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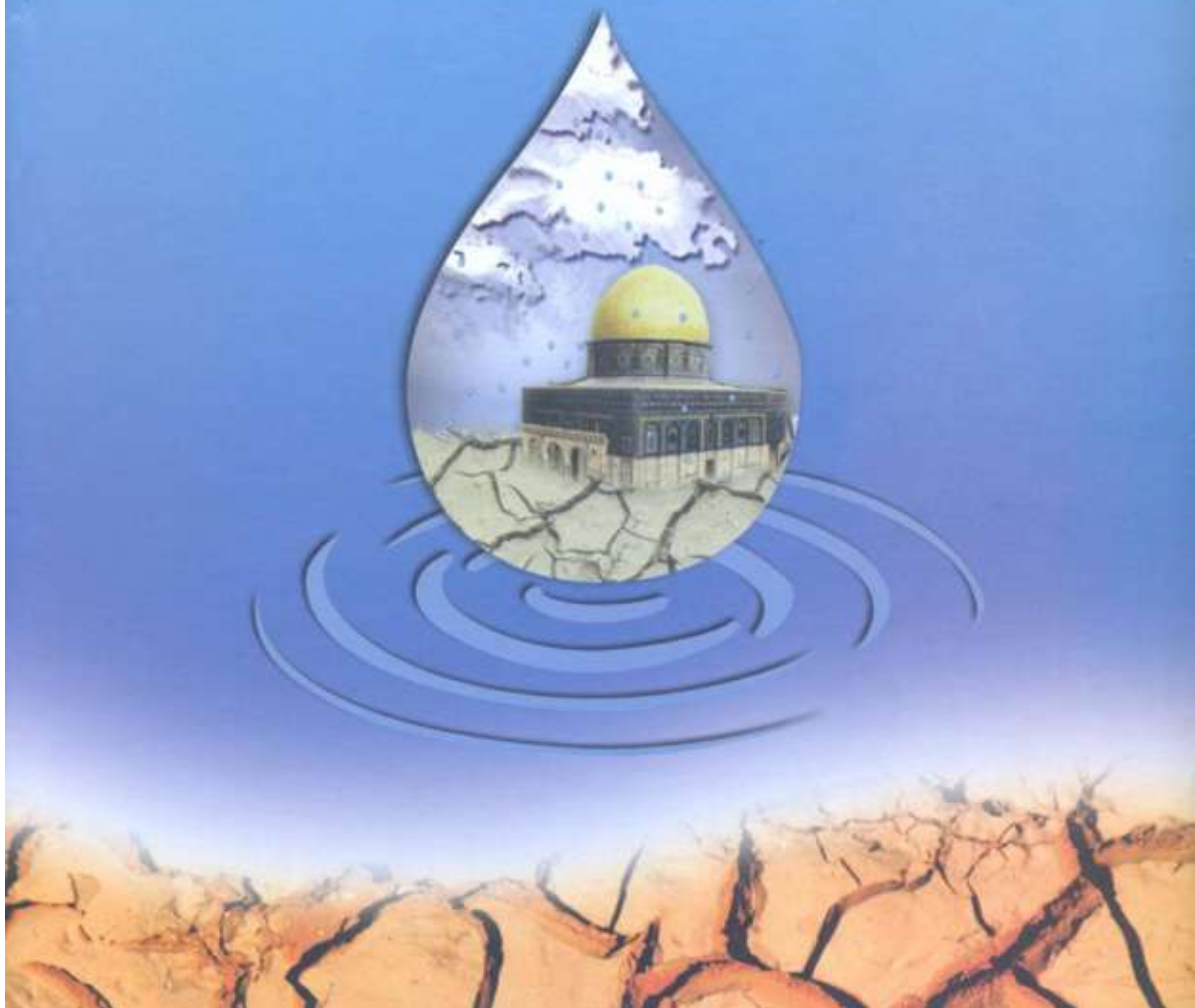
Sustainable Development and Management of Water in Palestine



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ISBN 978-9950-8501-5-6

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Health Risks from Microbial Growth and Biofilms in Drinking Water Distribution Systems in Palestine

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Abstract

Drinking water samples and swabs (n=24) from the inside of the distribution systems were collected at different locations in Ramallah District, Palestine. Samples were tested for total and fecal coliform, heterotrophic plate count (HPC), Pseudomonas auroginosa count, fecal streptococci, sulfite reducing anaerobes, residual chlorine, turbidity, ammonium and nitrate.

Four samples out of 12 (33%) contained too many to count for HPC. The remaining 8 samples contained an average of 26 CFU/100 ml. Five samples out of 12 (42%) were found to contain total coliform. The number ranged between 0 and 80 CFU/100 ml (average 14 CFU/100 ml). Summer samples were found to have more total coliforms than winter ones. Neither winter nor summer samples were found to contain fecal coliforms. Residual chlorine ranged between 0.080.55- mg/L (average 0.24mg/L) and nitrate concentrations in drinking water samples ranged between 4.7916.26- mg/L (average 9 mg/L).

Our results show that the drinking water in the distribution system of Ramallah District is of good quality and intermittent water supply should be avoided when possible, as this was associated with an increase in total coliform and turbidity.

Keyword: Microbial Biofilm, Drinking Water, Distribution Systems, Palestine.

Symbols and Abbreviation

APHA	American Public Health Association
CFU	Colony Forming Unit
DNA	Deoxyribonucleic acid
EPS	Extracellular Polymeric Substances
HPC	Heterotrophic Plate Count
PCR	Polymerase Chain Reaction

Introduction

Water in the distribution systems is not sterile, regardless of the treatment process used. Some microbes resist the treatment processes; others can enter water distribution systems due to leakage or damage in the pipes. Potable water of good bacteriological quality is generally regarded as that containing less than one total coliform per 100 ml of water sample. Deterioration of drinking-water quality during storage or in distribution systems remains one of the major difficulties experienced by

potable water suppliers. Pathogenic and toxigenic microbiological agents in drinking water have long been known to cause disease and death in consumers [1]. The health risks associated with these pathogens range from viral and bacterial gastroenteric diseases to infections such as hepatitis A and giardiasis.

Biofilms have been given many definitions [2, 3, 4, 5]. A definition states that a water distribution system biofilm is a complex mixture of microbes, organic and inorganic material accumulated amidst a microbially produced organic polymer matrix attached to the inner surface of the distribution system. The inner surface of a water pipe may have a continuous biofilm, but usually biofilms are quite patchy [6, 7].

While water produced in the treatment plant may be of high biological quality, the treated water may be subject to conditions in the distribution network that adversely affect it. The reasons why bacterial numbers increase during distribution are not yet fully understood but two of the main factors have been studied in detail. The first factor is described as mechanical failure [8] while the second refers to the situation where the increase of bacteria is due to internal regrowth or aftergrowth of bacteria and the associated formation of biofilms [9]. The occurrence of biofilms or encrustations that harbor various types of microorganisms has been described extensively [10, 11]. The most alarming results are the presence and multiplication of pathogenic and opportunistic pathogens such as *Pseudomonas, Mycobacter, Campylobacter, Klebsiella, Aeromonas, Legionella spp., Helicobacter pylori and Salmonella typhimurium* occurring within the biofilms [12, 13, 14, 15].

The primary intestinal bacterial waterborne pathogens include *Shigella, Salmonella, Yersinia enterocolitica, Campylobacter jejuni, and Escherichia coli O157*. The potential for these bacteria to attach to biofilms exists, and limited growth in some circumstances cannot be ruled out. One primary pathogen that may be waterborne, *Helicobacter pylori*, was found to survive at least 192 hours on stainless steel coupons (inserts used to monitor biofilm buildup) in a chemostat [15]. Park, et al. [16] have also noted the presence of *H. pylori* in biofilms of drinking water mains. In another study, two non-pathogenic E. coli strains injected into a pilot distribution system with a biofilm (20°C) grew slightly in the biofilm before eventually dying out [17]. The building plumbing system may act either as a direct conduit for pathogens sloughed off from distribution system biofilms or as an amplifier of these pathogens. A waterborne disease outbreak caused by E. coli O157 persisted for weeks after the suspected source– contaminated water meters and main breaks were replaced or repaired [18]. Although a biofilm was not implicated, the potential exists for biofilms to prolong the survival of some microbes. In another study, *Salmonella typhimurium* was able to grow for a short time at 24°C in non-sterile tap water [19]. Much more information is available on the presence of opportunistic bacterial pathogens that have been associated with both distribution system biofilms and disease.

Attachment is a first step in the process of microbial colonization of any surface and may initially limit the rate of the process [20]. Under suitable conditions a biofilm develops, initially through the accumulation of organic matter on the metal surface, which is then colonized by bacteria [21]. Bacteria subsequently develop into a consortium of species within the polysaccharide matrix which imparts the slimy nature to the biofilm. The formation of glycocalyx has been reported to be critical for cells to attach to exposed surfaces and survive shear forces [22]. Moreover, cells which are continually intimately associated with interior walls of water mains may be less susceptible to chemical disinfection processes due to boundary layer effects and the secretion of extracellular coatings [23]. As a result, the extent to which microbes become associated with the inner surfaces of pipes or with suspended particulate matter within the water column could significantly enhance their survival and regrowth potential in distribution lines and reservoirs [22].

The microbial composition of potable water reflects the microflora characteristics of the raw water source. These may be broadly classified into four groups: bacteria, viruses, protozoa and fungi. Biofilm serves as a focal point where bacteria and other microorganisms interact. A large variety of different heterotrophic bacteria have been isolated from the biofilm both in chlorinated and non-disinfected water distribution systems [24]. The presence of *E. coli, Pseudomonas, Aeromonas, Artrobacter, Caulobacter, Klebsiella Bacillus, Enterobacter, Citrobacter, Acinetobacter, Prosthescomicrobium, Alcaligenes, Serratior and Actinolegionella* has

been reported in many studies [10, 22, 25, 26].

The present research aims at collecting water samples and swabs from the drinking water distribution network in Ramallah District and analyzing the microbial, chemical and physical quality of the drinking water and the possible presence of bacterial biofilm on the inside surface of the pipes.

2. Materials and Methods

2.1. Summer and winter sampling

Drinking water samples (Volume=1000 ml, n=12) were collected in sterile bottles from different regions of the distribution system in Ramallah District. Immediately, samples were transferred into an ice box and brought back to the laboratory for analysis. At the same time, swabs (n=12) from the inside of the water distribution system of the same region were taken, kept in sterile transport tubes containing peptone water and sterile saline for bacterial and protozoal analysis, respectively and brought back to the laboratory.

2.2 Processing of samples

In the laboratory, samples were processed using standard microbiological techniques according to APHA [27]. The following tests were conducted on one half of each water sample: total coliform, fecal coliform, heterotrophic plate count, *Pseudomonas auroginosa* count, fecal streptococci, sulfite reducing anaerobes, residual chlorine, turbidity, ammonium and nitrate. The second half (500 ml) of each water sample was filtered through 0.45µM membrane (Millipore) to be enriched in peptone water (Merck) for further investigations. Similarly, biofilm swabs were enriched in peptone water.

R_2A agar (media used for enumeration of water microbes) was used for isolation of biofilm microbes directly from the biofilm swab for biochemical analysis. Standard biochemical tests for microbes› identification and confirmation of microbes were conducted (including the RapID ONE for oxidase negative Enterobacteriaceae, and Remel USA). For protozoa, microscopic examination was done for the swabs transported in saline from the biofilms within the same day of sampling. The protozoa examined were Cryptosporidium, Giardia lamblia and Entamoeba histolytica.

2.3. DNA Extraction

From enriched peptone water, 1.5 ml (previously enriched with the swabs and the 0.45µM filters) and 1.5 ml peptone water (without enrichment) were mixed and centrifuged for 4 min at maximum speed. The pellet was suspended in 0.5 ml sterile water, boiled for 10 min, centrifuged for 1 min at high speed and 3µl of supernatant were used in all PCR reactions.

2.4 PCR Analysis

Identification of *Shigella spp.* was conducted according to Islam *et al.* [28] using target sequence of invasive plasmid antigen (ipa) H-locus. This was amplified by PCR using the two primers H8 and H15 (Table 1). The PCR reaction was done using Hybaid Omnigene thermocycler for 35 cycles of 1 minute each at 94 °C (for denaturation), 1.5 minute each at 60 °C (for annealing of primers to single-stranded DNA), and 0.25 to 1 minute each at 72 °C (for DNA polymerase-mediated extension). The amplified products (700bp) were then separated by 1% agarose gel electrophoresis. Identification of *Salmonella spp.* was done according to Rahn et al. [29]. A 284bp fragment of invA gene was amplified using the two primers 139F and 141R (Table 1). The amplification profile consisted of 95 °C for 1 min and 35 cycle of 95 °C for 30 sec, 64 °C for 30 sec and 72 °C for 30 sec followed by 4 min at 72 °C. The 284bp product was separated by 1% agarose gel electrophoresis. Identification of Campylobacter spp. was done according to Denis et al. [30]. Amplification reactions were performed in a 50 µL mixture containing 1.25 U Taq polymerase (Promega), 0.2 mM of each dNTP, 0.2 µM of MD16S1 and MD16S2 primers (Table 1) and 1.5 mM $MgCl_2$. Amplification reactions were according to the following program: one cycle of 10 min at 95 °C, 35 cycles each consisting of 30 sec at 95 °C, 1 min 30 sec at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The 857bp product was separated by 1% agarose gel electrophoresis.

For Protozoa, multiplex PCR was conducted according to Verweij et al. [31]. The amplification reactions were performed in a 50 µl with PCR buffer containing 5 mM $MgCl_2$, 6.25 pmol of each E. histolytica primer, 6.25 pmol of G. lamblia primer and 25 pmol of Cryptosporidium primer

(Table 1) in addition to 3 µl of the DNA sample. The amplification consisted of 15 min at 95 °C followed by 40 cycles of 15 sec at 95 °C, 30 sec at 60 °C and 30 sec at 72 °C. The product was detected by 2% agarose gel electrophoresis.

3. Results and Discussion

Results of the physical, chemical and microbial analysis of the water samples collected in summer and winter period from drinking water distribution system in Ramallah District are summarized in Tables 2 and 3. The HPC values ranged between 0 and too many to count (TMC). Four samples out of 12 (33%) were containing TMC. The remaining 8 samples were containing an average of 26 CFU/100 ml. Tokajian and Hashwa [32] found HPC levels between 30-100 CFU/1ml in drinking water samples from Beirut, Lebanon. According to their results, intermittent water supply (which is common in Palestine too) increased the HPC values to reach as high as 7000 CFU/1ml. This seems to be much greater than the results obtained in this study. When comparing the HPC in summer and winter samples of the present study, summer samples seem to have more HPC than winter ones (Tables 2 and 3). Tokajian and Hashwa [32] reported no significant difference between summer and winter samples from Lebanon. Five samples out of 12 (42%) were found to contain total coliform. The number ranged between 0 and 80 CFU/100 ml (Tables 2 and 3). The average number of total coliform was 14 CFU/100 ml drinking water. Summer samples were found to have more total coliforms than winter ones. This is in agreement with the results obtained by Olstadt *et al.* [33] and Colbourne et al. [24] that more coliform occurrence have been noted in the distribution systems during the summer months when water temperatures are at their highest. According to the 1998 EU standards, the total coliform in drinking water should not exceed 0 CFU/100ml. According to the Palestinian Standards, total coliform should not exceed 3 CFU/100 ml. Accordingly, water total coliforms in 5 samples out of 12 (42%) were found to exceed the Palestinian and the EU Standards. Neither winter nor summer samples were found to contain fecal coliforms (Tables 2 and 3). This is in accordance with the Palestinian Standards that fecal coliform should be 0 CFU/100 mL drinking water. Similarly, none of the samples were found to contain Fecal Streptococci, *P. aeruginosa*, or Sulfite reducing anaerobes (Tables 2 and 3). This also generally confirms the good quality of the drinking water samples concerning microbial analysis.

Table 1: Primers used in this study for the identification of bacteria and protozoa.

Primer	Pathogen	Sequence 5' 3'	Reference
H8 H15	*Shigella*	GTT CCT TGA CCG CCT TTC CGA TAC GCC GGT CAG CCA CCC TA	[28]
139F 141R	*Salmonella*	GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C	[29]
MD16S1 MD16S2	*Campylobacter*	ATC TAA TGG CTT AAC CAT TAA AC GGA CGG TAA CTA GTT TAG TAT T	[30]
Ehd239 Ehd88	*E. Histolytica*	ATT GTC GTG GCA TCC TAA CTC A GCG GAC GGC TCA TTA TAA CA	[31]
CrF CrR	*Cryptosporidium*	CGC TTC TCT AGC CTT TCA TGA CTT CAC GTG TGT TTG CCA AT	[31]
80F 127R	*G. lamblia*	GAC GGC TCA GGA CAA CGG TT TTG CCA GCG GTG TCC G	[31]

Table 2: Physical, chemical and microbial analysis of water distribution system samples collected during the summer period of 2005 from Ramallah District.

Test	Sample #					
	1	2	3	4	5	6
HPC **	TMC*	120	32	0	TMC	20
Total Coliform**	65	80	7	0	0	8
Fecal Coliform**	0	0	0	0	0	0
Fecal Streptococci**	0	0	0	0	0	0
P. aeruginosa (CFU/250 ml)	0	0	0	0	0	0
Sulfite reducing anaerobes (CFU/50 ml)	0	0	0	0	0	0
Residual chlorine (mg/L)	0.08	0.19	0.16	0.08	0.37	0.55
Turbidity (NTU)	15	0.77	1.24	20.5	0.37	0.84
Ammonium (mg/L)	0.49	0.66	0.77	0.82	0.22	0.95
Nitrate (mg/L)	6.87	7.17	4.79	5.19	16.26	13.60

*: Too many to count, **: (CFU/100 ml)

Some chemical and physical properties of drinking water were tested for the summer samples (Table 2). Residual chlorine ranged between 0.08-0.55 mg/L (average 0.24mg/L). These values are much less than the health-based guideline by the WHO of 5 mg/L. Free chlorine thresholds varying from 0.05-0.5 mg/L in full scale distribution systems have been quite useful in keeping the rate of occurrence of coliforms in drinking water low [34]. However, coliform-positive samples have been reported to occur in distribution systems with free chlorine residuals ranging from 0.6-4.0 mg/L [35]. Even though, increasing chlorine residuals has helped to control coliform occurrence in some cases, such chlorine increase may not always be an acceptable solution because of the formation of potentially carcinogenic disinfection by-products [36]. Turbidity measurements of summer samples ranged between 0.37-20.5 NTU (average 6.45 NTU). This average is above the preferable WHO guideline of < 5.0 NTU. However, 2 samples out of 6 (33%) were found to exceed this concentration. The first sample (#1) was obtained from the network when water supply was disconnected and as a result water flow was very slow and water turbidity was high. The other sample (#4) was collected at a dead end of a pipe in the network of drinking water in Ramallah city. Ammonium concentrations in drinking water samples ranged between 0.22-0.95 mg/L. According to the EU standards, ammonium in drinking water should be less than 0.5 mg/L. Four of our samples (67%) were having ammonium level that exceeds this limit. Nitrate concentrations in drinking water samples ranged between 4.79-16.26 mg/L (average 9 mg/L). This is below the health-based guidelines by the WHO of 50 mg/L. Because nitrate is converted to a very toxic substance (nitrite) in the digestive systems of human infants and some livestock, nitrate-contaminated water is a serious problem.

Table 3: Microbial analysis of water distribution system samples collected during the winter period of 2005/2006 from Ramallah District.

Test	Sample #					
	1	2	3	4	5	6
HPC**	0	8	2	24	TMC*	TMC
Total Coliform**	0	0	0	8	0	0
Fecal Coliform**	0	0	0	0	0	0
Fecal Streptococci**	0	0	0	0	0	0
P. aeruginosa (CFU/250 ml)	0	0	0	0	0	0
Sulfite Reducing anaerobes (CFU/50 ml)	0	0	0	0	0	0

*: Too many to count, **: (CFU/100 ml)

The presence of (opportunistic) pathogenic mirobes in water samples from distribution systems and tanks in addition to biofilm swabs was tested with/without enrichment. After culturing on R_2A agar, the result showed the presence of about 5 different types of bacterial colonies dominated by *Bacillus spp*. Further studies on these colonies revealed that these colonies were belonging to bacterial types that are found normally in water and none of them was belonging to the pathogenic microbes investigated in this study.

Enriched samples were tested in the laboratory and revealed the presence of 4 different bacterial types in addition to the previous types. Further investigations showed that these four were *Shigella spp*, *Citrobacter freundii*, *Enterobacter agglomerans and Aeromonas*

spp. The identification of the first three types was confirmed later by RapID ONE Kit. Tokajian and Hashwa [32] repeatedly isolated *Citrobacter freundii and Enterobacter agglomerans* from drinking water network in Lebanon. They also reported the isolation of *E. cloacae* and *E. skazakii*. They attributed their presence to inadequate chlorination of drinking water. The four bacterial species isolated in this study could have entered the water network accidently through accidental breakage of the network, for example. Parts of the network are very old and are subjected to frequent leakage. In addition, the water distribution system was subjected to destruction by the Israelis during their operations in Ramallah. However, the four bacterial species found were isolated only after enrichment only.

DNA from a total of 24 samples of different origins (pipes water and biofilm swabs) was extracted and analysed by Polymerase Chain Reaction (PCR) as described in the material and method section. None of the pathogenic microbes (bacteria and protozoa) considered in this study was detected in any of the water or swab samples.

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