Evidence suggests that the fecal streptococci are more practical and reliable indicators of fecal contamination of water than the coliforms. The author concludes that their use as an index of sewage pollution of water should receive more attention from federal, state, and local water testing laboratories.

DETECTION AND SANITARY SIGNIFICANCE OF FECAL STREPTOCOCCI IN WATER

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WHILE the coliform bacteria continue to be accepted as the indicator organisms of choice to establish the presence of sewage or fecal contamination of water, there has been a renewed interest in recent years in revaluating the fecal streptococci as indicators of such pollution. This has been due to the development of more efficient technics for the enumeration of these organisms in water or sewage with the subsequent demonstration, as was suggested by bacteriologists[1-4] at the beginning of this century, that the presence of fecal streptococci in water is positive and definite evidence of fecal contamination of human or animal origin.

Improvement in technics for the detection of fecal streptococci in water probably began with the report of Mallmann[5] in 1940 that sodium azide could be used as a selective agent for detection of these organisms. Hajna and Perry[6] in 1943 modified the Mallmann medium by the addition of brom cresol purple. Acid production at 45.5° C in this medium was indicative of Streptococcus fecalis. Winter and Sandholzer[7] employed a sodium azide broth as a presumptive test for fecal streptococci in water or sewage and confirmed the presence of these organisms using the same medium to which they added 0.5 per cent NaCl and 650 units of pencillin. Litsky, Mallmann, and Fifield[8] in 1953 described an ethyl violet azide broth for use as a confirmatory medium for fecal streptococci. For water analysis, they proposed a multiple tube test using dextrose azide broth as a presumptive medium and the ethyl violet azide broth for confirmation. They reported this procedure confirmed 100 to 1,000 times as many enterococci as did the Hajna-Perry SF and the Winter and Sandholzer methods.

The use of a membrane filter technic for the enumeration of fecal streptococci in water was reported by Slanetz, Bent, and Bartley[9] in 1955 and by Slanetz and Bartley[10] in 1957. In the latter report, a medium designated as M-Enterococcus Agar was described for use with membrane filters which provided higher counts of fecal streptococci in water, sewage, or feces than were obtained by any of the tube dilution methods tested. This medium was essentially 100 per cent selective for fecal streptococci, and based on the arithmetic mean counts the ratio of enterococci to coliforms was 1.9:1 for the water samples tested, 1:1.7 for the

sewage samples, 1:1.6 for fecal samples from human beings, and 15:1 for fecal samples from animals. These are the largest ratios of fecal streptococci to coliforms so far reported for such samples. Sureau[11] in 1958 reported that this M-Enterococcus Agar yielded highly satisfactory results for the isolation and enumeration of fecal streptococci in water samples tested by the membrane filter technic and he noted that this procedure has been adopted at the Pasteur Institute in Madagascar for the routine bacterial analysis of water. Leclerc and Catsaras[12] also reported favorable results with the M-Enterococcus Agar using membrane filter procedures and found that its sensitivity for fecal streptococci was better than the Litsky, et al.,[8] procedure.

Croft[13] in 1959 published the results of a comparative study of media for detection of enterococci in water carried out in eight different laboratories using tube dilution and membrane filter technics. The AD-EVA tube dilution method of Litsky, et al.,[8] and the buffered azide glycerol glucose broth (BAGG) of Hajna[14] were used for the MPN procedures, and the M-Enterococcus Agar of Slanetz and Bartley[10] was used for the membrane filter technic. These media were supplied in dehydrated form to the participating laboratories. A total of 185 water samples were examined for enterococci by the eight laboratories. The AD-EVA broth and the membrane filter M-Enterococcus Agar methods were found to give the same order of productivity, but the BAGG broth gave a lower yield of enterococci in 70.8 per cent of the water specimens tested. The multiple tube dilution procedure using the AD-EVA broth and the membrane filter technic using the M-Enterococcus Agar were included for the detection of fecal streptococci in the eleventh edition (1960) of "Standard Methods for the Examination of Water and Wastewater."[15]

Kenner, Clark, and Kabler[16] in 1961 described a new medium for the growth of fecal streptococci which, with minor modifications, could be used in a multiple tube (MPN) method, with a membrane filter, or by agar pour plate technic. This medium was compared to the M-Enterococcus Agar for the membrane filter technic, and to the BAGG broth and AD-EVA broth for tube dilution tests on 25 water samples of varying degree and type of pollution. They reported that the KF medium yielded higher results in the recovery of fecal streptococci from this series of polluted waters than were obtained by the other media tested. They recommended the use of the membrance filter method over the multiple tube procedure where applicable.

Comparative studies on the productivity and selectiveness of the KF medium and the M-Enterococcus Agar have recently been made in our laboratory using the membrane filter technic. Some of the results obtained with the KF broth (Difco) are listed in Table 1. While the recovery of streptococci from the fecal samples with the KF and M-Enterococcus media are comparable, the numbers obtained from the sewage and water samples were appreciably higher on the M-Enterococcus Agar membranes. Colonies of the fecal streptococci on the membranes incubated on the KF broth saturated pads also were smaller and not as distinctly colored. Better growth of the fecal streptococci was obtained when agar was added to the KF broth when used with membrane filters. In a personal communication, the originators of this medium had also made this observation and they now recommend its use with the agar added.

In a recent survey of coliforms and fecal streptococci in shellfish waters and in oysters, a comparative study was made of the KF agar and the M-Enterococcus Agar for detecting fecal streptococci using membrane filters for the

water samples and a plating technic for the oysters. Isolation and identification of selected numbers of red and pink colonies from these cultures revealed that the KF agar was not highly selective for fecal streptococci in salt water or oysters. As is indicated in Table 2, for the shellfish water samples only 28 per cent of the colonies growing on membranes with the KF agar medium were fecal streptococci as against 98.5 per cent confirmation on the M-Enterococcus Agar membranes. Only 42 per cent of the red and pink colonies were fecal streptococci from the samples of oysters using the KF agar, while all colonies were fecal streptococci on the M-Enterococcus Agar plates. The contaminating organisms on the KF agar were mainly Pseudomonas, filamentous Gram-negative rods and micrococci. Thus, the KF agar does not appear suitable for the selective detection of fecal streptococci in sea water or sea foods. While this medium is more complex in composition than the M-Enterococcus Agar, we have found it no more productive for the recovery of fecal streptococci from water and sewage samples tested in our laboratory. We have also noted that the KF agar does not support good growth of Streptococcus bovis on membrane filters. We still feel that, for all waters with suitable physical qualities. the membrane filter technic using M-Enterococcus Agar is a more direct and efficient procedure for the quantitation of fecal streptococci than tube dilution methods. The M-En-

Table 1—Comparison of M-Enterococcus Agar (BBL) and KF Streptococcus Broth (Difco) for the Recovery of Fecal Streptococci on Membrane Filters

Source	Sample Number	M-Enterococcus Agar	KF Broth
Feces		Colonies per Gram	
Human	1	21,000	19,000
	2	50,000	40,000
Hog	1	3,600,000	5,000,000
	2	2,760,000	3,113,000
Sewage effluent		Colonies per 1 ml	
	1	4,500	2,600
	2	4,300	2,300
	3	1,520	430
	4	2,000	1,400
	5	2,000	900
	6	9,400	470
	7	8,900	270
	8	8,000	500
	9	8,000	300
River water		Colonies per 100 ml	
Oyster River	1	264	140
	2	376	196
Lamprey River	1	2,200	900
	2	490	158

Experiments based on freshly prepared media using 8 ml agar and 2.2 to 2.5 ml of broth per 60 mm Petri plate. Incubation 48 hours, the KF medium in water bath and M-Enterococcus medium in hot air incubator at 35° C.

Table 2—Comparison of the Efficiency of KF Agar and M-Enterococcus Agar for the Detection of Fecal Streptococci

Sample Number	Numbers of Streptococcus-like Colonies per 100 ml of Sample		Number of Isolates Not Fecal Streptococci	
	KF Agar	M-Enterococcus Agar	KF Agar	M-Enterococcus Agar
		Shellfish Waters		
1	8	7	3/8	0/32
2	349	60	27/34	2/48
3	128	33	43/63	0/48
4	22	3	21/24	0/3
5	185	10	8/11	0/4
		Oysters		
	Colonies per Gram			
1	24	12	19/22	0/3
2	27	9	6/15	0/12
3	187	33	13/28	0/22

NOTE: Membrane filter technic was used for the shellfish water samples and plating in Petri dishes for the oyster samples.

terococcus Agar can also be used as an effective plating medium for samples with high turbidity content.

As previously indicated, one of the chief advantages of using fecal streptococci as an index of fecal or sewage contamination is based on the recognition that these organisms normally are found only in the intestinal tract of man and animals. Suckling[17] stated that these bacteria were present in feces, sewage, and known polluted waters, whereas they could not be detected in unpolluted waters, virgin soils, and sites not exposed to human or animal life. In a recent study, Medrek and Litsky[18] compared the incidence of coliform bacteria and enterococci in undisturbed soil using the dextrose azide broth-ethyl violet azide broth technic for the detection of enterococci. Three hundred and sixty-nine soil samples were taken from areas adjacent to four water supply reservoirs in which past fecal contamination was either nonexistent or very remote due to the sanitary control of the watershed. Of these samples, 270 (73.4 per cent) contained coliform bacteria as indicated by presence of gas in lactose seeded from eosin methylene blue plates and/or presence of gas in brilliant green lactose bile broth. Five samples (1.4 per cent) yielded typical Escherichia coli colonies on eosin methylene blue agar. Enterococci were found in only 8 (2.2 per cent) of the 369 soil samples examined. While Medrek and Litsky note that enterococci and E. coli may show equal results for the determination of fecal pollution, they feel that when factors of experience and economy of materials are considered, the recommendation for using the enterococcus group as the preferred indexes of pollution seems justified.

Another reason for giving serious consideration to the use of fecal streptococci as indicators of pollution is the possibility that, if the species or types are identified in a sample of water, it may permit a qualitative interpretation of the possible source of pollution. For example, Cooper and Ramadan,[19] Bartley and Slanetz,[20] and Kenner, et al.,[21] and others have demonstrated that certain species or groups predominate in human feces and other species or groups in animal feces. These workers agree that the

presence of typical S. fecalis strains would indicate pollution of human origin while the presence of S. bovis strains would point to pollution of animal origin. In our studies, the raffinose fermenting, tellurite resistant fecal streptococci also appear to be of animal origin, particularly from cows and sheep.

As has been noted recently by Slanetz[22] and Niven,[23] there is still no final agreement on the definition or use of the term enterococcus or on the nomenclature and classification of fecal streptococci. The term enterococcus has been used by some authors to include only the fecal streptococci meeting the so-called Sherman criteria. These are the streptococci that grow at 45° C and 10° C, at a pH of 9.6, and grow in 6.5 per cent sodium chloride broth and in 0.1 per cent methylene blue milk. At times, the term enterococcus has been used to include fecal streptococci that do not meet all of these criteria although they do occur in human or animal feces in large numbers. These organisms are sensitive to 1:2,500 potassium tellurite and, as reported by Niven, et al.,[24] they do not hydrolyze arginine with the production of ammonia. Streptococcus equinus and S. bovis are two well recognized species of fecal streptococci in this group. As Shattock[25] and Papavassiliou[26] have reported, all of these streptococci appear to belong to Lancefield's serological Group D. Thus, if there is merit in retaining the vernacular name enterococcus for certain types of fecal streptococci, then the term Group D streptococcus could be used as a common name to designate all streptococci normally inhabiting the intestinal tract of man and animals. This would exclude such streptococci as Streptococcus salivarius but this organism has not been established as a normal inhabitant of the intestinal tract.

While further studies are needed to elucidate the nomenclature and taxonomy of the fecal streptococci, we do have efficient and relatively simple procedures for the detection and enumeration of these organisms in water. Their presence in water is positive evidence of fecal contamination and, by use of proper qualitative tests, it may be possible in certain instances to establish whether the contamination is of human or animal origin. There is much evidence to suggest that the fecal streptococci are more practical and reliable indicators of fecal contamination of water than the coliforms and their use as an index of sewage pollution of water should be given much more attention by federal, state, and local water testing laboratories.

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This paper was presented before the Laboratory Section of the American Public Health Association at the Ninetieth Annual Meeting in Miami, Florida, October 16, 1962.

The research reported in this paper was supported by a Public Health Service research grant WP-9(C6).

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