Holy springs and holy water: underestimated sources of illness?

Alexander K. T. Kirschner, Michael Atteneder, Angelika Schmidhuber, Sonja Knetsch, Andreas H. Farnleitner and Regina Sommer

ABSTRACT

Use of holy springs and holy water is inherent in religious activities. Holy spring water is also used extensively for personal drinking water, although not assessed according to drinking water standards. Holy water in churches and chapels may cause infections via wetting of lips and sprinkling on persons. Our aim was to assess the microbiological and chemical water quality of holy springs and holy water in churches and hospital chapels. Of the holy springs investigated, only 14% met the microbiological and chemical requirements of national drinking water regulations. Considering results from sanitary inspections of the water catchments, no spring was assessed as a reliable drinking water source. All holy water samples from churches and hospital chapels showed extremely high concentrations of HPC; fecal indicators, *Pseudomonas aeruginosa* and *Staphylococcus aureus* occurred only in the most frequently visited churches. We conclude that it is highly necessary to include holy springs in programs for assessment and management of water quality. Public awareness has to be raised to perceive holy springs as potential sources of illness. Holy water can be another source of infection, especially in hospital chapels and frequently visited churches. Recommendations are made for proper water quality management of both water types.

Key words | *Campylobacter*, drinking water, fecal indicator bacteria, heterotrophic plate counts, *Pseudomonas*, *Staphylococcus*

Alexander K. T. Kirschner* (corresponding author)
Michael Atteneder
Angelika Schmidhuber*
Sonja Knetsch*
Regina Sommer*
Medical University Vienna,
Center for Pathophysiology,
Infectiology and Immunology,
Institute for Hygiene and Applied Immunology,
Water Hygiene,
Kinderspitalgasse 15,
A-1095, Vienna,
Austria
E-mail: *alexander.kirschner@meduniwien.ac.at*

Andreas H. Farnleitner*
Vienna University of Technology,
Institute for Chemical Engineering,
Environmental Microbiology and Molecular
    Ecology Group,
Vienna,
Austria

*Interuniversity Cooperation Center Water &
    Health
(*www.waterandhealth.at*)

INTRODUCTION

Austria is a country in which a large part of its citizens are deeply engrained in Catholicism. Inhabitants have many ancient customs and ceremonies, which often involve contact with holy springs and holy water. Whereas holy water is used throughout life in Catholic ceremonies, holy springs are visited for pilgrimage or, in particular cases, when someone is drinking it because of illness or firm belief. Additionally, water from such holy springs is taken simply to use as drinking water by people who believe that it is of higher quality than normal tap water. Despite the fact that some Christian holy springs have been widely used since the 15th century (Hirsch & Ruzicka 2002) and despite their obviously high importance for public health, to the best of our knowledge, no papers have been published before on the microbiological and chemical quality of the water of holy springs. Moreover, holy springs are usually not under surveillance and control by local authorities.

Some information has been published on the quality of holy water (see below). The field of application of holy water is large and comprises wetting the fingers in a font after entering a church and making the sign of the cross on the forehead, lips and chest and blessing food or persons by sprinkling them. Holy water is usually made only once a year at Easter time, when tap water is blessed and stored afterwards in tanks. In case of running out of holy water during the year, it may be diluted with tap water. Furthermore, the Catholic Church recommends adding an unregulated amount of blessed salt (sodium chloride) to

the water during the blessing ceremony, resulting in different salt concentrations of holy waters. In some hospitals, cases of infections have been reported, because severely injured patients had been sprinkled with holy water. For example, according to Greaves & Porter (1992) *Pseudomonas aeruginosa* was detected from a multiply injured patient because of contamination with holy water which caused pneumonia, tachypnea and fever. Rees & Allen (1995) reported a case in which holy Lourdes water was the reason for an infection with *Acinetobacter baumanii.* Research on the microbiological quality of holy water was performed in temples in Thailand (Phatthararangrong *et al.* 1998) where *Escherichia coli* was isolated. In Freiburg, Germany, Daschner (1997) detected massive contamination with *Pseudomonas* spp. In Seville, Spain, where seven churches were investigated (Jurado *et al.* 2002), high concentrations of diverse opportunistic pathogenic bacteria were recorded during Easter Holy Week, including – among others – representatives of the genera *Pseudomonas, Staphylococcus, Acinetobacter* and *Stenotrophomonas*.

The aims of our study were to investigate, for the first time, the microbiological and chemical quality of a variety of holy springs and holy waters. As mentioned above, holy springs as widely used drinking water sources have been a neglected topic. In contrast to published studies on holy water, we investigated a wider range of churches from frequently visited ones to less popular ones, by taking samples at least twice at different seasons. In total, 50 samples of 21 holy springs and 53 samples of holy water fonts in 16 churches and two hospital chapels were taken in Eastern Austria. Based on our findings, recommendations are made for proper water quality management of both water types.

MATERIALS AND METHODS

Sampling holy spring water

The 21 investigated holy springs are located in Eastern Austria in the provinces of Lower Austria and Burgenland. To preserve anonymity, no map of the exact sampling locations is shown; holy springs were thus expressed as HS in the text and numbered consecutively. At each sampling day a sanitary inspection of the water catchment was performed to assess the susceptibility of the water source to contamination. Furthermore, the odor and appearance of the water samples taken were checked on site. Because of possible infection risk the taste of the water samples was not assessed. Samples were taken at least twice from each holy spring in June, July and August 2010, and in April 2011. A volume of 3,000 ml water was collected into sterile glass bottles. Immediately after sampling, water temperature and air temperature were measured. The bottles were transported within 6 h in the dark to our accredited laboratory by using an ice-box with ice-packs guaranteeing storage below 8 °C. After arrival in the laboratory, electrical conductivity and pH values were determined immediately.

Sampling holy water

Holy water samples were taken from holy water fonts in 18 churches in Vienna, including frequently and less frequently visited ones as well as chapels of two large hospitals in Vienna. To preserve anonymity, no map of the exact sampling locations is shown; holy water fonts were thus expressed as HF in the text and numbered consecutively. With a minimum of two holy water samples per church, in total 53 samples were collected between July 2010 and January 2011. The fonts were located near to the major entrance of each church. With a sterile pipette a minimum of 50 ml water was retrieved from each font, added to a sterile glass bottle and transported within 4 h as described above. Extraction of a larger volume of water was impossible because the water volume in the fonts was too low. Water temperature, air temperature, electrical conductivity and pH values were determined as described above.

Bacteriological parameters

Heterotrophic plate counts

For holy water and holy spring water samples, heterotrophic plate counts (HPCs) were determined according to ISO 6222 (ISO 1999a). Parallel yeast agar plates derived from 1 ml subsamples and appropriate dilutions were incubated for 24 h at $36 \pm 2\,°C$ (HPC37) and for 48 h at $22 \pm 2\,°C$ (HPC22).

Fecal indicator bacteria

Escherichia coli, coliform bacteria and intestinal enterococci were determined via the membrane filtration technique in 10 ml of holy water and 250 ml of holy spring water and appropriate dilutions according to ISO 9308-1 (ISO 2000a) for *E. coli* and coliform bacteria, and ISO 7899-2 (ISO 2000b) for intestinal enterococci.

Other bacterial species

P. aeruginosa was determined according to ISO 16266 (ISO 2008); 10 ml subsamples were used for holy water and 250 ml subsamples were used for holy spring water.

Staphylococcus aureus was determined in 10 ml subsamples of holy water only, following ISO 6888-1 (ISO 1999b).

Salmonella spp. were determined in 250 ml subsamples of holy spring water only, following ISO 19250 (ISO 2010).

Campylobacter spp. were determined in 250 ml subsamples of holy spring water only, following the protocol recommended by the Health Protection Agency (2007). After a final microscopic examination (Nikon Labophot-2) for species identification, a MALDI-TOF analysis (Bruker-Daltonik, MALDI Biotyper; Bruker, Bremen, Germany) was used for suspected colonies following the manufacturer's instructions.

Chemophysical parameters of holy water and holy spring water

Water and air temperature were measured with a calibrated digital thermometer. Electrical conductivity was measured with an Ino Lab conductivity meter (WTW, Weilheim, Germany). The pH value was measured with a pH 531 pH-meter (WTW).

Chemical parameters of spring water

Total organic carbon (TOC) was determined with a Phoenix 8000 TOC Analyzer (Tekmar-Dohrmann, JCT, Wiener Neustadt, Austria) according to the manufacturer's instructions.

Anions (Cl^-, SO_4^{2-}, NO_3^-) and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were measured with a IC DX-120 ion chromatograph (DIONEX, Sunnyvale, CA) according to ISO 10304-1 (ISO 2007) for anions and ISO 14911 (ISO 1998) for cations. Total hardness was calculated from the sum of Mg^{2+} and Ca^{2+} cations.

Nitrite (NO_2^-) was measured with a Lambda 40 UV/VIS Spectrometer (Perkin Elmer, Waltham, MA) according to DIN EN 26777 (DIN 1993).

Ammonium (NH_4) was determined photometrically (Lambda 40 UV/VIS) according to ISO 7150-1 (ISO 1984).

For determination of iron and manganese the flame-AAS methodology (AAS 5000, Perkin Elmer) was used, following DIN38406-32 (DIN 2000a) and DIN 38406-33, (DIN 2000b), respectively.

Alkalinity was measured by titration with methyl orange. The HCO_3^- concentration was calculated from alkalinity values according to ISO 9963-1 (ISO 1995).

Statistical analysis

All statistical analysis was performed with SPSS 17.0. Correlations between all variables were calculated according to Spearman's rank test; p values <0.05 were regarded as significant. Only statistically significant correlations are reported in the text. For comparison of means, Students t-test was used.

RESULTS

Holy spring water

Visual inspection

Most of the springs are located next to or are part of a chapel, situated adjacent to residential buildings. The surrounding of the water catchment was usually cleaned up. The construction of water catchments (i.e. the structures that collect the water from the spring), installations and outlets, though, were often in bad condition and built in a way that contamination from outside was likely to occur. Burning candles, fresh flowers and pictures of saints put up next to the holy springs demonstrated frequent use of the spring water within organized pilgrimages and ceremonies celebrated in the chapels. During sampling, we observed people filling up several bottles of water, and parents,

trying to convince their children of the high quality of the water.

Bacteriological parameters

Concentrations of HPC22 ranged from 0 to 1.7×10^4 CFU ml^{-1}, HPC37 ranged from 0 to 3.2×10^2 CFU ml^{-1} (Figures 1(a) and 1(b)). In total, 19 samples from 12 springs exhibited HPC values above recommended indicator values (100 CFU ml^{-1} for HPC22 and 20 CFU ml^{-1} for HPC37).
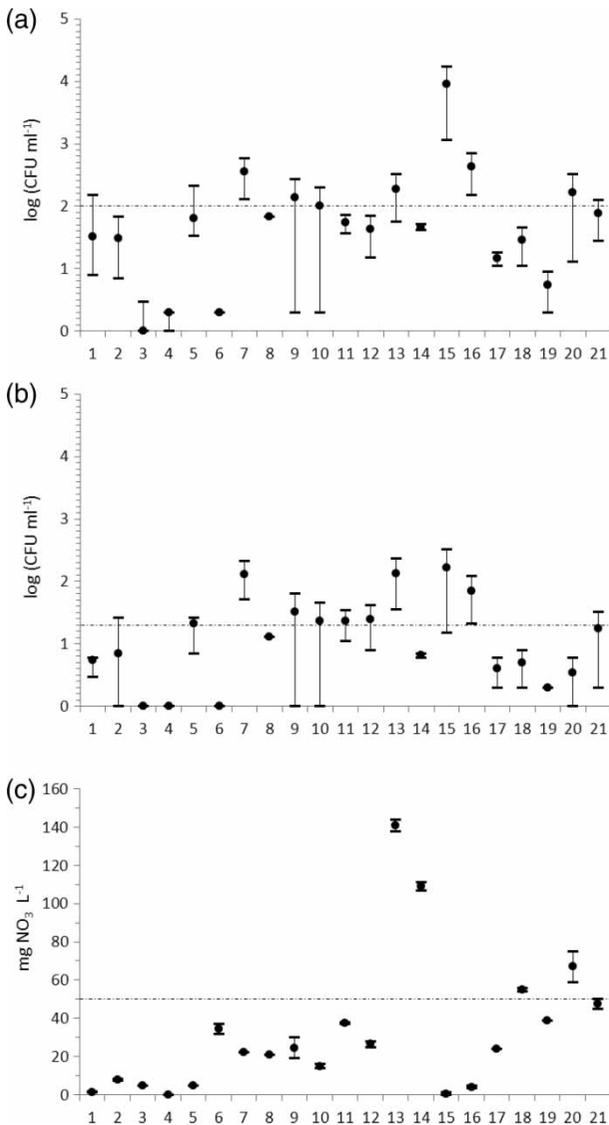
Highest concentrations of both HPC22 and HPC37 were observed at HS15. This spring is equipped with a hand-operated pump made of wood, in which biofilms could grow very well. HS3, HS4 and HS6 water samples had lowest CFU values.

A high percentage of the 50 samples was contaminated with fecal indicator bacteria (Figure 2): 38 samples (76%) were contaminated with coliform bacteria, 19 samples (38%) with *E. coli* and 16 samples (32%) with enterococci. *P. aeruginosa* was found four times (8%; data not shown). In a single sample, collected from HS7, *Campylobacter jejuni* was detected. Here, also the highest concentration of total coliform bacteria, 3.25×10^3 in 250 ml, was found. This spring is not protected by any artificial structure, thus allowing animals or surface water to contaminate the
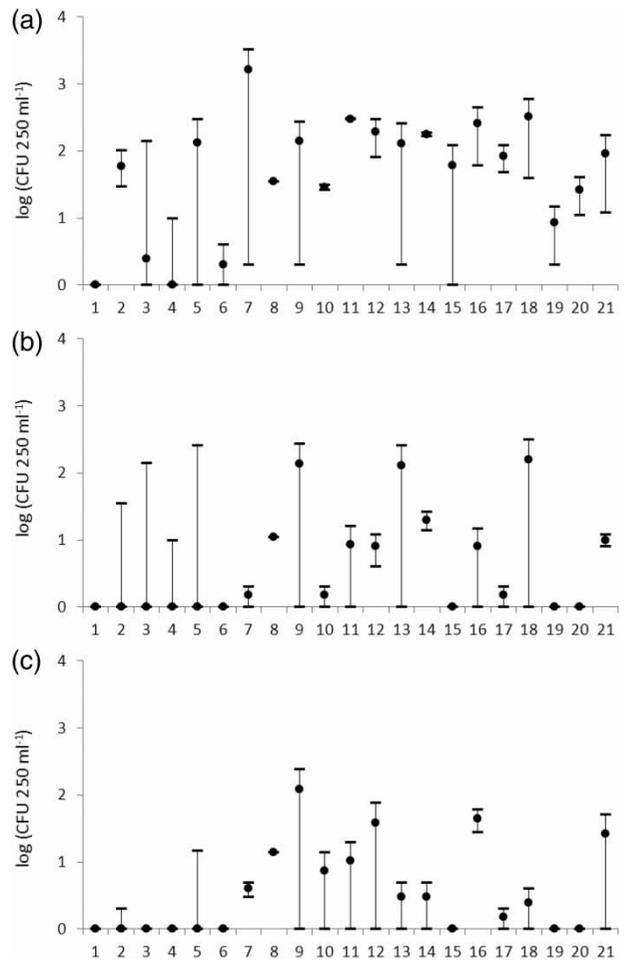

Figure 1 | HPC22 (**a**), HPC37 (**b**) and NO$_3$ (**c**) concentrations in 21 holy water springs (HS1–HS21). Values represent median, minimum and maximum values of 2–5 measurements. Dotted lines represent indicator values (HPC) and parametric values (NO$_3$), according to the Austrian Drinking Water Directive (2001).

Figure 2 | Coliform bacteria (**a**), *E. coli* (**b**) and enterococci concentrations (**c**) in 21 holy water springs (HS1–HS21). Values represent median, minimum and maximum values of 2–7 measurements.

spring. Highest *E. coli* concentrations were found in HS18, where the catchment of the spring is integrated into the wall on the outside of a little church. In one sample, 310 *E. coli* CFU per 250 ml were detected. The highest concentration of enterococci (240 per 250 ml) was detected in a sample from HS9, which also was highly polluted with *E. coli* (275 per 250 ml). In this sample also *P. aeruginosa* (4 CFU per 250 ml) was found. *Salmonella* spp. did not occur in any of the samples. Altogether, only nine samples out of 50 (18%) met the microbiological requirements of the Austrian Drinking Water Directive (2001).

Chemophysical and chemical parameters

A complete chemical analysis with all parameters listed above was conducted twice per sampling point. The springs investigated exhibited a wide range in conductivity and total hardness (Table 1), attributable to their specific geological situation. Highest hardness and conductivity values were recorded in HS2, located within the foothills of the Northern Calcareous Alps, with 49.1° GH (GH, German Hardness) and 1.760 μS cm^{-1}, respectively. Lowest values were measured in HS15, located within a granitic mountainous formation, with 2.2 °GH and 100 μS cm^{-1}, respectively. The samples were taken in July, August and April, therefore temperature varied between 6.5 and 14.7 °C (Table 1). The pH values ranged between 6.5 (HS15) and 8.2 (HS13) (Table 1). TOC showed highly significant correlations with both HPC22 (rho = 0.61; $p < 0.001$) and HPC37 (rho = 0.60; $p < 0.001$) values, with a maximum of 4.0 mg l^{-1} in a sample of HS16, which was also highly contaminated with total coliforms (450 per 250 ml). Lowest TOC levels (0.23 mg l^{-1}) were recorded in HS4, where also lowest HPC values were found. A significant number of samples

(nine out of 41, Figure 1(c)) exhibited nitrate levels above the parametric value of 50 mg l^{-1} of the Austrian Drinking Water Directive (2001). Highest concentrations were found in HS13 (144 mg l^{-1}). The median of 25 mg l^{-1} (Table 1) indicates a rather high contamination of the water samples; 18 samples were above this value. Lowest values around 1 mg l^{-1} were found in HS1, HS4 and HS15.

Holy water

Bacteriological parameters

Holy water samples showed extremely high concentrations of heterotrophic plate counts in all churches investigated. HPC22 and HPC37 reached maximal values of 6.2×10^7 and 3.0×10^7 CFU ml^{-1}, respectively, both of them registered in the most frequently visited church (HF4, Figure 3). With one exception (HF14), HPC22 were always above 10^4 CFU ml^{-1}, HPC37 were – with two exceptions (HF11, HF14) – always above 10^2 CFU ml^{-1}. Interestingly, in HF14, HPC22 values
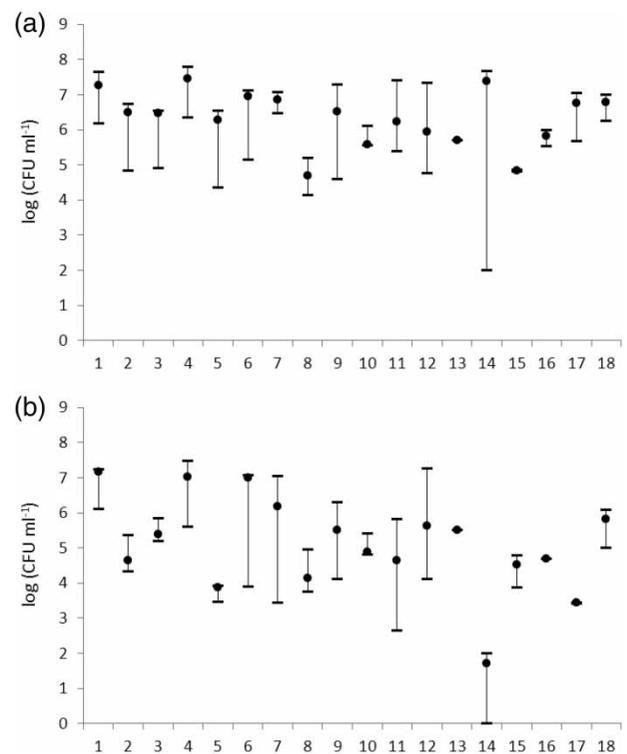
Figure 3 | HPC22 (**a**) and HPC37 (**b**) concentrations in the holy water of 18 churches (HF1–HF18). Values represent median, minimum and maximum values of 2–7 measurements.

Table 1 | Median, minimum and maximum values of chemophysical and selected chemical parameters in holy spring water samples. Temp: water temperature, Cond: electrical conductivity, TOC: total organic carbon, TH: total hardness, expressed in degrees of German Hardness (GH)

	Temp. (°C)	Cond. (μS cm^{-1})	pH	TOC (mg l^{-1})	NO$_3$ (mg l^{-1})	TH (GH)
Median	9.8	660	7.4	1.5	25	21.1
Min	6.5	100	6.5	0.23	1.1	2.2
Max	14.7	1,760	8.2	4.0	144	49.1

dropped from 4.8×10^7 to 1×10^2 CFU ml^{-1} within 1 week in January, most probably due to a preceding water change. A significant correlation of HPC37 but not of HPC22 with air temperature (rho = 0.36; $p < 0.05$) and water temperature (rho = 0.48; $p < 0.01$) was found. Water samples were assessed for their appearance by visual examination and judged as non-turbid ($n = 29$) or turbid ($n = 24$), when a homogeneously distributed visible turbidity was absent or present, respectively. Samples judged as turbid contained 0.6 log higher concentrations of HPC22 than non-turbid ones (Students t-test; $t = 2.0$; $p < 0.05$). Surprisingly, holy water in glass containers ($n = 13$) had on average 0.8 log higher HPC22 concentrations than holy water in the original stone bowl ($n = 40$; Students t-test; $t = 2.3$; $p < 0.01$). No significant differences were found for HPC37.

Among fecal indicators (Table 2), enterococci were the most frequently found group (13 out of 53 samples) with a maximum concentration of 180 CFU per 10 ml (HF4). Total coliforms were recorded five times (max. conc. 100 CFU per 10 ml) and *E. coli* was recorded only twice (HF4; max. conc. 3 CFU per 10 ml). *P. aeruginosa* and *S. aureus* were also only found in the most frequently visited church (HF4) with maximum concentrations of 16 CFU and 350 CFU per 10 ml, respectively.

Chemophysical and chemical parameters

Conductivity values varied markedly between churches but also between sampling dates within the same church.

Table 2 | Minimum, maximum and median values (in brackets) of coliform bacteria, *E. coli* and enterococci concentrations in holy water samples. Data of those holy water fonts are presented where at least one sample was positive for either parameter

	Coliforms (CFU 10 ml^{-1})	E. coli (CFU 10 ml^{-1})	Enterococci (CFU 10 ml^{-1})
HF1	0	0	0–1 (0)
HF2	0	0	0–1 (0)
HF3	0	0	0–2 (0)
HF4	0–100 (1)	0–3 (0)	0–80 (8)
HF7	0	0	0–1 (0)
HF10	0–9 (0)	0	0
HF12	0	0	0–2 (0)
HF17	0	0	0–1 (0)

The highest value was measured in the HF12 with 14.590 μS cm^{-1}, whereas in HF15 the conductivity amounted to 293 μS cm^{-1}, like normal Vienna tap water (Table 3). Water temperature varied during the sampling periods (August, September, January) between 3.8 and 23.4 °C (Table 3) and closely followed air temperature (rho = 0.97, $p < 0.001$). Although it can be assumed that the same original water (Vienna water supply) was used for production of the holy water in each of the churches and hospital chapels, pH values varied between 7.6 and 8.6 (Table 3) and were surprisingly negatively correlated with HPC22 (rho = -0.39, $p < 0.01$). The pH was obviously affected by the specific material of the original stone bowl while all pH-values of holy water which was stored in glass containers were constantly between 8.1 and 8.3.

DISCUSSION

Holy spring water

In our study, the majority of springs did not meet the requirements of the Austrian drinking water regulations. Only three springs (HS1, HS6, HS19) both fulfilled microbiological criteria and exhibited nitrate concentrations below 50 mg l^{-1}. But even in HS1, HPC22 exceeded the indicator guideline value of 100 CFU ml^{-1} at one sampling date (150 CFU ml^{-1}), and in HS6 and HS19, nitrate levels were rather high with 37 and 39 mg l^{-1}, respectively. According to Austrian and European regulations, the guideline value for nitrate is 50 mg l^{-1}, and especially for infants below 1 year, water with less than 50 mg l^{-1}(10 mg NO$_3$-N) is recommended (Greer *et al.* 2005). Nitrate has been associated with methemoglobinemia of infants (Ward *et al.* 2005), and with bladder cancer, colon cancer (Weyer *et al.* 2001) and non-Hodgkin's lymphoma (Law *et al.* 1999). Because

Table 3 | Median, minimum and maximum values of chemophysical parameters in the 53 holy water samples

	Water temperature (°C)	Conductivity (μS cm^{-1})	pH-value
Median	19.3	869	8.2
Min	3.8	293	7.6
Max	23.4	14,590	8.6

water from holy springs is used for drinking in private homes, its consumption by infants below 1 year cannot be excluded. In addition, nitrate levels above 3 mg l^{-1} indicate a possible contamination of the spring (Rogan et al. 2009).

With the exception of a few springs (HS3, HS5, HS18, HS20), the construction of the catchment and the installation was in nearly all cases of poor quality. It can thus be expected that the contamination with fecal indicators, P. aeruginosa and C. jejuni is a result from these construction deficiencies. But also those spring waters meeting the microbiological and chemical requirements cannot be recommended as reliable drinking water source due to constructional defects detected by sanitary inspection. Thus, the contamination with fecal indicators may result from the susceptibility of the water source to contamination. Most of the springs are not located in defined water protection areas. Some of the springs investigated are located in a water protection area but, nevertheless, did not meet quality standards. In HS3, for example, 138 E. coli were identified in one sample.

Apart from poor quality of the water source itself, the majority of people who regularly collect water from such springs use impure bottles and store the water at room temperature, with a further negative impact on water quality. Despite the fact that the quality of nearly all holy springs in our study did not meet drinking water standards, the opinion of the public is still positive. For an explanation it has to be considered, that in the 15th century, when holy springs became popular, they were of much greater importance than nowadays. For example, it is reported that even the King of Denmark in 1639 was convinced of the healing effects of holy springs (Johansen 1997). The water supply of cities and villages in those days was problematic and waterborne epidemics were abundant. In comparison to the medieval cities lacking sanitary infrastructure, such holy springs, far away from densely populated areas, were of likely significantly better quality than the water within the cities. This may be an explanation for the relevance of holy springs for the public in the past which continues up to now.

Holy water

The data collected showed high to extremely high contamination with saprophytic bacteria of all holy water fonts with values up to 62×10^6 HPC ml^{-1} and significant correlation of HPC22 with temperature was observed. Pathogenic species (P. aeruginosa and S. aureus) were only identified in the most frequently visited church (HF4). It has to be mentioned, that the presence of pathogenic microorganisms may be underestimated, because the examined volume per species was only 10 ml due to limitation by available sample volume. Also fecal indicators were present in all seven samples of HF4. Enterococci were the most frequently detected fecal indicator group, most probably because they tolerate higher salt concentrations than E. coli (Hanes & Fragala 1967; Kirschner et al. 2004), as a variable amount of salt (NaCl) is added to the holy water. Jurado et al. (2002) recommended the use of a concentration of at least 20% sodium chloride to prevent proliferation and survival of pathogenic and non-pathogenic bacteria.

The observed high contamination of HF4 could be explained by the popularity of the church and the high abundance of its visitors. Extremely high contamination of holy water fonts was also observed by Jurado et al. (2002) in churches in Seville (Spain) during Easter Holy Week. Our study showed that other, less frequently visited, churches were much less contaminated, although HPC22 values were, with one exception, always above 10^4 CFU ml^{-1}. This indicates that the frequent dipping of fingers is the main reason for microbial growth in the fonts. On the one hand bacteria (and other potential pathogens) from the skin are transferred into the holy water and on the other hand, nutrients for bacterial growth arrive in the font via this route. Frequent use of the holy water increases the turbidity and we found a significantly higher bacterial contamination in holy water fonts judged as turbid as in fonts with clear water. Surprisingly, glass containers positioned in the original stone bowls did not exhibit better water quality than holy water stored directly in the stone bowls, indicating again, that the frequency of dipping is the main factor for contamination of holy water.

The risk for public health emanating from holy water must, however, not be overestimated. Touching the water just with the fingers and not getting in contact with open wounds should not cause any disease in a person with a healthy immune system. It is a different matter when it comes to children, elderly people, severely injured patients or persons with a suppressed immune defence due to

chemotherapy. Especially for the last two groups of persons the condition of the holy water fonts in hospital chapels is of importance. There are some reports on hospitalized injured patients (Greaves & Porter 1992; Rees & Allen 1995) who got severe complications after contact with holy water from the hospital. In our study, the holy water samples from both hospitals had very high values of HPC22 and HPC37 with values ranging from 1.2×10^6 to 4.5×10^7 CFU ml^{-1}, and in one sample, enterococci could also be detected. These findings indicate that hospital holy water fonts should not be neglected as a possible source of nosocomial infection of religious patients.

CONCLUSIONS

Water from holy springs is frequently contaminated with fecal bacteria or nitrate and cannot be recommended as a drinking water source for the public. Even if microbiological and chemical requirements were met, sanitary inspection of the water catchment revealed that most of the springs have severe hygienic deficiencies in their construction and the installations. Raising public awareness is highly necessary to prevent people from using these waters without programs for assessment and management of water quality. Clear warning signs have to be installed stating that the holy springs are not suitable for drinking water, especially for infants below 1 year of age. Alternatively, these springs have to be surveyed by local authorities and reconstructed to meet the hygienic standards. Hazard analyses and risk assessment as well as epidemiological studies are desirable to substantiate our findings.

For holy water in churches addition of 20% sodium chloride has been recommended to prevent proliferation of potentially harmful microorganisms (Jurado *et al.* 2002). However, so far there is no sufficient proof of the microbicidal efficacy of this measure. In order to protect the original stone bowls from attack by chemically aggressive saline solution, glass dishes may be positioned in the stone bowl. An even better solution for decreasing infection risk is the dispensers equipped with a light sensor, which dispense holy water from a small tank by just putting a hand under it. Due to the fact that holy water is produced by the priest only once a year, appropriate large tanks have to be used

for long-term storage. For hospital chapels, specifically, where injured or immunosuppressed patients have access, it may be considered wise to completely avoid the use of holy water.

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