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Bacteriological analysis of drinking water from shallow wells on a tropical, limestone island (Mactan, Philippines)

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Contents

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Summary and conclusions

Bacteriological analyses of well water samples from a tropical island were performed using membrane filtration techniques and subsequent identifications of pure cultures. Thermotolerant coliforms are found to be a reliable index of faecal contamination, because a high proportion proved to be Escherichia coli. In wells with low numbers of thermotolerant coliforms sometimes high numbers of total coliforms or group D streptococci could be found. This may reflect either proliferation in the environment or a better survival, compared to thermotolerant coliforms. In additional experiments, it was demonstrated that group D streptococci are not reduced in numbers during desiccation of faeces, while thermotolerant coliforms are reduced by a factor of ca 1000.

1. Introduction

In May 1975 the University of San Carlos (USC), Cebu City, Philippines organized the Hydrologic Research and Training Unit, later renamed the Water Resources Centre (WRC). The objectives of WRC were to conduct research on the water resources of the entire province of Cebu thus also providing the basis for a training program for students. In January 1977 a cooperation was established between the WRC and the Delft University of Technology, Holland in which an exchange of information was to take place, mainly by carrying out joint projects in the Philippines with the help of Dutch experts and by training Filipino students in Delft. In the first years the attention was mainly directed towards quantitative aspects of water management (hydrology, geology etc.), but it was soon realized that also water

quality aspects should be taken into account. In cooperation with the other departments of USC a water laboratory was established in 1978. The laboratory started by performing chemical and physical water analysis, and in January 1979 the equipment necessary for microbiological analyses was added to the laboratory inventory. To start with, a project was initiated in which two Dutch students would perform standard analyses of the bacteriological quality of drinking water from shallow wells at the island Mactan, situated opposite Cebu-city. Details of this study will be published elsewhere. This report describes studies aimed to assure the reliability of the analytical methods used by the Water Laboratory of the USC under the existing conditions.

2. Materials and methods

2.1 Sampling

The wells that have been included in this study can be divided into three categories:

- Open wells (O) consisting of a square, manually dug shaft, usually about 60 x 60 cm wide and dug to 0,5 - 1 m below the ground water level. Generally a concrete casing and a concrete apron of varying size are constructed to prevent pollution by surface run-off or washing water. Sometimes, the wells are covered by a wooden or metal hood to prevent pollutants to fall into the water. Water is abstracted from these wells by lowering and pulling up a container, that can be anything like a used basketball, an oil drum or a galvanized bucket. All open wells are private property and used by a limited number (1-10) of families.
- Pumped wells (P) basically having the same construction as open wells but the casing is always covered by a concrete hood. A suction pipe has been lowered in the standing ground water that is lifted by a hand pump. Pumped wells also are private property and used by a limited number of families.

- Government wells (G), drilled tube wells that generally abstract water at a few metres below ground water level. Water is abstracted by means of a deep-well pump. The wells are owned and maintained by the government and in some instances used for the drinking water supply of a great number of families.

  Samples from open wells were taken by means of an alcohol-flame sterilized stainless steel bucket. Pumped wells were sampled by flaming the mouth of the pump and abstracting the sample directly from the outflow after a few minutes of pumping. Samples were collected in sterilized glass-bottles that were kept in a wooden box, isolated by polystyrene foam. A sampling trip took about 4 hours, after which analyses were started directly. Generally, a rise in temperature of at most 2°C was reached in the samples (initial temperatures were 28-30°C).

2.2 Isolation techniques

A volume of sample expected to give 20 - 80 typical colonies was sucked through a Gelman GN 6 membrane filter (0,45 µm). If the sample volume was less than 10 ml, it was suspended in at least 10 ml of distilled water before filtration.

The filter was brought onto a selective solid medium that was incubated at the appropriate temperature, depending on the analysis. A small circular (50 mm) glass fibre filter impregnated with 0.5 ml of glycerin was introduced into the lid of each Petri dish to prevent excessive moisture on the agar surfaces during incubation. The filter was removed after incubation, lids were rubbed dry and flame-sterilized.

2.3 Coliforms

Membrane filters were incubated on Endo-agar (Difco B6) for 24 h at 37°C. All colonies with a metal sheen or deep red in appearance were counted as presumptive coliforms. A number of these colonies was selected for further identification. They were inoculated into two liquid

confirmatory media, brilliant green bile lactose broth (BGBL, Difco B7) and tryptone lactose broth (TLB, see annex 1). The tubes were incubated at 37°C and read for growth and gas formation after 24 and 48 h. In addition, pure cultures were prepared by streaking on tryptone agar (see annex 1), incubated at 37°C for 24 h. If necessary, a second plate was streaked. Pure cultures were maintained on Dorset egg-medium slopes (see annex 1).

2.4 Thermotolerant coliforms

Membrane filters were incubated on 0,4% enriched Teepol agar (Oxoid MM369 solidified with 15 g/l agar Oxoid L15) for 24 h at 44°C. All yellow colonies were counted as presumptive thermotolerant coliforms. A number of colonies was selected for further identification. These colonies were inoculated into two liquid confirmatory media, brilliant green bile lactose broth (BGBL, Difco B7) and tryptone lactose broth (TLB, see annex 1). The tubes were incubated at 44°C and read for growth and gas formation after 24 and 48 h. In addition, pure cultures were prepared by streaking on tryptone agar (see annex 1) and incubated at 37°C for 24 h. If necessary, a second plate was streaked. Pure cultures were maintained on Dorset egg medium slopes.

2.5 Identification of coliforms and thermotolerant coliforms

Some cell material from a slant was suspended in sterile distilled water and identified in the API 20E gallery (Analytab Products Incorporated).

2.6 Group D streptococci

Membrane filters were incubated on KF streptococcus agar (Difco B496) for 48 h at 37°C. All red or pink colonies were counted as presumptive group D streptococci. A number of colonies was selected for further identification. These colonies were pure cultured on bile esculin azide agar

(Difco B525), incubated at 37°C for 48 h. Growth on this medium was recorded as positive only if a tan coloured zone appeared around the colonies. These colonies were subsequently subjected to a catalase test by suspending in a 3% (w/v) solution of hydrogen peroxide. Bile esculin positive and catalase negative colonies were further tested for growth at 44°C in Todd-Hewitt broth (annex 1) and gelatin liquefaction in Todd-Hewitt gelatin (annex 1). Reactions in these media were recorded after 5 days. Pure cultures were maintained on Dorset egg medium slopes.

3. Results

The presumptive counts of the three indicator bacteria are given in Table 1. Due to limited resources in the laboratory, it was not possible to analyze decimal dilutions of the sample, only one volume was chosen. Owing to faulty estimation of the number of bacteria, indeterminate results (i.e. > 100) were obtained in some cases. Almost all membranes, incubated on Endo-agar showed heavy growth of non-coliform bacteria, frequently leading to a confluent growth. Therefore, the number of coliforms reported in Table 1 should be regarded as an indication of the lower limit only. From these membranes, 32 colonies were inoculated into the two confirmatory media. From Table 2b it can be seen that 66% of typical colonies on Endo-agar were able to form gas in BGBL and were thus confirmed as coliform bacteria. Upon further identification of 13 pure cultures (Table 2a) it was found that all strains showing gas formation in BGBL indeed belonged to the four genera that make up the coliform group (i.e. Escherichia, Citrobacter, Enterobacter and Klebsiella) while among the bacteria not showing gas formation only one belonged to these genera. Gas formation in the non-inhibitory medium TLB occurred less frequently than in BGBL.

From membranes incubated on enriched Teepol agar, 32 colonies were inoculated into the confirmatory media and also identified. The results

are given in Table 3. It can be calculated that 71% of the yellow colonies (regardless of size) were formed by Escherichia coli. A regrouping of the results in Table 4 shows that neither BGBL nor TLB gave accurate indications of the identity of a yellow colony as E.coli. Only with the indol test (in this study performed as a part of the API 20E profile) it was possible to distinguish between E.coli and other thermotolerant lactose fermenters.

The results of further testing of 50 presumptive group D streptococcus colonies isolated from KF-agar are given in Table 5. Further work on a smaller number of pure cultures (Table 6) showed that all strains giving a positive bile-esculin reaction also posessed the group D antigen. Based on the biochemical reactions most strains are related to Streptococcus faecium. From Table 7 it can be read that a very high proportion of colonies with a diameter of 0.5 mm or more belong to the group D streptococci. The overall selectivity of KF-agar under these circumstances was 83%.

4. Discussion

The aim of this study was to evaluate the reliability of commonly used indicator systems for fecal contamination in the analysis of tropical ground waters. To reach this goal, it has to be investigated firstly whether commonly used techniques and media will perform as well under tropical conditions as they do in temperate climates.

This study has demonstrated that counting coliform bacteria by the membrane filtration technique is virtually impossible due to the presence of a heavy background flora that could not even be suppressed on a highly selective medium like Endo-agar. If the enumeration of coliform bacteria is considered necessary, the use of liquid enrichment techniques should be tested.

Thermotolerant coliforms on the contrary could very well be counted by membrane filtration, even on the low selectivity medium Teepol-agar. Apparently, the higher incubation temperature of 44°C inhibits growth of almost all background flora. Moreover, a very high proportion of yellow (i.e. lactose-fermenting) colonies on the medium proved to be Escherichia coli, so that the counts bear a direct relationship to fecal pollution. Further investigation of yellow colonies by inoculation into a liquid medium and subsequent reading of gas formation from lactose at 44°C - the usual way of confirming the presence of thermotolerant coliforms - gave confusing results. A number of strains that were later identified as E.coli did not produce gas in tryptone lactose or brilliant green bile broth when analysed in the Philippines. Upon further investigation in Holland they were found to be gas-producing strains nevertheless, so that these observations have probably been caused by some inhibitory factors in the media. It must be born in mind that all work was done in a small laboratory with only basic equipment, and that especially brilliant green bile broth is a very delicate medium to prepare. If it is felt necessary that the identity of yellow colonies on Teepol-agar is known, the API system appears to be very useful. Alternatively, the indole-test alone can also indicate whether a culture belongs to E.coli but at a considerably lower cost (Table 4).

For the analysis of group D streptococci, membrane filtration using KF-streptococcus agar was found to be suitable. Usually, no further identification of typical red to pink colonies will be necessary. If in some cases (like in the presence of large numbers of pin-point colonies) confirmations should be done, reliable results can be obtained by subculturing on bile esculin azide agar. All strains that develop colonies on the medium, surrounded by the typical tan-coloured to black halo caused by

esculin hydrolysis, and giving a negative catalase-reaction, can be considered group D streptococci.

Apart from theses technical considerations it has to be evaluated what information can be derived from counts of indicator organisms and their numerical ratio's. Apparantly the conclusions that can be reached in this report will be limited to a comparison of the three different indicator groups. There are no data on survival of pathogens in the water or on the incidence of diseases in relation to the water quality. The results in Table 1 indicate that there may be large differences in numbers of indicator-organisms compared to each other. This is even more clearly shown by Table 8, which is based on the previously mentioned four-months study by two Dutch students (Smet and Negenman, 1979). This table is based on 4-6 observations per well. From comparison with Table 1 it follows that the numbers of indicator bacteria in a well may vary considerably with time. In some wells high numbers of coliforms or group D streptococci were found, while only low numbers of thermotolerant coliforms were present (i.e. well numbers 5, 7, 22, 23 and others). At present thermotolerant coliforms are considered the principal sanitary indicator for water supplies that may be subject to direct fecal contamination (Evison and James, 1978). In their absence the presence of other indicator organisms must be explained by either proliferation in the environment or greater survival.

As far as coliform bacteria are concerned they are long known to be able to multiply in surface water in temperate climates (Kittell and Furfari, 1963; Dutka, 1973). As the temperature of the wells investigated in this study ranged from 28 to 30°C, multiplication of coliforms in this water is very likely. This implies that this parameter probably has little sanitary significance. This is especially important in relation to the WHO International Standards for Drinking Water (1971) that specifically stated that water form open wells should contain not more than 10 coliforma bacteria in 100 ml. If these standards were applied to the wells at Mactan (Table 8) 40 out of 55 should be condemned although in

24 of these 40 weeks the medium number of thermotolerant coliforms did not exceed 5 per 100 ml.

Some variants of group D streptococci are also known to be able to multiply on plants, in soils or in water. Identification of a number of strains isolated from wells at Mactan (Table 7) showed that none of the isolates was related to these ubiquitous variants, as described a.o. by Mundt (1961, 1973, 1975). It can therefore not be excluded that these streptococci are of true fecal origin. Possibly the high numbers of streptococci compared to thermotolerant coliforms may be explained by a better survival of the former during the desiccation of faeces (annex 2). This process will usually happen before the faeces are transported to the ground water by occasional rain-showers.

Only by comparison with the survival of important pathogens a choice between the different indicator-organisms can be made on solid grounds. At present this information is lacking and will be very hard to obtain, as in only one-third of cases of gastro-enteritis the responsible pathogen can be identified (Echeverria et al., 1979). It therefore seems logical to regard the presence of thermotolerant coliforms as an indication of recent fecal pollution and that of coliforms of group D streptococci in the absence of thermotolerant coliforms as the remainders of an intermittent or remote pollution. Health risks will be smaller in the latter case as only the more resistant pathogens will have survived.

Great care should be taken in condemning a water supply on the basis of counts of indicator organisms. As has been stated clearly by Feachem (1978) the quality of a water supply is but one factor to promote community hygiene, water quantity and accessibility being other factors of prime importance. Condemning a supply because of suspected fecal contamination may even lead to an increase of certain infectious diseases because limited water supplies cause a breakdown of general hygiene.

With regard to the situation at Mactan, it should also be realized that each well is generally used by only a small number of families. If pathogens are introduced in a well by faeces, it is also very likely that the same pathogens will reach the users of this well by other ways (contaminated food or surfaces, direct contact with feces etc.) so that water may play only a minor role in the transmission of the diseases. Care should be taken however if this contaminated water is transported to other communities by piped supplies or by trucks. In such a case, the water should obey to much more rigid standards.

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Table 1

Presumptive counts of coliforms (CF), thermotolerant coliforms (TC) and group D streptococci (FS) per 100 ml water.

Sample number	Well[1] number	Well[2] type	CF	TC	FS
1	9	O	65	>100	20
2	10	P	>100	>100	34
3	26	O	20	>100	70
4	22	O	n.c.[3]	30	25
5	19	O	n.c.	103	>100
6	18	P	n.c.	99	20
7	32	G	>100	0	6
8	33	O	>100	107	22
9	35	O	80-100	20-40	20
10	37	O	95	4	50
11	46	P	7	2	15
12	50	O	>100	4	>>100
13	8870	G	1	0	37
14	7228	G	60	1	15
15	2698	G	45	70	6
16	2162	G	n.c.	50	>100
17	2725	G	15-30	6	46
18	7052	G	10	0	0
19	2724	G	>100	0	0
20	8872	G	21	0	>100
21	8871	G	12	0	3
22	18-62-112	G	30	0	97
23	10-23-47	G	2	0	0

1) See text

2) O = open well P = pumped well G = government well

3) n.c. = not countable due to heavy background of non-lactose fermenting bacteria

Table 2

a. Identification of colonies from Endo agar (24 h 37°C)

Culture no.	Sample no.	Colony type	BGBL 48h 37°C	TLB 48h 37°C	API number	Identification
C1	1	sheen	g	t	5144572	E.coli
C2	1	sheen	g	t	5146572	E.coli
C3	7	deep red, slimy	g	t	5215772	Kl.aerogenes
C4	7	deep red, slimy	g	t	5215773	Kl.aerogenes
C5	7	deep red	-	t	0207127	-
C6	10	sheen	t	t	3046524	Ae.hydrophila
C7	10	sheen	-	t	0011206	-
C8	10	sheen	-	t	2200004	Pseudomonas sp.
C9	10	deep red	-	t	5217773	Kl.aerogenes
C10	11	sheen	g	t	1404773	Ci.freundii
C11	11	sheen	t	t	3044125	Ae.hydrophila
C12	11	sheen	-	t	2211004	Ps.aeruginosa
C13	11	sheen, small	g	t	1504712	Ci.freundii

Legend: see Table 1.

b. Confirmation of typical (sheen or deep red) colonies on Endo (includes C1 - C13)

Confirmatory medium / No. of tubes with	BGBL 48h 37°C	TLB 48h 37°C
gas formation	21	13
growth only	6	19
no growth	5	0

Table 3

Identification of colonies from 0,4% enriched Teepol agar (24h 44°C)

Culture no.	Sample no.	BGBL[1] 48h 44°C	TLB[1] 48h 44°C	API number	Identification
T1	1	t	t	5144572	E.coli
T2	1	-	t	5144572	E.coli
T3	2	-	g	1104543	Citrobacterspec.
T4	2	t	g	1106543	-
T5	3	g	g	5144572	E.coli
T6	3	-	t	non-viable	
T7	4	-	g	5044553	E.coli
T8	4	-	g	5144572	E.coli
T9	5	t	g	5144572	E.coli
T10	5	t	g	5144572	E.coli
T11	6	g	g	1144552	E.coli
T12	6	t	g	2201000	Pseudomonas sp.
T13	6	g	g	5144542	E.coli
T14	8	g	g	5144572	E.coli
T15	8	t	-	5144552	E.coli
T16	9	-	-	2305173	En.sakazakii
T17	9	-	t	2305173	En.sakazakii
T18	10	-	g	5144572	E.coli
T19	10	t	g	5144572	E.coli
T20	11	-	t	3305373	En.sakazakii
T21	11	t	t	3305173	En.cloacae
T22	12	t	t	3305373	En.sakazakii
T23	12	g	t	4344572	E.coli
T24	14	-	g	1044552	E.coli
T25	15	t	g	5144572	E.coli
T26	15	-	-	1007127	Pseudomonas sp.
T27	15	-	-	5144572	E.coli
T28	15	-	-	5144552	E.coli
T29	16	-	t	5144572	E.coli
T30	16	-	g	5144572	E.coli
T31	16	-	g	5355773	Kl.oxytoca[2]
T32	17	-	t	5144572	E.coli

1) - no growth
t growth
g growth and gas formation

2) in further work identified as a mixed culture of E.coli and Kl.aerogenes

Table 4

Comparison of confirmatory tests for thermotolerant coliforms

Brilliant green bile broth

g 5 x E.coli

t 6 x E.coli, 2 x Enterobacter, 2 x ?

- 11 x E.coli, 3 x Enterobacter, 2 x ?, 1 x Klebsiella, 1 x Pseudomonas

Tryptone lactose broth

g 12 x E.coli, 3 x ?, 1 x Klebsiella

t 5 x E.coli, 4 x Enterobacter, 1 x ?

- 3 x E.coli, 1 x Enterobacter, 1 x Pseudomonas

Indole test

+ 21 x E.coli, 1 x Klebsiella*

- 5 x Enterobacter, 4 x ?, 1 x Pseudomonas

Yellow colonies on Teepol-agar: $\frac{22}{31}$ = 71% is E.coli

* in further work identified as a mixed culture of E.coli and Kl.aerogenes

Table 5

Identification of colonies on KF-agar, 48 h at 37°C

Culture number	Well[1] number	Well[2] type	Colony[3] type	BEA[4] 37°C	Catalase	Growth at 44°C [5]	Gelatin hydr. 5 d 37°C [6]
S1	9	0	R	+	-	-	-
S2	9	0	R	+	-	(+)	-
S4	10	P	P	+	-	+	-
S5	10	P	P	+	-	-	-
S6	10	P	R	+	-	+	+
S7	26	0	R	+	-	(+)	-
S8	26	0	P	+	-	+	-
S9	26	0	(r)	-	-		
S10	22	0	R	-	+		
S11	22	0	P	-	-		
S12	22	0	r	-	-		
S13	19	0	r	-	-		
S14	19	0	r	+	-	-	-
S15	19	0	r	+	-	(+)	-
S16	18	P	P	+	-	+	-
S17	18	P	R	+	-	+	-
S18	18	P	r	+	-	-	-
S19	32	P	r	+	-	-	-
S20	32	P	r	+	-	-	-
S21	32	P	r	+	-	+	-
S22	33	0	P	+	-	+	-
S23	33	0	r	+	-	-	-
S24	33	0	r	+	-	-	-
S25	35	0	P	+	-	+	-
S26	35	0	R	±	-	-	-
S27	35	0	r	+	-	+	-
S28	37	0	P	+	-	+	-
S29	37	0	R	+	-	+	-
S30	37	0	r	±	+		
S32	46	0	r	+	-	+	-
S34	50	0	r	+	-	+	-
S35	50	0	(r)	+	-	-	-
S37	Naga		R	+	-	+	-
S38	(8870)	G	r	-	-		
S39	(2698)	G	R	+	-	+	-
S40	-	G	r	±	+		
S41	-	G	r	+	-	-	-
S42	-	G	(r)	+	-	-	-
S43	-	G	R	+	-	+	+
S44	-	G	R	+	-	+	-
S45	(8872)	G	R	+	-	-	-
S46	(8872)	G	R	+	-	+	-
S47	(2162)	G	R	+	-	-	-
S48	(2162)	G	r	+	-	+	-
S49	(7228)	G	R	+	-	-	-
S50	(7228)	G	P	+	-	+	-

1), 2) see Table 1

3) R = large red > 2 mm P)
r = medium red 0,5-2 mm p) same but pink
(r)= small red < 0,5 mm (p))

4) BEA = bile esculin azide agar (Difco)
\+ = growth + esculin hydrolysis
\- = growth, no esculin hydrolysis
$\pm$ = growth + esculin hydrolysis only after 48 h

5) + : growth within two days
(+): growth within five days
\- : no growth within five days

6) + : gelatin liquefaction within 2 days
\- : no gelatin liquefaction within 5 days

Table 6 Serological and biochemical reactions of selected streptococcal strains, and reaction patterns typical for S.faecium and S.faecalis.

Culture no.	Group D-antigen	BEAA[1]	BE[2]	10% bile	40% bile	45°C	30 min. 60°C	6.5% NaCl	bacitracin	Voges-Proskauer	arabinose	glucose	lactose	saccharose	maltose	trehalose	raffinose	starch	inuline	glycerol O[3]	glycerol ø[4]	mannitol	sorbitol	salicin	aesculin	arginin	hippurate	melibiose	melezitose
1	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	-
4	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	+	-
8	+	+	+	+	+	+	(+)	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-
14	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	-	-	+	-
15	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	-	-	+	-
17	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	-
19	+	+	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-
22	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	-
25	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-
28	+	+	+	+	+	+	(+)	+	-	-	+	+	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	+	-
32	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+	-	+	-	-	-	-	-	+	+	-	-	+	-
39	+	+	+	+	+	+	(+)	+	-	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	+	+	-	-	-
42	+	+	+	+	+	-	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+	-
43	+	+	+	+	+	(+)	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+	-
45	+	+	+	+	+	-	-	+	-	-	-	+	+	+	+	+	-	-	-	+	-	+	-	+	+	+	+	-	-
48	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+	-	-	-	-	+	-	+	-	+	+	-	-	+	-
50	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-
S.faecium	+	+	+	+	+	+	+	+	-	D	+	+	+	+	+	+	D	-	-	-	-	+	-	+	+	+	D	+	-
S.faecalis	+	+	+	+	+	+	+	+	-	D	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+

1) BEAA: bile esculin azide agar

2) BE: 40% bile esculin agar

3) O: aerobic

4) ø: anaerobic

Table 7

Identification of colonies on KF-agar, 48 h at 37°C

Colony type	Group D	Non group D	because of	
			cat+	BEA-
R	15 (94%)	1	1	0
r	15 (75%)	5	2	3
(r)	1 (50%)	1	0	1
P	8 (100%)	0	0	0
p	0 (0%)	1	0	1

Overall selectivity for group D streptococci: 83%

Legend: see table 5.

Table 8

Median value for the period of 17 February 1979 till 24 April 1979 of presumptive counts of coliforms (CF), thermotolerant coliforms (TC) and group D streptococci (FS) per 100 ml

Well[1] number	Well[2] type	CF	TC	FS
1	P	0	0	32
2	P	20	0	0
3	O	>1000	12	140
4	O	100	6	>100
5	O	100	2	>1000
6	P	125	4	1
7	O	>1000	3	140
8	P	200	3	157
9	O	10	5	600
10	P	350	1	700
11	O	120	0	120
12	P	100	0	60
13	P	19	0	85
14	P	360	1	119
15	P	120	0	17
16	P	10	13	100
17	P	220	8	124
18	P	195	1	129
19	O	1	1	78
20	P	16	0	3
21	G	0	0	0
22	O	75	2	1000
23	P	2500	1	62
24	O	220	57	273
25	P	52	0	5
26	O	380	100	1432
27	O	300	21	470
28	P	8	0	1
29	O	70	0	64
30	O	135	0	>100
31	P	540	>50	>100
32	G	0	0	28
33	O	800	66	680
34	O	600	9	280
35	O	300	75	200
36	G	0	0	0
37	O	1120	116	930
38	O	255	12	>1000
39	O	48	0	>100
40	G	3	0	0

Table 8 (continued)

Well[1] number	Well[2] type	CF	TC	FS
41	P	15	0	47
42	P	20	4	9
43	O	260	>50	400
44	P	0	0	50
45	O	>1000	94	>100
46	P	20	0	>100
47	P	180	27	72
48	P	20	0	4
49	P	0	0	0
50	O	70	0	>100
51	P	0	0	0
52	P	0	0	0
53	P	1	0	0
54	P	0	0	0
55	O	>150	18	40
56	O	no data	0	>100

1) See text

2) O = open well, P = pumped well, G = government well

Annex 1

Preparation of culture media

Tryptone lactose broth (TLB)

Tryptone (Oxoid L 42)	10 g
Lactose	10 g
Sodium chloride	5 g
Distilled water	1000 ml

Dissolve by heating
Dispense in 10 ml aliquots in culture-tubes containing a Durham-vial
Sterilize 15 min. at 121°C

Tryptone agar (TA)

Tryptone (Oxoid L 42)	10 g
Sodium chloride	5 g
Agar (Oxoid L 13)	15 g
Distilled water	1000 ml

Dissolve by heating
Sterilize 15 min. at 121°C, pour into Petri-dishes

Todd-Hewitt broth (TH)

Fat-free minced beef	450 g
Tryptone (Oxoid L 42)	20 g
Glucose	2 g
Sodium bicarbonate	2 g
Sodium chloride	2 g
Disodium phosphate (anhydrous)	0,4 g
Distilled water	1000 ml

Prepare an infusion from the meat by gently boiling in the water for 1 h.
Filter through cotton-gauze
Dissolve other components and dispense in culture-tubes
Sterilize 10 min. at 115°C

Todd-Hewitt gelatin

Todd-Hewitt broth	1000 ml
Gelatin	150 g

Dissolve gelatin, dispense into culture-tubes
Sterilize 10 min. at 115°C

Dorset egg medium

Whole eggs	3 parts
Physiological saline	1 part

Clean the eggs thoroughly
Disinfect by immersion into 96% alcohol for 5 min.
Break the eggs and add physiological saline
Mix well by shaking
Distribute into culture-tubes
Solidify by placing into an incubator set at 80°C for 2 h., repeat if necessary the next day for 1 h.

Annex 2

Survival of thermotolerant coliforms and group D streptococci during desiccation of faeces.

Procedure

Freshly taken human faecal samples were well mixed by hand-kneading and 7 parts of 2 g each were taken from each sample. One part was used to be analysed for indicator bacteria, the other parts were smeared onto the surface of microscopic slides in layers of ca. 2 mm. The slides were placed in glass containers over either a saturated solution of sodium hydroxide or silicagel.

For each desiccant, three containers were prepared in such a way that all containers held one slide of each faecal sample. The containers were closed with a glass lid, sealed with vaseline and placed in an incubator at 30°C. After 24, 72 and 168 h respectively, one container with each desiccant was opened and the dried faeces were examined for numbers of indicator organisms.

Faecal samples were suspended in 198 ml peptone-physiological saline (dried faeces were soaked for 1 h and then scraped from the slide). The mixture was homogenized by mechanical action (Ultraturax) and serially diluted in peptone-physiological saline. These dilutions were analyzed in duplicate by membrane filtration, using Gelman GN-6 Metricel filters placed on 0,4% enriched Teepol agar (incubation 6 h at 30°C, followed by 12 h at 44°C) and KF streptococcus agar (incubation 48 h at 37°C).

Following incubation, typical colonies were counted and the numbers on membranes showing 20-80 colonies were used to calculate the bacterial densities of the original faecal samples.

Results

The number of thermotolerant coliforms in the fresh samples varied considerably (see Table).

In the first 24 h of desiccation over sodium hydroxide, numbers fell about a factor 1000, they remained at that level for the additional desiccation period. Die-off of thermotolerant coliforms during drying over silicagel (a more hygroscopic substance) was more gradually, but the reduction after 1 week was more or less the same.

Group D streptococci on the other hand showed no significant reduction during desiccation up to 1 week.

Behavior of indicator bacteria during desiccation of human faeces under laboratory conditions (^{10}log presumptive counts per g faeces).

a. Thermotolerant coliforms

Faecal sample number	Fresh	Dried over sodium hydroxide			Dried over silicagel		
		24 h	72 h	168 h	24 h	72 h	168 h
1	6,9	3,5	2,9	2,8	4,8	3,7	3,8
2	8,5	4,8	3,7	5,0	5,6	3,1	5,0
3	7,4	3,6	3,1	4,3	5,5	5,1	3,5
4	7,6	5,3	5,0	4,6	6,3	5,8	5,0
5	5,6	3,5	3,0	3,6	4,1	2,4	3,4
6	5,3	4,1	4,0	4,6	6,7	4,8	4,8
Mean reduction		2,8	3,3	2,7	1,4	2,5	2,6

b. Group D streptococci

Faecal sample number	Fresh	Dried over sodium hydroxide			Dried over silicagel		
		24 h	72 h	168 h	24 h	72 h	168 h
1	6,6	6,4	6,1	6,3	6,8	6,3	6,5
2	6,9	6,5	6,7	6,7	6,2	6,6	6,9
3	7,3	6,0	6,7	7,2	7,3	6,8	7,4
4	5,4	4,7	5,0	5,2	5,3	5,1	5,3
5	7,6	7,8	7,6	7,7	7,8	7,7	7,9
6	5,5	5,6	5,7	5,8	6,8	6,6	5,4
Mean reduction		0,4	- 0,2	0,2	0,0	0,1	0,0