THE TYPE OF COLIFORM BACILLI PREVALENT IN URINE AND THEIR SIGNIFICANCE, WITH SPECIAL REFERENCE TO THE SANITARY ASPECTS.

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For many years past, the classification of the colon-aerogenes group of bacteria, and the sanitary significance of their presence outside the body has engaged the attention of a large number of bacteriologists. The work undertaken and the conclusions arising therefrom are now too well known to require further description: it is sufficient to state that the investigations have been concerned largely with the types of coliform bacteria met with in faeces, sewage soil and water. On the basis of the different tests employed, it has been found convenient to classify the bacteria broadly into "excretal" and "non-excretal" varieties from a sanitary standpoint, according as to whether they are normally more prominent in excreta or outside the body. It is, however, remarkable that with a few exceptions these studies have not included coliform bacteria isolated from urine. The latter have indeed been studied extensively and with great detail by several workers (Dudgeon *et al.*, 1922; Hill *et al.*, 1929), but their researches have dealt almost entirely with the clinical applications.

It would appear that for a sanitary study of the colon-aerogenes group to be complete an examination of their incidence in urine is essential. So long as this is omitted, the classification into "excretal" and "non-excretal" varieties is inaccurate, and should more properly be described as "faecal" and "non-faecal"—a very different matter when one considers the potential dangers of urine infected with pathogenic intestinal bacilli. Once a group of bacteria have been proved to be "non-excretal," it may be taken that their presence is not a sign of excretal contamination. If, however, they are only shown to be "non-faecal," the possibility is still present that urine teeming with typhoid bacilli may have been a recent source of pollution. It may be argued that so far as the bacteriological examination of water supplies is concerned this danger is ever present no matter what criterion is employed, since there is no specific bacterial indicator of urine, and water showing no coliform bacteria whatever might still be contaminated with typhoid bacilli of urinary origin. This argument cannot, however, be regarded as unassailable until the premisses have been proved correct, for if it can be shown that certain bacteria are typical or at any rate reasonably suggestive of urinary contamination, the classification of coliform bacteria into "excretal" and "non-excretal" must undergo certain modification.

Thus the study of coliform bacteria isolated from urine would seem to have some value from the point of view of tracing the fate of *B. coli* after it has left the intestine. Certain workers have shown that the characters of *B. coli* isolated from urine are not those of faecal *B. coli*, nor again those of *B. coli* occurring in soil. Hill and his co-workers (1929), in a study of Gram-negative bacilli isolated from urological infections, found that 44·1 per cent. of 179 true Colon group infections were due to strains of the *aerobacter* variety. The present writer (1932) employing the indol, methyl-red and citrate tests obtained the following results:

Source	No.	Percentage			
		Indol	Coli	Aerogenes	Intermediate
Faeces	278	84	96	1	2
Urine	130	34	29	63	7
Cesspits	130	78	47	33	4
Soil unpolluted	40	8	—	100	—
„ remotely polluted	24	42	4	67	29
„ recently polluted	36	90	81	11	8
Water unpolluted	204	17	—	87	13
„ polluted	6	83	83	17	—

and in a chosen series of well-waters obtained the following percentage results:

Sanitary opinion	Strains	Indol	Coli	Aerogenes	Intermediate
Good	409	25	12	67	14
Suspicious	315	41	20	56	15
Bad	345	63	46	35	13

The atypical results, *i.e.* MR negative-citrate negative, are not included in the tables.

It is apparent that the strains from urine and cesspit showed less "faecal" characters than those from faeces, whilst those from unpolluted soil and water were predominantly non-faecal in character. In other words, the farther removed from the normal habitat the less obvious were the faecal characters of the strains. This would seem to suggest that in the urinary strains we have a method of studying the biological behaviour of extra-intestinal coliform bacteria in a manner free from the difficulties which surround a similar study applied to these bacteria as they occur in the soil and water. It is almost impossible to say that any sample of natural soil, vegetation or surface water is free from all possibility of faecal pollution. It is possible with a considerable degree of certainty to say that *B. coli* isolated from a sample of urine under aseptic conditions has gained access to the urine from the faeces without leaving the body, whatever the route followed. Thus possible sources of error are restricted at least as regards the ultimate origin of the bacteria. These considerations give rise to a number of questions requiring answers based on experimental evidence and which may be briefly summarised thus:

(1) Do the coliform bacteria predominant in urine commonly show typical "faecal" characters, as judged by recognised tests, *e.g.* indol, methyl-red and citrate?

(2) If the "non-faecal" types prevail, is this to be regarded as an ascendancy

of the few non-typical strains normally present in faeces or as an environmental change in typical "faecal" *B. coli*?

(3) What bearing would the finding of "non-faecal" strains in urine have upon the sanitary classification of coliform bacteria into "excretal" and "non-excretal" varieties?

Hill and his colleagues (1929) in their important paper suggested that the predominance of the *aerobacter* strains in urine was due either to a response to some selective action in the genito-urinary tract or else to the more resistant *aerobacter* bacteria normally present in small numbers in the intestine outnumbering the *Escherichia* type when transplanted into surroundings unfavourable to the latter. The present writer (1932) suggested that in urine a transition from the typical "faecal" *B. coli* occurred as a metabolic necessity when they were divorced from their normal habitat, and that this would help to explain the even less typical *B. coli* found extra-corporeally. If it can be shown that the "non-faecal," or "aerogenes" types constantly predominate in infected urine, then their significance as indicators of the absence of faecal pollution must undergo revision. It must be admitted that grounds for such modification are already forthcoming. Skinner and Brudnoy (1932) have recently shown that citrate utilisation and cellobiose fermentation, upon which the classifications are so frequently based, are not altogether reliable for distinguishing coliform bacteria in water. With a doubt thrown on the value of the tests, any classifications based upon such tests must be regarded with some suspicion.

Experimental.

The experimental work consisted in a study of a number of strains of coliform bacilli isolated from specimens of urine, and tested by the indol, methyl-red and citrate tests. In order to conform with the investigations previously reported (1932) McConkey's grouping on the basis of saccharose and dulcite fermentation was also employed. Little information of any sanitary value was, however, obtained by the latter means. For the purposes of this investigation, 1000 strains of coliform bacteria isolated from 126 specimens of urine were examined. Care was taken to exclude outside contamination. Conditions of race, sex and clinical features were studied, but as no information of value to the primary purposes of the investigation was obtained, these are not recorded here. For comparative purposes a further 500 strains from 55 samples of human faeces were also examined. The method of expressing results was that employed in a previous communication (1932).

Results.

500 *strains from faeces. Percentage of total strains found in each group.*

McConkey's groups				Indol	Methyl-red-citrate			
1	2	3	4		Coli	Aero-genes	Inter-mediate	Atypical
3	–	70	27	94	87	8	4	1

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It will be noted that the vast majority of stains corresponded to the *B. coli communior* group. Not one strain of *B. coli communis* was isolated. This striking rarity of *B. coli communis* in the tropics was referred to in a previous paper and has long since been discussed by Castellani (1910) in Ceylon and Clemesha (1912) in India.

As judged by the methyl-red and citrate tests, the number of *aerogenes* strains was 8 per cent., *i.e.* rather higher than in previous experiments. The *coli* type was, however, overwhelmingly predominant and the majority of strains produced Indol.

1000 *strains isolated from urine. Percentage of total strains found in each group.*

McConkey's groups					Methyl-red-citrate			
1	2	3	4	Indol	Coli	Aero-genes	Inter-mediate	Atypical
18	7	27	48	43	33	52	10	5

The *B. lactis aerogenes* type proved the commonest by McConkey's grouping.

By the methyl-red-citrate method the *aerogenes* variety proved to be over 50 per cent. of the whole, whilst the intermediate strains were 10 per cent. Indol formations only took place with 43 per cent. of strains.

The first question is therefore answered in the negative, for it is evident that the predominant strains correspond to those which are commonly adjudged as "non-faecal." As regards the second question, the writer is impelled to recant from his original opinion that these strains represented variants of faecal *B. coli* as a result of unfavourable environmental change. It is difficult to conceive of such a rapid change of characters taking place, and the alternative explanation of an outnumbering of the "faecal *B. coli*" by the *aerogenes* type is a more acceptable conclusion. If then it is accepted that, owing to unfavourable conditions, the *aerogenes* type outnumbers the *coli* type outside the intestine and yet inside the body, it might be reasonable to regard a preponderance of *aerogenes* outside the body as being a completion of the same process. In other words the farther the bacteria are removed from their faecal habitat, the less obvious their faecal characters. This would seem to imply that the change is less one of time than of environment. One is therefore inclined to doubt whether the interpretation of the *aerogenes* type as being indicative of "remote" pollution is wholly justifiable, since no characters can justly be regarded as suggesting remote excretal pollution which are found to apply to a large group of bacteria present in the body. The most therefore that can be said is that if the *aerogenes* group predominate at the expense of the *coli* group both in the urine and outside the body, the finding of them in soil or water would probably suggest that the majority of the *coli* type had died off, even though the pollution was recent. By inference one might fairly conclude that the equally delicate pathogenic intestinal bacteria had perished also. As Gray (1932) has pointed out, the important point to be considered is the preponderance rather than the complete absence of the various groups, for the distribution of both *coli* and *aerogenes* in Nature is variable and wide.

As regards our third question, it is evident that there is no specific difference between the characters of the *aerogenes* and intermediates of urine and those found in soil and water, on the basis of the tests used. The finding of these bacteria as the principal coliform strains in urine may therefore be judged primarily as additional evidence in favour of the susceptibility of faecal *B. coli* to environmental change, and secondly as evidence that the so-called "non-excretal" *aerogenes* types are not confined to an extracorporeal habitat, though they predominate in an extra-intestinal habitat. What we are concerned with, however, from a sanitary standpoint is not so much the type of coliform bacteria predominant in the intestine, for few will dispute the position of true *B. coli* in this respect whatever tests are employed, but rather the type predominating after the intestinal habitat has been lost. Thus we return to Clemesha's original contention that the most delicate group, the group that dies most rapidly when exposed to external conditions is the surest indicator of recent or at any rate dangerous faecal pollution when it is found in any considerable numbers in a water supply. Unfortunately the commonest duty of the water bacteriologist is to pronounce upon the absence of pollution, not to detect it, and in this respect the known tests appear to be unreliable. It would appear that such tests as those described are of most value when they identify the typical "faecal" *B. coli*, and that their value is least in determining the significance of the atypical strains. It may probably be assumed that the farther removed from the typical faecal characters, the less does the strain suggest dangerous pollution. What it does signify in these circumstances, and whether it may be taken to represent remoteness of pollution seems more difficult to decide. In conclusion it may be asserted that by the employment of the combined methyl-red, citrate and indol tests we have a useful method of deciding whether dangerous faecal pollution is present in a water sample: we are on less certain ground when attempting to pronounce that it is not.

Summary and conclusions.

1. 1000 urinary and 500 faecal cultures of coliform bacteria were studied by means of the methyl-red, citrate and indol tests and also by their fermentation reactions upon saccharose and dulcite.

2. 8 per cent. of the faecal strains and 52 per cent. of the urinary strains were of the *aerogenes* type, *i.e.* methyl-red negative and citrate positive. Indol was produced in 94 per cent. and 43 per cent. of cases respectively.

3. This would suggest additional evidence that the *coli* group are quickly outnumbered by the *aerogenes* group once they leave the faeces, and also that the presence of the *aerogenes* type is not confined even in large numbers to an extra-corporeal habitat.

4. In water bacteriology the constancy or change in the relative proportions of *coli* and *aerogenes* strains present is the goal at which to aim. The *aerogenes* variety cannot of itself be regarded as non-excretal or even non-faecal.

5. From a sanitary standpoint, the classification of coliform bacteria by means of the methyl-red and citrate tests cannot be regarded as entirely free from error so long as (*a*) the *aerogenes* strains are found in faeces, in however small numbers, (*b*) these strains occur as the predominant type in urine, and (*c*) intermediate strains are found in the faeces, urine, soil and water.

6. The principal value of these tests lies in their comparative specificity in identifying *B. coli* of immediate faecal origin. The presence of such an organism, interpreted together with the indol test, may be said to suggest dangerous faecal pollution if found prominently in a water sample. The presence of other types identified by the same tests cannot be regarded as having the same significant value in regard to a negative opinion.

7. A plea is made for a further study of coliform bacteria by means of the tests described in relation to the biological behaviour of the bacteria under different environmental conditions.

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