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Control of microbial contamination in drinking water from microfiltering dispensers by dialysis ultrafilters

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Abstract. Tap water filtering devices are widely employed to improve odor and taste of tap water, or to obtain refrigerated or sparkling drinking water. The presence of disinfectants-resistant bacteria in tap water is responsible of the biofilm formation inside tubes and tanks. The consequent contamination of dispensed water is a well-known hygiene problem because of the quite constant presence of potentially pathogenic bacteria likes *P. aeruginosa*. In this study, we tested the technical feasibility and effectiveness of the addition to different commercial devices of a packaged polysulphone fibers filter. We aimed to find a simple solution to implement the quality of the delivered water. Water contamination levels were determined in a wide selection of microfiltered water dispensers and we selected among them a representative group of 10 devices, new or in use. The packaged ultrafilter was introduced in about half of them, to monitor, when possible, in parallel the contamination levels and flow rate of a couple of identical units, with and without the filter. The placement of the dialysis filters resulted feasible at different positions along the water circuits of the variously designed filtration units. Delivered water resulted completely free from bacteria when the filter was placed exactly at, or very close to, the outlet in spite of the inner surfaces contamination. This performance was not obtained in presence of a more or less long tract of water circuits downstream the ultrafilter: a significant but not complete reduction of the plate count numbers was observed. The filters worked in continue over the whole study period, ten months, showing exactly the same efficiency. Moreover, the flow rate in presence of the filter was quite unaffected. The addition of this kind of filter to already in use water dispensers was technically easy, and its use can be recommended in all cases a simple but reliable water sanitization is requested.

Keywords: water microfiltration devices; drinking water quality; sterile dialysis filters; *P. aeruginosa*.

1. Introduction

The organoleptic and compositional characteristics of tap water can greatly differ, but in most cases the consumers feel them are not satisfying. As an alternative to the bottled water consumption, the number of microfiltered water dispensers (MWDs), coolers, and soda fountains installed in private residences and public places has increased considerably.

All these devices suffer the same hygiene problems noticed in other devices dispensing potable water and having a tap water input, such as dental units [1]. Tap water contains aerobic, heterotrophic, bacteria which inside the MWDs circuits create multispecies adherent biofilm, source of the undesired, bacterial contamination in the dispensed water, often higher with respect to the input one [1]. The microorganisms detected in tap water are those particularly resistant to disinfecting agents [2]. Inside the microfiltration units, their vitality is favored by the activated carbon filters that remove chlorine and by the slow flow or stagnation inside tanks. *P. aeruginosa*, the most frequently detected one [3-5], and other bacteria are well-known opportunistic pathogens, noxious for human health especially in case of vulnerable individuals [6].

The EU rules concerning the quality of drinking water [7] set restrictive limits to the presence of microorganisms, creating the need for a careful monitoring of drinking water and for its effective sanitization, as indicated by the number of publications suggesting a solution to this problem [5, 8-13]. The awareness about the not good hygiene of the water from MWDs is recently growing among the consumers, too.

Nowadays, in most cases the solution applied to the in use devices is a periodical sanitization by chemicals, which can offer only temporary and not satisfactory results. Alternatively, UV-rays sources (low-pressure bulbs or UV-LEDs) have been added to WMDs in order to exploit their bactericide effect [14-16] but to our knowledge, these solutions achieve completely different, sometime negligible, results.

The aim of this work was to investigate the effectiveness of the addition into some of the most common commercial MWDs of a packaged fibers ultrafilter as a physical sanitization tool for drinking

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water. Such a kind of packaged filter was designed for use in dialysis hospital units' and we supposed it was suitable to remove the bacterial contamination from dispensed water, in spite of the biofilm amount on the circuits' surfaces. We evaluated the technical adjustments required for its placement in already assembled, in use devices as well as its performance with respect to the position along the water circuits, the influence on the water flow rate, and the sanitization effect over time.

2. Experimental

2.1. Water Microfiltration Devices

Ten water filtration units were selected among several different models on the market and included in this study. A local agent of these devices kindly put the units at our disposal. The selected devices represented, in our opinion, the two main categories of WMDs designed for private residences or public places, and in Table 1 we summarized the main information about their features. The units from 5 to 9 were completely new; the other ones were in use. Depending on the availability of the devices, we tried to work with couples (or triplicates, as the devices No. 5, 6, 7) of identical units in order to obtain representative data. Otherwise, we monitored the same device with and without the ultrafilter, like in case of the bench top filtration device No.10. This particular device was designed with a quartz-made, spiral-shaped tube representing the outlet and an UV lamp placed in the center of the spiral, switched on starting the water flow. Because the lack of a second device, after a first monitoring period we switched off the UV lamp and we attached the packaged ultrafilter to the outlet. Concerning the other devices, in the No. 4 the filter was fitted in the water circuit quite far from the outlet because physical hindrance. In the undersink units No. 5 and No.7, we inserted the packaged filter just before the outlet.

Table 1. Structural and functional features of the water filtration units included in this study.

Assigned Number	Characteristics	Water filtration system	Water sterilization system
1, 2	Twin undersink filtration units dispensing still, sparkling, chilled filter water (3 water lines, 1 outlet) IN USE	Activated carbon(ACF)	None.
3, 4	Twin benchtop filtration units dispensing still water. IN USE	ACF + Osmotic membrane	**3**: None **4**: Packaged ultrafilter
5, 7	Twin Undersink filtration units dispensing still water. NEW	ACF + Osmotic membrane	Packaged ultrafilter on the water line before the outlet
6	Undersink filtration unit dispensing still water NEW	ACF + Osmotic membrane	UV LEDs before the outlet
8, 9	Twin benchtop coolers dispensing still, sparkling, chilled water (3 water lines, 1 outlet) NEW	ACF	None
10	Benchtop cooler dispensing still, sparkling, chilled water (3 water lines, 1 outlet) NEW	ACF	*1st group of experiments:* UV inside the outlet. *2nd group:* a packaged ultrafilter at the outlet, the UV lamp was switched off.

2.2. Packaged dialysis ultrafilter

The dialysis sterile ultrafilters were made by polysulphone fibers packaged in a plastic cylindrical container of about 12 cm length x 5 cm ∅ (Culligan Pure Filter, Culligan Italiana SpA, Cadriano, BO, Italy). The filter can block all contaminants with molecular weight higher than 15 kDalton. Adapters supplied together to the filter allow to easily connecting the filter to plastic tubes of different diameter.

We checked the influence of the ultrafilter on the delivered water flow rate by evaluating it at regular time intervals: the time required to collect a five liters volume was recorded over all the investigation period.

2.3. Samples

We collected the water samples (100 mL) directly from the outlet of the MWDs or from the tap supplying the devices, in plastic sterile containers (IDEXX Laboratories, Inc., Westbrook, ME, USA).

We followed a four-day sampling cycle according to the procedure indicated by the Watercoolers Europe Association [17].

Briefly: the first, second and third day the whole content of the tank, when it was present, was drained off two times per day. In case of coolers without tanks we performed water draining off for 10 min, six times per day. In the afternoon of the third day, we collected a sample from the water inlet to the cooler. The fourth day we collected a sample from the water inlet, drained off half of the tanks' content and then we collected water samples from each circuit, through the same outlet.

2.4. Microbiological analysis: total aerobic plate count

We determined the total number of colony forming units (CFU) in the water samples by aerobic plate count according to the National Legislation rules for microbiological analysis [18]. Shortly, four aliquots of 1 ml each one were obtained from each water sample and placed in four Petri plates, 9 cm diameter, and immediately added with about 15 mL of Plate Count Agar medium (Sigma Italia, MI, Italy) maintained fluid till use in a 45°C water bath. We incubated a couple of plates at 22°C and the second one at 37°C for 48 and 72 h, respectively.

The presence of *Pseudomonas aeruginosa* was qualitatively revealed by following the development of blue-green, fluorescent, or red-brown colonies on the plates incubated at 37°C.

2.5. Microbiological analysis: colorimetric-fluorescent test kits

We determined the presence of *Enterococcus spp, Escherichia coli*, *Pseudomonas aeruginosa*, and the Total Coliforms number by using three specific colorimetric/fluorescent kit tests included among the official assays for water microbiological quality assessment [18]. The kits (IDEXX Laboratories, Inc.,

Westbrook, ME, USA), based on the Most Probable Number (MPN) methodology, were employed according to the producer's instructions.

By the "Colilert-18" multiwell kit were determined both the total number of coliforms and the number of *Escherichia coli* colonies. The water sample, 100 mL, was added to the growth substrate. Incubation was performed at 44°C to reveal the presence of *E. coli* by measuring the fluorescence emission and at 35°C to obtain the colorimetric determination of the total number of coliforms. Both results were obtained after 18 hours.

The "Enterolert" fluorescent kit was employed to evaluate the total amount of *Enterococcus spp*. in half time with respect to the standard methods [19]. The US EPA, FDA, and APHA including it in the Manual of "Standard Methods for Examination of Water and Wastewater" [20] already approved its use.

By using the "Pseudoalert" kit we quantified the presence of *Pseudomonas aeruginosa* by the fluorescence emission of a dye included in the incubation medium, after 24 h incubation at 36°C.

3. Results and Discussion

3.1. Drinking water microbiological quality monitoring: the starting set of MWDs

During the first six months, we repeated weekly the CFU/mL determination in water dispensed by the selected devices and in municipal tap water. The plate counts for samples from cooler No. 10 were always equal to zero, since UV disinfection occurred exactly during drawing. The same results were obtained for the two exactly new devices (No. 5 and 7) equipped with the ultrafilter all over this first period.

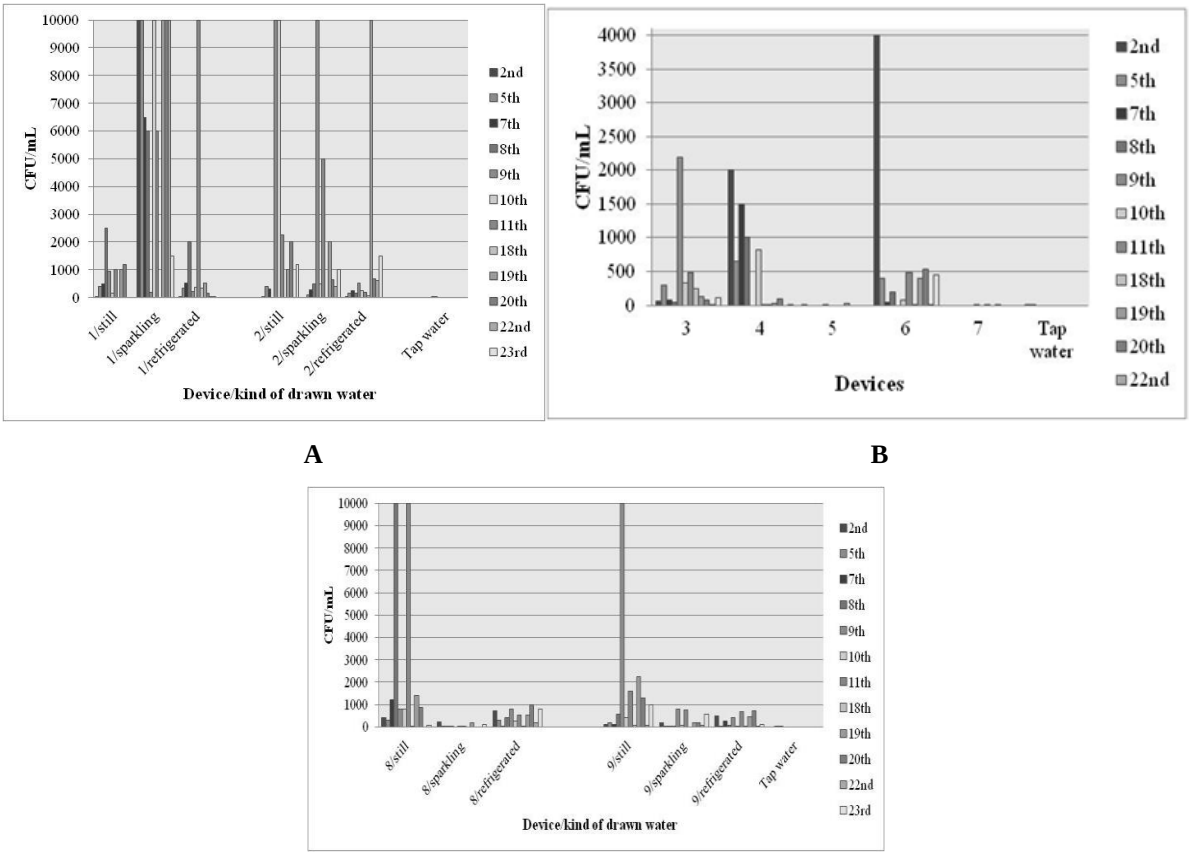

Figure 1. Aerobic plate count data obtained after incubation at 37°C of delivered water samples collected during the weeks, from the start of investigation, reported in the caption. A: samples from devices 1 and 2. B: samples from devices 3-7. C: samples from devices 8-10.

Water samples from all other filtration units resulted more or less seriously contaminated. To reduce the contamination by chemical disinfection procedures obtained only temporary results.

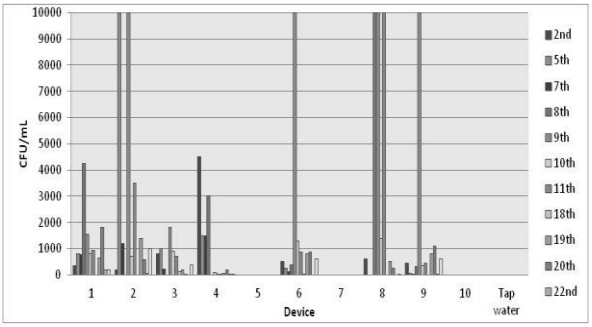

Figure 2. Aerobic plate count data obtained after incubation at 22°C of delivered water samples. To make understanding easier we reported for all devices only results concerning the still water

We reported in Figure 1 examples of the plate count data obtained after incubation at 37°C and in Figure 2 those at 22°C just to show the general trend of the contamination levels. The too high CFU/mL values were cut at 10,000.

3.3. Luminescent-colorimetric tests

Concerning the devices never used before this study the number of samples resulting positive to the specific microbiological tests was very limited.

Tap water at our laboratories was free from *P. aeruginosa*, *E. coli*, coliforms bacteria or Enterobacteriaceae all through the investigation.

The filtration unit No. 1, already in use before our study, was the only one resulting from the beginning contaminated by *P. aeruginosa* and later on by Enterobacteriacae or coliforms bacteria The most affected water line was the sparkling one. Some of those data were reported in Figure 3. Lower values were recorded in the refrigerated water samples.

As expected, all tests gave negative results when applied to refrigerated water samples after we inserted the ultrafilter on this waterline.

Table 2. Plate count data obtained after incubation at 37 and 22°C, respectively, the water samples collected from the devices 1 and 6, now equipped with the packaged filter, 5 and 7 provided with the packaged filter from the beginning of the investigation. These data can be compared with those in Figure 1 A and B.

Device/ kind of sampled water	**CFU/ml**			
	Sampling day			
	1st day	**18th day**	**25th day**	**56th day**
	(37/22°C)	**(37/22°C)**	**(37/22°C)**	**(37/22°C)**
1/ still	600/1200	400/800	1300/1550	539/1120
1/ sparkling	20/110	70/170	14/59	109/340
1/ refrigerated	0/0	1/1	0/0	0/1
5	2/0	3/6	0/0	23/2
6	2/0	1/1	0/0	5/2
7	1/0	0/0	0/0	3/9
Tap water	0/0	0/0	0/1	0/0

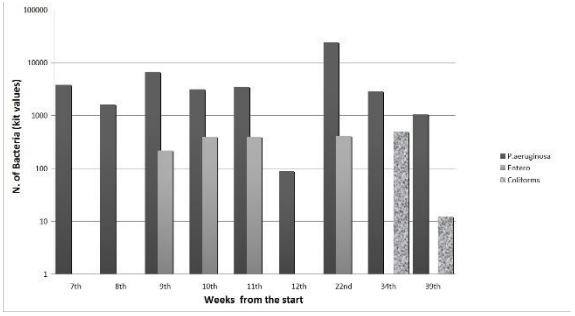

Figure 3. Amounts of specific bacteria evaluated by colorimetric-fluorescent kits on samples from No. 1.

3.4. Dispensed water flow rate

A possible drawback consequent to the addition of an ultrafilter was a loss in the flow rate of the dispensed water. To this regard, we monitored regularly the flow rates until the end of the investigation. As an example, we reported in Figure 4 a set of these values concerning the device No. 10 and recorded during a 45 days period of intensive use, i.e. water flowed continuously during the daily working time at the laboratory.

In Figure 5 the trend of the flow rate values for each filtration unit during the whole period was represented by showing the mean values recorded for five on the ten months.

The results of the microbiological analysis we performed on the selected devices (Figures 1 and 2) and on a wider number of WMDs (data not shown) confirmed the literature data: water dispensed by devices supplied with tap water and lacking in effective sanitizing solutions will be more or less heavily contaminated by opportunistic, disinfectants-

resistant bacteria. Many water samples showed CFU/mL higher than 10,000 and the presence of *P. aeruginosa*, enterobacteriaceae, and coliforms was detected (Figure 3). Such a kind of drinking water not in agreement with the current rules should not be administered in public places. UV-rays emitting lamps represent an effective disinfecting tool, but they must be correctly employed, as confirmed by the data we obtained for the devices 6 and 10.

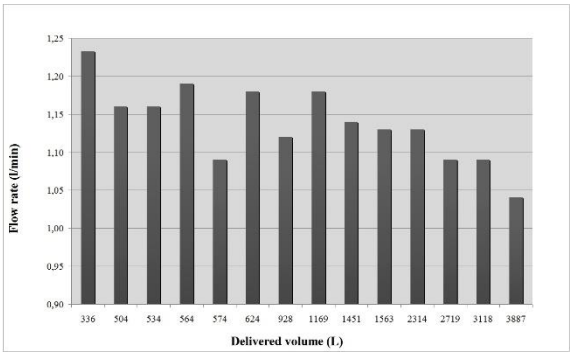

Figure 4. Delivered water flow rate values for the benchtop cooler No. 10. over a 45 days period of intensive use (total dispensed volume: 3887 liters) the water flow rate was reduced of the 15.5%.

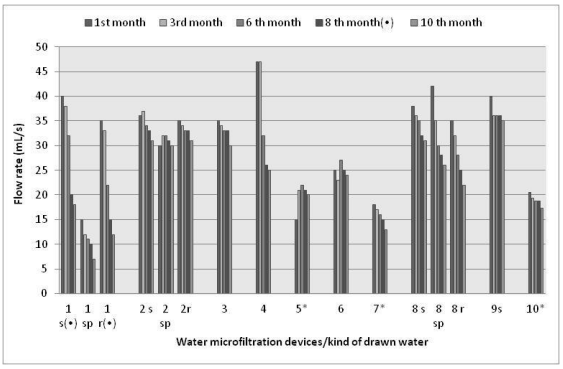

Figure 5. The trend of the delivered water flow rate for all the devices included in this study was here represented by showing some representative data. ../s=still water; ../sp=sparkling water; r=refrigerated water. (●) = on these waterlines the ultrafilter was added in October; (*) = devices equipped with the same ultrafilter from the beginning to end of the study.

Only the second one was completely effective in sanitizing the dispensed water thanks to a particular combination of materials and design. In device No. 6, as well as in other WMDs tested in preliminary assays, the best result was a reduction of the bacterial charge with respect to that measured before the irradiation system. After sanitization water must avoid contact with any contaminated surface of the device, on the contrary to use UV rays is useless.

The unsatisfactory results obtained from UV-lamp equipped WMDs must alert about the possible health risks connected with the use of these systems. The presence of a disinfecting lamp surely produces a drop in consumers' guard on the quality of the water they drink.

The aims of our work, *i.e*. to place sterile filters for medical use in already assembled WMDs and test their effectiveness of as an alternative solution to the presence of UV irradiation systems was quite easily achieved. Our proposal to sanitize water by filtration was obviously not new, but it was, to our knowledge, never applied before to ensure good hygiene conditions of water from microfiltration devices.

The sterile dialysis ultrafilter presented all the favorable characteristics: it was not expensive and equally effective for long time; it was highly effective in sterilizing large volumes of fluids in quite short time thanks to the large surface of the packaged fibers; it had small dimensions and accessories to fit it to tubes of several dimensions. In case it was not possible to place it close to the outlet (device No. 4) we experienced the same situation of device No. 6: the not correct placement of the sanitizing tool greatly affected its performance, because of the downstream presence of contaminated circuit tracts.

The practical applicability of this solution was tested monitoring the time interval during which the filter maintained its efficiency and the influence of filter addition on the flow rate of delivered water.

Once applied, regardless of the bacterial activity present inside the WMD, the ultrafilter maintained water sanitized until the end of the investigation period (10 months) and longer. This performance was unexpected and it clearly indicates that this kind of filter will be a very convenient solution greatly reducing the maintenance activities on the WMDs.

To discuss the ultrafilters' impact on the flow rate it must be done taking into account that a multifactorial influence exists. The devices showed different initial flow rate because of differences in their designs or in the number of filtration systems (activated carbon filter alone or coupled to an osmotic membrane). The in use devices can suffer a reduction in the flow rate due to the fouling effect produced by water particulate. We observed a remarkable reduction of the flow rate following the introduction of the packaged filter only in case of the undersink device No. 1. After six-months of continuous use and one month after the packaged filter addition the percent of reduction in the flow rate was important, being around the 50±10%. In all other cases, the changes in flow rate values can be considered not significant and, in our opinion, six months of safe water production with minimal decay of the flow rate can be considered a good result. In case the flow rate is not an important parameter, the lifetime of these ultrafilter can be at least the double of this period.

4. Conclusions

The data here reported indicate that the introduction of an effective sanitization tool downstream to the usual filtration system is strongly

recommended in the most part of WMDs on the market. The contamination levels detected in drinking water were in most of cases unacceptable. It must be underlined that the supplying water was of medium-high quality. The use of such WMDs in situations with scarce tap water quality can result in a serious risk of waterborne diseases.

The sterile dialysis filters here tested are cheaper than an UV lamp and easier to be installed in any already in use WMD. Further studies are in progress to ascertain the performance of these packaged fibers filter when applied to different filtration devices or in obtaining sanitized water in on field situations.

Conflict of interests

The authors declare that there is no conflict of interests.

Acknowledgements

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