Characterization of *Klebsiella* Isolates from Natural Receiving Waters and Comparison with Human Isolates

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Received for publication 20 May 1974

Two hundred sixty-six strains of *Klebsiella pneumoniae* isolated from natural water sources in geographically diverse areas (Florida, Massachusetts, and Oregon) were analyzed to determine the serotype, biochemical, virulence, and antimicrobial susceptibility differences between these natural strains and human *Klebsiella* isolates. Sixty of 72 defined serotypes were found among 210 typable strains. Geographic patterns were present, but in general were not pronounced among serotypes. Reactions with 28 biochemical tests showed percentage responses which were very similar to the summaries of primarily human *Klebsiella* isolates (as reported by Edwards and Ewing, 1972) and that represented diverse geographic sampling. Virulence studies in representative strains showed no geographic variability and little difference from comparable hospital patient-obtained isolates. In contrast to human hospital isolates, strains demonstrated 90% or greater susceptibility to all antibiotics except ampicillin and carbenicillin; and in further contrast, there was little multiple antibiotic resistance beyond that with ampicillin and carbenicillin.

*Klebsiella* strains constitute a significant portion of the organisms that cause serious gram-negative infections in humans. These organisms may be endogenously acquired, as part of the natural host flora, or they may be exogenously acquired within the hospital environment. The *Klebsiella* which are associated with infections pose therapeutic problems for the physician because of the potential for antimicrobial resistance among these infection-related isolates. A study was undertaken to determine the degree of natural *Klebsiella* resistance to antibiotics, to assess other characteristics of *Klebsiella* strains isolated from natural receiving waters in geographically divergent areas of the United States, and to compare these strains with characteristics of human clinical *Klebsiella* isolates.

**MATERIALS AND METHODS**

**Organism isolation.** *Klebsiella* strains, as characterized by indole, methyl red, Voges-Proskauer, and citrate biochemical reactions, were isolated from fresh and saline water sources in Oregon (88 strains), Massachusetts (68 strains), and Florida (110 strains). The water source was classified as clean if there was no identifiable source of human contamination, or as contaminated if human contamination was thought or known to have occurred in any way. Organisms were collected by researchers of the National Council of the Paper Industry for Air and Stream Improvement and mailed to the University of Minnesota on either stabbed agar deeps or streaked agar slants. These were subcultured to plates of sheep blood agar and MacConkey agar to assure purity prior to characterization.

**Biochemical testing.** Tests for the presence of indole were done by using Kovacs reagent on 48-h cultures incubated at 37°C in a broth containing 1% tryptone (Difco Laboratories, Detroit, Mich.) and 0.5% NaCl. Methyl red determinations were carried out with MR-VP medium (Difco), in which organisms were grown for 5 days at room temperature and tested by the method of Edwards and Ewing (4). The Voges-Proskauer test was also performed with MR-VP medium. Organisms were grown in 1 ml of broth at 37°C for 48 h, at which time 0.2 ml of 40% potassium hydroxide and 0.6 ml of 5% alpha-naphthol in absolute ethyl alcohol were added, and positives were read after 4 h. The utilization of citrate was determined by using Simmons citrate agar (Difco) slants on which organisms were streaked and incubated for 4 days at 37°C. Negative citrate tests were repeated.

Decarboxylase and dihydrodase tests were carried out with decarboxylase base Moeller (Difco) containing 1% concentrations of the amino acids. A control broth without amino acids was also inoculated, and all tubes were overlaid with mineral oil. Final readings were made after 4 days of incubation at 37°C.
Malonate broth (modified, Difco) was used to detect utilization of malonate. Readings were made at 48 h, and all negative malonate tests were repeated.

Deoxyribonuclease production was tested on deoxyribonuclease test agar (Difco) containing 2.5 ml of a 2% toluidine blue solution per liter. The organisms were grown for 18 to 24 h at 37 C, and a positive reaction was indicated by the development of a pink color.

All other biochemical tests were carried out as previously described (8) except that carbohydrate broth cultures were incubated at 37 C for 5 days and observed daily. Cultures were then held an additional 25 days at room temperature and observed for acid and gas production.

**Susceptibility tests.** Susceptibility testing was carried out by the method of Bauer et al. (1) as previously described (9).

**Serologic testing.** All strains were tested with type-specific Klebsiella antisera produced at the University of Minnesota (8), which employed 72 stock type cultures supplied originally by W. H. Ewing of the Center for Disease Control, Atlanta, Ga. Typing was carried out by the slide agglutination and Quellung reactions for capsular antigens as described by Edwards and Ewing (4).

**Virulence testing.** Six-hour cultures of Klebsiella strains were serially diluted to give organism counts ranging from 10^1 to 10^7/ml in trypticase soy broth (Difco) containing 5% hog gastric mucin. Groups of five Swiss Webster mice weighing from 23 to 25 g were injected intraperitoneally with 1.0 ml of these dilutions. Virulence was assessed in reaction to the number of mice that died within 48 h after injection. The mean lethal dose determinations were made by the method of Reed and Muench (13).

**RESULTS**

Two hundred sixty-six strains of Klebsiella were received for complete evaluation and analysis by the methods described. Of these strains, 88 were collected in Oregon, 68 were collected in Massachusetts, and 110 were collected in Florida. Table 1 defines not only the geographic distribution of these strains, but outlines also the water source according to type of water where the isolates were collected. Seventy-two strains were derived from clean freshwater, and 135 strains were derived from contaminated freshwater. Fewer strains were collected from seawater, 33 strains from clean saline water sources and 26 from saline water sources considered to be contaminated.

In Table 2, the results of the biochemical studies are compared with those of Edwards and Ewing (4). The sources of strains studied by Edwards and Ewing were primarily human Klebsiella isolates and represented diverse geographic sampling. There were very few significant differences in these biochemical reactions. Study strains demonstrated 98.1, 89.5, and 71.4% positive reactions, as compared to 92.8, 97.2, and 87.7%, which Edwards and Ewing reported as positive reactions for mucate, lysine decarboxylase, and adonitol fermentation tests, respectively. All other reactions were very similar. When biochemical reactions were analyzed according to geographic area of isolation, few differences occurred. However, Oregon and Massachusetts isolates gave 17% negative reactions.

### Table 1. Geographic and water-type sources of Klebsiella

| Water source          | No. found in: |   |
|-----------------------|---------------|---|
|                       | Oregon | Massachusetts | Florida |
| Clean, fresh          | 29     | 12             | 31      |
| Contaminated fresh    | 49     | 45             | 41      |
| Clean, saline         | 6      | 5              | 22      |
| Contaminated saline   | 4      | 6              | 16      |

### Table 2. Biochemical comparisons of Klebsiella strains

| Biochemical test                | Percent positive | Receiving waters | Edwards-Ewing (4) |
|---------------------------------|------------------|------------------|-------------------|
| Indole                          | 4.4±             | 6.0              |                   |
| Voges-Proskauer                 | 95.9±            | 91.1             |                   |
| Simmons citrate                 | 99.6±            | 97.2             |                   |
| Malonate                        | 95.1             | 92.5             |                   |
| Urease                          | 98.1             | 94.5             |                   |
| Mucate                          | 98.1             | 92.8             |                   |
| Arginine dihydride              | 4.1              | 0.9              |                   |
| Lysine decarboxylase            | 89.5             | 97.2             |                   |
| Gelatin                         | 0.8              | 3.3              |                   |
| Nitrate                         | 97.7             | 99.9             |                   |
| Motility                        | 0                | 0                |                   |
| Oxidase                         | 0                | 0                |                   |
| Deoxyribonuclease               | 0                | 0                |                   |
| Phenylalanine deaminase         | 0                | 0                |                   |
| Ornithine decarboxylase         | 0                | 0                |                   |
| Sugar fermentations:            |                  |                  |                   |
| Lactose                         | 98.5             | 99.6             |                   |
| Adonitol                        | 71.4             | 87.7             |                   |
| Inositol                        | 95.5             | 98.7             |                   |
| Glycerol                        | 98.1             | 99.9             |                   |
| Arabinose                       | 99.2             | 99.9             |                   |
| Raffinose                       | 98.1             | 99.7             |                   |
| Rhamnose                        | 98.1             | 99.7             |                   |
| Glucose                         | 100.0            | 100.0            |                   |
| Sorbitol                        | 98.1             | 99.7             |                   |
| Salicin                         | 98.9             | 100.0            |                   |
| Cellobose                       | 98.9             | 100.0            |                   |

*Comparisons not valid because of the sampling bias of the organisms received, i.e., organisms sent for characterization were selected on the basis of classical Klebsiella indole, Voges-Proskauer, and citrate reactions.
tions in each group with the lysine decarboxylase test, but strains from Florida gave 0.9% (only 1 strain) negative reactions.

Susceptibility patterns of Klebsiella isolates indicated that all strains were susceptible to gentamicin, and 97 to 99% were susceptible to cephalothin, cephaloridine, chloramphenicol, kanamycin, nalidixic acid, and sulfisoxazole.

Table 3 shows susceptibility, intermediate susceptibility, and resistance where there was variability of results. Few strains of the 266 tested were susceptible to ampicillin (13%, or 35 strains) or carbenicillin (5%, or 14 strains), whereas most were susceptible to colistin (94%, or 252 strains), polymyxin B (94%, or 251 strains), nitrofurantoin (87%, or 234 strains) streptomycin (93%, or 250 strains), and tetracycline (90%, or 240 strains). A total of 10.5% of the strains showed resistance to at least one antibiotic other than ampicillin or carbenicillin.

There was little difference when the susceptibility of Klebsiella strains to ampicillin and carbenicillin was analyzed according to water source, except that 27% of strains from clean saline water sources were susceptible to ampicillin, as compared to only 6 to 13% susceptibility from the other source classifications (Table 4). In addition, more strains from clean saline water sources were susceptible to carbenicillin (12%) than from the other sources (3 and 4%).

Table 5 shows the geographic differences in susceptibility to ampicillin and carbenicillin. Strains from Oregon demonstrated 20.5% susceptibility to ampicillin, whereas Florida isolates only showed a 4.5% susceptibility.

In a statistical approach to the differences in ampicillin susceptibility and nonsusceptibility, \( P < 0.01 \) when Massachusetts isolates are similarly compared to the Florida patterns. However, if these same comparisons are approached through analysis of ampicillin resistance and nonresistance then \( P < 0.01 \) when Florida and Oregon isolates are compared, but there is no significant difference \( (P = 0.1528) \) when Massachusetts and Florida strains are compared as to resistance.

A statistical analysis of carbenicillin results shows no significant differences in the susceptible category for the various geographical groups, but does show differences in the intermediately susceptible category for Oregon and Florida \( (P < 0.05) \), Massachusetts and Florida \( (P < 0.05) \) and for Massachusetts, and Oregon and Florida \( (P < 0.05) \). Similarly, differences are present in the resistant category: Oregon and Florida \( (P < 0.05) \), and Massachusetts and Florida \( (P < 0.01) \), indicating differences in the degree of susceptibility of Florida strains.

Table 3 shows the variable antibiotic susceptibility patterns with 266 Klebsiella strains isolated from receiving waters.

| Antibiotic       | Resistant (%) | Intermediate (%) | Susceptible (%) |
|------------------|---------------|-----------------|----------------|
| Ampicillin       | 79            | 8               | 13             |
| Carbenicillin    | 84            | 11              | 5              |
| Colistin         | 0.4           | 6               | 94             |
| Polymyxin B      | 0             | 7               | 94             |
| Nitrofurantoin   | 3             | 10              | 87             |
| Streptomycin     | 0.7           | 6               | 93             |
| Tetracycline     | 5             | 5               | 90             |

Table 4. Susceptibility of 266 Klebsiella strains isolated from receiving waters to ampicillin and carbenicillin

| Klebsiella source | No. of Strains | Ampicillin (%) | Carbenicillin (%) |
|-------------------|---------------|----------------|-------------------|
|                    | S+ I+ R+      | S+ I+ R+       | S+ I+ R+          |
| Clean fresh water | 72            | 6              | 11               | 83               | 3               | 7               | 90               |
| Contaminated fresh| 135           | 13             | 7               | 80               | 4               | 13              | 83               |
| Clean saline water| 33            | 27             | 6               | 67               | 12              | 18              | 70               |
| Contaminated saline water | 26 | 8 | 12 | 80 | 4 | 8 | 88 |

* S, Susceptible; I, intermediate; R, resistant.

Table 5. Geographic differences in susceptibility to ampicillin and carbenicillin

| Geographic source | Susceptible | Intermediate |
|-------------------|-------------|--------------|
|                   | Ampicillin | Carbenicillin | Ampicillin | Carbenicillin |
|                   | No. %      | No. %        | No. %     | No. %        |
| Oregon (88)*      | 18         | 20.5         | 5         | 5.7          | 8            | 9.1           | 13             | 14.8            |
| Massachusetts (68)*| 10         | 14.7         | 6         | 8.8          | 5            | 7.4           | 11             | 16.2            |
| Florida (110)*    | 5          | 4.5          | 2         | 1.8          | 9            | 8.2           | 6              | 5.5             |

* Total number of strains tested from each area.

In addition, differences were demonstrated in the resistance category: Oregon and Florida \( (P < 0.05) \), and Massachusetts and Florida \( (P < 0.01) \), indicating differences in the degree of susceptibility of Florida strains.
with human Klebsiella isolates. The human population used for comparison purposes was the 1970 University of Minnesota annual patient susceptibility tabulation. Ninety-seven percent of water strains were susceptible to cephalothin, but only 76% of human Klebsiella hospital isolates were similarly susceptible. Ninety-nine percent of water strains were susceptible to chloramphenicol, kanamycin, and sulfisoxazole; human hospital isolates showed 87, 75, and 65% susceptibility, respectively. A large difference in susceptibility was shown in the case of nitrofurantoin, in which 87% of water strains were susceptible, although the comparable human isolate figure was only 50%. The greatest antibiotic resistance among water strains, other than with ampicillin and carbenicillin, was found to occur with tetracycline (only 90% susceptible). Human isolates were 76% susceptible.

There were 210 isolates on which typing could be carried out serologically. Of these, 60 out of 72 possible serotypes were represented. There were 23 strains (8.6%) which had no capsule and 36 strains (13.5%) which had insufficient capsule for typing. One hundred three typable strains (49%) fell within 11 serotypes (8, 11, 22, 28, 30, 55, 56, 60, 61, 62, 64). The comparison of these and other serotypes is shown in Table 7 in relation to geographic source.

Mouse virulence testing is summarized in Table 8. The breakdown was made between geographic source of water isolates; also shown are results of virulence testing with Klebsiella strains isolated from receiving waters as compared to multiple antibiotic-resistant human Klebsiella isolates from patients at the University of Minnesota Hospitals. The virulence of the two groups was essentially the same; a mean of $5.3 \times 10^4$ human Klebsiella organisms was required to kill 50% of the mice, and $4.5 \times 10^4$ water Klebsiella were required to give the same lethal effect.

### DISCUSSION

Klebsiella isolates from natural receiving waters were not evenly distributed with respect to geographic location or origin from saline or freshwater sites. The total number of saline water isolates was rather limited and precludes placing a great deal of significance on the results of the analysis of these isolates. The reactions with the 28 biochemical tests showed percent responses very similar to the summaries of primarily human Klebsiella isolates (as reported by Edwards and Ewing [4]) and repre-

### Table 6. Klebsiella susceptibility comparisons

| Antibiotic       | Water strains* (%) | U. of Minnesota strains* (%) |
|------------------|--------------------|-----------------------------|
| Ampicillin       | 13                 | 6                           |
| Cephalothin      | 97                 | 76                          |
| Chloramphenicol  | 99                 | 87                          |
| Kanamycin        | 99                 | 75                          |
| Nitrofurantoin   | 87                 | 50                          |
| Sulfisoxazole    | 99                 | 65                          |
| Tetracycline     | 90                 | 76                          |

* 266 Strains isolated from receiving waters.

* 1972 University of Minnesota annual susceptibility tabulation (570 strains).

### Table 7. Serotype distribution of 266 Klebsiella strains isolated from natural receiving waters

| Serotype | No. found in: | Serotype | No. found in: |
|----------|---------------|----------|---------------|
|          | Florida       | Massachusetts | Oregon       | Florida       | Massachusetts | Oregon       |
| 1        | 37            | 1        | 1            | 1            | 1            |
| 2        | 35            | 1        | 2            | 1            | 1            |
| 3        | 39            | 1        | 1            | 1            | 1            |
| 4        | 40            | 1        | 1            | 1            | 1            |
| 5        | 41            | 1        | 1            | 1            | 1            |
| 6        | 42            | 1        | 1            | 1            | 1            |
| 7        | 43            | 1        | 1            | 1            | 1            |
| 8        | 44            | 1        | 1            | 1            | 1            |
| 9        | 45            | 1        | 1            | 1            | 1            |
| 10       | 46            | 2        | 4            | 2            | 4            |
| 11       | 47            | 1        | 4            | 2            | 4            |
| 12       | 48            | 1        | 4            | 2            | 4            |
| 13       | 49            | 2        | 1            | 1            | 1            |
| 14       | 50            | 1        | 1            | 1            | 1            |
| 15       | 51            | 1        | 1            | 1            | 1            |
| 16       | 52            | 2        | 2            | 2            | 2            |
| 17       | 53            | 1        | 1            | 1            | 1            |
| 18       | 54            | 1        | 1            | 1            | 1            |
| 19       | 55            | 2        | 3            | 3            | 3            |
| 20       | 56            | 1        | 1            | 1            | 1            |
| 21       | 57            | 2        | 1            | 1            | 1            |
| 22       | 58            | 1        | 1            | 1            | 1            |
| 23       | 59            | 1        | 1            | 1            | 1            |
| 24       | 60            | 3        | 3            | 3            | 3            |
| 25       | 61            | 4        | 13           | 1            | 1            |
| 26       | 62            | 1        | 3            | 6            | 6            |
| 27       | 63            | 1        | 1            | 1            | 1            |
| 28       | 64            | 3        | 3            | 3            | 3            |
| 29       | 65            | 2        | 1            | 2            | 2            |
| 30       | 66            | 1        | 1            | 1            | 1            |
| 31       | 67            | 1        | 1            | 1            | 1            |
| 32       | 68            | 1        | 1            | 1            | 1            |
| 33       | 69            | 1        | 1            | 1            | 1            |
| 34       | 70            | 1        | 1            | 1            | 1            |
| 35       | 71            | 1        | 1            | 1            | 1            |
| 36       | 72            | 1        | 1            | 1            | 1            |
sented diverse geographic human sampling. In assessing these biochemical tests, there appeared to be a greater homogeneity among receiving-water strains with respect to indole, Voges-Proskauer, Simmons citrate, malonate, urease, and mucate testing. However, these organisms were initially selected on the basis of a homogenous pattern of reaction with the indole, methyl red, Voges-Proskauer, and Simmons citrate tests. There is greater heterogeneity with arginine dihydrolase, lysine decarboxylase, nitrate, and among certain sugars. In general, however, there was a rather high degree of correlation between the Edwards and Ewing results and the results with the *Klebsiella* isolates from natural receiving waters.

In comparing the results of biochemical testing between the geographic sections represented in this particular study, there were few geographic differences. Excluding the indole and Voges-Proskauer reactions because of the sampling bias, only one obvious geographic difference was present. In that instance, the Oregon and Massachusetts isolates showed 16 and 17%, respectively, lysine decarboxylase negative results, although there was only one strain (0.9%) from the Florida isolates which showed a similar negative result. Results with other testing showed only minor discrepancies.

Antibiotic susceptibility testing was carried out with 14 of the commonly used antibiotics, resulting in 21% susceptible or intermediately susceptible to ampicillin and 16% to carbenicillin. Otherwise, strains demonstrated 90% or greater susceptibility to the other 12 agents used, except the 87% susceptibility results found with nitrofurantoin. The greatest resistance to both ampicillin and carbenicillin was found among those strains which were isolated from clean freshwater areas (Table 4). Additionally, strains from Florida demonstrated a much lower susceptibility rate for ampicillin and for carbenicillin (*P < 0.01*) than strains isolated in Oregon or Massachusetts (Table 5). It was of interest that only 12% of fresh water isolates, either from water having no contamination or from contaminated water, carried resistance to any of the 12 antibiotics other than carbenicillin or ampicillin. The high percentage of natural resistance of *Klebsiella* to ampicillin and carbenicillin was necessarily taken into consideration when assessing previously published work relating to antibiotic resistance among coliform or lactose-positive, gram-negative bacilli isolated from fresh or salt water, and the degree of human contamination (12, 6) also had to be assessed. Due to the low numbers of saline water isolates, comparison was difficult; there appeared to be no differences.

However, when the total number of susceptibilities done (over 1,200 tests in each of the two freshwater categories) were considered, then a difference did appear. Contaminated freshwater isolates, when resistant to any antibiotic other than carbenicillin or ampicillin, were more likely to be resistant to additional antibiotics. The same trend appeared in the intermediate results.

Ninety-seven percent or greater of all of the receiving waters *Klebsiella* strains isolated were susceptible to six of the antibiotics tested (cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, and sulfisoxazole). When these results were compared with those from the University of Minnesota Hospital patient population (Table 6), there appeared to be a distinctly higher susceptibility to these antibiotics among the water strains. For example, the high degree of susceptibility to cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, and sulfisoxazole (and the significant percentage of hospital isolates which show re-

### Table 8. Comparison of lethal effect in mice by intraperitoneal injection of *Klebsiella* strains from receiving waters and human hospital sources

| Source               | No. of organisms tested | LD$_{50}$* |
|----------------------|-------------------------|------------|
|                      |                         | Mean       | Median    | Range       |
| Florida              | 5                       | $5.3 	imes 10^4$ | $1.3 	imes 10^4$ | $7.9 	imes 10^3$ to $2.3 	imes 10^4$ |
| Massachusetts        | 5                       | $1.3 	imes 10^4$ | $7 	imes 10^3$ | $1.5 	imes 10^4$ to $4.5 	imes 10^4$ |
| Oregon               | 5                       | $7 	imes 10^4$ | $5.1 	imes 10^4$ | $5.4 	imes 10^4$ to $1.9 	imes 10^4$ |
| Total of water isolates | 15                     | $4.5 	imes 10^4$ | $5.1 	imes 10^4$ | $7.9 	imes 10^4$ to $4.5 	imes 10^4$ |
| Multiply antibiotic-resistant human hospital isolates | 22 | $5.3 	imes 10^4$ | $2.1 	imes 10^4$ | $1.0 	imes 10^4$ to $6.2 	imes 10^4$ |

* Number of organisms required to kill 50% of the mice injected.
sistance to these antibiotics), would indicate the really significant differences outlined in this particular study with respect to receiving-water and hospital-associated Klebsiella isolates. In other words, both kinds of isolates appeared to be biochemically similar, and there is no significant difference with respect to virulence studies (Table 8). In addition, there appeared to be no great differences with respect to the serotypes isolated. The real difference therefore appeared to be the fact that the Klebsiella water strains demonstrated greater susceptibility to antibiotics.

In a study recently completed in our laboratory (T. J. Davis and J. M. Matsen, J. Infect. Dis., in press), in which the prevalence of Klebsiella in various populations was determined, adults from each of four Minneapolis-St. Paul areas, University of Minnesota doctors, nurses, laboratory technologists, and newly admitted hospital patients were compared with patients who had been in the hospital longer than 15 days. Klebsiella were isolated from 37% of the Minneapolis-St. Paul residents sampled. The prevalence was not significantly different in any of the three hospital-associated populations. All but one of the non-hospital isolates were resistant to only ampicillin and carbenicillin, but approximately one of every five strains isolated in the greater-than-15-day hospital patient population was multiply resistant. R factors were present. Fifty-one of 72 possible Klebsiella serotypes were found, but no predominant types were revealed in any of the population groups. Biochemical test results were comparable to the results reported by Edwards and Ewing (4), except that one-third of the Klebsiella isolated were indole positive. The isolates of Klebsiella from natural receiving waters differed biochemically from those Klebsiella isolated from the non-hospital-associated humans only in the aspect of production of indole. Other characteristics appeared very similar.

It would appear from the investigation of Klebsiella isolated from natural receiving waters, in comparison with the investigation of Klebsiella samples from both the three non-hospital and non-patient human populations described in the preceding paragraph, that the Klebsiella isolated from natural receiving waters are essentially similar in broad serotype distribution, in lack of antibiotic resistance except for resistance to ampicillin and carbenicillin, and in biochemical characteristics. Chronic (over 15 days in the hospital) patients and the overall clinical isolates from the University of Minnesota Hospitals did show a greater incidence of overall and multiple antibiotic resistance (Table 6).

Eickhoff (5) indicates, in his monograph reviewing the topic of the epidemiologic significance of water-borne Klebsiella, that there is no evidence that the presence of Klebsiella in recreational waters has been a factor in the epidemiology of K. pneumoniae infections in humans. A review of the public health aspects of bacterial pollution of both coastal and inland waters by Moore (10) would tend to support the Eickhoff thesis. However, it was apparent from the current study that there appeared to be little difference between the biotypes of Klebsiella isolated from natural receiving waters and those isolated from human sources (T. J. Davis and J. M. Matsen, J. Infect. Dis., in press), and that there indeed was a broad biotype duplication.

Further investigations are necessary to work out the complexities of the relationships between water-borne Klebsiella and human Klebsiella colonization. A safe assumption would appear to be that Klebsiella are widely distributed in nature (5, 7) and that human colonization occurs from a variety of sources (2, 11; Davis and Matsen, in press). Furthermore, studies in our laboratory would indicate that multiple antibiotic resistance from human strains, and similarly multiple antibiotic resistance from nonhuman sources, would carry with it a significant percentage of resistance transfer factor possession. The source of these multiple antibiotic-resistant strains in the environment requires further study.

ACKNOWLEDGMENTS

We would like to acknowledge the technical assistance rendered by Doris A. Goldman, Evelyn Busch, William Spring, Robert Mathews, and the excellent secretarial assistance of Marian Wallfred.

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