

The Microbiological Quality of Sabil (Free) Drinking Water in Makkah Al-Mukarramah during Ramadan 2007

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Abstract. Eighty six samples of Sabil water were collected during 19-29 of Ramadan 1428A.H. (1-10 October 2007) and examined using standard methods of analyses for drinking water, which include viable counts for total viable bacterial count (TVB), coliform bacteria count and presence of *E.coli*. In addition, water samples were examined for the presence of potential pathogens, and tested for their resistance to antibiotics by the disc diffusion method. Also, the occurrence of filamentous fungi together with bacteriological parameters was assessed in this study. Sabil water has a high bacterial contamination. Coliforms (39.71%), *E. coli* (13.24%) and other pathogenic bacteria were widely represented in the investigated Sabil water. In a first step to screen the waters for potentially pathogenic properties, 163 (47.8%) of the isolates showed α - or γ -haemolysis on human blood agar media. Among the haemolytic isolates, 45.1% were resistant to clindamycin and 52.3% to ampicillin. The most commonly isolated genera with these potentially pathogenic features were *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp., *E.coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Aeromonas veronii*, *Acinetobacter* spp. and *Aeromonas hydrophila*. While the most frequently isolated fungal species were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Epidermophyton* spp., *Microsporium* spp., *Penicillium* spp. The high bacterial contamination in Sabil water is related to human skin transmission and, probably to misuse of the water, such as using the same cups for drinking to perform the ritual washing, *i.e.* wudow (ablution).

Introduction

One of the most common forms of charity in Makkah is to establish public drinking fountain for Sabil - free - water inside or outside mosques, where fresh Zamzam water – accessible only from a special well in the holy city – is made available to worshipers and passersby. But, in the holy month of Ramadan, most of the mosques in Makkah establish small tankers containing Zamzam water in their dooryard. The water is treated before distribution to consumers by a series of sand filters, micro filters and ultraviolet disinfection. Therefore, it is generally of good quality for drinking from its source, but if not properly protected during filling up, could be subjected to contamination. Human activities have a strong impact on the environment, especially on water sources, which are a reservoir for pathogens. Potable water has been described as a reservoir because several non-coliform bacteria (*e.g. Pseudomonas aeruginosa, Serratia marcescens, Flavobacterium meningosepticum, Aeromonas hydrophila, etc.*) can grow in relatively pure water (Black *et al.*, 1979; Millership & Chattopadhyay, 1985). These microorganisms may, be present in drinking water that has acceptable levels of coliform bacteria (*i.e.* one coliform bacterium per 100 ml) (Jurado *et al.*, 2002). Water contamination is of particular concern in hospitals, where potable water in sinks, showers, toilets, dialysis water, ice and ice machine, water baths, *etc.*, has been found to be a source and reservoir of pathogens, and control practices are directed to interrupt their transmission to the patient (Rutala *et al.*, 1997). However, outside hospitals, humans are subjected to many other, rather uncontrolled, sources of pathogenic bacteria.

Some social activities involving the use of water are also potential sources of pathogenic bacteria. The microbiology of these waters is less known and rarely studied, but should be considered deserving special interest; because of its high pathogenic potential. One of the most extended social activities common in Makkah during the holy month of Ramadan, is the use of Sabil water dispensers, such as water fountain to offer drinking water near most of the mosques.

We have addressed our attention to Sabil water, because these waters might present a source of potential risk to health. Therefore the objectives of this project are to determine the:

1. Bacteriological quality of Sabil water in Makkah.

2. Potential health risk of viable bacterial isolates of Sabil water.
3. Susceptibility of these isolated bacteria to the commonly used antibiotics.

Methods

Samples (1000 ml) were collected using sterile bottles from 68 tankers of Sabil water in Makkah during 19-29 of Ramadan 1428A.H (1-10 October 2007). Samples were transported to the laboratory on ice for analysis and processed within 6h of collection. All water samples were tested for the presence and enumeration of total coliforms, *E. coli* and total viable bacterial count (TVB).

Single samples of Sabil water were mixed with Colilert[®] media (IDEXX Labs, Westbrook, Maine) in sterile 120-mL polycarbonate containers and then sealed in QuantiTray/2000[®] containers. Generally, undiluted samples (100 mL) were used. QuantiTrays were incubated for 24 h at 35°C. After incubation, QuantiTrays were examined under long wave (366 nm) ultraviolet light, and wells that both turned yellow and fluoresced were counted as *E. coli* positive. Combinations of numbers of large and small yellow, fluorescing wells were used to determine total coliforms and *E. coli* density. All enumerations were performed using a most probable number (MPN)-based system with a quantification range between <1 and >2419.6 colony forming units (CFU) per 100 ml.

For the enumeration of TVB pour plate count method was chosen, using 1 ml of Sabil water sample and mixing with melted Water Plate Count Agar (Oxoid) tempered at 44°C. Two sets of plates were prepared for all samples. One set was incubated aerobically at 37°C for 48 h and the other set at 22°C for 72 h. All colonies were counted as CFU per milliliter of the water sample.

The first step in screening the TVB isolates for potentially pathogenic features consisted of testing their ability to grow on human-blood agar media. Pure 24-h bacterial cultures were streaked aseptically on blood agar plates and incubated at 37°C for 24 h (Atlas, 1997). The observation of clear zones around the bacterial colonies indicated β -haemolysis; whereas green zones around the colonies suggested α -haemolysis and no reference was made to as γ -haemolysis (Atlas, 1997).

The isolated microorganisms were also preliminarily examined for oxidative of fermentative metabolism (oxidation- fermentation basal medium with 1% glucose), cytochrome oxidase, pigmentation, Gram stain reaction, and morphology. Gram-negative, non-fermentative isolates were further identified with the API 20E and API 20NE (BioMérieux, 69280 Marcy-l'Étoile, France). Furthermore, *E. coli* isolates were tested whether they belonged to the O157 serogroup. The diagnostic reagent used was *E. coli* O157 Latex agglutination test (Oxoid). Fungal species were isolated on Malt Extract Agar (MEA) and incubated for 5 days at 25°C. Isolates were identified according to Pitt and Hocking (1997).

The antibiotic susceptibility of the bacterial isolates was tested using the Kirby-Bauer quality-controlled disk diffusion method (Raphael *et al.*, 1983; and Atlas, 1997). Antibiotic disks (Oxoid) were placed on the inoculated plates with sterile forceps. The following antibiotics were tested: ampicillin, augmentin, cefoxitin, cephalothcin, clindamycin, cotrimoxazole, erythromycin, gentamicin, penicillin G. The Minimum Inhabitation Concentration (MIC) values of six selected anti-pseudomonal antibiotics (amikacin, aztreonam, ceftazidime, ciprofloxacin, imipenam, piperacillin) were used to test the antibiotic susceptibility of *Pseudomonas* spp. isolates. Organisms were reported as either resistant or sensitive to tested anti-microbial (Raphael *et al.*, 1983; and Atlas, 1997). Iron, lead and manganese concentrations were measured in water samples using standard atomic absorption spectrophotometric methods (Perkin Elmer 5100PC, Perkin Elmer, Boston, MA, USA) (Greenberg *et al.*, 1992).

Results

In total, 68 samples of Sabil water were tested for the presence and enumeration of total coliforms, *E. coli* and total viable bacteria count. According to Saudi regulations, the total coliforms in 100 ml of untreated potable water must not exceed 3 CFU after 48 h incubation at 37°C, while the number of *Enterococcus*, *E.coli* and related coliform organisms (including *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp.) in 100 ml of water sample should be zero (SASO, 2000). Thirty six (52.94%) of Sabil waters samples were positive for total coliforms ranging from 1 to 1011 CFU. In 27 out of the 36 samples (39.71 %), the total coliforms

exceeded the acceptable level, while nine (13.24%) of water samples were positive for *E. coli*.

Table 1. Total viable bacterial count (CFU ml⁻¹) of the samples tested at 22 and 37 °C, and incubated for 48h.

TVB (CFU ml ⁻¹)	No. (%) of samples	
	22 °C	37 °C
TVB= 0	20 (29.41)	18 (26.47)
TVB ≤ 20	6 (8.82)	5 (7.35)
20 < TVB ≤ 100	11 (16.18)	3 (4.41)
100 < TVB ≤ 1000	18 (26.47)	11 (16.18)
TVB > 1000	13 (19.12)	31 (45.59)
Total	68 (100)	68 (100)

Table 1 shows the prevalence of aerobic bacteria in the samples tested, counted as CFU ml⁻¹ at 37 and 22°C. Because the Saudi legislation do not mention the limitation of TVB in potable water, the study used the EU standards for TVB, which state that the total number of bacteria in 1 ml of potable water must not exceed 20 CFU after 24 h incubation at 37°C, and 100 CFU after 72 h incubation at 22°C (CEU, 1998). From the 68 water samples tested, 54.41% had TVB < 100 CFU/ml at 22°C and 33.28% had TVB < 20 CFU/ml at 37°C. A total of 341 isolated bacteria were randomly isolated and purified. Different colony morphologies were observed among the TVB isolates such as flat, convex, smooth, circular, irregular, finely or coarsely granulated, pinhead or with larger dimensions. Some of the TVB isolates produced orange and yellow pigments, whereas red and pink bacteria constituted a small number of the pigmented isolates.

After streaking the purified bacterial isolates on human-blood agar plates, a total of 163 (47.8%) bacterial isolates were α- or β-haemolytic and consisted of 60.74% (99 isolates) Gram-negative and 39.26% (64 isolates) Gram-positive isolates. α-Haemolysis was indicated by 33.13% (54 isolates) of the total bacterial isolates, of which 19.63% (32 isolates) were Gram-negative and 13.5% (22 isolates) Gram-positive bacteria. β-Haemolysis was observed for 66.87% (109 isolates) of the TVB isolates, from which 29.45% (48 isolates) were Gram-negative and 37.42% (61 isolates) Gram-positive bacteria. Table 2 shows the most abundant species of Gram-positive in water samples from positive species, 32 isolates were *Bacillus* spp. (19.63%), 14 *Streptococcus* spp. (8.59%) and 6 *Staphylococcus* spp. (3.68%) followed by *Corynebacterium* spp.

(0.61%). While, however the most abundant genera of Gram- negative in Sabil water samples were 24 isolates of *E.coli* (14.72%), 17 *Proteus mirabilis* (10.43%), 17 of *Proteus vulgaris* (10.43%), 10 of *Pseudomonas aeruginosa* (6.13%) followed by 2 isolates of *Aeromonas veronii* (1.84%) and one isolate of *Aeromonas hydrophila* (0.61%) and *Acinetobacter* spp. (0.61%). All isolates of *E. coli* were negative for 0157 strains. Many isolates could not be identified with the API system due to the limitations of the database, which are often mentioned, especially when we are referring to environmental isolates (Armas and Sutherland, 1999).

Table 2. Most abundant species isolated from Sabil water.

species	Number	Percentage (%)
<i>Bacillus</i> spp.	32	19.63
<i>Corynebacterium</i> spp.	1	0.61
<i>Staphylococcus</i> spp.	6	3.68
<i>Streptococcus</i> spp.	14	8.59
<i>Acinetobacter</i> spp.	1	0.61
<i>Aeromonas hydrophila</i>	1	0.61
<i>Aeromonas veronii</i>	2	1.23
<i>Escherichia coli</i>	24	14.72
<i>Proteus mirabilis</i>	17	10.43
<i>Proteus vulgaris</i>	17	10.43
<i>Pseudomonas aeruginosa</i>	10	6.13
Unidentified isolates	38	23.31
Total	163	100

The antibiotic resistance of bacterial isolates is presented in Table 3. The most effective antibiotics were gentamicin, augmentin and cefoxitin, where detected the resistance rates were 7.44%, 12.4% and 13.2%, respectively among 121 isolates of Sabil water. However, resistance against cotrimoxazole, erythromycin, penicillin G and cephalothcin rates of isolates were 29.4%, 29.5%, 30.5% and 35.3% respectively, while the resistance rates of isolates of clindamycin and ampicillin were 45.1% and 52.3%, respectively. The susceptibility of *P. aeruginosa* to different values of six selected anti-pseudomonal antibiotics was also tested as shown in Table 3. Of the 10 isolates of *P. aeruginosa*, ciprofloxacin displayed the best inhibitory activity (100% of strains susceptible), followed by amikacin (30%), ceftazidime (30%) and aztreonam (10%), while 70% and 60% of *P. aeruginosa* isolates were resistant to piperacillin and imipenam, respectively.

Table 3. The resistance of bacterial isolates from Sabil water to selected broad band antibiotics and anti-pseudomonal antibiotics.

Isolates from Sabil water		broad band antibiotics										Anti-pseudomonal antibiotics					
species	No. of isolates	Ampicillin	Augmentin	Cefoxitin	Cephalothcin	Cindamycin	Cotrimoxazole	Erythromycin,	Gentamicin	Penicillin G	Amikacin	Aztreonam	Ceftazidime	Ciprofloxacin	Imipenam	Piperacilin	
<i>Bacillus</i> spp.	32	23	—	—	18	27	9	5	—	18	—	—	—	—	—	—	
<i>Streptococcus</i> spp.	14	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	
<i>Staphylococcus</i> spp.	6	6	0	0	0	0	0	0	0	6	—	—	—	—	—	—	
<i>Corynebacterium</i> spp.	1	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	
<i>E.coli</i>	24	18	12	12	0	12	12	—	6	—	—	—	—	—	—	—	
<i>Proteus mirabilis</i>	17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
<i>Proteus vulgaris</i>	17	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	
<i>Pseud. aeruginosa</i>	10	—	—	—	—	—	—	—	—	—	3	1	3	0	6	7	
<i>Aeromonas veronii</i>	2	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	
<i>Aeromonas hydrophila</i>	1	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	
<i>Acinetobacter</i> spp.	1	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	
Unidentified isolates	38	33	3	4	24	30	16	23	3	5	—	—	—	—	—	—	
Total of tested isolates	163	153	121	121	119	153	153	95	121	95	10	10	10	10	10	10	
Resistances of isolates	No.	80	15	16	42	69	45	28	9	29	3	1	3	0	6	7	
	%	52.3	12.4	13.2	35.3	45.1	29.4	29.5	7.44	30.5	30	10	30	0	60	70	

Fungal species were detected in 29 water samples (42.65%) where most positive samples contained only one fungus species. The fungal isolates of water samples included species of *Epidermophyton* spp. from 11 samples (16.18%), *Penicillium* spp. from 8 samples (11.76%), *Aspergillus* spp. from 5 samples (7.35%), *Alternaria* spp. from 2 samples (2.94%), *Cladosporium* spp. from 2 samples (2.94%), and *Microsporum* spp. from 1 sample (1.47%).

The levels of iron, lead and manganese found in this study were all within the Saudi recommended standard (SASO, 2000). The detection limit for lead was 0.05 mg l^{-1} , for iron 0.05 mg l^{-1} and for manganese 0.01 mg l^{-1} .

Discussion

One of the most common forms of charity in Makkah is to establish public drinking fountain of Sabil inside or outside the mosques, where fresh water is distributed freely to worshipers and passersby. But in the Holy Month of Ramadan, most of the mosques in Makkah establish small tankers containing Zamzam water in their dooryard. Therefore the microbial quality of Sabil water is of great interest as many consumers use it in Ramadan as an alternative to municipal water. The tankers of Sabil water are usually filled up by illegal vendors, who carry Zamzam water in plastic containers of 20 or 30- liters size, which they fill themselves from filling point at the King Abdul Aziz station; then transport these plastic containers to fill up the tankers of Sabil.

Zamzam water before distribution to consumers at the King Abdul Aziz station filling point is treated by a series of sand filters, micro filters and ultraviolet disinfection; therefore the source of Sabil water is generally of good quality for drinking. But if water is not properly protected during filling up, it could be subjected to contamination.

The current study used TVB tests as one of several methods available for monitoring Sabil water overall quality. They do not deal with the problem of water safety and therefore, do not indicate the possible presence of human pathogens. However, a monitor guideline value has been established for TVB levels in drinking water, but effective treatments including disinfection can yield water with a concentration of viable bacteria as low as 20 CFU ml^{-1} after incubation at 37°C for 24h,

and 100 CFU ml⁻¹ after incubation at 22°C for 72h. The results of this study show that 45.59% and 61.76% of samples exceeded the acceptable recommendation level at 22°C and at 37°C respectively. Some specific isolates present in water have been shown to be opportunistic pathogens in immune-compromised individuals, or through routes of exposure other than consumption.

There is no evidence, neither from epidemiological studies nor from correlations with the occurrence of opportunistic pathogens, that TVB values alone directly relate to health risk (WHO, 2001). Consequently, the presence of microorganisms in drinking water is not a health concern for the general public. Of 68 samples of Sabil water, 27 (39.71 %) exceeded the acceptable level of total coliforms, while nine (13.24%) were positive for *E. coli*. However, coliform bacteria are a natural part of the flora of the intestinal tract of warm-blooded mammals, including man, and can be found in their wastes. Therefore coliform bacteria are no longer regarded as indicators of faecal contamination, but are of use as indicators of general microbial quality. The presence of total coliforms and *E. coli* indicates incidence of contamination and potential presence of pathogenic enteric microorganisms (Bharath *et al.*, 2003). The same results have been reported by Ghenghesh *et al.* (2007) who isolated *E. coli* from 14% of water samples obtained from 50 mosques in Tripoli, Libya. These results are also in line with the findings of some studies, which have examined the contamination of water used for drinking and for rituals in houses of worship of other religion. For example, Phatthararangrong *et al.* (1998) detected *E. coli* from 29% of 76 water samples when they examined the bacteriological quality of Holy Water from Thia temples in Songkhia Province.

The presence of potential pathogens in this study was proved by the identification of some pathogenic microorganisms. Of the original 341 TVB isolates, 163 (47.8%) bacteria showed α - or β -haemolytic and the identified bacterial isolates were associated with bacterial pathogenesis including: *Prot. mirabilis*, *Prot. vulgaris*, *Streptococcus* spp., *P. aeuginosa*, *Staphylococcus* spp., *A. veronii*, *A. hydrophila*, *Acinetobacter* spp. and *Corynebacterium* spp. Antibiotic analysis showed that TVB isolates were more resistant to clindamycin and ampicillin respectively, while isolated *P. aeuginosa* were more resistance to piperacillin and imipenam respectively.

Furthermore the presence of the genera *Staphylococcus* spp., *Streptococcus* spp., *Acinetobacter* spp. and *Pseudomonas* spp. in Sabil waters is an indication for hand contamination and inoculation from human skin (Holland *et al.*, 1985 and Holland, 1993). Transmission occurs by contact with infected hands and fingers, although some other water misuses cannot be ruled out. In the case of Sabil water, the use of bare hands, at every stage in filling up the tankers and filling the plastic containers from filling points is a probable source of bacterial contamination. The contributions made by hands to the contamination of drinking water, have been emphasized by research in South America, where the quality of vended polythene-bagged water was much improved by filling the bags through a funnel instead by hand. These findings are in line with those reported previously from Ghana by Obiri-Danso *et al.* (2003).

Although filamentous fungi in water are commonly thought to pose no potential public health problems, some of the fungi isolated from Sabil water, such as *Alternaria* spp. and *Penicillium* spp. (*i.e.* *P. citrinum*), have some toxigenic potential, and could constitute some health risk. It is therefore advisable to count fungal propagules in routine microbiological studies of water and to establish baselines.

Lead, iron and manganese are the most common sources of consumer complaints on water. The tankers of Sabil water are mainly made of iron, many have frequently undergone considerable corrosion and have been shown to readily contaminate the water supply with metal particulates or turbidity-producing materials (Pelig-Ba *et al.*, 1991). High iron concentrations are not directly a health risk, but can cause unpleasant odor and taste (Smedley, 1995). The levels of iron, lead and manganese found in this study were all within the Saudi recommended standard (SASO, 2000).

Our findings show that most samples of Sabil water are in unacceptable quality and may pose health hazards to worshipers and passersby, particularly to children, elderly and immunocompromised consumers. The author suggests that:

1. The health and environmental authorities should have an important role in providing guidance and supervision to personnel in charge of mosques, to ensure that the appropriate quality of drinking water is provided to worshipers and passersby.

2. Illegal vendors should be prohibited.
3. The legal vendors must receive education in food hygiene, and a similar case must be made for other street vended waters.

Conclusions

The findings of this study show that most samples of Sabil water are in unacceptable quality conditions. The health and environmental authorities should take proper measures to ensure that good quality of drinking water is provided for worshipers and passersby in mosques and in front of some private homes.

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الجودة الميكروبيولوجية لمياه السبيل بمكة المكرمة خلال

موسم رمضان ٢٠٠٧م

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جامعة أم القرى - ص. ب. ٤١٢٣ مكة المكرمة - المملكة العربية السعودية

المستخلص. تعتبر مياه السبيل أحد أنواع الصدقة المنتشرة بأحياء مكة المكرمة، حيث توزع هذه السبل مياهًا عذبة للمصلين وعابري السبيل، وخصوصاً في شهر رمضان المبارك. لذلك تهدف هذه الدراسة إلى تحديد الجودة البكتريولوجية لماء السبيل بمكة المكرمة، إضافة إلى تحديد مخاطر الأنواع البكتيرية المعزولة من هذه المياه، ومدى حساسيتها للمضادات الحيوية المعروفة. ولتحقيق أهداف الدراسة المنشودة فقد تم جمع ٦٨ عينة من مياه السبيل خلال الفترة من ١٩-٢٩ رمضان ١٤٢٨هـ، الموافق ١-١٠ أكتوبر ٢٠٠٧م. وتم تحليل جميع العينات طبقاً للطرق البكتريولوجية للقياسات المعتمدة لمياه الشرب، وشملت التحاليل على العد الكلي للبكتريا، ومجموعة القولون، والكشف عن وجود بكتيريا *E. coli*، وبعض الأنواع البكتيرية الممرضة، إضافة إلى الكشف عن الأنواع الفطرية. أظهرت نتائج الدراسة وجود نسبة تلوث بكتيري ظاهرة في مياه السبيل، حيث أن نسبة ٤٧,٨% من المجموع الكلي للبكتريا المعزولة محللة للدم من نوع ألفا أو بيتا، وكما أن نسبة ٥٢,٨% و ٤٧,٨% من المجموع الكلي للبكتريا المحللة للدم مقاومة للمضادين الحيويين ampicillin و clindamycin على التوالي. كما أظهرت

النتائج أيضاً أن معظم العزلات البكتيرية تنتمي إلى الأجناس المرضية التالية: *Streptococcus* spp. ، *Bacillus* spp. ، *E.coli* ، *Corynebacterium* spp. ، *Staphylococcus* spp. ، *Pseudomonas* ، *Proteus vulgaris* ، *Proteus mirabilis* ، *Acinetobacter* spp. ، *Aeromonas veronii* ، *aeuginosa* و *Aeromonas hydrophila*. بينما معظم العزلات الفطرية تنتمي إلى الأجناس التالية: *Aspergillus* spp. ، *Alternaria* spp. ، *Microsporium* spp. ، *Epidermophyton* spp. ، *Cladosporium* spp. و *Penicillium* spp. هذا وقد خلصت الدراسة إلى أن معظم الأنواع البكتيرية المعزولة لها علاقة بانتقال الأنواع البكتيرية عن طريق الجلد، ونتيجة الاستخدامات غير السليمة للمياه. لذلك يجب على المسؤولين بالصحة وصحة البيئة اتخاذ الوسائل المناسبة، والتي تضمن سلامة جودة مياه السبيل.