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Seroepidemiology of Clinical Isolates of *Klebsiella* in Connecticut

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The distribution of capsular serotypes of 200 clinical isolates of *Klebsiella pneumoniae* and *Klebsiella oxytoca* from four Connecticut hospitals was determined. Serotyping was done by an indirect fluorescent-antibody technique. Hospitals included three community hospitals from the Hartford area and one university hospital in New Haven. During the test period, epidemiological surveillance did not detect any nosocomial epidemic involving *Klebsiella* species. Ninety-two percent of the isolates were typable. Of the 72 possible serotypes, 62 were represented among these strains. Forty-two percent of the typable strains were distributed among 10 serotypes. The predominant serotypes were types 31, 22, and 18 representing 19% of the typable strains (8, 6, and 5%, respectively). No one particular serotype was associated exclusively with a specific site of infection.

Klebsiella pneumoniae and *Klebsiella oxytoca* are among several members of the *Enterobacteriaceae* that have been prominently associated with morbidity and mortality in hospitalized patients (6, 9, 26, 28). Colonization with gram-negative bacilli seems to be a prerequisite for infection and increases with hospitalization, antimicrobial therapy, and the level of patient care required (13). For example, in one survey 9% of apartment residents were colonized with gram-negative bacilli compared with 60% of patients in acute hospital wards, and *Klebsiella* was the most common species isolated (27). In 1975 *Klebsiella* was the most common cause of nosocomial respiratory infection and second only to *Escherichia coli* as a cause of bacteremias and urinary tract infection (3).

Despite this major role in nosocomial infection, there has been limited investigation into the seroepidemiology of these organisms in the hospital setting, especially if one excludes nosocomial epidemics of specific types. Epidemiological studies of *Klebsiella* have been hampered by the difficulty and expense of capsular serotyping by the quellung method. An indirect fluorescent-antibody (IFA) technique for capsular typing of *Klebsiella* first described by Riser et al. (20, 21) and later evaluated in our laboratory (17) has simplified the study of these bacteria. Using the IFA, we compared the distribution of capsular serotypes of *Klebsiella* from diverse clinical sources. Included in the study were three large community hospitals in the Hartford, Conn., area as well as a university hospital in New Haven, Conn.

MATERIALS AND METHODS

Organisms and media. Two hundred clinical isolates were identified as *Klebsiella pneumoniae* or *Klebsiella oxytoca* in the clinical microbiology laboratories of Saint Francis Hospital and Medical Center (120 isolates), Hartford Hospital (30 isolates), the Newington Veterans Administration Hospital (20 isolates), and the Yale-New Haven Hospital (30 isolates) over a period of 6 months. Isolates from St. Francis Hospital were collected consecutively, whereas those from Hartford, Newington Veterans Administration, and Yale-New Haven Hospitals were collected 1 day per week and do not represent all *Klebsiella* isolates during this period. Species identification was based on the API 20E system (Analytab Products Inc.) in all these laboratories with the exception of Yale-New Haven Hospital, where conventional biochemical tests were used. Sources of these isolates included urine, sputum, blood, spinal fluid, and wounds. In our laboratory all the isolates were subcultured to blood agar and then stored in brain heart infusion agar deeps at 4°C. Isolates were subcultured to Worfel-Ferguson agar (Difco) before serotyping.

IFA procedure. Antisera, slide preparation, and antiserum and conjugate dilutions as well as specific details of the IFA procedure were previously described by Murcia and Rubin (17). Fluorescence was graded as negative, 1+, 2+, 3+, or 4+. Strong reactions (3+ to 4+) were clearly distinguishable from weak reactions (2+). Cross-reactions $\geq$ 3+ fluorescence were easily resolved by titration with type-specific antisera.

RESULTS

Distribution of serotypes. The distribution of *Klebsiella* capsular serotypes found in the four Connecticut hospitals is outlined in Table 1. There was no known nosocomial epidemic involving *Klebsiella* at any hospital during the test period. Repeat isolates from the same patient were not included in Table 1 unless they were of different serotypes. Although no serotypes predominated, serotypes 31, 22, and 18 were the most common and were found in several of the four hospitals. Types 31 (13 isolates)

TABLE 1. *Distribution of Klebsiella capsular serotypes isolated from four Connecticut hospitals*[a]

Capsular type	No. of serotypes from:				Capsular type	No. of serotypes from:			
	SFHMC[b]	HH	NVAH	YNHH		SFHMC[b]	HH	NVAH	YNHH
1	3	1	1		38	1			1
2	5	1			39	1			
3	3			1	40	1	1		
4	4	1	1		41				
5	3			2	42				
6	3			1	43				
7	2	1			44		1		
8	3	1		2	45				
9	2				46	1			4
10	1				47		1	1	
11	2		1	1	48	2			
12				1	49		1		
13	1				50				
14	1				51				
15					52		1		
16	2		1		53	1			
17					54		1		
18	7	1			55	2	2	1	
19	1			1	56				1
20	1			1	57				
21	1	1			58	1	1		
22	7	2		1	59	1			
23	1	1	1		60	1			
24	1	1	1	1	61	1		1	
25		1			62	2		2	
26	1		1		63				1
27				1	64	1			
28	1		1		65	1			
29	1			1	66				3
30		1			67	1	1		
31	7	2	3	1	68	1			
32					69				
33					70	2		1	1
34				1	71		1		
35	1	1			72		1	1	
36	1				Untypable	9	2	1	3
37	1		1						

[a] Repeat specimens from the same patient were included only if the isolates were different serotypes.
[b] SFHMC, Saint Francis Hospital and Medical Center; HH, Hartford Hospital; NVAH, Newington Veterans Administration Hospital; YNHH, Yale-New Haven Hospital.

and 24 (4 isolates) were found in all four hospitals and types 1, 4, 8, 11, 23, 55, and 70 were present in three hospitals. There was no observed relationship between *Klebsiella* species and serotype.

Serotype in relation to specimen source. Table 2 summarizes the distribution of capsular serotypes among various clinical sources at St. Francis Hospital and Medical Center. No one particular serotype was identified with any one source. Urine isolates accounted for 51%, sputa accounted for 28%, and other sources accounted for 21% of all isolates submitted. Although both urine and sputum isolates were associated with many different serotypes, the lower-numbered serotypes were more frequently represented in both groups. Twenty-nine percent of the isolates were serotypes 1 through 10.

TABLE 2. *Distribution of Klebsiella capsular serotypes among various clinical sources at Saint Francis Hospital and Medical Center*[a]

Capsular types	No. of serotypes from:				
	Urine	Respiratory tract	Wound	Other	Total
1-10	14	14		1	29
11-20	9	4		2	15
21-30	8		12	2	13
31-40	4	2	1	6	13
41-50	1		1	1	3
51-60	2	2	2		6
61-72	6	2	1		9
Nontypable	5	2	2		9
Total	49 (51)[b]	27 (28)	9 (9)	12 (12)	97 (100)

[a] Excludes duplicate serotypes from the same patient.
[b] Numbers in parentheses represent the percentage of total isolates.

Serotype distribution in duplicate specimens. Nineteen patients had *Klebsiella* isolated more than once from the same or a different site. In 13 patients the repeat serotypes were the same (11 patients) or untypable (2 patients). Six patients had *Klebsiella* isolated more than once with capsular serotypes that were typable and not identical (Table 3). Only patient 3 was shown to have different serotypes from the same source, but these cultures were taken 3 months apart. The time interval between cultures otherwise varied from 1 and 30 days. One patient (no. 6) had 3 different *Klebsiella* serotypes from three different sources in a 5-day period.

DISCUSSION

Epidemiological studies of common bacteria require some means of subtyping. Previously, only the quellung method of capsular serotyping was reliable for serotype determination of *K. pneumoniae* and *K. oxytoca*. The IFA method provides a simple, inexpensive, and reproducible means of determining capsular serotypes of these bacteria. Unfortunately, antisera against only types 1 to 6 are now commercially available. However, once prepared, antisera are stable for long periods, and the IFA procedure requires very small amounts (0.02 ml of a 1:32 dilution of antiserum). We feel this is the preferred method for subtype determination of *K. pneumoniae* and *K. oxytoca*.

Among the 72 *Klebsiella* capsular serotypes for which type-specific antibody was available, we found no predominating serotypes, although several appeared more frequently. Overall, the six most common capsular serotypes were types 31, 22, 18, 2, 4, and 8. Two of these serotypes (2 and 18) were also found among the most common serotypes from two other seroepidemiological surveys (14, 18). Types representing at least 5% of the isolates in one of the three surveys included types 18, 22, and 31 in Connecticut; 7, 24, 27, 43, 55, and 61 in Toronto (18); and 2, 3, and 35 in Minnesota (14). Surprisingly, only four of these serotypes (types 2, 3, 22, and 24) have been implicated in nosocomial outbreaks (1, 4, 5, 16, 19, 22, 26). Type 2 has been involved in five reported nosocomial outbreaks due to *Klebsiella*. Types 21, 22, and 30 have been associated with two outbreaks each (10, 19, 22, 25, 25, 26). Serotypes causing one outbreak each include types 3, 8, 9, 16, 19, 24, 25, 26, 33, and 68 (2, 11, 12, 16, 23, 29). The majority of these outbreak strains were uncommon or did not occur at all in seroepidemiological surveys (7, 14, 18).

TABLE 3. *Variation of Klebsiella capsular serotypes isolated more than once from the same patient*[a]

Patient	Source	Time between cultures	Capsular serotypes
1	Sputum	30 days	13
	Urine		18
2	Urine	24 h	28
	Rectum		31
3	Urine	3 mos	35
	Urine		22
4	Sputum	18 days	1
	Urine		67
5	Urine	7 days	29
	Wound		31
6	Sputum	5 days[b]	18
	Wound		48
	Abdominal fistula		26

[a] Excludes identical serotypes from the same patient.

[b] Multiple cultures from each site were obtained within a 5-day period.

Most of the serotypes associated with outbreaks also appear to be uncommon in the nonhospital environment. Matsen et al. (15) determined the capsular type of 210 *Klebsiella* strains isolated from natural receiving waters in three different areas of the United States. They found 60 of the 72 possible serotypes were represented but 103 (49%) fell within 11 serotypes (types 8, 11, 22, 28, 30, 55, 60, 61, 62, and 64). Only three types (8, 22, and 30) have been involved in outbreaks. The remaining 11 serotypes involved in outbreaks were rare or did not occur.

Thus, during a nonepidemic period, there is a broad serotype distribution among strains found in or out of the hospital. No particular serotypes predominate, and those associated with outbreaks are not commonly isolated.

The ability of certain serotypes, particularly type 2, to cause more widespread infection may, in part, be due to enhanced virulence. However, mouse virulence testing with type 26 did not show any increase in virulence compared with control strains (12). Virulence testing of other outbreak strains has not been done.

A major contributing factor for the involvement of these strains in epidemic infection is increased antibiotic resistance. Almost all outbreak strains were resistant to kanamycin and, when tested, gentamicin. Strains not involved in outbreaks tend to be susceptible to aminoglycosides (7, 14, 17). However, the presence of plasmid-mediated resistance alone does not appear to confer increased ability to survive or persist. Rennie and Duncan (19) described the patterns of infection with gentamicin-resistant *Klebsiella* in their hospital. Although *Klebsiella* of at least six different serotypes were gentamicin resistant, only type 22 spread to several wards and persisted in the hospital for 2 years.

Half of our sputum isolates were found among

the first 10 serotypes, and 9 (64%) of these were types 1, 3, 4, and 5. Distribution of the isolates from other sources was more diverse. Several previous studies suggest that types 1, 3, 4, and 5 are associated with the respiratory tract, whereas type 2 is more commonly isolated from urine (7, 8, 14). Although types 1, 3, 4, and 5 were primarily associated with the respiratory tract, we did not find type 2 exclusively in urine. Two of the five type 2 isolates were isolated from sputum. Riser et al. (21) obtained somewhat different results in that serotypes 3, 25, 31, 51, 64, and 68 were primarily associated with respiratory isolates whereas serotypes 21, 28, and 30 were usually found in urine specimens.

Variation of serotypes from repeat specimens from the same patient has been observed in several studies. In the present survey, 6 of 19 patients (32%) with repeat specimens had more than one serotype isolated. In a previous report (17) 2 of 9 patients (22%) had repeat isolates of *K. pneumoniae* of different serotypes, whereas Rennie and Duncan (18) found 8 of 37 patients (22%) with isolates of different *Klebsiella* serotypes present in at least two separate cultures. We have also seen this variation with repeat isolates of *Serratia marcescens* occurring with the same frequency as *Klebsiella* in that 2 of 7 (29%) patients had repeat isolates with different serotypes (24). It is difficult to compare these results since sources and time between cultures are only available from our studies. Of the eight patients in our two studies, two had *Klebsiella* of different serotypes isolated from the same source. The time interval between isolations was 4 days and 3 months. Isolates from the other 6 patients were from different sources, with time intervals ranging from 24 h to 1 month. In the present study, an uncommon serotype was replaced by a common serotype in four of six patients (patients 1, 2, 3, and 5). Rennie and Duncan suggest that these variations in serotype are due to either more than one serotype in an infection or a secondary infection or colonization with different subtypes of the same species. Since we could demonstrate no colony-to-colony variation in serotype when five colonies each of 55 clinical isolates were tested by IFA, the former suggestion seems unlikely, at least for isolates from the same source (17). We are presently testing the serotypes of sequential isolates from individual patients to examine the latter suggestion.

The ability of *Klebsiella* to colonize the respiratory tract and wounds, the relative ease with which it causes nosocomial urinary tract infection, its propensity for stool carriage in hospitalized patients, and the acquisition of antibiotic resistance make it a formidable enemy. Accurate and reproducible capsular serotyping of *Klebsiella* strains is an important epidemiological tool in recognizing and understanding a difficult nosocomial problem.

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