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Source and Extent of *Klebsiella pneumoniae* in the Paper Industry

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Three pulp and paper mill processing plants were evaluated for fecal coliform and *Klebsiella pneumoniae* bacterial concentrations. Freshwater consumed by paper industries contained minimum detectable levels of *K. pneumoniae*, <10 organisms per 100 ml. Elevated concentrations of *K. pneumoniae* could be traced from early pulping stages to water processing reuse systems. Concentrations of *K. pneumoniae* (thermotolerant and thermointolerant) ranged from 40,000 organisms per 100 ml to an estimated 3×10^6 organisms per 100 ml. *K. pneumoniae* biotyping provided evidence for the selective growth and persistence of *K. pneumoniae* from the initial wood washing stages through to the final effluent discharge. Wastewater treatment had limited effects in reducing *K. pneumoniae* concentrations. *K. pneumoniae* levels ranged from 40 organisms per 100 ml to an estimated 10^6 organisms per 100 ml. The presence of *K. pneumoniae* in water indicates degraded water quality, and its significance with regard to human health effects has yet to be examined.

Industrial organic wastes from textile, sugarcane, sugar refining, kelp, and pulp and paper mill processing have been shown to contain high concentrations of coliform bacteria (5, 13). Recently, because of bacterial taxonomic clarifications, the opportunistic pathogen *Klebsiella pneumoniae* has been isolated and identified as the predominant coliform bacterium from such industrial waste sources (7, 11).

Isolations of *K. pneumoniae* from the pulp and paper mill industry have been well documented. Duncan and Razzell (6) evaluated samples of water, soil, and bark from three different forest environments and effluent samples from a pulp and paper mill for total and fecal coliform bacteria. Only a small percentage of the bacteria isolated were found to be *Escherichia coli*, whereas up to 71% of the isolates were identified as *K. pneumoniae*. Bauer (2) examined pulp and paper mill effluents for total and fecal coliform bacteria. Coliform densities were found to be as high as 1.6×10^8 organisms per 100 ml, and fecal coliform densities were as high as 4.2×10^6 organisms per 100 ml. Of 300 isolated fecal coliforms, 68% corresponded biochemically to organisms of the *Klebsiella-Enterobacter* group. Further evaluation of the isolates showed 98% to be identified as *K. pneumoniae*. Knittel et al. (9) examined total coliform bacteria in pulp and paper mill effluents and found levels in excess of 10^5 organisms per 100 ml. Further evaluation showed 60 to 80% of these isolates to be *K. pneumoniae*. Huntley et al. (8) further confirmed the presence of large numbers of *K. pneumoniae* in waters receiving pulp mill effluents. A total of 60% of the isolates were identified as *K. pneumoniae*.

Attention has recently been focused on the reduction of the pollution load of wastes discharged from the pulp and paper mill industries (14). This effort has promoted methods for developing the reuse of water within the industry. Though the reuse of water has been beneficial to the industry by conserving chemicals and fibrous material suspended in the water, utilizing less raw water, conserving heat, and minimizing receiving water pollution, there have been indications that such reuse of water enhances coliform bacteria growth (4, 14).

K. pneumoniae is an opportunistic pathogen that is extremely virulent and has quickly developed immunity to almost all antibiotics. *K. pneumoniae* causes 2% of all cases of bacterial pneumonia, and as a result of the antibiotic resistance of this organism, it causes 60 to 70% of the deaths from this disease (10). *K. pneumoniae* was first isolated from infected lungs and has caused genitourinary infections, bacteremia, osteomyelitis, and meningitis (12). Colonization of the human intestinal tract has been related to hospital-acquired infections and, in fact, precolonizations may be the preliminary factor for hospital-acquired clinical infections (10). Symptomless colonized patients have been

shown to be four times more susceptible to hospital-acquired infections than are noncolonized patients (2).

It was the purpose of this study to establish the source and extent of fecal coliform and subsequent thermotolerant and thermointolerant *K. pneumoniae* concentrations from three different pulp and paper mills in Wisconsin. Bacterial concentrations were to be traced from the initial freshwater supplies to plants, through the processing system of the mill for recycling water, to the final, treated effluent water that was disposed into receiving waters downstream from the plants.

MATERIALS AND METHODS

Study area. Water samples were obtained from pulp and paper mills located predominantly in the northcentral and northeastern sections of Wisconsin. The sampling points examined for bacterial densities included freshwater supplies, recycled water within the mills, treated effluent wastewater, and waters receiving effluent wastes downstream. The mill types included a groundwood pulp mill, a sulfite pulp and fine paper plant, and a deink pulp and fine paper plant. Mill types bacteriologically examined for the effects of wastewater treatment included the above mentioned and a tissue paper (glassine) plant, a deink pulp and tissue paper plant, a sulfite pulp mill, a groundwood pulp and book paper plant, and a semichemical-mechanical pulp plant.

Sample collection. Samples were collected in sterile 250-ml screw-cap bottles and stored at approximately 4°C during transport to the laboratory. Samples containing residual chlorine were collected in sterile bottles containing the dechlorinating agent sodium thiosulfate (approximate concentration of 100 mg/liter per sample bottle; enough to neutralize approximately 15 mg of residual chlorine per liter). Bacteriological examination was initiated within 24 h of sample collection.

Bacteriological analyses. Determination of fecal coliform bacterial densities was made by a membrane filter technique. The membrane filter procedure calls for an enriched lactose medium that depends on an incubation temperature of 44.5 ± 0.2°C for selectivity. The membrane-fecal coliform (M-FC) medium utilized for this test was prepared according to the medium specifications in *Standard Methods for the Examination of Water and Wastewater* (1).

The volume of the water sample examined by the membrane filter technique involved filtering serial dilutions of sample with 0.45-μm membrane filters (type HC; Millipore Corp., Bedford, Mass.) to yield a countable filter, usually between 20 and 60 fecal coliform colonies.

Prepared culture plates were incubated in a 44.5 ± 0.2°C water bath for 24 h. All fecal coliform isolates (blue colonies on M-FC medium) were verified by subculturing isolated blue colonies into elevated coliform broth at 44.5°C for 24 ± 2 h. Gas production in the fermentation tube within 24 h was considered a positive reaction indicating fecal origin.

The membrane filter method for the enumeration of *K. pneumoniae* in freshwater and marine water developed by Dufour and Lupo (A. P. Dufour and L. B. Lupo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, Q5, p. 262) was used. The early stages in the development of this method, using a simple growth medium with antibiotics, was found satisfactory (5) and led to its use in later research (A. Dufour, M. Kanarek, R. Marino, and N. Caplenas, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q75, p. 213). Appropriate volumes of water samples were filtered with 0.45-μm Millipore HC membrane filters and incubated at 35°C for 48 h on the specifically developed medium. All nonpink colonies were counted for the total *K. pneumoniae* concentration. (At 35°C both the thermointolerant and the thermotolerant strains of *K. pneumoniae* can be evaluated, i.e., total *K. pneumoniae* concentration.)

Presumptive identification of the isolated *K. pneumoniae* as to species was made by using triple sugar iron agar, urea slants, Simmons citrate agar, lysine and ornithine decarboxylase broths, motility, and the indole, methyl red, Voges-Proskauer, citrate tests. Further identification and characterization of *K. pneumoniae* included the API 20E system and the bacteriocin (klebocin) sensitivity typing of *K. pneumoniae* according to Buffenmyer et al. (3). A 13-test battery for klebocin biotyping was performed (Dufour et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q75, p. 213).

Fecal coliform-positive *K. pneumoniae*, i.e., thermotolerant *K. pneumoniae*, was identified by subculturing isolated *K. pneumoniae* colonies from MK agar into elevated coliform tubes as previously described.

In cases in which total *K. pneumoniae* counts were not obtainable, underestimated counts of thermotolerant *K. pneumoniae* were enumerated by using the M-FC plates, which evaluated the water samples for fecal coliform levels. Typically large, raised, mucoid blue fecal coliform colonies from M-FC plates were subcultured onto eosin methylene blue plates for incubation at 35°C for 24 h. Typically large, raised, mucoid pink colonies with purple centers were isolated and underwent the series of biochemical reactions as previously described for the presumptive identification of thermotolerant *K. pneumoniae*. Presumptive *K. pneumoniae* colonies were then picked from the eosin methylene blue plates into elevated coliform tubes for thermotolerant response.

RESULTS

Table 1 summarizes the fecal coliform and the thermotolerant and thermointolerant *K. pneumoniae* concentrations of the three sample plants. The findings indicate that water consumed by the paper mills had minimum detectable levels of *K. pneumoniae* (<10 organisms per 100 ml). Support for *K. pneumoniae* maintaining a wood, bark, or soil reservoir was evident since detectable levels of *K. pneumoniae* were apparent after raw wood chips or samples from the debarker were analyzed. Elevated con-

TABLE 1. *Concentrations of fecal coliform and thermotolerant and thermointolerant Klebsiella bacteria*

Plant type	Source of sample	Fecal coliforms/100 ml	Thermotolerant *Klebsiella* bacteria/100 ml	Total *K. pneumoniae* (thermotolerant plus thermointolerant *Klebsiella* bacteria/100 ml)	% Fecal coliforms identified as *K. pneumoniae*
Groundwood pulp mill	Influent water to plant	<10	<10	<10	
	Raw wood from debarker[a]	8.5×10^2	6×10^2	6×10^2[b]	71
	Grinder white water[c]	3.5×10^3	1.6×10^3	1.6×10^3[b]	46
	White water chest[c]	4×10^5	2.4×10^5	2.4×10^5[b]	60
	Hydrapulper[c]	4×10^6 EST[d]	3×10^6 EST	3×10^6 EST[b]	75
	Wet machine	3.3×10^5	2.3×10^5	2.3×10^5[b]	70
	Clarified white water	6×10^1	4×10^1	4×10^1[b]	67
Sulfite pulp and fine paper plant	Influent water to plant	<10	<10	<10	
	Wood chips[e]	2×10^2	1×10^2	1×10^2[b]	50
	White water[c]	5.5×10^2	4×10^2	4×10^2[b]	73
	Water to primary treatment	1.7×10^3	1×10^3	4×10^4	59
	Primary treatment[f]	4.8×10^4	4×10^4	6.7×10^5 EST	83
	After nutrients	4.7×10^4	3.7×10^4	9×10^5 EST	79
	Effluent	5×10^4	4.5×10^4	2×10^5	90
Deink pulp and fine paper plant	Influent water to plant	4.9×10^2	<10	<10	
	Effluent chest water[c]	1.45×10^5	1.4×10^5	1.4×10^5[b]	97
	Water to tile tank[c]	4.2×10^4	3.9×10^4	5×10^4	93
	Sidehill washers	2×10^5 EST	1×10^5 EST	1×10^5 EST[b]	50
	Stock to decker chest	1.9×10^4	1.4×10^4	1.4×10^4[b]	74
	Decker chest	4×10^3	3.5×10^3	3.5×10^3[b]	88
	Primary treatment[g]	3×10^5 EST	2×10^5 EST	2×10^5 EST[b]	67
	Secondary treatment[h]	2×10^6 EST	1×10^6 EST	1×10^6 EST[b]	50
	Effluent	2×10^6 EST	1×10^6 EST	1×10^6 EST[b]	50

[a] Aspen wood.
[b] Underestimation of total *K. pneumoniae* since only thermotolerant *K. pneumoniae* counts were obtainable.
[c] Integral portion for reuse of water within plant.
[d] EST, Estimation.
[e] Hemlock, balsam, and birch.
[f] Primary clarifier, trickling filter, secondary clarifier, and mechanized sludge dewatering.
[g] Primary clarifier.
[h] Two-stage activated sludge system with holding lagoon for sludges.

centrations of *K. pneumoniae* can be traced from early pulping stages to the water reuse systems. The reuse of water within the plants apparently enables fecal coliform bacteria, predominantly *K. pneumoniae*, to enter the mill repeatedly at the freshwater influent stage, thereby inoculating fresh pulp with *K. pneumoniae*, which then continues to contaminate the next production stages. The presence of high *K. pneumoniae* counts in the fecal coliform analyses of the study in the absence of *E. coli* suggests that bacterial regrowth, initially considered to occur only among nonfecal coliforms (5), now can be applied to certain biotypes of *K. pneumoniae* that meet the criterion for fecal coliforms. Since *K. pneumoniae* proliferation occurs in recycled wa-

ter portions of the plant, treatment stages are loaded with elevated bacterial concentrations. In addition to the results shown in Table 1, Table 2 also indicates that industrial wastewater treatment has limited effects in the reduction of bacterial counts, especially for *K. pneumoniae*.

Results for the bacteriocin sensitivity typing of *K. pneumoniae* are shown in Table 3. It is apparent that the reuse water systems in plants serve as a reservoir for the maintenance of *K. pneumoniae* biotypes initially inoculated from washed wood sources and that these biotypes are distributed further into subsequent processing stages which can be persistent through to the final effluent stage. The closed cycle of bacterial enhancement and proliferation in the recycle system is exemplified by the presence of identical *K. pneumoniae* biotypes in the washed wood sources and the effluent water.

DISCUSSION

Figure 1 shows a typical flow diagram for a groundwood pulp mill. As apparent from Table 1 for the groundwood pulp mill, the high fecal coliform and *K. pneumoniae* concentrations from within the plants, grinder white water, white water chest, and hydrapulper (with correspondingly low bacterial counts entering the plant via a freshwater inoculum), there is an association between enhanced bacterial growth and the use of recycled water systems. From the samples taken, it can be interpreted that the source of *K. pneumoniae* was from the wood chips and soil that initially came into the mill system. The logs utilized were primarily an aspen wood located in close proximity to the mill. Soil can readily contaminate the wood because of the "dragging-out" process of moving wood from cutting areas to yarding areas for loading preparations. The highest *K. pneumoniae* concentration found in the mill corresponded to the excess water source which overflowed into the hydrapulper before water reuse. The retention period in this tank (from 24 to 48 h), a temperature range of 15 to 25°C, and the presence of wood fibers and particulate matter providing nutrient sources provided the needed enrichment for *K. pneumoniae* growth. Thermotolerant *K. pneumoniae* comprised up to 75% of the fecal coliform bacteria.

Fecal coliform bacteria, of which *K. pneumoniae* predominated, were consistently isolated from other mill process types, indicating the ubiquity of *K. pneumoniae* in the pulp and paper

TABLE 2. *Fecal coliform and thermotolerant and thermointolerant K. pneumoniae distributions in plant treatment waters and waters receiving effluent discharge*

Plant type	Source of sample	Fecal coliforms/100 ml	Thermotolerant *Klebsiella* bacteria/100 ml	Total *K. pneumoniae* (thermotolerant plus thermointolerant *Klebsiella* bacteria/100 ml	% Fecal coliforms identified as *K. pneumoniae*
Tissue papers (glassine)	Influent water to treatment[a]	7.5×10^3	3.6×10^3	3.6×10^3[b]	48
	Effluent	4.8×10^3	2.9×10^3	2.9×10^3[b]	60
Deink pulp and tissue paper	Influent water to treatment[c]	7.5×10^3	3.5×10^3	1.5×10^4	50
	Effluent	3.1×10^3	1.3×10^3	1.2×10^4	42
Sulfite pulp	Influent water to treatment[d]	2×10^6 EST[e]	5×10^5	1×10^6 EST	25
	Effluent	2×10^6	8×10^5	1×10^6 EST	40
Groundwood pulp and book paper	Influent water to treatment[c]	5.5×10^3	4.6×10^3	4.6×10^3[b]	84
	Effluent	1.5×10^3	1×10^3	3×10^3	67
Chemical mechanical pulp, deink pulp, and fine papers	Influent water to treatment[f]	1.1×10^4	9×10^3	1.5×10^4	82
	Effluent	3.3×10^2	2.5×10^2	5×10^3	76

[a] Primary clarifier plus settling lagoon.
[b] Underestimation of total *K. pneumoniae* since only thermotolerant *Klebsiella* counts were obtained.
[c] Primary clarifier plus two-stage activated sludge with centrifuges for sludges.
[d] Primary clarifier plus secondary activated sludge system.
[e] EST, Estimation.
[f] Primary clarifier and pure O_2 activated sludge system.

TABLE 3. *Klebocin biotypes for the three sampled mills*

Plant type	Source of sample	Klebocin biotype code[a]	Frequency of isolation (%)
Groundwood pulp, mill	Raw wood	8172	50
		8174	25
		4076	10
	White water chest	8174	35
		4076	20
		4078	10
	Clarified white water	4076	25
Sulfite pulp and fine paper plant	Raw wood	6143	100
	Slush pulp	1792	6
		2030	12
	White water	2030	60
	Water to treatment	2030	60
		6126	30
	Effluent	1792	30
Deink pulp and fine paper plant	Effluent chest water	7936	4
		8174	58
		8175	8
	Sidehill washers	7936	8
		8174	42
	Decker chest	8174	40
	Water to treatment	8174	11
	Effluent	7936	10

[a] Assigned numbers from 0 to $2^{13} - 1$. (Dufour et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, Q75, p. 213).

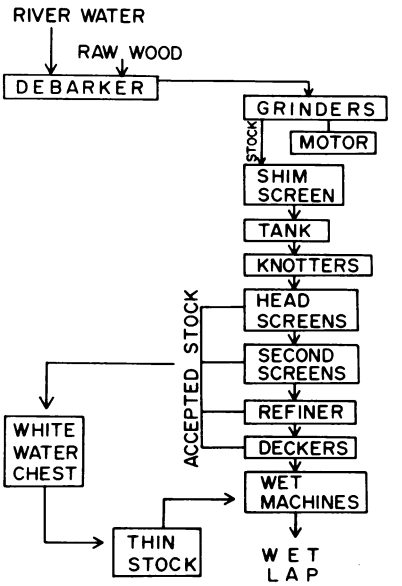

FIG. 1. *Schematic flow diagram of a basic groundwood pulp mill.*

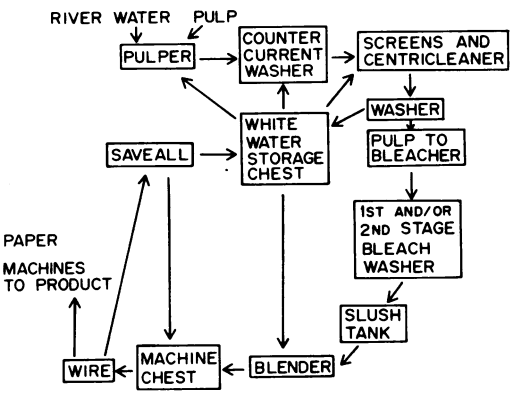

FIG. 2. *Schematic flow diagram of a sulfite pulp and fine paper plant.*

mill industry. Figure 2 shows a schematic flow diagram depicting a typical sulfite pulp and fine paper plant. It was apparent from the results of the fecal coliform and *K. pneumoniae* concentrations of the sulfite pulp and fine paper plant that raw wood with probable soil contamination was the originating source of the bacteria. Results from this plant indicated that treated wastewater had a minimal effect in lowering bacterial levels and that, in fact, elevating bacteria occurred before the final discharge of the wastewater into a receiving body of water. Reuse of any of this treated water would serve as a continuous inoculum of *K. pneumoniae* within the plant.

Thermotolerant *K. pneumoniae* concentrations accounted for up to >90% of the fecal coliform concentration in the sampled deink pulp and fine paper plant. Figure 3 shows a schematic flow diagram of the sampled deink pulp and fine paper mill. Complications concerning the control of bacterial pollution become apparent when the levels of *K. pneumoniae* are examined from the stock going into the decker chest (which originates from the bleach tower) and from the decker chest itself. Bleach treatment of stock alone (typically single-stage hypochlorite bleaching at a terminal pH range between 6 and 7 with temperature ranges between 25 and 35°C) was apparently not sufficient for bacterial reduction.

From the mills sampled, it is concluded that once *K. pneumoniae* originates and proliferates

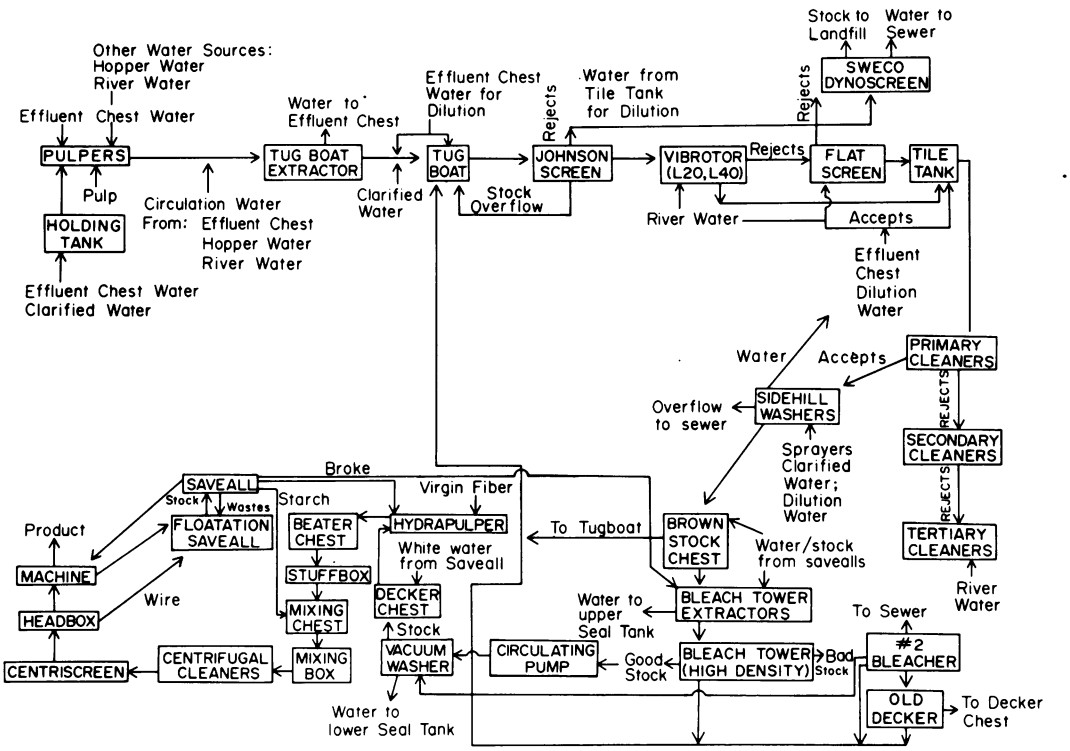

FIG. 3. *Schematic flow diagram of a deink pulp and fine paper mill.*

from the early pulping stages, the reuse of water within plants aids in maintaining elevated *K. pneumoniae* levels throughout the treatment stages of each plant. Current wastewater treatment does not significantly reduce *K. pneumoniae* concentrations in waters receiving pulp and paper mill effluents.

The study findings indicate that the paper industry is a source for the enhanced growth of *K. pneumoniae* in levels exceeding the only surface water standard promulgated, i.e., the fecal coliform standard of no more than 200 organisms per 100 ml. Clearly, the levels of fecal coliform bacteria and thermotolerant and thermointolerant *K. pneumoniae* violate this standard. Since *K. pneumoniae* is an opportunistic pathogen, there is concern with regard to the health consequences for individuals exposed to such high levels of bacteria both within the industry and via the recreational use of water exposed to plant effluents.

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