A STUDY OF THE CULTURAL AND SEROLOGICAL REACTIONS OF SOME TYPICAL AND ATYPICAL COLIFORM ORGANISMS

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by

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INTRODUCTION

Some years ago Bergey and his colleagues (5) described organisms of the genera Escherichia and Aerobacter as aerobic, Gram negative, non-spore forming short rods which ferment lactose and dextrose with the production of acid and gas.

These organisms, now collectively designated as coliform, (7) have risen in importance. For a number of years the coliform group has been used as the criterion of polluted water in routine laboratory analysis all over the country and, indeed, all over the world. For this reason the coliform organisms have been and are being studied more extensively than probably any other one group of organisms. As a result of these wide detailed studies, observers are continually finding organisms apparently coliform but which vary in some characteristic which has been accepted as standard for the colon-aerogenes group.

Various authors have designated those organisms as "irregulars" which ferment lactose but do not fall into either the Escherichia or Aerobacter group according to the citrate, methyl red and Voges Proskauer tests for the differentiation of Escherichia coli from Aerobacter aerogenes. Those organisms which are seemingly coliform but do not ferment lactose with the production of acid or acid and gas in 48 hours will be called atypical.

In addition to a discussion of "irregular" and atypical organisms, a consideration of The Standard Methods for the Examination of Water and Sewage (2) from the point of view of the efficiency of eosin methylene blue agar as a means of differentiating E. coli from A. aerogenes. will be presented.

HISTORICAL

"Irregular" Coliform Organisms

Coliform organisms which could not be classified as Escherichia or Aerobacter according to the methyl red, Voges Proskauer, and citrate tests were mentioned by MacConkey (24) as early as 1905.

France (10) isolated E. coli from both feces and polluted water. He found that 28.7 per cent of the organisms from water gave irregular results as compared with 2.2 per cent from feces.

Others have found also a large percentage of "irregular" coliform organisms outside of the animal body. Bahlman (3) states that in water he found 15.3 per cent "irregular" out of 1223 strains tested. From a total of 4297 strains Bradsley (6) found that 581 or 12 per cent were "irregular." Koser (18) stated that "irregular" forms were found mostly among soil strains but were absent in feces. Again in 1926 he (19) found 31.9 per cent of the organisms in non-polluted pasture soil to be "irregular" and a very small number in polluted pasture soil. Yale (39) isolated 204 colon-aerogenes cultures from milk. He reported that 33 per cent fell into the "irregular" group. Hicks (13) found 10.6 per cent "irregular" from human feces, Carpenter and Fulton (8) 13.3 per cent, and Parr (28) found 7.7 per cent from fresh human fecal samples.

These researches indicate that large numbers of "irregular" organisms are commonly present in soil and water samples.

The Accuracy of Eosin Methylene Blue Agar

The accuracy with which eosin methylene blue agar can be used to differentiate E. coli and A. aerogenes has been contested.

Levine (22) believed it to be highly satisfactory. He stated that in 96.8 per cent of the cases eosin methylene blue agar gave typical colonies from E. coli, 82.4 per cent were typical from A. aerogenes and that "irregular" colonies showed characteristics similar to A. aerogenes.

Georgia and Morales (11) concluded that in general the coliform organisms could be differentiated on E. M. B. agar. They reported, however, that in a number of instances colonies which were picked for one type proved to be another.

Poe (31) testing the dependability of the E. M. B. agar in 195 cultures belonging to the colon group found that 88.2 per cent gave typical Escherichia colonies. 23 of these cultures were "irregular" and 13 had sufficient characteristics to put them in the colon group. He stated that there was a 94.9 per cent correlation for Escherichia. 4.3 per cent of the 164 aerogenes cultures were not characteristic, giving an accuracy of 95.7 per cent.

Ruchhoft (33) in a study of both pure and mixed cultures stated that macroscopic interpretation of first streaked isolation plates is unscientific and in many cases conjecture.

In this paper an attempt has been made to determine the accuracy of this widely used method for differentiating E. coli and A. aerogenes.

Atypical Coliform Organisms

Atypical coliform organisms have been referred to as non-lactose fermenters, slow lactose fermenters or late lactose fermenters.

Some workers believe that atypical coliform organisms are not derived from E. coli and therefore are of no sanitary significance. Klein and Houston (14) found atypical organisms on grain. Savage (35) stated that atypical E. coli were of little sanitary significance. He showed that holding typical E. coli in mud did not change its characteristics. MacConkey (24) showed that E. coli retained all of its characteristics unchanged after an unfavorable environment of 258 days.

Later some evidence was brought forth to show the importance of atypical coli. Karstrom (17) demonstrated that the enzyme lactase in coliform organisms is adaptive. It therefore may vary in amount depending upon the conditions for growth or synthesis of protoplasm.

Stokes, Weaver and Scherago (36) reported the conversion of late lactose fermenters to rapid lactose fermenters and again to late lactose fermenters. They concluded that the strains studied were variants of different members of the colon-aerogenes group. Kriebel (20) agreed that such organisms as these are as definitely fecal contamination as E. coli.

That same year Lewis (23) studied the phenomenon of dissociation in mutable strains of Escherichia and Aerobacter by the use of synthetic media containing lactose. He stated that non-lactose fermenting variants gave rise to lactose fermenting strains.

Ziegler (40) in 1939 suggested that since lactase is adaptive, a reversal may have taken place in late lactose fermenters. In a polluted

water the concentration of nutrient material is usually too low to permit active multiplication of cells. Conditions may exist which would cause a decrease in lactase activity even in the absence of cell multiplication.

In this paper an attempt has been made to identify these atypical organisms by the use of cultural reactions such as the fermentation of various carbohydrates.

Serological Relationship Among Coliform Organisms

Many investigators have tried using serological methods to determine the relationship between atypical coliform organisms and Escherichia coli. A rather startling fact was mentioned by Pfaundler (30) in 1898 when he stated that there was no serological homogeneity among typical coliform cultures. He also noted that sera of the immunizing animals did not always agglutinate their homologous strains. In other words, he found it difficult to produce antibodies for these organisms.

One year later Radzrevsky (32) concluded that the colon bacteria can be divided into a large number of serological groups. Jatha (15), Mackie (25), Van Loghem (38), Herrold (12), and Magheru (26) confirmed the work of Radzievsky.

Strunz (37) immunized rabbits with 14 out of 23 strains of fecal coliform organisms and by cross agglutination classified these 23 strains into 3 major groups independent of their origin. Meyer (27) obtained similar results. He divided E. coli into 3 types on the basis of type-specific and species-specific antigens.

Even though the various strains of E. coli were shown to be serologically heterogeneous many tried to show serological homogeneity between the non-lactose fermenters or atypical coli. Fothergill (9) showed that non-lactose fermenters were serologically heterogeneous and Abdoosh (1) confirmed this.

Jones and Little (16) found that the slow lactose fermenters showed no immunological specificity for paratyphoids. Kriebel (20) and Sandiford (34) confirmed this and Sandiford also stated that the atypical coli are heterogeneous with a small degree of common antigen between individual strains.

Kriebel (20) attempted to classify the atypical coli from feces. Antisera were used from 2 Escherichia types, 4 Salmonella, 3 Shigella and 1 Eberthella type. Over 65 per cent of the strains were negative and the positive ones agglutinated non-specifically.

Parr (29) using slow lactose, non-lactose and non-dextrose fermenters tested them serologically with antigens of E. typhosa, Salmonella sonnei, S. paradysenteriae, S. paratyphi, S. schottmulleri, S. enteritidis, S. cholerae, S. aertrycke, Proteus $^{X}2$, and Alcaligenes fecalis. He (54) found that cross reactions occurred slightly for only E. typhosa and S. paradysenteriae.

It is easily seen that none of these researches has succeeded in linking the atypical coli with any other one group of organisms serologically. All workers do agree upon one fact, i.e., that various typical strains of E. coli are not serologically homogeneous.

Apparently, very few workers, if any, have determined the serological relationship between various strains of A. aerogenes nor have they attempted to determine the relationship between A. aerogenes and the atypical coliform organisms.

EXPERIMENTAL

The cultures with which this work was done were obtained from various bacteriological laboratories over the country. They were isolated from water supplies and were identified as coliform organisms according to The Standard Methods for the Examination of Water and Sewage (2).

The experimental work which follows is divided into two parts. First, an analysis was made of the typical cultures (those coliform organisms which ferment lactose and dextrose with the production of acid and gas in 24 or 48 hours). These organisms were classified as E. coli, A. aerogenes, or an "irregular" type according to the citrate, methyl red and Voges-Proskauer tests. A correlation was made between the original classification of these cultures on eosin methylene blue agar by workers in various laboratories according to results obtained with the citrate, M. R., and V. P. tests.

Secondly, the atypical coliform organisms (slow lactose fermenters or non lactose fermenters) were considered. They were tested for acid and gas production on the following media: inositol, inulin, dextrin, xylose, mannose, sorbitol, raffinose, mannitol, dulcite, trehalose, galactose, levulose, and salicin broths. Gelatin liquefaction and the ability to produce hydrogen sulfide were noted. A correlation was also made between the nature of the organism isolated and the source of the water sampled.

In addition an attempted classification was made by serological methods. Antiserum for typical E. coli and A. aerogenes cultures was obtained from rabbits. Agglutination and absorption tests were run with typical antigens of E. coli, A. aerogenes, and atypical coliform organisms.

TECHNIQUE

The coliform cultures received were checked for purity and were tested on the Koser's citrate medium, and for methyl red and Voges Proskauer reactions on dextrose phosphate medium. Koser's citrate test and the methyl red tests were carried out in the usual manner. A newer modification of the Voges Proskauer test was used. To 1 ml. of the culture, 0.6 ml. of 5 per cent alpha napthol in absolute ethyl alcohol and .2 ml. of 40 per cent KOH were added. The tubes were incubated at 37°C and the results were read from 3 to 4 hours later. A positive reaction was designated by the formation of a red ring (4).

The atypical cultures were tested on various carbohydrates for the production of acid and gas, on gelatin for liquefaction, and on iron citrate agar for the production of hydrogen sulfide. The following carbohydrates were used: inositol, inulin, dextrin, mannose, sorbitol, raffinose, mannitol, dulcite, trehalose, galactose, levulose, and salicin. Five tenths per cent of each of these was added to nutrient broth and autoclaved at 10 pounds pressure for 10 minutes. One tenth per cent Andrade's indicator was used to designate a fall in pH. Durham tubes were used to check gas production. Observations were made after 24 and 48 hours incubation. Gelatin liquefaction was tested by incubating the cultures for 3 days at 37°C and chilling to determine whether the gelatin would solidify. Hydrogen sulfide production was determined by using stab cultures of iron citrate agar and observing a blackening along the line of inoculation. The medium contained 2 per cent Difco proteose peptone, 0.1 per cent K_2HPO_4 and 0.05 per cent iron citrate in agar.

The serological work was carried on with antisera from rabbits. The antigens used for the production of the antisera were phenolized (.5 per cent) suspensions in physiological saline but were not heat killed. The antigens were of a density comparable to a No. 2 McFarland Nepholometer. Intravenous injections were made on three successive days of each week until the maximum titre was obtained. The quantity of antigen was increased from .5 ml. to 3.0 ml. over a period of three weeks. The antiserum was preserved by refrigeration. The antigens used in the agglutination reactions were made in the same manner as those for injections. The agglutination reactions were made in the following manner. The antigen in saline was added to 8 tubes, 2 ml. in the first and 1 ml. in the rest. One tenth of the antiserum was added to the first tube containing 2 ml. of antigen. Serial dilutions were made by taking 1 ml. from the first tube and adding it to the second. In this way, dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280 were obtained. The eighth tube served as a control.

Tests for species-specific and group-specific antigens were run. The H or species-specific antigen was prepared as follows: the twenty-four hour growth from beef-infusion agar in pint Blake bottles was suspended in 20 to 30 ml. of .85 per cent salt solution containing 0.2 per cent formalin. After the micro-organisms had been killed the turbidity was adjusted to correspond to $BaSO_4$ standard No. 3 by the addition of salt solution containing 2.0 per cent formalin.

The O or group-specific antigen was prepared by washing the growth in 10 ml. of 0.85 per cent salt solution containing 0.5 per cent phenol. The growth from several bottles was combined and one-half the volume of absolute

alcohol or a proportional amount of 95 per cent alcohol was added slowly, while the suspension was constantly stirred. It was then allowed to remain at a temperature of 37°C for about 18 hours, after which the supernatant fluid was decanted and tested for bacterial growth and agglutinability. After determining the dilution necessary to secure a density equal to that of $BaSO_4$ standard No. 3, sufficient alcohol was added to the concentrated suspension to give 2.5 per cent in the diluted antigen, which should contain not more than 0.04 per cent phenol.

The agglutination reactions with these antigens were made in the same manner as those previously described.

Typical Coliform Organisms

"Irregular" Coliform Organisms

A total of 254 coliform cultures which ferment lactose and dextrose with the production of acid and gas in 24 to 48 hours was classified according to the methyl red, Voges Proskauer and Koser's citrate tests. As shown in tables 1 and 2, a total of 89 (34.6 per cent) of these organisms was found to be E. coli, 94 (36.5 per cent) A. aerogenes, and 71 (28.8 per cent) were "irregular" to these tests.

This relatively high percentage of "irregular" organisms confirms the work of France (10) who found 28.7 per cent of the "irregular" coliform organisms in water. Previously, Bahlman (3) had found only 15.3 per cent to be "irregular" of the cultures isolated from water. The variation in the results found by France (10) and the author as compared with that of Bahlman (3) is probably caused by a basic difference in the source of the water from which the samples were taken. For example, it is believed that there is a smaller percentage of "irregular" organisms in fresh feces than in cultures isolated from water supplies. Koser (19) found a smaller number of "irregular" organisms in polluted pasture soil than in virgin soil, and Parr(28) in 1936 stated that there were less "irregular" organisms in fresh feces than in water.

Table 3 shows that the majority (54.9 per cent) of the 71 "irregular" organisms found in this group isolated from various water supplies were citrate positive, methyl red positive and Voges Proskauer negative. As high as 35.3 per cent were positive to all three tests. Seven per cent were

Table 1

Classification of coliform organisms which ferment lactose and dextrose *

Culture	Koser's citrate	Methyl red	Voges-Proskauer
1	-	+	-
3	+	+	-
5	-	+	-
7	+	-	+
8	+	-	+
9	+	-	+
10	-	+	-
11	-	+	-
12	-	+	-
13	-	+	-
14	+	-	+
15	-	+	-
16	-	-	+
17.	-	+	-
19	-	+	-
20	-	+	-
21	+	-	+
23	+	+	-
25	+	+	-
26	+	-	+
33	-	+	-
35	+	+	+

Culture	Koser's citrate	Methyl red	Voges-Proskauer
40	+	+	-
41	+	+	-
42	+	+	-
43	+	-	+
44	-	+	-
46	+	-	+
55	-	+	-
56	-	+	-
61	+	+	-
62	-	+	-
70	-	+	-
75	-	+	-
90	+	-	-
91	+	-	+
92	+	-	+
102	-	+	-
104	-	+	-
107	+	+	+
108	+	+	-
109	-	+	-
115	-	+	+
132	+	-	-
142	+	-	+
143	+	-	+

Culture	Koser's citrate	Methyl red	Voges-Proskauer
144	+	-	+
145	+	-	+
146	+	-	+
149	+	-	+
160	+	-	+
164	+	+	+
165	+	-	+
167	+	-	+
171	+	-	+
172	+	-	+
176	+	+	+
178	+	-	+
179	+	-	+
182	+	-	+
185	+	-	+
189	+	-	+
203	+	-	+
205	-	+	-
208	+	-	+
215	+	-	+
229	-	-	+
221	+	-	+
223	+	-	+
239	+	+	+

Culture	Koser's citrate	Methyl red	Voges-Proskauer
226	+	+	-
245	+	-	+
278	+	+	-
291	+	-	+
241	+	-	+
292	+	+	+
299	+	+	-
231	+	-	+
298	-	+	-
295	+	+	-
258	+	+	+
296	+	+	-
294	+	+	+
261	+	-	+
267	-	+	-
260	+	+	-
281	-	+	-
283	-	+	-
297	+	-	+
279	+	-	+
280	+	-	+
285	+	+	-
287	+	+	+
286	+	+	-

Culture	Koser's citrate	Methyl red	Voges-Proskauer
288	+	+	+
289	+	+	+
290	-	+	-
277	-	+	-
276	-	+	-
242	+	-	+
275	-	+	-
268	+	+	-
269	-	+	-
274	-	+	-
238	-	-	+
192	+	-	+
193	+	-	+
342	-	+	-
343	-	+	-
344	-	+	-
345	-	+	-
346	-	+	-
348	+	-	-
349	-	+	-
350	-	+	-
351	-	+	-
352	-	+	-
353	-	+	-

Culture	Koser's citrate	Methyl red	Voges-Proskauer
354	-	+	-
355	+	-	+
356	-	+	-
357	+	+	-
358	+	-	+
359	+	-	+
360	+	+	-
361	-	+	-
362	+	-	+
363	+	-	+
364	+	-	+
365	-	+	-
368	-	+	-
367	+	+	+
369	+	-	-
370	+	+	-
371	-	+	+
372	+	+	+
374	-	+	-
375	+	+	-
376	+	+	+
377	-	+	-
278	-	+	-
279	+	-	+

Culture	Koser's citrate	Methyl red	Voges-Proskauer
380	+	+	+
381	-	+	-
382	+	-	+
384	+	+	-
385	+	-	+
386	+	+	-
387	+	+	-
388	+	+	-
389	+	+	-
390	+	+	-
391	+	+	-
392	+	+	-
393	+	+	-
399	+	-	+
400	+	+	-
301	-	-	+
302	+	-	+
304	-	+	-
306	-	+	-
307	+	+	-
308	-	+	-
310	-	+	-
311	+	-	+
300	-	+	-

Culture	Koser's citrate	Methyl red	Voges-Proskauer
312	+	-	-
313	+	+	-
314	-	+	-
315	-	+	-
316	+	+	-
318	-	+	-
319	-	+	-
320	-	+	-
317	+	+	-
322	-	+	-
323	-	+	-
324	-	+	-
325	-	+	-
326	-	+	-
328	+	+	+
327	-	+	-
329	+	+	+
330	-	+	-
331	+	+	-
321	+	+	-
340	-	+	-
339	-	+	-
338	-	+	-
337	-	+	-

Culture	Koser's citrate	Methyl red	Voges-Proskauer
336	-	+	-
335	-	+	-
334	-	+	-
333	-	+	-
332	-	+	-
513	-	+	-
512	-	+	-
510	-	+	-
509	-	+	-
507	-	+	-
504	-	+	-
523	+	-	+
518	-	+	-
517	-	+	-
516	-	+	-
522	-	+	-
532	+	+	+
531	-	+	-
530	+	-	-
529	-	-	+
528	+	-	+
527	-	+	-
526	-	+	-
542	+	+	-

Culture	Koser's citrate	Methyl red	Voges-Proskauer
544	+	-	+
545	+	+	-
303	+	-	+
597	+	-	+
585	+	+	+
593	+	-	+
588	+	+	+
594	+	-	+
583	+	-	+
595	+	+	+
596	+	-	-
607	+	-	+
612	+	+	+
625	+	-	+
629	+	+	+
601	+	-	+
600	+	-	+
620	+	-	+
592	+	-	+
590	+	-	+
589	+	-	+
615	+	-	+
631	+	-	+
609	+	-	+

Culture	Koser's citrate	Methyl red	Voges-Proskauer
622	+	+	+
602	+	-	+
614	+	+	+
599	+	-	+
628	+	-	+
623	+	+	+
618	+	+	+

* Source of cultures used:

Connecticut State Department of Health, Hartford, Connecticut.

Detroit Board of Water, Detroit, Michigan.

The Flint Water Filtration Plant, Flint, Michigan.

Highland Park Filtration Plant, Highland Park, Michigan.

Indianapolis Water Board, Indianapolis, Indiana.

Maryland State Department of Health, Baltimore, Maryland.

Minnesota State Department of Health, Minneapolis, Minnesota.

Quebec Province of Health, Montreal, Quebec.

Pennsylvania State College, State College, Pennsylvania.

Saginaw Water Filtration Plant, Saginaw, Michigan.

Department of Bacteriology, Stanford University, California.

Department of Bacteriology, University of Pennsylvania, Philadelphia, Pennsylvania.

West Virginia State Department of Health, Charleston, West Virginia.

Table 2

Classification by percentages of the coliform organisms which ferment lactose and dextrose (summarized from table 1)

Organism	Total number	Per cent
Escherichia coli	89	34.6
Aerobacter aerogenes	94	36.5
"Irregulars"	71	28.8

Table 3

Relative percentages for the different types of "Irregular" organisms (summarized from Table 1)

Tests used			Number of	
Citrate	Methyl red	Voges-Proskauer	cultures	Per cent
+	+	-	39	54.9
+	+	+	25	35.3
-	+	+	2	2.8
-	-	+	5	7.0

citrate negative, methyl red negative and Voges Proskauer positive. Very few (2.8 per cent) were citrate negative, methyl red positive and Voges Proskauer positive.

This confirms the work of Parr (29) who in 1938 also found the citrate positive, methyl red positive and Voges Proskauer negative group to be the one in which most "irregular" organisms are classified.

The Accuracy of Eosin Methylene Blue Agar

A correlation was made between the original classification of the typical coliform organisms by the eosin methylene blue agar plate method and the results obtained by the citrate, methyl red and Voges Proskauer tests. According to tables 4 and 5, out of a total of 89 typical E. coli cultures only 60 (67.5 per cent) formed characteristic colonies as observed by various technicians in laboratories over the country. Out of a total of 75 A. aerogenes cultures only 34 (45.3 per cent) had produced characteristic colony formations on E. M. B. agar.

These results are much lower than those found by Levine (22) in 1921. He stated that E. coli could be identified in 96.9 per cent of the times in contrast to 67.5 per cent as found by the author. Levine (22) also found an accuracy of 82.4 per cent for A. aerogenes in contrast to the 45.3 per cent reported here.

Levine (22) reported that eosin methylene blue agar could be used to identify colonies of E. coli and A. aerogenes with almost 100 per cent accuracy. This may easily be the case with the experienced worker. However, this differential medium is being used by laboratory technicians all over

the country and, as the data of these workers compiled here show, they are not getting the accurate results that Levine (22) obtained. These technicians were 67.5 per cent accurate in the identification of E. coli, which is only 17.5 per cent better than pure speculation. In the identification of A. aerogenes colonies they were accurate in 45.3 per cent of the cases, which is not as good as guessing.

Since the data are compiled from the work of many technicians, it clearly shows that eosin methylene blue agar should not be used routinely in the laboratory as a means of differentiating E. coli from A. aerogenes.

Table 4

Comparison of the designation of coliform organisms on eosin methylene blue agar and their complete identification

Organisms	Original identification	Final identification
1	E. coli	E. coli
5	E. coli	E. coli
7	E. coli	A. aerogenes
8	A. aerogenes	A. aerogenes
9	E. coli	A. aerogenes
10	E. coli	E. coli
11	E. coli	E. coli
12	E. coli	E. coli
13	E. coli	E. coli
14	?	A. aerogenes
15	?	E. coli
17	A. aerogenes	E. coli
19	E. coli	E. coli
20	E. coli	E. coli
21	Confluent	A. aerogenes
26	E. coli	A. aerogenes
33	Atypical	E. coli
43	Confluent	A. aerogenes
44	E. coli	E. coli
46	Atypical	A. aerogenes
55	E. coli	E. coli

Organisms	Original identification	Final identification
56	E. coli	E. coli
62	E. coli	E. coli
70	E. coli	E. coli
75	A. aerogenes	E. coli
91	A. aerogenes	A. aerogenes
92	Atypical	A. aerogenes
102	E. coli	E. coli
104	E. coli	E. coli
109	Atypical	E. coli
142	E. coli	A. aerogenes
143	Atypical	A. aerogenes
144	Atypical	A. aerogenes
145	Atypical	A. aerogenes
146	Atypical	A. aerogenes
149	E. coli	A. aerogenes
160	E. coli	A. aerogenes
165	E. coli	A. aerogenes
167	E. coli	A. aerogenes
171	A. aerogenes	A. aerogenes
172	Atypical	A. aerogenes
178	Atypical	A. aerogenes
179	A. aerogenes	A. aerogenes
182	E. coli	A. aerogenes
205	A. aerogenes	E. coli

Organisms	Original identification	Final identification
267	A. aerogenes	E. coli
278	Confluent	E. coli
279	Confluent	A. aerogenes
281	A. aerogenes	E. coli
298	E. coli	E. coli
300	E. coli	E. coli
302	A. aerogenes	A. aerogenes
303	E. coli	A. aerogenes
304	E. coli	E. coli
306	E. coli	E. coli
308	E. coli	E. coli
310	A. aerogenes	E. coli
311	A. aerogenes	A. aerogenes
314	A. aerogenes	E. coli
315	A. aerogenes	E. coli
318	E. coli	E. coli
319	E. coli	E. coli
320	A. aerogenes	E. coli
322	E. coli	E. coli
323	E. coli	E. coli
324	E. coli	E. coli
325	Confluent	E. coli
326	E. coli	E. coli
327	E. coli	E. coli

Organisms	Original identification	Final identification
330	E. coli	E. coli
332	E. coli	E. coli
333	E. coli	E. coli
334	E. coli	E. coli
335	Atypical	E. coli
336	A. aerogenes	E. coli
337	E. coli	E. coli
338	Atypical	E. coli
339	E. coli	E. coli
340	E. coli	E. coli
382	Atypical	A. aerogenes
399	E. coli	A. aerogenes
504	E. coli	E. coli
507	E. coli	E. coli
509	E. coli	E. coli
510	E. coli	E. coli
512	E. coli	E. coli
513	E. coli	E. coli
516	Atypical	E. coli
517	A. aerogenes	E. coli
518	E. coli	E. coli
522	E. coli	E. coli
523	E. coli	A. aerogenes
526	E. coli	A. aerogenes

Organisms	Original identification	Final identification
527	E. coli	A. aerogenes
528	Atypical	E. coli
531	E. coli	E. coli
544	A. aerogenes	A. aerogenes
583	A. aerogenes	A. aerogenes
592	A. aerogenes	A. aerogenes
594	A. aerogenes	A. aerogenes
597	A. aerogenes	A. aerogenes
599	Dew drop colonies	A. aerogenes
600	A. aerogenes	A. aerogenes
601	A. aerogenes	A. aerogenes
602	A. aerogenes	A. aerogenes
605	A. aerogenes	A. aerogenes
607	A. aerogenes	A. aerogenes
615	Confluent	A. aerogenes
620	A. aerogenes	A. aerogenes
628	A. aerogenes	A. aerogenes

Table 5

Comparison of the designation of coliform organisms on eosin methylene blue agar and complete cultural identification (Summarized from table 4)

Organisms	Number of cultures	Cultures correctly identified	Per cent correctly identified
E. coli	89	60	67.5
A. aerogenes	75	34	45.3

Atypical Coliform Organisms

Cultural Characteristics

A number of so-called atypical coliform organisms were studied both culturally and serologically in an attempt to discover whether these organisms should be considered important from a public health standpoint. Atypical coliform organisms are unlike the true coliform organisms in that they either do not ferment lactose with the production of acid and gas or they ferment it slowly (after 48 hours).

The 38 atypical organisms studied showed in general the same cultural reactions on the carbohydrates as that for the typical organisms. Gelatin was liquified by only two of the cultures and none produced H_2S.

Table 6 shows that some of the cultures react the same as E. coli or A. aerogenes to the carbohydrates tested. However, some of the strains in addition to lactose do not attack one or more of the carbohydrates which are usually fermented by the coliform organisms. The results were more irregular on sorbitol and xylose than on any of the other media tested.

After studying the reactions of these organisms on the carbohydrates it is conceivable that all of these organisms are typical coliform organisms in different stages of losing their fermentative abilities. They have not only lost their ability to ferment lactose but many of them have lost the ability to ferment also such things as sorbitol and xylose.

The reason for this gradual loss in fermenting power is evident when the source of these atypical cultures is noted. It was found that

Table 6

Cultural characteristics for atypical coliform organisms on various media*

Organisms	Lactose	Dextrose	Inositol	Inulin	Dextrin	Xylose	Mannose	Sorbitol	Raffinose	Mannitol	Dulcite	Trehalose	Galactose	Levulose	Salicin	Gelatin	H_2S
514	-	⊕	+	⊕	-	-	⊕	-	-	⊕	-	+	⊕	⊕	⊕	-	-
80	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	+	-	-
250	-	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
201	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
515	-	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
183	-	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
556	-	⊕	-	⊕	⊕	⊕	⊕	-	-	⊕	-	⊕	⊕	⊕	-	-	-
524	-	⊕	-	⊕	-	-	⊕	-	-	+	-	-	⊕	⊕	⊕	-	-
540	-	⊕	-	⊕	+	+	+	+	+	+	-	+	⊕	⊕	+	-	-
541	-	⊕	-	⊕	⊕	⊕	⊕	-	-	⊕	-	⊕	⊕	⊕	-	-	-
6	-	⊕	+	+	+	-	+	+	-	+	-	⊕	⊕	+	-	-	-
555	-	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
4	-	⊕	+	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	+	-
573	-	⊕	-	⊕	⊕	-	⊕	-	-	⊕	-	+	+	+	-	-	-
366	-	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-

Organisms	Lactose	Dextrose	Inositol	Inulin	Dextrin	Xylose	Mannose	Sorbitol	Raffinose	Mannitol	Dulcite	Trehalose	Galactose	Levulose	Salicin	Gelatin	H_2S
251	-	⊕	-	⊕	⊕	⊕	⊕	-	-	⊕	-	⊕	⊕	⊕	⊕	-	-
536	-	⊕	-	⊕	⊕	⊕	⊕	-	-	⊕	-	⊕	⊕	⊕	-	-	-
84	-	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	-
48	-	⊕	-	⊕	+	⊕	⊕	⊕	-	⊕	-	⊕	⊕	⊕	⊕	-	-
568	+	⊕	-	⊕	⊕	⊕	⊕	-	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
577	+	⊕	-	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	-
563	+	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
567	+	⊕	-	⊕	+	⊕	⊕	-	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
578	+	⊕	-	⊕	⊕	⊕	⊕	-	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
566	+	⊕	-	⊕	⊕	⊕	⊕	⊕	+	⊕	-	⊕	⊕	⊕	⊕	+	-
572	+	⊕	-	⊕	⊕	⊕	⊕	-	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
570	+	⊕	+	⊕	+	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
564	+	⊕	+	⊕	+	⊕	⊕	⊕	⊕	⊕	-	-	-	⊕	⊕	-	-
565	+	⊕	+	⊕	+	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
562	+	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
576	+	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	⊕	⊕	⊕	⊕	-	-
561	+	⊕	-	⊕	⊕	⊕	⊕	⊕	-	⊕	-	⊕	⊕	⊕	⊕	-	-

Organisms	Lactose	Dextrose	Inositol	Inulin	Dextrin	Xylose	Mannose	Sorbitol	Raffinose	Mannitol	Dulcite	Trehalose	Galactose	Levulose	Salicin	Gelatin	H_2S
273	+	+	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	-
265	-	+	-	⊕	⊕	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	-
37	-	-	-	+	+	-	+	⊕	-	⊕	-	+	-	+	+	-	-
50	-	-	-	+	+	-	⊕	-	-	-	-	-	-	+	+	-	-
72	-	-	-	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	-	-
150	-	⊕	+	⊕	⊕	⊕	⊕	⊕	-	⊕	-	⊕	⊕	⊕	⊕	-	-

* - denotes no reaction

\+ denotes acid production

⊕ denotes acid and gas production

the samples from which these atypical organisms were obtained were taken from deep wells or chlorinated water supplies. Raw polluted water supplies seldom showed atypical organisms. These atypical organisms are probably forms which have degraded after a long absence from their natural habitat. The idea of a degraded form of E. coli and A. aerogenes is not new for it was mentioned by Parr (29) in 1936. He suggested that A. aerogenes changes more than E. coli. This was confirmed by the author and is shown in tables 7 and 8. According to the citrate, methyl red and Voges Proskauer tests 57.1 per cent of the atypical cultures were found to be A. aerogenes, 2.9 per cent E. coli and 40.0 per cent "irregular".

Serological Relationship Among Coliform Organisms

In order to show more conclusively that the non-lactose fermenters are truly coliform, serological methods were used. First of all, the serological relationship between various strains of typical E. coli and A. aerogenes was determined in order to know what could be expected from the atypical cultures. It was discovered that various strains of E. coli are serologically heterogenous with a high degree of common antigen shown only in 35 per cent of the cultures. The same was found true among typical A. aerogenes cultures.

Next, agglutination reactions were run with atypical antigens and antiserum from various strains of typical E. coli and A. aerogenes. Using E. coli culture No. 1 and 42 atypical antigens, agglutination was obtained in a dilution of 1:80 or higher in only 17.6 per cent of the

Table 7

Cultural identification of atypical coliform organisms

Organisms	Koser's citrate	Methyl red	Voges-Proskauer
514	+	-	+
80	+	-	-
250	+	-	+
201	+	-	+
515	+	-	+
183	+	-	+
556	-	-	+
524	-	+	-
540	+	+	-
541	-	-	+
6	+	-	+
555	+	-	-
4	-	-	+
573	+	-	-
366	+	-	-
251	+	-	+
536	-	-	+
568	+	-	+
577	+	-	+
563	+	-	+
567	+	-	+
578	+	-	+

Organisms	Koser's citrate	Methyl red	Voges-Proskauer
566	+	-	+
572	+	-	+
570	+	-	+
564	+	-	-
565	+	-	+
562	+	-	+
576	+	-	+
561	+	-	+
273	-	+	+
265	+	-	+
37	-	-	+
50	-	-	-
72	-	-	-

Table 8

Cultural identification by percentages of atypical coliform organisms (summarized from Table 7)

Organisms	Number of cultures	Per cent
A. aerogenes	20	57.1
E. coli	1	2.9
Irregular	14	40.0

cultures. Using other typical E. coli antisera the percentage was even lower. On the other hand, A. aerogenes was found to have a much closer relationship. In table 9 it is shown that A. aerogenes culture No. 1 with as high as 19 cultures out of 42 tested (45.2 per cent) showed agglutination in 1:80 or higher. Table 10 shows that A. aerogenes culture No. 43 showed 10 out of 42 or 23.8 per cent of the cultures with close antigenic relationship.

This shows that in general the atypical coliform organisms are much more closely related to A. aerogenes than E. coli. On an average, atypical coliform organisms are agglutinated in 34.5 per cent of the time by typical A. aerogenes antiserum. This is the same relationship which was found to exist between A. aerogenes serum and typical aerogenes antigens.

A macroscopic agglutinin absorption test was run with the atypical antigens which agglutinate the A. aerogenes No. 1 antiserum. Species specific or formalin treated antigens and group specific or alcohol treated antigens were used. According to table 11 both species specific and group specific antigens were found to be present in 10 out of the 16 cultures tested.

Table 9

Agglutination tests showing the antigenic relationship between typical Aerobacter aerogenes No. 1 and atypical coliform organisms *

Antigens of atypical cultures	Dilution titre of A. aerogenes No. 1 antiserum							
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control
Control	-	-	++++	++++	++++	++++	++++	-
572	++	++	+	+	-	-	-	-
50	-	-	-	-	-	-	-	-
564	-	-	-	-	-	-	-	-
570	++++	+++	+	-	-	-	-	-
560	-	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-	-
567	++++	++++	++++	++++	++++	+++	++	-
72	-	-	-	-	-	-	-	-
563	-	-	-	-	-	-	-	-
577	++++	++++	++++	++++	++++	+++	++	-
568	++++	++++	++++	++++	++++	++++	+++	-
265	-	-	-	-	-	-	-	-
273	-	-	-	-	-	-	-	-
561	++++	++++	++++	++++	++++	++++	+++	-

Antigens	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control
565	-	-	-	-	-	-	-	-
562	++++	++++	++++	++++	++++	++++	+++	-
576	++++	++++	++++	++++	++++	++++	+++	-
578	++++	++++	++++	++++	++++	++++	+++	-
566	-	-	-	-	-	-	-	-
514	-	-	-	-	-	-	-	-
505	-	-	-	-	-	-	-	-
80	++++	++++	++++	+++	+++	+++	+++	-
83	++++	++++	+++	++++	++++	++++	++++	-
541	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
45	++++	++++	+++	+++	++	+	-	-
540	++	+	-	-	-	-	-	-
524	-	-	-	-	-	-	-	-
506	-	-	-	-	-	-	-	-
556	-	-	-	-	-	-	-	-
515	+	++	+++	+	-	-	-	-
250	++++	++++	+++	+++	+++	++	-	-
574	-	-	-	-	-	-	-	-

Antigens	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control
183	+++	+++	+++	+++	+++	++	-	-
201	+	+	-	-	-	-	-	-
555	++	++	+++	++++	++++	++++	+++	-
4	-	-	-	-	-	-	-	-
366	+++	++++	++++	++++	++++	++++	++++	-
251	+++	+++	++++	++++	+++	-	-	-
536	-	-	-	-	-	-	-	-
84	-	-	-	-	-	-	-	-
571	+++	+++	++++	++++	++++	++++	++++	-

* ++++ complete agglutination

+++, ++, + lesser degrees of agglutination

- no agglutination

Table 10

Agglutination tests showing the antigenic relationship between typical *Aerobacter aerogenes* No. 43 and atypical coliform organisms*

Antigens of atypical cultures	Dilution titre of A. aerogenes No. 43 antiserum							
	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control
Control	++++	++++	+++	+++	+++	++	-	-
564	-	-	-	-	-	-	-	-
570	-	-	-	-	-	-	-	-
572	+++	++	++	++	+	+	+	-
50	-	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-	-
567	++++	+++	++	++	++	-	-	-
72	-	-	-	-	-	-	-	-
563	+	+	-	-	-	-	-	-
577	+++	+++	++	-	-	-	-	-
568	-	-	-	-	-	-	-	-
265	-	-	-	-	-	-	-	-
273	-	-	-	-	-	-	-	-
561	-	-	-	-	-	-	-	-
565	-	-	-	-	-	-	-	-

Antigens	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control
562	+++	+++	+	-	-	-	-	-
576	++++	++++	++++	+++	+++	++	-	-
578	+++	+++	+++	+++	+	-	-	-
566	-	-	-	-	-	-	-	-
514	-	-	-	-	-	-	-	-
560	+++	+++	+++	++	-	-	-	-
505	-	-	-	-	-	-	-	-
80	-	-	-	-	-	-	-	-
83	+++	+++	+++	+++	++++	+++	+++	-
250	-	-	-	-	-	-	-	-
201	+	-	-	-	-	-	-	-
515	-	-	-	-	-	-	-	-
183	+	+	-	-	-	-	-	-
556	-	-	-	-	-	-	-	-
574	++	-	-	-	-	-	-	-
524	-	-	-	-	-	-	-	-
45	-	-	-	-	-	-	-	-
506	-	-	-	-	-	-	-	-

Antigens	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Control
540	-	-	-	-	-	-	-	-
541	-	-	-	-	-	-	-	-
6	++	+++	+++	++	+	-	-	-
555	++	++	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
366	-	-	-	-	-	-	-	-
251	++++	++++	++++	++++	++++	+++	++	-
536	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-
84	-	-	-	-	-	-	-	-
508	+	-	-	-	-	-	-	-
573	-	-	-	-	-	-	-	-

* ++++ complete agglutination

+++, ++, + lesser degrees of agglutination

- no agglutination

Table 11

Agglutination absorption reactions of atypical coliform antigens and typical A. aerogenes antiserum *

Antigens of atypical cultures	Dilution titre of A. aerogenes No. 1 antiserum															
	1/20		1/40		1/80		1/160		1/320		1/640		1/1280		Control	
	O	H	O	H	O	H	O	H	O	H	O	H	O	H	O	H
572	-	+	-	+	-	+	-	++++	-	+++	-	+++	-	+++	-	-
570	+++	++++	+++	++++	++++	++++	++++	++++	+++	++++	-	++++	-	++++	-	-
251	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
366	-	-	-	+	-	+	-	++	-	-	-	-	-	-	-	-
183	-	-	-	-	-	-	-	++	-	++	-	++	+++	++	-	-
515	-	-	-	-	-	-	++	++	+++	++	++++	++	++++	++	-	-
83	-	+	-	+	-	+	-	++	-	-	-	-	-	-	-	-
80	-	++	-	++	-	++	-	++	-	++	-	+	-	-	-	-
568	++	++++	++	++++	+++	++++	+++	++++	+++	++++	+++	++++	+++	++++	-	-
577	+	++++	+	++++	++	++++	++	++++	++	++++	+++	++++	+++	++++	-	-
567	+	+++	+	+++	-	++++	-	++++	-	++++	-	++++	-	++++	-	-
562	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	-	-
576	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++	+++	+++	-	-	-

Antigens of atypical cultures	Dilution titre of A. aerogenes No. 1 antiserum															
	1/20		1/40		1/80		1/160		1/320		1/640		1/1280		Control	
	O	H	O	H	O	H	O	H	O	H	O	H	O	H	O	H
561	+	+	-	+	-	+	-	++	-	++	-	-	-	-	-	-
578	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
250	+++	++	+++	+++	-	++	-	+	-	+	-	-	-	-	-	-

* ++++ complete agglutination

+++, ++, + lesser degrees of agglutination

- no agglutination

CONCLUSION

A total of 254 typical coliform cultures were classified according to the citrate, methyl red and Voges Proskauer tests. As high as 28.8 per cent were found to be irregular. This almost exactly confirms the work of Parr (29). The "irregular" organisms most commonly found are of the citrate positive, methyl red positive, and Voges Proskauer negative group.

The accuracy of eosin methylene blue agar for the differentiation of E. coli from A. aerogenes by laboratory technicians was determined. It was found that E. M. B. agar is not satisfactory for the differentiation of these organisms in routine laboratory analyses.

Most of the atypical coliform fultures were found to be identical culturally with the typical organisms except for their fermentation of lactose. Some of the strains have also lost the ability to ferment one or more of the other carbohydrates. It was concluded that these so-called atypical coliform organisms are merely a degraded form of the typical organisms and, therefore, have just as much sanitary significance.

According to the citrate, methyl red and Voges Proskauer tests, it was found that these atypical organisms largely react as A. aerogenes. That atypical coliform organisms are mainly A. aerogenes was also shown by using serological methods. In addition, agglutinin absorption tests showed both group specific and species specific antigens present for A. aerogenes.

SUMMARY

1. 29 per cent of the coliform organisms in water supplies are "irregular" types.
2. The citrate positive, methyl red positive and Voges Proskauer negative organisms are the most common group found.
3. Eosin methylene blue agar cannot be accurately used by laboratory technicians as a means of differentiating E. coli from A. aerogenes.
4. Atypical coliform cultures are probably degraded coliform organisms.
5. Serologically, atypical cultures are frequently shown to be A. aerogenes and seldom E. coli.

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