A SURVEY OF THE BACTERIOLOGICAL CONDITION OF THE WATER SUPPLIES OF THE RURAL SCHOOLS OF RILEY COUNTY

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1943

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1949

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INTRODUCTION

The bacteriological condition of water used for human consumption has been the object of extensive research ever since John Snow's classical studies of the dissemination of Asiatic Cholera through drinking water. This topic is of especial interest from a public health standpoint, because polluted water may become a potential health hazard, particularly in the transmission of enteric diseases. Since, in general, a rural water supply serves only a limited number of individuals, little attention has been paid to such waters as a public health problem. However, rural school supplies are in a somewhat different category, and since they have been known to serve as foci of serious epidemics they warrant more consideration as a public health problem than they have received. This survey of the bacteriological condition of the water supplies of the rural schools of Riley County, Kansas, was carried out in an effort to determine the quality of water supplied to children of school age in this limited area.

REVIEW OF LITERATURE

Introduction

The utilization of the test for the coli-aerogenes group of bacteria as a criterion of the sanitary quality of water is so widely employed, and the mechanics of the test so thoroughly standardized as to need no comment. The actual procedures to be followed are incorporated in "Standard Methods for the Examination of Water and Sewage", prepared jointly by the American Public Health Association and the American Water Works Association, first issued in 1917, and last brought up to date in 1946.

It was recognized as early as 1885 that two distinct types of coli-aerogenes bacteria existed. These two types were described by Escherich (1) and named B. coli (Escherichia coli) and B. lactis aerogenes (Aerobacter aerogenes). Escherich and his contemporaries believed the presence of these organisms in water to be a true indication of fecal contamination. In due time, however, it was found by other workers in the same field, that organisms possessing the same basic characteristics of these two types were widely distributed in nature. With this discovery, the significance of the test was questioned. To gain much needed information two major lines of research were initiated and have continued unabated ever since. These involved, first, devising ways and means of differentiating between the various members of the coli-aerogenes group, and second, evaluating the relative sanitary significance of the different forms as indicators of pollution.

Development of Methods Employed for Differentiation

Indole. Blumenthal (2) in 1895 showed that *Escherichia coli* was capable of producing indole from a peptone medium, and many authorities since that time have regarded the production of indole as indicative of the presence of *Escherichia coli*. Chen and Rettger (3) did not place much emphasis on this test, and reported in 1920, "the indole test is of little value and should be used with caution. While all strains of *B. coli* (*Escherichia coli*) produce indole, the percentage of positive tests with "aerogenes" type is too large to make the test a practical one." Despite this fact, the indole test has come to occupy an important place in the differentiation of the various coli-aerogenes types.

Voges-Proskauer and Methyl Red Reactions. Voges and Proskauer (4) in 1898 first described a characteristic reaction occurring in a glucose broth culture containing coli-aerogenes bacteria. They noted production of an eosin-red color when caustic potash was added to the culture and allowed to stand for 24 hours. This reaction was studied by several individuals, among them Harden and Walpole (5), who in 1906 showed this color reaction to be due in part to an end product of bacterial metabolism, acetyl methyl carbinol. Bacteria of the "aerogenes" type produced acetyl methyl carbinol from glucose broth while bacteria of the "coli" type were incapable of producing this compound. Clark and Lubs (6) in 1916 pointed out that pH changes in glucose broth could be determined easily by use of methyl red as an indicator. They, and other workers, confirmed this and in 1918 found that a rather consistent relationship existed between the methyl red and Voges-Proskauer tests. The true "coli" types were consistently methyl red positive (produced large amounts of acid), but gave negative results with the Voges-Proskauer test, while the typical "aerogenes" types were methyl red negative and Voges-Proskauer positive.

Utilization of Organic Salts. Unfortunately, it soon became evident that the aforementioned tests were not conclusive evidence of fecal or non-fecal type organisms. When this became apparent, emphasis was placed on the utilization of salts of organic acids by these bacteria. Koser (7) found that Escherichia coli was unable to use the nitrogen from uric acid while Aerobacter aerogenes grew in uric acid and other simple salts. Chen and Rettger (3) just two years later, 1920, found an almost perfect correlation between methyl red, Voges-Proskauer, and uric acid tests. Brown (8) grew Aerobacter aerogenes on sodium citrate, but could not produce growth with Escherichia coli in the same medium. Koser (9) later confirmed Brown's findings and in 1924 reported a close correlation of citrate, methyl red, and Voges-Proskauer reactions with fecal cultures.

Importance of the Above Reactions in Determining Source of Bacteria

MacConkey (10) was one of many research workers who realized that the Voges-Proskauer reaction was rarely demonstrated in the case of coli-aerogenes from feces. Rogers, Clark and Evans (11) upon examination of 166 cultures from 33 samples of dried grains from various states, found that none of the types on grains agreed with the characteristic flora of bovine feces. They based their conclusions upon results obtained from indole fermentation and comparison of hydrogen and carbon dioxide gas ratios in glucose broth. Levine (12) was first to notice any connection between the Voges-Proskauer reaction and source of the bacteria. In 1916 he reported, "the natural habitat of coli-like bacteria which form acetyl-methyl-carbinol (V-P positive) from glucose and other carbohydrates is probably the soil. They are also rare in feces." Levine in 1918 (13) reported that he was able to isolate *Aerobacter aerogenes* and *Aerobacter cloacae* from soil and sewage but not from any animal feces. *Escherichia coli* was isolated from soil, sewage and animal feces, but a rather distinct correlation with the source was observed as indicated by the following figures--1.1 percent in soil, 2.6 percent in sewage, and 20 percent in human feces. Greenfield (14) made an extensive study in 1916 of water sources in Kansas. Her results seemed to point to the fact that *Escherichia coli* type bacteria predominated in the sources studied. She also found perfect correlation between the methyl red and Voges-Proskauer reactions. Stovall and

Nichols (15), in the routine analysis of 68 water samples from private and public supplies, isolated 200 cultures which gave positive completed tests. Of these, 120 were methyl red positive, 38 Voges-Proskauer positive, and 42 gave irregular reactions--supposedly corresponding to those cultures from the soil which are methyl red positive and citrate positive. These are classed as intermediates. Rogers, Clark and Lubs (16) in 1918 conducted a study to establish the characteristics of "coli" type cultures from the human intestine. Of 177 cultures, 131 gave reactions characteristic of Escherichia coli, and 46 cultures were definitely Aerobacter aerogenes. Emphasis was placed upon the fact that by far the great majority of coli-aerogenes type organisms found in feces proved to be Escherichia coli.

Winslow and Cohen (17) attempted to summarize results obtained by earlier researchers concerning correlation between source and biological reactions of coli-aerogenes type bacteria. Their summary, published in 1918, indicated that the percentage of Voges-Proskauer positive and methyl red negative organisms in feces is not constant, and as a rule is small. The evidence also pointed to the fact that on grains and cereals, Aerobacter aerogenes is the predominating type. Stokes (18), in a paper published in 1919, states "aerogenes" (Aerobacter aerogenes) come probably from grains or cereals, and "coli" (Escherichia coli) from human contamination." This statement was made with reference to coli-aerogenes in water. Young and Greenfield (19) in 1923 concluded that the difference between soil and fecal strains was of little practical significance to the sanitarian.

Following the work done by these two individuals, other authors, including Koser (20), Lewis (21), Clark (22), Bahlman and Sohm (23), Bardsley (24), and Lewis and Pittman (25) found the existence of intermediate type bacteria and these studies indicated that differentiation in routine work was not simple and that results were seldom clear cut enough to make interpretation easy. In 1931, however, despite the fact that earlier authors were skeptical about interpretation, Ruchhoft, Kallas, Chinn and Coulter (26) attempted to correlate biological reactions with source of organisms and their method of interpretation is used today by many in the differentiation of coli-aerogenes type bacteria found in water. A modified table based on their original interpretation may be found in "Standard Methods for the Examination of Water and Sewage" (27). The reaction combinations obtained with the four tests used by these authors are very constant and serve as a superior criterion of differentiation. O'd Burke-Gaffnew (28), in attempting to classify coli-aerogenes type bacteria, found Aerobacter aerogenes rare in feces and common outside the intestine. Escherichia coli proved overwhelmingly fecal, while the "aerogenes" type predominated in urine and soil and was present in water. Intermediate types were also found in soil and water. Practically no fecal strains were intermediates. They believed that the methyl red positive organisms of soil had not the significance of the methyl red positive organisms of feces and represented at the most, remote pollution. Gray (29), reporting on the significance of Aerobacter aerogenes in water, states "B. aerogenes (Aerobacter aerogenes) is practically universal, although in small numbers in the stools of normal healthy adult humans. Preponderence of aerogenes over coli in a water supply may, for practical purposes, be regarded as an indication of freedom on the part of the water from pathogenic organisms."

It is apparent from the above review that there is a definite correlation between source and types of coli-aerogenes bacteria. It is also evident that various biological reactions may be used as a criterion for differentiation of various types of coli-aerogenes found in nature. In this paper an attempt will be made to differentiate the coli-aerogenes types isolated from the water supplies of the rural schools of Riley County, Kansas.

EXPERIMENTAL PROCEDURES

Water samples were collected from 33 different sources, collections being made on an average of four times for each source, twice in the fall and twice during the spring season. Bacteriological examinations, therefore, were performed on a total of 138 standard samples. All tests were performed in accordance with specifications listed in "Standard Methods for the Examination of Water and Sewage", Ninth Edition, 1946.

Collection of Samples

Samples of water were collected directly from the well when possible, and if not possible, from faucets, drinking fountains, buckets, or other storage receptacles. Collections were made as aseptically as conditions would permit, sterilization being accomplished by first swabbing the water outlet with Clorox solution and then flaming this dry with an alcohol burner. All samples were kept refrigerated in an insulated iced box where the temperature maintained was approximately 10°C. Samples were carried from the source to the laboratory in this container and kept there until inoculation into cultural media. In no instance did the time of holding at 10°C. exceed six hours.

Slight deviation in this routine was introduced after the project was underway. It was suggested that it might be of interest to determine what effect temporary refrigeration might have on the viability of coli-aerogenes organisms. To determine this, 50 ml samples were inoculated directly into 50 ml double strength lactose broth at the site of collection. These samples were never refrigerated. In view of the fact that in actual practice water samples from rural sources usually do not reach the testing laboratory within less than 24 hours, it was thought worth while to determine whether holding samples for 24 hours at room temperature would have any effect upon viability of coli-aerogenes bacteria therein. With this thought in mind, samples which gave positive presumptive tests were retested for coli-aerogenes after standing at room temperature for 24 hours.

Methods of Cultivation

Briefly, the methods employed were as follows: one 0.1 ml, one 1.0 ml, and five 10 ml portions of sample were inoculated into Durham fermentation tubes containing lactose broth made according to specifications in "Standard Methods." Those samples which produced gas within 24 hours were considered as positive presumptives. Those producing gas during the second 24 hours only were termed doubtful presumptives, and those producing no gas at the end of 48 hours were considered to be negative for coli-aerogenes bacteria. All positive and doubtful presumptive reactions were confirmed on Endo's medium. Colonies which gave reactions typical of coli-aerogenes were fished from all Endo plates and transferred to nutrient agar slants and lactose broth for the completed test. Only those cultures which were gram negative, non-spore forming rods and produced gas from lactose in 48 hours were regarded as coli-aerogenes and were saved for differentiation. All other cultures were discarded.

The above tests were performed to determine whether or not true coli-aerogenes types were present. Various differential tests were then performed on all coli-aerogenes isolations to determine exactly which types were present. A comparison of the importance of the various differential tests has been given in the review of literature. The following differential tests were employed because they are recommended by "Standard Methods", and also because a review of the literature points to the fact that these are the most valuable tests for differential purposes: methyl red test, Voges-Proskauer reactions, growth in citrate medium, indole production, and gelatin liquefaction. By recording and correlating the results obtained with these tests, it is possible, generally, to distinguish between the so-called fecal and non-fecal types of bacteria.

Poured plates of 1.0 ml and 0.1 ml portions of water were prepared to determine plate counts, using nutrient agar as a medium. A single 10 ml portion was planted in sterile skimmed milk, sealed under a vaseline-paraffin mixture and heated at 80°C. for 20 minutes to test for the presence of spore-bearing anaerobes. All incubating was done at 37°C. for the length of time designated by "Standard Methods" for each of these individual tests.

RESULTS

A detailed record of results obtained from the above examinations will be found in Tables 1 and 2.

Table 1. Results from completed tests, plate counts, and "stormy fermentation" tests. Most probable number (MPN) calculated from positive completed tests.

School source	Date	Completed tests: 50 ml.	Completed tests: 5-10 ml.	Completed tests: 1-1.0 ml.	Completed tests: 1-0.1 ml.	MPN	Plate counts	Stormy fermentation
Hunter's Island	10- 5-48		3	1	0	12	2600	-
	10-28-48		5	1	1	240	2100	+
	3-31-49	+	2	0	0	5	1960	-
Ashland Bottoms	10- 5-48		0	0	0	0	20	-
	10-28-48		0	0	0	0	12	+
	3-31-49		0	0	0	0	100	-
	4-12-49		0	0	0	0	5	+
Ashland Bottoms (Container in school room)	4-12-49		0	0	0	0	19	-
McDowell Creek	10- 5-48	0	0	0	0	0	16	-
	10-28-48	0	0	0	0	0	100	-
Ogden	10- 5-48	0	0	0	0	0	0	-
	4-12-49	0	0	0	0	0	0	-
College Hill	10- 5-48		2	1	0	7.6	8	-
	12- 7-48	0	0	0	0	0	0	-
	3-31-49	0	0	0	0	0	2920	-
	4-12-49	0	0	0	0	0	0	-
Strong	10- 5-48		0	0	0	0	13	-
	12- 7-48	+	0	0	0		2	-
	3-31-49	0	0	0	0	0	24	-
	4-12-49	0	0	0	0	0	6	-
Keats Grade	10-11-48	0	0	0	0	0	2	-
	11-17-48	+	2	0	0	5.0	140	-
	3-19-49	+	0	0	0		100	-
	4-17-49		2	0	0	5.0	30	-

Table 1 (cont.).

School source	Date	Completed tests: 50 ml.	5-10 ml.	1-1.0 ml.	1-0.1 ml.	MPN	Plate counts	Stormy fermentation
Keats High	11-17-48		3.0	1.0	0.0	5.0	30.0	-
	4-19-49	0.0	0.0	0.0	0.0	0.0	0.0	+
	4- 7-49	0.0	0.0	0.0	0.0	0.0	2.0	+
Magic	10-11-48		3.0	0.0	0.0	8.8	54.0	-
	11-17-48	0.0	2.0	0.0	0.0	5.0	6.0	-
	4-19-49	0.0	0.0	0.0	0.0	0.0	14.0	-
	4- 7-49	0.0	0.0	0.0	0.0	0.0	1.0	-
Myersdale	10-11-48	0.0	0.0	0.0	0.0	0.0	6.0	+
	11-17-48	0.0	0.0	0.0	0.0	0.0	3.0	-
	4-19-49	0.0	0.0	0.0	0.0	0.0	0.0	+
	4- 7-49	0.0	0.0	0.0	0.0	0.0	2.0	-
Bala	10-11-48	0.0	0.0	0.0	0.0	0.0	2.0	+
	11-17-48		1.0	0.0	0.0	2.2	17.0	+
	3-19-49	0.0	0.0	0.0	0.0	0.0	23.0	-
	4-17-49		2.0	0.0	0.0	7.5	0.0	+
Laurel Hill	10-11-48		1.0	0.0	0.0	2.2	16.0	-
	11-17-48		2.0	1.0	0.0	7.6	4200.0	-
	4- 7-49		2.0	0.0	0.0	7.5	6.0	+
Pleasant Hill (container in school room)	10-11-48	0.0	0.0	0.0	0.0	0.0	31.0	-
	11-17-48	+	0.0	0.0	0.0		4300.0	-
	4- 7-49	+	5.0	0.0	0.0	38.0	790.0	+
	4-19-49		1.0	1.0	0.0	4.0	TNTC	-
Farm well used as source for Pleasant Hill	10-11-48	0.0	0.0	0.0	0.0	0.0	3.0	-
	11-17-48	0.0	0.0	0.0	0.0	0.0	0.0	+
	4- 7-49	0.0	0.0	0.0	0.0	0.0	0.0	-
	4-19-49	0.0	0.0	0.0	0.0	0.0	4.0	-

Table 1 (cont.).

School source	Date	Completed tests				MPN	Plate count	Stormy fermentation
		50 ml.	5-10 ml.	1-1.0 ml.	1-0.1 ml.			
Alert	10-11-48		0.0	0.0	0.0	0.0	15.0	-
	11-17-48		0.0	0.0	0.0	0.0	27.0	-
	4- 7-49		0.0	0.0	0.0	0.0	8.0	≠
	4-19-49		0.0	0.0	0.0	0.0	80.0	≠
Alert (container in school room)	4-19-49		0.0	0.0	0.0	0.0	170.0	≠
Leonardville Grade and High	10-11-48	0.0	0.0	0.0	0.0	0.0	18.0	-
	11-17-48	0.0	0.0	0.0	0.0	0.0	2.0	-
	4- 7-49	0.0	0.0	0.0	0.0	0.0	0.0	-
	4-19-49	0.0	0.0	0.0	0.0	0.0	0.0	-
Riley Grade and High	10-11-48	0.0	0.0	0.0	0.0	0.0	0.0	-
	11-17-48	0.0	0.0	0.0	0.0	0.0	0.0	-
	4- 7-49	0.0	0.0	0.0	0.0	0.0	0.0	≠
	4-19-49	0.0	0.0	0.0	0.0	0.0	0.0	
Grandview	10-11-48		1.0	0.0	0.0	2.2	14.0	≠
	11-17-48		0.0	0.0	0.0	0.0	1520.0	≠
	4- 7-49	0.0	0.0	0.0	0.0	0.0	7.0	-
	4-19-49	0.0	0.0	0.0	0.0	0.0	1.0	-
Grandview (container in school room)	4-19-49	0.0	0.0	0.0	0.0	0.0	41.0	≠
Stockdale	10-11-48		0.0	0.0	0.0	0.0	0.0	≠
	11-28-48		3.0	1.0	0.0	12.0	60.0	≠
	11-17-48		1.0	0.0	0.0	2.0	60.0	≠
	4-18-49		1.0	0.0	0.1	2.2	96.0	

Table 1 (cont.).

School source	Date	Completed tests: 50 ml.	5-10 ml.	1-1.0 ml.	1-0.1 ml.	MPN	Plate counts	Stormy fermentation
Winkler	11-21-48	+	4.0	0.0	0.0	15.0	100	+
	11-24-48		1.0	0.0	0.0	2.2	0.0	+
	4-18-49		3.0	1.0	1.0	16.0	6.0	+
	4-21-49		3.0	1.0	0.0	1.0		-
Winkler (container in school room)	4-14-49	0	0.0	0.0	0.0	0.0	17.0	+
May Day	10-21-48		5.0	1.0	0.0	240.0	900.0	+
	11-24-48		1.0	1.0	0.0	4.4	380	+
	4-21-49		0.0	0.0	0.0	0.0	TNTC	+
Farm well used as source for May Day	11-24-48		0.0	0.0	0.0	0.0	0.0	+
	4-18-49		1.0	1.0	1.0	6.7	560.0	+
	4-21-49		0.0	0.0	0.0	0.0	4.0	-
Farm spring used as source for May Day	4-14-49	+	0.0	0.0	0.0	0.0	3.0	-
Star	10-21-48		2.0	0.0	0.0	5.0	5.0	-
	11-24-48		1.0	0.0	0.0	2.2	3.0	+
	4-21-49		0.0	0.0	0.0	0.0	0.0	+
	4-22-49	0.0	0.0	0.0	0.0	0.0	20.0	-
Star (container in school room)	4-22-49		0.0	0.0	0.0	0.0	20.0	-
Ober	10-21-48		0.0	0.0	0.0	0.0	1900	-
	11-24-48		2.0	0.0	0.0	5.0	70	+
	4-18-49		2.0	0.0	0.0	5.0	1880	+
	4-21-49		1.0	1.0	0.0	4.4	TNTC	+

Table 1 (cont.).

School source	Date	Completed tests: 50 ml.	5-10 ml.	1-1.0 ml.	1-0.1 ml.	MPN	Plate counts	Stormy fermentation
Farm well used as source for Ober	4-18-49		4.0	1.0	0.0	21.0	44	+
	4-21-49	+	0.0	0.0	0.0	0.0	62	-
Peach Grove	10-21-48		0.0	0.0	0.0	0.0	5.0	+
	11-24-48		0.0	0.0	0.0	0.0	10.0	+
	4-18-49		0.0	0.0	0.0	0.0	13.0	+
	4-21-49		0.0	0.0	0.0	0.0	86.0	+
Swede Creek	10-21-48		0.0	0.0	0.0	0.0	0.0	-
	11-24-48		0.0	0.0	0.0	0.0	3.0	-
	4-18-49		1.0	0.0	0.0	2.2	5.0	-
Cleburne Grade	10-21-48		2.0	0.0	0.0	5.0	30.0	+
	11-24-48		0.0	0.0	0.0	0.0	14.0	+
	4-18-49		0.0	0.0	0.0	0.0	6.0	+
Cleburne (container inside school room)	4-18-49		1.0	0.0	0.0	2.2	150.0	-
Cleburne High	10-21-48		2.0	1.0	1.0	10.0	2.0	+
	11-24-48		3.0	0.0	0.0	8.8	6.0	+
	4-18-49		0.0	0.0	1.0	2.0	38.0	-
Rose Hill	10-21-48		0.0	0.0	0.0	0.0	260.0	+
	11-24-48		1.0	0.0	0.0	2.2	3.0	+
	4-19-49		2.0	0.0	0.0	5.0	2840.0	+
Farm well used as source for Rose Hill	4 -19-49		1.0	0.0	0.0	2.2	180.0	+

Table 1 (concl.).

School source	Date	Completed tests: 50 ml.	5-10 ml.	1-1.0 ml.	1-0.1 ml.	MPN	Plate counts	Stormy fermentation
Columbus	10-21-48		0.0	0.0	0.0	0.0	1.0	+
	11-24-48		0.0	0.0	0.0	0.0	0.0	+
	4-18-49		0.0	0.0	0.0	0.0	19.0	+
	4-21-49		0.0	0.0	0.0	0.0	3.0	-
Randolph Grade and High	11-21-48		0.0	0.0	0.0	0.0	0.0	+
	11-24-48	+	1.0	0.0	0.0	2.2	120.0	-
	4-18-49		0.0	0.0	0.0	0.0	200.0	-
	4-21-49	-	0.0	0.0	0.0	0.0	3.0	-
Tabor Valley	10- 5-48		2.0	1.0	0.0	7.6	2680	+
	10-28-48		1.0	0.0	0.0	2.2	1500	+
	3-31-49	-	0.0	0.0	0.0	0.0	740	+
	4-12-49	-	1.0	0.0	0.0	2.2	190	+
Oak Grove	10- 5-48		1.0	0.0	0.0	2.2	1020	+
	10-28-48		0.0	0.0	1.0	2.0	500	+
	3-31-49		0.0	0.0	0.0	0.0	444	-
	4-12-49		0.0	0.0	0.0	0.0	0.0	-
Zeandale	10-28-48		1.0	0.0	0.0	2.2	50	-
	12- 7-48	+	3.0	0.0	0.0	8.8	22	+
	3-31-49	-	3.0	0.0	0.0	8.8	680	+
	4-12-49	-	0.0	0.0	0.0	0.0	0.0	+
Sherman	11-17-48		0.0	0.0	0.0	0.0	3.0	-
	12- 7-48		0.0	0.0	0.0	0.0	0.0	-
	4-19-49		0.0	0.0	0.0	0.0	49.0	+
	4- 7-49		0.0	0.0	0.0	0.0	1.0	+
Sherman (container in school room)	4-19-49		1.0	0.0	0.0	2.2	8.0	-

Table 2. Differentiation of coli-aerogenes isolated from water samples.

School source	Portions tested	Portions + for G.G.	Cultures isolated	Differentiation					
				E. coli	A. aerogenes	A. cloacae	Intermediates I	Intermediates II	Unclassified
Hunter's Island	15	9	14	2	7		1	2	2
College Hill	20	3	3			2	1		
Keats Grade	20	4	5			1	4		
Keats High	15	3	4		3				1
Magic	20	4	4				2		2
Bala	20	3	3				3		
Laurel Hill	15	5	6	1	1	1	1	1	1
Pleasant Hill	40	6	9		8		1		
Grandview	20	1	1			1			
Stockdale	20	5	7				4		3
Winkler	20	11	18	1	1		14		2
May Day	31	8	13	2	9		1		1
Star	20	3	4				4		
Ober	30	9	13	10	1			2	
Cleburne Grade	20	1	1				1		
Cleburne High	15	5	10		2		7		1

Table 2 (concl.).

School source	Portions tested	Portions + for g.s.	Cultures isolated	Differentiation					
				E. coli	A. aerogenes	A. cloacae	Intermediates I	Intermediates II	Unclassified
Rose Hill	20	3	3	2			1		
Randolph	20	1	2				2		
Tabor Valley	20	4	5				5		
Oak Grove	20	1	2	2					
Zeandale	20	6	8				7	1	
Strong	20	1	1				1		
Sherman (container in school room)	5	1	1				1		

DISCUSSION OF RESULTS

Waters used for drinking purposes were examined from 33 individual schools or school systems in Riley County. Four of these were from towns having public water supplies and were found to be satisfactory in every respect. These cannot be considered as rural waters; therefore, 29 rural school waters were examined.

It is a practice in Riley County for the teachers to bring water from their homes or some other source for drinking purposes when the local school well water is found to be unsatisfactory. During the past year there were five schools in which this was done. In such cases samples were taken from the source when practicable. In the case of one school, Laurel Hill, no samples were obtained from the source.

In some schools water used for drinking purposes is carried from the well and placed in a container in the school room. Samples were taken from these containers as well as from the well in some instances. In the following overall analysis all samples from a given school will be treated as a single source.

A detailed record of the results obtained from these analyses will be found in Table 1. Examination of these data will reveal coli-aerogenes types were isolated from one or more samples from 22 school supplies, that is 75 percent of the sources contained coli-aerogenes type bacteria. When applying percentages to samples rather than sources, it was found that 36 percent of all the samples examined contained coli-aerogenes bacteria, that is, 36 percent positive completed tests were obtained. The MPN (most probable numbers of coli-aerogenes per 100 ml of water) for these positive samples ranged from 2.2 to 240.

Certain standards have been adopted by the U. S. Treasury Department (30) in connection with purity of drinking water used on interstate carriers which may well be used in the interpretation of results obtained from the completed tests. These standards are as follows:

> (1) Of all the standard (10 ml.) portions examined in accordance with the procedure specified below, not more than 10 percent shall show the presence of organisms of the B. coli (coli-aerogenes group). (2) Occasionally three or more of the five equal (10 ml.) portions, constituting a single standard sample may show the presence of B. coli. This shall not be allowable if it occurs in more than (a) five percent of the standard samples when 20 or more samples have been examined; (b) one standard sample when less than 20 samples have been examined.

Application of these standards to the data in Table 1 would classify 59 percent of all the school supplies as unsatisfactory for human use. There are several instances in which extenuating circumstances might well alter such rigid interpretations. In the case of Oak Grove Rural School according to the Treasury Department Standards the water was satisfactory; however, plate counts ranging from 444/ml to 1020/ml in water from the well certainly indicate an unsatisfactory condition. On the other hand in two cases, Pleasant Hill and May Day, the water coming directly from the well was entirely satisfactory, but when samples were taken from the container in the school room the water was, on occasions, found to contain coli-aerogenes and the number of colonies developing from the 0.1 ml poured plate were too numerous to count--in excess of 5000. Such situations could be easily remedied through proper cleansing and care of the storage receptacle.

Little correlation was found between plate counts and presence of coli-aerogenes bacteria. It is worthy of mention, however, that 23 percent of all the samples tested contained more than 100 bacteria per ml. At one time, state health authorities of Kansas considered such water undesirable for human consumption.

Results obtained from the "stormy fermentation" test revealed that 36 percent of the samples or 75 percent of the sources having no coli-aerogenes contained spore bearing anaerobes. In contrast to this, 67 percent of the samples or 86 percent of the sources positive for coli-aerogenes also showed indication of spore bearing anaerobes. This indicates a definite correlation between the presence of spore bearing anaerobes and coli-aerogenes bacteria in water.

There has been a tendency in America in recent years to classify the types of coli-aerogenes isolated from waters with the aid of differential media and tests, and to attach more significance to the presence of Escherichia coli than to other types as an indicator of dangerous pollution.

A summary of results obtained from the differential classification of the coli-aerogenes group (see Table 2, page 18) provided the following information: of 141 cultures isolated, 14 percent proved to be Escherichia coli, 24 percent Aerobacter aerogenes, 3.5 percent Aerobacter cloacae, 44 percent Intermediate type I, 4 percent Intermediate type II, and 10 percent unclassified.

The British system of differentiation of the coli-aerogenes group was used for this interpretation, an outline of which is given in Table 3.

It is evident from these data that Intermediate type I coli-aerogenes is responsible for the majority of positive completed tests obtained from these waters. This type is supposedly found normally inhabiting the soil. Aerobacter aerogenes and Escherichia coli are next in number, the former being considered a non-fecal type and the latter as normally coming from human or animal intestinal tracts.

If Escherichia coli alone is employed as a criterion of pollution, then only 28 percent or 8 of the 29 rural school supplies would be classed as unsatisfactory.

The data obtained in the study of the effect of a delay in the inoculation of lactose broth upon the MPN of coli-aerogenes detected in water samples are recorded in Table 4. There were 25 samples initially giving a positive presumptive test which were held at room temperature for 24 hours and upon which a second presumptive test was carried out. Of these 6, or 24 percent, failed to give a positive presumptive test; 17, or 68 percent, showed decreases in MPN ranging from 56 percent to 99 percent; while only 2, or 8 percent, failed to show any decrease in total number.

When inoculations were carried out at the well and compared with inoculations upon reaching the laboratory, it was found, that

Table 3. Differentiation of the coliform (coli-aerogenes) group.
(Direct copy of table 21, p.238, Standard Methods.)

Bacterium	MR	VP	Growth in citrate	Indole	Gas in MacConkey at 44°C	Gelatin liquefaction	Probable Habitat
Bact. coli, type I, faecal	+	-	-	+	+	-	Human and animal intestine
Bact. coli, type II	+	-	-	-	-	-	Doubtful, probably not intestinal
Intermediate, type I	+	-	+	-	-	-	Mainly soil
Intermediate, type II	+	-	+	+	-	-	Mainly soil
Bact. aerogenes, type I	-	+	+	-	-	-	Mainly vegetation
Bact. aerogenes, type II	-	+	+	+	-	-	Mainly vegetation
Bact. cloacae	-	+	+	-	-	+	Mainly vegetation
Irregular, type I	+	-	-	+	-	-	Human and Animal intestine
Irregular, type II	+	-	-	-	+	-	Doubtful
Irregular, other types	Reactions variable						Doubtful

Table 4. The effect of delay in inoculation of lactose broth upon MPN of coli-aerogenes bacteria, based on positive presumptive tests.

Source	MPN/100ml. after standing 2-6 hrs. at 10°C.	MPN/100ml. after standing at room temperature for 24 hrs.
Hunter's Island	240 38	5.0 8.8
McDowell Creek	2.2	0.0
Keats Grade	5.0	0.0
Bala	38.0	0.0
Laurel Hill	38.0 15.0	8.8 8.8
Pleasant Hill	38.0	8.8
Stockdale	240.0 240.0 240.0	5.0 0.0 2.2
Winkler	240.0	0.0
May Day	240.0 240.0 240.0	240.0 15.0 240.0
Star	8.8	2.2
Ober	5.0 240.0 240.0	2.2 15.0 0.0
Cleburne High	240.0 240.0	5.0 2.2
Rose Hill	240.0 5.0	5.0 2.2
Tabor Valley	5.0	2.2
Oak Grove	21.0	5.0

of the 66 samples giving a positive presumptive test for coli-aerogenes when inoculated at the well, 9 samples, or 14 percent, failed to show any evidence of lactose fermenters when inoculations were made after reaching the laboratory or after being held at a temperature of 10°C. for 2 to 6 hours. Three of these samples gave positive completed tests.

The results from these delayed inoculation studies indicate that many samples of water in which testing is delayed for 24 hours, or even shorter lengths of time, may be reported as free of coli-aerogenes when actually the organisms were present in appreciable numbers in the sample when collected.

SUMMARY

Based upon the Treasury Department Standards for interpretation of the bacteriologic findings in waters used for domestic purposes, 59 percent of the rural school waters of Riley County were found unsatisfactory. If the somewhat more liberal criterion of the presence or absence of _Escherichia coli_ were applied, then only 28 percent of the rural school supplies were found non-potable.

ACKNOWLEDGMENT

The author wishes to express her appreciation to Dr. P. L. Gainey, Head of the Department of Bacteriology, for his valuable advice and encouragement throughout the course of this problem, for his assistance in collection of water samples from the rural schools, and for the thorough checking and correcting of this paper.

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