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Bacterial Quality of River Nile Water at Cairo Region in Egypt

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The aim of is study was to elucidate the bacterial quality of the River Nile water at Cairo region in Egypt. The microorganisms such as heterotrophic bacteria and faecal coliforms were examined. The bacterial isolates were identified using different keys of identification and Biolog metabolic fingerprint system. The results showed the presence of different genera of microorganisms, which were. *Alcaligenes, Escherichia, Aeromonas, Pseudomonas, Klebsiella, Enterobacter, Rahnella, Xanthobacter, Streptomyces, Rhodococcus* and *Arthrobacter*. The significance of the results was shortly discussed.

Key words: bacteria, water pollution, taxonomy, coliform

Introduction

Water is essential to sustain life, and a satisfactory supply must be made available to consumers. No source of water that is intended for human consumption can be assumed to be free from pollution. Polluted water is an important vehicle for the spread of diseases. It has been estimated that 50 000 people die daily in the world as a result of water related disease (Schalekamp 1990).

Not only the developed countries have been affected by environmental problems, but also the developing nations suffer the impact of pollution due to disordered economic growth associated with the exploration of virgin natural resources (Listori 1990).

The River Nile forms very important aquatic and wetland ecosystems and it supplies about 97% of Egypt's water reserves (Abdel-Shafey & Aly 2002). The riverine floodplains and delta are subjected to slight flooding and *Cyperus papyrus* swamps, which play a significant role in elimination of pathogenic microorganisms. The area of the swamps have been decreased, however.

Rare published information is, however, available on microbiological characteristics of the Nile water and all of them deal with the bacterial indicators only. Payment et al. (1985) mentioned that faecal indicators bacteria failed to judge the water safety because different opportunistic and/or pathogenic bacteria were found in the absence of such indicators.

El-Abagy et al. (1999) studied the quality of River Nile water at Beni-Suif area. They detected different genera of pathogenic bacteria in the water.

Ali et al. (2000) studied the biological characteristics of the River Nile water to evaluate the trophic and autotrophic state of the River. They revealed the presence of faecal bacterial indicator, high number of pathogenic bacteria and yeasts because the River body receiving big quantities of domestic, industrial and agricultural wastes.

El-Taweel & Shaban (2001 a,b) studied the classical bacterial indicators of the River Nile water at Cairo segment and Ismailia canal. They reported that the total coliforms, faecal coliforms and faecal *Streptococcus* were high in the water. They also detected the presence of *Aeromonas*, *Staphylococcus* and *Listeria* genera which revealed the high pollution of the water.

The present study aimed to examine the bacterial quality of River Nile water from Cairo region in order to evaluate how the water supply may influence infection and disease health implications.

Materials and methods

Sampling collection

Thirty six water samples were collected under the surface layer of the water in the middle part of the River Nile from the three water stations at Cairo (El-Gezera (El-G), Rod El-Farag (R) and El-Kanater (El-K)) during the period from January till December in 2006. The samples were taken into 1 litre sterilised conical flasks. The flasks were dipped into water with opening counter current, recovered strongly, covered with cotton and aluminium foil.

Isolation of bacterial cultures

The composite samples were serially diluted in sterile physiological saline and 0.1 ml aliquots from the appropriate dilutions were spread on the surface of four different agar media: 1) nutrient, 2) eosin, 3) endo and 3) starch casein agar. The inoculated plates were incubated for 48 hrs at 35 ^{o}C. Colonies were isolated and purified on the adequate medium.

The use of endo agar medium is important for its restricted selectivity of some genera important for hygienic parameter of water as *Pseudomonus*, *Aeromonus* and *Alcaligens* as well as eosin agar for coliform microbes and characteristic autochthonous. Nutrient medium is an undefined medium that most bacteria can grow on it. The use of starch casein medium is important to monitor the presence of actinobacteria in water.

Morphological, physiological and biochemical tests

The following examinations were performed: Gram stain reaction, Japanese test, amino peptidase test, oxidase test, catalase test, NO_3^- reduction, H_2S production, utilization of citrate, gelatine hydrolysis and utilization of different carbon sources as glucose, arabinose, xylose, fructose, mannitol and mannose (Bergey's Manual of Systematic Bacteriology 1989).

Numerical analysis

The results of the diagnostic tests were coded for cluster analysis. On the basis of their characteristics, computer aided comparative numerical analysis was performed using SPSS for Window 6.0 software. For the calculation of similarities, the simple matching coefficient was chosen. The cluster and dendrogram were generated by UPGMA (average linkage) according to Sneath & Sokal (1973). The phenograms were evaluated using different systematic and determinative bacteriological manuals of Bergey's Manual of Systematic Bacteriology (1989), Bergey's Manual of Determinative Bacteriology (1994) and the prokaryote of Falkow et al. (1981).

Biolog metabolic fingerprint system

The Biolog identification system is based on the simultaneous examination of utilisation of 95 different carbon sources. The utilisation of the carbon source in a well was demonstrated by formation of purple colour or turbidity changes as compare to a negative control. Computer software and a database enabled the species level identification. The carbon source utilisation pattern of a strain investigated was compared with the results in database. The computer displays the closest matches on screen along with a numerical similarity index. A strain can be clustered in relation to its closest relatives, or entire user-constructed library can be clustered.

The strains were streaked on nutrient agar and grown for 12–18 hours. With cotton swab 1–2 colonies were suspended in physiological saline, and by using the Biolog turbidimeter, the desired cell density of about 3×10^8 cells ml^{-1} was adjusted. The pre- warmed microplate was used for Gram-negative strains, were inoculated promptly by a multichannel repeating pipette with precisely 150 µl suspension. The inoculated microplates were covered, and incubated at 28 °C for 4 hours, read using a Uniscan II (Labsystem, Finland) microplate reader and the results were evaluated, and clustered by using the Micro Log software of Biolog (Winding 1994).

Results and discussion

The results of morphological, biochemical and physiological characteristics of the studied water samples (Table 1) indicated that the ratio of metabolically active/inactive isolates within a sample and the number of facultative anaerobes showed an increasing tendency with the rate of bacterial load. This latter reveals the growing of organic pollution, with its increasing biochemical or chemical oxygen demand, which renders the sample more frequently microaerophilic or even anaerobic. Moreover, the results revealed the presence of high populations of typical aquatic and oligotrophic bacteria. The same results were obtained by Obiri-Danso et al. (2005) who found that the River Subin in Ghana became grossly polluted. In addition, Djuikom et al. (2006) reported that there is greet potential risk for human use or primary contact of Mfoundi River water in Cameroon.

The profile of the bacterial community was illustrated in Fig. 1, from which it is clear that the number of identified strains, on the genus level, increases with growing pollution load. The obtained results revealed the presence of nine phenons. The first one, including 5 isolates from water of El-Gezera and Rod El-Farag, showed positive catalase with a characteristic capability to reduce nitrates to nitrites (Table 1). The suitability of nitrates as terminal electron acceptors enabled us to delineate the species level position of the phenon as *Alcaligenes eutrophus*. This type of bacterium can thrive in the presence of millimolar concentrations of several heavy metals (Mergeay et al. 1985). Furthermore, it use a variety of substrates as carbon sources, or it can grow chemo-lithotropically using molecular hydrogen as the energy source and carbon dioxide as a carbon source.

Only one strain from Rod El-Farag water was identified as *Escherichia coli*. It is catalase positive and oxidase negative with rod shaped type, nitrate reduction positive and highly biochemical activities (Table 1). It lives in the lower intestines of mammals. The presence of this bacterium in water is a common indicator of faecal contaminations (El-Taweel & Shaban, 2001a).

The third phenon of Rod El-Farag samples comprises two isolates representing the genus *Aeromonas*. Such genus is Gram-negative, rod-shaped with rounded ends, oxidase and catalase positive and reduce nitrate to nitrite (Table 1). Szczuka & Kaznowski (2004) indicated that these bacteria are widely spread in the environment, especially in surface water and sewage. The presence of different *Aeromonas* species indicates increasing pollution. Additionally, the existence of the single members phena is the possible representatives of different sewage originating microbes.

The fourth group, representing water from El-Gezera, is identified as members of the genus *Pseudomonas* which are described as Gram-negative, non-spore forming and straight or slightly curved rods (Table 1). They are typically motile by means of one or more polar flagella. It was assumed that their position were the facultative H_2-autotrophic methylotrophic species, which were delineated into new genera like *Hydrogenophaga*, *Acidovorax* and *Hydrogenomonas* (Stover et al. 2000). Members of the genus are found abundantly as free-living organisms in soils, fresh water and marine environments and in many other natural habitats (Purohit et al. 2003).

The fifth isolate from El-Gezera water was identified as *Klebsiella pneumoniae*. It is a Gram-negative bacterium with cylindrical rods (Table 1). It is widely distributed in nature, in soil and water. Also it found in respiratory, intestinal and urinogenital tracts of animals and man. The presence of *Klebsiella* indicates increasing water pollution.

The sixth large cluster of samples, comprising water from the three studied localities, forms

Table 1. Results of morphological, biological and physiological tests of the isolated strains examined in the samples of Nile water from three areas in the region of Cairo.
Taulukko 1. Testituloksia Niilin vedestä otettujen vesinäytteiden mikrobi-isolaattien morfologisista, biologisista ja fysiologisista ominaisuuksista Kairossa.

Sample No. *	Gram stain	Gapanese reaction	Amino-peptidase test	Motility	Oxidase test	Catalase test	Shape	NO3 - reduction	H2S production	Utilisation of citrate	Gelatin hydrolysis	Utilisation of carbon sources (1% w/v)					
												Glucose	Arabinose	Xylose	Fructose	Mannitol	Mannose
El-G 1	-	+	+	-	-	+	Rods	+	-	+	-	+	+	+	+	+	+
El-G 2	-	+	+	+	+	+	Rods	+	-	-	-	±	-	-	+	-	-
El-G 3	-	+	+	+	+	+	Rods	+	-	+	-	-	-	-	+	-	-
El-G 4	-	+	+	+	+	+	Rods	+	±	+	-	±	-	-	+	-	-
El-G 5	-	+	+	+	+	+	Curved rods	+	-	+	+	+	-	-	+	-	-
El-G 6	-	+	+	+	+	+	Long rods	±	+	+	-	±	+	-	+	+	+
El-G 7	-	+	+	+	+	+	Long rods	+	-	+	-	+	+	+	+	-	±
El-G 8	-	+	+	+	+	+	Rods	+	±	-	+	+	±	-	+	-	-
El-G 9	-	+	±	+	+	+	Rods	±	+	-	-	+	±	-	+	-	-
El-G 10	-	+	+	-	-	+	Small rods	-	-	+	-	+	+	+	+	+	+
El-G 11	-	+	+	+	-	+	Long rods	+	-	+	-	+	±	±	+	-	+
El-g 12	-	+	+	+	-	+	Rods	+	-	+	-	+	+	+	+	+	±
R 1	-	+	+	+	-	+	Rods	+	-	-	±	+	+	+	+	+	+
R 2	-	+	+	+	+	+	Rod-coccus	+	-	+	-	-	-	-	+	-	-
R 3	-	+	+	+	+	+	Rod-coccus	+	+	+	-	-	-	-	+	-	-
R 4	±	+	+	+	+	+	Rods	-	-	+	-	±	-	-	±	+	+
R 5	-	+	±	-	+	+	Rods	-	-	+	+	-	-	-	+	±	+
R 6	-	+	+	-	+	+	Rods	±	+	+	-	-	+	±	±	+	+
R 7	-	+	+	+	+	+	Long rods	+	-	+	-	-	+	+	+	+	+
R 8	±	+	+	+	+	+	Rods	-	±	+	-	-	+	+	+	-	-
R 9	±	+	-	-	+	+	Rods	-	-	+	-	-	+	-	±	±	±
R 10	-	+	+	+	+	+	Rods	+	+	+	+	-	+	+	+	+	+
R 11	-	+	+	+	+	+	Rods	+	-	+	+	-	+	+	+	+	+
R 12	-	+	+	+	+	+	Long rods	+	+	+	-	-	-	+	±	+	+
R 13	-	+	+	+	+	+	Long rods	+	-	+	-	-	-	±	±	+	+
El-K 1	+	-	-	-	-	-	Filamentus	+	+	+	-	+	+	±	+	±	±
El-K 2	+	+	-	-	-	-	Rods-branched mycelium	+	-	-	+	+	+	+	+	+	+
El-K 3	+	-	-	-	-	-	Rods-branceh mycelium	+	-	-	+	+	+	+	+	+	+
El-K 4	+	-	-	-	-	-	Irregular rods	-	-	-	±	+	±	+	+	+	+
El-K 5	-	±	-	+	+	+	Long rods	±	-	+	-	+	-	+	±	+	+

*) El-G= EL-Gezera, R= Rod El-Farag, El-K= El-Kanater

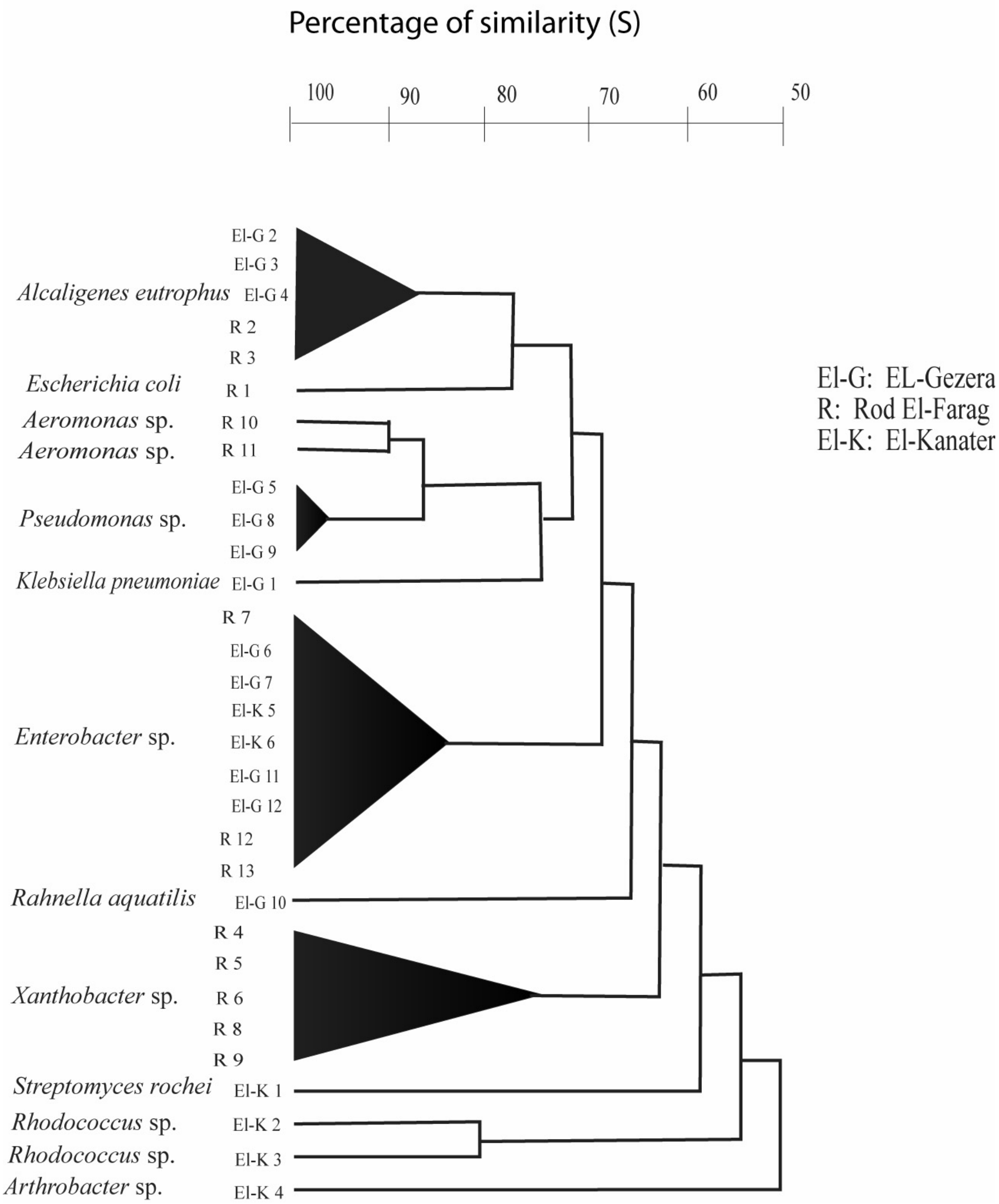

Fig 1. Dendrogram of the analysed bacterial community of water from River Nile in Cairo region.

the facultative fermenting metabolic type of long rods shaped Gram-negative bacteria. These strains are members of the genus *Enterobacter*. They are metabolically inactive and motile bacteria (Table 1). It can be found of human skin and plants as well as in soil, water, sewage, intestinal tracts of human and animals and some dairy products.

Also one strain from El-Gezera water was identified as *Rahnella aquatilis*. It is Gram-negative bacteria, catalase positive, oxidase negative and small rod-shaped (Table 1). Typically, it exists in fresh water reservoirs, plant rhizosphere, agriculture soil and patients (Varbanets et al. 2004).

In samples from Rod El-Farag, the members of the genus *Xanthobacter* characterised water (Table 1). *Xanthobacteria* have pleomorphic cell morphology together with certain biochemical feathers. This genus may play a role in the degradation of organochlorine compounds (Ensign et al. 1998). The decrease of facultative H_2 autotrophic methylotrophic bacteria (*Xanthobacter* and *Pseudomonas*) indicate increasing water pollution (Green 1992).

The water samples of El-Kanater revealed the presence of four strains as Gram-positive bacteria, which composed the end of the dendrogram. One of them was identified as *Streptomyces rochei*; two strains were *Rhodococcus* sp., while the last strain was *Arthrobacter* sp. All strains were highly metabolic active (Table 1). *Streptomyces rochei* have the ability to produce metabolites having antimycotic property (Augustine et al. 2005). The genus *Rhodococcus* is a unique taxon consisting of microorganisms that exhibiting broad metabolic diversity, particularly to hydrophobic compounds such as hydrocarbons, chlorinated phenolic, steroids, lignin, and petroleum (Jing-Liang et al. 2006). They have been found to degrade agricultural pesticides in conjunction with several strains of *Streptomyces* in synergistic relationship (Hayatsu et al. 1999).

Conclusions

Water contaminated with microbiological constituents can cause a variety of diseases. Water intended for human consumption should be safe, palatable and aesthetically pleasing. Water sources have different qualities influenced by natural or anthrobiological pollution. In Egypt, the availability of safe and clean water is a serious problem.

Investigation of the River Nile water at Cairo region revealed that coliform and autochthonous microbes in water grow out usually together on the surface of Endo and Eosin-methylene blue agar. As sewage pollution increases, the facultative H_2 autotrophs (*Xanthobacter* sp. and *Pseudomonas* sp.) were present and the diversity of members of genus *Aeromonas* increase.

The presence of different bacterial genera in the water of the River Nile at Cairo is due to direct contamination caused by human activities and indirect effect caused by ecological disturbances. People discharge their domestic and/or agricultural wastes as well as human body wastes into rivers. In addition, using a river as a source for traffic travel as well as birds and some animals inhabiting the water can also contaminate the water through direct defecation and urination. In addition, the presence of organic suspended materials promotes the growth of microorganisms.

The risk of population exposure to water-related diseases is often underestimated. The presence of different kinds of pathogenic bacteria in the studied water samples indicated the necessity to deal with water quality issues as a matter of urgency. Emphasis should be placed on the need to control microbial disease agents, develop sanitation and agricultural practices as well as other activities that can contribute to the degradation of water quality and cycle disease agents back to human population.

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Tiivistelmä

Tutkimuksessa selvitettiin Niilin veden mikrobiologista laatua Kairon alueella Egyptissä. Niili on egyptiläisille erittäin tärkeä juoma- ja talousveden lähde ja puhtaan veden saanti muodostaa erittäin merkittävän ongelman.

Selvityksen kohteena olivat heterotrofisten ja koliformisten bakteerien esiintyminen jokivedessä. Bakteerilajien tunnistamisessa käytettiin geneettistä sormenjälkimenetelmää.

Tulokset osoittivat, että vedestä löytyi useita bakteerilajeja, joista monet ovat merkittäviä batogeenejä ja veden laatua heikentäviä eliöitä. Löydetyt bakteerit kuuluivat *Alcaligenes, Escherichia, Aeromonas, Pseudomonas, Klebsiella, Enterobacter, Rahnella, Xanthobacter, Streptomyces, Rhodococcus* ja *Arthrobacter* -sukuihin. Bakteerit olivat pääsääntöisesti peräisin jätevesistä ja toissijaisesti ekosysteemeissä tapahtuneiden häiriöiden seurausta. Tulosten merkitystä tarkasteltiin lyhyesti vesivarojen käyttökelpoisuuden näkökulmasta.

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