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Brief report

Holy water fonts are reservoirs of pathogenic bacteria

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Summary

The holy water fonts from the churches of Seville, Spain have a strong bacterial contamination. Coliforms, *Enterobacteriaceae* and other pathogenic bacteria are widely represented in the fonts investigated. Thirty out of the 37 different species isolated from holy waters are known human pathogens. The high bacterial contamination is related to human skin transmission and, probably, to misuse of the water.

Introduction

Human activities have a strong impact in 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, Burkholderia cepacia, Serratia marcescens, Flavobacterium meningo-septicum, Aeromonas hydrophila*, etc.) can growth in relatively pure water (Black *et al*., 1979; Millership and 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).

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 and Weber, 1997). However, outside the hospitals, humans are subjected to many other, rather uncontrolled, sources of pathogenic bacteria.

Some social and religious 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 of interest because of its high pathogenic potential. Among the religious activities, one of the most extended is the use of holy water in Roman Catholic churches, which is offered in fonts near the doors so that the faithful may bless themselves with it on entering. In Seville, Spain, churches are widely visited in the days before, during and after Holy Week, and the holy water fonts are extensively used. We have addressed our attention to these fonts, because there was increasing evidence that holy water could represent a potential risk to health (Greaves and Porter, 1992; Rees and Allen, 1996; Payne, 2001).

Results and discussion

In Seville we have observed that when the faithful enter and leave church, they introduce their fingers into the font and make the sign of the cross on their foreheads, ending by touching their lips with their fingers. In some cases, during mass celebration, infants play with the holy water and, subsequently, put their fingers into their mouths. As there was a high possibility of transmission of pathogens, inoculated by the hands, in this study we have investigated the microbiology of the holy water fonts located in some of the most visited churches of Seville, Spain. Samples were collected from La O and Cachorro churches on January 28th 2001, and checked for environmental bacteria using trypticase soy agar medium (Oxoid, Basingstoke, England). The presence of pathogenic bacteria in one of these samples (La O) alerted us and additional samples were collected from one of the fonts of El Salvador and Esperanza Triana churches, and from two fonts from Gran Poder, Macarena, and Santa Ana churches between 9th and 17th April, 2001. These were processed as for pathogenic bacteria, inoculated on Columbia III agar with 5% sheep blood stacker plates (Becton Dickinson, Meylan, France) and incubated at 37°C for 24–48 h.

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. While generally considered to be a discomfort to health, these infections can prove fatal for infants, the elderly and those who are

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Table 1. Most abundant genera isolated from holy water fonts from Sevillian churches[a] (in percentage of isolates).

Genus	Sta Ana	Gran Poder	Macarena	Salvador	Esperanza Triana	Cachorro[b]	La O[b]
Acinetobacter	–	1.7	4.9	–	–	–	–
Aeromonas	–	3.3	–	8.7	–	–	–
Aureobacterium	–	–	–	–	–	–	4.8
Bacillus	5.5	3.3	17.1	56.5	–	33.3	19
Brevibacterium	–	–	–	–	–	–	4.8
Brevundimonas	–	5	–	–	–	–	9.5
Delftia	1.8	10	9.8	4.3	–	–	–
Gemella	–	–	–	4.3	–	–	–
Haemophilus	–	–	–	–	–	–	4.8
Paenibacillus	–	–	–	4.3	–	–	–
Pseudomonas	50.9	15	29.3	–	57.1	–	–
Sphingobacterium	–	10	–	–	–	–	9.5
Staphylococcus	5.5	5	9.8	4.3	–	–	14.3
Stenotrophomonas	–	–	4.9	–	–	–	–

a. Genera *Enterobacter*, *Microbacterium*, *Neisseria*, *Pantoea*, *Ralstonia*, *Salmonella and Streptococcus* represented less than 4% of isolates.
b. Only searched for environmental bacterial (TSA medium).

already ill. The fonts were also sampled using Petrifilm plates (3M, St. Paul, USA) for total aerobic bacteria, coliforms and *Enterobacteriaceae* counts, which were inoculated directly with 1 ml water. In the fonts, coliforms were higher than 10^3 per 100 ml of water and of the same range were *Enterobacteriaceae*. In one church (Gran Poder), total aerobic bacteria and coliforms were 'too numerous to count'. The presence of potential pathogens was proved by the identification of species of *Acinetobacter, Aeromonas, Haemophilus, Neisseria, Salmonella, Staphylococcus*, etc. MIDI (Microbiol Identification System, Newark, DE, USA) clinical library CLIN40 and BIOLOG (Hayward, CA, USA) automatic systems were used for isolate identification.

The presence in the holy waters of the genera *Staphylococcus, Streptococcus, Acinetobacter, Pseudomonas* and a few others is related to hand contamination and inoculation from human skin (Holland and Kearney, 1985; Holland, 1993). Transmission occurs by contact with infectious hands and fingers, although some other water misuses cannot be ruled out.

The most abundant genera in holy waters were *Pseudomonas* and *Bacillus*, followed by *Staphylococcus, Sphingobacterium* and *Delftia* (Table 1). However, biodiversity was very different from one church to another. In fact, some waters showed a majoritary colonization by *Pseudomonas* (Esperanza Triana, Santa Ana, Macarena, Gran Poder), *Bacillus* (El Salvador, Cachorro, La O, Macarena), *Staphylococcus* (La O, Macarena) or *Delftia* (Gran Poder, Macarena).

A community-level assay based on carbon source utilization (BIOLOG) was used to characterize and classify the microbial communities (Garland and Mills, 1991). Principal-components analysis revealed distinctive patterns among churches (Fig. 1). In fact, the Gran Poder and El Salvador separated well from all other churches. The Gran Poder is probably the most visited church in Seville which explains the higher bacterial counts. Also biodiversity was higher in this church where 15 genera and 20 species were identified. Other churches presented a maximum of six genera or 10 species. This indicates a direct influence of the number of visitors on the bacterial contamination of the holy water fonts.

The water from El Salvador church had an extremely high conductivity 2700 μS/cm, as well as high sulphate, nitrate, calcium, magnesium, potassium and sodium contents. El Salvador church was in restoration when the samples were taken, which could explain the high content of some elements and the abundance of a *Bacillus* population. *Bacillus* species are common in terrestrial environments and deteriorated monuments and their osmotic adaptation would explain their abundance in El Salvador water (Saiz-Jimenez and Laiz, 2000).

Thirty of the 37 different species isolated from holy water were reported to be human pathogens, e.g. *Brevibacterium otitidis, Bacillus thuringiensis*, *Delftia acidovorans*, *Gemella haemolysans, Sphingobacterium multivorum,* etc. (Reller, 1973; Chatelain *et al.*, 1982; Samples and Buettner, 1983; Horowitz *et al.*, 1990; Reina *et al.*, 1992). However, the majority of these bacteria only rarely cause disease in predisposed individuals.

This together with previous reports (Greaves and Porter, 1992; Rees and Allen, 1996; Payne, 2001) stresses the fact that religious activities involving the use of water are sources of pathogenic bacteria and should be carefully controlled. This bacterial contamination, far from being restricted to certain churches or cities must be extended to all countries where holy water fonts are used, as human skin is populated by a high number of bacteria, fungi, viruses, protozoa and mites (Holland and Kearney, 1985).

Transmission of pathogenic bacteria from holy water fonts can be prevented by daily removal of waters and font

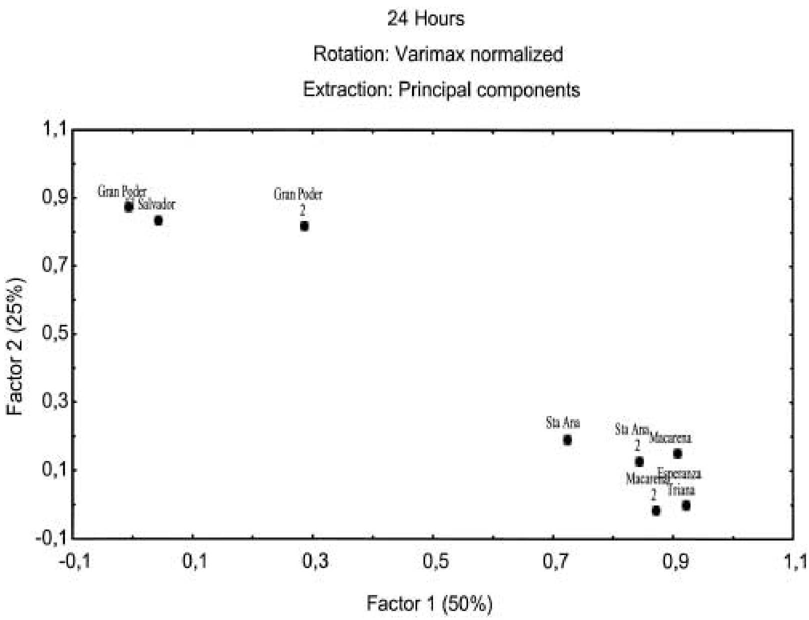

Fig. 1. Principal components analysis plots of substrate utilization patterns from churches bacterial communities. PC1 and PC2 accounted for 50 and 25% of the total variance respectively.

cleaning or by adding high sodium chloride concentrations if the waters are not periodically cleaned. Holland (1993) reported that most cutaneous bacteria grew up to 10% sodium chloride, but only a few up to 15% (*Staphylococcus* spp., *Brevibacterium* spp.). Table 2 shows the 48 h viability of selected strains at increasing sodium chloride concentrations. The bacteria were inoculated in trypticase soy broth (TSB) medium in microplates and tested for viability (CFU) and growth (absorbance at 750 μm) at different times. In TSB medium with 5% sodium chloride concentration *Brevundimonas diminuta*, *D. acidovorans, Pseudomonas fluorescens*, *Pseudomonas alcaligenes* and *Stenotrophomonas maltophilia* were inhibited. At 10% concentration eight strains were inhibited, *B. otitidis, S. multivorum* and the three *Staphyloccocus* spp. being the only viable strains. At 15% salt concentration only *Staphylococcus xylosus* showed a low growth, while at 20 and 25% concentrations all strains were inhibited. Therefore, it is expected that at 20% sodium chloride pathogenic and non-pathogenic bacteria will lyse, as only halobacteria (*Archaea*), not expected in the holy water fonts, should be able to survive at this high salt concentration.

Table 2. Viability of selected bacterial strains at increasing concentrations of sodium chloride (48 h).

	TSB + % sodium chloride					
Strain	0	5	10	15	20	25
Acinetobacter johnsonii	+ + +[a]	+ +	–	–	–	–
Bacillus cereus	+ + +	+	–	–	–	–
Brevibacterium otitidis	+ + +	+ + +	+ +	–	–	–
Brevundimonas diminuta	+ + +	–	–	–	–	–
Delftia acidovorans	+ + +	–	–	–	–	–
Haemophilus paracuniculus	+ + +	+ +	–	–	–	–
Pseudomonas alcaligenes	+ + +	–	–	–	–	–
Pseudomonas fluorescens	+ + +	–	–	–	–	–
Sphingobacterium multivorum	+ + +	+ + +	+	–	–	–
Staphylococcus lugdunensis	+ + +	+ +	+	–	–	–
Staphylococcus saprophyticus	+ + +	+ +	+	–	–	–
Staphylococcus xylosus	+ + +	+ + +	+ +	+	–	–
Stenotrophomonas maltophilia	+ + +	–	–	–	–	–

a. + + + , optimum growth; + + , medium growth; + , low growth; –, no growth.

The addition of high amounts of sodium chloride is respectful with the Catholic tradition as this salt is used in the preparation of holy water. However, high concentrations of this salt could result in deterioration of the marble fonts due to salt crystallisation into the calcitic matrix (Lazzarini and Laurenzi Tabasso, 1986), and the use of sodium chloride should be carefully considered from the point of view of cultural heritage protection. The removal of water from the fonts, as adopted in some Dublin churches (Payne, 2001), could be another way of fighting against bacteria contamination from holy water fonts.

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