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Bacterial Contamination of Drinking Water Supplies in a Modern Rural Neighborhood†

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On six occasions during a 15-month period, the private well and spring water supplies in a modern rural neighborhood of 78 households were examined for total coliforms, fecal coliforms, *Staphylococcus aureus*, and standard plate count bacteria. More than one-third of the water supplies were unsatisfactory on at least one occasion as judged by standard plate counts over 10^3/ml and the presence of coliforms, fecal coliforms, and/or *S. aureus*. *Citrobacter freundii*, *Klebsiella pneumoniae*, and *Escherichia coli* were the most frequently isolated total coliforms. At least 12 other genera of bacteria were identified from standard plate count agar. Coliform contamination was found to be higher after periods of rainfall, and high standard plate counts were more prevalent during warmer weather. These observations probably reflect leakage of surface water into improperly sealed wells or aquifer contamination during winter and the lack of chlorination to control microbial regrowth during the warm season. An inverse correlation was found between the presence of high standard plate counts and incidence of coliforms. Consumer education and at least a twice yearly monitoring of private water supplies (winter and summer) are suggested methods to signal that treatment may be necessary to reduce the risk of waterborne disease.

Interest in the quality of drinking water supplies has been stimulated by the enactment of the Safe Drinking Water Act of 1974 and the dramatization of recent waterborne epidemics which occurred in recent years in the United States. The goal of this act is to improve the quality of drinking water supplies throughout the nation (16). To date, most of the research on this topic has been devoted to municipal supplies (10, 18) since the population at risk from any treatment failure is much greater for public water supply systems than for smaller, private water supplies. However, 69% of the reported outbreaks have been from private supplies (8), which serve more than 30 million Americans (2). It has been estimated that consumption of untreated, contaminated groundwater, faulty well construction, and improper well location are the primary causes of these waterborne outbreaks (7). It is indeed unfortunate that the act does not offer any benefit to the rural homeowner served by an individual water supply.

In the present study, an intensive bacteriological investigation was made of the incidence, numbers, and bacterial species present in the drinking water of private wells and springs which serve a modern rural community. Conventional indicator bacteria were not just monitored, but all coliforms present were identified to species. In addition, total plate count bacteria were isolated and identified. Although other rural drinking water supplies have been sampled (20, 27), the present report is the first seasonal study of drinking water supplies conducted in a recently populated rural neighborhood zoned by modern methods.

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MATERIALS AND METHODS

Study area. A map of the study area is shown in Fig. 1. The area consists of a recently zoned development covering approximately 1.3 km^2. It is located in the foothills of western Oregon, where the slope ranges from 3 to 50%.

The soil in the area belongs mainly to the Dixonville and Philomath series. The Dixonville series consists of well-drained, moderately deep soils of the fine, mixed, mesic family of Pachic Ultic Argixerolls. Dixonville soils are underlaid by weathered basalt bedrock at about 37 inches (about 92.5 cm). The Philomath series is a shallow, well-drained silty clay of the clayey, montmorillonitic, mesic, shallow family of Vertic Haploxerolls. These soils are underlaid by a partly weathered basalt bedrock at a depth of about 18 inches (about 45 cm) (22).

There are about 78 households in the study area, most of which were built within the last 5 years. Lots are 1 to 2 acres in size, with single-family dwellings. Water is obtained from groundwater supplies (private wells 80 to 200 feet [about 2.4 to 61 m] deep) or from surface springs. Waste is disposed of by septic tanks. There is a minimum distance requirement of 100 feet (about 30 m) between septic tank and well.

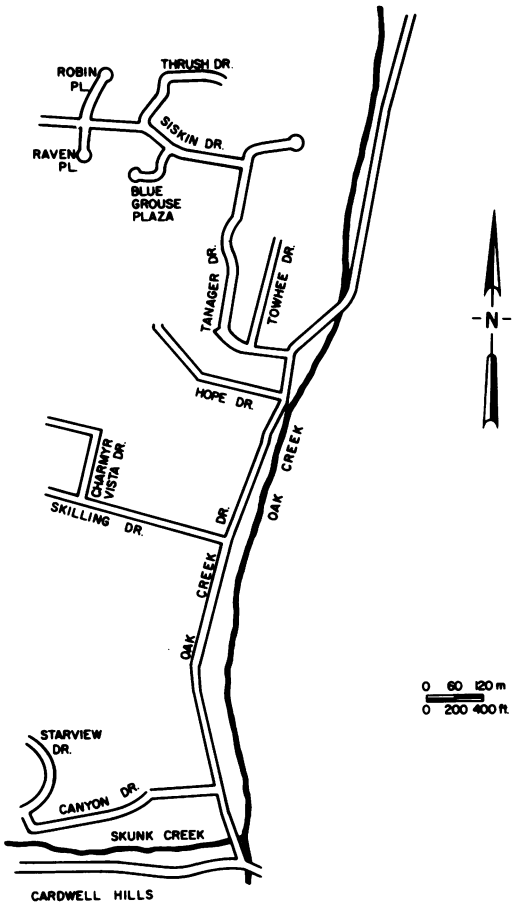

FIG. 1. *Map of the study area.*

Eunumeration and identification techniques. Water samples were collected from each household, placed on ice, and processed within 4 h.

Total and fecal coliforms were enumerated by the five-tube most-probable-number technique through the completed step (3). All coliforms from each contaminated sample were identified by the API 20E system (Analytab Produces, Inc., Plainview, N.Y.).

Standard plate count organisms were enumerated on standard plate count agar (Difco Laboratories, Detroit, Mich.) after incubation at 35°C for 48 h. Selected colonies of all morphological types were picked from standard plate count agar, streaked for purification, and maintained on slants of plate count agar. The cultures were identified according to *Bergey's Manual of Determinative Bacteriology* (5) and the scheme of Shayegani et al. (21). Ten percent of the gram-negative cultures were confirmed by the API 20E system.

Standard plate count bacteria were identified by using carbohydrate fermentation broth, tryptone broth, gelatin hydrolysis medium, standard plate count medium with 3 IU of penicillin G, and oxidase test reagent (14). All were prepared according to standard procedures. Moeller arginine dihydrolase and lysine decarboxylase broths, phenylalanine agar, and oxidation-fermentation test media were prepared from dehydrated media (BBL Microbiology Systems, Cockeysville, Md.). Triple sugar iron agar slants, Simmons citrate slants, deoxyribonuclease agar, and nitrate broth were prepared from Difco products. The inoculated media were incubated for up to 5 days at 35°C.

Staphylococcus aureus was enumerated by membrane filtration through 0.45-μm membrane filters (Gelman Instrument Co., Ann Arbor, Mich.) which were placed onto *Staphylococcus* 110 medium (Difco) and incubated at 35°C for 48 h. Typical staphylococcal colonies were inoculated into coagulase plasma (Difco), and the coagulase reaction was interpreted according to the method of Sperber and Tatini (23). Coagulase-positive bacteria were further identified as *S. aureus* by oxidase test (16), catalase test, Gram stain, and morphology. The ability of the bacteria to ferment glucose and mannitol anaerobically was determined by using the media and procedures recommended by the Subcommittee on Taxonomy of Staphylococci and Micrococci (24).

Staphylococcal enterotoxins A, B, and C were obtained from M. S. Bergdoll, Food Research Institute, Madison, Wis. The antiserum was obtained from the Foods and Nutrition Department, Oregon State University. The enterotoxins were assayed by the microslide technique described by Casman and Bennett (6).

RESULTS

During the survey, 27 (35%) of the households were found to be using water contaminated with coliforms, fecal coliforms, or *S. aureus*, or with standard plate counts exceeding 500/ml. Table 1 presents a summary of each sampling period, listing all incidences of total plate counts exceeding 500/ml and those samples which contained *S. aureus*, coliforms, or fecal coliforms. There was a seasonal trend in the quality of the water. For example, surveys conducted in October 1977, January 1978, and December 1978 were preceded within the previous 24 h by periods of rainfall. During these periods, the highest incidence of coliform contamination was noted. The level of significance of the difference between coliform levels during periods of rain and no rain was $P = 0.10$. Another seasonally related parameter was the increased occurrence of high total plate counts during the warmer spring and summer months compared with the cool months. This difference was significant at a level of $P = 0.05$.

The identity and incidence of isolates obtained from the completed tests for total and fecal coliforms, along with the number of samples containing each species, are listed in Table 2. In those samples with total coliforms exceeding 2/100 ml, only two yielded just one species of coliform. The other 10 samples yielded mixed cultures of at least two species of coliforms. Nine different species of enteric bacteria were identi-

TABLE 1. *Incidences of total coliform, fecal coliform, high standard plate count, and S. aureus in rural drinking water supplies*[a]

Date	Sample source	SPC/ml	TC/100 ml	FC/100 ml	*S. aureus*/ 100 ml	Date	Sample source	SPC/ml	TC/100 ml	FC/100 ml	*S. aureus*/ 100 ml
10/1/77	1	3.7×10^3	27	0	NT	6/9/78	14	5.0×10^1	0	0	2
	15	5.7×10^3	0	0			25	1.9×10^3	0	0	0
	24	1.6×10^3	0	0			29	1.9×10^4	0	0	600
	30	1.1×10^1	34	0			36	3.0×10^1	2	0	0
	38	1.3×10^1	8	0			47	5.7×10^2	0	0	0
	39	1.2×10^2	8	0			50	3.1×10^2	13	0	0
	47	1.7×10^3	0	0			56	3.0×10^1	2	0	0
	48	3.1×10^1	5	0			63	7.2×10^2	2	0	0
	56	1.0×10^1	8	2			65	9.5×10^2	0	0	0
							73	5.7×10^2	0	0	0
1/4/78	30	7.7×10^3	0	0	NT						
	48	1.0×10^1	2	0		10/4/78	39	1.5×10^2	2	0	NT
	56	4.0×10^1	49	5			56	2.0×10^1	2	2	
	70	1.6×10^1	33	0			78	7.0×10^1	2	0	
3/28/78	17	1.0×10^2	0	0	1	12/29/78	30	5.4×10^2	0	0	NT
	40	5.1×10^2	0	0	0		42	7.3×10^2	0	0	
	42	8.4×10^3	0	0	0		48	1.4×10^2	2	0	
	48	2.0×10^2	0	0	4		56	7.0×10^1	5	0	
	49	1.0×10^1	0	0	12		70	6.0×10^1	33	2	
	54	6.0×10^1	2	0	0		72	1.0×10^1	33	0	
	55	1.2×10^3	0	0	0						
	63	1.0×10^3	0	0	0						
	67	1.0×10^1	0	0	38						
	73	3.5×10^3	0	0	0						

[a] Samples are tabulated only for those specimens containing indicator organisms, *S. aureus*, and/or standard plate count bacteria in excess of 500/ml. SPC, standard plate count bacteria; TC, total coliforms; FC, fecal coliforms; NT, not tested.

TABLE 2. *Total and fecal coliform species isolated from rural drinking water supplies*

Identification	No. of isolates	% of total	No. of samples
Total			
Citrobacter freundii	34	46	11
Enterobacter agglomerans	9	12	5
Enterobacter cloacae	3	4	3
Enterobacter hafniae	2	3	2
Escherichia coli	10	14	4
Klebsiella pneumoniae	13	18	6
Serratia liquefaciens	1	1	1
Fecal			
Citrobacter freundii	1	9	1
Escherichia coli	8	73	2
Serratia liquefaciens	2	18	1

fied during the study. *Escherichia coli* comprised 13% of the total coliforms and 73% of the fecal coliforms. *Citrobacter freundii* and *Klebsiella pneumoniae*, on the other hand, were the most commonly isolated total coliforms. On one occasion, *Yersinia enterocolitica* was isolated as a "typical gas producing coliform" (19). Fecal coliforms were isolated from two water supplies which used surface springs as the raw water source.

Table 3 presents the organisms isolated and identified from the standard plate count agar. There was a large variety of species isolated, and a surprising number were opportunistic pathogens. It was, in fact, the presence of *S. aureus* in the total plate counts that led to their being monitored in the subsequent samples. All of the *S. aureus* isolates exhibited coagulase activity, and half of them produced enterotoxin A.

Table 4 compares the bacterial plate counts and coliform incidence. It can be seen that incidence of coliform occurrence decreased as standard plate count exceeded 500/ml. Fecal coliform occurrence appeared to be even more inhibited by high bacterial levels, since there is no occurrence of fecal coliform at standard plate counts exceeding 100/ml.

DISCUSSION

In previous studies of rural drinking water supplies, as many as 90% of systems studied have been found to be contaminated with coliforms (20, 27). However, many questions have remained unanswered. It has not been demonstrated to what degree modern zoning practices would alleviate the problem of contaminated potable water supplies. Also needed are data on how seasonal variations affect such contamination. Finally, further emphasis on the threat posed by total plate count bacteria is necessary, regarding both coliform masking and the indication of the presence of nonfecal pathogens (10).

Total coliforms are the primary indicator organisms enumerated in investigations of drink-

TABLE 3. *Bacteria identified from standard plate count agar in samples of rural drinking water*

Gram positive	Gram negative
Corynebacterium spp.	*Aeromonas hydrophila*
Arthrobacter spp.	*Pseudomonas acidovorans*
Actinomycetes	*P. alcaligenes*
Bacillus	*P. mallei*
Staphylococcus aureus	*P. maltophilia*
S. epidermidis	*Acinetobacter calcoaceticus*
S. saprophyticus	*Alcaligenes denitrificans*
Micrococcus luteus	*Flavobacterium* spp.
M. roseus	*Moraxella bovis*
	M. kingii
	CDC[a] group M-1
	CDC group M-3
	CDC group M-4
	CDC group M-5
	Group IIk biotype 1
	Group IVe

[a] Center for Disease Control.

TABLE 4. *Standard plate count and the incidence of total and fecal coliform detection*

Standard plate count density range/ 1 ml	No. of samples	Total coliform occurrences	Total coliform incidence	Fecal coliform occurrences
≤10	61	1	1.6%	1
11-100	177	14	7.9%	3
101-500	52	4	7.7%	0
>500	19	1	5.2%	0

ing water supplies since they indicate surface runoff contamination. In addition; they themselves may be opportunistically pathogenic (4, 17) or capable of enterotoxin production (13). *E. coli* in drinking water represents the certain presence of recent fecal contamination (9) and the possible presence of bacterial pathogens, enteric viruses, or intestinal parasites. So it is significant that in 15% of the households the drinking water was contaminated with coliforms in spite of the up-to-date standards used in installing such wells.

Previous research has demonstrated the necessity of concurrent enumeration of standard plate count bacteria along with that of coliforms to properly evaluate the potability of a water supply (10; E. E. Geldreich, paper presented at the 1st American Water Works Association Conference on Water Quality Technology, Cincinnati, Ohio, 2-4 December 1973). Because they can mask coliform presence in both most-probable-number and membrane filtration techniques (10; Geldreich, 1973), failure to consider the total plate count could cause one to underestimate the health hazard of a given water supply. An example of this masking problem is reported in a study in Karachi, Pakistan: in 16 of 22 instances of pathogen occurrence in the absence of coliform detection, the standard plate count was greater than 500/ml (1). Other investigators have shown that *Bacillus* sp., pseudomonads, *Flavobacterium* sp., *Actinomyces*, and *Micrococcus* sp. can inhibit coliform growth (11, 12, 25, 26). Table 3 shows that all of these antagonists were isolated in rural drinking water. Table 4 further demonstrates that high standard plate counts may also have been important in the assessment of the water supplies. As the total plate count rises from less than 10 bacteria per ml to between 11 and 500, the coliform incidence rises from 2 to 8%. However, as the total bacterial population rises above 500/ml, the detection of coliforms drops to 5%. This supports a previous statement that standard plate counts greater than 500/ml adversely affect detection of coliform organisms (10; Geldreich, 1973).

In addition to their importance as coliform antagonists, many of the total plate count organisms isolated are pathogens, such as *S. aureus* and *Aeromonas hydrophila*. Most of the other species have been implicated at one time or another as opportunistic pathogens (15). Because of the unexpected isolation of pathogens in drinking water, the presence of *Staphylococcus* was investigated more thoroughly. Other studies from this laboratory have demonstrated that *S. aureus* probably arise most commonly from contaminated faucet aerator screens (14). In this study, 8% of the sampled households harbored *S. aureus*, high levels of which were accompanied by excessive standard plate counts. When *S. aureus* reached 600/100 ml, the total plate count exceeded 1,000/ml, whereas coliforms remained undetected. It therefore appears that high standard plate counts may be useful for indicating the general drinking water quality and the presence of other pathogens or opportunistic pathogens when coliforms are not even detected.

This research also demonstrated the importance of education for the consumers who use individual supplies for their drinking water. Much needs to be done to increase awareness of the hazard of drinking contaminated water and of ways to prevent contamination. Improper placement of wells, lack of sanitary seals, proximity of grazing animals to the well, and lack of knowledge of the significance of contaminated water were all found to be factors contributing to the poor-quality water supply in this study.

More frequent monitoring was helpful in detecting contaminated water supplies which otherwise would have gone unsuspected, particularly during and after rainfall periods. Consumers need to know that testing water only

upon installation of a well is an inadequate measure of potability of a water supply.

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