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Enumeration and Characterization of Standard Plate Count Bacteria in Chlorinated and Raw Water Supplies†

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Nearly 700 standard plate count (SPC) bacteria were isolated from drinking water and untreated surface water and identified according to a scheme developed to permit the rapid, simple classification of microorganisms to genus, species, or group. Actinomycetes and *Aeromonas* species were the two most common groups of SPC bacteria in chlorinated distribution water. *Aeromonas* spp. and *Enterobacter agglomerans* were the two most common groups of SPC bacteria in raw water. Identification of bacterial populations before and after contact with chlorine (1 to 2 mg/liter) for 1 h revealed that chlorination selected for gram-positive bacteria. Water that contained high densities of bacteria known to be antagonistic to coliforms had low coliform isolation rates. The membrane filtration technique for enumerating SPC bacteria recovered significantly higher numbers ($P < 0.001$) than the standard pour plate technique.

Much attention is focused on the role of standard plate count (SPC) bacteria in potable water. The SPC is considered by some to be a better indicator of potable water quality than the coliform index (10, 19, 21). Mood (18) indicated that the 35°C agar plate count was the best indicator of the quality of swimming pool water. The presence of opportunistic pathogens in the total SPC population can pose a threat to the young, the old, and the infirm (6, 7, 9, 12). The control of the aesthetic quality of potable water has also been attributed to the control of SPC bacteria in distribution lines (26). Large densities of SPC bacteria have been reported to interfere with the detection of coliforms (1, 10).

Despite these significant observations, little quantitative or qualitative research has been reported on the incidence, distribution, and kinds of SPC bacteria present in chlorinated drinking water. The greatest barrier to obtaining information on SPC populations has been the almost overwhelming task of identifying this diverse group of bacteria. It is the purpose of this report, therefore, to propose a simple scheme to rapidly identify SPC bacteria and to examine the incidence and distribution of these organisms in the chlorinated drinking water and raw surface water supplies of an Oregon coastal community.

MATERIALS AND METHODS

Sampling area. Samples were collected from the finished drinking water supply of an Oregon coastal community serving 14,000 residents and from the two coast range streams supplying the raw water to the city. The watershed does not receive industrial or domestic waste, but previous logging operations have left some of the upstream slopes barren of sufficient ground cover to prevent erosion. During precipitation periods, runoff occasionally carried material into the streams, leaving drinking water turbid.

The intake points of the raw water supplies are located behind small concrete retention dams about 1.2 to 1.5 m (4 to 5 f) high. At one intake, water flows by gravity into a 30.5-cm (12-inch) main line and, after 15-min flow time, receives gaseous chlorine injection by a flow proportional chlorinator resulting in an average 1.5-mg/liter (1.5-ppm) initial dose. At the other intake, water is pumped from the diversion dam into a settling reservoir. Reservoir effluent receives a similar dose of gaseous chlorination before entering the distribution system. Both water supplies receive about a 30-min contact period in the main lines before reaching the first service connections. On occasion, water was experimentally chlorinated in the laboratory with a stock solution of calcium hypochlorite (Fischer Scientific Co.) to simulate field chlorination of raw water.

Collection of samples. Raw surface water and finished drinking water samples were collected in 4-liter sterile polypropylene containers. Sodium thiosulfate was added to neutralize any chlorine residual in the drinking water samples. The temperature of all water samples was determined upon collection with a YSI Tele-thermometer, 400 series. The turbidity of the samples was measured using a Hach model 2100 A turbidimeter. Formazin turbidity standards were prepared weekly according to standard methods (2). The nitrate content of water was determined by the cadmium reduction method according to standard methods (2). Water samples were placed on ice and transported to the laboratory within 3 h after collection, and analyses were completed within 7 h after collection.

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Enumeration of coliforms. Total coliforms were enumerated by both the membrane filtration (MF) and most-probable-number (MPN) techniques according to standard methods (2). Typical coliform colonies from the MF technique were verified by using lauryl tryptose broth (Difco Laboratories). Coliforms recovered by the MPN technique were carried through the completed step (2). Fecal coliforms were enumerated on m-FC medium (Difco) according to standard methods (2). *Aeromonas* spp. were enumerated on m-*Aeromonas* medium (23). *Klebsiella* spp. were enumerated on MacConkey-inositol-carbenicillin medium (3).

Enumeration and identification of SPC bacteria. SPC bacteria were enumerated by filtering suitable volumes through a gridded 0.45-μm membrane filter (Gelman GN-6). The filter was placed on m-SPC agar (25) and incubated at 35°C for 48 h. Colonies on all membrane filters were counted with the aid of a dissecting microscope at 15× magnification. The SPC bacterial density was also determined by the pour plate technique (2).

SPC bacteria were selected for identification by picking colonies from a membrane filter quadrant (m-SPC agar) until 20 isolates (where possible) were obtained. Isolates were purified by streaking onto agar composed of tryptic soy broth (Difco) supplemented with 0.3% yeast extract (TSB-YE; Difco) and 1.5% agar (Difco) and incubating at 35°C for 48 h. Pure cultures were maintained on slants of TSB-YE agar at 5°C.

All isolates were placed into genera or groups by noting colony and cell morphology, Gram stain, catalase and oxidase reactions, motility, urease, and indole and glucose fermentation or oxidation (Fig. 1 and 2). Media and reagents were prepared according to standard procedures (16). Hugh Leifson O/F medium with 1% glucose or 1% mannitol, methy red-Voges-Proskauer medium, Moeller decarboxylase broth, triple sugar iron (TSI) agar slants, Simmons citrate, and urease test agar were prepared from Difco products. Lysine, ornithine, and arginine amino acids were obtained from Sigma Chemical Co. Triple sugar iron agar slants were incubated for 24 h. The Voges-Proskauer test was performed after 48 h of incubation. The Hugh Leifson methyl red, decarboxylase, dihydrolase, citrate, tryptophan, and urease media were incubated for up to 5 days at 35°C. Further identification of *Pseudomonas* spp. and *Pseudomonas*-like and *Moraxella*-like bacteria (group M bacteria) was performed with reference to the scheme of Shayegani et al. (24), and enteric bacteria were identified by using the tests listed in Fig. 2 and the *Manual of Clinical Microbiology* (16).

Physical, chemical, and microbiological parameters were entered in the Oregon State University CYBER 70/73 computer equipped with a statistical interactive programing system (SIPS) for data storage and statistical analyses. Statistical comparisons were made on the basis of the paired *t*-test on logarithmically transformed data.

RESULTS

SPC bacteria were enumerated by using two methods, the standard pour plate technique (SPC-pour) and the MF technique (M-SPC). A comparison of these two procedures indicated that the M-SPC technique (geometric mean of 2.0 bacteria/ml) was superior ($P < 0.001$) to the SPC-pour technique (geometric mean of 0.37 bacteria/ml) in recovering bacteria from chlorinated drinking water samples. However, no significant difference ($P > 0.5$) was observed between the M-SPC (geometric mean of 49 bacteria/ml) and the SPC-pour (geometric mean of 60 bacteria/ml) techniques in raw water.

The number of SPC bacteria (M-SPC technique) in drinking water ranged from <0.02 to over 1×10^4 bacteria/ml. Dead-end distribution lines in which no free residual chlorine could be detected contained 23 times the number of SPC bacteria (geometric mean of 17 bacteria/ml) compared with distribution lines with a free chlorine residual. Total coliform numbers (MF technique) in distribution water ranged from 4 to 440 coliforms/100 ml with a geometric mean of 1.5 coliforms/100 ml. The geometric mean number of coliforms in dead-end lines which contained no free chlorine was 1.9 coliforms/100 ml. SPC bacteria (M-SPC technique) in raw water ranged from 2.5 to 5.0×10^3 bacteria/ml with a geometric mean of 73 bacteria/ml. Coliforms in raw water (MF technique) ranged from 1.3 to 680/100 ml with a geometric mean of 51/100 ml.

Nearly 700 bacteria enumerated by the M-SPC technique from raw and finished drinking water have been identified by the application of the protocol depicted in Fig. 1 and 2. The use of these procedures permitted bacterial isolates to be rapidly identified as representing 1 of 13 genera or groups. Over 80% of the isolates from chlorinated water, 82% of the isolates from raw water, and 97.5% of distribution system isolates collected during a chlorination failure were identified according to the protocol. Once the organism was placed into a category, further identification to species could be accomplished by routine identification procedures presented in *Bergey's Manual* (5) or by the scheme of Shayegani et al. (24).

A total of 347 bacteria have been identified from chlorinated drinking water samples (Table 1). Data were combined from two to four sample sites collected seven times over a 1-year period. The actinomycete group comprised the largest portion of the M-SPC population (10.7%), whereas *Aeromonas* spp. represented the second largest group, comprising 9.5% of the total population. *Acinetobacter* was the most commonly isolated organism, being present in every sample except during the month of September. Bacteria

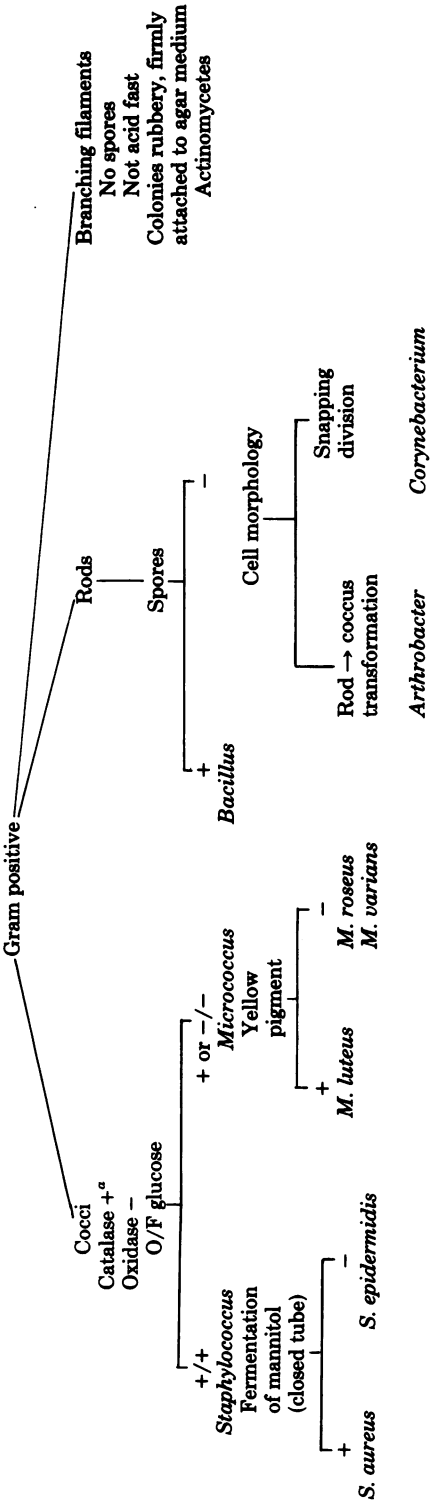

FIG. 1. *Proposed scheme for identification of gram-positive SPC bacteria from potable and raw drinking supplies. a, Excludes catalase-negative streptococci.*

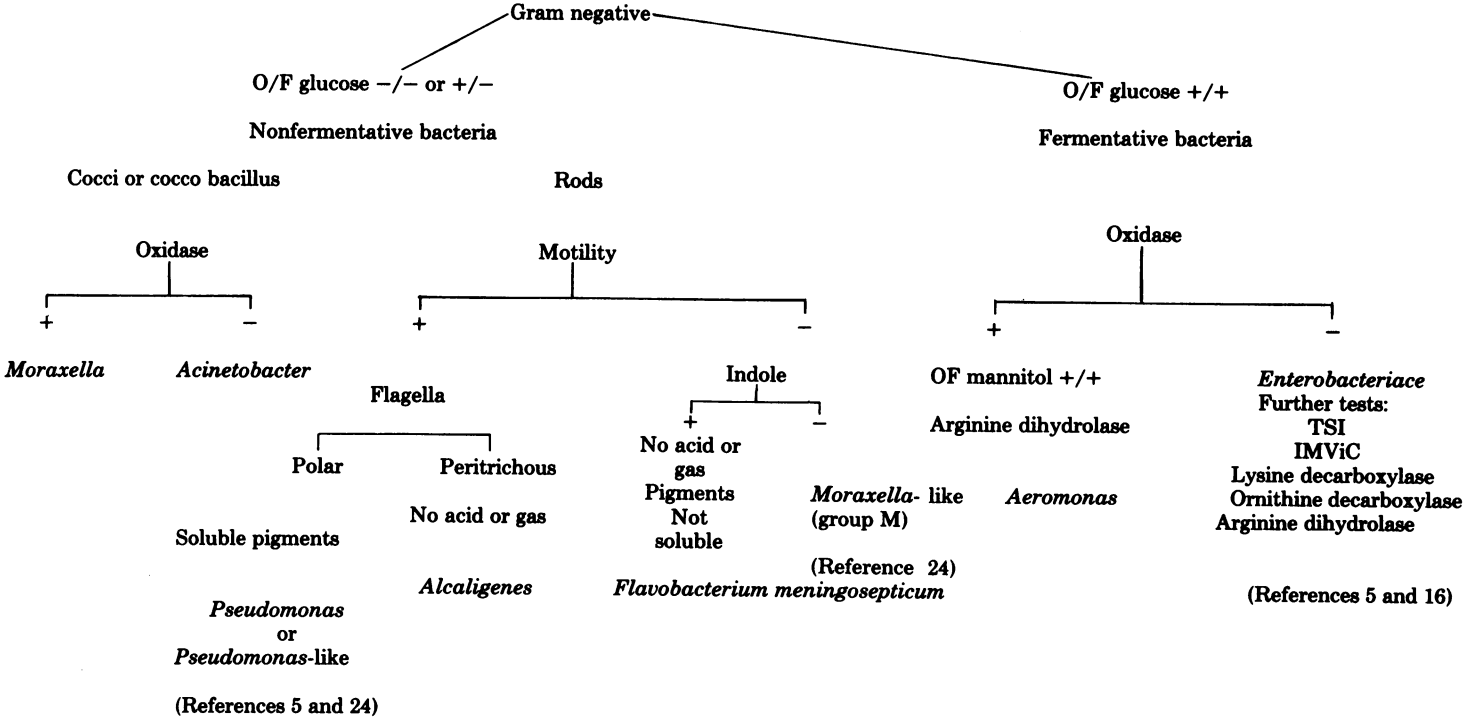

FIG. 2. *Proposed scheme for identification of gram-negative SPC bacteria from potable and raw water supplies.*

that may be primary or opportunistic pathogens (*Pseudomonas maltophila, P. fluorescens, P. cepacia, P. mallei, Acinetobacter* spp., *Klebsiella pneumoniae, Staphylococcus aureus, Moraxella* spp., *Serratia liquefaciens*; 5, 16, 20) comprised 30% of the total population identified. Bacteria considered antagonistic to coliforms (*Pseudomonas, Micrococcus, Flavobacterium, Bacillus, Actinomyces*; 13, 22, 27) comprised 35% of the SPC bacteria identified.

Aeromonas spp. comprised the largest portion of the M-SPC population (15.9%) isolated from the raw water intake to the distribution system. *Enterobacter agglomerans* was the second most common group and comprised 11.5% of the population (Table 1). Bacteria that may be opportunistic pathogens and coliform antagonists comprised 51 and 14% of the M-SPC population, respectively.

The dominant bacterial population identified in the distribution samples taken during a chlorination failure (Table 1), as anticipated, was similar to the population found in raw water. Opportunistic pathogens comprised 55.0% and coliform antagonists comprised 15.0% of the total population.

Over the entire study period, gram-positive bacteria comprised nearly 36% of the M-SPC bacteria isolated from chlorinated drinking water. Gram-positive bacteria in the raw water and distribution water during the chlorine failure averaged only 13.5% of the SPC population. Because of this difference in percentage of gram-positive organisms in the two types of water specimens, bacteria were identified before and after experimental chlorination of three water samples (Table 2). Samples received 1.5 mg of chlorine per liter for a 1-h contact period. The average decrease in the SPC count was 200-fold after chlorination. The average level of gram-

TABLE 1. *Identification of SPC bacteria in distribution water, raw water, and distribution water during a chlorine failure*

Organism	Distribution water		Raw water		Distribution water during chlorine failure	
	Total[a]	% of total	Total	% of total	Total	% of total
Actinomycete	37	10.7	0	0	1	1.3
Arthrobacter spp.	8	2.3	2	1.3	7	8.8
Bacillus spp.	17	4.9	1	0.6	0	0
Corynebacterium spp.	31	8.9	3	1.9	0	0
Micrococcus luteus	12	3.5	5	3.2	4	5.0
Staphylococcus aureus	2	0.6	0	0	0	0
S. epidermidis	18	5.2	8	5.1	0	0
Acinetobacter spp.	19	5.5	17	10.8	12	15.0
Alcaligenes spp.	13	3.7	1	0.6	3	3.8
F. meningosepticum	7	2.0	0	0	0	0
Group IVe	4	1.2	0	0	0	0
Group M5	9	2.6	2	1.3	0	0
Group M4	8	2.3	2	1.3	0	0
Moraxella sp.	1	0.3	1	0.6	0	0
Pseudomonas alcaligenes	24	6.9	4	2.5	0	0
P. cepacia	4	1.2	0	0	3	3.8
P. fluorescens	2	0.6	0	0	1	1.3
P. mallei	5	1.4	0	0	0	0
P. maltophilia	4	1.2	9	5.7	4	5.0
Pseudomonas spp.	10	2.9	0	0	0	0
Aeromonas spp.	33	9.5	25	15.9	17	21.3
Citrobacter freundii	6	1.7	8	5.1	1	1.3
Enterobacter agglomerans	4	1.2	18	11.5	7	8.8
Escherichia coli	1	0.3	0	0	0	0
Yersinia enterocolitica	3	0.9	10	6.4	3	3.8
Group IIK biotype I	0	0	1	0.6	8	10.0
Hafnia alvei	0	0	9	5.7	3	3.8
Enterobacter aerogenes	0	0	1	0.6	2	2.5
Enterobacter cloacae	0	0	1	0.6	0	0
Klebsiella pneumoniae	0	0	0	0	2	2.5
Serratia liquefaciens	0	0	1	0.6	0	0
Unidentified	65	18.7	28	17.8	2	2.5
Total	347	100.2	157	99.7	80	100.5

[a] Number of strains identified for the entire sampling period of 1 year.

TABLE 2. *Changes in SPC populations after exposure to chlorine*[a]

Organism	No. before chlorine addition	% of isolates	No. after contact with 1.5 mg of chlorine/liter	% of isolates
Actinomycete	0		2	4.6
Arthrobacter spp.	4	7.5	2	4.6
Bacillus spp.	0		11	25.6
Corynebacterium spp.	0		6	14.0
Micrococcus spp.	3	5.7	2	4.6
Staphylococcus epidermidis	1	1.9	0	
Group M5	1	1.9	0	
Group M4	6	11.3	0	
Group IVc	0		4	9.3
Group IIK biotype I	1	1.9	0	
Acinetobacter	6	11.3	6	14.0
Alcaligenes	0		1	2.3
Pseudomonas spp.	20	37.8	6	13.9
Enterobacter agglomerans	1	1.9	0	
Aeromonas spp.	2	3.8	0	
Hafnia alvei	2	3.8	0	
Yeast	0		1	2.3
Unidentified	6	11.3	2	4.6
Total	53	100.1	43	99.9

[a] Data represent the combined results from three water samples. Chlorine contact was at 10°C, pH 7, for 1 h; the average SPC before chlorination was 1,030 bacteria/ml; the average SPC after chlorination was 5 bacteria/ml.

positive bacteria in the water before chlorination was 15.0%, and this increased to 54% after chlorination. The percentages of coliform antagonists in the water before and after chlorination changed very little. Opportunistic pathogens comprised 53.3% of the population before chlorination and were reduced to 27.5% of the population after chlorination.

On 23 occasions, the investigators identified and compiled the percentage of coliform antagonists in chlorinated drinking water samples and on 11 occasions determined their percentage in raw water samples (Table 3). The results illustrated that 16 of 23 drinking water samples contained SPC populations with over 20% of the isolates considered coliform antagonists, and only 3 (19%) of the 16 samples contained detectable coliforms. When the coliform antagonists were fewer than 20% of the population, the incidence of coliform occurrences increased to 57% (Table 3). In raw water samples, coliforms were always detected and the percentage of the SPC population considered coliform antagonists was always below 20%.

Table 4 summarizes those significant correlations observed between the numbers of SPC organisms and one or more of the additional 13 parameters measured in each water sample. All raw water samples were removed from the point of intake to the distribution system, whereas the distribution water represented only chlorinated samples removed from over 40 locations throughout the water supply system. The number of SPC bacteria was found to correlate very well with the number of *Aeromonas* and *Klebsiella* in both raw and distribution water. However, numbers of SPC bacteria correlated with coliform density (both MPN and MF) in only the raw water. Turbidity ranged from less than 1 to 28 nephelometric turbidity units (NTU) with a geometric mean of 1.2 NTU and was significantly correlated with SPC numbers in distribution water. Increases in the range of 1 to 3 logs of SPC bacteria were common when turbidities exceeded 5 NTU. Temperature also correlated positively with numbers of SPC bacteria, and highest cell densities coincided with late summer water temperatures, which approached 20°C. Nitrate correlated inversely with SPC numbers, suggesting that nitrate plays a nutritional role in influencing the extent of bacterial regrowth in distribution systems.

TABLE 3. *Relationship between percentage of coliform antagonists and the presence of coliforms*

Sample	No.	No. with coliforms	Occurrence (%)
Distribution			
>20%[a]	16	3	3/16 (19)
<20%	7	4	4/7 (57)
Raw water			
>20%	0	0	0/0
<20%	11	11	11/11 (100)

[a] Percentage of coliform antagonists in sample.

TABLE 4. *Correlation coefficients for numbers of SPC bacteria (MF and pour) in raw and distribution water*

Variable	Raw water						Distribution water					
	M-SPC			SPC-pour			M-SPC			SPC-pour		
	r	df	*P*	*r*	df	*P*	*r*	df	*P*	*r*	df	*P*
Temp	0.698	29	<0.01	0.422	29	<0.05	0.188	161	<0.05	0.164	161	<0.05
Turbidity	−0.130	28	>0.05	0.394	28	<0.01	0.380	161	<0.01	0.567	161	<0.01
Nitrate	−0.680	19	<0.01	−0.742	19	<0.01	−0.308	86	<0.01	−0.238	86	<0.05
SPC-pour	0.570	31	<0.01	1.000	31		0.988	159	<0.01	1.000	159	
Aeromonas[a]	0.689	23	<0.01	0.042	23	>0.05	0.720	128	<0.01	0.604	128	<0.01
Klebsiella	0.651	20	<0.01	0.311	20	>0.05	0.544	119	<0.01	0.204	119	<0.05
TC-MF[b]	0.654	29	<0.01	0.500	29	<0.01	0.003	61	>0.05	−0.002	61	>0.05
MPN[c]	0.455	31	<0.01	0.867	31	<0.01	0.040	165	>0.05	0.046	165	>0.05
Precp[d]	−0.099	31	>0.05	0.473	31	<0.01	−0.069	165	>0.05	−0.051	165	>0.05

[a] Enumerated by MF technique.
[b] Verified total coliforms enumerated by the MF technique.
[c] Total coliforms enumerated by MPN technique through completed step, using lauryl tryptose broth as presumptive medium.
[d] Precipitation recorded on sampling day.

DISCUSSION

In chlorinated drinking water supplies, significantly more SPC organisms were detected by the M-SPC technique than by the SPC-pour method. The M-SPC technique permitted greater flexibility in drinking water supplies since volumes greater than 1 ml could be processed. Counting colonies by the M-SPC technique also proved easier with a dissecting microscope at 15× magnification than counting SPC-pour plates with a colony counter. These observations are consistent with those of Taylor and Geldreich (25).

The schema presented in Fig. 1 and 2 summarize rapid and convenient procedures needed to document the SPC diversity in a water sample. It is possible by performing only the initial tests (colony morphology, Gram stain, motility by wet mount, O/F Hugh Leifson, catalase test, and oxidase test) to place many of the bacteria into a genus or group. Identification of 100 isolates can be conveniently completed by examining some 20 characteristics. Because the bacteria can be identified so rapidly with little subculturing, culture maintenance and loss of viability was not a major problem. Overall, some 84% of the SPC isolates were identified by use of these schema.

Many of the bacterial groups identified in chlorinated municipal water in this study were already shown to exist in unchlorinated rural drinking water (15).

Identification of SPC bacteria in potable drinking water permitted an assessment of the bacterial diversity present, whereas enumeration of SPC bacteria provided a threefold evaluation of the water quality in a distribution system: (i) elevated SPC levels indicated a potential health risk posed by opportunistic pathogens; (ii) high SPC levels could increase the potential for coliform suppression by coliform antagonists and; (iii) increased SPC levels indicated deterioration of general water quality and inadequate disinfection.

Several trends were apparent in the seasonal distribution and species diversity of the SPC population present in distribution water. For example, there was greater species diversity in the warmer summer period and in the fall after the first major precipitation than during the cold winter months. Of the 25 identifiable bacterial groups, an average of 8 were detected in drinking water during November, December, January, and March compared with an average of 14 groups detected in July and October. Gram-positive bacteria predominated the SPC population during the cooler months (November through January).

Raw water at the intake contained an average of nine different groups throughout the year, with a range of only 7 to 11 species for all reporting periods. The changes in the SPC population in the distribution system were therefore not a simple function of bacterial quality in the raw water. It can be hypothesized that interactions of water temperature, fall precipitation, and disinfection efficiency all influenced the species diversity in drinking water derived from surface sources which are only chlorinated and not filtered.

Identification of bacteria in distribution water indicated that over 30% of the total bacterial populations were opportunistic pathogens. *Aeromonas* spp. was the second most prevalent bacterial species in chlorinated distribution water

(9.5%) and the most common species in raw water (15.9%). *A. hydrophilia* is known to have a widespread distribution, being able to inhabit a variety of aquatic habitats (11). *Aeromonas* spp. have also been associated with a wide range of human infections, indicating that these organisms may act as primary pathogens (14). Opportunistic pathogens such as *Flavobacterium*, *Pseudomonas* spp., and *Acinetobacter* comprised some 25% of the bacterial population in distribution water. Populations of *Pseudomonas* and *Flavobacterium* have been recognized as health risks to patients in hospitals, clinics, nurseries, and rest homes (9, 12). Nosocomial infections caused by *P. cepacia* have resulted from contamination of materials thawed in a water bath (6). *Acinetobacter* spp., which comprised up to 15% of the SPC bacteria in drinking water collected during a chlorination failure, have been reported as causing nosocomial infections in stressed individuals at a rate of 3.54 per 10,000 patients discharged (7). Since the present drinking water quality is based on the absence of coliforms, the opportunistic pathogens and secondary invaders comprising the SPC go unrecognized even though their numbers often greatly exceed those of the coliform group (21).

Some species and strains of *Pseudomonas*, *Sarcina*, *Micrococcus*, *Flavobacterium*, *Proteus*, *Bacillus*, actinomycetes, and yeasts have been shown to suppress coliform detection (13, 22, 27). The significance of coliform suppression in municipal and rural water supplies has been previously presented (1, 4, 10, 15), and it has been speculated that suppression can at least partly be attributed to elevated SPC numbers. For example, increased SPC densities have been noted before, or have been associated with, waterborne outbreaks (1, 10, 17, 19). Pathogen occurrences have also been noted to occur in the absence of detectable coliforms in samples with high SPC numbers (1). Previous studies, however, did not identify the kinds of SPC organisms present, so the potential role and significance of genera representing coliform antagonists could not be assessed.

Identification of the SPC population in this study indicated, on an average, that nearly three times more bacteria that are potential coliform antagonists were present in distribution water compared with raw surface water (35 versus 14%). Observations consistent with a possible role of antagonists in coliform suppression were provided by the lack of coliforms in 81% of the drinking water specimens which contained 20% or more antagonists in the SPC population. Conversely, when the antagonist population was less than 20% of the SPC, a greater coliform incidence (57%) was recorded. Since SPC levels varied greatly in samples containing and not containing coliforms (less than 1 to over 500/ml in both types of samples), the species components of SPC populations as well as cell densities (10) may be important in assessing the propensity for interference with coliform detection. In addition, experimental chlorination of the raw water did not significantly alter the relative percentage of coliform antagonists over the level found in untreated water. Therefore, one can assume that factors other than disinfection will determine the relative abundance of these genera in the distribution system.

Gram-positive bacteria in chlorinated drinking water comprised nearly three times the number found in the raw water. It is presumed that the thicker peptidoglycan layer of the wall enables gram-positive bacteria to be more chlorine resistant. It has already been suggested that the outer wall components of *Mycobacterium* species enable these organisms to resist chlorination (8).

Increasing densities of SPC bacteria correlated significantly with increasing levels of turbidity, *Aeromonas*, *Klebsiella*, and water temperature. Runoff after storm events will therefore carry turbidity and increased numbers of bacteria into the distribution system, and some detectable increment will survive chlorination. These observations collectively indicate the potential consequences in a distribution system which uses surface water as a source water and does not have filtration facilities. The SPC method was a sensitive indicator of the factors which contribute to the deterioration of water quality.

In summary, enumeration of SPC bacteria was a useful tool to indicate the presence of opportunistic pathogens, the potential for coliform suppression, and drinking water quality deterioration in a distribution system.

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LITERATURE CITED

1. **Ahmed, Z., I. A. Poshni, and M. Siddiqui.** 1967. Bacteriological examination of drinking water of Karachi and isolation of enteric pathogens. Pakistan J. Sci. Ind. Res. 7:103-110.
2. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Inc., New York.
3. **Bagley, S. T., and R. J. Seidler.** 1978. Primary *Klebsiella* identification with MacConkey-inositol-carbenicillin agar. Appl. Environ. Microbiol. **36**:536-538.

4. **Boring, J. R., III, W. T. Martin, and L. M. Elliott.** 1971. Isolation of *Salmonella typhimurium* from municipal water, Riverside, Calif., 1965. Am. J. Epidemiol. **93**:49–54.
5. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
6. **Center for Disease Control.** 1979. Follow-up on nosocomial *Pseudomonas cepacia* infection. Morbid. Mortal. Weekly Rep. **28**:409.
7. **Center for Disease Control.** 1979. Nosocomial infections caused by *Acinetobacter calcoaceticus*-United States, 1978. Morbid. Mortal. Weekly Rep. **28**:177–178.
8. **Engelbrecht, R. S., B. F. Severin, M. T. Masarik, S. Farooq, S. H. Lee, C. N. Hass, and A. Lalchandani.** 1977. New microbial indicators of disinfection efficiency. Environmental Protection Technology Series EPA-600/2-77-052. U.S. Environmental Protection Agency, Cincinnati, Ohio.
9. **Fierer, J., P. M. Taylor, and H. M. Gezon.** 1967. *Pseudomonas aeruginosa* epidemic traced to delivery room resuscitation. N. Engl. J. Med. **276**:991–996.
10. **Geldreich, E. E., H. D. Nash, D. J. Reasoner, and R. H. Taylor.** 1972. The necessity of controlling bacterial populations in potable waters; community water supply. J. Am. Water Works Assoc. **64**:596–602.
11. **Hazen, T. C., C. B. Fliermans, R. P. Hirsch, and G. W. Esch.** 1978. Prevalence and distribution of *Aeromonas hydrophilia* in the United States. Appl. Environ. Microbiol. **36**:731–738.
12. **Herman, L. S., and C. K. Himmelsbach.** 1965. Detection and control of hospital sources of *Flavobacteria*. Hospitals **39**:72–76.
13. **Hutchinson, D., R. H. Weaver, and M. Scherago.** 1943. The incidence and significance of microorganisms antagonistic to *Escherichia coli* in water. J. Bacteriol. **45**: 29.
14. **Joseph, S. W., O. P. Daily, W. S. Hunt, R. J. Seidler, D. A. Allen, and R. R. Colwell.** 1979. *Aeromonas* primary wound infection of a diver in polluted waters. J. Clin. Microbiol. **10**:46–49.
15. **Lamka, K. G., M. W. LeChevallier, and R. J. Seidler.** 1980. Bacterial contamination of drinking water supplies in a modern rural neighborhood. Appl. Environ. Microbiol. **39**:734–738.
16. **Lennette, E. H., E. H. Spaulding, and J. P. Truant (ed.).** 1974. Manual of clinical microbiology, 2nd ed. American Society for Microbiology Washington, D.C.
17. **McCabe, L. J., J. M. Symons, R. D. Lee, and G. G. Robeck.** 1970. Study of community water supply systems. J. Am. Water Works Assoc. **62**:670–687.
18. **Mood, E. W.** 1977. Bacterial indicators of water quality in swimming pools and their role, p. 239–246. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicator/hazards associated with water. American Society for Testing and Materials, Philadelphia.
19. **Muller, G.** 1977. Bacterial indicators and standards for water quality in the Federal Republic of Germany, p. 159–167. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/hazards associated with water. American Society for Testing and Materials, Philadelphia.
20. **Prier, J. E., and H. Friedman (ed.).** 1974. Opportunistic pathogens. University Park Press, Baltimore.
21. **Ptak, D. J., and W. Ginsburg.** 1977. Bacterial indicators of drinking water quality, p. 218–221. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/hazards associated with water. American Society for Testing and Materials, Philadelphia.
22. **Reitler, R., and R. Seligmann.** 1957. *Pseudomonas aeruginosa* in drinking water. J. Appl. Bacteriol. **20**: 145–150.
23. **Rippey, S. R., and V. J. Cabelli.** 1978. Membrane filter procedure for enumeration of *Aeromonas hydrophila* in fresh waters. Appl. Environ. Microbiol. **38**:108–113.
24. **Shayegani, M., A. M. Lee, and L. M. Parsons.** 1977. A scheme for identification of non-fermentative bacteria. Health Lab Sci. **14**:83–94.
25. **Taylor, R. H., and E. E. Geldreich.** 1979. A new membrane filter procedure for bacterial counts in potable water and swimming pool samples. J. Am. Water Works Assoc. **71**:402–406.
26. **Victoreen, H. T.** 1969. Soil bacteria and color problems in distribution systems. J. Am. Water Works Assoc. **61**: 429–431.
27. **Weaver, R. H., and T. Boiler.** 1951. Antibiotic producing species of *Bacillus* from well water. Trans. Ky. Acad. Sci. **13**:183–188.