JOURNAL OF CLINICAL MICROBIOLOGY, May 1988, p. 950–953
0095-1137/88/050950-04$02.00/0

Vol. 26, No. 5

Composition and Density of Microflora in the Subungual Space of the Hand

K. J. McGINLEY,[1]* E. L. LARSON,[2] AND J. J. LEYDEN[1]

Department of Dermatology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104,[1] and School of Nursing, The Johns Hopkins University, Baltimore, Maryland 21205[2]

Accepted 15 April 1987/Accepted 15 January 1988

There were significant quantitative differences in the composition and density of microflora in different areas of the hands of 26 adult volunteers. The subungual spaces had an average $\log_{10}$ CFU of 5.39, compared with a range from 2.55 to 3.53 for other hand sites. In quantitative cultures from five subungual spaces in 26 subjects, coagulase-negative staphylococci were the dominant organisms, with *Staphylococcus epidermidis*, *S. haemolyticus* and *S. hominis* being the most frequently isolated species. Other bacteria recovered from subungual spaces included gram-negative bacilli in 42.3% of subjects, with *Pseudomonas* species composing 31.3% of this group, and coryneforms in 42.3% of subjects, with multiply resistant JK group coryneforms making up 12.5%. Yeasts were isolated from 69.0% of subjects sampled, with 51.3% of the yeasts identified as *Candida parapsilosis*. The subungual coagulase-negative staphylococci were susceptible to most antibiotics, with resistance to penicillin, ampicillin, and erythromycin detected in 23 to 38% of isolates.

The hand has long been recognized as an important vector in the transmission of microorganisms associated with nosocomial infections. Frequent hand washing, often with antimicrobial agents in detergent vehicles, has become standard practice for health care personnel. It has also been known since the classic work of Price in 1938 (15) that it is not possible to sterilize the hand, even with frequent hand washing with either detergents or agents containing antimicrobial substances. It was this inability to rid the hand of bacteria that led Price to propose the concept of transient and resident bacteria, with the latter being those organisms which could not be washed away. Hann, in 1973 (7), reevaluated the work of Price and demonstrated that the continued recovery of bacteria from the hand despite serial washings could be eliminated by covering the fingertips. Hann suggested that the majority of bacteria on the hand exists in and around the fingernails. Very little work has been done to further define the number and kinds of bacteria which may be found in and around the fingernail region (6).

In this study, we have determined the numbers and types of bacteria found on various portions of the hand, as well as the total numbers of bacteria recovered after serial washings of the entire hand. Our results indicate that substantial numbers of bacteria are found under each fingernail and that this region of the hand is a significant bacterial niche from which germ removal is difficult.

MATERIALS AND METHODS

A sample of 26 healthy adult volunteers, primarily secretaries, research assistants, and other employees at the study institution, served as the study population. There were 13 males and 13 females, with ages from 17 to 63 years (mean age, 37.2 years). None of the subjects had used antimicrobial cleansing agents for at least 1 month prior to this study, and none had contact with patients.

Serial hand cultures. To examine the consecutive harvest of organisms from the hands in a series of samplings, three subjects inserted each hand separately into a sterile polyethylene bag containing 50 ml of sampling solution (0.075 M phosphate buffer [pH 7.9] containing 0.1% Tween 80 [BBL Microbiology Systems, Cockeysville, Md.]). The entire hand surface was rubbed vigorously through the wall of the bag for 3 min (11). Both hands were sampled for six consecutive times on three consecutive days (18 samples per subject; total number of samples, 108). No hand washing was done between samplings. Samples were processed by making three 10-fold dilutions in half-strength scrub fluid and plating on Trypticase (BBL) soy agar with 0.5% sheep blood, 0.5% yeast extract, and 0.5% Tween 80. Plates were incubated aerobically for 3 days at 37°C. CFUs were counted, and total counts from the hand are reported as $\log_{10}$ CFU.

Density of flora at various sites on the hand. A total of 23 sites on each hand of three individuals were sampled. These sites included the base of each digit (five sites), each interdigital space (four sites), the middorsum of the hand (one site), the right and left sides and center of the palm (three sites), each subungual space (five sites), and the outer surface of each fingernail (five sites). On the soft tissue, a sterile cotton-tipped swab premoistened with half-strength scrub fluid was rubbed vigorously for 30 s (base of fingers, dorsum, and palm of hand) or 10 s (interdigital spaces) in an area of approximately 4 cm^2. The tip of the swab was then inserted into 2 ml of the same sampling solution, and the solution was vigorously mixed on a Vortex mixer and processed as described below. To extract subungual material, a sterile stainless steel microspatula (3.2 mm wide and 20 cm long) was used. Subjects were directed to make no particular preparation of the nails. Prior to sampling, hands were soaked in sterile water for 3 min to soften the horny layer of the nails. Extraction was performed by a sterile-gloved investigator to the point at which all visible material was removed from under each fingernail. Harvested material was placed aseptically into 0.5 ml of 0.1% Tween 80, and the mixture was mixed vigorously and processed as described below. For three subjects, subungual scrapings from five fingers were pooled and weighed on an electronic balance so that the number of CFUs of microorganisms could be expressed in CFUs per microgram of subungual debris.

The entire surface of each fingernail was scraped gently by a sterile-gloved investigator with a sterile scalpel. Again,

* Corresponding author.

scrapings were placed in 0.5 ml of 0.1% Tween 80, and the mixture was mixed vigorously and processed in the same manner. For three subjects, the subungual flora of both hands was examined on three occasions (90 samples) to determine whether hands differed in any quantitative or qualitative fashion. The subungual flora of five nails on the dominant hand of 26 subjects was studied (total number of samples, 130). With the extraction technique described above, samples were diluted 10-fold in 0.05% Tween 80, and a 40-μl portion of the suspension was plated onto each of the following media: a general nutrient medium consisting of Trypticase soy agar with 5% sheep blood and 0.5% yeast extract (BBL); the same agar with 0.5% Tween 80 (B80), a general nutrient medium for lipophilic and large-colony diphtheroids; B80 agar with 100 μg of phosphomycin (Sigma Chemical Co., St. Louis, Mo.) for selective isolation of diphtheroids; B80 agar with 100 μg of phosphomycin and 100 μg of ticarcillin (BBL) for selective isolation of *Corynebacterium* JK group; MacConkey agar (BBL) for selective isolation of gram-negative rods; phenylethyl alcohol agar with 5% sheep blood (BBL) for selective isolation of gram-positive rods; mannitol salt agar (BBL) for selective isolation of *Staphylococcus aureus*; Mycosel agar (BBL) for selective isolation of dermatophytes; and Sabouraud dextrose agar with 0.005% chloramphenicol (Sigma) for selective isolation of fungi.

Plates were incubated aerobically at 37°C for 72 h and then stored at room temperature for 4 days to enhance pigment production and colony morphology. Representative colonies were analyzed by Gram stain and identified by using the following API systems (Analytab Products, Plainview, N.Y.): API Staph-Ident System, API 20E Enteric System, and API 20C Clinical Yeast System. Corynebacteria were identified as described by McGinley et al. (13), and other organisms were identified by standard techniques (4, 17). Gram-positive and gram-negative bacteria and yeasts or fungi were identified to species level. Representative colonies of gram-positive cocci, coryneforms, and yeasts were tested for antimicrobial susceptibilities by using the disk diffusion technique (17). Mueller-Hinton agar (BBL) was used to test all the isolates, except for the lipophilic diphtheroids, which grow poorly on this medium and for which testing was performed on B80 agar. Gram-positive isolates were tested by using disks containing 10 μg of ampicillin, 30 μg of cephalothin, 30 μg of chloramphenicol, 2 μg of clindamycin, 15 μg of erythromycin, 10 μg of gentamicin, 5 μg of methicillin, 1 μg of oxacillin, 10 U of penicillin, 30 μg of tetracycline, and 30 μg of vancomycin.

Statistical analysis. The Wilcoxon rank sum test, the Student *t* test, or analysis of variance was used to compare mean differences between groups. The Spearman rank correlation coefficient was calculated to examine the correlation between length of nails and density of flora (2).

RESULTS

The data obtained from six consecutive samplings of each hand of three subjects on three occasions demonstrated a mean $\log_{10}$ CFU of 6.37 ± 0.26 prior to washing. After six consecutive washings, the mean $\log_{10}$ CFU was 5.25 ± 0.21. No significant difference in mean $\log_{10}$ CFU was found between the right and left hands of each subject.

For the subjects from whom 23 sites were cultured, the average $\log_{10}$ CFUs ranged from 2.55 ± 0.42 for fingernail scrapings to 5.39 ± 0.18 for the subungual region (Table 1). The number of bacteria recovered from the subungual region was significantly higher than those from all other sites (analysis of variance; $P < 0.01$).

Tables 2 and 3 show the numbers and kinds of organisms recovered from the subungual areas of the dominant hands of 26 subjects. The $\log_{10}$ CFU from each subungual space (averaged for five fingers for each subject) ranged from 2.47 to 6.93 (mean, 4.66 ± 0.71). Coagulase-negative staphylococci were present in 100% of samples, yeasts or fungi were present in 65.4% of samples, both gram-negative bacteria and coryneforms were present in 42.3% of samples, and *S. aureus* was present in 7.7% of samples (Table 2). Coagulase-negative staphylococci made up 92.0% of the total flora.

A total of 236 isolates were identified (Table 3). *S. epidermidis* and *S. haemolyticus* were the most frequently isolated staphylococci. Lipophilic diphtheroids were the most frequently isolated aerobic diphtheroids; *Pseudomonas* and *Enterobacter* species were the most frequently isolated gram-negative bacteria, and *Candida parapsilosis* was the most frequently isolated yeast.

The antibiotic susceptibility patterns of coagulase-negative staphylococci demonstrated that isolates were generally susceptible to nafcillin, methicillin, cephalosporins, gentamicin, vancomycin, and tetracycline, whereas 38, 26, and 23% of isolates were resistant to penicillin, ampicillin, and erythromycin, respectively (Table 4).

For the three subjects for whom an assessment of the quantity of subungual material was made and for whom the numbers and types of bacteria in the subungual area and the hand were compared, the mean $\log_{10}$ CFUs from the hands and from the subungual regions were 6.36 (±0.71) and 6.20 (±0.16), respectively. *S. aureus* was isolated from the subungual region, but not from the hand, of one individual. Individuals who had gram-negative bacteria or yeasts, or both, carried them at both sites. The subungual material harvested from five nails of three subjects weighed 388, 456, and 547 μg, with a mean $\log_{10}$ CFU of 6.17/100 μg of subungual debris. For the three subjects whose nail subungual areas were sampled on three occasions, there was no significant difference in the numbers of organisms recovered from the right (5.30 ± 0.48) and left (5.11 ± 0.51) hands.

For a given individual, there were no significant differences in the numbers of organisms isolated from the subungual spaces of the five fingernails. There was no significant difference in the total numbers of organisms isolated from the subungual spaces of females and males (means, 5.82 ± 0.47 and 5.47 ± 0.81, respectively). Although women had significantly longer fingernails than men did, there was no significant correlation between nail length and the density of bacteria found in the subungual areas.

DISCUSSION

Our results from serial cultures of the hand obtained after consecutive washings with a detergent are in good agree-

TABLE 1. Density of total aerobic flora at various sites (n = 46) on the hand

Site	Density of flora (mean $\log_{10}$ CFU [SD])
Subungual region	5.39[a] (0.18)
Palm	3.53 (0.04)
Interdigital spaces	3.42 (0.32)
Dorsum of hand	3.00 (0.79)
Base of fingers	2.95 (0.38)
Fingernail	2.55 (0.42)

[a] Value for subungual region significantly higher than those for other sites ($P < 0.01$), as determined by analysis of variance.

TABLE 2. Composition of subungual flora[a]

Subject no.	No. of indicated organisms (log_{10} CFU [%]) recovered from subject						Total no. of organisms (log_{10} CFU) recovered[b]
	Coagulase-negative staphylococci	*S. aureus*	Coryneforms	Gram-negative bacteria	Yeasts and fungi	Others	
1	5.04 (66.7)	4.74 (33.2)	—	1.22 (0.01)	1.17 (0.009)	—	5.22
2	5.30 (99.9)	—[c]	—	0.78 (0.003)	—	—	5.31
3	5.04 (100)	—	—	—	—	—	5.04
4	4.75 (87.3)	3.79 (9.7)	—	—	3.28 (3.0)	0.11 (0.002)	4.81
5	4.18 (88.6)	—	3.29 (11.3)	—	1.51 (0.19)	0.84 (0.04)	4.24
6	4.41 (91.5)	—	—	3.34 (7.8)	0.22 (0.006)	2.29 (0.69)	4.45
7	4.77 (87.0)	—	—	3.90 (11.6)	0.84 (0.01)	2.95 (1.3)	4.84
8	5.12 (99.8)	—	2.17 (0.11)	—	1.43 (0.02)	—	5.13
9	4.89 (100)	—	—	—	—	—	4.89
10	4.63 (99.9)	—	—	—	0.64 (0.01)	—	4.64
11	5.31 (96.3)	—	3.89 (3.7)	—	1.17 (0.007)	1.63 (0.02)	5.33
12	5.10 (89.5)	—	4.16 (10.3)	2.45 (0.2)	0.75 (0.004)	0.15 (0.001)	5.15
13	3.67 (100)	—	—	—	—	—	3.67
14	4.78 (99.0)	—	2.78 (0.98)	0.63 (0.007)	0.09 (0.002)	—	4.79
15	5.34 (52.9)	—	5.22 (39.9)	4.47 (7.1)	0.92 (0.002)	—	5.62
16	5.60 (99.8)	—	2.81 (0.16)	0.61 (0.001)	1.45 (0.007)	—	5.61
17	3.65 (92.2)	—	—	2.10 (2.6)	2.28 (3.9)	1.80 (1.3)	3.69
18	6.08 (73.5)	—	5.64 (26.4)	1.22 (0.001)	—	1.22 (0.001)	6.22
19	3.83 (99.5)	—	—	—	—	1.5 (0.5)	3.84
20	4.58 (73.5)	—	4.13 (26.3)	—	1.62 (0.08)	1.92 (0.16)	4.72
21	3.61 (99.7)	—	—	—	0.31 (0.05)	0.92 (0.20)	3.62
22	3.96 (99.9)	—	—	—	0.92 (0.09)	—	3.97
23	3.39 (100)	—	—	—	—	—	3.39
24	4.20 (99.1)	—	2.07 (0.74)	0.51 (0.02)	1.11 (0.08)	—	4.21
25	4.32 (99.7)	—	1.77 (0.28)	—	—	—	4.33
26	4.45 (95.7)	—	3.06 (3.9)	—	2.01 (0.35)	—	4.47

[a] Values are means of data from five fingers per subject.
[b] Mean value, 4.66 ± 0.70.
[c] —, None detected.

ment with those of Price (15) and Hann (7). Hann concluded that the fingernail region of the hand accounted for the continued recovery of bacteria on serial sampling. Our results show that the subungual region can be an important site for bacterial growth on hands. Samples from multiple sites of the hand show only hundreds to a few thousands of bacteria, compared with hundreds of thousands of bacteria per subungual site. The continued recovery of bacteria with consecutive samplings may represent continued release of organisms from the subungual area. The subungual region is an occluded area, and presumably, sufficient amounts of moisture exist to support the proliferation of bacteria and fungi. The number of bacteria recovered from other hand areas correlates well with that reported for the extremities (12). The subungual region appears to be an important reservoir of bacteria on the hand and may be important in the transmission of microbes from hands. With accumulated moisture, such as can occur with prolonged gloving during

TABLE 3. Species isolated from subungual regions of 26 subjects[a]

Organism group (no. of isolates)	Species	% of total isolates in group
Staphylococci (109)	*S. aureus*	1.8
	S. haemolyticus	32.1
	S. epidermidis	23.8
	S. hominis	17.4
	S. cohnii	8.2
	S. saprophyticus	7.3
	Others	9.1
Gram-negative bacteria (48)	*Pseudomonas* species	31.3
	Enterobacter species	27.1
	Serratia species	14.6
	Others	27.0
Coryneforms (40)	Lipophilic diphtheroids	55.0
	JK group coryneforms	12.5
	C. minutissimum	25.0
	C. xerosis	7.5
Yeasts and fungi (39)	*Candida parapsilosis*	51.3
	Rhodotorula rubra	23.1
	Others	25.6

[a] Total number of isolates, 236.

TABLE 4. Antibiotic susceptibilities of subungual coagulase-negative staphylococci from 26 subjects[a]

Drug	% of organisms resistant to drug
Penicillin	38.5
Ampicillin	26.2
Methicillin	5.9
Oxacillin	5.9
Cefoxitin	3.2
Cefalothin	1.1
Gentamicin	1.6
Clindamycin	1.6
Erythromycin	23.0
Tetracycline	9.1
Chloramphenicol	13.4
Vancomycin	0

[a] Total number of isolates, 187.

surgical procedures or when hands are washed frequently or immersed in fluid, release of subungual microorganisms may be facilitated.

The species of organisms isolated from the subungual region are similar to those reported on the hand. In a previous study (W. A. Horn, E. L. Larson, K. J. McGinley, and J. J. Leyden, Infect. Control, in press), coagulase-negative staphylococci were found on hands of all subjects; *S. aureus* was present in 7%. In the present study, we found aerobic coryneforms and gram-negative bacteria in the subungual region of 42% of subjects and yeast and/or fungi in 69.2% of subjects. The recovery of yeast and/or fungi and gram-negative bacteria from the subungual areas probably is the result of the occlusion of the area with retention of sufficient moisture to permit the survival and growth of these organisms, which are sensitive to dessication. The presence of penicillin-resistant staphylococci in 38% of subjects and multiply resistant aerobic coryneform bacteria (group JK) in 12.5% of subjects demonstrates that the subungual region may be a reservoir of drug-resistant organisms.

The finding of many investigations (1, 3, 5, 8–10, 12, 16) that scrubbing the hand with detergents or antimicrobial agents in detergent vehicles does not sterilize the hand and our finding that there are significant numbers of bacteria in the subungual compartment suggest that this hand region may be relatively inaccessible to antimicrobial agents during normal hand-washing procedures. As suggested by the work of Gross et al. (6), studies of the effect of antimicrobial agents in reducing the bacterial flora of the hand have not considered the subungual area. Our results indicate the need for a systematic evaluation of the subungual flora in other populations, e.g., health care personnel, and the need for a systematic study of the effects of various antimicrobial agents and regimens on the significant reservoir of bacteria in the subungual space.

ACKNOWLEDGMENTS

We thank Arlene Foglia for her expert technical assistance.

This work was supported in part by a grant from Avon Products, Inc.

LITERATURE CITED

1. **Altemeir, W. A.** 1983. Surgical antiseptics, p. 493–504. *In* S. S. Block (ed.), Disinfection, sterilization and preservation, 3rd ed. Lea & Febiger, Philadelphia.
2. **Armitage, P.** 1971. Statistical methods in medical research. Blackwell Scientific Publications, Ltd., Oxford.
3. **Ayliffe, G. A. J.** 1984. Surgical scrub and skin disinfection. Infect. Control **5**:23–27.
4. **Barry, A. L., and C. Thornsberry.** 1985. Susceptibility tests: diffusion test procedures, p. 978–987. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Geelhoed, G. W., K. Sharpe, and G. L. Simon.** 1983. A comparative study of surgical skin preparation methods. Surg. Gynecol. Obstet. **157**:265–268.
6. **Gross, A., D. E. Cutright, and S. M. D'Allessandro.** 1979. Effect of surgical scrub on microbial population under the fingernails. Am. J. Surg. **138**:463–467.
7. **Hann, J. B.** 1973. The source of the "resident" flora. The Hand **5**:247–252.
8. **Larson, E. L., P. I. Eke, and B. A. Laughon.** 1986. Efficacy of alcohol-based hand rinses under frequent-use conditions. Antimicrob. Agents Chemother. **30**:542–544.
9. **Larson, E. L., J. J. Leyden, K. McGinley, G. Grove, and G. H. Talbot.** 1986. Physiologic and microbiologic changes in skin related to frequent handwashing. Infect. Control **7**:59–63.
10. **Larson, E. L., K. McGinley, G. Grove, J. J. Leyden, and G. H. Talbot.** 1986. Physiologic, microbiologic, and seasonal effects of handwashing on the skin of health care personnel. Am. J. Infect. Control **14**:51–59.
11. **Larson, E. L., M. S. Strom, and C. A. Evans.** 1980. Analysis of three variables in sampling solutions used to assay bacteria of hands: type of solution, use of antiseptic neutralizers, and solution temperature. J. Clin. Microbiol. **12**:355–360.
12. **Leyden, J. J., K. McGinley, and G. Webster.** 1983. Cutaneous microbiology, p. 1153–1165. *In* L. H. Goldsmith (ed.), Biochemistry and physiology of the skin. Oxford University Press, Oxford.
13. **McGinley, K. J., J. S. Labows, K. M. Nordstrom, G. F. Webster, and J. J. Leyden.** 1985. Pathogenic "JK" group corynebacteria and their similarity to human cutaneous lipophilic diphtheroids. J. Infect. Dis. **152**:801–806.
14. **Meers, P. D., and G. A. Yeo.** 1978. Shedding of bacteria and skin squames after handwashing. J. Hyg. **81**:99–105.
15. **Price, P. B.** 1938. The bacteriology of normal skin: a new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. J. Infect. Dis. **63**: 301–318.
16. **Rotter, M. L.** 1984. Hygienic hand disinfection. Infect. Control **5**:18–22.
17. **Schoenknecht, F. D., L. D. Sabath, and C. Thornsberry.** 1985. Susceptibility tests: special tests, p. 1000–1008. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.