A scoping study on the prevalence of *Escherichia coli* and *Enterococcus* species in harvested rainwater stored in tanks

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ABSTRACT

Rainwater harvesting (RWH) is a relatively inexpensive technology that has the potential to provide safe water in communities where conventional technologies are difficult to implement. In this study, the microbiological quality of rainwater harvested from rooftops and ground-surface runoff was evaluated based on the concentrations of *Escherichia coli,* total coliforms and enterococci. Samples were collected from 15 roof-harvested rainwater (RHRW) tanks, 4 ground-surface runoff rainwater harvesting (GRWH) tanks, 3 rivers and 1 spring water source in the Eastern Cape Province of South Africa, and 14 RHRW tanks in Gauteng Province. In the Eastern Cape Province *E. coli* and enterococci were detected in 7 and 4 of the 15 RHRW tanks, respectively. Enterococci were detected only from one river whereas *E. coli* was detected in all three rivers; in spring water neither enterococci nor *E. coli* were detected. Samples from GRWH tanks were positive for *E. coli* and enterococci in 2 and 3 of the 4 tanks, respectively. In Gauteng Province, *E. coli,* coliforms and enterococci were detected from 6, 6 and 9 of the 14 rainwater tanks, respectively. On average, *E. coli* and enterococci were detected in 44.8% of the RHRW tanks, although enterococci concentrations were several times higher than those for *E. coli.* We further evaluated the significance of urban pigeons as the likely sources of contamination by isolating 156 enterococci from 30 pigeon faecal samples and 208 enterococci from RHRW samples collected from Gauteng Province. Matrix-assisted laser desorption ionisation identification of the various enterococci revealed 4 species – *E. faecalis* (20.5%), *E. mundtii* (20.51%), *E. faecium* (23.1%) and *E. casseliflavus* (17.3%) – to be dominant in faecal samples, whereas *E. casseliflavus* (34.6%) and *E. mundtii* (33.2%) were dominant in RHRW.

Keywords: rainwater harvesting, contamination, indicator bacteria, health risks

INTRODUCTION

Roof-harvested rainwater (RHRW) is one of the major alternative water sources used in South African rural communities without access to piped water (Kahinda and Taigbenu, 2011). The water is generally considered to be clean and is used without prior treatment. This perception is supported by a number of studies which showed that RHRW poses no increased risk when compared to municipal piped water (Heyworth et al., 2006). In contrast, a number of other studies on the microbial quality of RHRW have reported the presence of specific zoonotic pathogens in individual or communal rainwater harvesting (RWH) systems (Ahmed et al., 2010; Mpogui and Mpogui, 2012).

The major sources of pollution in RHRW are faeces of animals. Animals can carry a wide range of human gastrointestinal pathogens either through being diseased or as healthy carriers (Cox et al., 2005). The pathogens can be excreted in their faeces and may include the bacteria *Campylobacter* spp., *Listeria* spp., *Salmonella spp.*, *Aeromonas* spp., *Vibrio* spp., *Yersinia*, spp. and *Escherichia coli* O157:H7, the protozoa *Giardia* spp. and *Cryptosporidium* spp., as well as the bacteria used as pollution indicators, including *E. coli,* total coliforms and enterococci (Curtis et al., 2000; Cox et al., 2005).

Households in rural communities practice free-range domestic animal rearing and faecal matter is a common feature on the ground around their homesteads. The presence of faecal matter is a significant contamination risk factor since dried faecal matter can be blown by wind onto roof surfaces. Following rain events, faecal droppings and other organic debris deposited on the roof and gutters can be transported into the tank with roof runoff. The actual level of risk from potential pathogens in RHRW can be influenced by several factors including the type and number of pathogens carried by the infected animals, the time between deposition of faecal matter on the roof and pathogens being flushed into the tank, the form of exposure (ingestion from drinking vs. exposure to droplets in the shower or toilet flushing), and the relative persistence of the different pathogens (Ahmed et al., 2011). However, in the South African context, most roofs in rural communities are made from metal sheets and temperatures are high during summer which influences the survival and persistence of pathogens in dust and faeces due to desiccation and solar radiation.

To determine the acceptability of RHRW for drinking, it is common practice to use drinking water guidelines. In most guidelines, this entails the non-detection of faecal indicator bacteria such as *E. coli* or enterococci (usually at numbers below 1 CFU/100 mℓ water), whose presence is used to indicate potential faecal contamination of the water (World Health Organisation, 2004).

Although *E. coli* is widely used to assess RHRW quality a number of recent studies have reported that *E. coli* may be of limited use for comprehensive evaluation of harvested rainwater microbial quality. In these investigations a number of samples yielded culturable *Enterococcus* spp. but not *E. coli* (Spinks et al., 2006; Sazakli et al., 2007; Ahmed et al., 2008) the microbiological quality of roof-harvested rainwater was assessed by monitoring the concentrations of *Escherichia coli*, enterococci, *Clostridium perfringens*, and *Bacteroides* spp. in rainwater obtained from tanks in Southeast Queensland, Australia. Samples were also tested using real-time PCR (with SYBR Green I dye. The half-life of *E. coli* in non-host environments has been reported to be approximately 1 day in water,

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1.5 days in sediments and 3 days in soil. These estimates imply that *E. coli* does not persist for long in non-host environments (Winfield and Groisman, 2003). Hence *E. coli* is most useful in identifying recent contamination since it is not as environmentally long-lived as many pathogens (i.e. viruses and protozoa). Consequently, additional complementary tests examining for the more robust enterococci and the spores of *Clostridium perfringens* have been used to shed light on less recent faecal contamination (Ahmed et al., 2011).

Pollution sources in RHRW include windblown dust and faecal droppings on the roof surface, where they are exposed to solar radiation and consequent drying. Since enterococci are highly resistant to drying they are more likely to persist while the more sensitive *E. coli* are likely to die out. Consequently, enterococci may serve as a better faecal pollution indicator and the pathogen of choice in microbial water quality evaluation of RHRW (Ahmed et al., 2010).

Enterococci are a diverse group of Gram-positive gastrointestinal colonisers with lifestyles ranging from intestinal symbionts and environmental persisters to multidrug resistant nosocomial pathogens (Kühn et al., 2003). The *Enterococcus* genus includes more than 20 species, most of which are part of the intestinal flora of mammals, reptiles, birds, and other animals, while some species have been isolated from non-faecal sources. Different groups of species predominate in different hosts, for example *E. faecalis* and *E. faecium* are dominant in the human digestive tract, whereas *E. cecorum, E. durans, E. faecalis, E. faecium* and *E. hirae* are dominant in poultry (Devriese et al., 1993).

A number of researchers have shown enterococci to be more prevalent in RHRW than *E. coli*, and enterococci have been suggested to be a better indicator for assessing faecal contamination (Ahmed et al., 2011). However, few studies have characterised *Enterococcus* spp. from RHRW (Ahmed et al., 2012a). In this study our aims were: (i) to investigate the prevalence of faecal indicator bacteria in harvested rainwater and alternative water sources used by rural households, and in the various environmental settings in which rainwater harvesting

(RWH) is practised in South Africa, and (ii) to evaluate the prevalence of *Enterococcus* sp. in RHRW and faecal droppings of pigeons, as the most likely source of RHRW contamination.

METHODOLOGY

Sampling site description and water sample collection

Sampling areas were comprised of farm, urban residential and business, and rural settlement settings in the Gauteng, North West and Eastern Cape Provinces (Table 1). The sampling environments were divided into 6 sampling areas and included Johannesburg, (JHB, 1 area) and Pretoria (PTA, 3 areas) in Gauteng Province, Brits (BTS, 1 area) in the North West Province and Port St Johns (PSJ, 1 area) in the Eastern Cape Province. Sites in Pretoria included the University of Pretoria Experimental Farm with 3 RWH tanks (PTA1, 3 tanks), the Plant Science Building, University of Pretoria Hatfield Campus (PTA2, 3 tanks), and a household in Sunnyside, Pretoria (PTA3, 1 tank). The Johannesburg sites included Thembisa township (JHB1, 6 tanks), and Weltvreden Park (JHB2, 1 tank) whereas the Brits site was Ifafi suburb near Harteesbeespoort (BTS1, 1 tank). The Port St Johns (PSJ) sites included 15 RHRW tanks from 15 households (PSJ1 to 15), 4 ground-surface runoff harvested rainwater (GHRW) storage tanks, 3 rivers and 1 spring water source.

The PTA1 site represents a typical farm setting; with a cattle pen within 50 m of the rainwater tanks where masses of doves and pigeons feed on cattle feed. The house on which rainwater tanks were installed has overhanging mulberry trees on two sides of the roof, where various kinds of birds feed on mulberries. The roof catchment surface on these two sides feeds water into two separate tanks. The third side of the roof is free of vegetation cover and feeds into a separate tank. Samples from this site were collected 3 times from the beginning of to the middle of the rain season, whereas the rest of the samples from other sites were collected on a once-off basis during mid-rain season (2012 to 2013).

TABLE 1
Summary of sampling sites and water samples collected

Province	Area	Location	Setting	*RHRW	*GHRW	River	Spring
Gauteng	Johannesburg	Thembisa township	Urban/residential	6	–	–	–
		Weltvreden park	Urban/residential	1	–	–	–
	Pretoria	University of Pretoria, Experimental farm	Farm	3	–	–	–
		University of Pretoria, Hatfield campus, Plant Science building	Urban/business	3	–	–	–
		Sunnyside (household)	Urban/residential	1	–	–	–
North West	Brits	Hartbeespoort, Ifafi	Urban/residential	1	–	–	–
Eastern Cape	Port St johns	Luthengele village	Rural	15	4	3	1

*RHRW: roof-harvested rainwater; *GHRW: ground-harvested rainwater*

The PTA2 site is located on the second floor of the Plant Science Building at the University of Pretoria Hatfield Campus. Three tanks were installed and the site represents a typical urban business setting where there is minimal vegetation and bird interference. Water harvested at this site is used to irrigate flowers and experimental plants. The Thembisa site (JHB1) represents a typical urban township. Schools in this township were provided with RWH systems for irrigation of the school garden for the school feeding programme. The Weltevreden Park (JHB2) and Hartebeespoort (BTS1) sites have modern RWH systems with first-flush diverters and a complex filtering system and were included for comparative purposes.

The PSJ site in the Eastern Cape Province, Port St Johns is located in Luthengele village, which is situated in a mountainous area. The terrain in this village is such that municipal water supply services would be too expensive to implement. Hence, the people rely mainly on river and harvested rainwater (HRW). The community benefited from government projects where rainwater tanks were installed for potable and domestic food-gardening purposes. In this village RHRW is stored in above-ground tanks and is used for potable purposes whereas GHRW is stored in underground tanks and used for domestic food gardening. Water from the local rivers and spring is used to supplement RHRW for potable purposes. To evaluate the influence of water handling practices, water collected from tanks and stored prior to use in kitchens (hereafter referred to as kitchen water) was also collected (in 2-ℓ containers) from 2 households (PSJ1 and PSJ2), and for the rest of the households (PSJ3-15) samples were collected from RWH tanks only. In addition, GHRW samples were also collected from 4 households that had installed tanks to harvest surface runoff at their homesteads.

Samples were collected in duplicate from the outlet taps located close to the base of the tanks, in sterilised 2-ℓ containers. Taps were wiped with 70% ethanol, and allowed to run for 30 to 60 s to flush out stagnant water from the taps before collecting water samples. Samples were transported to the laboratory and processed within 24 h.

Microbiological analysis of water samples

Undiluted water samples were assayed directly for densities of total coliforms, *E. coli*, and enterococci with Colilert and Enterolert chromogenic substrate tests kits and Quantitray 2000 trays (Idexx, Westbrook, Maine), as per the manufacturer's instructions. Water samples (100 mℓ) were poured into Quantitrays which were then sealed and incubated at 35°C (Colilert for total coliforms and *E. coli*) and 40.5°C (Enterolert for enterococci) for 24–28 h. Following incubation, the Colilert Quantitray wells were read for yellow colour and fluorescence (total coliforms and *E. coli*, respectively), and Enterolert Quantitrays were read for fluorescence only (enterococci). A bench-top ultraviolet (UV) light (366 nm) was used to identify fluorescent wells. The manufacturer-provided (Idexx, Westbrook, Maine) most probable number (MPN) table of colony forming units (CFU) was used to generate microbial density estimates based on the proportion of positive reactions in each tray.

Faecal sample collection

Thirty fresh faecal droppings were collected from pigeons that came to feed at the cattle feedlot of the University of Pretoria

Experimental Farm. Indicator bacterial density in faecal material was measured by first diluting 1 g of faeces in 9 mℓ distilled water, vortexed and allowed to stand for 5 min to allow debris to settle. A 1-mℓ sample of the supernatant was consequently extracted and serially diluted for microbial isolation and quantification. Densities of total coliforms, *E. coli* and enterococci were determined with Colilert-18 and Enterolert-18 chromogenic substrate tests kits and Quantitray 2000 trays (Idexx, Westbrook, Maine), as per the manufacturer's instructions. The diluted sample (1 mℓ) was mixed with 99 mℓ sterile water to meet the 100 mℓ requirement for Quantitray 2000 trays for quantification.

Recovery of isolates and presumptive identification

Following incubation, the backing material of each Quantitray was disinfected by application of 70% ethanol with a sterile swab. After the residual ethanol evaporated, sterile razor blades were used to pierce the backing material of 3 fluorescence-positive wells per tray and 3 trays were processed per water sample. One loop full of well content was streaked onto *Enterococcus* spp. selective agar (Merck, Johannesburg). Presumptive enterococci positive colonies were sub-cultured twice on nutrient agar (Merck, Johannesburg) and re-inoculated into 200 µℓ of Enterolert chromogenic media (Idexx, Westbrook, Maine) in sterile 96 microwell plates and incubated for 18 h to confirm fluorescence before matrix-assisted laser desorption ionisation (MALDI-TOF-MS) analysis.

Matrix-assisted laser desorption ionisation time of flight mass spectroscopy identification and characterisation of bacterial isolates

Bacterial strains were sub-cultured twice on nutrient agar (Merck, Johannesburg) before matrix-assisted laser desorption ionisation (MALDI-TOF-MS) analysis. Samples were processed as previously described and the whole process from MALDI-TOF-MS measurement to species identification was performed automatically without user intervention (Bittar et al., 2009; Pinto et al., 2011). MALDI Biotyper 3.0 software (Bruker Daltonics, Germany) was used to analyse raw spectra of the bacterial isolates, with default settings. The software compares acquired sample spectra to reference spectra in the provided database. The degree of spectral pattern matching is expressed as a logarithmic identification score and interpreted according to the manufacturer's instructions. Results are expressed as log (score) values ranging from 0 to 3 levels. Scores ≥2.300 indicate species identification with a high level of confidence, ≥2.000 indicates species identification, 1.700–1.999 indicates genus identification, and <1.700 no identification (Romanus et al., 2011).

Statistical analysis

All statistical analyses were carried out using Statistica 10 (Statsoft, USA). Data for the microbial concentrations were separated into areas: JHB, PTA, BTS and PSJ. Water samples were divided into RHRW, GHRW and alternative sources (spring and river). To compare differences in microbial concentration graphical representations of mean values of the various indicator bacteria were used. The various enterococci species were grouped by their sources to compare their respective percentage prevalences.

RESULTS

Samples from Gauteng Province (Johannesburg and Pretoria)

Fourteen RWH tanks were sampled from Johannesburg and Pretoria sites. *Escherichia coli* were detected in 9 tanks from 5 of the 6 sites: JHB1 (3 tanks), PTA1 (3 tanks), PTA2 (2 tanks) and PTA3 (1 tank). The concentrations of *E. coli* from two of the tanks from JHB1 were 3.1 and 15.3 CFU/100 mℓ, whereas the concentrations in the other tank were >2 149.6 CFU/100 mℓ. Although *E. coli* were detected in all three tanks from site PTA1 the concentrations varied with the presence of overhanging tress as detailed below. At site PTA2 *E. coli* counts were 103.6 and 145 CFU/100 mℓ in the two positive tanks. Total coliforms were detected in all of the tanks at site PTA2 and the concentrations ranged from 10.9 to >2419.6 CFU/100 mℓ with an average of 716.5 CFU/100 mℓ. Enterococci were detected in the tanks from the sites JHB1 (3 tanks), JHB2 (1 tank) PTA2 (2 tanks) PTA3 (1 tank) and BTS1 (1 tank). The concentration of entero-cocci detected ranged from as low as 1.7 to >2 419.6 CFU/100 mℓ , with an average of 715.1 CFU/ mℓ.

Samples were collected from 2 sites (JHB2 and BST1) that use modern RWH systems for all their domestic purposes, including drinking, bathing and washing. The RWH systems were installed with first-flush diverters and multiple filtration systems. Water samples were collected directly from the tank before filtration and at the point of use after filtration, including water from the geyser. Water samples collected after filtration points from both sites tested negative for *E. coli*, total coli-forms and enterococci. Water samples collected directly from the tank tested positive for enterococci at both sites (JHB2 and BST1), whereas only the JBH2 site tested positive for total coli-forms. The detected enterococci concentration observed for the JHB2 site was 31.8 CFU/100 mℓ and that for the BST1 site was 7.5 CFU/100 mℓ, with a detected faecal coliform concentration of 8.6 CFU/100 mℓ.

The three tanks from PTA1 site (PTA1 to 3) were installed on a house located close to a cattle-feeding pen where masses of pigeons come to feed on the cattle feed. At the house where the tanks were installed there were overhanging mulberry trees on two sides of the house from which two of the tanks (PTA1-1 and PTA1-2) received roof runoff. However, the third tank (PTA1-3) was installed on the side where there were no overhanging trees. Although *E. coli* was detected from all of the tanks during the three sample collections, the concentrations were less than 25 CFU/100 mℓ, except for the samples collected from tanks PTA1-1 and PTA1-2 during the third sampling event, when the counts were >2 419.6 CFU/100 mℓ for both tanks. Concentrations detected from tank PTA1-3 during the three samplings were 4.1, 220 and 387.3 CFU/100 mℓ for enterococci; 31.8, 574 and 688.4 CFU/100 mℓ for total coliforms, whereas those for tanks PTA1-1 and PTA1-2 were >2 419.6 CFU/100 mℓ for all of the samples, for both ente-rococci and total coliforms. It is evident that samples from tanks on the roof sides with overhanging trees had consist-ently higher concentrations of indicator bacteria compared to the samples from the tank on the side without overhanging trees.

Prevalence of *Escherichia coli* and enterococci in roof-harvested rainwater samples from Luthengele village, Eastern Cape

The quality of water used by households in Port St Johns, Luthengele village (PSJ site), was evaluated based on *E. coli* and enterococci. *Escherichia coli* were detected in 7 of the 15 roof-harvested rainwater storage tanks and ranged from 1 to 8.6 CFU/100 mℓ (Fig. 1). Kitchen water was collected from 2 households (PSJ and PSJ2), and notable differences were observed when it was compared to the source tank water qual-ity. At Household PSJ1, enterococci were not detected in tank water but were detected in kitchen water at values of 10 46.2 CFU/100 mℓ. When all RHRW tanks were considered entero-cocci were detected in 4 of the 15 tanks (PSJ5, 6, 12 and 14) and the highest concentrations were observed from tank PSJ12 (>2 419.6 CFU/100 mℓ).

Three rivers and one spring water source used by house-holds were sampled and analysed (Fig. 2). Enterococci were only detected from one river (River 1) (1 299 CFU/100 mℓ).
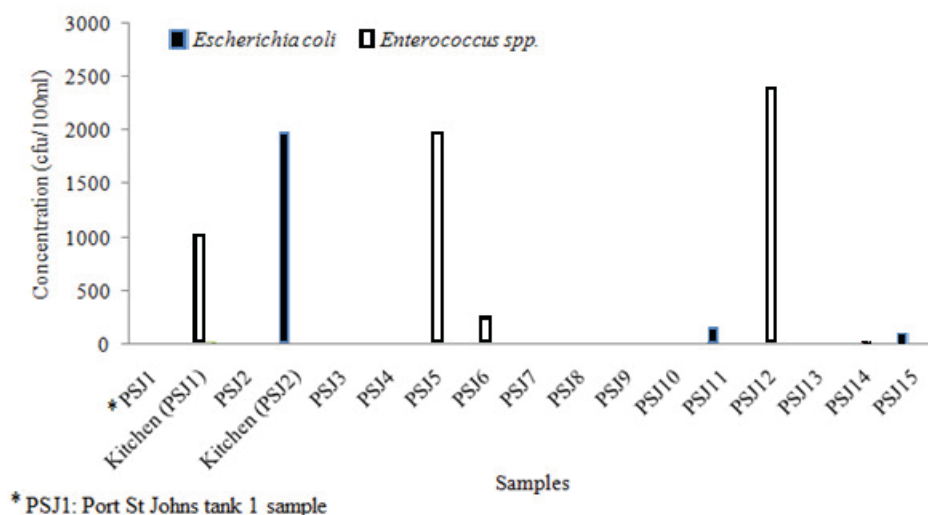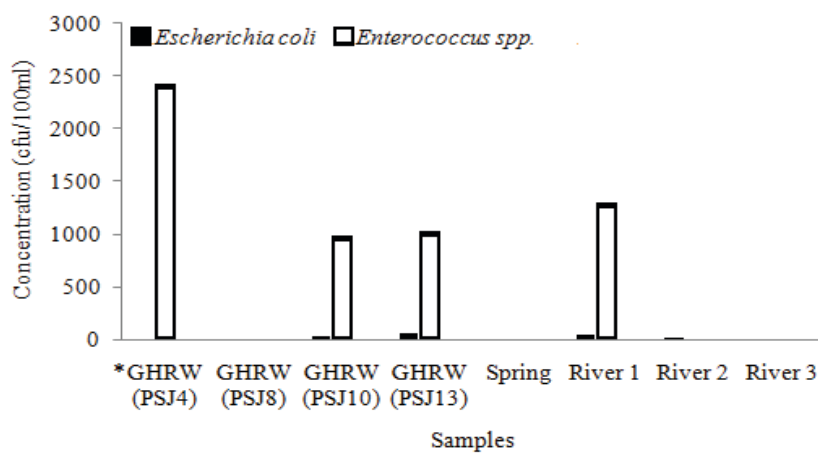

Figure 1
Prevalence of bacterial species in tank and kitchen water samples. Tank water samples were collected directly from rainwater harvesting tanks and kitchen water samples were collected from water fetched from the rainwater harvesting tanks but stored in the kitchen prior to use.

E. coli were detected in all three rivers at 3 to 16 CFU/100 mℓ. Rainwater harvested from ground-surface runoff and stored in underground tanks was positive for *E. coli* in 2 of the 4 tanks at 33.6 CFU/100 mℓ (PSJ10) and 64 CFU/100 mℓ (PSJ13). Three tanks (PSJ4, 10 and 13) tested positive for enterococci (980 to >2 419.6 CFU/100 mℓ). Interestingly, PSJ10 had the highest concentration observed for *E. coli* (64.7 CFU/100 mℓ) and enterococci (1 011 CFU/100 mℓ).

Prevalence of enterococci in roof-harvested rainwater and bird faecal samples

Considering the 30 fresh urban pigeon faecal samples collected from the cattle feeding lot close to site PTA1, 19 and 30 of the samples tested positive for *E. coli* and enterococci, respectively. A total of 364 enterococci were isolated from 30 pigeon faecal samples (156 isolates) and 11 RHRW tanks (208 isolates) from sites PTA1, PTA2 PTA3 and JHB1.

MALDI-TOF-MS was used for the identification of the various enterococci species. In total 7 enterococci species were identified (Fig. 3). Four species *E. faecalis* (20.5%), *E. mundtii* (20.51%), *E. faecium* (23.1%) and *E. casseliflavus* (17.3%) were prevalent in faecal samples whereas *E. casseliflavus* (34.6%) and *E. mundtii* (33.2%) were prevalent in RHRW samples (Fig. 3), although the concentrations of *E. faecalis* (18.7%) in RHRW were similar to those observed in faecal samples. The least abundant species were *E. durans* (2.5%) in both sample sources and *E. hadei* (4.5% in faeces and 2.9% in RHRW). Although *E. galinarium* was observed in low proportions in RHRW (0.96%), it constituted 11.5% of faecal isolates. Significant differences in the relative abundance of enterococci species were observed for *E. casseliflavus* and *E. hadei*. The prevalence of *E. casseliflavus* and *E. mundtii* in faecal samples was almost half of the relative abundances detected in RHRW. *Enterococcus faecium* was 3 times more abundant in faecal samples (23.1%) than in RHRW samples (7.2%).

Figure 2

Prevalence of bacterial species in ground-harvested rainwater (ground-surface runoff rainwater collected in tanks) used for irrigation and 3 rivers and 1 spring water source used for potable purposes in Luthengele village Eastern Cape Province, South Africa
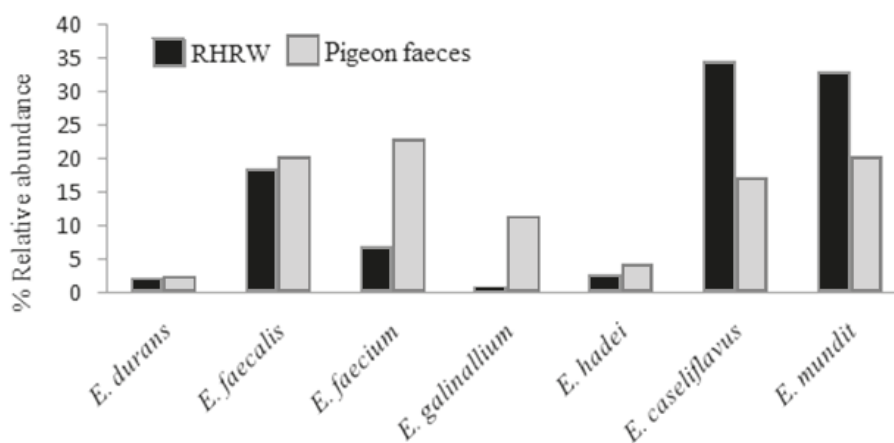

Figure 3

Enterococcus spp. distribution among 364 enterococci isolated from pigeon faeces collected from pigeons that gather at the University of Pretoria Experimental Farm cattle feedlot, and from roof-harvested rainwater from rainwater harvesting tanks in Pretoria (University of Pretoria Experimental Farm, Hatfield Campus Plant Science Building, and a household in Sunnyside) and Johannesburg (Thembisa Township and Weltevreden Park).

DISCUSSION

Rainwater quality from different technological and environmental settings

During our sampling visit we observed that the nature of the currently employed RWH systems varied from rudimentary to sophisticated. The most basic systems included a roof, gutter, and tank. A variety of makeshift containers from used drums to baths tubs, clay pots, etc., are used by villagers as storage containers. In such situations, neither first-flush diverter nor filtration system is employed. However, in modern RWH systems such as the ones from sites JHB2 and BTS1, first-flush diverters were installed together with a filtration system. Consequently, these samples had the lowest level of contamination and the residents at the households where the tanks were installed appeared to be better informed about RWH system management and potential health risks compared to their rural counterparts. While the use of modern rainwater systems would be ideal in terms of guaranteeing the water quality, the installation of such systems would be too expensive for rural households who can hardly afford the cost of purchasing a RWH tank.

Households in Luthengele village in the Eastern Cape Province of South Africa use river water, RHRW and spring water for potable purposes. The water from these sources is generally used without prior treatment. The water from the rivers flows directly from the groundwater table and therefore should be relatively free of faecal contamination. The water is collected at some distance after the points where it comes off the ground and these collection points are shared by both people and livestock. Animal faeces are a common feature at these collection points. However, analysis of the microbiological quality of river water did not reveal it to be worse than roof-harvested rainwater in terms of the observed microbiological counts. Of the river water collection points, 1 and 3 of the 3 collection points tested positive for enterococci and *E. coli,* respectively. Since these rivers are constantly flowing, the dilution effect play a significant role in determining the microbiological counts observed.

While the observed microbiological counts may suggest the water to be relatively clean, river water is an open system that is subject to many uncertain factors of contamination. Domestic animals drink water early in the morning and late in the afternoon at the same points from which the people fetch water. Since we collected our samples during midday from these points the observed microbial quantities may not give a true representation of the levels of contamination and potential risks. Hence, we cannot make a general statement concerning the microbial quality of these water sources. There is therefore a need to evaluate the variation in the microbial quality of the water over the course of the day in order to suggest appropriate times for people to fetch water when levels of contamination will be at their lowest.

Water in South African rural communities is fetched from the source (tank, river, borehole, etc.) using a variety of containers, and stored prior to use. Although the water might be clean at the source the process of fetching and storing prior to use exposes it to potential contamination. Comparison of the microbiological quality of RHRW sampled directly from the tanks and that which had been stored temporarily prior to use showed significant variations in microbiological quality. While we did not detect any significant quantities of indicator bacteria in the RHRW from both tanks tested, we detected high concentrations of enterococci in kitchen water from 1 of the 2

households. These findings suggest the containers used to store the water, or the process of fetching it, to be responsible for the contamination detected. It would be advisable therefore that the containers be thoroughly cleaned. However the most-common types of containers used to fetch water from RHRW tanks for storage prior to use are narrow-mouthed 20 or 25 ℓ containers which are difficult to clean inside. Consequently bacteria may accumulate in the form of biofilm creating a perennial source of contamination (Camper et al., 1998).

The microbiological counts observed in this water, especially with respect to *E. coli* may suggest the water to not be highly contaminated. However, consideration of alternative faecal indicator bacteria represented by enterococci suggests otherwise. The ground-harvested rainwater is stored for relatively long periods of time in the tanks before use. It is therefore most likely that *E. coli* will die with time, while the more resilient enterococci and other pathogens persist (Ahmed et al., 2012b). Hence, there is a need to evaluate the water quality by targeting specific pathogens and to evaluate the survival and persistence of various pathogens in relation to the presence of the commonly-used faecal indicators throughout the rainy season (Ahmed et al., 2010).

Roof-harvested rainwater quality

The microbiological quality of roof-harvested rainwater stored in tanks varied significantly from one household to another. While the water quality in some tanks was within drinking water standards, the microbiological quality of water from other tanks was so poor that treatment would be necessary before the water could be used for potable purposes. This variation supports the previous findings from a number of studies which have provided contradictory results on the quality of RHRW (Evans et al., 2006b; Ahmed et al., 2010). While some researchers report that RHRW microbial quality is within the acceptable drinking water quality range, others have reported the presence of indicator bacteria including pathogens and recommended pre-treatment before the water can be used for potable purposes (Ahmed et al., 2011). Our findings suggest that the quality of harvested rainwater stored in tanks is site-specific and depends on the management practices implemented by the households, as previously reported (Kus et al., 2010).

Faecal material on the roof surface has been cited as one of the major sources of contamination in RHRW. Faeces of birds, insects, mammals, and reptiles that have access to the roof can potentially contain a wide array of pathogens (Ahmed et al., 2011, 2012b). During rain events, debris on the roof surface or gutter, including animal droppings, can be washed by the roof runoff into the tank (Evans et al., 2007). Our results from the experimental farm are in agreement with this notion. The microbial quality of water from the roof side with overhanging mulberry trees was poor, with high concentrations of indicator bacteria, in contrast with that of the tank from the roof side without overhanging trees. The presence of high concentrations of indicator bacteria in samples suggests the presence of a direct source of faecal contamination.

From our observations, faecal contamination of RHRW appears to be limited to improperly designed systems, as well as systems that are not well maintained. Hence, guidance on RHRW systems will encourage good maintenance practices, including ensuring the cleanliness of the systems before rainfall events, especially roofs and gutters, which should be cleaned frequently, while the receiving tanks should be cleaned at least twice per year to improve the water quality (Cunliffe, 1998). The

roof should be kept clear of overhanging trees, which may provide access to the roof by animals and birds. Indeed, the high numbers of bacteria in bird faecal samples indicate the need for good maintenance of roofs and gutters and elimination of overhanging tree branches to minimise faecal contamination (Ahmed et al., 2012b).

The overall observation from the results of our study is that there are a number of critical factors responsible for the observed variations in roof-harvested rainwater quality. It is clear that further data relating to the prevalence of microbial contamination throughout the year, including indicator bacteria and specific pathogens and their persistence in rainwater tanks, is needed. Considering the survival and persistence of bacterial species in RHRW, previous studies have suggested disinfection before use as potable water, especially for drinking (Ahmed et al., 2010). It is however important that, before treatment can be implemented, the levels of actual risk of contamination and infection should be established so that informed decisions can be made on management and mitigation practices that will be instituted in regulatory or guidance information.

Indicator bacteria in roof-harvested rainwater

A number of studies have reported higher prevalence of enterococci in RHRW tanks compared to *E. coli,* similarly to our findings (Spinks et al., 2006; Ahmed et al., 2011). In consideration of these findings and other published research, Ahmed et al. (2008) concluded that *E. coli* could not be detected in a number of the water samples that were positive for other indicators of potential faecal origin. However, most of the promising alternative faecal indicators, including *Bacteroides* spp., *Bifidobacterium* spp., *Clostridium perfringens*, and viruses, have also been shown to have similar limitations to those of the conventional *E. coli* and enterococci (Ahmed et al., 2008). Hence, the current traditional faecal indicators, despite their flaws, may be the only practical option in assessing microbial quality of stored harvested rainwater (Evans et al., 2007).

In testing of RHRW quality, the use of *E. coli* and enterococci as dual sources of evidence on potential faecal contamination is recommended, rather than relying on their performance as individual indicators (Ahmed et al., 2011; McFeters et al., 1974; Savichtcheva and Okabe, 2007; Schets et al., 2010. The observed differential persistence rates between *E. coli* and enterococci may serve as a practical perfect fit in RHRW quality evaluation. This would require the creation of a scheme that integrates the short-lived nature of *E. coli* in stored harvested rainwater to show recent contamination, with the longer persistence of enterococci to show historical contamination. In the scheme it will be expected that enterococci will tend to accumulate whereas although *E. coli* may accumulate during frequent contamination events the extent will be of relatively lower magnitude. Hence it can be concluded that, in the absence of *E. coli*, higher values of enterococci concentrations suggest historical high levels of frequent contamination. In cases where both *E. coli* and enterococci are detected the relative proportions between them will show how recently the contamination occurred. The scheme would however need laboratory simulation and field testing before it can be adopted in RHRW quality evaluation and possibly risk assessment.

Enterococci in roof-harvested rainwater

We used MALDI-TOF-MS to identify enterococci isolates from RHRW and bird faecal samples, so as to obtain information on their ecology, diversity and potential sources. The presence of a variety of enterococci including *E. casseliflavus, E. faecalis, E. faecium, E. hirae* and *E. mundtii* in both RHRW and pigeon faecal samples suggests that the birds may have contributed to the enterococci detected in rainwater tanks. It is also possible that other sources of enterococci not tested in this study, including dust, vegetation and animals such as lizards, rats or frogs may have contributed to the enterococci detected in RHRW tanks (Evans et al., 2006a; Ahmed et al., 2012b)212 Enterococcus isolates from 23 rainwater tank samples in Southeast Queensland (SEQ.

The detection of *E. casseliflavus* as one of the dominant enterococci both in RHRW and bird faecal samples supports the previous finding which suggested that, although the species is considered to be mainly epiphytic (Mundt and Graham, 1968), its presence in the environment cannot be attributed exclusively to non-faecal sources. The presence of *E. casseliflavus* in faeces could be attributed to it being incorporated into the microbiota of the digestive tract of birds after consumption (Layton et al., 2010).

Previous studies have attributed the high prevalence of *E. faecalis* in rainwater tank samples to *E. faecalis* being ubiquitous in nature (Ahmed et al., 2012a) and that it may not have limited host specificity as previously reported (Wheeler et al., 2002). Furthermore, enterococci have been reported to be present in the faeces of non-human animals, including birds, which is similar to our findings (Kühn et al., 2003; Layton et al., 2010).

The presence of *E. mundtii* and *E. casseliflavus* in 10 of the 11 tanks sampled is not surprising, considering that these species have been reported to be associated with soil, plants and non-human animal hosts (Pinto et al., 1999). In this study, *E. mundtii* and *E. casseliflavus* constituted 67.8% of RHRW isolates and 37.8% of bird faecal isolates, supporting the notion that although birds are the most likely source of contamination, they may not be the sole source of these bacteria. These findings show the significance of enterococci speciation for its use as an indicator of faecal contamination. Furthermore, the lack of one dominant *Enterococcus* species in both faecal and RHRW samples suggests that no single *Enterococcus* is a reliable indicator of the host faecal source. However, strain diversity characterisation of enterococci has been suggested to provide supporting evidence for bacterial source identification, whether it be faecal, vegetation or soil (Layton et al., 2010)

CONCLUSIONS AND RECOMMENDATIONS

The contamination of RHRW appears to be strongly influenced by the environmental setting, especially the presence of a faecal source in the form of animal housing. While little can be done to avoid the presence of a faecal source around rural households practising animal husbandry, appropriate RWH system maintenance should be in place to lessen the levels of contamination. This should include system cleanliness, especially of the roof and gutters, before rainfall events.

Although we observed significant levels of contamination in RHRW it is our opinion that some level of indicator bacteria presence in RHRW may be tolerated. However, tolerable levels of contamination will depend on use and can only be established where good rainwater harvesting practices are being implemented. That is, after everything possible has been done to harvest clean rainwater, contaminant levels detected in the water may be what is normal for the area. However, this would need a proper risk assessment to evaluate the potential

risks and remediation measures, if necessary. Since the presence of pathogens cannot be correlated to faecal indicators in RHRW, we recommend the development of a risk assessment system based on the dual prevalence of *E. coli* and enterococci as sanitary indicator bacteria. Further research should focus on establishing the applicability of *E. coli* and enterococci dual use to show levels of contamination and potential health risk as applied to stored harvested rainwater.

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