Distribution of Bacteria in Feces of Swine

GLORIA D. RALL, ARLETTA J. WOOD, R. B. WESCOTT, AND A. R. DOMMERT
Department of Veterinary Microbiology, School of Veterinary Medicine, University of Missouri, Columbia, Missouri 65201

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A new technique is described for evaluating bacterial cell distribution in fecal samples. Spatial relationships of cells within an area rather than number of cells per unit volume or weight are measured by this technique. Measurements of cell distribution by this method indicated that bacteria occurred in freshly voided swine feces as pure, discrete colonies rather than as single cells distributed randomly or uniformly throughout the sample.

The usual method of measuring bacterial populations in fecal material has been to dilute samples quantitatively (8, 10, 12). Values obtained are adjusted to numbers of bacteria per gram of feces and are assumed to reflect the bacterial population of the lower intestinal tract. The method is valid if bacteria are randomly distributed throughout samples and in proportion to the types present in the lower intestinal tract. If these conditions are not met, quantitative dilution may yield misleading bacterial counts. The techniques reported in this paper were developed to investigate patterns of bacterial cell distribution in fecal samples to improve understanding of the ecology of intestinal microbes.

MATERIALS AND METHODS

Freshly voided fecal samples were collected into sterile, paper containers from miniature swine ranging in age from 6 to 30 months. Immediately after collection, samples were taken to the laboratory for processing. The bacterial populations in subsamples of each fecal specimen were determined as follows. The sterile tip (0.0025 cm²) of an inoculating needle was brought in apposition to the specimen surface; it was then rinsed in a drop of phosphate-buffered water (8) deposited on the surface of an agar medium in a standard petri dish, and the drop was immediately streaked across the agar surface with a sterile glass spreader. This procedure was repeated 100 times, providing 100 subsamples plated on the same medium in separate petri dishes, each representing a different exterior area of the fecal sample. The interior of the specimen then was exposed by gently separating the fecal sample and was similarly tested. Therefore, approximately 200 subsamples, evenly divided between the interior mass and exterior surface, were taken at random from each fecal specimen. After incubation, the colonies from each subsample (the .0025-cm² area of the tip of the inoculating needle) were counted. One fecal specimen was used for each taxonomic group studied except for the coliforms for which two specimens were utilized.

The bacteria identified in this study were lactose-fermenting coliforms (predominantly Escherichia coli), nonlactose-fermenting coliforms, Staphylococcus sp., Clostridium sp., enterococci and Lactobacillus sp. The coliform bacteria were cultured on Tergitol-7 agar (Difco), with 10 ml of 0.4% solution of 2,3,5-triphenyltetrazolium chloride added per liter of medium. Staphylococci were cultured on mannitol-salt agar (BBL). Clostridia were cultured on reinforced clostridia media (Consolidated Laboratories, Chicago Heights, Ill.) with 10 ml of 0.1% hemin and 15 mg of neomycin sulfate added per liter of medium. Enterococci were cultured on buffer peptone glucose agar (Difco) with 2.5 ml of glycerol, 10 g of saccharose, and 15 g of agar added per liter of medium. Lactobacilli were cultured on Rogosa SL agar (Difco). Media used for coliforms, staphylococci, and enterococci were incubated aerobically. Reinforced clostridia media was incubated in anaerobic jars with 10% carbon dioxide in hydrogen, and Rogosa SL agar was incubated in candle jars. All media were incubated at 37 C. Times of incubation were 18 hr for coliforms, 24 hr for clostridia, and 48 hr for all other organisms. The identification of these organisms, using these media and incubation methods, have been reported in detail (8).

RESULTS

Total numbers of bacteria in 100 subsamples and percentage frequency (%F) of viable bacteria for each of the fecal specimens examined are shown in Table 1. The %F varied widely among specimens for the same organisms and different taxonomic groups. However, %F and numbers of bacteria for most groups of organisms were lower from the exterior than from the interior of samples for each fecal sample. Tables 2 and 3 show actual frequency distribution data which were compared with the distribution expected if cell distribution was...
random. Significant \((P < 0.005)\) Chi-square (3) values were obtained in all comparisons, indicating nonrandom distribution of the bacteria studied.

Figures 1 and 2 are typical bacterial cell distributions for the groups of organisms studied.

**Table 1. Percentage frequency of viable bacteria in fecal samples from swine**

| Sample no. | Organism group       | Location of subsamples | \(X^a\) | \(\%F^b\) |
|------------|----------------------|-------------------------|---------|-----------|
| 1          | Lactose fermenters   | Exterior                | 1,466   | 33.7      |
| 1          | Lactose fermenters   | Interior                | 1,936   | 78.5      |
| 2          | Lactose fermenters   | Exterior                | 3,771   | 60.0      |
| 2          | Lactose fermenters   | Interior                | 7,892   | 89.7      |
| 3          | Nonlactose fermenters| Exterior                | 280     | 10.2      |
| 3          | Nonlactose fermenters| Interior                | 339     | 39.7      |
| 4          | Nonlactose fermenters| Exterior                | 759     | 46.9      |
| 4          | Nonlactose fermenters| Interior                | 2,386   | 83.6      |
| 5          | Clostridium sp.      | Exterior                | 2,669   | 95.0      |
| 5          | Clostridium sp.      | Interior                | 3,290   | 68.0      |
| 6          | Lactobacillus sp.    | Exterior                | 3,656   | 79.2      |
| 6          | Lactobacillus sp.    | Interior                | 6,052   | 87.0      |
| 7          | Enterococci          | Exterior                | 119     | 30.6      |
| 7          | Enterococci          | Interior                | 239     | 51.0      |
| 8          | Staphylococcus sp.   | Exterior                | 19      | 7.0       |
| 8          | Staphylococcus sp.   | Interior                | 36      | 4.1       |

\(a\) \(X\) = total number of bacterial cells in 100 subsamples.

\(b\) \(\%F\) = (total subsamples containing organisms of a particular group/total subsamples made for group) \(\times 100\).

Two distinct patterns of distribution are apparent in these figures: (i) a regular distribution (5), characterized by having the highest frequency groups nearest the mean (Fig. 1), and (ii) a contagious distribution (5), characterized by having the highest frequency groups without bacteria or containing large numbers of bacteria (Fig. 2).

**DISCUSSION**

A random distribution of bacterial cells in feces should have produced a normal or a Poisson distribution of cells in the 100 subsample groups. These types of distribution were not observed for any of the organisms studied. Therefore, the distribution of bacterial cells, of the taxonomic groups studied, must be considered as nonrandom throughout the samples. This nonrandom distribution indicated that the organisms studied must have occurred as discrete microcolonies rather than as individual cells in samples. Bacteria in soil occur in colonies (7), as do bacteria on the walls of the gastrointestinal tract in rodents (9), and it is suspected that in most habitats bacteria occur in colonies (1). Thus, their appearance in fecal samples as colonies rather than individual cells is consistent with the normal ecological behavior of bacteria.

Kolacz et al. (8) studied the fecal microflora of miniature pigs by using conventional dilution techniques and noted more variation among samples than among animals for several groups of organisms. The conclusions reached from the present study may explain in part the reasons for the variation among samples. Colonies apparently were not of equal size and their number varied from one location to another within the fecal mass. Hence, the number of bacteria within a single subsample of material may not reflect the mean of the sample. Furthermore, the colonies present are probably not sufficiently dispersed in the fecal suspension to achieve a precise cell count (2, 4, 6).

**Table 2. Frequency of occurrence of bacteria in subsamples from exterior of fecal samples**

| Organism group           | No. of colonies per subsample |
|--------------------------|-------------------------------|
|                          | 0    | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | >9   |
| Lactose fermenters      | 40   | 4    | 2    | 3    | 0    | 1    | 2    | 1    | 1    | 0    | 46   |
| Lactose fermenters      | 67   | 3    | 2    | 1    | 3    | 1    | 0    | 0    | 1    | 2    | 18   |
| Nonlactose fermenters   | 88   | 4    | 0    | 0    | 0    | 0    | 1    | 0    | 0    | 0    | 1    |
| Nonlactose fermenters   | 52   | 11   | 3    | 2    | 3    | 4    | 0    | 1    | 0    | 0    | 22   |
| *Clostridium* sp.       | 5    | 7    | 5    | 3    | 1    | 3    | 2    | 2    | 3    | 3    | 66   |
| *Lactobacillus* sp.     | 20   | 7    | 3    | 4    | 4    | 3    | 0    | 6    | 0    | 1    | 48   |
| Enterococci             | 68   | 10   | 8    | 3    | 3    | 1    | 1    | 1    | 1    | 2    | 1    |
| *Staphylococcus* sp.    | 93   | 5    | 1    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    |
TABLE 3. Frequency of occurrence of bacteria in subsamples from interior of fecal samples

| Organism group          | No. of colonies per subsamples |
|-------------------------|--------------------------------|
|                         | 0   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | >9   |
| Lactose fermenters      | 21  | 3   | 6   | 8   | 5   | 1   | 3   | 2   | 3   | 1    | 45   |
| Lactose fermenters      | 12  | 2   | 2   | 1   | 0   | 0   | 0   | 0   | 0   | 0    | 83   |
| Nonlactose fermenters   | 16  | 1   | 3   | 2   | 3   | 1   | 1   | 0   | 1   | 2    | 52   |
| Nonlactose fermenters   | 59  | 10  | 8   | 6   | 3   | 2   | 1   | 1   | 1   | 1    | 6    |
| Clostridium sp.         | 13  | 1   | 1   | 1   | 1   | 3   | 0   | 0   | 0   | 1    | 77   |
| Lactobacillus sp.       | 13  | 5   | 5   | 3   | 3   | 0   | 1   | 1   | 3   | 0    | 66   |
| Enterococci             | 49  | 16  | 9   | 6   | 3   | 5   | 2   | 2   | 1   | 0    | 7    |
| Staphylococcus sp.      | 82  | 11  | 2   | 1   | 0   | 0   | 0   | 0   | 1   | 0    | 1    |

FIG. 1. Frequency distribution of cells of enterococci in fresh fecal specimens from miniature swine.

The concept of bacteria occurring in colonies in fecal samples makes meaningful ecological interpretation of data derived from conventional dilution techniques difficult because such information constitutes absolute density data (5) and does not indicate the number of colonies present. Since a count of the number of colonies in feces may be as important as the total number of cells in ecological relationships, the dilution technique has definite limitations. If fecal material represents a habitat in which bacteria grow and die, evidence from other reports (3, 5, 11) suggests colony distribution also will be found to be non-random. The possibility that environmental factors affecting colony numbers may not influence cell numbers implies that a nonlinear relationship may exist between numbers of cells and numbers of colonies in feces.

FIG. 2. Frequency distribution of cells of Lactobacilli in fresh fecal specimens from miniature swine.

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