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Studies on Microbial Barrier Faucets for Sterile Solutions. III.[1] An Improved Microbial Barrier Faucet

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A microbial barrier faucet with dual pipes, having a multiple capillary nozzle attached to the internal pipe and with clean air blown between the internal and external pipes, was manufactured and its performance was tested. This faucet prevented airborne microbial contamination when clean air was blown at a velocity of 4.5 m/sec.

Water splashes occurring at initial water flow through the faucet were easily subject to contamination by direct contact with the contaminated external pipe end, and should be removed before subsequent use of the faucet.

The principle of this faucet should be applicable for supplying not only distilled water but also sterile pharmaceutical solutions. Further application to sterile bottling in the beverage industry may also be possible.

Keywords——microbial contamination; distilled water; faucet; airborne microbes; sterile bottling; clean air system

In the previous paper,[1] it was shown that distilled water supplied through faucets in a hospital pharmacy was often microbially contaminated.[3] Further, it was confirmed that one of the causes of this was contamination at the air-water interface of the faucets by airborne microbes and their subsequent multiplication in the water. In addition, so-called clean rooms often show heavy airborne microbial contamination,[4] including pathogenic organisms.[5] Microbial contamination of distilled water supplied through faucets has been discussed not only as a pharmaceutical problem,[1] but also as a clinical problem,[6] including iatrogenic infections involving contaminated distilled water[7] and infection resulting from contaminated disinfectant solution.[8] This problem is of particular concern in the field of surgery,[9] and methods for decontamination have been investigated.[10] Yoshiyama[11] has reported a device for dealing with contamination of source water, but a device for decontamination at the faucet terminal is not yet available.

We therefore developed two sorts of microbial barrier faucets and investigated their performance[1] by using air artificially contaminated with bacteria.[12] The results suggested

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that a microbial barrier faucet with dual pipes was effective. However, water flowing through the faucet occasionally suffered contamination by contact with the contaminated external guard pipe of the faucet as a result of splashing when the water contained air bubbles at the start of flow. Such bubbles might form as a result of water evaporation at the water-air interface of the faucet. In addition, the clean air velocity necessary to prevent contact of room air with the inner faucet nozzle required further investigation for practical use.

In this work, water evaporation from the faucet terminal was confirmed, faucet nozzles which reduced splashing were test-manufactured and a clean air velocity appropriate for practical use was investigated.

Experimental

Materials——Biofermin, a *Streptococcus faecalis* powder preparation, was obtained from Biofermin Pharmaceutical Co., Ltd., and used as a contaminant for artificial contamination tests. This powder preparation was placed on thioglycollate (TGC) agar medium[12] and cultured to produce *Streptococcus faecalis* colony paste. This paste was used as a contaminant of the outer pipe of the microbial barrier faucet with dual pipes.

Culture Medium and Culture Method——As described in the previous paper,[12] TGC agar medium was used as a culture medium and was cultured aerobically at 32° for 48 hr after sampling.

Instruments——Fig. 1 shows a test device for determining the evaporation of distilled water in a microbial barrier faucet. A multiple capillary nozzle A was fixed to a test tube B with an air-tight joint C and the device was positioned vertically. Nozzle A (made of unsaturated polyester plastic) was manufactured by the authors. The nozzle was 8 mm in diameter and 10 mm in length, and had 30 capillaries, of which had a diameter of 0.7 mm.

The faucet used in this experiment was similar to the microbial barrier faucet with dual pipes described in the previous paper,[1] except for the attachment of various nozzles to the internal pipe.

Fig. 2 shows the structures of four sorts of nozzles tested, that is, nozzle A described in Fig. 1, nozzle B where a guard was attached to nozzle A, nozzle C possessing a single hole and nozzle D where a guard was attached to nozzle C.

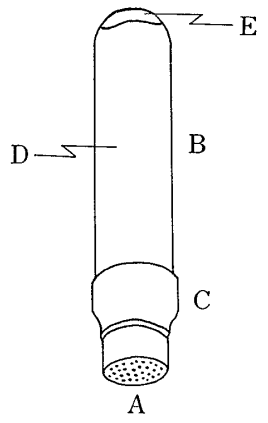

Fig. 1. Device for Testing Distilled Water Evaporation from a Microbial Barrier Faucet

A : Multiple capillary nozzle for the internal pipe of the microbial barrier faucet (diameter, 8 mm; length, 10 mm; containing 30 capillaries with a diameter of 0.7 mm). B : Test tube with an inside diameter of 10 mm. C : Air-tight joint. D : Distilled water. E : Air intake resulting from water evaporation.

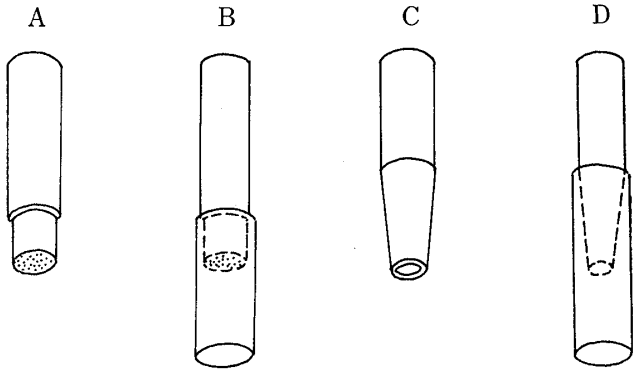

Fig. 2. Four Types of Nozzles for the Internal Pipe of the Microbial Barrier Faucet

A : Nozzle with multiple capillary described in Fig. 1. B : Nozzle A with a guard of 8 mm inside diameter and 15 mm length from the nozzle end. C : Nozzle with a single hole of 4 mm inside diameter. D : Nozzle C with a guard of 8 mm inside diameter and 15 mm length from the nozzle end.

Fig. 3 shows an instrument for splash testing with distilled water. Nozzle A was fixed to internal pipe B with the holder C. *Streptococcus faecalis* paste D was applied to the end of the external pipe as a contaminant. A polyethylene bottle E containing distilled water G was connected with a pipe to the upper end of the internal pipe. On applying a force F, the water (including air bubbles H) produced splashes I leading to direct contact of water drops with the external pipe end; these drops fell into a Petri dish containing TGC agar medium.

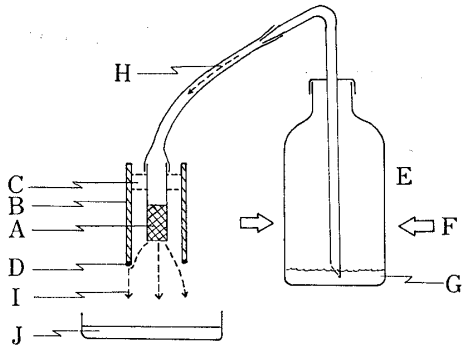

Fig. 3. Instrument for Splash Contamination Testing

A : A test nozzle for the internal pipe of the microbial barrier faucet. B : External pipe of the faucet. C : Internal pipe holder. D : Contaminant paste of *Streptococcus faecalis*. E : Polyethylene bottle. F : Force applied to produce splashing. G : Decontaminated distilled water. H : Flowing water containing air bubbles. I : Splashes of distilled water. J : Thioglycollate agar medium in a Petri dish.

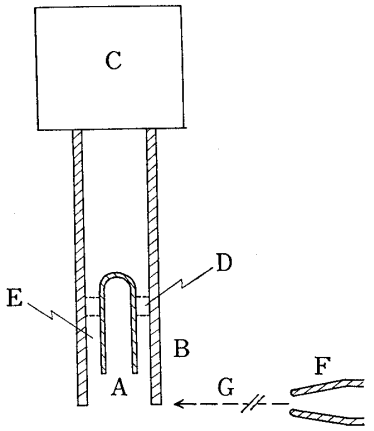

Fig. 4. Diagram of Dummy Microbial Barrier Faucet with Dual Pipes for Testing Effectiveness as an Airborne Microbial Barrier

A : Dummy internal pipe which can be changed and moved to vary the distance from the end of the external pipe. B : External pipe. C : Clean air blower with variable air velocity. D : Holder for internal pipe. E : Space for ventillation. F : Nozzle of microbe-air blower. G : Microbe jetting axis.

A dummy microbial barrier faucet with dual pipes was manufactured by the authors to test the barrier effect against airborne microbes (Fig. 4). That is, a dummy internal pipe A (12 mm in outside diameter) was fixed to the external pipe B (21 mm in inside diameter) through a holder D. The depth of the internal pipe end was freely adjustable. The jetting distance between the nozzle F and external pipe B, and the clean air velocity from the blower C were both variable. The blower could eliminate 99.97% of airborne particles 0.3 μm in diameter.

Measurement of Air Intake Resulting from Evaporation of Distilled Water——The device shown in Fig. 1 was filled with distilled water and was positioned vertically. The total weight was determined every 24 hr to calculate the weight losses due to evaporation.

Tests of Various Nozzles——Each nozzle shown in Fig. 2 was attached to the device shown in Fig. 3 and distilled water was passed into a Petri dish containing TGC agar medium, as shown in Fig. 3. The medium was cultured to examine whether or not colonies appeared. After splashing distilled water through the above device ten times, distilled water was passed normally through the faucet, collected and cultured by the method described above. The decontamination ratio was presented as the percentage contamination frequency of the samples relative to that of the total test runs. The decontamination ratio was designated as the protection ratio from the contamination within the faucet system itself.

Test of Microbial Barrier Faucet with Dual Pipes using Airborne Microbial Contamination——One g of Biofermin was jetted at the dummy faucet shown in Fig. 4; the clean air velocity and the depth of the dummy internal pipe end were each varied for different runs. After jetting the contaminant, the end of the dummy internal pipe was brought into contact with TGC agar medium and the medium was cultured to examine whether or not colonies appeared. Protection ratios were determined in the same way as decontamination ratios.

Results

Measurement of Air Intake Resulting from Evaporation of Distilled Water

Distilled water was lost at a rate of 0.191 ml/day (SE=0.005, $n=29$) from the device shown in Fig. 1. This evaporation test was carried out at an average temperature of 26.9° and an average relative humidity of 63.3%.

Decontamination Ratios of Various Nozzles

Table I shows decontamination ratios with various internal pipes. The multiple capillary nozzle was not significantly different from guarded nozzles ($n_1=95$, $n_2=140$, $p<0.08$), but

was superior to the single hole nozzle ($n_1=95$, $n_2=74$, $p<0.005$). Further, an internal pipe end depth of 5 mm was more effective for decontamination than 10 mm ($n_1=171$, $n_2=138$, $p<0.05$). The faucet with a multiple capillary nozzle at a depth of 5 mm gave the highest decontamination ratio (97%) under conditions of splashing.

In normal water flow after splashing, all of the faucets with nozzles at a depth of 5 mm gave complete decontamination, but the faucets with nozzles at a depth of 10 mm showed inferior decontamination ratios, except in one case.

Table I. Decontamination Ratios of Distilled Water Flowed through Four Types of Nozzles with Splashing at Flow Initiation

Nozzle	Splashing water		Normal water flow		(depth of internal pipe end)
	5 mm	10 mm	5 mm	10 mm	
Multiple capillary	97[a)]	87	100[b)]	85	
Multiple capillary with guard	83	77	100	100	
Single hole	57	30	100	96	
Single hole with guard	83	67	100	92	

The data represent at least 10 runs.
a), *b*) Thirty runs.

Protection Ratio of Microbial Barrier Faucet with Dual Pipes in the Case of Airborne Contamination

The protection ratio of the faucet with dual pipes from microbial contamination depended on the contaminated air velocity, the clean air velocity and the depth of the internal pipe end. As shown in Table II, increases of clean air velocity increased the protection ratio. That is, the faucet with 4.5 m/sec clean air velocity was superior to that with 3.5 m/sec ($n_1=111$, $n_2=120$, $p<0.001$) and that with 1.8 m/sec ($n_1=111$, $n_2=97$, $p<0.001$). An increase in the depth of the internal pipe end increased the protection ratio ($n_1=160$, $n_2=168$, $p<0.001$).

On the other hand, increases of contaminated air velocity lowered the protection ratio. Thus, a 3.0 m/sec contaminated air velocity resulted in a lower ratio than 1.8 m/sec ($n_1=91$, $n_2=114$, $p<0.01$) or 1.0 m/sec ($n_1=91$, $n_2=123$, $p<0.001$). Complete protection was attained at less than 1.5 m/sec contaminated air velocity and more than 3.5 m/sec clean air velocity, when the internal pipe terminated 10 mm before the end of the external pipe. Complete protection was also attained at less than 1.0 m/sec contaminated air velocity and more than

Table II. Protection Ratios of the Microbial Barrier Faucet with Dual Pipes related to Clean Air and Contaminated Air Velocity

Clean air velocity (m/sec)	Contaminated air velocity (m/sec)	Protection ratio (%)		(depth of internal pipe end)
		5 mm	10 mm	
4.5	1.0	100	100	
4.5	1.5	100	100	
4.5	3.0	73	94	
3.5	1.0	100	100	
3.5	1.5	80	100	
3.5	3.0	40	90	
1.8	1.0	52	100	
1.8	1.5	50	86	
1.8	3.0	0	80	

The data represent at least 10 runs.

1.8 m/sec clean air velocity, with the same pipe end positions. When the internal pipe ended 5 mm above the external pipe, complete protection was attained at less than 1.0 m/sec contaminated air velocity, or at less than 1.5 m/sec contaminated air velocity and more than 4.5 m/sec clean air velocity.

Discussion

As reported in the previous paper,[1] it was found that a microbial barrier faucet with dual pipes was contaminated *via* two routes. That is, direct contact of splashing water (when flow is started) with the external pipe end, which might be microbially contaminated, and contact of the water surface within the faucet with airborne microbes in the room air.

Regarding the former, evaporation of distilled water from the faucet nozzle was tested and the resulting air intake was measured quantitatively. The results showed that splashing was inevitable. To reduce this, directional nozzles were test manufactured for attachment to the internal pipe end. A multiple capillary nozzle was the most effective, as shown in Table I. Table I also shows that the multiple capillary nozzle inset at a depth of 5 mm from the end of the external pipe gave a 97% decontamination ratio for water splashing and 100% for normally flowing water; this was superior to the same nozzle positioned at a depth of 10 mm. This suggests that too great a depth allows drops to form by splashing onto the contaminated external pipe end, causing contamination.

To check contamination by airborne microbes in the room air, the performance of the microbial barrier faucet with a dummy internal pipe was examined using a microbe-air blower.[10] Even when the contaminated air was blown at a velocity of 1.5 m/sec, which is higher than the air velocity caused by usual human actions, the microbial barrier faucet was found to provide complete protection.

Judging from the data shown in Tables I and II, a practical microbial barrier faucet with dual pipes should have a 5 mm difference in the depth of nozzles with a 4.5 m/sec clean air flow to provide complete protection from microbial contamination. Water splashes occurring when flow is switched on should be removed before subsequent use of the faucets because complete protection could not be ensured in the case of splashing under any operating conditions. A dummy microbial barrier faucet (21 mm inside diameter of the external pipe and 12 mm outside diameter of the internal pipe) was used to study contamination by airborne microbes for experimental convenience. If these dimensions are altered, the operating conditions would reqire modification, but the modified conditions could be easily determined by means of the microbe-air blower.

On the basis of these results, this faucet will prevent water contamination from airborne microbes. It should be suitable not only for supplying distilled water and bottling sterile solutions in hospital pharmacies and in the pharmaceutical industry, but also for supplying hand washing water for surgical operations. Furthermore, the principle of the faucet may also be applicable for sterile bottling in the beverage industry.

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