

Gram-Negative Bacilli as Nontransient Flora on the Hands of Hospital Personnel

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The possibility that gram-negative bacilli (GNB) are part of the nontransient flora on hands was examined by using a broth rinse technique to detect low titers of GNB after a hygienic hand wash with soap and water. A total of 100 nurses who had direct patient contact and 40 controls without patient contact had a similar rate of recovery of GNB (46 and 55%, respectively). GNB persisted on the hands of 10 nurses throughout five successive hand washes with soap and water. Hand cultures were obtained daily from 12 nurses before and after a work shift in a surgical intensive care unit. GNB were recovered from 57% of individuals before patient contact and from only 24% after the work shift. Nontransient GNB on the hands of hospital personnel are a potential reservoir for hospital strains, and patient contact is not an obvious source for the acquisition of nontransient GNB.

Transmission of microbial flora via the hands of hospital personnel with close patient contact has been implicated as a likely mode of spread of nosocomial pathogens. Hand washing is considered to be the most important measure in limiting the frequency of hospital-acquired infections, and antiseptic hand-washing agents are commonly used in critical care units and on wards with immunocompromised patients. Price described two mutually exclusive categories of microbial hand flora, transient and resident (8). Transient flora consists of organisms loosely attached to the outer epithelium and is lost spontaneously or is removed by a simple wash with soap and water. Resident flora, which lives in or on the skin, forms a relatively stable population in both size and composition when not under the influence of antiseptic agents (1, 3, 4). *Staphylococcus*, *Micrococcus*, *Corynebacterium*, and *Streptococcus* species predominate, and their numbers recover rapidly after removal by mechanical means (1, 5).

Gram-negative bacilli (GNB) cause more than 50% of hospital-acquired infections. These organisms are not thought to be part of the resident flora of the skin, perhaps because of influences such as desiccation, inhibition by skin lipids, or interference by normal cutaneous bacterial flora (Center for Disease Control, *Isolation Techniques for Use in Hospitals*, 1st ed., U.S. Government Printing Office, Washington, D.C., 1970). Most studies have assumed that GNB are part of the transient flora only and easily removed by hygienic hand washing with soap (2-4, 8, 10, 11). GNB remaining after removal of transient hand flora with soap and water have not been studied extensively.

The purpose of this study was to determine the prevalence of GNB as a part of the nontransient flora on the hands of hospital personnel during a period when an epidemic of nosocomial infections caused by GNB was not present. We examined the persistence of GNB after successive hand washes and the influence of working a shift in an intensive care unit on hand carriage of GNB by nurses.

MATERIALS AND METHODS

Subjects. Personnel at the University of Virginia Hospital who responded to a request to participate and who had no evidence of dermatitis were sampled between March and July of 1983. A total of 100 nurses with direct patient contact from eight wards and five critical care units were cultured while on duty. Fourteen (14%) of the nurses reported that they used antibacterial soaps, either 4% chlorhexidine-gluconate (Hibiclens; Stuart Pharmaceuticals, Wilmington, Del.) or povidone iodine (Clinipad Corp., Guilford, Conn.) for hand washing while on duty. The remainder used a nonmedicated soap (Kindest-Kare; Calgon Corp., St. Louis, Mo.). Four bottles of Kindest-Kare obtained from the nursing units were cultured for aerobic bacteria and found to be free of GNB. The use of medicated soaps outside the workplace was not determined. Forty control subjects with clerical or administrative duties were also sampled while at work. The controls were matched for age and sex to the study population. The mean age (\pm standard deviation) for nurses and controls was 34 ± 9.2 and 36 ± 11.6 years, respectively ($P = 0.2$). Five members of the study group and three of the controls were male ($P = 0.4$).

Sampling of hands. A hygienic hand wash (15 to 20 s) for removal of transient flora was performed by each subject (7). An unused bar of Ivory soap (Proctor and Gamble, Cincinnati, Ohio) was provided for each wash, and hands were dried with brown paper towels (Crown Zellerbach Corp.). No GNB were detected from five samples of the bar soap or five samples of the paper towels. The samples were incubated at 37°C overnight in 10-ml samples of broth, and 1 ml of each broth was inoculated onto MacConkey agar plates.

The broth rinse technique of Sprunt et al. (9) was used to sample the nontransient bacterial flora of the hands after the hands were washed. The fist was opened and closed five times while immersed in 10 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) in a sterile polyethylene bag (Dow Chemical Co., Indianapolis, Ind.). The procedure was repeated with the other hand using the same bag. Each rinse sample was inoculated on agar either within

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TABLE 1. Hand cultures from 12 nurses paired according to patient contact interval

Hand-culture results for each pair	No. (%) of cultures in interval		P value
	With patient contact ^a (n = 58)	Without patient contact ^b (n = 54)	
Both negative for GNB	20 (34)	17 (31)	NS ^c
Both positive, same GNB	4 (7)	4 (7)	NS
Lost 1 GNB	24 (41)	2 (4)	0.000001
Gained 1 GNB	2 (3)	18 (33)	0.000026
Both lost ≥ 1 organism and gained ≥ 1 organism	8 (14)	13 (24)	NS

^a Samples taken before and after a work shift.

^b Samples taken after completion of a shift and before the start of the next shift.

^c NS, Not significant.

1 h or after storage at 4°C for up to 5 h. Neutralizers were not used in the broth, since the majority (86%) of people did not use medicated soap while on duty.

Bacterial counts. The total number of aerobic bacteria per milliliter of broth was determined by using Trypticase-soy-agar pour plates which were read after 48 h of incubation at 37°C. GNB in the broth rinse were quantitated by plating 1.0 and 0.1 ml of the broth onto MacConkey agar plates. The plates were examined after 24 and 48 h of incubation. GNB were identified by the API-20E system (Analytab Products, Plainview, N.Y.) with supplementary tests for non-glucose fermenters.

Study design. The prevalence of nontransient GNB on the hands of nursing personnel and controls was first determined. In a second study, the persistence of the GNB on the hands of 10 nurses identified in the first study was evaluated by culturing their hands after each of five successive hand washes with nonmedicated soap and water. In the third study, the effect of patient contact on the presence of nontransient GNB during a 2-week period was determined by twice-daily culturing of the hands of 12 nurses who worked in a surgical intensive care unit. Their hands were sampled after a hygienic hand wash with bar soap before and after a work shift. None of these nurses had dermatitis, and none used a medicated soap during the work shift.

Statistics. A 95% confidence interval for the number of colonies in the original volume of broth was determined, assuming a Poisson distribution. Differences in the age of the control and study populations were tested by using the Student *t* test. Log₁₀ counts of GNB from both groups were compared by using a two-sample *t* test. The proportion of GNB and of individual genera and species of organisms were examined by using the chi-square test. The chi-square test was also used to compare culture results from before and after patient contact. When expected values dropped below 10, a Fisher exact test was used.

RESULTS

Prevalence of GNB as nontransient hand flora. The total number of aerobic bacteria on the hands of nurses after a routine hand wash with nonmedicated soap was $(1 \pm 1.1) \times 10^5$ CFU/ml of hand rinse. Total bacterial counts on the hands of controls were similar, with $(1 \pm 0.4) \times 10^6$ CFU/ml of rinse.

GNB were detected in 46% of the hand cultures from 100 nurses and in 55% of the cultures from the 40 controls ($P = 0.6$). GNB were found in the hand cultures of 44% of nurses who worked on a ward and 49% of nurses who worked in intensive care units. Fourteen nurses reported that they used antimicrobial hand-washing agents while at work. GNB were recovered from the hands of 10 (71%) of these nurses, whereas GNB were recovered from only 36 (42%) of the 86 nurses who used a nonmedicated soap while on duty ($P = 0.04$). Titers of GNB in broth rinses from both nurses and controls were low ($\leq 10^3$ CFU/ml of wash). The organisms recovered were *Acinetobacter calcoaceticus* (subsp. *lwoffii* and *anitratus*), *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Serratia marcescens*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Hafnia alvei*, *Serratia species*, and *Pseudomonas species*. The genera of GNB detected on the hands of nurses were similar to those on the hands of controls, except for *E. coli* and *A. calcoaceticus* subsp. *anitratus*; both were recovered more often from the control group ($P = 0.005$ and $P = 0.03$, respectively). *Acinetobacter species* (19%), *Klebsiella species* (10%), and *Enterobacter species* (8%) predominated on the hands of nurses, and *Acinetobacter species* (27%) and *Klebsiella species* (15%) were most common on the hands of controls.

Ten nurses who had GNB on their hands were cultured after five consecutive hand washes with nonmedicated bar soap to determine whether the GNB were merely transient flora which had not been effectively removed by a single hygienic hand wash. The GNB were persistent in all 10, since GNB detected after wash 1 or 2 were always recovered in broth rinses after washes 3 to 5. The organisms isolated were *K. pneumoniae* (60%) and *A. calcoaceticus* subsp. *anitratus* (40%).

Effect of work activities on GNB carriage. Twelve nurses from intensive care units were cultured twice daily for 2 weeks to see whether patient contact altered nontransient hand flora. Cultures were obtained at the beginning of the work shift and again at the completion of the work shift. The hand-washing agent available to these nurses at work was a nonmedicated soap (Kindest-Kare). A mean of 11 (range, 10 to 17) hand cultures was obtained per nurse. Of the 134 cultures collected, 44% were positive for GNB. The number of positive cultures per nurse ranged from one to seven. All 12 nurses had GNB recovered from their hands at least once during the 2-week period. Surprisingly, 41 (57%) of 72 hand cultures before patient contact contained GNB, whereas only 15 (24%) of 62 hand cultures obtained after completion of the work shift were positive for GNB. In further analysis, hand cultures from each individual before and after a work shift were paired and compared with culture pairs from the completion of one shift and before the next shift (Table 1). Loss of one species of GNB was greater during a work shift than during time spent off shift (41 versus 4%, respectively; $P = 0.000001$). In contrast, acquisition of a GNB on the hands was greater while off duty than while on duty (33 versus 3%, respectively; $P \leq 0.0001$).

Acinetobacter or *Enterobacter species* predominated in hand cultures obtained before patient contact. *Acinetobacter species* were the predominant organism recovered from cultures obtained at the completion of the shift. Of the 12 nurses, 2 were considered true carriers of GNB, since the same species was recovered from at least 40% of their hand cultures over the 2 weeks of observation (6). *A. calcoaceticus* subsp. *lwoffii* was carried by one of these nurses (46%

of 13 cultures were positive), and *E. agglomerans* was carried by the other (47% of 15 cultures were positive).

DISCUSSION

GNB were detected with a broth rinse technique on the hands of approximately 50% of nurses and controls after hygienic hand washing with nonmedicated bar soap. The GNB did not seem to be transients which had been left behind after one wash, since they were not removed by five consecutive hygienic hand washes.

Our finding that GNB persisted on the hands of 10 nurses after five successive hand washes yet were apparently lost by some nurses from intensive care units by the end of the work day suggests that a cumulative number of hand washes (more than five) may be required for removal. Larson reported that the frequency of hand washing correlated inversely with the prevalence of gram-negative bacterial colonization in various hospital personnel and a control group (6). She defined frequent hand washing as hand washing eight times a day and found a lower recovery of GNB from hands of hospital personnel who washed their hands frequently than from those who did not (16 versus 33%). Thus, there may be a threshold for the total number of hand washes or exposure time with the hand-washing agent after which some of the nontransient flora is effectively removed.

The finding of GNB more frequently on the hands of nurses entering the work unit from home suggests that while nurses are away from the hospital (and perhaps the frequent hand washing practice), nontransient GNB could grow to numbers sufficient for recovery. Alternatively, GNB could have been acquired at home and later removed during hand washing with nonmedicated soap at the hospital.

We did not test the persistence of GNB on hands during repeated hand washing with an antiseptic-containing soap. Whether an antiseptic would facilitate suppressors of growth or killing of these nontransient GNB is not known. However, it is possible that repeated hand washes with antiseptic soap may alter the resident flora of the hand and select for eventual growth of GNB. Some support for this concept was

found in our prevalence study, in which GNB was recovered more frequently from nurses who used medicated soaps than from nurses who used nonmedicated soap.

The biology of the bacterial flora of the hands is not simple. An expanded definition beyond transient and resident flora is required for precise description. We prefer the term transitional to define those bacteria which remain after a simple hygienic hand wash yet could be removed after a number of repeated washes. It remains for future studies to determine whether transitional organisms are important as reservoirs of nosocomial infection, whether transient organisms could become transitional flora, and whether medicated hand-washing agents, which may alter the normal ecology of hand flora, have any effect on transitional bacteria.

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