A COMPARATIVE STUDY OF VARIETIES OF B. COLI ISOLATED FROM "TYPHOID" AND NORMAL DEJECTA.

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The want of success which has so persistently attended the efforts of most bacteriologists to isolate the *B. typhosus* from water supplies suspected to have caused enteric fever, suggested a study of the varieties of *B. coli* which are associated with the *B. typhosus* in the dejecta of patients suffering from enteric fever. It was hoped that the organisms in question might show cultural characteristics or reactions to specific sera, which would enable them to be distinguished from the varieties of *B. coli* present in the dejecta of healthy people; so that even if the *B. typhosus* were not detected, the presence of these special organisms might afford reasonable grounds for the belief that the water under examination had been fouled by the specific dejecta of cases of enteric fever. With this object in view 150 organisms have been examined; of these 80 were isolated from the stools of cases of enteric fever and 70 from the stools of healthy men. The enteric fever cases were five in number, one being a severe relapse, and the other four severe cases which terminated fatally. The stools were obtained during the third and fourth weeks of the disease and also, in the fatal cases, from the intestines after death had occurred. In order to isolate the organisms, one c.c. of each of the liquid stools was diluted 1—10,000 and 1—100,000 with distilled water, and $\frac{1}{10}$ c.c., $\frac{1}{4}$ c.c., and $\frac{1}{2}$ c.c. of these dilutions were plated out in gelatine. This method was not very satisfactory as the plates liquefied too rapidly. The use of carbolic acid might have helped to restrain the growth of liquefying organisms, but as this acid nearly always interferes with the perfect development of colonies, I thought it would be better to avoid its use if possible. After several trials, I found that the best results were obtained by taking a loopful of the liquid stool and then stroking it over the surface of a series of plates containing solidified gelatine. The first two or three plates usually liquefied, but the fourth, fifth and sixth plates showed discrete colonies which

https://doi.org/10.1017/S0022172400000176 Published online by Cambridge University Press

developed satisfactorily. Typical colonies were fished and planted out on agar for further study.

Agglutination experiments. A twenty-four hours' agar growth of each organism was made into an emulsion with broth and then mixed with an equal bulk of horse's "anti-typhoid serum," also diluted with broth, so as to make final dilutions of the mixed emulsion and serum, of 1—50, 1—100, 1—200, 1—500 and 1—1000. At first the results were judged by the microscopic appearances in a hanging-drop at the end of two hours and the macroscropic appearances in capillary tubes at the end of twenty-four hours. Later, the hanging-drop method was omitted as a prolonged comparison of the two methods enabled the results obtained by the hanging-drop to be judged from the appearance of the capillary tube. The investigation of the 150 organisms extended over a period of four months, consequently it was necessary to employ different batches of horse's anti-typhoid serum. In order to make the results strictly comparable, the various specimens of serum were standardised week by week with two stock cultures of *B. typhosus* (K obtained from Dr Král's laboratory, and 13 P obtained from Prof. Wright of Netley). All through the experiments the sera used gave a complete agglutination in a dilution of 1—1000 and a marked reaction in a dilution of 1—10,000, with the two stock cultures.

TABLE A.

Varieties of B. coli isolated from typhoid stools.

+, = complete agglutination. ±, = marked agglutination.
−, = traces of agglutination. 0, = no agglutination.

	1—50	1—100	1—200	1—500	1—1000
1	+	+	±	0	0
2	+	−	0	0	0
3	+	−	0	0	0
4	+	+	±	0	0
5	0	0	0	0	0
6	+	+	±	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	+	+	±	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	+	+	±	0	0
16	+	+	±	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0

TABLE A. (*cont.*)

	1—50	1—100	1—200	1—500	1—1000
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	0	0	0	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	+	+	±	−	0
28	+	+	±	−	0
29	+	+	±	−	0
30	+	+	±	−	0
31	+	+	±	−	0
32	+	+	±	−	0
33	−	0	0	0	0
34	−	0	0	0	0
35	−	0	0	0	0
36	+	+	±	0	0
37	+	+	±	0	0
38	+	±	±	0	0
39	+	+	−	0	0
40	+	+	±	0	0
41	+	+	±	0	0
42	+	+	±	0	0
43	+	+	±	0	0
44	+	+	±	0	0
45	0	0	0	0	0
46	0	0	0	0	0
47	0	0	0	0	0
48	0	0	0	0	0
49	0	0	0	0	0
50	+	−	0	0	0
51	+	±	0	0	0
52	+	±	±	0	0
53	+	±	±	0	0
54	+	±	±	0	0
55	+	±	±	0	0
56	+	±	−	0	0
57	+	+	±	0	0
58	+	+	+	−	0
59	+	±	0	0	0
60	+	+	+	0	0
61	+	+	+	0	0
62	+	+	+	+	+
63	+	+	+	+	−
64	+	+	+	+	−
65	+	+	+	+	−
66	+	+	+	+	±
67	+	+	+	+	±
68	+	+	+	−	0
69	+	+	+	+	−
70	0	0	0	0	0
71	0	0	0	0	0
72	0	0	0	0	0
73	0	0	0	0	0
74	0	0	0	0	0
75	0	0	0	0	0
76	0	0	0	0	0
77	0	0	0	0	0
78	0	0	0	0	0
79	0	0	0	0	0
80	0	0	0	0	0

https://doi.org/10.1017/S0022172400000176 Published online by Cambridge University Press

Control tubes of the emulsions of all these organisms were kept under observation for 24 hours, but none of them showed the slightest trace of agglutination.

Note. Complete agglutination in a capillary tube means that all the bacilli were precipitated in a *firm globular mass* at the bottom of the tube. Marked agglutination means that the bacilli were precipitated in several firm globular masses scattered through the column, the portions of fluid between the masses being quite clear. Traces of agglutination means that a few globular masses were seen, but the remaining portions of the fluid were still opaque.

The results obtained with the 80 specimens of *B. coli* isolated from typhoid stools are shown in Table A. It will be seen that seven cultures (Nos. 62, 63, 64, 65, 66, and 69) were completely agglutinated by the typhoid serum diluted 1 in 500, two cultures (66 and 67) showed a marked reaction with the serum diluted 1 in 1000, and one culture (62) was completely agglutinated by the serum in this dilution. This last culture (62) was again tested at once with the serum in still higher dilutions, and it was found to be completely agglutinated when the serum was diluted 1—2500.

TABLE B.

Cultures of B. typhosus isolated from the spleens of fatal cases of enteric fever.

	1—50	1—100	1—200	1—500	1—1000	1—10,000
G	+	+	+	+	–	0
G* (1)	–	0	0	0	0	0
G* (2)	+	+	+	+	+	–
M	+	+	+	+	±	0
A	+	+	+	+	+	0
K	+	+	+	+	+	±
13 P	+	+	+	+	+	±

G* (1) tested immediately after isolation from the spleen.

G* (2) re-tested after being preserved for six months in milk.

In Table B are recorded the results obtained when the specimens of *B. typhosus* isolated from the spleens of the four cases of enteric fever, from which the stools were obtained, and the two stock cultures were tested with the same anti-typhoid serum. It will be observed that all

the specimens were completely agglutinated by the serum in a dilution of 1—500. Culture (G) *B. typhosus* and culture (62) *B. coli* were obtained from the same case, and it will be noticed that the *B. coli* was completely agglutinated by the serum in a higher dilution than was effective with the *B. typhosus*. If the serum used had been obtained from the patient it would have been easy to explain the result by assuming that the patient had suffered from a "mixed infection." The serum employed, however, was prepared by injecting a horse with *B. typhosus*, so a "secondary coli infection" is out of the question. It therefore appears that varieties of *B. coli* in typhoid stools may become agglutinated by a highly dilute anti-typhoid serum. That the reaction is only the result of environment, and is not truly specific as in the case of *B. typhosus*, is shown by the fact that when the cultures were removed from their associations and preserved for several months on agar, they gradually became incapable of reacting to a dilute serum. At the end of six months the results shown in Table C were obtained.

TABLE C.

Varieties of B. coli (from enteric stools) re-tested after being preserved for six months on agar.

	1—50	1—100	1—200	1—500	1—1000
62	±	±	0	0	0
63	±	0	0	0	0
64	+	+	0	0	0
65	+	+	0	0	0
66	+	±	0	0	0
67	+	±	0	0	0
68	−	0	0	0	0
69	+	+	±	−	0

The cultures of *B. typhosus* (G, M, A, K and 13 P), however, when tested at the end of six months showed no change in their reaction to the specific serum; the results recorded in Table B were again obtained. The *B. typhosus* culture G* was, however, peculiar; when first isolated from the spleen it showed only traces of agglutination with the specific serum diluted 1—50, six months later it was completely agglutinated by the serum diluted 1—1000. The cultural characteristics of this organism were carefully studied and compared with the other races of *B. typhosus*. The results are shown in the following Table D.

TABLE D. *Races of B. typhosus.*

	G	G*	A	M	K	13 P
Agar	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth	Thin greyish-white growth
Broth	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface	Turbid, no film on the surface
Glucose or lactose-gelatine (shake)	No gas formation	No gas	No gas	No gas	No gas	No gas
Witte's peptone and salt solution, after 7 days at 37° C.	No indol	Traces of indol	No indol	No indol	Traces of indol	No indol
Potato	Moist, colourless growth	Colourless growth	Colourless growth	Colourless growth	Colourless growth	Colourless growth
Milk, 3 weeks at 37° C.	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Litmus-whey, 7 days at 37° C., acidity equals	5·7 % $\frac{N}{10}$ alkali	5·1 % $\frac{N}{10}$ alkali	5·6 % $\frac{N}{10}$ alkali	5·4 % $\frac{N}{10}$ alkali	5·6 % $\frac{N}{10}$ alkali	5·5 % $\frac{N}{10}$ alkali
Proskauer & Capaldi's medium, No. I., 24 hours at 37° C.	No growth or change in reaction	Growth, but no change in reaction	No growth or change in reaction	No growth or change in reaction	No growth or change in reaction	Growth, but no change in reaction
Ditto, No. II., 24 hrs. at 37° C.	Strongly acid	Strongly acid	Strongly acid	Strongly acid	Strongly acid	Strongly acid
Gelatine plates	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow	Colonies small and growth slow
Motility (24 hours in broth)	Highly motile	Highly motile	Highly motile	Highly motile	Highly motile	Highly motile
Stained by Gram's method	No	No	No	No	No	No

TABLE E.

Varieties of B. coli isolated from typhoid stools.

	No. 62	No. 63	No. 64	No. 65	No. 66
Agar slope	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth
Broth	Turbid, no surface pellicle	Turbid, slight surface pellicle	Turbid, no surface pellicle	Turbid, no surface pellicle	Turbid, marked surface pellicle
Peptone (Witte) and salt solution, 7 days at 37° C.	No indol reaction	Traces of indol reaction	Marked indol reaction	Marked indol reaction	No indol reaction
Glucose and lactose-gelatine (shake)	Marked gas formation	Marked gas formation	Marked gas formation	Marked gas formation	Marked gas formation
Potato	Yellowish-brown growth	Thick yellowish-white growth	Thick yellowish-white growth	Thick yellowish-white growth	Thick yellowish-white growth
Milk (37° C.)	Coagulated in 7 days	Coagulated in 24 hours	Coagulated in 24 hours	Coagulated in 24 hours	Coagulated in 24 hours
Litmus-whey, 7 days at 37° C.	Acidity = 30 per cent. $\frac{N}{10}$ alkali	Acidity = 37·8 per cent. $\frac{N}{10}$ alkali	Acidity = 29 per cent. $\frac{N}{10}$ alkali	Acidity = 34·5 per cent. $\frac{N}{10}$ alkali	Acidity = 38 per cent. $\frac{N}{10}$ alkali
Proskauer & Capaldi's No. I. medium, 24 hours at 37° C.	Strongly acid	Strongly acid	Strongly acid	Strongly acid	Strongly acid
Ditto, No. II., 24 hrs. at 37° C.	Faintly acid	Neutral	Faintly acid	Faintly alkaline	Neutral
Gelatine plates	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic
Motility (24 hrs. in broth at 37° C.)	Not motile	Not motile	Not motile	Not motile	Not motile
Stained by Gram's method	No	No	No	No	No

TABLE E. (*cont.*)

	No. 67	No. 68	No. 69	No. 70
Agar slope	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth	Thick greyish-white growth
Broth	Turbid, slight surface pellicle	Turbid, slight surface pellicle	Turbid, no surface pellicle	Turbid, no surface pellicle
Peptone (Witte) and salt solution, 7 days at 37° C.	Traces of indol reaction	Marked indol reaction	Traces of indol reaction	Traces of indol reaction
Glucose and lactose-gelatine (shake)	Marked gas formation	Marked gas formation	Marked gas formation	Marked gas formation
Potato	Yellowish-white, rather dry growth	Thick yellowish-white growth	Thick yellow growth	Thick yellowish-brown growth
Milk (37° C.)	Coagulated in 24 hours	Coagulated in 24 hours	Coagulated in 48 hours	Unchanged after 19 days
Litmus-whey, 7 days at 37° C.	Acidity = 32·3 per cent. $\frac{N}{10}$ alkali	Acidity = 30 per cent. $\frac{N}{10}$ alkali	Acidity = 30 per cent. $\frac{N}{10}$ alkali	Neutral after 19 days
Proskauer and Capaldi, No. I., 24 hours at 37° C.	Strongly acid	Strongly acid	Strongly acid	Growth, but no change in reaction
Ditto, No. II., 24 hours at 37° C.	Neutral	Faintly acid	Neutral	Growth, but no change in reaction
Gelatine plates	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic	Colonies grow rapidly, characteristic
Motility (24 hours in broth at 37° C.)	Not motile	Not motile	Not motile	Highly motile
Stained by Gram's method	No	No	No	No

It will be seen that culture G* agrees with the other members of the Table in most of the tests and must be pronounced an undoubted *B. typhosus.*

The cultural characteristics of the organisms (62), (63), (64), (65), (66), (67), (68), (69) and (70), were then carefully studied. It was thought that as most of them came within the "typhoid range" as regards agglutination, they might show an approximation to the cultural reactions of the *B. typhosus.* Table E gives the results obtained and shows that these cultures, with the exception of No. 70, presented all the chief typical reactions of *B. coli.* Cultures No. 62 and No. 66 only varied from the type by failing to produce indol. Culture No. 70, which was not agglutinated by the anti-typhoid serum, showed, however, considerable aberrations from the type; it was highly motile, did not produce acid in litmus-whey nor the characteristic reactions in Proskauer and Capaldi's media.

Forty-five other cultures (shown in Table A) were also examined as to the production of indol in peptone-and-salt solutions, acid in litmus-whey, souring of milk, and gas-formation in glucose-media. Twelve of these cultures failed to produce indol, and sour milk, and the amount of acid formed in litmus-whey was small, requiring only from 8 to 16 per cent. of decinormal alkali to neutralise it; the colonies, however, were typical and gas was produced in sugar-media. As regards the three tests usually considered typical of *B. coli*, viz., the production of indol, the formation of gas in sugar-media and the souring of milk, 64 per cent. of the colonies isolated gave all three reactions, 4·5 per cent. failed to produce indol, 4·5 per cent. failed to sour milk, and 27 per cent. gave only one reaction, viz., the production of gas in sugar-media, and were specially characterised by the small amount of acid formed in litmus-whey.

The results obtained show that *B. coli* from typhoid stools may be agglutinated by a highly dilute anti-typhoid serum, but this reaction is not necessarily nor usually associated with an approximation to the cultural characteristics of *B. typhosus.* The cultures which, according to their growths on the various media, approached most nearly to the *B. typhosus* were not agglutinated by a dilute anti-typhoid serum. On the other hand, culture G* shows that it is also possible for the *B. typhosus* to be present and yet show, when first isolated, no reaction to the specific serum.

Seventy colonies derived from the stools of healthy men were next investigated as to their cultural characteristics and reactions to anti-typhoid serum. The same procedure was followed as before.

As regards agglutination with anti-typhoid serum, Table F shows that not one of the varieties of *B. coli* derived from healthy stools was agglutinated by the serum in a dilution of 1—500, traces were seen but this reaction was so slight as to have no practical value. A complete reaction was only obtained twice with the serum diluted 1—100, and a marked reaction ten times with a dilution of 1—200. An anti-typhoid horse-serum certainly acts more strongly on *B. coli* than a normal horse-serum, but the same thing happens with water-organisms such as the *B. fluorescens liquefasciens* and *B. fluorescens putidus*. None of the anti-typhoid sera that I have examined when diluted 1—500 have ever completely agglutinated these organisms, no matter whether the experiment was performed in a capillary tube or a hanging-drop. Beco has isolated varieties of *B. coli*, from healthy stools, and *B. fluorescens liquefasciens* which were completely agglutinated by an anti-typhoid serum diluted 1—10,000. I have never obtained these results, though in one case I worked with a serum which when diluted 1—2,000,000, completely agglutinated the stock *B. typhosus* (13 P).

TABLE F.

Varieties of B. coli isolated from normal stools.

	1—50	1—100	1—200	1—500	1—1000
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	+	+	–	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0
16	0	0	0	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	+	+	–	0	0
24	0	0	0	0	0
25	0	0	0	0	0
26	0	0	0	0	0
27	0	0	0	0	0

TABLE F. (*cont.*)

	1—50	1—100	1—200	1—500	1—1000
28	0	0	0	0	0
29	0	0	0	0	0
30	+	+	–	0	0
31	0	0	0	0	0
32	0	0	0	0	0
33	0	0	0	0	0
34	0	0	0	0	0
35	0	0	0	0	0
36	0	0	0	0	0
37	0	0	0	0	0
38	0	0	0	0	0
39	0	0	0	0	0
40	0	0	0	0	0
41	0	0	0	0	0
42	+	±	0	0	0
43	+	±	±	–	0
44	0	0	0	0	0
45	0	0	0	0	0
46	+	±	±	–	0
47	+	±	±	–	0
48	+	±	±	–	0
49	0	0	0	0	0
50	0	0	0	0	0
51	0	0	0	0	0
52	±	–	0	0	0
53	0	0	0	0	0
54	0	0	0	0	0
55	0	0	0	0	0
56	±	±	±	–	0
57	0	0	0	0	0
58	±	±	±	–	0
59	±	±	±	–	0
60	0	0	0	0	0
61	±	±	±	–	0
62	±	±	–	0	0
63	±	±	±	–	0
64	–	0	0	0	0
65	±	±	±	–	0
66	–	0	0	0	0
67	0	0	0	0	0
68	±	–	0	0	0
69	0	0	0	0	0
70	0	0	0	0	0

The cultural characteristics of the varieties of *B. coli* isolated from normal stools were briefly as follows:—48 per cent. gave the three typical reactions, 52 per cent. gave only two reactions, usually the souring of milk was absent. The acidity produced in litmus-whey varied from 27 to 47 per cent. of decinormal alkali. There were no constant characteristics by which the varieties of *B. coli* which reacted to the specific serum could be distinguished from those which showed no agglutination.

Conclusions.

(1) As regards the cultural characteristics on the various media employed, there appear to be no types of *B. coli* in typhoid stools which display sufficiently constant characters to enable them to be distinguished from the varieties of *B. coli* found in normal stools.

(2) As regards reaction to anti-typhoid horse-serum, the varieties of *B. coli* isolated from typhoid stools show much greater sensibility to agglutination than the varieties of *B. coli* isolated from healthy stools. Consequently, if varieties of *B. coli* isolated from a water-supply are found to be agglutinated with anti-typhoid horse-serum diluted 1—500, it would appear that there are reasonable grounds for the assumption that the water-supply in question has been fouled with the specific dejecta from cases of enteric fever.