ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 1, 29–34
www.aaem.pl

Occurrence of non-fermenting gram negative bacteria in drinking water dispensed from point-of-use microfiltration devices

Franza Zanetti[1], Giovanna de Luca[1], Erica Leoni[1], Rossella Sacchetti[2]

[1] *Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy*
[2] *Department of Education Sciences, University of Bologna, Italy*

Zanetti F, De Luca G, Leoni E, Sacchetti R. NF-GNB in drinking water dispensed from point-of-use devices. Ann Agric Environ Med. 2014; 21(1): 29–34.

Abstract

Introduction and objective. Many devices have been marketed in order to improve the organoleptic characteristics of tap water resulting from disinfection with chlorine derivates. The aim of the presented study was to assess the degree of contamination by non-fermenting Gram-negative bacteria (NF-GNB) of drinking water dispensed from microfiltration devices at point-of-use.

Methods. Water samples were collected from 94 point-of-use water devices fitted with a filter (0.5µm pore size) containing powdered activated carbon. The microbiological contamination of water entering and leaving the microfiltered water dispensers was compared. The NF-GNB loads were correlated to Total Heterotrophic Counts (HPCs) at 37 and 22 °C, residual chlorine, and some structural and functional features of the devices.

Results. NF-GNB were detected from 23% of supply water samples, 33% of still unchilled water, 33% of still chilled water and 18% of carbonated chilled water. The most frequent isolates were Pseudomonadaceae: *Steno.maltophilia* 30.2% of isolates, *Pseudomonas* 20.5%, *Delftia acidovorans* 13.4%, while the species more largely distributed was *Ps. aeruginosa* recovered from 13% of samples. The distribution of the various NF-GNB was different in the water entering and in that leaving the devices. *Ps.aeruginosa* and *Steno.maltophilia* were the predominant species in water leaving the microfiltration dispensers, probably due to their capacity to colonize the circuits and to prevail over the others. Recovery of NF-GNB was favoured by the reduction in residual chlorine of the supply water, occasional use, the absence of a bacteriostatic element in the filter and inadequate disinfection of the water lines.

Conclusions. The presence of high concentrations of potentially pathogenic species of NF-GNB (*Ps.aeruginosa, Steno. maltophilia, Burkhol.cepacia*) in the water dispensed from microfiltration devices represents a risk of waterborne infections for vulnerable individuals. When these devices are used in environments such as hospitals, nursing homes for the elderly, etc., microbiological monitoring for the detection of NF-GNB is advisable.

Key words

Drinking water, point-of-use microfiltration devices, non-fermenting Gram negative bacteria

INTRODUCTION

The misgivings expressed by many consumers towards tap water due to the unpleasant smell and taste often resulting from disinfection with chlorine derivates, is one of the main reasons that has led to the notable rise in the consumption of bottled water, with negative repercussions on environmental pollution correlated to the production of the bottles, road transport and the disposal of waste. In an attempt to reduce such drawbacks, devices have been marketed that treat municipal tap water at the point-of-use, in order to improve its organoleptic characteristics and remove any possible suspended material through the process of microfiltration. These devices are equipped with filters and waterlines of small diameter, on whose surfaces the formation of biofilm is favoured by the stagnation of the water during periods of non-use and the intermittent flow of the dispensed water [1]. Also, the material from which the circuits are made, such as silicone, enhances the adhesion of bacteria to the walls and can even be a suitable nutrient for many species of bacteria [2]. Biofilm can be the source of undesirable levels of opportunistic and pathogen bacteria of aqueous habitats [3, 4]. Non-fermenting Gram negative bacteria (NF-GNB) are among the species able to colonize water systems and form biofilm which may represent a health risk in vulnerable subpopulations (infants, the elderly, immune-compromised individuals). Wang et al. (2009) found a significant correlation between the overall occurrence of NF-GNB in tap aerators and the prevalence of infection of exposed patients in intensive care units [5].

The NF-GNB most frequently detected belong to the family of Pseudomonadaceae, including the genera *Pseudomonas*, *Stenotrophomonas*, *Burkholderia*, *Delftia, Ralstonia, Comamonas,* and *Brevundimonas*. *Ps. aeruginosa* is believed to be a major cause of high mortality in hospital-acquired infections (septicaemia and meningitis), through skin and wound contact, the inhaling of aerosol droplets and drinking water exposure [6, 7]. Many strains are resistant to a range of anti-microbial agents, resulting in an increase in the significance of the microorganism in medical facilities or nursing homes. *Stenotrophomonas maltophilia* is an emerging multi-drug resistant opportunist pathogen which can cause a wide variety of nosocomial and community-acquired infections, including bacteraemia and pneumonia, especially

Address for correspondence: Erica Leoni, University of Bologna, Department of Biomedical and Neuromotor Science, Via S. Giacomo 12, Bologna, Italy
e-mail: erica.leoni@unibo.it

Received: 16 January 2013; accepted: 19 February 2013

in debilitated or immune-compromised individuals [8, 9, 10]. *Burkholderia cepacia* is a common environmental Gram negative bacterium which, according to reports in the literature, may be highly pathogenic and responsible for serious nosocomial infections in cystic fibrosis patients [11, 12].

Ps. putida, Ps. stutzeri, *Ps. fluorescens*, *Delftia acidovorans* (formerly *Comamonas acidovorans*), *Ralstonia pickettii (*formerly *Burkholderia pickettii)* are known as less virulent organisms. However, they may be responsible for infections such as pneumonia, bacteraemia, meningitis, septic arthritis, endocarditis, keratitis and osteomyelitis in immune-compromised individuals [13, 14, 15, 16, 17]. Recently, a *Ps. fluorescens* outbreak was reported in a bone marrow transplant unit, due to contaminated drinking water following the pharyngeal colonization of patients with haematologic disorders, confirmed by genotypic analysis of the isolated strains [18].

Other potentially pathogenic NF-GNB belong to the family of Flavobacteriaceae. *Cryseobacterium* (formerly *Flavobacterium*) spp can survive in chlorine-treated municipal water supplies and colonize water taps. *Chryse. meningosepticum* and *Chryse. indologenes* are the etiological agents of meningitis in neonates and of a wide variety of infections in immune-compromised subjects (sepsis, bacteraemia, pneumonia, endocarditis) [19, 20]. Finally, *Weeksella virosa* is sporadically associated with nosocomial invasive infections [21].

Other NF-GNB frequently detected in drinking water are *Moraxella* spp and *Alcaligenes* (formerly *Achromobacter*) *xylosoxidans*, occasionally able to cause infections mainly in elderly and in immune-compromised hosts (conjunctivitis, otitis, sinusitis, upper and lower respiratory-tract infections, meningitis, bacteraemia, endocarditis) [22, 23, 24].

Various studies on the occurrence of NF-GNB in drinking water have been performed on municipal water supply systems [7, 25, 26], a few studies have focused on dispensers such as soda fountains [27] and water coolers [28, 29], while there are no studies on point-of-use water devices fitted with filters with a composite structure containing powdered activated carbon. Furthermore, to date, no specific bacteriological requirements or monitoring protocols have been established for drinking water dispensed from these devices.

The aim of the presented study was to assess the degree of contamination by NF-GNB of drinking water entering and leaving microfiltered water dispensers. The NF-GNB loads were correlated to the Total Heterotrophic Counts (HPC at 37 and 22 °C) and to some physico-chemical parameters of the dispensed water. In addition, the influence of certain structural and functional characteristics of the devices on the presence of NF-GNB in the dispensed water was also investigated.

MATERIALS AND METHODS

Equipment. The study was carried out on water samples collected from 94 point-of-use water microfiltration devices, installed in private and public structures (habitations, offices, bars and restaurants, nursing homes, etc.) served by the same water distribution network in a city of northern Italy. The devices, of varying brands available on the market, were all fitted with a filter with a composite structure (0.5 µm pore size) containing powdered activated carbon and, in some cases, also a bacteriostatic element (copper or silver salts). 82 devices had a water cooling system and 56 also a carbonation system.

The different types of drinking water were collected from each device – a total of 299 samples: 94 of Supply Water (SW), 67 of Still Unchilled Water (SUW), 82 of Still Chilled Water (SCW) and 56 of Carbonated Chilled Water (CCW).

Collection and processing of water samples. The water samples (1 liter) were collected in sterile bottles containing 1 mL of a sterile sodium thiosulfate solution (10% w/v) to neutralize any residual chlorine, and were kept at 4 °C and analysed within 3 hours. At the moment of sampling, the water temperature and the residual chlorine were measured, using, respectively, a mercury thermometer and the DPD (N,N-dietil-p-fenilendiammina) colorimetric method [30]. Information concerning certain structural and functional characteristics of the devices was also recorded.

The Heterotrophic Plate Counts (HPC) at 37 °C and 22 °C were determined by the pour plate method using Plate Count Agar (Oxoid). The mean value of three replicates was calculated. The detection limit was 1 cfu mL^{-1}. In each sample, the NF-GNB were counted and identified following the techniques proposed in the Standard Methods [30]. 250 mL was filtered through a cellulose acetate filter with a porosity of 0.45 µm (Millipore); the filter was placed on Pseudomonas CFC agar (Oxoid) and incubated at 30 °C for 24–48 h. All colonies grown were differentiated, counted and sub-cultured on Tryptone Soya Agar (TSA- Oxoid) (at least 5 colonies of each type, or all, if less than 5). Finally, they were identified by the multitest API 20NE system (BioMérieux). The detection limit was 1 cfu mL^{-1}.

Statistical analysis. The HPC and NF-GNB values were converted into $Log_{10}x$. For all negative samples, the detection limits were used. A simple correlation test was used to evaluate the correlations between NF-GNB and HPC at 22 °C, HPC at 37 °C, temperature and residual chlorine. The paired t test was applied to compare the NF-GNB in the various types of water. The same analysis was made on the HPCs. Multiple logistic regression analyses with a forward stepwise procedure were carried out to predict the presence of NF-GNB as a function of a number of characteristics. The following parameters were considered: NF-GNB in supply water, residual chlorine in input water, temperature, age of device, presence of bacteriostatic element in the filter, everyday use, liters of water dispensed per day, adequate disinfection, frequency of disinfection, frequency of filter change. Separate models were fit for each of the three kinds of dispensed water. The significance level chosen for all analyses was $p<0.05$. All descriptive and statistical calculations were made using the SPSS programme 20.0.

RESULTS

Table 1 summarizes the microbiological results for the different types of water samples examined, and shows the heterotrophic bacteria counts at 22 and 37 °C, the frequency of detection, and the means and standard deviations of the NF-GNB. All samples of water leaving the devices had higher mean values of HPCs compared to the Supply Water

Table 1. Microbial contamination of drinking water dispensed from water microfiltration devices at the point of use

	37 °C HPC		22 °C HPC		NF-GNB		
Types of water	mean	SD	Mean	SD	Positive samples	mean	SD
	(Log cfu mL^{-1})	(Log cfu mL^{-1})	(Log cfu mL^{-1})	(Log cfu mL^{-1})	%	(Log cfu 250 mL^{-1})	(Log cfu 250 mL^{-1})
Supply Water (n=94)	1.08	0.71	0.68	0.61	23.4	0.31	0.67
Still Unchilled Water (n=67)	1.67	0.68	1.33	0.70	32.8	0.56	0.93
Still Chilled Water (n=82)	1.55	0.66	1.17	0.72	32.9	0.50	0.99
Carbonated Chilled Water (n=56)	1.92	0.84	1.51	0.88	17.9	0.30	0.80

(p=0.0001). Comparing the various types of water dispensed from the microfiltration devices, significant differences were seen only between the Still Chilled Water and the Carbonated Chilled Water (HPC-37 °C, p<0.001; HPC-22 °C, p<0.01).

Overall, 41.5% of the devices studied dispensed at least one type of water contaminated by NF-GNB. Around 23% of samples of incoming water were contaminated by NF-GNB; in the dispensed water, the percentages of positivity and the mean values were higher in the Still Unchilled (32.8%) and Still Chilled Water (32.9%) and lower in the Carbonated Chilled Water (17.9%). However, no statistically significant difference was found when compared to the values of the incoming water. The presence of NF-GNB in the water entering the devices was not always associated with their recovery from the dispensed water. Only in 8 devices were NF-GNB recovered from both the incoming and dispensed water. Moreover, a direct statistically significant association (p=0.0001) between NF-GNB and HPCs was only observed in Still Chilled Water. HPCs were not correlated with any particular species/genera of NF-GNB in any types of water examined.

In all the various types of water, the NF-GNB and HPCs were seen to increase in correspondence to an increase in water temperature and decrease in residual chlorine, although these associations were not statistically significant. The mean temperature was 17.2 °C for SW, 21.9 °C for SUW, 9.9 °C for SCW and 10.1 °C for CCW. The mean residual chlorine content was 0.18 mgL^{-1} for SW, 0.10 mgL^{-1} for SUW and 0.08 mgL^{-1} for SCW.

Table 2 correlates he presence of NF-GNB in the dispensed water (in at least one sample for each device) to some parameters of the supply water and to certain functional and structural characteristics of the devices. The multivariate analysis failed to reveal any significant associations between the selected characteristics and the presence of NF-GNB. However, this lack of association should be interpreted with caution because logistic regression analyses were conducted on the small sub-samples with complete data. NF-GNB were isolated from the dispensed water when the mean concentrations of these bacteria were higher in the incoming water and when the residual chlorine was lower. Furthermore, NF-GNB were isolated with lower frequency from the everyday used devices, as well as from devices fitted with a filter with a bacteriostatic element and by adequate disinfection of the circuits. However, no significant differences were observed. Similarly, the presence of NF-GNB was not statistically correlated to the frequency of disinfection and filter substitution, or to the quantity of water dispensed each day or the age of the devices.

Table 2. Presence/absence of NF-GNB in water dispensed from water microfiltration devices in relation to certain characteristics of incoming water and of the devices

Variables		NF-GNB	
		yes (n=39)	no (n=55)
Supply Water NF-GNB (Log cfu 250 mL^{-1})	mean (SD)	0.34 (0.75)	0.27 (0.60)
Supply Water residual chlorine (mg L^{-1})	mean (SD)	0.16 (0.10)	0.19 (0.13)
Supply Water temperature (°C)	mean (SD)	16.8 (3.8)	18.6 (3.8)
Age (months)	mean (SD)	49 (40)	50 (38)
Filter+bacteriostatic element (Ag or Cu)	%	55.0	64.7
Everyday use	%	54.7	60.6
Daily use (hours)	mean (SD)	7.5 (4.9)	8.0 (4.5)
Water dispensed per day (liters)	mean (SD)	58 (63)	71 (157)
Adequate disinfection*	%	45.4	56.8
Frequency of disinfections (number/year)	mean (SD)	2.6 (1.2)	2.2 (1.5)
Filter changes (number/year)	mean (SD)	1.9 (1.4)	1.8 (1.6)

* adequate disinfection: compliance with the means and frequency of disinfection suggested by the manufacturer

Table 3 shows all species of NF-GNB identified and, for each one, the percentage of positive samples and the number of isolates in the different types of water examined. The highest counts were for *Steno. maltophilia* (other 'Pseudomonadaceae') and *W. virosa* (Flavobacteriaceae), recovered exclusively from the dispensed water. 20.5% of the isolates belonged to the genus *Pseudomonas,* with a clear prevalence of *Ps. aeruginosa*, found in 39 samples (13%) in concentrations of up to 4750 cfu mL^{-1}. Other Pseudomonadaceae isolated, important from the health viewpoint, were *Delftia acidovorans* (isolated 4 times of which 2 in SW) and *Burkhol. cepacia* (once in CCW). It was not possible to identify 1,837 isolates (around 3.9%) due to the poor growth on subculture.

Figure 1 shows the distribution percentage of NF-GNB in the different types of water examined. In the supply water, a greater heterogeneity of isolates is evident, a low percentage belonging to *Pseudomonas* spp, and higher percentages to other Pseudomonadaceae (in particular *Delftia acidovorans*), and more especially to 'other species/genera' (in particular *Moraxella*), the latter never being found in the dispensed water. In Still Unchilled Water, the highest levels are for *Pseudomonas* spp (especially *Ps. aeruginosa*) and other Pseudomonadaceae (in particular *Steno. maltophilia).* The Still Chilled Water is characterized by the presence of Flavobacteriaceae (in particular *W. virosa*), not found in the two types of water previously mentioned. In Carbonated Chilled Water there is a clear prevalence of Pseudomonadaceae, especially *Steno. maltophilia.*

Table 3. Prevalence of NF-GNB species

Species	Supply Water (n=94)		Still Unchilled Water (n=67)		Still Chilled Water (n=82)		Carbonated Chilled Water (n=56)		Total isolates	
	positive samples	isolates	positive samples	isolates	positive samples	Isolates	positive samples	isolates	n	%
	%	n	%	n	%	n	%	n		
Stenotrophomonas maltophilia	0	-	4.5	1654	3.7	1550	1.8	11175	14379	30.222
Weeksella virosa	0	-	0	-	2.4	11250	1.8	2500	13750	28.899
Pseudomonas aeruginosa	6.4	29	19.4	3569	17.1	5493	10.7	54	9145	19.222
Delftia acidovorans	2.1	635	0	-	2.4	5750	0	-	6385	13.420
Moraxella spp	4.3	945	1.5	1	2.4	4	0	-	950	1.997
Pseudomonas aureofaciens	1.1	4	3.0	29	4.9	315	1.8	2	350	0.736
Aeromonas salmonicida	1.1	250	0	-	0	-	0	-	250	0.525
Ralstonia pickettii	1.1	6	1.5	150	2.4	22	0	-	178	0.374
Pseudomonas stutzeri	1.1	1	0	-	2.4	64	0	-	65	0.137
Pseudomonas mendocina	1.1	64	0	-	0	-	0	-	64	0.134
Pseudomonas putida	3.2	21	3.0	30	2.4	2	0	-	53	0.111
Pseudomonas fluorescens	1.1	5	4.5	14	2.4	2	1.8	30	51	0.107
Comamonas testosteroni/Ps.alc	1.1	50	0	-	0	-	0	-	50	0.105
Acaligenes xylosoxidans	1.1	4	0	-	0	-	1.8	27	31	0.065
Pseudomonas mesophilica	0	-	0	-	0	-	1.8	14	14	0.029
Burkholderia cepacia	0	-	0	-	0	-	1.8	13	13	0.027
Cryseobacterium indologenes	1.1	11	0	-	0	-	0	-	11	0.023
Brevundimonas vesicularis	0	-	1.5	2	0	-	0	-	2	0.004
Cryseobacterium meningosepticum	1.1	1	0	-	0	-	0	-	1	0.002
Unidentified strains		77		0		1760		0	1837	3.861
Total		2103		5449		26212		13815	47579	100

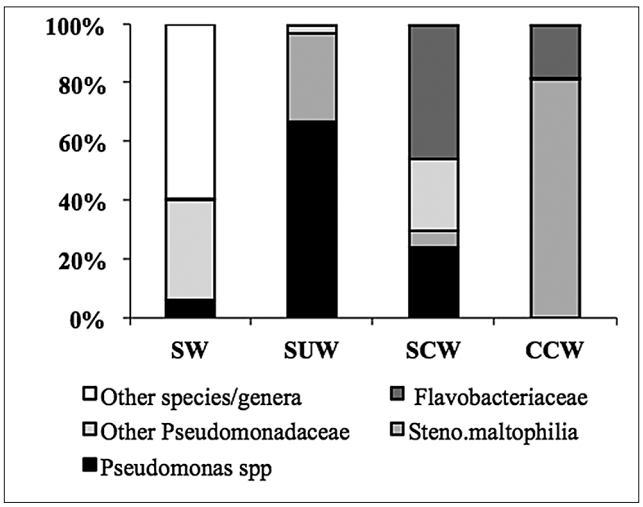

Figure 1. Distribution percentage of NF-GNB in different types of water (SW: supply water; SUW: still unchilled water; SCW: still chilled water; CCW: carbonated chilled water)

DISCUSSION

In the still waters (both chilled and unchilled) dispensed from the devices the mean concentrations of NF-GNB were higher than in the supply water. The reduction of the residual chlorine in water entering the devices, the stagnation of the water in the circuits due to infrequent use of the devices, the absence of a bacteriostatic element in the filter and inadequate disinfection of the waterlines were the main conditions seen to favour the multiplication of NF-GNB within the circuits. The same conditions can explain the increase in HPCs observed in the dispensed water, compared to the supply water. On the other hand, the generally lower concentrations of NF-GNB found in the Carbonated Chilled Water could be due to the acid pH, as well as to the potentially toxic action of the carbon dioxide itself.

Considering all the samples, 19 NF-GNB species and/or genera were isolated. As in other studies performed on similar conditions [7, 25, 26], the predominant isolates belonged to the Pseudomonadaceae family: *Stenotrophomonas* (30.2%),

Pseudomonas (20.5%) and *Delftia* (13.4%). The distribution of the various NF-GNB species was different in the water entering and in that leaving the devices. The dispensed water often contained species not isolated from the supply water, and *vice versa*. This could be explained by the greater capacity of some species, in particular the psychrophilic, to colonize the devices and to prevail over the other species, even though they were present only occasionally in the supply water and in low concentrations. This was observed for *Ps. aeruginosa* and other Peudomonadaceae such as *Steno. maltophilia, Delftia acidovorans,* and *Ps. aureofaciens*. It is also possible that before their installation the circuits were already colonized by bacteria from the water used for testing the devices in the factory.

At least 4 of the Pseudomonadaceae isolated (*Ps. aeruginosa, Steno. maltophilia, Burkhol. cepacia and Ralst. pickettii*) have been reported as important opportunistic agents of waterborne infections transmitted through contact with the skin and wounds, but also through the inhalation of aerosol droplets and the consumption of drinking water in highly immune-compromised patients [6, 18]. *Ps. aeruginosa* was the species isolated from the highest number of samples (SW: 6%; SUW: 19%; SCW: 17%; CCW: 11%), sometimes at high concentrations which could represent a health risk for susceptible subjects (children, the elderly, immune-compromised subjects), subsequent to colonization by ingestion [31]. High levels of *Ps. aeruginosa* also provoke changes in the organoleptic characteristics of the water, giving it an unpleasant smell and taste and increasing its turbidity [32]. The highest number of isolates, however, was of *Steno. maltophilia,* recovered only from the dispensed water, testifying to its capacity to colonize and form biofilm in the water circuits of the devices. The clear prevalence of *Steno. maltophilia* in the Carbonated Chilled Water would appear to point to a greater resistance of this species to the negative effects of the carbon dioxide compared to *Pseudomonas* spp. In a previous study, *Steno. maltophilia* was seen to be more resistant than *Ps. aeruginosa* also to disinfection [33]. The presence of high concentrations of *Steno. maltophilia* in water destined for human consumption is of particular interest in light of recent studies on the multidrug resistance of this microorganism and the still uncertain role that chronic infection by *Steno. maltophilia* may play in the evolution of lung disease in patients affected by cystic fibrosis [34, 35]. The isolation of *Burkhol. cepacia,* an opportunistic bacteria with a high pathogenicity [32], in the Carbonated Chilled Water is of particular concern, even though this occurred only once and with few isolates. Other opportunistic pathogens belonging to the Pseudomonadaceae, such as *Ps. mendocina, Ps. mesophilica*, *Ps. putida, Ps. stutzeri,* and *Brev. vesicularis,* of lesser clinical significance, were only found sporadically and at low levels.

Flavobacteriaceae were occasionally recovered in the SW samples (*Cryse. meningosepticum* and *Cryse. indologenes* only once and in very few units), while they were isolated in higher concentrations in the samples of SCW and CCW, including the species *W. virosa* which has rarely been associated with nosocomial invasive infections [21]. The proliferation of some species of Pseudomonadaceae and Flavobacteriaceae in the dispensed waters, and their inhibiting action on other bacteria, probably explains the lack of 'other species/genera' (e.g. *Moraxella*) which were present at high levels in the supply water.

In Italy, the legislation regulating the quality of water destined for human consumption (D. Lgs. 31/2001, application of Directive 98/83/EC) [36], specifies the usual indicators of faecal contamination (*Escherichia coli,* enterococci) as microbiological parameters, while *Ps. aeruginosa* is included among the 'supplementary' parameters to be applied only if deemed necessary by the Health Authority responsible for the control and vigilance of the quality of potable water. However, no standard has yet been established for *Ps. aeruginosa* in unbottled drinking water. Moreover, there are currently no standards set by the European Community for the microbiological quality of water dispensed by point-of-use microfiltration devices.

The results of the presented study suggest that the use of microfiltration devices in sensitive environments such as hospitals, nursing homes for the elderly, etc., may represent a new source of emerging nosocomial infections. In this context, the traditional testing for water quality is of little relevance, although it would be useful as a measure of control and prevention to associate the determination of HPCs with a quantitative search for *Ps. aeruginosa* (already required for bottled water) and for other non-fermenting Gram negative bacteria, since there is not always a direct correlation between HPCs and NF-GNB. HPCs are important indicators of bacteria re-growth in the circuits and of changes in the water quality, whereas the detection of NF-GNB would allow the identification of species with higher potential pathogenicity, and thus provide a useful tool for assessing the risk of infection and determining the possible disinfection measures to be undertaken.

Acknowledgements
The authors express their thanks to the staff of the Food Hygiene and Nutrition Service directed by Dr. Emilia Guberti (Bologna Local Health Authority) for their much appreciated contribution.

REFERENCES

1. Zanetti F, De Luca G, Sacchetti R. Control of bacterial contamination in microfiltered water dispensers (MWDs) by disinfection. Int J Food Microbiol. 2009; 128: 446–452.
2. Sacchetti R, De Luca G, Zanetti F. Influence of material and tube size on DUWL contamination in a pilot plant. New Microbiol. 2007; 30: 29–34.
3. Leoni E, Sacchetti R, Zanetti F, Legnani PP. Control of *Legionella pneumophila* contamination in a system for respiratory hydrotherapy with sulphurous spa water. Infect Control Hosp Epidemiol. 2006; 27(7): 716–721.
4. Szymanska J. Bacterial decontamination of DUWL biofilm using Oxigenal 6. Ann Agr Environ Med. 2006; 13: 163–167.
5. Wang JL, Chen ML, Lin YE, Chang SC, Chen YC. Association between contaminated faucets and colonization or infection by nonfermenting Gram-negative bacteria in intensive care units in Taiwan. J Clin Microbiol. 2009; 47(19): 3226–3230.
6. Leclerc H, Schwartzbrod L, Dei-Cas E. Microbial agents associated with waterborne diseases. Critical Rev Microbiol. 2002; 28: 371–409.
7. Stojek NM, Szymanska J, Dutkiewicz, J. Gram-negative bacteria in water distribution systems of hospitals. Ann Agr Environ Med. 2008; 15: 135–142.
8. Aydemir C, Aktas E, Eldes N, Kutsal E, Demirel F, Ege A. Community-acquired infection due to *Stenotrophomonas maltophilia*: a rare cause of septic arthritis. Turk J Pediatr. 2008; 50: 89–90.
9. Brooke JS. *Stenotrophomonas maltophilia:* an emerging global opportunistic pathogen. Clin Microbiol Rev. 2012; 25: 2–41.
10. Fihman V, Le Monnier A, Corvec S, Jaureguy F, Tankovic J, Jacquier H, et al. *Stenotrophomonas maltophilia* – the most worrisome treat among unusual non-fermentative gram-negative bacilli from hospitalized patients: a prospective multicenter study. J Infection. 2012; 64: 391–398.

11. Mahenthiralingam E, Baldwin A, Dowson CG. *Burkholderia cepacia* complex bacteria: opportunistic pathogens with important natural biology. J Appl Microbiol. 2008; 104: 1539–1551.
12. Zanetti F, De Luca G, Stampi S. Recovery of *Burkholderia pseudomallei* and *B. cepacia* from drinking water. Int J Food Microbiol. 2000; 59: 67–72.
13. Lee SM, Kim MK, Lee JL, Wee WR, Lee JH. Experience of *Comamonas acidovorans* keratitis with delayed onset and treatment response in immunocompromised cornea. Korean J Ophthalmol. 2008; 22: 49–52.
14. Stampi S, Zanetti F, Bergamaschi A, De Luca G. *Comamonas acidovoran*s contamination of dental unit waters. Let Appl Microbiol. 1999; 29: 52–55.
15. Ryan MP, Pembroke JT, Adley CC. *Ralstonia pickettii:* a persistent gram-negative nosocomial infectiou organism. J Hosp Infect. 2006; 62: 278–284.
16. Yoshino Y, Kitazawa T, Kamimura M, Tatsuno K, Ota Y, Yotsuyanagi H. *Pseudomonas putida* bacteraemia in adult patients: five case reports and a review of the literature. J Infect Chemotherapy. 2011; 17: 278–282.
17. Sunbul M, Zivalioglu M, Tasdelen Fisgin N. Community-acquired *Pseudomonas stutzeri* meningitis in immunocompetent patient. Mikrobyol Bul. 2009; 43: 159–162.
18. Wong V, Levi K, Baddal B, Turton J, Boswell TC. Spread of *Pseudomonas fluorescens* due to contaminated drinking water in a bone marrow transplant unit. J Clin Microbiol, 2011; 49: 2093–2096.
19. Ceyhan M, Celik M. *Elizabethkingia meningosepticum (Chryseobacterium meningosepticum)* infections in children. Int J Ped. 2011; doi: 10.1155/2011/215237.
20. Shah S, Sarwar U, King EA, Lat A. *Chryseobacterium indologenes* subcutaneous port-related bacteraemia in a liver transplant patient. Transpl Infect Dis. 2012; 14: 398–402.
21. Martinez MA, OValle A. *Weeksella virosa.* Rev Chil Infectol. 2011; 28: 429–430.
22. Manckoundia P, Mazen E, Coste AS, Somana S, Marilier S, Duez JM, et al. A case of meningitis due to *Achromobater xylosoxidans* denitrificans 60 years after a cranial trauma. Med Sci Monitor. 2011; 17: 63–65.
23. Epling J. Bacterial conjuctivitis. Clin Evid. 2012.
24. Gupta N, Arora S, Kundra S. *Moraxella catarrhalis* as a respiratory pathogen. Ind J Path Microbiol. 2011; 54: 769–771.
25. Stojek NM, Dutkiewicz J. *Legionella* and other Gram-negative bacteria in potable water from various rural and urban sources. Ann Agr Environ Med. 2006; 13: 323–335.
26. Volker S, Schreiber C, Kistemann T. Drinking water quality in household supply infrastructure. A survey of the current situation in Germany. Int J Hyg Environ Health. 2010; 213: 204–209.
27. Chaberny IF, Kaiser P. Sonntag HG. Can soda fountains be recommended in hospitals? Int J Hyg Environ Health. 2006; 209: 471–475.
28. Baumgartner A, Grand M. Bacteriological quality of drinking water from dispensers (coolers) and possible control measures. J Food Prot. 2006; 69: 3043–3046.
29. Lévesque B, Simard P, Gauvin D, Gingras S, Dewailly E, Letarte R. Comparison of the microbiological quality of water coolers and that of municipal water systems. Appl Environ Microbiol. 1994; 60: 1174–1178.
30. Standard Methods for the Examination of Water and Wastewater. 21th edn Washington, DC: APHA-AWWA-WEF, APHA 2005.
31. Mena KD, Gerba CP. Risk assessment of *Pseudomonas aeruginosa* in water. Rev Environ Contam Toxicol. 2009; 201: 71–115.
32. Guidelines for drinking water quality, fourth Ed. WHO, Geneva 2011.
33. Sacchetti R, De Luca G, Zanetti F. Control of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* contamination of microfiltered water dispensers with peracetic acid and hydrogen peroxide. Int J Food Microbiol. 2009; 132: 162–166.
34. Hansen CR. *Stenotrophomonas maltophilia*: to be or not to be a cystic fibrosis pathogen. Curr Opin Pulm Med. 2012; 18: 626–631.
35. Waters V, Atenafu EG, Salazar JG, Lu A, Yau Y, Matukas L et al. Chronic *Stenotrophomonas maltophilia* infection and exacerbation outcomes in cystic fibrosis. J Cyst Fibros. 2012, 11: 8–13.
36. D.Lgs 31/2001. Attuazione della Direttiva 98/83/CE relativa alle acque destinate al consumo umano. G.U. Repubblica Italiana. Supplemento n°52 del 3 marzo 2001.