

Survival of Gram-Negative and Gram-Positive Bacteria Artificially Applied on the Hands

PAULO P. GONTIJO FILHO,* MARTHA STUMPF, AND CELSO L. CARDOSO

Department of Medical Microbiology, Institute of Microbiology, Federal University, Rio de Janeiro, RJ, Brazil 21944

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We evaluated the survival of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Escherichia coli*, and *Staphylococcus aureus*, derived from either hospitalized patients or culture collections, on the fingertips of human volunteers. Over 99% of the bacteria died within 2 min of the application, and about 10^5 cells remained on the fingers for up to 90 min.

About 5% of all patients admitted to hospitals develop a nosocomial infection (5). Contaminated hands of health care personnel are reported as a major route for the spread of nosocomial infections (4, 8, 11, 15, 17, 18). Nevertheless, little importance has been given to relevant factors which control the survival of microorganisms on the hands.

The degree of hydration of the epidermis is one of the most significant factors in the control of bacterial flora on the skin (12). Gram-negative bacteria such as *Pseudomonas* spp. or members of the family *Enterobacteriaceae* are more sensitive to drying than are gram-positive *Streptococcus pyogenes* or *Staphylococcus aureus* (1, 10, 16). There are few reports on the survival of these organisms when artificially applied to hands (2, 3). The present study evaluated differences in the survival rates of bacteria applied to the fingertips of human volunteers.

Strains of *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 27736, *Serratia marcescens* CDC 1724, *Escherichia coli* ATCC 11229, and *S. aureus* ATCC 6538 obtained from culture collections were used. Strains of the same species were isolated from hospitalized patients and studied. Gram-negative rods were confirmed by the Analytab API 20E system (Analytab Products, Plainview, N.Y.), and staphylococci were confirmed by the coagulase, DNase, and mannitol tests. Five colonies from a 24-h Trypticase soy agar plate were transferred into 3.0 ml of Trypticase soy broth and incubated for 18 h at 37°C. All media were obtained from BBL Microbiology Systems, Cockeysville, Md.

The hands of four volunteers were washed with soap and water (social wash) and dried thoroughly with sterile paper towels. Each volunteer received 0.02 ml of the broth culture on each of the four fingertips of the left hand, with the palm facing upward and the fingers outstretched; the culture was spread by rubbing together opposite fingertips for 40 s. The process was completed by drying the fingers in air, without rubbing, for another 80 s before sampling. Thereafter, the eight fingers were sampled individually at 2, 5, 10, 15, 30, 60, and 90 min after the application of bacteria by rubbing for 3 min against 10 g of sterile glass beads (3 to 5 mm in diameter) in short, flat-bottomed glass tubes (28 by 70 mm for the first, second, and third finger and 28 by 65 mm for the fourth finger) containing 5.0 ml of 0.1% peptone water.

Duplicate material for each finger was obtained concomitantly from the opposite finger. Viable counts of the sampling fluids were determined by the drop-counting method of

Miles and Misra (14). Thus, 10-fold dilutions (10^0 to 10^4) of the sampling fluids were prepared, 0.1 ml was added to 0.9 ml of 0.1% peptone water, and six 0.02-ml drops of each dilution were applied to the surface of an agar plate. After incubation at 37°C for 24 to 48 h, the number of colonies was estimated from the mean of the six counts of dilution that showed the largest numbers of colonies without signs of confluence or gross diminution in colony size as a result of overcrowding. The following culture media were used for the recovery of test bacteria: Trypticase soy agar containing 32 µg of penicillin G per ml for culturing the hospital-collected gram-negative rods and *P. aeruginosa* ATCC 15442, mannitol salt agar (BBL) for culturing staphylococci, and eosin methylene blue agar (BBL) for growth of the other culture collection strains. An interval of 7 days between tests was observed to allow the restoration of the normal level of skin flora.

The microbial population applied to the fingertips of the volunteers declined rapidly, with a loss of up to 99% during the period of drying for 5 min. Thereafter, the decline was less pronounced, but populations of up to 10^5 cells per ml remained after 90 min (Fig. 1). The use of Trypticase soy agar with penicillin for the recovery of test bacteria (transient flora) facilitates the counting of applied bacteria by inhibiting the resident microorganisms. However, on mannitol salt agar or eosin methylene blue, some aliquots showed colonies representing resident flora (micrococci or staphylococci) that were easily differentiated from the test bacteria by colonial morphology.

Among hospital strains *K. pneumoniae* and *S. aureus* showed a much greater survival rate: 8.0×10^5 and 2.8×10^5 CFU/ml, respectively, after 90 min (Fig. 1a). *Serratia marcescens* and *P. aeruginosa* strains showed an intermediate survival (10^4 CFU/ml), and *E. coli* was the most sensitive to drying. The survival of the culture collection strains is shown in Fig. 1b. *S. aureus* ATCC 6538 and *Serratia marcescens* CDC 1724 were the most resistant, with 1.7×10^5 and 5.5×10^5 viable cells per ml, respectively, left after 90 min. *K. pneumoniae* ATCC 27736 and *P. aeruginosa* ATCC 15442 strains were less resistant to drying (10^2 CFU/ml), and *E. coli* ATCC 11229 was not recovered after 90 min, therefore, being the least hardy of the bacterial species tested.

The large number of bacteria recovered from the fingers of volunteers in this study may be the result of heavy initial artificial contamination (up to 10^8 bacteria per finger). Such a high number of bacteria was also used by Ayliffe et al. (2) and Lilly and Lowbury (9) and should be found in clinical

* Corresponding author.

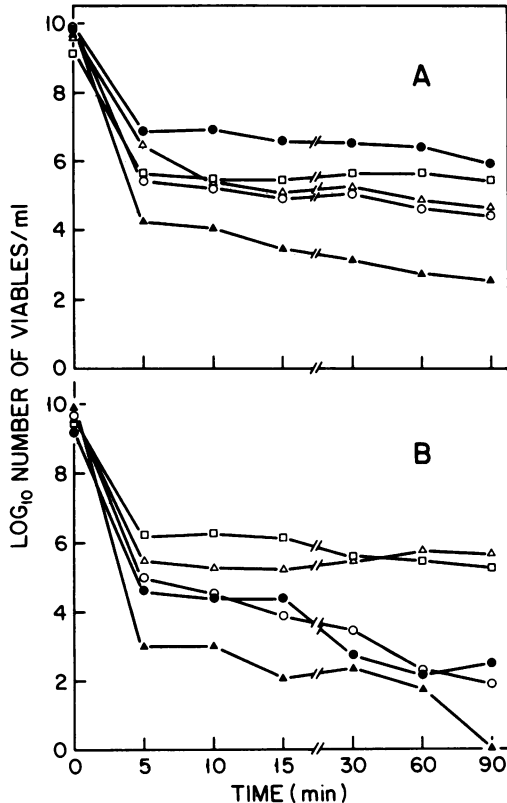


FIG. 1. Survival of hospital and culture collection strains on fingertips. Each point represents the mean value of four experiments on four different volunteers. (A) Hospital-collected strains; (B) strains from the culture collections. Symbols: ○, *P. aeruginosa*; ●, *K. pneumoniae*; △, *Serratia marcescens*; ▲, *E. coli*; □, *S. aureus*.

specimens. Pathogens are generally not present in high numbers on the fingers. However, up to 10^7 cells of *S. aureus* and gram-negative bacilli have been recovered from the hands of nurses (2). It is also established that casual or accidental contamination of hands of health care personnel handling patient specimens could contain from 10^5 bacteria per ml (urine) to 10^9 bacteria per g (fecal matter [6]).

Although some authors have reported the survival of *S. aureus* or gram-negative bacilli after artificial contamination of fingertips (2), palmar surfaces (3), or forearms (13), our paper describes the first attempt to compare the survival of hospital-collected and culture collection strains of nosocomially important bacteria on fingertips. As expected, *S. aureus* showed a much greater survival among the bacteria tested, and the number of cells (about 10^5 cells per ml) in both hospital-collected and culture collection strains remained almost unchanged after 5 min. The number of gram-negative bacilli showed a relatively rapid decline, but all bacteria tested showed a significant decrease in number during the drying process.

Our results clearly show that freshly isolated strains are more resistant than those from stock cultures and that the number of *Serratia marcescens*, *K. pneumoniae*, and *P. aeruginosa* cells is almost unchanged after drying. Our results do complement those of Hart et al. (7), who demonstrated that *Klebsiella* strains isolated from the environment are less resistant than strains from hospitalized patients.

Although it does not seem easy to assess the clinical and epidemiological implications of the hands on the transmis-

sion route by laboratory tests (6), our results could emphasize the fact that whereas drying reduces the number of bacteria on the hands it may not contribute to their removal from the finger surfaces. The relatively large bacterial population that is resistant to drying and so is left on the fingers may therefore represent a risk for patients and points to the importance of full implementation of handwashing for the prevention of nosocomial infections.

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LITERATURE CITED

- Aly, R., and H. I. Maibach. 1981. Factors controlling skin bacterial flora, p. 29-39. In H. I. Maibach and R. Aly (ed.), *Skin microbiology relevance to clinical infection*. Springer-Verlag, New York.
- Ayliffe, G. A. G., J. R. Babb, and A. H. Quoraishi. 1978. A test for "hygienic" hand disinfection. *J. Clin. Pathol.* **31**:923-928.
- Casewell, M., and I. Phillips. 1977. Hands as a route of transmission for klebsiella species. *Br. Med. J.* **2**:1315-1317.
- Dixon, R. E. 1980. Control of nosocomial and other infections acquired in medical-care institutions, p. 934-938. In E. H. Lennette, A. Balows, W. E. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
- Eickhoff, T. C. 1981. Nosocomial infections—a 1980 view: progress, priorities and prognosis. *Am. J. Med.* **70**:381-388.
- Greene, V. W. 1977. Hospital sterilization practices, p. 415-444. In D. Seligson (ed.), *CRC handbook series in clinical laboratory science, section E: clinical microbiology*. CRC Press, Inc., Cleveland, Ohio.
- Hart, C. A., M. F. Gibson, and A. M. Buckles. 1981. Variation in skin and environmental survival of hospital gentamicin-resistant enterobacteria. *J. Hyg.* **87**:277-285.
- Knittle, M. A., D. V. Eitzman, and H. Baer. 1975. Role of hand contamination of personnel in the epidemiology of gram-negative nosocomial infections. *J. Pediatr.* **86**:433-437.
- Lilly, H. A., and E. J. L. Lowbury. 1978. Transient skin flora. Their removal by cleansing or disinfection in relation to their mode of deposition. *J. Clin. Pathol.* **31**:919-922.
- Lowbury, E. J. L., and J. Fox. 1953. The influence of atmospheric drying on the survival of wound flora. *J. Hyg.* **51**:203-214.
- Maki, D. G. 1978. Control of colonization and transmission of pathogenic bacteria in the hospital. *Ann. Intern. Med.* **89**:777-780.
- Marples, M. J. 1974. The normal microbial flora of the skin, p. 7-12. In F. A. Skinner and J. G. Carr (ed.), *The normal microbial flora of man*. Academic Press, Inc. (London), Ltd., London.
- McBride, M. E., W. C. Duncan, and J. M. Knox. 1975. Physiological and environmental control of gram-negative bacteria on skin. *Br. J. Dermatol.* **93**:191-199.
- Miles, A. A., and S. S. Misra. 1938. The estimation of the bactericidal power of the blood. *J. Hyg.* **38**:732-749.
- Mortimer, E. A., P. J. Lipsitz, E. Wolinsky, A. J. Gonzaga, and C. H. Rammelkamp, Jr. 1962. Transmission of staphylococci between newborns—importance of the hands personnel. *Am. J. Dis. Child.* **104**:289-295.
- Pettit, F., and E. J. L. Lowbury. 1968. Survival of wound pathogens under different environmental conditions. *J. Hyg.* **66**:393-406.
- Sprunt, K. W., W. Redman, and G. Leidy. 1973. Antibacterial effectiveness of routine hand washing. *Pediatrics* **52**:164-171.
- Steele, C. A., and G. F. Mallison. 1975. Handwashing practices for the prevention of nosocomial infections. *Ann. Intern. Med.* **83**:683-690.