A PRELIMINARY REPORT ON THE FECAL MICROFLORA OF HEALTHY ADULTS

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Abstract

Quantitative bacteriological methods were used to evaluate the numbers and kinds of aerobic and anaerobic fecal micro-organisms in 23 healthy adults. Of the cultivable flora anaerobes outnumbered the aerobic flora by 1 to 2 logs. E. coli was the predominant aerobe. Direct microscopic counts were higher than counts obtained by culture. The different groups of organisms were arranged in high, intermediate and low count categories. Total anaerobes, Total aerobes, Coliforms Veillonella, Streptococci

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were placed in the high count group, while Lactobacilli, Staphylococci and Yeasts were placed in the intermediate count group. No significant difference between males and females for each group of organisms was observed. Direct microscopic examination of feces revealed the presence of parasites in more than half of the subjects studied.

Introduction

The influence of the balance of normal flora of the intestinal tract on health and well being of the host is well documented (Gall 1970). A great many different bacterial species are present in the intestinal tract of man. They have been reported to affect the natural resistance of the host (Zubrzycki and Spaulding, 1962). Suppression of certain components of the normal flora by antibiotics increases susceptibility to enteric pathogens such as Salmonella (Miller and Bohnhoff, 1963) and Shigella (Freter 1956). Drug susceptible micro-organisms are replaced by drug resistant ones particularly Staphylococci, Proteus, Pseudomonas and Yeasts. Studies conducted by Dubos et al. (1963) indicated that almost all the components of the normal flora can exert pathogenic effects under certain conditions. Symptoms suggestive of vitamin B deficiency also appear as a result of the disturbance in the intestinal flora. The intestinal flora have also been implicated in the metabolism of cholesterol, hormones and vitamin (Goldsmith 1965).

The present investigation deals with the quantitative data on the organisms present in normal feces of 23 healthy adults and the presence of parasites as revealed by direct microscopic examination.

Material and Methods

Samples of feces were collected in clean hard paper receptacles from 23 healthy individuals between 20 to 50 years of age. No antibiotics or other drugs were administered during the period of study.

Direct Examination of Feces:

Fecal specimens were emulsified in iodine and wet preparations were examined under the microscope for ova and cyst.

Cultural Methods:

Each fecal sample was studied in the following manner. One gram of feces was thoroughly mixed in 9 ml sterile saline and serial ten fold dilutions were carried out upto a concentration of 10^{-9} gm/ml. From appropriate dilutions 0.1 ml samples were spread on the surface of 4" agar plates. The medias used are listed in table I with the period and temperature of incubation mentioned against each. After 24 hours incubation, colonies were counted on each plate with the help of a colony counter.

Total Microscopic Count:

An appropriate dilution of fecal material was mixed with 0.1% congo red solution and bacteria were counted in WBC counting chamber and the number calculated and multiplied by dilution factor to obtain counts per gram of feces.

Identification Procedures:

Representative colonies were picked up from different media and subcultured on slants of Tripticase Soy agar to get the isolates in pure cultures. The organisms were identified on the basis of colonial morphology on selective media, cellular morphology, gram staining and biochemical reactions. Micro-organisms identified on the above grounds were total anaerobes, total aerobes, Streptococci, Lactobacilli, Coliforms, Neisseria, Veillonella, Staphylococci and Yeasts.

Table I: Selective Media and Methods of Incubations

Organisms Selected	*Medium*	*Incubation*	*Feces Dilution (-log 10)*
Total Anaerobes	Trypticase Soy Agar + 5% sheep blood	37°C—24 hours Anaerobic	7, 8, 9, 10
Total Aerobes	m. Plate count medium	37°C—24 hours Aerobic	6, 7, 8, 9
Streptococci	Blood Agar	37°C—24 hours Aerobic	6, 7, 8, 9
Neisseria	Muller Hinton Agar	37°C—48 hours Aerobic	1, 2, 3
Veillonella	Veillonella Medium (Rogosa, 1956)	37°C—48 hours Anaerobic	5, 6, 7, 8
Lactobacilli	Rogosa S.L. Agar (Rogosa et al., 1951)	37°C—72 hours Aerobic	4, 5, 6, 7
Coliforms	MacConkey Agar	37°C—24 hours Aerobic	6, 7, 8, 9
Staphylococci	Staphylococci medium 110	37°C—48 hours Aerobic	2, 3, 4, 5
Yeasts	Sabouraud's dextrose Agar	28°C—72 hours Aerobic	1, 2, 3, 4

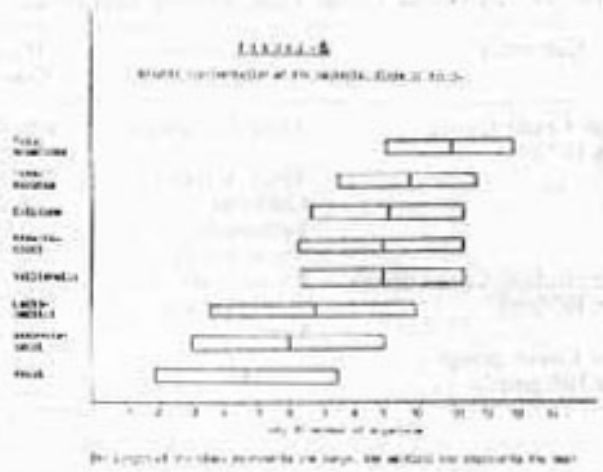
Results

Table II shows a comparison of the total microscopic and viable counts of fecal microflora. Trypticase Soy agar (with 5% sheep blood) incubated anaerobically yielded the highest viable counts and averaged 3.8×10^{10} organisms.

Aerobic count averaged 0.3 x 10^{10}, while the total microscopic count had a mean count of 181 x 10^{10} per gram of feces. The mean total microscopic count was almost 50 times higher than the mean total anaerobic and aerobic counts.

Table II: Total Microscopic and Viable Count Per Gram of Feces

Samples	*Total Microscopic Count* ($n \times 10^{10}/gm$)	*Total Anaerobic* ($n \times 10^{10}/gm$)	*Viable Count Aerobes* ($n \times 10^{10}/gm$)
1.	480	0.9	.36
2.	96	12.0	.31
3.	160	59.0	.28
4.	160	2.45	15.2
5.	90	51.0	10.0
6.	100	62.0	.0078
7.	256	0.295	.12
8.	128	150.0	.29
9.	192	0.54	.4
10.	192	0.70	.168
11.	96	12.5	9.0
12.	224	.0075	.077
Mean	181.17	3.8	.3
Standard Deviation	108.75	48.8	5.2



fecal samples were placed into high, intermediate and low count categories according to their mean total viable counts. This is shown in table IV. Results of direct microscopic counts for the cyst and ova of parasites are shown in table V. Out of 23 fecal samples entamoeba coli which is supposed to be the normal inhabitant

Table III. Distribution of Bacteria in Samples of Freshly Voided Feces

Organisms	*VIABLE COUNT PER GRAM*													*mean Viable Count (log 10)*	*S.D. (log 10)*
	0	1 10	2 10	3 10	4 10	5 10	6 10	7 10	8 10	9 10	10 10	11 10	12 10		
Total Anerobes (23)	—	—	—	—	—	—	—	—	—	4	6	11	2	11.0	1.0
Total Aerobes (23)	—	—	—	—	—	—	—	2	4	9	5	3	—	9.7	1.0
Coliforms (23)	—	—	—	—	—	—	2	1	8	6	5	1	—	9.1	1.2
Streptococci (23)	—	—	—	—	—	—	—	4	9	5	4	1	—	8.9	1.0
Veillonella (23)	—	—	—	—	—	2	—	1	5	12	3	—	—	8.9	1.3
Lactobacilli (23)	5	—	—	—	4	3	2	3	6	—	—	—	—	8.9	1.6
Staphylococci (23)	3	—	—	—	4	9	3	1	1	2	—	—	—	6.8	1.5
Yeasts (23)	8	1	2	—	5	3	3	1	—	—	—	—	—	6.0	1.4
Neisseria (23)	23	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Figures in paranthesis indicate total number of fecal samples tested

Table III shows the distribution of micro-organisms in feces. Anaerobes outnumbered the aerobic flora by 1 to 2 logs. Among the individual micro-organisms of the aerobic flora Coliforms and Streptococci were present in higher numbers, while the other micro-organisms such as Lactobacilli, Staphylococci and Yeast were present in lower numbers. Among the anaerobic flora Veillonella was present in all the specimens and in high numbers.

Fig 1 illustrates the fecal microbial counts in healthy adults. The length of the blocks represent the range in 95% confidence limits and the vertical lines represent the mean.

The mean counts of various groups of micro-organisms and their standard deviations are shown in fig 2. The high standard deviations for most of the groups reflect the variability of samples for individual groups of organisms. Different groups of organisms recovered from of intestinal tract was present in ten cases while Giardia was present in four cases. Ascarias lumbricoides and Hymenolipis nana were seen in one subject each.

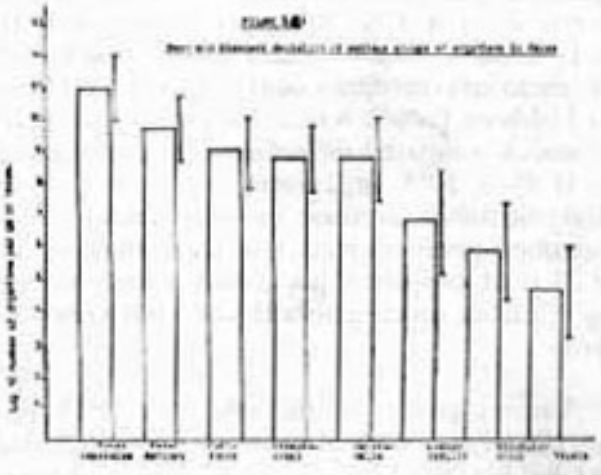


Table IV: Microbial Count Categories in Feces

Categories	*Organisms*	*Mean Count*
High Count Group ($n \times 10^9$/gm)	Total Anaerobes	100.00
	Total Aerobes	5.0
	Coliform	1.3
	Veillonella	0.8
	Streptococci	0.8
Intermediate Count group ($n \times 10^5$/gm)	Lactobacilli	70.0
	Staphylococci	10.0
	Yeast	0.5
Low Count group ($n \times 10^4$/gm)	—	—

Table V: The Distribution of Parasites in 23 Samples of Feces

Parasites	*No. of positive cases*
Entamoeba histolytica	5
Entamoeba coli	10
Giardia lamblia	4
Ascaris lumbricoides	1
Hymenolipis nana	1
Negative for protozoa or helminths	8

Discussion

Qualitative as well as quantitative studies were used for estimation of normal microbial flora of feces. Total viable counts for feces were found to be much less than the total microscopic count. This was probably because favourable growth conditions for all the organisms were not present. Moreover using direct microscopic count dead as well as viable bacteria are counted. Another reason for this, as also suggested by Richardson and Jones (1958), may be that direct counting includes each organism of a chain or cluster whereas in viable count the total chain or cluster is manifested as only one colony and therefore is counted as one organism. According to Richardson and Jones (1958) the true population of bacteria probably lies somewhere between values obtained by the two methods of counting.

The mean microscopic count was 181×10^{10} organisms per gram of feces in this study as compared to 4.1×10^{10} per gram for total viable counts, i.e. 50 times more (Table II). The mean microscopic count reported by Houte and Gibbons (1966) was 3.16×10^{11} per gram of feces as compared to mean viable count which was 0.85×10^{10} organisms per gram thereby getting 40 times decrease in total viable counts. Anaerobes predominated the fecal flora in all the 23 fecal samples. This confirms the observation of earlier investigators (Houte and Gibbons, 1966).

Various groups of bacteria were recovered from the feces of normal persons. These included total acrobes, Streptococci, Veillonella, and Coliforms. Other types such as lactobacilli, staphylococci and yeasts were present in decreased numbers. Among the aerobic flora predominance of streptococci in the fecal samples was observed. Similar findings were reported by Houte and Gibbons (1966). Coliforms were consistently present in high numbers among the fecal samples. Veillonella was obtained frequently in feces, and was isolated in high counts. Similar findings were reported by Cregan and Hayward (1953) and Bishop and Allock (1960) while Smith and Crabb (1961) found it only in one out of twelve fecal samples studied. Lactobacilli were isolated from 18 out of 23 individuals and in intermediate count range. This is in confirmation with Levison and Kaye (1969). Staphylococci were isolated in 20 out of 23 subjects examined. Similar findings were reported by Smith and Crabb (1961). Yeasts in feces were isolated in 65% of the samples examined. Levison and Kaye (1969) reported it to be present in 40% of the specimens. Drasar et al. (1969) recovered Neisseria in feces in lower numbers, but it was not recovered from any of the fecal samples in the present study.

Different groups of organisms arranged in various categories, i.e. high, intermediate and low count groups indicated that total anerobes, total aerobes, Streptococci, Coliform, Veillonella were present in high count group. Other organisms such as Lactobacilli, Staphylococci and Yeasts were present in intermediate group. Similar studies were performed by Drasar et al. (1969) in England and Bhat et al. (1972) in India.

Independent t-test was calculated for each group of organisms in males and females (Table VI). These calculations confirm the hypothesis that no significant difference exists between the two sexes for the microflora studied.

Table VI: Difference in Microbial Counts Between Males and Females (T. test) for Feces

Groups	*Sex*	*No of cases*	*Mean*	*Standard Deviation*	*Significance*
Total Anaerobes	M	8	10.47	0.79	$P > 0.05$
	F	15	11.28	1.03	
Total Aerobes	M	8	9.49	0.89	$P > 0.05$
	F	15	9.85	1.19	
Coliform	M	8	8.86	0.77	$P > 0.05$
	F	15	9.28	1.49	
Streptococci	M	8	9.0	1.07	$P > 0.05$
	F	15	8.96	1.01	
Veillonella	M	8	8.9	0.89	$P > 0.05$
	F	15	9.0	1.63	
Lactobacilli	M	5	6.20	1.44	$P > 0.05$
	F	13	7.06	1.59	
Staphylococci	M	7	5.91	1.36	$P > 0.05$
	F	13	6.10	1.68	
Yeasts	M	5	5.52	0.92	$P > 0.05$
	F	9	4.34	1.49	

Parasites were found in the feces of 15 out of 23 of the subjects studied. *Entamoeba coli* was present in 10 out of 23 subjects. Some

individuals harboured both *Entamoeba coli* and *Entamoeba histolytica*. Giardia was found either alone or in combination with either of the two parasites mentioned. *Ascarias lumbricoides* and Hymenolipis nana were recovered from 2 out of the 23 subjects studied. Prevalence of parasites was also reported by various other workers. Shrivastav et al. (1953) performed a survey on the incidence of *Entamoeba histolytica* in healthy adults in the population of Bombay and found 20.3% positive for this parasite. Amin et al. (1962) reported 10.3% positive for *E. histolytica*. Kuntz et al. (1958) reported 36% positive for *E. histolytica*, 20% for *E. coli* and 20% for Giardia. Ascarias were present in 52% of cases, H. nana were present in 8% of cases. The samples negative for both protozoa and helminth were 16% while in the present study the samples negative for parasites were 8, i.e. 34%.

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