APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1984, p. 361–366
0099-2240/84/080361-06$02.00/0

Vol. 48, No. 2

Isolation of *Aeromonas hydrophila* from a Metropolitan Water Supply: Seasonal Correlation with Clinical Isolates

VALERIE BURKE,[1,2] JENNIFER ROBINSON,[1] MICHAEL GRACEY,[1,2]* DENNIS PETERSON,[3] AND KIM PARTRIDGE[4]

Princess Margaret Children's Medical Research Foundation,[1] Department of Child Health, University of Western Australia,[2] State Health Laboratories,[3] and Metropolitan Water Authority,[4] Perth, Western Australia, Australia

Received 18 October 1983/Accepted 14 May 1984

The occurrence of *Aeromonas* spp. in the metropolitan water supply of Perth, Western Australia, Australia, was monitored at several sampling points during a period of 1 year. Water within the distribution system conformed to international standards for drinking water but contained *Aeromonas* spp. in numbers comparable to those in raw surface water, although this water was free of *Escherichia coli*. Coliforms and *E. coli* were found in raw surface waters, and *Aeromonas* spp. were found in raw water from surface and underground sources. Chemical treatment, followed by chlorination at service reservoirs, resulted in water free of *E. coli* and a decrease in the number of *Aeromonas* spp. *Aeromonas* spp. were found in the greatest numbers in summer. Multiple regression analysis showed that growth of *Aeromonas* spp. in chlorinated water was related to water temperature, residual chlorine, and interaction between these variables. The incidence of *Aeromonas*-associated gastroenteritis, determined from isolates referred to us for enterotoxin testing, paralleled the pattern of isolation of *Aeromonas* spp. in water within the distribution systems. We suggest that the presence of *Aeromonas* spp. in drinking water needs public health appraisal and that further work should be undertaken to permit reevaluation of standards for the quality of drinking water.

Aeromonas spp. occur widely in soil and surface waters (9, 14, 24) and have been isolated from drinking water, even after chlorination, in Canada (5), the United States (20), and India (26). *Aeromonas* spp. have been accepted as opportunistic pathogens in immunologically compromised hosts (6), and there is increasing evidence for their role as enteric pathogens in patients without known immunological abnormality (4, 12).

There have been several reports of non-gastrointestinal infections in humans after exposure to water contaminated with *Aeromonas* spp. (6, 13). Studies of toxigenicity of *Aeromonas* spp. isolated from drinking water (20) and from an estuary (18) have shown that such strains are capable of toxin production and may therefore be possible enteric pathogens in humans.

In the present study, isolation of *Aeromonas* spp. from the metropolitan water supply was monitored at sources and at several points throughout the distribution system for 1 year. Isolation of *Aeromonas* spp. from the water supply was compared with the incidence of cases of *Aeromonas*-associated gastroenteritis during the same period in the corresponding area.

MATERIALS AND METHODS

Source of water samples. Samples were collected at weekly intervals during the period from November 1981 to November 1982 from the drinking water supply of Perth, Western Australia, a city of 900,000 people on the west coast of Australia. Raw water from surface sources was treated with chlorine initially and then was rechlorinated at the service reservoirs. Water from underground sources was given conventional treatment involving alum coagulation, sedimentation, and rapid sand filtration to improve color and turbity and then was chlorinated at the service reservoirs. Water from all sources entered unroofed service reservoirs and was rechlorinated before distribution to consumers.

* Corresponding author.

Samples were collected from sites shown in Fig. 1. There were raw surface water and raw groundwater (level 1), surface water and groundwater samples immediately after chemical treatment (level 2), water sampled after chemical treatment but before entry into reservoirs (level 3), water in service reservoirs before rechlorination (level 4), water from service reservoir outlets immediately after chlorination (level 5), and water from service reservoir distribution systems beyond the site of chlorination (level 6).

Collection of samples. About 60 sites (range, 30 to 103) were sampled each week, and a total of 3,224 samples were examined during the year of study. Water temperature and free residual chlorine levels were recorded at the time of collection. Samples of 200 ml were collected into sterile, screw-capped glass bottles containing sodium thiosulfate. In further accordance with Australian standards (1), samples were chilled at the time of collection and during transfer to the laboratory. All samples were processed within 5 h of collection.

Microbiological methods. Membrane filtration techniques used were based on those recommended for the bacteriological examination of water supplies (15). Membrane enriched teepol broth (Oxoid Ltd., Basingstoke, England) was modified to contain 0.2% teepol (BDH 610; BDH, Poole, England). Table 1 summarizes the current protocol applied to the routine examination of drinking water in Western Australia.

Isolation of *Aeromonas* spp. Colonies of presumptive *Aeromonas* spp. were counted on the total coliform group membrane. Characteristic colonies were picked from the enriched teepol and subcultured onto blood agar containing 10 μg of ampicillin per ml. Oxidase-positive colonies were inoculated into Kaper medium (17) for identification. In our experience, results with this method are concordant with those obtained with multitest systems such as MBE 20 (Disposable Products, Adelaide, South Australia) or API 20E (Carter-Wallace, Sydney, Australia).

Colonies were enumerated when accurate counting was

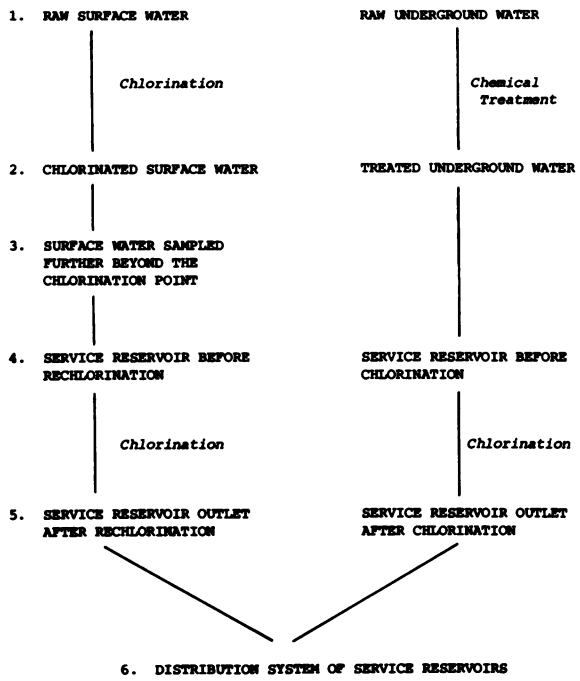

FIG. 1. Water sampling sites at stages in the processing of the Perth water supply.

possible, but some samples gave confluent growth so that colonies could not be counted adequately. For this reason, the following scale was adopted unless otherwise specified for quantitating *Aeromonas* spp. and was also applied to coliforms and *Escherichia coli* to facilitate comparison: 1, 1 to 9 colonies per 100 ml; 2, 10 to 49 colonies per 100 ml; 3, 50 to 99 colonies per 100 ml; 4, 100 to 149 colonies per 100 ml; 5, >149 colonies but not too numerous to count; 6, too numerous to count. The mean score was calculated for each time of sampling in relation to the number of sites sampled.

Chlorine concentration. Free and total chlorine levels were assayed by the Palin method (21) with *N,N*-diethyl-*p*-phenylenediamine (Lovibond 1000 comparator), and mean levels of free chlorine were calculated for each time of sampling.

***Aeromonas* spp.-associated gastroenteritis.** The laboratory of the Gastroenterology and Nutrition Research Unit, Princess Margaret Hospital, Perth, Australia, is used as a reference laboratory for enterotoxin testing of *Aeromonas* spp. isolated from patients with diarrhea. All such strains of *Aeromonas* spp. isolated from patients in Princess Margaret Hospital, which is the only children's hospital in Western Australia, and two major general hospitals in Perth were referred for enterotoxin testing. In addition, all *Aeromonas* spp. isolated from one private pathology laboratory in Perth were referred to us. The sample included two of the three major hospitals for adults in Perth but excluded patients treated privately, except for those whose specimens were sent to one of the above laboratories for fecal culture.

Statistical analysis. Stepwise multiple regression was used to analyze results involving the variable coliform group count, *E. coli* count, *Aeromonas* spp. score, number of cases of *Aeromonas* spp.-associated gastroenteritis, temperature of water, and concentration of free chlorine. Models were compared using the F test with a significance level of $P < 0.05$.

RESULTS

Distribution of bacteria and source of water samples. Raw water (level 1). Raw water from surface sources showed little variation in content of coliforms and *E. coli* throughout the year (Fig. 2). Means calculated with actual colony counts instead of scores were 19.7 (standard deviation, 40.5) for coliforms and 14.7 (standard deviation, 0.2) for *E. coli*. No fecal streptococci were isolated during this study. Untreated water from underground sources contained no coliforms or *E. coli*.

Weekly samples of untreated surface water were free of *Aeromonas* spp. on only two occasions during the year-long study. The colony count of *Aeromonas* spp. showed marked seasonal variations, and for this reason a mean value was not calculated.

Aeromonas spp. were isolated less frequently from samples of untreated water from underground sources. *Aeromonas* spp. were present on 10 occasions in the weekly samples of raw underground water. Three positive samples

TABLE 1. Routine bacteriological examination of drinking water by membrane techniques

Type of water	Sample preparation procedure					
	Total coliforms[a]	*E. coli*[a]	*Salmonella* spp.[b]	Fecal streptococci[c]	Total counts[d]	
					22°C	35°C
Raw surface, raw ground, clarified surface, or clarified ground	50 ml sample, 4 h at 30°C, 14 h at 35°C	100 ml sample, 4 h at 30°C, 14 h at 44°C	Membranes from enriched teepol broth[a] subcultured to SB broth[b] 48 h at 43°C	50 ml sample, 4 h at 37°C, 44 h at 45°C		
Chlorinated drinking (domestic)	100 ml sample, 6 h at 25°C, 18 h at 35°C	100 ml sample, 6 h at 25°C, 18 h at 44°C	Subculture 48 h[e]	Membrane to esculin iron agar; 30 min at 41°C	1.0 ml sample, 96 h	1.0 ml sample, 24 h

[a] Enriched teepol broth was modified to contain 0.2% teepol.
[b] Strontium chloride B broth (60 ml).
[c] Membrane enterococcus agar (27).
[d] Triphenyltetrazolium chloride in heart infusion broth; local modification.
[e] Bismuth sulfite agar-desoxycholate citrate agar.

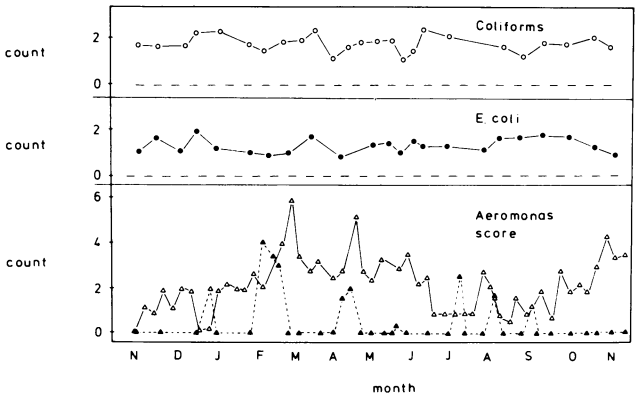

FIG. 2. *Aeromonas* spp., *E. coli*, and coliforms in raw water (level 1) from surface (solid lines) and underground (broken lines) sources during the year of study.

were found in consecutive weeks during late summer, and two consecutive samples were positive in midautumn. Otherwise, positive samples were sporadic, and non-*Aeromonas* spp. were found in the following weekly samples.

Water after initial chemical treatment (level 2). After chlorination, water from surface sources was free of *E. coli* and showed colony counts of one or two coliforms on only six occasions. No coliforms or *E. coli* were isolated from chemically treated underground water.

Aeromonas spp. were isolated on 36 occasions from the weekly samples of surface water after chlorination. Counts were lower than those in untreated surface water and showed little seasonal variation. Free chlorine levels exceeded 3 mg/liter on all but two occasions, but those occasions were associated with a fourfold increase in *Aeromonas* count. No *Aeromonas* spp. were isolated from treated underground water, which consistently showed higher levels of free chlorine than treated surface water did.

Water from surface sources was also sampled from sites beyond the point of chlorination (level 3) but before entry to service reservoirs and showed a further decrease in bacterial contaminants.

Service reservoirs (level 4). Service reservoirs containing previously treated water from surface sources before rechlorination (Fig. 3) showed levels of coliforms and *E. coli* similar to those found in untreated water, but water from

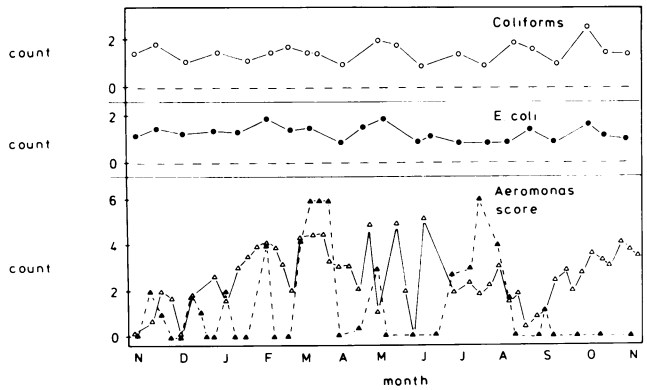

FIG. 3. *Aeromonas* spp., *E. coli*, and coliforms in water from service reservoirs before chlorination (level 4) during the year of study. Solid and broken lines represent values from surface and underground sources, respectively.

underground sources remained free of coliforms and *E. coli*. *Aeromonas* counts in water from surface sources were also similar to those in raw water and also showed seasonal variation. *Aeromonas* spp. were again isolated in water from underground sources. Levels were higher than in raw water, and on 17 occasions during the year, weekly samples contained *Aeromonas* spp.

Water at outlets from service reservoirs (level 5). Water from underground and surface sources sampled at the outlets of service reservoirs immediately after chlorination contained no coliforms or *E. coli*. *Aeromonas* spp. decreased in numbers when compared with prechlorination samples in both underground and surface waters. There was no obvious seasonal variation in *Aeromonas* counts, and most increases in the number of *Aeromonas* spp. appeared to be directly related to a temporary decrease in free chlorine levels. This was particularly noticeable with water samples from underground sources (Fig. 4).

Water from distributions of service reservoirs (level 6). Water sampled at sites throughout the distribution systems of service reservoirs showed no difference between water from surface and underground sources, and these samples are therefore considered in a single group. All samples were free of *E. coli*, and coliforms were detected on only four occasions. Colony counts did not exceed 3 in any sample.

Counts of *Aeromonas* spp. showed levels and seasonal variation very similar to the pattern seen with samples of raw surface water. Mean levels of free chlorine in these samples did not exceed 0.3 mg/liter, and there was no obvious relationship between *Aeromonas* counts and fluctuations in free chlorine levels (Fig. 5). The percentage of samples positive for *Aeromonas* spp. in general paralleled the mean count of *Aeromonas* spp.

Thus, initial chemical treatment effectively removed *E. coli* and coliforms from surface water samples and decreased *Aeromonas* counts, which became zero in water from underground sources. However, in service reservoirs before chlorination, coliforms and *E. coli* were again found in surface water samples; *Aeromonas* spp. were present in levels similar to those in raw water from both surface and underground sources. At the outlets of service reservoirs immediately after chlorination, no coliforms or *E. coli* were isolated, and *Aeromonas* levels decreased. Fluctuations in *Aeromonas* counts, particularly in water from underground sources, were related to changes in free chlorine levels. Beyond these sampling points, water from underground and surface sources remained free of *E. coli*, but *Aeromonas* spp. showed mean colony counts and seasonal variation similar to the pattern in untreated surface water.

Distribution of *Aeromonas* spp.-associated gastroenteritis. Figure 5 shows the distribution of isolations of *Aeromonas* spp. from patients with gastroenteritis seen during the year of the study. These patients do not include all patients with *Aeromonas* spp.-associated gastroenteritis during this period but represent only those patients whose fecal *Aeromonas* spp. were referred to our laboratory for enterotoxin testing. However, the distribution of these cases closely paralleled the variation in mean number of *Aeromonas* spp. in water sampled beyond the chlorination points of service reservoirs (level 6).

Relation between *Aeromonas* counts, free chlorine, and temperature. Seasonal variation in mean *Aeromonas* counts closely paralleled mean water temperature in samples which were either unchlorinated or had free chlorine values consistently less than 0.3 mg/liter (Fig. 2, 4, and 5). The relationship between these variables was less obvious in chlorinated

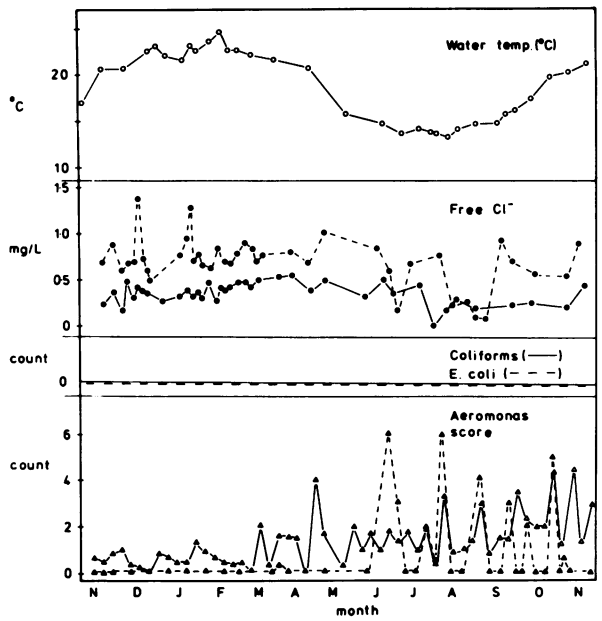

FIG. 4. *Aeromonas* spp., *E. coli*, and coliforms in relation to month, temperature, and free chlorine levels in water at outlets from service reservoirs immediately after chlorination (level 5). Water from underground sources (not shown) and surface sources was free of *E. coli* (---) and coliforms (—). The solid lines and broken lines in the free chlorine and *Aeromonas* score panels represent surface and underground sources, respectively.

samples (Fig. 4). The association among water temperature, free chlorine, and *Aeromonas* counts becomes clearer when the samples from a single point throughout the year are considered. Figure 6 shows the relationship between water

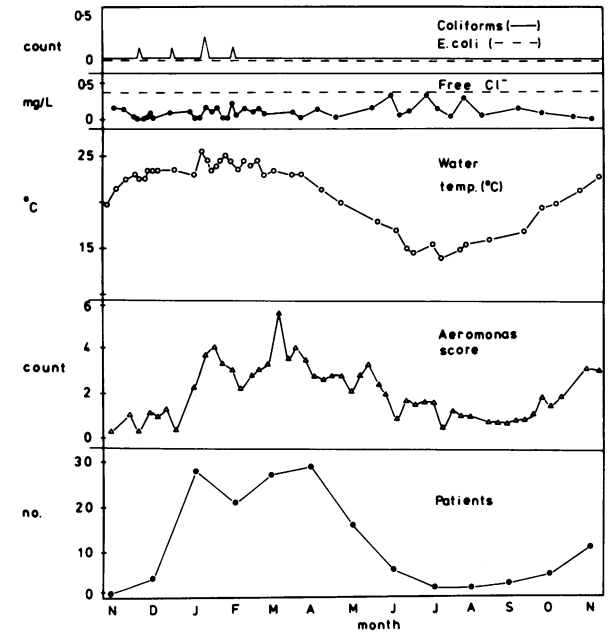

FIG. 5. *Aeromonas* spp., *E. coli*, and coliforms in water sampled from distributions beyond chlorination at service reservoirs (level 6), both surface and underground sources, in relation to month, temperature, and free chlorine. The distribution of cases of *Aeromonas* spp.-associated gastroenteritis is shown for the same period.

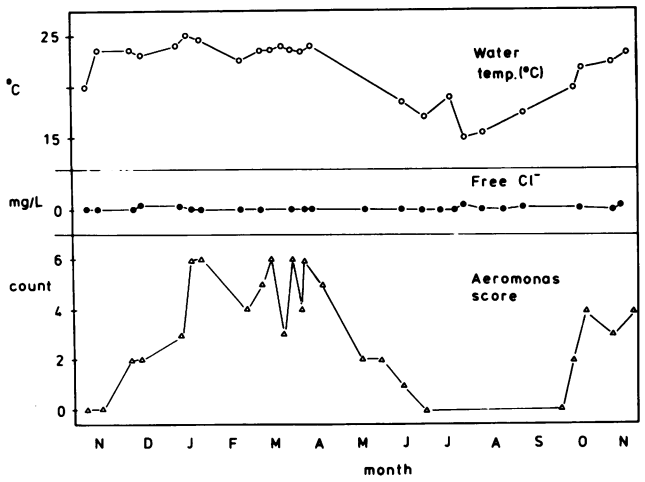

FIG. 6. *Aeromonas* spp. in relation to water temperature in samples from a single site (level 6) during the year of study. *Aeromonas* counts were clearly related to variations in temperature in samples which were almost free of chlorine.

temperature and *Aeromonas* counts in water sampled at a single point in the service reservoir distribution (level 6), where free chlorine levels were zero for most of the year. Figure 7 shows the more direct relationship between free chlorine levels and *Aeromonas* counts in surface water sampled at a single point beyond the site of initial chemical treatment but before entry into the service reservoir (level 3). In this sample, peaks of *Aeromonas* counts throughout the year corresponded with periods in which free chlorine levels were low.

Multiple regression analysis. *Aeromonas* counts in samples from untreated surface water required only temperature as an independent variable in the regression equation ($P < 0.05$). *E. coli* or coliform counts were not significantly related to *Aeromonas* counts.

Similarly, in water from service reservoirs and from distributions beyond the chlorination points of service reser-

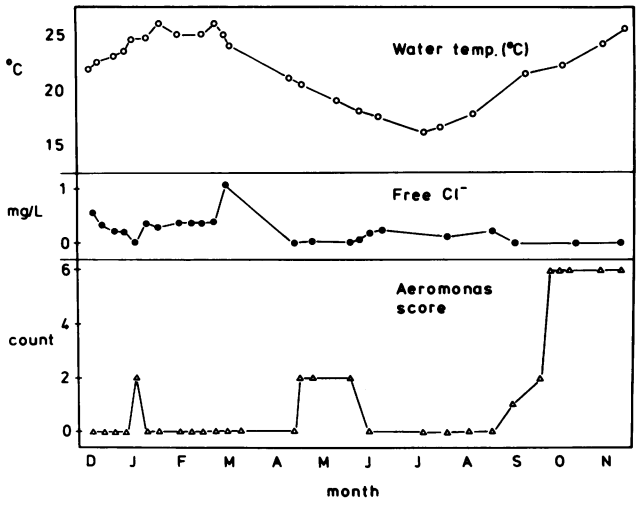

FIG. 7. *Aeromonas* spp. in relation to chlorine levels in samples from a single site (level 3) during the year of study. Variations in *Aeromonas* counts were related to a decrease in free chlorine levels.

voirs, where levels of free chlorine were consistently less than 0.3 mg/liter, temperature was the only independent variable required in the regression equation. Deletion of free chlorine levels as an independent variable from the regression equation resulted in a decrease of 1.6 in the F value, which was not statistically significant ($P > 0.05$).

Water samples in which free chlorine levels were mainly greater than 0.3 mg/liter showed *Aeromonas* scores (A) to be predicted by the equation $A = 7.29 + 0.28T - 17.6C + 0.76$ TC, where T is water temperature, C is free chlorine, and TC is a term which accounts for interaction between free chlorine and temperature. F tests showed that all three independent variables were required in the regression equation ($P < 0.05$).

Multiple linear regression relating the number of cases of *Aeromonas* spp.-associated gastroenteritis to *Aeromonas* counts and water temperature in the samples from the distributions of service reservoirs showed that only *Aeromonas* counts were required as the independent variable in the regression equation, although temperature and number of cases were correlated (correlation coefficient, 0.73). This reflects the high correlation (correlation coefficient, 0.804) between water temperature and *Aeromonas* counts in these samples. The F value for deletion of temperature from the larger model was 0.06, which is not statistically significant ($P > 0.05$). The univariate model relating numbers of patients (P) with *Aeromonas* spp.-associated gastroenteritis and *Aeromonas* counts (C) in water samples was $P = -6.69 + 7.94C$.

DISCUSSION

This study has shown that drinking water supplies in Perth, although conforming to recommended international standards for drinking water (29), contained large numbers of *Aeromonas* spp. These organisms were present in raw water, and although their numbers decreased temporarily after chlorination, water distributed to consumers contained *Aeromonas* spp. in concentrations similar to those in untreated water. In contrast, drinking water in distributions beyond the service reservoir contained no *E. coli*, and coliforms were present rarely, with colony counts not greater than 3.

Aeromonas spp. were found most frequently and in greatest numbers during summer. This association has previously been found with *Aeromonas* spp. isolated from chlorinated drinking water (20) as well as from surface waters in the United States (9, 14, 25). Isolation of *Aeromonas* spp. from drinking water in Perth was associated, in general, with water temperatures greater than 14.5°C in unchlorinated samples.

Multiple regression analysis showed that growth of *Aeromonas* spp. in chlorinated samples was related to water temperature, content of free chlorine, and interaction between these two variables.

The number of patients with *Aeromonas* spp.-associated gastroenteritis whose fecal *Aeromonas* isolates were referred to our laboratory for enterotoxin testing closely followed the distribution of *Aeromonas* spp. in drinking water. This association must be interpreted with caution since the patients do not represent a random sample, nor do they include all patients with *Aeromonas* spp.-associated gastroenteritis during the period of the study. Moreover, the apparent relationship between the distribution of *Aeromonas* spp. in drinking water and the isolation of fecal *Aeromonas* spp. from patients with diarrhea may in fact be explained by their association with another variable such as temperature. In a similar study of an unchlorinated domestic water supply in a Western Australian country center, fecal isolation of *Aeromonas* spp. also showed temporal correlation with isolation of *Aeromonas* spp. from water. However, *Aeromonas* isolation did not correlate with environmental temperature (3). This suggests that temperature is not the determining factor.

Aeromonas spp. isolated from chlorinated drinking water in the United States have been shown to possess virulence factors (20) possibly associated with enteric disease. Although we have found differences in the pattern of biochemical characteristics, toxin production, and hemagglutination in *Aeromonas* spp. isolated from water and from feces, many of the strains from water show properties identical to those of fecal isolates (2). For example, about 91% of fecal isolates were enterotoxigenic, compared with 70% of strains from water. We suggest that such strains in water may be potential enteric pathogens. We are currently investigating the epidemiology of *Aeromonas* spp.-associated diarrhea in Perth in relation to biotype and virulence factors of *Aeromonas* spp. in domestic and recreational waters to which individual patients are exposed.

Aeromonas spp. must also be considered in relation to nonenteric disease. Nonenteric infections resulting from exposure to water contaminated with *Aeromonas* spp. have been reported frequently (10, 13, 16) and are particularly hazardous in patients who are immunologically compromised (28). Contaminated water is also a potential risk for patients undergoing dialysis (8, 23).

For these reasons, we suggest that the presence of *Aeromonas* spp. in water supplied for domestic use needs public health appraisal. The coliform count did not correlate with the *Aeromonas* count in our study or in reports from North America (5, 20) and therefore cannot be used as an index of contamination with *Aeromonas* spp. Several authors have questioned the suitability of coliforms as an index of water quality (7, 11, 19, 22). Various indicator organisms have been suggested to replace or supplement this index, and measurement of chlorine residuals has also been proposed as an indicator of the quality of drinking water (22), but none of these alternatives has gained universal acceptance. The finding of large numbers of potentially pathogenic organisms in drinking water in our study and in North America (5, 20) suggests that standards for the quality of drinking water need to be reevaluated.

ACKNOWLEDGMENTS

This work was supported financially by the TVW Telethon Foundation, Perth, and the Wellcome Trust, London.

We thank the Commissioner of Public Health and the Managing Director of the Metropolitan Water Authority of Perth for permission to undertake this study, and we thank their staffs for technical advice and assistance.

LITERATURE CITED

1. **Australian Standards Association.** 1977. Recommendations for the selection of containers and preservation of water samples for chemical and microbiological analysis (Australian Standard 2031). Part 2, Microbiological. Sydney, Australia.
2. **Burke, V., J. Robinson, M. Cooper, J. Beaman, K. Partridge, D. Peterson, and M. Gracey.** 1984. Biotyping and virulence factors in clinical and environmental isolates of *Aeromonas* species. Appl. Environ. Microbiol. **47**:1146–1149.
3. **Burke, V., J. Robinson, M. Gracey, D. Peterson, N. Meyer, and V. Haley.** 1984. Isolation of *Aeromonas* spp. from an unchlorinated domestic water supply. Appl. Environ. Microbiol. **48**:367–370.
4. **Chatterjee, B. D., and K. N. Neogy.** 1972. Studies on Aeromonas and Plesiomonas species isolated from cases of choleraic diar-

rhoea. Indian J. Med. Res. **60**:520–524.
5. **Clark, J. A., G. A. Burger, and L. E. Sabatinos.** 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can. J. Microbiol. **28**:1002–1013.
6. **Davis, W. A., J. G. Kane, and V. F. Garagusi.** 1978. Human Aeromonas infections: a review of the literature and a case report of endocarditis. Medicine (Baltimore) **57**:267–277.
7. Dutka, B. J. 1973. Coliforms are an inadequate index of water quality. J. Environ. Health **36**:39–46.
8. **Favero, M. S., N. J. Peterson, and K. M. Boyer.** 1974. Microbial contamination of renal dialysis systems and associated health risk. Trans. Am. Soc. Artif. Intern. Organs **20A**:175–180.
9. **Fliermans, C. B., R. W. Gorden, T. C. Hazen, and G. W. Esch.** 1977. *Aeromonas* distribution and survival in a thermally altered lake. Appl. Environ. Microbiol. **33**:114–122.
10. **Fulghum, D. D., W. R. Linton, and D. Taplin.** 1978. Fatal *Aeromonas hydrophila* infection of the skin. South. Med. J. **71**:739–741.
11. **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.
12. **Gracey, M., V. Burke, and J. Robinson.** 1982. Aeromonas-associated gastroenteritis. Lancet **ii**:1304–1306.
13. **Hanson, P. G., J. Standridge, F. Jarrett, and D. G. Maki.** 1977. Freshwater wound infection due to *Aeromonas hydrophila*. J. Am. Med. Assoc. **238**:1053–1054.
14. **Hazen, T. C., C. B. Fliermans, R. P. Hirsh, and G. W. Esch.** 1978. Prevalence and distribution of *Aeromonas hydrophila* in the United States. Appl. Environ. Microbiol. **36**:731–738.
15. **Her Majesty's Stationery Office.** 1969. The bacteriological examination of water supplies. Reports on public health and medical subjects, no. 71. London, England.
16. **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.
17. **Kaper, J., R. J. Seidler, H. Lockman, and R. R. Colwell.** 1979. Medium for the presumptive identification of *Aeromonas hydrophila* and *Enterobacteriacae*. Appl. Environ. Microbiol. **38**:1023–1026.
18. **Kaper, J. B., H. Lockman, R. R. Colwell, and S. W. Joseph.** 1981. *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. J. Appl. Bacteriol. **50**:359–377.
19. **Kott, Y.** 1977. Current concepts of indicator bacteria, p. 3–13. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazards associated with water. ASTM STP 635. American Society for Testing and Materials, Philadelphia, Pa.
20. **Le Chevallier, M. W., T. M. Evans, R. J. Seidler, O. P. Daily, B. R. Merrell, D. M. Rollins, and S. W. Joseph.** 1982. *Aeromonas sobria* in chlorinated drinking water supplies. Microb. Ecol. **8**:325–333.
21. **Palin, A. T.** 1957. The detection of free and combined chlorine in water by the use of diethyl-*p*-phenylene-diamine. J. Am. Water Works Assoc. **49**:873–880.
22. **Ptak, D. J., and W. Ginsberg.** 1977. Bacterial indicators of drinking water quality, p. 218–221. *In* A. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazards associated with water. ASTM STP 635. American Society for Testing and Materials, Philadelphia, Pa.
23. **Ramsay, A. M., B. J. Rosenbaum, C. L. Yarbrough, and J. A. Hotz.** 1978. *Aeromonas hydrophila* sepsis in a patient undergoing hemodialysis therapy. J. Am. Med. Assoc. **239**:128–129.
24. **Schubert, R. H. W.** 1967. Das Vorkommen der Aeromonaden in oberirdischen Gewässern. Arch. Hyg. **150**:688–708.
25. **Seidler, R. J., D. A. Allen, H. Lockman, R. R. Colwell, S. W. Joseph, and O. P. Daily.** 1980. Isolation, enumeration, and characterization of *Aeromonas* from polluted waters encountered in diving operations. Appl. Environ. Microbiol. **39**:1010–1018.
26. **Sen, R., and B. Jacobs.** 1969. Pathogenic intestinal organisms in the unfiltered water supply of Calcutta and the effect of chlorination. Indian J. Med. Res. **57**:1220–1227.
27. **Slanetz, L. W., and C. H. Bartley.** 1957. Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J. Bacteriol. **74**:591–595.
28. **Wolff, R. L., S. L. Wiseman, and C. S. Kitchens.** 1980. *Aeromonas hydrophila* bacteremia in ambulatory immuno-compromised hosts. Am. J. Med. **68**:238–242.
29. **World Health Organization.** 1971. International standards for drinking water, 3rd ed. World Health Organization, Geneva.