Bull. Environ. Contam. Toxicol. (1988) 41:828–831

Environmental Contamination and Toxicology

Klebsiella spp. in Prairie Aquifer

S. Huong Wong*

Department of Biology, University of Regina, Regina, Saskatchewan, Canada

Coliform Klebsiellae are widely recognized as agents of bacteremia, genitourinary, respiratory and other infections, primarily in stressed patients; (Burnett and Scherp, 1968; Cruickshank, et al. 1975; Eickhoff, 1971) in outbreaks of mastitis in cattle and a wide variety of diseases in other domestic and wild animals (Martin, et al. 1971; Crouch et al. 1972; Orskov, 1974; Fox, and Rohosvsky, 1975). There has been an increasing interest in these bacteria not only for their role as an antibiotic resistant opportunistic pathogens, but also because of their ubiquitous distribution in environments (Wong et al. 1985). Their presence in prairie aquifer is not well documented. This study shows that some deep wells in Swift Current area are contaminated with Klebsiella.

MATERIALS AND METHODS

Thirty farms in Swift Current area were randomly selected. Water samples were collected from the drinking water wells of these farms. The temperatures of the well waters were recorded at the time when samples were taken. These samples were kept refrigerated before analyzing. The holding time was not more than 4 hours. The whole exercise was done in the early August of 1984.

Membrane filtration, spread-plate and seeded pour-plate techniques were used to detect and enumerate total bacteria on plate count agar, (Difco, Detroit, Michigan), total coliforms on M-Endo agar (Difco) and Klebsiella on Wong's modified medium (WM). Wong's modified medium consisted of the following compositions (grams per litre of triple-distilled deionized water): Na_2HPO_4, 0.7; NaH_2PO_4, 0.3; $MgSO_4.H_20$, 0.2; $MnSO_4$, 0.1; CoCl, 0.005; $FeCl_2$, 0.005; MoO_3, 0.005 ; $ZnCl_2$, 0.005; KNO, 1.08; lactose, 5.0; sodium desoxycholate, 1.0; Noble

*Present address: 630 Broadway Avenue, Regina, Saskatchewan, Canada S4N 1C1

agar, 15; neutral red 0.03 and crystal violet 0.004. It was prepared in according to Wong et al. (1984). Incubation temperature for the inoculated WM and M-Endo plates was at 35 °C and that of plate count agar (PCA) plates was at 28 °C. Viable cell (v.c) counts were performed after 36 and 48 hours of incubation respectively. Mucoid-pink red or more watery light-red with dark red center colonies of sizes ranging from 1 - 2 mm (diameter) or larger were treated as Klebsiella positive.

All the colonies appeared on each WM or M-Endo plates were restreaked onto the same medium to check for purity. Each pure isolate was then identified using standard laboratory procedure for the identification of coliforms in according to Cruickshank et al. (1975) and were cross-checked with data obtained from using Minitek system (BBL, Becton, Dickinson and Company, Cockeysville, Maryland).

RESULTS AND DISCUSSION

The results in Table 1 show that all samples were contaminated by bacteria. The total bacterial counts in some of these samples were extremely high (over 100,000 v.c./ml). There were more than four million viable cells in every ml of water in Well No. 18. Out of these 30 wells, 73% (22/30) were contaminated by coliforms and 23% (7/30) by Klebsiella respectively. However, the densities of viable cells of Klebsiella in these water samples were low. Only two of the seven samples had the cell counts exceeded 10 v.c./ml. No other coliforms except Klebsiella was found in four of the coliform-positive wells. This suggests that the presence of these bacteria in these deep wells were not related to faecal contamination. All the contaminated wells were over 100 feet deep, except well No.13 (83 feet). But, in well 13, no other coliforms were found in the water and the total bacterial count was low (5,000/ml). It seemed that this organism is of soil origin.

K. pneumoniae was not found in these water samples. Every single strain of Klebsiella isolated was identified as K. oxytoca. This could be due to the low temperatures of these well waters. The results in other separate studies show that K. oxytoca can grow in ground water at temperature below 10 °C (4-6 °C), but not K. pneumoniae. Most strains of K. pneumoniae tested did not grow at this temperature. At the time of sampling, the temperature of these well waters was below 15 °C, except well No. 29. This was done in late summer. In cold Saskatchewan winter, the water temperatures of these wells could drop quite considerably. The low temperatures could be the prime factor affecting the growth of K. pneumoniae in these wells.

There was no co-relationship between the total bacterial count and the presence of Klebsiella and total coliforms

Table 1. Bacterial counts in well waters

Sample No.	Total bacteria	Total coliforms	Klebsiella	Well depth	Temperature C
1	25,000	4	0	560	7.0
2	1,300	14	0	200	10.0
3	91	0	0	65	12.0
4	2,500	0	7	285	10.0
5	39,000	5	0	185	10.0
6	93	0	0	136	8.0
7	960	2	0	345	12.0
8	13,500	410	0	250	12.0
9	260	2	0	325	12.0
10	250	440	0	243	12.0
11	980	2	0	80	12.0
12	3,900	19	0	--	10.5
13	4,200	0	3	83	14.0
14	6,100	0	0	185	11.0
15	9,900	490	0	365	15.0
16	8,800	0	0	95	14.5
17	3,700	24	0	58	16.0
18	4,100,000	0	0	84	15.5
19	600,000	1	0	--	11.0
20	2,600	5	0	67	14.5
21	8,700	28	0	360	12.5
22	5,000	400	0	200	13.0
23	4,800	560	1	30	14.0
24	66	12	12	180	10.0
25	770	100	0	180	11.0
26	1,100	30	0	280	12.0
27	31,000	51	16	175	14.5
28	3,600	2	0	220	13.0
29	37	4	4	85	17.0
30	120	0	0	180	0.0

in the well water. The high total bacterial counts did not affect the number of viable cells of total coliforms or Klebsiella; nor were the cell densities of Klebsiella in these wells elevated by the high cell densities of total coliforms. Neither coliforms nor Klebsiella were found in the well water (No. 18), which had the highest total bacterial count.

When M-Endo agar was used in this study to detect total coliforms, some strains of Klebsiella were not detected. Similarly, klebsiella was found in two of the coliform-negative samples. When Wong's modified medium was used, only Klebsiella were detected. Other coliforms did not grow on Wong's modified medium. On surface-spread and seeded plates, pin-point colonies of Klebsiella appeared

on the agar from the positive samples after 18 hours of incubation at 35 °C. No growth was found on the WM plates of all the negative samples, even though the plates were incubated for further 48 hours. However, when membrane filtration technique was used on WM medium, the incubation period was increased to 48 hours. The colonies were light-colored, mucoid and watery. There is no distinct difference between the colonies of Klebsiella from surface-spread plates and seeded plates. But there was a considerable decrease in viable cells in seeded plates. In most cases, there was a drop of 60% or more of viable cells. The medium is extremely good to detect Klebsiella in well water using spread-plate technique. It is capable of detecting Klebsiella in water as low as 1 viable cell per ml. Besides, there was no interference from the presence of other coliforms and other soil microorgansms. These organisms do not grow on this medium at 35 °C.

Acknowledgments. The author wish to thank Mr. Grant Duke, a former technician of Regina Water Research Institute for helping in taking the water samples and the University of Regina for the financial support through Herzberg scholarship.

REFERENCES:

Burnett GW, Scherp HW (1968) Oral Microbiology and Infectious Diseases. 3rd ed., Williams and Wilkins Co., Baltimore. pp. 769-789.

Crouch JRF, Atherto JG, Platt H (1972) Venereal transmission of Klebsiella aerogenes in a thorough bred stud from a persistently infected stallion. Vet Rec 90:21-24.

Cruickshank R, Duguid JP, Marmion BP, Swain RHA (1975). Medical Microbiology. 12th Ed. Vol. 2. Churchill Livingstone, Edinburgh, London.

Eickhoff TC (1971) Nosocomial infections due to Klebsiella pneumoniae: Mechanisms of intra-hospital spread. In: Proceedings of the International Conference on Nosocomial Infections. (Brachman PS, Eickhoff TC eds.). Am Hosp Assoc Chicago. PP. 117-125.

Fox JG, Rohosvsky MV (1975) Meningitis causes by Klebsiella spp. in two Rhesus monkeys. J Am Vet Med Assoc 67:634-636.

Martin WJ, Yu PKW, Washington JA II (1971) Epidemiological significance of Klebsiella pneumoniae. A 3 months study. Mayo Clinic Proceed. 46:786-793.

Orskov I (1974) Klebsiella In Bergey's Manual of Determinative Bacteriology. 8th ed. (Buchanan RE, Gibbons NE eds.) The Williams & Wilkins Company, Baltimore. pp. 321-324.

Wong SH, Cullimore DR, Bruce LR (1985) Selective medium for isolation and enumeration of Klebsiella spp. Appl Environ Microbiol 49:1022-1024.

Received December 15, 1987; accepted May 10, 1988.