Random survey of the microbial quality of bottled water in South Africa

Marthie M Ehlers*, Walda B van Zyl, Dobromir N Pavlov and Etienne E Müller
Department of Medical Virology, Institute of Pathology, University of Pretoria, PO Box 2034, Pretoria, 0001, South Africa

Abstract

Due to the increased demand and consumption of bottled water in South Africa, there has been a growing concern about the microbiological quality of this product. Retail outlets sell local as well as imported bottled water to consumers. The microbiological quality of 10 different (8 local and 2 imported) bottled water products were tested over a period of three months on days 1, 30 and 90. Tests for the detection of heterotrophic plate count (HPC) bacteria, total and faecal coliform bacteria, spore-forming *Clostridium perfringens*, somatic and F-RNA coliphages were performed on the samples. In addition samples were analysed for three selected enteric viruses, caliciviruses, enteroviruses and rotaviruses using the reverse transcriptase-polymerase chain reaction (RT-PCR). The results indicated that 8/10 of the bottled water samples analysed, met the requirements set by the South African Bureau of Standards (SABS) for HPCs in bottled water of less than 100 counts per mℓ. However, in two bottled water samples the average HPC bacteria counts were 2.64×10^2 cfu·mℓ$^{-1}$ and 8.89×10^3 cfu·mℓ$^{-1}$ respectively which exceeded the recommended SABS guideline. HPC counts showed a slight variation during the three-month period in the bottled water samples. Total and faecal coliform bacteria, enterococci, *C. perfringens*, bacteriophages or enteric viruses were not detected in any of the ten bottled water samples analysed. It can be concluded that the microbial quality of eight of the ten selected bottled water samples analysed was within the acceptable limits set by the SABS guidelines and therefore, was safe for human consumption.

Keywords: bottled water, bacteria, coliphages, indicators, enteric viruses

Introduction

Bottled water can be defined as any potable water that is bottled and distributed or offered for sale and specifically intended for human consumption. The source water may be springs, municipal systems or other sources, which are considered to be of safe and sanitary quality and fit for human consumption. However, consumers should be aware that bottled water is not necessarily safer than tap water. Bottled and municipal water may contain the same micro-organisms since both can originate from the same sources (Warburton, 2000). Under improper and/or prolonged storage of bottled water, bacteria can grow to levels that may be harmful to human health (Warburton, 2000). Consequently, when offered for sale to the consumer, these bottled water products should comply with all of the regulations as set by the South African Natural Bottled Water Association (SANBWA). According to SANBWA any harmful bacteria must be absent; however, these bacteria were not specified. Water from springs, wells, boreholes, municipal supplies, bottled water and other sources are known vehicles for enteric pathogens such as bacteria, parasites and viruses (Manaia et al., 1990). The presence of these micro-organisms can have an impact on the health of travellers, immuno-compromised persons and infants if bottled water is used for formula preparations.

Bottled water has been implicated as the source of outbreaks of cholera, typhoid fever as well as traveller's disease in countries such as Portugal and Spain (Blake et al., 1977; Mavridou, 1992; Warburton et al., 1992; Warburton, 2000). Recently, Norovirus (previously known as Norwalk-like viruses) sequences were detected in three European brands of mineral water (Beuret et al., 2002). However, controversy surrounded the accuracy of the results obtained by Beuret and co-workers (2002).

In Canada and other overseas countries stringent regulations have been implemented for the microbial quality of bottled water (Warburton et al., 1992; Warburton et al., 1998). However, in South Africa little is known about the microbiological quality of bottled water.

Heterotrophic plate count bacteria (HPC) are commonly used to assess the general microbiological quality of bottled water (WHO, 2001). Drinking water quality specifications world-wide recommend HPC limits from 100 to 500 cfu·mℓ$^{-1}$ in tap water and 50 cfu·mℓ$^{-1}$ in bottled water (WHO, 2001). The South African Bureau of Standards (SABS) specifies an HPC limit of less than 100 cfu·mℓ$^{-1}$ for bottled water (SABS, 1996). This limit is endorsed by the Department of Health and Water Affairs. Total and faecal coliform bacteria demonstrate faecal pollution in water and food and the counts of these indicator bacteria should be 0 cfu·100 mℓ$^{-1}$ in bottled natural water for consumption purposes (SABS, 1996).

Bacterial indicators do not always indicate the presence of pathogenic viruses and protozoa (Payment et al., 1985; Grabow et al., 1993; Grabow, 1996). Compared to coliform bacteria, viruses and protozoan cysts are known to be more persistent in the environment and more resistant to water treatment processes (Sobsey et al., 1995). The isolation of human enteric viruses in water meeting coliform standards, demonstrated the inadequacy of coliform bacteria to ensure virus-free water (Sobsey et al., 1995). The inability of these bacterial indicators to indicate virus-free water has prompted interest in developing an indicator system more appropriate to the human enteric viruses (Sobsey et al., 1995). Thus, coliphages (phages) have been suggested as potential indicators of the presence of enteric viruses, because of their similar structure and persistence in the environment (Kott et al., 1974; Kott, 1981; Simkova and Cervenka, 1981; Grabow et al., 1984;

* To whom all correspondence should be addressed.
☎+2712 319 2534; fax: +27 12 325 5550; e-mail: marthie.ehlers@up.ac.za
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Stetler, 1994, Grabow, 1996). There are many potential applications of phages as indicators or model viruses, which include their use as indicators of sewage contamination, efficiency of water and wastewater treatment and survival of enteric viruses and bacteria in the environment (Gerba, 1987).

Enteric viruses are transmitted by the faecal-oral route and infection can be acquired through contaminated water, food or vomitus and can cause diseases such as gastro-enteritis and hepatitis (Zaoutis, 1998). Enteroviruses are among the most important viral pathogens of humans and can cause an estimated 30 million infections in the US each year (Donaldson et al., 2002). The spectrum of diseases ranges from a mild febrile illness to aseptic meningitis, hand, foot and mouth disease, myocarditis, meningoencephalitis, poliomyelitis, neonatal multi-organ failure and gastroenteritis (Muir et al., 1998; Caro et al., 2001). Rotaviruses are the most common cause of acute viral gastroenteritis in infants and young children in both developing and industrialised countries (Bishop, 1994). The symptoms associated with rotavirus infections include fever, vomiting, chronic watery non-bloody diarrhoea and abdominal pain (Bishop, 1994). Caliciviruses are a major cause of acute viral gastroenteritis, also known as winter vomiting disease, in all age groups (Berke et al., 1997). Symptoms include nausea, vomiting and diarrhoea, lasting for one to three days (Berke et al., 1997). Although the symptoms are usually mild, the large numbers of people affected have economical implications in terms of medical costs and productivity (Berke et al., 1997).

Information about the survival, type and pathogenicity of micro-organisms associated and isolated from bottled water in South Africa is crucial to public health and for the evaluation of possible health risks. Although the market for bottled water at this stage is not so large, it is the fastest growing industry in the country (Neall, 2000). Bottled water quality is of major importance for the tourist industry. People tend to see bottled water as a first-world product but it sells even better in third-world countries with their suspect potable water supplies (Neall, 2000). The objective of this study was to evaluate the microbial quality of eight local and two imported brands of bottled water using bacterial indicators, coliphages as well as selected enteric viruses.

Materials and methods

Samples

Ten different commercial bottled water (non-carbonated) products that originated from springs or boreholes were randomly selected from various retail outlets. Bottled water of the same batch was bought (9 x 1.5 ℓ or 3 x 5 ℓ) of each type. Samples were stored at room temperature (25°C) and analysis was conducted on Day 1, after 1 month, and after 3 months.

Sample analyses

pH

The pH of each sample was determined before analysis (Crison Basic 20, Lasec).

Bacterial indicators

The detection of heterotrophic plate count bacteria

HPC bacteria present in bottled water samples were determined by the pour plate (1 mℓ in triplicate) method using Plate Count Agar (PCA) (Biolab) media (WHO, 2001). HPC plates were incubated at 37°C for 48 h. The number of bacteria colonies were determined and reported as colony-forming units per millilitre (cfu·mℓ$^{-1}$).

The detection of total and faecal coliform bacteria, enterococci and *Clostridium perfringens*

Total and faecal coliform bacteria were determined by passing 100 mℓ volumes of each sample (in triplicate) through Gelman filter units (Millipore) (0.45 µm pore size, 47 mm diameter). The filter membranes were placed on selective media, specific for each bacterial indicator: for the detection of total coliform bacteria on mENDO agar LES (Bacto), faecal coliform bacteria on mFC agar (Bacto), enterococci on mENTEROCOCCUS agar (Bacto) and *C. perfringens* on Perfringens agar (Oxoid). The Perfringens agar was supplemented with perfringens (OPSP) selective supplement A and B (Oxoid) for the selection of *C. perfringens* bacteria. The agar plates were aerobically incubated at 37°C for 24 h except for *C. perfringens*, which was incubated anaerobically.

Bacteriophage indicators

The qualitative presence-absence (P/A) test for the qualitative detection of low numbers of somatic and F-RNA phages in water was used for sample analysis (Grabow et al., 1993; ISO, 1995; ISO, 1998a; ISO, 1998b). Presence-absence medium was added to 500 mℓ of the bottled water samples for coliphage replication. Samples were incubated at 37°C for 24 h. This amplification method resulted in an increase in the numbers of coliphages to a level where they could readily be detected by a standard direct plaque assay (Grabow, 2001). Samples were handled aseptically, since contamination with a single viable phage particle would lead to a false positive result.

The following host cultures were added to the P/A enrichment media and incubated at 37°C for 24 h (Grabow et al., 1997):

Somatic coliphages

- *Escherichia coli* strain C (ATCC 13706) mutant (WG5) (ISO, 1997)

Male-specific coliphages

- *Salmonella typhimurium* strain WG49 phage type 3 Nal^r(F'lac::Tn5) Reference: NCTC 12484 (ISO, 1995)

Assays of phages by plaque assays were conducted by the double-agar layer method (Adams, 1959) with subsequent modifications such as performing direct qualitative spot tests of P/A on the solidified host-bacteria lawn (Grabow et al., 1997).

Concentration of viruses from water samples

Viruses were concentrated from 1 ℓ of bottled water according to the SiO_2 method described by Baggi and Peduzzi (2000). The SiO_2 (Sigma) was prepared as described by Boom et al. (1990). After 1 ℓ of the bottled water was acidified to pH 3.5 with 1 M acetic acid (Merck), 200 µℓ of SiO_2 (Sigma) and 1 mℓ of 0.5 M $AlCl_3$ (Sigma) were added. The samples were stirred on a magnetic stirrer for 30 min at room temperature (25°C) and the SiO_2 was allowed to settle for 24 h at 4°C. The supernatant (900 mℓ) was aspirated and the remaining solution (100 mℓ) was centrifuged at 7 500 x g (Sorvall Super T21) for 10 min to pellet the SiO_2. Viruses were recovered from the pellet with 1 mℓ of beef extract glycine buffer (GBEB) [0.05 M glycine (Merck) and 0.5% beef extract (Merck), pH 9]. Samples were homogenised and incubated in an oven at 64°C for 10 min. After centrifugation at 16 000 x g (Eppendorf 5415D) for

TABLE 1
Primers used for the detection of selected enteric viral nucleic acids in bottled water samples

Virus	Reaction (product size, bp)	Primer (nucleotide position)	Sequence (5' - 3')	Reference
Calici	RT-PCR: Norovirus (319) Sapovirus (331)	P290 (4568-4590) P289 (4865-4886)	GATTACTCCAAGTGGGACTCCAC TGACAATGTAATCATCACCATA	Jiang et al., 1999
Entero	RT-PCR (414)	EP1 (64-83) EP4 (459-478)	CGGTACCTTTGTGCGCCTGT TTAGGATTAGCCGCATTCAG	Gow et al., 1991
	nPCR (297)	E1 (166-182) E2 (447-463)	AAGCACTTCTGTTTCCC ATTCAGGGGCCGGAGGA	Kuan, 1997
Rota	RT-PCR (1,062)	R1 (1-28) END9(1036-1062)	GGCTTTAAAAGAGAGAATTTCCGTCTGG GGTCACATCATACAATTCTAATCTAAG	Le Guyader et al., 1994
	nPCR (189)	R3 (51-71) Rp (220-239)	GTATGGTATTGAATATACCAC TCCATTGATCCTGTTATTGG	Le Guyader et al., 1994

2 min to pellet the SiO_2, the supernatant was centrifuged at 125 000 x g (Beckman TL-100 ultracentrifuge) for 1 h at 4°C to pellet the viruses. The pellet was dissolved in 120 µℓ nuclease-free water (Promega Corp., Madison, WI) and immediately used for RNA extraction.

Extraction of viral RNA

Viral RNA was extracted from the water samples using TRIzol® reagent (Invitrogen, Life Technologies, Paisley, Scotland). A total of 120 µℓ of each concentrated water sample were mixed with 500 µℓ of TRIzol® (Invitrogen) and incubated at room temperature (25°C) for 5 min to permit complete dissociation of the nucleoprotein complex. Following the addition of 100 µℓ chloroform (Merck), each sample mixture was incubated for 3 min at room temperature (25°C) and centrifuged at 12 000 x g for 15 min at 4°C(Eppendorf 5402D). The aqueous phase (300 µℓ) was transferred to each of the 1.5 mℓ Eppendorf tubes (Eppendorf), containing 30 µℓ of 3 M sodium acetate (pH 5.2) and 600 µℓ of absolute ethanol. The samples were incubated at -20°C overnight. The samples were centrifuged at 12 000 x g for 15 min at 4°C (Eppendorf Centrifuge 5402D). Each RNA pellet was washed with 300 µℓ of 70% ethanol (Merck) and centrifuged at 12 000 x g for 5 min at 4°C (Eppendorf Centrifuge 5402D). The pellets were briefly air-dried and dissolved in 30 µℓ of nuclease-free water (Promega). The extracted viral RNA was frozen at -70°C for further analysis.

Molecular detection of viruses

Extracted RNA was used for the molecular detection of calici-, entero- and rotaviruses. All RT-PCR reagents were obtained from Promega. The amplified RT-PCR and nested PCR products were separated using 2% agarose (Seakem LE Agarose, Bioproducts, USA) gel electrophoresis in Tris-borate-EDTA (TBE) buffer (Medicell Primo gel apparatus, Holbrook, NY). A 100 bp DNA ladder (Promega) was used to determine the size of the products.

Caliciviruses: The RT-PCR method of Jiang et al. (1999) was used to determine the presence of both genera, Noro- and Sapoviruses (previously known as Norwalk-like and Sapporo-like, respectively) of caliciviruses in the bottled water samples. 10 µℓ of extracted RNA was added to the following reverse transcription polymerase chain reaction (RT-PCR) mix: 10 mM Tris-HCl (pH 9), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTP's, 100 pmol each of primers P289 and P290 (Table 1), 10 U AMV-reverse transcriptase and 2.5 U Taq DNA polymerase. The reaction was incubated at 42°C for 1 h and at 94°C for 3 min. The cycling programme (Techne Genius thermocycler) included 40 cycles at 94°C for 30 s, 49°C for 80 s and 72°C for 60 s. A final extension of 72°C for 10 min was included.

Enteroviruses*:* The possible presence of enteroviruses in the bottled water samples was determined according to the methods of Gow et al. (1991) and Kuan (1997). A Promega Access RT-PCR System (Promega) was used for the reverse transcription and PCR amplification of enteroviral RNA. A 50 µℓ reaction volume, containing the following was prepared: AMV/Tfl reaction buffer (1x), 0.2 mM dNTP mix, 50 pmol each of primers EP1 and EP4 (Table 1), 1.5 mM $MgSO_4$, 5 U of AMV reverse transcriptase and 5 U of Tfl DNA polymerase. The reaction was incubated at 49°C for 45 min and then subjected to 30 amplification cycles consisting of 60 s at 94°C, 60 s at 55°C and 60 s at 72°C (Hybaid OmniGene Thermocycler). The final extension step was performed for 7 min at 72°C. A nested PCR was conducted by adding 1 µℓ of the amplified product to 49 µℓ of the PCR mixture. The PCR mixture contained the following: 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 50 pmol each of primers E1 and E2 (Table 1) and 2.5 U of Taq DNA polymerase. Cycling was carried out 35 times with denaturation at 94°C for 60 s, annealing at 45°C for 60 s and extension at 72°C for 60 s (Hybaid OmniGene thermocycler).

Rotaviruses*:* The presence of rotaviruses was determined according to the method of Baggi and Peduzzi (2000). Primers R1, END9, R3 and Rp were used for the amplification of sequences from the VP7 gene of Group A rotaviruses (Le Guyader et al., 1994) (Table 1). After denaturation of extracted RNA at 97°C for 5 min, the samples were immediately cooled on ice. The denatured RNA

TABLE 2
Bacterial analysis of bottled water samples A-J during the 3-month period

Sample	Days	pH	HPC (cfu·mℓ$^{-1}$)	Entero-cocci (cfu·100 mℓ$^{-1}$)	Total coliform bacteria (cfu·100 mℓ$^{-1}$)	Faecal coliform bacteria (cfu·100 mℓ$^{-1}$)	*Clostri-dium per-fringens* (cfu·100 mℓ$^{-1}$)
A	1	6.36	2	0	0	0	0
	30	6.80	0	0	0	0	0
	90	6.08	2	0	0	0	0
B (Imported)	1	7.64	6	0	0	0	0
	30	7.64	3	0	0	0	0
	90	7.00	5	0	0	0	0
C	1	6.77	6	0	0	0	0
	30	6.91	0	0	0	0	0
	90	6.71	2	0	0	0	0
D (Imported)	1	7.59	10	0	0	0	0
	30	7.74	5	0	0	0	0
	90	7.10	3	0	0	0	0
E	1	7.45	11	0	0	0	0
	30	7.58	1	0	0	0	0
	90	7.50	1	0	0	0	0
F	1	7.53	1	0	0	0	0
	30	7.67	3	0	0	0	0
	90	7.40	1	0	0	0	0
G	1	8.19	240	0	0	0	0
	30	8.05	540	0	0	0	0
	90	7.37	11	0	0	0	0
H	1	7.18	13 100	0	0	0	0
	30	6.95	2 580	0	0	0	0
	90	7.17	11 000	0	0	0	0
I	1	7.71	3	0	0	0	0
	30	7.76	0	0	0	0	0
	90	7.50	6	0	0	0	0
J	1	7.21	2	0	0	0	0
	30	7.00	2	0	0	0	0
	90	7.41	4	0	0	0	0

cfu = colony forming units; HPC = Heterotrophic plate count

was added to the following PCR mix: 10 mM Tris-HCl (pH 9), 50 mM KCl, 1 mM $MgCl_2$, 0.2 mM dNTP's, 25 pmol each of primers R1 and End9 (Table 1), 10 U AMV-reverse transcriptase and 2.5 U Taq DNA polymerase. The RT-PCR reactions were incubated for 30 min at 42°C followed by 60 s at 94°C, 120 s at 55°C and 60 s at 72°C, for a total of 25 cycles. A final extension at 72°C for 7 min concluded the RT-PCR amplification step. A 2 µℓ volume of the RT-PCR amplification products was added to the following PCR mix for the nested PCR: 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP's, 25 pmol each of primers R3 and Rp (Table 1) and 2.5 U Taq DNA polymerase. After initial denaturation of 120 s at 94°C, the following cycling conditions were performed: 30 s at 94°C, 30 s at 50°C and 30 s at 72°C for 3 cycles, followed by 27 cycles of 15 s at 94°C, 15 s at 50°C and 20 s at 72°C. A final extension at 72°C for 7 min concluded the nested PCR amplification step (Techne Genius thermocycler).

Results and discussion

The microbial quality of bottled (non-carbonated) water is of great interest as many consumers use it as an alternative to municipal water and consider it to be better and safer (Mavridou, 1992). Bottled water is generally of good quality for drinking, but if not properly protected during bottling and transit, could be a subject of

contamination (Mavridou, 1992).

Despite the potential for contamination of source water, there have been only a few waterborne outbreaks associated with bottled water (Warburton, 1993; Warburton et al., 1998). According to epidemiological evidence the waterborne exposure route is important in the dissemination of viruses to human hosts (Biziagos et al., 1988; Beuret et al., 2002). However, to date bottled water has not been clearly identified as a source of infection with enteric viruses such as hepatitis A or Noroviruses (Biziagos et al., 1988; Beuret et al., 2002).

The bottled water examined in this study showed heterotrophic plate counts (HPCs) which were within the established quality guidelines for bottled water of less than 100 cfu·mℓ^{-1} (SABS, 1996) (Table 2). Bottled water samples marked A, B, C, F, I and J had HPCs less than 10 cfu·mℓ^{-1}, whereas samples D and E had HPCs less than 20 cfu·mℓ^{-1}. However, two local brands (G and H) displayed HPCs that were above the excepted limit indicated by the SABS standard (SABS, 1996). Heterotrophic plate counts for bottled water brand G ranged from 1.1 x 10^2 cfu·mℓ^{-1} to 5.4 x 10^2 cfu·ml^{-1} with a mean value of 2.64 x 10^2 cfu·mℓ^{-1}. The HPCs for bottled water brand H ranged from 2.58 x 10^3 cfu·mℓ^{-1} to 1.3 x 10^4 cfu·mℓ^{-1} with a mean value of 8.89 x 10^3 cfu·mℓ^{-1}.

The South African Department of Health has a health risk range for HPC bacteria of no risk for counts less than 100 cfu·mℓ^{-1} and greater risk for counts above 10 000 cfu·mℓ^{-1}. Thus, the South African Bureau of Standards (SABS) specifies a HPC limit of less than 100 cfu·mℓ^{-1} for bottled water (SABS, 1996). This limit is endorsed by the South African Departments of Health and Water Affairs and is in line with specifications and recommendations in the rest of the world (WHO, 2001).

Bottled (non-carbonated) water generally have high HPCs, since the elimination of micro-organisms by disinfection or sterilisation is not permitted (Anon, 1980; Manaia et al., 1990). The presence of high numbers of heterotrophic bacteria in bottled water may be due to the natural microbial flora of the source water. These bacteria can multiply after bottling, resulting in high numbers of HPCs (Li et al., 2001). While bottled water may initially meet bacteria standards, the lack of a residual disinfectant (chlorine) and prolonged periods of storage at room temperature or higher may result in elevated HPC bacteria counts by the time the water is consumed (Li et al., 2001). Microbial numbers reach a peak after a week of storage and remain fairly constant thereafter (Li et al., 2001).

Recent studies have identified several heterotrophic bacteria as being common in bottled water; these included genera such as: *Achromobacter, Acinetobacter, Aeromonas, Alcaligenes, Arthrobacter, Caulobacter, Corynebacterium, Flavobacterium* and *Pseudomonas* (Manaia et al., 1990). However, some of these genera contain species that have been known as opportunistic pathogens. Although HPC bacteria have been considered harmless, several epidemiological studies conducted in countries such as Canada and the USA suggested the potential health risk associated with HPC bacteria present in drinking water, which comply to water quality standards (Payment et al., 1994; Edberg et al., 1996; Grabow, 1996; Rusin et al., 1997; Pavlov et al., 2004). In studies on filtered and non-filtered tap water conducted by Payment et al. (1991a; 1991b), the researchers found an association between high numbers of HPC bacteria and gastroenteritis. According to a study conducted on treated drinking water in South Africa, some members of the HPC bacteria produce virulence factors and therefore, may act as opportunistic pathogens (Pavlov et al., 2004). The components of the community that are specifically at risk of HPC infections include the very young and the elderly with weakened immune systems, as well as individuals with immuno-compromising diseases such as AIDS, and patients that underwent organ transplantation, chemotherapy, and pregnant women. However, it was not part of this study to identify the heterotrophic bacteria isolated from the bottled water samples but it is possible that the samples may include potentially pathogenic species of the above-mentioned genera.

Strict standards exist that prohibit the sale of bottled water containing coliforms, faecal coliforms, faecal streptococci, sulphide-reducing clostridial sporeformers, *Pseudomonas aeruginosa* and pathogens (Anon, 1980). The fact that neither *Clostridium perfringens*, enterococci, total coliform bacteria nor faecal coliform bacteria were found in any of the ten bottled water samples in this study indicated the absence of faecal contamination in these samples (Table 2). However, studies have indicated that high counts of *E. coli* (10^5 to 10^7 cfu·mℓ^{-1}) in bottled water could not be detected within four days (Ducluzeau et al., 1976) or 20 to 30 d (Moreira et al., 1994). This absence of coliform bacteria might be due to die-off in the bottles during storage.

The bottled water samples were tested for the presence of bacteriophages such as somatic and F-RNA coliphages. The presence of phages, which is typically associated with human and animal excreta, indicates the potential presence of enteric viruses (Grabow, 1986; Grabow, 2001). However, in this study somatic and F-RNA coliphages were not detected (Table 3), which indicated the possible absence of enteric viruses in the tested bottled water samples.

The WHO Scientific Group has concluded that the presence of even a few enteric viruses in drinking water poses a human health threat (WHO, 1979). According to the SABS standard (2001) for drinking water, enteric viruses must be absent from 100 ℓ of water. Therefore, the ten bottled water samples were further analysed for the presence of enteric viruses such as caliciviruses, enteroviruses and rotaviruses. Recent developments in molecular detection technology made it possible to identify viruses by non-culture-based methods such as the polymerase chain reaction (PCR) (Muir et al., 1998; Donaldson et al., 2002). Reverse transcription-polymerase chain reaction (RT-PCR) assays have been developed to detect viral RNA directly from samples because of the high sensitivity for small amounts of RNA (Casas et al., 2001).

The presence of enteroviruses in raw and treated drinking water, which comply with specifications for treatment and counts of indicator organisms has been documented (Abbaszadegan et al., 1999; Grabow et al., 2000). The presence of caliciviruses in European bottled water brands (Beuret et al., 2002) and of rotaviruses in drinking water supplies in Colorado, USA has been described (Hopkins et al., 1984). However, no calici-, entero- or rotaviruses were detected with PCR in this study in any of the bottled water samples that were analysed (Table 3).

Conclusions

The study indicated that eight local and two imported brands of bottled water were free of total and faecal coliform bacteria, enterococci, *C. perfringens*, bacteriophages and selected enteric viruses. Levels of HPC bacteria that exceeded the specifications of the SABS were, however, detected in two of the ten brands analysed (Brands G and H). The results obtained in this study were comparable and in agreement with similar reports by other researchers such as Manaia et al. (1990), Mavridou (1992), Tsai and Yu (1997) indicating that bottled water generally complied with current drinking water legislations. Microbial contamination of bottled natural water is most likely to occur due to improperly

TABLE 3
Bacteriophage and selected enteric viral analysis of bottled water samples A-J during the 3-month period

Sample	Days	pH	Coliphages P/A in 500 mℓ		PCR per 1 litre		
			Somatic	F-RNA	Calici-virus	Entero-virus	Rota-virus
A	1	6.36	A	A	A	A	A
	30	6.80	A	A	A	A	A
	90	6.08	A	A	A	A	A
B (Imported)	1	7.64	A	A	A	A	A
	30	7.64	A	A	A	A	A
	90	7.00	A	A	A	A	A
C	1	6.77	A	A	A	A	A
	30	6.91	A	A	A	A	A
	90	6.71	A	A	A	A	A
D (Imported)	1	7.59	A	A	A	A	A
	30	7.74	A	A	A	A	A
	90	7.10	A	A	A	A	A
E	1	7.45	A	A	A	A	A
	30	7.58	A	A	A	A	A
	90	7.50	A	A	A	A	A
F	1	7.53	A	A	A	A	A
	30	7.67	A	A	A	A	A
	90	7.40	A	A	A	A	A
G	1	8.19	A	A	A	A	A
	30	8.05	A	A	A	A	A
	90	7.37	A	A	A	A	A
H	1	7.18	A	A	A	A	A
	30	6.95	A	A	A	A	A
	90	7.17	A	A	A	A	A
I	1	7.71	A	A	A	A	A
	30	7.76	A	A	A	A	A
	90	7.50	A	A	A	A	A
J	1	7.21	A	A	A	A	A
	30	7.00	A	A	A	A	A
	90	7.41	A	A	A	A	A

P/A = Presence-absence; P = Present; A = Absent

cleaned equipment and bottles, failure of ozonation or UV equipment or due to contamination of the water by workers. Future studies may address the microbiological quality of source water used for the bottling of local mineral water brands as well as the detection of virulence factors associated with potentially pathogenic HPC bacteria

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