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

Zeinab J. Abdel Daim [1], Awad M. Abdel-Rahim [2] and Abdulwahab Ali Ahmed [3]

[1] Al Gedarif State, Water Corporation Laboratory.

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

[3] Faculty of Agriculture, University of Al Gedarif

ABSTRACT

In this study, the possible aerobic Gram-negative bacteria were isolated from the main sources of 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 (*Neisseria*, *Aeromonas*, *Vibrio* and *Pseudomonas*), in addition to Enterobacteria which include *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Salmonella*, *Shigella*, *Erwinia*, *Providencia*, *Proteus*, *Serratia* and *Yersinia*. However, the biochemical tests identified the isolates up to the species level. The bacterium *E. coli* was the most predominant one among the Gram-negative isolates. Moreover, different species of other genera were also identified. The standardized system (Analytical Profile Index- API 20 E) was used to confirm the identification of the Gram-negative rod species. The study also included the distribution of the identified species in the different sources. It was found that *E. coli* was 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, many species were not detected in any of the underground sources.

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

INTRODUCTION

Although water is essential for life, yet it can be a source of danger and may at times jeopardize animal life and particularly that of man (Abdel-Daim, 2009). Water borne illness remains a major source of worldwide human morbidity and mortality and it has been estimated that one-half of the world population has suffered from diseases caused by polluted water (Mc Feters and Singh, 1991).

Numerous pathogenic agents have been found in fresh waters used as sources of water supplies, recreational bathing and irrigation. These agents include bacterial pathogens, enteric viruses and several protozoans more common to tropical waters (Geldreich, 1998; Hazen. 2006; Al-Sainary, 2007). The total coliform group of bacteria includes both Gram- positive and Gram-negative groups as well as many bacteria of non-fecal origin. In water quality testing, both groups are identified by their ability to ferment lactose and selective media are used to inhibit the growth of Gram-positive organisms (Horsnell *et al*., 2006). Intestinal Gram-negative bacteria which are known to occur in contaminated drinking water include species of the genera; *Salmonella*, *Shigella, Vibrio*, *Yersinia, Sampylobacter and Escherichia coli (*Internet, 2008*).*

Heterotrophic Plate Count (HPC) of bacteria in drinking-water often include isolates of the genera; *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Aeromonas* and *Xanthomonas*. The three non-fermentative Gram-negative rods most frequently isolated in the clinical laboratories are *Pseudomonas aeruginosa*, *Xanthomonas maltophillia* and *Acinetobacter sp.* (WHO, 1993).

WHO (1993) also reported that some opportunistic pathogens are able to cause disease in people with impaired local or general defence 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. Examples of such agents are *Pseudomonas aeruginosa* and species of *Flavobacterium, Acinetobacter, Klebsiella, Serratia, Aeromonas* and certain "slow growing" *Mycobacterium*. A certain serious illness results from the drinking of water in which the causative organism has multiplied because of warm temperatures and the presence of nutrients, is the Legionnaires' disease (*Legionella* spp.).

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, IMViC tests (Indole, Methyl red, Voges–Proskauer and Simmon citrate tests), Motility, Urease activity, Nitrate reduction, Pigment production, H_2S production, citrate utilization, Growth at 22º C and 42º C, Starch hydrolysis, Gelatin hydrolysis and Acid from carbohydrate tests (Sugar fermentation test). The Analytical Profile Index 20 Enterobacteria (API 20 E – bioMerieux, Inc. Hazelwood, MO, France) was applied for all Gram-negative rods of the bacterial isolates, to confirm the identification of the enterobacteriaceae and associated organisms. The API 20 E system consists of a plastic strip holding twenty mini-test tubes. Each tube was inoculated with a saline suspension of a pure culture. Some of the tubes were completely filled), while the other tubes were overlaid with paraffin oil, so that anaerobic reactions could take place. After incubation at 37º C for 18 – 24 hours, the color reactions were read. However, reagents were added to reaction tubes of the tryptophan deaminase, indole and Voges–Proskauer. The reactions were converted to a seven-digit code, and then read from the API 20 E manual, to obtain the identification as genus and species (manufacturer's directions).

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. Four of the genera appeared as rod shaped, one was cocci. All the genera were none spore forming, however, all the isolates were catalase positive and three of them was oxidase positive, while one of the tested genera showed positive oxidation–fermentation reaction. However, most of them were motile (Table, 1). The isolates of each genus were further identified to the species level by the biochemical tests.

Table 1. Primary tests of Gram negative 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	5
Shape	Rod	Cocci	Rod	Rod	Rod

Spore forming	–	–	–	–	–
Catalase test	+	+	+	+	+
Oxidase test	-	+	+	+	+
Oxidation–Fermentation	+	-	ND	ND	ND
Motility test	-/+	-	-/+	+	+
Genus	Enterobacteria*	*Neisseria*	*Aeromonas*	*Vibrio*	*Pseudomonas*

ND: Not Done - : Negative +: Positive

*they include many genera: *Escherichia, Enterobacter, Citrobacter, Klebsiella, Salmonella, Shigella, Erwinia, Providencia, Proteus, Serratia* and *Yersinia*.

Results in table (2.a and 2.b) illustrate the biochemical tests of the Gram-negative – non-oxidative bacteria (enterobacteria). They included; *Escherichia coli, Enterobacter aerogenes, Ent. cloacae, Citrobacter freundii, Cit. koseri, Klebsiella oxytoca, Klb. pneumonia, Klb. ozaenae, Salmonella typhi, Salmonella spp., Shigella spp., Erwinia herbicola, Providencia rettgeri, Proteus mirabilis, Prot. vulgaris, Serratia liquefaciens, Ser. marcescens* and *Yersinia pestis.* The Gram-negative – oxidative bacteria and their biochemical reactions are recorded in tables (3.a and 3.b). They included; *Neisseria* spp., *Aeromonas hydrophila, Aeromonas sp., Vibrio cholerae* and some species of *Pseudomonas* (*Ps. aeruginosa, Ps. cepacia, Ps. fluorescens, Ps. pseudomallei, Ps. vesicularis, Ps. paucimobilis and Ps. maltophilia*).

The identification results obtained from the API 20 E standardized system were typical to that of the manual tests. The study also included distribution of the identified bacterial species, in the different sources of Al Gedarif city drinking-water.

Table (4) shows the distribution of the Gram-negative – non-oxidative bacteria (enterobacteria) in the surface sources. The Bacterium *E. coli* was found in all sources. *Enterobacter aerogenes* and *Ent. Cloacae* were found in the main reservoir, zeer waters of Atbara River and the elevated tanks of Al Saraf and Dalassa dams. The bacteria

Citrobacter freundii and *Cit. koseri* were detected in the treated water and zeer waters of Atbara River.

Table 2.a. Biochemical tests for Gram-negative – non-oxidative (enterobacteria) species isolated from the different sources of Al Gedarif city drinking-water.

Biochemical tests / Species	IMViC tests				Motility	Urease	H_2S production
	Indole	Methyl red	Voges - Proskaur	Citrate utilization			
E. coli	+	+	-	-	+	-	-
Ent. Aerogenes	-	+	-	+	+	-	-
Ent. Cloacae	-	+	-	+	-	+	-
Cit. Freundii	-	+	-	+	+	+	+
Cit. Koseri	+	+	-	+	+	+	+
Klb. Oxytoca	+	-	+	+	-	+	-
Klb. Pneumonia	-	-	+	-	-	+	-
Klb. Ozaenae	-	+	-	+	-	-	-
Sal. Typhi	-	+	-	+	+	-	+
Salmonella spp.	-	+	-	+	+	-	+
Shigella spp.	-	+	-	-	-	-	-
Erw .herbicola	-	+	-	+	+	-	-
Pro. Rettgeri	+	+	-	+	+	+	+
Prot. Mirabilis	-	+	-	+	+	+	+
Prot. Vulgaris	+	+	-	+	+	+	+
Ser. Liquefaciens	-	+	-	+	+	-	-
Ser. Marcescens	-	-	+	+	+	+	+
Y. pestis	-	+	-	-	-	-	-

+: Positive - : Negative

Table 2.b. Sugar fermentation tests by Gram-negative – non-oxidative (enterobacteria) species isolated from the different sources of Al Gedarif city drinking-water.

Sugar / Species	Glucose	Lactose	Sucrose	Mannitol	Maltose
E. coli	+	+	+	+	+
Ent. Aerogenes	+	+	+	+	+
Ent. Cloacae	+	-	+	+	+
Cit. Freundii	+	+	+	+	+
Cit. Koseri	+	+	-	+	+
Klb. Oxytoca	+	+	+	+	+

Klb. Pneumonia	+	+	+	+	+
Klb. Ozaenae	+	+	+	+	-
Sal. Typhi	-	-	-	+	+
Salmonella spp.	+	-	-	+	+
Shigella spp.	-	-	-	+	+
Erw .herbicola	-	-	+	+	-
Pro. Rettgeri	-	-	+	-	+
Prot. Mirabilis	+	-	-	-	-
Prot. Vulgaris	+	-	+	-	+
Ser. liquefaciens	+	-	+	+	+
Ser. Marcescens	+	-	+	+	+
Y. pestis	-	-	-	+	-

+: Positive - : Negative

Table 3.a. Biochemical tests for Gram-negative – oxidative species isolated from the different sources of Al Gedarif city drinking-water.

+: Positive - : Negative ND: Not Done

Biochemical tests / Species	Motility	Pigment production	Growth at 22° C	Growth at 42° C	Citrate	Urease	H_2S	Nitrate reduction
Neisseria spp	-	-	+	ND	ND	ND	-	-
Aer. Hydrophila	+	-	-	-	ND	ND	ND	+
Aerococcus sp.	-	-	-	-	ND	ND	ND	+
V. cholera	+	-	-	-	ND	ND	ND	+
Ps. Aeruginosa	+	Green	+	+	+	+	-	+
Ps. Cepacia	+	-	+	+	+	+	-	-
Ps. Fluorescens	+	-	+	-	+	+	-	-
Ps. Pseudomallei	+	-	+	+	+	+	-	-
Ps. Vesicularis	-	Orange	+	-	-	-	-	-
Ps. Paucimobilis	-	Yellow	+	-	-	-	-	-
Ps. Maltophilia	+	-	+	+	-	-	+	-

The former was found also in the main stream of Atbara River, the elevated tank and zeer water of Al Saraf dam, and zeer water of Dalassa dam, however, the later was also found in the elevated tank of Dalassa dam. Regarding the genus *Klebsiella*, three species were identified; *Klb. oxytoca* which was found in zeer waters of Atbara River and Dalassa dam, and the elevated tank of Al Saraf dam, *Klb. pneumonia* which was found in the main stream,

Table 3.b. Starch and gelatine hydrolysis and sugar fermentation by Gram-negative - oxidative species isolated from the different sources of Al Gedarif city drinking-water.

Biochemical tests / Species	Starch hydrolysis	Gelatin hydrolysis	Sugar fermentation					
			Glucose	Lactose	Fructose	Sucrose	Mannitol	Maltose
Neisseria spp.	ND	ND	+	-	+	-	-	-
Aer. Hydrophila	ND	ND	+	-	ND	+	ND	ND
Aerococcus sp.	ND	ND	-	-	ND	+	ND	ND
V. cholera	+	ND	-	-	ND	+	ND	ND
Ps. Aeruginosa	-	+	+	-	+	-	+	-
Ps. Cepacia	-	+	+	+	+	+	+	+
Ps. Fluorescens	-	+	+	-	+	+	-	+
Ps. pseudomallei	-	+	+	+	+	+	+	+
Ps. Vesicularis	-	+	+	-	-	-	-	+
Ps. paucimobilis	+	-	+	+	+	+	+	+
Ps. Maltophilia	+	+	-	-	+	-	-	+

+: Positive - : Negative ND: Not Done

Table 4. Distribution of the Gram-negative – non-oxidative – bacteria (enterobacteria) in the surface sources of Al Gedarif city drinking-water.

Treated water and zeer waters of Atbara River, and the elevated tank and zeer water of Al Saraf dam, and *Klb. ozaenae*, which was found in the elevated tank of Al Saraf dam.

Sources / Bacterial species	ARMS	ARTW	ARMV	ARZ	SDET	SDZ	DDET	DDZ
E. coli	+	+	+	+	+	+	+	+
Ent. Aerogenes	+	-	+	+	+	-	+	-
Ent. Cloacae	-	+	+	+	+	-	+	-
Cit. Freundii	+	+	-	+	+	+	-	+
Cit. Koseri	-	+	-	+	-	-	+	-
Klb. Oxytoca	-	-	-	+	+	-	-	+
Klb. Pneumonia	+	+	-	+	+	+	-	-
Klb. Ozaenae	-	-	-	-	+	-	-	-
Sal. Typhi	+	-	-	-	-	-	-	-
Salmonella spp.	+	+	+	+	+	+	+	+
Shigella spp.	-	-	-	+	-	+	-	+
Erw. Herbicola	-	+	-	-	-	-	-	+
Pro. Rettgeri	-	-	-	+	-	-	-	+
Prot. Mirabilis	+	+	-	+	-	-	-	-
Prot. Vulgaris	-	-	-	-	+	-	-	-
Ser. Liquefaciens	-	-	-	+	-	-	-	+
Ser. Marcescens	-	-	-	+	-	+	-	-
Y. pestis	+	+	-	-	-	-	+	+

The bacterium *Salmonella typhi* was detected only in the main stream of Atbara River. In contrast, the other unknown species of *Salmonella* were found in all sources. Some species of the genus *Shigella* were isolated from zeer waters of all sources. The bacterium *Erwinia herbicola* was found in two sources; the treated water of Atbara River and the

zeer water of Dalassa dam, while *Providencia rettgeri* was detected in zeer waters of Atbara River and the zeer water of Dalassa dam. Although, *Proteus mirabilis* was detected in the main stream, treated water and zeer waters of Atbara River, *Prot. vulgaris* was detected only in the elevated tank of Al Saraf dam. The two species of *Serratia (Ser. liquefaciens* and *Ser. marcescens*) were isolated from zeer waters of Atbara River. The last species of enterobacteria identified, is *Yersinia pestis*. It was detected in the main stream and treated water of Atbara River, and the elevated tank and zeer water of Dalassa dam.

The distribution of the Gram-negative – non-oxidative bacteria (enterobacteria) in the underground sources is shown in Table (5). The Bacterium *E. coli* and *Shigella spp.* were found in all sources. *Enterobacter aerogenes* was found in the collection tanks of Abu Al Naja and Al Azaza boreholes. The bacteria *Citrobacter freundii* and *Salmonella spp.* were detected in the collection tank and zeer water of Abu Al Naja boreholes, while, *Klebsiella pneumonia* and *Yersinia pestis* were found in the collection tank and zeer water of Al Azaza boreholes. Regarding the genus *Klb. Ozaenae*, was detected in zeer waters of Abu Al Naja and Al Azaza boreholes. However *Cit. Koseri* was found only in zeer water of Abu Al Naja boreholes. The other species of enterobacteria identified, were not detected in the underground sources.

Table (6) illustrates the distribution of the Gram-negative – oxidative bacteria in the surface sources. Species belonging to four different genera were identified; *Neisseria* spp.

Also in the treated water of Atbara River. However, only one species of *Vibrio* (*V. cholerae*) was detected in zeer waters of the all sources. On the other hand, seven species of the genus *Pseudomonas* were detected. The bacteria *Ps. Aeruginosa* and *Ps. paucimobilis* were found in all of the sources, *Ps. cepacia* was found only in the main reservoir, *Ps. fluorescens* was found in all sources, except in the main reservoir of Atbara

River and elevated tank of Al Saraf dam. However, *Ps. Pseudomallei* was found only in the main stream of Atbara River. While, *Ps. vesicularis* was detected in the main reservoir and the elevated tanks of Al Saraf and Dalassa dams. Moreover, *Ps. maltophilia* was found in zeer waters of Atbara River and the zeer waters of Al Saraf and Dalassa dams.

Table 5. Distribution of the Gram-negative – non-oxidative – bacteria (enterobacteria) in the underground sources of Al Gedarif city drinking-water.

Sources / Bacterial species	ZBCT	ZBZ	NBCT	NBZ
E. coli	+	+	+	+
Ent. Aerogenes	+	-	+	-
Ent. Cloacae	-	-	-	-
Cit. Freundii	-	-	+	+
Cit. Koseri	-	-	-	+
Klb. Oxytoca	-	-	-	-
Klb. pneumonia	+	+	-	-
Klb. ozaenae	-	+	-	+
Sal. typhi	-	-	-	-
Salmonella spp.	-	-	+	+
Shigella spp.	+	+	+	+
Erw. Herbicola	-	-	-	-
Pro. Rettgeri	-	-	-	-
Prot. Mirabilis	-	-	-	-
Prot. Vulgaris	-	-	-	-
Ser. Liquefaciens	-	-	-	-
Ser. Marcescens	-	-	-	-
Y. pestis	+	+	-	-

+: Positive - : Negative

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

Sources / Bacterial species	ARMS	ARTW	ARMV	ARZ	SDET	SDZ	DDET	DDZ

Neisseria spp.	+	-	-	+	-	+	+	+
Aer. Hydrophila	+	-	+	+	+	+	-	+
Aerococcus sp.	+	+	+	+	-	-	-	+
V. cholerae	-	-	-	+	-	+	-	+
Ps. Aeruginosa	+	+	+	+	+	+	+	+
Ps. Cepacia	-	-	+	-	-	-	-	-
Ps. Fluorescens	+	+	-	+	-	+	+	+
Ps. Pseudomallei	+	-	-	-	-	-	-	-
Ps. Vesicularis	-	-	+	-	+	-	+	-
Ps. Paucimobilis	+	+	+	+	+	+	+	+
Ps. Maltophilia	-	-	-	+	-	+	-	+

+: Positive - : Negative

Were found in the main stream of Atbara River, the elevated tank of Dalassa dam, and zeer waters of Atbara River, Al Saraf and Dalassa dams. Two species of *Aeromonas* were isolated; *Aer. hydrophila* and an unknown species which were found in the main stream, the main reservoir and zeer waters of Atbara River, and zeer water of Dalassa dam. The former was detected also in the elevated tank and zeer water of Al Saraf dam, while the latter was found

The distribution of the Gram-negative – oxidative bacteria in the underground sources was shown in table (7). Most of the species were not detected in the underground sources. However, *V. cholerae* was detected in the all sources, except in the collection tank of Abu Al Naja boreholes. The bacteria and *Ps. Aeruginosa Ps. fluorescens* were found the collection tank and zeer water of Abu Al Naja boreholes. While, *Ps. vesicularis* and *Ps. paucimobilis* were detected only in zeer water of Abu Al Naja boreholes.

DISCUSSION

The present study included identification studies for the unknown Gram negative 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-negative were in general belonged to four bacterial genera (*Neisseria*, *Aeromonas*, *Vibrio* and *Pseudomonas*) in addition to

Enterobacteria which include *Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Salmonella*, *Shigella*, *Erwinia*, *Providencia*, *Proteus*, *Serratia* and *Yersinia*. 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. 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) investigated the microbial quality of Port-Sudan drinking-water and Ahmed Alhag (2005) studied the microbial quality of water in different areas in Khartoum. They confirmed the presence of isolates belonging to the following genera; *Aerococcus*, *Bacillus*, *Micrococcus*, *Enterobacteria*, *Staphylococcus* and *Streptococcus*. On the other hand, Alcamo (1997) also stated that the non-coliform bacterial isolates, which were common in his study, were belonged to the genera; *Streptococcus*, *Proteus* and *Pseudomonas*.

Table 7. Distribution of the Gram-negative – oxidative – bacteria in the underground sources of Al Gedarif city drinking-water.

Sources / Bacterial species	ZBCT	ZBZ	NBCT	NBZ
Neisseria spp.	-	-	-	-
Aer. Hydrophila	-	-	-	-
Aerococcus sp.	-	-	-	-
V. cholerae	+	+	-	+
Ps. Aeruginosa	-	-	+	+
Ps. Cepacia	-	-	-	-
Ps. Fluorescens	-	-	+	+
Ps. pseudomallei	-	-	-	-
Ps. Vesicularis	-	-	-	+
Ps. paucimobilis	-	-	-	+
Ps. Maltophilia	-	-	-	-

+: Positive - : Negative

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الملخص

تم في هذا البحث عزل البكتريا السالبه لصبغة جرام من المصادر الرئيسية لمياه الشرب بمدينة القضارف (مياه طبيعية ، مياه معالجه ومياه أزيار لكل المصادر). وتم تصنيف العزوات بإستخدام الاختبارات الأولية والكيميائية ، حيث استخدمت الاختبارات الأولية للتصنيف حتى مرحلة الحبس . أثبتت النتائج أن العزولات تتبع لأربعة أجناس رئيسية هي : (*Neisseria, Aeromonas, Vibrio* و *Pseudomonas*) بجانب اجناس البكتريا البرازية (*Escherichia, Enterobacter, Citrobacter,* و *Klebsiella, Salmonella, Shigella, Erwinia, Providencia, Proteus, Serratia Yersinia*) . هذا وقد أستخدمت الاختبارات الكيميائية لتصنيف العزلات لإلى أربعة انواع ، حيث كانت بكتريا القولون (*E .coli*) هي السائدة بين البكتريا السالبة لصبغة جرام . وقد استخدم نظام (*ApI 20 E*) لتأكيد تصنيف تلك البكتريا . وشملت الدراسة كذلك إنتشار تلك العزلات في مصادر المياه المختلفة. فكانت بكتريا القولون (*E .coli*) تواجده في جميع المصادر تقريباً ، في حين كانت الأنواع الاخرى متواجده في بعض المصادر وغير متواجده في بعضها الاخر . ومن ناحية أخرى لم يتم عزل كثير من هذه الأنواع من مصادر المياه الجوفية .