An ecological study of the coliform group, with special reference to the status of *Escherichia coli* I as an indicator organism of faecal pollution of water

by

P.A.J. Brand, B.Sc. (Hons)

Thesis presented in fulfilment of the
requirements for the degree of MAGISTER SCIENTIAE
at the Potchefstroom University for
Christian Higher Education

February, 1966

CONTENTS

4.8/...

ERRATUM

Cover, for "Escherichicia"
read "Escherichia"

1. INTRODUCTION

1.1 The discovery of the coliform bacteria and the development of differential media

The two classical coliform bacilli, viz. Bacterium coli commune (Escherichia coli) and Bacterium lactis aerogenes (Klebsiella aerogenes), were isolated from the faeces of breast-fed infants by Escherich in 1885 (Prescott, Winslow and McCrady, 1946; Windle Taylor, 1949, 1958; Wilson and Miles, 1955, 1964; Breed, Murray and Smith, 1957; Burrows, 1959, 1963). Escherich described the former as fairly long motile rods which clotted milk slowly and the latter as short plump non-motile rods which clotted milk more actively (Wilson and Miles, 1964). Both were Gram-negative, gave characteristic colonies on gelatin, agar and potato and formed gas in glucose (Bardsley, 1926).

Since the discovery of the first two coliforms (vide supra)many workers have contributed to their classification and more coliforms have been discovered. In 1890 Jordan identified another coliform from sewage, Bacillus cloacae (Klebsiella cloacae) (Breed et al. 1957; Burrows, 1959; Smith and Conant, 1960; Wilson and Miles, 1964). This bacillus could be differentiated from Bacterium lactis aerogenes by virtue of its ability to liquefy gelatin. (Jordan, 1903; MacConkey, 1905). He described this bacillus further in 1903 (Jordan, 1903).

Fermentation tests provided a useful method of distinguishing certain species of Enterobacteriaceae from others. Smith introduced the fermentation of lactose in 1890 to differentiate Bact. coli commune from other Enterobacteriaceae (Wilson and Miles, 1964). Later this became an important primary distinguishing test for all the coliforms. In 1896 Refik added the fermentation of lactose and the production of indole to the existing fermentation of glucose and the clotting of milk to divide the coliforms into groups (Bardsley, 1926).

More tests for differentiating the coliforms followed soon, viz. gas-ratio (i.e. the ratio of CO_2:H_2 produced - Bact. coli commune 1:1, Bact. lactis aerogenes and Bact. cloacae 2:1) - Smith in 1895; Voges-Proskauer (V.P.) reaction - Voges and Proskauer in 1898, and the Methyl-red (M.R.) test - Clark and Lubs in 1915 (Bardsley, 1926; Wilson and Miles, 1964). By utilising the gas-ratio, the Methyl-red test and the Voges-Proskauer reaction, the coliforms could be divided into two main groups, i.e. the Bacterium coli (commune) group and the Bacterium lactis aerogenes - cloacae group. (Bardsley, 1926, Wilson and Miles, 1964). In the latter

group/...

group Bacterium cloacae could be differentiated from Bacterium lactis aerogenes by means of the gelatin liquefaction test (vide supra).

Several classification systems have been worked out for coliforms, e.g. Durham in 1901 (Bardsley, 1926) and Jordan (1903). These were followed by the preliminary classification of MacConkey (1905), which was revised (MacConkey, 1909). This last detailed classification was based on motility, indole production, Voges-Proskauer reaction, gelatin liquefaction and the fermentation of inositol, adonitol, sucrose, dulcitol and inulin. By means of these reactions he classified the coliforms isolated from faeces, sewage, water, soil, various grains, etc. Many of the reactions used by MacConkey are, however, today of subsidiary importance in water bacteriology (Windle Taylor, 1958).

During the same decade Houston employed the so called "flaginac" tests for the classification of the coliforms which produced acid and gas in MacConkey (dextrose) broth (Thresh and Beale, 1925; Bardsley, 1926). These tests, used for nearly a quarter of a century in Britain, consisted of the production of fluorescence (fl) in neutral red broth, acid and gas (ag) in lactose peptone, indole (in) in peptone water, acid and clot (ac) in litmus milk, and acid and gas (s) in saccharose. According to these, Bacillus coli, i.e. Bact. coli could be classified into "flaginac", "saginac","aginac", "sagin" or "agin B.coli" (Thresh and Beale, 1925). The first three belonged to "the true Bacillus coli group."

In 1919 Winslow, Kligler and Rothberg employed only five tests, for the classification of coliforms, viz. motility, adonitol, sucrose, salicin and dulcitol (Windle Taylor, 1949). With the aid of these they confirmed only seven of MacConkey's twelve Bact. coli strains (vide supra).

Koser (1923) introduced a citrate medium by which he provided another differential test between Bacterium coli and Bacterium lactis aerogenes (Bacterium aerogenes). In the following year he studied the use of this citrate medium further (Koser, 1924). For the first time he could separate the coliforms into three groups, viz. Bacterium coli, Bacterium aerogenes-cloacae and the Intermediates (Bacterium freundii). Koser (1926 a, 1926 b) described this new group of coliforms further. The existence of Bact. freundii (Citrobacter freundii) was confirmed by Bardsley (1926, 1934) and, according to Windle Taylor (1958) and Wilson and Miles (1964), by Pawan in 1925.

Employing the M.R., the V.P., the citrate and gelatin liquefaction tests, the coliforms could then be differentiated as follows :

Bact. coli/...

ERRATUM

p 3, lines 34 and 35, for "conformation" read "confirmation"

Bact. coli	(+ - - -),
Bact. freundii	(+ - + -),
Bact. aerogenes	(- + + -), and
Bact. cloacae	(- + + +).

In water bacteriology, however, Thresh and Beale (1925) still followed the same lines of Houston's classification (vide supra). Although they added the tests for motility and gelatin liquefaction, they still stressed the importance of the production of : acid and gas within 48 hours in a bile-salt glucose broth and in a bile-salt lactose broth, indole in peptone water, and acid and clot in milk within three days.

As early as 1926, Bardsley gave an excellent summary of all the methods previously employed, out of which she selected several tests for the classification of the coliforms and which are still considered important for the classification of coliforms. She employed MacConkey bile-salt lactose broth (acid and gas within 24-48 hours at 37°C.); 1% peptone water (indole production after 48 hours at 37°C., later within 5 days); Clark and Lub's glucose-phosphate medium (Methyl-red and Voges-Proskauer tests after 5 days at 30°C.); citrate medium (Koser's reaction after 5 days at 30°C.), gelatin stabs (liquefaction after 5 days at 20°C.), and litmus milk (acid and clot within 48 hours or 3 weeks at 37°C.) (Bardsley, 1926).

Later, in 1935, Wilson, Twigg, Wright, Hendry, Cowell and Maier drew attention to a fifth group of coliforms, which they could not satisfactorily place in the existing genera, and consequently named them the Irregulars (Batty-Smith, 1942 b; Wilson and Miles, 1955; Windle Taylor, 1958).

Still another group, the so-called Intermediates from faeces, was described by Parr (1936 a, 1936 b, 1937, 1938). According to their biochemical reactions, some of these Intermediates corresponded to Koser's Intermediates (vide supra), some to Bacterium aerogenes, others to certain of the Irregulars (vide supra), while the remainder were newly described coliforms.

Another of the numerous biochemical tests developed was that for H_2S production. This test was ultimately finalised by Vaughn and Levine (1936). Materially this test assisted only in additional conformation of the classification of the Intermediates (i.e. Koser's Intermediates or Bacterium freundii) and later replaced the litmus milk test of Bardsley's classification (vide supra) (Windle Taylor, 1958; Wilson and Miles, 1964).

The mnemonic/...

ERRATUM

p 4, line 31, for "Taylor, 1949" read "Windle Taylor, 1949"

The mnemonic IMViC was introduced by Parr (1938) to designate four reactions, viz. indole (I), Methyl-red (M), Voges-Proskauer (V) and citrate (C). This still forms the main reaction of coliform differentiation in the United States of America (Prescott et al. 1946; American Public Health Association et al. 1960; Salle, 1961; U.S. Department of Health et al. 1962; McKee and Wolf, 1963).

A test, in which a 1.4% glucose medium was used and in which the cultures were incubated at 46°C., was introduced by Eijkman in 1904 (Batty-Smith, 1942 b; Wilson and Miles, 1955). For 30 years anomalous results were obtained in employing this technique. Then a comparison between incubation temperatures 43° to 44°C. and 44.5° to 46°C. was made by Levine, Epstein and Vaughn (1934). Finally in 1935 Wilson and his co-workers standardised the incubation temperature at 44°C. (Batty-Smith, 1942 b). During this study of temperature relationships glucose was replaced by lactose in the medium to render it more specific (Wilson and Miles, 1955).

As a result of these earlier experiments on the optimum temperature, the specified incubation temperatures for this "modified Eijkman test" still vary from 43° to 46°C., viz. 43°C., 43.5°C., 44°C., 44.5°C., 45°C., 45.5°C. and 46°C. (Levine et al. 1934; Perry, 1938; Hajna and Perry, 1939; Stuart, Zimmerman, Baker and Rustigian, 1942; Levine, Tanimoto, Minette, Arakaki, Fernandes, 1955; Pederson and Skinner, 1955). It was, however, undoubtedly proved by Batty-Smith (1942 b), Sherwood and Clegg (1942) and Taylor (1945) that the higher the incubation temperature rose above 44°C., the more Bact.coli strains would be unable to produce acid and gas, whilst below 43.5°C. some Bact. aerogenes strains would generate acid and gas. By means of this test, however, the genus Bacterium and the Irregular group could be differentiated into various strains important in water bacteriology, i.e. Bact.coli, type I, Irregular, type II and Irregular, type VI which produce acid and gas at 44°C., while all other coliforms are unable to do so (Taylor, 1949; Wilson and Miles, 1955) (vide 4.2).

Although the usefulness of this 44°C. test was immediately realised by British authorities (Great Britain Ministry of Health et al. 1956; Public Health Laboratory Service, 1957; Windle Taylor, 1958; Wilson and Miles, 1964), many American authors have always been reluctant in accepting it as of real significance (Prescott et al. 1946; American Public Health Association et al. 1960; U.S. Department of Health et al. 1962;

McKee and/...

McKee and Wolf, 1963). This attitude is probably based on the anomalous results obtained by employing different temperatures in the 43° to 46°C. range (vide supra). As a standard test at 44°C., as indicated by Batty-Smith (1942 b), Sherwood and Clegg (1942) and Taylor (1945), it is, however, a valuable differentiating tool. The value of the modified Eijkman test has now been amply proved by Bardsley (1938, 1948), Perry (1938), Raven, Peden and Wright (1940), Ferramola (1940), Sherwood and Clegg (1942), Batty-Smith (1942 b), Stuart, Zimmerman et al. (1942), Taylor (1945), Mackenzie, Taylor and Gilbert (1948) and Windle Taylor (1955).

A final test which should be mentioned is the production of acid and gas in a cellobiose medium. In 1924 Jones drew attention to cellobiose as an aid in differentiating the coliforms (Batty-Smith, 1942 a). Koser made an extensive study of its possible uses in 1926 (Batty-Smith, 1942 a). The value of this test was again confirmed by Jones and Wise (1926). In later years various workers used this test, or a modification of it. Stuart, Wheeler and Griffin (1938), Stuart, Griffin and Baker (1938), and Griffin and Stuart (1940) employed it successfully in extensive coliform studies. Yet Taylor (1941), working on waters from lakes and streams, found that no additional information could be obtained by this test. With this anomaly in mind Batty-Smith (1942 a) studied the use of cellobiose and found that it was inferior to the citrate utilisation test as a result of which it was abandoned.

1.2 The ecology of the coliforms

The young and dynamic science, known as ecology, initiated as "scientific natural history" (Kendeigh, 1961; Macfadyen, 1963). In the early 1920's it implied the recording of the occurrence or distribution of organisms, and sometimes it was attempted to link these observations to environmental factors (Macfadyen, 1963).

Today this science overlaps with many other sciences or branches thereof (Odum, 1959; Kendeigh, 1961; Reid, 1961; Macfadyen, 1963). It is usually defined as "the study of the relation of organisms or groups of organisms to their environment, or the science of the interrelations between living organisms and their environment" (Odum, 1959). Odum (1959) pointed out that the following definitions will also be acceptable : "the study of structure and function of nature", "the science of the living environment" or "environmental biology". To this Kendeigh (1961) added "the study of biotic communities" and "the science of community populations".

When/...

When dealing with the higher organisms only, Kendeigh (1961) defined ecology as "a study of animals and plants in their relation to each other and to their environment". From this it follows that there are bioecology, i.e. ecology of all organisms, animal ecology, and plant ecology (Kendeigh, 1961). To this can then be added microbial ecology, i.e. the ecology of micro-organisms, or, specifically, bacterial ecology.

Ecology is commonly divided into "autecology", i.e. the study of individual organisms or an individual species, and "synecology", i.e. the study of groups of organisms which are associated together as a unit (Odum, 1959; Kendeigh, 1961; Reid, 1961). In synecology the populations, communities (or associations), ecosystems, etc. are studied (Odum, 1959; Reid, 1961; Macfadyen, 1963).

According to the environment, ecology is often subdivided into marine (i.e. oceanography), fresh-water (i.e. limnology) and terrestrial (Odum, 1959; Kendeigh, 1961).

From these few cursory remarks, it is obvious that ecology covers a wide scope. Unfortunately microbial ecology, and here especially bacterial ecology, is still in its infant stage, i.e. where animal and plant ecology were in the early 1920's (vide supra). Consequently in a bacteriological sense, ecology still implies the recording of the occurrence or distribution of organisms, and sometimes attempts are made to link these observations to environmental factors. This will be obvious when the ecology of the coliforms is discussed (vide infra).

It is, however, clear why bacterial ecology is still in the initial stages, viz. the problem of working with minute organisms makes ecology a formidable task. For this reason the present study was more concerned with habitat and the distribution of coliforms, than with "the inter-relations between" bacteria "and their environment" (also vide Griffin & Stuart, 1940; Gray, 1951).

Because of their isolation from faeces (vide 1.1), Escherich thought that both his classical coliform bacilli were typical faecal organisms (Prescott et al. 1946; Windle Taylor, 1949, 1958; Breed et al. 1957; Burrows, 1959). His work can be regarded as one of the first attempts at the ecology of the coliforms.

In 1899 Russell and Bassett (Windle Taylor, 1949, 1958; Wilson and Miles, 1955, 1964) confirmed Kruse's observation of 1894 that Klebsiella aerogenes i.e. Bact. aerogenes, was not exclusively found in faeces but also in water and soil, while in 1907 Winslow and Walker concluded that

Escherichia coli,/...

Escherichia coli, i.e. Bact. coli was essentially an intestinal organism and that K.aerogenes was more frequently found in soil and vegetation (Windle Taylor, 1949, 1958; Wilson and Miles, 1955, 1964). The value of these observations is clear, because this laid the basis for defining E.coli as an indicator organisms of faecal pollution (vide 1.3).

MacConkey (1905, 1909), Koser (1923, 1924, 1926 a, 1926 b), Skinner and Brudnoy (1932); Burke-Gaffney (1932); Parr (1936 a, 1936 b, 1937, 1938), Bardsley (1926, 1934, 1938, 1948); Malcolm (1938), Stuart, Griffin and Baker (1938), Griffin and Stuart (1940), Prescott et al. (1946), Windle Taylor (1949, 1958) and Wilson and Miles (1955, 1964) established clearly that E.coli I (i.e. the indole positive, 44°C. positive strain of E.coli, vide 4.2) is the predominant coliform in faeces, sewage, polluted soils, etc.

It was further indicated by Skinner and Brudnoy (1932), Burke-Gaffney (1932), Bardsley (1934, 1938, 1948), Parr (1938), Malcolm (1938), Windle Taylor (1958) and Wilson and Miles (1964) that Citrobacter freundii (i.e. Bacterium freundii or Koser's Intermediates) occurs in faeces, but in small numbers; while Koser (1924,1926 a, 1926 b), Bardsley (1934) and Randall (1956) proved that Cit.freundii was far more numerous than E.coli I in unpolluted soils and waters.

These findings led to the assumption that soils are the natural habitat of Cit.freundii (Great Britain Ministry of Health, Ministry of Housing and Local Government Reports on Public Health and Medical Subjects No 71, 1956; Windle Taylor, 1958). Taylor (1942), Bardsley (1948) and Taylor (1951), however, found that Cit.freundii could not live in soils and ultimately died off. Taylor (1951) even found E.coli I to preponderate over Cit.freundii in all kinds of soil. Consequently he could not support the earlier views that Cit.freundii has the soil as a primary habitat. He thus suspected that these bacteria, when found in soil, were also derived from faeces. Randall (1956), however, proved that Cit.freundii preponderates over E.coli I in unpolluted soils. He also found that soil cannot support the growth of Cit.freundii, but that its primary habitat could not be faeces. In the U.S.A. Cit.freundii was found to be typical of unpolluted soils (Geldreich, Huff, Bordner, Kabler & Clark, 1962).

According to MacConkey (1905, 1909), Koser (1924, 1926 a, 1926 b), Burke-Gaffney (1932), Gray (1932), Skinner & Brudnoy (1932), Bardsley (1934, 1938), Malcolm (1938), Stuart, Griffin et al. (1938), Griffin and Stuart (1940), Windle Taylor (1958) and Wilson and Miles (1964) small numbers of K.aerogenes and Klebsiella cloacae, i.e. Bacterium cloacae,

may occur/...

may occur infrequently in faeces. On the other hand Koser (1926 b), Skinner and Brudnoy (1932), Burke-Gaffney (1932), Gray (1932), Windle Taylor (1958), Burrows (1959), and Wilson and Miles (1964) decided that K.aerogenes and K.cloacae outnumbered E.coli I in unpolluted soils, waters, grasses, grains and cereals. In the tropics Burke-Gaffney (1932) concluded that these organisms in soil and water are derived from sources other than recent faecal pollution. Malcolm (1938), however, regarded the occurrence of K.aerogenes and K.cloacae in soil, water, fodder, grain, etc., in absence of recent faecal pollution as indicative of remote faecal pollution.

Windle Taylor (1958) concluded that the coliforms present in faeces can be distributed to the soil, air, water, vegetation, etc., but that not all these coliforms were able to adapt themselves to their new environment. He alleged that E.coli I could survive in its new habitat for a considerable time, but because of its inability to multiply under such conditions, it ultimately died off. This was illustrated by the work of Gray (1932) who found that "in water contaminated with faeces, the proportion of Bact. aerogenes to B.coli is relatively low but is rapidly reversed on storage". Bardsley (1934) also stated that in "very heavily polluted waters the ratio", i.e. E.coli I to Intermediate-aerogenes-cloacae (I.A.C.) group, "appears to diminish again - presumably owing to actual growth of organisms of the I.A.C. group in the water". According to Windle Taylor's citations certain coliforms, such as K.aerogenes and K.cloacae,could adapt themselves to their new environment.

Irregular VI, a variant of K.aerogenes (differing from K.aerogenes only in being 44°C. positive (vide 4.2)) found on jute, jute packing, jute yarn, hemp, sacking, string, leather washers, etc., is able to pollute water in water-mains, reservoirs, wells and boreholes (Windle Taylor, 1958). As soon as these fibres come in contact with water, enough nutrient is available for this organisms to multiply enormously (Windle Taylor, 1958). Mackenzie, Taylor and Gilbert (1948) determined that decaying wood in wells could also provide the necessary nutrients for the ample proliferation of Irregular VI. This organism, in symbiosis with the cellulose-utilising bacteria, is thus able to flourish on decomposing cellulose in water, metabolising by-products such as cellobiose (Windle Taylor, 1958).

In southern England it was found that K.aerogenes multiplied in the filter beds of drinking-water purification works, while during a similar investigation it was found that algal growth (Enteromorpha intestinalis)

favoured the/...

favoured the growth of K.aerogenes (Thresh and Beale, 1925; Windle Taylor, 1958).

Windle Taylor (1958) also drew attention to some evidence that various coliforms, including E.coli I, were able to grow and multiply in several kinds of waters and under various conditions. Although he merely referred to them in a cursory manner, some of these authors have been consulted, namely Bigger (1937), Bigger and Nelson (1941, 1943), and Nelson (1942), and their observations will be discussed later (vide 4.8).

Moreover, Pivnick and Fabian (1954) isolated citrate-utilising coliforms, i.e. Klebsiella and Citrobacter spp., from industrial soluble-oil emulsions, although E.coli was not among these.

Some investigators concentrated on the isolation of various types of coliforms other than E.coli from faeces, so as to prove that all coliforms are typical faecal types. Gray (1932) found K.aerogenes to be present in 92.5% of the faecal specimens which he examined after enrichment in citrate. In human faeces 61% contained the I.A.C. group, as isolated by Bardsley (1938). One of the interesting cases is that of Malcolm (1938), who, while working with the faeces of cattle, selectively inhibited the growth of E.coli I by employing a medium which contained brilliant green in order to promote the growth of a greater variety of other coliforms. Consequently he recovered many strains of C.freundii, K.aerogenes and K.cloacae. K.aerogenes and K.cloacae were especially abundant. (Unfortunately a description of this selective inhibitory medium was not given in this article. Malcolm referred to it as being described in one of his earlier publications, but this could not be obtained). Parr (1938) stressed the importance of his own findings and those of Carpenter and Fulton in 1937. In both these studies a considerable number of human faecal samples was found to contain Intermediates, viz. 21.6% and 13.3% respectively. At the same time Parr stressed the occurrence of K.aerogenes in human faeces. Prescott et al. (1946) found that C.freundii and K.aerogenes constituted "a small but rather persistent proportion of the coliforms in human and animal faeces ...."

It could, therefore, be concluded that occasionally some faecal specimens may contain other coliforms in addition to E.coli I. When E.coli I, however, suffers a numerical reduction or is totally absent from its natural habitat, i.e. the human or animal intestine, it is usually due to a certain amount of seasonal flux, species variation, disease, drugs, antibiotics, etc. (Windle Taylor, 1958 ; Burrows, 1959). The

exact habitat/...

exact habitat of the other coliforms (e.g. Klebsiella spp., Citrobacter spp. and Irregulars) is still doubtful at the moment. It appears as if the intestine is not their primary habitat. From the literature survey, however, it is apparent that they outnumber E.coli in unpolluted soils, water, plants, cereals, etc. It has been clearly established that Irregular VI has fibres, especially that of jute and hemp, as its natural habitat. Because of this it is often referred to as the "yarn organism" (Windle Taylor, 1958). Coliforms, other than E.coli I, are regarded as "organisms of passage" (Bardsley, 1948) and as "adventitious" (Griffin & Stuart, 1940) when present in faeces.

1.3 Bacteriological evidence of water pollution

As water is liable to be polluted by pathogenic micro-organisms, it is necessary to have reliable means of testing the bacteriological quality of water intended for human consumption or for other domestic purposes, as well as for swimming.

Bacteria presenting the greatest danger to public health are almost invariably of human origin, especially those from human excreta. Unfortunately, direct testing for the presence of pathogenic intestinal organisms in water is impeded by various factors such as : the fact that there is no generally accepted quantitative method of determination available; that the pathogens are greatly outnumbered by the saprophytic "water bacteria" and the intestinal commensals; that the time-lag before the onset of intestinal diseases after the consumption of polluted water can be considerable; etc. (Galloway and Burgess, 1950; Windle Taylor, 1949, 1958; American Public Health Association, American Water Works Association, Federation of Sewage and Industrial Wastes Associations, 1955; Gainey and Lord, 1956; Great Britain Ministry of Health, Ministry of Housing and Local Government Reports on Public Health and Medical Subjects No. 71, 1956; Burrows, 1959; World Health Organization, 1958, 1961, 1963; Cruickshank, 1960). Admittedly good progress has been made with selective and enrichment media for the isolation of intestinal pathogens, e.g. Salmonellae and Shigellae (Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Wilson and Miles, 1964). Recently methods have been improved for the isolation of these intestinal pathogens from water and polluted waters, viz. Moore swab (Moore, Perry and Chard, 1952), cotton wool filtration method (McCoy, 1962), and an improved method for isolating Salmonellae from water (Livingstone, 1965), employing the Selenite brilliant green medium of Stokes and Osborne (1955). These methods are, however, only qualitative, and they have so far not yet been fully evaluated for use as parameters of pollution.

The acknowledged alternative for avoiding this problem is to select an organism which is normally a common intestinal commensal in man and

animals/...

animals. The presence of such an organism is assumed to serve as an indicator of faecal pollution of human or animal origin. In such an instance the water is regarded as suspect, the assumption being that if faecal commensals are present, pathogens and their inherent threat to public health could also be present. Moreover, their presence indicates faecal pollution, whether pathogens are present or not. [In referring to bacteria as intestinal commensals it does not imply that they are always harmless. Sometimes they are pathogenic to man and animals (Lovell, 1937; Charter and Taylor, 1952; Gillespie, 1952; Taylor and Charter, 1952; Kauffmann, 1954; Breed et al. 1957; Windle Taylor, 1958; Edwards and Ewing, 1962; Burrows, 1963; Wilson and Miles, 1964).]

Indicator organisms for this purpose are E.coli, Streptococcus faecalis, Clostridium perfringens (welchii) and the coliform group of which the former is by far the most universally accepted (Windle Taylor, 1949, 1958; McEwen, 1949; Prescott et al.1946; Galloway and Burgess, 1950; American Public Health Association et al. 1955, 1960; Strell, 1955; Gainey and Lord, 1956; Great Britain Ministry of Health et al. 1956; Coetzee, 1962 b, and Cruickshank, 1960). In addition Pseudomonas aeruginosa has also been suggested as an indicator organism of faecal pollution (Reitler and Selegmann 1957; Windle Taylor, 1958; Coetzee, 1962 b; Bonde, 1963). Recently the bacteriophages of certain specific intestinal pathogens and E.coli were also suggested to be used as indicator organisms (Dienert, 1947; Cocioba, 1948; Guelin, 1948, 1954; Hruby, 1954; Coetzee, 1962 a; Carstens, 1963).

The pathogens are greatly outnumbered by normal intestinal flora such as E.coli. Moreover these commensals usually outlive the majority of the pathogens, so that water which is free from E.coli is normally assumed to be free from pathogens (Windle Taylor, 1949, 1958; Prescott et al. 1946; Gainey and Lord, 1956; Great Britain Ministry of Health et al. 1956). In addition, due to the fact that E.coli tends to die when removed from its natural habitat, its presence is thus regarded as an indication of recent faecal pollution. In view of this, bacteriologists to date have tended to be more concerned about whether pathogens could be present (indicated by the presence of E.coli), than whether they are actually present (Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Coetzee, 1962 b).

Notwithstanding the considerable amount of research which has been done on the ecology of the coliforms resulting in the acceptance of E.coli I as the prime indicator organism (vide 1.2), much attention is still being devoted to the role of the coliform group as a whole in the faecal pollution of water. In many of the specifications for the standards of drinking-water

the coliform group is considered to be important (Windle Taylor, 1949, 1958; Prescott et al. 1946; South African Bureau of Standards, 1951; American Public Health Association et al. 1955; Great Britain Ministry of Health et al. 1956; World Health Organization, 1958, 1961, 1963). The American Public Health Association et al. 1955, for example, presenting the American Standards for drinking-water, assumed that ".... all types of coliform organisms may occur in feces ...." and " .... there is little or no evidence that coliform bacteria multiply on fresh grasses or grains; nor is there evidence that they multiply in soil". Windle Taylor (1949, 1958) and the Great Britain Ministry of Health et al. (1956), however, are more cautious about the coliforms. Although notice is taken of the importance of the coliforms as a group, the superiority of E.coli I as an indicator organism is stressed. Other opinions on this subject are those of Gray (1932) and Mackenzie and Hilton-Sergeant in 1938 (cited by Windle Taylor, 1958), that the presence of the coliforms other than E.coli, is an indication of such remote pollution that it is epidemiologically insignificant.

But the question naturally arises whether the recovery of coliforms from water, invariably indicates faecal pollution. If, by an ecological study, the presence of coliform bacteria in the faeces of humans and animals is proved to be of such frequency, then the American view should be accepted without reserve. However, should the coliforms prove not to be abundant, as opposed to E.coli, then the general practice of accepting the coliform group as an indicator of faecal pollution may be shown to be insufficient. The present investigation was undertaken with the view to obtain more information about the ecological distribution of the coliform bacteria and to establish whether these organisms, and especially E.coli I, can unquestionally be regarded as indicators of faecal pollution of water. To this end various sources, such as faeces of fowls, sheep, cattle and man, which could contribute to bacterial pollution of water, were examined for the presence of such organisms. In addition a river was also sampled in the same area where most of the faecal specimens were obtained, in order to determine how the water was affected by faecal pollution and by the incidence of coliform bacteria.

2. MATERIALS AND METHODS

2.1 Planning of the experimental work

This investigation, which was carried out in and around Potchefstroom, was confined to the faeces of man and animals. Representative groups of each, especially those which were most likely to contribute to the pollution of water, were selected. Each group was subdivided on the basis of the type of diet, viz. unbalanced or balanced. The terms balanced and unbalanced diets need closer description. In connection with man it is general knowledge that a diet should consist of a balanced ratio of protein, carbohydrate, fat, vitamin and certain mineral salts in order to combat malnutrition and deficiency diseases. In the same way animals need a balanced diet (vide infra for description of unbalanced and balanced diets in connection with fowls, sheep, cattle and man). A striking difference was, however, not expected between the subgroups of each main group as the faecal specimens could not at the time be studied by quantitative methods (vide infra).

Sampling and examination of faecal specimens commenced in 1962. At the time it was impossible to get the survey statistically planned so as to assess the number of samples required for each subgroup in order to obtain reliable and representative results. Unfortunately most statistical handbooks refer to the number of samples to be taken in a cursory manner, without giving a real solution and in any case no standard work consulted has dealt with the statistics of the type of experiment utilised in the present study. For these reasons it was decided to collect whenever possible at least 20 samples for each subgroup - usually 25 samples were collected.

The following animal groups were selected and divided into subgroups :

A Fowls with an unbalanced diet, viz. mainly dependent on the dry veld, with an occasional ration of mealies and human waste food and infrequent access to water.

A^1 Fowls with a balanced diet, viz. rations consisting of a fixed ratio of yellow mealie meal, lucerne meal, bran, pollard, fish meal, peanut meal, bone meal, oyster shell powder, fine sodium chloride, brewer's yeast, manganous sulphate and/or magnesium sulphate and a vitamin mixture, with free access to water. (Consequently vital substances such as protein, calcium, phosphorous, fibre,

fat/...

fat, carbohydrates, and vitamin are supplied). (Potchefstroom Experimental Farm Poultry Section, 1963).

B Sheep with an unbalanced diet, viz. mainly dependent on the dry veld, with infrequent access to a mixture of sodium chloride, bone meal and flowers of sulphur and to water.

B^1 Sheep with a balanced diet, viz. rations consisting of a fixed ratio of lucerne hay, Eragrostis curvula hay and yellow mealie meal, with free access to a mixture of sodium chloride, bone meal and flowers of sulphur and to water. (Consequently vital substances such as protein, calcium, phosphorous, fibre and fat are supplied). (Potchefstroom Experimental Farm Stock-breeding Section, 1963).

C Cattle with an unbalanced diet, viz. mainly dependent on the dry veld, with infrequent access to a mixture of sodium chloride and bone meal and to water.

C^1 Cattle with a balanced diet, viz. ad lib rations consisting of a fixed ratio of teff hay, lucerne meal, mealie meal and oil cake, with free access to a mixture of sodium chloride and bone meal and to water. (Consequently vital substances such as protein, calcium, phosphorous, fibre and fat are supplied). (Potchefstroom Experimental Farm Stock-breeding Section, 1963).

According to analyses done at the Potchefstroom Experimental Farm some of their main fodder included in many of the balanced diets, showed the following mean composition (Potchefstroom Experimental Farm Stock-breeding Section, 1963):

Ingredients	Eragrostis curvula hay	Lucerne hay	Yellow mealies
% Fat	2.04	2.30	4.71
% Nitrogenous substances	1.56	2.78	1.59
% Non-nitrogenous substances	47.71	44.52	81.54
% Fibre	35.45	28.66	2.00

From/...

From these it is obvious where the proteins, carbohydrates, fats and fibre (roughage) in the balanced diets come from.

Subgroups A and A^1 were included, not because they were regarded as major contributors to water pollution, but to obtain some knowledge of the coliforms from birds. Wild birds always have access to many water sources, but their faeces are too difficult to sample. Furthermore, it was borne in mind that the normal body temperature of birds is higher than that of the mammals and consequently might affect the coliform flora. The specimens from subgroup A were collected in the vicinity of Potchefstroom, Western Transvaal, exclusively from fowls belonging to Bantu owners, as they were considered to constitute the best example of fowls receiving an unbalanced diet. These samples were furthermore taken during the winters of 1962 and 1963 since this was regarded as the season offering optimum conditions favouring an unbalanced diet.

The A^1 subgroup was collected from the fowl run of the Potchefstroom Experimental (Agricultural) Farm, from those fowls which received a fully balanced diet only (vide supra).

The specimens of the subgroups B and C were sampled in the vicinity of Potchefstroom on the farms of white farmers, during the winters of 1962 and 1963. The B^1 and C^1 subgroups were obtained from the pens of the Experimental Farm, from animals fed on a fully balanced diet (vide supra).

Man, considered to be of prime importance in the faecal pollution of water, was added to the previous groups. The following three subgroups were investigated:

D Man with an unbalanced diet - Bantu, viz. poor in vitamin, protein and fat.

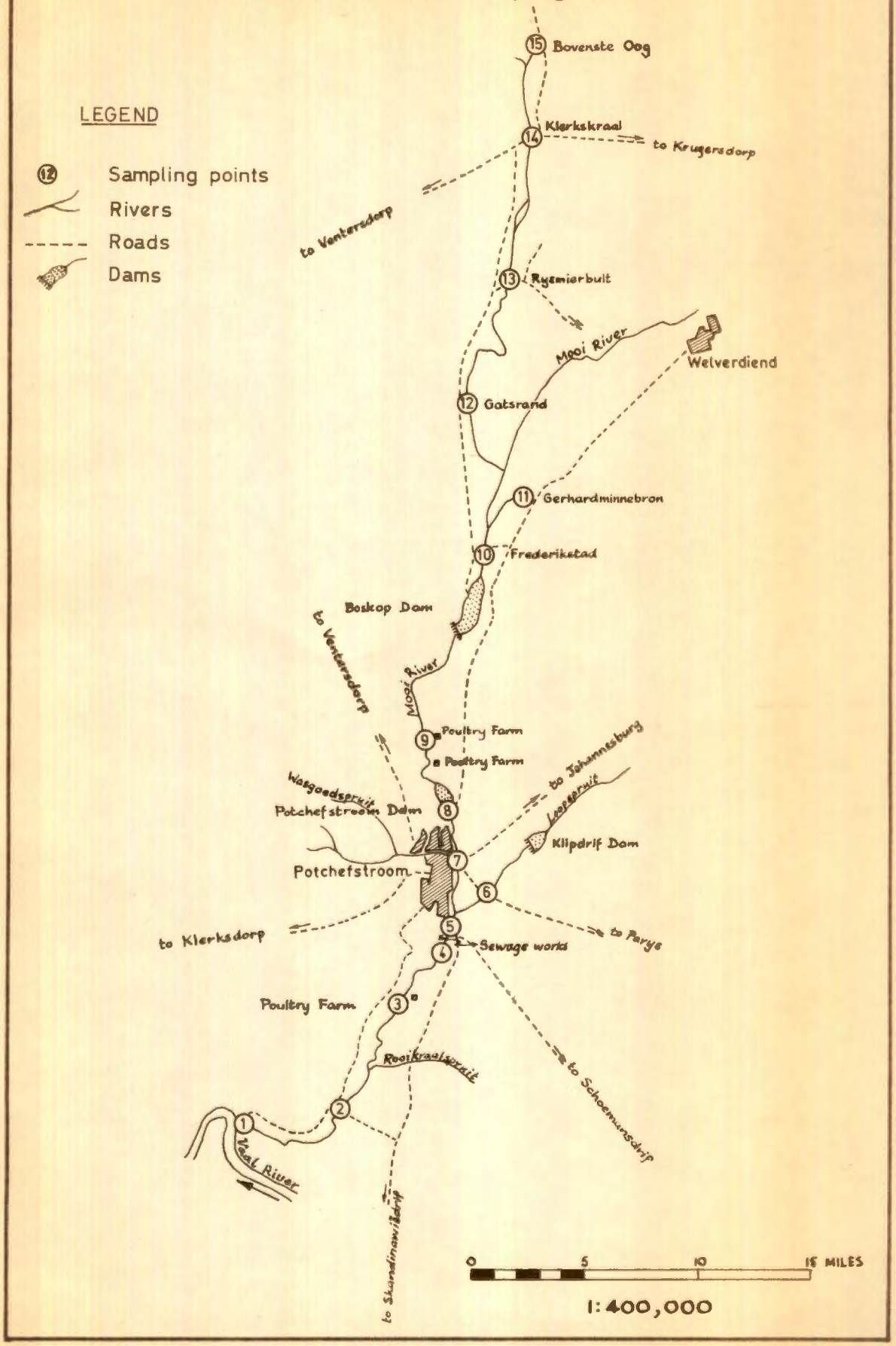
D^1 Man with a balanced diet - Whites, viz. balanced protein, carbohydrates, etc.

D^2 Man differing in diet from D and D^1 - Indians, viz. containing enormous quantities of spices, and often also lacking in some of the main diet ingredients.

It was extremely difficult to obtain enough specimens from the Bantu and Indians, as they were suspicious and superstitious. For this reason fewer specimens of subgroups D and D^2 were studied than in the case of the other subgroups. Unfortunately specimens from hospitals could not

be/...

Map of the Mooi River Catchment Area showing Towns, Communications and Sampling Points.



be included, because of the possibility of patients having been treated with antibiotics, sulphonamides, etc.

Each faecal specimen was examined for the presence of the various bacteria constituting the coliform group. For the following reasons this was done by qualitative rather than quantitative methods :

(1) Several authors (e.g. Prescott et al. 1946; Windle Taylor, 1949, 1958; Wilson and Miles, 1955, 1964) quoted the numbers of coliforms occurring in 1 gram of faeces, without mentioning the methods employed.

(2) It was felt that the methods then available, viz. the pour-plate (MacConkey and Eosin methylene blue agar) and the modified Most Probable Number technique, were far too inaccurate and intricate to manipulate and employ successfully, especially for comparative purposes.

It was therefore considered more important to undertake a qualitative survey and attempt to obtain the greatest variety of coliforms possible rather than to attempt unsatisfactory quantitative work.

In the final stages of the investigation two faecal specimens were, however, examined by quantitative methods using a modification of the membrane filtration technique, described by Kjellander (1960) (vide 2.6).

Samples from the Mooi River, Potchefstroom, were included because of the variety of coliforms which might be encountered in this habitat. There were many sources from which the coliform bacteria could reach the water. The Mooi River (vide map) originates south west of Randfontein, and nearly 45 miles north of Potchefstroom. It flows mainly through agricultural areas. The main crops planted are maize, kaffir-corn and ground-nuts, while the live-stock farming includes cattle, sheep, pigs and poultry. On its way to the Vaal River it is impounded twice - firstly by the Boskop Dam, approximately 9 miles north of Potchefstroom, and secondly by the Potchefstroom Dam, on the northern outskirts of the town. [Impoundment, i.e. damming or storage, usually reduces the

numbers/...

numbers of E.coli (Prescott et al. 1946; Taylor, 1958; Wilson and Miles, 1964). 7 From here it winds through the town and the old location, passing the sewage works from which it receives the maturation pond effluent. It continues through intensively irrigated plots and joins the Vaal River (approximately 15 miles south of Potchefstroom).

Samples were taken from 15 sampling points (vide map), viz. No 1 the Mooi River immediately above confluence with the Vaal River; Nos 2 and 3 between 1 and sewage works; No 4 after entry of maturation pond effluent; Nos 5, 7 and 8 in Potchefstroom below the Potchefstroom Dam; No 6 on the Loopspruit tributary; No 9 below Boskop Dam; No 10 above Boskop Dam; No 11 Gerhardminnebron (spring); Nos 12, 13 and 14 above 11, and No 15 Bovenste Oog (spring).

The river water was analysed thrice :

E Water from the Mooi River, collected during May, 1963,

E^1 Water from the Mooi River, collected during July, 1963,

E^2 Water from the Mooi River, collected during October, 1963.

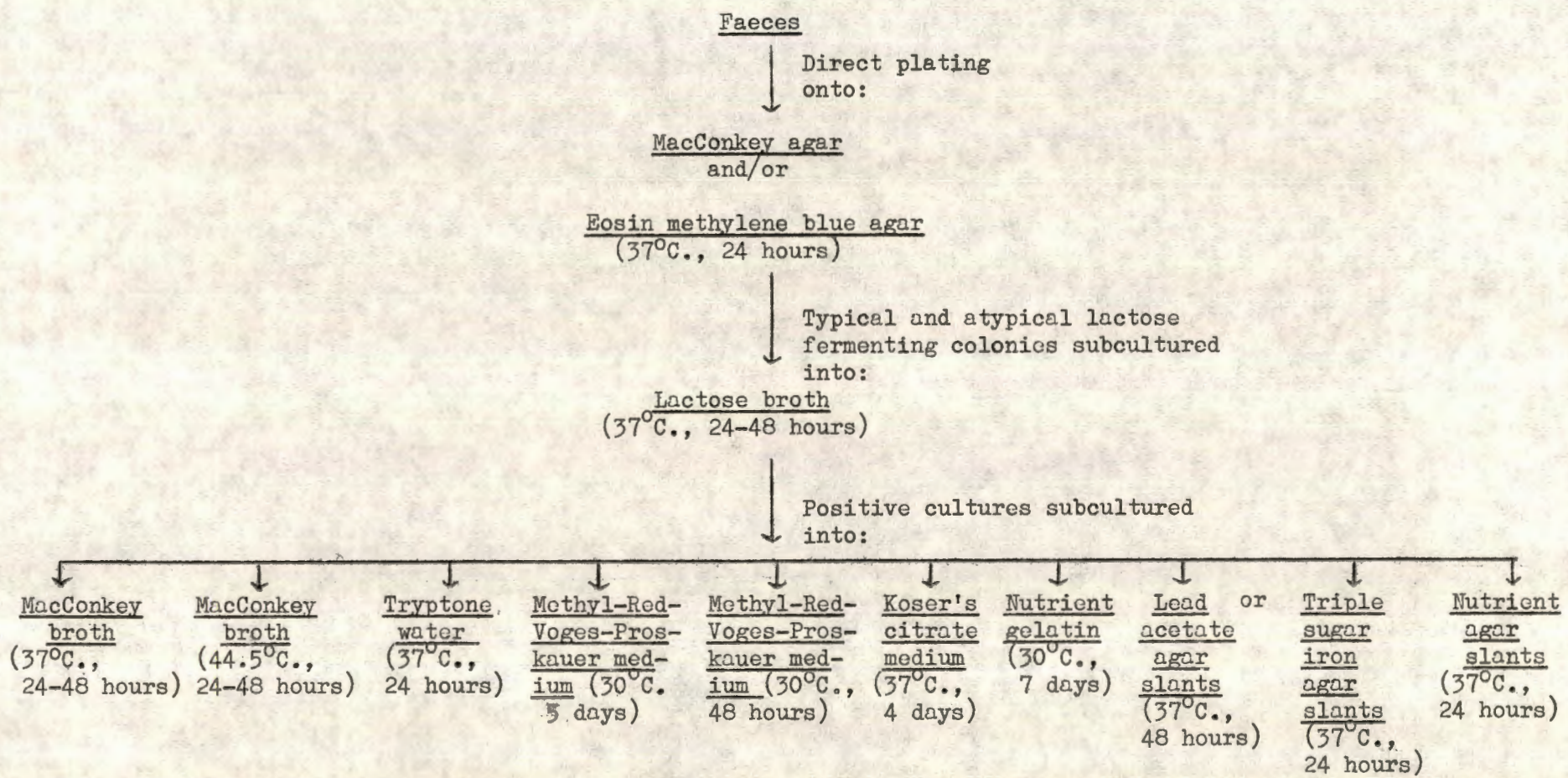
In the first two series (E and E^1) special attention was devoted to the presence of E.coli I, while in the third series (E^2) the plates were inspected more carefully for other members of the coliform group, so as to ascertain whether the media, usually employed, favoured the recovery of all the coliform bacteria or of E.coli I only.

2.2 Collection of faecal specimens

Fresh human faeces were collected and transferred into sterile wide mouth screw-capped 2 oz. bottles (commonly known as "stool jars" or "pomade pots", Cruickshank,1960) by means of wooden spatulas (tongue depressors) supplied wrapped and sterilised by the manufacturer (Winthrop). Fresh animal faeces were similarly sampled into either 2 oz. bottles or sterile 1 oz. McCartney (Universal) bottles. The specimen bottles, wrapped in cotton wool to protect them against temperature loss and breakage, were transported to the laboratory within one hour of collection, and the material was immediately plated. Preserving (Kolmer, Spaulding and Robinson, 1951; Silverton and Anderson 1961), artificial means of temperature control or cooling of the specimens were thus not employed.

2.3/...

2.3 Scheme for the isolation and differentiation of coliforms present in faeces



2.4 Techniques employed in isolating and differentiating the coliforms present in faeces.

Each faecal specimen was plated out directly on to 3 to 5 MacConkey agar plates (Bardsley, 1926, 1934, 1938, 1948; Windle Taylor, 1958; Wilson and Miles, 1955, 1964; The Oxoid Division of Oxo Limited , 1961) and/or Eosin methylene blue (E.M.B.) agar plates (Stuart, Wheeler and Griffin, 1938; Stuart, Griffin and Baker, 1938; Griffin and Stuart, 1940; Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Wilson and Miles, 1955, 1964; Salle, 1961). The plates were incubated at 37°C. for 24 hours (Prescott et al. 1946; Windle Taylor, 1949, 1958; American Public Health Association et al. 1955; Wilson and Miles, 1955, 1964; Great Britain Ministry of Health et al. 1956; Salle, 1961). The following day all the plates were examined and well-isolated typical and atypical lactose fermenting colonies (vide 3.4) were picked off with a straight wire and transferred to Lactose broth tubes supplied with Durham fermentation tubes (Stuart, Wheeler et al. 1938; Stuart, Griffin et al. 1938; Griffin et al. 1940; Prescott et al. 1946; Difco Laboratories Incorporated, 1949, 1953; American Public Health Association et al. 1955; Wilson and Miles, 1955, 1964; Windle Taylor, 1958; Salle, 1961). Where the growth was too luxuriant subcultures were first made onto MacConkey agar or E.M.B. agar. From here separate colonies were subcultured into Lactose broth. The latter were incubated at 37°C. for 24-48 hours (Prescott et al. 1946; American Public Health Association et al. 1955; Wilson and Miles, 1955, 1964; Great Britain Ministry of Health et al. 1956; Society of American Bacteriologists, 1957; Windle Taylor, 1958; Salle, 1961; The Oxoid Division of Oxo Limited, 1961). Depending on the morphological appearance of the lactose fermenting colonies (vide 3.4), 5 to 10 subcultures were made into Lactose broth, in order to obtain the widest possible variety of lactose fermenting strains. In practice this meant that every morphologically different lactose fermenting colony was picked off. All the plates (vide supra) were retained at room temperature for a few days for later reference, if necessary. Because of the inhibitory action of gall and dyes on some of these organisms (Prescott et al. 1946; Allen, Pasley and Pierce, 1952 a; Windle Taylor, 1958), Lactose broth was initially preferred to MacConkey broth or Brilliant green lactose 2% bile broth as primary fermentation medium to obtain maximum recovery of coliforms. Later this practice was discontinued and MacConkey broth was used since :

(1) all/...

(1) all the cultures that were positive in Lactose broth were invariably positive in MacConkey broth (vide 3.5);

(2) the primary plating media (vide supra) contained these inhibitory substances;

(3) in Lactose broth only gas production could be observed;

(4) it was indicated that Klebsiella spp. and Citrobacter spp. tend to overgrow E.coli in Lactose broth (Levine, Tanimoto, Minette, Arakaki and Fernandes, 1955) while it is an inferior medium for the maximum recovery of coliforms when compared with other media (McCrady, 1937; Hajna and Perry, 1943; Perry and Hajna, 1944), and

(5) this procedure accorded better with the definition that was accepted and formulated for the present study (vide infra, 4.1).

As soon as gas production was observed, subcultures were made from every positive Lactose broth culture. Those cultures that evidenced no gas after 48 hours, were discarded (Prescott et al. 1946; American Public Health Association et al. 1955). The following media were employed for subculturing all positive Lactose cultures : 2 tubes of MacConkey broth, 1 tube of Tryptone water, 2 tubes of Methyl-Red - Voges-Proskauer (M.R.-V.P.) medium, 1 tube of Koser's citrate medium, 1 tube of Nutrient gelatin, and 1 tube of Lead acetate agar or Triple sugar iron agar slants (with butts) and 1 Nutrient agar slope.

One tube with MacConkey broth was incubated at 37°C. for 48 hours and the other in a constant temperature waterbath at 44.5°C. for 48 hours, in each case to determine whether acid and gas were produced (Wilson and Miles, 1955, 1964; Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Keller, 1959, 1960; Harrison, Keller, Dimovic and Cholnoky, 1960; The Oxoid Division of Oxo Limited, 1961; Cruickshank, 1960). The incubation temperature of 44.5°C. (Keller, 1959, 1960) was the only digression from the ordinary standard incubation procedure, which operates at 44 °C. (Wilson and Miles, 1955, 1964; Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Cruickshank, 1960). In the present study the temperature was carefully regulated between 44 °C. and 44.5°C. The use of Brilliant green lactose 2% bile broth (Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Wilson and Miles, 1955, 1964; Windle Taylor, 1958)

in the/...

in the earlier stages was later discontinued because of its lack of an acid indicator.

Following incubation at 37°C. for 24 hours the Tryptone water cultures were tested for the presence of indole by adding 0.2 - 0.3 ml. of Kovacs's reagent (Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955). The tubes were shaken and, if not showing a positive reaction immediately, left to stand for a few minutes and examined again. A dark red ring at the surface of the culture was regarded as a positive reaction, while the original colour of the reagent was taken as indole negative (Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Great Britain Ministry of Health et al. 1956; Society of American Bacteriologists, 1957; The Oxoid Division of Oxo Limited, 1961). Tryptone was preferred in this medium instead of peptone, as the former was proved to have a higher quality tryptophan than the latter (Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955, 1960; Society of American Bacteriologists, 1957; The Oxoid Division of Oxo Limited, 1961). The reagent of choice was that of Kovacs, because it was proved superior to others (American Public Health Association et al. 1955, 1960; Society of American Bacteriologists, 1957), like that of Gnezda, Gore, Ehrlich (Koser and Galt, 1926), Böhme (Wilson and Miles, 1964), Salkowski and Ehrlich-Böhme (Levine, 1954).

The cultures in the M.R.-V.P. medium were incubated at 30°C. (Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Great Britain Ministry of Health et al. 1956; Society of American Bacteriologists, 1957; Salle, 1961; The Oxoid Division of Oxo Limited, 1961; Wilson and Miles, 1964). After 48 hours one of the cultures was tested for the Voges-Proskauer (V.P.) reaction by the sensitive Barritt test, i.e. the addition of 0.6 ml. of 5% α- naphthol and 0.2 ml. of a 40% KOH solution to 1 ml. of the culture (Barritt, 1936). A crimson ruby colour forming within 4 hours (usually within 30 minutes) was regarded as a positive V.P. result, while no colour change within 4 hours was considered as negative (American Public Health Association et al. 1955, 1960). After 5 days (120 hours) incubation the Methyl-red (M.R.) test was performed on the remaining culture by adding 5 drops of a 0.0125% Methyl-red solution to 5 ml. of the culture (Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Salle, 1961; The Oxoid Division of Oxo Limited, 1961; Wilson and Miles, 1964).

A distinct/...

A distinct red colour was taken as positive, and yellow as negative.

On inoculating the tube containing Koser's citrate medium, care was taken to transfer the smallest possible inoculum to minimize the possibility of a false interpretation (Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Great Britain Ministry of Health et al. 1956; Society of American Bacteriologists, 1957; Windle Taylor, 1958; Salle, 1961; The Oxoid Division of Oxo Limited, 1961; Wilson and Miles, 1964). This difficulty was later overcome by incorporating 0.016% brom-thymol blue indicator in the Citrate medium. By means of this a colour change from green to blue-green or blue could be noted when growth occurred (Batty-Smith, 1942 b; Prescott et al. 1946; The Oxoid Division of Oxo Limited, 1961). All inoculations were still performed by means of a straight wire, as it was found that minute quantities of organic material could lead to the growth of E.coli (Allen, Pasley and Pierce, 1952 b). Metal test tube caps were used on the Citrate tubes instead of cotton wool plugs, in order to avoid the introduction of extraneous carbon into the medium and thus resulting in the growth of E.coli (Silverton and Anderson, 1961). The tube was incubated at 37°C. for 4 days (96 hours) after which it was examined for any evidence of growth (Prescott et al. 1946; American Public Health Association et al. 1955; Society of American Bacteriologists, 1957).

To test for the ability to liquefy gelatin the tube of Nutrient gelatin was inoculated by the stab technique and incubated at 30°C. (Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; Society of American Bacteriologists, 1957; Windle Taylor, 1958; The Oxoid Division of Oxo Limited, 1961). After 7 days the tube was placed in ice-water or in the refrigerator to test for liquefaction. This normally manifests itself as a loss of the gelatin's faculty to solidify at low temperatures (Prescott et al. 1946; Society of American Bacteriologists, 1957; The Oxoid Division of Oxo Limited, 1961).

Hydrogen sulphide (H_2S) production was tested for on Lead acetate agar (Difco). The butt of the agar was stab-inoculated while the slant was streaked and the tube was incubated at 37°C. for 48 hours (Difco Laboratories Incorporated, 1948, 1953). A blackening or a definite brown colour was taken as an indication of H_2S production. Triple sugar iron (TSI) agar (Baltimore Bacteriological Laboratories) was later employed. It was inoculated as for the Lead acetate agar and incubated at 37°C. for 24 hours. A black colour indicated hydrogen sulphide production.

After/...

ERRATUM

p 23, line 5, for "Taylor (1949)" read "Windle Taylor (1949)"

After inoculation the Nutrient agar slant was incubated at 37°C. for 24 hours (Prescott et al. 1946; Difco Laboratories Incorporated, 1948, 1953; American Public Health Association et al. 1955; Windle Taylor, 1958). Gram's staining method (Jensen's Modification) was performed on a smear from this culture, employing the technique described by Taylor (1949) and Gurr (1957). The remainder of the Nutrient agar culture was kept for reference purposes.

All cultures manifesting mixed growth were purified on MacConkey or E.M.B. agar.

2.5 Methods employed for determining coliforms present in river water

Although the Most Probable Number (MPN) technique is suitable for evaluating the quality of water for public health purposes (Prescott et al. 1946; McEwen, 1949; South African Bureau of Standards, 1951; Galloway and Burgess, 1950; American Public Health Association et al. 1955, 1960; Wilson and Miles, 1955, 1964; Great Britain Ministry of Health. et al. 1956; Klein, 1957; Pelczar and Reid, 1958; Public Health Laboratory Service, 1957; Windle Taylor, 1958; World Health Organization, 1958, 1961, 1963; Burrows, 1959, 1963; Keller, 1959; Coetzee, 1962 b; Cruickshank, 1960, 1965; Department of Water Affairs, Republic of South Africa, 1962; McKee and Wolf, 1963; U.S. Department of Health, Education and Welfare, Public Health Service, 1962) it is ineffective for comparing the coliform flora of different waters because the statistical confidence limits fluctuate too greatly (American Public Health Association et al. 1955, 1960; World Health Organization, 1958, 1963 ; Allen, Pasley and Pierce, 1952 a).

Unfortunately facilities for the membrane filter technique were not available for the major part of the present investigation. The water samples were therefore of necessity examined by the MPN technique, but this was employed in a qualitative rather than a quantitative manner. For this purpose five 10 ml., five 1 ml., and five 0.1 ml. quantities of each sample were tested (Prescott et al. 1946; McEwen, 1949; South African Bureau of Standards, 1951; Galloway and Burgess, 1950; American Public Health Association et al. 1955, 1960; Wilson and Miles, 1955, 1964; Great Britain Ministry of Health et al. 1956; Klein, 1957; Pelczar and Reid, 1958; Public Health Laboratory Service, 1957; Windle Taylor, 1958; World Health Organization, 1958, 1961, 1963; Burrows, 1959, 1963; Keller, 1959, 1960; Coetzee, 1962 b; Cruickshank, 1960, 1965; Department of Water Affairs, Republic of South Africa, 1962; McKee and Wolf, 1963; U.S. Department of Health, Education and Welfare, Public Health Service, 1962). The

last/...

last set was made from a tenfold dilution of the sample. MacConkey broth was employed and all tests were carried out in duplicate. The first series of tubes was incubated at 37°C. for a maximum of 48 hours, to determine the Total coliform index and the second series was pre-incubated for 2 hours at 37°C. and then incubated at 44.5°C. for a maximum of 46 hours (total maximum of 48 hours) to evaluate the Presumptive E.coli I index per 100 ml. As soon as acid and gas were formed the positive cultures from both series were subcultured onto MacConkey agar or E.M.B.agar(Bardsley, 1926, 1934, 1938, 1948) and further tests were performed just as for the faecal specimens (vide supra, 2.4).

All water specimens were collected in sterile 250 ml. glass stoppered reagent bottles, according to the general prescribed procedures (Prescott et al. 1946; South African Bureau of Standards, 1951; American Public Health Association et al. 1955, 1960; Great Britain Ministry of Health et al. 1956; Public Health Laboratory Service, 1957; Windle Taylor, 1958; World Health Organization, 1958, 1961, 1963; Cruickshank, 1960, 1965; Department of Water Affairs, Republic of South Africa, 1962). The samples were transported to the laboratory within two hours and the MPN test was performed immediately. The results were read off from the MPN tables as revised by Swaroop (World Health Organization, 1958, 1963) to compute the Most Probable Number.

2.6 Quantitative method for determining coliforms present in faeces

As pointed out in 2.1 it was only towards the final stages that two specimens were examined quantitatively, employing a modification of the method used by Kjellander (1960). Membrane filtration (molecular filter membrane, molecular filter, millipore filter) is a recognised quantitative method for testing water accurately for the presence of coliforms (Goetz, 1952; Slanetz and Bartley, 1955; Great Britain Ministry of Health et al. 1956; Pelczar and Reid, 1958; Windle Taylor, 1958; American Public Health Association et al. 1960; Salle, 1961; Burrows, 1963; McKee and Wolf, 1963; World Health Organization, 1963; Wilson and Miles, 1964; Cruickshank, 1965). It was satisfactorily adapted for faeces, although this was a laborious task. By means of a sensitive chemical balance 1.0 g. of fresh faeces was weighed into a sterilised glass-stoppered 10 ml. measuring cylinder. The faeces were transferred with two sterile straight nichrome wires, of which the points were drawn out to form minute spatulas. The faeces were deposited on the bottom of the measuring cylinder only, so as to facilitate accurate measuring of the diluent. Sterile distilled water was carefully measured into the cylinder up to the 10 ml. mark, in order to obtain a 10% faecal suspension. The cylinder was stoppered and shaken vigorously by hand to obtain a

thorough/

thorough suspension. From this tenfold dilutions were made into sterile 1 oz. McCartney bottles, using a separate sterile pipette for each dilution.

A sterilised filter membrane (The Oxoid Division of Oxo Limited, 1961; Windle Taylor and Burman, 1964) - 5 cm. (diameter) Oxoid grid-lined type - was assembled in a sterile 100 ml. Oxoid metal filter holding unit connected to an electric vacuum pump (Clark and Kabler, 1952; Task Group Report, 1953; The Oxoid Division of Oxo Limited, 1961). The funnel was rinsed with sterile distilled water and the membrane soaked well (The Oxoid Division of Oxo Limited, 1961).

Approximately 30 ml.of sterile distilled water was measured into the filtering funnel (Burrows, 1963) and the appropriate dilution of the faecal suspension added. The funnel was gently swirled to obtain mixing and suction was applied. After filtration the funnel was rinsed 2 to 4 times with 10 to 30 ml. of sterile distilled water and the membrane sucked dry again (Clark and Kabler, 1952; Kabler and Clark, 1952; American Public Health Association et al. 1960).

The membrane was removed from the filter holder by means of sterile forceps and transferred, grid-side up to a small petri dish containing a soaked resuscitation pad (i.e. two sterilised circles of Whatman No 2, 5.5 cm. filter paper). Care was taken not to entrap any air bubbles and that the entire membrane contacted the pad (Clark and Kabler, 1952; Kabler and Clark, 1952; Task Group Report, 1953; McKee and McLaughlin, 1958; The Oxoid Division of Oxo Limited, 1961; Windle Taylor and Burman, 1964) .

Each dilution, viz. 0.001, 0.0001, 0.00001, 0.000001 and 0.0000001 ml, was done in duplicate. To obtain the Total coliform count, half of the duplicates were "pre-incubated" on Resuscitation membrane broth (Oxoid) for one hour at 37°C. Immediately after one hour the membranes were transferred without any delay to soaked MacConkey membrane (broth) pads and returned to the incubator for a further 17 hours, i.e. total incubation time of 18 hours (The Oxoid Division of Oxo Limited, 1961; Windle Taylor and Burman, 1964). For the Presumptive E.coli I counts the resuscitation plates were "pre-incubated"for two hours at 37°C. The membranes were then transferred to MacConkey membrane broth (Oxoid) and the plates packed in water-tight canisters, incubated in a waterbath at 44.5°C. for a further 16 hours, i.e. total incubation time of 18 hours

(The/...

(The Oxoid Division of Oxo Limited, 1961; Windle Taylor and Burman, 1964).

After 18 hours total incubation all plates were counted while the membranes and pads were still warm. All lactose fermenting colonies were yellow (or shades of yellow) and were easily counted by the naked eye against the light blue background (The Oxoid Division of Oxo Limited, 1961).

From the Total coliform and Presumptive E.coli I membranes having 3 to 300 colonies (Task Group Report, 1953) 10 to 40 lactose fermenting colonies were subcultured into the set of differential media given in 2.3 and 2.4. Whenever necessary colonies were purified on MacConkey agar.

2.7 Media

The primary plating media were made up by mixing the ingredients according to the recipes given by The Oxoid Division of Oxo Limited (1961) for the neutral red crystal violet modification of MacConkey agar and by Difco Laboratories Incorporated (1948, 1953) for E.M.B. (Levine) agar. Likewise the primary fermentation medium, viz. Lactose broth was made up by adding 0.5% lactose to Difco Nutrient broth. MacConkey broth (purple) and Brilliant green lactose 2% bile broth (i.e. the secondary fermentation media) were made up according to the methods described by the Oxoid Division of Oxo Limited (1961) and Difco Laboratories Incorporated (1948, 1953) respectively.

The differential media were accordingly made up : Tryptone water (Difco Laboratories Incorporated, 1948, 1953), M.R.-V.P. medium (at first Difco dehydrated medium and later made up according to the recipe of Difco Laboratories Incorporated, 1948, 1953), Koser's citrate medium (first Difco dehydrated medium, later brom-thymol blue added to this according to the Oxoid Division of Oxo Limited, 1961), dehydrated Difco Nutrient broth plus 12% gelatin, Lead acetate agar (dehydrated Difco medium), T.S.I. agar (dehydrated Baltimore Bacteriological Laboratories medium) and dehydrated Difco Nutrient broth plus 2% agar.

The media given in 2.6, viz. Resuscitation membrane broth and MacConkey membrane broth were dehydrated products of Oxoid.

In all cases the directions given by the Oxoid Division of Oxo Limited (1961), Difco Laboratories Incorporated (1948, 1953) and those on the label of the product of the Baltimore Bacteriological Laboratories were strictly followed as far as preparation, autoclaving and distribution were concerned.

The/...

The ingredients used for making up media, were as follows : dehydrated Nutrient broth (Difco), lactose (Bacto), peptone (Bacto), commercial sodium taurocholate (Hopkin and Williams Ltd.) sodium chloride (Protea), brom-cresol purple (Merck), dehydrated oxbile (Bacto), brilliant green (Gurr), tryptone (Bacto), dextrose (Bacto), K_2HPO_4 (Merck), gelatin (Bacto), brom-thymol blue (Merck), eosin, yellow (Riedel De Haën), methylene blue (Merck), neutral red (Merck), crystal violet (Merck) and agar (Bacto).

2.8 Reagents and stains

The following reagents were used for Kovacs's test : paradimethyl-aminobenzaldehyde (Merck), amyl alcohol, Analar (Hopkin and Williams Ltd.), and hydrochloric acid, concentrated, A.R. (Riedel De Haën).

For the M.R. test Merck products were employed (i.e. methyl-red and ethyl alcohol, absolute). Merck products were also utilised for the V.P. test, viz. α - naphthol, ethyl alcohol (absolute) and KOH.

In Gram's staining method the following Merck dyes and reagents were employed : methyl violet 6 B, iodine, potassium iodide, neutral red and glacial acetic acid.

3. Results/...

3. RESULTS

3.1 Introductory remarks

The results of the present study are tabulated in 3.5 (vide infra). In 3.4 (vide infra) an explanation is given of the symbols and abbreviations which are used in those Tables showing the coliforms isolated from the various subgroups(vide 3.5). In the last column of each of the Tables giving the coliform flora of the various subgroups the names of all the coliforms isolated, other than typical E.coli I are given. Where no name is shown, it implies that the organism is typical E.coli I. This was done in order to facilitate the reading of these Tables.

3.2 Morphological characteristics

All coliform cultures isolated and purified were Gram-negative, non-sporing bacilli, mostly extremely short rods. Capsules were not seen among the E.coli strains, but sometimes Irregular II bacilli were encapsulated, confirming the work of Windle Taylor (1958). Among the Klebsiella and Irregular VI strains the occurrence of capsules was common.

3.3 Purification of cultures

A phenomenon which should be mentioned here is the problem of obtaining purified coliform cultures. It was pointed out by Parr (1938) that Ruchhoft, Kallas, Chinn and Coulter emphasized the problem of purification in 1931. Consequently Parr, in his work, subjected "a number of strains" to "detailed purification involving 28 serial transplants on various mediums including seven platings and pickings." This necessity for repeated replating to obtain purification was again stressed by Frank and Skinner (1941).

During the present study this problem was often encountered, both in faecal specimens and water samples. In faecal specimens, E.coli I and Irregular II cultures, yielding citrate positive reactions, were most common. Invariably this was due to a mixed growth with short Gram-positive chains, most probably strains pertaining to the Streptococcus faecalis group. This amazingly persistent co-growth occurred even after many replatings and subcultures in Lactose and MacConkey broth.

From water samples, mixed growths of various coliform organisms and of coliforms and streptococci occurred. Because of these mixed growths Parr's Intermediate VIII and X (vide 4.2) were encountered. After careful purification, sometimes after 20 subcultures, more than one strain of coliform and/or of streptococcus could be separated.

3.4/...

3.4 Explanation of symbols and abbreviations used in Tables in 3.5

+ on E.M.B. agar =	Typical E.coli appearance, i.e. dark, almost black, centred colonies, with a typical greenish metallic sheen, while neighbouring colonies do not tend to become confluent.
- on E.M.B. agar =	Typical K.aerogenes appearance, i.e. colonies usually bigger than E.coli, brown centred (not as dark as E.coli), without metallic sheen, while neighbouring colonies tend to become confluent.
+ on MacC. agar =	Typical E.coli appearance, i.e. non-mucoid, dark (brick) red colonies, sometimes with a paler periphery.
- on MacC. agar =	Typical K.aerogenes appearance, i.e mucoid, pink colonies.
G in Lact. broth =	Fermentation of Lactose with the production of gas.
- in Lact. broth =	Lactose not fermented.
AG in MacC. broth =	Fermentation of Lactose with the production of acid and gas.
Ag in MacC. broth =	Fermentation of Lactose with the production of acid and a bubble of gas only.
A - in MacC. broth =	Fermentation of Lactose with the production of acid only.
- in MacC. broth =	Lactose not fermented.
+ Indole =	Indole produced in Tryptone water.
- Indole =	Indole not produced in Tryptone water.
+ M.R. =	Acid produced from dextrose in M.R.-V.P. medium.
- M.R. =	Acid not produced from dextrose in M.R.-V.P. medium.
+ V.P. =	Acetylmethylcarbinol produced from dextrose in M.R.-V.P. medium.
- V.P. =	Acetylmethylcarbinol not produced from dextrose in M.R.-V.P. medium.
+ Citrate =	Able to grow in Citrate medium, i.e. citrate utilisation.
- Citrate =	Unable to grow in Citrate medium.
+ Gelatin =	Able to liquefy Gelatin.
- Gelatin =	Unable to liquefy Gelatin.
+ H_2S =	Able to produce H_2S in the appropriate medium.
- H_2S =	Unable to produce H_2S in the appropriate medium.

E.M.B. agar =	Eosin methylene blue agar.
MacC. agar =	MacConkey agar.
MacC. broth =	MacConkey broth.
Lact. broth =	Lactose broth.
M.R. =	Methyl-red test.
V.P. =	Voges-Proskauer test.
M.R.-V.P. medium =	Methyl-Red-Voges-Proskauer medium.
H_2S =	Test for the production of hydrogen sulphide.
T.S.I. agar =	Triple sugar iron agar.
hrs =	hours.

3.5 Results

TABLE 1/...

TABLE 1

COLIFORM FLORA OF SUBGROUP A

Sample no	Strain no	E.M.B. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a	+	G	AG	AG	+	+	-	-	-	+	E.coli I (?)
1	b-j	+	G	AG	AG	+	+	-	-	-	-	
2	a-j	+	G	AG	AG	+	+	-	-	-	-	
3	a-j	+	G	AG	AG	+	+	-	-	-	-	
4	a-j	+	G	AG	AG	+	+	-	-	-	-	
5	a-e	+	G	AG	AG	+	+	-	-	-	-	
6	a	+	G	AG	AG	-	+	-	-	-	-	Irregular II
6	b-e	+	G	AG	AG	+	+	-	-	-	-	
7	a	+	G	AG	AG	-	+	-	-	-	-	Irregular II
7	b-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-e	+	G	AG	AG	+	+	-	-	-	-	
9	a-e	+	G	AG	AG	+	+	-	-	-	-	
10	a-e	+	G	AG	AG	+	+	-	-	-	-	
11	a-e	+	G	AG	AG	+	+	-	-	-	-	
12	a,b	+	G	AG	AG	-	+	-	-	-	-	Irregular II
12	c-e	+	G	AG	AG	+	+	-	-	-	-	
13	a-e	+	G	AG	AG	+	+	-	-	-	-	
14	a-e	+	G	AG	AG	+	+	-	-	-	-	
15	a-e	+	G	AG	AG	+	+	-	-	-	-	
16	a-e	+	G	AG	AG	+	+	-	-	-	-	
17	a-e	+	G	AG	AG	+	+	-	-	-	-	
18	a-e	+	G	AG	AG	+	+	-	-	-	-	
19	a-e	+	G	AG	AG	+	+	-	-	-	-	
20	a-e	+	G	AG	AG	+	+	-	-	-	-	
21	a-e	+	G	AG	AG	+	+	-	-	-	-	
22	a	+	G	AG	AG	+	-	-	-	-	-	Atypical E.coli I
22	b-e	+	G	AG	AG	+	+	-	-	-	-	
23	a-e	+	G	AG	AG	+	+	-	-	-	-	
24	a	+	G	AG	AG	-	+	-	-	-	-	Irregular II
24	b-e	+	G	AG	AG	+	+	-	-	-	-	
25	a-e	+	G	AG	AG	-	+	-	-	-	-	Irregular II

TABLE 2

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP A

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	24	96	133	91.72
E.coli I H_2S+	1	4	1	0.69
Atypical E.coli I	1	4	1	0.69
Irregular II	5	20	10	6.90
E.coli I only	18	72		
Irregular II only	1	4		
E.coli I and E.coli I H_2S+	1	4		
E.coli I and atypical E.coli I	1	4		
E.coli I and Irregular II	4	16		
TOTAL	25	100	145	100.00

TABLE 3

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP A,

GROUPING ALL ATYPICAL AND TYPICAL STRAINS TOGETHER.

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	24	96	135	93.1
Irregular II	1	4	10	6.9
E.coli I only	20	80		
Irregular II only	1	4		
E.coli I and Irregular II	4	16		
TOTAL	25	100	145	100.0

The strain of E.coli I which produced hydrogen sulphide (vide Table 1), only caused a browning of the medium, but enough colour was visible to record it as positive.

TABLE 4

COLIFORM FLORA OF SUBGROUP A[1]

Sample no	Strain no	E.M.B. agar 37°C. 24 hrs	Lact.broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30.°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a-g	+	G	AG	AG	+	+	-	-	-	-	
2	a,b,d,e	+	G	AG	AG	+	+	-	-	-	-	
2	c	+	G	AG	AG	-	+	-	-	-	-	Irregular II
3	a-e	+	G	AG	AG	+	+	-	-	-	-	
4	a,c-f	+	G	AG	AG	+	+	-	-	-	-	
4	b	+	G	AG	AG	-	+	-	-	-	-	Irregular II
5	a-f	+	G	AG	AG	+	+	-	-	-	-	
6	a-f	+	G	AG	AG	+	+	-	-	-	-	
7	a-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-f	+	G	AG	AG	+	+	-	-	-	-	
9	a-e	+	G	AG	AG	+	+	-	-	-	-	
10	a-f	+	G	AG	AG	+	+	-	-	-	-	
11	a-e	+	G	AG	AG	+	+	-	-	-	-	
12	a-e	+	G	AG	AG	+	+	-	-	-	-	
13	a-e	+	G	AG	AG	+	+	-	-	-	-	
14	a-e	+	G	AG	AG	+	+	-	-	-	-	
15	a-e	+	G	AG	AG	+	+	-	-	-	-	
16	a-e	+	G	AG	AG	+	+	-	-	-	-	
17	a-e	+	G	AG	AG	+	+	-	-	-	-	
18	a	+	G	AG	-***	+	+	-	-	-	-	E.coli III
18	b-e	+	G	AG	AG	+	+	-	-	-	-	
19	a-e	+	G	AG	AG	+	+	-	-	-	-	
20	a-g	+	G	AG	AG	+	+	-	-	-	-	
21	a-g	+	G	AG	AG	+	+	-	-	-	-	
22	a-f	+	G	AG	AG	+	+	-	-	-	-	
23	a-e	+	G	AG	AG	+	+	-	-	-	-	
24	a-e	+	G	AG	AG	+	+	-	-	-	-	
25	a-e,g	+	G	AG	AG	+	+	-	-	-	-	
25	f	-	G*	AG**	-***	-	-	+	+	-	-	K.aerogenes I

* G in 48 hrs

** AG in 48 hrs

*** Negative AG in 48 hrs

TABLE 5

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP A[1]

Coliform strains	Nc of Samples	% of Samples	No of Strains	% of Strains
E.coli I	25	100	135	97.12
E.coli III	1	4	1	0.72
K.aerogenes I	1	4	1	0.72
Irregular II	2	8	2	1.44
E.coli I only	21	84		
E.coli I and *E.coli* III	1	4		
E.coli I and *K.aerogenes* I	1	4		
E.coli I and Irregular II	2	8		
TOTAL	25	100	139	100.00

TABLE 6

DISTRIBUTION OF COLIFORM STRAINS IN FOWLS.

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	49	98	268	94.37
Irregular II	7	14	12	4.23
E.coli I H_2S+	1	2	1	0.35
Atypical *E.coli* I	1	2	1	0.35
E.coli III	1	2	1	0.35
K.aerogenes I	1	2	1	0.35
E.coli I only	39	78		
Irregular II only	1	2		
E.coli I and Irregular II	6	12		
E.coli I and *E.coli* I H_2S+	1	2		
E.coli I and atypical *E.coli* I	1	2		
E.coli I and *E.coli* III	1	2		
E.coli I and *K.aerogenes* I	1	2		
TOTAL	50	100	284	100.00

TABLE 7

DISTRIBUTION OF COLIFORM STRAINS IN FOWLS,
GROUPING ALL ATYPICAL AND TYPICAL STRAINS TOGETHER.

Coliform strains	Nc of Samples	% of Samples	Nc. of Strains	% of Strains
E.coli I	49	98	270	95.07
E.coli III	1	2	1	0.35
K.aerogenes I	1	2	1	0.35
Irregular II	7	14	12	4.23
E.coli I only	41	82		
Irregular II only	1	2		
E.coli I and E.coli III	1	2		
E.coli I and K.aerogenes I	1	2		
E.coli I and Irregular II	6	12		
TOTAL	50	100	284	100.00

TABLE 8

COLIFORM FLORA OF SUBGROUP B

Sample no	Strain no	E.M.B.agar 37°C. 24 hrs	Lact.broth 37°C. 24 hrs	MacC.broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a-j	+	G	AG	AG	+	+	-	-	-	-	
2	a-j	+	G	AG	AG	+	+	-	-	-	-	
3	a-j	+	G	AG	AG	+	+	-	-	-	-	
4	a-c,e-j	+	G	AG	AG	+	+	-	-	-	-	
4	d	-	G	AG	-*	+	-	+	+	-	-	K.aerogenes II
5	a	+		AG	A-**	-	+	-	-	-	-	E.coli II (?)
5	b-e	+		AG	AG	+	+	-	-	-	-	
6	a-e	+		AG	AG	+	+	-	-	-	-	
7	a-e	+		AG	AG	+	+	-	-	-	-	
8	a-e	+		AG	AG	+	+	-	-	-	-	
9	a-e	+		AG	AG	+	+	-	-	-	-	
10	a-e	+		AG	AG	+	+	-	-	-	-	
11	a-e	+		AG	AG	+	+	-	-	-	-	
12	a-e	+		AG	AG	+	+	-	-	-	-	
13	a-e	+		AG	AG	+	+	-	-	-	-	
14	a-e	+		AG	AG	+	+	-	-	-	-	
15	a-e	+		AG	AG	+	+	-	-	-	-	
16	a	+		AG	AG***	-	+	-	-	-	-	Irregular II
16	b-e	+		AG	AG	+	+	-	-	-	-	
17	a-e	+		AG	AG	+	+	-	-	-	-	
18	a-e	+		AG	AG	+	+	-	-	-	-	
19	a-e	+		AG	AG	+	+	-	-	-	-	
20	a-e	+		AG	AG	+	+	-	-	-	-	
21	a-e	+		AG	AG	+	+	-	-	-	-	
22	a-e	+		AG	AG	+	+	-	-	-	-	
23	a-e	+		AG	AG	+	+	-	-	-	-	
24	a-e	+		AG	AG	+	+	-	-	-	-	
25	a-e	+		AG	AG	+	+	-	-	-	-	

* Negative AG in 48 hrs
** A in 36 hrs, negative G in 48 hrs
*** AG in 48 hrs

TABLE 9

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP B

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	25	100	142	97.93
E.coli II	1	4	1	0.69
K.aerogenes II	1	4	1	0.69
Irregular II	1	4	1	0.69
E.coli I only	22	88		
E.coli I and *E.coli* II	1	4		
E.coli I and *K.aerogenes* II	1	4		
E.coli I and Irregular II	1	4		
TOTAL	25	100	145	100.00

The *E.coli* II strain (*vide* Table 8) was atypical, as it produced acid in MacConkey broth at 44.5°C., while the *K.aerogenes* II strain formed more gas than this type usually does (Windle Taylor, 1958). (Usually this strain only forms a bubble of gas.)

TABLE 10

COLIFORM FLORA OF SUBGROUP B[1]

Sample no	Strain no	E.M.B.agar 37°C. 24 hrs	Lact.broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a-j	+	G	AG	AG	-	+	-	-	-	-	Irregular II
2	a-e	+	G	AG	AG	+	+	-	-	-	-	
3	a-e	+	G	AG	AG	+	+	-	-	-	-	
4	a-e	+	G	AG	AG	+	+	-	-	-	-	
5	a-e	+	G	AG	AG	+	+	-	-	-	-	
6	a-e	+	G	AG	AG	+	+	-	-	-	-	
7	a-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-e	+	G	AG	AG	+	+	-	-	-	-	
9	a-e	+	G	AG	AG	+	+	-	-	-	-	
10	a-e	+	G	AG	AG	+	+	-	-	-	-	
11	a-e	+	G	AG	AG	+	+	-	-	-	-	
12	a-e	+	G	AG	AG	+	+	-	-	-	-	
13	a-e	+	G	AG	AG	+	+	-	-	-	-	
14	a-e	+	G	AG	AG	+	+	-	-	-	-	
15	a-e	+	G	AG	AG	+	+	-	-	-	-	
16	a-e	+	G	AG	AG	+	+	-	-	-	-	
17	a-e	+	G	AG	AG	+	+	-	-	-	-	
18	a-e	+	G	AG	AG	+	+	-	-	-	-	
19	a-e	+	G	AG	AG	+	+	-	-	-	-	
20	a-e	+	G	AG	AG	+	+	-	-	-	-	
21	a-e	+	G	AG	AG	+	+	-	-	-	-	
22	a-e	+	G	AG	AG	+	+	-	-	-	-	
23	a-e	+	G	AG	AG	+	+	-	-	-	-	
24	a-e	+	G	AG	AG	+	+	-	-	-	-	
25	a-e	+	G	AG	AG	+	+	-	-	-	-	

TABLE 11

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP B[1]

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	24	96	120	92.3
Irregular II	1	4	10	7.7
E.coli I only	24	96		
Irregular II only	1	4		
TOTAL	25	100	130	100.0

TABLE 12

DISTRIBUTION OF COLIFORM STRAINS IN SHEEP

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	49	98	262	95.2
E.coli II	1	2	1	0.4
K.aerogenes II	1	2	1	0.4
Irregular II	2	4	11	4.0
E.coli I only	46	92		
Irregular II only	1	2		
E.coli I and E.coli II	1	2		
E.coli I and K.aerogenes II	1	2		
E.coli I and Irregular II	1	2		
TOTAL	50	100	275	100.0

TABLE 13

COLIFORM FLORA OF SUBGROUP C

Sample no	Strain no	E.M.B. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a-j	+	G	AG	AG	+	+	-	-	-	-	
2	a-j	+	G	AG	AG	+	+	-	-	-	-	
3	a-j	+	G	AG	AG	+	+	-	-	-	-	
4	a-j	+	G	AG	AG	+	+	-	-	-	-	
5	a-e	+	G	AG	AG	+	+	-	-	-	-	
6	a-e	+	G	AG	AG	+	+	-	-	-	-	
7	a-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-e	+	G	AG	AG	+	+	-	-	-	-	
9	a-e	+	G	AG	AG	+	+	-	-	-	-	
10	a-e	+	G	AG	AG	+	+	-	-	-	-	
11	a-e	+	G	AG	AG	+	+	-	-	-	-	
12	a-e	+	G	AG	AG	+	+	-	-	-	-	
13	a-e	+	G	AG	AG	+	+	-	-	-	-	
14	a-e	+	G	AG	AG	+	+	-	-	-	-	
15	a-e	+	G	AG	AG	+	+	-	-	-	-	
16	a	+	G	AG	AG	-	+	-	-	-	-	Irregular II
16	b-e	+	G	AG	AG	+	+	-	-	-	-	
17	a-e	+	G	AG	AG	+	+	-	-	-	-	
18	a,c,e	+	G	AG	AG	+	+	-	-	-	-	
18	b	+	G	AG	AG	-	+	-	-	-	-	Irregular II
18	d	-	G*	AG**	-***	-	-	+	+	-	-	K.aerogenes I
19	a-e	+	G	AG	AG	+	+	-	-	-	-	
20	a-e	+	G	AG	AG	+	+	-	-	-	-	
21	a-e	+	G	AG	AG	+	+	-	-	-	-	
22	a-e	+	G	AG	AG	+	+	-	-	-	-	
23	a-e	+	G	AG	AG	+	+	-	-	-	-	
24	a-e	+	G	AG	AG	+	+	-	-	-	-	
25	a-e	+	G	AG	AG	+	+	-	-	-	-	

* G in 48 hrs
** AG in 48 hrs
*** Negative AG in 48 hrs

TABLE 14

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP C

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	25	100	142	97.93
K.aerogenes I	1	4	1	0.69
Irregular II	2	8	2	1.38
E.coli I only	23	92		
E.coli I, K.aerogenes I and Irregular II	1	4		
E.coli I and Irregular II	1	4		
TOTAL	25	100	145	100.00

TABLE 15

COLIFORM FLORA OF SUBGROUP C^1

Sample no	Strain no	E.M.B. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a-e	+	G	AG	AG	+	+	-	-	-	-	
2	a-e	+	G	AG	AG	+	+	-	-	-	-	
3	a-e	+	G	AG	AG	+	+	-	-	-	-	
4	a-e	+	G	AG	AG	+	+	-	-	-	-	
5	a-e	+	G	AG	AG	+	+	-	-	-	-	
6	a-e	+	G	AG	AG	+	+	-	-	-	-	
7	a-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-e	+	G	AG	AG	+	+	-	-	-	-	
9	a-e	+	G	AG	AG	+	+	-	-	-	-	
10	a-e	+	G	AG	AG	+	+	-	-	-	-	
11	a-e	+	G	AG	AG	+	+	-	-	-	-	
12	a-e	+	G	AG	AG	+	+	-	-	-	-	
13	a-e	+	G	AG	AG	+	+	-	-	-	-	
14	a-e	+	G	AG	AG	+	+	-	-	-	-	
15	a-e	+	G	AG	AG	+	+	-	-	-	-	
16	a-e	+	G	AG	AG	+	+	-	-	-	-	
17	a-e	+	G	AG	AG	+	+	-	-	-	-	
18	a-e	+	G	AG	AG	+	+	-	-	-	-	
19	a-e	+	G	AG	AG	+	+	-	-	-	-	
20	a-e	+	G	AG	AG	+	+	-	-	-	-	
21	a-e	+	G	AG	AG	+	+	-	-	-	-	
22	a-e	+	G	AG	AG	+	+	-	-	-	-	
23	a	+	G	AG	-*	+	+	-	-	-	-	E.coli III
23	b-e	+	G	AG	AG	+	+	-	-	-	-	
24	a-e	+	G	AG	AG	+	+	-	-	-	-	
25	a-e	+	G	AG	AG	+	+	-	-	-	-	

* Negative AG in 48 hrs

TABLE 16

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP C^1

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	25	100	124	99.2
E.coli III	1	4	1	0.8
E.coli I only	24	96		
E.coli I and E.coli III	1	4		
TOTAL	25	100	125	100.0

TABLE 17

DISTRIBUTION OF COLIFORM STRAINS IN CATTLE

Coliform strains	No of Samples	% of Samples	No of Strains	%. of Strains
E.coli I	50	100	266	98.52
E.coli III	1	2	1	0.37
K.aerogenes I	1	2	1	0.37
Irregular II	2	4	2	0.74
E.coli I only	47	94		
E.coli I and E.coli III	1	2		
E.coli I, K.aerogenes I and Irregular II	1	2		
E.coli I and Irregular II	1	2		
TOTAL	50	100	270	100.00

TABLE 18

COLIFORM FLORA OF SUBGROUP D

Sample no	Strain no	MacC. agar 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S +++ 37°C. 24 hrs	Micro-organism
1	a-e	+	AG	AG	+	+	-	-	-	-	
2	a-e	+	AG	AG	+	+	-	-	-	-	
3	a,b	+	AG	AG	+	+	-	-	-	-	
3	c-e	-	AG	-+	+	+	-	-	-	-	E.coli III
4	a,b,d,e	+	AG	AG	+	+	-	-	-	-	
4	c	+	AG	AG**	+	+	-	-	-	-	
5	a,b	+	AG	-+	-	+	-	-	-	-	E.coli II
5	c-e	+	AG	AG**	-	+	-	-	-	-	Irregular II
6	a-e	+	AG	AG	+	+	-	-	-	-	
7	a,b	+	AG	AG	+	+	-	-	-	-	
7	c-e	-	AG	AG**	-	-	+	+	-	-	Irregular VI
8	a	+	AG	-+	+	+	-	-	-	-	E.coli III
8	b-e	+	AG	AG	+	+	-	-	-	-	
9	a-e	+	AG	AG	+	+	-	-	-	-	
10	a,b	+	AG	AG	+	+	-	-	-	-	
10	c-e	-	AG	-+	-	-	+	+	-	-	K.aerogenes I
11	a,b,e	+	AG	AG	+	+	-	-	-	-	
11	c,d	-	AG	-+	-	-	+	+	-	-	K.aerogenes I
12	a	-	AG	-+	-	-	+	-	-	-	Irregular V
12	b,e	-	AG	-+	-	-	+	+	-	-	K.aerogenes I
12	c,d	-	AG	AG	-	-	+	+	-	-	Irregular VI
13	a	+	AG	A-++	+	+	-	-	-	-	E.coli III (?)
13	b-e	+	AG	AG	+	+	-	-	-	-	
14	a-e	+	AG	AG	+	+	-	-	-	-	
15	a,b,d	+	AG	AG	+	+	-	-	-	-	
15	c,e	-	AG*	-+	-	-	+	+	-	-	K.aerogenes I
16	a,b	+	AG	AG	+	+	-	-	-	-	
16	c,e,f	-	AG	-+	-	-	+	+	-	-	K.aerogenes I
16	d	-	AG	AG**	-	-	+	+	-	-	Irregular VI
17	a,b,e	+	AG	AG	+	+	-	-	-	-	
17	c	-	AG	AG	-	-	+	+	-	-	Irregular VI
17	d	-	AG	-+	-	+	-	+	+	-	Irregular IV
18	a,c	+	AG	-+	+	+	-	-	-	-	E.coli III
18	b	+	AG	AG	+	+	-	-	-	-	
18	d,e	+	AG	AG**	+	+	-	-	-	-	
19	a-e	+	AG	AG	+	+	-	-	-	-	
20	a	+	AG	AG**	+	+	-	-	-	-	
20	b-e	+	AG	AG	+	+	-	-	-	-	
21	a,b	+	AG	AG	+	+	-	-	-	+	E.coli I (?)
21	c-e	+	AG	AG	+	+	-	-	-	-	
22	a-e	+	AG	AG	+	+	-	-	-	-	

* AG in 48 hrs
** AG in 48 hrs

\+ Negative AG in 48 hrs
++ A in 24 hrs, negative G in 48 hrs
+++ H_2S production tested on T.S.I. agar instead of Lead acetate agar.

TABLE 19

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP D

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	20	90.9	76	68.47
E.coli I H_2S+	1	4.6	2	1.80
E.coli II	1	4.6	2	1.80
E.coli III	4	18.2	7	6.31
K.aerogenes I	5	22.7	12	10.81
Irregular II	1	4.6	3	2.70
Irregular IV	1	4.6	1	0.90
Irregular V	1	4.6	1	0.90
Irregular VI	4	18.2	7	6.31
E.coli I only	9	40.90		
E.coli I and E.coli I H_2S+	1	4.55		
E.coli I and E.coli III	4	18.17		
E.coli I and K.aerogenes I	3	13.63		
E.coli I, K.aerogenes I and Irregular VI	1	4.55		
E.coli I, Irregular IV and Irregular VI	1	4.55		
E.coli I and Irregular VI	1	4.55		
E.coli II and Irregular II	1	4.55		
K.aerogenes I, Irregular V and Irregular VI	1	4.55		
TOTAL	22	100.00	111	100.00

TABLE 20

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP D, GROUPING ALL ATYPICAL AND TYPICAL STRAINS TOGETHER

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	20	90.9	78	70.27
E.coli II	1	4.6	2	1.80
E.coli III	4	18.2	7	6.31
K.aerogenes I	5	22.7	12	10.81
Irregular II	1	4.6	3	2.70
Irregular IV	1	4.6	1	0.90
Irregular V	1	4.6	1	0.90
Irregular VI	4	18.2	7	6.31
E.coli I only	10	45.45		
E.coli I and E.coli III	4	18.17		
E.coli I and K.aerogenes I	3	13.63		
E.coli I, K.aerogenes I and Irregular VI	1	4.55		
E.coli I, Irregular IV and Irregular VI	1	4.55		
E.coli I and Irregular VI	1	4.55		
E.coli II and Irregular II	1	4.55		
K.aerogenes I, Irregular V and Irregular VI	1	4.55		
TOTAL	22	100.00	111	100.00

TABLE 21

COLIFORM FLORA OF SUBGROUP D[1]

Sample no	Strain no	E.M.B. agar 37°C. 24 hrs	MacC. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 24-48 hrs	Micro-organism
1	a-e	+		G	AG	AG	+	+	-	-	-	-	
2	a-b		+		AG	AG	+	+	-	-	-	-	
2	c		+		AG	-+	-	+	-	+	-	+	Cit.freundii I
2	d-f		-		AG	-+	-	-	+	+	-	-	K.aerogenes I
3	a-e	+		G	AG	AG	+	+	-	-	-	-	
4	a-e	+		G	AG	AG	+	+	-	-	-	-	
5	a,c,d	+		G	AG	AG	+	+	-	-	-	-	
5	b,e	+		G	AG	AG	+	+	-	-	-	-	Irregular II
6	a,b,d,e	+		G	AG	AG	+	+	-	-	-	-	
6	c	-		G	AG	AG	-	-	+	+	-	-	Irregular VI
7	a-e	+		G	AG	AG	+	+	-	-	-	-	
8	a,b,d,e	+		G	AG	AG	+	+	-	-	-	-	
8	c	-		G	AG	-+	-	-	+	+	-	-	K.aerogenes I
9	a-e	+		G	AG	AG	+	+	-	-	-	-	
10	a-e	+		G	AG	AG	+	+	-	-	-	-	
11	a-e	+		G	AG	AG	+	+	-	-	-	-	
12	a-e	+		G	AG	AG	+	+	-	-	-	-	
13	a-e	+		G	AG	AG	+	+	-	-	-	-	
14	a-e	+		G	AG	AG	+	+	-	-	-	-	
15	a-e	+		G	AG	AG	+	+	-	-	-	-	
16	a-e	+		G	AG	AG	+	+	-	-	-	-	
17	a-i		+		AG	AG	+	+	-	-	-	-	
18	a-e		+		AG	AG	+	+	-	-	-	-	
19	a-e		+		AG	AG	+	+	-	-	-	-	
20	a,b		+		AG	AG	+	+	-	-	-	-	
20	c,d		-		Ag	-+	+	-	+	+	-	-	K.aerogenes II
20	e		-		Ag***	A-++	+	+	-	-	-	-	E.coli III (?)
21	a-e		+		AG	AG	+	+	-	-	-	-	
22	a-d		+		AG	AG	+	+	-	-	-	-	
22	e-h		+**		AG	AG	+	+	-	-	-	-	
23	a,b,d,e		+		AG	AG	+	+	-	-	-	-	
23	c		+		AG	-+	+	+	-	-	-	-	E.coli III
24	a-e		+		AG	AG	+	+	-	-	-	-	
25	a-e		+		AG	AG	+	+	-	-	-	-	
26*	a-e		-		AG	-+	-	-	+	+	-	-	K.aerogenes I

* Not included in the general survey (vide 4.5)

** Red colour faded within 18 hrs

*** A in 24 hrs, g in 5 days

\+ Negative AG in 48 hrs

++ A in 24 hrs, negative G in 48 hrs

+++ H_2S production for No 2, 17-26 tested on T.S.I. agar instead of Lead acetate agar.

TABLE 22

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP D^1

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	25	100	121	90.97
E.coli III	2	8	2	1.51
Cit.freundii I	1	4	1	0.75
K.aerogenes I	2	8	4	3.00
K.aerogenes II	1	4	2	1.51
Irregular II	1	4	2	1.51
Irregular VI	1	4	1	0.75
E.coli I only	19	76		
E.coli I and E.coli III	1	4		
E.coli I, E.coli III and K.aerogenes II	1	4		
E.coli I, and Cit.freundii I and K.aerogenes I	1	4		
E.coli I and K.aerogenes I	1	4		
E.coli I and Irregular II	1	4		
E.coli I and Irregular VI	1	4		
TOTAL	25	100	133	100.00

The atypical E.coli III strain of subgroup D (vide Table 18, Sample No 13) formed acid at 37 °C. and 44.5°C., while only a bubble of gas was produced after 5 days. According to the definition of coliforms (vide 4.1) this should be regarded as a paracolon organism.

In subgroup D^1 (vide Table 21) an E.coli III strain formed acid at 44.5°C. (vide Sample No 20).

In subgroup D^2 (vide Table 23) quite a few of the E.coli III strains produced acid at 44.5°C., while the K.aerogenes I strain showed the same characteristic.

TABLE 23

COLIFORM FLORA OF SUBGROUP D^2

Sample no	Strain no	MacC. agar 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S ++ 37°C. 24 hrs	Micro-organism
1	a-d	+	AG	-*	+	+	-	-	-	-	E.coli III
1	e	+	AG	AG**	+	+	-	-	-	-	
2	a-c	+	AG	AG	+	+	-	-	-	-	
2	d-e	-	AG	AG	-	-	+	+	-	-	Irregular VI
3	a-e	+	AG	AG	+	+	-	-	-	-	
4	a-e	+	AG	AG	+	+	-	-	-	-	
5	a-e	+	AG	AG	+	+	-	-	-	-	
6	a-e	+	AG	AG	+	+	-	-	-	-	
7	a-c	+	AG	AG	-	+	-	-	-	-	Irregular II
7	d-e	+	AG	AG	+	+	-	-	-	-	
8	a-e	+	AG	AG	+	+	-	-	-	-	
9	a-e	+	AG	AG	+	+	-	-	-	-	
10	a,c,d	+	AG	AG	+	+	-	-	-	-	
10	b	+	AG	-*	+	+	-	-	-	-	E.coli III
10	e	+	AG	A-+	+	+	-	-	-	-	E.coli III (?)
11	a,c,d	+	AG	A-+	+	+	-	-	-	-	E.coli III (?)
11	b	-	AG	A-+	-	-	+	+	-	-	K.aerogenes I (?)
11	e	+	AG	AG	+	+	-	-	-	-	
12	a,c,e	+	AG	A-+	+	+	-	-	-	-	E.coli III (?)
12	b,d	+	AG	AG	+	+	-	-	-	-	
13	a-e	+	AG	A-+	+	+	-	-	-	-	E.coli III (?)
14	a-e	+	AG	-*	+	+	-	-	-	-	E.coli III

* Negative AG in 48 hrs

** AG in 48 hrs

\+ A in 24 hrs, negative G in 48 hrs

++ H_2S production tested on T.S.I. agar.

TABLE 24

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP D^2

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	12	85.71	42	60.00
E.coli III	6	42.86	22	31.43
K.aerogenes I	1	7.14	1	1.43
Irregular II	1	7.14	3	4.28
Irregular VI	1	7.14	2	2.86
E.coli I only	6	42.86		
E.coli III only	2	14.29		
E.coli I and E.coli III	3	21.43		
E.coli I, E.coli III and K.aerogenes I	1	7.14		
E.coli I and Irregular II	1	7.14		
E.coli I and Irregular VI	1	7.14		
TOTAL	14	100.00	70	100.00

TABLE 25

DISTRIBUTION OF COLIFORM STRAINS IN MAN

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	57	93.4	197	72.43
E.coli I H_2S+	1	1.6	2	0.73
E.coli II	1	1.6	2	0.73
E.coli III	12	19.7	31	11.40
Cit.freundii I	1	1.6	1	0.37
K.aerogenes I	8	13.1	17	6.25
K.aerogenes II	1	1.6	2	0.73
Irregular II	3	4.9	8	2.94
Irregular IV	1	1.6	1	0.37
Irregular V	1	1.6	1	0.37
Irregular VI	6.	9.8	10	3.68
E.coli I only	34	55.74		
E.coli III only	2	3.28		
E.coli I and E.coli I H_2S+	1	1.64		
E.coli I and E.coli III	8	13.11		
E.coli I, E.coli III and K.aerogenes I	1	1.64		
E.coli I, E.coli III and K.aerogenes II	1	1.64		
E.coli I, Cit.freundii I and K.aerogenes I	1	1.64		
E.coli I and K.aerogenes I	4	6.55		
E.coli I, K.aerogenes I and Irregular VI	1	1.64		
E.coli I and Irregular II	2	3.28		
E.coli I, Irregular IV and Irregular VI	1	1.64		
E.coli I and Irregular VI	3	4.92		
E.coli II and Irregular II	1	1.64		
K.aerogenes I, Irregular V and Irregular VI	1	1.64		
TOTAL	61	100.00	272	100.00

TABLE 26

DISTRIBUTION OF COLIFORM STRAINS IN MAN, GROUPING ALL ATYPICAL AND TYPICAL STRAINS TOGETHER

Coliform strains.	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	57	93.4	199	73.16
E.coli II	1	1.6	2	0.73
E.coli III	12	19.7	31	11.40
Cit.freundii I	1	1.6	1	0.37
K.aerogenes I	8	13.1	17	6.25
K.aerogenes II	1	1.6	2	0.73
Irregular II	3	4.9	8	2.94
Irregular IV	1	1.6	1	0.37
Irregular V	1	1.6	1	0.37
Irregular VI	6	9.8	10	3.68
E.coli I only	35	57.38		
E.coli III only	2	3.28		
E.coli I and E.coli III	8	13.11		
E.coli I, E.coli III and K.aerogenes I	1	1.64		
E.coli I, E.coli III and K.aerogenes II	1	1.64		
E.coli I, Cit.freundii I and K.aerogenes I	1	1.64		
E.coli I and K.aerogenes I	4	6.55		
E.coli I, K.aerogenes I and Irregular VI	1	1.64		
E.coli I and Irregular II	2	3.28		
E.coli I, Irregular IV and Irregular VI	1	1.64		
E.coli I and Irregular VI	3	4.92		
E.coli II and Irregular II	1	1.64		
K.aerogenes I, Irregular V and Irregular VI	1	1.64		
TOTAL	61	100.00	272	100.00

TABLE 27

TOTAL DISTRIBUTION OF COLIFORM STRAINS IN FAECES

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	205	97.2	993	90.1
E.coli I H_2S+	2	1.0	3	0.3
Atypical E.coli I	1	0.5	1	0.1
E.coli II	2	1.0	3	0.3
E.coli III	14	6.6	33	3.0
Cit.freundii I	1	0.5	1	0.1
K.aerogenes I	10	4.7	19	1.6
K.aerogenes II	2	1.0	3	0.3
Irregular II	14	6.6	33	3.0
Irregular IV	1	0.5	1	0.1
Irregular V	1	0.5	1	0.1
Irregular VI	6	2.8	10	1.0
E.coli I only	166	78.6		
E.coli III only	2	0.9		
Irregular II only	2	0.9		
E.coli I and E.coli I H_2S+	2	0.9		
E.coli I and atypical E.coli I	1	0.5		
E.coli I and E.coli II	1	0.5		
E.coli I and E.coli III	10	4.7		
E.coli I, E.coli III and K.aerogenes I	1	0.5		
E.coli I, E.coli III and K.aerogenes II	1	0.5		
E.coli I, Cit.freundii I and K.aerogenes I	1	0.5		
E.coli I and K.aerogenes I	5	2.4		
E.coli I, K.aerogenes I and and Irregular II	1	0.5		
E.coli I, K.aerogenes I and Irregular VI	1	0.5		
E.coli I and K.aerogenes II	1	0.5		
E.coli I and Irregular II	10	4.7		
E.coli I, Irregular IV and Irregular VI	1	0.5		
E.coli I and Irregular VI	3	1.4		
E.coli II and Irregular II	1	0.5		
K.aerogenes I, Irregular V and Irregular VI	1	0.5		
TOTAL	211	100.0	1,101	100.0

TABLE 28

TOTAL DISTRIBUTION OF COLIFORM STRAINS IN FAECES, GROUPING ATYPICAL AND TYPICAL STRAINS TOGETHER

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	205	97.2	997	90.5
E.coli II	2	1.0	3	0.3
E.coli III	14	6.6	33	3.0
Cit.freundii I	1	0.5	1	0.1
K.aerogenes I	10	4.7	19	1.6
K.aerogenes II	2	1.0	3	0.3
Irregular II	14	6.6	33	3.0
Irregular IV	1	0.5	1	0.1
Irregular V	1	0.5	1	0.1
Irregular VI	6	2.8	10	1.0
E.coli I only	169	80.0		
E.coli III only	2	0.9		
Irregular II only	2	0.9		
E.coli I and E.coli II	1	0.5		
E.coli I and E.coli III	10	4.7		
E.coli I, E.coli III and K.aerogenes I	1	0.5		
E.coli I, E.coli III and K.aerogenes II	1	0.5		
E.coli I, Cit.freundii I and K.aerogenes I	1	0.5		
E.coli I and K.aerogenes I	5	2.4		
E.coli I, K.aerogenes I and Irregular II	1	0.5		
E.coli I, K.aerogenes I and Irregular VI	1	0.5		
E.coli I and K.aerogenes II	1	0.5		
E.coli I and Irregular II	10	4.7		
E.coli I and Irregular VI	3	1.4		
E.coli I, Irregular IV and Irregular VI	1	0.5		
E.coli II and Irregular II	1	0.5		
K.aerogenes I, Irregular V and Irregular VI	1	0.5		
TOTAL	211	100.0	1,101	100.0

COMPOSITION OF THE COLIFOR

Coliform Strains usually recognised	FOWLS			SHEEP		
	No of strains studied	% of coliform flora in fowls	% of total coliform flora	No of strains studied	% of coliform flora in sheep	% o tot colif flo
E.coli I	268	94.37	24.3	262	95.2	23.
E.coli II				1	0.4	0.
E.coli III	1	0.35	0.1			
Cit.freundii I						
Cit.freundii II						
K.aerogenes I	1	0.35	0.1			
K.aerogenes II				1	0.4	0.
K.cloacae						
Irregular II	12	4.23	1.0	11	4.0	1.
Irregular III						
Irregular IV						
Irregular V						
Irregular VI						
Irregular VII						
Irregular VIII						
Additional strains isolated						
Atypical E.coli I	1	0.35	0.1			
E.coli I H_2S+	1	0.35	0.1			
Total number of strains	284			275		
% of total coliform flora	25.8			25.0		

TABLE 29

FLORA ISOLATED FROM FAECAL SPECIMENS

	CATTLE			MAN			Total number of strains	% of total coliform flora
n	No of strains studied	% of coliform flora in cattle	% of total coliform flora	No of strains studied	% of coliform flora in man	% of total coliform flora		
	266	98.52	24.2	197	72.43	17.9	993	90.2
				2	0.73	0.2	3	0.3
	1	0.37	0.1	31	11.40	2.7	33	2.9
				1	0.37	0.1	1	0.1
							0	
	1	0.37	0.1	17	6.25	1.5	19	1.7
				2	0.73	0.2	3	0.3
							0	
	2	0.74	0.2	8	2.94	0.7	33	2.9
							0	
				1	0.37	0.1	1	0.1
				1	0.37	0.1	1	0.1
				10	3.68	1.0	10	1.0
							0	
							0	
							1	0.1
				2	0.73	0.2	3	0.3
	270			272			1,101	
	24.5			24.7			100.0	100.0

TABLE 30

COLIFORM FLORA OF SUBGROUP E

Sample no	Strain no	MacC. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a-e	+	G	AG	AG	+	+	-	-	-	-	
2	a-e	+	G	AG	AG	+	+	-	-	-	-	
3	a,c-e	+	G	AG	AG	+	+	-	-	-	-	
3	b	+*	G	AG	AG	+	+	-	-	-	-	
4	a,c	+	G	AG	AG	+	+	-	-	-	-	
4	b,d,e	-	G	AG	AG	-	-	+	+	-	-	Irregular VI
5	a,c	+	G	AG	AG	+	+	-	-	-	-	
5	b,d,e	-	G	AG	-**	-	-	-	+	-	-	Atypical K.aerogenes I
6	a-e	+	G	AG	AG	+	+	-	-	-	-	
7	a-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-e	+	G	AG	AG	+	+	-	-	-	-	
9	a,c-e	+	G	AG	AG	+	+	-	-	-	-	
9	b	-	G	AG	AG	-	-	+	+	-	-	Irregular VI
10	a,c,e	+	G	AG	AG	+	+	-	-	-	-	Irregular II
10	b,d	+	G	AG	AG	+	+	-	-	-	-	
11	a-e	+	G	AG	AG	+	+	-	-	-	-	
12	a-e	+	G	AG	AG	+	+	-	-	-	-	
13	a,b	+	G	AG	AG	+	+	-	-	-	-	
13	c-e	+*	G	AG	-**	+	+	-	-	-	-	E.coli III
14	a,e	+	G	AG	AG	+	+	-	-	-	-	
14	b-d	+	G	AG	-**	-	-	-	-	-	-	Irregular VIII
15	a,b,e	+	G	AG	AG	+	+	-	-	-	-	
15	c,d	+*	G	AG	-**	-	+	-	+	+	-	Irregular IV

* Red colour faded within 18 hrs
** Negative AG in 48 hrs

TABLE 31

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP E

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	15	100	57	76.0
E.coli III	1	6.7	3	4.0
Atypical K.aerogenes I	1	6.7	3	4.0
Irregular II	1	6.7	3	4.0
Irregular IV	1	6.7	2	2.7
Irregular VI	2	13.3	4	5.3
Irregular VIII	1	6.7	3	4.0
E.coli I only	8	53.32		
E.coli I and E.coli III	1	6.67		
E.coli I and atypical K.aerogenes I	1	6.67		
E.coli I and Irregular II	1	6.67		
E.coli I and Irregular IV	1	6.67		
E.coli I and Irregular VI	2	13.33		
E.coli I and Irregular VIII	1	6.67		
TOTAL	15	100	75	100.0

The atypical K.aerogenes I strains (vide Table 30), differed from typical K.aerogenes I strains in being Voges-Proskauer negative.

TABLE 32

COLIFORM FLORA OF SUBGROUP E[1]

Sample no	Strain no	MacC. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. Broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a,c	+	G	AG	AG	+	+	-	-	-	-	
1	b,d,e	+	G	AG	AG	-	+	-	-	-	-	Irregular II
2	a-e	+	G	AG	AG	+	+	-	-	-	-	
3	a-e	+	G	AG	AG	+	+	-	-	-	-	
4	a-e	+	G	AG	AG	+	+	-	-	-	-	
5	a-e	+	G	AG	AG	+	+	-	-	-	-	
6	a-e	+	G	AG	AG	+	+	-	-	-	-	
7	a-e	+	G	AG	AG	+	+	-	-	-	-	
8	a-e	+	G	AG	AG	+	+	-	-	-	-	
9	a-e	+	G	AG	AG	+	+	-	-	-	-	
10	a-e	+	G	AG	AG	+	+	-	-	-	-	
11	a,c	+	G	AG	-*	-	+	-	-	-	-	E.coli II
11	b,d,e	-	G	AG	-*	-	-	+	+	+	-	K.cloacae
12	a,b	+	G	AG	AG	+	+	-	-	-	-	
12	c-e	-	G	AG	-*	-	-	+	+	-	-	K.aerogenes I
13	a-e	+	G	AG	AG	+	+	-	-	-	-	
14	a-e	+	G	AG	AG	+	+	-	-	-	-	
15	No coliforms could be demonstrated to be present.											

* Negative AG in 48 hrs

TABLE 33

DISTRIBUTION OF COLIFORM STRAINS IN SUBGROUP E[1]

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	13	86.7	59	84.28
E.coli II	1	6.7	2	2.85
K.aerogenes I	1	6.7	3	4.29
K.cloacae	1	6.7	3	4.29
Irregular II	1	6.7	3	4.29
E.coli I	11	73.2		
E.coli I and *K.aerogenes* I	1	6.7		
E.coli I and Irregular II	1	6.7		
E.coli II and *K.cloacae*	1	6.7		
Samples containing no coliforms	1	6.7		
TOTAL	15	100.0	70	100.0

TABLE 34

COLIFORM FLORA OF SUBGROUP E^2

Sample no	Strain no	E.M.B. agar 37°C. 24 hrs	Lact. broth 37°C. 24 hrs	MacC. broth 37°C. 24 hrs	MacC. broth 44.5°C. 24 hrs	Indole 37°C. 24 hrs	M.R. 30°C. 5 days	V.P. 30°C. 48 hrs	Citrate 37°C. 4 days	Gelatin 30°C. 7 days	H_2S 37°C. 48 hrs	Micro-organism
1	a,b,d	+		AG	AG	+	+	-	-	-	-	
1	c	-		AG	-**	-	-	+	+	-	-	K.aerogenes I
1	e,f	-	G	AG	A-+	-	-	+	+	+	-	K.cloacae (?)
2	a-d,g	+		AG	AG	+	+	-	-	-	-	
2	e,f	-		AG	-**	-	-	+	+	-	-	K.aerogenes I
3	a,b,d,e	+		AG	AG	+	+	-	-	-	-	
3	c,f,g	+		AG	AG	+	+	-	-	-	+	E.coli I (?)
4	a,d,e	+		AG	AG	+	+	-	-	-	-	
4	b	+		AG	AG	-	+	-	-	-	-	Irregular II
4	c	-		AG	-**	-	-	+	-	-	-	Irregular V
5	a,b,d,f	+		AG	AG	+	+	-	-	-	-	
5	c	-		AG	AG	-	-	+	+	-	-	Irregular VI
5	e	+		AG	A-+	-	+	-	-	-	-	E.coli II (?)
5	g	-		AG	-**	-	-	+	+	-	-	K.aerogenes I
6	a,b,d-f	+		AG	AG	+	+	-	-	-	-	
6	c	+		AG	-**	-	+	-	-	-	-	E.coli II
6	g	-		AG	-**	-	-	+	+	-	-	K.aerogenes I
7	a,d	-		AG	-**	-	-	+	+	-	-	K.aerogenes I
7	b,e	-		AG	Ag++	-	-	+	+	-	-	Irregular VI (?)
7	c,f	+		AG	AG	+	+	-	-	-	-	
8	a-c,e	+	G	AG	AG	+	+	-	-	-	-	
8	d,f	-	G	AG	AG	-	-	+	+	-	-	Irregular VI
9	a,b,f	+	G	AG	AG	+	+	-	-	-	-	
9	c-e	-	G	AG	-**	-	-	+	+	-	-	K.aerogenes I
10	a,e	+	G	AG	AG	+	+	-	-	-	-	
10	b	+	G	AG	AG	-	+	-	-	-	-	Irregular II
10	c,d	-	G	Ag*	-**	-	-	+	+	-	-	K.aerogenes I
11	No coliforms could be demonstrated to be present											
12	Dried up											
13	a,b,d	+	G	AG	AG	+	+	-	-	-	-	
13	c,e	-	G	AG	AG	-	-	+	+	-	-	Irregular VI
14	a,b,e	+	G	AG	AG	+	+	-	-	-	-	
14	c,d	-	G	Ag*	-**	-	-	+	+	-	-	K.aerogenes I
15	a,b,e	+	G	AG	AG	+	+	-	-	-	-	
15	c,d	-	G	AG	-**	-	-	+	+	-	-	K.aerogenes I

* A in 24 hrs, g in 48 hrs + A in 24 hrs, negative G in 48 hrs
** Negative AG in 48 hrs ++ A in 24 hrs,, g in 48 hrs

TABLE 35

DISTRIBUTION OF COLIFORMS IN SUBGROUP E^2

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	13	92.9	44	57.1
E.coli I H_2S+	1	7.2	3	3.9
E.coli II	2	14.3	2	2.6
K.aerogenes I	9	64.3	16	20.8
K.cloacae	1	7.2	2	2.6
Irregular II	2	14.3	2	2.6
Irregular V	1	7.2	1	1.3
Irregular VI	4	28.6	7	9.1
E.coli I only	0	0		
E.coli I and E.coli I H_2S+	1	7.2		
E.coli I, E.coli II and K.aerogenes I	1	7.2		
E.coli I, E.coli II, K.aerogenes I and Irregular VI	1	7.2		
E.coli I and K.aerogenes I	4	28.3		
E.coli I, K.aerogenes I, and K.cloacae.	1	7.2		
E.coli I, K.aerogenes I, and Irregular II	1	7.2		
E.coli I, K.aerogenes I, and Irregular VI	1	7.2		
E.coli I, Irregular II Irregular V	1	7.2		
E.coli I and Irregular VI	2	14.1		
Samples containing no coliforms	1	7.2		
TOTAL	14	100.0	77	100.0

TABLE 36

DISTRIBUTION OF COLIFORMS IN SUBGROUP E^2, GROUPING ATYPICAL AND TYPICAL STRAINS TOGETHER.

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	13	92.9	47	61.0
E.coli II	2	14.3	2	2.6
K.aerogenes I	9	64.3	16	20.8
K.cloacae	1	7.2	2	2.6
Irregular II	2	14.3	2	2.6
Irregular V	1	7.2	1	1.3
Irregular VI	4	28.6	7	9.1
E.coli I only	1	7.2		
E.coli I, *E.coli* II and *K.aerogenes* I	1	7.2		
E.coli I, *E.coli* II, *K.aerogenes* I and Irregular VI	1	7.2		
E.coli I and *K.aerogenes* I	4	28.3		
E.coli I, *K.aerogenes* I and *K.cloacae*	1	7.2		
E.coli I, *K.aerogenes* I and Irregular II	1	7.2		
E.coli I, *K.aerogenes* I and Irregular VI	1	7.2		
E.coli I, Irregular II and Irregular V	1	7.2		
E.coli I and Irregular VI	2	14.1		
Samples containing no coliforms	1	7.2		
TOTAL	14	100.0	77	100.0

Various atypical strains were recovered from subgroup E^2 (*vide* Table 34). The *K.cloacae* strains (*vide* Sample No 1) produced acid at 44.5°C., while some *E.coli* I strains formed hydrogen sulphide (*vide* Sample No 3), The *E.coli* II strain produced acid at 44.5°C. (*vide* Sample No 5) and the atypical Irregular VI strains formed only a bubble of gas at 44.5°C. (*vide* Sample No 7).

TABLE 37

DISTRIBUTION OF COLIFORMS IN WATER

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	41	93.2	160	72.1
E.coli I H_2S+	1	2.3	3	1.3
E.coli II	3	6.8	4	1.8
E.coli III	1	2.3	3	1.3
K.aerogenes I	10	22.7	19	8.6
Atypical K.aerogenes I	1	2.3	3	1.3
K.cloacae	2	4.6	5	2.3
Irregular II	4	9.1	8	3.6
Irregular IV	1	2.3	2	0.9
Irregular V	1	2.3	1	0.5
Irregular VI	6	13.6	11	5.0
Irregular VIII	1	2.3	3	1.3
E.coli I only	19	43.1		
E.coli I and E.coli I H_2S+	1	2.3		
E.coli I, E.coli II and K.aerogenes I	1	2.3		
E.coli I, E.coli II, K.aerogenes I and Irregular VI	1	2.3		
E.coli I and E.coli III	1	2.3		
E.coli I and K.aerogenes I	5	11.3		
E.coli I, K.aerogenes I and K.cloacae	1	2.3		
E.coli I, K.aerogenes I and Irregular II	1	2.3		
E.coli I, K.aerogenes I and Irregular VI	1	2.3		
E.coli I and atypical K.aerogenes I	1	2.3		
E.coli I and Irregular II	2	4.5		
E.coli I, Irregular II, Irregular V	1	2.3		
E.coli I and Irregular IV	1	2.3		
E.coli I and Irregular VI	4	9.0		
E.coli I and Irregular VIII	1	2.3		
E.coli II and K.cloacae	1	2.3		
Samples containing no coliforms	2	4.5		
TOTAL	44	100.0	222	100.0

TABLE 38

DISTRIBUTION OF COLIFORMS IN WATER, GROUPING ATYPICAL AND TYPICAL STRAINS TOGETHER

Coliform strains	No of Samples	% of Samples	No of Strains	% of Strains
E.coli I	41	93.2	163	73.4
E.coli II	3	6.8	4	1.8
E.coli III	1	2.3	3	1.3
K.aerogenes I	11	35.0	22	9.9
K.cloacae	2	4.6	5	2.3
Irregular II	4	9.1	8	3.6
Irregular IV	1	2.3	2	0.9
Irregular V	1	2.3	1	0.5
Irregular VI	6	13.6	11	5.0
Irregular VIII	1	2.3	3	1.3
E.coli I only	20	45.4		
E.coli I, E.coli II, and K.aerogenes I	1	2.3		
E.coli I, E.coli II, K.aerogenes I and Irregular VI	1	2.3		
E.coli I and E.coli III	1	2.3		
E.coli I and K.aerogenes I	6	13.6		
E.coli I, K.aerogenes I and K.cloacae	1	2.3		
E.coli I, K.aerogenes I and Irregular II	1	2.3		
E.coli I, K.aerogenes I and Irregular VI	1	2.3		
E.coli I and Irregular II	2	4.5		
E.coli I, Irregular II and Irregular V	1	2.3		
E.coli I and Irregular IV	1	2.3		
E.coli I and Irregular VI	4	9.0		
E.coli I and Irregular VIII	1	2.3		
E.coli II and K.cloacae	1	2.3		
Samples containing no coliforms	1	4.5		
TOTAL	44	100.0	222	100.0

TABLE 39

QUANTITATIVE ANALYSIS OF TWO FAECAL SAMPLES FOR THE PRESENCE OF COLIFORMS (MODIFIED MEMBRANE FILTER TECHNIQUE)

Sample No	Total Coliforms per gram	Presumptive *E.coli I* per gram	Confirmed *E.coli I* per gram	Confirmed Irregular II per gram
24 *	11,170,000	11,170,000	10,890,750	279,250
25 **	52,000,000	52,000,000	52,000,000	0

* All 20 coliform colonies subcultured from the Total coliform membranes were *E.coli I* and of the 20 colonies from the Presumptive *E.coli I* membranes 19 were *E.coli I* and 1 was Irregular II.

** All 10 coliform colonies subcultured from the Total coliform membranes were *E.coli I* and all 10 colonies from the Presumptive *E.coli I* membranes were *E.coli I*.

TABLE 40/...

TABLE 40

TOTAL COLIFORM INDEX, PRESUMPTIVE E.COLI I INDEX AND ESTIMATED DIFFERENTIATED COLIFORM INDEX OF SUBGROUP E AS DETERMINED BY THE MPN METHOD

Sample No	Total Presumptive Coliforms		Presumptive E.coli I		Confirmed E.coli I per 100 ml. of sample	Confirmed E.coli III per 100 ml. of sample	Confirmed atypical K.aerogenes per 100 ml. of sample	Confirmed Irregular II per 100 ml. of sample	Confirmed Irregular IV per 100 ml. of sample	Confirmed Irregular VI per 100 ml. of sample	Confirmed Irregular VIII per 100 ml. of sample
	Code	MPN/100ml. of sample	Code	MPN/100ml. of sample							
1	554	1,609	542	221	221						
2	555	1,800+	543	278	278						
3	555	1,800+	550	240	240						
4	554	1,609	551	348	139					209	
5	554	1,609	553	918	918		691				
6	554	1,609	542	221	221						
7	553	918	500	23	23						
8	554	130	520	49	49						
9	551	348	541	172	138					34	
10	554	1,609	554	1,609	643			966			
11	330	17	400	13	13						
12	552	542	541	172	172						
13	552	542	552	542	542	813					
14	552	542	541	172	172						370
15	510	33	510	33	33				13		

TABLE 41

TOTAL COLIFORM INDEX, PRESUMPTIVE E.COLI I INDEX AND DIFFERENTIATED COLIFORM INDEX OF SUBGROUP E^1 AS DETERMINED BY THE MPN METHOD

Sample No	Total Presumptive coliforms		Presumptive E.coli I		Confirmed E.coli I	Confirmed E.coli II	Confirmed K.aerogenes I	Confirmed K.cloacae	Confirmed Irregular II
	Code	MPN/100 ml. of sample	Code	MPN/100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample
1	555	1,800+	530	79	27				52
2	554	1,609	530	79	79				
3	553	918	520	49	49				
4	552	542	540	221	221				
5	552	542	541	172	172				
6	554	1,609	431	33	33				
7	553	918	500	23	23				
8	520	49	420	22	22				
9	440	34	410	17	17				
10	552	542	540	130	130				
11	521	70	000	0	0	346		175	
12	551	348	511	46	46		465		
13	550	240	540	130	130				
14	540	130	410	17	17				
15	000	0	0	0	0				

TABLE 42

TOTAL COLIFORM INDEX AND ESTIMATED DIFFERENTIATED COLIFORM INDEX OF SUBGROUP E^2 AS DETERMINED BY THE MPN METHOD

Sample No	Total Presumptive coliforms		Confirmed E.coli I	Confirmed E.coli II	Confirmed K.aerogenes I	Confirmed K.cloacae	Confirmed Irregular II	Confirmed Irregular V	Confirmed Irregular VI
	Code	MPN/100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample	per 100 ml. of sample
1	555	1,800+	900+		300+	600+			
2	555	1,800+	1,386+		514+				
3	555	1,800+	1,800+						
4	555	1,800+	1,080+				360+	360+	
5	555	1,800+	1,029+	257+	257+				257+
6	554	1,609	873	268	268				
7	555	1,800+	600+		600+				600+
8	554	1,609	1,073						536
9	555	1,800+	900+		900+				
10	554	1,609	644		644		321		
11	000	0							
13	555	1,800+	1,080+						720+
14	554	1,609	965		644				
15	310	11	7		4				

4. DISCUSSION

4.1 Definition

The term "coliform" is generally used as a collective name for a group of lactose fermenting bacilli belonging to the Enterobacteriaceae (Thresh and Beale, 1925; Prescott et al. 1946; McEwen, 1949; Windle Taylor, 1949, 1958; Galloway and Burgess, 1950; American Public Health Association et al. 1955, 1960; Wilson and Miles, 1955, 1964; Great Britain Ministry of Health et al. 1956; Gainey and Lord, 1956; Klein, 1957; Public Health Laboratory Service, 1957; Pelczar and Reid, 1958; World Health Organization, 1958, 1961, 1963; Burrows, 1959, 1963; Cruickshank, 1960, 1965; Smith and Conant, 1960; Salle, 1961; U.S. Department of Health et al. 1962; McKee and Wolf, 1963). Gainey and Lord (1956) defined this rather large group as "aerobic and facultative anaerobic Gram-negative non-spore-forming bacilli which ferment lactose with gas formation". They further pointed out that the group consists of thirty three "species" and "varieties", without naming them.

An almost identical definition was given by Pelczar and Reid (1958) and American Public Health Association et al. (1960), while various other authors, such as Cruickshank (1960), expressed similar views.

Windle Taylor (1949, 1958) gave the following definition of the group : "The coli-aerogenes bacteria are Gram-negative, non-sporing, rod-shaped micro-organisms, capable of growing in the presence of bile-salt (i.e. 'bile-tolerant') and of fermenting glucose and lactose with the production of acid and gas. They are easily cultivated, either aerobically or anaerobically ....".

According to Great Britain Ministry of Health et al. (1956) coliform bacteria refer to "bacilli capable of producing acid and gas from lactose at 37°C., within 48 hours, and of growing aerobically on an agar medium containing bile salt".

One of the more vague definitions, quoted by Prescott et al. (1946), is that of Breed and Norton of 1937, viz. "all lactose fermenting aerobic bacteria used as a measure of pollution of water".

The World Health Organization (1963) defined this group as including "all aerobic and facultative anaerobic Gram-negative non-spore-forming rods capable of fermenting lactose with the production of acid and gas at 35 - 37°C. in less than 48 hours". This is materially identical to the definition accepted by the World Health Organization (1961) for Europe.

For/...

For the present study the following definition was adopted as a working hypothesis : Coliforms are aerobic and facultative anaerobic non-sporing Gram-negative bacilli, capable of fermenting lactose with the production of acid and gas at 37°C. within 48 hours, and of growing in the presence of a bile-salt and/or certain dye(s) included in the appropriate lactose medium.

4.2 Classification

In order to facilitate any ecological study, attention should be given to the classification of the relevant bacteria. The classification of the coliforms is still unsatisfactory (Windle Taylor, 1958; Burrows, 1963). The application of serological reactions has, so far, proved of no practical value in their differentiation. Consequently the biochemical characteristics alone have to be used (Windle Taylor, 1958). Fortunately the coliforms can be sorted into "groups" by such means, while E.coli can easily and distinctly be separated from the rest (Windle Taylor, 1958; Burrows, 1963).

The only mutual biochemical characteristic of the coliforms is that they ferment lactose rapidly (vide 4.1). It is apparent that the more biochemical tests are employed, the more strains will be obtained for a specific species (Windle Taylor, 1958). This is obvious from the historical review (vide supra, 1.1) and can easily be seen by comparing the biochemical tests shown in Tables 43 and 44 (vide infra). It can further be demonstrated by comparing the biochemical classification in Table 44 (vide infra) with that of Stuart, Griffin et al. (1938), Stuart, Wheeler et al. (1938) and Griffin and Stuart (1940). These workers employed cellobiose in addition to the tests given in Table 44 (vide infra). By means of this they could more or less subdivide every strain, given in Table 44 (vide infra), into three substrains. However, these subdivisions have, so far, proved of no practical value in the detection of faecal pollution of water.

The background to the tests given in Tables 43 to 46 (vide infra) was discussed in 1.1, while the methods for performing them were given in 2.4. The symbols used in these Tables (vide infra) are as explained in 3.4.

It will suffice to pay attention to four schemes by which coliforms can be classified. In the first instance the relatively simple classification, usually employed by American investigators, is given in Table 43 (vide infra). This Table was summarised from Stuart, Zimmerman, Baker

and/...

and Rustigian (1942); Prescott *et al.* (1946); Galloway and Burgess (1950); Pelczar and Reid (1958); American Public Health Association *et al.* (1960) and Burrows (1963). This scheme was not employed in the present study.

TABLE 43

AMERICAN COLIFORM CLASSIFICATION ON THE BASIS OF BIOCHEMICAL REACTIONS

Name and variety of organism	Lactose 37°C. 48 hours	Indole	M.R.	V.P.	Citrate
Escherichia coli, variety I	G	+	+	-	-
Escherichia coli, variety II	G	-	+	-	-
Citrobacter freundii, variety I	G	-	+	-	±
Citrobacter freundii, variety II	G	+	+	-	+
Aerobacter aerogenes, variety I	G	±	-	+	±
Aerobacter aerogenes, variety II	G	±	-	+	+

The second classification is that commonly used in Britain (*vide* Table 44). This classification was developed by Wilson and his co-workers in 1935 (Bardsley, 1938, 1948; Taylor, 1942, 1945, 1951; Batty-Smith, 1942 b; Windle Taylor, 1958; Wilson and Miles, 1964), and was partially based on that used by Bardsley (1926). Table 44 (*vide infra*) is a compilation from schemes presented by Bardsley (1938, 1948); Taylor (1942, 1945, 1951); Batty-Smith (1942 a, 1942 b); Great Britain Ministry of Health *et al.* (1956); Windle Taylor (1958); Cruickshank (1960) and Wilson and Miles (1964).

This classification (Table 44) was used for the present study as it is clear-cut and authoritative, although naturally there were a few atypical forms which could not be classified according to this scheme (*vide infra*, 4.5). This classification can be regarded as the classical type of work, i.e. four groups - *Escherichia*, *Citrobacter*, *Klebsiella* and Irregulars (Bardsley, 1938; Batty-Smith, 1942 a, 1942 b).

The third classification was proposed in 1949 by the Sub-Committe on the classification of the coliforms of the Society for Applied Bacteriology.

TABLE 44

BRITISH CLASSIFICATION OF THE COLIFORMS ACCORDING TO THEIR BIOCHEMICAL REACTIONS

Previous name and type of organism	Present name and type of organism	Acid and gas lactose bile salt medium 37°C. 48 hrs	Acid and gas lactose bile salt medium 44.5°C. 48 hrs	Indole production 37°C. 24 hrs	M.R. test 30°C. 5 days	V.P. test 30°C. 48 hrs	Citrate utilising 37°C. 4 days	Gelatin liquefaction 30°C. 7 days	H_2S production 37°C. 24 hrs
Bact.coli type I	E.coli type I	+	+	+	+	-	-	-	-
Bact.coli type II	E.coli type II	+	-	-	+	-	-	-	-
Irregular type I	E.coli type III	+	-	+	+	-	-	-	-
Bact. (Escherichia)freundii type I	Cit.freundii type I	+	-	-	+	-	+	-	+
Bact. (Escherichia)freundii type II	Cit.freundii type II	+	-	+	+	-	+	-	+
Bact.aerogenes type I	K.aerogenes type I	+	-	-	-	+	+	-	-
Bact.aerogenes type II	K.aerogenes type II	+	-	+	-	+	+	-	-
Bact.cloacae	K.cloacae	+	-	-	+	-	+	-	-
Irregular type II	Irregular type II	+	-	-	+	-	+	-	-
Irregular type III	Irregular type III	+	-	-	+	-	+	-	-
Irregular type IV	Irregular type IV	+	-	-	+	-	+	-	-
Irregular type V	Irregular type V	+	-	-	+	-	+	-	-
Irregular type VI	Irregular type VI	+	-	-	+	-	+	-	-
Irregular type VII	Irregular type VII	+	-	-	+	-	+	-	-
Irregular type VIII	Irregular type VIII	+	-	-	+	-	+	-	-

This has since been revised (Coli-aerogenes Sub-Committee, 1956) as shown in Table 45 (vide infra). It is evident that this classification is basically the same as that of Table 44 (vide supra) except that it also includes the related plant pathogens (Erwinia) which grow at 30°C.

The fourth classification is that of the American worker, Parr (1938). Following an extensive study of the coliforms, he concluded that the classification scheme, given in Table 46 (vide supra) was inadequate, Consequently he worked out a new scheme, which is shown in Table 46 (vide infra). This was not employed in the present study. In fact, Parr's classification only tended to be confusing and materially produced no solution to the problem of taxonomy. Although constant watch was kept for Parr's newly differentiated bacteria, none was isolated (vide 3.5).

From a taxonomic point of view the definition (vide 4.1) and the classification (vide supra, Table 44) accepted for the present study, suffer from the fact that no allowance has been made to include :

(1) the paracolon bacteria, i.e. the so-called Paracolobactrum genus (Breed, Murray and Smith, 1957),

(2) the plant pathogens, viz. the Erwinia genus (vide Table 45), and

(3) the causative agent of pneumonia, i.e. Klebsiella pneumoniae (vide infra).

An arbitrary time limit of 48 hours for fermenting lactose is not sufficient, from a taxonomic point of view, for separating the coliforms from the related late lactose fermenting or even non-lactose fermenting strains (Prescott et al. 1946; MacPherson, 1950; Kauffmann, 1954; Coli-aerogenes Sub-Committee, 1956; Cowan, 1956; Breed et al. 1957; Pelczar and Reid, 1958; Windle Taylor, 1958; Smith and Conant, 1960; Salle, 1961; Edwards and Ewing, 1962; Burrows, 1963; Wilson and Miles, 1964). These related organisms should be included in the existing genera and species of the coliform group, viz. Escherichia coli, Citrobacter freundii and Klebsiella aerogenes-cloacae instead of in the so-called Paracolobactrum genus of Breed et al. (1957) (Kauffmann, 1954; Cowan, 1956; Edwards and Ewing, 1962; Burrows, 1963; Wilson and Miles, 1964). Further reason for the inclusion of the paracolons in the existing genera was supplied by workers who found that slow lactose fermenting bacteria could, through "training", acquire the ability to ferment lactose rapidly, whereafter it was impossible to distinguish between these new mutants and

TABLE 45

THE RELATIONSHIP OF DIFFERENT CLASSIFICATIONS OF THE COLI-AEROGENES GROUP

Coliform Sub-Committee (1949)		Reactions							Wilson et al. (1935)	Coli-aerogenes (1956) Sub-Committee		
Name	Group	Lactose 37°C.	Lactose 44°C.	Indole	M.R.	V.P.	Citrate	Gelatin	Name	Scientific name	Descriptive name	Abbreviation
Bacterium coli type I	Ia	+	+	+	+	-	-	-	Bacterium coli type I	Escherichia coli	Escherichia coli (indole-positive 44°C. positive)	E.coli I
Bacterium coli type I 44°C. neg.	Ib	+	-	+	+	-	-	-	Irregular I coli-like I	Escherichia coli	Escherichia coli (indole-positive 44°C. neg.)	E.coli III
Bacterium coli type II	IIa	+	-	-	+	-	-	-	Bacterium coli type II	Escherichia coli	Escherichia coli (indole-negative)	E.coli II
Intermediate type I	IIIa	+	-	-	+	-	+	-	Intermediate type I	Citrobacter freundii	Citrobacter freundii	Cit.freundii I
Intermediate type II	IVa	+	-	+	+	-	+	-	Intermediate type II	Citrobacter freundii	Citrobacter freundii (indole-positive)	Cit.freundii II
Bacterium aerogenes type I	Va	+	-	-	-	+	+	-	Bacterium aerogenes type I	Klebsiella aerogenes	Klebsiella aerogenes	K.aerogenes I
Bacterium cloacae	Vb	+	-	-	-	+	+	+	Bacterium cloacae	Klebsiella cloacae	Klebsiella cloacae	K.cloacae
Bacterium carotovorum *	Vc	+**	-	-	-	+	+	+		Erwinia carotovora		Erw.carotovora
Bacterium aerogenes type II	VIa	+	-	+	-	+	+	-	Bacterium aerogenes type II	Klebsiella aerogenes	Klebsiella aerogenes (indole-positive)	K.aerogenes II
									Irregular types II - VIII	Cannot be named without further investigation; at present should be described by use of suffixes.		

* Characterized by pectin liquefaction. ** Incubated at 30°C.

TABLE 46

PARR'S CLASSIFICATION OF COLIFORM TYPES, EMPLOYING FOUR TESTS, AND THE OCCURRENCE OF THESE 16 POSSIBLE TYPES AS DETERMINED BY VARIOUS WORKERS

Name of Parr's organism	Reactions IMViC	Found by*	Present Name	Remarks
Coli I	+ + - -	2 - 9	E.coli I or III	
Coli II	- + - -	3 - 9	E.coli II or Irregular II	
Coli III	+ - - -	0	atypical E.coli I	
Intermediate I	+ + + -	5,9	?	Existence doubtful
II	+ + - +	3 - 9	Cit.freundii II	Koser's classical Intermediate II
III	- + - +	2 - 9	Cit.freundii I	Koser's classical Intermediate I
IV	+ - + -	0	?	Existence doubtful, not even found by Parr
V	- - - -	8	Irregular VIII	
VI	+ - - +	3,4,8	Irregular VII	
VII	- + + -	3,7,9	?	Existence doubtful
VIII	+ + + +	4,7,9	?	Existence doubtful
IX	+ - + +	2-4,6, 8,9	K.aerogenes II	
X	- + + +	3,4,7,9	?	Existence doubtful
Aerogenes I	- - - +	3,4,7	Atypical K.aerogenes	
Aerogenes II	- - + -	3,6-9	Irregular V	
Aerogenes III	- - + +	2 - 9	K.aerogenes I and/or Irregular VI	

* 0 Not found isolated up to 1938.
2 Minkewitsch
3 Kline
4 Ruchhoft et al.
5 Skinner and Brudnoy
6 Bardsley
7 Bigger
8 Wilson et al.
9 Parr

the ordinary rapid fermenting strains by cultural and serological characteristics (Hitchner, Donogan and Alpert, 1938; Ziegler, 1939; Parr and Friedlander, 1942). The specifications for drinking-water and water, however, specify an incubation time limit of 48 hours (South African Bureau of Standards, 1951; Great Britain Ministry of Health et al. 1956; Public Health Laboratory Service, 1957; Windle Taylor, 1958; American Public Health Association et al. 1960; U.S. Department of Health et al. 1962; McKee and Wolf, 1963). Because the present study was undertaken with reference to the faecal pollution of water and drinking-water, no attention was given to the related paracolons.

Similarly the genus Erwinia should be included in the coliform group since one species, Erw. carotovora, has the same biochemical characteristics as Klebsiella cloacae (vide Table 45). This plant pathogen is of economic importance but has no importance in water bacteriology. (With an incubation temperature of 37°C. Erwinia could not be detected.) Consequently no attention was paid to this genus in the present study.

The last organism which was mentioned (vide supra), is Klebsiella pneumoniae (Friedländer's bacillus, Bacillus pneumoniae, Bacillus mucosus capsulatus, Bacterium friedländeri, Bacterium pneumoniae crouposae, Encapsulatus pneumoniae, Hyalococcus pneumoniae, Klebsiella crouposa) (Breed et al. 1957; Smith and Conant, 1960; Burrows, 1963; Wilson and Miles, 1964). This bacillus, isolated by Friedländer in 1882, is one of the aetiological agents of pneumonia. Although this pathogen is generally not regarded as a coliform, it is closely related to Klebsiella aerogenes (Coli-aerogenes Sub-Committee, 1956; Breed et al. 1957; Smith and Conant, 1960; Burrows, 1963; Wilson and Miles, 1964). Cabelli (1955) showed that K.pneumoniae could give rise to lactose positive mutants, which could, biochemically, not be distinguished from K.aerogenes. Edwards and Fife (1955) and Edwards and Ewing (1962) determined that it is impossible, both biochemically and serologically (capsule serotyping), to distinguish between K.pneumoniae and K.aerogenes. Reviewing all the evidence, Kauffmann (1954), Edwards and Fife (1955), Coli-aerogenes Sub-Committee (1956), Cowan (1956), Edwards and Ewing (1962), Burrows (1963) and Wilson and Miles (1964) regarded K.aerogenes and K.pneumoniae as the same species, preferring the latter name. In the present survey, however, K.aerogenes was still used, because of the long association of the "aerogenes" species name in water bacteriology (Coli-aerogenes Sub-Committee, 1956).

4.3 Nomenclature/...

4.3 Nomenclature

In the course of time the generic and the specific names of the main groups of coliforms have undergone several changes. Breed et al. (1957) gave the following summary of Escherichia coli (Migula) Castellani and Chalmers : Bacterium coli commune of Escherich was changed to Bacillus escherichii - Trevisan in 1889; Bacillus coli - Migula in 1895; Bacterium coli - Lehmann and Neumann in 1896; Escherichia coli - Castellani and Chalmers in 1919. The name Bacterium coli was, however, used until recently.

Klebsiella aerogenes (Kruse) Trevisan was originally described as Bacterium lactis aërogenes by Escherich in 1885 and was altered to Bacillus aerogenes - Kruse in 1896; Bacterium aerogenes - Chester in 1897, and Aerobacter aerogenes - Beijerinck in 1900 (Breed et al. 1957). The name Bacterium aerogenes was used until recently by British authors, e.g. Wilson and Miles (1955), while American workers preferred Aerobacter aerogenes, e.g. Breed et al. (1957). As described previously, the name K.aerogenes came into use recently (vide supra, 4.2).

Another coliform, Klebsiella cloacae (Jordan) Trevisan, Jordan's Bacillus cloacae of 1890, was changed to Bacterium cloacae - Lehmann and Neumann in 1896; Bacillus cloacae - Jordan (1903), and Aerobacter cloacae - Bergey and co-workers in 1923 (Breed et al. 1957). Here again, until recently, the name Bacterium cloacae was used in Britain (e.g. Wilson and Miles, 1955), while Aerobacter cloacae was used in America (e.g. Breed et al. 1957). Kauffmann (1954) and others even preferred the name Cloaca cloacae (Jordan) Castellani and Chalmers for this bacillus. Furthermore, French authors used the name Enterobacter cloacae (Jordan) Hormaeche and Edwards (Leclerc, 1961). This name was later also suggested by the International Bulletin of Bacteriological Nomenclature and Taxonomy (1963).

The Intermediates, Citrobacter freundii (Braak) Werkman and Gillen, discovered by Koser (1923, 1924) were named Bacterium freundii - Braak in 1928; Citrobacter freundii - Werkman and Gillen in 1932, and Escherichia freundii - Yale in 1939 (Breed et al. 1957). Although Kauffmann (1954) and Breed et al. (1957) accepted the latter name, many other workers were reluctant to do so (Windle Taylor, 1949; Wilson and Miles, 1955; Coli-aerogenes Sub-Committee, 1956; Great Britain Ministry of Health et al. 1956). Consequently the name Bacterium freundii Braak was used until recently (Windle Taylor, 1949; Wilson and Miles, 1955; Great Britain Ministry of Health et al. 1956). It was felt, however, that

the/...

the nomenclature of Citrobacter freundii (Braak) Werkman and Gillen should be the one of choice (Coli-aerogenes Sub-Committee, 1956; Windle Taylor, 1958; Burrows, 1959, 1963; Edwards and Ewing, 1962; Wilson and Miles, 1964; Cruickshank, 1965).

In the present study the following generic and specific names were used for the coliforms : Escherichia coli, Citrobacter freundii, Klebsiella aerogenes and Klebsiella cloacae, while the Irregulars were differentiated by using Roman numerals, e.g. Irregular II, III, etc.

The literature also reveals a confusing general terminology for describing the coliforms in water bacteriology. The "coli-aerogenes group" refers to the coliform bacteria (Windle Taylor, 1949, 1958; Great Britain Ministry of Health et al. 1956; Public Health Laboratory Service, 1957; Coetzee, 1962 b). The subject is complicated because some authors use names, such as Escherichia coli, E.coli, Bacterium coli, Bact.coli, Bacillus coli or B.coli to refer to the whole coliform group (Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Coetzee, 1962 b).

While "typical Bact.coli" is synonymous with "typical E.coli", i.e. E.coli I, "atypical Bact.coli" or "atypical E.coli" refers to the rest of the coliforms, sometimes known as the "intermediate-aerogenes-cloacae" or "I.A.C." group. The term "faecal coli" or "faecal E.coli" or "faecal Bact.coli" is used as a synonym for "typical E.coli" or "typical Bact.coli". "Faecal coli" or "faecal E.coli" is often used to stress the importance of E.coli I, but the rest of the coliforms cannot be described as "non-faecal coli" or "non-faecal E.coli". The same applies to "typical faecal bacilli" (Great Britain Ministry of Health et al. 1956; Public Health Laboratory Service, 1957; Windle Taylor, 1958; Coetzee, 1962b). Cruickshank (1960) used the terms "typical coliform bacilli" for E.coli I, and "atypical coliform bacilli" for the rest of the coliforms.

In addition Gainey and Lord (1956) employed the terms "colon bacilli", "B.coli group" and "colon group" as synonyms for coliforms. They furthermore used the terms "grain"or "non-fecal" and "fecal types" to differentiate between K.aerogenes and E.coli.

In the present study these terms have, as far as possible, been avoided. The terms "coliforms", "coliform group" or "coliform bacteria" were used as defined previously (vide 4.1). The names of the various coliform strains, or their abbreviations, as given in Tables 44 and 45 (vide 4.2), were used rather than some of the terms attached to them.

When/...

When the terms "typical" and "atypical" were employed they invariably referred to strains which showed a biochemical digression.

4.4 Direct plating versus enrichment

In studying the faecal coliform flora, four methods may usually be employed :

(1) direct plating,
(2) enrichment of specimens in one of the lactose media or in a citrate medium,
(3) pour-plate method, and
(4) membrane filtration technique.

Parr (1938) concluded "that direct plating is the most accurate method for determining the actual flora present. The various coliform bacteria have different metabolic demands and responses. It is improbable that any enrichment method yields an entirely accurate picture of the material enriched". Some workers, such as Bardsley (1926, 1934, 1938, 1948) and Malcolm (1938) employed enrichment of the specimens, but Burke-Gaffney (1932) commented : "On the one hand, the use of liquid media in the initial stages tends to encourage the overgrowth of B.aerogenes at the expense of B.coli..." The third and fourth methods have been referred to in 2.1 and the fourth was discussed in 2.6.

Accordingly, all faecal specimens were plated directly during the present study.

4.5 Distribution and occurrence of coliforms in faeces as revealed by the present study

The distribution of coliforms in subgroup A is given in Table 2 (vide 3.5). Two atypical E.coli I strains were isolated in this subgroup. With the usual routine methods employed in water analysis, i.e. 37°C. and 44°C. incubation in MacConkey broth and the subsequent confirmation of the positive 44°C. cultures in Tryptone water (Windle Taylor, 1955, 1958; Department of Water Affairs, Republic of South Africa, 1962; Wilson and Miles, 1964; Windle Taylor and Burman, 1964; Cruickshank, 1965) the E.coli I H_2S + strain and the atypical E.coli I strain would have been classified as typical E.coli I strains. Consequently these atypical strains might be grouped together with the typical E.coli I strains, resulting in the occurrence of E.coli I and

Irregular II/...

Irregular II strains only (vide 3.5, Table 3).

E.coli I was the predominant strain in the fowl group (vide 3.5, Tables 1 to 7). It should be stressed that this organism was absent from only one sample, in which Irregular II occurred (vide 3.5, Tables 1 to 7).

Although a few different coliform strains were isolated from sheep (vide 3.5, Tables 8 to 12) and from cattle (vide 3.5, Tables 13 to 17) E.coli I was again the preponderant strain. It should be pointed out that the one sample of subgroup B^1, from which E.coli I was absent, contained Irregular II. Consequently it is obvious that E.coli I, was the predominant strain in the animal groups (vide 3.5, Tables 6,7,12, 17 and 29), and when absent Irregular II was present. Cit.freundii, K.cloacae and Irregular III to VIII were never isolated (vide 3.5, Tables 6,7,12,17 and 29; also vide 4.2, Table 44).

It was impossible to obtain 25 samples for each of subgroupsD and D^2 (vide 2.1). Nevertheless, subgroup D (vide 3.5, Tables 18 to 20) and subgroup D^2 (vide 3.5, Tables 23 and 24) yielded a greater variety of coliform strains than the animal subgroups(vide 3.5, Tables 6,7,12, 17 and 29). E.coli I, however, was the predominant strain in both these subgroups. Cit.freundii, K.aerogenes II, K.cloacae, Irregular III, Irregular VII and Irregular VIII were not isolated from subgroup D (vide 4.2, Table 44), but the occurrence of E.coli III, K.aerogenes I and Irregular VI was striking. In the two specimens of this subgroup from which E.coli I was absent, mixed growths of E.coli II and Irregular II and of K.aerogenes I, Irregular V and Irregular VI occurred (vide 3.5, Tables 19 and 20). The occurrence of E.coli III was striking in subgroup D^2 and this organism was also isolated from the two specimens which yielded no E.coli I strains. E.coli II, Cit.freundii, K.aerogenes II, K.cloacae, Irregular III to V, Irregular VII and Irregular VIII however, were not recovered from this subgroup (vide 3.5, Tables 19 and 20; also vide 4.2, Table 44).

When compared with subgroup D^1 (vide 3.5, Table 21 and 22), a considerable number of E.coli III strains was isolated from D and D^2. It thus appears as if these results suggest that E.coli III might have Bantu and Indians as their natural habitat.

Only the first 25 specimens of subgroup D^1 were used for the general survey (vide 3.5, Table 21). E.coli I was again the preponderant strain and was recovered from all samples (vide 3.5, Table 22). The paracolon-like organism was included as an E.coli III strain (vide 3.5, Table 21, Sample No 20)./...

Sample No 20). This, however, illustrates the superfluity of placing a slow lactose fermenting bacterium in a different genus (vide 4.2).

The 26th specimen of subgroup D^1 was included merely out of interest (vide 3.5, Table 21), since this phenomenon could not be studied in detail during this survey. This specimen came from an apparently healthy "patient" who was being treated with penicillin, administered orally. Only a few coliform colonies were recovered, all of which were K.aerogenes I. Unfortunately no further specimens could be obtained from this "patient" to study the succession of coliform flora during antibiotic therapy. It is, however, well known that antibiotics eliminate E.coli from the intestine, while some other coliforms can still persist (Windle Taylor, 1958; Burrows, 1963). In all the other samples from which E.coli I was not recovered, it was impossible to determine whether antibiotic treatment had been given previously (vide especially 3.5, Table 18, Sample Nos 5 and 12).

The distribution of coliform strains in human faeces (vide 3.5, Tables 25, 26 and 29) thus showed a wider range than in that of fowls (vide 3.5, Tables 5 and 6), sheep (vide 3.5, Table 12) and cattle (vide 3.5, Table 17). The occurrence of K.aerogenes I and Irregular VI in human faeces (vide 3.5, Table 29), was striking when compared with Irregular II, but this might be ascribed to several reasons. It is well known that human food is usually suitable for bacterial growth, especially for many coliform strains (Pelczar and Reid, 1958; Burrows, 1963; Wilson and Miles, 1964). Furthermore, it is known that bacteria require moisture for growth (Pelczar and Reid, 1958; Burrows, 1963; Wilson and Miles, 1964; Cruickshank, 1965). When comparing human food with that of animals (vide 2.1), it can generally be seen that the human food contains more moisture, thus resulting in the prevalence of more coliform strains. Consequently the higher incidence of coliforms, other than E.coli, in human faeces appears to confirm the assumption that they are "organisms of passage" (Bardsley, 1948) or "adventitious" (Griffin and Stuart, 1940), due to the fact that human food initially may contain a larger variety of coliforms than animal food.

The final classification of the 1,101 purified strains is shown in Table 29. Tables 2,3,5,6,7,9,11,12,14,16,17,19,20,22 and 24 to 28 give an accurate picture of the percentage occurrence of coliforms in the samples. A true picture of the distribution of coliform strains in faeces, expressed in terms of percentage of the total coliform flora cannot, however,

be/...

be derived from these Tables. It is to some extent biased, viz. they tend to over-emphasise the strains which occur rarely, while the predominant E.coli I strain is under-emphasised. The reason for this is that every colony which differed from E.coli I was subcultured, while only a few of the E.coli I colonies were treated similarly. This can be illustrated by considering one example. In subgroup D (vide 3.5, Table 19), 76 strains of E.coli I were studied, comprising 68.47% of the total of 111, while K.aerogenes I comprises 10.81%. In this case, however, only 12 K.aerogenes I strains could be isolated, while 760 E.coli I strains could easily be isolated. Consequently the percentage distribution would then have reflected a more exact picture than it actually does. From a practical point of view, however, the number of the preponderant strain investigated had to be limited to such an extent as to bring them within the limits of reasonable handling.

Table 39 (vide 3.5) shows the results of the quantitative analysis of the last two faecal specimens of subgroup D^1. Irregular II was isolated from Sample No 24, while all the coliforms isolated from Sample No 25 were E.coli I. By means of qualitative methods no Irregular II could be recovered (vide 3.5, Table 21). Prescott et al. (1946) quoted figures of 92,800,000 coliforms, of which at least 200,000 strains were Klebsiella or Citrobacter types, per gram human faeces. Employing the membrane filter technique Broderick and Loughlin (1963), Geldreich, Bordner, Huff, Clark and Kabler (1962) found E.coli I to be the predominant strain in animal faeces. Unfortunately more quantitative data are required before any conclusions can be drawn as to the quantitative distribution of coliforms in faeces.

4.6 Distribution and occurrence of coliforms in water examined

The object was to establish whether E.coli I could be found in the water, although the presence of all other coliforms was carefully tested for.

The presence of E.coli I in every sample of subgroup E^2 (vide 3.5, Tables 30 and 31) could possibly be accounted for by faecal material having been washed into the river, since sampling was done during a rainy period (Prescott et al. 1946; Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Wilson and Miles, 1964). Furthermore, since animals and human beings had access to most parts of the river and the surrounding area, the possibility of direct faecal pollution

cannot/...

cannot be excluded. An estimate of the levels of the coliforms isolated was given in Table 40 (vide 3.5).

Five different strains were recovered from subgroup E^1 (vide 3.5, Tables 32 and 33). Only one sample did not contain any coliforms, i.e., water from a spring (vide 2.1 and 3.5, Table 32). Attention should be drawn to the occurrence of K.cloacae, as this organism was never isolated from faeces. During the present survey unseasonal rains occurred which could wash faecal material into the river from the land surface (Prescott et al. 1946; Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Wilson and Miles, 1964), while direct pollution from man and animals could also have taken place. Consequently E.coli I was recovered from thirteen of the sampling points. The estimated levels of the coliforms recovered, were given in Table 35 (vide 3.5).

Extensive plating, at least 5 plates per sample, was performed in examining subgroup E^2. These samples were collected during a dry period. The result was that sampling point No 12 (vide 2.1) was dry and in general the flow of the river was much lower than during the collection of samples in the previous two subgroups. Therefore faecal pollution could only have taken place directly from man and animals and from effluents, e.g. sewage works (vide 2.1). E.coli I was again the predominant strain, while a great variety of other coliforms was also isolated (vide 3.5, Tables 34 to 35). During this survey the incidence of K.aerogenes I was striking. It should be kept in mind that no rain fell during this survey to dilute the river or to wash in faecal material from outside (vide supra). Gray (1932) found a rapid increase of K.aerogenes in stored waters and the increase of K.aerogenes I during this survey appears to suggest that the same might have occurred during the dry period (vide also 3.5, Table 42). In general it appears as if the levels of coliforms were higher during this survey.

From the results on the river water (vide 3.5, Tables 37 and 38) it appears that a greater variety of coliforms was isolated than from faeces (vide 3.5, Tables 27 to 29).

In Tables 40 to 42 (vide 3.5) the estimated quantitative distribution of the coliforms in the three subgroups was given. This was estimated from the MPN results and should not be regarded as definite figures. By means of this, however, some idea can be obtained as to the levels at which coliforms occurred in the water samples. In many samples (vide 3.5, Tables 40 and 41) the Total presumptive coliform index was higher than the

Presumptive/...

Presumptive E.coli I index, but it was impossible to demonstrate the presence of other coliforms in subsequent plating. This might be due to the inherent inaccuracy of the MPN method (Bardsley, 1934, 1938, 1948; Allen, Pasley and Pierce, 1952 a; Hoather, 1952). It was attempted to overcome this problem in the third subgroup by estimating the coliform levels from the Total presumptive coliform index only, after subsequent plating of the positive cultures (vide 3.5, Table 42). It was, however, still unsuccessful.

4.7 Statistical analysis

Biologists often attempt to analyse their data statistically in order to give more weight to their findings. Such statistics are of tremendous value to the biological sciences, for as Siegel (1956) said "Statistics provides tools which formalize and standardize our procedures for drawing conclusions." Furthermore, Dixon and Massey (1951) have stated that statistics can be seen "as the science of experimentation."

Dixon and Massey (1951) pointed out that it is often erroneously maintained that "You can prove anything with statistics." Such a false conclusion is usually based on a neglect of certain requirements that experimental data must fulfil before a statistical analysis can be validly applied. As indicated, the present study could not be planned statistically (vide 2.1). This is a major drawback for applying statistical analysis. It was further explained that, for the most part, quantitative analysis of samples could not be undertaken (vide 2.1, 2.4 and 2.6). For these reasons it was attempted to utilise at least 20 faecal specimens of each subgroup. (As a rule 25 specimens were employed; it was, however, impossible to obtain 25 specimens from subgroups D and D^2 (vide 2.1 and 3.5).) Although Siegel (1956) indicated that nonparametric tests are useful to the researcher who has to work with a small number of samples, it was felt that this was not relevant to the present study (vide infra).

Normally statistical tests require quantitative data so as : to obtain means, medians, standard deviations, etc. (Dixon and Massey, 1951, Siegel, 1956), to apply correlational methods, e.g. Pearson and Fisher tests (Dixon and Massey, 1951; Siegel, 1956), to work out significance levels, e.g. by applying the null hypothesis or the research hypothesis (Siegel,1956) or to work out sampling distribution, etc. (Siegel, 1956).

As the present study had to employ qualitative methods only, its nature was clearly of such a kind that the statistical tests mentioned (vide supra), could not be applied (Calitz, 1966).

Nevertheless, although the present study could not be interpreted statistically, it provides results which are needed in the field of public health in South Africa.

4.8/...

4.8 Difficulties in comparing the results with earlier work

Because of the "revolution" which biochemical techniques, for the identification of the coliforms, have undergone (vide 1.1) and the increased knowledge gained of the taxonomy of these organisms (vide 4.2) it is often difficult to compare recent results with earlier findings. Thresh and Beale (1925) summarised this problem as follows : "Unfortunately, much that has been written with reference to the presence of the Bacillus coli in water has no importance, since observers so often failed to describe the processes they employed, and the characteristics of the bacterium which they have regarded as the Bacillus coli. In other cases the descriptions given show, beyond doubt, that more than one organism has been regarded as the Bacillus coli".

This problem can best be illustrated by the following example. An important question which has always been asked (and is still unsolved), is : Are coliforms able to grow and multiply in water or anywhere outside the intestine and which strains, if any, will be able to do so? According to Bigger (1937) the first "marked" and "unquestionable" indication of the "multiplication of coliform bacilli in water" was found by Savage and Wood in 1917-18. This was further investigated by Bigger (1937). He found that some strains of "coliform bacilli, when inoculated into raw water which was kept at air temperature, showed evidence of multiplication, but the increases recorded were not striking. In two experiments in which boiled, filtered and sterilised tap water was used as culture medium, very striking increases in the numbers of coliform bacilli were obtained". Bigger further indicated that "The bacillus used in the majority of the experiments is called 'E'. It is a coliform bacillus, isolated from human faeces. Its characteristics are given in full ...., but here it may be stated that it differs from a typical Bact.coli only in being non-motile and in failing to ferment dulcitol".

He gave the biochemical reactions of "bacillus E" and of 8 other coliforms (Nos 4,6,14,17,19,88,97 and NC 744) isolated from human faeces and urine, which were able to grow in autoclaved Vartry water. He concluded that "Any attempt to allocate to these bacilli specific names is, in the present state of our knowledge, rather a waste of time. It may be remarked, however, that No.6 appears to be a typical Bact.coli. No typical Aerobacter aerogenes is present although a number were tested, but No.4 might be classed as an atypical Aerobacter aerogenes. E, 19 and NC 744, owing to their failure to ferment dulcitol, may be regarded as atypical Bact.coli. The rest are intermediates".

It should/...

It should be noted that the majority of Bigger's experiments were carried out by using laboratory cultures. Naturally laboratory cultures differ from bacteria occurring in their natural habitat. Admittedly, he also tested this phenomenon with cultures freshly isolated from faeces, but "bacillus E", which was mostly employed, was a laboratory culture. Furthermore, he paid very little attention to natural waters.

He followed the classification of coliforms on the basis of that of MacConkey (1909) (vide 1.1) but it appears as if he also mingled this with that of Parr (1936 b, 1938), (vide 4.2) in referring to some types of "intermediates".

Bigger's work suffers mainly from the fact that he ignored the classification introduced by Bardsley in 1926 (vide 1.1). That classification, as later improved (vide 1.1 and 4.2), was mainly for use in water bacteriology. It is therefore difficult to decide whether his E.coli strains, i.e. E, Nos 6,19 and NC 744 were E.coli I or III. Furthermore, his atypical Aerobacter aerogenes suggested a mixed growth (vide 3.3 and 4.2), while some of his so-called "intermediates" could easily have been E.coli strains.

The work of Bigger was followed by more extensive investigations, viz. Bigger and Nelson (1941, 1943) and Nelson (1942) (vide 1.2). In these studies "bacillus E" was mostly used. Although all this led to a better knowledge of general bacterial physiology and metabolism, nothing regarding the possible multiplication of coliforms in natural waters was proved, because multiplication was found to take place in "boiled, filtered and sterilised tap water". The possibility that such types of waters may occur in Nature, is questionable.

4.9 Comparison with previous work

Notwithstanding the difficulties in connection with comparing the present results with previous work (vide 4.8), some comparison is possible. In the comprehensive investigation of MacConkey (1909) the citrate test and the 44°C. test (vide 1.1) had not yet been devised. Windle Taylor (1958), however, rearranged these data carefully to obtain the various coliform bacteria isolated. Comparing MacConkey's data (as revised by Windle Taylor) with the present study (vide 3.5, Tables 29 and 38) Table 47 is obtained .

TABLE 47/...

TABLE 47

COMPARISON OF COLIFORM STRAINS ISOLATED FROM VARIOUS SOURCES

Strains isolated	Human faeces		Animal faeces		Water	
	MacConkey (1909) % of strains	Present study % of strains	MacConkey (1909) % of strains	Present study % of strains	MacConkey (1909) % of strains	Present study % of strains
E.coli I	89.9	84.56	94.2	96.51	22.4	74.7
E.coli II	2.2	3.67	0	3.12	4.1	5.4
Cit.freundii I	1.1	0.37	0.6	0.00	2.0	0.0
Cit.freundii II	0.6	0.00	0	0.00	8.2	0.0
K.aerogenes I	5.1	6.25	1.3	0.24	4.1	9.9
K.aerogenes II	1.1	0.73	0	0.13	0	0.0
K.cloacae	0.0	0.00	3.9	0.00	59.2	2.3
Other coliforms	0.0	4.42	0	0.00	0	7.7

For this comparison the data of the present study were grouped together as for E.coli I and E.coli III, and E.coli II and Irregular II, as it was impossible for MacConkey to differentiate between these organisms. Judged on the percentage of the E.coli I strains, the water examined in the present study appears to have been subjected to more faecal pollution than that which MacConkey examined. There is a general agreement between the results of the two studies, except that Cit.freundii was isolated only once during the present study, viz. from human faeces. Furthermore K.cloacae was never isolated from faeces in the present study, while its occurrence in water was rare when compared with the data of MacConkey, in whose investigation it was the most prevalent strain.

In some of the following comparisons strains of the same species, isolated during the present investigation, had to be grouped together, in order to have comparable data, e.g. sometimes authors reported E.coli instead of its three strains, while in the present study all the coliforms were differentiated into their various strains (vide supra, also vide 4.2, Table 44).

When he discovered Citrobacter freundii, Koser (1924) also analysed 31 human and 88 animal faecal specimens. It was impossible for him to differentiate coliform species into strains, as the indole test was not employed. Nevertheless a comparison can be made (vide Table 48).

TABLE 48/...

TABLE 48

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES

Strains isolated	Human faeces		Animal faeces	
	Koser (1924) % of strains	Present study % of strains	Koser (1924) % of strains	Present study % of strains
E.coli	96.8	88.23	86.4	99.6
Cit.freundii	0.0	0.37	10.2	0.0
K.aerogenes	3.2	6.98	3.4	0.4
Other coliforms	0.0	4.42	0.0	0.0
Total	100.0	100.00	100.0	100.0

At the time when Koser did his analysis the present classification of coliforms (vide 4.2, Table 44) was not yet worked out. A striking difference is the absence of Cit.freundii in the animal faeces of the present study.

Particularly interesting is the work done in the tropics (India) by Raghavachari in 1926, as cited by Bardsley (1934). Unfortunately only water samples were analysed. At the time Raghavachari could not differentiate K.aerogenes from Irregular VI (vide Table 49).

TABLE 49

COMPARISON OF COLIFORM STRAINS ISOLATED FROM INDIAN AND SOUTH AFRICAN WATERS

Strains isolated	Raghavachari in 1926 India % of strains	Present study South Africa % of strains
E.coli	69.2	80.1
Cit.freundii	0.7	0.0
K.aerogenes	30.1	17.2
Other coliforms	0.0	2.7
Total	100.0	100.0

It is/...

It is impossible to compare the occurrence of E.coli strains, as this depends on the degree of faecal pollution to which the water has been subjected, but the occurrence of K.aerogenes and the rareness of Cit.freundii are significant in both studies.

It is too difficult to compare the data of Bardsley (1926), as she later discontinued the citrate test. Her more recent work is, however, comparable (vide Table 50).

TABLE 50

COMPARISON OF COLIFORM STRAINS ISOLATED FROM VARIOUS SOURCES

Strains isolated.	Human faeces		Animal faeces		Water		
	Bardsley (1938) direct plating % of strains	Present study direct plating % of strains	Bardsley (1948) direct plating % of strains	Present study direct plating % of strains	Bardsley (1938) % of strains	Bardsley (1948) % of strains	Present study % of strains
E.coli I	83.5	73.16	91.3	96.3	56.6	63.99	73.4
E.coli II	3.9	0.73	0.0	0.1	2.2	3.48	1.8
E.coli III	3.1	11.40	0.0	0.2	0.7	2.95	1.3
Cit.freundii I	4.3	0.37	0.5	0.0	18.9	13.90	0.0
Cit.freundii II	1.5	0.00	0.0	0.0	1.3	6.80	0.0
K.aerogenes I	3.4	6.25	5.8	0.2	15.3	5.51	9.9
K.aerogenes II	0.3	0.73	0.0	0.1	1.7	1.40	0.0
K.cloacae	0.0	0.00	0.0	0.0	0.9	0.46	2.3
Irregular II	0.0	2.94	2.4	3.1	0.9	0.27	3.6
Irregular III	0.0	0.00	0.0	0.0	0.0	0.04	0.0
Irregular IV	0.0	0.37	0.0	0.0	0.1	0.30	0.9
Irregular V	0.0	0.37	0.0	0.0	0.2	0.23	0.5
Irregular VI	0.0	3.68	0.0	0.0	0.1	0.04	5.0
Irregular VII	0.0	0.00	0.0	0.0	0.0	0.04	0.0
Irregular VIII	0.0	0.00	0.0	0.0	0.0	0.59	1.3
Unclassified Irregulars	0.0	0.00	0.0	0.0	1.1	0.00	0.0
Total	100.0	100.00	100.0	100.0	100.0	100.00	100.0

A striking difference between the two surveys is the rareness of Citrobacter freundii in the present study. The absence of K.cloacae in the faecal analyses is noteworthy.

In one of her investigations Bardsley (1934) also gave the distribution of coliforms in the samples. A comparison between this and the present study is shown in Table 51,

TABLE 51/...

TABLE 51

COMPARISON OF THE DISTRIBUTION OF COLIFORM STRAINS IN VARIOUS SAMPLES

Strains isolated	Human faeces				Water			
	Bardsley (1934)		Present study		Bardsley (1934)		Present study	
	% of samples	% of strains	% of samples	% of strains	% of samples	% of strains	% of samples	% of strains
E.coli	94	88	98.4	88.23	86.3	78.1	93.0	80.1
Cit.freundii	16	8	1.6	0.37	11.6	10.9	0.0	0.0
K.aerogenes	9	3	21.0	10.66	7.7	7.5	36.6	17.2
Irregulars	6	1	3.2	0.74	5.3	3.5	4.6	2.7
Total	-	100	-	100.00	-	100.0	-	100.0

The rareness of Cit.freundii in the present study is again striking.

An interesting comparison was made by Burke-Gaffney (1932) between the incidence of coliforms in the tropics (Dar-es-Salaam, East Africa) and in Europe, regarding faeces, unpolluted waters and polluted waters. In Table 52 this is compared with the results of the present investigation.

TABLE 52

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES AND WATER IN DIFFERENT GEOGRAPHICAL AREAS

Strains isolated	Faeces			Water		
	Burke-Gaffney (1932)		Present study South Africa % of strains	Burke-Gaffney (1932)		Present study South Africa % of strains
	Tropics % of strains	Europe % of strains		Tropics Unpolluted % of strains	Tropics Polluted % of strains	
E.coli	96	87	96.7	0	83	80.1
K.aerogenes	1	9	3.0	87	17	17.2
Cit.freundii	2	3	0.1	13	0	0.0
Other coliforms	1	1	0.2	0	0	2.7
Total	100	100	100.0	100	100	100.0

As far as the faecal specimens are concerned there is much resemblance between the results of the investigation in the tropics and that of the present investigation with the exception that the incidence of Cit.freundii

was/...

was low in the present study. Compared with the work of Burke-Gaffney, the water in the present study falls in the polluted group.

Skinner and Brudnoy (1932) made a survey of the occurrence of coliforms in faecal samples. Table 53 shows the comparison between his results and that of the present investigation.

TABLE 53

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES

Strains isolated	Faeces	
	Skinner & Brudnoy (1932) % of strains	Present study % of strains
E.coli I	87.4	93.5
E.coli II	1.4	3.3
Cit.freundii I	5.1	0.1
Cit.freundii II	1.0	0.0
K.aerogenes I	2.9	1.6
K.aerogenes II	1.9	0.3
Other coliforms	0.3	1.2
Total	100.0	100.0

This comparison reveals close similarity between the two sets of results except that in the present study , Cit.freundii occurred to a lesser extent.

The present results cannot easily be compared with that of Parr (1937, 1938) (vide Table 54). He used a broader definition for the Intermediates, than was used for the classical Koser's Intermediates, now known as Cit.freundii (vide 4.2, Table 46).

TABLE 54

COMPARISON OF COLIFORM STRAINS ISOLATED FROM HUMAN FAECES

Strains isolated	Parr (1937) % of samples	Present study % of samples
E.coli	92.3	98.3
E.coli H_2S+	0.5	1.6
E.coli liquefying gelatin	0.5	0.0
Intermediates	20.0	3.2
K.aerogenes	25.2	21.1
K.cloacae	3.8	0.0
Irregulars	0.0	1.6

During/...

During the present study no K.cloacae and gelatin liquefying E.coli strains were isolated, while the Intermediates were rare. Interesting is the occurrence of E.coli I H_2S+ strains in both studies. These strains were also isolated by Leclerc (1961).

A comparison with the results of Malcoln (1938) is shown in Table 55.

TABLE 55

COMPARISON OF COLIFORM STRAINS ISOLATED FROM CATTLE FAECES

Strains isolated	Malcoln (1938) Direct plating % of strains	Malcoln (1938) Inhibiting E.coli I % of strains	Present study Direct plating % of strains
E.coli I	97.3	0	98.89
Irregular II	0	0	0.74
Cit.freundii I	0.7	16.6	0.00
Cit.freundii II	0	7.2	0.00
K.aerogenes I	0.9	33.2	0.37
K.aerogenes II	0.7	12.3	0.00
K.cloacae	0.4	30.7	0.00
Total	100.0	100.0	100.00

Except for Irregular II, Cit.freundii and K.cloacae there is considerable agreement between both studies. As explained earlier (vide 1.2), Malcoln's method for inhibiting E.coli I and promoting the growth of the other coliforms could not be studied, as the paper concerned could not be obtained. Apparently his methods included enrichment in a brilliant green broth, in which case only one organism of each strain, promoted in that way, could multiply at such a rate to give the percentages obtained.

Another survey in America, of which the results are compared with that of the present investigation in Table 56, was done by Stuart, Griffin and Baker (1938). With the exception of Cit.freundii the results of these two surveys showed an amazing correspondence. The similarity between the bacteria found in faecal specimens from cattle is striking.

TABLE 56/...

TABLE 56

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES

Strains isolated	Human faeces		Cattle faeces	
	Stuart, Griffin and Baker (1938) % of strains	Present study % of strains	Stuart, Griffin and Baker (1938) % of strains	Present study % of strains
E.coli I	78.4	84.56	96.6	98.89
E.coli II	0.7	3.67	4.4	0.74
Cit.freundii I	3.6	0.37	0.0	0.00
Cit.freundii II	2.2	0.00	0.0	0.00
K.aerogenes I	14.0	9.93	0.0	0.37
K.aerogenes II	1.1	0.73	0.0	0.00
Irregular IV	0.0	0.37	0.0	0.00
Irregular V	0.0	0.37	0.0	0.00
Total	100.0	100.00	100.0	0.00

In Argentine Ferranola (1940) applied the British system of classification (vide 4.2, Table 44) to a very large number of water samples. The waters which were analysed were obviously faecally polluted, when judged on the occurrence of E.coli I strains (vide Table 57).

TABLE 57

COMPARISON OF COLIFORMS STRAINS ISOLATED FROM WATER

Strains isolated	Argentine water Ferranola (1940) % of strains	South Africa Present study % of strains
E.coli I	89.2	73.4
E.coli II	0.0	1.8
E.coli III	0.6	1.3
Cit. Klebsiella spp.	9.9	12.2
Irregular II	0.3	3.6
Irregular VI	0.0	5.0
Other coliforms	0.0	2.7
Total	100.0	100.0

It was/...

It was thought that waters of Argentine would correspond roughly to South African, Indian and other tropical and subtropical waters (vide Boizot, 1941). This, however, is not confirmed by Ferranola's data. His data correspond better to that for British waters (vide supra, Bardsley, 1938) and Australian waters (vide supra, Bardsley, 1948), especially as far as Irregular II and Irregular VI are concerned. Yet, it should be indicated that Kenpny (1948) made a study of the coliforms from 100 human beings in Buenos Ayres (Argentine). According to these results E.coli I predominated (90.7% of the strains), while 3.8% of the strains were of the "aerogenes-cloacae group," in which Irregular VI was the preponderant strain. Consequently Irregular VI was isolated in Argentine in 1948, and there is a possibility that it will be present in Argentine waters too today.

The results of a further survey on human faeces in America by Griffin and Stuart (1940) are compared with the present results in Table 58.

TABLE 58

COMPARISON OF COLIFORM STRAINS ISOLATED FROM HUMAN FAECES

Strains isolated	Griffin and Stuart (1940) % of strains	Present study % of strains
E.coli I	76.4	84.56
E.coli II	2.3	3.67
Cit.freundii I	7.4	0.37
Cit.freundii II	2.7	0.00
K.aerogenes I	9.4	9.93
K.aerogenes II	1.8	0.73
Other coliforms	0.0	0.74
Total	100.0	100.00

Except for Cit.freundii there is a remarkable agreement between the two sets of results.

An important study on the coliform flora of tropical waters was made by Boizot (1941) in Singapore. From the comparison in Table 59 it is evident that, although Cit.freundii was not isolated in the present study, the occurrence of K.aerogenes I and Irregular VI is significant in both studies. Boizot further indicated that according to Wilson and his co-workers in 1935, only 0.7% of the water samples in Britain contained Irregular VI, while according to Raghavachari and Iyer in 1938-9, 63.3% of Indian water samples contained Irregular VI.

TABLE 59/...

TABLE 59

COMPARISON OF COLIFORMS ISOLATED FROM WATER

Strains isolated	Boizot (1941) % of samples	Present study % of samples
E.coli I	65.6	93.2
E.coli II	3.3	6.8
Cit.freundii I	36.1	0.0
Cit.freundii II	13.1	0.0
K.aerogenes I	63.9	37.3
K.aerogenes II	4.9	0.0
E.coli III	4.9	2.3
Irregular II	1.6	9.1
Irregular VI	11.4	13.6
Other coliforms	0.0	6.9

Mackenzie, Taylor and Gilbert (1948) studied the occurrence of coliform flora in human faeces and in filtered and chlorinated waters in Britain.

TABLE 60

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES AND WATER

Strains isolated	Human faeces		Water	
	Mackenzie, Taylor and Gilbert (1948) % of strains	Present study % of strains	Mackenzie, Taylor and Gilbert (1948) % of strains	Present study % of strains
E.coli I	92.3	85.29	52.9	73.4
E.coli II	-	-	1.2	1.8
E.coli III	-	-	2.4	1.3
Cit.freundii	3.8	0.37	20.2	0.0
K.aerogenes	1.0	6.98	14.6	12.2
K.cloacae				
Irregular II	2.6	2.94	6.9	3.6
Irregular VI	0.3	3.68	7.8	5.0
Other coliforms	0.0	0.74	0.0	2.7
Total	100.0		100.0	100.0

From the comparison between these and the present results, in Table 60, it is again obvious that Cit.freundii was isolated rarely in the present study. The incidence of Irregular II and Irregular VI in the

treated/...

treated waters of Britain is important, as it is usually maintained that these two organisms rarely occur in British raw waters (Bardsley, 1938; Boizot, 1941; Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Wilson and Miles, 1964).

More recently Windle Taylor (1958) gave data of faecal analysis for coliforms.

TABLE 61

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES

Strains isolated	Windle Taylor (1958) % of strains	Present study % of strains
E.coli I	87.19	90.5
E.coli II	1.82	0.3
E.coli III	3.65	3.0
Cit.freundii	3.65	0.1
K.aerogenes	0.97	1.9
K.cloacae	0.00	0.0
Irregular VI	0.24	1.0
Other coliforms	0.00	3.2
Total	100.00	100.0

Table 61 shows that these results differ from the present mainly in the lower incidence of Cit.freundii and the higher incidence of Irregular VI in the present study.

Recently Geldreich, Bordner, Huff, Clark and Kabler (1962) undertook an extensive investigation on the occurrence of coliforms in faecal specimens obtained from man, cattle, pigs, sheep, fowls, turkeys and ducks in the U.S.A. Employing the membrane filtration technique several thousand coliform strains were isolated (vide Table 62). They did not specify the number of specimens which was examined. There is a general agreement between the investigation of Geldreich et al. and the present survey, regarding the incidence of E.coli I and some of the other strains. The rare occurrence of other coliforms in both studies is apparent. It is noteworthy that Geldreich et al. were able to recover four of Parr's Intermediates. Yet, Stuart, Griffin et al. (1938) and Griffin and Stuart (1940) failed to obtain similar results in the U.S.A. None of Parr's Intermediates was isolated in pure culture during the present investigation (vide supra, 3.3 and 4.2),

TABLE 62/...

TABLE 62

COMPARISON OF COLIFORM STRAINS ISOLATED FROM FAECES

Coliform strains isolated	Human				Livestock				Poultry			
	Geldreich et al.		Present study		Geldreich et al.		Present study		Geldreich et al.		Present study	
	No of strains	% of strains	No of strains	% of strains	No of strains	% of strains	No of strains	% of strains	No of strains	% of strains	No of strains	% of strains
E.coli I	3,932	87.2	230	84.56	2,237	95.6	529	97.0	1,857	97.9	270	95.07
E.coli II	99	2.2	10	3.67	14	0.6	14	2.6	20	1.1	12	4.23
Cit.freundii I	50	1.1	1	0.37	1	- *	0	0.0	5	0.3	0	0.00
Cit.freundii II	35	0.8	0	0.00	27	1.2	0	0.0	11	0.6	0	0.00
K.aerogenes I	245	5.4	27	9.93	0	0.0	1	0.2	1	0.1	1	0.35
K.aerogenes II	14	0.3	2	0.73	0	0.0	1	0.2	0	0.0	0	0.00
Atypical E.coli I	2	- *	0	0.00	0	0.0	0	0.0	2	- *	1	0.35
Atypical K.aerogenes I	2	- *	0	0.00	0	0.0	0	0.0	0	0.0	0	0.00
Parr's Intermediate I	106	2.4	0	0.00	59	2.5	0	0.0	0	0.0	0	0.00
Parr's Intermediate VII	0	0.0	0	0.00	1	- *	0	0.0	0	0.0	0	0.00
Parr's Intermediate VIII	6	0.1	0	0.00	0	0.0	0	0.0	0	0.0	0	0.00
Parr's Intermediate X	21	0.5	0	0.00	0	0.0	0	0.0	0	0.0	0	0.00
Other coliforms	0	0.0	2	0.74	0	0.0	0	0.0	0	0.0	0	0.00
Total	4,512	100.0	272	100.00	2,339	100.0	545	100.0	1,896	100.0	284	100.00

* Insufficient number of cultures examined.

It can be concluded that the results of the present investigation confirm those of the above-mentioned authors, viz. that E.coli I is the predominant strain in faeces and polluted waters and that there is a higher incidence of Irregular II and Irregular VI in waters of the warmer climates. The incidence of Cit.freundii in South Africa is rare when compared with data from abroad.

4.10 Status and significance of the various members of the coliform group as indicators of faecal pollution of water

The various members of the coliform group usually do not carry the same weight as indicators of faecal pollution of water (vide 1.2 and 1.3). Their status and significance as indicators of faecal pollution of water are summarised in Table 63 (Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Wilson and Miles, 1964).

The status and the significance, as given in Table 63, however, have been debated fiercely. American authorities do not accept this, as has been indicated (vide 1.3) : "....there is little or no evidence that coliform bacteria multiply on fresh grasses or grains; nor is there evidence that they multiply in soil" (American Public Health Association et al. 1955). It is, however, striking that this sentence was omitted in the Eleventh Edition of American Public Health Association et al. (1960).

From the foregoing (vide 4.9) it is apparent that E.coli I was the predominant organism in the majority of faecal specimens examined as well as in water which was subjected to faecal pollution. This undoubtedly suggests that the general acceptance of this organism as a reliable indicator of recent faecal pollution is soundly based (also compare Uhl, 1948). From the present study and from the literature (Great Britain Ministry of Health et al. 1956; Windle Taylor, 1958; Wilson and Miles, 1964) it is also apparent that the only other organisms of importance are E.coli III and Irregular II. Consequently, wherever E.coli I fails to be present a special survey can be carried out for the presence of E.coli III and Irregular II. As far as Irregular II is concerned, it should be indicated that it has been suggested to be included as an Escherichia strain, because of the biochemical and cultural characteristics which this organism has in common with the other Escherichia strains (Windle Taylor, 1958). By employing American classification schemes it is impossible to distinguish between this strain and E.coli II (vide 4.2, Table 43). In standard works on biochemical and serological characteristics of the Enterobacteriaceae this organism is included as an Escherichia strain, e.g. Kauffmann (1954), Ewing and Edwards (1962). Previously E.coli III was known as Irregular I, but for the same reasons it was renamed E.coli III

(Windle/...

TABLE 63

STATUS AND SIGNIFICANCE OF THE VARIOUS MEMBERS OF THE COLIFORM GROUP

Type of organism	Probable habitat	Indication of faecal pollution
E.coli I	Human and animal intestine	Supreme
E.coli II	Doubtful, probably not primarily intestinal	Suspicious, probably remote
E.coli III	Human and animal intestine	Possible
Cit.freundii I	Mainly soil	Suspicious, probably remote
Cit.freundii II	Mainly soil	
K.aerogenes I	Mainly vegetation	Suspicious, probably remote
K.aerogenes II	Mainly vegetation	
K.cloacae	Mainly vegetation	
Irregular II	Doubtful, probably human and animal	Possible
Irregular III	Doubtful	Suspicious, probably remote
Irregular IV	Doubtful	
Irregular V	Doubtful	
Irregular VI	Doubtful	
Irregular VII	Doubtful	
Irregular VIII	Doubtful	

(Windle Taylor, 1958; Wilson and Miles, 1964).

To get around this problem and to assure the highest quality of water, attention is being given to the significance of the coliforms as a group in drinking-water practice. The World Health Organization (1961) summarised this problem as follows : "All the members of the coliform group may be of faecal origin, and the worst possible interpretation should, therefore, be attached to their presence in water; thus from a practical point of view it should be assumed that they are all of faecal origin. Quite apart from the question of their being indicative of faecal pollution, organisms of the coliform group as a whole are foreign to water and must at least be regarded as indicative of pollution in its widest sense".

This last part is important. Coliforms may be regarded as indicators of pollution of water with terrigenous material. Another interpretation should, however, be attached to this. At present it is well known that coliforms, other than E.coli I, viz. Cit.freundii, K.aerogenes, K.cloacae and some of the so-called Irregulars, are usually more resistant to chlorination than E.coli I and intestinal pathogens, e.g. Salmonellae and Shigellae (Prescott et al. 1946; Great Britain Ministry of Health et al. 1956; Kabler, 1951; Windle Taylor, 1958; Kabler and Clark, 1960; McKee and Wolf, 1963; Coetzee and van Duuren, 1964; Wilson and Miles, 1964).

It is therefore obvious that when a survey is made on water and raw waters, E.coli I is the prime indicator of faecal pollution, but that the coliforms other than E.coli I are important as a controlling index in drinking-water practice.

A final point which should be mentioned is the occurrence of Irregular II and Irregular VI in natural waters. It is generally accepted that the occurrence of these two organisms in faeces and water is rare in Britain, Australia and North and South America (vide supra, 4.9). Attention, however, was drawn to the fact that treated water in Britain usually contains these organisms (vide 4.9) and that Irregular VI occurred in faeces in South America (vide 4.9 - Kempny, 1948). Furthermore, it is known that Irregular VI occurs in large numbers in the tropical waters of India and Singapore (vide 4.9). Little, however, was known about South Africa and the rest of Africa. When Burke-Gaffney (1932) undertook his survey of Eastern Africa (vide 4.9) he could not differentiate Irregular VI from K.aerogenes I. It was known in Europe and America that so-called "infections" of water with Irregular VI could occur (Prescott et al. 1946;

Mackenzie, Taylor and Gilbert, 1948; Windle Taylor, 1958; Wilson and Miles, 1964) and that these organisms are variants of K.aerogenes I (Windle Taylor, 1958; Wilson and Miles, 1964) (vide 1.2 and 4.2, Table 44). Furthermore, Taylor (1948) found that both Irregular VI and K.aerogenes were able to multiply on jute yarn and this might cause "infections"of water-mains.

Until the end of 1963 it was the practice in South Africa not to confirm Presumptive E.coli I counts. This is best demonstrated by the work of Keller (1959) : "MacConkey broth at 44.5°C. for 48 hr. to determine the number of typical faecal Escherichia coli" and this ".... is specific for Esch.coli ....". It was thus assumed that the occurrence of Irregular II and Irregular VI is also rare in South African waters. Consequently all surveys, viz. Gaillard (1959), Keller (1959, 1960), Harrison et al. (1960), Schoonbee (1962) and Harrison et al. (1963) used Presumptive E.coli I counts as an equivalent to E.coli I counts.

During the present survey, however, it was found that Irregular II and Irregular VI do occur in raw waters in the Potchefstroom region (vide 3.5, Tables 30,31,34-38, 40 and 42). No reference as to the occurrence of these organisms in South Africa could be found anywhere. Enquiries brought to light that occasionally they do occur in small quantities in the Pretoria region (Coetzee, 1964). During the years following 1963, extensive river and ocean surveys were carried out in Natal under the auspices of the South African Council for Scientific and Industrial Research (National Institutefor Water Research). From these it was obvious that Irregular II and Irregular VI occur in enormous numbers in some Natal waters (Kemp, Brand and Pretorius, 1966; Livingstone, 1966). In many cases the origin of the Irregular VI organisms could be traced back to fibre pollution, e.g. from sugar and paper mills.

This immediately suggests that it will be unsafe to regard Presumptive E.coli I counts as sufficient. It is therefore obvious that confirmative and differential methods should always be applied to all Presumptive E.coli I tests.

Apart from Total coliform tests, World Health Organization (1963) also advocated tests for the "faecal coliform group" which is stated as to be "all of definite faecal origin and are indicative of recent faecal pollution". This so-called "faecal coliform group" is the same as the Presumptive E.coli I count. Because it also includes Irregular VI, which is mainly derived from fibres, the above statement is not acceptable.

4.11/...

4.11 Conclusions

From the results obtained, it can be concluded that E.coli I is the predominant coliform occurring most frequently in the human and the animal intestine, and this organism is usually manifest in the recently passed faeces, as well as being normally detectable in faecally polluted river water.

Whereas, very few coliforms other than E.coli I were to be found in animal faeces apart from Irregular II, a number of strains other than E.coli I was isolated from human faeces. E.coli I without doubt, is the preponderant strain in man, but other strains whose presence is worth noting are E.coli III, K.aerogenes I, Irregular VI and Irregular II.

As expected, the river examined showed evidence of faecal pollution. This was revealed by the frequent recovery of E.coli I.

The higher incidence of K.aerogenes I and Irregular VI in river water, suggests that these coliforms have their natural habitat elsewhere than within the human and the animal intestine. As K.cloacae was recovered from the water samples only and not from faeces it appears safe to assume that this coliform has a habitat outside the human and the animal intestine.

Cit.freundii was isolated only once from faeces and never from water. This suggests that the intestine and river water are not the natural habitat of this coliform. Because of the uncommonness (or even total failure) with which E.coli II, K.aerogenes II, Irregular III to V, Irregular VII and Irregular VIII were recovered from faeces and river water it would appear that these coliforms have habitats other than warm-blooded intestines and natural fresh waters, or that these strains normally occur only rarely in nature.

In view of Irregular II and Irregular VI being present in South African waters, the Presumptive E.coli I test should never be taken as specific enough to demonstrate conclusively the presence of E.coli I.

Finally it may be concluded that in conformity with the aim of the present investigation, the data reported, showed that not all the members of the coliform group invariably occur in faeces and should consequently be regarded as mere organisms of passage when present in the human and the animal intestine. Therefore these coliform strains cannot be considered to be altogether acceptable and reliable indicators of recent faecal pollution

of/...

of water. On the other hand the frequent occurrence of E.coli I in faeces is noteworthy. The superiority of this coliform as a parameter of faecal pollution is thus confirmed. It appears to be the only practical and reliable indicator organism for the detection of faecal pollution of water in South Africa. Where relevant the occurrence of E.coli III and Irregular II may also be considered as supplementary parameters.

5. SUMMARY

An ecological study of the coliform bacteria, with special attention to their status as indicators of faecal pollution of water, was undertaken. Altogether 211 faecal specimens, from human and animal origin, were examined and 1,101 purified coliform strains were isolated and studied. Water samples were examined by the MPN method and from the positive tests of 44 samples, which confirmed the presence of coliform bacteria, 222 strains were isolated and studied in detail.

While the occurrence of the members of the coliform group in human and animal faeces was established, E.coli I proved to be the predominant strain in both faeces and faecally polluted waters. These results confirmed those of previous workers in this field. Cit.freundii was rearely recovered from faeces and not at all from water, while K.cloacae was isolated from water only.

The results reported show that not all the members of the coliform group can be considered as indicative of recent faecal pollution of water. The general acceptance of E.coli I as a reliable indicator of such pollution, however, was confirmed.

In addition to the above the membrane filtration technique usually employed for the quantitative determination of coliforms in water, was modified for the quantitative examination of coliform bacteria present in faeces.

6. ACKNOWLEDGEMENTS

The author wishes to express his gratitude and appreciation to the following people :

Staff of the Potchefstroom Experimental Farm for their assistance in obtaining faecal specimens from fowls, sheep and cattle.

Farmers and owners of live-stock and fowls who permitted sampling on their properties.

Mr K. de Kock for collecting the water samples.

Messrs P.H. Kemp and D.J. Livingstone for their advice and criticism.

Dr O.J. Coetzee for his guidance and advice throughout this study.

Dr D. van Eeden for his constructive criticism and advice.

Mesdames B.A. Warren-Hansen and F.D. Buckle for their assistance in the compilation of Tables and proof reading.

Mr J.Prinsloo for drawing the map of the Mooi River Catchment.

The author's wife for her assistance in preparing the manuscript and to her and Elsabé for their moral support.

Mrs O.D. de Goede for typing this manuscript.

7. REFERENCES

ALLEN, L.A., PASLEY, SHEILA, M. and PIERCE, MARGARET S.F. (1952 a) Conditions affecting the growth of Bacterium coli on bile salt media. Enumeration of this organism in polluted waters. J.gen. Microbiol. 7,257.

ALLEN, L.A., PASLEY, SHEILA M. AND PIERCE, MARGARET, A.F. (1952 b) Some factors affecting the viability of faecal bacteria in water. J.gen. Microbiol. 7,36.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, FEDERATION OF SEWAGE AND INDUSTRIAL WASTES ASSOCIATIONS (1955) Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. 10th ed., New York, American Public Health Association, Inc.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION AND WATER POLLUTION CONTROL FEDERATION (1960) Standard Methods for the Examination of Water and Wastewater. 11th ed., New York, American Public Health Association, Inc.

BARDSLEY, DORIS A. (1926) B.coli as an index of faecal pollution of water supplies. J.Hyg., Camb. 25, 11.

BARDSLEY, DORIS A. (1934) The distribution and sanitary significance of B.coli, B.lactis aerogenes and the intermediate types of coliform bacilli in water, soil, faeces and ice-cream. J.Hyg., Camb. 34, 38.

BARDSLEY, DORIS A. (1938) A comparison of two methods of assessing the number of different types of coliform organisms in water. J.Hyg., Camb. 38, 309.

BARDSLEY, DORIS A. (1948) A study of coliform organisms in the Melbourne water supply and in animal faeces, with observations on their longevity in faeces and in soil. J.Hyg., Camb. 46, 269.

BARRITT, M.M. (1936) The intensification of the Voges-Proskauer reaction by the addition of α - naphthol. J.Path. Bact. 42, 441.

BATTY-SMITH, C.G. (1942 a) Fermentation of cellobiose by the coli-aerogenes group of bacteria. J.Path. Bact. 54, 45.

BATTY-SMITH, C.G. (1942 b) The Eijkman test for faecal coli in the bacteriological examination of water supplies. A survey and discussion of the experimental work from 1929 to the present day with a study of 104 water samples and 602 cultures. J.Hyg., Camb. 42, 55.

BIGGER, J.W. (1937) The growth of coliform bacilli in water. J. Path. Bact. 44, 167.

BIGGER, J.W. AND NELSON, J.H. (1941) The growth of coliform bacilli in distilled water. J.Path.Bact. 53, 189.

BIGGER, J.W. AND NELSON, J.H. (1943) The metabolism of coliform bacilli in distilled water, J.Path.Bact, 55, 321.

BONDE, G.J./...

BONDE, G.J. (1963) Bacterial indicators of water pollution. A study of quantitative estimation. Wat.Poll.Abstr. 36,10.

BRODERICK, J.J. AND LOUGHLIN, A. (1963) Determination of Escherichia coli in fresh faeces of farm animals, using the MF technique. Wat. Poll.Abstr. 36, 44.

BREED, R.S., MURRAY, E.G.D. AND SMITH, N.R. (1957) Bergey's Manual of Determinative Bacteriology. 7th ed., Baltimore, The Williams and Wilkins Company.

BURKE-GAFFNEY, H.J. O'D. (1932) The classification of the colon-aerogenes group of bacteria in relation to their habitat and its application to the sanitary examination of water supplies in the tropics and in the temperate climates. A comparative study of 2500 cultures. J.Hyg., Camb. 32,85.

BURROWS, W. (1959) Textbook of Microbiology. 17th ed., Philadelphia and London, W.B. Saunders Company.

BURROWS, W. (1963) Textbook of Microbiology. 18th ed., Philadelphia and London, W.B. Saunders Company.

CABELLI, V.J. (1955) Lactose utilization in Klebsiella pneumoniae : The slow utilization of lactose by resting cells of lactose fermenting strains. J.Bact. 70,15.

CALITZ, F. (1966) Personal communication.

CARSTENS, ELIZABETH M.J. (1963) Bacteriophages and their possible use in sewage purification. J.Proc. Inst. Sew. Purif. 5, 467.

CHARTER, RUTH E. AND TAYLOR, JOAN (1952) Cultural and serological reactions of strains of Bact.coli isolated from babies. J.Path.Bact. 64,729.

CLARK, H.F. AND KABLER, P.W. (1952) The membrane filter in water quality tests. Am.J.publ.Hlth 42,385.

COCIOBA, J. (1948) The occurrence of bacteriophage in water. Its epidemiological importance. Wat.Poll.Res. 21,226.

COETZEE, O.J. (1962 a) Bakteriophagen als Indikator fäkaler Wasserverunreinigung. Gesundheitsingenieur 85,371.

COETZEE, O.J. (1962 b) Coliform Index - its use and limitations. Publ. Hlth, Johannesburg 62,16.

COETZEE, O.J. (1964) Personal communication

COETZEE, O.J. AND VAN DUUREN, F.A. (1964) The hygienic control of water distribution systems. Publ.Hlth, Johannesburg 64,31.

COLI-AEROGENES SUB-COMMITTEE (1956) The nomenclature of coli-aerogenes bacteria (Report). J.appl. Bact. 19,108.

COWAN, S.T. (1956) Taxonomic rank of Enterobacteriaceae 'Groups'. J.gen. Microbiol. 15,345.

CRUICKSHANK, R/...

CRUICKSHANK, R. (Editor) (1960) Mackie and McCartney's Handbook of Bacteriology a Guide to the Laboratory Diagnosis and Control of Infection. 10th ed., Revised Reprint 1962, Edinburgh and London, E. & S. Livingstone Limited.

CRUICKSHANK, R. (Editor) (1965) Medical Microbiology a Guide to the Laboratory Diagnosis and Control of Infection. 11th ed., Edinburgh and London, E. & S. Livingstone Limited.

DEPARTMENT OF WATER AFFAIRS, REPUBLIC OF SOUTH AFRICA (1962) Government Notice No. R.969 Regional standards for industrial effluents : Methods of testing. Regulation Gazette No. 90., Extraordinary Government Gazette (No. 266) 4,3, Pretoria, Government Printer.

DIENERT, E. (1947) Hygiene of bathing places. Wat.Poll.Res. 20,249.

DIFCO LABORATORIES INCORPORATED (1948) Difco Manual of dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. 8th ed., Detroit, Difco Laboratories Incorporated.

DIFCO LABORATORIES INCORPORATED (1953) Difco Manual of dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. 9th ed., Reprinted 1963, Detroit, Difco Laboratories Incorporated.

DIXON, W.J. AND MASSEY, F.J. (1951) Introduction to Statistical Analysis. 1st ed., New York, Toronto, London, McGraw-Hill Book Company, Inc.

EDWARDS, P.R. AND EWING, W.H. (1962) Identification of Enterobacteriaceae. 2nd ed., Minneapolis, Burgess Publishing Company.

EDWARDS, P.R. AND FIFE, MARY, A. (1955) Studies on the Klebsiella-Aerobacter group of bacteria. J.Bact. 70,382.

FERRAMOLA, R. (1940) Wilson method for bacteriological examination of water. Am.J.publ.Hlth 30,1083.

FRANK, N.F. AND SKINNER, C.E. (1941) Coli-aerogenes bacteria in soil. J.Bact. 42,143.

GAILLARD, J.R. (1959) A bacteriological survey of Durban coastal and harbour waters. Paper delivered at the Annual General Meeting of the Institute of Sewage Purification (South African Branch).

GAINEY, P.L. AND LORD, T.H. (1956) Microbiology of Water and Sewage. Second Printing, Englewood Cliffs, Prentice-Hall, Inc.

GALLOWAY, L.D. AND BURGESS, R. (1950) Applied Mycology and Bacteriology. 3rd ed., Reprinted 1952, London, Billing and Sons, Ltd.

GELDREICH, E.E., HUFF, C.B., BORDNER, R.H., KABLER, P.W. AND CLARK, H.F. (1962) The faecal coli-aerogenes flora of soils from various geographical areas. J.appl.Bact. 25,87.

GELDREICH, E.E., BORDNER, R.H., HUFF, C.B., CLARK AND KABLER, P.W. (1962) Type distribution of coliform bacteria in the feces of warm-blooded animals. J.Wat.Pollut. Control Fed. 34,295.

GILLESPIE, W.A./...

GILLESPIE, W.A. (1952) Biochemical mutants of coliform bacilli in infections of the urinary tract. J.Path.Bact. 64,551.

GOETZ, A. (1952) Discussion (of paper by Clark and Kabler, 1952). Am.J. publ.Hlth 42,389.

GRAY, E. (1951) The ecology of the bacteria of Hobson's Brook, a Cambridgeshire chalk stream. J.gen.Microbiol. 5,840.

GRAY, J.D.A. (1932) The significance of Bact.aerogenes in water. J.Hyg., Camb. 32,132.

GREAT BRITAIN MINISTRY OF HEALTH, MINISTRY OF HOUSING AND LOCAL GOVERNMENT REPORTS ON PUBLIC HEALTH AND MEDICAL SUBJECTS No. 71 (1956) The Bacteriological Examination of Water Supplies. 3rd ed., Reprinted 1960, London, Her Majesty's Stationery Office.

GRIFFIN, A.M. AND STUART, C.A. (1940) An ecological study of the coliform bacteria. J.Bact. 40,83.

GUELIN, A. (1948) Quantitative study of bacteriophage of the sea. Wat. Poll.Res. 21,204.

GUELIN, A. (1954) Bacteriophage and enteric bacteria in sea fish, and the problems of polluted waters. Wat.Poll.Abstr. 27,287.

GURR, G.T. (1957) Biological Staining Methods. 6th ed., London, George T. Gurr Limited.

HAJNA, A.A. AND PERRY, C.A. (1939) Optimum temperature for differentiation of Escherichia coli from other coliform bacteria. J.Bact. 35,275.

HAJNA, A.A. AND PERRY, C.A. (1943) Comparative study of presumptive and confirmation media for bacteria of the coliform group and for faecal streptococci. Am.J.publ.Hlth 33,551.

HARRISON, A.D., KELLER, P., DIMOVIC, D. AND CHOLNOKY, B.J. (1960) Ecological studies on Olifantsvlei near Johannesburg. (Separatum). Hydrobiologia 15,89.

HITCHNER, E.R., DONAGAN, E.A. AND ALPERT, S. (1938) A comparison of the biological activities of certain slow lactose fermenting bacteria and of rapidly fermenting strains derived from them. J.Bact. 36,260.

HOATHER, R.C. (1952) The bacteriological examination of water with particular reference to the stability of the numbers of coli-aerogenes, including Bact.coli, during storage of samples overnight. J.Instn.Wat.Engrs. 6,426.

HRUBY, J. (1954) An epidemic of typhoid fever in Slanj in 1951-1952. Wat.Poll.Abstr. 27,322.

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY (1963) Opinion 28 on the Nomenclature of Cloaca cloacae. Int. Bull.bact.Nomencl.Taxon. 13,38.

JONES, H.N./...

JONES, H.N. AND WISE, L.E. (1926) Cellobiose as an aid in the differentiation of members of the colon-aerogenes group of bacteria. J.Bact. 11,359.

JORDAN, E.O. (1903) The kinds of bacteria found in river water. J.Hyg., Camb. 3,1.

KABLER, P.W. (1951) Relative resistance of coliform organisms and enteric pathogens in the disinfection of water with chlorine. J.Am. Wat.Wks Ass. 43,553.

KABLER, P.W. AND CLARK H.F., (1952) The use of differential media with the membrane filter. Am.J.publ.Hlth 42,390.

KABLER, P.W. AND CLARK, H.F. (1960) Coliform group and fecal coliform organisms as indicators of pollution in drinking water. J.Am. Wat.Wks Ass. 52,1577.

KAUFFMANN, F. (1954) Enterobacteriaceae. 2nd ed., Copenhagen, Ejnar Munksgaard Publisher.

KELLER, P. (1959) Bacteriological aspects of sewage purification and river pollution. J.Hyg., Camb. 57,410.

KELLER, P. (1960) Bacteriological aspects of pollution in the Jukskei - Crocodile river system in the Transvaal, South Africa (Separatum). Hydrobiologia 14,205.

KEMP, P.H., BRAND, P.A.J. AND PRETORIUS, S.J. (1966) Surveys in the Three Rivers Region of Natal, South Africa. C.S.I.R. publication in preparation.

KEMPNY, J.C. (1948) The distribution of coliform bacteria in human faeces. Wat.Poll.Res. 21,233.

KENDEIGH, S.C. (1961) Animal Ecology. Second Printing 1962, Englewood Cliffs, Prentice-Hall, Inc.

KJELLANDER, J. (1960) Enteric streptococci as indicators of fecal contamination of water. Thesis, Supplementum 136, Acta path. microbiol. scand. 48,1.

KLEIN, L. (1957) Aspects of River Pollution. New York, Academic Press Inc., Publishers, London, Butterworths Scientific Publications.

KOLMER, J.A., SPAULDING, E.H. AND ROBINSON, H.W. (1951) Approved Laboratory Technic. 5th ed., New York, Appleton-Century-Crofts, Inc.

KOSER, S.A. (1923) Utilization of the salts of organic acids by the colon-aerogenes group. J.Bact. 8,493.

KOSER, S.A. (1924) Correlation of citrate utilization by members of the colon-aerogenes group with other differential characteristics and with habitat. J.Bact. 9,59.

KOSER, S.A. (1926 a) Differential tests and their relation to habitat of the coli-aerogenes group. J.Bact. 11,77.

KOSER, S.A./...

KOSER, S.A. (1926 b) Further observations on utilization of the salts of organic acids by the colon-aerogenes group. J.Bact. 11,409.

KOSER, S.A. AND GALT, R.H. (1926) The oxalic acid test for indol. J.Bact. 11,293.

LECLERC, H. (1961) Etude de Bacilles a Gram negatif, présentant une activité β galactosidesique, isoles des eaux. These, Faculte de Medecine et de Pharmacie de Lille.

LEVINE, M. (1954) An Introduction to Laboratory Technique in Bacteriology. 3rd ed., New York, The MacMillan Company.

LEVINE, M., EPSTEIN, S.S. AND VAUGHN, R.H. (1934) Differential reactions in the colon group of bacteria. Am.J.publ.Hlth 24,505.

LEVINE, M., TANIMOTO, R.H., MINETTE, H., ARAKAKI, J. AND FERNANDES, G.B. (1955) Simultaneous determination of coliform and Escherichia coli indices. Appl.Microbiol. 3,310.

LIVINGSTONE, D.J. (1965) An improved method for isolating Salmonellae from polluted waters. Publ.Hlth, Johannesburg 65,87.

LIVINGSTONE, D.J. (1966) Personal communication.

LOVELL, R. (1937) Classification of Bacterium coli from diseased calves. J.Path.Bact. 44,125.

MACKENZIE, E.F.W., TAYLOR, E.W. AND GILBERT, W.E. (1948) Recent experiences in the rapid identification of Bacterium coli type I. J.gen. Microbiol. 2,197.

MACCONKEY, A. (1905) Lactose-fermenting bacteria in faeces. J.Hyg., Camb. 5,333.

MACCONKEY, A. (1909) Further observations on the differentiation of lactose-fermenting bacilli with special reference to those of intestinal origin. J.Hyg., Camb. 9,86.

MACFADYEN, A. (1963) Animal Ecology. 2nd ed., London, Sir Isaac Pitman & Sons Ltd.

MACPHERSON, C.R. (1950?) Paracolons. Thesis, University of Cape Town.

MALCOLM, J.F. (1938) The classification of coliform bacteria. J.Hyg., Camb. 38,395.

MCCOY, J.H. (1962) Salmonellae in crude sewage, sewage effluent and sewage-polluted natural waters. Advances in Water Pollution Research, Proceedings of the First International Congress held in London, 1,205, B.A. Southgate (Editor). Oxford, London, Pergamon Press, published in 1964.

MCCRADY, M.H. (1937) A practical study of procedures for the detection of the presence of coliform organisms in water. Am.J.publ.Hlth 27,1243.

MCEWEN, W.W.W./...

MCEWEN, W.W.W. (1949) Bacteriological Technique. London, J. & A. Churchill Ltd.

MCKEE, J.E. AND MCLAUGHLIN, R.T. (1958) Application of molecular filter techniques to the bacterial assay of sewage. Sewage ind. Wastes 30,129,245.

MCKEE, J.E. AND WOLF, H.W., (Editor) (1963) Water Quality Criteria. Prepared with Assistance from Division of Water Supply and Pollution Control U.S. Public Health Service, Department of Health, Education and Welfare. 2nd ed., Sacramento, The Resources Agency of California State Water Quality Control Board.

MOORE, B., PERRY, E.L. AND CHARD, S.T. (1952) A survey by the sewage swab method of latent enteric infection in an urban area. J.Hyg., Camb. 50,137.

NELSON, J.H. (1942) The growth of coliform bacilli in water containing various organic materials. J.Path.Bact. 54,449.

ODUM, P. (1959) Fundamentals of Ecology. 2nd ed., Reprinted 1964, Philadelphia and London, W.B. Saunders Company.

PARR, L.W. (1936 a) Culture characters, relationships and occurrence of coli-aerogenes intermediates, with particular reference to feces, fresh and stored at various temperatures. J.Bact. 31,23.

PARR, L.W. (1936 b) Sanitary significance of the succession of coli-aerogenes organisms in fresh and in stored feces. Am.J.publ.Hlth 26,39.

PARR, L.W. (1937) Succession of colon-typhoid organisms in normal human feces. J.Bact. 33,75.

PARR, L.W. (1938) Coliform intermediates in human feces. J.Bact. 36,1.

PARR, L.W. AND FRIEDLANDER, H. (1942) Studies on aberrant coliform bacteria. Am.J.publ.Hlth 32,381.

PEDERSON, H.T. AND SKINNER, C.E. (1955) A comparison of standard lactose broth with lauryl sulphate broth and with the Eijkman method for demonstrating fecal coliform bacteria. Appl.Microbiol. 3,55.

PELCZAR, M.J. AND REID, R.D. (1958) Microbiology. New York, Toronto, London, McGraw-Hill Book Company, Inc.

PERRY, C.A. (1938) Escherichia coli versus the coliform group of bacteria as an indicator of fecal pollution in shell-fish and the value of the Eijkman test as a primary test for Escherichia coli. J.Bact. 36,451.

PERRY, C.A. AND HAJNA, A.A. (1944) Further evaluation of E.C. medium for the isolation of coliform bacteria and Escherichia coli. Am. J.publ.Hlth 34,735.

PIVNICK, H. AND FABIAN, F.W. (1954) Coliform bacteria in soluble oil emulsions. Appl.Microbiol. 2,107.

POTCHEFSTROOM EXPERIMENTAL FARM POULTRY SECTION (1963) Personal communication.

POTCHEFSTROOM/...

POTCHEFSTROOM EXPERIMENTAL FARM STOCK-BREEDING SECTION (1963) Personal communication.

PRESCOTT, S.C., WINSLOW, C.E-A., MCCRADY, M.H. (1946) Water Bacteriology with special Reference to Sanitary Water Analysis. 6th ed., Reprinted 1950, New York, John Wiley and Sons, Inc., London, Chapman and Hall, Limited.

PUBLIC HEALTH LABORATORY SERVICE (1957) The bacteriological control of drinking water. (Memorandum No. 3 of the Public Health Laboratory Service). Mon.Bull.Minist.Hlth. 16,8.

RANDALL, J.S. (1956) The sanitary significance of coliform bacilli in soil. J.Hyg., Camb. 54,365.

RAVEN, CLARA, PEDEN, D. AND WRIGHT, H.D. (1940) The bacteriological examination of water supplies. J.Path.Bact. 50,287.

REID, G.K. (1961) Ecology of Inland Waters and Estuaries. Third Printing 1964, New York, Reinhold Publishing Corporation, London, Chapman and Hall, Ltd.

REITLER, R. AND SELEGMANN, R. (1957) Pseudomonas auruginosa in drinking water. J.appl.Bact. 20,145.

SALLE, A.J. (1961) Fundamental Principles of Bacteriology. 5th ed., New York, Toronto, London, McGraw-Hill Book Company, Inc.

SCHOONBEE, H.J. (1962) Hydrobiology of the Umgeni estuary and Zeekoe River. Thesis, Potchefstroom University for C.H.E.

SHERWOOD, H.P. AND CLEGG, L.F.L. (1942) Further studies of incubation at 44°C. as a test for 'faecal coli'. J.Hyg., Camb. 42,45.

SIEGEL, S. (1956) Nonparametric Statistics for the behavioral Sciences. New York, Toronto, London, McGraw-Hill Book Company, Inc.

SILVERTON, R.E. AND ANDERSON, M.J. (1961) Handbook of Medical Laboratory Formulae. London, Butterworths.

SKINNER, C.E. AND BRUDNOY, H.G. (1932) The utilisation of citrates and the fermentation of cellobiose by strains of Bacterium coli isolated from human faeces. J.Hyg., Camb. 32,529.

SLANETZ, L.W. AND BARTLEY, CLARA H. (1955) Evaluation of membrane filters for the determination of numbers of coliform bacteria in water. Appl.Microbiol. 3,46.

SMITH, D.T. AND CONANT, N.F. (1960) Zinsser Microbiology. 12th ed., New York, Appleton - Centuary - Crofts, Inc.

SOCIETY OF AMERICAN BACTERIOLOGISTS (1957) Manual of Microbiological Methods. New York, Toronto, London, McGraw-Hill Book Company, Inc.

SOUTH AFRICAN BUREAU OF STANDARDS (1951) Specification for Water for Domestic Supplies. Pretoria, Published by the Council of the South African Bureau of Standards.

STOKES, J.L. AND OSBORNE, W.W. (1955) A selenite brilliant green medium for the isolation of Salmonellae. Appl. Microbiol. 3,217.

STRELL, M. (1955) Wasser und Abwasser Reinhaltung der Gewässer. München, R. Oldenbourgh Verlag.

STUART, C.A., GRIFFIN, A.M. AND BAKER, MURIEL E. (1938) Relationships of coliform organisms. J.Bact. 36,391.

STUART, C.A., WHEELER, K.M. AND GRIFFIN, A. (1938) Coliform organisms in certified milk. J.Bact. 36,411.

STUART, C.A., ZIMMERMAN, A., BAKER, MURIEL E., AND RUSTIGIAN, R.(1942) Eijkman relationships of the coliform and related bacteria. J.Bact. 43,557.

TASK GROUP REPORT (1953) Technique of bacterial examination of water with molecular filter membranes. J.Am.Wat.Wks Ass. 45,1196.

TAYLOR, C.B. (1941) Bacteriology of fresh water. II The distribution and types of coliform bacteria in lakes and streams. J.Hyg., Camb. 41,17.

TAYLOR, C.B. (1942) The ecology and significance of the different types of coliform bacteria found in water. A review of the literature. J.Hyg., Camb. 42,23.

TAYLOR, C.B. (1945) The effect of temperature of incubation on the results of tests for differentiating species of coliform bacteria. J.Hyg., Camb. 44,109.

TAYLOR, C.B. (1951) Coli-aerogenes bacteria in soils. J.Hyg., Camb. 49,162.

TAYLOR, E.W. (1948) Bacteriology of water samples from mains. Wat.Poll. Res. 21,109.

TAYLOR, JOAN AND CHARTER, RUTH E. (1952) The isolation of serological types of Bact.coli in two residential nurseries and their relation to infantile gastro-enteritis. J.Path.Bact. 64,715.

THE OXOID DIVISION OF OXO LIMITED (1961) The Oxoid Manual of Culture Media including Ingredients and other Laboratory Services. 2nd ed., London, The Oxoid Division of Oxo Limited.

THRESH, J.C. AND BEALE, J.F. (1925) The Examination of Waters and Water Supplies. 3rd ed., London, J. and A. Churchill.

U.S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE PUBLIC HEALTH SERVICE (1962) Public Health Service Drinking Water Standards. Washington, U.S. Department of Health, Education and Welfare Public Health Service.

UHL, E. (1948) The rôle of the coli content in water in estimating its hygienic quality. Wat.Poll.Res. 21,182.

VAUGHN, R. and LEVINE, M. (1936) Hydrogen sulfide production as a differential test in the colon group. J.Bact. 32,65.

WILSON, G.S/...

WILSON, G.S. AND MILES, A.A. (1955) Topley and Wilson's Principles of Bacteriology and Immunity. 4th ed., London, Edward Arnold (Publishers) Ltd.

WILSON, G.S. AND MILES, A.A. (1964) Topley and Wilson's Principles of Bacteriology and Immunity. 5th ed., London, Edward Arnold (Publishers) Ltd.

WINDLE TAYLOR, E. (1949) The Examination of Waters and Water Supplies (Thresh, Beale and Suckling). 6th ed., London, J. & A. Churchill Ltd.

WINDLE TAYLOR, E. (1955) Comparison of 6-hour and 24-hour incubation periods at 44°C. as a confirmatory test for Bacterium coli type I. J.Hyg., Camb. 53,50.

WINDLE TAYLOR, E. (1958) The Examination of Waters and Water Supplies (Thresh, Beale and Suckling). 7th ed., London, J. & A. Churchill Ltd.

WINDLE TAYLOR, E. AND BURMAN, N.P. (1964) The application of membrane filtration techniques to the bacteriological examination of water. J.appl.Bact. 27,294.

WORLD HEALTH ORGANIZATION (1958) International Standards for Drinking-Water. Geneva, Palais des Nations.

WORLD HEALTH ORGANIZATION (1961) European Standards for Drinking-Water. Geneva, Palais des Nations.

WORLD HEALTH ORGANIZATION (1963) International Standards for Drinking-Water. Geneva, 2nd ed., Palais des Nations.

ZIEGLER, N.R. (1939) Late lactose fermenting organisms of the coli-aerogenes group. Am.J.publ. Hlth 29,257.