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Klebsielleae in Drinking Water Emanating from Redwood Tanks

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A survey was made of the bacteriological quality and chlorine content of 33 public and private water systems that utilize redwood storage tanks. Coliforms of the genera *Klebsiella* and *Enterobacter* were isolated from 9 of 10 private drinking water systems and from 11 of 23 water systems in state and federal parks. Total coliform counts in the private systems exceeded federal membrane filter guidelines by as much as 10- to 40-fold. Coliform counts were highest in the newer reservoirs. Factors contributing to poor water quality are: lack of automated chlorination equipment or an insufficient supply to maintain a residual, common inlet/outlet plumbing design, and lengthy average retention periods. The latter two factors contribute to improper mixing and stagnation of the water, whereas the former allows microbes to multiply on the water-soluble nutrients that leach from the wood. Wooden reservoirs exert a high chlorine demand, and 0.4 ppm of chlorine residual in the incoming tank water proves inadequate. It is suggested that specific water-soluble nutrients in redwood (and in numerous other types of botanical material) induce a natural nutritional selection for coliforms of the tribe *Klebsielleae*.

In the summer of 1974 the cause of excessive coliform counts in the drinking water of a rural Oregon community was investigated. The point of origin was traced to a 50,000-gallon (ca. 189,250-liter) wooden storage reservoir constructed of redwood. The coliforms present were *Klebsiella pneumoniae* and *Enterobacter* species. Further surveys of similar water systems indicated that this was not an isolated situation; more extensive studies of this coliform contamination problem are reported in this paper.

Redwood tanks are in wide use throughout the western United States, Canada, South America, and the Pacific islands. Their primary function is to store water for drinking purposes. These tanks serve the water needs in recreational areas, motels, mobile home parks, and communities and cities in the above-mentioned locations. Redwood is also used for construction of whirlpool baths used in hotel/motel establishments throughout the United States. Redwood water storage tanks are often used in state and federal camping areas in western states, and in Oregon alone about 30 tanks serve over 1,000,000 annual day visitors and over 600,000 annual camper nights.

Much has been written in the literature concerning the ubiquitous distribution of *K. pneumoniae* in the environment, especially its association with industrial effluents containing botanical milieu (pulp and paper and textile mills and potato processing plants [4, 6, 10, 12–14]). *K. pneumoniae* has also been isolated from the surfaces of fresh vegetables and from within living white fir trees (1, 5, 7). Although the health significance of environmental *Klebsiella* strains is still being debated, human and animal clinical isolates are pathogens of documented importance. Studies have indicated that isolates from receiving waters and other habitats are indistinguishable from human and animal pathogenic isolates in terms of their biochemical reactions and virulence in mice (6, 11, 15).

Although a descriptive report appeared in 1964 on coliforms in redwood storage tanks (9), the present study is the first to document a problem specifically linking wood-associated *K. pneumoniae* with water used for drinking purposes. Regardless of the uncertain significance of the opportunistically pathogenic *Klebsiella* in the environment, federal and state drinking water standards are being exceeded in many situations. In this report a study is presented of the quantitative aspects of the association of *Klebsielleae* in drinking water emanating from wooden reservoirs. In addition to the identification of the coliforms present, the levels of coliform contamination are correlated with improper and abusive use of these reservoirs in terms of inadequate or no chlorination, im-

properly designed plumbing systems, and excessively long water retention periods.

MATERIALS AND METHODS

Sampling of field tanks. Operational redwood water storage systems serving a variety of private and public water supply systems were examined at 33 locations throughout Oregon. Whenever possible, the water sample was removed from the interior of the tank by submerging a sterile, 1-liter flask under the water surface. When the tank was not accessible (as in most of the public water systems), the sample was taken from the most available location on the service line. Under such circumstances, the water was run full force for 60 s prior to taking the sample.

Combined chlorine concentration (parts per million) was determined by the orthotolidine (OTO; 2) technique (early survey, see Table 1), and free chlorine was determined by the *N,N*-diethyl-*para*-phenylenediamine (DPD) method (2) in all other determinations. The minimum sensitivity to chlorine concentration was about 0.1 ppm. Sodium thiosulfate was routinely added to all samples to remove any free chlorine (2). Water samples were stored on ice and transported to the laboratory for processing. Samples were processed generally within 6 h after collection; two samples were processed after 24 h.

Information concerning tank size and age and chlorination and engineering designs were obtained from questionnaires mailed to the various purveyors.

Processing of water samples. Water samples from each tank were examined for the following parameters: (i) total coliforms, (ii) species of coliforms present, (iii) fecal coliforms (public water systems only), and (iv) chlorine content.

Total coliforms were enumerated on m-Endo agar LES (Difco) by the membrane filter technique (2). For comparative purposes, total coliforms in some samples were also enumerated by the most probable number (MPN) technique (2). Ten-, one-, and one-tenth-ml water samples were inoculated into five replicate lactose broth tubes, incubated at 35°C, and examined for gas production after 24 and 48 h; positive tubes were streaked onto EMB agar (Difco) for confirmation. Fecal coliforms were enumerated by the membrane filter procedure (2), using mFC agar (Difco).

Laboratory redwood tanks. Small, 65-liter laboratory redwood tanks were used in an attempt to simulate contamination problems observed in field tanks. Three tanks were studied: one (tank A) was of kiln-dried wood, whereas the staves in the two other tanks were air-dried (tanks B and C). The tanks were constructed by National Tank and Pipe Co., Portland, Ore. All were made from redwood heartwood and measured 23 inches (ca. 58.4 cm) in height, with a 24-inch (ca. 61-cm) diameter. Each tank contained 22 staves of 2.5 by 3.5 inches (ca. 6.3

TABLE 1. *Field survey of private drinking water emanating from wooden reservoirs*[a]

Site	Capacity (gallons)	Use	Age (mo)	Automated chlorination	Cl_2 residual (ppm)	Common inlet/outlet	No. of user households	Estimated ART[b] (days)	No. of total coliforms/100 ml	Coliform
1	25,000	MH	17	Yes	0.3	No	20	4–6	0/MF Coliforms in slime	*K. pneumoniae*
2	25,000	R	10	Yes	0.3	No	30	1–2	0/MF	NCD
3	6,000	R	48–72	—[c]	NC	Yes	4	4–5	2/MF	*Enterobacter*
4	50,000	R	24–48	—[c]	NC	Yes	50	3	2/MF	*K. pneumoniae*, *Enterobacter*
5	50,000	R	10	—[c]	NC	Yes	50	3	15/MF	*K. pneumoniae* *Enterobacter*
6	50,000	R	20	No[d]	NC	Yes	68	2	150/MF	*K. pneumoniae*
			29						0/MF[e]	NCD
7	50,000	R	19	No[d]	NC	Yes	50	3	200/MF	*K. pneumoniae* *Enterobacter*
			28		NC				14/MF[e]	*K. pneumoniae* *Enterobacter*
8	150,000	R[f]	3	No	NC	Yes	None		200/MF	*K. pneumoniae*, *Enterobacter*
			12		NC				60/MF[e]	*K. pneumoniae*, *Enterobacter*
9	50,000	R	3	Yes	NC	Yes	4	40	80/MF	*K. pneumoniae*
			12		NC				1/MF[e]	*K. pneumoniae*
10	50,000	R	5	Yes	NC	No	5	30	150/MF	*K. pneumoniae*
			14		NC				0/MF[e]	NCD

[a] Abbreviations: MH, mobile home park; R, residential use; NC, no chlorine (combined) at a detection limit of 0.1 ppm as determined by the OTO method (2); NCD, no coliforms detected; MF, membrane filtration.

[b] Estimated average retention time (ART) obtained from purveyor or assuming 350-gallon daily usage per home.

[c] Water entered the tank via the city line at 0.2 to 0.4 ppm of chlorine residual. There was no further chlorination at tank site.

[d] Tank was "occasionally" hand chlorinated with tablets.

[e] Results of second sampling.

[f] At the time of the first sampling, the tank was not in service.

by 8.9 cm). The tank tops were sealed with 0.75-inch (ca. 1.9-cm) plywood.

Bacteriological and chlorine monitoring was accomplished by removing samples from within the tanks and testing as indicated for the field tanks. Total bacterial counts were made on nutrient agar (Difco) incubated at 30°C for 24 h. Qualitative bacterial counts were also made by lowering the water and scraping with a sterile microscope slide an approximately 15-cm² area of selected staves that had been immersed below the water level. Any slime material was dispersed in 0.01 M tris(hydroxymethyl)aminomethane buffer (pH 7) and plated onto either nutrient agar or m-Endo agar LES.

Media and coliform identification. Media preparation and procedures used for the identification of coliform organisms were those recommended by Edwards and Ewing (8).

RESULTS

The field survey results are presented in Tables 1 and 2 for private and public water systems, respectively. Drinking water from only two of the ten redwood reservoirs serving private residences was free from coliforms (Table 1). The two coliform-free systems were the only samples with detectable chlorine in the water. Counts in most other systems far exceeded federal and Oregon regulations for total coliform counts. Coliform isolates were always of the genera *Klebsiella* and *Enterobacter*; *Escherichia coli* was never isolated during this survey.

In general, the tank systems less than 1 year old at sites 5, 8, 9, and 10 yielded the highest coliform counts. Two older systems (sites 6 and 7) yielded high counts, but have no automated chlorination system. Samples from sites 1 and 2, with 17- and 10-month operational systems, respectively, were potable, but the water contained a chlorine residual. The two oldest systems (sites 3 and 4) yielded coliform counts under the allowable limit, even though there was no detectable chlorine.

Figures 1 and 2 show a portion of the staves below water level as seen in a redwood reservoir less than 1 year old. The light staves on the right of Fig. 1 illustrate the microbial slime matrix that gradually accumulates on the wood surface. The slime contains bacteria embedded in mycelial stages of fungal growth. Note that all staves are not coated with macroscopically obvious growth. This may be due to differences in the water-soluble extractive content of the individual staves (J. Behrens, National Tank and Pipe, personal communication). Figure 2 shows a close-up view of the matrix that has been partly scraped off to demonstrate its depth of accumulation.

At 9 months after the initial survey, five of the original sites were resampled. In each case there was a significant reduction in the coliform counts. Most significant was the fact that three samples previously exceeding standards now yielded satisfactory water (sites 6, 9, and 10). *K. pneumoniae* and *Enterobacter* spp. were again isolated from three of the five tanks.

Of the four sites with automated chlorination systems, it was possible to examine the residual in the incoming tank water only at site 1. The total and free chlorine (DPD method) was in the range of 1 to 1.5 ppm for several samples taken over about 1 year. The residual on the service lines and in the tank water was about 0.3 ppm. The chlorine demand of the wooden reservoir is, therefore, very high and undoubtedly accounts for the lack of chlorine in the other systems. Sampling results from tank sites 3 to 5 serve to confirm this high chlorine demand. Although all three tanks are on city water lines and receive water with a residual of

TABLE 2. *Field survey of public drinking water emanating from wooden reservoirs*[a]

Site	Cl_2 residual (ppm)	No. of total coliforms/ 100 ml	No. of fecal coliforms/ 100 ml	Coliform
1	NC	0/MF, 2/MPN	0	*K. pneumoniae, Enterobacter*
2	NC	5/MF, 8/MPN	0	*K. pneumoniae, E. coli, Enterobacter*
3	NC	1/MF, <2/MPN	1	*Enterobacter*
4	NC	1/MF, 5/MPN	0	*Enterobacter*
5	NC	0/MF, 2/MPN	0	*Enterobacter*
6	NC	0/MF, 5/MPN	1	*K. pneumoniae, E. coli, Enterobacter*
7	NC	1/MF, <2/MPN	0	*Enterobacter*
8	NC	2/MF, 2/MPN	0	*Enterobacter*
9	NC	2/MF, 6/MPN	2	*K. pneumoniae, E. coli, Enterobacter*
10	0.2–0.6	3/MF, 14/MPN	1	*K. pneumoniae, E. coli, Enterobacter*
11	0.2	1/MF, 2/MPN	0	*K. pneumoniae, Enterobacter*

[a] The chlorine concentration was measured by the DPD method. NC indicates no detectable chlorine (free or combined); detectable chlorine (parts per million) refers to free chlorine residual. A total of 23 parks were surveyed. The 11 parks summarized above yielded coliforms, whereas the remaining 12 were coliform-free. MF, Membrane filtration.

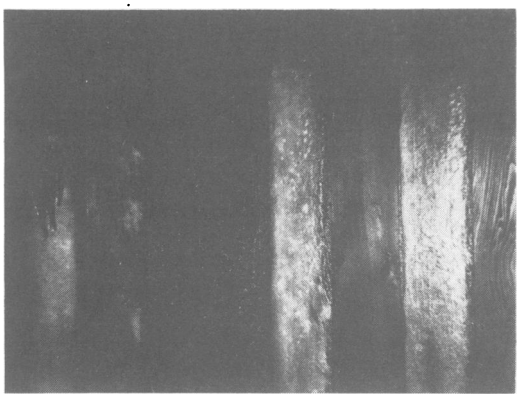

FIG. 1. *Inside of a redwood tank that had been in use for less than 1 year. The lighter staves on the right are coated with slime consisting of bacteria and fungi. All staves are not evenly colonized.*

approximately 0.2 to 0.4 ppm, samples removed from the tank contained no detectable chlorine.

At seven of these ten test sites serving homes, the water enters and leaves the tank through the same pipe, which is commonly located in the bottom of the reservoir (common inlet/outlet). During periods of simultaneous tank filling and high water demands, the water, in effect, enters the homes directly from the source (city lines, wells, etc.). This minimizes mixing of the tank water, increases the average retention time, and further aggrevates the chlorine demand.

In a separate survey conducted during the summer of 1975, drinking water samples were examined from 23 Oregon state parks. All parks in this survey use redwood water storage reservoirs. In each park, three to five samples were taken from separate faucets. Table 2 shows the highest coliform counts in each system yielding coliforms. Unfortunately, detailed information on plumbing, chlorination, and tank age was not readily available. Of those tanks actually observed, all would be considered approximately 3 years of age or older. In all cases, the coliform counts were significantly lower than those observed in the newer residential tank systems. Nevertheless, sites 2 and 10 exceeded federal limits for a single sampling. At 9 months after the first survey, 10 parks were resampled. None of these samples exceeded the coliform limits, and neither *Klebsiella* nor *E. coli* was isolated.

A significant difference from the water survey of residential systems was indicated by the isolation of *E. coli* from four parks. Samples containing *E. coli* had MPN total coliform counts of 5 to 14 per 100 ml. However, site 4, with an MPN count of 5, contained only *Enterobacter*. *K. pneumoniae* was isolated from six parks, four times in association with *E. coli*. It was discovered that the water at site 10 was contaminated from a sewage line break, and the facilities were closed shortly after our sampling. The influx of sewage may account for the high counts at this site even though a chlorine residual (DPD test) was detected in the water.

In an attempt to gain an understanding of the origin of *Klebsielleae* in wooden reservoir water, three small, 65-liter redwood tanks were constructed for laboratory experiments. The tanks were filled with municipal water containing 0.4 ppm of chlorine residual, covered, and monitored for up to 50 days. A summary of the bacteriological monitoring is provided in Table 3. As in the field tanks, coliforms of the genera *Klebsiella* and *Enterobacter* were recovered from the tank water and staves. Tanks constructed of kiln-dried staves accumulated fewer coliforms than did the air-dried tank, but the coliform counts in the former were nevertheless significant. Periodic monitoring of 1-liter samples of municipal water entering the tanks indicated that it was coliform-free during the entire course of the experiments. One would have to assume that the coliforms entered as air contaminants when the lid was removed for sampling and then rapidly multiplied in the water and on the stave surface, or, perhaps, the coliforms were originally present in or on the wood surfaces. Recent contamination of tank water by rodents, birds, or insects was therefore not necessary for *Klebsielleae* to be present in considerable numbers.

A significant feature of these monitoring experiments was the observation that a very small fraction of the total coliform population was being detected on m-Endo agar LES. For

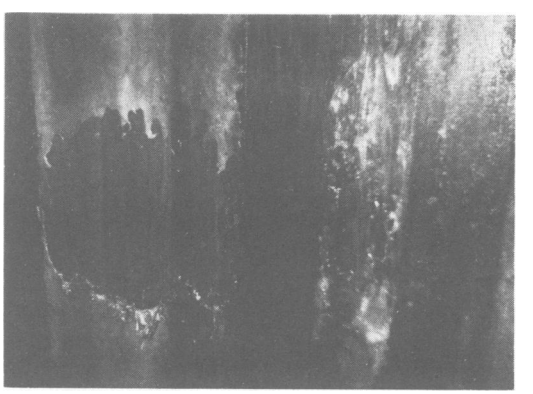

FIG. 2. *Partially removed microbial accumulation illustrating depth of slime mass.*

TABLE 3. *Bacteriological monitoring of 65-liter laboratory redwood tanks*

Tank	Day	Test performed	Observation	Coliform
A (kiln-dried wood)	1	1-liter MF[a]	No coliforms	
	2	Drain and refill with 0.4 ppm of chlorine		
	4	1-liter MF	11 coliforms	*K. pneumoniae*
	10	1-liter MF	No coliforms	
	14	Slime scraped	Heavy coliform growth	
	14	Drain, scrub with 200 ppm of chlorine, and refill		
	16	500-ml MF	No coliforms	
	21	1-liter MF	43 coliforms	*K. pneumoniae*
	21	Drain and refill with 0.4 ppm of chlorine		
	30	100-ml MF	1 coliform	*K. pneumoniae*
	42	100-ml MF, nutrient agar plate counts	No coliforms; total count, 3×10^3/ml	
	48	100-ml MF, nutrient agar plate counts	No coliforms; total count, 4.5×10^3/ml	
	50	Nutrient agar plate counts	Total count, 9.5×10^3/ml	*K. pneumoniae* from 10^{-2} and 10^{-3} plate dilutions
C (air-dried wood)[b]	1	1-liter MF	7 coliforms	
	3	Staves scraped, drained, and refilled with 0.4 ppm of chlorine	Coliforms present	
	5	1-liter MF	No coliforms	
	6	500-ml MF	Coliforms present	
	8	500-ml MF	1,000 coliforms	*E. agglomerans*
	22	100-ml MF	3 coliforms	*K. pneumoniae* and *E. agglomerans*
	34	100-ml MF, nutrient agar plate counts	300 coliforms; total count, 24×10^3/ml	
	35	25-ml MF	120 coliforms	
	40	25 ml MF, nutrient agar plate counts	150 coliforms; total count, 35×10^3/ml	
	42	Nutrient agar plate counts	Total count, 16.5×10^3/ml	*K. pneumoniae* from 10^{-2} and 10^{-3} plate dilutions

[a] MF, Membrane filtration.

[b] The air-dried tank was filled with water and held in this condition for 7 days prior to shipping to the laboratory at Oregon State University.

example, in water from tank A (Table 3), the total coliform counts never exceeded 4.3 per 100 ml. Nevertheless, on day 50 it was possible to easily recover *K. pneumoniae* isolates from the 10^{-2} and 10^{-3} dilutions on nutrient agar. Similarly, on day 40, tank C contained 600 coliforms per 100 ml, whereas a total count on day 42 indicated the *K. pneumoniae* was also present at 10^{-2} and 10^{-3} dilutions. Additional enumeration experiments on all laboratory tanks indicated that approximately 2,000-fold-higher coliform counts were obtained on nutrient agar as compared with m-Endo agar LES. This ratio was estimated by isolating and identifying total colonies growing on nutrient agar, determining the coliform fraction, and comparing this count with that obtained on m-Endo agar LES. Coliform counts on m-Endo agar LES were not obscured by overgrowth of noncoliforms. Therefore, it would appear that only a small fraction of the total coliform population was capable of growing upon initial plating on the selective/differential medium.

A surprising observation was the repeated occurrence of coliforms after refilling the small tanks with chlorinated municipal water and even after scrubbing with a solution of 200 ppm of chlorine (tank A, Table 3). This recurrence could only occur if the coliforms were uniquely chlorine resistant or if the chlorine demand of the wood was sufficiently high to rapidly reduce its effectiveness.

A lack of unique chlorine resistance was demonstrated in laboratory experiments in which seven isolates of *K. pneumoniae* (five of redwood origin and two of human origin) were studied. In these experiments cell densities ranging from approximately 1,000 to 3,000 per 100 ml were exposed in glass containers to municipal water containing 0.4 to 0.7 ppm of chlorine or distilled water (no chlorine). Cell counts in the distilled water remained constant over the 60-min study period, whereas all municipal water samples sterilized the inoculum within 30 to 60 s. The continuing occurrence of chlorine-sensitive coliforms after the addition of chlorinated water (up to 200 ppm) could indicate that a continual source of *Klebsielleae* is entering the tank water from wood microenvironments not accessible to dissolved chlorine.

Chlorine demand studies were carried out in tank B by adding a concentrated stock solution of calcium hypochlorite. Chlorine concentration was monitored by using the OTO procedure (Fig. 3). An initial chlorine concentration of 4 ppm was reduced to 0.5 ppm in a static water situation in 2 days and to undetectible levels in 4 days. The use of a polyvinyl chloride (PVC) tank liner confirmed that the chlorine demand was due to the wood and not to chlorine diffusion from the water. In two experiments using a PVC liner, an initial chlorine concentration of 0.6 and 0.7 ppm was retained at effective levels for 10 to 25 days (Fig. 3). No coliforms were recovered from water held in the tank with a liner, and no apparent slime accumulated on the liner surface during the brief study period.

DISCUSSION

Redwood water storage reservoirs represent a flexible alternative to certain types of water storage needs. Redwood tanks are less expensive than concrete or steel, readily available, easy to transport and assemble in a variety of locations, and long lasting. The bacteriological studies reported here would seem to seriously restrict their usefulness. However, the problems that we have documented, although severe in terms of the coliform burden in drinking water, result from obvious cases of abuse involving improperly constructed and maintained systems. Several interacting factors appear to compound the deteriorated water quality: lack of automated chlorination or an insufficient supply to maintain a detectable residual, tank age, a common inlet/outlet plumbing design, and lengthy average retention periods during which the chlorine residual is consumed.

Coliform counts were recorded that exceeded federal membrane filtration guidelines by as much as 10- to 40-fold (Table 1). Highest counts were generally associated with newer tanks not equipped with an automated chlorination system (or failure to supply adequate chlorine to retain a free residual) and with lengthy average retention periods. Excluding sites 1 and 2, which maintained adequate residuals (Table 1), only the two oldest tanks (sites 3 and 4) were under the coliform limit. The tank at site 5, which has the same plumbing configuration and identical water source as do sites 3 and 4, had coliform counts about sevenfold higher. This tank differs only in its time of installation (10 months as compared with 24 to 48 months for sites 3 and 4).

Coliform counts dropped significantly between resampling trips. In the private drinking water systems, the average coliform counts for the five systems resampled dropped nearly 10-fold during the 9-month interval. The only change in these systems during the sampling interval was the bringing on line of the 150,000-gallon (ca. 567,750-liter) tank at site 8.

Based on our interpretation of the field surveys, suggestions can be made that should insure the potability of water emanating from new redwood tanks. Whenever possible, the tank should be installed several months before anticipated domestic use. This would permit aging or curing of the tank and allow water-soluble matter to be leached out and consumed by microbes. Prior to use, the manufacturer's recommended 24-h sal soda soak should be extended to 1 week to further facilitate removal of colored matter and other water-soluble material. The tank should be drained and filled with at least 200 ppm of chlorine and held for about 7 days. If possible, it would seem advisable to occasionally circulate the water during this pe-

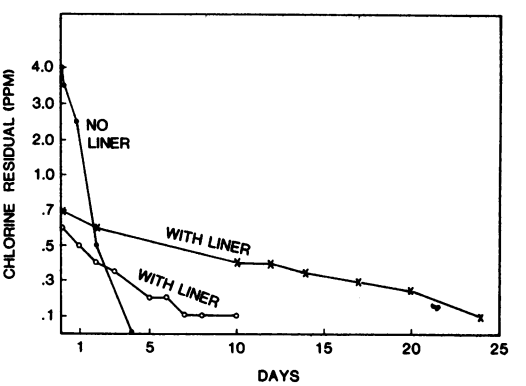

FIG. 3. *Kinetics of chlorine demand in static water held in 65-liter redwood tanks. Two independent experiments were run with liners in the tank.*

riod to maintain a chlorine residual at the wood-water interface. The tank would be drained and then put into service. The average retention time should always be as short as possible. One-day retention times would be optimal and could be achieved by lowering the water level to some small percentage of the tank capacity. The short retention time might also facilitate the maintenance of a chlorine residual. All tank systems must have adequate mixing of incoming water. Under no circumstances should a common inlet/outlet arrangement be used.

Little doubt remains as to the importance and nature of the chlorine demand exerted by wooden reservoirs. The 65-liter-capacity laboratory reservoirs consume approximately 1 ppm of chlorine residual per day (slope, Fig. 3). This compares with a daily reduction of 0.03 to 0.06 ppm in the same tanks lined with PVC. These results are compatible with the data obtained from two field tanks at sites 1 and 2 (Table 1). The chlorine concentration in the incoming tank water ranged from 1 to 1.5 ppm, and, with average retention times of 1 to 4 days, a residual of 0.2 to 0.4 ppm is retained in the service lines. The customary 0.2- to 0.4-ppm residual entering most field tanks was clearly inadequate in maintaining a chlorine residual in the service lines.

The origin of *Enterobacter* and *Klebsiella* in these water systems is an important question, but virtually impossible to delineate with certainty. In this context the absence of *E. coli* in private water systems and its presence in some public supplies require comment. Total coliform counts in the private water systems generally exceeded those in the parks by about 40-fold, and ordinarily with such high coliform counts in drinking water one would expect to isolate *E. coli*. In some samples, nearly all coliform colonies on plates were picked for identification. Thus, one cannot discount the high *Klebsiella* counts as masking the presence of *E. coli* in the private water systems. Furthermore, *E. coli* was never isolated from the three laboratory tanks. It seems likely that specific nutrients in wood, trees, vegetables, and other plant matter selectively support coliforms of the tribe *Klebsielleae*. In an earlier study we commonly obtained *Enterobacter* as well as an occasional *K. pneumoniae* isolate from within living white fir trees (1). Knittel has demonstrated that *K. pneumoniae* grow in pulp mill wastewater to levels exceeding 10^5 per ml (10), and others have isolated *K. pneumoniae* (10^3 cells and more per g) in unused sawdust bedding and in textile mill effluents (6, 14). *E. coli* is only rarely, if ever, isolated from such habitats. Therefore, the occasional *E. coli* isolate in the park systems is most likely due to more immediate exposure to fecal contamination and not to the use of wooden reservoirs per se. This is supported by the discovery of a sewage line break in one of the four parks where *E. coli* was isolated in this survey.

The mass of microbial growth that accumulates on some staves is obvious to the unaided eye (Fig. 1 and 2). It is impossible that this level of microbial biomass is supported by nutrients, sediment, etc., in the incoming water. This would be inconsistent with the diversity of source waters for the various systems studied and the absence of uniform colonization of all stave surfaces. It has been shown that redwood heartwood contains about 15% (dry weight basis) water-extractable materials (3). Included in this extract are free sugars that comprise approximately 0.1% of the dry weight of redwood. The six common carbohydrates (arabinose, glucose, fructose, rhamnose, sucrose, and raffinose) are all utilizable by the coliforms isolated from these habitats. Although these carbohydrates may supply adequate levels of nutrients for *Klebsiella* and *Enterobacter* to colonize these habitats, their presence does not explain the absence of other coliforms that also ferment these carbohydrates.

It may soon be possible to demonstrate that botanical-associated *K. pneumoniae* represent the reservoir for the evolution of clinical isolates, and this environment also serves as a milieu to support the multiplication of strains of fecal origin as well. Biochemical and serological studies on *K. pneumoniae* have demonstrated, for the most part, that no differences exist among human pathogenic strains and those obtained from most natural environments. Also, recent studies in our laboratory have shown that 13 of 75 *K. pneumoniae* isolates associated with redwood are fecal coliform positive. The presence of fecal coliform-positive *Klebsiella* in the absence of *E. coli* is common in environments containing botanical milieu. This is probably a reflection of natural nutritional selection pressures that allow for the multiplication of *Klebsielleae* and the consequent dilution of *E. coli* and perhaps other enteric bacteria. The 13 fecal coliform-positive cultures represent the progeny that probably emanated from animal, human, or avian fecal material. The remaining fecal coliform-negative cultures may represent a "natural" reservoir of nonfecal *K. pneumoniae* strains or reflect environmentally induced phenotypes in strains of older fecal/clinical origin. In view of

these possibilities, we recommend that *Klebsielleae* emanating from wooden water reservoirs be controlled as any other coliform should when federal drinking water standards are exceeded. Coliforms should be controlled by proper engineering systems that maintain satisfactory chlorine residuals and by compensating water levels to accommodate the shortest retention times as practicable.

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LITERATURE CITED

1. **Aho, P. E., R. J. Seidler, H. J. Evans, and P. N. Raju.** 1974. Distribution, enumeration, and identification of nitrogen-fixing bacteria associated with decay in living white fir trees. Phytopathology 64:1413–1420.
2. **American Public Health Association.** 1974. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Inc., New York.
3. **Anderson, A. B.** 1961. The influence of extractives on tree properties. 1. California redwood (*Sequoia sempevirens*). J. Inst. Wood Sci. 8:14–34.
4. **Bordner, R. H., and B. J. Carroll.** 1972. Proceedings of seminar on the significance of fecal coliform in industrial wastes. U.S. Environmental Protection Agency, Denver, Colo.
5. **Brown, C., and R. J. Seidler.** 1973. Potential pathogens in the environment: *Klebsiella pneumoniae*, a taxonomic and ecological enigma. Appl. Microbiol. 25:900–904.
6. **Dufour, A. P., and V. J. Cabelli.** 1976. Characteristics of *Klebsiella* from textile finishing plant effluents. J. Water Pollut. Control Fed. 48:872–879.
7. **Duncan, D. W., and W. E. Razzell.** 1972. *Klebsiella* biotypes among coliforms isolated from forest environments and farm produce. Appl. Microbiol. 24:933–938.
8. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
9. **Jones, F. E., and A. E. Greenberg.** 1964. Coliform bacteria in redwood storage tanks. J. Am. Water Works Assoc. 66:1489–1493.
10. **Knittel, M. D.** 1975. Taxonomy of *Klebsiella pneumoniae* isolated from pulp/paper mill wastewater. Environmental protection technology series. Environmental Protection Agency, Corvallis, Ore.
11. **Matsen, J. M., J. A. Spindler, and R. O. Blosser.** 1974. Characterization of *Klebsiella* isolates from natural receiving waters and comparison with human isolates. Appl. Microbiol. 28:672–678.
12. **Newman, L. E., and J. J. Kowalski.** 1973. Fresh sawdust bedding—a possible source of *Klebsiella* organisms. Am. J. Vet. Res. 34:979–980.
13. **Nunez, W., and A. R. Colmer.** 1968. Differentiation of *Aerobacter-Klebsiella* isolated from sugarcane. Appl. Microbiol. 16:1875–1878.
14. **Rendos, J. J., R. J. Eberhart, and E. M. Kesler.** 1975. Microbial populations of teat ends of dairy cows, and bedding materials. J. Dairy Sci. 58:1492–1500.
15. **Seidler, R. J., M. D. Knittel, and C. Brown.** 1975. Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. Appl. Microbiol. 29:819–825.