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O-Antigen Seroepidemiology of *Klebsiella* Clinical Isolates and Implications for Immunoprophylaxis of *Klebsiella* Infections

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To provide a database for the development of an O-antigen-polysaccharide-containing vaccine against *Klebsiella* spp., we examined the O-antigen seroepidemiology of 378 *Klebsiella* clinical isolates collected prospectively in two university centers. Strains were typed by competitive enzyme-linked immunosorbent assay with rabbit antisera specific for serogroups O1 to O12 and monoclonal antibodies (MAbs) specific for serogroups O1, O2ab, O2ac, and the genus-specific core antigen. The numbers of isolates (percentages) of individual O serogroups were as follows: 148 (39.2) for serogroup O1, 40 (10.6) for serogroup O2ab, 4 (1.1) for serogroup O2ac, 89 (23.6) for serogroup O3, 2 (0.5) for serogroup O4, 32 (8.5) for serogroup O5, none for serogroups O7, O9, and O12, and 21 (5.6) for serogroup O11. Forty-two (11.1) of the strains were non-O-typeable. O-serogroup distributions were virtually identical between isolates from invasive infections and those from noninvasive infections or colonizations. A vaccine containing the O-specific polysaccharides of serogroups O1, O2ab, O3, and O5 would cover 82% of clinically occurring O-antigen specificities. Three hundred thirty-eight of 378 isolates (89.4%) reacted with the genus-specific MAb V/9-5, which recognizes an epitope of the outer core region of *Klebsiella* lipopolysaccharide. Antibodies directed against this epitope may represent a further alternative for O-antigen-targeted immunoprophylaxis of *Klebsiella* infections. These data support further experimental investigations on the protective potential of O-antigen-based vaccines and/or hyperimmune globulins in *Klebsiella* infection.

Organisms of the *Klebsiella* group, in particular *Klebsiella pneumoniae* and *Klebsiella oxytoca*, are frequently isolated nosocomial pathogens (18, 19). Typical infections associated with *Klebsiella* spp. are nosocomial urinary tract infections, wound infections, pneumonia, and septicemia (3, 18, 27, 33). The fact that pulmonary and bloodstream infections caused by klebsiellae result in high rates of mortality despite the use of potent antimicrobial agents (3, 27) has acted as a stimulus to researchers to develop immunologic strategies for their prevention and treatment. Since it has been shown that the *Klebsiella* capsule (K antigen) plays an important role in pathogenicity (12, 41), Cryz and coworkers developed a capsular antigen-based vaccine containing the purified polysaccharides of 24 capsular serotypes (7, 15). A hyperimmune intravenous immunoglobulin (Ig) preparation made from the postvaccination plasma of volunteers immunized simultaneously with the *Klebsiella* capsular antigen and *Pseudomonas* vaccines has recently undergone a randomized clinical trial with intensive-care patients (9). In that study, the *Klebsiella*-capsule-specific Ig exerted significant protection; however, as expected, the protective effect was limited to those *Klebsiella* isolates that belonged to capsular serogroups included in the vaccine. The fact that only about 70% of the *Klebsiella* isolates examined in that study were covered by the capsule-specific vaccine indicated a need to broaden the antibacterial activity of this product (9). However, in a previous analysis of *Klebsiella* capsular serotypes causing bacteremia, it had been shown that as many as 69 of the 77 recognized capsular types of *Klebsiella* could be isolated from blood cultures, with many serotypes causing only 1 to 2% of all infections (8). Based on that study, the addition of 10 more capsular antigens to the 24 serotypes currently included in the vaccine would broaden the coverage of the vaccine by only 13% (8). Therefore, it may be desirable to develop a composite *Klebsiella* vaccine containing other surface determinants whose seroepidemiology is less complex than that of the capsular antigens (6).

For several reasons, the lipopolysaccharide (LPS) (O antigen) of *Klebsiella* is an attractive candidate for such a second-generation *Klebsiella* vaccine. (i) There are only 12 recognized *Klebsiella* O-antigen serogroups, and recent data indicate that the actual number may be even lower because of serological similarity or identity between groups (for a review, see reference 46). (ii) The *Klebsiella* O1 antigen, which is known to occur in a significant proportion of clinical isolates (1), has been shown to contribute significantly to the pathogenicities of *Klebsiella* strains in animal models of infection (42, 43). (iii) In a recent study, we were able to show that a monoclonal antibody (MAb) directed against the O1 serogroup antigen was protective in animal models of septicemia and peritonitis caused by a highly virulent *Klebsiella* strain (39). Thus, it appears that a vaccine containing selected, clinically relevant O-antigen polysaccharides in addition to the capsular antigens mentioned above may provide superior coverage against clinical *Klebsiella* strains.

As a basis for vaccine development, seroepidemiological studies which analyze the spectrum of O-antigen serotypes occurring in clinical material are required. In the past, such

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TABLE 1. O antigens of *Klebsiella* O serogroup reference strains

Strain designation	Previously assigned antigen formula[a]	Extended and revised O-antigen formula[b]
Friedländer 201	O1:K− (21)	O1/2a/core
7380	O2ab:K− (21)	O2a/core
5053	O2ac:K− (21)	O2ac/core
390	O3:K11 (36)	O3/core
1702	O4:K42 (36)	O4/core
4425/51	O5:K57 (36)	O5/core
NCTC 8172	O6:K64 (10)	O1/2a/core
264(1)	O7:K67 (10)	O7/−
889	O8:K69 (10)	O1/2a/core[c]
1205	O9:K72 (10)	O2a/O9/core
337	O10:K73 (29)	Not *Klebsiella*[d]
378	O11:K78 (11)	O4/O11/core
708	O12:K80 (11)	O12/core

[a] References for the original descriptions appear in parentheses.
[b] The presence of the O2a antigen was demonstrated by reaction with MAb IV/4-5 or serum O2ab raised against strain 7380. The presence of the genus-specific core antigen was demonstrated by reaction with MAb V/9-5.
[c] See the text for the existence of a separate O8 antigen.
[d] This strain was found to belong to the genus *Enterobacter* (see the text).

investigations have been hampered by technical problems with O-antigen typing of klebsiellae caused by a masking effect of the capsule which covers the O antigen (21, 38). To our knowledge, only one large study, which included more than 300 isolates and took place in Japan 13 years ago, has been performed (13), and no up-to-date data on significant numbers of strains from the Western hemisphere are available. Recently, we developed a competitive enzyme-linked immunosorbent assay (ELISA) method which allowed us to reliably O serotype a small number of clinical strains (46). Here we report the use of this technique for typing 378 clinical *K. pneumoniae* and *K. oxytoca* isolates collected in two university hospitals by means of O-antigen-specific polyclonal antisera and MAbs.

MATERIALS AND METHODS

Clinical isolates. *Klebsiella* isolates obtained from routine clinical material were collected in two 1,000-bed university hospitals (Virchow-Klinikum, Humboldt University, Berlin, Germany, and Ulm University Hospital, Ulm, Germany). The origin of each strain was noted, and only one isolate per patient was retained. Urinary tract isolates were from samples exhibiting significant numbers of bacteria ($\geq 10^5$ organisms/ml). Pulmonary isolates were either from open lung biopsies or bronchoalveolar lavage fluid containing significant numbers of bacteria ($\geq 10^4$ organisms/ml). Species identification was based on their typical appearance on nutrient and MacConkey agars, the absence of motility in soft agar, and biochemical profiles generated by means of the API20E system. Only strains yielding an identification probability of ≥95% were included. Strains were stored frozen at −25°C in Microbank vials (Pro-Lab Diagnostics, Austin, Tex.).

Bacteria and LPSs. The *Klebsiella* O serogroup reference strains were described in our previous study (46). A list of these strains, together with their extended O-antigen formulas based on the findings of this study, is given in Table 1. LPS preparations were obtained from these strains and from the control strains *Escherichia coli* Bort (5), *E. coli* O19ab (21), and *Serratia marcescens* O16 by the hot-phenol-water method (48).

MAbs. MAb Ru-O1, specific for *Klebsiella* O1 LPS, and MAb V/9-5, specific for a *Klebsiella* genus-specific antigen located in the outer core region of LPS, have been described previously (39, 45). MAb IV/4-5 was obtained by the same methods except that strain 7380 (O2ab:K−) was used for immunization of mice. The antibody (mouse IgG3) reacted with O2ab and O1/2ab LPSs as described in detail in Results. MAb O2-67.1 is a mouse IgM specific for the O2c antigen (50).

Competitive ELISA. Strains were typed by competitive ELISA as described previously (46). This system allowed us to distinguish the O serogroups O1 to O5, O7, and O12, which are recognized as distinct O serogroups (38). The O1 serogroup was defined by its reaction with antiserum raised against *E. coli* 8188/41 (O19ab), since the *Klebsiella* O1 antigen is immunologically identical to the O19b portion of this *E. coli* O serogroup (21). Use of the *E. coli* serum avoids potential false-positive reactions due to K-antigen homology of *Klebsiella* strains (21).

The O serogroups O8 and O9 were not determined in the previous study (46), because they had been reported to be identical or highly related to O serogroups O1 (22, 24, 32) and O2ab (24, 38), respectively. In order to clarify the relationships between these groups, we produced additional rabbit antisera against strains 889 (O8:K69) and 1205 (O9:K72), using previously described immunization schedules and cross-absorption procedures (46). Similarly, the relationship between serogroups O4 and O11, which have been suggested to be closely related or identical (25, 34), was clarified by the production of an antiserum raised against strain 378 (O11:K78) and its absorption by strain 1702 (O4:K42). All strains were typed with all sera and MAbs available, with the exception of MAb O2-67.1, which was used only for confirmation of strains in which the O2ac antigen had been identified by conventional serology.

Gel electrophoresis and immunoblotting of *Klebsiella* LPS. Purified LPS (10 to 20 μg per lane) was electrophoresed in two identically laden 16.0% acrylamide gels containing no sodium dodecyl sulfate. One of the gels was stained with silver according to the method of Tsai and Frasch (47), while the LPS bands from the second were transblotted to an Immobilon-P membrane with a transblotting cell (Bio-Rad, Richmond, Calif.). The membrane was reacted with rabbit antisera or murine MAbs as described previously, and the reactions were developed by adding alkaline phosphatase-conjugated secondary antibodies and the appropriate substrates (45).

RESULTS

Reexamination of O-serogroup reference strains. The O1 serogroup reference strain, Friedländer 201, was found to exhibit three antigenic specificities defined by MAb Ru-O1 or antiserum raised against *E. coli* O19ab (both of which recognize a high-molecular-weight LPS component in this strain), MAb IV/4-5 (which recognizes a lower-molecular-weight smooth LPS fraction), and MAb V/9-5 (which recognizes a genus-specific core epitope). The reaction patterns of the MAbs with strain Friedländer 201 are shown in Fig. 1. Based on these reactions, the extended O-antigen formula of this strain may be given as O1/2a/core (Table 1). The lower- and higher-molecular-weight components correspond to two distinct galactose-containing polysaccharides which have been analyzed previously and called D-galactan I and II, respectively (26, 49).

The O2 serogroup has been shown to comprise a number of antigenic factors that may be described by extended antigen formulas (36). Structural analyses performed by Whitfield et al. have shown that the major antigenic component of O2 serogroup strains is the D-galactan I polysaccharide, which corresponds to the O2a antigen (23, 50). Additional residues that form the bases for serologically distinguishable partial or subantigens may be attached to the D-galactan I polysaccharide (23, 24, 50). In the present study, we distinguished the O2a (D-galactan I) antigen by its reaction with MAb IV/4-5 or antiserum raised against strain 7380 (O2ab) and the O2ac antigen by its additional reactivity with antiserum raised against strain 5053 (O2ac) and absorbed by strain 7380 (O2ab) and with MAb O2-67.1.

Antigens of the O-antigen reference strains O3 to O5, O7, and O12 were confirmed previously to represent distinct O serogroups (46) (Table 1).

The O10 antigen was not considered previously because its reference strain was relocated to the genus *Enterobacter* (37), and the O6 antigen is known to be identical to the O1 antigen (20, 25, 34). Therefore, antisera to these antigens were not included in the present study.

Chemical analyses (22, 24) indicate that the O1 and O8 polysaccharides have identical glycan backbones containing both D-galactan I and D-galactan II. However, the D-galactan I of serotype O8 is distinguished by partial O acetylation (22). For reexamination of serogroup O8, a high-titer rabbit antiserum was produced by immunization with the O8 serogroup reference strain 889 (O8:K69). Absorption of this serum by strain Friedländer 201 (O1:K−) resulted in complete removal of LPS-specific antibody. Conversely, strain 889 was able to remove all homologous antibody from serum produced with strain Friedländer 201, suggesting a high degree of similarity

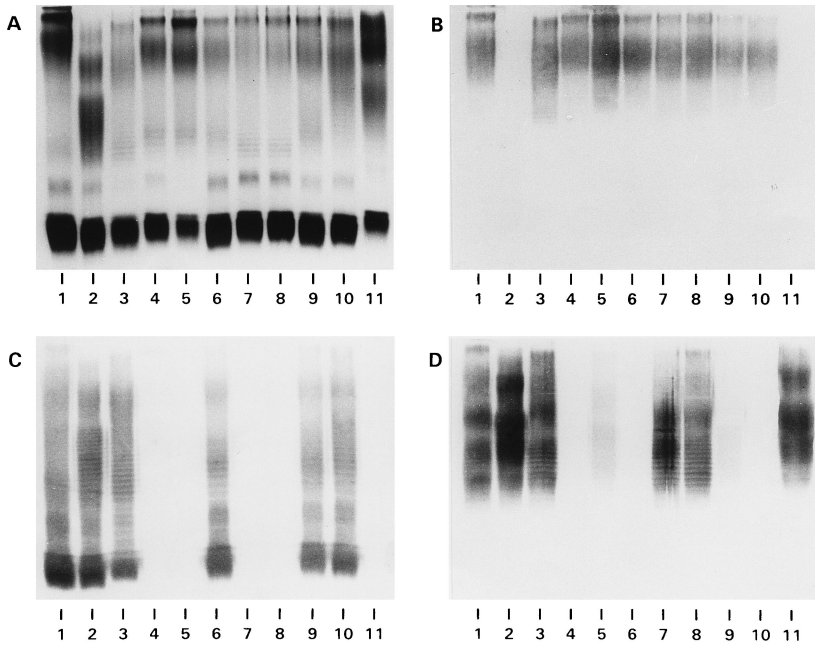

FIG. 1. Heterogeneity of the *Klebsiella* O1 serogroup demonstrated by Western blot analysis of LPSs derived from representative O1 strains. LPS from a strain of *S. marcescens* was run as a control for full expression of the antigen recognized by MAb IV/4-5. The O16 serotype of *S. marcescens* has been shown to harbor the same D-galactan I polysaccharide as *Klebsiella* strain 7380 (O2ab) (49, 50). LPS preparations were electrophoresed in a 16% acrylamide gel. (A) Silver-stained gel; (B) Western blot reacted with MAb Ru-O1 at 10 μg/ml; (C) Western blot reacted with MAb V/9-5 at 10 μg/ml; (D) Western blot reacted with MAb IV/4-5 at 10 μg/ml. Gels contained LPS samples from the following strains (subtype designations from Table 4 appear in parentheses): lanes 1, Friedländer 201 (subtype 1); lanes 2, 7380 (O2a/core [Table 1]); lanes 3, U9 (subtype 1); lanes 4, B205 (subtype 6); lanes 5, 23 (subtype 4); lanes 6, B106 (subtype 5); lanes 7, U102 (subtype 3); lanes 8, B46 (subtype 3); lanes 9, U40 (subtype 2); lanes 10, U66 (subtype 5); and lanes 11, *S. marcescens* O16 as a positive control for MAb IV/4-5.

between the O1 and O8 antigens (data not shown). Furthermore, MAb Ru-O1, specific for the O1 antigen, also reacted strongly with LPS of strain 889 as was predicted by the identity of the D-galactan II components (22). We cannot exclude the possibility that another MAb might be able to detect a minor antigenic difference between the O1 and O8 antigens as suggested by the chemical analyses of Kelly et al. (22); however, we were unable to separate these two antigens by serological methods. By contrast, the O9 and O11 serogroups were confirmed to have true O-antigen specificities. Cross-absorption studies performed with antisera raised against the O2a, O9, O4, and O11 reference strains showed that strain 1205 (O9) possesses the complete O2a antigen but, in addition, exhibits an antigenic factor not present in strain 7380 (O2ab) (data not shown). The correct O-antigen formula of this strain may thus be given as O2a/O9 (Table 1). These data are consistent with the nonstoichiometry of addition to the D-galactan I backbone (2a antigen) of side-branch α-D-galactopyranosyl residues which define the O9 antigen (23, 28). Similarly, the O antigen of strain 378 (O11) was found to harbor a particular antigenic factor in addition to the entire O4 antigen and may thus be described as O4/O11 (Table 1). Sera specific for the O9 and O11 partial antigens were obtained by thorough absorption by strain 7380 (O2ab) and strain 1702 (O4), respectively.

O-antigen distribution in clinical isolates. The data obtained by typing with polyclonal sera are summarized in Table

TABLE 2. Distribution of O-antigen serogroups in clinical *Klebsiella* isolates

O serogroup	No. of isolates from indicated source							Total (%)
	Blood	Lung	Abdominal cavity	Upper respiratory tract	Wound	Urine	Miscellaneous	
O1	29	4	2	32	18	36	27	148 (39.2)
O2ab	10			6	5	9	9	40 (10.6)
O2ac			1			1	2	4 (1.1)
O3	24	2	4	14	15	12	18	89 (23.6)
O4						2		2 (0.5)
O5	5	1	1	7	3	7	8	32 (8.5)
O7								
O9								
O11	4		1	5	4	6	1	21 (5.6)
O12								
NT[a]	7		4	11	3	7	11	42 (11.1)
Total	79	7	13	75	48	80	76	378 (100)

[a] NT, non-O-typeable.

TABLE 3. O-serogroup distribution in invasive versus noninvasive *Klebsiella* isolates

O serogroup	No. of invasive isolates (%)	No. of noninvasive isolates (%)
O1	35 (35.4)	113 (40.5)
O2ab	10 (10.1)	30 (10.8)
O2ac	1 (1.0)	3 (1.1)
O3	30 (30.3)	59 (21.2)
O4		2 (0.7)
O5	7 (7.1)	25 (9.0)
O7		
O9		
O11	5 (5.1)	16 (5.7)
O12		
NT[a]	11 (11.1)	31 (11.1)
Total	99 (100)	279 (100)

[a] NT, non-O-typeable.

2. The O1 antigen was found in 39.2% of the strains, followed by 23.4% of strains exhibiting the O3 antigen. Thus, 62.8% of all strains expressed either one of these antigens. Four O groups, namely, O1, O2ab, O3, and O5, accounted for 82% of the isolates.

A comparison between strains from the two university centers showed nearly identical distributions of O antigens (data not shown). A total of 99 isolates was considered to be involved in invasive infections, because these strains were obtained from blood cultures (n = 79), from open-lung-biopsy specimens (n = 7), and from patients' abdominal cavities during septic surgery (n = 13). A comparison of the O-serogroup distribution of these strains and that of strains obtained from other samples did not show significant differences (Table 3). Also, no significant differences were found between O-serogroup distributions of *K. pneumoniae* subsp. *pneumoniae* (n = 290) and *K. oxytoca* (n = 86) isolates. Two isolates identified as *K. ornithinolytica* with API20E profiles 5355773 and 1355773 (identification probability, >99%) belonged to the O1 serogroup and the O2ab serogroup, respectively.

Reactivities of MAbs. All isolates belonging to the O1 serogroup (n = 148) were recognized by MAb Ru-O1, which in turn did not react with any isolate belonging to other serogroups or with non-O-typeable isolates (sensitivity and specificity, 100%). MAb IV/4-5, raised against LPS of strain 7380 (O2ab), reacted with 100 of 121 O1/2ab strains (82.6%) and 30 of 40 O2ab strains (75%) and reacted weakly with 3 of 4 O2ac strains (all of which reacted with MAb O2-67.1). MAb IV/4-5 did not react with strains not harboring the O2ab epitope. These findings confirm earlier observations by Ørskov, who showed that strains in which only parts of the O2a antigen are expressed may exist (36). Three hundred thirty-eight of 378 isolates (89.4%) reacted with MAb V/9-5, which confirms our previous observation that ~10% of clinical *Klebsiella* strains lack the genus-specific core epitope (45). Nineteen of 40 nonreactive strains were found in the O1 serogroup (Table 4).

Heterogeneity of the O1 serogroup. As described above, the reference strain of the O1 serogroup, strain Friedländer 201, contains three antigenic specificities. Together, these factors may be regarded as representing the complete O1 antigen. An analysis of all clinical isolates belonging to the O1 serogroup showed that most of them also contained these three antigenic factors. However, as shown in Table 4, antigenic variations with loss of one or two factors were found in a portion of strains in this O serogroup. A number of strains showed only weak expression of D-galactan I, and others lacked this antigen completely. Similar observations from experiments using a limited collection of O1 strains expressing reference capsule types were reported previously (31). Furthermore, some strains also did not react with MAb V/9-5, which recognizes the genus-specific core antigen. Examples for the Western blot binding patterns found in these variant O1 strains are depicted in Fig. 1.

DISCUSSION

In previous studies, it has been shown that O-antigen-specific polyclonal antibodies and MAbs may protect against experimental infection with encapsulated *Klebsiella* strains (39, 42). The mechanism of this protection has not been fully elucidated, but our own data obtained in a murine sepsis model have shown that a MAb specific for the O1 antigen was able to reduce the level of bacteremia as well as bacterial dissemination to the lungs, liver, and spleen (39). Although these data have to be confirmed for other O serotypes and a larger number of encapsulated strains, it appears that protection against *Klebsiella* infection by O-antigen-specific antisera or MAbs is feasible.

Before further experimental and clinical studies extending this concept can be planned, it must be known which of the 12 O-antigen serogroups initially described for the genus *Klebsiella* are truly distinct O serogroups and which of these occur in a relevant proportion of clinical isolates. Based on the reevaluation of O-serogroup reference strains performed in this study, the O serogroups O1 to O5, O7, O9, O11, and O12 can be accepted as distinct groups. From our research, the O8 antigen was indistinguishable from the O1 antigen and was thus included in the latter O group for the purpose of this study. Both serotypes O1 and O8 contain chemically identical D-galactan II (the O1 epitope), but they differ in showing partial O acetylation of D-galactan I in serotype O8 (22). The *O*-acetyl groups do not prevent expression of the 2a epitope (reference 22 and this study), and we have been unable to produce O8-specific rabbit polyclonal antisera. Since chemical analysis has been performed only on the reference O8 strain and distinguishing antiserum is not available, the precise distribution of O-acetylated D-galactan I is unclear. MAb technology will probably be required to show whether the distinction of a separate O8 serogroup is justified.

Compared to the results of the Japanese study (13), our data confirmed the relevance of the O1, O2ab, O3, and O5 sero-

TABLE 4. Distribution of partial antigens in *Klebsiella* O1 serogroup strains

O1 serogroup subtype	Expression of partial antigen[a]:			No. of isolates (%)
	O1	O2a	Core	
1	+	+	+	108 (73)
2	+	(+)	+	10 (6.8)
3	+	+	−	13 (8.8)
4	+	(+)	−	2 (1.4)
5	+	−	+	11 (7.4)
6	+	−	−	4 (2.7)
Total				148 (100)

[a] Partial antigen O1 was defined by reaction with MAb Ru-O1 or antiserum raised against *E. coli* 8188/41 (O19ab). Partial antigen O2a was defined by reaction with MAb IV/4-5 or antiserum raised against strain 7380 (O2ab). Weak reactions indicated by (+) were characterized by incomplete inhibition in the competitive ELISA and a veil-like reaction pattern with MAb IV/4-5 and serum 7380 in Western blots (Fig. 1). The partial core antigen was defined by reaction with MAb V/9-5.

groups, which together accounted for 82% of strains collected in the two university centers (84% of strains in the Japanese study). Surprisingly, the individual percentages of strains harboring these O antigens were very similar in the two studies. A prominent role for the O1 serogroup has also been described by Albertí et al., who detected this antigen in 32% of 124 clinical isolates (1). The O7 and O12 antigens were not detected in our material, and these antigens also occurred very rarely in Japan (1 and 8 of 361 strains, respectively). In addition to O serogroups examined by the Japanese authors, we prepared antisera against O groups O9 and O11. Both of these antigens were found to be partial or decorative antigens, since the O9 antigen was found to contain the O2a epitope and the O11 antigen was found to contain the O4 epitope (Table 1). No strain harboring the O9 antigen was detected in this clinical material, but 5.6% of strains expressed the O11 partial antigen. Non-O-typeable isolates accounted for 11.1% of the strains (Table 2). In this respect, it is of interest that a new *Klebsiella* O antigen which has never been described before has recently been identified (2). Some of the isolates that were non-O-typeable in this study may harbor this antigen and should be included in future studies.

A subanalysis of the strains definitively associated with invasive disease revealed an O-antigen distribution not significantly different from that of other isolates which either colonized the skin or pharynx or were associated with noninvasive disease, such as wound and urinary tract infections. This finding is compatible with the hypothesis that the source of invasive infection is the normal pharyngeal, cutaneous, or bowel flora which is known to harbor klebsiellae in a proportion of healthy individuals (33). These data are also comparable to data from seroepidemiology analyses of *E. coli* O antigens, which indicate that the distribution pattern of O serotypes in the normal human bowel flora is the same as that found in bacteremic infections (14, 30, 35).

Since the O1 serogroup was found to account for the largest proportion of isolates, a detailed examination of the subantigens of this serogroup was performed. It was determined that 73% of the strains exhibited three antigenic factors as represented by strain U9 (Fig. 1, lanes 3). The extended O-antigen formulas of the majority of the O1 strains can therefore be given as O1/2a/core (Table 4). However, some strains failed to react with either MAb IV/4-5 or MAb V/9-5 or both MAb IV/4-5 and MAb V/9-5. Furthermore, a small number of strains demonstrated partial reactivity with MAb IV/4-5, as with antiserum O2ab, and this partial reactivity corresponded to faint binding visible on Western blots (e.g., Fig. 1D, lane 5). Chemical analysis of the O1 LPSs from a wild-type strain (26, 49) and a mutant (49) indicates that D-galactan I (the 2a antigen) is attached directly to the lipid A core. In contrast, D-galactan II is attached to the distal end of D-galactan I glycan chains and is found only in the high-molecular-weight LPS (26, 49). Nuclear magnetic resonance experiments indicate that those polysaccharide chains containing D-galactan II contain only small amounts (~5%) of D-galactan I. The apparent antigenic heterogeneity in O1 LPSs can be explained by the process of biosynthesis of the O1 polysaccharide. The same argument pertains to any of the *Klebsiella* O antigens whose structures are based on D-galactan I, such as O2ac and O9 (included here) and the O2 group of O antigens, whose structures have been described previously (23).

The D-galactan I component is synthesized by the products of genes located at the *rfb* (O-antigen-biosynthesis) locus (4, 24). Expression of the *rfb* genes in *E. coli* shows that D-galactan I formation is independent of any of the decorations attached to it in different serotypes (e.g., D-galactan II in serotype O1) (4, 24). The gene(s) for modification of the D-galactan I backbone resides elsewhere at an undetermined location on the chromosome. D-Galactan II expression requires an additional locus but is also dependent on the *rfb* gene products (48a). Since D-galactan I synthesis can occur independently of D-galactan II (but not vice versa), the LPS population can contain a mixture of O side chains; some of these comprise only D-galactan I (2a epitope), and others comprise a small amount of D-galactan I but predominantly comprise D-galactan II (O1 epitope). The efficiencies of the D-galactan II gene products will then determine the relative distribution of the two types. In almost all cases, the modifications of D-galactan I are nonstoichiometric, but high-efficiency modification might reduce the amounts of unmodified D-galactan I below the limits of detection by MAb IV/4-5 and by silver staining. This modification might be affected by growth conditions, by the precise genetic background of the strain (the D-galactan I *rfb* region is clonally diverse on the basis of its nucleotide sequence and DNA hybridization data [24]), or by mutation or loss of the modifying locus.

The molecular basis for variation in the core epitope is not yet clear. The only structural data for the *Klebsiella* core oligosaccharide are a partial structure from an O1 isolate (44) and a complete structure from a related O8 isolate (40). The larger O8 structure contains the region first identified in O1. Both structures are from strains that react with MAb V/9-5 (45). A complete and characterized set of core mutants is not available, so the unique epitope recognized by this MAb has not been identified. There are two potential explanations for the lack of reactivity of some isolates: (i) the MAb recognizes one of the novel side branches identified in the core oligosaccharide structure, and this epitope can potentially be lost without affecting elongation of the core backbone to provide a site to which O antigen can be attached, and (ii) a minority of *Klebsiella* isolates have core structures that are substantially different, as with the multiple core types in *E. coli* (17). Resolution of this issue will require further chemical analyses of the nonreactive cores.

Since all O1 strains harbored the epitope recognized by MAb Ru-O1 and since this MAb has been shown to be protective in animal experiments, this antigenic specificity appears to represent the most relevant target antigen for an O-antigen-based *Klebsiella* vaccine. Further antigens that may be considered are the O2a, O3, and O5 antigens. MAbs directed against these antigens should be produced and examined for their ability to protect against experimental infections caused by encapsulated *Klebsiella* strains harboring the homologous O antigens. Provided that protection can be shown, a human polyclonal hyperimmune globulin comprising these antigenic specificities would offer potential protection against ~80% of clinical *Klebsiella* isolates. It should, however, be emphasized that K-antigen-specific antibodies provide superior protection on a weight basis (16, 39). Therefore, as outlined above, an O-antigen-based vaccine might be best used in conjunction with the existing K-antigen-specific vaccine as a composite, second-generation O-K vaccine (6).

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