THE ENUMERATION OF STAPHYLOCOCCUS AUREUS ON SEVERAL TELLURITE-GLYCINE MEDIA [1] [2]

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A study of 50 coagulase-positive staphylococcus cultures on four Tellurite-glycine formulas shows some inhibition of these organisms by the media. This inhibition can be removed partially by altering and enriching the nitrogen source, increasing mannitol and yeast extract contents and lowering the glycine concentration. Tellurite formulas that showed the greatest inhibition with pure cultures behaved similarly when coagulase-positive staphylococci were determined from raw milk.

Dairy products have been implicated from time to time in staphylococcal food intoxications *(1, 3, 7, 8)*. Thus far the enterotoxigenic strains of staphylococci causing these outbreaks have not been shown to differ from other pathogenic members of the species in any feature that would allow for their selection from a mixed flora. The coagulation of blood plasma is regarded by many authorities as the most reliable "in vitro" criterion for potential pathogenicity *(2, 5, 6)*. Evans and Niven *(4)* reported that most enterotoxigenic staphylococci were members of the coagulase-positive group. Consequently in this investigation, coagulase activity was regarded as a suitable characteristic for selection of potentially enterotoxigenic strains. Tellurite-glycine Agar, among the several media devised for the selection of staphylococci, was designed especially for the selection of coagulase-positive staphylococci *(9)*. Thus, in an investigation concerning the enumeration and incidence of these organisms, this was the medium of choice.

Materials and Methods

All of the cultures tested were isolated from Grade A raw milk of producers in the Iowa State University milkshed during the period January through April, 1960. The isolations were made from the four Tellurite-glycine media employed in this investigation.

The composition of these media is listed in Table I. The media were autoclaved at 15 pounds pressure for 20 minutes and cooled to approximately 50°C.

Table 1—Tellurite-glycine Media[a]

A	g/liter
Trypticase	10.0
Yeast extract	5.0
d-Mannitol	5.0
Glycine	10.0
Lithium chloride	5.0
Dipotassium phosphate	5.0
Agar	16.0
pH = 7.2	
B	
Tryptone	10.0
Yeast extract	5.0
d-Mannitol	5.0
Glycine	10.0
Lithium chloride	5.0
Dipotassium phosphate	5.0
Agar	20.0
pH = 7.2	
C	
Soytone	3.5
Tryptone	10.0
Yeast extract	6.5
d-Mannitol	5.0
Glycine	10.0
Dipotassium phosphate	5.0
Lithium chloride	5.0
Agar	17.5
pH = 7.2	
D	
Proteose peptone	10.0
Yeast extract	5.0
d-Mannitol	15.0
Glycine	8.0
Dipotassium phosphate	5.0
Lithium chloride	5.0
Agar	15.0
pH = 7.1	

[a]Twenty ml of sterile aqueous 1-% solution of potassium tellurite added to each 1000 ml of medium

Twenty ml of a sterile 1-% aqueous stock solution of potassium tellurite (Baltimore Biological Laboratory) were added aseptically to 1000 ml of medium. The agar was poured into petri dishes in approximately 15-ml amounts. The surface of the solidified agar was dried by overnight incubation at 37°C in an inverted position.

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A surface plating technique was used in which 0.1-ml aliquots were spread uniformly over the agar surface by means of a sterile glass rod bent into the shape of a triangle.

After inoculation, the plates were incubated at 37°C for 48 hours. Coagulase-positive staphylococci produce smooth, convex, jet-black colonies. A few of these colonies were picked from each of the tellurite media to Tryptose-phosphate broth and incubated at 37°C for 18-24 hours. Tube coagulase tests using Warner-Chilcott diagnostic plasma (Warner-Chilcott Laboratory Supply Division) were performed on these broth cultures.

Plate Count Agar (Difco) inoculated by surface streaking, was used as a non-selective medium and served as the basis of comparison for all recovery determinations on pure cultures.

TABLE 2—PURE CULTURE COUNTS[a] OF COAGULASE-POSITIVE STAPHYLOCOCCI

Sample No.	Plate count agar	Tellurite-glycine agars			
		A	B	C	D
1	78	18	68	70	75
2	44	10	39	40	52
3	276	15	211	187	212
4	106	68	92	99	93
5	66	36	45	56	53
6	75	70	59	71	71
7	160	116	127	167	180
8	64	25	50	56	65
9	179	68	80	119	75
10	141	49	59	90	66
11	115	20	20	60	45
12	270	76	119	138	111
13	152	105	109	139	140
14	61	61	74	70	78
15	84	35	49	74	54
16	37	30	33	37	34
17	133	70	99	103	117
18	87	53	41	64	66
19	49	24	35	44	38
20	113	39	65	63	53
21	156	91	132	129	140
22	108	84	94	97	86
23	57	46	48	50	49
24	31	14	21	24	23
25	38	24	29	31	34
26	202	144	181	187	173
27	166	110	136	160	144
28	132	77	119	113	115
29	115	24	44	51	49
30	105	51	60	85	81
31	137	81	124	122	123
32	52	21	42	60	46
33	187	96	132	145	139
34	105	62	75	75	80
35	86	47	61	64	59
36	84	63	68	60	73
37	162	83	124	116	101
38	157	10	111	101	102
39	64	34	53	53	64
40	212	70	113	111	139
41	218	39	82	80	88
42	129	99	86	120	99
43	187	136	171	187	171
44	283	70	121	173	210
45	161	41	107	132	116
46	311	139	185	187	177
47	159	83	115	115	136
48	300	196	225	212	235
49	144	35	58	86	108
50	135	102	117	107	91

[a]All counts x 10^7

RESULTS AND DISCUSSION

Composite counts of the pure culture studies are shown in Table 2 for 50 staphylococcus isolates. The mean counts for the media were as follows: 133.46 for Plate Count Agar, 63.20 for A, 90.16 for B, 99.76 for C and 98.58 for D. Using the count obtained with Plate Count Agar as 100 percent, the percentage recoveries on the various tellurite media were 47.36, 67.56, 74.75, and 73.86 for A, B, C and D, respectively.

Preliminary work with pure cultures of coagulase-positive staphylococci showed considerable inhibition by Tellurite-glycine Agar as employed with medium A. In only three instances did it yield a comparable count to that given by Plate Count Agar. For this work, two counts were considered quantitatively comparable if the number of colonies produced by equal dilutions fell within ± 10 of one another. Medium B differs from A in the nitrogen source and agar concentration; the concentrations of inhibitors are the same. The number of cultures yielding quantitative recovery on this medium compared to the non-selective medium was increased to eight and the degree of inhibition seen with other cultures was considerably less than that obtained with A.

In the hope of modifying these two basic formulas and obtaining a medium that would yield a better recovery with a greater percentage of the cultures and still maintain selectivity, other nitrogen sources and increased concentrations of Trypticase and Tryptone were inserted into the basic formula. Higher concentrations of these compounds did not produce any significant improvement in recovery. The substitution of Proteose peptone in conjunction with the same concentration of inhibitors did not give a medium of greater advantage than Medium B. A decrease in the concentration of potassium tellurite sharply lessened the selectivity and allowed for the growth of coagulase-negative organisms within 24 hr of incubation. On the other hand, decreasing the concentration of another inhibitor, glycine, from 1.0 to 0.8%, while not removing selectivity to any detectable extent did not increase recovery. However, by increasing the concentration of mannitol

from 0.5 to 1.5% together with this lower glycine concentration, a greater percentage of the cultures were quantitatively recovered while selectivity against coagulase-negative organisms was maintained.

A newer commercial preparation which became available shortly thereafter also was employed. This preparation, medium C, made use of two nitrogen sources and an increased amount of yeast extract. A comparison of formulas C and D shows that the number of cultures having a quantitative recovery to Plate Count Agar was 18 and 12, respectively.

In no fewer than 32 of the cultures, there was inhibition on all four media that precluded consideration of their counts comparable to those of the non-selective medium by the standard adopted. T-test analyses of each mean on tellurite as compared to the non-selective medium show all of the differences to be highly significant. In the absence of any nutritional studies, the reasons for the varied responses of these cultures to the tellurite cannot be stated with any certainty.

Data for the Grade A milk samples are recorded in Table 3. The formula giving the highest mean yield of coagulase-positive organisms for the 50 samples was C. The percentage yield of the other media expressed in relation to C were 67.17, 83.96, 89.00 for A, B and D, respectively. The use of these media with milk samples, in general, showed the same effects. The same tendency for the lowest number of coagulase-positive organisms on medium A was obtained again, while C produced the highest yield. There was no attempt made to estimate the number of coagulase-positive staphylococci present in each sample by other means, for example, coagulase testing a proportionate number of staphylococcus colonies appearing on a non-selective medium. The efficiency of each tellurite medium for recovery was estimated approximately from pure culture results. We are aware of the limitations to which comparisons can be made and conclusions drawn on such a basis. Consequently, medium C which gave the highest mean yield was taken as the standard against which the other media were compared for recovery of coagulase-positive organisms from milk. On such a basis, the percentage efficiencies of the other tellurite media to C were those stated above. The differences noted among these formulas were shown to be highly significant at the 1% level by analysis of variance tests.

TABLE 3—COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED FROM GRADE A RAW MILK ENUMERATED ON TELLURITE-GLYCINE MEDIA

	Coagulase-positive staphylococci per ml			
Sample No.	A	B	C	D
1	190	840	810	880
2	210	350	470	510
3	410	2,900	2,500	1,200
4	200	390	690	310
5	260	1,400	1,400	410
6	400	1,700	1,500	1,500
7	260	1,400	1,500	1,300
8	280	690	1,300	710
9	180	1,700	2,000	1,700
10	1,500	1,900	3,100	2,500
11	190	300	320	350
12	530	390	670	1,100
13	690	1,400	1,800	1,400
14	450	750	560	660
15	510	1,300	1,300	760
16	2,000	3,500	3,800	2,800
17	200	390	530	400
18	1,400	1,800	2,000	2,000
19	2,500	2,800	2,500	2,800
20	2,000	2,500	2,200	2,500
21	700	420	620	640
22	270	180	450	320
23	810	1,300	750	1,200
24	1,500	720	1,800	1,000
25	1,100	1,000	1,400	1,000
26	600	600	630	720
27	1,300	1,800	2,100	1,900
28	1,900	2,400	3,200	2,100
29	1,500	1,400	2,600	1,500
30	4,900	5,800	8,600	6,700
31	780	960	750	1,000
32	960	900	1,400	1,100
33	1,400	1,800	2,100	1,700
34	600	1,600	1,200	1,100
35	810	840	810	1,000
36	1,900	2,100	2,400	2,600
37	7,900	7,000	9,000	8,100
38	1,300	1,000	960	1,400
39	2,000	2,000	3,100	2,100
40	2,900	3,000	2,600	3,500
41	4,000	2,800	3,000	3,500
42	1,400	1,200	1,400	1,400
43	870	1,400	1,500	1,000
44	2,100	2,800	2,400	2,600
45	1,600	2,100	3,500	2,600
46	900	1,600	1,500	1,100
47	11,000	11,000	14,000	14,000
48	1,300	1,100	2,000	1,800
49	1,700	2,100	3,600	2,800
50	2,300	4,500	3,800	4,300

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NEWS AND EVENTS

NOTICE

The announcement of the Sanitarian's Award and the rules of eligibility as shown in the January 1962 issue of the Journal contained two points which appear to have caused some confusion. We hope the following will clarify them.

1. Any sanitarian employed by a local official regulatory agency within the United States or Canada is eligible. The sanitarian may be engaged in a general, milk, or food program.

2. A good basic criterion to determine whether or not the sanitarian is employed by a local agency will be its source of revenue. If 51% or more of its financial support comes from local tax money, it may be considered local. If 51% or more of its tax money comes from State or Federal sources, it would not be considered as local for purposes of this award. In Canada, the same rule would be applied, with predominantly Provincial and Federal Government financial support being considered non-local.

If there is some worthy sanitarian you would like to nominate, don't delay obtaining entry forms and submitting them for consideration. Closing date of the competition is June 1, 1962.

Committee on Recognition and Awards

MICHIGAN ASSOCIATION HOLDS SUCCESSFUL MEETING

Michigan Association of Milk and Food Sanitarians held its annual meeting on the Campus of Michigan State University, March 6 and 7, 1962. Programs and arrangements were in charge of Dr. Frank Peabody of the Department of Microbiology and Public Health of the University, and Armin Roth, President of the Michigan Association.

Mr. Phil Shirley of the Ingham County Health Department was selected as Sanitarian of the Year for his outstanding contributions and leadership in securing adequate public health protection for residents of Ingham County and for his devotion in raising the quality and quantity of sanitation services in this county.

A year's subscription to the Journal of Milk and Food Technology was presented to the following 1962 recipients of the Kaufmann-Peabody Award: The names are Zelmer Bothic, Jr., Detroit Department of Health; Kenneth Cotter, Detroit Department of Health; John Robinson, Barry County Health Department; Peter Hrit, Detroit Department of Health; Robert Anan, Grand Rapids Health Department; and Pembleton Cochran, Detroit Department of Health.

A special session was held at Kellogg Center with the guest speaker Mr. George Blincoe, Office of Civil Defense, Battle Creek, Michigan. Mr. Blincoe gave a very stimulating talk on the radiological decontamination and related techniques. The roll of the sanitarian in the event of this type of disaster was also discussed.

New officers of the Michigan Association of Sani-