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BACTERIA WE DRINK

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Water is considered unsafe to drink without boiling when it contains bacteria that live naturally in the intestines of man or other warm-blooded animals, such as chickens, horses, cows, pigs, etc. These various intestinal bacteria are commonly referred to, collectively, as the coliform group. Fortunately, most of the coliform bacteria are not disease-producing bacteria. However, the fact that they are present in water indicates that an avenue of contamination is open for disease-causing bacteria to get into the water supply in the same manner that the coliform group gained entrance.

The coliform group of bacteria is considered more technically to include all aerobic and facultative anaerobic gram-negative, non-spore-forming bacilli which ferment lactose with gas formation. The genera most often encountered are *Escherichia* and *Aerobacter,* and they have become known as the indicators of pollution. Water is considered unsafe to drink without boiling or treating chemically when either or both are found.

The first step in a standard bacteriological water analysis as prescribed by the American Public Health Association is the inoculation of lactose broth fermentation tubes and is referred to as the "presumptive test" (Norton, 1946). If gas is formed in lactose broth, it is presumed that the coliform group is present, and the result is called a "positive presumptive". However, the "confirmed test", which requires the inoculation of a more selective medium than lactose, is necessary to prove that the coliform group is definitely present. Thus, it is not unusual to find water in which there are bacteria that ferment lactose to produce gas and then fail to produce gas in a confirmatory medium. Such a result is called a "false positive presumptive". Very little attention has been given to the organisms responsible for such results; therefore, it is with the occurrence and significance of the false positive presumptives in Charleston drinking water that the writers are primarily concerned.

Review of Literature

Advanced student projects by Jones (1948) and Loftin (1949) were concerned with the Charleston, Illinois, drinking water. The occurrence of false positive presumptives and the effect of pre-chlorination on the reduction of bacteria responsible for false positive presumptives were studied. Little or no attention was given to species determinations.

It is rather generally agreed that false positive presumptives may result from either of two possibilities.

First, species of the genera *Clostridium, Bacillus, Klebsiella, Erwinia,* and *Serratia* have been reported by Salle (1954:523-524) as being capable of fermenting lactose and then failing to produce a positive confirmation. Second, a type of biological association known as synergism, which involves the joint action of two or more organisms on a substance, has been known to result in lactose fermentation that produced gas but yet this same joint action would not ferment a confirmatory medium. The exploring of these two possibilities for an explanation of Charleston's drinking water problem was the major objective of this study.

Methods

Numerous water samples were collected at the filtration plant water intake in Lake Charleston and at various points throughout the city during September and October, 1955. Raw lake water was water which had no chemical treatment whatsoever. Drinking water samples had pre-chlorination, chemical treatment, sedimentation, filtration, and post-chlorination. Each sample was submitted to the presumptive and confirmed tests as prescribed (Norton *et al.,* 1946). Pure culture isolations were made from the nutrient agar dilution plates and were submitted to standard procedures for the identification of species (Breed, 1948).

Results

Of a total of 50 portions of raw lake water inoculated into lactose broth, 40 (80%) produced gas in the presumptive test. Thirty-nine (78%) of the presumptive portions confirmed the presence of coliform bacteria when inoculated into brilliant green bile. Thus, only 2% of all the raw lake water portions tested were found to be false positive presumptives (Table 1).

In chlorinated drinking water only 5 (4%) of the 125 portions inoculated into lactose were found presumptive. None of the positive presumptive portions produced gas in the confirmed test. Thus, all of the portions found presumptive in chlorinated drinking water were false positive presumptives (Table 1).

This relationship of low percentage values for false positive presumptives in Charleston's raw lake water and chlorinated drinking water has not always existed. Jones (1948) reported 74% of all chlorinated drinking water tested as false positive presumptives. Following the adoption of pre-chlorination, Loftin (1949) reported less than 9% false positive presumptives. The Lipousky (1955) data (Table 1) show only 4% of all drinking water portions as false positive presumptives. This evidence seems to support the suggestion that the application of chlorine prior to the addition of other chemicals (pre-chlorination) would be effective in reducing the false positive presumptives (Damann, 1950). The reduction has been found to be 70% when comparing the 74% reported by Jones in 1948 to the 1955 Lipousky data of 4%.

As a means of determining the species responsible for the false positive presumptives, more than 30 pure cultures were picked from nutrient agar plates for taxonomic study.

TABLE 1.—A Comparison of the Presumptive and Confirmed Results of Charleston, Illinois, Drinking Water Before and After Chlorination.

	Raw water (before chlorination)		Drinking water (after chlorination)	
	Number	Percent	Number	Percent
Portions tested	50	100	125	100
Portions presumptive in 48 hours	40	80	5	4
Portions confirmed in brilliant green bile	39	78	..	..
Portions false positive presumptives	1	2	5	4

The raw lake water selections yielded eight different species while only four species could be positively identified from the chlorinated drinking water (Table 2). The coliform species that indicate pollution, *Aerobacter aerogenes* and *Escherichia coli*, were encountered in the raw lake water isolations only. All of the *Bacillus* spp. reported (Table 2), except *B. polymyxa*, are considered to be widely distributed in nature in the water, soil, and dust. Thus, they are accorded little significance in matters of sanitation. *B. polymyxa* differed from the other species in that it fermented lactose, producing gas, but failed to confirm in brilliant green bile which marked it as a false positive presumptive.

To investigate the possibilities that a synergic reaction might also be responsible for false positive presumptives, each of the non-lactose fermenting species isolated from chlorinated water, *Bacillus cereus*, *Bacillus subtilis*, *Proteus mirabilis*, was inoculated into lactose broth in all possible combinations. Under none of the conditions could gas be produced in lactose broth unless *B. polymyxa* was also introduced. Thus, it appears evident that *B. polymyxa* must be responsible for the false positive presumptives in both the raw lake water and the chlorinated drinking water at Charleston, Illinois.

DISCUSSION

Specimens of *Bacillus polymyxa* have been found in both chlorinated and unchlorinated water in Maryland, Kentucky, Iowa, and in Illinois from Lake Michigan (Prescott, 1945: 82). Even though the species is common in soil from cultivated fields and pastures and has been found in abundance in decaying vegetables, its significance in drinking water has not been clearly determined. In the laboratory it has been found to produce the antibiotic polymxin.

Proteus mirabilis has been found in putrid meat, infusions, and in abscesses, which makes its appearance in the chlorinated drinking water a matter of concern. Cherry, Lentz, and Barnes (1946) reported this species as a possible cause of gastroenteritis, which definitely

TABLE 2.—Species of Bacteria Isolated from Lake Water Before and After Chlorination. September and October, 1955, Charleston, Illinois.

Lake water	Chlorinated water
Aerobacter aerogenes	none*
Bacillus brevis	none*
Bacillus cereus	Bacillus cereus
Bacillus coagulans	none*
Bacillus firmus	none*
Bacillus laterosporus	none*
Bacillus polymyxa	Bacillus polymyxa
none*	Bacillus subtilis
Escherichia coli	none*
none*	Proteus mirabilis

* Indicates that the corresponding species was not found.

makes its presence undesirable in drinking water. The fact that it was not found in the raw lake water but was found in the chlorinated drinking water suggests the possibility of secondary contamination in the distribution system occurring after the water had passed through the filtration treatment.

There is no direct evidence to support the view that *Proteus mirabilis* is a causative agent for any of the gastro-intestinal cases that occur in Charleston.

SUMMARY

A bacteriological investigation of the cause of the high percentage of false positive presumptive tests occurring in drinking water in Charleston, Illinois, was conducted during September and October, 1955. *Bacillus polymyxa* was isolated as the species responsible for the false positive presumptive results. *Proteus mirabilis,* which has been associated with gastroenteritis at a naval base in eastern United States, was also found in the chlorinated drinking water of Charleston, Illinois.

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