'-GCGGTTTTATTTGCATTAGC-3'), Stx2-b-R (5'-TCCCGTCAACCTTCACTGTA-3'), Stx2c-a-F (5'-GCGGTTTTATTTGCATTAGT-3'), Stx2c-b-R (5'-AGTACTCTTTTCCGGCCACT-3'), Stx2d-a-F (5'-GGTAAAATTGAGTTCTCTAAGTAT-3'), Stx2d-b-R (5'-CAGCAAATCCTGAACCTGACG-

3') [13]. Dream Taq™ Green PCR Master Mix (Fermentas) was used for PCR amplification. PCR reaction mixture with melting temperature, was incubated in thermal cycler at specific conditions. It included initial denaturation at 95ºC for 5 min, denaturation 95ºC for 30s, annealing at 59ºC for 2 min, extension at 72ºC for 4 min and final extension at 72ºC for 10 min. Samples were stored at -20ºC after completion of 35 PCR cycles. Amplified PCR products were detected after running agarose gel electrophoresis.

2.4 Serotyping of Bacterial Strains

For the serotyping of bacterial strains, Prolex™ *E. coli* O157 Latex Test Reagent Kit (Pro-lab diagnostics) was used. Cultures were prepared by inoculating them on sorbitol mackonkey agar to differentiate between sorbitol positive and sorbitol negative strains. Sorbitol negative strains indicated potential O157. Plates were incubated at 37ºC for 24 h and sorbitol negative colonies were selected. Before use all the reagents were allowed to cool to room temperature. Then using a sterile loop, 0.2 ml normal saline was transferred to eppendroff. Turbidity of 5 Mcfarland Standard by the strains were achieved by making saline suspension of isolated strains. One drop of Prolex™ *E. coli* O157 Latex Reagent was placed in a test circle on one of the test cards provided. Using a Pasteur pipette one drop of the test suspension was added in the same test circle and was mixed with sticks provided. Agglutination was observed after two min and the results were compared to positive control provided in the kit.

2.5 Antibiotic Sensitivity Testing

Plates of Mueller-Hinton (MH) agar were prepared and inoculated with bacterial strains (Mcfarland standard 0.5) to ensure the confluent growth. The antibiotic discs (Bioanalyse®) for amoxicillin (25 µg), Tobramycin (30 µg) tetracycline (30 µg), streptomycin (25 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), amikacin (30 µg), norfloxacin (10 µg), cephalexin (30 µg), nitrofurantoin (30 µg), Tobramycin, and gentamicin (10 µg) were aseptically placed to the surface of agar plates at well-spaced intervals. The plates were then incubated at 37ºC for 24 h. After incubation, the plates were observed for the presence of clear zones of inhibition around the antibiotic disc. Zones were measured in millimeter (mm) using the Inhibition Zone Ruler provided by the manufacturer. The zones were then compared with the standardized chart for antibiotics (M100-S23) given by clinical laboratory standard institute (CLSI, 2013).

2.6 Microtiter Plate Assay for Determination of Biofilm Forming Potential

Twelve random bacterial strains of *E. coli* were examined by microtiter plate assay for biofilm formation. Each strain was grown in 5 ml Tryptic Soy Broth (TSB) at 37ºC under agitation (150 rpm) for 24 h. The optical density of cell suspension was adjusted to 0.5 at 600 nm. The microtiter plate test was modified to determine the biofilm forming potential of single and double combinations of selected strains [21]. For single cultures, each well was filled with 200 µl of cell suspension in TSB. In co cultures (two strains) 100 µl of each strain was added aseptically to a sterile-well flat tissue culture plate. The biofilm was also observed in the presence of glucose and tricalcium phosphate that was finally adjusted to 0%, 1% and 5% concentrations.

To promote biofilm formation, the plates were incubated aerobically on a shaker at 150 rpm for 72 h at 37ºC. After 72 h, the content of each well was discarded and washed three times with 250 µl of sterile distilled water to remove any non-adherent and weakly adherent bacteria. Negative controls were prepared by incubating the wells only with TSB without adding any inoculum. All combinations were performed in triplicate. The bacterial biofilm in the 96-well microtiter plates was fixed with 250 µl/well of 98% methanol for 15 min. Afterwards, the plates were emptied and allowed to dry. Then, the fixed bacteria were stained for 5 min with 200µl/well of crystal violet. Excessive stain was rinsed out by placing the plate under low-running tap water. Crystal violet bound to the adherent cells was re-solubilized by 200 µl/well of 33% (v/v) glacial acetic acid. The optical density (OD) of the obtained solution was measured at 570 nm using a microtiter plate reader (Model 680XR; BIO-RAD).

3. RESULTS

3.1 Isolation of Bacterial Strains

Escherichia coli O157 was isolated from sewage waste water from different localities of Lahore on Hichrome EC O157 agar. Different shades of purple colored bacteria were passed from several rounds of quadrant streaking (Fig. 1). Sample 5 and 9 gave the highest CFU/ml i.e., $3x10^6$ and $3.2x10^6$, respectively The lowest

CFU/ml was observed in the sample 3 i.e., 5×10^1. On the selective media Hichrome EC O157 agar, 56 bacterial strains were isolated.

3.2 Sequence Analysis

Sequences of 56 bacterial strains were compared with online sequences submitted to GenBank. After comparison, 51 strains showed good quality peaks and around 99 % similarity to *Escherichia coli.* Sequences of the strains were deposited to GenBank under accession numbers KY765032 to KY765085 (Table 1). (Fig. 2) showed phylogenetic relationships among the strains of *E. coli.*

3.3 Detection of Shiga Toxin Related Genes

Different variants of *stx1* and *stx2* genes of *E. coli* such as *stx1*, *stx2*, *stx2c*, *stx2d* were aimed to isolate from different sewage samples of Lahore. After PCR, gel electrophoresis was accomplished to visualize the bands. Analysis indicated that specific size of bands of shiga toxin genes was not detected. Rather non-specific bands of approximately 400 bp, 800 bp and 1000 bp were visualized for the strains E101 and E114 in the presence of primer stx2d. Strains E34, E35 E151 and E141 produced 1000 bp band by primer stx1 as compared to the 1500 bp ladder.

3.4 Serotyping

Isolated colonies were picked and suspended in autoclaved water and the cell density was compared with 5 Mcfarland standards. Agglutination test was performed for all the strains to check the serotype *E. coli* O157 by using Prolex™ *E. coli* O157 Latex Test Reagent Kit. All the strains were negative for the serotype *E. coli* O157 as agglutination was not observed with any sample as compared to control (Fig. 3).

3.5 Antibiotic Sensitivity Testing

Seven random bacterial strains isolated from waste water were checked for susceptibility towards different antibiotics including amoxicillin, ampicillin, tetracycline, streptomycin, nalidixic acid, chloramphenicol, ciprofloxacin, norfloxacin,

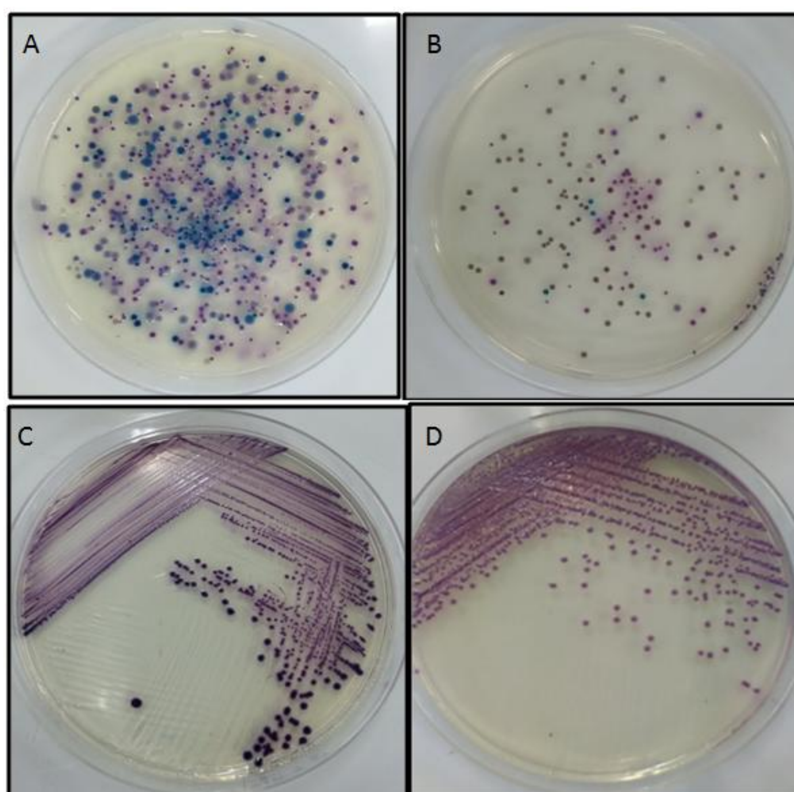

Fig. 1. Isolation of potential *E. coli* O157 on Hichrome EC O157 from sewage water. (A-B) Growth of spreading on EC O157 medium. (C-D) Quadrant streaking on EC O157 medium

Table 1. 16S rRNA gene sequencing *of E. coli* strains isolated from sewage water samples

S. no.	Isolates	Homology (%)	Identified as	Accessions
1	E35	99	*E. coli* E35	KY765032
2	E63	99	*E. coli* E63	KY765033
3	E101	94	*E. coli* E101	KY765034
4	E84	99	*E. coli* E84	KY765035
5	E62	98	*E. coli* E62	KY765036
6	E71	99	*E. coli* E71	KY765037
7	E141	98	*E. coli* E141	KY765038
8	E142	100	*E. coli* E142	KY765039
9	E143	99	*E. coli* E143	KY765040
10	E111	97	*E. coli* E111	KY765041
11	E135	98	*E. coli* E135	KY765042
12	E134	99	*E. coli* E134	KY765043
13	E133	99	*E. coli* E133	KY765044
14	E132	91	*E. coli* E132	KY765045
15	E23	100	*E. coli* E23	KY765046
16	E121	97	*E. coli* E121	KY765047
17	E114	100	*E. coli* E114	KY765048
18	E16	99	*E. coli* E16	KY765049
19	E22	99	*E. coli* E22	KY765050
20	E162	99	*E. coli* E162	KY765051
21	E131	97	*E. coli* E131	KY765052
22	E124	99	*E. coli* E124	KY765053
23	E144	99	*E. coli* E144	KY765054
24	E151	96	*E. coli* E151	KY765055
25	E152	98	*E. coli* E152	KY765056
26	E153	98	*E. coli* E153	KY765057
27	E154	99	*E. coli* E154	KY765058
28	E163	100	*E. coli* E163	KY765059
29	E164	99	*E. coli* E164	KY765060
30	E112	99	*E. coli* E112	KY765061
31	E113	99	*E. coli* E113	KY765062
32	E33	99	*E. coli* E33	KY765063
33	E21	100	*E. coli* E21	KY765064
34	E82	99	*E. coli* E82	KY765065
35	E17	99	*E. coli* E17	KY765066
36	E61	99	*E. coli* E61	KY765067
37	E93	79	*E. coli* E93	KY765068
38	E15	99	*E. coli* E15	KY765069
39	E13	99	*E. coli* E13	KY765070
40	E83	99	*E. coli* E83	KY765071
41	E43	100	*E. coli* E43	KY765072
42	E11	99	*E. coli* E11	KY765073
43	E93	100	*E. coli* E93	KY765074
44	E92	100	*E. coli* E92	KY765075
45	E103	99	*E. coli* E103	KY765076
46	E51	100	*E. coli* E51	KY765077
47	E102	100	*E. coli* E102	KY765078
48	E52	100	*E. coli* E52	KY765079
49	E44	97	*E. coli* E44	KY765080
50	E32	100	*E. coli* E32	KY765081
51	E34	99	*E. coli* E32	KY765082

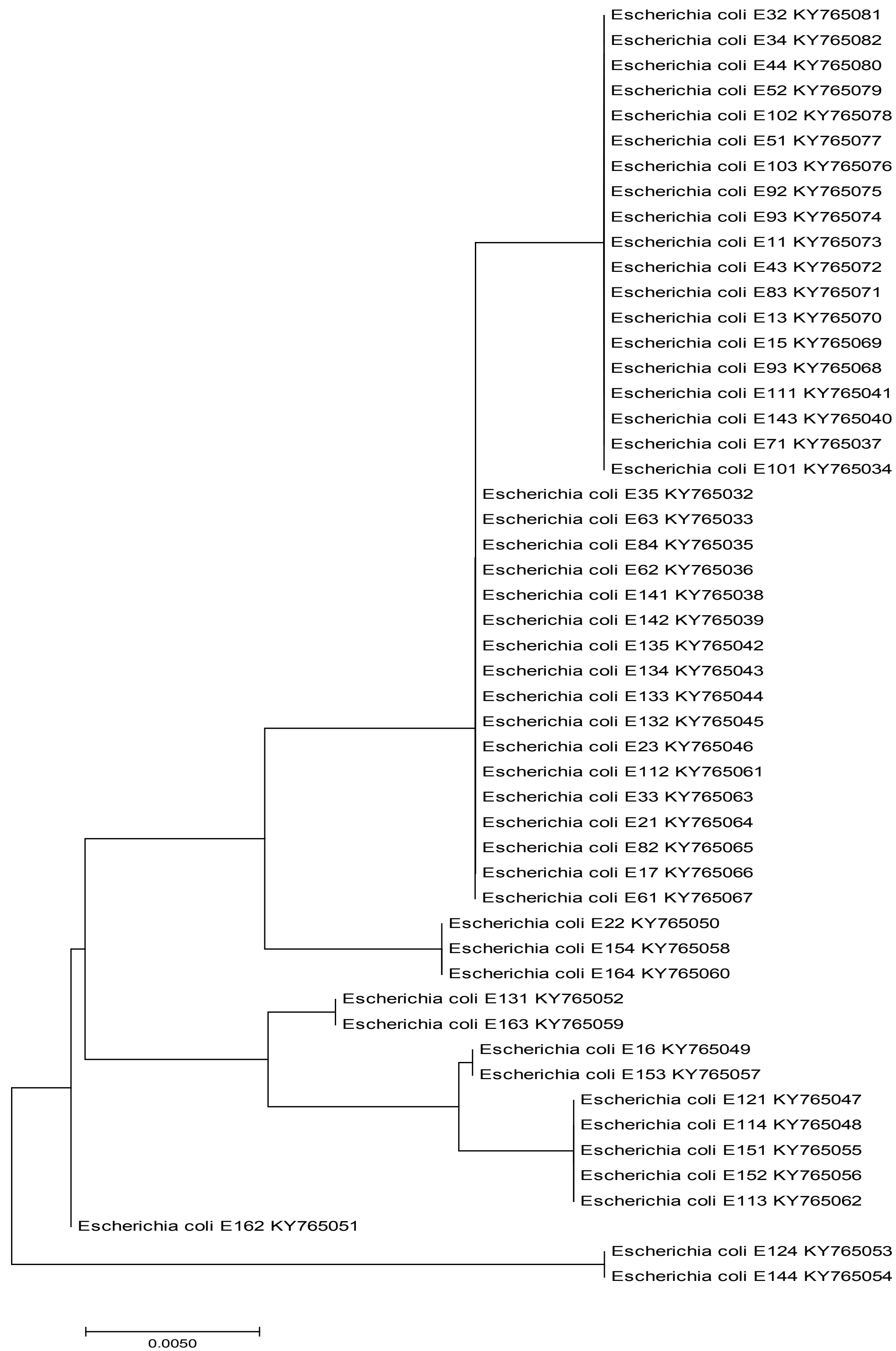

Fig. 2. Combined phylogenetic tree showing the relationship among 51 strains of *E. coli* isolated from sewage water samples. The scale bar represents mutations per nucleotide position

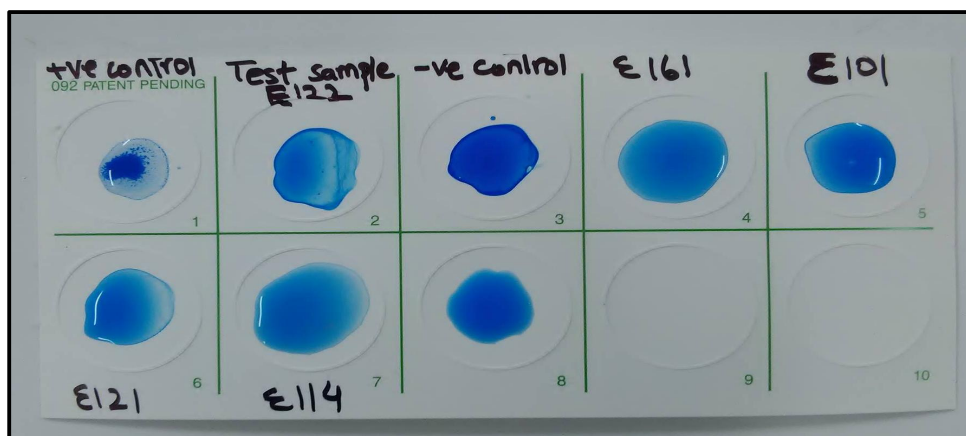

Fig. 3. Agglutination test using Prolex™ *E. coli* O157 latex test reagent kit

cephalexin, nitrofurantoin, gentamycin. The inhibition zones measured in millimeter (mm) by using Inhibition Zone Ruler, were compared with the standard zone sizes provided by the clinical laboratory institute (CLSI, 2013). All the strains were resistant against amoxicillin, tobramycin, tetracycline and nitrofurantoin. While, chloramphenicol was found to be resistant for strains E42 and E121. The rest of the strains E161 (21 mm), E15 (18 mm), E151 (20 mm), E34 (24 mm), E114 (23 mm) were sensitive for chloramphenicol (Table 2). Norfloxacin was found to be highly sensitive for all the *E. coli* strains giving zone of inhibition 19 to 26 mm (Fig. 4).

3.6 Biofilm Formation

Single bacterial isolates were examined by micro titer plate assay for biofilm forming potential. Bacterial strains E124, E111, E62 and E34 were observed as strong biofilm producers when used as monocultures. The bacterial strain E124 showed highest biofilm production, while E34 was weak biofilm producer. The cultures used in combinations of bacterial strains showed weak biofilm production. When combination of bacterial strains were used strain D.C E35, E101 produced good biofilm.

3.7 Effect of Salt and Sugar on Biofilm Production

In the presence of carbon source, all bacterial strains showed variation in its ability to produce biofilm. In the presence of glucose, good biofilm was produced by bacterial strains in the order E124>E34>E61>E35>E101>E61at concentration of 1% while at 5% strain E34 was active as compared to others. In the presence of tricalcium phosphate, biofilm activity was suppressed at 1% and 5% concentrations of salt. Strain E34 produced good biofilm at 1% but when the concentration of salt was increased to 5% its activity to produce exopolysaccharide was suppressed. Strain E35 produced maximum biofilm at 5% salt concentration (Figs. 5 to 7).

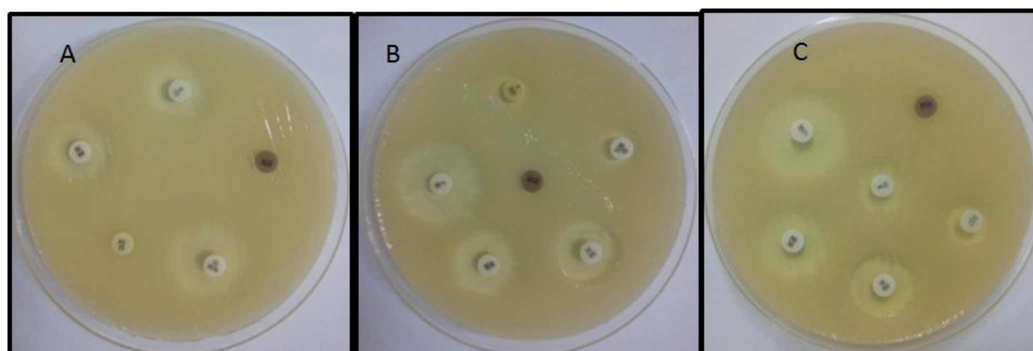

Fig. 4. Antibiotic sensitivity pattern of *E. coli* strains

(A) = E34, (B) = E121, (C) = E15

Table 2. Antibiotic susceptibility pattern of different bacterial isolate

Sr. no.	Strains	Zones of Inhibition (mm)										
		AX	CL	TOB	AK	TE	S	NA	CN	F	NOR	C
1	E161	0(R)	10(R)	11(R)	18(S)	10(R)	10(R)	18(I)	15(S)	10(R)	22(S)	21(S)
2	E15	0(R)	10(R)	10R)	18(S)	0(R)	15(S)	15(I)	15(S)	10(R)	20(S)	18(S)
3	E151	0(R)	8(R)	7(R)	11(R)	0(R)	5(R)	17(I)	17(S)	8(R)	19(S)	20(S)
4	E34	0(R)	0(R)	10(R)	22(S)	5(R)	10(R)	23(S)	18(S)	6(R)	26(S)	24(S)
5	E114	0(R)	8(R)	8(R)	11(R)	0(R)	11(R)	12(R)	15(S)	0(R)	25(S)	23(S)
6	E42	0(R)	18(S)	11(R)	16(I)	0(R)	10(R)	8(R)	13(I)	0(R)	23(S)	12(R)
7	E121	0(R)	10(R)	10(R)	18(S)	0(R)	16(S)	18(I)	18(S)	12(I)	25(S)	24(R)

Letters in parenthesis indicate level of sensitivity of respective antibiotics. R: Resistant, I: Intermediate, S: Sensitive. Antibiotics: AX: Amoxicillin; CL: Cephalexin; AK: Amikacin; TOB: Tobramycin; TE: Tetracycline; S: Streptomycin; NA: Nalidixic acid; CN: Gentamycin; F: Nitrofurantoin; NOR: Norfloxacin; C: Chloramphenic

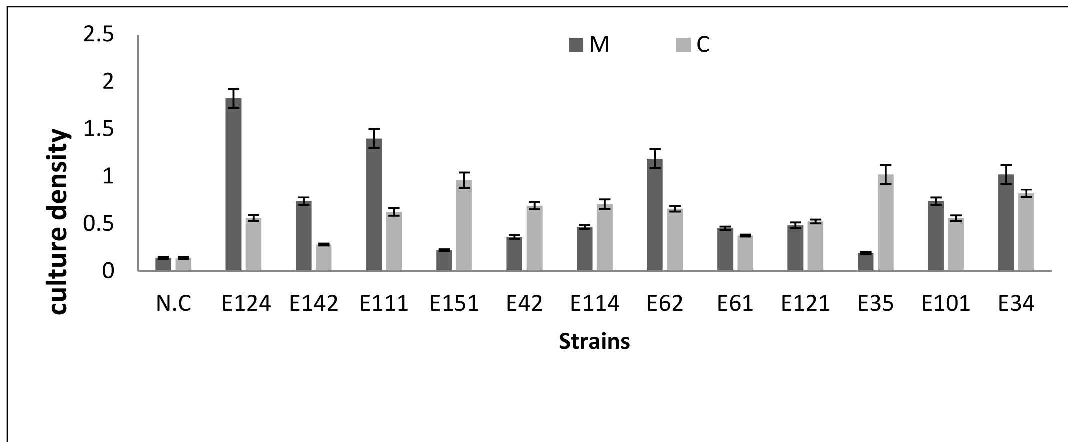

Fig. 5. Biofilm forming potential of strains in the presence of (M=monocultures) and (C=cocultures) in tryptic soy broth (TSB), N.C= Negative control (Mean ± S.E of 3 replicates)
C1=E124+E142, C2= E142+E111, C3=E111+E151, C4=E151+E42, C5= E42+E114, C6= E114+E62, C7= E62+E61, C8= E61+E121, C9= E121+E35, C10= E35+E101, C11= E101+E34, C12= E34+E124

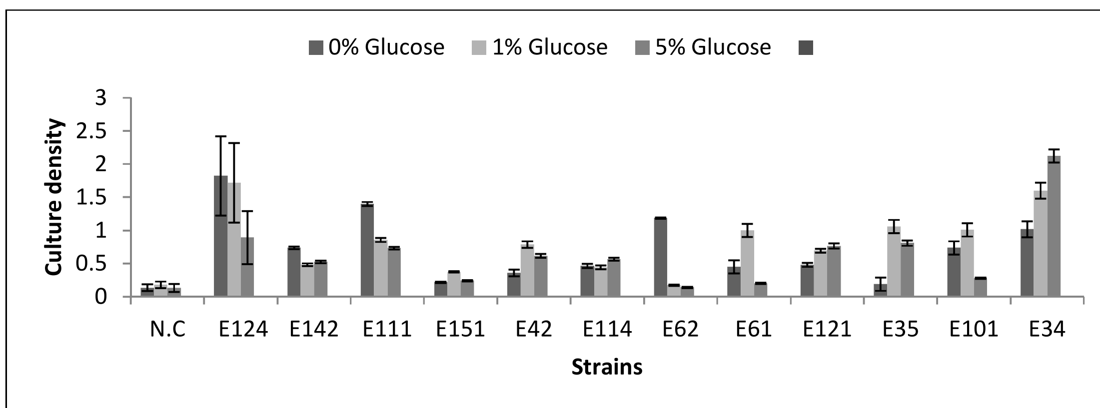

Fig. 6. Biofilm forming potential of single *E. coli* strains in the presence of different concentration of glucose in TSB, N.C= Negative control (Mean ± S.E of 3 replicates)

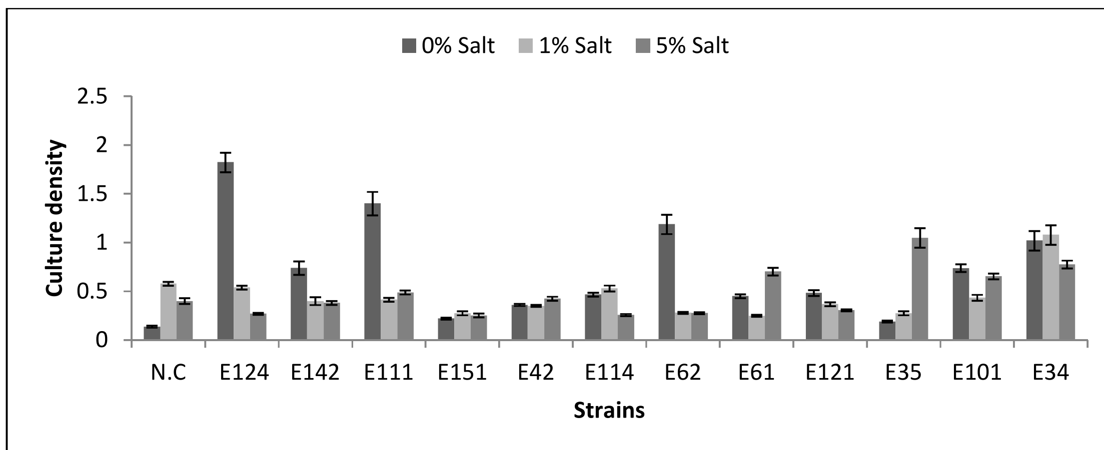

Fig. 7. Biofilm forming potential of *E. coli* strains in the presence of different concentrations of tricalcium phosphate in TSB, N.C= Negative control (Mean ± S.E of 3 replicates)

4. DISCUSSION

The main aim of the present study was to evaluate the genetic diversity of *E. coli* from the waste water of different towns of Lahore. For this purpose, sixteen samples of sewage waste water were collected from different regions. Selective media Hichrome EC O157 agar was used for the isolation. On the basis of pigment production, purple colored colonies were considered as *E. coli* O157. In total, 56 strains were isolated from sewage waste water. These strains were subjected to DNA isolation, following sequencing of the strains to confirm it as *E. coli* O157. 51 sequences retrieved after NCBI Blast search showed resemblance to *E. coli*. The strains were also confirmed through biochemical tests as *E. coli*. The strains were positive for catalase and methyl red test while, negative for oxidase and voges prausker test, To check the serotype, agglutination test was performed by Prolex^{TM} *E. coli* O157 Latex Test Reagent Kit, but all the strains were negative for *E. coli* O157 serotype. According to Wells J, et al. [21] *E. coli* was isolated and biochemically confirmed from cattle feaces. Another study held by [13] (2005) reported *E. coli* O157 from aquatic environment. Gastrointestinal symptoms are widely reported worldwide due to ingestion of polluted water or swimming in feacally polluted water [13]. Shiga toxin producing *E. coli* is widely associated with diseases in human like hemorrhagic uremic syndrome due to production of *stx1* and *stx2* toxins [22]. According to a study more protein expression was observed for the *E. coli* producing shiga toxin genes in animals than human samples this study suggest that cattle may be the primary source of HUS [11]. As these genes are highly toxic they are present in open environment. Therefore, there was a need to check its prevelance in our environment. PCR is a rapid detection method to diagnose enteric infections. *E. coli* O157 can detected from environmental samples through PCR. Shiga toxin genes (*Stx1, Stx2, Stx2c* and *Stx2d*) were amplified with the help of forward and reverse primers [13] (2005) through PCR in order to check the genetic diversity of these genes in our local environment. On the basis of melting temperature of primers, gradient PCR was set with temperatures range 50-65ºC. Conditions for the PCR were changed many times variated from published sources to get the required band length, but no specific band was observed with any primer set used. Almost all the strains were used for the detection of shiga toxin genes. The results obtained after gel electrophoresis were highly promising as no bands of desire length was obtained [23] (2016) reported prevelance of shiga toxin genes isolated from waste water in USA.

The ability to form biofilm by the bacterial cultures in the presence of monocultures as well as combination of bacterial strains was examined. The strains giving cell mass OD equal or above 1 were considered good biofilm producers. Strain E124 showed maximum biofilm formation. However when used as combination with strain E142 the biofilm forming ability was decreased to 0.56. This may be due to ability of self-interaction of bacterial strains and availability of the substrates to them. The possible reason is may be due to synergestic effect of the strains, its activity was decreased. A study showed that *Arthrobacter* can form biofilms of the surfaces of different polymers [24]. Biofilms causes diseases in human body, it cause harm to human health and cause dental carriers form biofilm on teeth [25].

Biofilm was also examined in the presence of carbon sources and salt concentrations. As a carbon source sugar glucose was used while, salt tricalcium phosphate was used. Strain E34 was very good biofilm producer at 5 % glucose. At 1% its activity was decreased while no biofilm was produced in the absence of glucose. Strain E111 and E62 were good biofilm producers in the absence of glucose, with the increase of glucose concentration, decrease in biofilm activity was observed. According to [26], deletion of *ompA* gene reduced biofilm by 80 %. This study suggested that increase of glucose concentration, biofilm ability was reduced. When salt tricalcium phosphate was used, remarkable variation in the results was observed. Mostly strains were good biofilm producers in the absence of salt. When salt was added along with the cultures, biofilm formation was inhibited. [27] reported biofilm formation in the presence of tricalcium phosphate which reduces the bacterial count and it can be an effective source of treatment for raw poultry.

Growth on Hichrome EC O157 agar indicated the presence of potential strains of O157. Previously reported studies isolated *E. coli* on different selective media like EMB, Mackonkey agar, rainbow agar etc. Although appearance of purple colored colonies on Hichrome EC O157 agar showed the presence of *E. coli* O157 but serotyping of the strains confirmed no agglutination for *E coli*. Therefore, *E. coli* O157

was not detected in our study. It was also confirmed through PCR where no bands of specific lengths were present after amplification.

5. CONCLUSION

This study indicated that our exposed environment is free from the presence of shiga toxin genes. Sewage water samples contained *E. coli* as it was confirmed through biochemical testing and sequencing. But its serotype O157 might not be present there because it is exclusively associated with the stool samples while sewage water contains different types of waste such as domestic waste, industrial waste etc. Several community hazards are related due to mixing of sewage waste water with community potable water.

ACKNOWLEDGEMENT

This study was supported by University of the Punjab.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Praveen PK, Ganguly S, Kumar K, Kumari K. Water pollution and its hazardous effects to human health: A review on safety measures for adoption. Int J. Sci Technol. 2016a;5:1559-1563.
2. Praveen PK, Ganguly S, Wakchaure R, Para PA, Mahajan T, Qadri K, Kamble S, Sharma R, Shekhar S, Dalai N. Water-borne diseases and its effect on domestic animals and human health: A Review. Int. J. Adv. Res. Technol. 2016b;6:242-245.
3. Abida B, Basharat A. 16S rRNA gene sequencing and culture dependent analysis of bacterial diversity associated with commercially processed salads. Res. J. Biotechnol. 2016;11:8-16.
4. Wardrop NA, Hill AG, Dzodzomenyo M, Aryeetey G, Wright JA. Livestock ownership and microbial contamination of drinking-water: Evidence from nationally representative household surveys in Ghana, Nepal and Bangladesh. Int J Hyg Envir Heal; 2017.
5. Akyıldız A, Köse ET, Yıldız A. Compressive strength and heavy metal leaching of concrete containing medical waste incineration ash. Construction and Building Materials. 2017;138:326-332.
6. Eze C, Nwagwe O, Ogbuene E, Eze H. Investigating groundwater contamination following the disposal of hospital wastes in a government reserved area, Enugu, Nigeria. Bull Environ Contam Toxicol. 2017;8:1-8.
7. Römer W, Berland L, Chambon V, Gaus K, Windschiegl B, Tenza D, Aly MR, Fraisier V, Florent JC, Perrais D. Shiga toxin induces tubular membrane invaginations for its uptake into cells. Nature. 2007;450: 670-675.
8. Badouei MA, Morabito S, Najafifar A, Mazandarani E. Molecular characterization of enterohemorrhagic *Escherichia coli* hemolysin gene (EHEC-hlyA)-harboring isolates from cattle reveals a diverse origin and hybrid diarrheagenic strains. Infect. Genet. Evol. 2016;39:342-348.
9. Rivas L, Coffey B, McAuliffe O, McDonnell MJ, Burgess CM, Coffey A, Ross RP, Duffy G. In vivo and ex vivo evaluations of bacteriophages e11/2 and e4/1c for use in the control of *Escherichia coli* O157: H7. Appl Environ Microbiol. 2010;76:7210-7216.
10. Srinivasan V, Nguyen LT, Headrick SI, Murinda SE, Oliver SP. Antimicrobial resistance patterns of Shiga toxin-producing *Escherichia coli* O157: H7 and O157: H7− from different origins. Microb Drug Resist. 2007;13:44-51.
11. Tahamtan Y, Hayati M, Namavari M. Prevalence and distribution of the *stx1*, *stx2* genes in Shiga toxin producing E. coli (STEC) isolates from cattle. *Iran* J Microbiol. 2010;2:9-14.
12. Noll LW, Shridhar PB, Dewsbury DM, Shi X, Cernicchiaro N, Renter DG, Nagaraja TG. A comparison of culture-and PCR-based methods to detect six major non-O157 serogroups of Shiga toxin-producing *Escherichia coli* in cattle feces. PloS one. 2015;10:1-8.
13. Garcia Aljaro C, Muniesa M, Blanco J, Blanco M, Blanco J, Jofre J, Blanch A. Characterization of Shiga toxin-producing *Escherichia coli* isolated from aquatic environments. FEMS Microbiol Lett. 2005; 246:55-65.
14. Islam MA, Mondol AS, De Boer E, Beumer RR, Zwietering MH, Talukder KA, Heuvelink AE. Prevalence and genetic characterization of shiga toxin-producing

Escherichia coli isolates from slaughtered animals in Bangladesh. Appl Environ Microbiol. 2008;74:5414-5421.

15. Fraser ME, Fujinaga M, Cherney MM, Melton-Celsa AR, Twiddy EM, O'Brien AD, James MN. Structure of Shiga toxin type 2 (Stx2) from *Escherichia coli* O157: H7. Jl. Biol Chem. 2004;279:27511-27517.
16. Kieckens E, Rybarczyk J, Barth S, Menge C, Cox E, Vanrompay D. Effect of lactoferrin on release and bioactivity of Shiga toxins from different *Escherichia* coli O157: H7 strains. Vet. Microbiol. 2016;202: 29-37.
17. Law D. Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli.* J. Appl Microbiol. 2000;88:729-745.
18. Losada L, DebRoy C, Radune D, Kim M, Sanka R, Brinkac L, Kariyawasam S, Shelton D, Fratamico PM, Kapur V. Whole genome sequencing of diverse Shiga toxin-producing and non-producing *Escherichia coli* strains reveals a variety of virulence and novel antibiotic resistance plasmids. Plasmid. 2016;83:8-11.
19. Maheux AF, Bouchard S, Bérubé È, Bergeron MG. Comparison of MI, Chromocult® coliform and Compass CC chromogenic culture-based methods to detect *Escherichia coli* and total coliforms in water using 16S rRNA sequencing for colony identification. J. Water Health. 2017;174.
20. Rappaport F, Henigh E. J. Clin Pathol. 1952;5:361.
21. Wells J, Shipman L, Greene K, Sowers E, Green J, Cameron D, Downes F, Martin M, Griffin P, Ostroff S. Isolation of *Escherichia coli* serotype O157: H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin Microbiol. 1991;29:985-989.
22. Wang G, Clark CG, Rodgers FG. Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157: H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J. Clin Microb. 2002;40:3613-3619.
23. Ibekwe A, Murinda SE, DebRoy C, Reddy GB. Potential pathogens, antimicrobial patterns and genotypic diversity of *Escherichia coli* isolates in constructed wetlands treating swine wastewater. FEMS Microbiol Ecol. 2016;92:1-14.
24. Maksimova YG. Microbial biofilms in biotechnological processes. Appl Biochem Microbiol. 2014;50:750-760.
25. Jongsma MA, Van der Mei HC, Atema-Smit J, Busscher HJ, Ren Y. *In vivo* biofilm formation on stainless steel bonded retainers during different oral health-care regimens. Int J. Oral Sci. 2015;7:42-48.
26. Barrios AFG, Zuo R, Ren D, Wood TK. Hha, YbaJ and OmpA regulate *Escherichia coli* K12 biofilm formation and conjugation plasmids abolish motility. *Biotechnol.* Bioeng. 2006;93:188-200.
27. Somers EB, Schoeni JL, Wong AC. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157: H7, Listeria monocytogenes and Salmonella typhimurium. Int J Food Microbiol. 1994; 22:269-276.

© 2019 Bano and Ali; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://sdiarticle4.com/review-history/52559