

THE DETECTION AND SIGNIFICANCE OF THE ESCHERICHIA-AEROBACTER  
GROUP OF BACTERIA IN MILK

By

M. Thomas Bartram  
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## INTRODUCTION

The *Escherichia*-*Aerobacter* group of bacteria has been used for many years as an indicator of pollution in determining the sanitary quality of water, and in this connection the group has received a great deal of study. In spite of extensive investigations, there still remains a lack of agreement in regard to the most satisfactory method for the isolation of this group and the significance of the various members of the group.

The prevalence of *Escherichia*, and in many instances of *Aerobacter*, in the intestinal tract of man and animals makes it of considerable importance in determining the sanitary quality of food products. However, it is evident that many types are to be found in soil and grain where no direct fecal contamination is apparent, and in or on anything coming in contact with man or animals. If it were possible to designate any one type or species as being of recent fecal origin, then the presence of this group in a product used as food would be of definite importance. Many have felt that *Escherichia* represent the true fecal type but there is considerable evidence to indicate that this is not necessarily the case and that *Aerobacter* may have definite significance as an indicator of fecal pollution.

Methods for the isolation of the *Escherichia*-*Aerobacter* group from milk have not received as much attention as in the case of water, and most investigators studying this group in milk have followed the trends used in water analysis. However, the presence of milk may alter the effectiveness of those substances used in inhibiting the growth of organisms not of the *Coli*-*Aerogenes* group. The prevalence and importance of this group in milk and milk products, while studied to a limited extent has not as yet been sufficient to permit definite conclusions. Many investi-

gators believe that the total bacterial count is in some instances insufficient to show completely the sanitary quality of the milk and in the case of pasteurized milk, careless handling or insufficient heating may result in significant contamination and yet the total count will not be markedly increased. In such cases, the examination for members of the Coli-Aerogenes group would be of great importance. However, it must be remembered that Beavans (5), and Minkin and Burgwald (31) have shown that strains resistant to pasteurization may occur.

In water, the chances of any definite increase in the numbers of the colon organisms are not great, but in milk the numbers may increase rapidly under instances of inadequate cooling, so that to standardize on any definite number as excessive becomes a pronounced problem.

## HISTORICAL

Methods of Isolation. The methods employed in the isolation of the coli-aerogenes groups from milk have been quite numerous. The American Association of Medical Milk Commission (2) recommended taurocholate agar, Kessler and Swenarton (20), employed 1:25,000 gentian violet containing 1 per cent bile, and found that of 1,010 positive samples, all but 10 confirmed on eosin methylene-blue agar; Kline (22) used Endo medium. McCrady and Langevin (26) employed gentian violet bile and the 2 per cent brilliant green bile of Jordan (19) with almost identical results. Brilliant green bile has been used for many years by the Quebec Provincial Laboratories and the Montreal Health Department according to Moldavan (33), who recommends that it be solidified with agar and stratified with 2 per cent agar. This method does not, however, lend itself to further study of the organisms. McCrady and Archanbault, (27) using 2 per cent brilliant green bile, obtained 99 per cent confirmation with raw milk and 86 per cent confirmation with pasteurized milk in amounts of 1 cc. to 0.001 cc. Demeter, Sauer and Miller (13) comparing Endo agar, crystal-violet broth of Salle (45), gentian violet bile broth, and Klimmer's (21) bromthymol blue-tryptaflavin-lactose agar found the latter to be most satisfactory. Kon (23) employed lactose bile medium for primary isolation with subsequent streaking on MacConkey's agar. Tonney and Noble (53), using an adaptation of ferrocyanide-citrate agar, found they could obtain a full yield of B. coli and B. aerogenes in 20 - 24 hours with dependable differentiation between types. Brown and Gott (9) found Endo medium satisfactory in locating B. coli contamination of milk. Stark and Curtis (50) in a comparative study of Salles' crystal violet broth, Dominick and Lauters' broth (14), gentian violet broth, and 2 per

cent brilliant green bile broth found that none were satisfactory due to the ability of "false test organisms" to grow in them, which lack of inhibition was increased by the addition of 1 cc. of sterile milk to the medium. Stark and England (51) recommend formate-ricinoleate broth as superior for isolation of the colon group from milk and water. Leifson (24), in the examination of milk, found desoxycholate agar "to give counts of colon bacilli as high as that obtained by the best of the other available media and methods".

Among media which have been frequently employed in the isolation of the coli-aerogenes group from water, but which have not been extensively used for that purpose with milk, only a few will be mentioned. Basic fuchsin lactose broth of Schreiner has been found by Ritter(40)(41) to give satisfactory results with water. Digestive Ferments Company, in a personal communication state, that they found Neutral Red Bile and Violet Red Bile experimental media to give 95 - 98 per cent confirmation for the coli-aerogenes group. Perry and Hajaa (37) have employed their modifications of Eijkman medium in the isolation of "fecal" colon organisms from shellfish and from a limited number of sewage samples, eggs, and milk.

Coli-Aerogenes Types in Milk. The determination of the type of the coli-aerogenes group found in milk products has received only limited attention and in most instances the species have not been determined. The earlier work along this line is, as Yale (56) points out, difficult to compare with more recent investigations because of variation in methods of classification and because of the use of technique now not employed.

Kline (22) found that of 407 cultures from milk, 35 per cent were Escherichia, 51 per cent were Aerobacter, and 14 per cent were intermediates. Pont (39), in a study of 128 cultures isolated from cream samples, found 76.5 per cent to be Escherichia, 11.0 per cent Aerobacter, and 12.5 per

cent intermediates. The species determined according to the classification suggested by Levine (25) were B. coli 34.4 per cent; B. communior 27.3 per cent; B. acidi lacticum 9.4 per cent; B. neapolitanum 3.5 per cent; B. coscorba 1.6 per cent; B. aerogenes 7.8 per cent; and B. cloacae 3.2 per cent.

Yale (56) found that *Escherichia* comprised 63 per cent, *Aerobacter* 26 per cent, and intermediates 11 per cent of the cultures from raw milk; from pasteurized milk *Escherichia* comprised 57 per cent, *Aerobacter* 10 per cent, and intermediates 33 per cent. The species isolated by this investigator will be discussed under section 3.

Malcolm (29) found that of the strains isolated during the winter and spring 71.0 per cent were of the B. coli type, 7.5 per cent of the B. lactis aerogenes, 8.6 per cent of the B. cloacae, and 7.6 per cent of the intermediate type. In the summer and autumn 40.4 per cent were B. coli, 22.4 per cent B. lactis aerogenes, 9.8 per cent B. cloacae, and 13.5 per cent intermediate.

Kon (23), using "lactose bile salt" for the isolation of this group from milk, found coli and aerogenes present in about equal numbers, 58:42 with direct isolation on MacConkey's agar, aerogenes outnumbered the coli 13:4. In feces the coli were found to predominate 44:1. This, the author concluded, "indicated that coliform organism in milk were not derived from faeces but from the utensils".

Significance of Colon-Aerogenes in Milk. Some early work, prior to 1925, on the isolation and significance of the colon bacilli in milk was done by Ayres, Cook and Clemmer (3); Hunter (18); and Finkelstein (16). The first mentioned investigators concluded that the colon group could be used to a limited extent in determining the efficiency of the cooling of the milk. Hunter observed that a close correlation existed between total count and the number of colon organisms. Finkelstein



found that the coli-aerogenes count would be under 100 per cc. in carefully handled raw milk. In pasteurized milk, this investigator found that the coli-aerogenes organisms were reduced to an average of 42 per cc. and in some cases none were found. However, the methods of isolating colon bacilli have changed since that time and the care used in the production of milk has improved. Later, Swenarton (52), using gentian violet lactose peptone bile medium, found that a high count of B. coli correlated with plants showing improper heating or cooling of milk. Kline, in studying the various grades of raw milk and pasteurized milk, found that there was little correlation between colon count and total count in low count milk, and that in high count milk the correlation was slight but insignificant. The same general consideration held true in pasteurized milk but in certified milk there was a definitely lower percentage of samples with a count of under 10,000 per cc. showing colon. Malcolm (29) found a definite correlation between the average bacterial count and the proportion of coliform-positive samples. Munchberg (34) observed a close relationship between the coli titre and tests for contamination.

Sherman and Wing (46) concluded that the colon test is of no special value as an index to the sanitary condition of the usual grade of raw milk, but in raw milk of under 10,000 total count, the colon test may act as a supplementary index of quality, where these organisms should be present in numbers less than 100 per cc. In certified milk they believed the recommendation of the American Association of Medical Milk Commission of less than 10 colon organisms per cc. to be satisfactory. Chalmers (11) considered it essential to differentiate between Bact. coli communis and Bact. lactis aerogenes in determining the significance of the colon group in certified milk, finding that the Bact. lactis aerogenes may result, in some instances, from infection of the teat canal but more frequently from the outside of the udder, where

they resisted removal by washing by reason of their capsulated and sticky nature. Barkworth (4), in a statistical examination of the interrelationship of the plate count, coliform content, and keeping quality, found that the variabilities were too great to permit forecasting of one term from the other, but that an increase of coliform organisms reduced the keeping quality of the milk to the same extent as a seven-fold increase in plate count. Moldavan (33) stated that "it is generally agreed that in most cases, positive results from samples of pasteurized milk are due to non-fecal organisms, the presence of which indicates faulty sterilization or improper pasteurization rather than fecal contamination". Minkin and Burgwald (31), in an examination of 350 pasteurized samples, found that approximately 40 per cent gave gas in presumptive tests and that of the organisms isolated 35 per cent were able to resist pasteurization temperatures. McCrady and Langevin (26) found the coli-aerogenes organism were practically absent in one cc. quantities at the pasteurizer but that contamination often occurred thereafter, although it appeared that properly pasteurized milk should not contain these organisms in one cc. portions in more than 10 to 20 per cent of the tests. Klimmer, Haupt and Borches (21) found that 57 per cent of market milk contained less than 10 colon-aerogenes organisms per cc., 23.5 per cent containing between 10 and 100, 10 per cent between 10 and 1000 and 23.5 per cent contained over 1000 of these organisms per cc. Moore and Fuller (32), in comparing the total bacterial count and the colon count of milk from farms scored by the State Board of Health dairy farm score card, found that the majority of farms producing milk free of colon organisms likewise had lower counts than the farms from whose milk colon organisms were isolated, yet there was no direct relationship between the score placed on the farm and the presence of colon organisms in the milk or between the score and average bacterial count. Davis (12)

concludes that the B.coli test is of no value as an indicator of gross contamination, but does serve to indicate faulty production and unsterilized utensils. He found that no growth of these organisms occurred in milk held below 10° C.

## EXPERIMENTAL

The experimental work will be divided into four main sections, (1) a comparison of the efficiency of several methods for the isolation of members of colon-aerogenes group from milk, (2) the relative productivity of these methods using pure cultures of Escherichia, Aerobacter and intermediate types, (3) the identification of the species isolated and, (4) correlation of total count and presence of coli-aerogenes group in raw and pasteurized milk.

### Section I

#### A Comparison of the Efficiency of Several Methods for the Isolation of the Coli-Aerogenes Group from Raw Milk.

Comparison using four media simultaneously. In this study, 275 samples of raw milk were obtained over a period of a year from three milk plants which received milk from approximately 100 individual shippers. These samples were divided into five groups of fifty samples each and one group of 25 samples, and planted simultaneously into four media. Throughout these experiments Endo's agar and 2 per cent brilliant green bile were used as standard, and one liquid and one solid medium employed for comparison until fifty samples had been examined; then another liquid and solid medium were used, Endo and brilliant green bile being retained.

The milk samples were obtained, by means of a sampling tube, directly from the cans of the shippers as they were received at the milk plants, and examined within one-half hour after being taken. The samples were plated in the solid media in 1 cc. and 0.1 cc. amounts in duplicate or triplicate; in liquid media 1 cc., 0.1 cc., and 1 cc. of a

1:100 dilution were planted in duplicate. All were incubated at 37° C. and examined after 24 and 48 hours. At the same time, standard plate counts were made of appropriate dilutions (usually 1:1000 and 1:10,000) and counted after 48 hours incubation at 37° C.

The following media were used for the isolation of the coli-aerogenes group; lactose taurocholate agar as recommended by the American Association of Medical Milk Commission, Inc., (2) ferrocyanide citrate agar (Noble (35)), brilliant green lactose bile dehydrated agar (Tonney and Noble (53)), Hexamine agar medium of Wilson (5b), Leifson's (24) desoxycholate agar (dehydrated), Klimmers' (21) bromthymol blue tryptaflavin agar, Ritter's (40) fuchsin lactose broth, Ritter's (41) modified fuchsin lactose broth, methylene blue-brom cresol purple dehydrated broth of Dominick and Lauter (14), Stark and Englands' (51) formate-ricinoleate broth (dehydrated), Endo agar (dehydrated), and 2 per cent brilliant green bile broth (dehydrated). All media were prepared, tubed, and sterilized according to the direction of the various authors. Where indicated, the dehydrated medium prepared by the Digestive Ferment Company was used except in the case of Leifson's desoxycholate agar, which was prepared by the Baltimore Biological Laboratory. Other media were prepared using ingredients accepted by the proponents of the medium.

Positive tubes or colonies were streaked on Levine's eosin methylene-blue agar (dehydrated) and from this, typical or most nearly typical colonies were picked to lactose broth and to agar slants, following the partially and fully confirmed procedure suggested in Standard Methods of Water Analysis (49). If gas was found in the lactose broth tubes, the agar slant cultures were restreaked on eosin methylene blue, repicked and restreaked, the process being repeated two to three times to ensure pure cultures. The isolated cultures were examined microscopically for gram negative short rods and identified as Escherichia, Aerobacter or

intermediate using Simmons' (47) citrate agar (dehydrated) and Methyl-red, Voges-Proskauer medium (dehydrated) the latter medium being tested according to Standard Methods of Water Analysis procedures. In most cases the indol reaction was determined using a 1 per cent solution of tryptophane (tryptone) and tested by means of the Gore method recommended in the "Manual of Methods for the Pure Culture Study of Bacteria" (28). In later studies, the indol reaction was omitted in making the genera classification.

Results. The results of these tests are shown in Table I. They can best be compared in series comprising 50 samples each, since identical shippers are not included in each series and seasonal variations in flora may occur which would invalidate a general comparison.

In the first series, the best results were obtained with methylene-blue brom-cresol purple which gave 89.5 per cent confirmation of positive presumptives and 38 per cent sample confirmation, brilliant green bile with 83 and 30 per cent, Endo with 72 and 26, and lactose taurocholate with 47 and 18 ranked in the order given. The order of ranking, based on a percentage relationship, taking the medium with the highest per cent of samples confirming as 100, is shown in the last column.

In series two, the order of arrangement, from the most efficient to the least, is formate ricinoleate with a positive presumptive confirmation of 95 per cent and 82 per cent of samples confirming, Endo with 94 and 64 per cent, ferrocyanide-citrate with 86.5 and 64 per cent, and brilliant green bile with 80.5 and 58 per cent. In series three, brilliant green bile gave a higher percentage of samples confirming (74 per cent) but showed more false presumptives than Endo which showed 100 per cent of presumptives confirming and 46 per cent of samples confirming brilliant green lactose bile agar with 82 and 46 per cent, and fuchsin lactose broth (original formulae) with 67 and 42 per cent followed. In

series four, Endo with 65.2 and 55.5 per cent again was most satisfactory, followed by brilliant green bile with 56 and 48 per cent, fuchsin lactose (modified) with 53 and 37 per cent, and hexamine with 57 and 29.6 per cent. In series five, desoxycholate gave 90 per cent confirmation of positive presumptives and 72 per cent of samples confirmed, Endo with 80 and 68, brilliant green bile with 40 and 33.4, and fuchsin lactose (modified) 46 and 26 followed. In this series, the results with brilliant green bile were unexplainably poor.

In series six, consisting of only 25 samples, tryptaflavin gave the highest confirmation (93.4 per cent) of presumptives, but Endo with 89 per cent and 64 per cent of samples confirming ranked best on the latter basis, brilliant green bile was poorest with 72 and 81 per cent.

Table I

Presumptive and Confirmatory Isolation of Coli-Aerogenes  
(Results on basis of 275 samples of raw milk inoculated simultaneously into four media)

Series	Medium	Positive Presumptive	Number Confirmed	Presumptive Confirmed Per Cent	Samples Confirmed Per Cent	Highest Confirmation Per Cent
* 1	Endo	18	13	72	26	76.5
	Taurocholate	19	9	47	18	53
	Brilliant green bile	18	15	83	30	88
	Methylene blue brom cresol purple	19	17	89.5	38	100
	Endo	34	32	94	64	78
2	Ferrocyanide-citrate	37	32	86.5	64	78
	Brilliant green bile	36	29	80.5	58	71
	Formate-Ricinoleate	43	41	95	82	100
	Endo	23	23	100	46	62
3	Brilliant green lactose bile	28	23	82	46	62
	Brilliant green bile	41	37	90	74	100
	Fuchsin lactose (old formula)	31	21	67	42	57
	Endo	23	15	65.2	55.5	100
4	Hexamine	14	8	57	29.6	53.4
	Brilliant green bile	23	13	56	48	86.6
	Fuchsin lactose (modified)	19	10	53	37	66.5
	Endo	21	17	80	68	94.4
5	Desoxycholate	20	18	90	72	100
	Brilliant green bile	22	9	40	33.4	50
	Fuchsin Lactose (modified)	15	7	46	26	39
	Endo	18	16	89	64	100
x 6	Trypaflavin	15	14	93.4	56	87.5
	Brilliant green bile	18	13	72	52	81

\* Comprising 50 samples each.

x Result of 25 samples.



Comparison using ten Media Simultaneously. Twenty-five samples of raw milk were obtained and inoculated as previously outlined into Endo, neutral red bile, violet red bile, brilliant green lactose bile, ferrocyanide-citrate, and desoxycholate agars and into 2 per cent brilliant green bile, fuchsin lactose (modified), formate-ricinoleate and methylene blue brom-cresol purple broths. The media were examined, and cultures isolated and identified as previously stated. Media used in first tests but omitted from this study were dropped either because of unsatisfactory results obtained with them in previous tests or in productivity studies to be described later. The neutral red and violet red bile are dehydrated media experimentally prepared by the Digestive Ferments Company, and kindly supplied by them for these experiments.

The results obtained in these tests are shown in Table II. The percentage of samples confirming show that neutral red bile, violet red bile, and brilliant green bile gave the best results with 32 per cent each; however, neutral red bile and brilliant green bile gave one and two false positive presumptives respectively. Methylene blue brom-cresol purple likewise showed satisfactory results with 28 per cent of samples confirming. Formate-ricinoleate, ferrocyanide-citrate, and desoxycholate gave unsatisfactory results. Endo and brilliant green lactose bile showed a low total sample confirmation of 24 per cent.

Table II

Presumptive and Confirmatory Isolation of Coli-Aerogenes  
 (Results on basis of 25 samples of raw milk inoculated simultaneously into ten media)

Medium	Positive Presumptive	Number Confirmed	Presumptive Confirmed Per Cent	Samples Confirmed Per Cent	Highest Confirmation Per Cent
Endo	11	6	54.5	24	75
Neutral red bile	9	8	89	32	100
Violet Red bile	8	8	100	32	100
Brilliant green lactose bile	9	6	66.8	24	75
Ferrocyanide citrate	7	4	57	16	50
Desoxycholate	9	5	55.6	20	62.5
Brilliant green bile	10	8	80	32	100
Fuchsin lactose (modified)	7	5	71.5	20	62.5
Formate-Ricinoleate	5	1	40	8	25
Methylene blue brom-cresol purple	7	7	100	28	81

Comparison using the media indicated as most satisfactory.

Thirty-one samples of raw milk which were to be pasteurized, 34 samples of pasteurized milk taken from various points in the cooling and bottling process and 25 samples of raw milk produced under conditions meeting all the requirements of certified milk, but sold as grade A raw, were procured and examined within one hour of sampling. The samples were either taken by means of sampling tubes, caught in sterile bottles on entering or leaving the cooler, or poured from the containers into sterilized sample bottles. The milk samples were inoculated into neutral red bile, violet red bile, 2 per cent brilliant green bile, modified Eijkman medium of Perry and Hajna (37) and into a solid medium prepared in this laboratory. This last medium had the following composition:

Peptone 0.5 per cent; lactose 0.5 per cent; sodium formate 0.25 per cent, sodium ricinoleate 0.05 per cent; methylene blue 1:50,000, neutral red 1:30,000 and agar 1.5 per cent.

Raw milk samples were inoculated in amounts previously employed. Pasteurized and certified samples were inoculated into solid media in 1 cc. amounts only and into liquid media in 1 cc. and 0.1 cc. amounts, the total counts being performed with dilutions of 1:100.

In these tests and in a portion of previous tests the positive tubes and colonies from positive plates were inoculated into formate-ricinoleate broth along with streaking onto eosin methylene blue agar, a similar technique being recently employed by Ruchhoft (43). Positive results with formate-ricinoleate were confirmed by streaking on eosin methylene blue agar and if both methods gave gram-negative, lactose fermenting rods the duplicate cultures were discarded.

The results obtained in this test are shown in Table III. Of the raw milk only 11 samples were tested on medium four, if all the media are considered on the basis of these samples, medium four and neutral

red gave 55 per cent sample confirmation each and 85.5 and 75 per cent confirmation of positive presumptives, while violet red bile gave 45 and 83 per cent, brilliant green bile 18 and 50 per cent and the medium of Perry and Hajna 9.1 and 100 per cent. On the basis of 31 samples, in which case medium four must be eliminated, the order of most satisfactory results remains the same with the percentages shown in the table. The 34 pasteurized samples gave the most satisfactory results on neutral red and violet red bile with 35 per cent of the samples confirming, brilliant green bile gave 32 per cent and medium four 29.4 per cent sample confirmation. All of the medium (except Eijkman) gave 100 per cent confirmation of the positive presumptive tests. The examination of 25 samples of "certified" milk showed violet red bile to be most efficient with 12 per cent of samples confirming, while neutral red bile and brilliant green bile showed 8 per cent samples confirmation and media four 4 per cent. However, the differences between these media as shown by these samples is, for reason to be discussed later, not as great as is indicated. Medium four gave 100 per cent, brilliant green bile 67 per cent, violet red bile 50 per cent and neutral red bile 40 per cent confirmation of presumptive tests. Eijkman medium showed no positive reactions although Escherichia were isolated in four scattered instances on the other media.

Table III

Presumptive and Confirmatory Isolation of Coli-Aerogenes  
 (Results on basis of 31 samples of raw milk, 34 samples  
 of pasteurized milk and 25 samples of "certified"  
 milk inoculated simultaneously into four media)

Medium	Positive Presumptive	Number Confirmed	Positive Presumptive Confirmed Per Cent	Samples Confirmed Per Cent	Highest Confirmation Per Cent
<u>Raw</u>					
Medium number four *	7	6	85.5	55	
Neutral red bile	15	13	86.5	42	100
Violet red bile	13	12	92	39	93
Brilliant green bile	14	12	85.5	39	93
Eijkman (modified)	3	3	100	9.7	21
<u>Pasteurized</u>					
Medium number four	10	10	100	29	84
Neutral red bile	12	12	100	35	100
Violet red bile	12	12	100	35	100
Brilliant green bile	11	11	100	32	91
Eijkman	1	0	0	0	0
<u>Certified</u>					
Medium number four	1	1	100	4	33
Neutral red bile	5	2	40	8	67
Violet red bile	6	3	50	12	100
Brilliant green bile	3	2	67	8	67
Eijkman	0	0	0	0	0

\* Includes 11 samples of raw milk only.

## DISCUSSION

It will be noted that the percentage confirmation of the media, which appear in two or more series or in the different portions of this experiment, varies considerably. This is probably due to errors which have frequently been mentioned in the literature concerned with the isolation of the members of the coli-aerogenes group. These are (1) false presumptives due to lactose fermenters not of the coliform group or due to symbiotic activity; (2) loss of the coliform organisms by overgrowth of other organisms. This would not seem a valid criticism where colonies were picked from solid media but where inoculations were made to formate-ricinoleate and to eosin methylene blue simultaneously, it was frequently noticed that the growth on the latter was negative or so atypical as to be considered negative and so confirmed, while the formate-ricinoleate would yield positive cultures. This has been noticed by Ruchhoft who used formate-ricinoleate broth, brilliant green bile broth, MacConkey's agar and standard procedure simultaneously for confirmation tests. The overgrowths by other organisms might be expected to vary with different samples as mentioned.

The results from the series studied in the first group of tests were used only to serve as a process for selecting the media to be used in the later tests and it is difficult to select the most promising medium from the results obtained. However, it would seem that lactose taurocholate, fuchsin lactose (old formulae) and hexamine could be eliminated from further consideration. The first medium has not received a great deal of consideration in recent procedure being replaced by other bile containing compounds. Fuchsin lactose has been considered favorably by numerous investigators, on the basis of probable numbers necessary to produce positive results, however no report has been found of its use for

milk examination. Hexamine medium gave definitely lower sample confirmation which indicates that it is inhibitive to the weaker members of the coliform group. Studies on the productivity of tryptaflavin medium show it to be likewise quite inhibitive, as will be shown in a later section. Demeter, Sauers and Miller, already mentioned, and Marshall (30) obtained satisfactory results with tryptaflavin medium using streak inoculations. Ritter (42), however, found the medium of Kessler and Swenarten to be more satisfactory than that of Klimmer.

From the results obtained in the simultaneous comparison of all media, although only relatively few samples were examined, the data obtained lends itself to more definite comparison. The samples in this series included a number from each of the plants studied and comprised milk with a total bacterial count ranging from under 10,000 to over 100,000 per cc. as will be shown later. It will be seen that neutral red bile, violet red bile, brilliant green bile and methylene blue brom-cresol purple were most satisfactory. Endo and desoxycholate agar showed several false positive presumptive tests which proved to be slow or non-lactose fermenters, which were atypical on eosin methylene blue and frequently fermented formate-ricinoleate. These organisms probably belong to the proteus group but no attempt was made to identify them. The one organism on neutral red which failed to confirm was not entirely typical on that medium and likewise may have belonged to the proteus group. All of the solid media showed considerable growth, which, while they were not at all typical colon-aerogenes colonies in appearance, made the counting and isolation of the colon organisms difficult, also it is possible that they repressed the growth of this group due to crowding of the plates. For that reason attempts to devise a new medium were made and several composed of various selective and differential substances were tried. The one designated as medium number 4

seemed to be the most promising and was tested along with the other media in the last series with the results to be discussed later.

It must be borne in mind that in this experiment something over 22 cc. of milk were used for the analysis so that where the organisms were not present in quantities of more than one per cc. it would be quite possible to obtain positive results with one medium and not with another, due to distribution probability. However in all these studies there were only a few instances, to be mentioned later, where duplicate plates did not show the same number of coli-aerogenes organisms. It thus seems most probable that the fewer positives occurring in some media are due to the inhibitive properties of that medium on weakened strains of members of the group.

It seems probable that the observations of Stark and Curtis (50), in comparing crystal violet broth, methylene-blue brose-cresol purple, gentian violet broth and brilliant green bile are correct in that the percentage confirmation, on those media which were used in the present experiment, are lower than have been found by numerous investigators using water specimens, indicating a lowering of inhibitive properties by the milk. From this experiment too, it would seem that neutral red bile and violet red bile are the most satisfactory while brilliant green bile with two false presumptives is next.

The results shown in table III, generally confirm this conclusion, Medium four, although used only on 11 samples of raw milk, is considered worthy of further investigation. The number of samples used being too few to seriously propose it as a new medium at present with so many media already having been proposed. This medium in only two instances (of the 90 samples) showed any considerable growth of organisms other than those of the coliform groups, also in all types of milk it showed good presumptive confirmation, the one false presumptive being a sample which likewise gave false presumptives on the other media. The sample



confirmation was equal to the most satisfactory in the raw milk, on the basis of the 11 samples in which it was studied. Platings with pasteurized and "certified" milk were not as conclusively good, the two pasteurized samples which failed to show positive colonies on this medium were identified as intermediates and appeared on one plate only of the neutral red and violet red bile medium and in one tube of the brilliant green bile in one sample and the other sample was negative in both tubes. Hence this may be an instance where distribution is important. The number of colonies isolated from other samples on medium four were equal to or better than neutral red and violet red bile. In this connection it was found that on four occasions (out of 12) violet red gave slightly higher counts than neutral red. With certified milk in only one case were coli-aerogenes organisms isolated on two media from the same sample and in this case one proved to be an intermediate and the other Escherichia. Likewise of the two cultures isolated on neutral red, one was a slow lactose fermenter though typical on eosin methylene blue, on violet red bile two of the three fell in this category and of the two in brilliant green bile, one only was a typically rapid lactose fermenter. Hence the differences between the media in these cases can be of little importance. Perry and Hajna's modification of Eijkman medium gave only one false presumptive from which no organism could be isolated, positive results were obtained in three samples of raw milk and Escherichia communior isolated. This same species was isolated from other media on eight occasions when negative results were obtained in Eijkman medium.

The results of this experiment would indicate neutral red and violet red bile to be the most satisfactory solid medium now obtainable, with brilliant green bile as the best liquid medium. However, none of these are entirely satisfactory, due to lack of inhibition for other lactose fermenting organisms and for non-lactose fermenters.

## SECTION II

### Comparative Productivity of Some Media for the Isolation of Pure Cultures of the Coli-Aerogenes Group

The methods followed in these tests were in general those described by Butterfield (10) and Hoskins (17) for determining the comparative productivity of media for coli-aerogenes. The method was modified in that 10 instead of 15 tubes of each medium were used and two dilutions instead of three were planted in the media. One cc. of sterile whole milk was added to all media except standard lactose before the organisms were added. With solid media the technique used was to determine the approximate titre of the buffer suspension of organisms using standard agar, on the day prior to the test, then on the test day plating two dilutions of the suspensions into the trial media in triplicate, such dilutions being used that the plates would show between 3 and 300 colonies per plate. One cc. of whole milk was added to each plate before adding the organisms. Both the liquid and solid media were made up in such concentrations that the addition of the one cc. of milk would serve to reduce the concentration of the media to the usual value. All tubes and plates were examined at the end of 24 and 48 hours. Two strains each of *Escherichia*, *Aerobacter* and intermediates recently isolated from milk were used in the test. The dilutions were so made that 1 cc. quantities were inoculated in each case, in all instances the media were inoculated alternately as specified.

## RESULTS

The results of section 2 are shown in table IV, the percentage productivity of the liquid media are based on standard lactose broth as 100 per cent and the solid media on standard agar plus milk as 100 per cent. On the basis of these results the liquid media may be placed in the following order with the most productive ranked first, (1) methylene blue brom-cresol purple (2) brilliant green bile (3) fuchsin lactose (4) formate-ricinoleate and (5) Perry and Hajnas' Eijkman medium. The solid media are arranged (1) neutral red bile (2) violet red bile (3) Endo (4) brilliant green lactose bile (5) lactose taurocholate (6) desoxycholate and (7) tryptaflavine.

Table IV

Comparison of Productivity, with Standard lactose and  
Standard Agar, of Colon-Aerogenes Group.

	M.P.N.*	M.P.N. x
Fuchsin Lactose (mod.)	52.5	
Brilliant Green Bile	63.1	
Methylene Blue Brom-Cresol Purple	71.7	
Formate-Ricinoleate	27.4	
Eijkman	0	
Desoxycholate		60.5
Taurocholate		86.6
Brilliant Green Lactose Bile		92.0
Endo		102.0
Tryptaflavine		18.0
Neutral Red Bile		121.5
Violet Red Bile		113.0

\* Basis of standard lactose as 100 per cent.

x Basis of standard agar as 100 per cent.

## DISCUSSION

The experiment was not sufficiently extensive to evaluate completely the various media and for this purpose a much more detailed examination is essential, as is pointed out by Noble (36), however the results may be used within these recognized limits especially since no "border-line" medium was indicated. The results are somewhat in disagreement with those obtained by other workers. Ruchhoft (43) in a summary of data received from co-operating laboratories, all using the method of Butterfield-Hoskins, obtained the following productivity from highest to lowest:- buffered lactose, fuchsin lactose, methylene blue brom-cresol purple, brilliant green bile, crystal violet and formate-ricinoleate. Ruchhoft and Norton (44) in a preliminary report found essentially the same results with the exception that the positions of buffered lactose and fuchsin lactose were reversed. Farrell (15) rated buffered lactose and brilliant green bile, in the order named, ahead of fuchsin lactose and placed tryptaflavin broth below formate-ricinoleate. The results here reported agree most closely with those of Farrell except that methylene blue brom-cresol purple is placed ahead of brilliant green bile and fuchsin lactose. It would be interesting to determine if the addition of 1 cc. of milk causes the change in relative productivity of these media. Black and Klinger (7) working in this laboratory observed buffered lactose, fuchsin lactose, methylene blue brom-cresol purple, brilliant green bile, crystal violet and formate-ricinoleate to rank in the order named. These results agree with those found by Ruchhoft.

So far as is known the productivity of the various solid media has not been reported so that no comparison is possible, however, the results in general confirm those obtained in section I, although desoxycholate fell below the results that might have been expected. This was due to

its low productivity with the strains of the intermediate group and may be a characteristic of the medium though this has not been determined with additional intermediate strains.

### SECTION III

#### Identification of Species of Escherichia-Aerobacter Organisms Isolated from Milk

The cultures isolated, in section I, from the 331 samples of raw milk, 34 samples of pasteurized milk and 25 samples of "certified" milk were identified as Escherichia, Aerobacter or intermediate as has been outlined. The species in these genera were determined according to the classification used in Bergey's Manual.

Motility was tested in one per cent dextrose broth after twenty hours incubation at 37° C.

The technique and media employed in physiological tests were as recommended in the "Manual of Methods for the Pure Culture Study of Bacteria" (28). Gelatin liquefaction was determined after 14 days incubation at 37° C., the cultures being placed in the icebox to determine liquefaction. Litmus milk was incubated for ten days at 37° C. Fermentation of dextrose, sucrose, salicin and dulcitol was determined in broth containing one per cent carbohydrate to which brom-thymol-blue had been added, results being read after 2 and 4 days incubation at 37° C.

#### RESULTS

Four hundred and eighty four cultures were isolated from the samples of raw milk. Of these 272 or 56 per cent were identified as Escherichia, 115 or 23.7 per cent were Aerobacter, and 96 or 20.3 per cent were intermediate (methyl-red positive, Voges-Proskauer negative, and citrate positive).

The cultures of Escherichia were found to be composed of 12 different species according to the results shown in table V. The organisms in the Aerobacter genus were further identified as comprising 5 species.

The pasteurized samples yielded 45 cultures of which 1 or 2 per cent was *Escherichia* and the same number *Aerobacter*. Intermediate comprised 43 or 96 per cent. The *Escherichia* culture proved to be *E. paragruenthali* and the *Aerobacter*, *A. cloacae*. The preponderance of one species is not surprising since the positive samples were all isolated from one pasteurizing plant where they probably resulted as contamination due to improper cleaning of the equipment or to faulty equipment.

Eight cultures were isolated from the "certified" milk, of these 4 or 50 per cent were *Escherichia*, 3 or 38 per cent *Aerobacter* and 1 culture or 12 per cent intermediate. Of the total cultures 12 per cent were *E. gruenthali*, 38 per cent *E. communior*, 12 per cent *A. aerogenes*, and 25 per cent *A. levans*.

Table V

## Per cent of Species Isolated in the Escherichia-Aerobacter Group

	Raw milk 484 cultures	Pasteurized 45 cultures	Certified 8 cultures
<b>E. coli</b>	8		
paragruenthami	7	2	
formica	4		
gruenthami	4		12
anaerogenes	.2		
enterica	5		
vesiculiferans	2		
communior (atypical)	20 3.1		38
pseudocoloides	1		
anindolica	1		
neapolitana	1		
leporis	.1		
<b>A. aerogenes</b>	5		12
oxytocolum	1		
cloacae	5	2	
hibernicum	12		
levans	.3		26
<b>Intermediate</b>	20.3	96	12



## DISCUSSION

The results obtained in this experiment do not truly lend themselves to comparison with the results obtained by other investigators in-so-far-as species identification is concerned, since the present classification was made according to the system given in a more recent edition of Bergey.

The preponderance of *Escherichia* over *Aerobacter*, 56:23.7, agrees in general with the results obtained by Pont (39), Yale (56), and Malcolm (29) although the ratio is not the same in every instance. Kline (22) found 34 per cent *Escherichia*, 57 per cent *Aerobacter* and 9 per cent intermediates.

The species identification of Yale based on the third edition of Bergey is shown in table VI reproduced from the report of this investigator.

Table VI

Percentages of cultures belonging to the different species  
of the Escherichia-Aerobacter group

Species	Dairy Products Investigated	
	Raw Milk (91 cultures)	Pasteurized Milk (21 cultures)
<i>E. coli</i>	13	10
<i>pseudocoloides</i>	10	24
<i>communior</i>	9	5
<i>paragrunthali</i>	9	10
<i>vesiculiformans</i>	7	5
<i>formica</i>	4	
<i>enterica</i>	4	
<i>anaerogenes</i>	3	
<i>grunthali</i>	2	5
<i>neapolitana</i>	1	
<i>A. aerogenes</i>	10	
<i>cloacae</i>	11	10
<i>oxytocum</i>	5	
Intermediate group*	11	33

\* Methyl red +, Voges-Proskauer-, Utilization of citrate +.

This table shows 10 species of Escherichia and three of Aerobacter with *E. coli* and *E. pseudocoloides* being the most frequently isolated in the first genera and *A. aerogenes* and *A. cloacae* being most prevalent in the latter. Kline found only four species of Escherichia with *E. neapolitana* being most common, two species of Aerobacter, *A. cloacae* and *A. aerogenes* were found by the investigator. The present results showed 12 species of Escherichia and 5 of Aerobacter, with *E. communior* and *A. hibernium* occurring most frequently. Yale observed *E. communior* to be only slightly less than *E. coli* and *E. pseudocoloides* in frequency, *A. hibernium* was not identified by this investigator since it is a new

species not included in the third edition of Bergey's Manual, but he isolated ten cultures comprising 37 per cent of the A. cloacae in which no gelatin liquefaction occurred, these might be similar to A. hibernicum.

Kline found E. neapolitana to be most common in the Escherichia section which Yale and the author found it one of the least common. Malcolm in a study of 800 cultures isolated from raw milk found B. coli to predominate in the winter with 71 per cent, B. lactis Aerogenes 7.5 per cent, B. cloacae 8.6 per cent and intermediate 7.8 per cent. During the summer months 40.4 per cent were B. coli, 22.4 per cent B. lactis aerogenes and the percentage of B. cloacae and intermediates were similar to those previously found. Of 100 cultures isolated from milk, Aichelburg (1) found B. coli communis, B. lactis aerogenes, B. neapolitanum and B. coli commune to occur most frequently, while B. pseudocoscroba, B. pseudocoloides, B. acid-lactici, and B. gruenthali were seldom isolated. Intermediates comprised 20-30 per cent.

Kon (23) concluded that the coliform organisms in milk were not derived from faeces contamination but from utensils, since in milk coli and aerogenes occurred nearly equally 58:42, while in faeces coli predominated 44:1. However the important consideration would seem the fact that contamination was occurring irrespective of the source. The addition of fecal types would seem of no more importance than non-fecal if they were added indirectly by contaminated utensils.

The results of Pont based on Levine's classification, as was mentioned earlier, cannot be compared with the present investigation, however the statement that "whilst the majority of the coliform organism isolated from cream in this study appear to be of fecal type, the possibility that they have been derived more immediately from contaminated utensils cannot be overlooked", is in line with the foregoing statement of the author.

## SECTION IV

### Relationship of Total Bacterial Count and Presence of the Coli-Aerogenes Group in Raw and Pasteurized Milk

Three hundred and thirty one samples of raw milk used in sections I and II were examined for total bacterial count using Standard Methods of Milk Analysis Procedures, the coli-aerogenes determination being the results of the earlier experiments. In addition 50 samples, obtained in the same manner as previously mentioned, were plated in neutral red bile agar in 1 cc. amounts and in dehydrated tryptone glucose milk medium of Bower and Hucker (8). Duplicate platings being made in each case. The latter medium was incubated for 48 hours at 32° C. The investigators cited and others have shown that higher counts may be obtained on a modified medium at lower temperatures, than can be obtained on standard agar at 37° C. These results have also been confirmed in this laboratory in unpublished data. Thirty one samples of pasteurized milk and 25 samples of "certified" milk are likewise recorded here.

## RESULTS

Table VII shows the results obtained with the 331 samples of raw milk, the samples being separated into different grades based on total count and the prevalence of colon organism in these grades being shown. The per cent of samples negative for colon organisms does not vary significantly in samples under or above 10,000 total count, however, 69.2 per cent of the samples with counts under 10,000 showed less than 10 colon organism per cc., while 29.1 per cent of the samples having a total count above 10,000 showed this number of colon organisms. It must be remembered that from 9.4 to 22 cc. of milk were used for inoculation, so that the actual number of coli-aerogenes per cc. might be lower than is indicated.

.33.

Table VII

Prevalence of Coli-Aerogenes in 331 Samples of Raw Milk Grouped on Basis of Total Count on Standard Agar

Total Count	Number of Coli-Aerogenes per cc.			
	0	1 - 9	10-99	100
Under 10,000	36.4*	32.8	23.8	7.0
Over 10,000	21.1	8.0	28.6	42.3
Under 50,000	31.8	22.6	27.9	17.7
Over 50,000	16.2	8.1	20.3	55.4

\* Per cent.

Of these samples 27.9 per cent were negative for colon in 1, 0.1 and 0.01 cc. amounts; 19.1 per cent were positive in 1cc, 26 per cent were positive in 1 cc. and 0.1 cc. and 27 per cent were positive in 1 cc., 0.1 cc. and 0.01 cc. Malcolm (loc. cite) in the examination of 21,569 samples of raw milk found 48.3 per cent to be negative in 0.1, 0.01 and 0.001 cc., 21.4 per cent positive with 0.1 cc., 14 per cent positive with 0.1 and 0.01 cc. and 16.3 per cent positive with 0.1, 0.01 and 0.001 cc. If the 1 cc. positives are eliminated from the results reported here, 47.0 per cent of samples would be negative in all amounts, thus corresponding closely with the cited results. Klimmer, Haupt and Borches (21) found 57 per cent of their samples to have less than 10 colon organisms per cc., 23.5 per cent from 10 to 100, 10 per cent from 100 to 1000 and 23.5 per cent to have over 1000. The results of these investigators are compared with the present investigations in table VIII.

Table VIII

Percentage of coli-aerogenes found in various amounts of raw milk by different investigators

	Malcolm (29)	Klimmer, Haupt & Borches (21)	Present Data
Negative in 0.1 cc.	48.3	57	47
Positive in 0.1 cc.	21.4	23.5	26
Positive in 0.01 cc.	14.0	10	27
Positive in 0.001 cc.	16.3	23.5	—

Slack and Maddeford (48), in examining 25 samples of raw milk as sold to pasteurizing plants, found 4 per cent to contain no colon organisms in 10 cc. of milk, 12 per cent positive in 10 cc., 12 per cent positive in 1 cc., 20 per cent positive in 0.1 cc., and 52 per cent positive in amounts less than 0.1 cc. In 100 samples of bottled raw milk from a tuberculosis free herd, these investigators found no colon organisms in 30 per cent of the 10 cc. samples, in 10 per cent colon were present in this amount, and in 20 per cent of 1 cc., 14 per cent of 0.1, and 26 per cent of less than 0.1 cc. portions.

The average total count of the colon positive samples was 140,500 per cc. or 2.9 times the average total count of the colon negative samples, which was 48,270. Malcolm, in the investigation cited, found an average count of 160,577 in coliform positive samples and 25,295 per cc. in the coliform negative samples, the positive samples having 6.3 times as many bacteria per cc. as the negative samples.

The 50 samples of raw milk, plated on neutral red bile and tryptone glucose milk medium, showed a quite pronounced relationship existing between colon titre and total count, however, the number of samples are not sufficient for the drawing of definite conclusion. The results given in table IX, show 93.5 per cent of the samples, with a total count of under 10,000, to be negative in all amounts while 6.5 per cent were positive in

1 cc. amounts. The samples with a total count of over 10,000 showed 22.2 per cent to be negative in all amounts and 77.8 per cent positive in 1 cc. amounts. The average count of the colon negative samples was 6,650 which the colon positive samples had an average total count of 80,000 or 12 times as many. The logarithmic average of the colon negative samples, computed by the method of the U. S. Public Health Service Milk Ordinance and Code (54), was 5,000 while the positive samples showed an average of 45,000 or 9 times as many.

Table IX

Prevalence of Coli-Aerogenes on Fifty Samples of Raw Milk of Different total Counts on Glucose Tryptone Agar at 32° C.

Total Count	Number of Coli-Aerogenes per cc.			
	0	1 - 9	10-99	100
Under 10,000	93.5*	6.5	0	0
Over 10,000	22.2	77.8	0	0
Under 50,000	85	15		
Over 50,000	0	100		

\* Per cent

The 34 samples of pasteurized milk, showed 38 per cent to contain colon organisms with an average total count of 17,900 while 62 per cent were negative and showed an average count of 4,171, the positive samples having 4.3 times as many bacteria per cc. All of the positive samples were obtained from one plant where coli-aerogenes organisms were found from the cooler, bottler, and from the bottled milk, this plant had an exceptionally high bacterial count even in the pasteurizing vat. The two other plants studied showed no colon organisms in the bottled milk and the count never exceeded 700 per cc. The frequency of the colon organism in relation to total count is shown in table X.

Table X

Prevalence of Coli-Aerogenes in 34 samples of Pasteurized Milk  
of Different total Counts on Standard Agar

Total Count	Number of Coli-Aerogenes per cc.			
	0	1 - 9	10-99	100
Under 1,000	100*			
1 - 10,000	40	40	10	10
Over 10,000	30	40	30	

\* Per cent

The samples of "certified" milk gave an average total count of 1,686 for the colon positive samples which was 1.6 times as great as 1,017 the average count of the colon negative samples. No colon organisms were found in 0.1 cc. of these samples thus fulfilling the requirements for this grade of milk. Since no samples were examined in which the total count exceeded 10,000, comparison to Kline's (22) results could not be made.



## DISCUSSION

It is felt, from the results obtained with pasteurized milk, as produced by satisfactory plants, that the standard proposed by Swenarten is not unduly stringent. This standard being "that not more than 20 per cent of 0.1 cc. portion shall show E. coli" or that "positive results shall not occur in more than 10 per cent of 0.1 cc. portions when 10 or more samples are examined". It would seem entirely possible for this standard to be met if based on the result of 1 cc. portions, which is the suggestion of McCrady and Langevin (26), who conclude that "properly pasteurized milk should not contain coli-aerogenes bacteria in 1 cc. portions in more than 10 to 20 per cent of tests". Slack and Maddeford (cited) found that colon bacilli in 0.1 cc. indicates recontamination due to faulty cooling or handling and that absence in 50 cc. may "reasonably be expected". A correlation of 38 per cent between low count and absence of colon bacilli was found by these investigators. It is believed that from these results and from those of other investigators, an accurate test for coli-aerogenes organisms in pasteurized and in certified milk would be a valuable aid in determining the proper production and sanitary quality of the milk.

This is well emphasized in the present investigation of the one pasteurizing plant from which samples positive for colon were obtained. As indicated, they were found present in samples taken from the milk at every point after it had gone over the cooler, at the same time little or no increase in total bacterial count was observed. Thus the colon examination indicated faulty methods or equipment while the total count failed to show any contamination occurring.

The work with raw milk tends to confirm the results obtained by other investigators but it is believed that no standard for this grade

can at the present time be set, however, it would seem possible that reasonably careful production should yield a product having fewer than 10 colon organisms per cc. in 70 to 80 per cent of examined samples and that in no instance should 100 per cc. be exceeded. Additional work using tryptone glucose agar or other modified agar at 32° C. for the comparison of total count and presence of the coli-aerogenes group is desirable.

## SUMMARY AND CONCLUSIONS

The examination of 331 samples of raw milk, 34 samples of pasteurized, and 25 samples of "certified" milk for members of coli-aerogenes organisms by inoculation in ten solid media and five liquid media, showed neutral red bile agar and violet red bile agar to be the most satisfactory solid media and 2 per cent brilliant green bile broth the most satisfactory liquid medium for the isolation of this group. A new solid medium is described which gives every indication of being equal to those tested for the isolation of the coli-aerogenes group.

A study of the relative productivity of these media, using coli-aerogenes cultures recently isolated from milk, indicated that none were as productive as standard lactose. Methylene blue brom-cresol purple and brilliant green bile, in the order given, were the most productive of the liquid media tested, while neutral red bile, violet red bile and Endo agar gave higher counts than standard agar.

Four hundred and eighty four cultures of the *Escherichia*-*Aerobacter* group were isolated from the raw milk. Of these 56 per cent were *Escherichia*, 23.7 per cent *Aerobacter* and 20.3 per cent were intermediates. Forty five cultures were isolated from pasteurized milk, composed of two per cent *Escherichia*, two per cent *Aerobacter* and 96 per cent intermediates. Eight cultures were isolated from "certified" milk of which 50 per cent were *Escherichia*, 38 per cent *Aerobacter* and 12 per cent intermediates.

Identification of the cultures from raw milk showed 12 species of *Escherichia* with *E. communior* being most prevalent. Five species of *Aerobacter* were isolated with *A. hibernium* being the most prevalent. Pasteurized milk yielded one species each of *Escherichia* and *Aerobacter*, while two species of *Escherichia* and two species of *Aerobacter* were isolated from certified milk.

A comparison of the total count on standard agar and presence of colon organisms showed the colon positive samples of raw milk to have a total count 2.9 times that of the colon negative samples. Less than 10 colon organisms per cc. were found in 69.2 per cent of samples with a total count of less than 10,000 bacteria per cc. No pasteurized samples with counts under 1,000 contained colon organisms, the count of colon positive samples being 4.3 times higher than colon negative samples. No colon organisms were found in 0.1 cc. of "certified" milk.

Total bacterial counts made on tryptone glucose milk medium at 32° C. gave excellent correlation with colon titre, 93.5 per cent of samples with a total count under 10,000 were negative for colon organisms, the average count of colon positive samples were 12 times higher than colon negative samples being 6,650 and 80,000 per cc. respectively.

The presence of the coli-aerogenes group in raw milk of high bacterial count, would seem to be of little significance, in milk of low count the number of colon bacteria might be limited to presence in 1 cc. amounts in a high per cent of samples examined. In order to determine the sanitary conditions under which the milk is produced, further work to determine a standard for these organisms is desirable.

The presence of coli-aerogenes organisms in 1 cc. of pasteurized milk in 10 to 20 per cent of samples would seem sufficient to indicate contamination, which as is shown in this investigation might not be indicated by total bacterial counts. This test in pasteurized milk is particularly desirable.

The present standards for certified milk in respect to colon content are quite lenient. Lactose taurocholate agar was found unsatisfactory in these experiments so that the adoption of a more satisfactory method for the detection of these organisms would be desirable and if this is realized a more stringent standard would seem possible. The value of a test to indicate contamination in a milk to be consumed raw can not be too highly stressed.

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## BIBLIOGRAPHY

1. Aichelburg, Di. Osservazioni su stipiti del gruppo coli-aerogenes isolati dal latte. Giorn Batter 12:481. 1934. Abst. in Zblt. Bakt. I Ref. 115:1/2, 24, 1934.
2. American Association of Medical Milk Commission. Methods and Standards for the Production of Certified Milk. 1935.
3. Ayres, S.H., Cook, L.B., and Clemmer, P.W. The Significance of the Colon Bacilli in Milk. Abst. of Bact. 1:52, 1917.
4. Barkworth, H. A Statistical Examination of the Interrelationship and Variability of Plate Count, Presumptive Colifora Content and Keeping Quality of Raw Milk. J. Dairy Res. 6:26, 1935.
5. Beavans, E.A., The Escherichia-Aerobacter Group as an Index to Proper Pasteurization. J. Dairy Sci. 13:91,94, 1930.
6. Bergey, D.H., Manual of Determinative Bacteriology, 4th Ed. Williams and Wilkins Co., Baltimore. 1934.
7. Black, L.A. and Klinger, Mary E., A Comparison of Media for the Detection of Escherichia-Aerobacter. J. Bact. 31:171, 1936.
8. Bower, C.S., and Hucker, G.J. The Composition of Media for the Bacteriological Analysis of Milk. Tech. Bul. # 228. New York Agri. Exp. Sta. Geneva.
9. Brown, L. A. and Gott, E.J. Use of Endo's Medium in Locating Milk Contamination. Am. Food. J. 18:295, 1923.
10. Butterfield, C.T. The Enumeration of Bacteria by means of Solid and Liquid Media. Pub. Health Rpts. 48:1292, 1933.
11. Chalmers, C.H. The Significance of True B. Coli (B. coli communis) and B. lactis aerogenes in Samples of Milk. Zent f. Bakt. II.Abt. 89:459, 1934.
12. Davis, G.O. The Significance of (a) Acid Fast Bacteria; (b) Bacillus coli in milk. Vet. Record 13:1046, 1933.
13. Dementer, K., Sauer, F. u Miller, M. Vergleichende Untersuchungen uber veschieden Methoden zur Colon-Aerogenes titrebestimmung in Milch. Milch Forsch. 15:265, 1933.
14. Dominick, J.F. and Lauter, C.J. Methylene Blue and Brown Cresol Purple in Differentiating Bacteria of the Colon-Aerogenes Group. J. Am. W. W. Assoc. 21:1067, 1929.
15. Farrell, M. A. Comparative Productivity Tests of Presumptive Test Media with Pure Cultures of the Colon-Aerogenes Group. J.Bact. 30:445. 1935
16. Finkelstein, R. Occurrence of the Colon-Aerogenes Group of Organisms in Raw and in Pasteurized Milk, and its Significance. J.Dairy Sci. 2:460 :

17. Hoskins, J.K. The Most Probable Numbers of B. Coli in Water Analysis. J. Am. W.W. Assoc. 25:867, 1933.
18. Hunter, O.W. The Colon-Aerogenes Group of Milk. J. Dairy Sci. 2:108, 1919.
19. Jordan, E.O. The Inhibitive Action of Bile upon B. Coli. J. Inf. Dis. 12:326, 1913.
20. Kessler, Mildred A., and Swenarton, J.C. Gentian Violet Lactose Peptone Bile for the Detection of B. Coli in Milk. J. Bact. 14:47, 1927.
21. Klimmer, M., Haupt, H., u Borches, F. Über der Vorkommen und die Bestimmung der Coli und Aerogenes Bakt. der Milch. Milch Forsch. 9:236, 1930.
22. Kline, E.K., The Colon Group of Bacteria in Milk. 19th Ann. Report Inter. Assoc. Dairy and Milk Inspectors. 68, 1930.
23. Kon, Phyllis, M. Coliform Organisms in Milk and Bovine Faeces. J. Dairy Res. 4:206, 1933.
24. Leifson, E. New Culture Media Based on Sodium Desoxycholate for the Isolation of Intestinal Pathogens and for the Enumeration of Colon Bacilli in Milk and Water. J. Path. and Bact. 40:581, 1935.
25. Levine, M. A Statistical Classification of the Colon-Cloacae Group. J. Bact. 3:253, 1918.
26. McCrady, M.H. and Langevin, E.M. The Coli-Aerogenes Determination in Pasteurization Control. J. Dairy Sci. 15:321, 1932.
27. McCrady, M.H. and Archambault, J. Examining Dairy Products for Members of the Escherichia-Aerobacter Group. Am. J. Pub. Health 24:122, 1934
28. Manual of Methods for the Pure Culture Study of Bacteria. Leaflet V, 5th Ed., 1934.
29. Malcolm, J.F. The Occurrence of Coliform Bacteria in Milk. J. Dairy Res. 5:15, 1933.
30. Marshall, H. Die praktische Auswirkung der Untersuchungsmethode auf die Höhe des ermittelten Coli-Aerogenes. Gehaltes von Milch und Milchprodukten. Osterr. Milchwirtsch Ztg. 41:313, 1934.
31. Minkin, J.L. and Burgwald, L.H. A Study of Escherichia-Aerobacter Organisms in Pasteurized Milk. J. Dairy Sci. 18:474, 1935.
32. Moore, H.C. and Fuller, J.M. Significance of Colon Organisms in Raw Milk. Tech. Bul. 57 Univ. of New Hampshire Agri. Exp. Sta.
33. Moldavan, A. A Modified Technic for the Detection of the Escherichia-Aerobacter Group in Milk. Am. J. Pub. Health 25:1032, 1935.
34. Munchberg, F. The Hygienic Estimation of Pasteurized Milk. Wien. tierärztl Mschr. 20:278, 1933.

35. Noble, R.E. Cyanide-Citrate Pour Plate Medium for Direct Enumeration of Colon-Aerogenes Content of Water and Sewage. J.Am.W.W.Assoc. 19:182 1928.
36. Noble, R.E. The Relative Productivity of Certain Culture Media. J. Am. W. W. Assoc. 27:1143, 1935.
37. Perry, C.A. and Hajna, A.A. A Modified Eijkman Medium. J. Bact. 26:419, 1933.
38. Price, W.H. The Significance of Bact. Coli in Ice Cream and Dairy Products. Ice Cream Trade Jr. 24:1928.
39. Pont, E.G. The Occurrence of Coliform Organisms in Cream and their Effect upon Cream Quality. J. Dairy Res. 6:146, 1935.
40. Ritter, C. The Presumptive Test in Water Analysis. J. Am.W.W.Assoc. 24:413, 1932.
41. Ritter, C. Personal Communication.
42. Ritter, W. Die quantitative bestimmung der Coli-Aerogenes Bakterien in Milch und Lab. Milchwirtsch Forschg. 15:154, 1933.
43. Ruchhoft, C.C. Comparative Studies of Media for the Determination of the Colon-Aerogenes Group in Water Analysis. J.AmW.W.Assoc. 27:1732, 1935.
44. Ruchhoft, C.C. and Corton, J.F. Study of Selective Media for Coli-Aerogenes Isolation. J. Am.W.W.Assoc. 27:1134, 1935.
45. Salle, A.J. A System for the Bacteriological Examination of Water. J. Bact. 20:381, 1930.
46. Sherman, J.M. and Wing, Helen, U. The Significance of Colon Bacteria in Milk, with Special Reference of Standards. J. Dairy Sci. 16:165, 1933.
47. Simmons, J.S. A Culture Medium for Differentiating Organisms of Typhoid-Colon-Aerogenes Group and for the Isolation of Certain Fungi. J. Inf. Dis. 39:209, 1926.
48. Slack, A.J. and Maddeford, C.W. The B. coli content of Raw and Pasteurized Milk. Canadian Pub. Health J. 23:574, 1932.
49. Standard Methods of Water Analysis 7th Ed., Am. Pub. Health Assoc. and Am. W. W. Assoc. 1933.
50. Stark, C.N. and Curtis, L.R. A Critical Study of Some Media Used for the Detection of Colon Organisms in Water and Milk. J. Bact. 29:27, 1935.
51. Stark, C.N. and England, C.W. Formate Ricinoleate Broth- A New Medium for the Detection of Colon Organisms in Water and Milk. J. Bact. 29:26, 1935.
52. Swenarton, J.C. Can B. coli be used as an Index of the Proper Pasteurization of Milk. J. Bact. 13:419. 1927.



53. Tonney, F.O. and Noble, R.E. A Solid Brilliant Green Lactose Bile Medium for Direct Plating with Results in Seventeen Hours. J. Am. W. W. Assoc. 27:108, 1935.
54. United States Pub. Health Service Milk Ordinance and Code. August, 1934.
55. Wilson, W.J. Selective Media for the Isolation, Cultivation and Differentiation of B. coli and B. lactis Aerogenes. J. Hyg. 33:404 1933.
56. Yale, M.W. The Escherichia-Aerobacter Group of Bacteria in Dairy Products. J. Dairy Sci. 16:481, 1933.