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Fimbriation, Capsulation, and Iron-Scavenging Systems of *Klebsiella* Strains Associated with Human Urinary Tract Infection

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Thirty-two strains of *Klebsiella pneumoniae* and seven strains of *Klebsiella oxytoca* isolated from urinary tract infections in elderly adults were analyzed for capsular antigens, iron-scavenging systems, and fimbriation. All strains were capsulated. Twenty-seven different K antigens were identified among the strains, with no particular antigen dominating. All strains produced the iron-scavenging system enterochelin as analyzed by bioassay and DNA hybridization. In contrast, the aerobactin iron-sequestering system was not detected in any of the strains. All strains caused hemagglutination of tannin-treated human erythrocytes and reacted with an anti-type 3 fimbriae antiserum as well as in DNA hybridization with a type 3 fimbria-specific probe, indicating that the *Klebsiella* strains possessed this fimbrial type. Possession of type 1 fimbriae was analyzed by agglutination tests and by hybridization with DNA probes from two distinct *Klebsiella* type 1 fimbria gene clusters. Phenotypic expression of the type 1 fimbriae was found in 29 of 32 *K. pneumoniae* strains, whereas 30 strains reacted with either of the two type 1 fimbrial cluster DNA probes. In *K. oxytoca*, however, only three of seven strains expressed type 1 fimbriae and reacted with the DNA probes. The type 3 fimbriae were found to bind to a fraction of epithelial cells exfoliated in normal human urine, whereas the type 1 fimbriae bound strongly to urinary slime. No inhibitors of type 3 fimbrial binding were detected in human urine.

Klebsiella pneumoniae and *Klebsiella oxytoca* are frequent causes of urinary tract infections in humans. These bacteria are associated with complicated urinary tract infections in male and female patients, especially those who have predisposing factors such as indwelling catheters or primary infections by other microorganisms (2, 4, 23, 33). These infections are commonly encountered in hospitalized patients, frequently leading to urosepsis as well as chronic or recurrent urinary tract infections (23, 33, 41).

Relatively little is known about bacterial factors that contribute to the pathogenetic mechanisms of urinary tract infections by klebsiellae. In animal models, fimbriae of *K. pneumoniae* have been found to be important for bacterial colonization of the lower urinary tract (8, 26), but the role of fimbriae in adhesion of *Klebsiella* cells to human cells has been questioned (39). Iron-regulated outer membrane proteins have been shown to be expressed by *Klebsiella* isolates collected directly from the urine of patients with acute urinary tract infection (24). Some uropathogenic *Klebsiella* isolates are able to form urease, which may contribute to formation of urinary stones (23).

Two types of fimbriae have been detected on *Klebsiella* strains. The type 1 fimbriae are characterized by their ability to bind to mannosides, and the type 3 fimbriae are characterized by their capacity to react with tannin-treated erythrocytes (7, 31, 32). The two fimbrial types differ morphologically and serologically. The *mrk* and the *fim* gene clusters encoding the type 3 and the type 1 fimbriae, respectively, have recently been cloned from clinical isolates of *K. pneumoniae* (10–13). As in a number of fimbrial types of *Escherichia coli* (17, 25), the adhesive capacity of the *Klebsiella* fimbrial filaments is located on a minor component, the MrkD and the FimH proteins (13). We recently showed that type V collagen serves as a renal target for the MrkD adhesive protein of type 3 fimbriae (45).

Adhesive capacity to specific epithelial elements of the human urinary tract is an important virulence factor for the uropathogenicity of *E. coli* (44). The major adhesive factor contributing to the uropathogenicity of *E. coli* is the P fimbria, which binds to globoseries of glycolipids on epithelial and endothelial cells in the human upper and lower urinary tract (for a review, see reference 22). P-fimbriated uropathogenic *E. coli* strains often harbor additional factors, such as hemolytic activity, acidic capsules, and iron-scavenging systems, that may contribute to the pathogenetic process (46). We report here an analysis of such factors on uropathogenic *Klebsiella* isolates.

MATERIALS AND METHODS

Bacteria. Thirty-two strains of *K. pneumoniae* were isolated from the urine of 9 male and 23 female patients with urinary tract infection. The mean age of the patients was 73 years. The seven strains of *K. oxytoca* were isolated from the urine of four boys less than 2 years old and of one male and two female patients 78 to 90 years old. The isolates were received in 1983 from a total of 25 health centers or clinics in the Helsinki region and were identified by the API 50E system (Analytab Products, Montalie Vergieu, France; scoring excellent identification). The recombinant strains *E. coli* HB101(pFK12), carrying the gene cluster for type 3 fimbriae

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of *K. pneumoniae* IA565, and HB101(pGG101), with the cloned type 1 fimbria genes from the same strain, were available from previous work (10–13). The double transformants HB101(pFK52/pDC17) and HB101(pFK53/pDC17), harboring either a complete (pFK52) or a truncated (pFK53) *mrkD* gene in association with a *papG*-deficient P-fimbria gene cluster of *E. coli*, have been described earlier (13). The bacteria were grown in static Luria broth for the detection of type 1 fimbriae and on Luria agar plates supplemented with antibiotics for the recombinant strains for the other analyses.

Iron-scavenging systems. Cross-feeding bioassays for siderophore production were performed on iron-restricted agar medium (containing 200 μM 2,2′-dipyridyl) inoculated with either of two indicator *E. coli* K-12 strains as previously described (3). Strain AN1937 was used to detect enterochelin production (49), and strain LG1522 (which carries a ColV-K30*iuc* mutant plasmid) was the indicator for aerobactin secretion (3). Isolates to be tested were spotted onto the agar surface; siderophore synthesis was indicated by a halo of growth in the lawn of bacteria around the point of inoculum. Strains were also tested for the presence of the genetic determinants of the aerobactin and enterochelin systems by colony hybridization (14). The DNA probe for the enterochelin system was a 3-kb *Eco*RI-derived restriction fragment of the recombinant plasmid pMS101 (5) containing part of the coding sequences of the *fepA* and *fes* genes of *E. coli* K-12. The aerobactin probes were a 2-kb *Ava*I fragment from within the aerobactin biosynthesis region and a 2,3-kb *Pvu*II fragment containing most of the ferric aerobactin receptor gene (3). Bacteria to be tested were grown overnight on Hybond nylon membranes (Amersham) laid on the surface of nutrient agar plates. Bacterial cells were lysed by exposure to alkali, and released nucleic acids were immobilized on the membranes by brief exposure to short-wavelength UV light. Membranes were incubated overnight at 65°C in the presence of DNA probes labeled with ^{32}P to high specific activity by the method of Feinberg and Vogelstein (9). After hybridization, filters were washed extensively at 65°C with three changes of 0.1 M sodium phosphate buffer (pH 7.2) containing 1% (wt/vol) sodium dodecyl sulfate (SDS) for 15 min each and finally with 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS for a further 15 min. Hybridization was detected by autoradiography of dried membranes.

K typing. K-antigen determination was done by the capsular swelling technique with the use of antisera against antigens K1 through K82 (35).

Fimbriation. The expression of the type 3 fimbriae was assessed by agglutination with a specific antiserum (20) and by hemagglutination of tannin-treated human erythrocytes (7) by standard methods (19). Mannose-sensitive agglutination of yeast cells (19) and bacterial agglutination in an antiserum raised against purified type 1 fimbriae from a *K. pneumoniae* strain (16) were used to determine the expression of type 1 fimbriae. Weak or negative results were confirmed by indirect immunofluorescence staining of bacteria with the antisera (38).

Possession of genetic elements for the two fimbrial types was analyzed by colony-blot hybridization with fimbria-specific DNA probes, using standard procedures (27) essentially as described previously (11). The P1 DNA probe for the type 3 fimbriae had been derived from a *Taq*I restriction enzyme fragment of plasmid pFK12 and contains most of the coding sequences of the *mrkA* gene of *K. pneumoniae* (11). Two probes were used to detect genes for the type 1 fimbriae. The IA551 and the IA565 *fimA* probes were derived from the middle or carboxy portion of the open reading frames of two distinct *K. pneumoniae* recombinant plasmids and have been described earlier (12, 40).

TABLE 1. K antigens of the *Klebsiella* strains

K antigen	No. of strains
K. pneumoniae	
K2	1
K3, K8	1
K9	1
K10, K61[a]	1
K14	2
K16	1
K23, K20	1
K24	1
K26	1
K27	1
K28	3
K28, K38	3
K30	2
K31	1
K35	1
K46	1
K47	1
K53	1
K54	2
K57	1
K68	1
K71	1
K80	1
Unidentified	2
K. oxytoca	
K6 related	1
K23, K41	1
K55	4
K62	1

[a] Indicates reactivity with two test sera.

Adhesion assays. Hemagglutination inhibition studies were performed on glass slides by routine methods (19). The urine sample and the fractions prepared from it have been described previously (42). The fractions had been obtained by serial ultrafiltration in Centricon centrifugal microconcentrators (Amicon Corp., Danvers, Mass.) with molecular weight cutoff sizes of 30,000, 10,000, and 3,000. The four fractions were tested for hemagglutination inhibition in twofold dilutions.

Adherence of fluorescein isothiocyanate-labeled bacteria to epithelial cells exfoliated in the urine of a healthy woman was tested as described previously (47). The bacteria were tested at a concentration of 2×10^8 cells per ml. Binding of the purified type 3 fimbriae to the uroepithelial cells was tested by indirect immunofluorescence as described earlier (47). The purified fimbriae were used at a concentration of 1.5 mg/ml. The antiserum raised in rabbits against purified type 3 fimbriae from *Klebsiella* isolates (20) was absorbed overnight at 4°C with homogenate of human kidney to remove possible nonspecific reactions with uroepithelial cells.

RESULTS

K antigens of the strains. A total of 23 different K types could be identified among the 32 strains of *K. pneumoniae*; 2 of the strains did not react with the available antisera (Table 1). Five of the strains reacted with two test sera, indicating

TABLE 2. Characteristics of the *Klebsiella* isolates

Bacteria	No. of strains	No. of strains with:						
		Enterochelin[a]	Aerobactin[a]	Type 3 fimbriae tested by:		YA[d]	Type 1 fimbriae tested by hybridization with probe:	
				HA[b]	DNA[c]		IA551[e]	IA565[e]
K. pneumoniae	32	32	0	32	32	29	8	29
K. oxytoca	7	7	0	7	7	3	3	2

[a] Identical results were obtained by bioassays and colony hybridization.
[b] Hemagglutination of tannin-treated human O erythrocytes.
[c] Colony hybridization with the P1 DNA probe comprising nucleotides from the *mrkA* gene.
[d] Mannose-sensitive yeast cell agglutination.
[e] *fimA* probes derived from two distinct type 1 fimbria gene clusters of *K. pneumoniae*.

that they had a capsular antigen cross-reacting with two previously assigned K antigens of *K. pneumoniae*. Most identified K types were represented by a single strain only. Four of the seven *K. oxytoca* strains had the K55 antigen.

Iron-scavenging systems of the *Klebsiella* strains. The strains were tested by bioassays for production of the iron-scavenging systems aerobactin and enterochelin as well as by DNA hybridization for the presence of the genetic elements of the two systems. None of the strains possessed the aerobactin system, whereas the enterochelin system was detected in all strains (Table 2). For each strain, the reactivity for an iron-scavenging system was identical in the bioassay and in the DNA hybridization.

Fimbriation of the strains. The expression of type 3 fimbriae by the strains was assessed by hemagglutination of tannin-treated erythrocytes and by bacterial agglutination in a type 3 fimbria-specific antiserum. The reactions were confirmed by indirect immunofluorescence with the antiserum. All strains were found to express this fimbrial type (Table 2). All 39 strains also reacted in colony blots with the *mrkA* DNA probe (Table 2).

Expression of type 1 fimbriae was detected in 29 of 32 *K. pneumoniae* strains (Table 2). In DNA hybridization, 29 of the strains reacted with the IA565 *fimA* probe and 8 strains reacted with the IA551 *fimA* probe (Table 2); 7 of these strains reacted with both probes. In all, 30 of 32 *K. pneumoniae* strains reacted with at least one of the *fimA* probes, and in one of these strains, no phenotypic expression could be detected. Only three of seven *K. oxytoca* strains showed expression of type 1 fimbriae and hybridized with the *fimA* probes (Table 2), and two of these strains reacted with both probes.

Binding of the fimbriated strains and the purified fimbriae to urinary material. The recombinant *E. coli* strains HB101 (pFK12) expressing type 3 fimbria genes cloned from *K. pneumoniae* IA565 (10) and HB101(pGG101) expressing type 1 fimbria genes cloned from the same strain (40) were assessed for binding to epithelial cells from urinary sediment. The type 3-fimbriated strain HB101(pFK12) efficiently adhered to sediment cells (Table 3; Fig. 1A and B). The high standard deviation in the assay is due to the fact that some of the epithelial cells did not bind any bacteria; for strains HB101 (pFK12) and HB101(pFK52/pDC17), the proportion of such epithelial cells varied at different test times between 5 and 20%. Conversely, only a low-level binding to sediment cells was observed with the type 1-fimbriated strain HB101(pGG101) (Table 3). α-Methylmannoside, a receptor analog for the type 1 fimbriae, had no effect on the observed adherence of HB101 (pGG101) (Table 3). As expected from work with *E. coli* type 1 fimbriae (34, 36), strain HB101(pGG101) adhered strongly to urinary slime (Fig. 1C and D), which was not recognized by strain HB101(pFK12). The binding of type 3 fimbriae to sediment cells was confirmed by indirect immunofluorescence staining with purified fimbriae (Fig. 1G to J).

Strain HB101(pFK52/pDC17), which expresses the *mrkD* adhesin gene on plasmid pFK52 in an association with *papG*-deficient P-fimbria genes on plasmid pDC17 (13), exhibited adherence to sediment cells (Table 3). In contrast, no adhesion was observed with strain HB101(pFK53/pDC17). Plasmid pFK53 carries a deletion of 145 nucleotides in the C-terminal end of the *mrkD* gene and fails to complement pDC17 to produce adherence fimbriae (13).

In addition to slime, or Tamm-Horsfall glycoprotein, human urine contains other factors that inhibit bacterial adherence (37). We therefore tested human urine and its four molecular weight fractions (the >30,000 fraction, the 10,000-to-30,000 fraction, the 3,000-to-10,000 fraction, and the <3,000 fraction) for the ability to inhibit hemagglutination by strain HB101(pFK12). No inhibition was found with any of the test materials. The highest concentrations of urine fractions that were used corresponded to two (the <3,000 fraction) or eight (other fractions) times the concentration in normal urine.

DISCUSSION

Our results show that type 3 fimbriae and the iron-scavenging system enterochelin are common features of *Klebsiella* isolates associated with human urinary tract infection. The mannose-binding type 1 fimbriae seem to occur less frequently, particularly on uropathogenic *K. oxytoca*. The similar occurrence of these two fimbrial types and the enterochelin system in urinary, environmental, and respiratory as well as bacteremic *Klebsiella* isolates (11, 15, 39)

TABLE 3. Adhesion of recombinant strains to epithelial cells from human urine sediment

Strain	Adhesion (bacteria/epithelial cell ± SD)
HB101	1.1 ± 1.1
HB101(pGG101)	1.4 ± 1.6
HB101(pGG101) + αMM[a]	1.8 ± 1.8
HB101(pFK12)	21.7 ± 16.0
HB101(pFK52/pDC17)	14.3 ± 12.6
HB101(pFK53/pDC17)	1.4 ± 2.0

[a] Adhesion in the presence of 0.5% (wt/vol) α-methylmannoside.

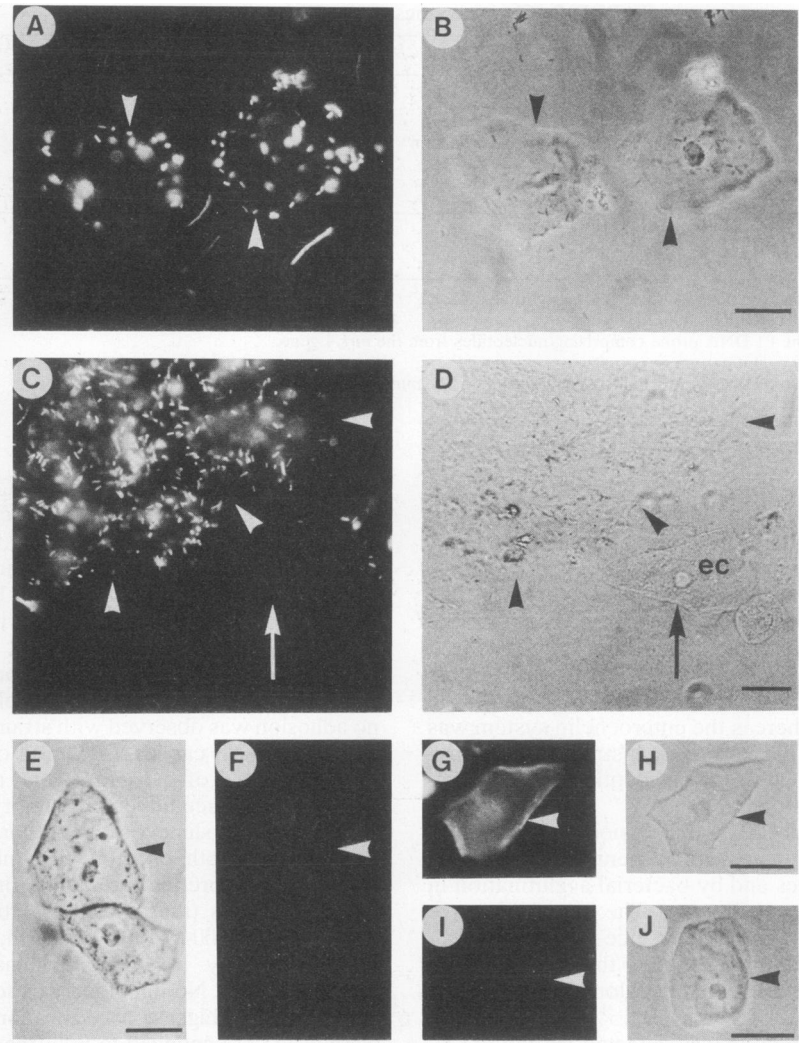

FIG. 1. Binding of type 3 fimbriae to epithelial cells from human urine. (A and B) Adhesion of fluorescein isothiocyanate-labeled, type 3-fimbriated *E. coli* HB101(pFK12) to sediment cells (A); the same microscopic field is shown by phase-contrast in panel B. Arrowheads point to edges of epithelial cells. Note adherent bacteria on the epithelial cells. (C and D) Fluorescence (C) and phase-contrast (D) micrographs of the adhesion of type 1-fimbriated strain HB101(pGG101) to urinary material. ec, epithelial cell. Arrowheads point to edges of urinary slime, and the arrow points to the edge of an epithelial cell. Note strong adhesion to urinary slime and lack of adhesion to the epithelial cells. (E and F) Phase-contrast (E) and fluorescence (F) micrographs of an adherence test with fluorescein isothiocyanate-labeled cells of the nonfimbriated strain HB101. Note lack of adhesion. Arrowheads point to the edge of an epithelial cell. (G and H) Binding of the purified type 3 fimbriae to uroepithelial cells tested by indirect immunofluorescence. (G) Fluorograph of the fimbriae binding; the same epithelial cell is shown by phase-contrast in panel H. (I and J) As in panels G and H, but the staining was without fimbriae. Arrowheads in panels G to J point to cell edges. Note strong staining of cell edges in panel G and lack of staining in panel I. Bars, 25 μm.

(Table 2) and the heterogeneity of capsular antigens among the *Klebsiella* strains of the present study suggest that uropathogenic *K. pneumoniae* and *K. oxytoca* isolates cannot be assigned to clonal groups by criteria similar to those used for the clonal groups characterized from *E. coli* strains associated with pyelonephritis (46) or newborn meningitis (1, 21). Klebsiellae cause urinary tract infections mainly in compromised or hospitalized patients, and the lack of an identifiable clonal substructure in uropathogenic klebsiellae probably reflects the opportunistic nature of the infections and their localization to the lower urinary tract (23).

We identified a total of 27 K types among the 39 uropathogenic *Klebsiella* isolates (Table 1). A similar heterogeneity of capsular antigens of uropathogenic or bacteremic klebsiellae has been reported in other studies as well (6, 33, 41, 43). Riser and Noone (41) identified 23 K types among 124 urinary *Klebsiella* isolates. The slightly higher heterogeneity in our study may be partially due to the fact that the strains studied by Riser and Noone (41) included multiple isolates of a type from one hospital ward and from different sites from a single patient.

The type 3 fimbriae bound effectively to epithelial cells in normal human urine (Fig. 1; Table 3). The finding that the double transformant HB101(pFK52/pDC17), but not HB101 (pFK53/pDC17), adhered to sediment cells indicates that this adhesion involves the MrkD minor protein of the type 3 fimbrial filament. MrkD has previously been identified as the hemagglutinin of the type 3 fimbrial filament (13) and shown to bind to type V collagen of renal extracellular matrix (45). Plasmid pFK53 expresses a truncated *mrkD* gene and is

unable to complement the *papG* deletion to produce P-fimbrial filaments with MrkD-specific binding (13). The binding of type 3 fimbriae to epithelial cells from the lower urinary tract may also take place in vivo, since we were unable to detect factors in normal human urine that inhibit the binding of type 3 fimbriae. Our results on type 3 fimbriae contradict those of Podschun et al. (39), who found no correlation between the expression of type 3 fimbriae and the adherence of *Klebsiella* strains to cultured HeLa and intestinal 407 cells and concluded that fimbriae are weakly or not at all involved in the binding of *Klebsiella* cells to eucaryotic cells.

K. pneumoniae and *K. oxytoca* cause urinary tract infections mainly in compromised patients, particularly in those subjected to instrumentation or with indwelling catheters (23). The importance of type 3 fimbriae for bacterial persistence in catheter-associated bacteriuria has recently been demonstrated (28). The tissue-binding properties of the type 3 fimbriae provide a possible basis for the association of type 3 fimbriae with infections in catheterized urinary tracts. It is thought that the most common site of catheter infection is urethral colonization (23). Type 3 fimbriae may facilitate this process by binding to the epithelia in the lower urinary tract. Binding of the type 3 fimbriae to type V collagen and to extracellular matrix (45) may be important in bacterial adherence to injured tissue and to catheter surfaces covered with a biofilm of host proteins. Interestingly, a similar tissue-binding tropism in the human urinary tract is exhibited by the O75X (also called the Dr) fimbriae of uropathogenic *E. coli*. This fimbrial type binds selectively to basement membranes in the kidney but does recognize epithelial cells of the lower urinary tract (22, 30, 48).

Expression of type 1 fimbriae was detected in 33 of the 39 *Klebsiella* isolates (Table 2). We could not detect any binding of the type 1 fimbriae of *Klebsiella* cells to human sediment cells (Fig. 1; Table 3), which agrees with earlier findings demonstrating a poor potential for the type 1 fimbriae to mediate adherence of *E. coli* to human uroepithelia (36, 47) and of *Klebsiella* cells to HeLa and intestinal cell lines (39). In contrast, the recombinant strain HB101 (pGG101), with type 1 fimbria genes from a *K. pneumoniae* strain, strongly bound to urinary slime (Fig. 1). Similar binding of the type 1 fimbriae of *E. coli* to urinary slime, or the Tamm-Horsfall glycoprotein, has been observed previously (34, 36, 37). In addition, normal human urine contains low-molecular-weight compounds that at similar concentrations to those found in urine inhibit binding mediated by the type 1 fimbriae of *E. coli* (37). It has recently been observed that clinical *Klebsiella* isolates harbor two distinct type 1 fimbria gene clusters, which can occur within the same strain (12). These gene clusters were originally described in *K. pneumoniae* IA565 and IA551 (12, 40). Our hybridization results (Table 2) showed that the IA565-like type 1 fimbria gene cluster was the predominant one among the uropathogenic isolates of *K. pneumoniae*, whereas the IA551-like gene cluster was more prominent among *K. oxytoca* strains. Nine *Klebsiella* strains reacted with both probes, indicating that they possessed portions of two type 1 fimbria gene clusters.

An interesting aspect of our data is that all the *Klebsiella* isolates made enterochelin but none of them made aerobactin (Table 2). This is in marked contrast to clonally derived isolates of *E. coli* from uncomplicated urinary tract infections (3), most of which make both aerobactin and enterochelin. It is assumed that structural or physiological advantages of aerobactin permit more effective sequestration of iron from iron-binding proteins of a normal host (18). This is supported by the study of Nassif and Sansonetti (29), who found that production of aerobactin, but not enterochelin, is correlated with virulence of *K. pneumoniae* K1 and K2 isolates in intraperitoneally infected mice. Nassif and Sansonetti (29) detected enterochelin in all of the nine bacteremic K1 and K2 isolates of their study and plasmid-encoded aerobactin in seven isolates. It remains to be determined whether bacteremic *Klebsiella* isolates harbor aerobactin genes more frequently than do urinary isolates.

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