RESEARCH ARTICLE Am. J. PharmTech Res. 2016; 6(1) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Investigation of bacterial pathogens in Meet Faris drinking water station, Egypt

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ABSTRACT

This study aims to study the diversity of bacteria in different water samples which taken from Meet Faris drinking water station, during the period from July, 2013 till June, 2014. The bacteriological analyses involved total bacterial counts (TBCs), total coliforms (TC) and estimation of fecal coliforms (FC), fecal streptococci and pathogenic bacteria. Fifty pathogenic bacterial isolates obtained from raw water samples. The most prevalent organisms were E.coli (30%), Klebsiella.spp (14%), Pseudomonasspp(16%), Proteus. Spp (10%), E. fecalis (14%) Shigellaspp.(8%), Staph.coccus (8%). According to antibiotic susceptibility test results, enrofloxacin and gentamicin were the most effective antibiotic on E.coli isolates. The obtained results revealed that, the River Nile water was subjected to sewage pollution. Detection of E.coli by PCR was found to be important for confirmation of the detected E.coli isolates.

Keywords: Pathogenic bacteria, Antibiotic test, PCR, Meet Faris, River Nile

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Received 24 December 2015, Accepted 31 December 2015

Please cite this article as: Bahgat MM *et al*., Investigation of bacterial pathogens in Meet Faris drinking water station, Egypt . American Journal of PharmTech Research 2016.

INTRODUCTION

The River Nile is considered the major source of water in Egypt.Drinking water must be safe and freefrom any harmfulpathogens but the Nile, unfortunately, polluted bya lot ofdomestic, industrial and agricultural wastes[1].

The most essential factor of water contamination is microbial pollution of water especially enteric pathogens which obtained from intestine of lived human populations and animals. The microbial contamination of drinking water affect the health of human badly and create a lot of disturbances in aquatic ecosystem[2].

The fecal coliforms and fecal streptococci used as indicators for detection of fecalwater pollution[3]**.** *E. coli* is one of the most important fecal indicators among the enteric bacteria which affect badly on water quality as well as the public health importance[4].

The pathogenicity of enteric microorganisms is mainly related to it is cell wall, especially the lipopolysaccharide (LPS) which called endotoxins[5]**.** So, reorganization of these enteric microorganisms in water is required for prevention of the harmful diseases.

The polymerase chain reaction (PCR) method used for amplifying specific target DNA sequences is an even more important procedure either for confirm diagnosis of the isolated microorganism or detection of specific genes that are responsible for production of the virulence factors of the organism[6]**.**

The aim of this study was to study the diversity of microbial pollution through Meet Farisdrinking water station

MATERIAL AND METHOD

Description of water supply plant:

Meet Faris water supply plant is fed by Nile water that comes through Bahr Tanah. Bahr Tanah water is taken to the system through an intake which is used to supply the system. The first step of purification in this system is the treatment of raw water with primary chlorine gas this step is called pre chlorination. This is followed by addition of alum, then water run to clarifying beds (flocculation and sedimentation).The clarified water is filtered through sandy filters, the filtered water runs to reservoirs in which the final chlorine dose is inoculated and then purified water is pumped to the distribution system. These reservoirs are opened once a year to be washed.

Sampling Sites:

The samples were taken monthly from different sites to cover all steps of water purification.

Sampling Technique:

For sampling water from different sites in the system, the weighted bottle sampler was used. This sampler consists of a sterile glass bottle, with rupper stopper, which has metallic bottle basket that serve as weight sinker. This technique could be summarized as follows: the bottle sampler was pushed down to a proper depth then the bottle stopper pulled out under water surface. The bottle allowed be filling and then capping under the water surface. The sampler was raised to remove the filled bottle which was then wiped off and labeled.

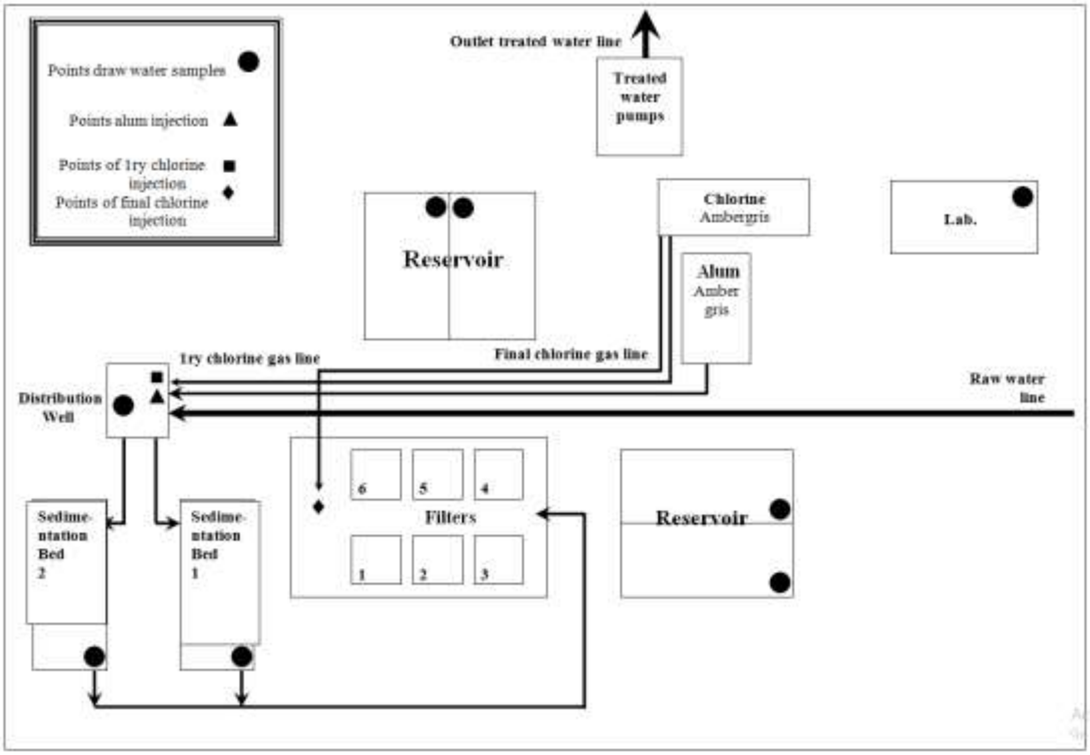

Figure 1 : Design of the Meet Faris drinking water station.

This figure explain that the raw water enter the plant to distribution well then primary chlorine is injected with suitable dose according to Break point test then alum is added with suitable dose according to Jar test , after the addition of suitable dose of primary chlorine that killed most of bacteria and alum the water is distributed to two sedimentation beds where water still about two hours that time is sufficient for clarification and sedimentation and also the achievement of suitable contact time is occurred.

After that the clarified water is directed to filters where filtration of water is occurred,then water is injected with final chlorine then purified water is directed to reservoirs to still about four hours to achieve suitable contact time.

Finally, this design is of that plant is successful because this plant received raw water that is contaminated with variety of pathogenic bacteria and produce purified water with high quality

Bacteriological examination:

The total bacterial counts were detected by the spread-plate method[3]The numbers of total and fecal coliforms were detected using the most probable number method. In that method samples is inoculated in tubes containing lauryl broth then incubated at 35°C for 24-48hr.

The result confirmed by inoculated 1 ml from the positive tubes in the Brilliant greenbroth and incubated at 35°C for 24-48hr. The result is used to determine total coliforms and by inoculation of1ml of the positive tubes in E.C broth and incubation in water bath at 44.5°C for 24-48hr we can determine fecal coliform.

The MPN of fecal streptococci was detected using azide dextrose broth at 37°C for 48hr. Positive tube was indicated by dense turbidity and confirmed using ethyl violet azide dextrose broth incubated at 37°C for 24hr. The presence of fecal streptococci is detected by formation of purple button at the bottom of the tube[3]**.** Bacterial colonies were identified morphologically and microscopically using Gram stain as well as biochemically using methods described by [7]**.**

Antimicrobial susceptibility test:

The sensitivity of *E.coli*isolates was tested according to[8] using disk diffusion method. Pure isolates from macconkey agar, suspended in 2mL sterile saline to a densitynearlyequal to McFarland opacity standard No. 0.5. A dry cotton wool swab was putted into the suspension and then inoculated on to Mueller-Hinton media with the swab in such a way that the whole surface of the media was covered. The plates were incubated at 37°C for 24 hr. Finally measure the inhibition zone according to the interpretive standards of[8]. The following 12 antimicrobial agents were assayed: ampicillin (10µg), cephalocin (30µg), amoxicillin (20µg), ciprofloxacin (5µg), erythromycin (15µg), enrofloxacin (5µg), gentamycin (10µg), norofloxacin (10µg), streptomycin (10µg), tetracycline (30µg), trimethoprim/sulphamethoxazole (25µg)and tobramycin (10µg).

PCR detection of *eaeA*. Gene:

the primer sequence was based on the gene sequence of ***eaeA***. This gene is responsible for pathogenicity and a species specific to *E. coli* [9].

Table 1: The primers used for *detection of E. coli*.

Gene	Name	Primer Sequence.	Size of PCR product (bp)
eaeA	Forward SK1	CCCGAATTCGGCACAAGCATAAGC	**894**
	Reverse SK2	CCCGGATCCGTCTCGCCAGTATTCG	

1- Extraction of DNA from *E. coli*isolates according to [10].

2- 2- Estimation of purity and concentration of DNA according to [11].

Using of spectrophotometer. The concentration was calculated as follows: OD260 = 50ug /ml. And purity of DNA = OD260 nm/ OD280 nm.

3-PCR Protocol:

Step 1: Initial Denaturation at 95°C for 2 min.

Step 2: Denaturation at 95°C for 30 second.

Step 3: Annealing at 42-65 °C for 30 Seconds.

Step 4: Extension at 72°C for 1 min

Cycles repeated for 35 times and proceeded by initial denaturation at 95°C for 2 min. and followed by final extension at 72 for 5 min.

4-Screening of PCR products:

By using 2 gagarose was added to 100 ml Tris acetate EDTA (TAE) buffer. The agarose was autoclaved for 10 minutes and 0.5ugethidium bromide/ml of agarose was added and then left to cool at room temperature.

5-Photographing of the gel: using photographic Camera (Kodak, USA).

RESULTS AND DISCUSSION

The total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) were high in raw water samples than sedimented water. While the treated water showed no any percentage of total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS).MPN of fecal indicators total coliforms (TC), fecal coliform (FC) and fecal streptococci (FS). MPN of TC varied from 14200 to 45000/100ml and from 12000 to 21000/100ml water for FC.

The highest bacterial indicators were recorded at Meet Faris station, might be explained by the effect of domestic and agricultural wastes from the surrounding area [12].

The ratio FC/FS points to the source of faeces whether it is human or animal [13], accordingly, the FC/FS ratio were in the range of 0.19-11, which means the mixed origin of fecal pollution. On the other hand, [14]reported that the data on absolute values of FC: FS may not reflect the source of contamination in CzarnaHancza River, Poland.According to the guideline criteria for fecal indicator organisms of [15], which accept the guide values of the investigated bacteria up to 500/100 ml for total coliforms and 100/100ml for both fecal coliforms and fecal streptococci. The survey of the indicator bacteria in waters revealed that the Nile waters at this branch is subjected to sewage pollution.

Table 2: The most probable number of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) /100ml water of the River Nile.

Month	Raw water			Sedimented water			Treated water		
	TC	FC	FS	TC	FC	FS	TC	FC	FS
July	45000	21000	2000	69	0	0	0	0	0
August	36000	18000	1900	60	0	0	0	0	0
September	33000	17000	1900	58	0	0	0	0	0

October	31000	16000	1800	49	0	0	0	0	0
November	17000	13000	1700	38	0	0	0	0	0
December	16000	14000	1400	29	0	0	0	0	0
January	14200	12000	1320	27	0	0	0	0	0
February	17200	13000	1390	21	0	0	0	0	0
March	19200	14500	1410	29	0	0	0	0	0
April	21000	15000	1500	36	0	0	0	0	0
May	31000	17000	1700	61	0	0	0	0	0
June	39000	20000	1900	72	0	0	0	0	0

Fifty pathogenic bacterial isolates obtained from raw water samples were identified according to result shown in Table 3 and Figure. 2. The most prevalent organisms isolated were ***E.coli, Klebsiella.spp, Pseudomonas.spp, Proteus.spp, Shigella.spp, E.fecalis, Staphcoccus***. In the present study, the isolates were differentiated and confirmed biochemically as *E. coli*, *E. coli*, the main indicator of fecal pollution, constitute 30% of the identified gram-negative bacteria in the examined water. This explained that the water is subjected to sewage pollution.

At present, the *Klebsiellaspp* isolates were identified morphological and biochemical characteristics according to [16]. *Klebsiellaspp* represented 14% of the identified gram-negative bacteria isolated from this branch. This pathogenic bacterium has been previously isolated from surface water [17,16]**.** The genus *Pseudomonas* is isolated from tested water of this branch of River Nile with the percentage (16%).

On the other hand, the *Proteus* isolates identified and represented in a percentage 10% of the identified gram-negative bacteria. Also *Shigella*. spp represented in a percentage 8% of the identified gram-negative bacteria. This pathogenic bacterium is an invasive pathogen which causes shigellasis or *Shigella*-related diarrhea.

On the other hand, the 7 isolates were identified as *E.fecalis* and represented 14% of the identified gram-negative bacteria. Finally, the 4 isolates were identified as *Staphylococcus. Spp* and represented about 8% of the isolated bacteria.

Table 3: Spp., number and percentage of isolated microorganisms.

Isolated spp.	**No. of isolates**	**%**
E.coli	15	30
Klebsiella.spp	7	14
Pseudomonas.spp	8	16
Proteus.spp	5	10
Shigella.spp	4	8
E.fecalis	7	14
Staphcoccus	4	8
Total	50	100

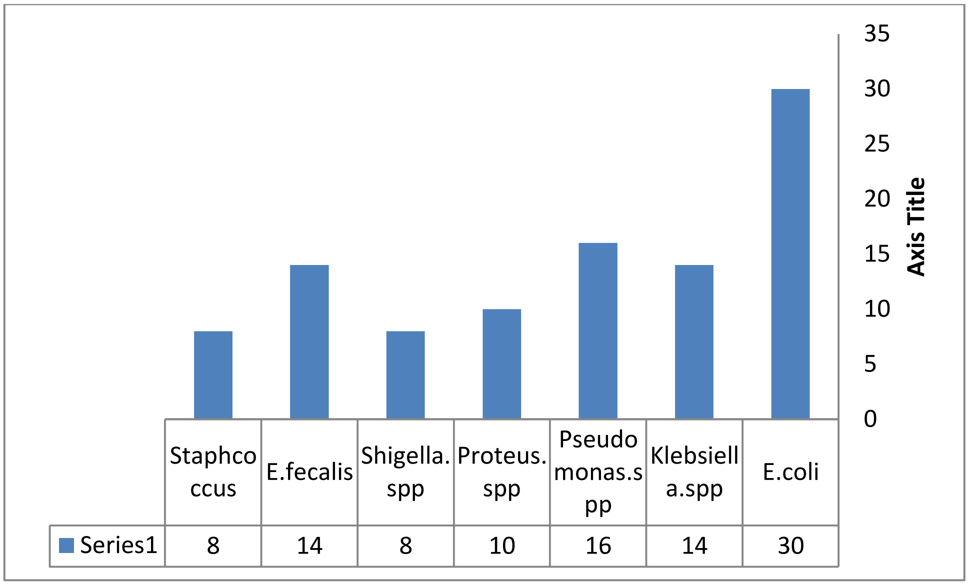

Figure 2: Percentage of isolated microorganisms from raw water

Results shown in Table 4 revealed that enrofloxacin, gentamicin, neomycine, ciprofloxacine, amikacin and norfloxacin were the most effective drugs against *E.coli* isolates (90%, 90%, 80%, 80%, 80% and 80% respectively).Meanwhile isolates were resistant to cephalocin (100%) and erythromycin, (80%) of each. The antimicrobial resistance pattern of *E. coli* isolates provided a wide range of resistance to erythromycin, flumequine (75%), trimethoprim-sulphamethoxazole (70%), streptomycin (60%) and enrofloxacin (50%)[18]. recorded that *E.coli* isolates recovered from calves were resistant to fluoroquinolone and enrofloxacin. Generally, no single antimicrobial agent was 100% effective against all *E. coli* isolates, this coincides with [19,20]who concluded that, multiple resistance to two or more drugs was observed in *E. coli* isolates.

Table 4: In-vitro antimicrobial sensitivity of *E.coli* (10 isolates).

Antimicrobial Agents	Disk Concentration	Resistant No.	Resistant %	Intermediate No.	Intermediate %	Sensitive No.	Sensitive %
Amikacin	30µg	0	0	2	20	8	80
Ampicillin	10µg	3	30	5	50	2	20
Cephalocin	30µg	10	100	0	0	0	0
Amoxycillin	20µg	3	30	3	30	4	40
Ciprofloxacin	5µg	0	0	2	20	8	80
Erythromycin	15µg	8	80	0	0	2	20
Enrofloxacin	5µg	0	0	1	0	6	90
Gentamycin	10µg	0	0	1	10	6	90
Neomycin	30µg	2	20	0	0	8	80
Norofloxacin	10µg	0	0	2	20	8	80
Streptomycin	10µg	6	60	1	10	3	30
Tetracycline	30µg	5	50	2	20	3	30

Photo (1) illustrated the positive amplification of 894bp fragment of **eaeA** gene from the extracted DNA of ***E.coli*** isolate. The PCR protocolused for amplification and detection of ***eaeA***gene of *E.coli* isolates as a confirm diagnosis of these isolates. The polymerase chain reaction used for amplifying specific target DNA sequences is an even more sensitive procedure either for confirm diagnosis of the isolated microorganism or detection of specific genes that are responsible for production of the virulence factors of the organism.

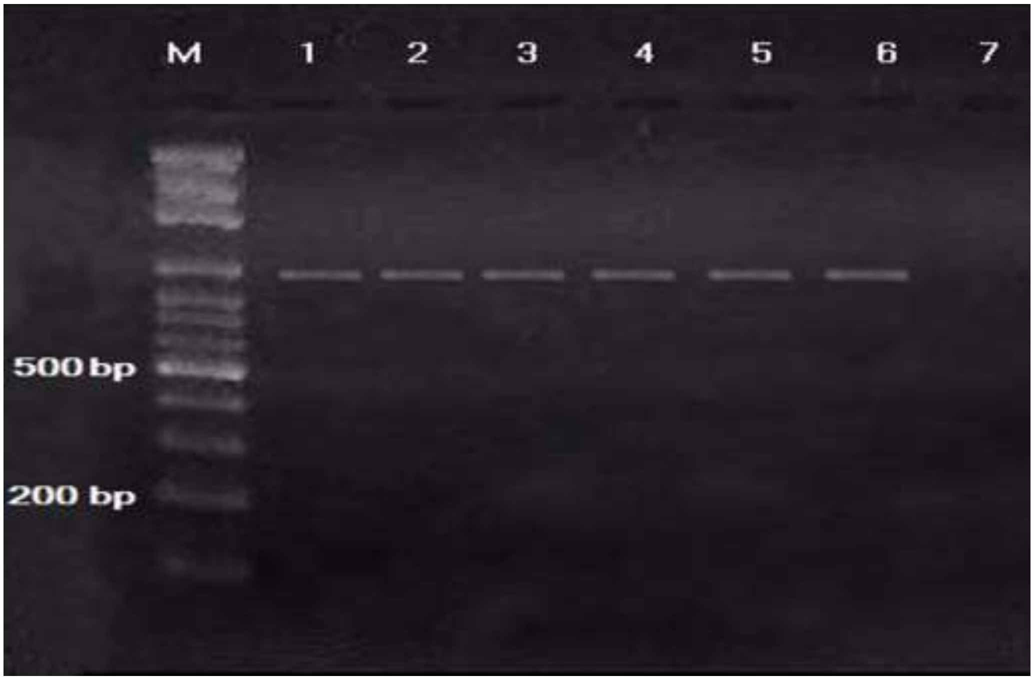

Photo 1: **eaeA gene PCR products from *E. coli* isolates.**

M: 100bp marker

Lane1: Control positive

Lane 2,3,4,5,6: Positive

Lane 7: Control negative

CONCLUSION

The water coming from the River Nile at this branch is subjected to microbial contamination so the country should doing it is best to control the spreading of pathogens through drinking water.

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