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Rapid Detection of Indicator Bacteria in Drinking Water Using Multiplex PCR Technique

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Abstract

For identifying fecal pollution in water, microbial indicators, particularly those from the coliform groups *Escherichia coli* and Enterococci, have been utilized, which are considered guidelines for the quality of drinking water. The multiple-tube fermentation (MTF) and membrane filter (MF) procedures for detecting indicator organisms are recognized conventional methods, and they are compared with the multiplex PCR method. Three sets of different primers were used in combination with the multiplex PCR procedure. The first set aims to amplify 876 base pairs from the *lacZ* gene, which is present in all coliform bacteria, including *E. coli*. The second set aims to amplify 147 base pairs from the *uidA* gene, which is unique to *E. coli*. While the final set yields a *tuf* gene with 112 base pairs that are unique to all Enterococci. The multiplex PCR technique has been found to be an effective, sensitive, and quick method for detecting these three microbial indicators in contaminated water. In Damietta County, Egypt, three water treatment stations (El-Rahmna - El-Adlyia - El-Bostan) were evaluated for their effectiveness at removing pollutants from the water using the optimized multiplex PCR.

Keywords: *Microbial indicators, PCR techniques, LacZ, uidA , tuf.*

Introduction

Environmental health preservation requires drinkable water safe and free of pathogenic bacteria. Microbial water quality is known as a measure of the microbiological water conditions associated with human and animal health requirements (Pachepsky *et al*., 2018).
The coliform group, particularly *E. coli*, was utilized as an indicator of the microbiological quality of water at the end of the 19th century. The presence of fecal coliform bacteria in water indicates that the water has been contaminated with the feces of warm-blooded animal (Sudip *et al.*, 2021). *E. coli* was used to identify water samples that may contain unacceptable levels of fecal contamination (Odonkor and Ampofo 2013).
Presence of total coliform bacteria can indicate treatment issues such as inefficiency,

disinfectant loss (McFeters *et al*., 1986), intrusion of contaminated water into the potable water supply (Geldreich *et al*., 1992; Clark *et al*., 1996), or regrowth issues in the distribution system (LeChevallier 1990). As a result, treatment should not be accepted if total coliform bacteria are present in treated water samples.

Other criteria, such as β-D-galactosidase-positive reactions, had been added to the definition of the coliform group (APHA 2017).

Fecal streptococci are considered to have advantages over coliform bacteria as indicators because they are more resistant to environmental stress and chlorination (Gebra and Pepper 2019). The two most common genera of fecal streptococci are *Enterococcus* and *Streptococcus*. *Enterococcus faecalis* and *Enterococcus faecium* are the pathogenic enterococci species in humans, with *E. durans, E. gallinarum, E. casseliflavus, E. avium, E. hirae, E. mundtii,* and *E. raffinosus* causing occasional infections (Murray 1990; Woodford *et al*., 1995).

The multiple-tube fermentation (MTF) and membrane filter (MF) techniques for coliform detection that based on the cultivation of bacteria (Deshmukh *et al*., 2016; Rompré *et al.,* 2002) have been approved by the US Environmental Protection Agency (EPA 1986). These techniques limitations include the length of the incubation period, interference from antagonistic organisms, a lack of group-specificity for the coliforms, and a poor level of detection of slow-growing or stressed coliforms. High levels of sensitivity and specificity can now be achieved using molecular techniques without the use of lengthy culture procedures or additional confirmation stages.

For the detection of coliform bacteria and *E. coli* in water samples, multiplex PCR and gene probes have been used in the past (Bej *et al.*, 1991; Juck *et al.,* 1996).

Tantawiwat *et al*., (2005) have been amplified the genes *lacZ, uidA*, and *plc* to detect total coliform, *Escherichia coli*, and *Clostridium perfringens* in drinking water.

E. coli groups that may exist in wastewater treatment plants were monitored and detected using multiplex PCR for the *mdh, eaeA, stx1, stx2, st, lt, ial*, and *eagg* genes (Omar and Barnard 2010).

The PCR-based assay was created to detect enterococci that target the elongation factor Ef-Tu encoding *tuf* gene (Ke *et al*., 1999; Maheux *et al*., 2009; Maheux *et al.,* 2011).

Hong *et al*., (2011) developed two types of PCR assays that could detect and quantify *Campylobacter* sp., *E. coli* O157:H7, and *Salmonella* sp., in watershed samples.

Additionally, Wose *et al*., (2012) created a different multiplex PCR technique that makes it possible to simultaneously detect water-borne *Salmonella, Shigella, E. coli*, and *Klebsiella* bacteria spp. from rivers in the North West region of South Africa utilizing the *IpaB, IpaH, Mdh*, and *GapA* genes, respectively.

Enterococcus faecalis and *Enterococcus faecium* strains used as indicators of fecal pollution were analyzed using Pulsed-Field Gel Electrophoresis (Furukawa and Suzuki 2013**)**.

Maheux *et al*., (2014) selected the *wecG* gene, encoding the enterobacterial common antigen, to create a PCR assay specifically for Enterobacteriaceae. The *wecG* gene was chosen since the family includes *Escherichia* and every coliform member, as well as about 20 different genera. *Salmonella, Shigella*, and *Yersinia* are also included as food-borne pathogens.

This study aimed to optimize a multiplex PCR technique using three sets of oligonucleotides specific for *lacZ, uidA* and *tuf* genes, which exist in coliform bacteria, *Escherichia coli* and Enterococci, respectively. This would help monitor the quality of potable water in terms of bacterial fecal contamination.

Materials and Methods

Water sampling

Water samples were collected from three different drinking water treatment stations (El-Adlyia, El-Bostan and El-Rahmna) present in Damietta County (Egypt). Samples were taken every three months from each treatment station alternatively for one year from June to May. The proposed samples were collected from four different places, input "before treatment", treatment basins, after treatment and output "some sources of water consumption" from each station. All used bottles, containers, and glassware were washed with nutrient-free detergent overnight, rinsed three times with distilled water, and then soaked in 10% (v/v) HCl for at least 24 hours. The items were then dried at room temperature after the HCl soak.

Method for multiple-tube fermentation

Multiple-tube fermentation (MTF) has been used as a method to count coliforms and track the quality of water. A series of tubes containing Lauryl tryptose medium (Biolife) are inoculated using the proper decimal dilutions of the water sample. After 48 hours at 37°C of incubation, the presence of gas production, acid generation in the test tubes is regarded as a positive presumptive reaction (Sudip *et al.*, 2021).
A confirmation test is subsequently performed on all tubes that have a positive presumptive result. Eosin-Methylene Blue Agar medium (EMB) was used for the confirmed test .Coliforms form colonies with dark centers in EMB within 48 hours at 37°C.These colonies were submitted to a completed test using MacConkey agar medium, which produce pink colonies within 24 hr. The positive colonies from the completed test were cultured on nutrient agar and then stained with Gram stain (AFNOR 1990; APHA 2017).
The MTF technique results are illustrated using the most probable number (MPN) of microorganisms present (Bachtia 2002).

Fecal coliform test

The EC medium, that is commercially produced as a powder (Conda, S.A) had been used for fecal coliform detection in contaminated testing water samples (APHA 2017). 0.1ml was added to the fermentation tube containing the EC broth medium from the positive presumptive tube. The development of turbidity in the fermentation tubes and the presence of gas in the Durham tubes were considered to be positive indicators of fecal coliforms in water samples. Inoculated EC broth tube(s) were incubated in a water bath at 44.5ºC for 24 hours.

Membrane filter technique for fecal coliform detection

A sterile 0.45 µm pore size filter was used to filter a 100 ml sample of water. A selective medium was used to incubate the filter that contained the bacteria. According to APHA (2017), filters should be cultured for 24 hours at 44.5°C on an enhanced lactose culture medium (m-FC). The development of blue or greyish colonies was regarded as a positive result.

Bacterial strains

The Microbiology Laboratory, Botany and Microbiology department, Faculty of Science, Damietta University, Egypt, generously donated reference strains (*Enterobacter* sp., *E. coli,* and *Enterococcus* sp.). Bergey's Manual of Systematic Bacteriology was used to identify the 13 bacterial strains that were recovered from collected water samples (Bernner and Farmer 1984; Schleifer 1984).

Genomic DNA Extraction

DNA was extracted either from a 1.5ml overnight nutrient broth culture of reference strain bacteria or directly from a 1 L water sample filtered through a sterile 0.45m pore size filter at each site. The filter paper was then washed with 1 ml of the SET buffer (20% sucrose, 50 mM Tris HCL, pH 7.6, 50 mM EDTA), and the microorganisms were separated by centrifugation at 6.500 rpm for 5 min. The process was modified to extract DNA from the bacterial pellets using phenol/chloroform and 70% ethanol precipitation (Ausubel *et al*., 1996). Bacteria were suspended in 100 µl of SL buffer (SET buffer plus lysozyme), to which 2 µl of RNase was then added. 500 µl of TE buffer (10 mM Tris HCl, pH 7.6, 0.2 mM EDTA) and 70 µl of 10% sodium dodecyl sulfate were added after 10 min of incubation at 37 °C.
Following isopropanol precipitation, DNA was extracted using a phenol, chloroform, and isoamyl alcohol solution (25:24:1). After being washed with 75% ethanol, the DNA was re suspended in water-free nuclease and kept at -20°C until it was used for PCR amplification.

The multiplex PCR primer and optimization

Table 1 shows the oligonucleotide sequence of the primers and the expected product sizes for each pathogen. 30pmole of each primer, 1 µl of extracted chromosomal DNA, and 12.5 µl of Dream Taq Green PCR Master Mix (Ferments) were employed to optimize the multiplex PCR amplification. With water free nuclease, the reaction mixture's final volume was adjusted to 25 µl. In a PCR system thermo cycler (TECHNE model TC-312, UK), all multiplex PCR reactions were performed using the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, primer annealing at 56°C for 30 sec, primer extension

at 72°C for 1 min, and the final extension at 72°C for 10 min.

Table 1. List of the primers used for water pathogens detection

Bacteria	Primer sets	Target gene	Product size (bp)	Ref.
Coliform bacteria	Set1: Forward: 5′ ATG AAA GCT GGC TAC AGG AAG GCC 3′ Reverse: 5′ CAC CAT GCC GTG GGT TTC AAT ATT 3′	*LacZ*	876	(Bej *et al*., 1990)
E. coli	Set2: Forward: 5′ TGG TAA TTA CCG ACG AAA ACG GC 3′ Reverse: 5′ ACG CGT GGT TAC AGT CTT GCG 3′	*UidA*	147	(Bej *et al*., 1991)
Enterococci Species	Set3: Forward: 5′ TAC TGA CAA ACC ATT CAT GAT G 3′ Reverse: 5′ AAC TTC GTC ACC AAC GCG AAC 3′	*tuf*	112	(Ke *et al*., 1999)

Multiplex PCR primers sensitivity

The sensitivity of the previous three sets of primers to the minimal cell count detection was tested. The reference strains (*Enterobacter* sp., *E. coli*, and *Enterococcus* sp.) were prepared from overnight cultures in 1 ml serial dilutions (10^0 to 10^{-7}). On nutrient agar plates, 100 µl aliquots of each dilution were cultivated to determine the colony forming unit (CFU) count. For the purpose of collecting bacterial cells, additional 100 µl aliquots from the same dilutions were centrifuged. Each pellets DNA was taken out, and the results of the PCR analysis were analyzed. For each dilution, the produced PCR products were compared to the corresponding CFU count.

Detecting amplified *DNA*

10 µl of the amplified product were electrophoresed on a 1.5% agarose gel in 1X TAE buffer (0.04M Tris-acetate, 0.001M EDTA) at pH 8 to check for the presence of the anticipated products. For DNA staining, 2 µl of Ethidium Bromide (10 mg/ml) were applied. 4µl of DNA ladder (Ferments) was run parallel to the samples. The run was performed at 150 volt. The gel was then visualized under 312-nm Transilluminator.

Results

Bacterial strain identification and distribution

Thirteen isolates were identified at the input stage of the three drinking water treatment stations. These isolates were classified into two groups. Group1: belongs to *Enterobacteriaceae* that include *Providencia* sp.1, *Providencia* sp.2, *Klebsiella* sp., *Serratia* sp., *Enterobacter* sp.1, *Enterobacter* sp.2 and *Citrobacter* sp. Group 2: was identified as Gram positive cocci which include 6 isolates of Streptococci. The distribution of the identified isolates within the input stage of El-Rahmna, El-Bostan and El-Adlyia stations were represented in Table 2. The most probable number (MPN) of bacteria was used to express the MTF method results (Table 2). MPN was calculated when five tubes were used per dilution (10ml, 1ml, 0.1ml of collected water samples) and inoculated on Lauryl tryptose medium. MPN was varied from 2 to ≥ 1600/100ml water. Generally, the highest values were observed in the input stage at El-Bostan, El-Rahmna and El-Adlyia stations. The highest values (1600/100ml) were detected in El-Bostan (September and December), in El-Rahmna (November, February and May) and in El-Adlyia (July and October). The lowest value (11/100ml) was recorded in El-Adlyia (January). The other treatment stages in all stations did not give a positive result for the MTF technique.

Fecal coliform test by EC broth

A fecal coliform test was then performed on the positive tubes in a presumptive reaction. After 24 hours of incubation at 44.5°C, the production of gas was considered a positive result (Table 3). Results showed that the highest counts of fecal coliform (50/100ml and 23/100ml) were observed in September and December, respectively for El-Bostan station (input stage).The lowest value (2/100ml) was reported in June for the El-Bostan treatment station. Interestingly, the other treatment stages for all stations gave a negative result on EC broth.

Table 2. Distribution of the identified bacterial strains within the input stage El-Rahmna, El-Bostan and El-Adlyia treatment stationsalong one year. (+) presence and (-) absence of the strain.

Water station	Time of sample collection	Combination of positives	MPN Index/100ml	*Providencia* sp.1	*Prvidencia* sp.2	*Serratia* sp.	Coliform bacteria				Streptococci
							Enterobacter sp.1	*Enterobacter* sp.2	*Citrobacter* sp.	*Klebsiella* sp.	
El-Rahmna	August	5-2-0	50	-	-	-	-	-	+	+	-
	November	5-5-5	≥1600	-	-	-	+	+	-	+	+
	February	5-5-5	≥1600	-	+	+	-	-	-	-	-
	May	5-5-5	≥1600	+	-	-	-	-	-	-	+
El-Bostan	June	5-5-0	240	-	-	-	-	-	+	+	-
	September	5-5-5	≥1600	+	+	-	+	+	-	-	-
	December	5-5-5	≥1600	+	-	-	+	+	+	+	-
	March	5-5-3	900	-	-	-	-	-	-	-	+
El-Adlyia	July	5-5-4	1600	+	+	-	-	-	-	-	+
	October	5-5-5	≥1600	-	-	+	-	-	-	+	-
	January	3-0-1	11	-	-	-	-	-	-	-	-
	April	5-4-2	220	+	-	-	+	-	-	-	-

Table 3. MPN Index for various combinations of positive results on fecal coliform (EC) medium for input stage of El-Rahmna, El-Bostan and El-Adlyia treatment planets.

Water station	Date	Combination of positives	MPN Index/100ml
El-Rahmna	August	2-1-0	7
	November	1-2-0	6
	May	2-2-0	9
El-Bostan	Jun	1-0-0	2
	September	5-1-1	50
	December	5-0-0	23
	March	2-0-0	4
El-Adlyia	October	0-2-0	4
	April	3-0-0	8

Membrane filter technique

The fecal coliform (FC) count using Millipore membrane filters showed two positive colonies found in El-Rahmna (November), while only one positive colony was recorded at El-Rahmna (August and May) and El-Adlyia (October and April) (Table 4). The other treatment stages in all stations gave a negative result on m-FC.

Table 4. Number of positive colonies of various stations/100ml of filtrated water samples using membrane filter technique on m-FC medium.

Water station	Date	Stage of treatment	No. of positive colonies
El-Rahmna	August	Input	1
	November	Input	2
	May	Input	1
El-Adlyia	October	Input	1
	April	Input	1
		basins Treatment	1

Molecular detection of indicator organisms using multiplex PCR

The three sets of oligonucleotide primers were tested separately using reference bacteria. Individual detection of amplified DNA fragments with expected molecular weights of 876 bp for total coliform bacteria, 147 bp for *E. coli*, and 112 bp for Enterococci is shown in Fig. 1, lanes 1-3.The combination of the three primer sets using a mixture of the reference bacteria produced the same expected size for each strain (Fig. 1, lane 4).

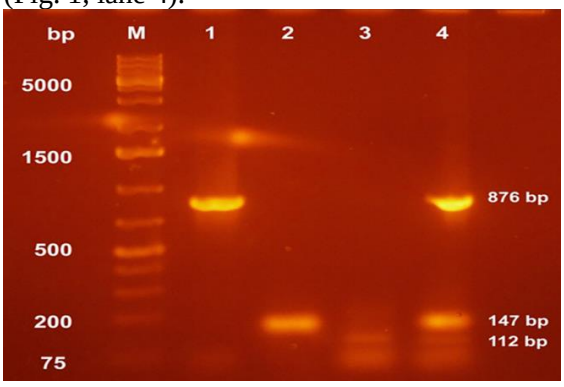

Fig. 1 PCR amplified products on 1.5% agarose gel for optimization the multiplex PCR condition. Lane M: 1Kb DNA ladder, lane 1: primer set1 for *Enterobacter* sp. detection, lane 2: primer set2 for *E. coli* detection, lane3: primer set3 for *Enterococcus* detection, lane 4: multiplex primers of "The three sets" with a mixture of the last previous strains.

Sensitivity of the optimized multiplex PCR

The sensitivity of the multiplex PCR was determined using *Enterobacter* sp., *E. coli*, and *Enterococcus* sp. (Fig. 2 A, B and C, respectively). The multiplex PCR assay detected cultivable pathogens up to 5-10 CFU bacterial count in dilutions of 10^{-6} for primer set 1 and

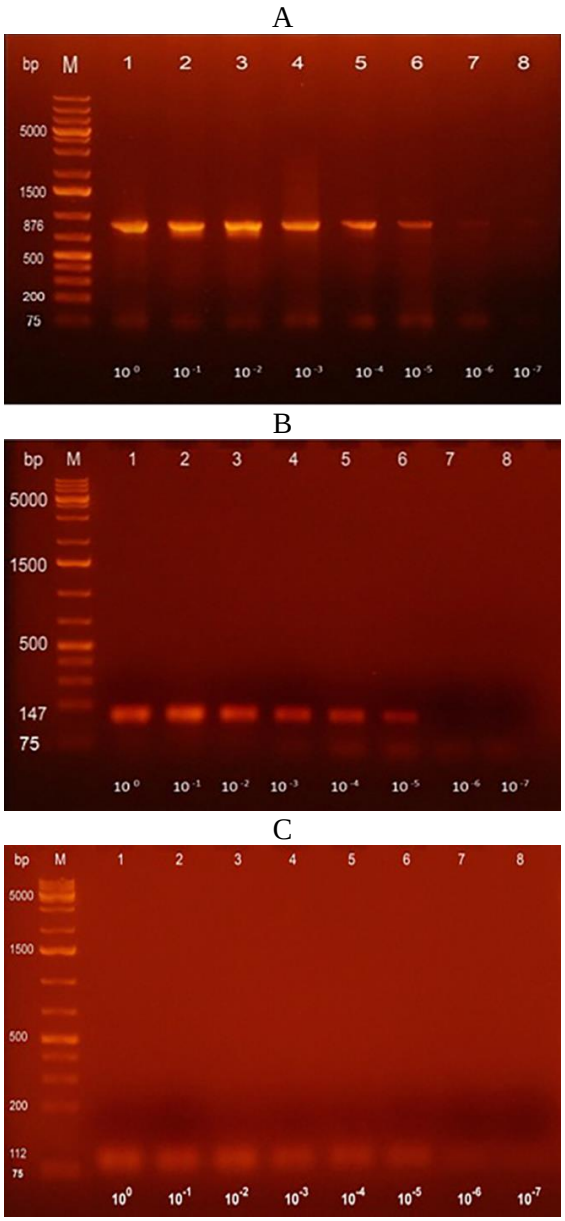

Fig. 2 Agarose gel (1.5%) showing sensitivity of PCR with bacterial dilution from 10^0 to 10^{-7} of **A**: *Enterobacter* sp. using *lacZ* primer set1, **B**: *E. coli* using *uidA* primer set2 and **C**: *Enterococcus* sp. using *tuf* primer set3. Lane M: molecular size marker (1kb DNA ladder), lanes 1 to 8, bacterial dilution, 10^0 - 10^{-7}, respectively

10^{-5} for primer sets 2 and 3. The tests reliability is comparable to that of existing culture-based techniques.

Detection of indicator organisms in polluted water using multiplex PCR

The multiplex PCR assay for the tested organisms that could be found in the studied sites produced a detectable DNA fragment of expected molecular weight only in the presence of their respective DNA templates. The *lacZ* gene of total coliform bacteria yielded a band of 876 bp on agarose gel electrophoresis, and the *tuf* gene of all Enterococci strains yielded a band of 112 bp on agarose gel electrophoresis (Fig. 3). For all of the sites examined, no *E. coli* strain was found.

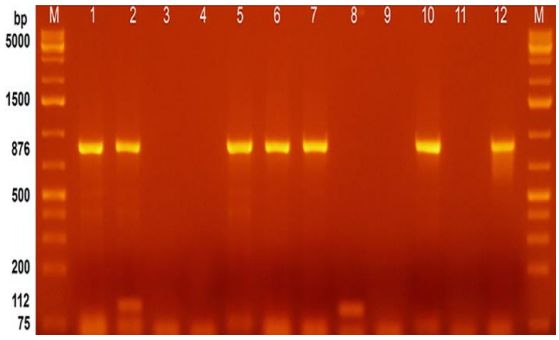

Fig. 3 Agarose gel (1.5%) electrophoresis of multiplex PCR amplified products of different water station samples. Lane M: molecular size marker (1kb DNA ladder), lane 1 to 4, El-Rahmna station (Input stage) August, November, February and May, respectively. Lane 5 to 8, El-Bostan station (Input stage) June, September, December and March, respectively. Lane 9 to 12, El-Adlyia (Input stage) July, October, January and April, respectively

Discussion

The multiplex PCR technique was used to test the presence of coliform bacteria (*E. coli* and *Enterococci*) in different drinking water treatment stations. The multiple-tube fermentation (MTF) and membrane filter (MF) procedures, which use different particular medium and incubation conditions, were significant compared to this assay. The El-Rahmna, El-Bostan, and El-Adlyia drinking water treatment stations typically recorded the highest count MPN of total coliform (TC) in the input stage because the Damietta branch receives a significant amount of wastes of mostly untreated agricultural domestic and

partially treated industrial wastewater (Zyadah 1996 ; Shawky and Saleh 2007; Amany *et al*., 2020). Additionally, the presence of organic suspended materials promotes microorganism growth (Rifaat 2007). Furthermore, high TC counts were observed in El-Rahmna (May), El-Bostan (March), and El-Adlyia (July) stations due to significantly high temperatures and discharged wastewater during these months; this result was consistent with (El Fadaly *et al*., 2001; Sabae 2004; Sabae and Rabeh 2007). In contrast, the MPN count of TC was low in El-Rahmna (August), El-Bostan (June) and El-Adlyia (April), It was due to the algal blooms in these months which, cause oxygen and nutrients depletion and also because of anti-microbial toxins (Sellner *et al*., 2003; Falconer 1999). However, The MPN count of total coliform was high in El-Rahmna (November and February), El-Bostan (September and December) and El-Adlyia (October) that might be due to the low water flow during autumn and winter. Furthermore, the Damietta branch receives the effluent of some wastes coming from several sources that may raise the microbial population (Henri 2009). Due to routine washing and cleaning of collecting basins during January, the MPN total coliform count was low in El-Adlyia treatment station. Migratory wildlife during the early winter increases the fecal coliform concentrations, so, the count of FC on EC and m-FC was high in El-Bostan (September) and El-Rahmna (November), respectively (Abu-Shady *et al*., 1996).

In this study, the multiplex PCR technique using three sets of primer detected the total coliform bacteria and Enterococci in drinking water with sensitivity reach to 5-10 CFU that collected directly from 1- liter water sample. Tantawiwat *et al*., (2005) were able to detect 1 to 10 CFU for *E. coli*, *Clostridium perfringens* and *Klebsiella pneumoniae* using multiplex PCR after pre-enrichment step by culturing for 6 hr. otherwise, the sensitivity was rather low to 10^4 CFU/ml. Maheux *et al.*, (2011) detected 4.5 Enterococcal CFU using real-time PCR. The results of the multiplex PCR were in agreement with those from the membrane filter (MF) and multiple-tube fermentation (MTF) procedures. Although MPN index of multiple-tube fermentation (MTF) technique was high in some stations like El-Rahmna (February and May) and El-Adlyia (July), multiplex PCR was not detected any tested organisms (total coliform and enterococci). It might be attributed to the false positive result with anaerobic lactose fermenters which made the count of MPN high *(Gary et al., 1995*).

E. coli was not detected using multiplex PCR, because it cannot survive well in the collecting basins outside intestinal tract, the population of *E. coli* in the collected samples is usually influenced by the degree of fecal pollution, absence of hygienic practices, or storage conditions (Krieg and Holt 1984). So, the test of presence of *E. coli* via a classical method does not absolutely confirm the absence or presence of fecal contamination. Multiplex PCR advantages in this study is to detect indicator bacteria and also to differentiate between the common three indicators (total coliform bacteria, *E. coli* and Enterococci) directly from a water sample in one step rather than the time-consuming presumptive and confirmatory procedures or even pre-enrichment culture step (Proctor and Hammes 2015). This would be useful as a quick alternative method for routine microbiological analysis of drinking water.

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الملخص العربي

عنوان البحث: الكشف السريع عن البكتريا الدالة علي تلوث مياه الشرب باستخدام تقنيه تفاعل البلمرة المتسلسل

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تهدف الدراسة إلى الكشف السريع عن الكائنات الدالة على تلوث المياه وذلك عن طريق المقارنة بين الطرق التقليدية للكشف عن تلوث المياه (تقنية الأنابيب عديدة التخمر وتقنية الفلترة) وبين الطرق الحديثة (تفاعل البلمرة السريع).
تم استخدام المؤشرات الميكروبية وخاصة الايشيريشياكولاى والانتيروكوكاى للكشف عن تلوث الماء بالبراز.
وقد تم تجميع العينات من ثلاث محطات لمعالجة مياه الشرب بمحافظة دمياط (محطة البستان – محطة العدلية – محطة الرحامنة) وتم ذلك من أربعة اماكن بالمحطة (المدخل "قبل المعالجة" – احواض المعالجة – بعد المعالجة – المخرج " مصادر الاستهلاك"). وذلك فى الفترة من ٢٠١٠/٦ الى ٢٠١١/٥.
العدد الأكثر احتمالا (MPN)من مجموع القولونيات لهذه المؤشرات البرازية تراوح بين ٢ الى ≥ ١٦٠٠/ ١٠٠مل.
وقد أظهرت هذه الطرق التقليدية وجود البكتريا القولونية الكلية والانتيروكوكاى في الثلاثة محطات وخصوصا في المرحلة الأولى (المدخل) من المعالجة.
ويستخدم تفاعل البلمرة المتسلسل عديد البادئات لتعيين البكتريا القولونية الكلية والايشيريشياكولاى والانتيروكوكاى في المياه الملوثة عن طريق تكبير جينات *tuf* and *lacZ, uidA* .

وبالفصل الكهربي لهلام الأجاروز أظهر قطعه من الحمض النووي طولها ٨٧٦ قاعدة نيتروجينية لجين *lacZ* في البكتريا القولونية الكلية.
وأظهر قطعتين من الحمض النووي طولهما ١٤٧ و٨٧٦ قاعده نيتروجينيه لجينى *lacZ and uidA* في الايشيريشياكولاى.
وأظهر قطعة اخرى طولها ١١٢ قاعدة نيتروجينية لجين *tuf* في الانتيروكوكاى.
كانت الفائدة الرئيسية من هذه الدراسة تطوير طريقة سريعة (تفاعل البلمرة المتسلسل عديد البادئات) للكشف عن أكثر من نوع من البكتريا في مياه الشرب.
هذه الطريقة يمكن استخدامها للكشف في وقت واحد عن الكائنات الحية الدقيقة الأخرى باستخدام البادئات المناسبة ويمكن استخدامها كطريقة تأكيدية للتحاليل الميكروبيولوجية الروتينية لمياه الشرب.