The prevalence of free-living amoebae in a South African hospital water distribution system

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The purpose of this study was to investigate the occurrence of free-living amoebae in the water system of a teaching hospital in Johannesburg (South Africa). Water and biofilm samples were collected from the theatres, theatre sterilisation service unit, central sterilisation service unit and endoscopy/bronchoscopy unit. The samples were filtered and seeded on non-nutrient agar spread with heat-killed *Escherichia coli*. Of the 71 samples collected, 63 (88.7%) were positive for free-living amoeba. *Acanthamoeba* spp., *Balamuthia* spp. and *Hartmanella* spp. were identified by morphology. The presence of free-living amoeba in the hospital water network may be a potential health risk.

Introduction

Free-living amoebae (FLA) are unicellular protozoans that are widely distributed in aquatic environments, including constructed water systems such as hospital water systems and swimming pools.[1,2] Although FLA are useful as predators of bacteria, algae, viruses and fungi in the environment, some species – *Naegleria fowleri, Balamuthia mandrillaris, Sappinia pedata* and *Acanthamoeba* species – have been implicated in infections of the central nervous system, eye and skin.[3,4] Some FLA also allow the survival and growth of bacterial pathogens linked to nosocomial infections such as *Legionella pneumophila, Mycobacteria, Pseudomonas aerugionosa* and *Acinetobacter baumannii*. These bacteria are able to infect, resist the digestive process of FLA, survive, multiply and exit FLA. Intracellular bacteria within FLA cysts are protected from hostile environmental conditions such as the presence of biocides used in water treatment.[5,6] FLA may also serve as vehicles for transmission of waterborne bacterial pathogens, enabling them to spread and colonise hospital water systems. Furthermore, bacteria that infect FLA can undergo morphological modifications within FLA and become more resistant to antibiotics and better adapted to survival in macrophages.[7] Therefore the presence of FLA in hospital water supplies may present a potential health concern for medical personnel and immunocompromised patients.

Methods

From February to April 2014, 71 samples were collected from a teaching hospital in Johannesburg, South Africa. A total of 35 tap water samples, 30 tap swab samples and 6 showerhead swab samples were collected. The samples were collected from theatres ($n=41$), the theatre sterilisation service unit ($n=4$), the central sterilisation service unit ($n=8$) and the endoscopy/bronchoscopy unit ($n=18$). Temperature, pH, residual chlorine and total dissolved solids were measured at the site of collection.

Samples (500 mL) were concentrated by filtration using a 0.45-μm pore size cellulose nitrate membrane. Swabs were vortexed for 30 s in 10 mL of Page's amoeba saline in individual sterile tubes and the suspensions were concentrated by filtration. The filter membrane was placed upside down onto a non-nutrient agar overlaid with heat-killed *Escherichia coli*. The plate was then incubated aerobically at 32 °C and examined daily under a light microscope for the appearance of amoebal trophozoites and cysts. Amoebae were sub-cultured on fresh non-nutrient agar–*E. coli* plates three to four times and harvested by scraping the agar surface and re-suspending in 2 mL of Page's amoeba saline. The suspensions were inoculated in microtitre wells before being observed under an inverted microscope for the presence of amoebae species.

Results

Water temperatures ranged between 19.0 °C and 27.0 °C (mean=23.1 °C), pH ranged between 7.5 and 8.0 (mean=7.9), total dissolved solids ranged from 110 mg/L to 187 mg/L (mean=109 mg/L) and residual chlorine ranged from 0.04 mg/L to 0.17 mg/L (mean=0.08 mg/L). Free-living amoebae were observed in 63 (88.7%) of the water and biofilm samples that were analysed using amoebal culture techniques. Of the samples collected, amoebae were recovered from 31 (43.7%) of the water and 32 (45.1%) of the swab samples taken from taps and showerheads. Typical *Acanthamoeba s*pp., *Balamuthia* spp. and *Hartmanella* spp. were observed in samples that were positive based on their morphology (Figure 1). Of the positive samples, 7 (11.3%) were *Acanthamoeba* species, 20 (32.3%) were *Hartmanella* species and 12 (19.4%) were *Balamuthia* species. The other 24 (38.7%) were not morphologically classified as belonging to any species (Table 1). Negative samples were from the theatre sterilisation service unit (2), the central sterilisation service unit (2), the endoscopy/bronchoscopy unit (2) and theatres (3).

Discussion

To our knowledge, this is the first report on the occurrence of FLA in a South African hospital water system. All physico-chemical parameters analysed in this study were within prescribed South African guidelines for drinking water.[8] Using amoebal enrichment, the prevalence of amoebae in this study – 88.7% – is higher than the prevalence found in previous studies done by Rohr et al.[9] and Lasheras et al.[10], in which 50% and 68.9% of samples, respectively, were positive for amoebae. In a study by Thomas et al.[11], amoebae were detected in 11.5% of water samples and 5.7% of taps and showerheads in a Swiss hospital, compared with the 43.7% of water and 43.7% of swab samples that were positive in this study. A more recent study by Ovrutsky et al.[1] recovered amoebae, mainly from biofilm, in 14.8% of hospital samples analysed. The higher prevalence of amoebae observed

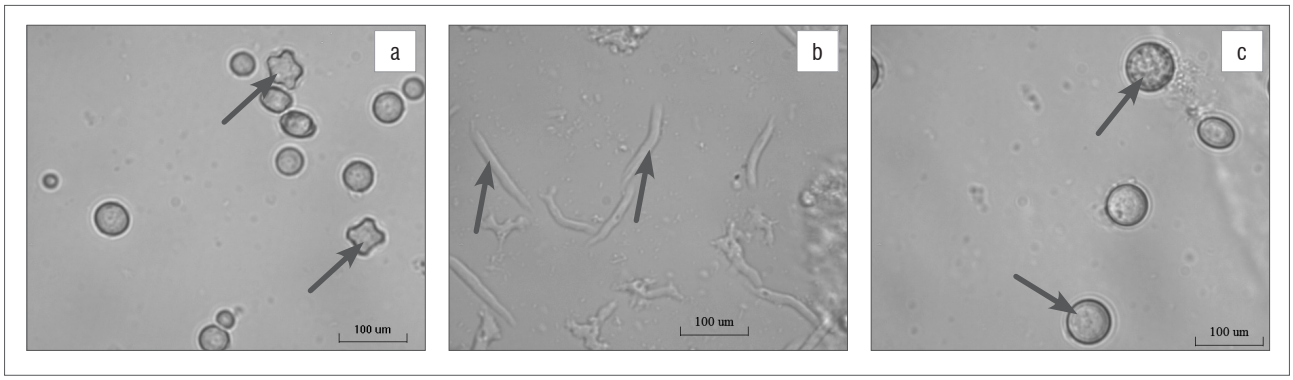

Figure 1: (a) *Acanthamoeba* cysts, (b) *Hartmanella* trophozoites and (c) *Balamuthia* cysts (all indicated by arrows) in water and biofilm samples from a hospital water system.

Table 1: Biodiversity of free-living amoeba from different sampling sites in a hospital water system

Sampling site	*Acanthamoeba* spp. (+) (%)	*Hartmanella* spp. (+) (%)	*Balamuthia* spp. (+) (%)	Other free-living amoeba† (+) (%)
Theatre and central sterilisation service units	3 (37.5)	3 (37.5)	0 (0)	2 (25.0)
Endoscopy/bronchoscopy unit	0 (0)	7 (43.8)	6 (37.5)	5 (31.3)
Theatre complex	4 (10.8)	10 (27.0)	6 (16.2)	17 (45.9)
Total	7 (11.3)	20 (32.3)	12 (19.4)	24 (38.7)

†*Not identified morphologically*

in our study could be a result of the relatively lower temperatures of cold water samples compared with the relatively high temperatures of hot water samples analysed in other studies. FLA with a low temperature tolerance would not survive the temperatures applicable in the other studies, which were above 45 °C.[1,9,10] High amoebae recovery rates may also be caused by increased water age in the distribution system, which encourages microbial re-growth.[12] Coşkun et al.[13] reports that 24 out of 33 detected FLA from 150 drinking water samples were of the genus *Hartmanella.* These data are consistent with our findings that 20 out of 63 detected FLA were *Hartmanella* spp., 7 were *Acanthamoeba* spp. and 12 were *Balamuthia* spp. (Table 1). The detection of FLA in this study indicates that the large population of immunocompromised individuals in this health-care setting might be exposed to these organisms. The pathogenic potential of *Acanthamoeba* spp. and *Balamuthia* spp. isolated in this study may play a significant role in hospital-acquired (nosocomial) infections in patients exposed through water systems. The presence of FLA may also present an added risk for a health-care setting, as FLA have been described as reservoirs and disseminators of opportunistic bacterial pathogens associated with water-related diseases. These pathogens have been implicated in nosocomial infections such as *Legionella pneumophila,* non-tuberculous mycobacteria, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[14]

Information on the prevalence of FLA in hospital networks may help identify connections between environment and patient infections. This identification will assist physicians to diagnose and treat amoebae-related infections in immunocompromised individuals. In addition, infection control staff can take precautions to prevent exposure to patients based on reported FLA data. Future work will focus on the molecular analysis of FLA using the polymerase chain reaction and sequencing to confirm FLA identified by morphology and to detect the other 24 isolates not identified in this study. The association between FLA and potential nosocomial bacterial pathogens will also be determined.

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Authors' contributions

P.M. performed the experiments and wrote the manuscript. C.D. was the project leader, made conceptual contributions and assisted in the data analysis. T.G.B. made conceptual contributions and assisted in project design.

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