Occurrence of *Klebsiella pneumoniae* in Surface Waters

M. D. KNITTEL

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

Received for publication 16 December 1974

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 10^8 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-PC 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 recipes published by Edwards and Ewing (4).

**Isolation and identification of coliforms.** Colonies appearing on M-Endo-LES agar were selected for identification. If the number of colonies were less than 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.
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           | -  | -  | +  | +  |
| H₂S (triple sugar iron) | -  | -  | +  | +  |
| Urease            | -  | -  | -  | +  |
| Motility          | +  | +  | -  | -  |
| Lysine decarboxylase | +  | +  | +  | +  |
| Arginine dihydrolase | -  | -  | -  | +  |
| Ornithine decarboxylase | -  | +  | +  | +  |
| Glucose (gas)     | +  | +  | +  | +  |
| Lactose           | +  | +  | +  | +  |
| Sucrose           | +  | +  | +  | +  |
| Mannitol          | +  | +  | +  | +  |
| Malonate          | -  | -  | +  | +  |

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

| Source                  | Coliforms/100 ml | Fecal streptococci/100 ml | FC/FS ratio | Enterobacteriaceae isolated* |
|-------------------------|-----------------|---------------------------|-------------|-------------------------------|
|                         | Total | Fecal |                    |               |                               |
| Alsea River*            | 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*           | Unknown coliforms            |
| Wiley Creek             | 14    | <1    | <1                 | ND            | Citrobacter, E. cloacae      |
| Willey Creek*           | TNTC* |       |                    |               |                               |
| Mary’s Creek            | 200   | 30    | <1                 | ND            | E. coli, Citrobacter         |
| Rittner Creek           | 315   | 18    | <1                 | ND            | E. coli                      |
| 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, Agricultural     | 228   | 24    | 15                 | 2.3           | E. coli, K. pneumoniae       |
|                         |       |       |                    |               | E. aerogenes, Proteus        |
| Cowiltz 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, K. pneumoniae       |
| Palouse River           | 390   | 230   | ND                 | ND            | Citrobacter, Providenciae,   |
|                         |       |       |                    |               | E. cloacae                   |
| Calapoolsa 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                      |

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, ornithine 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...
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 bacte-
rium, 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 repres-
ent a significant portion of the natural coliform
population. It is not present in all water envi-
ronments and its association with E. coli sug-
gests 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 domes-
tic, 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 reiter-
ate 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.

ACKNOWLEDGMENTS
I would like to thank B. Boese for his able assistance
during this study. Also J. Greene for collection of some of the
water sample reported here.

LITERATURE CITED
1. American Public Health Association. 1971. In Michael J.
Taras, Arnold E. Greenberg, R. D. Hoak, and M. C.
Rand (ed.), Standard methods for examination of
water and wastewater, 13th ed. American Public
Health Association, Washington, D.C.
2. Cruickshank, R. 1965. A guide to laboratory diagnosis
and control of infection. pp. 257-258. In Medical
Microbiology, 11th ed. The Williams and Wilkins Co.,
Baltimore.
3. Duncan, D. E., and W. E. Razzell. 1972. Klebsiella
biotypes among coliforms isolated from forest environ-
ments and farm produce. Appl. Microbiol. 24:933-938.
4. Edwards, P. R., and W. H. Ewing. 1972. Identification of
Enterobacteriaceae, 3rd ed. Burgess Publishing Co.
Minneapolis.
5. Ewing, W. H., and J. G. Johnson. 1960. The differentia-
tion of Aeromonas and C-27 cultures from Enterobacte-
riaceae. Int. Bull. Bacteriol. Nomencl. Taxon.
10:223-230.
6. Gaby, W. L., and C. Hadley. 1957. Practical laboratory
test for the identification of Pseudomonas aeruginosa.
J. Bacteriol. 74:356-358.
7. Goldreich, E. E., and B. A. Kenner. 1969. Concepts of
fecal streptococci in stream pollution. J. Water Pollut.
Control Fed. 41:R336-R352.
8. Knittel, M. D. 1972. Review of research regarding
coliforms in pulp and paper mill wastes. In R. H.
Borner and B. J. Carrol (ed.), Proc. Seminar on the
Significances of Fecal Coliforms in Industrial Wastes.
TR-3 U.S. Environmental Protection Agency, Denver.
9. McCoy, R. H., and R. J. Seidler. 1973. Potential patho-
gens in the environment: isolation and identification of
seven genera of intestinal bacteria associated with
small green pet turtles. Appl. Microbiol. 25:534-538.
10. Montgeromerie, J. Z., P. B. Doak, D. E. M. Taylor, J. D.
K. North, and W. J. Martin. 1970. Klebsiella in fecal
flora of renal transport patients. Lancet 2:787-791.
11. Nunez, W. J., and A. R. Colmer. 1968. Differentiation
of Aerobacter-Klebsiella isolated from sugarcane.
Appl. Microbiol. 16:1875-1878.
12. Ptak, D. J., W. Ginburg, and B. F. Willey. 1973.
Identification and incidence of Klebsiella in chlori-
ated water supplies. J. Am. Water Works Assoc.
65:604-608.
13. Selden, R., S. Less, W. L. Low, and T. C. Eickhoff. 1971.
Nosocomial Klebsiella infections: intestinal coloniza-
tion as a reservoir. Ann. Int. Med. 74:657-664.
14. Thone, B. T. 1970. Klebsiella in faeces. Lancet 2:1033.