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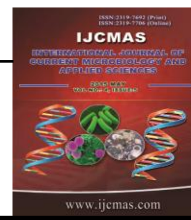

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Original Research Article

Bacterial Diversity and Biofilm Formation in Drinking Water Distribution System in Lebanon

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Keywords

Drinking water distribution system, STX-EC, coliforms, thermotolerant coliforms, biofilm

A B S T R A C T

Microorganisms that grow in the environment may enter the drinking-water and attach to and grow on drinking-water pipes and other surfaces, forming biofilms as the most common means of growth in the environment. In the present study bacterial diversity and the prevalence of Shiga Toxin-producing *E.coli* (STX-EC) were detected in drinking water distribution system in Lebanon. Bacterial isolates belonging to the coliform and thermotolerant coliforms including *E.coli* were the most prevalent isolates. Different *E. coli* isolates (7 isolates) were then tested for the presence of *stx1*, *stx2*, *eaeA*, *ehlyA*, and *fliC* virulence genes. The seven *E. coli* isolates were considered as STX-EC serotypes, carrying virulence marker genes. However none of these isolates were positive for the *fliC* gene and thus are H7-negative, and could not be classified as O157:H7. Biofilm production was quantitatively investigated using the method of adherence to polystyrene microtiter plates. 32.26% of the isolates were detected as strong, 51.61% as moderate and 16.13% as weak biofilm producers. Pre-coating the microtiter plate with tap water showed a significant increase in biofilm production in all the isolates.

Introduction

Drinking water is an important resource all around the globe (Martiny et al., 2005), but waterborne disease is still a major cause of death in many parts of the world (Fawell & Nieuwenhuijsen, 2003). Nevertheless, little research has focused on identifying the bacteria in water distribution systems (Martiny et al., 2005).

Microorganisms that grow in the environment may enter the drinking-water and attach to and grow on drinking-water pipes and other surfaces, forming biofilms (WHO, 2014) as the most common means of growth in the environment. The microbiota of biofilms in drinking water systems constitute mainly of non-pathogenic microorganisms of source water (Farkas et al., 2013), however, quite a few opportunistic bacterial pathogens naturally occur in aquatic and soil environments are able to persist and grow in biofilms of

drinking water systems (Wingender & Flemming, 2011).

The contamination of drinking water by *E. coli* that only come from human and animal fecal waste (USEPA, 2009) is an important aspect of drinking water quality (WHO, 2011).

Five groups of *E. coli*-causing diarrhea in humans and other warm-blooded animals have been identified (Brook et al., 1994 and Wasteson, 2001), that include enterotoxinogenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohaemorrhagic *E. coli* (EHEC). The latter includes Shiga Toxin (Stx)-Producing *E. coli* (STX-EC) (Harake et al., 2006).

Shiga Toxin - producing *E. coli*, also known as Verotoxin-producing *E. coli*, that is associated with infant diarrhea, haemorrhagic colitis, thrombiotic thrombocytic purpura, and hemolytic uremic syndrome in humans (Griffin & Tauxe, 1991). *E. coli* O157:H7, which belongs to STX-EC group, has been recognized as an emerging pathogen in drinking water systems (Szewzyk et al., 2000). Several virulence factors have been described in STX-EC, the major one being shiga toxins encoded by the genes *stx1* and *stx2* (Gyles, 1992). Some STX-EC strains can tightly attach to epithelial cells of the intestine through an adhesin called intimin encoded by the gene *eaeA* (Boerlin et al., 1999) in addition to the plasmid located enterohemolysin gene *ehlyA*, which has been suspected to play a role in pathogenicity of STX-EC infections (Ito et al., 2007) and the *fliC* genetic marker encoding the H7 flagellar protein (Reischl et al., 2002)

Antimicrobial products, particularly chlorine, have been the main agents used to disinfect drinking water. Although this strategy is widespread, there are not yet standardized disinfection strategies with reliable efficacy (Simões & Simões, 2013). Microorganisms may develop resistance and persist due to the indiscriminate use of disinfectants, and drinking water quality may then deteriorate in the distribution system.

Guidelines recommend monitoring of water in order to characterize bacterial counts and identify organisms (Beale et al., 2013). The primary goal for this monitoring is to identify the conditions that promote biofilm growth and observe changes in bacterial trends. It is considered more important to identify changes in the bacterial composition of the biofilm than to identify increased bacterial numbers (Beale et al., 2012).

The aim of the present study is to detect:

- The bacterial diversity and prevalence of STX-EC in drinking water distribution system in Lebanon.
- The ability of biofilm formation.

Materials and Methods

Sample collection

Water samples

Water samples (147) were collected during winter of 2014, from Beit-Din station which receives water from Al-Safa spring and supplies Al-Iklim with drinking water, and from 20 households tap water in Barja-Al Chouf. Samples were collected in 1L clean and sterile HDPE bottles containing 0.8 ml of a 10% (w/v) sterile sodium thiosulphate solution for neutralization of chlorine (USEPA, 2010), and were transferred under ice cold conditions immediately to the lab. Samples were tested within two hours of collection.

Walls of the water canal surfaces

Samples were also taken from the walls of the water canal in Beit-Din station using sterile swabs.

Microbiological analysis

Bacterial isolation from samples was carried out:

- For water samples: by using membrane filtration technique (USEPA, 2012) where water samples (1L) were filtered through 0.45µm cellulose nitrate filters. Filters were then placed on different culture media for the detection of bacterial diversity.
- For wall surfaces of water canal: the swabs were rubbed over different culture media and at a time for the detection of bacterial diversity as follows:

Detection of heterotrophic bacteria: membrane filters were transferred onto, and swabs were rubbed against R2A minimal medium and incubated at 28ºC for 7 days (Tokajian & Hashwa, 2004). Preliminary identification of gram negative bacteria was carried out using cultural features followed by biochemical characterization using commercially available kits API 20E and API 20NE (BioMérieux, France). Gram positive staphylococci were preliminary identified using cultural features followed by catalase and coagulase tests.

Detection of *Salmonella*: membrane filters and swabs were transferred separately into 50 mL pre-enrichment lactose broth and incubated at 37ºC for 24 hours, of which 1 mL was transferred into tetrathionate broth and incubated at 37 ºC. After 18-24 hours samples were streaked onto Xylose Lysine Desoxycholate (XLD) agar. Typical colonies were then preliminary identified using cultural features followed by biochemical characterization using commercially available kits API 20E (BioMérieux, France).

Detection of *Listeria monocytogenes*: membrane filters and swabs were transferred separately into a 100 mL Listeria enrichment broth (LEB) and incubated for 24-48 hours at 30ºC. 100 µL of LEB was streaked onto OXFORD agar and incubated for 48 h at 30°C (Sauders et al., 2012). Typical colonies were preliminary identified using cultural features followed by catalase production, motility test and haemolysis on 5% sheep blood agar (Nwachukwu & Orji, 2012).

Detection of *Pseudomonas* sp.: membrane filters were transferred onto, and swabs were rubbed against cetrimide agar plates and incubated for 24-48 hours at 37ºC. Typical colonies were preliminary identified using cultural features followed by oxidase and catalase production. Single colonies that were confirmed as putative *Pseudomonas* sp. (Wiedmann et al., 2000) were further identified by biochemical characterization using commercially available kits API 20E (BioMérieux, France).

Detection of coliforms and thermotolerant coliforms: membrane filters were transferred onto, and swabs were rubbed against lauryl sulfate agar plates and incubated for 24 h at 37°C and 44°C for isolation of total coliforms and fecal coliforms respectively. Yellow colonies on lauryl sulfate agar were considered as presumptive coliforms and were further purified on nutrient agar and identified using commercially available API 20E kits (BioMérieux, France) (Tokajian & Hashwa, 2004).

Detection of *E. coli* O157:H7: Occurrence of *E.coli* O157: H7 was detected by ELISA with the commercial kit VIDAS® ECO O157 (BioMérieux, France).

Detection of STX-EC: Different *E.coli* were analysed by PCR for the presence of *stx1*, *stx2*, *stx* $_+$ *stx2*, *eaeA*, and *ehlyA* virulence markers. *E.coli* were also tested for *fliC* genetic marker responsible for the production of the H7 *E. coli* flagellar protein (Harake et al., 2006).

Detection of Enterococci: membrane filters were transferred onto, and swabs were rubbed against Bile Esculin Agar plates and incubated at 37ºC for 24-48 hours. Typical black colonies were preliminary identified using cultural features followed by biochemical analysis using commercially available test strips API 20 Strep (BioMérieux, France) (Kurup et al., 2001).

Antibacterial activity of commonly used antibiotics against the isolated *E.coli*

Antibacterial activity against all the detected *E.coli* isolates was carried out using the disc-diffusion method (Murray et al., 1995). The test cultures (25µl), equivalent to 0.5 McFarland, were swabbed on the top of the solidified Müeller Hinton agar and allowed to dry for 10 min. The antibiotics (µg/disc) tested in the present study were : gentamicin (CN,10); levofloxacin (LEV,5); norfloxacin (NOR,10); piperacillin / tazobactam (TZP,100/10); amoxicillin / clavulanic acid (AMC,20/10); tetracycline (TE,30); cefotaxime (CTX,30); ceftriaxone (CRO,30); cefepime (FEP,30); sulphamethoxazole / trimethoprim (SXT, 23.75/1.25); cefpodoxime (CPD,10); azetreonam (ATM,30) and imipenem (IPM,10). The antibiotic discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 hours at 37°C.

Shiga toxin gene detection using polymerase chain reaction (PCR)

DNA extraction

DNA was extracted from *E.coli* isolates grown on EMB agar plates following the Manufacturer's protocol (GenEluteTM Bacterial Genomic DNA kits, SIGMA Aldrich, Germany), extracted DNA was stored at -20 C. 1µl DNA template was used per 25 µl Polymerase chain reaction (PCR) reaction (Ozpinar et al., 2013).

PCR assays

For the detection of STX-EC, primers were developed within the regions coding for five genes that are highly conserved in STX-EC strains. Primers for shiga toxins 1 and 2 (*stx1* and *stx2*) genes amplification, were selected as shown in table 1. The three other genes that were targeted for amplification were *eaeA* gene which encodes for Intimin gamma, *ehlyA* that encodes for enterohemolysin and *fliC* which encodes for H7 flagellar protein (Osek & Gallein, 2002 and Reischl et al., 2002) (table 1). STEC primers were used to amplify both *stx1* and *stx2* in combination, as described by Reischl et al. (2002).

Amplification of bacterial DNA was performed in 25 µL volumes containing 1 µL DNA template; 10.5 µL water ; 0.5 µL forward primer; 0.5 µL reverse primer and 12.5 µL of (RED Taq Ready mix) DNA polymerase. Amplification of *stx1*, *stx2*, *eaeA* and *ehlyA* was performed using a DNA thermal cycler (BioRad) of 35 cycles for 3 min at 93ºC, 30 s at 65ºC, followed by 2 min at 72ºC, and a final extension at 75 ºC for 10 min. *stx1*+ *stx2* and *fliC* were amplified using the same conditions except

for the annealing temperature that was performed at 55ºC for 30 s (Osek & Gallein, 2002). 10 µL of PCR products were analyzed by electrophoresis on a 2% (wt/vol) agarose gel (containing 10 µL ethidium bromide) at 100 mV for 60 min. The DNA samples analyzed by agarose gel electrophoresis, were then visualized by UV illuminator and photographed.

Detection of biofilm formation

Biofilm production was quantitatively investigated using the method of adherence to polystyrene microtiter plates proposed by Christensen et al. (1985), with some modifications. All the bacterial isolates were cultivated on tryptic soy broth and one at a time and incubated to initiate growth for 18 hours to reach an OD≤1 at 600 nm. The cultures were then diluted 1:100 with fresh medium (Hassan et al., 2011), and 200 µl were used to inoculate sterile 96-well polystyrene microtiter plates. After incubation for 24 hours at 37ºC, the cultures were discarded and microtiter plates were washed with distilled water to eliminate the unattached cells. Attached cells were then fixed at 60ºC for 1 hour and stained with 1% crystal violet solution. Excess stain was removed by successive washings. The crystal violet in each well was solubilized with 200 µL of 96% ethanol, and microplate reader was used to measure the absorbance at 540 nm (Sosa & Zunino, 2009). Negative control wells contained sterile broth. The experiment was performed in triplicates and repeated three times. The interpretation of biofilm production was done according to the criteria of Stepanovic et al. (2007) (table 2).

Effect of pre-coating microtiter plates with tap water on biofilm formation

Wells were pre-coated with 200 µl of tap water (sterilized by filtration against PVDF 0.45µm membrane filters) for 24 hours at 37ºC and then excess water was withdrawn from the wells. Pre-coated wells were inoculated with cultures of the isolated bacterial strains, incubated at 37 ºC for 24 hours and then biofilm formation was measured. Negative control pre-coated wells contained sterile broth. The experiment was performed in triplicates and repeated three times.

Data Analysis

The average biofilm formation was calculated and statistically significant increase in biofilm formation was determined using paired sample test. The criteria for statistical significance was based on a (p<0.05).

Results and Discussion

Bacterial diversity

Regarding the bacterial diversity, 25 different bacterial isolates were detected from all the tested sources. Most of the isolates were detected as Gram-negative bacteria, most belonging to the Coliform group. Gram-positive bacterial isolates detected represented about 8% of the total population. 91.3% of bacterial isolates detected in Beit-Din station were isolated mainly from canal walls. 86.95% of the isolates detected in Beit-Din station and other isolates were then detected each with different level within the 20 households tap water (table 3).

Farkas et al. (2013) proved that approximately 95% of bacterial cells were attached to the pipe walls forming biofilms and less than 5% were found in the water phase. These biofilms were a source of planktonic bacteria, which remain present in the water when delivered through a consumer's tap (Simöes & Simöes, 2013).

The detected bacterial isolates in all samples were: *E.coli, Citrobacter youngae, Citrobacter braakii, Enterobacter cloacae, Enterobacter intermedius, Klebsiella oxytoca, Klebsiella terrigena, Klebsiella pneumoniae ozaenae, Stenotrophomonas maltophilia, Pantoea spp., Hafnia alvei, Serratia marcescens, Serratia fonticola, Serratia plymuthica, Yersinia enterocolitica, Pseudomonas flourescens/ putida, Pasteurella pneumotropica/haemolytica, Pasteurella multocida, Chryseomonas luteola, Aeromonas hydrophila, flavimonas oryzihabitans*, *Acinetobacter, Alcaligenes, Staphylococcus aureus* and coagulase negative *Staphylococcus*. Faria et al. (2009) reported the presence of coagulase negative staphylococci (CNS) isolated from a drinking water distribution network. However no *Salmonella* sp., *Listeria* sp. nor Enteroccci were detected (table 3). Lleo et al. (2005) reported that *Escherichia coli*, *Enterococcus faecalis* and *Salmonella typhi*, once released into freshwater bodies, enter into a viable but non-culturable (VBNC) state and express different sets of activities, including virulence traits. Therefore, a molecular detection method is needed, since such methods are highly specific and sensitive (Kinge et al., 2012).

Thermotolerant bacteria namely: *E. coli*, *Klebsiella, Enterobacter* and *Citrobacter* as well as *Pantoea* spp., *Stenotrophomonas maltophilia, Serratia marcescens* and *Hafnia alvei* (48% of the total population) were isolated on Lauryl sulfate agar at 44ºC. These results are in accordance with those shown by Alonso et al. (1999) and Hachich et al. (2012). Thermotolerant coliforms other than *E. coli* may originate from organically enriched water such as industrial effluents or from decaying plant materials and soils (WHO, 2003). It was worth mentioning that coliforms showed the ability to grow under low nutrient conditions as those in drinking water distribution systems and remain within biofilms successfully competing with other bacteria (Camper et al., 1996).

Detection of *E. coli* O157:H7

E. coli O157:H7 was not detected in any of the tested samples using the commercial kit VIDAS® ECO O157 (BioMérieux, France).

Antibacterial activity of commonly used antibiotics against the isolated *E.coli*

The antibiogram analysis revealed that all seven *E. coli* isolates (7 isolates) (table 4) were proved to be of different strains. Each of the isolates showed different susceptibility to all the tested antibiotics.

Molecular characterization of STX-EC using PCR

Seven *E. coli* isolates were analysed by PCR for the presence of *stx1*, *stx2*, *stx1+stx2*, *eaeA*, *ehlyA* and *fliC* virulence markers with the specific sets of primers (table 1).

The results showed different gene profiles of the isolated strains. None of the seven *E. coli* isolates were positive for *stx1* and *stx2*, however, six isolates were positive for *stx1+stx2* (figure 1A). Four out of seven isolates were positive for the *eaeA* gene (figure 1B). Sandhu et al. (1996) reported that the presence of the *eaeA* gene in STX-EC strains is associated with some O groups such as O26, O103, O111, O157. Although *eaeA* carrying STX-EC strains are frequently associated with severe infections, outbreaks of HUS by *eaeA* negative STX-EC strains have already been reported (Paton & Paton, 1998).

Six *E. coli* isolates were positive for *ehlyA* gene (figure 1C), while four out of the six isolates were are also proved to be *eaeA*

positive (table 5). As reported by several authors (Beutin et al., 1989 ; Karch et al., 1989 ; Bielaszewska et al., 1998 and Paton & Paton, 1998), the combination of *ehlyA* and *eaeA* pathogenic marker genes proved to be an important indicator of pathogenicity of STX-EC for humans than either factor alone. The present study revealed that all the *E. coli* isolates (7 isolates) were considered as STX-EC serotypes, carrying virulence marker genes. It was reported that STX-EC can survive for long periods in water (Farrokh et al., 2013) and STX-EC was detected in drinking water supplies in Brazil (Lascowski et al., 2013). However none of these isolates were positive for the *fliC* gene and thus are H7-negative. Osek & Gallein (2002), reported that H7-negative strains have recently been identified that represent a distinct clone within *E. coli* O157serogroup. However, it shares several virulence characteristics with other STX-EC of the O157:H7 serotype.

Table.1 Primers used for STX-EC strain identification

Target gene	**Primers**	**Sequence**	**Reference**	**Predicted size of the amplified product (bp)**
stx1	stx1	F: CAGTTAATGTCGTGGCGAAGG R: CACCAGACAATGTAACCGCTG	Osek and Gallein (2002)	**348**
stx2	stx2	F: ATCCTATTCCCGGGAGTTTACG R:GCGTCATCGTATACACAGGAGC	Osek and Gallein (2002)	**584**
stx1+stx2	STEC	F: gA(Ag) C(Ag)A AAT AAT TTA TAT gTg R: TgA TgA Tg(Ag) CAA TTC AgT AT	Reischl et al. (2002)	**520**
eaeA	eaeA	F: gAC CCg gCA CAA gCA TAA gC R: CCA CCT gCA gCA ACA AgA gg	Reischl et al. (2002)	**383**
fliC	fliC H7	F: GCTGCAACGGTAAGTGAT R: GGCAGCAAGCGGGTTGGT	Osek and Gallein (2002)	**984**
ehlyA	ehlyA	F: gCA TCA TCA AgC gTA CgT TCC R: AAT gAg CCA AgC Tgg TTA AgC T	Reischl et al. (2002)	**532**

Table.2 Interpretation of biofilm formation

Average OD value	Biofilm production
≤ ODc	Non
ODc < ~ ≤ 2x ODc	Weak
2x ODc < ~ ≤ 4x ODc	Moderate
> 4x ODc	Strong

Table.3 Diversity of bacterial isolates in water system under test

Isolate	Water supply (Beit-Din)	Water canal	20 Households tap water
Salmonella	-	-	-
Listaeria	-	-	-
Enterococci	-	-	-
E.coli	-	+	+
Citrobacter youngae	+	-	+
Citrobacter braakii	-	+	-
Enterobacter cloacae	-	+	+
Enterobacter intermedius	-	+	+
Klebsiella oxytoca	-	+	+
Klebsiella terrigena	+	+	+
Klebsiella pneumoniae ozaenae	-	+	+
Stenotrophomonas maltophilia	-	+	+
Pantoea spp.	-	+	-
Hafnia alvei	+	+	+
Serratia marcescens	-	+	+
Serratia fonticola	-	+	+
Serratia plymuthica	+	-	+
Yersinia enterocolitica	-	+	+
Pseudomonas Flourescens/putida	-	-	+
Pasteurella pneumotropica/haemolytica	-	+	+
Pasteurella multocida	-	+	+
Chryseomonas luteola	-	+	+
Aeromonas hydrophila	-	+	+
Flavimonas oryzihabitans	-	+	+
Acinetobacter	-	+	-
Alcaligenes	-	-	+
Staphaphylococcus aureus	+	+	+
Coagulase negative *Staphylococcus*	-	+	+

Table.4 Sources of detection of *E.coli* isolates

***E. coli* isolates**	**Source**
E.coli 1	Water canal
E.coli 2	Household tap water $_1$
E.coli 3	Household tap water $_2$
E.coli 4	Household tap water $_3$
E.coli 5	Household tap water $_4$
E.coli 6	Household tap water $_5$
E.coli 7	Household tap water $_6$

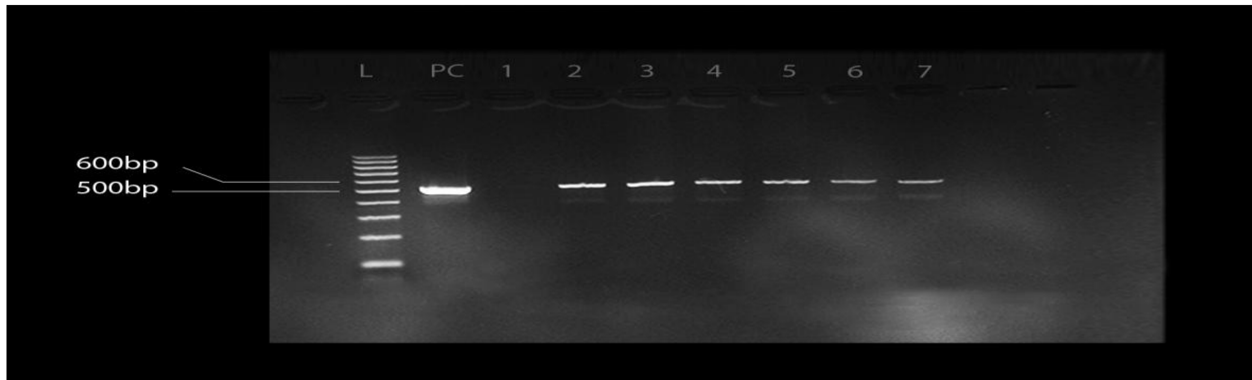
Table.5 Prevalence of virulence marker genes in *E.coli* isolates under test using PCR

Isolate	Virulence markers					
	stx1	*stx2*	*stx1+stx2*	*eaeA*	*ehlyA*	*fliC*
1	-	-	-	+	+	-
2	-	-	+	+	+	-
3	-	-	+	+	+	-
4	-	-	+	+	+	-
5	-	-	+	-	-	-
6	-	-	+	-	+	-
7	-	-	+	-	+	-

Table.6 Effect of pre-coating microtiter plates with tap water on biofilm formation

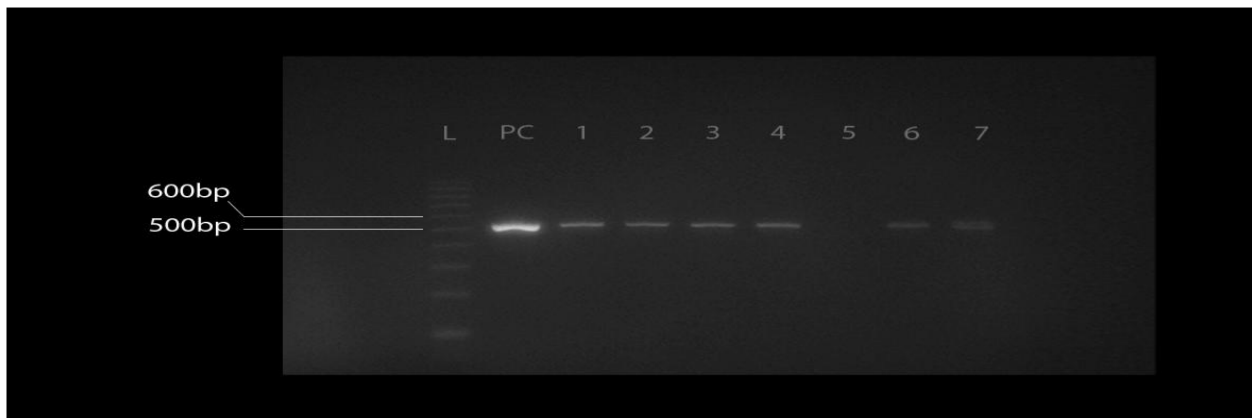
Isolate	Biofilm formation before pre-coating with tap water ± SD	Biofilm formation after pre-coating with tap water ± SD
E.coli 1	3.11 ± 0.11 (moderate)	4.13 ± 0.1 (strong)
E.coli 2	3.41 ± 0.1 (moderate)	3.81 ± 0.08 (moderate)
E.coli 3	5.77 ± 0.4 (strong)	6.10 ± 0.2 (strong)
E.coli 4	2.24 ± 0.23 (moderate)	3.42 ± 0.31 (moderate)
E.coli 5	2.14 ± 0.08 (moderate)	2.76 ± 0.1(moderate)
E.coli 6	2.73 ± 0.11(moderate)	3.18 ± 0.12 (moderate)
E.coli 7	4.15 ± 0.09 (strong)	4.20 ± 0.1 (strong)
Citrobacter youngae	1.85 ± 0.3 (week)	3.02 ± 0.2 (moderate)
Citrobacter braakii	1.63 ± 0.2 (week)	2.49 ± 0.09 (moderate)
Enterobacter cloacae	1.42 ± 0.5 (week)	2.49 ± 0.3 (moderate)
Enterobacter intermedius	3.08 ± 0.09 (moderate)	3.26 ± 0.11 (moderate)
Klebsiella oxytoca	4.37 ± 0.16 (strong)	4.67 ± 0.1 (strong)
Klebsiella terrigena	2.31 ± 0.2 (moderate)	2.80 ± 0.12 (moderate)
Klebsiella pneumoniae ozaenae	2.65 ± 0.07 (moderate)	3.18 ± 0.13 (moderate)
Stenotrophomonas maltophilia	1.88 ± 0.12 (week)	2.16 ± 0.09 (moderate)
Pantoea spp.	3.41 ± 0.05 (moderate)	3.59 ± 0.1 (moderate)
Hafnia alvei	2.75 ± 0.13 (moderate)	3.11 ± 0.2 (moderate)
Serratia marcescens	5.14 ± 0.14 (strong)	6.70 ± 0.07 (strong)
Serratia fonticola	2.81 ± 0.2 (moderate)	3.20 ± 0.15 (moderate)
Serratia plymuthica	2.59 ± 0.06 (moderate)	2.90 ± 0.18 (moderate)
Yersinia enterocolitica	3.78 ± 0.24 (moderate)	4.74 ± 0.17 (strong)
Pseudomonas Flourescens/putida	2.52 ± 0.31 (moderate)	2.93 ± 0.09 (moderate)
Pasteurella pneumotropica /haemolytica	3.76 ± 0.12 (moderate)	3.95 ± 0.1 (moderate)
Pasteurella multocida	3.40 ± 0.07 (moderate)	4.04 ± 0.05 (strong)
Chryseomonas luteola	8.51 ± 0.2 (strong)	8.81 ± 0.5 (strong)
Aeromonas hydrophila	1.67 ± 0.42 (week)	2.01 ± 0.27 (moderate)
Flavimonas oryzihabitans	4.61 ± 0.31 (strong)	5.62 ± 0.19 (strong)
Acinetobacter	8.51 ± 0.2 (strong)	8.81 ± 0.37 (strong)
Alcaligenes	4.87 ± 0.08 (strong)	5.12 ± 0.16 (strong)
Staphaphylococcus aureus	6.86 ± 0.26 (strong)	7.52 ± 0.09(strong)
Coagulase negative *Staphylococcus*	4.48 ± 0.13 (strong)	5.83 ± 0.07 (strong)

Figure 1: DNA fragments observed with specific primers for *stx1+ stx2* gene (A), *eaeA* gene (B) and *ehlyA* gene (C) detection in the seven *E.coli* isolates following PCR. L: Ladder; PC: Positive control; 1-7: *E.coli* isolates .



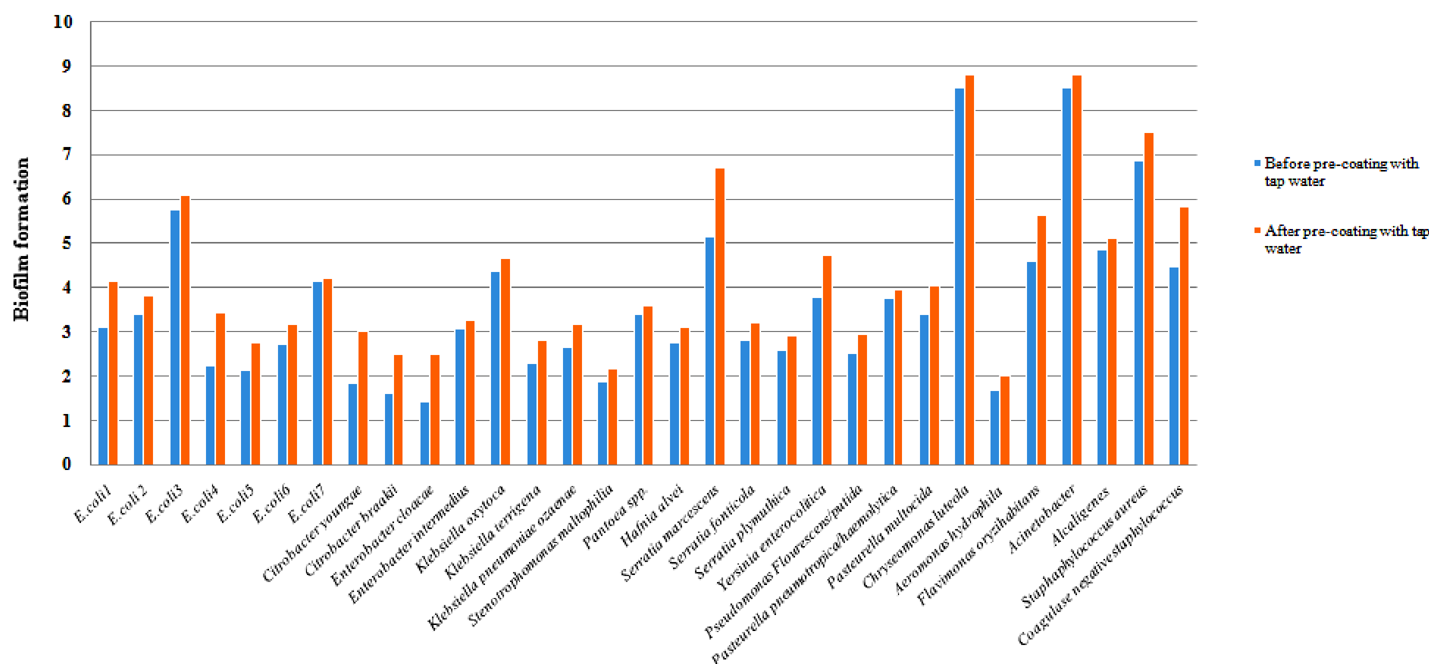
A

B



C

Figure.2 Effect of pre-coating microtiter plates with tap water on biofilm formation



Biofilm formation

Out of 31 tested isolates, 10 were detected as strong (32.26%), 16 as moderate (51.61%) and 5 as weak (16.13%) biofilm producers (table 6). Strong biofilms were produced by *Acinetobacter* and *Chryseomonas luteola* followed by *Staphylococcus aureus*, *E.coli* 3, *Serratia marcescens*, *Alcaligenes*, *Flavimonas orizyhabitans*, coagulase negative *Staphylococcus*, *Klebsiella oxytocae* and *E.coli* 7 individually and one at a time. Most of these strong producers were detected attached to the wall of the water canal. Strong biofilm producers that were not detected attached to the water canal but instead appeared in the water samples; maybe detached from the surface due to various circumstances, such as changes in the flow rate or desorption of reversibly attached cells within the biofilm, that represent a potential source of water contamination (Breyers & Ratner, 2004 and WHO, 2014).

Effect of pre-coating microtiter plates with tap water on biofilm formation:

Pre-coating the monitored microtiter plate with tap water showed a highly significant increase in biofilm production with all the isolates one at a time ($p<0.05$) (table 6 and figure 2), where all weak turned into moderate and some moderate turned into strong biofilm producers, so that 41.94% of the tested isolates appeared as strong and 58.06% appeared as moderate biofilm producers. It was shown that attachment will occur most readily on surfaces that are rougher, more hydrophobic, and coated by surface conditioning films (Millsap et al., 1997; Donlan, 2002; Chae et al., 2006; Patel et al., 2007; Oulahal, et al., 2008 and Simões et al., 2008). Pre-conditioning of the adhesion surface is due either to macromolecules present in the water or intentionally coated on the surface (Breyers & Ratner, 2004).

In conclusion, the present study provides an important baseline for the diversity of bacterial populations, and confirmed the prevalence of STX-EC in drinking water distribution system in Lebanon which poses public health problems. The present findings suggested that the microbiological quality of drinking water should be continuously monitored from the source to the tap using adequate disinfection processes. This also opens the door for finding novel strategies for the treatment of biofilms within the drinking water distribution system in Lebanon.

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