Identification of a *Klebsiella pneumoniae* Strain Associated with Nosocomial Urinary Tract Infection

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To differentiate between relapse of infection and reinfection of the urinary tract due to *Klebsiella pneumoniae*, 33 *K. pneumoniae* isolates collected from 20 patients with spinal cord injury (SCI) over 2 years were typed by genomic fingerprinting by repetitive-element PCR. Clinical isolates obtained from the same patients with recurrent episodes of urinary tract infection (UTI) revealed identical genomic fingerprints indicating relapse of UTI due to *K. pneumoniae*, despite appropriate antibiotic therapy. Seventeen isolates obtained from 8 of the 20 SCI patients shared a common genotype, termed RD6. Among non-SCI patients residing in other nursing units, the RD6 genotype was found in 5 of 10 patients with *K. pneumoniae* UTI but in only 1 of 20 patients with *K. pneumoniae* infection that did not involve the urinary tract, suggesting a strong association of this genotype with UTI. All RD6 isolates exhibited strong adherence ($\geq$50 adherent bacteria per cell) to HEp-2 cells, whereas other *K. pneumoniae* isolates generally did not adhere to or adhered very weakly to HEp-2 cells ($\leq$5 adherent bacteria per cell). Adherence was inhibited either by 4% D-mannose or by anti-type 1 fimbrial rabbit serum. These results suggest that the capacity of *K. pneumoniae* RD6 isolates to cause UTI may be mediated by its striking adherence to mammalian cells.

Klebsiella pneumoniae, a member of the family *Enterobacteriaceae*, has become one of the most common causes of urinary tract infection (UTI) in patients with spinal cord injury (SCI) (2, 5, 13). Although the incidence of UTI has been reduced by the use of intermittent bladder catheterization, recurrence of UTI continues to be a troublesome problem in many patients. Recurrence of bacteriuria may indicate either relapse of infection caused by the same bacterial strain which can persist at sites adjacent to the bladder, despite seemingly appropriate antibiotic therapy for UTI, or reinfection with a different bacterial strain (18). The differentiation between relapse of infection and reinfection has significant management implications. Patients with a documented relapse of UTI may need to be investigated for anatomic abnormalities such as renal stone, urethral stricture, and abscess or functional alterations such as vesicoureteral reflux that may potentially impede complete eradication of the bacteria.

Adherence of *K. pneumoniae* to mammalian epithelial cells, regarded as a prerequisite for colonization or infection of mammalian tissues (12), is mediated by the adhesins FimH and MrkD that are associated with type 1 and type 3 fimbriae, respectively (4, 8, 16), and by other R-plasmid-encoded adhesins (3). The genes for type 1 fimbriae are conserved among most species of the family *Enterobacteriaceae* (1), while the genes for type 3 fimbriae are not (14). Type 1 fimbriae facilitate mannose-sensitive adherence of *K. pneumoniae* to rat bladder cells (7) and ciliated hamster tracheal cells (6), whereas type 3 fimbriae facilitate adherence to trypsinized human buccal and tracheal cells in a mannose-resistant manner (9). Host receptors for the FimH adhesin of type 1 fimbriae and for the MrkD adhesin of type 3 fimbriae are believed to involve a D-mannose-containing glycoprotein (10) on epithelial surfaces and type V collagen of basal membrane (17), respectively.

The objectives of this study were to (i) differentiate between a relapse of *K. pneumoniae* UTI caused by the same bacterial strain and reinfection with a different bacterial strain by PCR-based DNA fingerprinting of clinical isolates and (ii) study the relationship between the development of UTI and the in vitro adherence of *K. pneumoniae* to human HEp-2 cells derived from a human laryngeal carcinoma.

MATERIALS AND METHODS

Design of study. Fifty patients hospitalized between August 1994 and July 1996 at the Veterans Affairs Medical Center, Houston, Tex., who developed infections caused by *K. pneumoniae* were studied. These 50 patients were divided into one experimental group (group A) and two control groups (groups B and C). Group A consisted of 20 patients in the SCI unit (nursing wards A and B) who had UTIs due to *K. pneumoniae*. Group B consisted of 10 non-SCI patients who had *K. pneumoniae* UTIs while residing in other nursing units of the same hospital. Group C consisted of 20 patients who had *K. pneumoniae* infections that did not involve the urinary tract while residing in other nursing units of the same hospital. Repeat urine cultures were obtained from the SCI patients who during hospitalization had recurrent UTIs after completing the first course of antibiotic therapy. Bacterial isolates from the agar plates were frozen directly in tryptic soy broth with 25% glycerol at −80°C without further passage until the time of study.

Antibiotic susceptibility testing. Antibiotic susceptibilities were determined in accordance with the guidelines established by the National Committee for Clinical Laboratory Standards by using the Vitek AMS system (BioMerieux, Hazelwood, Mo.) and the agar disc diffusion technique.

PCR-based DNA fingerprinting. PCR was performed as described previously (19) with REP1R-I and REP2-I primers that are derived from repetitive extragenic palindromic (REP) elements. Bacterial genomic DNAs were isolated from 1-ml samples of the overnight culture as described previously (8) and were used as templates for PCR. The reaction mixture was subjected to initial denaturation (95°C, 7 min), followed by 30 cycles of denaturation (90°C, 30 s), annealing (40°C, 1 min), and extension (65°C, 8 min) and then a single final extension (65°C, 16 min). Amplified DNA segments were visualized after running samples on 1% agarose gels for 2 h. The fingerprints were compared visually for the reproducible portion of the pattern below the 6.6-kb size marker. Patterns were considered different if they differed by one or more amplification bands, regardless of band intensity.

Adherence assay. The in vitro adherence assay was performed by the method reported previously (2). HEp-2 cells obtained from a human laryngeal carcinoma

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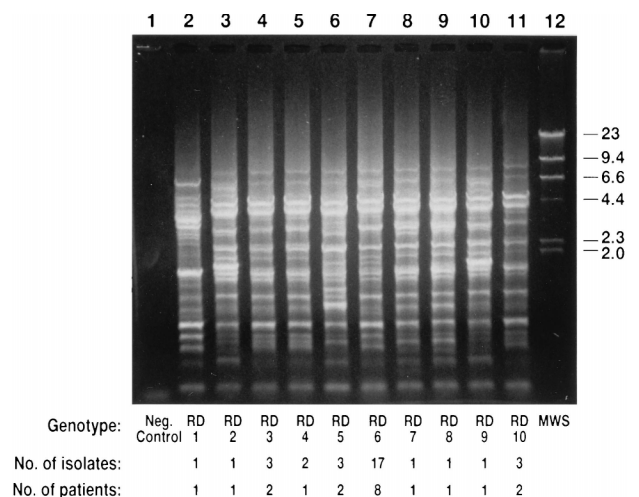
FIG. 1. DNA fingerprinting of 10 different representative isolates of *K. pneumoniae*. Lane 1, negative control with no template DNA; lanes 2 to 6 (genotypes RD1 to RD5, respectively) and lanes 8 to 11 (genotypes 7 to 10, respectively), *K. pneumoniae* isolates associated with transient UTIs; lane 7 (genotype RD6), *K. pneumoniae* isolates associated with recurrent UTI; lane 12, restriction enzyme *Hin*dIII-digested bacteriophage λ DNA (molecular weight standard [MWS]) indicating sizes (in kilobases).

were grown in minimum essential medium (Sigma, St. Louis, Mo.) supplemented with Earle's salt, 25 mM HEPES, NaHCO$_3$ (2.2 g/liter), 2 mM L-glutamine, 100 U of penicillin per ml, 0.1 mg of streptomycin per ml, and 10% fetal bovine serum until they formed a monolayer on top of cover glasses in six-well plates, usually in 2 to 3 days. Two-milliliter suspensions of *K. pneumoniae* (10^6 CFU/ml in phosphate-buffered saline with or without 4% D-mannose and with antifimbrial serum) were preincubated at room temperature for 10 min and were then placed on top of the monolayer in each well. After another 1 h of incubation at 37°C, the plates were washed twice with phosphate-buffered saline. Cells on the cover glasses were Gram stained, permanently mounted, and observed at ×630 or ×1,000 magnification under a microscope. Bacterial isolates were classified as either strongly adherent (≥50 adherent bacteria per cell) or weakly adherent (≤5 adherent bacteria per cell) on the basis of the average number of bacteria that adhered to epithelial cells (a total of 40 epithelial cells were studied to determine the average number of adherent bacteria per cell). The level of bacterial adherence was classified into these two categories because the average number of adherent bacteria was never between 5 and 50 bacteria per cell and because in the case of highly adherent bacteria it was difficult to count the exact number of bacteria if >50 bacteria adhered to each epithelial cell.

Antisera. Antifimbrial sera raised against the purified type 1 or type 3 fimbriae of *K. pneumoniae* in rabbits were kindly provided by T. Korhonen (Department of Biosciences, University of Helsinki, Helsinki, Finland).

RESULTS

DNA fingerprints. *K. pneumoniae* was isolated during this 2-year study on 33 occasions from a total of 20 SCI patients with UTIs. These 33 isolates, together with 20 *K. pneumoniae* isolates from 20 non-SCI patients with UTIs and 10 nonurinary *K. pneumoniae* isolates from 10 non-SCI patients, were studied for their genotypes. Ten distinct patterns of amplified DNA were detected (Fig. 1). The most common pattern, designated genotype RD6, was found in 17 of 33 urinary isolates in SCI patients (8 of 20 patients) and in 5 of 10 urinary isolates from non-SCI patients. In contrast, the RD6 genotype was found in only 1 of 20 nonurinary isolates from non-SCI patients.

Epidemiologic findings. Nine of 20 SCI patients had recurrent UTIs due to *K. pneumoniae* (Fig. 1). From each of these nine patients with recurrent *K. pneumoniae* UTIs only one strain of *K. pneumoniae* was isolated during this study, with the RD6 genotype accounting for recurrent infection in five of the nine patients (Fig. 2, patients 2, 7, 10, 13, and 20). One of those five patients (patient 7) had a total of five episodes of recurrent
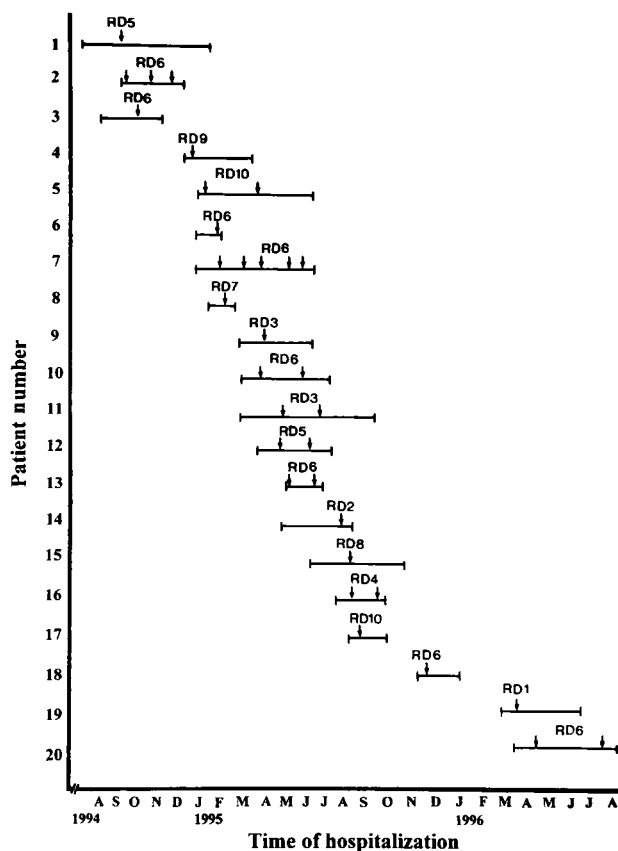

FIG. 2. Periods of time during which a total of 33 *K. pneumoniae* isolates were cultured from 20 hospitalized SCI patients who had UTIs during this 2-year study. The genotypes of the bacterial isolates are indicated above each bar representative of individual patients.

UTI due to the *K. pneumoniae* RD6 strain over a period of 4 months. Moreover, patient 7 resided in a different nursing unit (SCI nursing ward A) than the unit (SCI nursing ward B) in which the other three patients (patients 6, 10, and 13) who developed UTIs due to the *K. pneumoniae* RD6 strain while hospitalized during periods of time that overlapped the time that patient 7 was hospitalized (Fig. 2). Residence in separate SCI nursing wards was also noted for the other patients who developed UTIs due to the *K. pneumoniae* RD6 strain while hospitalized during similar periods of time. For instance, patient 2 resided in a different location (SCI nursing ward B) than patient 3 (SCI nursing ward A), and patient 19 resided in a different location (SCI nursing ward B) than patient 20 (SCI nursing ward A).

Bacterial adherence. All three isolates of the RD3 genotype (Fig. 3A) and all 17 isolates of the RD6 genotype (Fig. 3B) adhered strongly to HEp-2 cells (≥50 adherent bacteria per cell). The remaining 13 isolates belonging to the other eight genotypes (Fig. 3C) either did not adhere or were weakly adherent to HEp-2 cells (five or fewer adherent bacteria per cell). In every instance, adherence was almost completely inhibited in the presence of 4% D-mannose (Fig. 3D) or 25 μg of antibody to type 1 fimbria per ml (Fig. 3E), but was not affected by antibody to type 3 fimbria (Fig. 3F). Bacterial adherence to HEp-2 cells correlated well with bacterial adherence to other sources of human cells, such as human buccal and uroepithelial cells (Table 1).
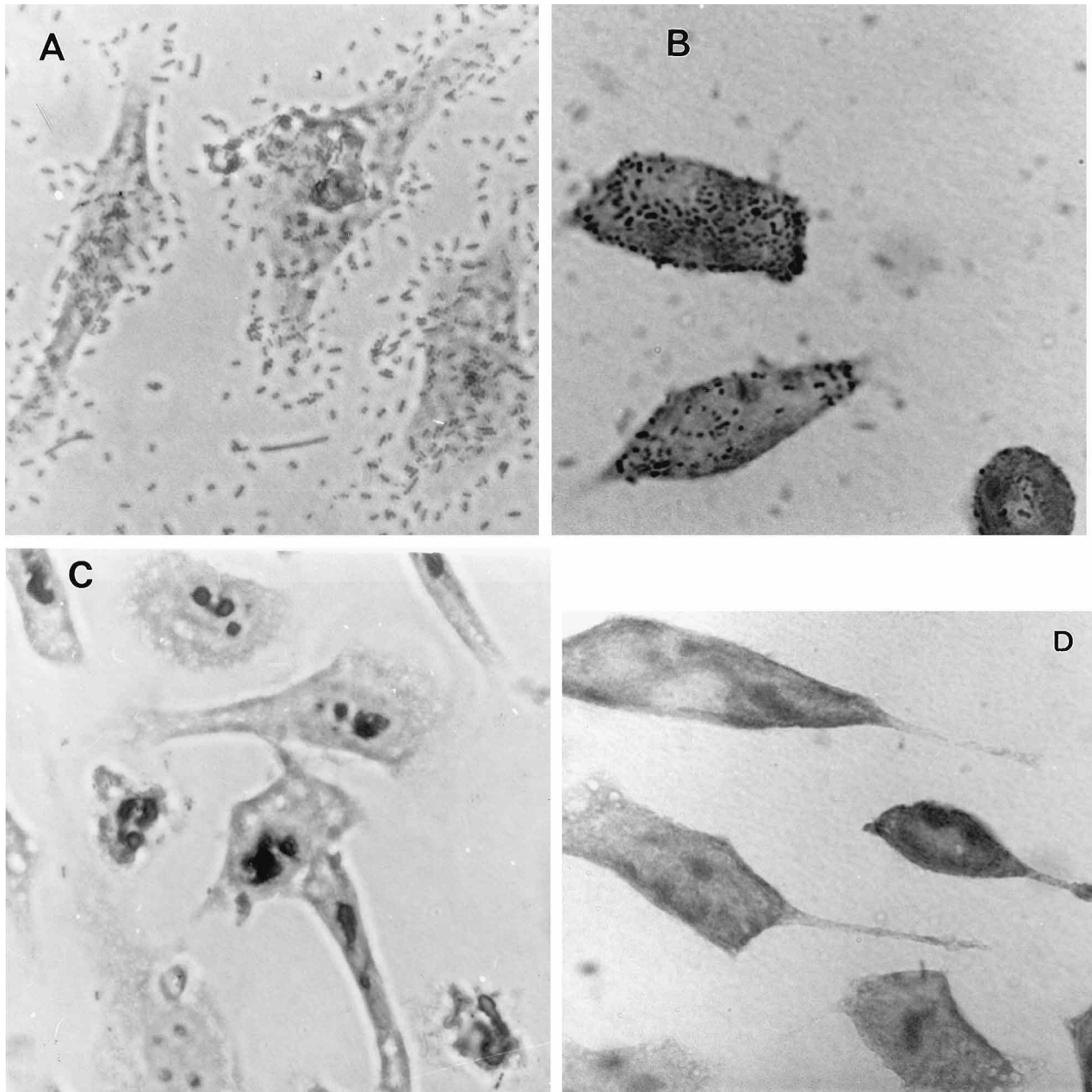
FIG. 3. Adherence of *K. pneumoniae* isolates to HEp-2 cells. (A) Genotype RD3; (B) genotype RD6; (C) genotypes RD1, RD2, and RD4, RD5, and RD7 to RD10; (D to F) genotypes RD3 and RD6 in the presence of 4% D-mannose (D), in the presence of anti-type 1 fimbrial serum (E) or in the presence of anti-type 3 fimbrial serum (F).

DISCUSSION

Klebsiella pneumoniae has emerged as the predominant urinary tract pathogen in SCI patients (2, 5, 13). In this study, genotype RD6 was the most common cause of UTIs due to *K. pneumoniae* and the most predominant genotype implicated in recurrent infection. When antimicrobial susceptibilities were tested with the Vitek system, all *K. pneumoniae* isolates of the RD6 genotype from both SCI and non-SCI patients were found to share the same pattern of broad resistance to nearly all commonly used antibiotics except cefotaxime and amikacin. In order to help examine whether these *K. pneumoniae* isolates produced extended-spectrum β-lactamases (ESBLs), agar disc

diffusion testing for cefotaxime susceptibility was done with high bacterial inocula ($\geq10^9$ CFU/ml; compared to ceftazidime and other cephalosporins, cefotaxime is a relatively weak inducer of ESBLs in vitro). Although it was observed that some of the RD6 isolates exhibited in vitro resistance to cefotaxime when high bacterial inocula were used in the agar disc diffusion test, this may have been caused by the production of ESBLs, and susceptibility to cefotaxime was rather similar among the RD6 urinary isolates that were recovered from some SCI patients at different times both during hospitalization and after discharge (data not shown). In addition, some SCI patients had recurrent episodes of UTI due to the *K. pneumoniae* RD6
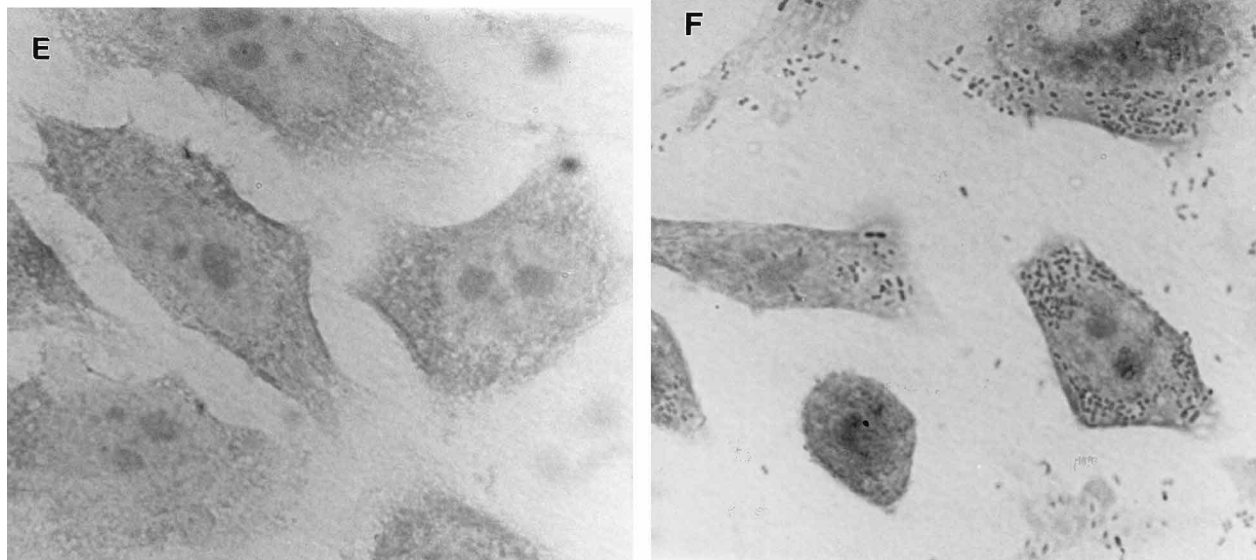
FIG. 3—*Continued.*

genotype while residing in a nursing ward with other patients with no episodes of UTI due to the *K. pneumoniae* RD6 strain. These findings are supportive of a true relapse of UTI by the same bacterial strain rather than reinfection with a highly prevalent nosocomial strain. The likelihood that the *K. pneumoniae* RD6 strain causes relapses of UTIs may be explained by the ability of this strongly adherent bacterial strain either to resist detachment due to urine flow from the mucosal lining of the urinary tract and to multiply after completion of antibiotic therapy and/or to persist in sites adjacent to the bladder, despite seemingly appropriate antibiotic therapy, and to migrate later to the bladder.

Not unexpectedly, the use of cefotaxime was regularly successful in treating episodes of recurrent UTI due to the *K. pneumoniae* RD6 strain, even those that could have potentially produced ESBLs because of the higher levels of cefotaxime achieved in urine compared to those achieved in blood (11). Since resistance to cefotaxime is usually conferred by plasmids, possible variations in the agar disc diffusion susceptibility to this drug among *K. pneumoniae* RD6 isolates recovered from different patients could not have been associated with differences in the PCR-based genomic fingerprints. It is possible that the nosocomial spread of a single clone within our medical center and selection by widespread use of antibiotics were responsible for the prevalence of this RD6 strain as a cause of UTI, as has been described previously (3, 12, 13).

All the RD6 and RD3 *K. pneumoniae* strains were found to

adhere strongly to mammalian cells in vitro. In each instance, adherence was inhibited both by mannose and by antibody to type 1 fimbria but not by antibody to type 3 fimbria. In contrast, other genotypes of *K. pneumoniae* that were implicated in two or fewer episodes of UTI exhibited minimal adherence in vitro. The lack of adherence may be the reason that these strains are not commonly identified as the cause of UTIs. The pattern of antibiotic resistance may also have been responsible for the difference in strain selection. It has been indicated that special adhesive properties associated with antibiotic resistance could account for the pathogenicities of certain nosocomial strains (12). In our study, the observation that the RD3 strain was more susceptible to antibiotics than the RD6 strain may help explain the finding that isolates with the RD3 genotype caused only occasional UTIs, despite their strong in vitro adherence.

PCR with primers derived from the *fimH* gene, the adhesin gene responsible for the type 1 fimbria-mediated adherence, revealed that all representative isolates of *K. pneumoniae* belonging to the 10 different genotypes produced a DNA band that was identical in size among the isolates (data not shown). Therefore, variations in the type 1 fimbria-mediated adherence among *K. pneumoniae* isolates could be due to either mutations in the *fimH* gene or alterations in the expression of the *fimH* gene. In *Escherichia coli*, differences in adherence result from structural differences in *fimH* genes (15). However, DNA sequencing of the *fimH* genes among *K. pneumoniae* isolates amplified by PCR showed no differences in the amino acid sequences, regardless of their adherence capacities (unpublished data). Therefore, the strong adherence of *K. pneumoniae* isolates with the RD6 genotype to HEp-2 cells may be due to the strong expression of the *fimH* gene. Another possibility is that the RD6 strain may possess another adhesin encoded in a 185-kb R plasmid which has been reported recently (3) to mediate the adherence of *K. pneumoniae* to HEp-2 cells in a way similar to that of type 1 fimbriae.

In summary, the *K. pneumoniae* strain with the RD6 genotype that is strongly adherent to epithelial cells and that is also multiresistant to antibiotics is associated with the recurrence of UTIs. Differences in the adherence of *K. pneumoniae* could result from alterations in the expression of the *fimH* gene or from the transmission of the R plasmid (185 kb) encoding

TABLE 1. Adherence of *K. pneumoniae* to human cells

K. pneumoniae genotype[a]	Average no. of adherent bacteria/cell[b]		
	HEp-2 cells	HBC[c]	HUC[d]
RD-6 (lane 6)	≥50	≥50	≥50
Others	≤5	≤5	≤5

[a] Genotype of *K. pneumoniae* refers to the DNA pattern shown in Fig. 1. For each genotype, all tested *K. pneumoniae* isolates showed similar results.
[b] A total of 40 epithelial cells were studied to determine the average number of adherent bacteria per cell.
[c] HBC, human buccal cells.
[d] HUC, human uroepithelial cells.

another adhesin. Further studies on *fimH* gene expression by clinical isolates along with cloning of the adhesin gene encoded in the 185-kb R plasmid will lead to an understanding of the virulence factors associated with nosocomial *K. pneumoniae* strains and will elucidate how they contribute to the recurrence of UTIs.

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