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Nested PCR and Conventional Techniques for Detection of *Salmonella* spp. in River Nile Water, Egypt

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THIS STUDY aimed to detect salmonellae group using most probable number (MPN), membrane filtration (MF) and nested PCR techniques from River Nile at Cairo segment and Rossita branch so, 74 samples were collected and analyzed to count the total viable bacteria/ml and total salmonellae. Total viable bacterial counts ranged from $1.0x10^2$ to $1.6x10^8$ cfu/m1 during the study. Using MPN technique salmonellae were detected in 64 samples out of 74 samples (86.4% recovery), with the MF technique, salmonellae were detected in 73 samples out of 74 samples and with PCR technique salmonellae were detected in all samples. It can be concluded that MF technique is more suitable to detect salmonellae as it allows to filter or pass relatively larger amount of water sample rather than MPN which measure salmonellae presence in fixed amount of water while nested PCR technique is more rapid, sensitive and specific than conventional techniques (*i.e.*, MF and MPN). Statistical analysis were done only between MF and MPN techniques due to PCR technique doesn't provide counts to be involved in statistical analysis (provide only positive and negative results). Results indicated that counts of *Salmonella* showed significant differences between sites using both techniques MF and MPN at Cairo segment. Wherever, no significant difference between sites at Rossita branch.

Keywords: River Nile, *Salmonella*, MPN technique, MF technique, PCR technique.

River Nile is the lifeblood of Egypt. The river is the main source of freshwater for household use and irrigation, a source of power from the hydroelectric facility at Aswan, and a mean of transportation for people and goods. According to the geographical features, the administrative boundaries and the human activities, the River Nile divided into seven segments. Cairo segment is considered the most important one because it represents the major cluster of drinking water treatment plants.

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Surface water may play an important role in the transmission of pathogenic microbes that arrive in this water via domestic wastewater and it can return to humans by various ways, such as the use of these water for recreation or sporting (swimming or fishing), for the irrigation of vegetables and fruit trees and as a source of drinking water (Kamel *et al.,* 2006).

Salmonella spp. are ubiquitous enteric bacteria. These Gram negative rods are the etiologic agents of waterborne salmonellosis and also the agents that cause typhoid and paratyphoid fevers. *Salmonella* is a prime example of a water transmitted pathogen. *Salmonella* is a leading cause of morbidity and mortality due to food and waterborne diseases in developed and developing world causes gastroenteritis and typhoid in humans (Malorny *et al.,* 2008). The typhoid caused by *Salmonella enterica* serotype Typhi remains an important public health problem in developing countries. In 2000, it was estimated that over 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths and that more than 90% of this morbidity and mortality occurred in Asia (Crump *et al.*, 2004). Further, Salmonellosis causes substantial medical and economic burdens worldwide (Voetsch *et al.,* 2004).

Salmonella is a large genus of bacteria including more than 2,300 serotypes, and diagnosis in the majority of laboratories relies on costly and laborious culture screening with both nonselective and selective media (Kilger & Grimont, 1993). *Salmonella* species are often detected in sewage, freshwater, marine coastal water, and groundwater. *Salmonella* spp. can survive for long periods in natural waters (El-Taweel, 1998; Baudart *et al.,* 2000; Shaban & El-Taweel, 2002 and Samhan, 2005).

A characteristic feature of this organism is its broad host spectrum, which comprises most animal species, including mammals, birds and cold-blooded animals, in addition to humans. Individuals infected with *Salmonella* shed the organism in their faeces, which enter the domestic sewage that, in turn, may contaminate drinking water sources. Although the concentration of *Salmonella* in water is low, ingestion of water can still cause infection, because the water may pass rapidly through the stomach into the intestines without stimulating digestion, and thereby escape the natural host defense mechanisms (Murray, 1991). There are several problems concerning the detection of *Salmonella* in water, such as their low numbers and sometimes intermittent presence, so to determine the presence of *Salmonella* in environmental and drinking water sources, sensitive and specific detection techniques are needed.

The traditional techniques currently in use are based on cultivation in selective media and identification of the organism by biochemical techniques followed by serotyping (D'Aoust, 1989). These procedures are time-consuming and laborious and several days are required for negative confirmation and at least 2–3 d for a positive confirmation. Further, increased public awareness related to health and economic impact of waterborne contamination and illness has resulted in greater efforts to develop more sensitive methods of pathogenic detection and identification. Therefore, efforts have been made by many workers to reduce time required and to increase the sensitivity of the methods to detect pathogenic bacteria (Carli *et al.,* 2001).

The uses of the polymerase chain reaction (PCR) to detect pathogenic micro-organisms in food, water and clinical specimens have become widely adopted during the last few years.

This study aims to compare between the two conventional techniques (MF and MPN) and the newest one (PCR) for investigation the presence of salmonellae group as pathogenic bacteria in raw River Nile water.

Materials and Methods

Sampling sites and collection

River Nile water samples were collected monthly intervals from five sites in Cairo segment from south to north as follow Kafr El-Elw, El-Maasara, El-Giza, Embaba and El-Galatma during one year and other additional sampling sites on Rossita branch (seven sites allocated along 60 km of Rossita branch which is a point source of pollution named as followed in the front of El-Rahawy drain, Abo El-Khawey (15 km next to El-Rahawy), El-Nigela (30 km next to El-Rahawy), Kafr El-Ziat (in the front of Menof drain), salt and soda production company (45 km, 50 km and 60 km next to El-Rahawy drain). Samples were collected from the middle of the river (30 cm deep from the surface) in a wide mouth sterile glass bottles. The samples were preserved in an ice box and examined within 2-4 hr.

Microbiological examination

Total bacterial counts were determined by pour plate technique according to APHA (2005). Detection of *Salmonella* was carried out by MPN and MF techniques according to ISO (2000) and APHA (2005), and nested PCR technique was carried out according to Waage *et al.* (1999). The samples were diluted with 1% NaCl up to 10^{-2}. One ml from each dilution was transferred into tube containing buffered peptone water (9 ml) (using five repetitions) and incubated at 37ºC for 24 hr, then 0.1 ml from each tube showing bacterial growth (turbidity) was transferred into 10 ml of Rappaport-Vassiliadis (RV) Broth (LAB M Co.) and incubated at 43.5±1ºC for 24 hr, then a loop from each tube was taken and streaked out onto bismuth sulfite agar (MERCK Co.). The plates were incubated for 48 hr at 37°C. While in case of MF technique, 10 ml from each sample were filtered through nitro-cellulose 0.45 µm membrane (*Whattman* Co.), then transferred onto bismuth sulfite agar and incubated for 48 hr at 37°C and suspected colonies were confirmed by PCR.

Preparation of water samples for PCR

The samples were prepared as follows: 1000-2000 ml were filtered with nitro-cellulose membrane (0.45 µm pore size and 47 mm in diameter (*Whattman* Co.)). The membrane filters were transferred to 10 ml tryptic soya broth (DIFCO Co.) with 10% glycerol plates and incubated overnight at room temperature with gently shaking. DNA extractions were carried out according to Kapperud *et al.* (1993) and Waage *et al.* (1999), from each overnight culture 100 µl were

transferred into Eppendorf tubes and centrifuged at 13000 rpm for 15 min in a microcentrifuge (Labfuge, 460). The resulting pellets were resuspended in 50µl PCR buffer with 1µl of Proteinase K (0.2 mg ml^{-1}). After incubation at 37^{o}C for 1 hr, the bacteria were lysed by boiling for 10 min. The samples were stored at 20^{o}C overnight prior to PCR. After thawing at room temperature and centrifugation at 14900 xg for 5 min, 5µl of supernatant were used for PCR reaction.

Selection and synthesis of primers

Two pairs of PCR primers were selected according to Waage *et al.* (1999) from published DNA sequences of a randomly cloned fragment of the *Salmonella typhimurium* chromosome, two outer primers for the first PCR step were as follows : SAL- 1F, (5'- GTA GAA ATT CCC AGC GGG TAC TG- 3'), SAL- 2R, (5'- GTA TCC ATC TAG CCA ACC ATT GC- 3') and the other two inner primers for second PCR step were as follows SAL- 3F, (5'- TTT GCG ACT ATC AGG TTA CCG TGG- 3'), SAL- 4R, (5'- AGC CAA CCA TTG CTA AAT TGG CGC A- 3'). Primers SAL-1F and SAL-2R were derived from the conserved sequences ST15 and ST11, respectively while Primers SAL-3F and SAL-4R were derived from the conserved sequences ST14 and ST11, respectively.

DNA amplification

The PCR amplification of the target sequence was performed according to Waage *et al.* (1999) by the following constituents 1x *Taq* buffer with $MgCl_2$, 200 µM dNTPs, 0.1 µM for primers and 1 U *Taq* DNA polymerase in total reaction mixture of 50 µl. The PCR protocol of first PCR was one cycle at 95°C for 3 min, then 40 cycles (95°C for 30 sec, 60°C for 1 min and 72°C for 1.5 min), one cycle at 72°C for 10 min and then maintain at 4°C. The nested PCR was carried out in a total volume of 50 µl. A 1.0 µl aliquot of the first PCR product was used as template for nested PCR. The PCR protocol of nested PCR was one cycle at 95°C for 3 min, then 20 cycles (95°C for 30 sec, 67°C for 1 min and 72°C for 1.5 min), one cycle at 72°C for 10 min and then maintain at 4°C. The PCR products were loaded on 1% agarose gel with ethidium bromide (Sambrook *et. al.,* 1989) with ΦX174 HaeIII DNA Marker (Promega Co.).

Statistical analysis

F ratio obtained by single factor *ANOVA* was calculated for salmonellae group at five sites at Cairo segment and seven sites at Rossita branch using Microsoft Windows XP, Excel (Office 2003) computer application.

Results and Discussion

In the present investigation, the total bacterial counts which used as a general picture of bacterial load and salmonellae group, as pathogenic bacteria, were determined in water samples collected from five sites (Kafr El-Elw, El-Maasara, El-Giza, Embaba and El-Galatma) of the River Nile at Cairo segment during one year. In addition of fourteen samples from Rossita branch.

Total bacterial counts

Table 1 represents the total bacterial counts at 22ºC and 37ºC in samples collected from Cairo segment. The densities of total bacterial counts at 22ºC and 37ºC ranged from $1.3x10^2$- $2.2x10^4$ cfu/ml in samples collected from Kafr El-Elw and $1.3x10^2$- $6.1x10^5$ cfu/ml in samples collected from other sites. Regarding the time of determination, the highest counts were recorded during August in samples collected from all sites. These data revealed a high incidence of microbial load in the Nile water especially; it is going to be used as a water supply for drinking water treatment plants. The higher microbial load in the Nile water at Cairo segment was due to dense population, human activities and industrial centers as a major pollution sources.

TABLE 1. Total bacterial count cfu/ml at 22°C and 37°C in River Nile water at Cairo segment.

Months	Kafr El-Elw		El-Maasara		El-Giza		Embaba		El-Galatma	
	22ºC	37ºC	22ºC	37ºC	22ºC	37ºC	22ºC	37ºC	22ºC	37ºC
December	$2.0x10^3$	$1.0x10^3$	$7.0x10^3$	$2.4x10^2$	$7.2x10^3$	$1.8x10^4$	$1.1x10^5$	$4.0x10^4$	$9.0x10^3$	$4.0x10^3$
January	$2.0x10^2$	$4.0x10^2$	$1.3x10^2$	$4.3x10^2$	$1.6x10^3$	$1.3x10^2$	$9.8x10^3$	$3.6x10^3$	$2.6x10^3$	$1.6x10^3$
February	$3.0x10^3$	$7.0x10^2$	$1.1x10^3$	$1.8x10^2$	$1.1x10^5$	$1.0x10^4$	$8.6x10^4$	$1.4x10^4$	$9.0x10^3$	$3.0x10^2$
March	$1.8x10^3$	$9.0x10^2$	$1.0x10^3$	$3.9x10^2$	$1.8x10^3$	$1.4x10^3$	$1.6x10^3$	$3.7x10^2$	$2.2x10^3$	$1.6x10^3$
April	$4.0x10^3$	$1.0x10^3$	$6.0x10^4$	$2.0x10^3$	$4.0x10^3$	$2.0x10^3$	$5.0x10^4$	$4.0x10^3$	$2.0x10^3$	$1.0x10^3$
May	$2.0x10^2$	$6.0x10^2$	$8.0x10^2$	$2.2x10^3$	$1.7x10^4$	$8.2x10^3$	$1.0x10^4$	$6.0x10^3$	$2.0x10^3$	$1.4x10^3$
June	$3.0x10^2$	$1.0x10^2$	$4.2x10^3$	$6.0x10^2$	$6.8x10^3$	$2.9x10^3$	$4.1x10^3$	$1.8x10^3$	$1.2x10^3$	$4.0x10^2$
July	$1.0x10^3$	$7.6x10^3$	$6.0x10^2$	$4.0x10^2$	$4.2x10^4$	$1.2x10^4$	$2.2x10^4$	$1.0x10^4$	$6.0x10^2$	$5.0x10^2$
August	$2.2x10^4$	$1.6x10^4$	$1.7x10^5$	$1.3x10^5$	$1.2x10^5$	$1.1x10^5$	$2.8x10^5$	$1.3x10^5$	$6.1x10^5$	$3.1x10^5$
September	$1.3x10^2$	$8.7x10^3$	$2.0x10^2$	$9.3x10^2$	$2.3x10^2$	$2.1x10^2$	$4.6x10^2$	$2.2x10^2$	$2.7x10^2$	$1.4x10^2$
October	$1.0x10^3$	$9.6x10^2$	$1.5x10^3$	$8.1x10^2$	$1.2x10^3$	$3.8x10^3$	$3.9x10^3$	$6.6x10^3$	$1.5x10^3$	$3.6x10^3$
November	$5.2x10^3$	$8.0x10^2$	$2.2x10^3$	$3.7x10^3$	$5.6x10^3$	$1.4x10^3$	$4.5x10^3$	$1.9x10^3$	$6.8x10^3$	$7.6x10^3$
Minimum	$1.3x10^2$	$1.0x10^2$	$1.3x10^2$	$1.8x10^2$	$2.3x10^2$	$1.3x10^2$	$4.6x10^2$	$2.2x10^2$	$2.7x10^2$	$1.4x10^2$
Maximum	$2.2x10^4$	$1.6x10^4$	$1.7x10^5$	$1.3x10^5$	$1.1x10^5$	$1.1x10^5$	$1.1x10^5$	$1.3x10^5$	$6.1x10^5$	$3.1x10^5$
Average	$3.4x10^3$	$3.2x10^3$	$2.0x10^4$	$1.1x10^4$	$2.6x10^4$	$1.4x10^4$	$4.8x10^4$	$1.8x10^4$	$5.3x10^4$	$2.7x10^4$

On the other hand, from Table 2 the results of fourteen samples collected from Rossita branch showed that, the average of total viable bacterial counts at 22°C and 37ºC was ranged from $4.0x10^4$- $1.6x10^8$ cfu/ml in all samples collected from all sites. The increase in total bacterial counts at Rossita branch sites were referred to the presence of two drains (El-Rahawy and Menof) receiving raw sewage and agriculture wastes of the surrounding area.

The average values of *Salmonella* at Cairo segment by MPN technique was ranged between 5.2x10- $2.3x10^2$ MPN-index/100ml. While the average values of *Salmonella* detected by MF technique was ranged between 3.1x10- $2.4x10^2$ cfu/100ml during the study period (Table 3). The highest densities of *Salmonella* were found at El-Giza and Embaba sites followed by El-Galatma site and the lower density of *Salmonella* was at El-Maasara site.

TABLE 2. Total bacterial count cfu/ml at 22°C and 37°C in River Nile water (Rossita branch).

Months	Site no. 1		Site no. 2		Site no. 3		Site no. 4		Site no. 5		Site no. 6		Site no. 7	
	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C	22°C	37°C
July	$2.6x10^6$	$3.1x10^6$	$1.6x10^8$	$1.6x10^8$	$3.4x10^6$	$3.3x10^6$	$3.5x10^5$	$3.8x10^5$	$2.9x10^6$	$2.3x10^6$	$2.2x10^6$	$2.3x10^6$	$4.1x10^6$	$1.5x10^6$
August	$4.0x10^5$	$7.2x10^5$	$6.0x10^4$	$6.5x10^4$	$3.0x10^5$	$3.2x10^5$	$8.0x10^4$	$2.8x10^5$	$4.0x10^4$	$4.0x10^4$	$1.0x10^5$	$9.5x10^4$	$4.0x10^4$	$3.8x10^7$
Average	$1.5x10^6$	$1.9x10^6$	$8.0x10^7$	$4.5x10^7$	$1.8x10^6$	$1.8x10^6$	$2.1x10^5$	$3.3x10^5$	$1.4x10^6$	$1.1x10^6$	$1.1x10^6$	$1.2x10^0$	$2.0x10^6$	$1.9x10^7$

TABLE 3. *Salmonella* counts by MPN (MPN-index/100ml) and MF techniques (cfu/100ml) from River Nile samples at Cairo segment.

Months	Kafr El-Elw		El-Maasara		El-Giza		Embaba		El-Galatma	
	MPN	MF	MPN	MF	MPN	MF	MPN	MF	MPN	MF
December	$3.4x10^2$	$2.6x10^2$	7.0x10	2.2x10	$9.0x10^2$	$1.8x10^2$	$1.6x10^2$	$2.2x10^2$	$1.6x10^2$	$1.3x10^2$
January	1.3x10	7.0x10	7.0x10	3.0x10	9.0x10	5.0x10	$1.7x10^2$	$2.7x10^2$	3.3x10	6.0x10
February	1.4x10	1.1x10	7.0x10	1.6x10	1.4x10	9.0x10	ND	1.6x10	2.0x10	1.4x10
March	ND	2.0x10	ND	1.1x10	ND	1.2x10	ND	1.1x10	ND	3.5x10
April	ND	1.0x10	2.0x10	1.9x10	$2.6x10^2$	$1.0x10^2$	8.0x10	1.1x10	$1.0x10^2$	$1.3x10^2$
May	4.0x10	2.4x10	$1.7x10^2$	2.8x10	$2.7x10^2$	$5.7x10^2$	$2.3x10^2$	$1.5x10^2$	$1.1x10^2$	$1.0x10^2$
June	7.0x10	ND	2.7x10	4.8x10	$2.3x10^2$	$2.6x10^2$	$2.4x10^2$	$1.4x10^2$	3.0x10	1.3x10
July	ND	3.2x10	ND	3.7x10	$5.0x10^2$	$9.0x10^2$	$4.4x10^2$	$9.2x10^2$	2.3x10	2.2x10
August	$1.1x10^2$	4.0x10	4.0x10	2.1x10	$1.2x10^2$	$1.3x10^2$	$3.0x10^2$	$2.4x10^2$	$2.6x10^2$	$1.5x10^2$
September	1.2x10	4.0x10	9.0x10	6.0x10	1.4x10	$1.8x10^2$	6.0x10	9.4x10	2.0x10	8.1x10
October	1.6x10	4.0x10	8.0x10	7.6x10	$3.3x10^2$	$1.0x10^2$	$1.1x10^2$	$3.2x10^2$	3.0x10	4.0x10
November	1.7x10	7.6x10	ND	1.2x10	$1.4x10^2$	$1.0x10^2$	$3.3x10^2$	$6.0x10^2$	1.7x10	1.2x10
Minimum	1.2x10	1.0x10	2.0x10	1.1x10	1.4x10	5.0x10	6.0x10	1.1x10	1.7x10	1.2X10
Maximum	$3.4x10^2$	$2.6x10^2$	$1.7x10^2$	7.6x10	$9.0x10^2$	$9.0x10^2$	$4.4x10^2$	$9.2x10^2$	$2.6x10^2$	$1.5x10^2$
Average	5.2x10	5.0x10	5.3x10	3.1x10	$2.3x10^2$	$2.1x10^2$	$1.7x10^2$	$2.4x10^2$	6.6x10	6.5x10

Salmonella spp. are constantly found in environmental samples, because they are excreted by humans, pets, farm animals and wild life. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in natural waters. It has been suggested that the survival capacity of environmental strains may depend on species and pollution sources (Polo *et al.*, 1998 and Baudart *et al.*, 2000).

While, the average values of *Salmonella* by MPN technique was ranged between 9.6x10- $1.7x10^3$ MPN-index/100ml, the average values by MF technique was ranged between 9.0x10- $1.8x10^3$ cfu/100ml at Rossita branch (Table 4). The highest density of *Salmonella* was found at site no. 2 followed by site no. 4 and the lower density of *Salmonella* at site no. 6. The obtained results of *Salmonella* were confirmed by the high density of total bacterial counts at the same sites.

The results showed that, *Salmonella* was detected in 64 out of 74 (86.4%) samples when using MPN technique and 73 out of 74 (98.6%) samples when using MF technique (including 14 samples of Rossita branch). El-Taweel (1998) studied the presence of salmonellae in water samples collected from River Nile at Cairo segment in eight sampling sites along 60 km during two years. He found that, the highest frequencies ($1.4x10^3$-$2.6x10^3$ cfu/100ml) of salmonellae isolation were obtained at El-Gezira site, Farouk Corner and Damietta branch sites. While the average counts recorded at the other sites were between $3.1x10^2$ and $5.8x10^2$ cfu/100ml and he added that salmonellae were detected in 25 out of 32 (78%) samples by MF technique during the study period. Ali *et al.* (2000), El-Taweel & Shaban (2001) and Kamel *et al.* (2006) recorded similar results and they concluded that the high counts of salmonellae may be referred to municipal and food processing waste discharge along the main stream of the River Nile and its branches.

Waterborne diseases have been a major global health problem throughout history. Pathogens are constantly released at variable concentrations from infected humans, pets, farm animals and wildlife (Geldriech, 1996). Municipal sewage and storm water runoff become the conduits for the passage of pathogens into surface waters (O'Shea & Field, 1992 and Irvine *et al.*, 1995). It was shown that the annual bacterial loads of *Salmonella* in rivers and coastal areas could be very important (O' Shea & Field, 1992 and Irvine *et al.*, 1995).

TABLE 4. *Salmonella* counts by MPN (MPN-index/100ml) and MF technique (cfu/100ml) from River Nile water at Rossita branch.

Months	Site no. 1		Site no. 2		Site no. 3		Site no. 4		Site no. 5		Site no. 6		Site no. 7	
	MPN	MF	MPN	MF	MPN	MF	MPN	MF	MPN	MF	MPN	MF	MPN	MF
July	$1.7x10^2$	$1.8x10^2$	$3.4x10^3$	$3.4x10^3$	$2.6x10^2$	$2.7x10^2$	$4.2x10^2$	$4.8x10^2$	$1.4x10^2$	$1.6x10^2$	8.2x10	9.0x10	$1.5x10^2$	$1.6x10^2$
August	5.5x10	6.0x10	$1.7x10^2$	$2.4x10^2$	$1.8x10^2$	$1.9x10^2$	$1.0x10^2$	$1.0x10^2$	$1.1x10^2$	$1.2x10^2$	$1.1x10^2$	$1.2x10^2$	$1.1x10^2$	$1.2x10^2$
Average	$1.1x10^2$	$1.2x10^2$	$1.7x10^3$	$1.8x10^3$	$2.2x10^2$	$2.3x10^2$	$2.6x10^2$	$2.9x10^2$	$1.2x10^2$	$1.4x10^2$	9.6x10	9.0x10	$1.3x10^2$	$1.4x10^2$

The first PCR step amplifies a fragment with an estimated size of 438 bp, while the size of the final PCR product is 312 bp. *Salmonella* was positive in 74 out of 74 (100%) samples by nested PCR where 10 out of 74 (13.5%) were positive by first PCR. All water samples were positive nested PCR (Fig. 1 and 2). 19 random isolates out of 19 (100%) were positive when confirmed by PCR.

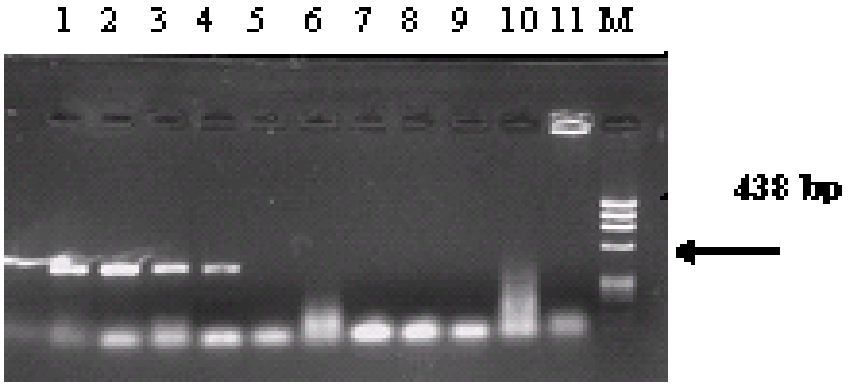

Fig. 1. Gel electrophoresis of first PCR lane1: positive control, lane 2: Kafr Elw, lane 3: El-Massara, lane 4: El-Giza, lane 5: Kafr El-Elw, lane6: El-Massara, lane 7: El-Giza, lane 8: Kafr El-Elw, lane9: El-Massara, lane10: El-Giza, lane11: Embaba, M: Marker (ØX 174).

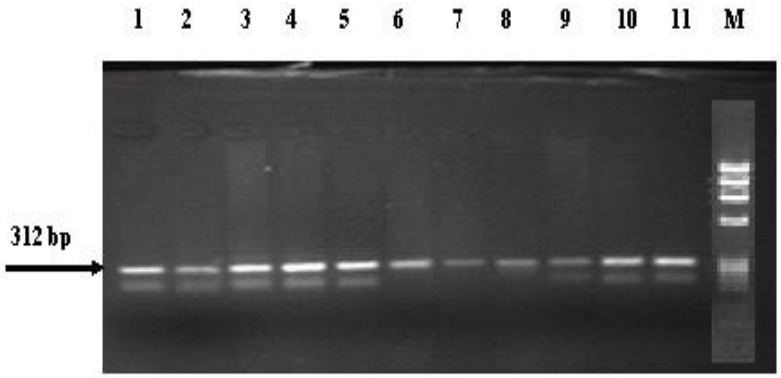

Fig. 2. Gel electrophoresis of nested PCR lane 1: positive control, lane 2: Kafr Elw, lane 3: El-Massara, lane 4: El-Giza, lane 5: Kafr El-Elw, lane 6: El-Massara, lane 7: El-Giza, lane 8: Kafr El-Elw, lane 9: El-Massara, lane 10: El-Giza, lane11: Embaba, M: Marker (ØX 174).

It can be conclude that, comparison between results of detection of *Salmonella* by MPN technique, MF technique and PCR techniques are difficult and puzzle. MPN technique is less sensitive (86.4%) than the two other techniques, it may due to presence of more than one selective constitutes in RV medium which inhibit for some species of *Salmonella* itself. On the other hand, there is no significant change between results of MF technique (98.3% & 100%) and nested PCR technique (100%), while first PCR (0.0% & 16.6%) which is less sensitive than nested PCR (Table 5), so the nested approach was chosen to improve the specificity of the assay (Steffan & Atlas, 1991). Any non-specific PCR products produced during the first step of the PCR will not be amplified during the second step due to lack of complementarily to inner primer sequences. The sensitivity of the assay is also improved by use of nested PCR. Sub-detectable levels of PCR products generated during the first step of the PCR are amplified to reach detectable levels during the second step. A 10000-fold increase in sensitivity has been reported (Arias *et al.*, 1995). In addition, any inhibitory substances present will be diluted, since only 1/100 of the reaction mixture of the first PCR step is transferred into the second reaction mixture.

TABLE 5. *Salmonella* presence in raw River Nile water by MPN, MF and nested PCR techniques.

Samples	Number of samples	MPN technique	MF technique	Nested PCR technique	
				First	Second
River Nile Cairo segment	60	50 (83.3%)	59 (98.3%)	10 (16.6%)	60 (100%)
Rossita branch	14	14 (100%)	14 (100%)	0.0 (0.0%)	14 (100%)

Jyoti *et al.* (2010) found that the site no. 3 (Nishat Ganj Bridge) exhibits highest number of the *Salmonella* spp. ($1.19x10^9$ cfu/100ml) harboring *invA* gene in comparison to other sampling locations in the river Gomti at Lucknow. Also, the surface water samples from the river Ganga, Yamuna and their confluence point at Allahabad were also positive for *Salmonella* spp. ($1.57x10^5$-$1.33x10^4$ cfu/100ml). The high vulnerability to waterborne *Salmonella* infections in Asia and other developing countries is due to scarcity of potable water and dependence of a large population on natural resources for daily water requirement (Moganedi *et al.*, 2007). The runoff from fields with animal husbandry, addition of untreated sewage from nearby civilization contributes *Salmonella* in natural water resources (Moganedi *et al.*, 2007 and Jenkins *et al.*, 2008).

Statistical analysis

Statistical analysis were occurred only between MF and MPN techniques due to PCR technique doesn't provide counts to involved in statistical analysis (provide only positive and negative results). F ratios obtained by single factor *ANOVA* for *Salmonella* determined by MF and MPN between different sites at

Cairo segment and Rossita branch were represented in Table 6. Results indicated that counts of *Salmonella* showed significant differences between sites using both techniques (MF and MPN) at Cairo segment. Wherever, no significant difference between sites at Rossita branch may be due to sampling number is low. From these results, it can be concluded that, significantly there is no difference when we compared MF with MPN techniques, it may be return to the ratio between negative to positive samples (Table 6).

TABLE 6. Analysis of Variance (*ANOVA*) for *Salmonella* detection by MF and MPN techniques from different sites at Cairo segment (during one year) and Rossita branch.

Sampling sites	Techniques	Sample source	df	F	P
Cairo segment	MPN	Between sites	4	*4.30	0.0042
	MF	Between sites	4	*4.26	0.0045
Rossita branch	MPN	Between sites	6	1.01	0.4857
	MF	Between sites	6	1.08	0.4527

*Significant at ≤5% level; other F ratios not significant.

Finally, MF technique is more suitable to detect salmonellae as it allows to filter or pass relatively larger amount of water sample rather than MPN which measure salmonellae presence in fixed amount of water while nested PCR technique is more rapid, sensitive and specific than conventional technique (MF and MPN). In the future the authors recommend using quantitative PCR technique for monitoring of pathogens in surface water which affects water treatment processes in drinking water treatment plants.

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الكشف عن مجموعة السالمونيلا بطريقة تفاعل البلمره المتسلسل التأكيدى وبالطرق التقليديه فى مياه نهر النيل , مصر

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تهدف هذه الدراسه إلى الكشف عن مجموعة السالمونيلا فى مياه نهر النيل بقطاع القاهره الكبرى وفرع رشيد وذلك بإستخدام كلاً من الطرق التقليديه (طريقة العد الأكثر احتمالاً وطريقة الأغشيه المرشحه) وبطريقة تفاعل البلمره المتسلسل التأكيدى, لذلك تم تجميع ٧٤ عينه من مياه نهر النيل تم فيها تقدير العد الكلى البكتيرى لكل واحد مل من عينة المياه وذلك لتحديد الصوره البكتيريولوجيه لمياه نهر النيل وكذلك تم الكشف عن مجموعة السالمونيلا بالطرق السابقه. أظهرت النتائج أن العد الكلى البكتيرى يتراوح بين ١,٠ x ١٠^{٢} إلى ١,٦ x ١٠^{٨} مستعمره/ واحد مل وذلك أثناء فترة الدراسه.

أما بالنسبة لمجموعة السالمونيلا فقد أظهرت النتائج ٦٤ عينة موجبه من ٧٤ عينة مختبره بنسبة ٧٦,٤٪ وذلك بإستخدام طريقة العد الأكثر احتمالاً, بينما أظهرت طريقة الأغشيه المرشحة ٧٣ عينة موجبة من ٧٤ عينة مختبره . أما بالنسبه لطريقة تفاعل البلمره المتسلسل التأكيدى فقد تم الكشف عن مجموعة السالمونيلا فى كل العينات (٧٤ عينة).

لذا فإن طريقة الأغشيه المرشحه تعتبر طريقه مناسبه للكشف عن مجموعة السالمونيلا وذلك لأنها تسمح بترشيح أو مرور كميه كبيره من عينة المياه المختبره إذ ما قورنت بطريقة العد الأكثر احتمالاً والتى تكشف عن مجموعة السالمونيلا فى كميه ثابته من عينة المياه لأنها مرتبطه بجداول احصائيه, وبالنسبة لطريقة تفاعل البلمره المتسلسل التأكيدى فهى طريقة سريعه, أكثر حساسيه, واكثر تخصصيه من الطرق التقليديه مثل (طريقة الأغشيه المرشحه وطريقة العد الأكثر احتمالاً).

وقد تم عمل تحليل أحصائى فقط بين طريقتى الأغشيه المرشحه وطريقة العد الأكثر احتمالا وذلك لأن طريقة تفاعل البلمره المتسلسل التأكيدى لا يعطى عدد لكى يتم ادراجها فى التحليل الأحصائى (يعطى فقط نتيجه سالبه أو موجبه). ولقد اوضح التحليل الأحصائى لأعداد مجموعة السالمونيلا المتحصل عليها بطريقتى الأغشيه المرشحه وطريقة العد الأكثر احتمالاً بأن هناك فرق واضح فى الأعداد بين المواقع فى قطاع القاهره الكبرى, بينما لا يوجد فرق واضح بين المواقع فى عينات المياه المجمعه من فرع رشيد وذلك ممكن رجوعه لقلة عدد العينات المأخوذه من هذا الموقع.

ولذلك توصى الدراسه بإستخدام تفاعل البلمره المتسلسل الكمى لتحديد الأعداد الفعليه لمجموعة السالمونيلا الموجوده فى نهر النيل حتى يتسنى للقائمين على محطات تنقية مياه الشرب سهولة التخلص من البكتيريا الممرضه باتباع أنسب طرق المعالجه.