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Microbiological Quality and Genotypic Speciation of Heterotrophic Bacteria Isolated from Potable Water Stored in Household Tanks

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Upon storage, a statistically significant increase in heterotrophic plate count (HPC) was detected in samples from household tanks. The most consistent correlate of regrowth was found to be storage time, however, temperature and the microbiological quality of the influent water were also important. Total and fecal coliforms were detected in the influent water and in the household storage tanks. Coliforms growing at 37ºC were a small but significant part (about 0.002%) of the total heterotrophic population. In the influent, 92% of the coliforms (11 colony forming units/100 mL) at 44ºC were *Escherichia coli*, while in the household tanks it was 86%. The majority of isolates from the influent and the household tanks recovered on R_2A agar formed pigmented colonies and were Gram-negative belonging to the α-, β-, and γ-subclasses of *Proteobacteria*. Overall, the α-subclass was the dominant type representing 61% of the recovered HPC, and 40 to 50% of the organisms based on 16S rDNA sequence analysis were members of the family *Sphingomonadaceae*. The presence of *E. coli* and the isolation of opportunistic pathogens as a significant part of the HPC population from both influent and household tanks indicated a potential health hazard to consumers.

Key words: **household, storage, *Escherichia coli*, 16S rDNA, *Sphingomonas*, *Aeromonas***

Introduction

Water transported through distribution systems is subject to both chemical and microbial quality changes. The hygienic quality of drinking water can be reduced if pathogens survive the water treatment process or if the condition in the distribution network enhances microbial regrowth (Miettinen et al. 1997). This quality is related to the effectiveness and the concentration of the disinfectant residual, the age of water in the system and the origin of the intake water (Edge and Finch 1987; Schreiber and Schoenen 1994; Le Chevallier et al. 1996; Momba et al. 2000). Decay in the disinfectant residual associated with long hydraulic retention and warm water conditions (Burlingame and Brock 1985), and the accumulation of sediments in drinking water reservoirs provides the needed environment for the establishment of a stable association between varieties of different organisms (Schreiber and Schoenen 1994). Storing treated drinking water in household tanks may lead to post-treatment contamination, introducing coliform bacteria and possible pathogens into the water supply (Geldreich 1996). Many countries in the Middle East, including Lebanon, suffer from chronic water shortage. As a result supplies generally operate on an intermittent basis, leaving the distribution system unpressurized for long periods of time (Tokajian and Hashwa 2003). Operating a distribution system in a non-continuous manner leads to conditions that favour the deterioration of the water quality, due mainly to infiltration, regrowth within pipes and the detachment of the bacterial biofilm following variations in pressure and velocity. Householders are also forced to store the water for several days providing further opportunity for regrowth of opportunistic pathogens, and thus contributing to water-quality problems.

In this work we report on the deterioration in the microbiological quality of water during storage in household tanks in Lebanon, and we present for the first time the isolation and identification of heterotrophic bacteria from the storage tanks based on 16S rDNA sequence analysis. We recognized, based on a preliminary survey (Tokajian et al. 2000; Tokajian and Hashwa 2003), that most of the isolates could not be assigned to a specific phylogenetic branch using conventional diagnostic means. The phylogenetic analysis was necessary to obtain a much more thorough picture of the distribution of bacterial species and to define any possible health hazards to consumers.

Materials and Methods

Sampling Program

Four covered household storage tanks (two polyethylene and two cast iron) of half-cubic metre size were installed in series on the campus of the Lebanese American University (LAU), Byblos, Lebanon. The influent water to the tanks, taken from LAU-owned artesian well, was treated

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with rapid sand filtration and ultraviolet light. The treated water is pumped to a reservoir and from there water flows by gravity into the distribution network. A bottom water-sampling tap was installed to control the effluent of each household water storage tank. The household tanks were drained and refilled repeatedly for a period of 12 months and samples were collected on a bimonthly sampling schedule as follows: tanks were filled on day 0 and samples from both types were collected immediately and after 2, 3, 4 and 7 days of storage. To mimic the water consumption in an ordinary household, 20% of the storage volume was wasted on each sampling day. Additionally, the tanks were completely drained on day 7 to be filled again at the beginning of the next sampling cycle. Temperature and pH values were determined using calibrated thermometers and pH metres. The natural design of the tanks did not allow for complete drainage and some sediment accumulated with storage time.

Enumeration of Heterotrophic Bacteria

Water samples for bacteriological analysis were collected according to the HMSO Report No. 71 (1994) to determine the heterotrophic plate count (HPC). HPC was enumerated on R_2A agar (Oxoid) (Reasoner and Geldreich 1985) by filtering 100 mL water through 0.45-µm Millipore membrane filters using Nalgene equipment. Filters were placed aseptically on R_2A agar plates. Tests were done in triplicate and plates were incubated at 28ºC for 7 days. Colonies were counted at the end of the 7-day incubation period.

DNA Extraction and 16S rDNA Amplification with Universal Primers

All colonies were further purified on R_2A agar and those showing phenotypic differences were chosen for further genotypic characterization. Recovered isolates in this study were designated as HST-1 through HST-128. DNA extractions were made from pure R_2A cultures using the Prepman protocols for Gram-negative and -positive bacteria as described by the manufacturer (Applied Biosystems, U.S.A.). DNA extraction was assessed by the amplification of the 16S rRNA gene using the gene sequence specific primers: forward primer 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') (Thermo Hybaid, Germany) (DeLong 1992; Suzuki and Giovannoni 1996). PCR products were visualized by ethidium bromide staining on agarose gel electrophoresis.

16S rRNA Gene Sequencing and Sequence Data Analysis

Only samples showing successful DNA extraction were used for 16S rDNA sequencing. A 500-bp 16S rDNA fragment was amplified using the MicroSeq 500 kit (Applied Biosystems, U.S.A.) in a reaction volume of 20 µL (10 µL of MicroSeq PCR master mix, 8.5 µL of molecular-grade water, 0.05 units of AmpliTaq Gold [Applied Biosystems, U.S.A.] and 1.5 µL DNA extract).

Prior to sequencing the amplified products were purified (0.2 units of shrimp alkaline phosphatase and exonuclease 1 [USB, Cleveland, Ohio] were mixed with 10 µL of the amplified product and incubated at 37ºC for 30 min, and then at 80ºC for 15 min).

Forward and reverse sequencing reactions were performed for each amplified product followed by ethanol precipitation. Sequences were analyzed on the ABI PRISM 310 (Applied Biosystems, U.S.A.) automated DNA sequencer. Using MicroSeq 500 microbial identification and analysis software, fragmentary sequence information was aligned and assembled, and the final consensus sequence was compared with over 1200 validated 16S ribosomal DNA sequences in the database (Patel et al. 2000; Tang et al. 1998). The sequences were also compared to published 16S rDNA sequences from the EMBL (FASTA version 3.3t09) (Pearson and Lipman 1988), and the RIDOM (Ribosomal Differentiation of Medical Microorganisms) (Turenne et al. 2001) databases. The 16S rDNA sequences and their accession numbers were derived from the GenBank. Classification of organisms into the different classes of *Proteobacteria* was done according to Stackebrandt et al. (1988). A phylogenetic tree was constructed using the TREECON software. Bootstrap analysis was also used to place confidence intervals on phylogenies (Van de Peer and Watcher 1994).

Enumeration and Identification of Coliforms

Total and fecal coliforms were enumerated by filtering 100 mL water samples through 0.45-µm Millipore membrane filters using Nalgene equipment. Filters were placed on membrane lauryl sulfate agar (Oxoid) and plates were incubated for 24 h at 37°C for total coliforms and at 44°C for fecal coliforms. Yellow colonies on lauryl sulfate agar were considered as presumptive coliforms and were further purified on nutrient agar (Oxoid) and identified using API-20E strips (bioMerieux, Marcy-L'Etoile, France) and confirmed with the Biolog identification system (Biolog, Inc., Hayward, California).

Statistical Analysis

Sample parameters were recorded onto a computer database using SPSS software. Statistical analyses were conducted, and log transformation was performed for the bacteriological data to decrease the variance and produce normal distribution population. Linear regression was used to study the effect on the HPC/mL for all studied criteria. Correlation analysis was performed to determine the effect of the different parameters on each other and

on the HPC/mL for water stored in the household tanks. One-way ANOVA was used to determine the presence of a significant difference in the HPC/mL upon storage. The presence of any differences in the heterotrophic bacterial counts between the tanks in general and as related to the season was determined using the t-test.

Results

Effect of the HPC in the Influent on the HPC in the Household Tanks

Regression analysis was used to assess the effect of the influent HPC on the total HPC in the household tanks upon storage. The impact of the influent water had its most significant effect on the HPC of the household water at day 0 (immediately when tanks are filled) with an R^2 value of 0.47. The R^2 dropped to 0.3 after 2, 3 and 4 days of storage and to 0.2 at day 7. The average temperature and log HPC/mL detected in the influent were 19.8°C and 3.15, respectively (Fig. 1).

Effect of the Length of Storage on Microbiological Water Quality

Data obtained showed that water stored in both types of household tanks underwent significant changes in the bacteriological quality during storage. Single factor ANOVA revealed the presence of a significant ($p \leq 0.05$) difference between the mean log HPC/mL in the samples collected from the household tanks at day 0 compared to the values after 2, 3, 4 and 7 days of storage (Fig. 2). The mean log HPC/mL for each storage day represented the mean value of the log HPC/mL detected upon storage in both tanks all through the study. Regression analysis revealed a significant positive correlation ($p \leq 0.05$) between the counts at day 0 and day 2 ($R^2 = 0.65$), day 2 and 3 ($R^2 = 0.87$), day 3 and 4 ($R^2 = 0.92$) and day 4 and 7 ($R^2 = 0.93$). The average log HPC/mL for the whole study period was 3.69 and 3.66 in the samples from the polyethylene and cast iron tanks, respectively. This difference however, was not significant at $p \leq 0.05$.

Seasonal Variation in the HPC and Temperature

The variations in the HPC/mL and water temperature were monitored in the household tanks through both the winter and the summer seasons (Fig. 1). The average log HPC/mL value in the household tanks during the whole study period was 3.675, while the average measured temperature was 21.7°C. A significant difference at $p \leq 0.05$ was detected in the HPC between the winter and the summer (two-tail t-test $P = 0.0003$). The average log HPC/mL was 3.59 during the winter, while it was 3.79 during the summer. Similar results were obtained when data from each type (polyethylene and cast iron) were considered separately. The average log HPC/mL in the winter was 3.63 for the polyethylene and 3.56 for the cast iron. The average in both tanks was higher during the summer with a mean log HPC/mL of 3.78 in the polyethylene and 3.82 in the cast iron tanks.

The log HPC/mL remained virtually constant (3.73–3.8) in the period between April to September, whereas the measured water temperature fluctuated between 21 to 28°C. However, abrupt changes in the log HPC/mL were registered during October and November at the beginning of the rainy season (Fig. 1).

Regression analysis revealed that the factor with the highest and the most significant effect on the HPC/mL in the household tanks was the length of storage in those tanks ($P = 4.03 \times 10^{-6}$). Temperature however, was the

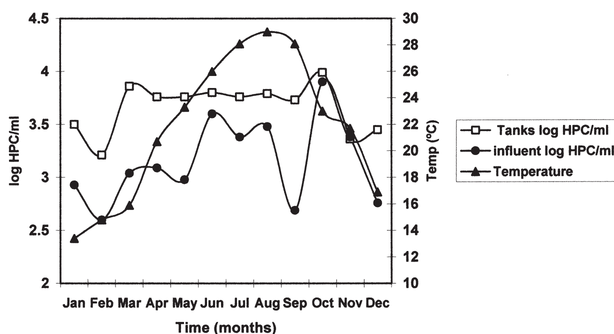

Fig. 1. Seasonal variation in the temperature and mean log HPC/mL for samples from the influent and household tanks. The mean log HPC/mL value for each month represents the mean of the log HPC/mL detected in all household tanks during the whole study period (each point represents mean of 40 values). The medium used was R_2A agar and the incubation temperature was 28°C.

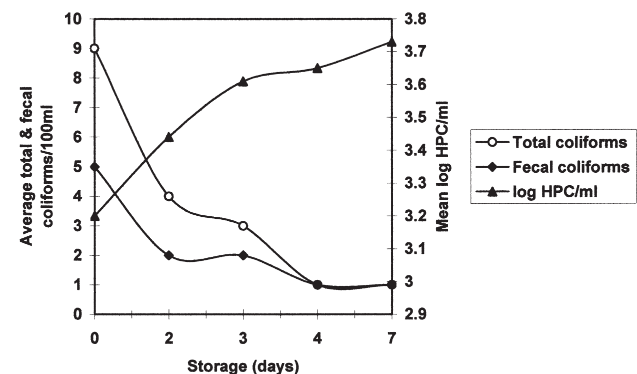

Fig. 2. Changes in the mean log HPC/mL and the number of total and fecal coliforms/100 mL upon storage in the household tanks. The mean log HPC/mL for each storage day represents the mean detected value in all household tanks during the whole study period (mean of 96 values). The total and fecal counts represent the mean value of counts reported on each storage day during the whole study period. The medium used for detection was lauryl sulfate agar and the incubation temperature was 37°C for total coliforms and 44°C for fecal coliforms.

next most important factor affecting the microbiological quality of water in the tanks ($P = 0.179$).

Total and Fecal Coliforms in the Influent and Household Tanks

Coliforms (fecal and total) were isolated from the influent and from the household tanks. Coliforms growing at 37ºC were a small but significant part (about 0.002%) of the heterotrophic count. The following members of the family *Enterobacteriaceae* were successfully recovered and identified from the influent water and the household tanks: *Escherichia coli*, *Enterobacter skazakii*, *Ent. agglomerans*, *Ent. amnigenus*, *Ent. intermedius*, *Ent. cloacae*, *Ent. aerogenes*, *Citrobacter freundii*, *Klebsiella ornithinolytica*, *Lecleria adecarboxylata* and *Serratia fonticola*. *E. coli* was the dominant type in the influent, representing 59 and 92% of the coliforms isolated at 37 and 44ºC, respectively. It was also the most numerous coliform species in the tanks isolated at 37 and 44°C, representing 54 and 86% of all the coliforms at both temperatures, respectively. The isolation of coliforms from the storage tanks was concomitant with their detection in the influent water.

Seasonal variation in the temperature did not have a significant impact on the frequency of the isolation of *E. coli* from the household tanks at 37ºC (51% in the summer and 49% in the winter). In contrast, such variation clearly affected the frequency of *E. coli* isolation at 44ºC, with 66% of all the isolates being recovered during the summer.

A decrease in the number of total and fecal coliforms was detected upon storage, contrary to the regrowth observed in the heterotrophic bacteria (Fig. 2).

Types of the HPC in the Household Tanks and the Influent

The majority of colonies recovered on R_2A agar from the household tanks (63%) and the source (56%) were pigmented. Most of the isolates in the household tanks and the influent were Gram-negative. In the household tanks the α-, β-, and γ-subclasses of *Proteobacteria* represented 85% of the HPC, whereas in the influent it was 60%. Gram-positive bacteria represented 10% of the population in the tanks and 25% in the influent. Additionally, acid-fast bacilli were detected and represented 5 and 25% of the HPC in the tanks and the influent, respectively. Overall, the α-subclass was the dominant type observed representing 61% of the recovered HPC in the storage tanks and 32% in the source.

Identification based on 16S rDNA sequence analysis revealed that around 40 to 50% of the organisms recovered from the storage tanks were members of the family *Sphingomonadaceae*. The phylogenetic tree shown in Fig. 3 reflects the phylogenetic relations between those isolates and their closest relatives in the Genbank. HST-80, which was the dominant and most persistent isolate recovered from the tanks, showed 98% similarity with *Sphingomonas rosa* (Fig. 3 and Table 1). However, isolates with a similarity of 97 to 98% to *Novosphingobium capsulatum* (HST-4), *Sphingomonas natatoria* (HST-1 and HST-3), *Sphingomonas adhaesiva* (HST-82, HST-91 and HST-103) and *Sphingobium yanoikuyae* (HST-18) were also detected (Fig. 3).

Other isolates with close similarities to *Methylobacterium* sp., *Brevundimonas* sp., *Dyadobacter fermentens* and *Caulobacter* sp. were also among the recovered isolates (Fig. 4 and Table 1).

In the γ-subclass HST-12 16S rRNA gene sequence showed high similarity with *Aeromonas* sp. CDC 715-84 and HST-33 with *Klebsiella oxytoca*, while in the β-subclass HST-31 showed high similarity with *Acidovorax* sp. “Smarlab 13305” (Fig. 5 and Table 1).

Acid-fast bacilli were also detected in the storage tanks with 99% similarity between HST-89 and HST-86 with *Mycobacterium porcinum* strain DSM 44242 and *Mycobacterium* sp. strain WF2, respectively (Fig. 6 and Table 1).

All the other Gram-positive isolates clustered into two major groups (Fig. 7). The 16S rDNA sequence simi-

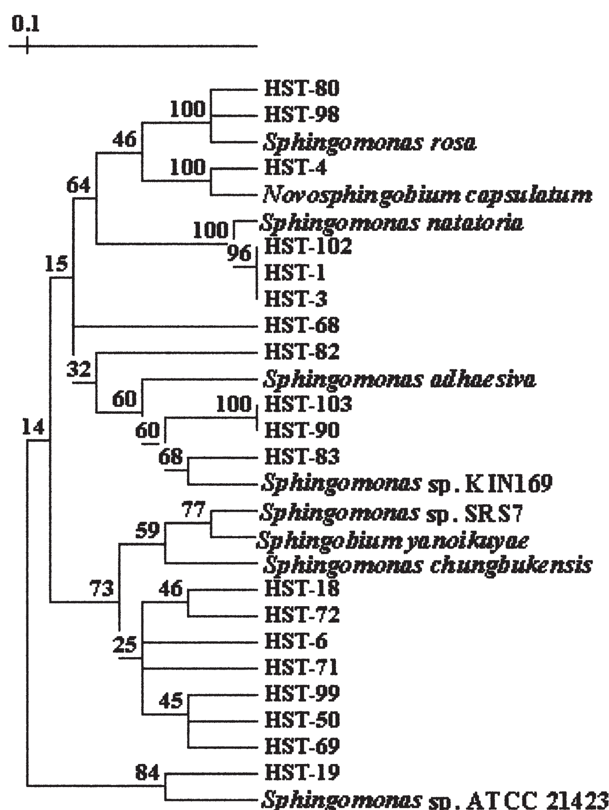

Fig. 3. Phylogenetic tree relating sequence-based identification of sphingomonads. The algorithm used to construct the tree is the unweighted pair group method using averages (UPGMA). Bootstrap values are displayed as percentages of 100 replicons.

TABLE 1. 16S rDNA-based identification of a collection of heterotrophic bacteria from the household storage tanks

Isolate	*16S rDNA-based identification*	*GenBank accession no.*
Isolates exhibiting 99% 16S rDNA sequence homology with a deposited sequence		
HST-59	*Mycobacterium frederiksbergense* WM0531	AF544629
HST-89	*Mycobacterium porcinum* DSM 44242	AF547958
HST-86	*Mycobacterium* sp.strain WF2	U90877
HST-79	*Aeromicrobium erythreum*	AF005021
HST-93	*Nevskia ramosa*	AJ001011
Isolates exhibiting 97-98% 16S rDNA sequence homology with a deposited sequence		
HST-4	*Novosphingobium capsulatum*	D16147
HST-69	*Sphingobium* sp. strain S7	AY190157
HST-99	*Sphingomonas* sp. strain SRS7	AY180075
HST-18	*Sphingobium yanoikuyae*	AF509480
HST-19	*Sphingomonas* sp.strain ATCC 21423	AF503277
HST-68	*Sphingomonas* sp. strain IFO 15917	AB033950
HST-83	*Sphingomonas* sp. strain KIN169	AY136096
HST-1	*Sphingomonas natatoria*	AY299222
HST-3	*Sphingomonas natatoria*	AJ250434
HST-80	*Sphingomonas rosa*	D13945
HST-54	*Bradyrhizobium* sp. strain ORS206	AF514798
HST-92	*Bosea thiooxidans* strain KB13-VS	AJ250800
HST-95	*Bosea thiooxidans* strain KB13-VS	AJ250800
HST-36	*Methylobacterium* sp. B28	AB027623
HST-51	*Caulobacter* sp. strain FWC41	AJ227775
HST-28	*Caulobacter* sp. strain FWC41	AJ227775
HST-25	*Brevundimonas* sp. strain FWC30	AJ227796
HST-97	*Brevundimonas vesicularis*	AY169433
HST-26	*Dyadobacter fermentens*	AF137029
HST-74	*Mycobacterium frederiksbergense*	AJ276274
HST-9	*Mycobacterium* sp.strain N110	AY215228
HST-106	*Rhodococcus fascians* DSM 20669	X79186
HST-63	*Nocardia corynebacteroides* strain QBTo	AY157677
HST-91	Soil bacterium strain NS15	AY039476
HST-47	*Brevibacterium casei*	AF510045
HST-96	*Aeromicrobium liquefaciens* strain L1/15	AJ516054
HST-107	*Microbacterium terregens*	AY039468
HST-16	*Micrococcus luteus* DSM 20030T	AJ536198
HST-87	*Arthrobacter* SMCC ZAT262	AF197055
HST-48	*Staphylococcus xylosus* strain BS20	AY126255
HST-45	*Brevibacillus* sp. AV-Pb	AJ47160
HST-17	*Stapylococcus warneri* strain PB1	AY186059
HST-11	*Bacillus thuringiensis*	AY170457
HST-41	*Bacillus pumilus* strain NR404	AJ458441
HST-64	*Bacillus licheniformis* strain KL-128	AF387514
HST-78	Ultramicrobacterium strain ND5	AB008506
HST-31	*Acidovorax* sp. strain "Smarlab 133815"	AY093698
HST-23	*Pseudomonas marginalis*	Z76663
HST-12	*Aeromonas* sp. strain CDC715-84	U88658
HST-22	Unidentified bacterium isolate SS5	AJ223456
HST-44	*Rhodanobacter lindanoclasticus*	AF039167
HST-14	*Pseudomonas saccharophilia* strain DSM 654T	ABO21407
Isolates exhibiting 95–96% 16S rDNAsequence homology with a deposited sequence		
HST-50	Shingobium sp. strain S10	AY190138
HST-71	*Sphingomonas chungbukensis*	AF159257
HST-6	*Sphingomonas chungbukensis*	AF159257
HST-72	*Sphingobium yanoikuyae* strain S10	AY190138
HST-82	*Sphingomonas adhaesiva*	D16146
HST-90	*Sphingomonas adhaesiva*	D16146
HST-103	*Sphingomonas adhaesiva*	D16146
HST-98	*Sphingomonas rosa*	D13945
HST-88	*Methylobacterium* sp. strain SY-2	AJ278347
HST-85	*Mycobacterium* sp. strain WF2	U90877
HST-100	*Ideonella* sp. strain B513	AB049107
HST-27	Uncultured bacterium WJGRT-78	AF175619
Isolates exhibiting between 90–94% 16S rDNA sequence homology with a deposited sequence		
HST-101	Uncultured eubacterium clone WR 169	AJ233561
HST-33	*Klebsiella oxytoca*	U78183

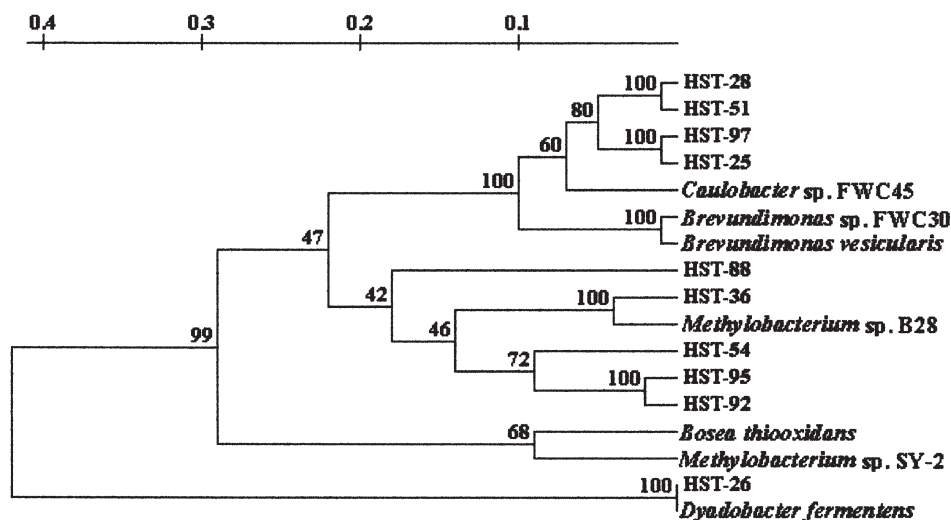

Fig. 4. Phylogenetic tree relating sequence-based identification of the alpha-subclass *Proteobacteria*. The algorithm used to construct the tree is the unwzzmethod using averages (UPGMA). Bootstrap values are displayed as percentages of 100 replicons.

larity between the drinking water isolates and their closest matches in the GenBank was in the range of 97 to 99% (Table 1). *Aeromicrobium*, *Nocardia*, *Arthrobacter*, *Micrococcus*, *Staphylococcus*, *Rhodococcus*, *Aeromicrobium*, *Brevibacillus*, *Microbacterium* and *Bacillus* represented the Gram-positive bacilli in the household storage tanks.

Discussion

The initial part of the work comprised an assessment of the microbiological quality of supplied and stored water and the factors affecting it. Total and fecal coliforms were detected in the influent water and in the household storage tanks. *E. coli* was the most frequent coliform isolated from the tanks followed by *Ent. skazakii* and *C. freundii*. It was therefore important to investigate the sources of bacteria in the storage tanks and their persistence.

A significant increase in the heterotrophic plate count was detected upon storage in the household tanks. The most consistent correlate of regrowth was found to be storage time. The mean log HPC/mL increased by almost one order of magnitude during the seven days of storage in the household tanks. In contrast, the number of total and fecal coliforms decreased over the 7 days. LeChevallier and McFeters (1985) have argued on the basis of similar evidence that because many members of the heterotrophic bacterial population can grow at low nutrient levels, whereas coliforms have high nutritional requirements, coliforms are susceptible to competition

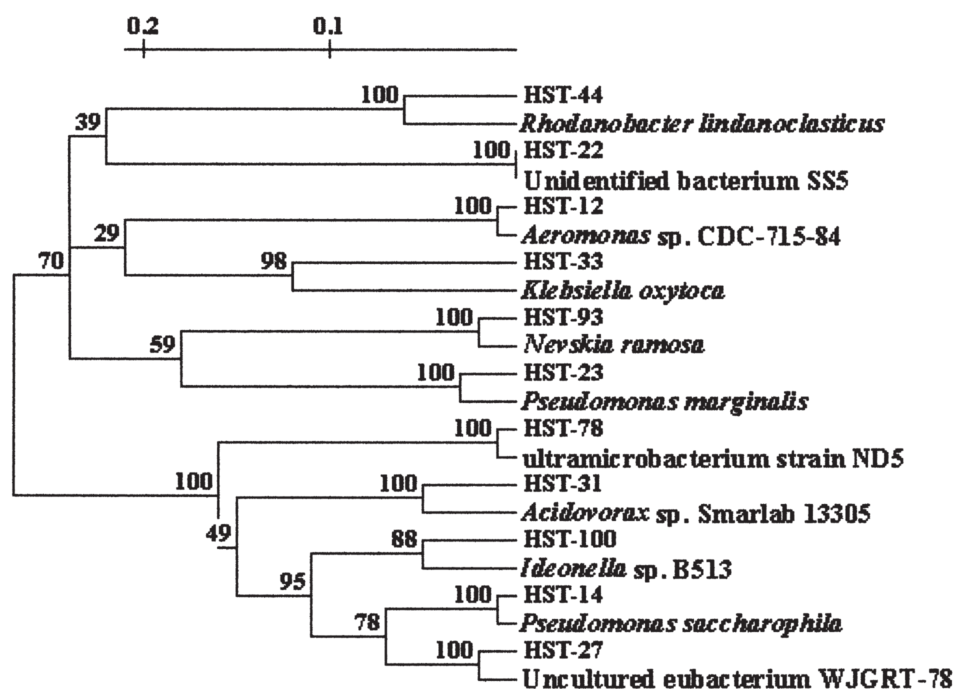

Fig. 5. Phylogenetic tree relating sequence-based identification of the beta- and gamma-subclasses *Proteobacteria*. The algorithm used to construct the tree is the unweighted pair group method using averages (UPGMA). Bootstrap values are displayed as percentages of 100 replicons.

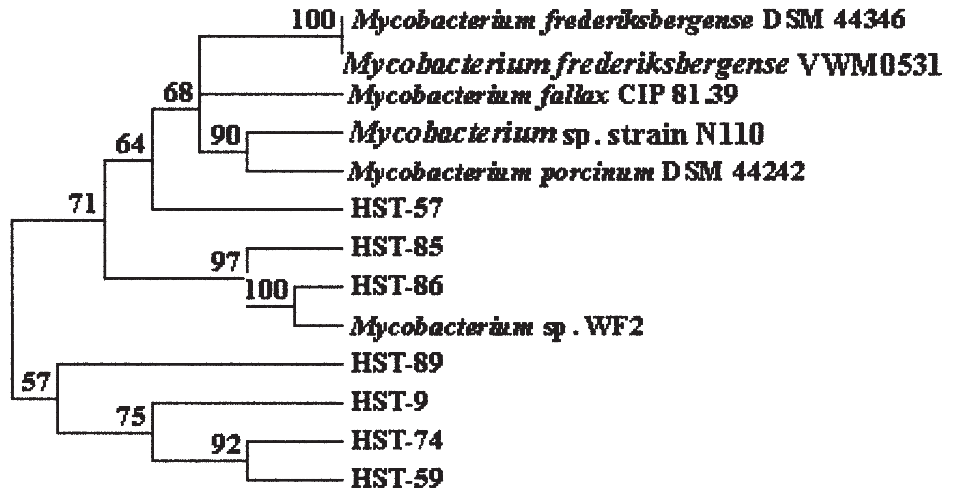

Fig. 6. Phylogenetic tree relating sequence-based identification of Mycobacteria species. The algorithm used to construct the tree is the unweighted pair group method using averages (UPGMA). Bootstrap values are displayed as percentages of 100 replicons.

for nutrients in oligotrophic environments such as drinking water. Additionally, McMath et al. (1999) demonstrated that environmentally derived coliforms cannot survive in the planktonic phase when introduced into low-flow region of a distribution system which has an established biofilm. McLellan et al. (2001) also did not detect *E. coli* replication in the environment, and reported that it was not yet clear whether *E. coli* grows or just survives and accumulates.

The inability to empty the household tanks completely allows for the remaining sediment to act as an inoculum for the incoming water and thus increases the regrowth potential. The possible contribution of these to the persistence of coliforms in the stored water is an area that merits further research.

The present study revealed a significant positive correlation between the level of heterotrophic bacteria in the household tanks and the temperature of the stored water. The highest HPC values and 66% of all *E. coli* isolates cultivable at 44ºC occurred during the summer season. A similar seasonal dependence was previously reported in samples collected from a coastal community

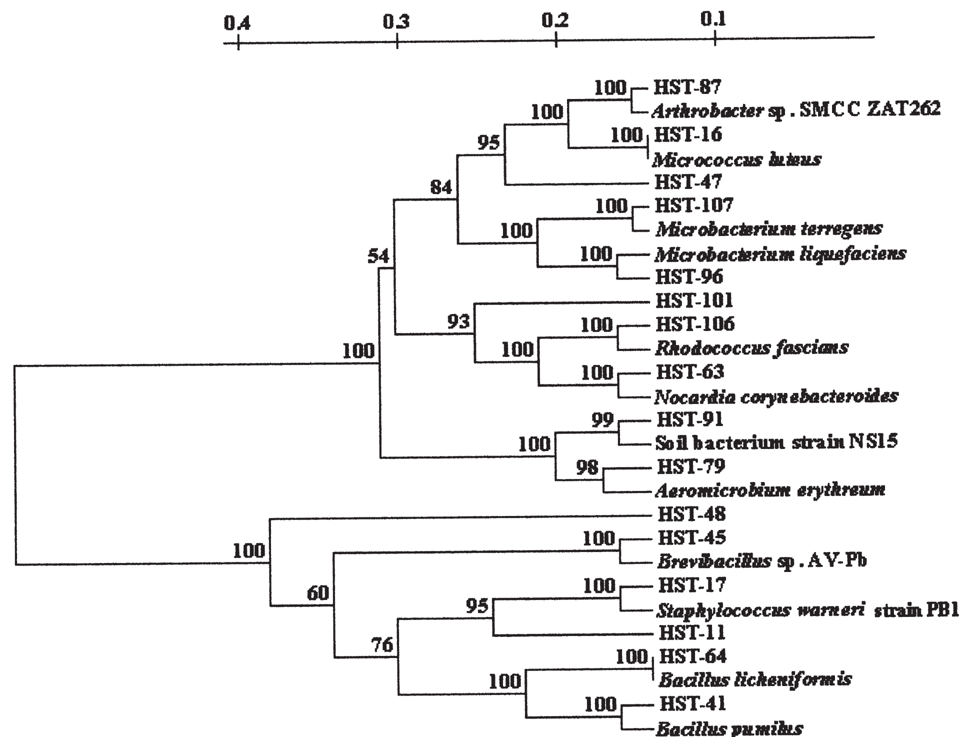

Fig. 7. Phylogenetic tree relating sequence-based identification of Gram-positive bacteria. The algorithm used to construct the tree is the unweighted pair group method using averages (UPGMA). Bootstrap values are displayed as percentages of 100 replicons.

in Oregon, where highest cell densities were enumerated late in the summer when the temperature approached 20°C (Le Chevallier et al. 1991; Lamka et al. 1980). Burke et al. (1984) also reported that the isolation of *E. coli* at 44ºC was mainly during the summer.

After storage time and temperature, the most important factor affecting regrowth was the microbiological quality of the influent water. The abrupt increase in the log HPC/mL detected in the storage tanks at the beginning of the rainy season (October) was associated with the deterioration observed in the microbiological quality of the influent water. It is known that heavy and prolonged rainfall increases the frequency of erosions in underground wells leading to abstraction of poor-quality water for treatment (Gavriel et al. 1998; Kerneis et al. 1995; Crowther et al. 2001). In addition to the quality, the source of the influent water could affect the HPC and the frequencies of indicator bacteria (Geldreich et al. 1975; Allen and Darby 1994; Clark et al. 1982). In a parallel study in Jordan, Evison and Sunna (2001) reported a four-log increase in the HPC upon the storage of conventionally treated surface water in household tanks, compared with a one order of magnitude increase in the present study. This difference could be attributed to the source of the water. Underground water is less polluted with organic compounds (Momba et al. 2000; Zacheus and Martikainen 1997). Particle counts, turbidity and coliform levels were all higher in surface water samples collected in the city of Los Angeles, compared to groundwater samples (McCoy and Olson 1986). On the other hand, other factors such as disturbance of accumulated sediment could have affected the recovered HPC in the water upon storage, especially at day 0. Although the HPC in the influent fluctuated, a steady count was established in the household tanks (March to September). The importance of the sediment on the overall water quality was also evident from the persistent occurrence of *Sphingomonas rosa* in the samples collected from the storage tanks, although it was rarely detected in the source feeding those tanks.

The majority of the colonies recovered from the storage tanks were pigmented. Guthrie and Cherry (1974) suggested that in non-potable water pigmented bacterial levels could be used as an indicator of the cleanliness of the water, with the percentage of pigmented forms present being inversely proportional to the pollution level of the source water. Pigmented bacteria are considered to be potentially useful markers that can help in interpreting changes in the microbiological quality of finished water (Rusin et al. 1997). Results of this study reaffirm this observation. Incidences of sudden increases in the HPC in water samples from the storage tanks were due to an increase in the number of the non-pigmented colonies recovered on R_2A agar. However, long retention times and warm temperatures favour pigmented subpopulations (Herman 1976; Reasoner et al. 1989; Carter et al. 2000). Pigmented bacteria on the other hand, could pose an increased health risk to immunologically compromised individuals (Reasoner et al. 1989).

Yellow pigmented colonies were the dominant type observed, and the 16S rDNA sequence analysis showed that the majority were members of the family *Sphingomonadaceae*. The high incidence of sphingomonads was consistent with their diverse metabolic capabilities, enabling them to inhabit a wide range of environments (Pollock and Armentrout 1999). The isolation of *Sphingomonas* from drinking water is not desirable, especially that some strains are considered as potential pathogens (Perola et al. 2002).

Other opportunistic pathogens including *Aeromonas*, *Methylobacterium*, and a number of acid-fast bacilli and other Gram-positive organisms (Gilardi and Faur 1984; Fernandez et al. 2000; Carson et al. 1978; Chang et al. 2002; Joseph and Carnahan 2000) were additionally recovered from the storage tanks. The presence of *E. coli* in the storage tanks and the increase in the numbers of opportunistic pathogens clearly demonstrated the potential role that such systems could play in the dissemination of infections, especially in immunocompromised hosts. Moreover, this study demonstrated that the heterotrophic population was very diverse, thus speciation (through 16S rDNA analysis) was important to effectively determine if they might be related to species known to be opportunistic pathogens. This, however, was in agreement with the work of other investigators (Fischeder et al. 1991; Lye and Dufour 1991).

Recently a WHO report on the HPC measurements in drinking water safety management, emphasized the lack of evidence linking HPC values alone to health risks or occurrence of waterborne pathogens. Regular cleaning, temperature management and maintenance of a disinfectant residual are important factors required in drinking water safety management (WHO 2002). In our opinion neither of these factors is properly controlled or maintained in Lebanon where household storage tanks are employed. In many Middle Eastern countries including Lebanon that have intermittent water supply systems, stored waters are not kept under controlled hygienic conditions. Residual chlorine values are negligible and often the variable source water is contaminated.

Conclusions

Storage time, temperature and the microbiological quality of the influent were the most important factors affecting the quality of water stored in household storage tanks. Most of the isolates recovered on R_2A agar from the influent and the household tanks formed pigmented colonies and were Gram-negative belonging to the α-, β-, and γ-subclasses of *Proteobacteria*. *E. coli* and some opportunistic pathogens like *Sphingomonas* and

Aeromonas were among the recovered isolates. Speciation of bacteria isolated from potable water could be useful to determine if opportunistic pathogens were present. Improving the microbiological quality of the influent and proper and continuous monitoring of the quality of water stored in household tanks would effectively reduce any potential health hazards to consumers in countries employing household storage systems to overcome water shortage problems.

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