Occurrence of bacterial diversity in surface and drinking waters in Damietta, Egypt

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Abstract: Bacterial pathogens in Egyptian water have been reported. While previous studies focused on raw water, correlation between raw and drinking waters quality including water treatment output and distribution networks bacterial pathogens were not explored. Therefore this study monitored the water quality parameters at drinking water treatment plant (DWTP), Damietta County, Egypt involving input, output, tap and distribution networks. Monthly changes in physiochemical parameters were analyzed for one year from (Jan. 2017 – Dec. 2017). All parameters were within the permissible limits of the Egyptian standards. Hence, the water is non-polluted chemically and can be used for drinking. Biochemical methods were conducted to identify bacterial pathogens. The distribution of the studied bacteria based on their site declared that the River Nile was subjected to different pollution sources as twenty four bacterial isolates obtained from the input were identified using biochemical reaction tests; however DWTP had the ability to remove it. On another hand, bacterial pathogens reoccurrence in distribution networks give a risk to health.

Key words: Bacterial pathogens; Drinking water treatment plant, *E. coli*, Non-virulent *Vibrio cholerae*, *Salmonella enterica*, water quality.

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1 Introduction

Water is a critical component of public health. The main source of Egyptian drinking water is the River Nile. Damietta branch is receiving huge amounts of pollutants [1].
These pollutants which are categorized as (inorganics, organics and microorganisms) can affect water quality and consequently the human health [2].
80 % of the disease cases are attributed to use of polluted water as reported by [3]. However, many studies investigated all types of water contaminants there has been no investigation exploring the relationship between the raw water quality parameters and the pathogenic bacteria. Water-borne pathogen contamination in water resources and related diseases are a major water quality concern throughout the world [4]. Also, no study has been conducted to evaluate the distribution water quality parameters in Damietta water.
Thus, the aim of the current study was to survey the water quality parameters of Nile surface water, output and networks of El-Bostan DWTP and to identify the bacterial species using biochemical tests during the period from January 2017to December 2017.

2 Materials and Methods

2.1 Sample collection protocol

To investigate the bacterial pathogens, 60 water samples were collected from a conventional drinking water treatment plant (DWTP)) ; 12 input sample, 12 output sample and 12 sample from each 3 different distribution networks in Damietta governorate monthly between (January and December, 2017) according to [5].

2.2 Physical and chemical analysis of water

2.2.1 Field measurements

Water temperature, electrical conductivity, TDS and pH value were measured on site, using Hydrolab, Model (Multi Set 430i WTW).

2.2.2 Laboratory analysis

The physico-chemical parameters were analyzed according to standard methods [5]. Cations and anions were measured using ion chromatography (Varian IC-3000); TOC was measured using ANA TOC analyzer. Iron and manganese were detected using atomic absorption (Varian).

2.3 Bacteriological analysis of water

The water samples were collected under sterile conditions. The output and distribution network samples were dechlorinated with 0.1 mL of 3% $Na_2S_2O_3.5H_2O$ at the time of sampling. The collected samples were stored at 4°C till analysis within 2 hours after collection. The numbers of total coliform, fecal coliform and fecal streptococci were quantified using Membrane Filtration Culture Method (MF).

2.3.1 Membrane Filtration Culture Method

100 mL of each sample were filtered within 2 hours of collection on 0.45µm Millipore membrane filter then transferred for culturing on specific selective chromogenic media. M-endo agar media was used for detection of total coliform. M-FC agar was used for fecal coliform detection. For fecal streptococcus we used m-enterococcus agar. Modified membrane thermo tolerant *E. coli* (m-TEC) agar media was used for detection and isolation of *E. coli*. After 2 hour incubation at 35±0.5°C, the m-TEC plates were transferred to a sealed plastic bag and placed onto a water bath rack at 44.5± 0.2°C for 22-24 hours. Salmonella-Shigella (S. S) media was used for *S. enterica* detection and isolation at 35±2°C for 18-24 hours. Thiosulfate citrate bile salt sucrose (TCBS) agar media was used for detection and isolation of Non-virulent *V. cholerae* at 35±2°C for 24-48 hours.

2.3.2. Biochemical tests for bacterial identification

Isolated bacteria were picked, purified and identified using growth characteristics and various physiological and biochemical activities according to [6], [7], [8].

2.4 Data analysis
Microsoft Excel 2010 and multivariate statistical analysis using SPSS, V.22 were used in data analysis.

3 Results

Table 1 shows the positive significant correlation between (temperature, turbidity, COD, TOC, alkalinity, total hardness, ammonia, magnesium and phosphate) and (total bacterial count at 37°C, total coliform, fecal coliform and fecal streptococcus).

In another hand, there are negative correlation between the tested bacteria and residual chlorine, EC, TDS, BOD, residual aluminium, iron, manganese, calcium, chloride and sulphate.
Table 2 shows bacterial diversity in the tested DWTP including some pathogenic bacteria such as *Aeromonas* sp.1**,** *E. coli***,** *klebsiella***,** *Enterobacter* sp.*1*, *Citrobacter***,** *Shigella***,** *Lactobacillus*, *Pseudomonas* sp.1**,** *Salmonella* sp, *E.coli*, Non-virulent *Vibrio cholerae*. *Staphylococcus*, *Pseudomonas* sp.2, *Aeromonas* sp.2, and *providencia*

The most frequent isolate was *E. coli*, while the least occurred was non-virulent *Vibrio cholerae*. So, we choosed them in addition to *Salmonella enterica* to quantify them in tested water samples. Moreover, these bacterial strainshave a hazard effect on the human health.
Table 3 illustrates the relation between tested pathogenic bacteria and physiochemical parameters. The 3three tested bacteria were positively correlated with nearly all parameters except residual chlorine, EC, TDS, Calcium, chlorides, residual aluminium, and residual sulphates.

Salmonella enterica is the most correlated bacteria to the temperature occurred only in raw water in July.

E. coli was significant to the other tested indicator bacteria; total coliform, fecal coliform and fecal streptococcus.
The most effective physical parameter related to tested bacteria was turbidity.

4 Discussion
Water is the medium of most biochemical reactions. Hence, its quality is very important for health. The most essential factor of water contamination is microbial pollution, so we studied the bacterial diversity in both influent and output water of Damietta drinking water treatment plant.
Our results declared the absence of bacteria in output water except *Bacillus* sp. in July and reoccurrence of some bacteria in distribution networks; this may be attributed to the prolonged residence time of water masses in the distribution systems that promote unexpected microbial re-growth as reported by [9]. Shen et al., [10] thought in his paper that algae may consume large quantities of chlorine and thus reduce the free chlorine available to control bacteria if they persist after treatment.
E. coli is the most frequent bacteria that had been detected in our study as it occurred in February, July and August; this is complying with [11] who reported that *E. coli* is the most frequent bacteria in the Egyptian River Nile Water. ***E.*** *coli* is highly positive significant with alkalinity, but non-virulent *Vibrio cholerae* negatively correlated with alkalinit.y. This may cause higher presence of *E. coli* than non-virulent *Vibrio cholerae.*

From correlation results, we observed the positive correlation with bacteria and residual aluminium. This mean that the water treatment with aluminium sulphates and its dose affect on remove these bacteria.

5 Conclusions
Pathogenic bacteria occurrence depends on the site, water quality parameters especially temperature. Absence bacteria in treated output water due to treatment processes. Reoccurrence the bacteria in distribution networks related to physical and chemical water quality.
Consequently, regular microbiological monitoring of raw and drinking water is undertaken as part of the regulatory frameworks used to control pathogenic bacteria.

TABLE1. THE CORRELATION COEFFICIENTS BETWEEN INDICATOR BACTERIA AND SOME PHYSIOCHEMICAL PARAMETERS IN STUDIED WATER SAMPLES OF DRINKING WATER TREATMENT PLANT (JANUARY, 2017 TO DECEMBER 2017).

Variables	**Temp.(°C)**	**pH**	**Residual chlorine ppm**	**TU. NTU**	**EC(µhoms\cm)**	**TDS (ppm)**	**DO(ppm)**	**BOD ppm**	**COD ppm**	**TOC ppm**	**Alk. ppm**	**T. H. ppm**	**Res. Al^{3+} ppm**	**Fe ppm**	**Mn ppm**	**Amm. ppm**	**Mg^{+2} ppm**	**Ca^{+2} ppm**	**Cl^{-} ppm**	**So_4^{-2} ppm**	**Po_4-3 ppm**	**TBC at 37⁰C CFU/mL**	**TBC at 22⁰C CFU/ML**	**TC CFU/100mL**	**FC CFU/100ml**	**F.S CFU/100ml**
TBC at 37^0C CFU/ML	0.018	0.61**	-0.50**	0.91**	-0.076	-0.076	0.235**	-0.108	0.693**	0.109	0.33**	0.243**	-0.39**	-0.21*	-0.21*	0.879**	0.0 66	-0.01	-0.15	-0.08	0.23*	1				
TBC at 22^0C CFU/ML	-0.006	0.61**	-0.51**	0.90**	-0.089	-0.089	0.247**	-0.107	0.708**	0.085	0.358**	0.244**	-0.407**	-0.22*	-0.23*	0.879**	0.068	-0.01	-0.13	-0.08	0.244**	0.993**	1			
TC CFU/100ML	0.044	-0.5**	-0.50**	0.92**	-0.021	-0.021	0.257**	-0.120	0.700**	0.050	0.288**	0.202*	-0.42**	-0.19*	-0.19*	0.914**	0.1	-0.02	-0.15	-0.10	0.245**	0.953**	0.957**	1		
FC CFU/100ML	0.038	0.61**	-0.50**	0.90**	-0.038	-0.038	0.261**	-0.070	0.698**	0.033	0.294**	0.198*	-0.43**	-0.22*	-0.22*	0.931**	0.086	-0.01	-0.12	-0.11	0.235**	0.941**	0.947**	0.960**	1	
FS CFU/100ML	0.056	0.61**	-0.48**	0.87**	-0.055	-0.055	0.171	-0.068	0.678**	0.088	0.294**	0.229*	-0.38**	-0.20*	-0.205*	0.934**	0.077	-0.06	-0.08	-0.11	0.244**	0.919**	0.928**	0.907**	0.950**	1

TU= Turbidity, **EC**= Electerical conductivity, **TDS**= Total dissolved solids, **DO= Diissolved oxygen,** BOD= biochemical oxygen demand, **COD**= Chemical oxygen demand,

TOC= Total organic carbon, Alk= Alkalinity. T.H=Total Hardness, Res. Al^{3+}= Residual Aluminium, TBC=Total bacterial count, TC= Total coliform, FC= Fecal coliform. F.S= fecal streptococcus

Table2. THE IDENTIFIED BACTERIAL ISOLATES FROM DIFFERENT SOURCES OF DWTP AND THEIR DISTRIBUTION NETWORS THROUGH THE YEAR:

Month	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
Raw water	*Aeromonas* sp.1	*E. coli*	*Klebsiella* sp.	*Enterobacter* sp.1, *Citrobacter*	*Shigella* sp.	*Lactobacillus Pseudomonas* sp.1	*E. coli Salmonella* sp.	*E. coli* Non-virulent *Vibrio* sp.	*Staphylococcus Pseudomonas* sp.2	*Aeromonas* sp.2	*Pseudomonas* sp.3	*Providencia*
Output							*Bacillus* sp.1					
Network 1				*Bacillus* sp.3		*Bacillus* sp.2		*Proteus* sp.1				
Network 2					*Bacillus* sp.4	*Proteus* sp.2						
Network 3						*Serratia* sp.	*Enterobacter* sp.2	*Micrococcus*				

TABLE3. THE CORRELATION COEFFICIENTS BETWEEN SOME PATHOGENIC BACTERIA AND SOME PHYSIOCHEMICAL PARAMETERS IN STUDIED WATER SAMPLES OF DRINKING WATER TREATMENT PLANT (JANUARY, 2017 TO DECEMBER 2017).

Variables	**Temp.(°C)**	**pH**	**Residual chlorine ppm**	**TU. NTU**	**EC(µhoms\cm)**	**TDS (ppm)**	**DO(ppm)**	**BOD (ppm)**	**COD(ppm)**	**TOC (ppm)**	**Alk. ppm**	**T. H. (ppm)**	**Res. Al^{3+} (ppm)**	**Fe (ppm)**	**Mn (ppm)**	**Amm. ppm**	**Mg^{+2} ppm**	**Ca^{+2} ppm**	**Cl^{-} ppm**	**So_4^{-2} ppm**	**Po_4-3 ppm**	**TBC at 37°C CFU/mL**	**TBC at 22°C CFU/ML**	**TC CFU/100mL**	**FC CFU/100ml**	**F.S CFU/100ml**	***E. coli* CFU/100mL**	***S. enterica* CFU/100mL**	**Non -virulent *V. cholerae* CFU/100mL**
***E. coli* CFU/100mL**	0.06	0.58**	-0.48**	0.87**	-0.007	-0.007	0.24**	-0.06	0.68**	0.02	0.27**	0.17	-0.42**	-0.20*	-0.19*	0.90**	0.06	0.10	-0.01	-0.17	-0.13	0.19*	0.89**	0.90**	0.94**	0.95**	0.88**	1	
***S. enterica* CFU/100mL**	0.20*	0.23*	-0.17	0.46**	0.101	0.101	-0.40	-0.14	0.25**	0.15	0.02	-0.01	-0.04	0.01	0.026	0.42**	0.06	0.09	-0.14	-0.15	-0.03	0.02	0.50**	0.46**	0.47**	0.42**	0.45**	0.42**	1
Non - virulent *V. cholerae* CFU/100mL	0.15	0.16	-0.13	0.33**	0.119	0.119	-0.05	-0.14	0.23*	0.13	-0.04	-0.01	-0.002	0.02	0.044	0.33**	0.01	0.11	-0.15	-0.16	-0.04	0.11	0.38	0.36**	0.38**	0.33**	0.34**	0.33**	0.78**

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