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Significance of Fecal Coliform-Positive *Klebsiella*[1]

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A total of 191 *Klebsiella pneumoniae* isolates of human clinical, bovine mastitis, and a wide variety of environmental sources were tested for fecal coliform (FC) response with the membrane filtration and most probable number techniques. Twenty-seven *Escherichia coli* cultures of human clinical and environmental origins were also tested. Eighty-five percent (49/58) of known pathogenic *K. pneumoniae* were FC positive, compared with 16% (19/120) of the environmental strains. *E. coli* results indicated 93% (13/14) of the clinical and 85% (11/13) of the environmental strains as FC positive. There was no significant difference in the incidence of FC-positive cultures between pathogenic *Klebsiella* and *E. coli*. pH measurements of *K. pneumoniae* and *E. coli* cultures growing in *m*-FC broth at 44.5°C revealed three distinct pH ranges correlating with colony morphology. β-Galactosidase assays of *Klebsiella* and *E. coli* cultures at 44.5°C indicated all were able to hydrolyze lactose, even if they were FC negative by the membrane filtration or most probable number techniques. The FC response pattern appears stable in *K. pneumoniae*. Three pathogenic cultures showed no change in FC responses after 270 generations of growth in sterile pulp mill effluent. Since *K. pneumoniae* is carried in the gastrointestinal tract of humans and animals and 85% of the tested pathogenic strains were FC positive, the isolation of FC-positive *Klebsiella* organisms from the environment would indicate their fecal or clinical origin or both. The added fact that *K. pneumoniae* is an opportunistic pathogen of increasing importance makes the occurrence of FC-positive environmental *Klebsiella*, particularly in large numbers, a potential human and animal health hazard.

The importance of *Klebsiella pneumoniae* as an opportunistic, multiply antibiotic-resistant human pathogen is well documented (11, 21, 33). It is the primary agent of bovine coliform mastitis (3) and causes serious infections in other animals (13, 17, 38). *K. pneumoniae* can also be routinely isolated from the environment, particularly from nutrient-rich industrial effluents such as pulp and paper mill wastes (9, 19), textile finishing plant effluents (8), and sugarcane wastes (27). Fresh vegetables (4, 9, 28), wood products (9), and natural receiving waters (18, 22) have been constant sources of this organism as well.

Due to its widespread occurrence in areas apparently free from obvious fecal contamination, *K. pneumoniae* has usually been grouped as a total coliform [IMViC type (±)−++] of no immediate health importance (2, 12, 29). In contrast, the fecal coliform (FC) elevated temperature test is considered indicative of recent fecal contamination; a positive test is generally equated with the presence of *Escherichia coli* (6, 11, 12, 14). *K. pneumoniae*, however, is normally carried in the intestinal tract of 30 to 40% of humans and animals (2, 6, 7). Environmental isolates of *Klebsiella* from a variety of sources have been reported as FC positive (2, 8, 9).

To assess the significance of FC-positive environmental isolates, known pathogenic *Klebsiella* isolates were tested for response to membrane filtration (MF) and most probable number (MPN) elevated temperature tests. A wide selection of human clinical and bovine mastitis cultures as well as environmental isolates of *K. pneumoniae* was examined. Clinical and environmental *E. coli* cultures were also included in this survey.

β-Galactosidase activity and pH changes due to growth in *m*-FC broth at 44.5°C were also studied in an effort to explain differences in colony growth or color or both at the elevated temperature in the MF FC tests.

MATERIALS AND METHODS

Bacterial cultures. The origin and source of *K. pneumoniae* and *E. coli* isolates used in this study

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are listed in Table 1. All drinking water-associated *K. pneumoniae* and *E. coli* were obtained from finished water emanating from Oregon public and private water systems (32). Organisms were initially isolated as either total or FCs using *Standard Methods* techniques (1). Ten *Klebsiella* cultures were isolated from finished drinking water held in 65-liter experimental redwood tanks at Oregon State University (OSU). One culture was isolated from a slat scraping of one of the tanks (32). Twelve of the clinical *E. coli* isolates were from urine, one was from a tracheal section, and one was from a cyst.

TABLE 1. *Origin and source of strains used in FC analyses*

Source of strain	No. of isolates	Reference
Klebsiella pneumoniae		
Human clinical	23	University of Oregon Medical School, Portland, Ore.
Human clinical	12	St. Luke's Hospital, Duluth, Minn.
Human clinical	11	University of Texas Medical Center, Houston, Tex.
Bovine mastitis	17	Pennsylvania State University, State College, Pa.
Bovine mastitis	4	Michigan State University, E. Lansing, Mich.
Guinea pig mastitis	1	University of Missouri, Columbia, Mo.
ATCC	3	13883, 13882, 15574
Redwood lab tanks	11	OSU
Drinking water	43	Public water systems
Drinking water	21	Private water systems
Vegetables	19	OSU
Pulp and paper mill effluent	5	B.C. Research, Vancouver, B.C., Canada
Textile mill effluent	4	NMWQL,[a] Environment Protection Agency, W. Kingston, R.I.
Potato process effluent	2	OSU
Redwood sawdust and chips	15	OSU
Escherichia coli		
Human clinical	8	Corvallis Clinic, Corvallis, Ore.
Human clinical	6	Good Samaritan Hospital, Corvallis, Ore.
Drinking water	13	Public water systems

[a] NMWQL, National Marine Water Quality Laboratory.

Biochemical testing for identification. All cultures isolated at OSU were tested biochemically according to the methods of Edwards and Ewing (10). Cultures received from other sources were verified as *K. pneumoniae* or *E. coli* by using a minimum of IMViC, lysine and ornithine decarboxylase, arginine dihydrolase, urease, and motility tests.

Determination of FC response. For MF FC tests, 24-h shake cultures grown at 35°C were diluted to approximately 10 cells/ml. Duplicate 3-ml volumes were filtered using 0.45-μm-pore-size membrane filters (GN-6, Gelman, Ann Arbor, Mich.) with cultivation on *m*-FC medium (Difco, Detroit, Mich.) for 24 h at 44.5 ± 0.2°C (1). Colony number and color were recorded on each plate after incubation.

All isolates were verified by subculturing a blue colony to phenol red lactose broth (Difco) for 24 h at 35°C. Tubes producing gas were subcultured in EC broth (Difco) for 24 h at 44.5 ± 0.2°C. Tubes producing gas were considered confirmed FC. Isolates having blue-green or light blue-gray colonies were not considered MF FC positive. These colony types had a low percent confirmation both in this and other reported surveys (2).

For MPN FC tests, cultures were first grown in phenol red lactose broth at 35°C. After 24 h, cultures were transferred to EC broth and incubated for 24 h at 44.5 ± 0.2°C. Tubes with growth and gas production were considered FC positive.

β-Galactosidase tests at 44.5°C. Selected isolates were streaked on brain heart infusion agar (Difco) with 1% added lactose. Plates were placed at 35°C for 2 h and then transferred to 44.5°C for 18 h of further incubation. Differentiation disks ONPG (*o*-nitrophenyl-β-D-galactopyranoside) were used to determine the presence of β-galactosidase. A loopful from the 20-h culture was suspended in 0.2 ml of 0.85% saline. Test suspensions were incubated with the ONPG disks at 44.5°C (not 35°C as directed by the manufacturer). Results were recorded at 20 min and after 4 h of incubation. Presence of a yellow color indicated β-galactosidase activity.

pH of cultures in *m*-FC broth at 44.5°C. Selected isolates of *K. pneumoniae* and *E. coli* were incubated in tubes containing 7 ml of *m*-FC broth (Difco) containing 1% rosolic acid (Difco) for 24 h at 44.5°C. Direct pH measurements of the broth were made with a combination triple-purpose electrode (no. 476022, Corning Glassware, Corning, N.Y.). Broth color was recorded immediately upon tube removal from the incubator.

Statistical testing of results. Results from FC tests for *K. pneumoniae* and *E. coli* isolates from various sources were analyzed for statistical differences using chi-square 2 × 2 contingency tables (35). Differences were considered significant if P values were less than 0.05.

RESULTS

A total of 191 cultures of *K. pneumoniae* and 27 *E. coli* were tested for MF and MPN FC response. The general test results are presented in Table 2.

With *m*-FC agar at 44.5°C for the MF technique, results indicated that 71.7% of the human clinical and 86.3% of the mastitis *K. pneumoniae* would be considered FCs. In contrast, 15.5% of the tested environmental isolates had blue colonies, indicating MF-positive results. Two of three American Type Culture Collection (ATCC) cultures (ATCC 13882 and 15574) would also be considered as FCs. Although able to grow at 44.5°C, the *K. pneumoniae* type culture, ATCC 13883, did not produce blue colonies. A greater percentage of known pathogenic strains were also able to grow at 44.5°C on *m*-FC agar as compared with those from the environment: clinical, 96.0%; mastitis, 86.3%; and environmental, 20.1%. *Klebsiella* FC-positive colony color and morphology ranged from flat and dark blue, with or without precipitated bile, to raised, mucoid, dark blue to raised with blue centers and cream-colored edges. About 40% of the FC-positive cultures exhibited flat, dark blue colonies with some orange coloration. This response is probably due to the interaction of the *Klebsiella* culture with the rosolic acid in the *m*-FC agar. Non-FC colony colors varied from cream and pink (definite negatives) to blue-green or light blue-gray. Representative colony types appeared from all sources of *K. pneumoniae* tested.

The number of *Klebsiella* FC positive by MPN techniques as opposed to MF varied slightly for the environmental isolates (16.7 to 15.8%) and not at all for the mastitis isolates (86.3%). The same two MF-positive ATCC cultures also produced gas in EC broth. The *K. pneumoniae* type culture again showed growth but negative results. Approximately 83% of the clinical isolates were MPN positive, whereas only 71.7% were positive in MF tests. Statistical tests, however, indicate no significant difference ($P > 0.05$) between the percentages for MF- and MPN-positive reactions within each of the three groups. Percent confirmation of all MF-positive isolates in EC broth was 93.2% for combined sources of *K. pneumoniae* cultures. This overall percent, and those for the separate sources, is well within the range of reported confirmation percentages for FCs in general (14, 15, 31).

TABLE 2. *MPN and MF FC-positive cultures*

Origin	No. of isolates	Positive FC response (%)		% Confirmation (MPN⁺/MF⁺)
		MPN	MF	
Klebsiella pneumoniae				
Human clinical	46	82.6	71.7	93.6[a]
Mastitis	22	86.3	86.3	100.0
ATCC	3	66.7	66.7	100.0
Environmental	120	16.7	15.8	89.5
Escherichia coli				
Drinking water	13	84.6	84.6	100.0
Human clinical	14	92.9	92.9	100.0

[a] Numbers indicate the percentage of MF FC-positive isolates confirmed in EC broth.

The only significant statistical differences between the incidence of FCs for any of the *Klebsiella* cultural origins reported in Table 2 were between clinical and environmental *K. pneumoniae* and between mastitis and environmental isolates (by both MF and MPN techniques). The *P* value was less than 0.05 for both comparisons.

Some 84.6% of *E. coli* from finished drinking water and 92.9% of clinical isolates were MPN and MF FC positive. All *E. coli* positive by MF were confirmed as FC in EC broth. The majority (87.5%) of the FC-positive colonies were flat blue with precipitated surface bile; the remainder (three environmental isolates) had dark blue colonies with lighter blue edges. Similar variations in colony color have been reported for *E. coli* isolated from pulp mill effluent receiving waters (2). The one FC-negative human *E. coli* culture (from urine) had cream-colored colonies at 44.5°C. The two FC-negative *E. coli* from environmental sources would not grow at 44.5°C with either MF or MPN techniques. There was no significant difference between the incidence of MF FC-positive pathogenic *K. pneumoniae* and *E. coli* ($P > 0.05$). As expected, there was a difference ($P < 0.05$) in the incidence of FC-positive *E. coli* and FC-positive environmental *Klebsiella*.

Based only on MF response of clinical *Klebsiella*, there is an apparent wide variation in the incidence of FC-positive cultures by source (Table 3). The results of the blood origin group may be biased because only four isolates were examined from this source. However, the incidence of FC-positive isolates from different clinical specimens was very similar when based on gas production in EC broth (MPN). A similar observation can be made in comparing the MPN FC-positive *Klebsiella* and *E. coli* urine isolates ($P > 0.05$).

A segregation of environmental *K. pneumoniae* isolates by source is presented in Table 4.

TABLE 3. *FC response of clinical K. pneumoniae isolates*

Origin	Source[a]	No. of isolates	Positive FC response (%)		% Confirmation (MPN+/MF+)
			MPN	MF	
Urine	UT, SL	28	85.7	64.3	88.9[b]
Blood	UO, UT	4	50.0	50.0	100.0
Sputum	UT, SL	8	87.5	87.5	100.0
Other[c]	UO, UT	6	83.4	66.7	100.0

[a] UT, University of Texas Medical School; SL, St. Luke's Hospital; UO, University of Oregon Medical School.

[b] See footnote *a*, Table 2.

[c] Includes pus (two), throat (one), stomach (one), wound (one), and abscess (one).

TABLE 4. *FC response of environmental K. pneumoniae isolates*

Source	No. of isolates	Positive FC response (%)		% Confirmation (MPN+/MF+)
		MPN	MF	
Redwood lab tanks	11	27.3	27.3	100.0[a]
Public drinking water	43	16.3	16.3	85.7
Private drinking water	21	0.0	4.8	0.0
Redwood sawdust and chips	15	33.3	33.3	100.0
Vegetables	19	21.1	15.8	100.0
Industrial effluents[b]	11	9.1	9.1	100.0

[a] See footnote *a*, Table 2.

[b] Includes pulp and paper mill (five), textile mill (four), and potato processing effluent (two).

Redwood lab tanks, sawdust and wood chips, vegetables, and finished public drinking water isolates account for the majority of FC-positive results, whether by MF or MPN techniques. The percent confirmation of MF-positive isolates as FCs was 100% for all but two sources (one of which had only one MF-positive culture). Analysis of the various groups showed no significant difference in the incidence of FCs from any of the environmental areas sampled ($P > 0.05$).

The increased numbers of FC-positive isolates obtained with EC broth (particularly with clinical *K. pneumoniae* isolates) and the diversity of colony morphology and color on *m*-FC agar led to investigations as to reasons for these observations. Direct measurement of pH change in *m*-FC broth at 44.5°C was studied since the *m*-FC medium relies on acid production from lactose fermentation to maintain a dark blue colony color (15). Results from testing 42 *Klebsiella* able to grow at 44.5°C (with varying FC responses) and 12 FC-positive *E. coli* are presented in Table 5. Distinct correlations were observed between pH reactions and the various types of FC responses with both MF and MPN techniques. The pH ranges in *m*-FC broth were pH <5.3, pH 5.3 to 5.8, and pH >5.8 for MF+,MPN(±), MF(−),MPN+, and MF(−),MPN(−), respectively [(−) indicates growth but a negative FC response]. All MF+,MPN+ cultures with pH <5.3 had dark blue- or blue-orange-colored colonies. Five *K. pneumoniae* cultures MF(−),MPN+ with broth pH between 5.3 and 5.8 had blue-centered colonies with cream-colored edges. Five of eight MF(−),MPN+ cultures (four clinical and one

TABLE 5. *pH in m-FC broth test incubated at 44.5°C versus FC response*

Origin	FC response		No. of isolates	pH range (% occurring)		
	MF	MPN		<5.3	5.3–5.8	>5.8
Klebsiella pneumoniae						
Clinical	+	+	9	66.7	33.3	0.0
	+	(−)[a]	3	100.0	0.0	0.0
	(−)	+	5	0.0	100.0	0.0
	(−)	(−)	4	0.0	0.0	100.0
Mastitis	+	+	8	100.0	0.0	0.0
Environmental	+	+	6	66.7	33.3	0.0
	+	(−)	1	100.0	0.0	0.0
	(−)	+	3	0.0	100.0	0.0
	(−)	(−)	3	0.0	0.0	100.0
Escherichia coli						
Human clinical	+	+	7	71.4	28.6	0.0
Environmental	+	+	5	100.0	0.0	0.0

[a] Parenthesis indicate growth but a FC-negative reaction.

environmental) had light blue-gray- or blue-green-colored colonies on *m*-FC agar. Cultures with broth pH >5.8 had pink or cream-colored colonies and produced no gas in EC broth. The MF+,MPN+ isolates of both *Klebsiella* and *E. coli* contained two biotypes with respect to acid production. These were the strong acid producers whose biotype FC configuration also was seen to be MF+,MPN(−), and the intermediate acid producers, which were aerogenic, and represented by the FC biotype MF(−),MPN+ (Table 5).

On the basis of pH range, there was no difference at 44.5°C between environmental, clinical, or mastitis MF+,MPN+ FC-positive *K. pneumoniae*. This pH range varied from 4.7 to 5.5, whereas the pH of *E. coli* FC-positive cultures was 5.0 to 5.5. All *E. coli* isolates had dinstinctive reactions in the *m*-FC broth: the medium was light pink or purple in color with some of the indicator dyes partially reduced. *m*-FC broth colors for all tested *K. pneumoniae* isolates roughly corresponded with their respective colony colors on *m*-FC agar [blue for MF+ and pink-purple for MF(−)].

β-Galactosidase activity (with ONPG disks [Difco]) was demonstrated in all 41 *K. pneumoniae* and 7 *E. coli* isolates able to grow at 44.5°C. Regardless of the FC biotype response, all had the ability to ferment lactose at the elevated temperature. Five of the β-galactosidase-positive *K. pneumoniae* isolates (three environmental, one mastitis, and one clinical) would grow on brain heart infusion agar with 1% lactose but were unable to grow on *m*-FC agar at 44.5°C. All five isolates would grow in EC broth at 44.5°C but produced no gas.

Of the conventional biochemical tests performed, the only variation in reactions by the *Klebsiella* isolates of various origins was indole production and urease activity (Table 6). Excluding the ATCC cultures, the percentage of indole-positive isolates, especially for environmental strains, was greater than reported by Edwards and Ewing (10). The environmental group also had a smaller number of urease-positive isolates (75%). Neither test was used for primary isolation of the test isolates.

TABLE 6. *Indole and urease test results of K. pneumoniae*

Origin	No. of isolates	Positive occurrence (%)	
		Indole	Urease
Clinical	46	15.2	100.0
Mastitis	22	9.1	95.5
ATCC	3	0.0	100.0
Environmental	120	33.3	75.0
Edwards and Ewing (10)		6.6	94.5

DISCUSSION

It is difficult to interpret earlier literature as to the importance and occurrence of *Klebsiella* in the environment, and as a FC in particular, due to its association with the former genus *Aerobacter*. Usually no attempt was made to identify total or FC-positive organisms beyond the IMViC pattern (−−++, +−++, and −+−+ for *Klebsiella-Enterobacter-[Aerobacter]*). However, any organisms with these IMViC patterns appearing as positive on elevated temperature tests were indeed considered as valid FCs (14). In this context, it should be pointed out that *m*-FC agar was developed for enumeration of FCs in general and not for *E. coli* in particular (2, 15).

During the 1970s, an increased use of the FC elevated-temperature test as an indicator of environmental quality has occurred. The significance and validity of such tests, however, have been challenged when *E. coli* is not present and other coliforms (namely *K. pneumoniae*) appear in the sample as FCs (2, 8, 11). When *E. coli* can be isolated from the same sample as *Klebsiella*, fecal contamination is considered to have been recent (19). *Klebsiella*, however, has not only routinely been isolated in large numbers from a wide variety of natural habitats, but often appears as the only FC-positive enteric genus (2). For example, Knittel and others (2, 8, 19) have reported that nutrient-rich industrial wastes yield high numbers of FC-positive *K. pneumoniae* giving MF colony morphology or MPN results indistinguishable from that expected of *E. coli*.

How is it possible to account for the presence of FC-positive *Klebsiella* in the absence of *E. coli*? Due to its unique nutritional capabilities, *Klebsiella* may not only survive but actually multiply in certain environments (2). This regrowth has been cited as the reason for negating the importance of FC-positive *Klebsiella* cultures (2, 12). Pathogens such as *Salmonella* have not been isolated in these situations, and this has been taken as an indication that any fecal pollution was not recent (8).

Other studies indicate that *Klebsiella* appears to have a differential survival rate over *E. coli*. Ptak et al. (29) found 40% of the FC in raw drinking water intake to be *Klebsiella* (as opposed to 60% *E. coli*); after treatment, 67% of the isolates were *K. pneumoniae*, whereas only 4% were *E. coli*. In examinations of receiving

waters below treated sewage outfalls, Schillinger and Stuart (J. E. Schillinger and D. G. Stuart, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, N12, p. 172) found increased isolation of *K. pneumoniae* and *Enterobacter aerogenes* over that of *E. coli*, indicating better recovery of the former from chlorination injury. As our own test results also indicate, not all *E. coli* of environmental or clinical origin are FC positive. Therefore, *E. coli* in some environmental samples may be unable to either grow at 44.5°C or to produce positive FC test results. In such situations, *Klebsiella* could appear as the only type of FC in the sample.

The 16% FC-positive rate among the environmental *Klebsiella* is significant because of the high incidence (85%) of FC-positive reactions among cultures of known pathogenic origin. The high percent confirmation of MF FC-positive *Klebsiella* from all sources indicates these would be true FCs, not false positives. Since the FC test is capable of detecting up to 85% of the *Klebsiella* of known pathogenic origins, the occurrence of FC-positive *Klebsiella* from environmental samples strongly supports their sanitary significance, and they must not be ignored.

Our data indicate 83% (38/46) of human clinical *Klebsiella* cultures are MPN FC positive, which is virtually identical with previously reported results of 83% (25/30) human clinical strains as MPN FC positive (8). It is also apparent that human clinical *K. pneumoniae* from any source (urine, blood, sputum, etc.) are equally likely to be FC positive. *K. pneumoniae* from animals can also be significant since over 86% of the pathogenic mastitis isolates were both MF and MPN FC positive.

FC-positive *Klebsiella* from experimental laboratory redwood tanks filled with finished drinking water and from redwood sawdust and chips comprised 36% of the total FC-positive environmental *K. pneumoniae* found in this survey. Although the original source of these FC-positive isolates has not been determined, it has been suggested that the high level of extractable nutrients from redwood provide the *Klebsiella* strains with a nutrient-rich growth medium (32). This environment is probably not unlike some industrial wastes, which also yield significant numbers of FC-positive *K. pneumoniae*, probably due to regrowth. Other FC-positive *Klebsiella* from finished public drinking water systems were probably transistory, recent fecal contaminants since *E. coli* was usually isolated as well (32).

Stability of the lactose fermentation phenotype (producing acid or gas or both at 44.5°C) was demonstrated in our laboratory by continual passage in sterile pulp mill waste of three pathogenic *Klebsiella* cultures. After 270 generations of growth (with 45 transfers) at 35°C, these strains retained their original FC response characteristics: (i) MF+,MPN+, (ii) MF(−),MPN(−), and (iii) MF−,MPN−. As fermentation ability at an elevated temperature is not selected for in most reported natural habitats of *K. pneumoniae*, it appears to be an inherent and stable characteristic of each organism. Dufour and Cabelli (8) reported that 60% of *Klebsiella* strains isolated from textile plant wastes seeded 2 years previously with sewage were still MPN FC positive. These studies indicate that even after generations of regrowth of *K. pneumoniae* of fecal origin in the environment, the organisms retained their FC response characteristics.

In the present study, 73% of the pathogenic *Klebsiella* strains were FC positive on both MF and MPN techniques. This FC biotype would be detected more readily in the environment if only MF or MPN techniques are used. Both MF+,MPN(−) and MF(−),MPN+ types of FC-positive *K. pneumoniae* appeared from human clinical sources. These same FC biotypes were found among the environmentally isolated *Klebsiella*, indicating their potential fecal origin as well. Since lactose fermentation appears to be genetically stable there is no reason to suspect that these types of FCs are of any less recent environmental deposition or of less importance than MF+,MPN+ strains.

Finding an intermediate class of *K. pneumoniae* isolates based on pH in *m*-FC medium (5.3 to 5.8) that are MF(−),MPN+ may explain the difference between the lower incidence by MF as compared with MPN FC results for some of the human clinical isolates. These intermediate clinical cultures appear as blue-green or light blue-gray colonies on *m*-FC agar at 44.5°C [MF(−)] but produce gas in EC broth (MPN+). Such atypical colonies appearing in environmental samples should be tested in EC broth for possible verification as FCs. These types of colonies on *m*-FC medium have been noticed in tests of pulp mill effluents and have generally been identified as *K. pneumoniae*, although MPN verfication was not reported (2).

Buras and Koh (5) reported a pH differential in *m*-FC broth between fecal *E. coli* (5.0–5.4) and nonfecal *A. aerogenes* (7.0 to 8.0). In our tests, there was no difference in pH produced between FC-positive *E. coli* and *K. pneumoniae*. The range for MF(−),MPN(−) strains was pH 5.9 to 6.6. All FC-negative *Klebsiella* strains tested had β-galactosidase activity at 44.5°C, but not necessarily the ability to ferment lactose so as to produce positive FC reac-

tions. A difference apparently exists between strains able to grow at the elevated temperature as to the amount and types of acid produced and not merely in the ability to hydrolyze lactose.

The percent variability in urease reaction between sources is similar to data reported by Henriksen (16) in which 96% of his pathogenic and fecal *Klebsiella* strains were urease positive and 69% of the water strains were positive. Positive urease activity has been suggested by Buttiaux (6) and Mossel (25) as indicative of *Klebsiella* strains of fecal origin. All FC-positive isolates in this study (human clinical, environmental, and mastitic) were urease positive. However, all but one of the FC-negative pathogenic strains (one from mastitis) and 68% of the environmental FC-negative strains were urease positive. Thus, in using only the current concept and techniques for FC detection, urease activity would not be sufficient to indicate that a *Klebsiella* isolate is of fecal origin.

Other studies of *Klebsiella* from vegetables (19) and textile finishing wastes (8) indicated no indole-positive *Klebsiella* IMViC types as EC positive; a similar observation was made in this survey. With only one exception (a human clinical strain), all indole-positive isolates from human clinical (7), mastitis (2), and environmental sources (40) were MPN FC negative. The indole-positive isolates, which appear otherwise to be *Klebsiella* by routine biochemical tests, resemble the oxytoca group, as proposed by Lautrop (20), Stenzel et al. (36), and Von Reisen (37). This group is characterized, in part, as indole positive and anaerogenic in EC broth at 45°C. The sanitary significance of these organisms should not be discounted, even if FC negative, since 34% of *Klebsiella* fecal isolates (7) and 17% of human clinical strains (21) have also been reported as indole positive. A similar rate (15%) was found among human clinical isolates in the present study.

Although only 16% of the environmental *K. pneumoniae* were FC positive, 49% of them grew in EC broth at 44.5°C. This trait was termed "thermotolerance" by Dufour and Cabelli (8) and was suggested by them to be indicative of coliforms of fecal origin. This designation may be based on their finding that all tested human clinical strains (30/30) were able to grow in EC broth at 44.5°C (as did 91% of our pathogenic strains). It is our conclusion that there are insufficient data at present to equate the mere ability of a coliform to grow in EC broth at 44.5°C (anaerogenic) with its fecal origin.

No distinction can be made between the type of FC response, health hazard, and time of entry of the organism into the environment, since all types appear among the pathogenic *K. pneumoniae* strains. If it is deemed necessary to identify organisms appearing as FCs on MF or MPN tests, *Klebsiella* should be considered as valid a FC as *E. coli*, with or without its concurrent isolation. Isolation of the two together, particularly if FC positive, should be indicative of recent fecal contamination. Occurrence of FC-positive *K. pneumoniae* alone should be indicative of fecal pollution at some point in time, which may have been recent or much earlier. The significance of the latter situation does not necessitate the implication that other pathogenic enteric bacteria be present. The deterioration of environmental quality rests on the opportunistic pathogenic nature of *Klebsiella* per se and recognition of the nature by which exposure and subsequent clinical manifestation are separated in time.

The ubiquitous distribution of FC-positive *Klebsiella* found in finished drinking water, foods, wood products, and industrial environments may already be manifested in the changing patterns seen in documented reports. Included are the increase in both cell densities and colonization rate of the human intestinal tract and the increase in human infection rates caused by *Klebsiella* during the last two decades (6). Several investigators have attributed increased infection rates in humans to prior colonization of the human gastrointestinal tract (12, 24). Colonization has been attributed to the ingestion of foods contaminated with *Klebsiella* (34). *Klebsiella* is also the primary agent of serious and sometimes lethal diseases of domestic animals such as dairy cattle, horses, and primates (3, 13, 23). Exposure of such animals to high densities of *Klebsiella* present in sawdust facilitates colonization of cow teats (26, 30). There is then sufficient evidence to warrant the statement that exposure to FC-positive *Klebsiella* should certainly be regarded as indicative of a potential human and animal health hazard.

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