

THE ECOLOGY AND SIGNIFICANCE OF THE DIFFERENT TYPES OF COLIFORM BACTERIA FOUND IN WATER

A REVIEW OF THE LITERATURE

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INTRODUCTION

In the examination of water for public supply there is a growing demand for the separation of the coliform group of bacteria into the *Bacterium coli* (the so-called faecal *Bact. coli*) and *Bacterium aerogenes* groups. The reason for the need for this distinction is that *Bact. coli* is recognized as the predominant type of coliform in human and animal faeces, while it is believed that *Bact. aerogenes* and intermediate types are the most common types in soils, grains, grasses, and decaying organic matter. Some authorities claim that *Bact. aerogenes* types are indigenous to those habitats. Thus Topley & Wilson (1931) state, 'and there seems equally little doubt that the *Bact. aerogenes*-*Bact. cloacae* group, so demarcated, consists of bacilli which live normally on plants or in the soil, and not in the intestines of man or animals', and, referring to *Bact. aerogenes*, 'This species is not a normal inhabitant of the intestine, but occurs on plants or grains, or in the soil.' It thus seems that many bacteriologists have come to regard *Bact. aerogenes* types as indicative of pollution from soil or plants and not from animal or human sources. *Bact. aerogenes* types are also considered to be indicators of 'remote' animal or human pollution by others who do not take such a rigid view, because some experiments on stored faecal material have shown that these types are present in relatively small proportions in faeces, and are more resistant and tend to outlive *Bact. coli*. The ambiguity of the term 'remote' will be discussed later.

In Britain the work of Wilson, Twigg, Wright, Hendry, Cowell & Maier

(1935) on milk has encouraged the separation of *Bact. coli* from *Bact. aerogenes* and intermediates by the incubation of inoculated MacConkey broth medium at 44° C., a temperature that has been found to be fairly specific for *Bact. coli*, in the test for the production of acid and gas. This method has the advantage of providing results in a shorter time than by the usual technique. The adoption of the method involving incubation at 44° C. without any reference to the usual method of incubation at 37° C. means the complete disregard of *Bact. aerogenes* and intermediate types.

In America and the tropics (Hajna & Perry, 1939; Raghavachari & Iyer, 1939) it has been found that the test with incubation at 44° C. is not so specific for the growth of *Bact. coli*, and that so many strains of *Bact. aerogenes* grow at that temperature as to make the test of little value. In general, the trend of experimental work in America is towards the development of media of such composition as to restrict the growth of bacteria other than coliforms. The American Public Health Association and the American Water Works Association (1936) are still cautious in evaluating the results of tests for *Bact. coli* and *Bact. aerogenes* in water, for they state: 'At the present time, any attempt to evaluate a drinking water on the basis of a distinction between these two types is regarded as unwarranted.'

The question therefore arises, on the evidence available, whether it is justifiable in this country to accept the statement that *Bact. aerogenes* is indigenous to soils, grasses, and grains, but not to faeces.

During an investigation of the types of coliforms in the waters of different lakes (Taylor, 1941) it was found that, contrary to expectation, the more polluted of the lakes examined gave a greater ratio for the numbers of *Bact. aerogenes* to *Bact. coli* types. This result was surprising in view of the fact that the drainage areas of the different lakes were of much the same character, the only significant differences being the degree of pollution by domestic sewage. In an attempt to explain this finding, the original papers dealing with the ecology of coliform bacteria have been studied. It is considered that a review of these papers is worth publishing, especially as the results presented in some of them appear to have been often misquoted. It is not intended to review the whole of the voluminous literature on this subject, for much of the older and pioneer work involved methods which are not comparable with those of to-day. Nor is it intended to duplicate the excellent review on coliform bacteria recently written by Parr (1939), but rather to study in more detail the results of the work on the ecology of the coliform bacteria and the technique employed.

THE METHODS ADOPTED BY VARIOUS WORKERS

The two main methods which have been used for the isolation of coliform bacteria from samples involve either preliminary enrichment or direct plating. The technique of enrichment has been invariably the addition of the material to a lactose broth, with or without bile salts, and it has usually, though not

always, been recognized that this enrichment might seriously disturb the balance of numbers in each of the original groups, and that consequently one group might overgrow the other in the culture medium. The lack of knowledge on the survival of mixed-cultures in liquid media is still evident by the contradictory statements in the literature. Hajna & Perry (1939) and Pawan (1931) state that *Bact. aerogenes* overgrows *Bact. coli*. On the other hand, Wilson *et al.* (1935) give figures to show that *Bact. coli* will overgrow *Bact. aerogenes* in milk. Twenty-four hours after inoculation of equal numbers of the two organisms into sterile milk they found that the ratio of numbers of *Bact. coli* to *Bact. aerogenes* was between 10 and 100.

Following the advent of the citrate test some workers have used primary enrichment in citrate medium in addition to a separate enrichment in a lactose broth, thus obviating overgrowth. From citrate tubes showing growth and from lactose broth tubes showing the production of acid and gas, smears were made on the surface of solid medium, usually Endo or MacConkey medium. After incubation, various numbers of colonies were picked off and slopes of beef-peptone agar were inoculated.

The method of direct plating has involved either smearing a suspension of the material over the surface of solid medium in a Petri dish or plating out definite volumes of suspensions of known strength with the object of obtaining quantitative results. After incubation, colonies were picked off and slopes inoculated.

The methods used for differentiation of types have been developed with the work on the different biochemical activities of the various types of coliforms. Following the work of Houston (1913) the classification was mostly based on the production of indol and the fermentation of sugars, and the lactose-positive, indol-positive organism was for some years the indicator of faecal pollution. Gradually the inclusion of the methyl red (M.R.), Voges-Proskauer (V.P.), and citrate differential tests have followed. Unfortunately, few workers until recent years have classified the organisms on the basis of the four tests, others merely listing the numbers as proportions of cultures positive or negative to each separate test; it is possible, however, in some instances to make some differentiation from the data given.

On the basis of the four most commonly used tests, indol production, and the M.R., V.P., and citrate tests, it is possible to divide the coliform organisms into three main groups (Wilson *et al.* 1935), the *Bact. coli* group, the *Bact. aerogenes* group, and the intermediates. Table 1 differentiates these groups expressed on their 'Imvic' characters (a mnemonic giving the reactions in the following order: indol, M.R., V.P., and citrate). The table includes only the main types considered in this review, though with these four tests sixteen types are theoretically possible, and further subdivision can be made almost indefinitely by increasing the number of tests. A number of types showing shifting reactions or delayed gas formation have been classified by various

the evidence so far available their ecology and sanitary significance are not yet clear.

Table 1. *The subdivision of the coliform organisms on the basis of their Imvic reactions*

	Indol	M.R.	V.P.	Citrate
<i>Bact. coli</i> group:				
Type I	+	+	-	-
Type II	-	+	-	-
Intermediate group:				
Type I	-	+	-	+
Type II	+	+	-	+
<i>Bact. aerogenes</i> group:				
Type I	-	-	+	+
Type II*	+	-	+	+

* This type is considered by Parr (1938) to be an intermediate.

On this grouping most bacteriologists now recognize faecal *Bact. coli* as a ++-- organism. It can be seen that the lactose-positive indol-positive organism of Houston would have included not only the present *Bact. coli* type I but also intermediate type II, and *Bact. aerogenes* type II.

FAECES

In view of the extent of the work on the coliform bacteria in faeces it is disappointing to find that very few quantitative examinations have been made. There are many reports giving characteristics of several hundred organisms, but without any data on the number per gram of faeces. Houston, Clemesha, MacConkey, and more recently Pawan (1931) studied the lactose-positive organisms isolated from human and bovine faeces and classified them according to their motility and reactions with sugars, thus subdividing them into *Bact. coli communis*, *Bact. coli mutabilis*, *Bact. grunthal*, *Bact. acidi lactici*, *Bact. coscoroba*, *Bact. schafferi*, etc. This method has been almost entirely abandoned, and all ++-- organisms are placed in one class irrespective of their reactions with sugars. All investigators are agreed that *Bact. coli* type I (++) is by far the most abundant type in faeces of man and other animals including birds, and that the proportion of the different types is less certain. Hill *et al.* (1929) summarized the results of many different workers and showed that the numbers of *Bact. aerogenes* and intermediates (M.R. - V.P. +) reported, varied from zero to 16.0% of the total. In general most of these figures are only approximations, as either the lactose-enrichment or direct-plating methods were employed.

Burke-Gaffney (1933) gave the results of an investigation, on the basis of the M.R. and citrate reactions, of 500 cultures isolated from human faeces and showed that 87% were *Bact. coli* types, 8% *Bact. aerogenes*, and 4% intermediates (?+ - +). Gray (1932) plated suspensions of faeces directly on to MacConkey and citrate agars and isolated five colonies from each plate. Of the 200 cultures isolated from MacConkey agar all proved to be of the

Bact. coli type (M.R. + V.P. -), but by the citrate method thirty-seven of the forty samples of faeces examined were found to contain *Bact. aerogenes* (M.R. - V.P. +), the three negative samples being all from the same individual. No quantitative results are given. It is mentioned that 'occasional faecal strains of *Bact. coli* were definitely citrate positive', an indication that these were intermediate type II. Ruchhoft, Kallas & Coulter (1931) examined thirty-two samples of faeces from eleven persons and ten animals; no quantitative results were obtained. After purification of the original cultures it was found that only four types were represented: *Bact. coli* type I, 83.2%; *Bact. coli* type II, 11.5%; intermediate type I, 2.0%; and *Bact. aerogenes* type I, 3.3%. The presence of intermediates in faeces is thus confirmed. Bardsley (1934) also found *Bact. aerogenes* type I and intermediates in relatively small numbers in faeces. With this point in mind the author concluded that the sanitary significance of these types could not be ignored, and that, as far as water examination in Great Britain is concerned, the indol, M.R., V.P., citrate, and other differential tests might perhaps be discarded. Bardsley (1938), by the use of primary incubation in MacConkey broth with subsequent inoculation from positive tubes into citrate media, found intermediates or *Bact. aerogenes* in sixty-one of 100 samples of human faeces examined. In eight cases the numbers were equal to or greater than those of *Bact. coli*.

A more intensive survey has recently been carried out by Reedy & Puncocar (1940), who determined the ratio of *Bact. coli* to citrate utilizers in 253 samples of faeces from 253 adult women. The faecal samples were diluted and duplicate plates were poured from several dilutions, using citrate and eosin methylene-blue agars. *Bact. aerogenes* or intermediates were found in 87% of the samples. The overall average ratio of citrate utilizers to *Bact. coli* is not given, but it was found that the most common ratio was 1:500, which occurred ninety times. In 166 samples *Bact. aerogenes* types, together with intermediates, were present in numbers of at least 10,000 per g. In an examination of bovine faeces Wilson *et al.* (1935) found that 98.4% of the bacteria isolated were *Bact. coli* types I and II, and only 1.6% could be classified as *Bact. aerogenes*.

STORED FAECES

The relative incidence of different types of coliform bacteria in stored faeces is of considerable importance, as seepage and overflow from cesspits, septic tanks, and sedimentation tanks are frequent sources of pollution of waters. It seems that considerable changes occur in the proportions of the types during the period in the tanks.

Clemesha (1912) exposed emulsions of faeces to the rays of the sun in India and found that certain types disappeared very rapidly and that others were more resistant; this led him to believe that the sun's rays were largely responsible for the rapid purification of stored waters in the tropics. Other investigations seemed to confirm these results, but recently Raghavachari & Iyer (1939), who made similar tests in India, did not reach the same conclusions.

Pawan (1931) also exposed suspensions of faeces to tropical sunshine. In two preliminary experiments it was found that suspensions of faeces which originally contained coliform bacteria in 0.01 and in 0.001 ml. gave negative results in 20 ml. after periods of exposure of 3 days and 26 hr. respectively. There seems to be no certainty that the diminution did not continue in the dark as well as in the sunlight. The more detailed experiments gave astonishing results; in one, after a period of exposure of $2\frac{1}{2}$ hr., all the organisms isolated were indol and V.P. negatives classified as *Bact. coscoroba* or *Bact. grüenthal*, but after a further hour all cultures were indol-positive and V.P.-negative. In other tests V.P.-positive, indol-negative coliform bacteria appeared after $1\frac{1}{2}$ – $2\frac{1}{2}$ hr., and after 5 hr. all except one of the cultures gave those reactions. After $5\frac{3}{4}$ hr. there were no lactose fermenters in 10 ml. of the suspension, a remarkably rapid purification. In general, *Bact. coli communis* (sucrose-negative, salicin-positive motile organism) disappeared in 1–2 hr., and at the end of the experiments no indol- and M.R.-positive coliform bacteria were isolated. There was no evidence to suggest that any multiplication took place, but rather that there was a rapid mortality of all types, some being more resistant than others. It should be mentioned that the temperature of the exposed suspensions varied between 27 and 31° C. The work of Browne (1915) did not confirm these results. Suspensions of human faeces were placed in duplicate sets of bottles; one set was incubated in the dark and the other on a bench in the window. The temperature of the room was approximately 20° C., that is, considerably lower than in the tests by the workers in the tropics. After a period of over 70 days there was no diminution in numbers of *Bact. coli communior* but rather a slight increase; and the decrease in *Bact. coli communis* (sucrose-positive, salicin-negative motile organism) was very gradual and slow. The presence or absence of light had no measurable effect on the ratio of numbers of the different groups.

Jordan (1926) studied the changes in numbers of bacteria in human faeces, during storage, by plate counts on beef-peptone agar and counts of coliform bacteria. Very large increases of colony and coliform counts took place both at room temperature and at 37° C. At 37° C. the increase in coliform bacteria was very rapid and reached a maximum after 24–48 hr., when the numbers were 500 times those at the beginning of the experiment. At room temperature the maximum occurred after 3 days, but at 10° C. multiplication was much slower, taking between 14 and 21 days to reach a maximum which was not so great as that obtained at the higher temperatures. In general the results showed that the higher temperatures favoured a more rapid multiplication to greater numbers followed by a sharper decline. Experiments on the longevity of coliform bacteria in faeces stored under soil or sand showed that after periods of between 8 and 19 weeks no coliform bacteria were present in the amount of sample taken, 0.01 g. In all the work no differentiation of the coliform types was made, and it is not possible to decide, therefore, whether multiplication and subsequent disappearance were confined to certain types.

Burke-Gaffney (1932) isolated a number of coliform bacteria from samples from cesspits and grouped them into *Bact. coli*, *Bact. aerogenes*, and intermediates on their M.R., V.P., and citrate reactions and found that the proportions were 47, 33, and 4% respectively. From the large percentage of the cultures which produced indol (78) it is probable that many of the *Bact. aerogenes* cultures were type II (+ - + +).

Gray (1932) inoculated 4 l. of Liverpool tap water with a spoonful of normal human faeces and determined the relative proportions of *Bact. coli*, *Bact. aerogenes*, and intermediates on the basis of the M.R., V.P., and citrate tests. It was found that the ratio of *Bact. aerogenes* to *Bact. coli* increased from an initial value of 0.5 at the time the faeces were added, to 3.3 after an incubation period of 56 days. A similar experiment on stored tap water without the addition of any faeces gave like results, and Gray was led to conclude that the presence of *Bact. aerogenes* only in water, without *Bact. coli*, was characteristic either of high sanitary quality or of remote pollution.

Parr (1936) studied the changes occurring in stored faeces and made a detailed examination of over 4000 cultures (1937, 1938). These three papers give valuable and detailed results; much of the previous work of other investigators was confirmed. It was found that the proportions of the different types of coliform bacteria in the faeces of the same individual could vary considerably from day to day. No coliform bacteria were found in the faeces of one subject on two successive occasions, but on the third occasion three of the MacConkey types were isolated. The faeces of another subject were found on one occasion to contain only intermediate I. The proportion of intermediates (Parr includes *Bact. aerogenes* type II in the intermediate group) was 7.7% in fresh faeces, 15.3% in faeces stored at 37° C., and 27.9% after storage at ice-box temperature. The most prevalent type of intermediate was intermediate I (- + - +) in all cases; types + + - +, + + + + and + - + + were present in approximately equal numbers. Parr (1936) found that faeces suspended in normal saline solution and incubated in the ice-box or at 37° C. showed marked changes in the coliform flora; at first *Bact. coli* were predominant, but after a few weeks a type of *Bact. aerogenes*, which produced little or no fermentation with lactose, became dominant. These results appear to confirm results obtained by Clemesha (1912), Pawan (1931) and Burke-Gaffney (1932).

URINE

The coliform bacteria in urine have received a considerable amount of attention from the medical profession, but in much of the earlier work no differentiation was made, and the cultures were called *Bact. coli* merely on the basis of their ability to ferment lactose. Dudgeon, Wordley & Bawtree (1922) reported that two types of coliform bacteria, haemolytic and non-haemolytic, occurred in urinary infections; they found no evidence of a direct relationship between the urinary haemolytic types and those of the intestinal tract. Hill, Seidman, Stadnichenko & Ellis (1929), who gave an excellent

summary of the previous literature, examined 200 cultures of Gram-negative bacteria isolated from 200 cases of genito-urinary infection. Of these Gram-negative cultures, 100 were stated to be *Bact. coli* on their M.R.-V.P. reactions, but from other data in the paper it can be seen that only 54% of these were true *Bact. coli* types, and the remainder were either intermediate type I or type II. Of the seventy *Bact. aerogenes* cultures more than 90% were type I and less than 10% type II. The rest of the cultures were *Proteus* and miscellaneous types. On the basis of the 177 cultures the proportions were *Bact. coli* types 30%, intermediate types 26%, and *Bact. aerogenes* types 44%.

The prevalence of *Bact. aerogenes* types in urine was noted by Burke-Gaffney (1932), who found that such types constituted 79% of the total number isolated from the urine of tropical subjects but only 8% of the total number from Europeans. This work was continued in more detail (Burke-Gaffney, 1933) and 1000 cultures were isolated from samples of urine obtained in the tropics. It was found that 53% of the cultures were *Bact. aerogenes* types, and only 33% *Bact. coli*. This led the author to point out the subtle difference that exists between the terms 'faecal' and 'excretal'.

No reference has been found to the absolute numbers of coliform bacteria per unit volume that may be found in the urine of cases of genito-urinary infections.

GRASSES, GRAINS, AND DECAYING ORGANIC MATTER

It has been known for many years that all green plants possess an epiphytic microflora which exists on the small amounts of carbohydrate, protein, and inorganic salts which are dissolved in the liquid exuding from the host. This microflora apparently consists of a few species of short non-spore-forming rods which, however, may be present in large numbers. It was at one time claimed by those who opposed examination for *Bact. coli* as a test for faecal pollution that these types were coliform bacteria, but it was later established that considerable differences existed between these types and true coliform bacteria. Two of the most common types were yellow pigmented organisms, *Pseudomonas trifolii* and *Bacterium coli* var. *luteoliquefaciens*.

One of the most commonly quoted papers on the coliform bacteria present on grains is that by Rogers, Clark & Evans (1915). The authors isolated 152 cultures from an unstated number of samples of dried grains of corn, barley, wheat, and oats obtained from freight cars. From two samples of green oats fourteen cultures were isolated; a third sample proved negative. The method of isolation consisted of implanting a few grains into tubes of dextrose broth and incubating overnight at 30° C. Positive tubes were plated on infusion agar and colonies were isolated in the usual manner. No quantitative results are given. The cultures were classified on the basis of their gas ratios (CO₂:H₂) and on indol production. Of the 166 cultures isolated seven need not be considered as they produced CO₂ only. Eight cultures were of the low gas ratio type, four produced indol and were therefore either *Bact. coli* type I or intermediate type II; the other four were thus either *Bact. coli* type II, or

intermediate type I. Of the remaining 151 cultures, which were of the high gas ratio type (M.R. -), forty were chromogenic, produced a yellow colour on agar, liquefied gelatin, and formed no indol. For the rest no comparative information is available. It is important to emphasize that the authors used an incubation temperature of 30° C. throughout their work.

A study giving more quantitative information was made by Allen & Harrison (1936) on the numbers and types of coliform bacteria in fresh grass and grass silage. Quantities of grass collected from pasture land, both grazed and not grazed, were ground up with saline solution, and 1 ml. amounts of different dilutions were inoculated into duplicate sets of tubes of bile-salt broth. One set was incubated at 30° C. and the other at 37° C. Results showed that from one thousand to one million organisms per gram of grass formed acid and gas at 30° C., numbers being higher in the grasses from the grazed pasture than in grasses from pastures not recently grazed. However, it was significant that of the six samples examined and tested at 37° C. only one showed the presence of coliform bacteria in a dilution of 1:100, the lowest dilution employed. In the case of the silage, fresh grass was packed into twelve experimental silos and separate samples from the top and the bottom of one silo were examined on each of twelve subsequent occasions. Activity was greatest during the first 8 days, and numbers of bacteria capable of fermenting lactose at 30° C. were as many as 10 million per g. of silage. At 37° C., however, numbers rarely exceeded 100 per g., although 10,000 per g. were found on one occasion in the top sample, and on three occasions in the bottom sample. Qualitative results were interesting, as they showed that the predominant type responsible for the fermentation of lactose at 30° C. was a capsulated organism (Imvic reactions - + + +), which very slowly liquefied gelatin and was peculiar in that it gave no growth at 37° C. Eleven strains were isolated from positive tubes of MacConkey broth at 37° C. and the types were three + + - ?, six - + - ? and two - - + ?. In the absence of citrate reactions it can only be deduced that the three cultures were either *Bact. coli* type I or intermediate type II, six cultures were *Bact. coli* type II or intermediate type I and two were probably *Bact. aerogenes* type I (one of these was gelatin-positive and therefore presumably *Bact. cloacae*).

Hunter (1921) studied the microflora of silage prepared from peas, oats, corn, and soya beans. Numbers of coliform bacteria were determined by inoculation of a lactose broth medium with serial dilutions of silage. Incubation was carried out at 37° C. The results were variable. In some instances numbers increased enormously and fluctuated from time to time. In one instance these were 710 million per g. after a period of 8 days, none were found after a period of 3 more days, and 100,000 per g. after a further period of 4 days. In one case no multiplication occurred and numbers decreased steadily. Sterilized pea and oat silage was heavily inoculated with coliform bacteria, but no growth took place and the organism steadily died out.

Wilson *et al.* (1935) examined ninety samples of straw, hay, grass, decaying

leaves, water, swedes, grains, meals, and feeding cakes. No quantitative determinations were attempted. A small amount of the material was inoculated into MacConkey broth, and after incubation for 18 hr. at 37° C. positive tubes were plated on MacConkey agar. Different types of colonies were picked off into broth medium. The results are shown in Table 2.

Table 2. *The distribution of coli-aerogenes strains according to type of foodstuffs examined (after Wilson and co-workers (1935))*

Material examined	No. of samples examined	No. of yielding coli-forms	No. of strains isolated	Percentage distribution of strains				
				Coli I, II and irregulars I, II and III	Inter-mediate I, II and irregular IV	Aerogenes I, II	Cloacae	Irregular VII
Straw, hay, grass, decaying leaves, water, swedes	25	11	23	56	22	9	13	nil
Grains, meals, and feeding cakes	65	36	57	23	5	46	23	3

One of the most interesting points brought out by the table is that 48% of the samples examined did not yield coliform bacteria. With the soil-contaminated materials *Bact. coli* types were the most prevalent, but in the grains, meals, and feeding cakes *Bact. aerogenes-cloacae* types were in the majority comprising some 69% of the total number isolated.

SOILS

Houston (1897-8) was among the first to study the occurrence of coliform bacteria in the soil. In some initial experiments he examined twenty-one soils taken from a variety of sources such as orchard, garden, and pasture. Although as much soil as 0.3 g. was examined coliform bacteria were only found in six samples. The only relevant information given concerning the six cultures isolated is that four produced indol. In two subsequent investigations on soil washings no coliform bacteria were found in eight virgin soils in amounts of 0.01 g. or less, but they were isolated from four out of seven highly polluted soils in amounts representing 0.0001 g. of soil. Chick (1900, 1901) found coliform bacteria in only one of twenty-nine samples of moorland and cultivated soils, but it should be pointed out that only small amounts of soil were tested (0.1 to 0.006 g.) and that a medium containing phenol was used.

Savage (1906) examined fifteen samples of surface soils and seven taken at a depth of 1 ft. The soils were of three classes, virgin, not recently manured, and manured within the previous 1½ years. In the virgin soils and soils not recently manured no excretal *Bact. coli* (lactose- and indol-positive) were found in 0.2 g. The results from the recently manured soils were discordant; in three garden soils manured within the previous 4 months no excretal *Bact. coli* were found in 0.2 g. of the surface layers, but 10-100 per g. were found at a depth

of 1 ft. In a cultivated field manured a year previously 100–1000 per g. were found at the surface and at a depth of 1 ft.

Johnson & Levine (1917) examined samples of soil from cropped and uncropped fields, most of which had been manured within 1 or 2 years. Although no quantitative data were obtained it was found that coliform bacteria were rare or absent in the fallow plots and more abundant in those which were cropped. An analysis of the data given shows that 80% of the total cultures isolated were V.P.-positive, and that one type (— — + ?), which liquefied gelatin, was probably *Bact. cloacae* and was the most prevalent type, making up 41% of the total. As the citrate test was not known at that time no accurate division of the different types is possible.

One of the papers often quoted is that by Chen & Rettger (1920) who examined 317 samples of soil from four localities. Although the authors state that one of the objects of the study was 'To determine the relative frequency of the colon and aerogenes types of bacteria in soils which from all appearances are free from animal pollution', no quantitative results are given, but approximations can be made. Amounts of 1.0, 0.5, 0.1 and 0.01 g. of soil were plated directly on to 'ordinary agar', and all coli-like colonies were isolated and inoculated into tubes of lactose broth. A temperature of 30° C. was employed. From 190 of the 317 soils (60%) no coliform bacteria were isolated, and from the 127 positive soils 467 cultures were isolated. Assuming that this number represents the total number of colonies appearing on plates, and there is no evidence to show that duplicate cultures were discarded, then only the 1.0 g. and occasionally the 0.5 g. amount of soil yielded colonies, and the average number of coliform bacteria must have been in the region of 4–5 per g. of soil. On the basis of the M.R.-V.P. reactions the cultures were grouped into *Bact. coli* and *Bact. aerogenes*. The cultures were assessed on their indol, M.R., V.P., and uric acid reactions, and if it may be taken that a negative correlation existed between the M.R. and V.P. tests and that citric and uric tests are equivalent, the following separation may be made: *Bact. aerogenes* type I formed 65.5% and type II 30%, and the remaining 4.5% were M.R.-positive types, the exact status of which cannot be determined on the available data.

Koser (1924), in testing his citrate method, examined soil samples from wooded hill tops with dense underbrush and from other places where the chance of human pollution was considered to be slight. Isolation of coliform bacteria was first attempted by preparing dilutions and plating direct, but owing to the scarcity of the organisms enrichment methods had to be employed. The procedure consisted of inoculating 75 ml. of lactose broth with 10.0–15.0 g. of soil, incubating at 30° C. and streaking plates of Endo's medium from positive cultures. Despite the large amount of soil taken, 24% of the samples yielded no coliform bacteria, and from 51% only one type was isolated. From the fifty-three positive soils seventy-two cultures were isolated. Of these, 50% were M.R.-negative and were either *Bact. aerogenes* type I or II, 22% were intermediate II, 15% were *Bact. coli* type I, and the remaining 15% gave indefinite M.R.-V.P.

reactions. It was noticed that fourteen of the seventy-two cultures gave a delayed lactose fermentation, the production of acid and gas taking from 3 to 4 days and in some cases as long as 7-8 days; six of these fourteen strains were of intermediate types.

Koser (1926) extended his studies to fifty-two samples of soil from central eastern Illinois. The samples were from cultivated fields under corn, oats, wheat, rye, alfalfa, and clover, and from pasture land. The soils were of a brown silt type and gave approximately neutral reactions. Samples from the cultivated fields were thought to be open only to chance pollution, and though the fertilization history was unknown, no manure had been applied recently. The pasture lands were highly polluted. A quantitative estimation was attempted by inoculating amounts of 10.0, 1.0, and 0.1 g. directly into lactose broth, and suspensions of smaller amounts representing 0.01 and 0.001 g. were likewise inoculated into lactose broth. Eosin methylene-blue agar plates were inoculated from positive tubes and only one culture was isolated from each plate. From Table 3 it can be seen that the results with the soils from the cultivated fields were erratic and that an appreciable number (17%) yielded no coliform bacteria even in 10 g. of soil. From the quantitative aspect there was surprisingly little difference between the cultivated and pasture soils, in view of the difference in degree of pollution.

Table 3. *Numbers of coliform bacteria isolated from different dilutions of samples of soils from pastures and cultivated fields (after Koser, 1926)*

Source	No. of samples	No. of soils yielding no coliform bacteria	Cultures obtained and weight of sample (g.)					Total no. of cultures
			10	1.0	0.1	0.01	0.001	
Cultivated field	41	7	24	43	25	3	9	104
Pasture	11	0	10	8	8	0	7*	33

* All from one sample.

It is significant that Koser found an apparent irregularity in the distribution of the organisms in different samples of soil, even from the same general locality. It was even noticed that from different parts of the same field some samples gave positive results in 10, 1, and 0.1 g., and in other samples no coliform bacteria could be found. The cultures were classified on the basis of their M.R.-V.P. and citrate reactions and the results obtained are shown in Table 4. Compared with the previous work (Koser, 1924) the outstanding

Table 4. *The types and numbers of coliform bacteria obtained from cultivated and pasture soils compared with those obtained from wooded hill tops (after Koser, 1924, 1926)*

Source	<i>Bact. coli</i> types I, II	Intermediates I, II	<i>Bact. aerogenes</i> types I, II	Irregulars
Koser (1926):				
Cultivated	23	8	67	2
Pasture	64	3	33	0
Koser (1924):				
Wooded hill tops	3	22	50	15

difference lies in the prevalence of intermediate types and the scarcity of *Bact. coli* types in the samples least liable to pollution, that is in those from wooded hill tops.

Cunningham & Raghavachari (1926) examined eighty-seven samples of Indian soils taken from the surface and at different depths down to 6 in. The sites from which the samples were taken were all subject to heavy animal and human pollution, the authors stating 'this being the natural state of affairs as far as India is concerned'. No mention is made of the technique employed nor are any quantitative results given. From the eighty-seven soils, 823 cultures were isolated which were classified on their M.R.-V.P. reactions; the relative proportion of M.R.-positive bacteria decreased with depth, whilst the M.R.-negative types showed a corresponding increase.

Raghavachari (1926) examined a further fifty-four samples of Indian soils taken at depths between 3 and 6 in., the surface soil being discarded as it was thought to be too polluted. No methods nor quantitative results are given, but from the fifty-four soils 518 cultures were isolated and their M.R.-V.P. and citrate reactions were tested. It was found that 93.5% were either *Bact. aerogenes* type I or II; 6.5% were M.R.-positive and V.P.-negative, eight of these thirty-four cultures being capable of growth in citrate medium.

Hicks (1927) in Shanghai studied the coliform content of local soils. From several samples taken from hillsides up country only two strains of lactose fermenters were isolated. Further samples were taken from local rifle-range butts, railway embankments, and grave mounds. The method consisted of the enrichment of an unstated amount of soil in lactose or dextrose broth with subsequent incubation at 20 or 37° C. Positive cultures were plated on MacConkey agar, and one culture was isolated from each plate. Though the indol M.R., V.P., and citrate reactions of the fifty cultures isolated were tested, the results are not presented in a comparable form. However, in contrast to the results of Raghavachari (1926), 76% were M.R.-positive and only 20% were negative. As 80% of the total were citrate-positive and only 32% indol-positive, it can be taken that many of the M.R.-positives were not *Bact. coli* but were intermediate I.

Gray (1932) tested six samples of soil from Perthshire, Scotland, which were considered unlikely to have been contaminated with human or domestic animal excreta. Emulsions of the soils were inoculated into MacConkey broth, and *Bact. aerogenes* was isolated from all samples, but 'nevertheless it was not present in large numbers'. *Bact. coli* was isolated from five of the six soils. Re-examination of the soils showed that a rapid decrease in numbers took place, and in a fortnight's time the samples had become 'sterile on aerobic culture'. In this work it is not clear how *Bact. coli* and *Bact. aerogenes* were differentiated, other than on their M.R.-V.P. reactions.

Bardsley (1934) investigated eighty-six samples of soils representative of a large proportion of the gathering grounds of north-west England. The soils were mostly of the acid moorland type, only seven of which had a pH greater

than 7.0 and thirteen greater than 6.0. Owing to the scarcity of organisms, preliminary enrichment was necessary; 15 g. of soil were mixed with 150 ml. of water and four other dilutions were prepared. Inoculations were made into MacConkey broth and positive tubes were plated on to MacConkey agar. No less than 75.6% of the soils yielded no coliform bacteria. Of the 152 organisms isolated from the twenty-one positive soils 31% were *Bact. coli* type I, 66.4% intermediate I and only 2.6% *Bact. aerogenes* types I and II. It is interesting to note that of one lot of thirteen soils false presumptive positives occurred in ten samples; this was found in all cases to be due to *B. welchii*.

Burke-Gaffney (1932) examined samples of some soils from Dar-es-Salaam, which he classified into unpolluted, remotely polluted, and recently polluted types. All the cultures isolated from unpolluted land were *Bact. aerogenes* types I or II; from remotely polluted land 67% were of those types and 29% were intermediates, probably type I. In the recently polluted samples 81% were *Bact. coli* types, 11% *Bact. aerogenes* types, and 8% intermediate types. From a series of European soils similar results were obtained. Of cultures isolated from soils considered to have been recently polluted, 80% were *Bact. coli* types, 6% intermediates, and only 3% *Bact. aerogenes* types. From those isolated from remotely polluted soils 29% were *Bact. coli* types, only 4% *Bact. aerogenes* types, but intermediates (mostly type II) constituted 58% of the total number.

Minkewitsch (1930) examined 704 samples of soils taken over a very wide area and representing many types and various degrees of pollution. They included samples from the Russian tundra, lake and river banks, mountains, and private and public gardens. Of the 704 soils only 40% yielded coliform bacteria in the amount examined, about 3 g. The 283 cultures isolated were tested for their indol, M.R., V.P. and citrate reactions and for their ability to form gas in Bulir's medium at 46° C. It was found that 85% were able to react at 46° C., and the majority of them were *Bact. coli* types I or II. Ignoring the test at 46° C., the proportions of the total were: *Bact. coli* types I and II 78%, intermediates I and II 7.7%, and *Bact. aerogenes* type I 4%. No coliform bacteria were found in the samples taken from tundra.

The prevalence of coliform bacteria in soil has been investigated from another aspect, that of the survival of different types. These studies had two main objects: to determine the time of survival with a view to finding which types represented recent pollution and which types remote pollution, and to follow up the interesting theory that by loss of some characteristics and gain of others *Bact. coli* could eventually turn into an intermediate form or into *Bact. aerogenes*, or vice versa. There have been considerable differences in the results obtained by various workers, but Kulp (1932), who inoculated separate portions of sterilized soils with fifteen cultures of *Bact. coli* and nine of *Bact. aerogenes*, found no reversion of characteristics other than that in a few cases a specific culture might be indol-positive on one occasion and negative on another. After storage for one year all cultures were living, and at the end

of the experiment, a period of 3 years and 7 months, six of the fifteen *Bact. coli* and two of the *Bact. aerogenes* cultures were still viable. The difficulties attending work which involves the use of sterilized soil will be recognized, and it should be emphasized that all sources of competition, bacterial, fungal, and particularly protozoan, are removed by sterilization and cannot be totally replaced without the risk of the introduction of coliform bacteria other than those to be inoculated. The type and reaction of the soils may affect the results.

Young & Greenfield (1923) made periodic observations of soil which was taken from a field not obviously contaminated and was placed in a galvanized steel tank. The soil was neither sterilized nor inoculated, but 'soil' and 'faecal' forms were known to be present. The relative abundance of the faecal type in the soil was determined at intervals. The faecal form was indol- and M.R.-positive and V.P.-negative (+ + - ?), and may have been intermediate type II. The results obtained showed that the organism was present in very variable numbers from time to time; when the soil was saturated with water or frozen the organism was not found in less than 10 g., but in warm dry weather large and varying numbers were recorded. It was claimed that after a period of 6 years the organism was still present in 0.01 g. of soil.

Laboratory experiments were carried out with bottles containing sterilized and unsterilized soils which were inoculated with coliform bacteria of the so-called soil and faecal types. The moisture content of the different samples was adjusted to from 10 to 100% of the saturation value. In both sterilized and unsterilized samples containing moisture equivalent to 100 and 60% of saturation the numbers of coliform bacteria dropped rapidly; after 18 months coliform bacteria were present in 10 g. of soil kept saturated with water, but they were absent from 10 g. of the soil containing moisture to 60% of saturation. At lower moisture contents the numbers dropped slowly in the inoculated sterilized soils, but in the unsterilized soils greater numbers were found after 3 months than at the start of the experiment. In this paper no details of sampling procedure and technique are given, and in consequence it is not possible to examine in detail the evidence on which the authors claim that coliform bacteria multiply in soil.

Skinner & Murray (1926) inoculated soil supposedly free from *Bact. aerogenes* with a suspension of cow faeces in which no *Bact. aerogenes* could be found. After incubation at an unstated temperature, numbers of coliform bacteria were determined at different times by inoculation of 1 ml. amounts of serial dilutions into a lactose broth medium. Although 600,000 per g. were present at the commencement of the experiment there was a steady decrease until after 122 days no coliform bacteria were found. A second experiment was carried out in which samples of soil were inoculated separately with broth cultures of *Bact. coli* and *Bact. aerogenes* and one sample with a mixture of the two. In the samples inoculated with separate cultures *Bact. aerogenes* was not found after 218 days and *Bact. coli* after 176 days, a difference of only 42 days. In the sample of soil containing the mixture of the two cultures the

result was similar to the results obtained where only one culture was employed. It is of importance to add that no multiplication of the added bacteria in the soils was found in any of the experiments by the authors.

DISCUSSION

From the review of the limited number of papers considered it is hoped that some rather more precise conception of the ecology of the different types of coliform bacteria may be obtained than that now prevalent in the minds of many concerned with the examination and purification of water for public supply.

Of the coliform bacteria in faeces there is overwhelming evidence to show that *Bact. coli* type I is the dominant type, but it is doubtful whether it is true, as is often stated, that *Bact. aerogenes* and the intermediate types are rare or few in number. Bardsley (1938) found *Bact. aerogenes* or intermediates in 61% of the samples examined, and Parr (1938) found *Bact. aerogenes* in 33% and intermediates in 31% of the samples he examined. It has been suggested that the presence of these types does not mean that they are indigenous to faeces; they may have been ingested and survived. Pawan (1931), who found intermediates in the faeces of a native child, concluded that the origin was soil on the child's body. The question of the significance of *Bact. aerogenes* and the intermediates is clearly whether they are more prevalent in any habitat other than faeces.

The figures obtained by different workers suggest that of the coliform cultures isolated from faeces, chiefly by means of lactose broth and enrichment, 5% or more of the total were types other than *Bact. coli*. Expressed numerically this may sometimes represent a million or more cells per gram of faeces, numbers which can scarcely be considered to be without significance. The investigations by Bardsley (1938) and Parr (1938) indicate that the numbers of these types may at times exceed those of *Bact. coli*, or they may even be the only types present. It is relevant to consider the prevalence of *Bact. aerogenes* and intermediate types in habitats other than fresh faeces in order to assess their significance, and to consider the survival of different types in those habitats.

In general, the results of different workers show that storage of faeces causes a more rapid disappearance of *Bact. coli* than of intermediate and *Bact. aerogenes* types, but little is known of the relative importance of different factors on the rate of change. Much more information is required on the effects of such factors as temperature, intensity of light, pH value, and availability of oxygen. From such work it should be possible to define more accurately the term 'remote' in reference to pollution. As the matter now stands the term 'remote' may signify only a few hours in the case of exposure to tropical sunlight, or several weeks or months in more temperate climates.

The distribution of coliform types in cases of genito-urinary infections

deserves more study than it has received. Though the subject is usually touched on in reviews of the subject, only Burke-Gaffney (1932, 1933) has stressed the possible significance. The results of his work showed that *Bact. aerogenes* was the most prevalent type in urine, thus confirming the results obtained by Hill *et al.* (1929). He pointed out that consideration of pollution by pathogenic organisms should not be confined to organisms of faecal origin but should include organisms from excreta in general; pathogenic organisms may be passed out in large numbers in the urine of persons suffering from typhoid and other diseases. Hill *et al.* (1929) state that in comparing their culture groups with different clinical infections one of the most significant facts was that 75% of the blood stream invasions were due to organisms of the *Bact. aerogenes* type. Of twelve cases in which the same organism was recovered from blood and urine, *Bact. coli* types appeared in only one case.

From different papers considered on the coliform content of grasses and grains certain points seem clear. First and most important is the finding that enormous numbers of lactose fermenters from grass can be detected if an incubation temperature of 30° C. is employed, but only negligible numbers when the temperature is 37° C. (Allen & Harrison, 1936). This indicates that most of the types developing at 30° C. are not coliform bacteria but members of other genera, in particular *Erwinia* (Bergey, 1939), a genus containing many members of plant pathogenic types which have optimum growth at temperatures between 25 and 30° C., and are incapable of growth or fermentation of lactose at 37° C. Many allied saprophytic types may also be considered. It is thus of interest to note that Rogers *et al.* (1915) used an incubation temperature of 30° C. throughout their work, and that a large proportion of the types isolated contained yellow pigment and liquefied gelatin. Nor is it by any means certain that the organisms isolated by those workers were the true epiphytic microflora of the grain, as the samples were taken from car loads and had been exposed to possible sources of contamination from the time of harvesting and through transportation and storage; only three samples of growing grain were examined and lactose fermenters were not isolated from one of the three samples. With incubation at a temperature of 37° C. Wilson *et al.* (1935) found coliform bacteria in only half of the ninety miscellaneous samples of straw, hay, grass, decaying leaves, water, grains, meals, and feeding cakes, which they examined. The samples which by nature of habitat had been in contact with soil and hence possibly with manure gave relatively large numbers of *Bact. coli*; the samples of grains, meals, and feeding cakes representing material long since in contact with soil were marked by the prevalence of *Bact. aerogenes* types.

The papers of Hunter (1921) and of Allen & Harrison (1936) on silage are unique in that they record evidence of multiplication of coliform bacteria in habitats other than faeces. Hunter, however, did not obtain multiplication when sterile silage was inoculated with a culture of coliform bacteria. It appears probable that as the temperature of the fresh silage approaches body

heat as a result of the activity of various organisms, conditions become favourable for the multiplication of colon types, and large numbers may be present until either the temperature becomes too high or the pH too low. The results of Allen & Harrison show that multiplication was not confined to any particular type.

It is incorrect to say that any soil is beyond doubt free from all pollution, as some workers have described their samples, for every portion of the globe is open to pollution from the faeces of birds and wild animals, or the washings therefrom, and it is well known that such faeces may contain several millions of coliform bacteria per gram.

The most noticeable point concerning the coliform content of the soil is the difficulty that various investigators have met in finding coliform bacteria in soils not obviously polluted. Statements like the following: 'As a result of the scarcity of the organisms direct plating of the soil was found to be impracticable and it was necessary to resort to enrichment by the addition of 10 to 15 g. . . ' appear in many of the papers on the subject. Thus Chen & Rettger (1920) found 60% of their samples of soils gave negative results, Bardsley (1934) 75%, Koser (1924) 24%, and Minkewitsch (1930) 60%. It thus appears that where there has been no pollution there are no coliform bacteria. It must be admitted, however, that such a statement may be questionable for the tropics where some investigators have found bacteria in unpolluted waters in as small a quantity as 0.01 ml. In fact, most of these workers have stated that the recognition of Houston's lactose-positive, indol-positive type would condemn the bulk of water supplies in those latitudes, but they have yet to show that such coliform bacteria originated from any material other than faeces. It is striking that Pawañ (1931) in Trinidad found poor negative correlation between the M.R. and V.P. tests, and it can be deduced from his results that a large proportion of his cultures isolated from waters and soils were indol-positive and M.R., V.P., and citrate negative (+ - - -). This type is very rare and Ruchhoft *et al.* (1931) found seven (0.33%) of the cultures examined by them to have these reactions and thought it possible that they were mixed cultures; Clegg (1941), who examined 107 cultures which gave anomalous results in MacConkey broth, found two cultures of this type. Where pollution of the soil has taken place at some time other than very recently, the results of different workers are in agreement that the most prevalent types are *Bact. aerogenes* and intermediates. The relative proportions of these types may vary according to the time which has elapsed since pollution occurred, the climate, and the type of soil and its reaction. Where pollution of the soil has been recent it has been generally found that *Bact. coli* types have been greatly in excess of other types, a result expected by reason of the similar state of affairs in faeces. After the contamination of the soil takes place a gradual mortality of all types occurs, but the evidence seems overwhelming that *Bact. coli* types are the first to die out and that intermediate and aerogenes types may survive much longer.

It still remains to be shown whether or not *Bact. coli*, *Bact. aerogenes*, or intermediates can normally multiply in soils, grasses, or grains, and whether they are found in those habitats in greater numbers than in the faeces of man and other animals. It does appear probable that in the decomposition of organic matter when a temperature approaching body heat is attained, coliform bacteria can multiply, possibly to as great an extent as in the human body. It is possible that the prevalence of coliform bacteria in tropical waters deemed unpolluted may be a result of the decomposition of organic matter under the naturally warm conditions. This problem offers a large field for research.

It is possible that the origin of *Bact. aerogenes* and the intermediates may be found to be other than intestinal; meanwhile these types must be considered for sanitary purposes to be indicators of faecal pollution, as stressed by Parr (1938) and Bardsley (1938). The available evidence shows that the preponderance of *aerogenes* and intermediates indicates pollution at some time other than recent, but owing to the complexity of the factors affecting survival of different types no reliable estimate of that time can be made.

SUMMARY

1. A review has been made of literature on the ecology of different types of coliform bacteria. The main object of the review has been to consider whether there is evidence to support the view that *Bact. aerogenes* and the intermediate types live normally on plants or in the soil and not in the intestines of man and other animals.

2. There is ample evidence that *Bact. coli* is by far the most common type of coliform in normal human faeces. On the other hand, there is evidence that *Bact. aerogenes* or intermediate types are usually present in faeces, may sometimes be present in greater numbers than *Bact. coli*, and on rare occasions may be the only type present. Both quantitatively and qualitatively the coliform flora of the faeces of an individual person may vary from day to day. There is insufficient evidence on the numbers of *Bact. aerogenes* and intermediate types in faeces to justify any more definite statement, but limited data suggest that such types may be absent or may be present in numbers of the order of a million per gram.

3. When fresh faeces are stored there is first a multiplication of such bacteria as will grow on ordinary laboratory media, including the coliform types. The rate of multiplication, as with the flora of soil, water, and milk, increases with an increase in the incubation temperature to 37° C., but the period of multiplication becomes shorter. In the literature consulted no evidence can be found to show which groups are prominent in the multiplication. Results are in agreement that on further storage the ratio of the numbers of *Bact. coli* to those of *Bact. aerogenes* and intermediates decreases, the typical *Bact. coli* flora dying off more rapidly than other coliform types. The rapidity

of decrease appears to depend partially at least upon the temperature of the environment, and the decrease may be accelerated by intense sunlight.

4. In urine from patients suffering from genito-urinary infections the dominant types of coliform are usually either *Bact. aerogenes* or intermediates. No data on the number of such organisms in urine have been obtained from the works consulted.

5. There is no evidence that coliform bacteria multiply on fresh grasses or grains. Few quantitative data on this question have been found. In some of the older work it is doubtful whether a large proportion of the cultures isolated were actually coliform bacteria or whether they were species of other genera capable of fermenting lactose at 30° C. but not at 37° C. In the decomposition of grasses and legumes during ensilage, a process involving a considerable increase in temperature, it would appear that multiplication of coliform bacteria may take place and counts may for a time equal those found in fresh faeces. No indication has been found that this multiplication is confined to *Bact. aerogenes* or intermediates.

6. Most workers who have studied the coliform bacteria in soil have ignored the quantitative aspects and no counts at intervals over long periods of coliform bacteria in any undisturbed soil appear to have been made. No evidence of any multiplication of coliform bacteria in soil has been found. Results, however, are in agreement that where pollution of the soil by animal excreta has taken place, the heavier the pollution the greater is the number of coliform bacteria; soils relatively free from human or other animal pollution either contain no coliform bacteria or only small numbers. It is generally agreed that the ratio of the numbers of *Bact. coli* to those of *Bact. aerogenes* and intermediates decreases with the increase of time which has elapsed since pollution of the soil. This change is similar to that which occurs in faeces during storage.

7. There is insufficient evidence to justify the definite statement often made that *Bact. aerogenes* and intermediates are normal inhabitants of soils, grasses, and grains.

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