Isolation, Identification and Distribution of the Gram-Positive Bacterial Isolates Contaminating the Drinking Water of Al Gedarif City, Sudan

Abdel-Rahim, A. M. [1], Abdel Daim, Z. J. [2], and Ahmed, A. A. [3]

[1]Center of Biosciences & Biotechnology, Fac. of Engineering & Technology, Univ. of Gezira.

[2] Al Gedarif State, Water Corporation Laboratory.

[3] Faculty of Agric.e, University of Al Gedarif

ABSTRACT

In this study, the possible aerobic Gram-positive bacteria were isolated from the main sources of the drinking water in Al Gedarif city (raw and treated waters of Atbara River, main reservoirs and zeer waters of all sources). The isolates were identified using the manual identification tests (primary and biochemical). The primary tests identified the isolates up to the genera level. The results indicated that the isolates belonged to four genera (*Staphylococcus*, *Micrococcus*, *Bacillus* and *Corynebacterium*). However, the biochemical tests identified the isolates up to the species level. The species identified included three of the genus *Staphylococcus*, four of the genus *Micrococcus* and eight of the genus *Bacillus*, as well as the bacterium *Corynebacterium diphtheriae*. The study also included the distribution of the identified species in the different sources. It was found that *Staphylococcus aureus* and *S. epidermidis* were found in almost all sources. However, the other bacterial species were detected in some of the sources and absent in the others. On the other hand, S. *saprophyticus*, *M. varians*, *M. kristinae*, *B. thuringiensis*, *B. pantothenticus* and *B. firmus* were not detected in any of the underground sources.

Key words: Al Gedarif city, Drinking water sources and Gram-positive bacteria.

INTRODUCTION

The total coliform group of bacteria includes both Gram - positive and Gram-negative groups as well as many bacteria of non-fecal origin, therefore, the fecal coliform

group, which is differentiated from the total coliform, only by its ability to grow at elevated temperature (44.5º C), has become the most important microbial indicator of water quality (American Public Health Association, 1970 and 1995).

Identification media contain nutrients and reagents that indicate, through some form of color formation, the presence of particular microorganisms. Fermentation of sugars, glycosides and polyhydric alcohols, is widely used in the diagnosis of bacteria. However, there are numerous types of selective and diagnostic media available to be used as a guide for identification. The application of simple stain such as the Gram-stain, can divide the numerous of genera of bacteria into two convenient broad groups; Gram-positive and Gram-negative (Stephen *et. al*, 2004). The process has become more simplified in recent years by the development of rapid identification methods and kits, such API 20 E– bio Merieux, Inc. Hazelwood, MO, France).

Various manuals such as the Bergey's manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) and the Coman and Steele's Manual for the Identification of Medically Important Bacteria (Barrow and Feltham, 1993), have been used for identification of bacteria.

Pathogenic microorganisms that are found in water (water-borne diseases), include both Gram-positive and Gram-negative groups. The ability to ferment lactose and the use of selected media are used to isolate the Gram-positive bacteria.

Heterotrophic Plate Count (HPC) of bacteria in drinking-water often includes isolates that are opportunistic pathogens which may cause serious diseases. WHO (1993) reported that the opportunistic pathogens are naturally present in the environment and are not formally regarded as pathogens. They are able to cause disease in people with impaired local or general defense mechanisms. Water used by patients for drinking or bathing, if it

contains large numbers of these organisms, can produce various infections of the skin and the mucous membranes of the eye, ear, nose and throat (Internet, 2008).

MATERIAL AND METHODS

Serial dilutions were made for each water sample. The sampling sites were as the following:

1- Cites of Atbara river (AR): ARMS = Main stream, ARTW = Treated water, ARMV= Main reservoir, ARZ= Zeer water.

2. Cites of Al Saraf dam (SD): SDET= Elevated tank, SDZ= Zeer water.

3. Cites of Dalassa dam (DD): DDET= Elevated tank, DDZ= Zeer water.

4. Cites of Al Azaza boreholes (ZB): ZBCT**=** Collection tank, ZBZ**=** Zeer water.

5. Cites of Abu Al Naja boreholes (NB): NBCT= Collection tank, NBZ**=** Zeer water.

About 1.0 ml was taken aseptically and spread onto a nutrient agar medium in Petri dishes. Dishes were incubated at 37º C for 24 hours. Each pure isolate was kept in a test tube containing brain heart infusion agar, in a slant form, given a code number, and then the tubes were incubated at 37º C for 24 hours and stored at 4º C for further study. The pure isolates were identified, using manual tests.

The manual tests included primary and biochemical (secondary) tests. The identification of all isolates was done according to Brenner (1984) and Barrow and Feltham (1993). The primary tests included; the Gram reaction, catalase test, oxidase test, Oxidation–Fermentation (O/F) test and motility test.

The biochemical tests included; Oxidase, Motility, Haemolysis, Coagulase, Novobiocin sensitivity, Voges–Proskauer, Urease activity, Nitrate reduction, Pigment production, citrate utilization, Casein hydrolysis, Starch hydrolysis, Gelatin hydrolysis,

Lecithovitellin (LV) reaction, growth in 10% NaCl, Growth at 50º C and Acid from carbohydrate tests (Sugar fermentation test).

RESULTS

The possible aerobic Gram-positive bacterial isolates obtained in the present investigation were identified, using manual techniques. The isolates were identified to the genera level by the primary tests, beginning with the Gram test. Two of the genera appeared as rod shaped, others were cocci, however, only one genus was spore forming. All the isolates were catalase positive and two of them were oxidase positive, while no one of the tested genera showed positive oxidation–fermentation reaction or motility test (Table, 1). The isolates of each genus were further identified to the species level by the biochemical tests.

Results in tables (2 and 3) illustrated the biochemical tests of the Gram-positive bacterial species other than *bacillus*. These included; some species of the genera *Staphylococcus* (*S. aureus*, *S. epidermidis* and *S. saprophyticus*), *Micrococcus* (*M. varians*, *M. nishinomiyaensis*, *M. kristinae* and *M. roseus*) and *Corynebacterium diphtheriae*. All three species of the genus *Staphylococcus* detected in the present study were positive with urease and Voge-Proskaur tests. Two of them were unable to reduce nitrate and two were sensitive to novobiocin (Table, 2). On the other hand, only one species of the genus *Micrococcus* was positive with Voge-Proskaur test (*M. kristinae*).

Table 1. Primary tests of Gram positive bacterial isolates in water taken from the different sources of Al Gedarif city drinking-water to the genera level.

Unknown isolates Tests	1	2	3	4
Shape	Cocci	Cocci	Rod	Rod

Spore formation	-	-	+	-
Catalase test	+	+	+	+
Oxidase test	-	+	-/+	_
Oxidation–Fermentation(O/F)	_	_	_	_
Motility test	-	-	-/+	-
Genus	*Staphylococcus*	*Micrococcus*	*Bacillus*	*Corynebacterium*

- : Negative +: Positive

Table (3) shows the sugar fermentation tests for the Gram-positive species other than *Bacillus* spp. It was found that all species of the genus *Staphylococcus* were able to use glucose, lactose, fructose, sucrose and maltose. However, only the bacterium S. *epidermis* was unable to utilize manitol. Glucose was used by all species of the genus *Micrococcus* as well as by the bacterium *C. diphtheria* (Table, 3).

On the other hand, the species of the genus *Bacillus* obtained in the present study and their biochemical reactions are shown in tables (4 and 5). All species of the genus *Bacillus* isolated in the present work were spore forming, all of them were able to reduce nitrate except *B. subtilis*, all were oxidase positive except *B. subtilis* and *B. firmus* and three of them were unable to utilize citrate (Table, 4).

Table 2. Biochemical tests for Gram-positive species other than *Bacillus* spp. isolated from the different sources of Al Gedarif city drinking-water**.**

Biochemical tests / Species	Pigment production	Urease	Voges - proskaur	Nitrate reduction	Hae moly sis	Coagul ase	Novo biocin
S. aureus	Yellow	+	+	+	+	+	S
S. epidermidis	Cream	+	+	+	-	-	S
S. saprophyticus	White	+	+	-	-	-	R
M. varians	Yellow	ND	-	+	ND	ND	ND
M.nishinomiyaensis	Orange	ND	-	+	ND	ND	ND
M. kristinae	Yellow	ND	+	-	ND	ND	ND
M. roseus	Red	ND	-	+	ND	ND	ND
C. diphtheriae	Cream	-	-	+	+	ND	ND

+: Positive - : Negative ND: Not Done S: sensitive R: resistant

However, different reactions were obtained by the different species of the genus *Bacillus* with regard to the motility and the haemolysis tests. From Table (5), it could be

seen that all the species of the genus Bacillus were able to hydrolyze starch except *B. pantothenticus* and all were able to ferment glucose, except B. subtilis. However, most of them were able to hydrolyze casein and to ferment mannitol (Table, 5). Four of them hydrolyzed lethocine, and fermented galactose while, a few were able to grow at 50^0 C.

Table 3. Sugar fermentation tests for Gram-positive species other than *Bacillus* spp. Isolated from the different sources of Al Gedarif city drinking-water.

Sugar Species	Glucose	Lactose	Fructose	Sucrose	Mannitol	Maltose
S. aureus	+	+	+	+	+	+
S. epidermidis	+	+	+	+	-	+
S. saprophyticus	+	+	+	+	+	+
M. varians	+	ND	+	+	ND	ND
M.nishinomiyaensis	+	ND	-	-	ND	ND
M. kristinae	+	ND	+	+	ND	ND
M. roseus	+	ND	+	-	ND	ND
C. diphtheriae	+	-	ND	-	-	+

+: Positive - : Negative ND: Not Done

Table 4. Biochemical tests for *Bacillus* species isolated from the different sources of Al Gedarif city drinking-water.

Biochemical tests / Species	Spore position and shape	Oxidase	Motility	Haemolysis	Voges-proskaur	Nitrate reduction	Citrate utilization
B. cereus	Central+subterminal/oval		+	-	+	+	+
B. anthracis	Central+subterminal/oval		-	+	+	+	+
B. mycoides	subterminal /oval		-	+	+	+	-
B. thuringiensis	subterminal /oval		+	+	+	+	+
B. lentus	subterminal /oval		+	+	-	+	+
B. subtilis	Central/oval		+	+	+	-	-
B.pantothenticus	Terminal/round+oval		+	-	+	+	+
B. firmus	Central+subterminal/oval		+	+	-	+	-

+: Positive

- : Negative

The study also included distribution of the identified Gram - positive bacterial species, in the different sources of Al Gedarif city drinking-water. Table (6) illustrates the distribution of the Gram-positive bacteria in the surface sources of Al Gedarif city drinking-water. The results showed that *Staphylococcus aureus* was found in all of the tested sources, except the elevated tank of the Al Saraf dam. However, *S. epidermidis* was detected in all sources, except in the main reservoir. While, *S. saprophyticus* was found in the main stream, the treated water and zeer waters of Atbara River, and zeer water of Dalassa dam. *Micrococcus varians* and *M. nishinomiyaensis* were found in the main stream, the treated water and zeer waters of Atbara River as well as the elevated tanks of Dalassa dam. The later was also found in the elevated tank of Al Saraf dam.

Table 5. More biochemical tests for *Bacillus* species isolated from the different sources of Al Gedarif city drinking-water**.**

Biochemical tests / Species	Growth at 10% NaCl	Growth at 50° C	Starch hydrolysis	Gelatin Hydrolysis	Lecithonase hydrolysis	Casien hydrolysis	Suger fermentation		
							Glucose	Galactose	Mannitol
B. cereus	+	-	+	+	+	+	+	-	+
B. anthracis	+	-	+	+	+	+	+	-	+
B. mycoides	+	-	+	-	+	+	+	+	-
B. thuringiensis	-	-	+	+	+	+	+	-	+

B. lentus	+	-	+	-	-	-	+	+	-
B. subtilis	+	+	+	-	-	+	-	+	-
B. pantothenticus	+	+	-	+	-	+	+	-	+
B. firmus	+	+	+	-	-	+	+	-	+

+: Positive - : Negative

On the other hand, *M. kristinae* was detected only in the main stream, treated water and zeer waters of Atbara River. The bacterium *M. roseus* was found in the main stream and zeer waters of Atbara Rive. Different *bacillus* spp. were detected generally in most of the sources. Although, *Corynebacterium diphtheriae* was isolated from the treated water and the main reservoir of Atbara River, the elevated tank and zeer water of Al Saraf dam. Results on Table (7) show the distribution of the Gram-positive bacteria in the underground sources of Al Gedarif city drinking-water. The results showed that *Staphylococcus aureus*, *S. epidermidis*, *B. anthracis* and *C. diphtheriae* were found in all of the tested sources. However, *S. saprophyticus*, *M. varians*, *M. kristinae*, *B. thuringiensis*, *B. pantothenticus* and *B. firmus* were not detected in any of the tested underground sources. The bacterium *B. subtilis* was detected in all of the tested sources, except in the collection tank of Al Azaza boreholes. While, *M. roseus* and *B. lentus* were found only in the collection tank of Al Azaza boreholes. On the other hand, *M. nishinomiyaensis* was found only in the collection tank of Abu Al Naja boreholes. The bacterium *B. cereus* was detected in the collection tank and zeer water of Al Azaza boreholes. However, *B. mycoides* was found in the collection tank and zeer water of Abu Al Naja boreholes.

Table 6. Distribution of the Gram-positive bacteria in the surface sources of Al Gedarif city drinking-water

Sources Bacterial species	ARMS	ARTW	ARMV	ARZ	SDET	SDZ	DDET	DDZ
S. aureus	+	+	+	+	-	+	+	+

S. epidermidis	+	+	-	+	+	+	+	+
S. saprophyticus	+	+	-	+	-	-	-	+
M. varians	+	+	-	+	-	-	+	-
M. nishinomiyaensis	+	+	-	+	+	-	+	-
M. kristinae	+	+	+	-	-	-	-	-
M. roseus	+	-	-	+	-	-	-	-
B. cereus	+	+	+	+	-	-	-	-
B. anthracis	-	+	+	-	-	+	+	+
B. mycoides	-	+	-	+	-	+	-	-
B. thuringiensis	+	-	-	-	-	-	-	-
B. lentus	+	+	-	-	-	-	+	+
B. subtilis	-	+	+	+	+	-	-	+
B. pantothenticus	+	-	+	-	+	-	-	+
B. firmus	-	+	-	+	-	-	-	-
C. diphtheriae	-	+	+	-	+	+	-	-

Table7. Distribution of the Gram-positive bacteria in the underground sources of Al Gedarif city drinking-water.

Sources / Bacterial species	ZBCT	ZBZ	NBCT	NBZ
S. aureus	+	+	+	+
S. epidermidis	+	+	+	+
S. saprophyticus	-	-	-	-
M. varians	-	-	-	-
M. nishinomiyaensis	-	-	+	-
M. kristinae	-	-	-	-
M. roseus	+	-	-	-
B. cereus	+	+	-	-
B. anthracis	+	+	+	+
B. mycoides	-	-	+	+
B. thuringiensis	-	-	-	-
B. lentus	+	-	-	-
B. subtilis	-	+	+	+
B. pantothenticus	-	-	-	-
B. firmus	-	-	-	-
C. diphtheriae	+	+	+	+

- : Negative +: Positive

DISCUSSION

The present study included identification studies for the unknown Gram positive bacterial isolates obtained from the main five sources of drinking water in Al Gedarif

city. The routine primary tests were applied for the identification up to the genera level. The results showed that the isolates of gram-positive were in general belonged to four bacterial genera (*Staphylococcus*, *Micrococcus*, *Bacillus* and *Corynebacterium*). Moreover, the biochemical tests were performed for the identification up to the species level. The results indicated that different species were detected for each genus. According to Brenner (1984), the Gram-positive form was the most important group of the aerobic heterotrophic micro flora in any water system. The two commonly genera found in water are: *Aerococcus* (a typical air-borne microorganism) and *Bacillus* (a typical soil-borne one). This author stated that the bacterial genera that are found in considerable numbers in water include; *Streptococcus*, *Lactobacillus*, *Micrococcus*, *Listeria* and *Corynebacterium*. Although, Bifid bacteria, Gram-positive bacteria, which constitute the most common part of the intestine of human and animal micro flora, and which are usually detected in water (Bonjoch *et al.*, 2004), they have not been isolated in the present study. However, most of the previous studies about the microbial quality of water done in the Sudan, were concentrating mainly on the enumeration of the bacteria and they did not give more attention for the identification tests (El Tom, 1997; and Ahmed Alhag, 2005).

REFERENCES

Ahmed Alhag, I. F. (2005). Microbiological Quality of Water in Some Food Factories Storage Cisterns in Khartoum North Industrial Area. Ph. D. Thesis, Department of Botany, Faculty of Science, University of Khartoum, Sudan.

/**Alcamo,** I. E. (1997). Fundamentals of microbiology. 5th Ed. By the Benjamin Cummings, an imprint of Addison Wesley Longman, Inc.

Barrow, G. I. and Feltham, R. K. A. (1993). Cowan and Steel's Manual for the identification of medical bacteria. 3rd Ed. Cambridge University Press. Cambridge, U.K.

Bonjoch, X.; Balleste, B. and Blanch, A. R. (2004). Multiplex PCR with 16srRNA Gene-Targeted Primers of *Bifidobacterium* spp. to Identify Sources of Fecal Pollution. *Applied and Environmental Microbiology*, 70: 3171 – 3175.

Brenner, D. J. (1984). Facultatively Anaerobic Gram-Negative Rods. Sec. 5 In: Bergey's Manual of Systematic Bacteriology. 1st Ed. Vol. 1, pp. 409 – 598.

Buchanan, R. E. and Gibbons, N. E. (1974). Bergey,s Manual of Determinative Bacteriology. 8th edition. Williams and Wilkins. Baltimore.

Elrofaei, N. A. (2000). Microbiological examination of drinking water for the displaced people living around Khartoum State. Ph. D. Thesis, Faculty of Agriculture, University of Khartoum, Sudan.

El Tom, A. M. (1997). Microbiology of Port-Sudan Water Supply. Ph. D. Thesis, Faculty of Agriculture, University of Khartoum, Sudan.

Internet (2008). Center for Disease Control and Prevention. Epidemiology Program Office. Morbidity and Mortality Weekly report. mmwr.html./mmwr/epo/www.cdc.gov//http:

Stephen, P. D.; Norman, A. H. and Sean, P. G. (2004). Pharmaceutical Microbiology. Blackwell Science Ltd. Oxford.

WHO (1993). Guidelines for Drinking-Water Quality. Vol. 2, World Health Organization, Geneva.

عزل وتصنيف وانتشار عزلات البكتريا الموجبة لصبغة جرام الملوثة لمياه شرب مدينة القضارف / السودان

الملخص

تم في هذا البحث عزل البكتريا الهوائية الموجبة لصبغة جرام من المصادر الرئيسية لمياه الشرب بمدينة القضارف (مياه طبيعية ، مياه معالجة ومياه أزيار لكل المصادر) . وتم تصنيف العزلات بأستخدام الإختبارات الأولية تحت مرحلة الجنس . أثبتت النتائج أن العزلات تتبع لأربعة أجناس (*Staphylococcus, Micrococcus, Bacillus* and *Corynebacterium*) . هذا وقد أكدت الاختبارات الكيميائية تصنيف العزلات إلى مستوى النوع . وأكدت النتائج أن ثلاثة عزلات تتبع الجنس *Staphghococeus* ، وأربعة تتبع الجنس *Micrcoccus* ، وثمانية تتبع الجنس *Bacillus* ونوع واحد هو البكتيريا *Corynebacterium dipltheriae* ، وشملت الدراسة كذلك انتشار تلك العزلات في المصادر المختلفة . لوحظ أن البكتريا *Staph aureus* و *Staph Epidermis* توجد في كل المصادر تقريباً . في حين كانت الأنواع الأخرى موجودة في بعض المصادر وغير موجودة في بعضها الأخرى.

ومن ناحية أخرى لم يتم عزل البكتريا أدناه من أي من مصادر المياه الجوفية.

pantothenticus and *B.) S. saprophyticus, M. varians, M. kristinae, B. thuringiensis, B.)*

firmus