

2 Title: 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 diarrhea and attempt to relate changes of fecal flora to the etiologic agent (s). In addition the influence of antimicrobial agents administered therapeutically to diarrhea patients or to non-diarrhea controls was determined in terms of the bacterial ecology of intestinal flora.

Description. Specimens consisted of stool and intestinal juices obtained by orally administered tubes or by direct aspiration at autopsy. The time required for processing specimens was usually less than one hour for anaerobic incubation and less than 2 hours for aerobic incubation. Serial dilutions and plate counts from 10^2 to 10^{10} were carried out on the 10 media listed in Table 6. In those instances when the same organism grew on more than one medium, the medium indicating the highest count was used. Routine bacteriologic and serologic procedures were utilized for final identification of organisms.

Progress. The bacterial flora of patients without diarrhea was as follows: Gastric aspirates consisted of organisms usually associated with throat flora and ranged from 10^2 to 10^7 /ml of aspirate. The bacterial counts of most organisms identified in duodenal aspirates were from 10^2 to 10^4 per ml. and the flora consisted of gram positive cocci, yeasts, coliforms, fecal streptococci and lactobacilli. Jejunal flora was similar to that seen in the duodenum except that bacterial counts often tended to be higher. Fecal flora consisted predominantly of coliforms mostly Escherichia coli, fecal streptococci, lactobacilli and yeasts, all usually present in numbers greater than 10^5 /gm of feces. Other organisms regularly present were Staphylococcus aureus, Staphylococcus epidermidis, clostridia (usually Clostridium perfringens), anaerobic gram negative bacilli, diphtheroids, Proteus sp. and Pseudomonas sp.

The flora of aspirates from diarrheal patients did not differ greatly from those from non-diarrheal patients. Stool specimens from patients with salmonellosis or shigellosis usually contained more lactobacilli and yeasts and the enteropathogen (s) were present in counts ranging from 10^5 to 10^8 /gm of feces.

Autopsy specimens were obtained from 6 infants expiring from gastrointestinal diseases. The following findings were from 4 of 5 infants from whom no enteropathogen was isolated. From 3 to 7 species were found in samples of duodenal contents at counts of 10^4 /ml or greater. Jejunal and ileal specimens contained fewer species and at lower counts. All specimens from the descending colon contained 6 or more species, most of them at counts of 10^6 /ml or greater. In contrast the flora of a fifth infant from whom no enteric pathogen was isolated was characterized by high counts of S. epidermidis, coliforms, S. faecalis, lactobacilli and yeasts in the duodenum, jejunum, ileum and colon. The sixth case was a one year old male with a clinical diagnosis of gastroenteritis and malnutrition. Specimens obtained within one hour after death, from the jejunum, ileocecal valve and descending colon each contained at least 9 species of bacteria with most of them at 10^7 /ml or greater. Specimens from the jejunum and ileocecal valve contained organisms whose biochemical patterns conformed to shigellae but they were nontypable.

Seventeen specimens were from an American adult female with a chronic diarrhea which became acute about every 10 days. She received no antibacterial therapy for the first fifteen days of sampling at which time her physician prescribed tetracycline for an ear infection. Ten specimens were received before and 7 after oral tetracycline therapy was started. The patient's clinical condition did not vary during the period of observation. Shortly after the last specimen was received a diagnosis of amebic dysentery was made. Presumably the data represent the bacterial flora of a patient with amebic dysentery before and after tetracycline therapy. No enteropathogens were isolated by either quantitative or routine procedures. Her quantitative flora was characterized by consistently moderate to high counts of coliforms, enterococci, anaerobic streptococci, diphtheroids, lactobacilli and gram negative nonsporulating anaerobes. Proteus sp. was present in moderate numbers and disappeared during tetracycline therapy. Other organisms sporadically present in low numbers were staphylococci, clostridia, Bacillus sp. and yeasts but their appearance or disappearance could not be related to tetracycline therapy.

Six normal children were given 750,000 units of oral colistin daily for one week. Quantitative bacterial analyses were carried out for at least 3 weeks starting one week before medication was given. In no instance did this regimen alter the stool flora to a significant degree. None had alterations in their lactobacilli count; 2 had transient changes in enterococci counts (there was a temporary increase in one and a temporary decrease in the other), lactose-positive enterobacilli remained constant as did the gram-negative nonsporulating anaerobic bacilli in five of the six children. In respect to nonsporulating anaerobic bacilli, the other child had fewer than 10^2 /gm of stool until receiving colimycin, after which the count increased to 10^8 /gm of stool, a normal count in most of the children studied. Other changes noted in the flora were the complete elimination of anaerobic streptococci from the stool of one child and the emergence of Proteus sp. during therapy and its disappearance three days after therapy in another child. Staphylococci were present in the stools of all children but at very low counts. Clostridia were eliminated by colistin from the fecal flora of two children but were unaffected in the other four. Yeasts were irregularly present in low counts. No side effects were noted and certainly no profound alterations of intestinal flora were induced by this regimen of colistin.

Five normal infants were given 40 mg/kg of pediatric oxytetracycline orally for one week without significant alteration of stool flora. There were instances of species of gram negative organisms becoming resistant to oxytetracycline during this experiment. In some instances the isolates remained resistant after therapy was discontinued but there were equally as many instances where their sensitivities to oxytetracycline reverted to pretreatment levels.

Three normal infants were given oral neomycin sulfate in a dosage of 50 mg/kg each day for 7 days. Coliforms, lactobacilli, fecal streptococci and diphtheroids, initially present in high numbers and resistant to neomycin, remained at high counts during the 3 weeks of the study. A neomycin-resistant Shigella sonnei I was present at a count of 5.4×10^6 /gm of stool of one patient 8 days after cessation of medication, but the child did not have diarrhea. There were no alterations other bacterial flora and the organism was not found in a stool specimen obtained 4 days later.

Summary - significant numbers of bacterial species were cultured from stomach, duodenal and jejunal aspirates of patients with or without diarrhea. Stool specimens from patients with salmonellosis or shigellosis usually contained more lactobacilli and yeasts and the enteropathogens were usually present in counts ranging from 10^5 to 10^8 /gm of feces. Duodenal, jejunal and cecal autopsy specimens from 6 infants expiring from gastrointestinal diseases contained 3 or more species of bacteria at count of 10^4 /ml or greater.

Oxytetracycline, colistin or neomycin sulfate administered therapeutically to diarrheal patients or experimentally to non-diarrheal controls failed to alter pre-existing bowel flora to any degree. There were instances of organisms initially sensitive becoming resistant to the antibiotics being studied.

Table I
 Enterobacteriaceae isolated from acute diarrhea cases in Thailand
 From 1 April 1965 through 31 March 1966

Month	No. of Specimens	Shigellae	Salmonellae	<u>V. cholerae</u> El Tor (Inaba)	NAGS	Paracolon
April 1965	281	1	26	0	2	108
May	237	3	29	0	2	105
June	211	3	31	0	0	108
July	436	6	29	4	5	219
August	194	4	10	0	0	105
September	196	1	32	0	0	94
October	311	10	31	0	2	105
November	318	12	31	0	0	126
December	279	13	41	0	0	96
January 1966	283	16	26	3	5	100
February	364	15	79	3	3	123
March	257	31	117	1	0	25
Total	3367	115	482	11	19	1314
Percent of total Specimens		3.4	14.3	0.3	0.6	39.0

Table II

Salmonella species isolated in Thailand from April 1965 through March 1966

Species	Group	Children	Adults	Unknown	Total
<u>Salmonella derby</u>	B	124	8	0	132
<u>S. paratyphi B</u>	B	204	0	2	206
<u>S. saint paul</u>	B	2	2	1	5
<u>S. stanley</u>	B	5	2	0	7
<u>S. typhimurium</u>	B	3	3	0	6
<u>S. heidelberg</u>	B	1	0	0	1
<u>S. montevideo</u>	C ₁	54	0	0	54
<u>S. tennessee</u>	C ₁	1	1	0	2
<u>S. newport</u>	C ₂	5	7	0	12
<u>S. tananarive</u>	C ₂	2	0	0	2
Salmonella group C ₂ species (untyped)	C ₂	0	1	0	1
<u>S. dublin</u>	D	2	0	0	2
<u>S. typhosa</u>	D	6	0	0	6
Salmonella group D species (untyped)	D	1	0	0	1
<u>S. anatum</u>	E ₁	6	8	0	14
<u>S. lexington</u>	E ₁	7	2	0	9
<u>S. meleagridis</u>	E ₁	3	1	0	4
<u>S. weltevreden</u>	E ₁	9	3	0	12
Salmonella group E species (untyped)	E	4	1	1	6
Total		439	39	4	482

Table III

Shigella species isolated in Thailand from April 1965 to March 1966

Species	Group	Children	Adults	Unknown	Total
<u>Shigella dysenteriae</u> 1	A	10	2	2	14
<u>Sh. dysenteriae</u> 2	A	1	2	0	3
<u>Sh. dysenteriae</u> 3	A	0	1	0	1
<u>Sh. flexneri</u> 1	B	3	3	2	8
<u>Sh. flexneri</u> 2	B	16	2	0	18
<u>Sh. flexneri</u> 3	B	30	1	1	32
<u>Sh. flexneri</u> 6	B	4	0	0	4
<u>Sh. boydii</u> 2	C	7	0	0	7
<u>Sh. boydii</u> 4	C	1	2	0	3
<u>Sh. sonnei</u> form I	D	13	5	0	18
<u>Sh. sonnei</u> form II	D	3	1	1	5
Alkalescens-dispar 04	A-D	0	1	0	1
Alkalescens-dispar 04	A-D	1	0	0	1
Total		89	20	6	115

Table 4

Enteropathogenic Escherichia coli from Acute Diarrhea cases in Thailand
from 1 April 1965 - 31 March 1966

	<u>Thai Nationals</u>	<u>Caucasians</u>
Number typed	2318	256
Rough	1283	123
Negative	913	118
Positive	122	15
Serotypes		
025: B19 - B23	30	7
026: B6	0	2
055: B5	5	0
086: B7	9	1
0111: B4	0	0
0112: B11	8	1
0119: B14	15	1
0124: B17	2	0
0125: B15	28	1
0126: B16	14	1
0127: B8	1	0
0128: B12	10	1

Table 6

CULTURE MEDIA 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	Yeasts	24	37	aerobic
Dextrose Agar				
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

General Information:

During the period covered by this report 3038 routine specimens processed were as follows:

Water samples	802
Urine specimens	442
Urethral swabs	276
Stool specimens	233
Dairy products	225
Pus specimens	176
Throat swabs	151
Vaginal swabs	69
Sputum specimens	55
Cerebrospinal fluid	39
Blood cultures	29
Miscellaneous	58

Sera for

Heterophile Test	197
Febrile Agglutination Test	156
VDRL	130

Table V
Survey of Enteric Bacterial Pathogens in Korat, Udorn, Ubol, Thailand
 (November 1965-January 1966)

Non-Diarrheal Group

	<u>Pathogenic E. coli</u>			<u>Shigellae</u>			<u>Salmonellae</u>		
	Korat	Udorn	Ubol	Korat	Udorn	Ubol	Korat	Udorn	Ubol
Thai adults	8/198	5/98	8/114	2/198	0/98	0/114	1/198	0/98	2/114
Thai children	8/147	3/107	6/111	2/147	5/107	3/111	1/147	1/107	2/111
Caucasian adults	1/23	2/25	5/47	0/23	0/25	0/47	0/23	0/25	0/47

Diarrheal Group

	<u>Pathogenic E. coli</u>			<u>Shigellae</u>			<u>Salmonellae</u>		
	Korat	Udorn	Ubol	Korat	Udorn	Ubol	Korat	Udorn	Ubol
Thai adults	0/12	—	0/3	1/12	—	1/3	0/12	—	1/3
Thai children	0/61	1/10	1/24	1/61	1/10	1/24	1/61	0/10	0/24
Caucasian adults	1/5	1/1	1/6	0/5	0/1	0/6	0/5	0/1	0/6