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Occurrence of *Klebisiella pneumoniae* in Surface Waters

M. D. KNITTEL[1]

Pacific Northwest Environmental Research Laboratory, U. S. Environmental Protection Agency, Corvallis, Oregon 97330

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The occurrence of *Klebsiella pneumoniae* in surface waters was not found to be ubiquitous. When it was isolated, *Escherichia coli* could also be found. The fecal coliform to fecal streptococci ratio suggest that its origin could be human, animal, or mixed sources.

Klebsiella pneumoniae is a well-known pathogenic bacterium causing such diseases as lobar pneumonia and urinary-tract infection in man and mastitis infections in cattle. It readily ferments lactose and is often confused with *Enterobacter aerogenes*. The organism has been found in large numbers (10^4 to 10^6 per ml) in pulp and paper mill wastewater effluents (5); and this finding has raised serious questions concerning possible health hazards. It has been reported by Duncan and Razzell (3) that *K. pneumoniae* can be isolated in large numbers in association with a variety of vegetation. These authors concluded that *K. pneumoniae* is a ubiquitous coliform in the environment and that because there are no documented cases of water-borne infection, little or no human health hazard exists.

It is not the purpose of this article to argue the question of pathogenicity of *K. pneumoniae* but to report: (i) the results of a survey of coliforms and presence of *K. pneumoniae* in surface water, rivers, streams, and lakes in the states of Oregon, Washington, and Idaho; (ii) the presence of *K. pneumoniae* is not as ubiquitous in surface waters as has been reported for vegetation; (iii) there is a correlation between occurrence of *K. pneumoniae* and *Escherichia coli*; and (iv) the ratio of fecal coliforms (FC) to fecal streptococci (FS) indicates that *K. pneumoniae* could be from human or animal sources.

MATERIALS AND METHODS

Collection of water samples. Water samples were obtained from streams, rivers, and lakes in various geographic areas of Oregon, Washington, and Idaho representing surface waters in highly developed and active agricultural lands and remote mountainous areas.

Samples were collected in sterile 500-ml, wide-mouth, screw-cap bottles and stored on ice for transport to the laboratory. In most cases, the time between sample collection and analysis was 6 h or less.

Bacteriological analysis of water samples. Volumes of each water sample or appropriate dilution in phosphate-buffered distilled water were vacuum filtered through 0.45-nm p membrane filters (Millipore Corp., Bedford, Mass.). Three membranes were prepared for each water sample and placed on one of three media for total coliform, fecal coliform, and fecal streptococci determinations.

Bacteriological media used. Total coliform determinations were done using M-Endo-LES agar and incubating the cultures at 37 C for 24 h. Fecal coliforms were grown on M-FC agar plates and incubated at 44.5 C for 24 h. Fecal streptococci were estimated by growing the cultures on K-F fecal streptococci agar at 37 C for 48 h. The above media were obtained from Difco and were prepared according to the recommendations listed by the manufacturer.

Media used to identify coliforms isolated from the M-Endo grown cultures was obtained either from Difco or BBL (Division of BioQuest, New York) or from recipies published by Edwards and Ewing (4).

Isolation and identification of coliforms. Colonies appearing on M-End-LES agar were selected for identification. If the number of colonies were less that 20, all of the well isolated colonies were selected for identification. However, if greater than 20 per plate, all of the well isolated colonies appearing within the center two rows of grids printed on the membrane and the two rows perpendicular to the first were selected. Both sheen and nonsheen colonies were transferred to triple sugar iron and nutrient agar slants and incubated for 24 h at 37 C.

The indolphenol oxidase reaction of each isolate was determined on the nutrient agar culture using the method of Gaby and Hadley (6) as modified by Ewing and Johnson (5). Those indophenol-oxidase negative cultures were stained for their gram reaction. All gram-negative rod-shaped bacteria were transferred from triple sugar iron to media listed in Table 1 and tested or observed for their reactions. The results were compared to the keys published by Edwards and Ewing (4) for identification. This procedure provided a profile of the species of coliform bacteria found in the various water samples.

[1] Present address: Western Fish Toxicology Station, U.S. Environmental Protection Agency, Corvallis, Ore. 97330.

TABLE 1. *Media and tests used to identify coliform culture*

Test or substrate	Coliform culture no.			
	1	2	3	4
Indole	+	+	−	−
Methyl red	+	−	−	−
Voges-Proskauer	−	−	+	+
Citrate	−	−	+	+
H_2S (triple sugar iron)	−	−	−	−
Urease	−	−	+	−
Motility	+	+	−	+
Lysine decarboxylase	+	+	+	+
Arginine dihydrolase	−	−	−	−
Ornithine decarboxylase	−	+	−	+
Glucose (gas)	+	+	+	+
Lactose	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Malonate	−	−	+	+

RESULTS AND DISCUSSION

The procedures used to identify coliforms and to assess the prevalence of *K. pneumoniae* in surface water resulted in data similar to those shown in Table 1. The reactions shown by isolate 1 would be *Escherichia coli* type I, i.e., both indole and methyl red positive. Isolate 2 would be also *E. coli* but type II with only indole being positive. Isolate 3 is a typical *K. pneumoniae* indole and methyl red negative and the Voges-Proskauer and citrate positive. It is nonmotile, ornithino decarboxylase negative, and urease positive. Isolate 4 is a typical *Enterobacter aerogenes* being motile, ornithine decarboxylase positive. These are the kind of data that were collected for each isolate from each water sample.

The coliforms that were isolated and identified in this study reveal that *E. coli* dominates

TABLE 2. *Occurrence of Klebsiella pneumoniae in lotic and lentic water samples*

Source	Coliforms/100 ml		Fecal streptococci/100 ml	FC/FS ratio	Enterobacteriacea isolated[a]
	Total	Fecal			
Alsea River[b]	108	34	29	1.2	*E. coli*
Mary's River	14	4	6	0.7	*E. coli, E. aerogenes*
Unknown Creek	192	117	65	1.8	*E. coli*
Rock Creek	128	16	30	0.5	*E. coli, K. pneumoniae*
Alder Creek	26	<1	3	ND[c]	Unknown coliforms
Wiley Creek	14	<1	<1	ND	*Citrobacter, E. cloacae*
Wiley[d] Creek	TNTC[e]	130	21	6.1	*E. coli, K. pneumoniae*
Mary's River	200	30	<1	ND	*E. coli, Citrobacter E. cloacae*
Ritner Creek	315	18	<1	ND	*E. coli, Citrobacter*
Unknown Creek	22	6	<1	ND	*E. coli, Proteus, Arizona*
Unknown Creek	52	16	<1	ND	*E. coli, Citrobacter*
Luckiamute River	38	11	5	2.2	*E. coli, Citrobacter*
Ditch,[f] Agricultural	228	24	15	2.3	*E. coli, K. pneumoniae E. aerogenes, Proteus*
Cowlitz River	30	2	<1	ND	*E. coli, K. pneumoniae Enterobacter*
Snoqualmie River	120	17	<1	ND	*K. pneumoniae*
Naches River	18	2	<1	ND	*E. coli*
Yakima River	32	<1	<1	ND	*E. coli*
Palouse River	390	230	ND	ND	*E. coli, K. pneumoniae, Citrobacter, Providenciae E. cloacae*
Calapoola River	4	2	ND	ND	*E. coli, Citrobacter*
China Lake	32	2	4	0.5	*E. coli, Unknown coliforms*
Cape Lake	28	8	<1	ND	*E. coli*

[a] Species of coliforms were isolated and identified from colonies appearing on M-ENDO LES agar.

[b] Streams and rivers flowing through populated or agricultural areas. All other sources from areas of limited access or minimal human activity.

[c] ND, Not determined.

[d] Sampling point below a housing development, foam on water surface.

[e] TNTC, Too numerous to count.

[f] Water draining off a field.

the enteric bacteria isolated, being found in all but two of the samples examined (Table 2). Six of the 21 water environments sampled yielded cultures of *K. pneumoniae* and in all cases, except one, *E. coli* was also present. The fecal origin of *K. pneumoniae* is indicated by its association with *E. coli* which has been used as an indicator of fecal pollution. The FC/FS ratios (7) show that the fecal origin can be either human (FC/FS > 4) or animal (FC/FS < 0.6), or from mixed sources (FC/FS < 4 but > 0.6). The data do not support the conclusion that *K. pneumoniae* is an ubiquitous coliform bacterium, because it was not found in all samples examined.

Surface water collected from three states from diverse geographic areas and water uses has shown that *K. pneumoniae* does not represent a significant portion of the natural coliform population. It is not present in all water environments and its association with *E. coli* suggests that it is of fecal origin. The FC/FS ratios support the conclusion that both human and animals could be its source.

There is little doubt in the literature that *K. pneumoniae* does occur in human intestinal tract (10, 11) and in some cases it has been found as the dominate coliform (2). McCoy and Seidler (9) have shown that intestinal contents of pet turtles contain *K. pneumoniae*. Nunez and Colmer (11) isolated *K. pneumoniae* from the gut of the sugar cane borer. The literature is sparse as to the occurrence of *K. pneumoniae* in the gut of other animals both wild and domestic, but what information is available suggest that *K. pneumoniae* in the environment may originate from the gut of man or animals. Future studies should be concerned with the origin of *K. pneumoniae* when it is encountered, not only in samples from the environment but also in human infections. This is only to reiterate what Ptak et al. (12) have already pointed out. Only in this way will it be possible to assess the hazard to human health that the presence of *K. pneumoniae* in the environment may offer.

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