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Rapid detection and enumeration of coliforms and *Escherichia coli* in River Nile using membrane filtration technique

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ABSTRACT

The purpose of this investigation was to compare Rapid Hicoliform Agar® (RHA, Himedia, India) with the conventional m-Endo Agar (DIFCO, Michigan, USA) for detection and enumeration of Total Coliforms (TC) and with the conventional Seven Hour Fecal Coliforms Agar (7hFC) for detection and enumeration of *Escherichia coli* using Membrane Filtration (MF) in River Nile water, Egypt. Sixty samples were collected (five samples per month) from five regions (Kafr El-Elw, El-Maasara, El-Giza, Embaba and El-Galatma) during one year (December 2005-November 2006). The results showed that total coliforms counts ranged from 10^3 to 10^4 CFU·100ml^{-1} using RHA but using m-Endo Agar the counts were around 10^4 CFU·100ml^{-1}. *Escherichia coli* counts ranged from 10^3 to 10^4 CFU·100ml^{-1} using 7hFC Agar, while using RHA the counts ranged from 10^3 to 10^4 CFU·100ml^{-1}. Statistical analysis showed that there was no significant difference ($p=0.423$) between total coliforms counts using m-Endo Agar and RHA media, also, there was no significant difference ($p=0.546$) between fecal coliforms (*E. coli*) counts using 7hFC Agar and RHA media. We conclude that RHA is a potential alternative medium for detection and enumeration of total coliforms and *E. coli* at the same time and the same Petri dish within 24h. Also, RHA medium was a very sensitive medium, which did not require any confirmatory tests and secured rapid recovery of total coliforms or *E. coli*.

INTRODUCTION

Correlations between enteric diseases and contaminated water supplies in the 1850s (Snow 1855) and the subsequent discovery of microbial disease agents in the 1880s led to the exploration of procedures to test for bacterial presence in water. Theodore Escherich (1885) suggested the use of *Bacillus coli* (later *Escherichia coli*) as a suitable indicator for fecal contamination since it was found in high densities in feces and was associated with the typhoid bacillus.

In the early 20th Century, microbiologists (Eijkman 1904; Leiter 1929) learned to detect *E. coli* by observing gas production in glucose broths incubated at elevated temperatures. This test, called multiple tube fermentation (MTF) used serially diluted test samples in lactose broth. MTF became known as the Standard Test for Water Analysis and was a universally applied test for fecal pollution of water.

Goetz and Tsuneishi (1951) proposed and developed a membrane filtration (MF) procedure that was found to be more accurate than MTF and offered a considerable savings in time, labor, and cost. A variety of defined media were formulated and tested for use with MF (Clark et al. 1951; Dufour et al. 1981; Geldreich et al. 1965; Levin et al. 1975; McCarthy et al. 1961; Messer and Dufour 1998; Presswood and Strong 1978; Rose et al. 1975; USEPA 2000, 2003) to isolate, enumerate, and identify indicator bacteria for water quality assessment. MF continues to be useful in monitoring drinking, natural, and wastewater samples and is a standard practice in many public health laboratories in the United States (APHA 2005).

Bacterial pollution indicators chosen for fresh waters are predominantly comprised of the total coliforms bacteria; a group of small, facultative, Gram-negative bacilli (Enterobacteriaceae), which produce acid and gas from lactose within 48h of incubation at 35°C (MTF technique) and develop a red colony with a metallic sheen within 24h at 35°C on an Endo-type medium containing lactose (MF technique) (APHA 2005). The use of 'total coliforms' narrowed to 'fecal coliforms' and then to the more restrictive *E. coli* norm currently approved for fresh water analysis by the U.S. Environmental Protection Agency (1986).

Although FC have traditionally been regarded as good indicators of fecal contamination of water, recent reviews suggest *Escherichia coli* to be a better indicator (Edberg et al. 2000; Fewtrell and Bartram 2001). The choice of *E. coli* as an indicator organism of fecal pollution is already accepted by various world organizations (APHA 1998, 2005; WHO 2001).

E. coli is considered to be superior to previous indicators (total coliforms and fecal coliforms) because: (a) it is the only "fecal coliform" bacteria of true fecal origin; (b) it is present in large numbers (approximately 10^9 colonies·g^{-1}) in the feces of warm-blooded animals; (c) it survives longer than some bacterial pathogens, yet is resistant to re-growth outside of the host under typical environmental conditions, and (d) it can be detected and quantified simply and affordably (Edberg et al. 2000; Leclerc et al. 2001). For these reasons, the United States Environmental Protection Agency (USEPA) encourages states to adopt *E. coli* as the basis of water quality standards for pathogens (USEPA 2002).

In the late 1980s another water assessment tool, defined substrate technology (DST), became available (Edberg et al. 1988). DST simultaneously detects total coliforms bacteria and *E. coli* by enzymatic hydrolysis of specific substrates. In DST, two active substrates, *O*-nitro-phenyl-β-D-galactopyranoside (ONPG) and 4-methyl-umbelliferyl-β-D-glucuronide (MUG) are combined to simultaneously detect total coliforms and *E. coli*. Total coliforms produce the enzyme β-galactosidase which hydrolyzes ONPG and thereby release *O*-nitrophenol which produces a yellow color. *E. coli* produce the enzyme β-D-glucuronidase which hydrolyzes MUG to form a fluorescent compound (4-methylumbelliferone) which produces fluorescence under long wave UV (366nm) (Anglés et al. 2000; Manafi, 2000; Olson et al. 1991; Rompré et al. 2002; Schraft and Watterworth 2005).

This study compares the DST medium (Rapid Hicoliform Agar) with a standard membrane filtration method using both m-Endo Agar and 7hFC Agar used for quantifying total coliforms and *E. coli*, respectively, in River Nile surface water.

MATERIAL AND METHODS

Samples

On the first Sunday of each month (12 months) from December 2005 to November 2006), River Nile water samples were collected from 5 different locations (n=60) in greater Cairo segment. The sampling sites were (from north to south of River Nile): Kafr El-Elw, El-Maasara, El-Giza, Embaba and El-Galatma.

Water samples were collected according to standard guidelines (APHA 1998). All samples were collected in sterile 1l glass bottle (Simax, Czech Republic) and stored on ice (4°C) for transportation to the laboratory. All samples were bacteriologically examined within 6h after collection. Additionally, one sample duplicate was randomly selected each month within each test category.

Bacteriological examination

The samples were analyzed for the detection and enumeration of total coliforms and *E. coli* using conventional membrane filtration (MF) technique according to APHA (1998) and DST.

Total coliforms were assayed using a 1% sample dilution (1ml test sample, 99ml sterile phosphate buffered dilution water) and processed according to APHA (1998). Two sample blanks were processed during the MF to insure quality of dilution water each month. The sterile cellulose nitrate membrane filter (Whatman®, 47mm diameter, 0.45μm pore size) was placed over a sterilized porous plate receptacle using sterile forceps, then sterilized funnel unit was placed over receptacle and locked. The samples were filtered through the membrane filter. After filtration the funnel was removed and the membrane filter was immediately placed on m-Endo Agar with a rolling motion to avoid entrapment of air, Petri dish inverted and incubated for 22 to 24h at 35±0.5°C. The colonies were counted using a low-power (magnification 10-15) binocular wide-field dissecting microscope. The typical total coliforms colony has a pink to dark-red color with a metallic surface sheen. Pink, blue, white or colorless colonies lacking sheen were considered non-coliforms. Total coliforms colonies were counted as CFU·100ml^{-1}. A negative control Petri dish was used for detection of any possible contamination and a positive control Petri dish was used for medium performance using *Salmonella typhi* (American Type Culture Collection, ATCC 14028).

Escherichia coli. Seven Hour Fecal Coliforms (7hFC) Agar was used for detection of fecal coliforms (*E. coli*) in water samples. The medium yields results in 7.0-7.5h. One liter of the medium contains 5.0g proteose peptone No. 3 (Difco), 3.0g yeast extract (Difco), 10.0g lactose, 5.0g d-mannitol, 7.5g NaCl, 0.2g sodium lauryl sulfate, 0.1g sodium desoxycholate, 0.35g bromocresol purple, 0.3g phenol red, 15.0g agar and reagent-grade water. Medium was heated in boiling water bath for dissolving ingredients, cooled to 55-60°C and their pH adjusted to 7.3±0.1 with 0.1N NaOH. Then medium was cooled to about 45°C, dispensed in sterile Petri dishes and stored at 2-10°C. The sample of appropriate volume was filtered through a cellulose nitrate membrane filter (Whatman®, 47mm diameter, 0.45μm pore size) and the filter was placed on the surface of 7hFC Agar medium. Inverted Petri dishes were incubated in water bath at 41.5°C for 7h. Fecal coliforms, yellow colonies of *E. coli* were counted as CFU·100ml^{-1}. A negative control Petri dish was used for detection of any possible contamination and a positive control Petri dish was used for medium performance using *E. coli* ATCC 11229.

DST for total coliforms and E. coli. Rapid Hicoliform agar (RHA) (Himedia, India) was used for detection of total

coliforms and fecal coliforms (*E. coli*) in water samples. This medium contains ortho-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolyzed by β-D-galactosidase enzyme which is produced by total coliforms, and also contains 4-methylumbelliferyl-β-D-glucuronide (MUG) hydrolyzed by β-glucuronidase enzyme produced by *E. coli*. The medium was prepared, sterilized by autoclaving at 121°C for 20min, and then poured on sterile Petri dishes and left to solidify. The appropriate volumes of water samples were filtered by membrane filtration technique as described above. Then the membranes were placed on the solidified media and incubated in inverted Petri dishes at 35°C for 24h. The yellow and blue colonies were counted as total coliforms while blue colonies were considered *E. coli*. All colonies were counted as CFU·100ml^{-1}. A negative control Petri dish was used for detection of any possible contamination and a positive control Petri dish was used for medium performance using *E. coli* ATCC 11229.

Statistical analysis (independent samples t-test) was performed with the Statistical Package for Social Sciences 11.5 for Windows software.

RESULTS

Total coliforms

The average counts of total coliforms using m-Endo Agar were around 10^4 CFU·100ml^{-1} at all sampling sites, while total coliforms counts ranged between 10^2 and 10^5 CFU·100ml^{-1}. However, total coliforms average counts ranged between 10^3 and 10^4 CFU·100ml^{-1} while using RHA media and between 10 and 10^5 CFU·100ml^{-1} while using RHA media. The average counts of total coliforms using standard membrane filtration medium (m-Endo Agar) ranged between logs 4 and 5 while using RHA medium and the average total coliforms counts ranged between logs 3 and 5 (Figure 1).

Fecal coliforms (*Escherichia coli*)

The average counts of fecal coliforms (*E. coli*) using 7hFC Agar ranged between 10^3 and 10^4 CFU·100ml^{-1} at all sampling sites, while fecal coliforms (*E. coli*) counts ranged between 10 and 10^4 CFU·100ml^{-1}.

Average counts of *E. coli* were around 10^2 CFU·100ml^{-1}, while *E. coli* counts ranged between 10^2 and 10^4 CFU·100ml^{-1} using RHA media.

The average counts of fecal coliforms (*E. coli*) ranged between logs 3 and 5 using both standard membrane filtration medium (7hFC Agar) and RHA medium (Figure 1).

Independent samples t-test

There was no significant difference (p=0.423) between number of total coliforms measured using m-Endo Agar and Rapid Hicoliform Agar media as well as there was no significant difference (p=0.546) between number of fecal coliforms (*E. coli*) measured using 7hFC Agar and Rapid Hicoliform Agar media.

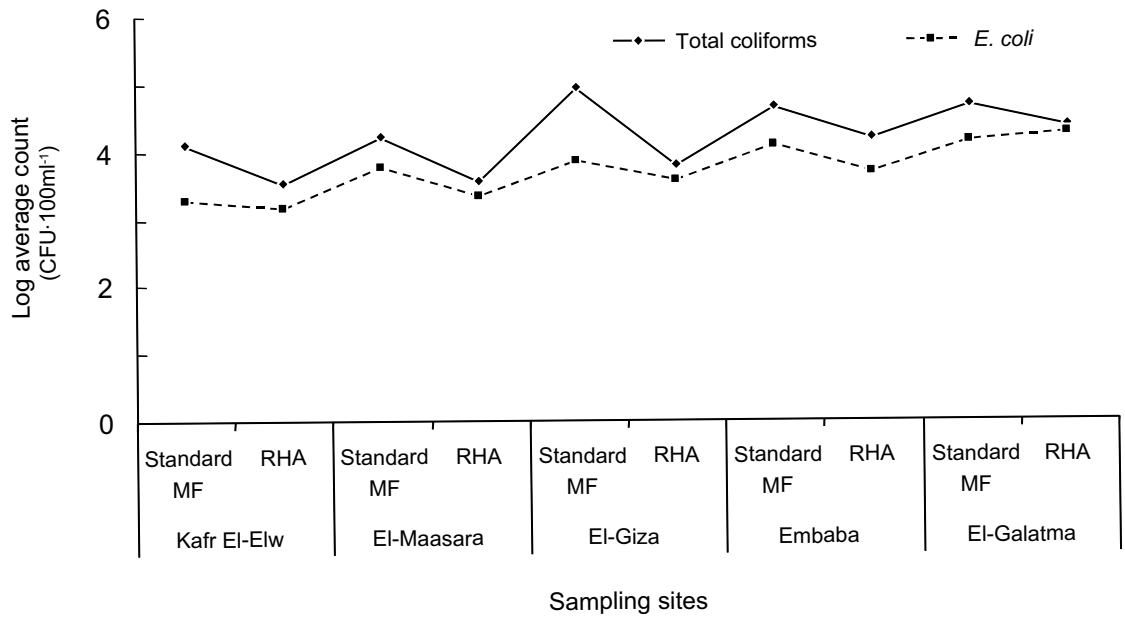

Figure 1. Total coliforms and fecal coliforms (*E. coli*) counts using standard membrane filtration media (m-Endo Agar in case of total coliforms, 7hFC Agar in case of *E. coli*) and RHA medium at all sampling sites.

DISCUSSION

Fresh waters polluted by fecal discharges from human and animals may transport a variety of human pathogenic microorganisms. Because the detection of all water borne fecal pathogens is very difficult, various indicators of fecal contamination are used to detect fecal pollution in natural waters.

Fecal coliform bacteria are known as one of the most important bacterial indicators of potential public health hazard due to fecal pollution. In recent years, *E. coli* is generally replacing the group of thermotolerant coliforms as an indicator of fecal contamination of drinking water (Edberg et al. 2000; WHO 2001).

This study indicates that the ability to measure total coliforms and fecal coliforms (*E. coli*) using DST is comparable to the standard MF media (m-Endo Agar and 7hFC Agar) while examining River Nile waters, providing that all parameters of collection, preservation, and testing are kept constant.

The sensitivity of the DST method to low bacterial numbers is well documented (McFeters et al. 1992; Niemela et al. 2003) and the test is noted for its ability to recover stressed cells in a variety of testing applications (Covert et al. 1992; Eccles et al. 2004; Eckner 1998; Jiang et al. 2002). DST shows a precision to 1 cell·100ml^{-1} due to the high sensitivity of MUG substrate to the presence of *E. coli* using the MPN estimate. Reports indicate that β-D-glucuronidase is found in more than 95% of *E. coli* strains (Berger 1994), is expressed globally among these strains (Venkateswaran et al. 1996), and remains stable at 44.5°C (Tryland and Fiksdal 1998). Similarly, DST sensitivity was also noted in tests of potable water (Cowburn et al. 1994; Fricker et al. 1997; Niemela et al. 2003). Reports indicate that identity confirmation is not required when using DST on freshwater samples (Fricker et al. 1997; Niemela et al. 2003).

Mounting evidence favors the use of DST for these bacteria over other assessment methods for testing freshwater (Cowburn et al. 1994; Eckner 1998; Edberg et al. 1991; Fiksdal et al. 1994; Fricker and Fricker 1996; Grasso et al. 2000; Niemela et al. 2003; Pisciotta et al. 2002; Shadix and Rice 1991; Yakub et al. 2002), drinking water (Cowburn et al. 1994; Edberg et al. 1988; Fricker et al. 1997; McFeters et al. 1992; Niemela et al. 2003), wastewater effluent (Eccles et al. 2004; Elmund et al. 1999; Kramer and Liu 2002), contaminated soils (Muirhead et al. 2004) and foodstuffs (Hara-Kudo et al. 2001; Venkateswaran et al. 1996).

At each sampling location, total coliforms and fecal coliforms (*E. coli*) counts obtained by DST were comparable with standard MF with no significant differences found between testing methods ($p=0.423$ for total coliforms and $p=0.546$ for *E. coli*). It can be concluded that the use of RHA for detection and enumeration of total coliforms and fecal coliforms (*E. coli*) is more recommendable than the use of traditional media such as m-Endo Agar and 7hFC Agar for detection and enumeration of total coliforms and fecal coliforms (*E. coli*), respectively, for the following reasons: (a) it is rapid, i.e. the result are obtained in less than 24h, (b) economic, i.e. measures total coliforms and fecal coliforms (*E. coli*) at the same time on the same Petri plate, and (c) it is sensitive, i.e. does not require any confirmatory test.

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