Health implications of lipopolysaccharide endotoxins in domestic container water used by rural households in South Africa

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ABSTRACT

This study assessed the occurrence of endotoxins, cyanobacteria and enterobacteria in untreated drinking water stored in domestic water containers by rural households in South Africa. Endotoxins, cyanobacteria, total coliforms and *Escherichia coli* were measured in the following numbers and ranges in container-water samples: 4–54 $\mu g\,l^{-1}$, 69–64,505 cells ml^{-1}, 9,000–280,000 CFU/100 ml and 90–1,100 CFU/100 ml, respectively, in source water and 0.23–24.7 $\mu g\,l^{-1}$, 1–501,187 cells ml^{-1}, 25–1,584,893 CFU/100 ml and 1–25,118 CFU/100 ml, respectively, in water from containers. The concentrations of these contaminants in water often exceeded guidelines. Container type, especially those that permit light into the vessel, played a significant role in the occurrence of these contaminants. Limited guidelines, as well as the absence of health evidence, make it uncertain whether the high levels of endotoxins in the containerised drinking water could cause a health effect in healthy persons. Most importantly, in the context of exposure to endotoxins potentially derived from high levels of cyanobacteria and enterobacteria such as coliforms in the water, a case is made for possible health effects in immune-compromised individuals exposed to water containing endotoxins and the bacteria that potentially produce it.

Key words | cyanobacteria, endotoxins, human health, total coliforms, water containers

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INTRODUCTION

Bacterial endotoxins are generally not a problem in a properly treated and reticulated drinking water supply (Jakubowski & Ericksen 1980; Wichmann *et al.* 2004), but they might be a problem in many developing areas of the world where households rely on remote and often untreated sources for their daily water supply. People use many different types of container to collect and store water in their homes. Endotoxins might be present in these container waters in sufficient concentrations to cause health effects.

Water stored in household water containers has been reported to adversely affect the health-related microbial quality of water, especially those waters that have been sourced from a good quality supply at communal taps (Momba & Kaleni 2002; Jagals *et al.* 2003). The potential health effect associated with the microbiological quality of the water in these containers, especially if intended for drinking, is often based on numbers of indicator bacteria such as *Escherichia coli* detected in the water. If no *E. coli* is found, the water is, at least from a health-related microbial quality perspective, deemed safe for drinking. However, these bacteria and their associated pathogens produce toxins and it can be assumed that the toxins might still be available in container water after the indicator bacteria have become undetectable and presumably died off (Jorgensen *et al.* 1979; Jagals *et al.* 2006). The question is whether the concentration of residual toxins in household container water could potentially affect the health of those who drink the water without further treatment.

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Among microbial toxins that may occur in water intended for drinking are lipopolysaccharide endotoxins produced by cyanobacteria and Gram-negative bacteria such as coliforms (Rapala *et al.* 2002). Lipopolysaccharides are essential for bacterial growth and occur in important areas of the bacterial structure such as the cell walls (Wiese *et al.* 1999). Bacteria lyse during and after die off, releasing cell-wall particles in the original medium (i.e. water) where they can remain for a long time (Jorgensen *et al.* 1979). These particles can partly consist of endotoxins that may cause excessive immunological responses in human cells in laboratory circumstances (Wichmann *et al.* 2004).

Although this implies the possibility that the presence of endotoxins can adversely affect the health of individuals, reports differ on the actual potential for enterobacterial and cyanobacterial endotoxins to affect immunologically competent populations. Endotoxins produced by enterobacteria (including the coliforms) can be quite potent and may have a greater potential for harmful effect on human health (Hunter 1998; Stewart *et al.* 2006). The health end-points of enterobacterial endotoxins range from acute reactions such as diarrhoea (Closs *et al.* 1998; Madigan *et al.* 2000) to more chronic effects from extended exposure such as intravascular coagulation (Braude 1982; Anderson *et al.* 2002). Jakubowski & Ericksen (1980) reported on the health significance of bacterial endotoxins ($1-10\,ng\,ml^{-1}$) in bulk drinking water supply and concluded that exposure to such water would not affect normal healthy individuals unless it is administered through intravenous injection. Endotoxins produced by cyanobacteria genera such as *Schizothrix*, *Microcystis* and *Anabaena* have been reported to be responsible for outbreaks of diarrhoea in areas where people ingested treated and untreated surface waters (Hindmann *et al.* 1975; Lippy & Erb 1976; Keleti *et al.* 1979; Hunter 1997).

Oral intake of surface waters could expose consumers to very high concentrations of endotoxins (Rapala *et al.* 2002; Wichmann *et al.* 2004). Many villages in rural settlements of the Limpopo province, South Africa, do not have access to a water supply system that delivers water in-house. The residents use plastic containers–usually of 20–25 l capacity–either to collect water from taps fed by untreated groundwater, or directly from mostly untreated sources that are occasionally groundwater but more often surface water. The containers are generally made of light- (light-permitting) or dark- (light-limiting) textured plastic, an important consideration as light availability inside these containers could influence the growth of cyanobacteria (Fosso-Kankeu *et al.* 2008) and therefore possibly influence the level of endotoxins.

These types of container have also been reported to be susceptible to the build-up of bacterial biomass in the form of biofilm on the inner side-walls, which can contain substantial numbers of enterobacteria such as coliforms (Momba & Kaleni 2002; Jagals *et al.* 2003) as well as cyanobacteria (Fosso-Kankeu *et al.* 2008). If this is the case in other geographical areas, this could expose many consumers directly to endotoxins that might have been sourced with the water (depending on the source) or produced by the bacteria in the containers after sourcing.

The purpose of this paper is, then, to determine endotoxins concentrations in water stored in domestic drinking water containers in poor and rural areas in a developing country. The bacterial producers of endotoxins targeted for this study were cyanobacteria and total coliforms including *E. coli*. The relationship between the occurrence of endotoxins and the bacterial producers in light and dark plastic containers is also reported. Finally the health implications of these findings are discussed.

METHODS

Study area

Water samples were collected from source points and households in two village groups (collectively renamed Village 1 and Village 2) of low socio-economic status in the Vhembe district of the Limpopo province, South Africa. The members of these villages collected water for their domestic use (including drinking) in a variety of light and dark plastic containers from two different types of water source and stored these in their houses for up to 4 days.

Water sources

In Village 1 water was collected from taps on a distribution system supplied from an untreated groundwater source. For Village 2, river water was the main water source for

domestic needs including drinking water. To include the influence of seasonal variation on bacterial occurrence as well as endotoxins concentration, samples were collected from the two water sources on two separate occasions in summer and two other occasions in winter.

Container water samples

Households in the study villages mostly use a mix of dark (D) and light (L) containers. Ten households from each village were randomly selected and water samples were collected from each: twice in summer and twice in winter within the same year on the same occasions when the sources were sampled. Samples were taken within four hours of water collection at each household from one dark and one light container, before and after dislodging biofilm from the inner side-walls of each container by brushing according to the method used by Jagals *et al.* (2003) and Fosso-Kankeu *et al.* (2008):

- Water was first sampled from the free volume of water (FV) of each container.
- Biofilm particles were dislodged from the container inner side-walls into the FV.
- A second sample (DB) was taken from the same container directly after dislodging biofilm into the FV.

This was done to determine the effect of the light-permitting containers on the side-wall biofilm and subsequently the concentrations of endotoxins that could be associated with the biofilm.

Microbiological water quality

Identification of cyanobacteria

Samples were prepared and cells identified according to the method described by Du Preez & Van Baalen (2006). This method includes the identification of cells with inverted light microscopy (40 × magnification) linked to a computer using the algal-counting software SCS® for enumeration of cells per ml.

Identification of enterobacteria

Water samples were then filtered through sterile 0.45 μm Millipore filters and the filter membrane plated on Chromocult® agar (Merck) and incubated according to the membrane filter method as described in Standard Methods (2005). Blue and purple colonies were counted as *E. coli* and pink colonies as other coliforms. The counts were expressed as colony-forming units (CFU) per 100 ml of water sample.

Endotoxins detection by LAL chromogenic assay

One ml of water sample was transferred into an Eppendorf tube and lyophilised to release endotoxins from the cell walls of any bacteria present in the sample especially the Gram-negative bacteria and cyanobacteria. Endotoxins concentrations were then measured according to the protocol provided by Cambrex (LAL chromogenic QCL 1000 120T SL, from Adcock Ingram, SA) for the limulus amoebocyte lysate (LAL) chromogenic test. Duplicates of the water sample, LAL reagent and chromogenic substrate were mixed in a pyrogen-free glass tube and incubated at 37°C in a water bath. The reaction was stopped after 16 min with 25% acetic acid and the absorbances read at 405 nm using a spectra-photometric microplate reader (Benchmark Plus from Bio Rad). Endotoxins concentrations were determined as endotoxins units per ml ($EU\,ml^{-1}$) and converted into $\mu g\,l^{-1}$, according to Cooper (1998) $1\,EU = 0.1\,\mu g\,l^{-1}$.

Biofilm measurement

Turbidity, expressed in nephelometric turbidity units (NTU), was used as a measure of the biofilm particle quantities dislodged from the inner side-walls of the containers. A Nephla turbidity meter (GmbH-Berlin, Germany) was used to measure the NTU.

Health considerations

After an intensive search of the literature, the only guideline value for health-related endotoxins concentrations in drinking water that could be found was that proposed by the New Zealand Ministry of Health (NZMH 2000) of $3\,\mu g\,l^{-1}$. For the purposes of this study the endotoxins content in water intended for drinking (the container water) was deemed unacceptable if it exceeded this level, with the

90th percentile as the level of compliance. For the bacteria, the World Health Organisation (WHO 1999) proposed, for cyanobacteria, a health primary alert guideline of 2,000 cells ml^{-1}. Maximum values of 1 and 10 CFU/100 ml for *E. coli* and coliforms, respectively, are recommended in drinking water at the 95th percentile (SABS 2005).

Data were recorded and analysed according to methods and statistical procedures reported by Fosso-Kankeu *et al.* (2008).

RESULTS AND DISCUSSION

Source water quality

Levels of endotoxins and their related bacteria in the water sources

Endotoxins were detected only in river water and at widely varying concentrations. The level of endotoxins was higher in summer than in winter but did not always reflect the numbers of the microorganisms (Table 1).

The endotoxins producers targeted for this study, namely the cyanobacteria (*Microcystis*, *Anabaena*, *Oscillatoria* and *Pseudo-anabaena*) and enteric bacteria (*E. coli* and total coliforms), were identified in river water in summer and winter. The absence of cyanobacteria from samples taken during the second sampling occasion in summer was probably because of excessive rain just before and during sampling. A river in flood can carry reduced numbers of cyanobacteria. As expected, the target bacteria could not be detected in groundwater during all of the sampling periods.

Container water quality

Endotoxins in drinking water from household storage containers

Endotoxins were detected in all water samples from the household containers, and the concentrations varied within the range 0.1–24.7 $\mu g l^{-1}$. Figure 1 shows that the median value of endotoxins in all sample types exceeded the NZMH (2000) guideline value (3 $\mu g l^{-1}$) at the 90th percentile. This suggests that the health-related quality of the water in containers made it unsuitable for drinking.

Further analyses using the rank-sum and signed-rank statistical tests showed that the levels of endotoxins in the FV water samples for both light and dark containers were not significantly different ($P = 0.739$). The concentration of endotoxins in DB water samples from light containers was significantly higher than the concentration of endotoxins in the dark containers ($P \leq 0.001$). The endotoxins levels in DB samples were significantly higher than endotoxins in the FV samples ($P \leq 0.05$).

These results show that the light-influencing properties of the container type play a significant role in the levels of endotoxins in these containers. It also suggests that the significantly higher concentration of endotoxins in DB samples (compared with FV) was because of higher levels of

Table 1 | Microbe numbers and related endotoxins concentrations in water sources

			Results presented at the 50th percentile				
Source	Season	Number of samples	Cyanobacteria cells (ml^{-1})	*E. coli* (CFU/100 ml)	Total coliforms (CFU/100 ml)	Endotoxin (EU ml^{-1})	Turbidity NTU
River water	Summer	1	64,505	100	22,000	47	25.7
		1	nd	1,100	280,000	54	28.3
	Winter	1	92	300	32,000	22	1.7
		1	69	90	9,000	4	1.4
Groundwater	Summer	1	nd	nd	nd	nd	1.0
		1	nd	nd	nd	nd	0.2
	Winter	1	nd	nd	nd	nd	0.2
		1	nd	nd	nd	nd	0.4

nd = not detected.

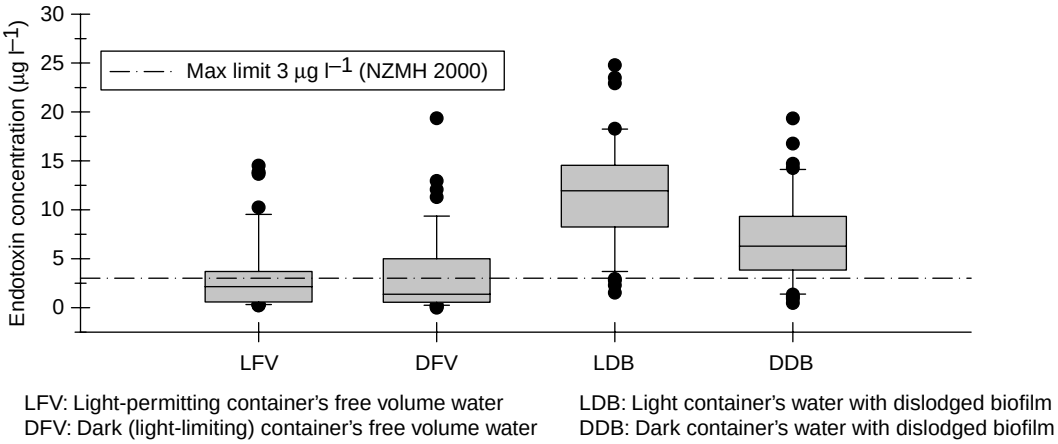

Figure 1 | Endotoxins concentration in drinking water stored in household containers (median value: solid black line in each box; 90th percentile: cap at the top end of the whisker).

biofilm in the containers. This indicates poor container hygiene; in short, people do not wash their vessels regularly before filling with fresh water supply, allowing biofilm–and with it endotoxins–to build up in the containers.

The source did not appear to play a role in the higher levels of endotoxins in containers. To demonstrate this, results of only the FV samples (excluding the biofilm-related activities demonstrated by the DB samples) are shown in Table 2. The FV samples of containers filled at groundwater sources contained significantly lower levels of endotoxins ($P < 0.001$) than those sourced from surface waters. This applied for both light and dark containers. The endotoxins in the FV for light and dark containers were not significantly different for each source ($P = 0.238$).

Turbidity

Turbidity levels of the water sampled from containers significantly increased ($P < 0.001$) in every sample after dislodging of biofilm particles from the inner side-walls of both light and dark containers–exceeding the 1 NTU standard of the South African National Standard: Drinking Water SANS 241 (SABS 2005). There were, however, no significant differences in the turbidity in LFV and DFV ($P = 0.822$) as well as those for LDB and DDB ($P = 0.565$). This supports the conclusion drawn above that containers had excessive biofilm forming on their inner side-walls which could be sources of endotoxin-producing bacteria.

Bacteria

Cyanobacteria. Analysis of the container water samples collected from surface and groundwaters showed the presence of three cyanobacteria genera with the potential to produce toxins, namely *Microcystis* sp., *Oscillatoria* sp. and *Anabaena* sp., notably lipopolysaccharide (LPS) endotoxins and also microcystin (Fosso-Kankeu *et al.* 2008). Water samples from the light-permitting containers after dislodging biofilm (LDB) contained significantly higher ($P = 0.004$) numbers of cyanobacteria than the biofilm suspension sampled from the DDB and the waters sampled from the LFV and DFV container groups–the latter three did not differ significantly in terms of cyanobacteria numbers ($P = 0.349$). This implied that cyanobacteria were more likely to occur in biofilm that forms in light-permitting containers.

Escherichia coli. *E. coli* occurred in substantial numbers (up to thousands of CFU/100 ml) in container water

Table 2 | Endotoxins concentration in container water filled from ground and surface water sources

	Mean values endotoxin (µg l^{-1})		
Sampling area	**LFV**	**DFV**	***P***
Groundwater area	1.69	1.52	0.697
Surface water area	3.28	2.76	0.305
P	<0.001	<0.001	

LFV: light free volume; DFV: dark free volume.

samples used for drinking purposes. The *E. coli* numbers from the free volume of water compared with the same water containing dislodged biofilm were significantly higher in light containers ($P = 0.048$). The numbers of *E. coli* in dislodged biofilm from dark (DDB) containers were not significantly different ($P = 0.109$) from the free volume in the same containers. This suggested that *E. coli* were more likely to accumulate in the biofilm of light-permitting containers than in the biofilm of dark containers.

Total coliforms. The numbers of total coliforms in the FV of both light and dark containers were not significantly different ($P = 0.563$). The numbers were also not different ($P = 0.058$) in the light and dark containers with biofilm suspension. However, the numbers of total coliforms were significantly higher ($P < 0.001$) in the samples of dislodged biofilm suspensions than those in samples from the FV containers. This implied that total coliforms did accumulate in all the drinking water containers' biofilm with light not playing a role.

Relationship between bacteria numbers and endotoxins concentrations in containers

Figure 2 shows that the concentrations of endotoxins increased in samples with dislodged biofilm essentially from light containers, which was concurrent with significantly higher numbers of total coliforms and *E. coli* as well as cyanobacteria in water sampled from the same type of container. The highest concentration of endotoxins was detected in LDB, which also contained the highest numbers of cyanobacteria, *E. coli* and total coliforms. Simple linear regression analyses (using SigmaPlot v10) of the data showed that endotoxins occurrence did not correlate meaningfully with the bacteria concentrations in the containers; the tendency was nevertheless that the light-permitting plastic containers were more conducive to sustaining higher numbers of the endotoxin-producing bacteria. For endotoxins versus cyanobacteria, $r = 0.133$, endotoxins versus *E. coli*, $r = 0.053$, while endotoxins versus total coliforms had the highest correlation ($r = 0.31$). The correlation between endotoxins and total coliforms, although weak, was stronger than with the other bacteria groups and close to the correlation ($r = 0.26$) reported by Rapala *et al.* (2002) for endotoxins and total coliforms.

GENERAL DISCUSSION

The differences in the numbers of microorganisms in water sources were not sufficiently significant to be attributed to the variation of season. As the studied area is subtropical but also semi-arid, with some parts on the lee side of a mountain range, the temperatures were quite similar during both seasons and were always sufficiently high to promote the growth of cyanobacteria and total coliforms. In terms of

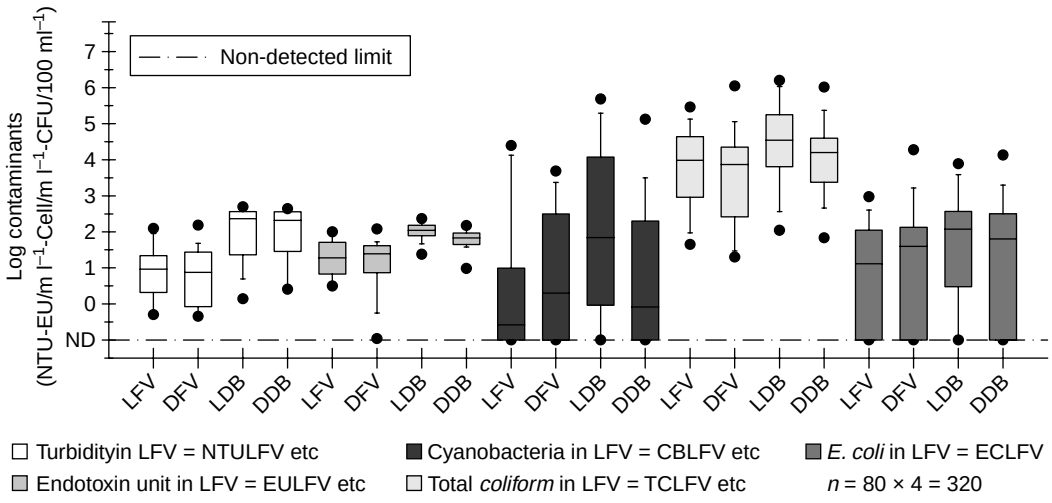

Figure 2 | The levels of turbidity, endotoxins, cyanobacteria, total coliforms and *E. coli* in light and dark containers.

source water, endotoxins and the bacteria groups were detected only in river water (Table 1) and not in the groundwater. At the 50th percentile, endotoxins concentrations in the surface water sources exceeded the proposed maximum acceptable limit of $3\,\mu g\,l^{-1}$ (NZMH 2000) in summer but not in winter which, in terms of the sources, suggested that seasons may have played a role. However, the sample size (four samples) was not sufficient for this to be meaningful.

Groundwater was significantly less contaminated. It was therefore expected that we would find significantly more of the target contaminants in the containers of households from the village using surface water more often. This could not be statistically demonstrated as will be discussed below.

The health-related quality of water in the drinking water containers was poor. More than 60% of the containers' water sample types analysed in this study showed unacceptably high concentrations of endotoxins in water intended for drinking regardless of the source, especially those samples with the dislodged biofilm suspension.

Although there was no significant correlation between the turbidity and the number of bacteria, significant increases ($P \leq 0.001$) in turbidity after brushing (NTU LV versus DB, Figure 2) demonstrated the release of biofilm from container inner side-walls as was also reported by Jagals *et al.* (2003) as well as Fosso-Kankeu *et al.* (2008). Bacteria from contaminated source points, essentially river water, as well as from improper handling of containers and their water content at home (Jagals *et al.* 2003; Moabi 2006), will attach to the inner side-walls of plastic containers and subsequently accumulate to form part of the biofilm. The condition of the containers as well as home storage and handling activities will therefore promote, on the plastic containers' inner side-walls, the formation of biofilm that, as was shown in this study, harboured or consisted of substantial numbers of cyanobacteria and enterobacteria.

De Philippis *et al.* (2005) showed that cyanobacteria have the capability to attach and form biofilm on solid surfaces at the water and solid material interface, using polysaccharide as the first step in the colonisation of the surfaces. Rapala *et al.* (2002) also reported that cyanobacteria are able to stimulate the growth of heterotrophic bacteria. Since enterobacteria are not light dependent, this implies that more cyanobacteria occurring in biofilm could support subsequent attaching of high numbers of enterobacteria including *E. coli*.

In our study, container biofilms were associated with the release of elevated concentrations of endotoxins into the containers' water content, especially when biofilm is washed off into the free volume of water during filling and handling of containers.

The co-occurrence of the highest concentrations of endotoxins and bacteria in dislodged biofilm from light containers further suggests that bacteria attached to the inner side-walls of containers contribute to increased levels of endotoxins in the containers' water. Despite the significant increase of bacteria and endotoxins concentrations in biofilm, the correlations between these parameters were weak as shown above, especially between endotoxins and cyanobacteria as well as between endotoxins and *E. coli*. A possible reason for this could be an underestimation of endotoxins in the water; previous studies have shown that cyanobacterial endotoxins are less active in the LAL test (Rapala *et al.* 2002). While the low numbers of *E. coli* were unlikely to markedly influence the concentration of endotoxins, the collective total coliform group appeared to have had some demonstrable association with the higher concentration of endotoxins, which suggested that this bacteria group might contribute more to LAL-measurable endotoxins than *E. coli* by itself. Assuming therefore that cyanobacteria and especially total coliforms are potent sources of endotoxins release into the container waters, the weak correlation between the bacteria numbers and the endotoxins levels in the test waters could be ascribed to the ability of the LAL chromogenic assay to also detect endotoxins from non-viable and/or dead bacteria (Jorgensen *et al.* 1979), which would be the reason for the disproportion between the endotoxins levels and viable bacteria numbers.

The quality of the source water does not appear to have influenced the occurrence of endotoxins in containers. This suggests other environmental contamination sources, possibly because of container management (handling and storage conditions) in the dwelling, contributing to numbers of bacteria and thereby indirectly contributing to the quantities of endotoxins in the container water.

The findings of this study suggest that collection, storage and handling of container water in rural households promote the accumulation of cyanobacteria and total coliforms (including *E. coli*) in container biofilm. Significantly larger numbers of bacteria accumulate in light-coloured plastic containers than in the other container types, thereby increasing the potential of releasing endotoxins into these waters. Waters in light-permitting plastic containers were of the poorest microbial quality as they contained significantly more biofilm and toxin. These containers appear to be more likely to contain high levels of cyanobacteria as well as total coliforms in the water (Fosso-Kankeu *et al.* 2008), which could also have contributed to higher concentrations of endotoxins.

While the community did experience consistent incidences of diarrhoea, the relationship with the diarrhoea incidence and levels of bacteria or endotoxins in their drinking water containers was not assessed since this was not part of the study design. Jakubowski & Ericksen (1980) reported that symptoms related to endotoxins ingestion by humans include nausea, asthenia, diarrhoea, vomiting and rash. No evidence could be found in literature that these effects could be expected as a result of ingesting excessive quantities of environmentally occurring endotoxins especially from contaminated container-stored drinking water.

The health significance of ingested endotoxins, especially at levels detected in this study, is therefore uncertain. However, there should be some consideration of the potential effects of ingesting high levels of enterobacteria from container water, as was the case in the study area. Frequent ingestion of such high numbers of enterobacteria could result in the excessive accumulation of some bacteria groups in the gut of consumers (Wiest & Rath 2003). In particular, pathogenic strains of *E. coli* have been reported by Satterwhite *et al.* (1978) to possess the ability to attach to the epithelium layer of the intestine. Apart from a realistic risk of diarrhoea (Closs *et al.* 1998; Madigan *et al.* 2000), chronic ingestion and therefore persistent accumulation of high concentrations of enterobacteria in the intestinal tract could promote translocation of viable bacteria as well as debris from died-off bacteria, especially LPS endotoxins (Berg & Garlington 1979), into the mesenteric lymph nodes and other organs causing liver cirrhosis, multiorgan failure, leucopenia, tachycardia, tachypnoea, hypotension and gastrointestinal disorders (Schletter *et al.* 1995; Wiest & Rath 2003). Whether these health problems are prominent in the study area was not investigated. However, bacterial translocation syndromes similar to those discussed here have been reported to claim substantial numbers of lives annually in the USA (Nogare 1991).

While healthy persons would possibly be unaffected by bacterial translocation or persistent ingestion of waterborne endotoxins, it is more likely to affect immunocompromised people such as those infected by HIV. Such individuals are likely to have impaired gut mucosal barriers, especially those with very low CD4 counts (Veazey *et al.* 1998; Brenchley *et al.* 2004, 2006; Shankar *et al.* 2007). Significantly high levels of plasma LPS were found by Brenchley *et al.* (2006) in chronically HIV-infected persons and, although these authors did not postulate on the potential sources, it is possible that persistent ingestion of endotoxins could be a risk to the health of immunocompromised individuals–a risk that could also be prevalent in the study area.

CONCLUSIONS

In terms of potential health consequences, ingesting endotoxins at the levels measured in samples of the waters used in the households of the study area, the water would not be suitable for drinking according to a guideline proposed by the New Zealand Ministry of Health (NZMH 2000). The numbers of cyanobacteria in most of the samples exceeded the critical levels contained in WHO guidelines. Total coliform numbers (including *E. coli*) measured in this study did not comply with health-related water quality guidelines (WHO 2004; SABS 2005) and it is plausible to suppose that such numbers could cause acute effects such as diarrhoea, or chronic effects after consistent ingestion of these bacteria and their related endotoxins especially through subsequent translocation of bacteria and endotoxins from the human gut into the body (Madigan *et al.* 2000; Wiest & Rath 2003). This bears further investigation especially when considering consumers such as children, the elderly and immuno-compromised people.

The levels of endotoxins could possibly be higher considering the reported shortcoming of the LAL test: that is, to underestimate bacterial endotoxins in an environmental sample. A more suitable test approach could be to use human cell-based immune-reactant tests as was reported by Wichmann *et al.* (2004) and Jagals *et al.* (2006).

The immediate need is for people in these circumstances to reduce the concentrations of endotoxins in biofilm in containers or introduce other measures to protect the health-related quality of water at the point of ingestion. Technologies generally effective for the removal of endotoxins from water such as chlorination and ultra-filtration are costly and/or require skill and techniques often not affordable by or available to rural and poor households. A practical approach to control the level of endotoxins at the point of the water's ingestion would be to implement a sustainable point-of-use treatment system such as filtration capable of removing microorganisms and, with that, also reducing potential endotoxins concentrations. However, the most effective solution could be management of container hygiene.

ETHICS

This particular study did not involve the direct participation of human subjects but access was required to the water stocks of households for which free and informed consent was obtained from the head of the household. Ethics approval was obtained (Approval number 19/06: July 2006) from the Research and Ethics Committee of the Faculty of Health Sciences, University of Johannesburg.

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