Bacterial flora in intestinal amoebiasis

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EDITORIAL SYNOPSIS The authors have studied 203 patients infected with *Entamoeba histolytica* and have cultured their stools for bacteria using standard methods. No attempt has been made to isolate pathogenic strains of *Escherichia coli*. No bacterial pattern has been found to be associated with the presence of *Entamoeba histolytica* in the gut.

Entamoeba histolytica was first cultured *in vitro* in 1925 (Boeck and Drbohlav, 1925) but has not yet been grown in the absence of living bacteria. Experimentally the dependence of *E. histolytica* on living bacteria in the intestine is well illustrated by the inability of *E. histolytica* to survive and invade the bowel wall of germ-free guinea-pigs (Phillips, Wolfe, Rees, Gordon, Wright, and Reyniers, 1955) and clinically by the observation that certain antibiotics halt acute amoebic dysentery by inhibiting the growth of intestinal bacteria (Elsdon-Dew, Armstrong, and Wilmot, 1952) but do not affect amoebic liver abscess (Wilmot, Armstrong, and Elsdon-Dew, 1952).

The mean size of *E. histolytica* cultured from bacteria-free human amoebic liver abscess varies according to the bacterial parasites provided (Freedman and Elsdon-Dew, 1958). However, the role of bacteria in the pathogenicity of *E. histolytica* is obscure; bacteria probably attack the tissues of the host and reduce the resistance or supply essential factors for the growth of amoebae. In the literature, evidence is provided to show that bacteria are necessary for the growth of amoebae in the colon, yet there are no studies to the best of our knowledge to show whether or not any specific bacteria are associated with the growth of amoebae in the intestine.

MATERIAL AND METHODS

In the study of the incidence of bacteria and parasites in 800 cases of dysenteric and non-dysenteric diarrhoea, different types of amoebae were detected on microscopic stool examination in 235 patients (Antia, Chaphekar, Chhabra, Swami, and Borkar, 1961). In 203 of these patients harbouring amoebae, stool was collected for bacterial culture during sigmoidoscopic examination.

BACTERIAL STUDIES The material from the colon collected with a spoon through a sigmoidoscope was transferred into nutrient broth and alkaline peptone water. Warm plates of salmonella and shigella agar, desoxycholate citrate agar, and MacConkey agar were kept ready in the incubator at 37°C. Primary plating from broth and peptone water was done immediately on all three plates.

After aerobic incubation for 24 hours at 37°C., the plates were observed carefully for pale colonies. At least three such colonies were selected and each was transferred to triple-sugar-iron agar. After 18 to 24 hours' incubation, the results of triple-sugar-iron slopes tentatively identified different organisms and were confirmed with appropriate biochemical and/or agglutination tests.

In some cases the plate culture showed greatly altered normal flora with a pure growth of a single organism. In such cases pink, pinkish white, pale grey, green, pale green mucoid or pale-spreading colonies were transferred on appropriate media, including triple-sugar-iron and the organisms identified with biochemical and/or agglutination tests.

RESULTS

The results of the bacteriological studies in 203 patients with different types of amoebae in stool are shown in Table I. A normal stool culture on MacConkey agar plate showed mixed colonies of *B. coli*, *Streptococcus faecalis*, *B. proteus*. Even though these bacteria are normal intestinal flora they are mentioned only when a MacConkey agar plate showed a predominant growth of one of these organisms. The growth of *B. coli* was considered significant when they were abundantly grown on salmonella and shigella agar as well as on desoxycholate citrate agar plates. Similarly *B. proteus* and *B. pyocyaneus* are mentioned only when they showed a prolific growth on all the primary plates.

TABLE I
PREPONDERANT BACTERIAL FLORA IN AMOEBIASIS¹

	<i>E. histolytica</i>	<i>E. coli</i>	<i>E. histolytica</i> + <i>E. coli</i>	<i>E. nana</i>	<i>I. butschlii</i>	Total
Shigella Group						
<i>Sh. Shigae</i>	1	1	1	1	—	4
<i>Sh. flexneri</i>	5	1	1	1	1	9
<i>Sh. boydii</i>	3	1	1	—	1	6
<i>Sh. sonnei</i>	1	—	2	—	—	3
<i>Sh. non-specific</i>	2	—	—	1	—	3
						= 25
Salmonella Group						
<i>S. typhi</i>	—	—	—	—	—	—
<i>S. paratyphi A</i>	—	—	—	—	—	—
<i>S. paratyphi B</i>	—	—	—	—	—	—
<i>S. non-specific</i>	—	—	1	2	—	3
						= 3
Providencia Group (<i>Paracolon bacilli</i>)	4	1	2	2	—	9
						= 9
B. Proteus Group (normal flora absent)						
<i>P. morganii</i>	2	1	2	2	—	7
<i>P. rettgeri</i>	1	—	—	—	—	1
<i>P. mirabilis</i>	2	—	—	—	—	2
<i>P. non-specific</i>	3	2	1	1	—	7
						= 17
B. Pyrocyanus Group (Normal flora absent)	—	—	2	2	—	4
						= 4
Escherichia coli						
<i>E. coli communior</i>	3	2	2	1	1	9
<i>E. coli communis</i>	7	1	2	—	—	10
<i>E. coli metabolis</i>	—	2	—	—	—	2
<i>E. coli non-specific</i>	12	7	4	1	—	24
						= 45
Aerobacter Group						
<i>Aerobacter cloacae</i>	1	—	—	—	—	1
<i>Aerobacter aerogenes</i>	2	1	—	—	—	3
						= 4
Non-bacillary Group (normal flora absent)						
<i>Streptococcus faecalis</i>	1	—	—	—	—	1
<i>Streptococcus viridans</i>	1	—	—	—	—	1
<i>Alkaligenes faecalis</i>	1	2	2	—	—	5
						= 7
More than one type of bacterial flora predominant	7	7	5	2	—	21
						= 21
Usual mixed flora in small numbers	43	14	7	2	2	68
						= 68
Total	102	43	35	18	5	203

¹ *Shigella schmitzii* and *Proteus vulgaris* were not grown in any case

DISCUSSION

The results show that there are no specific bacteria in the gut associated with clinical intestinal amoebiasis. However, it must be pointed out that with the limited bacteriological methods employed only certain intestinal bacteria were studied and this particularly applies to the pathogenic and non-pathogenic *Escherichia coli*. The

question then arises, is *E. histolytica* by itself a pathogen?

Simple observations cast doubt whether *E. histolytica* by itself is a pathogen because, even in asymptomatic individuals, trophozoites or cystic forms may be present in the colon. Blumenthal, Dutra, Paschal, and Kuhn (1947) reported *E. histolytica* on stool examination in 24% of American troops suffering from acute diarrhoea, while even

in asymptomatic soldiers the incidence was 20%. The incidence of *E. histolytica* on stool examination in about 10% in Great Britain (Hoare, 1950) as well as in the United States (Johnstone, David, and Reed, 1933; Hardy and Spector, 1935) and yet the incidence of clinical amoebiasis is remarkably low in both these countries. In Durban, amongst Africans, Indians, and Europeans the incidence of *E. histolytica* on stool examination is approximately the same (Freedman, 1958). However, the incidence of clinical amoebiasis is highest amongst Africans and lowest amongst Europeans. The variable factor in the three groups is diet (Elsdon-Dew, 1949). An Indian harbouring *E. histolytica* may be asymptomatic in Europe and usually develops symptoms after returning to India (unpublished observations). Broad-spectrum antibiotics, which have no direct action on amoebae in amoebic hepatitis or abscess (Wilmot *et al.*, 1952), are highly effective in acute intestinal amoebiasis, as they probably act on the associated secondary organisms in the large bowel (Elsdon-Dew, 1949; Elsdon-Dew *et al.*, 1952).

As amoeba alone is unlikely to be a pathogen, certain other factors must be partly contributing to the pathogenesis of intestinal amoebiasis. It is possible that amoebae remain dormant in the colon and may act as pathogens only when the environment is favourable with altered bacterial flora as, for the growth of amoebae, bacterial flora is necessary (Chinn, Jacobs, Reardon, and Rees, 1942; Phillips *et al.*, 1955). However, there are hardly any reports in the literature to show whether or not any specific group of bacteria is necessary for the growth of amoebae in the human intestine. The results of the present study with limited bacteriological methods do not show any specific group of bacterial responsible for the growth of amoebae in the human intestine.

SUMMARY AND CONCLUSIONS

Though bacteria are necessary for the growth of amoebae there are no studies to show the type of organisms associated with clinical amoebiasis. In this study of 203 patients with clinical amoebiasis, stool was collected through a sigmoidoscope for bacterial culture. In 135 patients there was a preponderant growth of only one type of organism and in 68 patients a normal mixed bacterial flora was obtained. No specific group of bacteria could be incriminated for the pathogenicity of amoebae.

REFERENCES

- Antia, F. P., Chaphekar, P. M., Chhabra, R. H., Swami, G. A., and Borkar, A. V. (1961). The incidence of bacteria and parasites in dysenteric and non-dysenteric diarrhoea. A study of 800 cases. *J. Ass. Physns India*, **9**, 723-739.
- Blumenthal, H. T., Dutra, F. N., Paschal, H., and Kuhn, L. R. (1947). The significance of *Endamoeba histolytica* in stools of individuals with acute diarrhea of moderate severity. *Amer. J. trop. Med.*, **27**, 711-721.
- Boeck, W. C., and Drbohlav, J. (1925). The cultivation of *Endamoeba histolytica*. *Amer. J. Hyg.*, **5**, 371-407.
- Chinn, B. D., Jacobs, L., Reardon, L. V., and Rees, C. W. (1942). The influence of the bacterial flora on the cultivation of *Endamoeba histolytica*. *Amer. J. trop. Med.*, **22**, 137-146.
- Elsdon-Dew, R. (1949). Endemic fulminating amoebic dysentery. *Ibid.*, **29**, 337-340.
- , Armstrong, T. G., and Wilmot, A. J. (1952). Antibiotics and amoebic dysentery. *Lancet*, **2**, 104-109.
- Freedman, L. (1958). The epidemiology of amoebiasis in Durban. *S. Afr. med. J.*, **32**, 797-798.
- and Elsdon-Dew, R. (1958). Size variation in *Entamoeba histolytica*. *Nature (Lond.)*, **181**, 433-434.
- Hardy, A. V., and Spector, B. K. (1935). The occurrence of infestations with *E. histolytica* associated with water-borne epidemic diseases. *Publ. Hlth Rep. (Wash.)*, **50**, 323-334.
- Hoare, C. A. (1950). Amoebiasis in Great Britain with special reference to carriers. *Brit. med. J.*, **2**, 238-241.
- Johnstone, H. G., David, N. A., and Reed, A. C. (1933). A protozoal survey of one thousand prisoners. *J. Amer. med. Ass.*, **100**, 728-731.
- Phillips, B. P., Wolfe, P. A., Rees, C. W., Gordon, H. A., Wright, W. H., and Reyniers, J. A. (1955). Studies on the amoeba-bacteria relationship in amoebiasis. *Amer. J. trop. Med. Hyg.*, **4**, 675-692.
- Wilmot, A. J., Armstrong, T. G., and Elsdon-Dew, R. (1952). Aureomycin in amoebic liver abscess. *Ibid.*, **1**, 429-435.