

## Coliform Organisms in Domestic Water Supplies in Ceylon

by

D. C. KATUGAMPOLA AND T. H. ASSIM

(from the Medical Research Institute, Colombo)

THE bacteriological examination of water supplies is conducted in accordance with the recommendations of the Ministry of Health, United Kingdom (1939). In routine control it is not practicable to search for the presence of specific pathogenic bacteria. The criterion of assessment of the purity of a water supply is the absence of faecal pollution, judged through bacteriological investigation for the indicator organism, *Bact. coli* type I.

The 44°C. MacConkey broth test has found acceptance as determining the presence of *Bact. coli* type I. Due to the interference of this test by lactose fermenting anaerobic bacteria Mackenzie, Taylor and Gilbert (1948) recommended the adoption of brilliant green bile broth (BGB) for this test as in this medium the anaerobes were suppressed and *Bact. coli* type I permitted to grow. BGB is widely used for this purpose now. A number of workers have reported on the specificity of the 44°C. BGB test for *Bact. coli* type I. Sometimes other organisms of the coliform group, most frequent being *Bact. aerogenes* type I, have been reported to produce acid and gas in lactose at 44°C.

### *Occurrence of 44°C. positive strains of Bact. Aerogenes type I in Water Supplies.*

Reference	No. of strains studied	Strains 44°C. +	
		No.	per cent.
Bardsley (1938)	2840	2	0.03
Garrard (1943)	126	1	0.8
Taylor (1945)	434	12	2.8
Bardsley (1948)	6986	3	0.04
Mackenzie et al (1948)	425	23	5.4
Thomas et al (1951)	204	1	0.5
Thomas et al (1955)	347	2	0.6

The occurrence of *Bact. aerogenes* type I had been so rare as to justify disregarding its presence in considering the specificity of the 44°C. test for *Bact. coli* type I. Sherwood and Clegg (1942) stated that incubation at 44°C. was the most suitable temperature for use with MacConkey broth for permitting the maximum number of *Bact. coli* to produce gas while inhibiting the maximum number of other coliforms. Bardsley (1948) found that, except for *Bact. coli* type I and other coliform organisms which had more of an affinity with *Bact. coli* type I rather than the intermediate-aerogenes-cloacae group, only a few organisms were able to produce acid and gas in MacConkey broth at 44°C.



On the contrary, Hajna and Perry (1939) reported that *Bact. coli*, *Citrobacter*, '*Aerobacter cloacae* and *aerogenes*' were able to produce acid and gas at 44°C. Taylor (1945) found 15% of *Bact. aerogenes* cultures studied producing acid and gas at 44°C. Raghavachari and Seetharama Iyer (1939) have shown that about 50% of *aerogenes*-like organisms isolated from 12 samples of Madras waters produced acid and gas in MacConkey broth at 44°C. These two workers later (1940) estimated that nearly 80% of the *aerogenes* organisms isolated by them grew at 44°C. Bancrjea and Sen (1940) found that a small number of *aerogenes*, intermediate and irregular forms were capable of producing acid and gas at 44°C. in MacConkey broth. Boizot (1941) reported that 9.6% of organisms of intermediate-*aerogenes*-*cloacae* group from 61 water samples fermented MacConkey broth at 44°C.

Mackenzie, Taylor and Gilbert (1948) found that *Bact. coli* type I could be differentiated from other coliforms both by fermentation of BGB and indole production at 44°C. According to them these two tests together were specific for *Bact. coli* type I.

*Indole Production at 44°C. by various Coliform Types*

(Mackenzie, Taylor and Gilbert, 1948)

Organism	No. of Strains	Strains producing indole at		Strains producing acid and gas in BGB at 44°C.
		37°C.	44°C.	
<i>Bact. coli</i> type I	59	59	59	59
Irregular type I	17	17	6	0
Intermediate type II	12	12	0	0
<i>Aerogenes</i> type II	19	19	0	0

*Differentiation of Coliform Types by Gas Production in BGB at 44°C. and Indole Production at 44°C.*

(Mackenzie, Taylor and Gilbert, 1948)

Gas in BGB at 44°C.	Indole at 44°C.	Organism
+	+	<i>Bact. coli</i> type I
+	—	Irregular type II and Irregular type VI
—	+	Other coliforms
—	—	do

Thomas et al (1955) found that the 44°C. indole test did not distinguish nine of ten 44°C. BGB positive strains of Intermediate type II.

In view of the divergent results reported by a number of workers the present study was undertaken to evaluate the 44°C. fermentation tests and the indole test at 44°C. for the differentiation of coliforms in water supplies in Ceylon.



The designation 'domestic' used in reference to water supplies is meant to indicate all supplies utilised for private domestic use other than those maintained by public bodies such as those under the Local Government Department and the Public Works Department.

### Experimental Methods

*Sources of Samples.* The results recorded in this paper were obtained from 153 water samples collected from various sources in different parts of the Island. They were considered representative of the domestic water supplies in Ceylon.

Source	No. of samples
Wells (all types)	100
Taps (private pipe-borne)	29
Tanks (domestic)	8
Reservoirs	10
Rivers	4
Springs	2
Total	153

*Examination of Samples.* All samples were collected by Public Health Inspectors who had been instructed on the procedure for the collection of water samples for bacteriological examination. The samples were collected into sterilised bottles of 250 ml. capacity and placed in special insulated boxes containing ice, immediately after collection. The boxes were despatched by special orderly to the Medical Research Institute. The time taken for transport varied, about 14 hours being the maximum. On receipt at the laboratory the samples were put up for the presumptive coli test at 37°C. in quintuplicate amounts of 10 ml., 1 ml., and 0.1 ml. in MacConkey broth according to the Ministry of Health (1939) technique. Tubes showing acid and gas production in 24 and 48 hours were sub-inoculated with a wire loop into (1) MacConkey broth, (2) brilliant green bile broth (BGB), for incubation at 44°C. in a water bath for 24 to 48 hours. From one tube each of (1) and (2) containing the least quantity of water and showing acid and gas production in 24 or 48 hours streak inoculations were made on MacConkey agar as advocated by Thomas et al (1955). These plates were incubated at 44°C. in a temperature controlled humidity cupboard for 24 hours. From each of the plates one to three acid producing colonies were picked into tubes of peptone water and these were incubated at 30°C. for 24 hours. The following studies were made on these cultures for purposes of differentiation, namely, morphology and Gram reaction, fermentation of lactose at 30°C./24 hours, fermentation of MacConkey broth at 37°C./24 hours, fermentation of MacConkey broth at 44°C./24-48 hours, indole production at 44°C./24 hours, and the IMViC reactions carried out according to the technique in the Report (1949) of the Coliform Subcommittee. Any cultures showing positive reactions in both the methyl red as well as V and P tests were, for all practical purposes, regarded as impure and discarded. Organisms were classified according to the Coliform Subcommittee recommendations (see Report, 1949).



## Results

(1) *44°C. positive MacConkey broths.* The results of the study made on lactose fermenting colonies picked from MacConkey agar sub-cultures at 44°C. are shown in Table I.\*

Of the 290 colonies studied, 145 (50%) were *Bact. coli* type I (44°C. positive), 109 (37.6%) other coliform colonies positive at 44°C., 36 (12.4%) subsequently failed to ferment MacConkey broth at 44°C. Of these 36, twenty-nine were *Bact. aerogenes* type I colonies. Of the 109 colonies of other coliforms than *Bact. coli* type I that on sub-culture were able to ferment MacConkey broth at 44°C., 84 (77%) were *Bact. aerogenes* type I, 10 (9.2%) *Bact. aerogenes* type II, 6 (5.5%) irregular forms of Wilson and others (1935).

(2) *44°C. positive BGB.* The results of the study made on lactose fermenting colonies picked from MacConkey agar sub-cultures at 44°C. are shown in Table II.\*

Of the 297 colonies studied, 165 (55.5%) were *Bact. coli* type I (44°C. positive), 111 (37.4%) other coliforms positive at 44°C., and 21 (7.1%) that failed to ferment lactose at 44°C. subsequently although 16 were *Bact. aerogenes* type I. Of the 111 colonies of other coliforms that continued to ferment MacConkey broth at 44°C., 64 (57.7%) belonged to *Bact. aerogenes* type I, 13 (11.7%) to *Bact. aerogenes* type II, 10 (9%) to Intermediate type II, and 19 (17%) to irregular forms.

*Indole production.* All colonies that fermented lactose at 44°C., and which were normally expected to produce indole, were tested for indole production at both 37°C. and 44°C. The results are shown in Table III.

TABLE III

Organism	Total No. of colonies studied	No. of colonies showing indole production at		
		37°C./24 hrs.	44°C./24 hrs.	44°C./48 hrs.
<i>Bact. coli</i> type I	310	310	308	2
<i>Bact. aerogenes</i> type II	28	23	23	not done
Intermediate type II	14	14	14	do
Irregular VII (Wilson et al, 1935)	10	10	10	do

Growth from all colonies examined produced indole at 37°C. in 24 hours. The 310 *Bact. coli* type I colonies fermented lactose at 44°C., and 308 of these produced indole at 44°C. in 24 hours, and 2 in 48 hours. All colonies of *Bact. aerogenes* type II, intermediate type II and irregular VII (Wilson et al, 1935) produced indole at 44°C. in 24 hours.

\* For tables I and II see end of article.



Contrary to the findings of Mackenzie, Taylor and Gilbert (1948) all normally indole producing organisms isolated by us in the present study were able to produce indole at 44°C. Thomas et al (1955) reported that the 44°C. indole test did not distinguish 9 of 10 BGB 44°C. positive strains of intermediate type II. Our results are in close agreement.

This study has emphasised the need for more knowledge on the coliform organisms found in water and their relationship to environment and excretal pollution. Standards for the evaluation of water supplies, specially in the Tropics, will not be satisfactory without this knowledge.

### Summary

153 water samples have been examined to determine the types of coliform organisms present in domestic water supplies in Ceylon.

The possibility of differentiation of organisms with the help of the MacConkey broth test at 44°C., BGB test at 44°C., and the indole test at 44°C. has been investigated.

The results of the study have been discussed in relation to the assessment of Tropical water supplies.

### Conclusions

BGB test at 44°C. is more advantageous for the isolation of Bact. coli type I than the MacConkey broth test at 44°C.

A large proportion of Bact. aerogenes type I colonies were found able to produce acid and gas both in MacConkey broth and BGB when grown at 44°C.

The indole test at 44°C. failed to differentiate Bact. aerogenes type II, intermedius type II and irregular type VII. All isolations of these organisms and Bact. coli type I produced indole at 44°C.

The present knowledge on the coliform organisms appears to be inadequate for a correct assessment of their importance especially in Tropical water supplies.



## REFERENCES

- BAKSHI, R. and SEN, A. K. (1940)—Ind. J. Med. Res. 28. 315.
- BAKSHI, D. A. (1938)—J. Hyg., Camb., 38. 309.
- BAKSHI, D. A. (1948)—J. Hyg., Camb., 46. 269.
- BOHART, G. E. (1941)—J. Hyg., Camb., 41. 566.
- GARRARD, E. H. (1943)—Canad. J. publ. Hlth. 34. 87.
- HARRIS, A. A. and PERRY, C. A. (1939)—J. Bact. 38. 275.
- MACKENZIE, E. F. W., TAYLOR, E. W. and GILBERT, W. E. (1948)—J. gen. Microbiol. 2. 197.
- MINISTRY OF HEALTH (1939)—Reports on Public Health and Medical Subjects, No. 71, London, H. M. S. O.
- RAJAGOPALACHARI, T. N. S. and SEETHARAMA IYER, P. V. (1939)—Ind. J. Med. Res. 26. 867.
- RAJAGOPALACHARI, T. N. S. and SEETHARAMA IYER, P. V. (1940)—Ind. J. Med. Res. 28. 55.
- Report (1949)—Classification of the Coli-aerogenes bacteria, Report of the Coliform Sub-committee, Soc. appl. Bact., London.
- SHERRWOOD, H. P. and CLEGG, L. F. L. (1942)—J. Hyg., Camb., 42. 45.
- TAYLOR, C. B. (1945)—J. Hyg., Camb., 44. 109.
- THOMAS, S. B., JONES, G. E. and FRANKLIN, P. M. (1951)—Proc. Soc. appl. Bact. 14. 45.
- THOMAS, S. B., CLEGG, L. F. L., CUTHBERT, W. A., OXLEY, C. D., SCARLETT, C. A. & WESTWATER, C. H. (1955)—J. appl. Bact. 18. 1. 9.
- WILSON, G. S., TWIGG, R. S., WRIGHT, R. C., HENDRY, M. P. and MAIER, I. (1935)—Med. Res. Comm. Spec. Rept. Ser. No. 206, London, H. M. S. O.

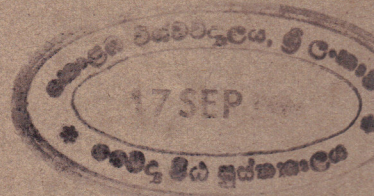




TABLE I  
LACTOSE FERMENTERS ISOLATED FROM MACCONKEY BROTH AT 44°C.  
Number of lactose fermenting colonies examined  
(picked from MacConkey agar at 44°C.)

Source	Number of samples	Total	<i>B. coli</i> I	<i>B. aerogenes</i> I	<i>B. aerogenes</i> II	Intermedius I	Intermedius II	Irregular forms			
44°C. + 44°C. + 44°C.— 44°C. + 44°C.— 44°C. + 44°C.— 44°C. + 44°C.—											
Wells	100	185	86	62	17	6	1	2	—	5	
Taps	29	52	29	8	9	3	—	—	—	1	
Tanks	8	19	12	4	3	—	—	—	—	—	
Reservoirs	10	21	9	7	—	—	—	3	—	2	—
Rivers	4	5	4	1	—	—	—	—	—	—	—
Springs	2	8	5	2	—	—	—	—	—	—	—
Total	153	290	145	84	29	10	1	5	4	—	6



TABLE II  
LACTOSE FERMENTERS ISOLATED FROM BGB AT 44°C.

Source	Number of samples	Number of lactose fermenting colonies examined (picked from MacConkey agar at 44°C.)					
		Total	<i>B. coli</i> I	<i>B. aerogenes</i> I	<i>B. aerogenes</i> II	<i>Intermedius</i> I	<i>Intermedius</i> II
				44°C.+ 44°C.+ 44°C.—	44°C.+ 44°C.—	44°C.+ 44°C.—	44°C.+ 44°C.—
Wells*	100	195	107	50	8	7	—
Taps	29	51	27	7	5	2	—
Tanks	8	18	12	4	2	—	—
Reservoirs	10	25	15	3	—	3	—
Rivers*	4	6	3	—	1	—	—
Springs	2	2	1	—	—	1	—
Total	153	297	165	64	16	13	1
						3	—
						—	10
						—	—
						—	—
						—	19
						—	4

\* 44°C.+Bact. coli type II isolated :—wells—1, rivers—1.