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Studies on Microbial Barrier Faucets for Sterile Solutions. II.[1] Trial Manufacture of Microbial Barrier Faucets

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Sterility tests of distilled water obtained through faucets in hospitals revealed that all of the samples were contaminated with microbes. Once the faucets became contaminated with airborne microbes, all distilled water obtained subsequently was contaminated. Two types of microbial barrier faucets were manufactured and tested by a method for microbial contamination testing using air artificially loaded with bacteria, as reported in the previous paper. One of the faucet was reasonably effective in preventing airborne microbial contamination, but further improvement is required for practical purposes.

Keywords——microbial contamination; distilled water; faucet; airborne microbes; artificial contamination

In the field of clinical medicine, it is generally recognized that liquid preparations for surgical treatment and distilled water for washing prior to surgical operations are liable to suffer microbial contamination[3] from airborne microbes which may induce iatrogenic infections in patients.[4] An assured supply of decontaminated piped water is therefore desirable.[5] In hospital pharmacies, similar contamination has been suspected in distilled water flowing through supposedly sterile faucets.[6] One cause of water contamination is contamination of the faucet terminal by airborne microbes, as reported in the previous paper.[1] Since a microbial barrier faucet is required to avoid this water contamination, a test method for microbial barrier performance of faucets using a microbe-air blower was developed in the previous paper.[1] In this paper, actual contamination of distilled water was first investigated and trial manufacture of microbial barrier faucets was attempted.

Experimental

Materials——Thioglycollate (TGC) medium I (Nissui Seiyaku Co., Ltd.) and glucose-peptone medium (Nissui Seiyaku Co., Ltd.) were used for sterility testing of distilled water. Biofermin containing *Streptococcus faecalis* 129 B 10 3B: AKU: MKU and *Bacillus subtilis* 129 B 10 H(α) obtained from Biofermin Pharmaceutical Co., Ltd. was used as a test contaminant. The particle size of this powder contaminant was less than 75 μm (diameter).

Microbial Barrier Faucet with Automatic Valves[7]——Fig. 1 shows a diagram of a trial faucet which consists of a filtered air blower A, a pipe for distilled water B, a tank for disinfectant solution C, automatic valves D, E and F, an internal pipe for distilled water G, an external pipe for filtered air H and a lid for the external pipe I. Valve D shuts off filtered air at the internal pipe and valve E shuts off disinfectant solution. Filtered

1) Part I: K. Takata, O. Fujishita, and S. Hokama, *Chem. Pharm. Bull.* (Tokyo), **27**, 1231 (1979).
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3) H. Usami, *Byoin Setsubi*, **15** (5), 19 (1973); Y. Hagihara and T. Muteki, *Ikakikaigaku*, **44**, 399 (1974).
4) R.G. Mitchell and A.C. Hayward, *Lancet*, **1**, 793 (1966).
5) T. Ashiyama, T. Hiraga, and Y. Suenaga, *Ikakikaigaku*, **40**, 403 (1970).
6) Japanese Pharmacopoeia 9th ed., 1976, p. 995.
7) K. Takata, Japan. Patent Kokai 51-148251 (1976).

air always blows into the space between the internal and external pipes. When distilled water is not flowing, only valve D is opened and filtered air is introduced inside the internal pipe G for drying. When disinfectant solution is passed to disinfect the terminals of both pipes, valve F is closed after putting the lid I tightly on the external pipe. All valves are operated automatically by functional push buttons.

The internal pipe has an inside diameter of 4 mm and its terminal end is situated at a distance of 20 mm from the end of the external pipe, which has an inside diameter of 40 mm. The air is filtered through a HEPA filter to eliminate 99.97% of particles of 0.3 μm in size. The air velocity of filtered air is 1.2 m/sec at a distance of 50 mm below the end of the external pipe. This faucet was designed by the authors and manufactured by Tokyo Shokai Co., Ltd., Tokyo.

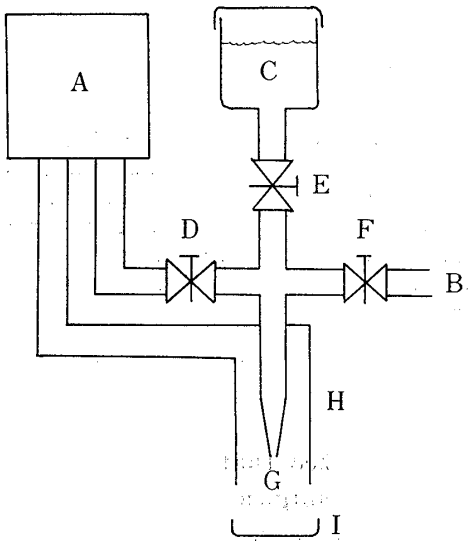

Fig. 1. Microbial Barrier Faucet with Automatic Valves

A, filtered air blower; B, pipe for distilled water; C, tank for disinfectant solution; D,E,F, automatic valves; G, internal pipe; H, external pipe; I, lid for external pipe.

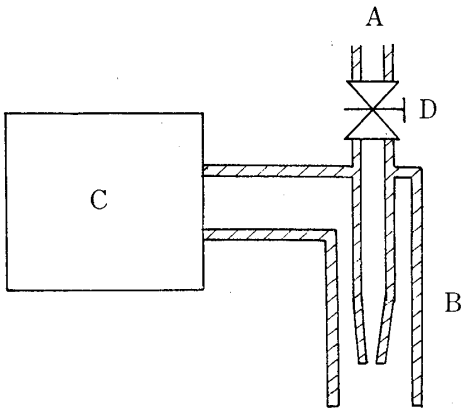

Fig. 2. Microbial Barrier Faucet with Dual Pipes

A, Internal pipe; B, external pipe; C, filtered air blower; D, valve.

Microbial Barrier Faucet with Dual Pipes[8]——Fig. 2 shows a diagram of a faucet consisting of an internal pipe A for distilled water, an external pipe B for filtered air, a blower C and a valve D. The end of the internal pipe has an inside diameter of 4 mm and is situated at a distance of 9 mm from the end of the external pipe, which has an inside diameter of 14 mm. The air velocity of the filtered air is 1.95 m/sec at a distance of 50 mm below the end of the external pipe. The air is first filtered through a HEPA filter to eliminate 99.97% of particles of 0.3 μm in diameter, and the resulting filtered air always flows independently of the distilled water. This faucet was designed and manufactured by the authors, and was sterilized in an autoclave before installation except for the filtered air blower.

Faucet with a Bell-shaped Dust Protector——This faucet consists of a pipe for distilled water and a bell-shaped dust protector with an inside diameter of 40 mm, fixed to the pipe. The end of the pipe is situated at a distance of 27 mm from the end of the protector.

Anemometer——An anemometer (model AM-2) manufactured by Oshitari Laboratory Inc. was used for the measurement of air velocity.

Preparation of Distilled Water——Distilled water was prepared in the authors' pharmacy using a Thermodrive TD-15 distillation system manufactured by Barnstead Sybron Corporation.

Collection of Samples of Distilled Water——Samples of distilled water were collected from the authors' Pharmacy, another department of the same hospital and a pharmacy of another hospital. Ordinary faucets equipped with membrane filters were installed in a clean room, where a pressurized filtered air blower was installed, and where cleanliness class 100000 was maintained according to NASA standards[9] when two persons were working there. The above distilled water was supplied *via* these faucets, and sterility tests were carried out on the distilled water obtained from the faucets.

8) K. Takata, Japan. Patent Kokai 53-40059 (1978).
9) "NASA Standards for Clean Room and Work Stations for the Microbially Controlled Environment," NHB 5340.2 August 1967 ed., National Aeronautics and Space Administration, Washington, D.C. 20546, 1967, p. 3.

Sterility Test——Contamination of bacteria and true fungi was examined in samples of distilled water obtained from the faucets in accordance with the sterility test of the Japanese Pharmacopoeia 9th ed.[10] For bacterial detection, 1 ml of the sample was added to 15 ml of TGC medium in a test tube and incubated at 32° for 7 days. For the detection of true fungi, 1 ml of the sample was added to 15 ml of glucose-peptone medium in a test tube and incubated at 25° for 7 days.

Faucet Contamination Tests with Artificially Contaminated Air——The microbe-air blower reported in the previous paper[1] was used to produce contaminated air, and Biofermin was used as the contaminant. The microbial barrier faucets were installed in an office room, and these faucets were supplied with distilled water filtered through a 0.22 μm membrane filter. One g of Biofermin was jetted towards the faucets at a distance of 10 cm or 20 cm from the jet nozzle, and distilled water obtained through the faucets was examined to determine whether contamination had occurred. In this contamination test, a faucet equipped with a protecting bell was used as a control.

Results

Sterility Tests of Various Samples of Distilled Water

Table I shows the results of sterility tests carried out with various samples of distilled water obtained through conventional faucets. Successive sterility tests revealed that all

Table I. Microbial Contamination of Distilled Water obtained through Ordinary Faucets

Day	I[a]				II[b]		III[c]			(origin of water sample)
	1	2	3	4[d]	1	2	1	2	3	(No. of faucet)
	B F	B F	B F	B F	B F	B F	B F	B F	B F	(bacteria and true fungi)
1	+	+	+	+	++	++	++	++	++	
14	+	+	+	+						
21	++	++	++	++						
120			++	++						
150			++	++						
180			−+	++						
210				++						

a) Authors' pharmacy.
b) Another laboratory of the authors' hospital.
c) A pharmacy of another hospital.
d) Faucet connected to a 0.45 μm membrane filter.

Table II. Microbial Contamination of Distilled Water after-Flow

Volume or time of water flow	1	2	3	4[a]	5[b]	(No. of faucet)
	B F	B F	B F	B F	B F	(bacteria and true fungi)
Beginning	+	+	+	++	++	
500 ml	+	+	+	+		
1000 ml	+	+	+	+		
2000 ml	+	+	+	+		
10 min				++		
20 min				+		
30 min				++	++	
40 min				+		
60 min					++	

a) Connected to a 0.45 μm membrane filter.
b) Connected to a 0.22 μm membrane filter.

10) Japanese Pharmacopoeia 9th ed., 1976, p. 695.

of the samples tested except one were contaminated with true fungi and bacteria. Similar contamination was noted in distilled water obtained through a faucet which was connected to a 0.45 μm membrane filter. Town water obtained from the authors' hospital was also contaminated as above, but the cultured medium was less turbid than in the case of the distilled water (data not shown).

Even when distilled water at the authors' pharmacy was allowed to flow through the faucets before testing (various volumes or times), the water did not become sterile. Decontamination was not achieved even after flowing for 60 min in addition to filtration through 0.45 μm and 0.22 μm membrane filters (Table II).

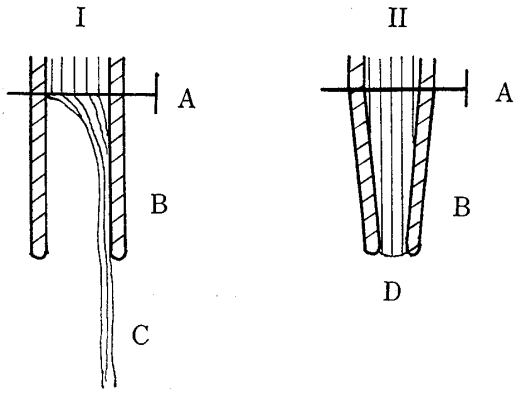

Fig. 3. Two Types of Faucets

I, faucet in which the terminal water is replaced by room air; II, faucet in which the terminal water is not replaced by room air. A, Valve; B, end of faucet; C, flowing water; D, retained water.

Airborne Microbial Contamination of Distilled Water obtained through a Faucet in an Office Room

Two types of faucets are shown in Fig. 3. In one, water flowed out of terminal portion B to produce a space when valve A was closed, and room air occupied the space; in the other, water always filled the terminal portion B, and replacement with air did not occur. Table III shows the time (days) which elapsed before the appearance of microbial contamination in these faucets installed in an office room. Rapid contamination with airborne microbes occurred. The faucet in which water in the terminal portion was easily replaced by room air was contaminated more rapidly than the other faucet. Once the faucets suffered contamination, the water obtained through them never became sterile again.

TABLE III. Period during which Faucets Protected Distilled Water from Microbial Contamination in an Office Room

Days after sterilization	Ordinary faucet 0.45 μm I[a]		Ordinary faucet 0.45 μm II[b]		Ordinary faucet 0.22 μm I[a]		Ordinary faucet 0.22 μm II[b]					Microbial barrier faucet 0.22 μm III[c]		Microbial barrier faucet 0.22 μm IV[d]	
	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F
0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
30									+	+	−	−	−	−	+	+

a) Faucet in which the terminal water is replace by room air.
b) Faucet in which the terminal water is not replaced by room air.
c) Faucet with automatic valves.
d) Faucet with dual pipes.

Contamination Tests of a Microbial Barrier Faucet with Automatic Valves

As shown in Table III, microbial contamination of the distilled water occurred 30 days after sterilization of the faucet. Table IV shows the results of airborne microbial contami-

nation tests carried out using the microbe-air blower. The microbial barrier faucet showed a high protecting ratio of 80% when microbes were jetted at a distance of 20 cm from the nozzle, but a low ratio of 30% when the microbes were jetted at a distance of 10 cm. This performance was inferior to that of the faucet equipped with a protecting bell. In addition, complicated procedures were required for sterilization of the microbial barrier faucet after it became contaminated with microbes.

TABLE IV. Protection Ratio of Faucets in Microbial Contamination Tests using Air artificially Contaminated with Bacteria

Faucet	Jet distance (cm)	No. of expt.	Protection ratio (%)
Control[a]	20	10	0
With protecting bell[b]	10	21	48
	20	10	50
With valves[c]	10	10	30
	20	15	87
With dual pipes[d]	10	50	90

a) Faucet of 4 mm inside diameter and no water replacement by room air.
b) The same faucet as above with a protecting bell of 40 mm inside diameter.
c) Microbial barrier faucet with automatic valves.
d) Microbial barrier faucet with dual pipes.

Contamination Tests of a Microbial Barrier Faucet with Dual Pipes

In this case the water remained sterile for 30 days or more in an office room, as indicated in Table III. When 1 g of the contaminant was jetted at a distance of 10 cm from the nozzle, this faucet showed a high protecting ratio of 90% on 50 runs, as shown in Table IV. However, when air at the end of the internal pipe A formed bubbles in the distilled water, at the beginning of water flow some water was found to come into contact with the external pipe F (Fig. 4).

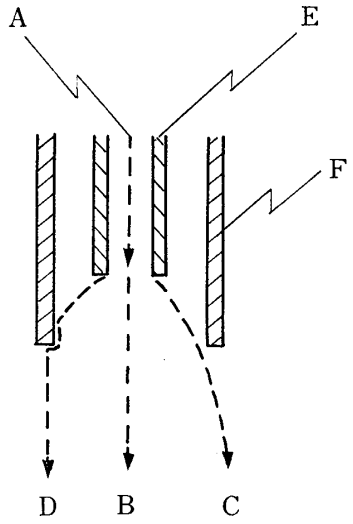

Fig. 4. Contamination by splashing of Distilled Water flowing through a Microbial Barrier Faucet

A, Flowing distilled water containing air bubbles; B, C, decontaminated splashing distilled water; D, splashing distilled water contacting the contaminated end ot the external pipe; E, internal pipe; F, external pipe.

Discussion

As an initial study prior to the development of microbial barrier faucets, actual contamination of distilled water flowing through faucets was investigated. Sterility tests revealed that all of the distilled water flowing through the faucets was contaminated with microbes, and that this contamination did not disappear even after water flow for 60 min. This suggests that the microbial contamination had reached not only the faucet terminal and vicinity but also the water source.[11]

Similarly, distilled water obtained through a faucet connected to a membrane filter never became decontaminated after microbial contamination had occurred. This contamination presumably arose from contamination of the terminal side of the filter housing, as Tsuzuki and his collaborators[12] suggested.

11) M. Furuhashi, *Geka Chiryo*, **26**, 407 (1972); T. Ashiyama, *Ikakikaigaku*, **42**, 703 (1972).
12) M. Tsuzuki, H. Kobayashi, H. Masuda, and D. Hayashi, *Ikakikaigaku*, **45**, 314 (1975).

Although the faucet which gave no air replacement was contaminated only at the outlet, the other faucet was easily contaminated throughout the terminal area as a result of direct contact with room air.[5] We therefore tested a microbial barrier faucet equipped with automatic valves. However, this faucet was no more effective than a conventional faucet equipped with a protecting bell, presumably due to inadequate air filtering. Further, complicated procedures would be required for sterilization of this microbial barrier faucet after contamination. Thus, a faucet of simple structure which could be autoclaved, that is, a microbial barrier faucet equipped with dual pipes, was tested. The results suggest that this faucet would be suitable to prevent airborne microbial contamination under normal conditions. However, air bubbles formed in the distilled water emerged with a splash, and water occasionally came into contact with the external pipe, the end of which was microbially contaminated. Consequently, further investigation is required to overcome this problem. Studies are also required to overcome this problem. Studies are also required to determine a clean air velocity sufficient to protect the distilled water completely from airborne microbial contamination.

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