[Chem. Pharm. Bull. 27(5)1231—1236(1979)]

UDC 576.85.093 : 578.085.2

Studies on Microbial Barrier Faucets for Sterile Solutions. I. A Method for Microbial Contamination Testing using Air artificially contaminated with Bacteria

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(Received August 16, 1978)

Since prone water surfaces of faucet terminals are liable to suffer contamination due to airborne microbes, development of a microbial barrier faucet is necessary. A new test method was developed to investigate faucet performance. The equipment could jet 7.8×10^7 microbes per 1 g of Biofermin, which was selected as a suitable contaminant, as calculated on the basis of colony formation.

Keywords——microbial contamination; fungi; bacteria; microbe-jetting equipment; Lactobacillus powder preparation; thioglycollate agar media; faucet terminal

When sterile preparations are manufactured in hospital pharmacies or by the pharmaceutical industry, it is important that distilled water and sterile solutions flowing through faucets should be protected from airborne microbial contamination.[2] In the field of clinical medicine, supplying decontaminated hand-washing water and liquid preparations for treatment is absolutely necessary to protect patients from iatrogenic infections.[3] Some clinical reports, however, have described microbial contaminations of distilled water and sterile preparations,[4] which may induce human infections.[5]

Pipe-supplied distilled water may be contaminated by two routes, that is, the source and the faucet terminal. Since the former is easily protected by reverse osmosis,[6] studies seem desirable on the latter; so far only a few reports[7] have dealt with this subject. Furthermore, no test method for microbial contamination is available except for the microbe culture method,[8] which takes a long time to confirm natural contamination.

In the present paper, an initial study was made on airborne microbial contamination in an office room. Subsequently, a new test method for airborne microbial contaminations was developed for further studies on microbial barrier faucets. A new microbe-air blower was devised to produce air contaminated with various microbes, and a suitable contaminant was selected for contamination tests. In addition, the contamination of prone surfaces was investigated.

Experimental

Materials——Biofermin containing *Streptococcus faecalis* 129B 10 3B: AKU: MKU and *Bacillus subtilis* 129B 10H(α) was obtained from Biofermin Pharmaceutical Co., Ltd. The particle size was less than 75 μm (diameter). Lac B containing *Lactobacillus bifidus* was obtained from Nikken Chemicals Co., Ltd., Polylacton

1) Location: *585 Yogi, Naha-shi, Okinawa, 902, Japan.*
2) Japanese Pharmacopoeia, 9th ed., 1976, p. 15.
3) Y. Hagihara, M. Sasaki, and T. Muteki, *Kurume Igakkai Zasshi*, **32**, 1166 (1969).
4) M.S. Favero, L.A. Carson, W.W. Bond, and N.J. Peterson, *Science*, **173**, 836 (1971); I. Phillips, S. Eykyn, and M. Laker, *Lancet*, **1**, 1258 (1972).
5) P.M. Last, P.A. Harbison, and J.A. Marsh, *Lancet*, **1**, 74 (1966); D.C.J. Bassett, K.J. Stokes, and W.R.G. Thomas, *Lancet*, **1**, 1188 (1970).
6) United States Pharmacopoeia, 19th ed., 1975, p. 539.
7) H. Usami, *Byoin Setsubi*, **15**, (5) 19 (1973); Y. Hagihara and T. Muteki, *Ikakikaigaku*, **44**, 399 (1974).
8) Japanese Pharmacopoeia 9th ed., 1976, p. 695.

containing *Lactobacillus acidophillus*, *Lactobacillus lactis* and *Streptococcus lactis* was from Green Cross Corporation; Enteronon R containing *Streptococcus faecalis* B 10-4R was from Morishita Pharmaceutical Co., Ltd. These materials were used as contaminants for the airborne microbial contamination test. Hydrogen peroxide solution (3%) was a Japanese Pharmacopoeia (JP) grade preparation.

Preparation of Culture Medium and Culture Method——The medium was prepared using thioglycollate (TGC) culture medium I (Nissui Seiyaku Co., Ltd.) according to the sterility test specified by JP (9th ed.).[8] Agar for bacteriological tests (Nissui Seiyaku Co., Ltd.) was added to make 15 w/v %. This was used as a plate medium in Petri dishes. The TGC agar medium was cultured aerobically at 32° for 48 hr after exposure to room air.

Equipment——A slit sampler (model FT) made by Takasago Netsugaku Co., Ltd. was used for counting microbes. A holder for inverted (prone) Petri dishes was made from steel wire fixed to an iron ring, as shown in Fig. 1. This holder was fixed to a stand at the height of 70 cm above the floor and an inverted Petri dish was mounted in the center of the ring. A microbe-air blower was manufactured to produce contaminated air (Fig. 2). This equipment consisted of an air pump (Fuji Kogyo Co., Ltd.), a metal aspirator (MA-2; Nishizawa Laboratory Co., Ltd.), a jet nozzle of 8 mm inside diameter, and a graduated glass containing the contaminant. An AM-2 anemometer made by Oshitari Laboratory Inc. was used for measuring air velocity.

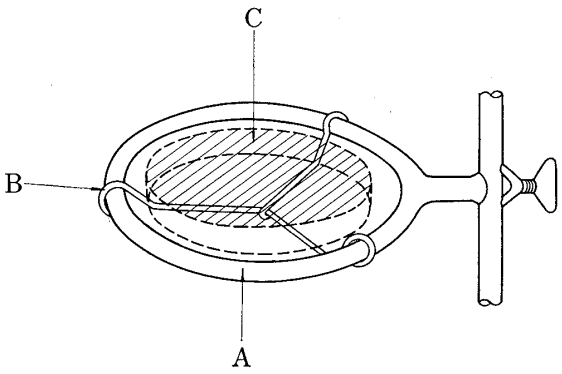

Fig. 1. Holder for an Inverted (Prone) Petri Dish

A: iron ring, B: steel wire, C: Petri dish.

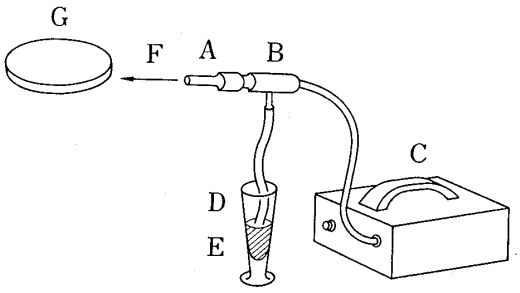

Fig. 2. Microbe-air Blower

A: jet nozzle, B: metal aspirator, C: air pump, D: glass measure, E: contaminant, F: contaminated air, G: target.

Airborne Microbe Counting using a Slit Sampler——Airborne microbes were counted in accordance with the NASA Standard Procedures.[9] A Petri dish containing 27 ml of the culture medium was placed on a turntable of the slit sampler, and the space between the slit and medium surface was adjusted to 2 mm. Room air was aspirated for 3 min at the rate of 33.3 l/min and the Petri dish was cultured to count colonies. The equipment was operated for 3 min at intervals of one hr, for 8 hr a day, for a period of 6 days. Bacteria and true fungi were distinguished by the appearance of the colonies, conventionally. Colony counting in all these experiments was carried out according to the above procedure. The office room comprised 42 m^3 with an outdoor louver of 3×150 cm, and one person was usually present in the closed room.

Airborne Microbe Counting in Supine and Prone Petri Dishes——A Petri dish was placed in the normal (supine) position on top of the inverted (prone) Petri dish mounted previously on the holder. Both dishes were exposed continuously to room air and changed every 12 hr. The exposed dishes were cultured to count colonies. The condition was the same as described in the experiments with the slit sampler.

Selection of Contaminants——Four sorts of contaminant were placed on sections on a Petri dish. The dish was cultured (TGC agar medium) at 32° for 72 hr to observe the growth of colonies.

Identification of Species and Microbe Counting——Hydrogen peroxide solution (3%) was added dropwise to the colonies to examine whether or not oxygen gas was produced. The microbes were counted by the decimal dilution method.[10]

Measurement of Air Velocity of the Microbe-air Blower——The anemometer was set up on the jet axis to measure maximal and minimal air velocities. Measurement was made for 30 sec at a distance of 10—100 cm from the jet nozzle.

Measurement of the Weight Distribution of Jetted Contaminant——1) Paper boxes with 24×24 mm open faces, were directed towards the nozzle in a crosswise arrangement, as shown in Fig. 3. After 10 g of

9) "NASA Standard Procedures for the Microbiological Examination of Space Hardware," NHB 5340.1 August 1967 ed., National Aeronautics and Space Administration, Washington, D.C. 20546, 1967, p. 12.
10) Standard Methods of Analysis for Hygienic Chemists, authorized by the Pharmaceutical Society of Japan, 1973, p. 33.

the contaminant had been jetted at a distance of 30 cm from the center box, the contaminant in the boxes was weighed. 2) The boxes, with their open faces (24×24 mm) turned upwards, were aligned on the jet axis at a distance of 10—100 cm from the nozzle. After 10 g of the contaminant had been jetted, the contaminant in the boxes was weighed.

Measurement of Jet Angle——A square plate medium (20×25 cm) was prepared and placed parallel to the jet axis. One g of the contaminant was jetted. The medium was cultured in a sterile bag at 32° for 48 hr, and the jet angle was determined from the colony formation.

Microbe Counting on Inverted Petri Dishes——A Petri dish was set up in an inverted (prone) position on the axis of the nozzle and 1 g of the contaminant was jetted at a distance of 20—60 cm from the nozzle. After culture, grown colonies were counted.

Microbe Counting on a Prone Water Surface——An eye-drop bottle with a mouth 9.7 mm in inside diameter was filled with sterile water and set up in a prone position on the jet axis to provide a prone water surface, then 1 g of the contaminant was jetted at a distance of 10—50 cm from the nozzle. The microbes were counted by the decimal dilution method.[10]

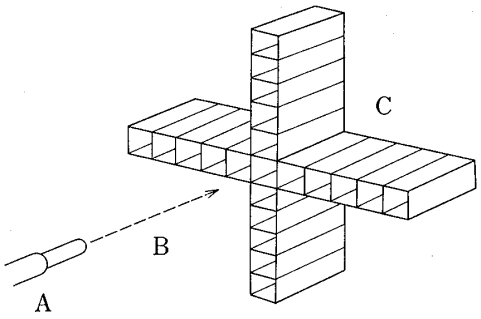

Fig. 3. Apparatus for Measuring the Perpendicular Distribution of Contaminant with Respect to the Jet Axis

A: jet nozzle, B: jet axis, C: paper boxes.

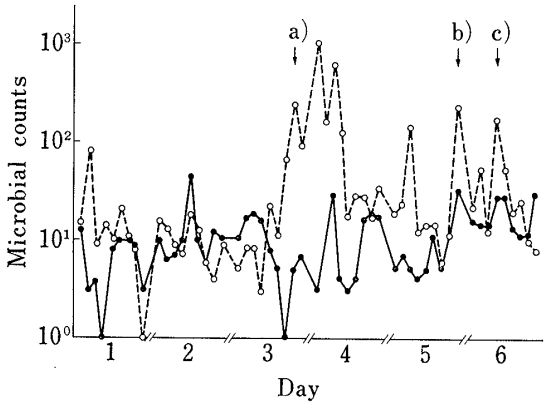

Fig. 4. Microbial Counts per 100 l of Air determined with a Slit Sampler in an Office Room

Microbes were counted for 3 min at intervals of one hr, for 8 hr a day, for 6 days. The room is 42 m³ and one person is usually present in the closed room. —●—: bacteria, ---○---: true fungi, a) trash disposal in the room, b) trash disposal in a neighboring room, c) presence of 7 persons.
Total microbes (max. 1003, med. 26, min. 4).
True fungi (max. 1000, med. 15, min. 1).
Bacteria (max. 43, med. 10, min. 1).

Results

Microbe Counting in an Office Room

1) **Counting with a Slit Sampler**——As shown in Fig. 4, there were no significant changes in the bacterial counts. However, the counts of true fungi increased to 275 colonies per 100 l of air when trash was disposed in the same room, and further increased to more than 1000 colonies by the next morning. In addition, counts of true fungi were influenced by trash disposal in a neighboring room, reaching 218 colonies per 100 l of air, and were also influenced by the number of persons present in the room; 163 colonies were counted in the presence of 7 persons. The reason for the high counts of true fungi which appeared in the first day and 5th day could not be determined. In terms of the medians, microbial counts were comparatively low under usual conditions.

2) **Microbial Counts in Supine and Prone Petri Dishes**——As shown in Fig. 5, microbial counts varied greatly in connection with events. Thus, when a sandy wind blew from Mongolia, 196 bacterial colonies were found in the supine Petri dish, but no colonies on the prone dish. Trash disposal in the room produced more than 190 colonies of true fungi in both

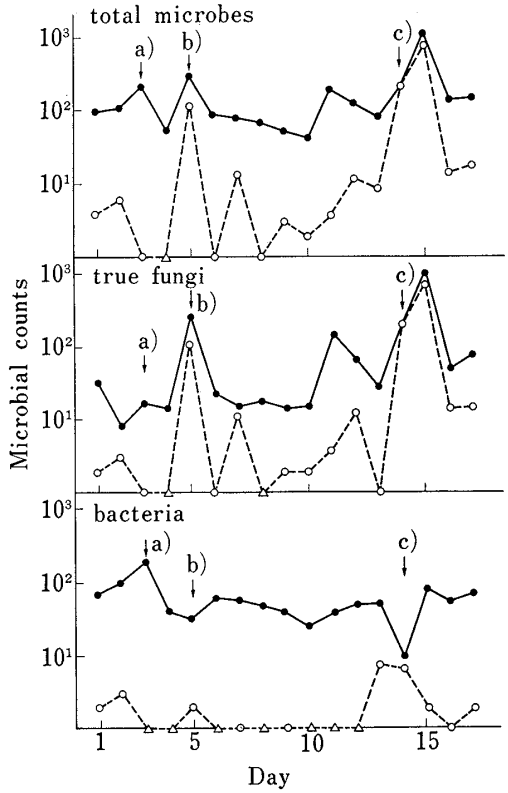

Fig. 5. Microbial Counts per 24 hr on Normal and Inverted Petri Dishes in an Office Room

—●—: normal (supine) Petri dishes, ---○---: inverted (prone) Petri dishes, △: zero count. a) A sandy wind blew from Mongolia, according to information from the meteorological observatory. b), c) trash disposal in the room.

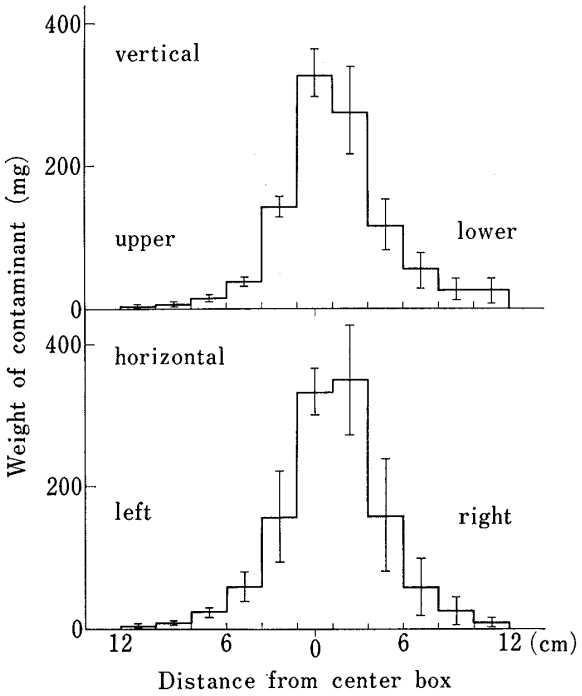

Fig. 6. Weight Distribution of Contaminant after Jetting 10 g of Contaminant at a Distance of 30 cm

The data are presented as means ±S.E. for 3 measurements.

Petri dishes. The day after trash disposal, the contamination of supine and prone Petri dishes by true fungi increased to give counts of 1025 and 800 colonies, respectively.

However, as shown by the medians of counts of true fungi (supine: $n=17$, med.$=28$; prone: $n=17$, med.$=3$), the counts were comparatively low under usual conditions. On the other hand, bacterial counts were always low in the prone Petri dishes (supine: $n=17$, med.$=54$; prone: $n=17$, med.$=1$), and did not vary greatly with circumstances. These results are very different from those of true fungi. There was a distinct correlation between counts of true fungi in supine and prone Petri dishes ($n=17$, $r=0.984$, $p<0.01$). The correlation was also noted in the total counts of microbes ($n=17$, $r=0.971$, $p<0.01$), but there was no correlation in the bacterial counts ($n=17$, $r=-0.240$).

Selection of Contaminant

Of four sorts of microbes cultured in the TGC agar medium, Biofermin grew best. Few colonies grew in the cases of Lac B and Enteronon R. Thus, Biofermin was selected as a suitable contaminant for the airborne microbial contamination tests.

Identification of Species of Bacteria and Bacterial Counts of Contaminant

When hydrogen peroxide solution was added dropwise to colonies of Biofermin, no gas was produced. Thus, the grown bacteria were assumed to be *Streptococcus faecalis*, which lacks catalase. When 1 g of Biofermin suspended in water was cultured, the number of colonies was calculated to be 7.8×10^7 ($\pm$S.E.$=2.6$, $n=11$).

Efficiency of Microbe-air Blower

1) **Air Velocity**——The air velocity on the jet axis of the equipment was inversely proportional to the distance within a range of 14—40 cm from the jet nozzle. The maximal horizontal velocity caused by human action (1.0 m/sec) corresponded approximately to a distance of 27 cm from the nozzle.

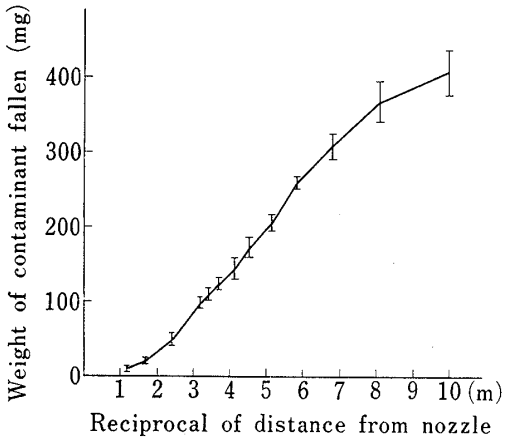

Fig. 7. Weight of Contaminant fallen after Jetting 10 g of Contaminant

The data are presented as means ± S.E. for 3 measurements.

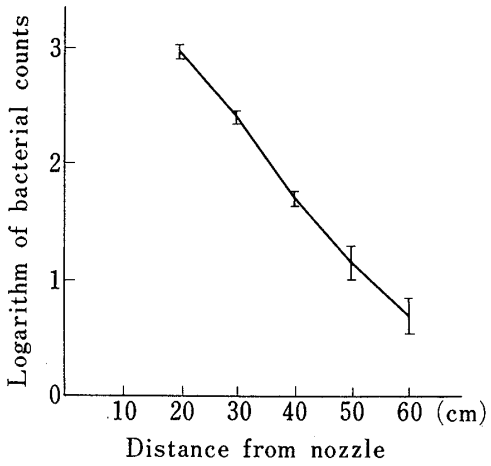

Fig. 8. Bacterial Counts on Prone Petri Dishes after Jetting Contaminant

The data are presented as means ± S.E. for 14 experiments.

2) **Weight Distribution of Jetted Contaminant** ——i) Weight distribution Perpendicular to the Jet Axis: The weight distribution was strongly directional in both the horizontal and vertical directions (Fig. 6). The vertical distribution was naturally affected by gravity. On the other hand, the horizontal distribution showed no significant deviation although it had a slight right-sided bias with respect to the jet axis.

ii) Weight Distribution along the Jet Axis: The weight distribution of the contaminant was inversely proportional to the distance within a range of 14—40 cm from the nozzle (Fig. 7). Jetting 1 g of contaminant required about 5 sec.

3) **Jet Angle**——The angle of colony growth was within the range of 12.5—15°. This corresponded to a normal jet angle.

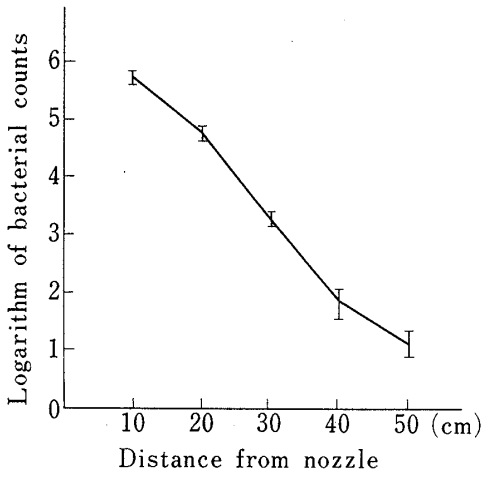

Fig. 9. Bacterial Counts on Prone Eye-drop Bottles after Jetting Contaminant

The data are presented as means ± S.E. for 10 experiments.

4) **Bacterial Counts of Prone Petri Dishes**—— The logarithm of bacterial counts fell proportionally to the distance (Fig. 8). On average, 251 bacterial colonies (log mean±S.E.=2.4±0.04, n=14) were counted at a distance of 30 cm. This count corresponded to a contamination density of 4.3 bacteria/cm^2.

5) **Bacterial Counts in Prone Water Surfaces**——On average, 1995 bacterial colonies (log mean±S.E.=3.3±0.12, n=10) were counted in a prone eye-drop bottle at a distance of 30 cm (Fig. 9). This count corresponded to a contamination density of 2696 bacteria/cm^2.

Discussion

Investigations of airborne microbial contamination in an office room were first carried out to provide basic data for the development of microbial barrier faucets. The microbial counts in an office room as determined with a slit sampler and using Petri dishes varied greatly according to circumstances. This is in agreement with the results obtained by Komoda and his collaborators.[11]

It is noteworthy that dense contamination was caused mainly by true fungi. They could adhere not only to supine but also to prone surfaces, and adherence to both surfaces was highly correlated. On the other hand, bacteria could adhere essentially only to the supine surface, as noted distinctly at the time when the sandy wind blew from Mongolia. This difference may be due to the fact that bacteria commonly adhere to dust particles. Temporary dense contamination with true fungi in an office room was caused not only by events in the same room but also in neighboring rooms, as observed in the case of trash disposal. In other words, the cleanliness of a room may be influenced temporarily by external factors. This suggests that a room without a positively pressurized clean air system would be easily subject to contamination. It is clear that the development of microbial barrier faucets is required. Thus, a method for microbial contamination testing was first investigated in these experiments to provide basic data for further studies.

To produce artificial contamination, a microbe-air blower using an aspirator system[12] was manufactured. Biofermin was selected as contaminant because it is pharmaceutically stable and contains *Streptococcus faecalis*, which grows well in TGC agar medium. The following performance characteristics are required for the microbe-air blower: 1) dense contamination must be obtained within a short time, 2) an air velocity higher than that caused by human movement must be obtainable, 3) the direction of the jet and the contamination density must be freely controllable, and 4) the equipment must be highly directional and show good reproducibility. The results indicated that the equipment fulfilled these criteria.

With regard to the density of artificial contamination, the logarithm of microbial counts on prone Petri dishes fell proportionally to the distance within a range of 20—60 cm from the jet nozzle.

In connection with the contamination densities under various conditions, bacterial counts on the prone water surface were 674 times higher than those on prone Petri dishes. This difference is presumably due to a protecting effect of the edge of the Petri dish, and the differing ease of adherence to the two surfaces. When 1 g of the contaminant was jetted at a prone Petri dish at the distance of 30 cm from the nozzle using the microbe-air blower, the contamination density reached one-fourth of the maximal microbial counts due to natural contamination in one day. Under the above conditions, the contamination density of the prone water surface was 169 times higher than the natural contamination. The exact significance of this in relation to the natural contamination level is not clear, but jetting at an air velocity of 1 m/sec gave a contamination density of 2696 bacteria/cm^2 at the prone water surface and this is sufficient for airborne microbial contamination testing. This test method should also be suitable for testing the performance of clean air systems.

In a forthcoming article, the development and performance testing of microbial barrier faucets will be reported, using this test method.

Acknowledgement The authors are grateful to Professor Hidetoshi Yoshimura, Faculty of Pharmaceutical Sciences, Kyushu University, for his review of the manuscript.

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