

BODY OF REPORT

SEATO Medic Study No. 68 Quantitative Changes in Fecal Bacterial
Flora During Diarrheal Diseases

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Objective: The objective of this study is to quantitate the fecal bacterial flora of patients with and without acute diarrhea and to attempt to relate changes of bowel flora to the etiologic agent. In addition the influence of antimicrobial agents administered therapeutically to patients or experimentally to controls is being determined in terms of the bacterial ecology of intestinal flora.

Description: Fecal specimens are obtained from hospitalized patients. The time required for processing the specimens for anaerobic incubation is less than one hour and less than 2 hours for aerobic incubation. Serial dilutions and plate counts from 10^2 to 10^{10} are carried out on the 10 media listed in Table I. In those instances when the same organism will grow on two different media, the media indicating the higher count is used. Routine bacteriologic and serologic procedures

Table I
CULTURE USED FOR ENUMERATION OF BOWEL FLORA

Medium	Specific for;	Incubation Condition		
		Time (Hrs.)	Temp. C	Environment
Blood Agar	Total aerobes	24	37	aerobic
MacConkey Agar	Total gram-negative aerobes	24	37	aerobic
Mannitol Salt Agar	Staphylococci	24	37	aerobic
SF Agar	Fecal Streptococci	96	45	aerobic
Tellurite*	Vibrios	24	37	aerobic
Sabouraud Dextrose Agar	Yeasts	24	37	aerobic
Blood Agar	Total Anaerobes	48	37	anaerobic
Egg Yolk Azide Agar	Clostridia	48	37	anaerobic
Neomycin Blood Agar	Total gram-negative anaerobes	96	37	anaerobic
Lactobacillus	Lactobacilli	48	37	anaerobic

*Alkaline lauryl sulfate tellurite

are utilized for final identification of organisms.

Three normal infants domiciled in the hospital were given 40 mg/kg of pediatric oxytetracycline orally for one week. Feces were analyzed before during and for two weeks after administration of antibiotic.

Progress: The bacterial flora of patients without diarrhea consisted predominantly of coliforms, mostly Escherichia coli, fecal streptococci, lactobacilli and yeast, all present in numbers greater than 10^5 /gm of feces. Other bacteria regularly present were Staphylococcus aureus and Staphylococcus albus. Organisms present in some patients were clostridia (usually Clostridium perfringens), anaerobic gram negative bacilli, anaerobic streptococci, diphtheroids, proteus and pseudomonas. Twenty specimens from patients with salmonellosis differed from the above in that there was a three log increase in the numbers of lactobacilli and yeasts and the presence of greater than 10^6 salmonellae/gm of feces in 18 of the 20 specimens. Both lactobacilli and yeasts were 3 logs lower in specimens from shigellosis patients. Shigellae isolated ranged from 10^4 to 10^9 /gm of feces. Both shigellae and salmonellae were isolated from the same specimen in only one instance and both at only 10^4 organisms/gm of feces.

Antibiotic therapy influenced the overall ecology of the feces less than might be expected. Numbers of coliforms remained high irregardless of therapy. There was a 2 log increase of S. fecalis when colimycine or the combination of sulfa

drugs and streptomycin were used, a 2 log increase of lactobacilli when sulfa drugs were used and a 5 log increase of yeasts when neomycin of the combination of sulfa drugs plus streptomycin were used. The presence of pseudomonas or proteus appeared to follow antibiotic therapy but neither ever predominated. Neither S. aureus nor S. albus increased significantly during or following the use of antibiotics.

No attempt was made to influence therapy in these patients unless sensitivity studies were requested. Thus it was possible to study stools of two diarrhea patients for over two months and relate changes in bacterial counts to the different therapeutic regimens employed. One patient was a 3 month old female from whom Salmonella montevideo was isolated in numbers greater than 10^6 /gm of feces. Quantitatively the patient responded to colimycine in that no organisms were found at the 10^2 dilution. However all but three of 27 routine specimens taken over a period of 73 days were positive for S. montevideo showing that the organism had been suppressed but not eliminated. It was noted that this organism was either not present at the 10^2 dilution or was present at greater than 10^6 organisms/gm of feces. Consistently high counts of coliforms, staphylococci, S. fecalis, lactobacilli and yeasts were noted. The counts of proteus, C. perfringens and bacteriodes were high when present but their presence or absence did not appear to relate to the counts of S. montevideo. The other patient was also 3 month old female from whom the initial enteropathogen isolated was the 025:B19 serotype of E. coli. This organism persisted in spite of antibiotic regimens of tetracycline for 3 days, streptomycin sulfate for 6 days and colimycine for 12 days. All during this time the routine cultures were positive for Salmonella derby but not until the 22nd hospital day was it countable. Both of the above organisms disappeared when a second course of colimycine was started on the 38th hospital day and continued through the 71st hospital day. On the 59th hospital day S. montevideo was isolated both by the routine technique and quantitatively. On the 72nd day of hospitalization the original serotype of E. coli and S. montevideo were isolated at 10^7 dilutions. The only change noted in the other bowel flora during this time was a 2 to 3 log decrease of S. fecalis at the time of emergence of S. montevideo.

The following observations were noted in the flora of the three normal infants receiving tetracycline. In the first infant coliform counts increased 2 to 3 logs and remained high for at least 12 days after tetracycline administration was stopped. S. aureus emerged during therapy and persisted in moderate counts for the remainder of the period of study. C. perfringens was virtually eliminated only to reappear in high numbers 10 days after tetracycline was stopped. There was a moderate but transient increase in the yeast count. Proteus and pseudomonas did not emerge during treatment. The second patient showed a similar increase in coliforms and yeasts, a decrease of C. perfringens and a moderate increase of lactobacilli. No S. aureus, pseudomonas or proteus were present. In the third patient there was a slight increase of coliforms and no other changes of note. The impact of this regimen of tetracycline did not appear to alter the normal flora of these infants to a significant degree.

Summary: Salmonellosis in these patients was characterized by counts of greater than 10^6 /gm of feces and up to 3 log increases of yeasts and lactobacilli. Shigellosis on the other hand showed decreases of a similar magnitude in the same two groups of organisms. Antibiotic therapy both in patients and normal controls had little lasting influence of the quantitative bacteriology of feces. There were no instances of overgrowth of the normal flora by S. aureus, yeasts, etc., during or following antibiotic therapy.

There were many examples of enteropathogens being present in the feces at counts of less than 10^2 at which level it is difficult to attribute their presence as the etiology of the disease. However their sudden emergence at counts of 10^6 /gm of feces indicates the importance of attempting to eliminate them.

Conclusion: Studies of the antibiotic sensitivities of intestinal flora before, during, and following antibiotic therapy are indicated. Also sampling at different levels of the intestinal tract are planned to learn the concomitant flora at each sampling site.