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Recovery of Microorganisms Shed by Humans into a Sterilized Environment

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An appartus and technique for quantitative comparison of the aerobic bacterial flora disseminated by human subjects has been developed. Dissemination from three healthy subjects was studied weekly for 3 weeks. Viable particles recovered ranged from 100,000 for one subject during a 30-min period to 620,000 for another subject during a 10-min period. One of the three subjects showed appreciably less variation in numbers of organisms shed than did the other two subjects. When the subjects were examined on consecutive days while wearing sterilized clothing, total particles recovered were reduced and variations in recoveries from run to run were slightly lessened. Three consistent nasal carriers of *S. aureus* were measured for dissemination. No viable *Staphylococcus aureus* was recovered from two of the carriers. However, 460,000 typable *S. aureus* particles were recovered during a 60-min period from the third carrier.

Humans have been shown to disseminate bacteria into their surrounding environment in the absence of fecal, genitourinary, respiratory, or purulent discharges (5, 10). Shedding of potentially pathogenic organisms has been demonstrated from persons revealing no lesions and no signs or symptoms of illness (6, 9, 10).

A person who harbors a specific infectious agent in the absence of discernible disease has been termed a "carrier" (APHA, 1965). Carriers of *Staphylococcus aureus* have been shown by epidemiological means to be associated with spread of staphylococcal disease to surgical patients and newborn infants (3, 4, 13, 14, 17, 18). The term "disseminator" has been applied to carriers who cast off detectable numbers of identifiable microorganisms from their bodies into their environment. Some individuals maintaining a carrier state have been shown to be disseminators or nondisseminators of their respective strains of organisms (6, 9, 10, 17).

Experimental staphylococcal disease has been shown to be associated, at least in part, with magnitude of dose of infectious organisms (7). It would, therefore, seem desirable to attempt quantitation of the discharge of these, and perhaps other, organisms from carriers. Dissemination of microorganisms from human carriers into nonsterile enclosures has been measured by sampling the aerial portion of the environment (1, 2, 5, 9-12, 15, 16). Only relative quantitative measurements of shedding were possible under the conditions of these studies.

Initial studies in our laboratories on the effect of personal hygiene measures upon dissemination of microorganisms into an enclosed space were difficult to interpret because of residual viable bacteria stirred up in the chamber by movements of the subject. This flora did not seem to be associated primarily with immediate dissemination from the subject, but came in large part from bacteria already present in the chamber (H. W. Wolf, *unpublished data*).

Therefore, we felt the need for information on total dissemination of microorganisms into an environment that could be sterilized before entrance of the carrier. It would be necessary to recover bacteria from the entire environment rather than from the air alone. An apparatus was built to fulfill these objectives. Preliminary results have demonstrated the operability and utility of the technique and apparatus.

MATERIALS AND METHODS

A simplified line drawing of the apparatus is shown in Fig. 1. The chamber in which the subject is enclosed is termed the "microbiotank" or, more simply, "tank." The tank is approximately 3 ft (0.914 meter) in diam-

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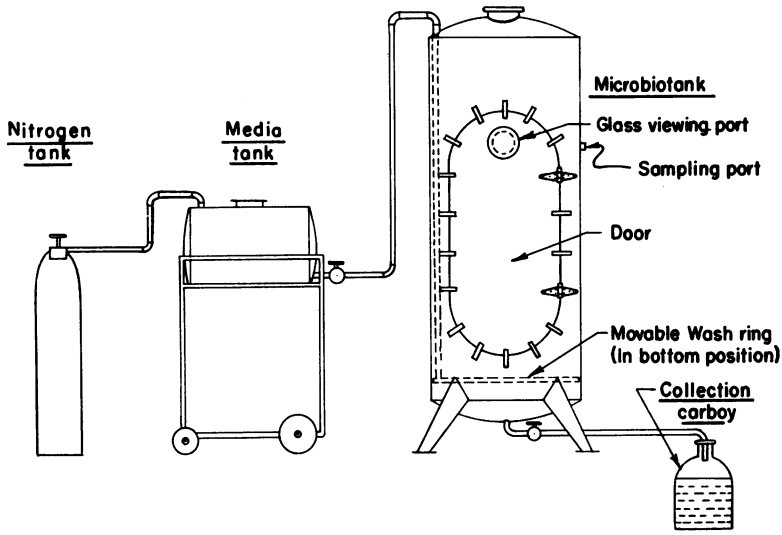

FIG. 1. *Microbiotank.*

eter and about 7 ft (2.13 meters) high. It is made of stainless steel. Both the upper and lower ends of the tank are internally concave. The volume of the tank is approximately 50 ft^3 (1.4 cu meters). All interior surfaces are highly polished and coated with a silicone compound.

A hinged entrance door is provided to the tank. The door is held in place by a series of latches that tighten it over a neoprene gasket. A 6-inch (15.24 cm) glass observation port is set in the wall of the tank opposite the hinged door. Four 1.75-inch (4.45 cm) diameter sampling ports that may be closed and sealed are located at various levels of the tank. A stainless-steel drain and valve are fixed at the center of the lower dished end of the tank.

The tank is fitted with a circular, horizontal wash-ring attached to a vertical supporting tube that passes through a port in the top of the tank. The ring and tube are made of stainless steel. A total of 312 outward-facing spray orifices, 0.0225-inch (0.572 mm) in diameter, are drilled in the wash-ring. The ring is slightly smaller in diameter than the tank, and is held equidistant from the interior walls by nylon guides. The ring can be moved up or down the inside of the tank by raising or lowering the supporting tube. For sterilization, the port for the tube can be sealed with the wash-ring and tube inside the tank.

The inside of the tank is sterilized by introducing steam at 15 psi (121 C). During the subsequent cooling period, replacement air is admitted to the tank from the outside through a stainless-steel ball valve and a sterilized tubular cotton filter. Condensate is drained off by gravity through the bottom drain and valve. The tank is then ready for the entrance of a human subject to be evaluated for shedding of microorganisms.

After the subject has left the tank, recovery of microorganisms shed is accomplished by rinsing the inside of the tank with sterilized, 0.25% peptone water under pressure. The reservoir of rinse-fluid is chilled to 5 C and then pressurized to 75 psi by nitrogen gas. The reservoir is attached to the top of the upright supporting tube of the wash-ring with a sterilized, flexible neoprene hose. The rinse-ring is drawn to the top of the tank by the supporting tube. A stainless-steel valve on the reservoir then is opened for approximately 15 sec. Throughout the rinsing, the ring is slowly lowered to its final position on the bottom of the tank. About 20 liters of fluid is used in the rinsing process. After flowing to the bottom of the microbiotank, the fluid passes through the drain and valve by gravity, and through sterilized flexible lines into a sterile, glass receiving vessel. The fluid then is passed through a continuous-flow, refrigerated, centrifuging unit. The portion of this unit which is in contact with the fluid is sterilized before use and assembled with precautions to maintain sterility. Centrifuging is carried out at 34,800 $\times$ g at 4 C, and flow of fluid through the unit is controlled at 200 ml/min.

Microbial particulates recovered from the centrifuged rinse fluid are collected in two 50-ml siliconized stainless-steel centrifuge tubes. After centrifugation, the contents of the two tubes are divided into four equal portions. The tubes are then placed on a wrist-action shaker for 10-min of agitation. All four tubes are then pooled. Dilutions are performed from 10^{-1} through 10^{-8}. The diluent is sterile, chilled, 0.25% peptone water.

Pour plates are prepared in triplicate for all dilutions. Trypticase Soy Agar (BBL) is used as the growth medium. Concurrent with the preparation of the pour plates, 0.2 ml of each dilution is flooded onto each of five Trypticase Soy Agar plates. Plates are incubated for 48 hr and are then counted. Pigmented colonies of staphylococci and colonies of all other organisms present are counted in these latter plates. The ratio between the number of pigmented staphylococci and all other colonies is determined. This ratio can be applied to the counts from the pour plates to estimate the number of staphylococci on these plates, when this information is desired.

RESULTS

Sterility of the system. Aerobic bacteria, recoverable after complete operation of the system without a known contaminating source, were measured. Assembly, sterilization, and rinsing of the system were carried out in a manner replicated in later experiments. The equipment was found to be essentially free of recoverable aerobic bacterial particles; less than 100 viable particles were recovered on repeated examinations at approximately every 20th run.

Recovery of known organisms from the system. Experiments were conducted to measure the recovery of viable bacterial particles artificially inoculated into the microbiotank. The comparison was between viable particles introduced at the beginning of the process and those recovered at its termination.

One ml of an 18-hr culture of a strain of *S. epidermidis* was introduced by a 1-ml pipette through the upper sampling port of the tank. Viable particles were allowed to settle for 5 min. Simultaneously with the seeding of the microbiotank, dilutions of the stock culture were made in Trypticase soy broth, 10^{-1} through 10^{-8}. Rinsing and recovery procedures were carried out on the tank as usual, as were centrifuging and dilution. All dilutions were plated in Trypticase Soy Agar, incubated 48 hr, and counted. Seeding with a culture of *Escherichia coli* O:126 was handled similarly. Results are shown in Table 1.

TABLE 1. *Seeding and recovery of Staphylococcus epidermidis and of Escherichia coli in the microbiotank*[a]

Experiment	Source	Counts from replicates			
		1	2	3	Average
S. epidermidis					
1	Culture	215	220	—	217
	Tank	76	86	—	81
2	Culture	122	135	142	133
	Tank	118	97	107	107
3	Culture	81	77	96	85
	Tank	59	54	65	59
4	Culture	124	103	112	113
	Tank	61	65	59	62
5	Culture	103	123	108	111
	Tank	93	95	87	91
E. coli					
1	Culture	187	177	173	179
	Tank	73	66	69	69
2	Culture	341	325	386	351
	Tank	50	42	42	45
3	Culture	130	137	140	136
	Tank	33	35	41	36
4	Culture	106	107	119	111
	Tank	60	54	58	57
5	Culture	242	245	262	248
	Tank	80	67	63	70
6	Culture	141	145	129	138
	Tank	36	45	38	40

[a] Dilution was 10^{-7} for each experiment reported.

It will be noted that all recoveries were within one logarithm of the number of organisms seeded. The range of counts found from run to run was relatively small. The recoveries were slightly higher with the staphylococci than with the *E. coli* which may have been due to breakup o clumps by the pressure rinse.

Dissemination from human subjects. Initially, shedding from three subjects was studied for periods of 10 to 40 min at 1-week intervals for 3 weeks. At the time of the study, C. S. was a 23 year old, 145 lb, white man. He had been a nasal carrier of *S. aeureus* for a period of many months. He had no known lesions attributable to staphylococcus during this period, and was apparently healthy in all respects. P. S. was an 18-year-old, 125-lb, white male college student, who had never revealed nasal or skin *S. aureus* on multiple examinations over a period of several months. D. H. was an 18-year-old, 140-lb, white male college student. Both of the latter subjects were apparently healthy in all respects.

About 2 hr before entrance into the microbiotank, the subjects bathed and shaved their faces. They wore street clothes, and their heads were uncovered and dry. The subject entered a portable still-air enclosure fitted to the microbiotank. He removed his shoes, opened the door of the tank, and stepped inside with minimal physical movement. The door of the tank was gently closed and sealed from outside. While inside, the subject simulated walking, but was careful not to touch the interior surface of the tank except with the feet. After the desired time interval, the door was opened from the outside, and the subject emerged into the portable enclosure without touching the inner walls of the tank. The door of the tank was gently closed and sealed. The microbiotank was then rinsed for assay of viable aerobic bacteria as previously described. Results are shown in Table 2.

Subject P. S. was then measured on 3 successive days under the following conditions: he bathed, shaved, and washed his hair prior to coming to the laboratory. No oil was applied to the hair or skin. Before entering the tank, he disrobed in the still-air room and donned a sterilized surgical scrub suit. He put on sterilized socks and stood on a sterilized towel prior to ingress. He immediately entered the tank for a 30-min period. All procedures were carried out as before. Uniform levels of shedding were found, with 110,000,

TABLE 2. *Viable aerobic bacterial particles removed from three subjects after differing time periods*

Subject	Time in tank (min)	Total viable particles recovered
C. S.	10	620,000
C. S.	20	116,000
C. S.	40	1,840,000
D. H.	15	98,000
D. H.	30	1,650,000
D. H.	30	100,000
P. S.	15	710,000
P. S.	30	583,000
P. S.	30	840,000

TABLE 3. *Staphylococcus aureus particles recovered from known carriers*

Subject	Time in tank (min)	Aerobic viable particles recovered	Particles of *S. aureus* recovered	Per cent *S. aureus*	Phage type shed
S. A.	60	8,960,000	460,000	5	80/81
C. S.	40	1,840,000	0	0	—
D. S.	30	196,000	0	0	—

91,000, and 105,000 viable particles recovered in the three runs.

Three subjects who were known consistent nasal carriers of *S. aureus* were measured for their capacity to disseminate. They entered the tank in their usual clothing, after removing their shoes. S. A. was an intern at a local hospital and had been found to be a carrier of an 80/81 strain 2 years earlier. On each of our multiple culturings of this man's nares over a period of several weeks, we found that he carried phage type 80/81. (Typing set included phages 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83, 81, 187, 42D.) C. S., referred to previously, was a carrier of a 79/83 strain. D. S. was an intern at the same hospital as S. A., and they were close associates. D. S. carried a nontypable (routine test dilutions) strain on each of the many occasions when his nares were cultured. None had lesions, and all were apparently healthy. Table 3 contains the data.

S. aureus was not recovered from either C. S. or D. S. It is possible that such organisms were shed, but that they were lost or rendered nonviable in the recovery process. However, results from S. A. indicate that naturally shed *S. aureus* is recoverable by the method. *S. aureus* colonies recovered from S. A. were typed, and each was found to be 80/81. The potential health significance of shedding of nearly 500,000 viable particles per hr of an 80/81 strain by a disseminator working as a hospital intern is unquestionable.

DISCUSSION

Duguid and Wallace (5) used a nonsterilized, oiled, unventilated, 100-ft^3 chamber for their work on dissemination of bacteria from humans. Air was the only portion of their environment that was sampled, and this was done by using volumetric air-sampling equipment. With "marching" activity by the ungowned subject, they had recoveries calculated at about 10,000 viable particles/min. Extrapolation of data from Speers and co-workers (15) and from Noble and Davies (11) also indicates shedding rates of the order of magnitude of 10^4 viable particles/min. Hare and Thomas (10) worked in their enclosures only with settling plates, and their experiments are less comparable.

It is interesting that our results, when expressed as numbers shed per unit time for the subject in the tank, are within about 1 log of the figures of Duguid and Wallace (5), Speers and co-workers (15), and Noble and Davies (11). Considering the great possibilities for variation in both shedding and recovery, agreement was far closer than might be expected. In this connection, however, subject P. S. showed appreciably less variation on three runs than did the other two persons measured. When possibilities for dissemination were reduced with sterile clothing, and examinations were made on consecutive days, the viable particles recovered from subject P. S. were diminished and variation from run to run was slightly lessened.

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