Nosocomial infections are an important cause of morbidity and mortality. Among gram-negative bacilli, which are the most common nosocomial pathogens, *Klebsiella pneumoniae* strains are the cause of many infections. Clinical isolates of *K. pneumoniae* are often resistant to multiple antibiotics including amnoglycosides, chloramphenicol, sulfonamide, tetracycline, amino-, carboxy-, and ureidopenicillins, and extended-spectrum cephalosporins (10, 24).

Most *K. pneumoniae* isolates express chromosomally mediated SHV-1 β-lactamase that mediates resistance to penicillins (23). Wider spectra of resistance to β-lactams, usually mediated by plasmid-encoded β-lactamases, are often encountered for clinical *K. pneumoniae* isolates. Of these, plasmid-encoded TEM-type β-lactamases mediate resistance to penicillins and to narrow-spectrum cephalosporins but do not hydrolyze the broad-spectrum cephalosporins (20). Other plasmid-encoded enzymes, called extended-spectrum β-lactamases (ESBLs), mediate resistance to broad-spectrum cephalosporins (for a review, see reference 26). Most are mutants of TEM-1, TEM-2, or SHV-1 with one or more amino acid substitutions (13). Since 1984, members of the family *Enterobacteriaceae* producing ESBLs derived from TEM (TEM-3, TEM-5, TEM-8, TEM-16, TEM-24) or SHV-1 (SHV-4) have been isolated from patients at the Clermont-Ferrand, France, teaching hospital (3, 28). Except for the TEM-5 enzyme, TEM-derived enzymes are often encoded by closely related 85-kb (Inc 7 or M) R plasmids, whereas SHV-derived enzymes are encoded by very high molecular weight R plasmids of 185 kb or higher (27). *K. pneumoniae* is the species in which the ESBL enzymes have been most commonly reported worldwide (26).

The reservoir for nosocomial strains of *K. pneumoniae* is the gastrointestinal tracts of patients (6, 25). Using intestinal cultured cells, we previously described the in vitro adhesive properties expressed by nosocomial isolates of *K. pneumoniae* and characterized the adhesive factors CF29K and KPF-28 (5, 8, 9, 11, 19). CF29K is a nonfimbrial adhesive factor encoded by a 185-kb R plasmid harboring genetic determinants encoding resistance to numerous antibiotics and the TEM-5 ESBL (5). KPF-28 is a fimbrial adhesive factor that is found in most of the *K. pneumoniae* strains producing the SHV-4 β-lactamase. The KPF-28 major subunit structural gene has been located on a >185-kb R plasmid encoding the SHV-4 β-lactamase; however, the plasmid has been shown to be not sufficient for expression of the KPF-28 fimbrial structure (9).

On the basis of the colonization capacity and the high level of occurrence of ESBLs in *K. pneumoniae*, we looked for a correlation between adhesion to human intestinal cells and antibiotic resistance. We compared the adhesion to Caco-2 cells of *K. pneumoniae* strains producing or not producing an R plasmid-encoded β-lactamase in bacterial strains isolated in the hospitals of Clermont-Ferrand. We also investigated the involvement of the R plasmids encoding β-lactamase in *Klebsiella* adhesiveness to Caco-2 cells.

**MATERIALS AND METHODS**

* Bacterial strains and culture conditions. A total of 61 nonduplicate *K. pneumoniae* strains isolated from 1984 to 1989 from different patients hospitalized in...
Adhesive to human Caco-2 cell line in vitro. Bacterial adherence to intestinal epithelial Caco-2 cells was assayed in 24-well Falcon tissue culture plates (Becton-Dickinson Labware, Oxnard, Calif.) as described previously (4). Monolayers of differentiated Caco-2 cells were used at postconfluence after 15 days of culture. A suspension of 10^6 bacteria per ml in the cell line culture medium containing 2% (wt/vol) d-mannose was added to the tissue culture, and the culture was incubated for 3 h at 37°C. After thorough washing with phosphate-buffered saline, the cells were fixed in methanol, stained with 5% Giemsa, and examined microscopically under oil immersion. Adhesion indices representing the mean numbers of bacteria per cell were calculated by examining 100 cells. Each mean number of bacteria per cell represents the results of three separate experiments. Bacterial strains were considered adherent when adhesion indices were greater than 2.

Transfer of R plasmids and curing. Conjugal experiments were carried out for the 26 K. pneumoniae strains that adhered to Caco-2 cells and for the CF144 strain that did not adhere to the cells, as described previously (18). The R plasmids were transferred to mutants of Escherichia coli K-12 C600 resistant to nalidixic acid. Transconjugants were selected on Mueller-Hinton agar containing nalidixic acid (150 mg/liter) and cephalaxine, cefotaxime, and cefoxitin (2 mg/liter), nalidixic acid, and ampicillin (40 mg/liter), streptomycin (25 mg/liter), kanamycin (25 mg/liter), tetracycline, and trimethoprim. Curing of R plasmids was done for nine strains selected from the different groups of β-lactamase-producing strains by successive bacterial subcultures at 44°C and we tested for the loss of plasmid-determined characteristics.

Preparation of plasmid DNA and restriction endonuclease analysis. Small-scale extraction of plasmid DNA from clinical isolates and E. coli transconjugants was done as described by Kado and Liu (16). The sizes of the plasmids were determined as described by Petit et al. (21).

For restriction endonuclease analysis, plasmid DNA was extracted from E. coli transconjugants by the method of Birnboim and Doly (1) and was digested with restriction endonucleases according to the manufacturer’s instructions (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Immunoblotting. Colony immunoblotting with the antisera raised against the CF29K or KPF-28 major subunits was performed as described previously (5, 9). Adsorbed antisera raised against CF29K or KPF-28 major subunits were applied to the nitrocellulose filters at a dilution of 1:200 or 1:300, respectively.

RESULTS

Adhesive properties of clinical K. pneumoniae isolates according to the type of β-lactamase produced. The antibiotic resistance patterns and the indices of adhesion to Caco-2 cells of the 61 clinical K. pneumoniae isolates are presented in Table 1. Of the 61 strains tested, 26 (42.6%) adhered to Caco-2 cells. None of the 61 strains reacted with the antisera raised against the major subunits of the CF29K and KPF-28 adhesive factors in colony immunoblotting experiments (data not shown). We compared the bacterial adhesiveness to Caco-2 cells of the strains producing only the chromosomally encoded SHV-1 β-lactamase with that of strains producing the SHV-1 enzyme and a plasmid-encoded β-lactamase (TEM-1 or ESBL). Of the 26 strains producing only SHV-1, 2 strains (7.7%) adhered to the Caco-2 cell line. These two adherent strains were both resistant to kanamycin, streptomycin, and tetracycline (Table 1). Forty-five percent of the 35 strains producing a plasmid-encoded β-lactamase were adhesive. The expression of adhesive properties was not restricted to a particular antibiotic resistance phenotype among these strains because adhesive properties were found whatever the plasmid-encoded β-lactamase type expressed. The TEM-1-producing strains CF088 and CF089 had the same antibiotic resistance properties; however, CF088 adhered to Caco-2 cells (adhesion index, 2.32) but strain CF089 did not (adhesion index, 0.34). Similar results were obtained for four CTX-1-producing strains (strains CF124, CF144, CF14, and CF314) and for two CAZ-2-producing strains (strains CF714 and CF784).

Involvement of the R plasmids in K. pneumoniae adhesive properties. Conjugal transfer experiments were performed with the 26 adherent K. pneumoniae strains. The plasmid contents, antibiotic resistance phenotypes, and adhesive properties of the E. coli transconjugants are presented in Table 1.

The CF055 and CF057 strains producing only the SHV-1 β-lactamase harbored two high-molecular-weight plasmids of 110 and 180 kb (Fig. 1). During conjugal transfer, selection for kanamycin resistance revealed the transfer of all the resistance characteristics of K. pneumoniae CF055 to the transconjugant E. coli CF055Tr. As analyzed by agarose gel electrophoresis, the transconjugant E. coli CF055Tr harbored a 180-kb plasmid (Fig. 1). The wild-type K. pneumoniae CF055 strain adhered to Caco-2 cells, with an adhesion index of 3.51 bacteria per cell. Transconjugant E. coli CF055Tr did not adhere: its adhesion index of 0.06 was similar to that of the recipient strain, E. coli K-12 C600 (adhesion index, 0.07).

Of the three TEM-1-producing strains, strain CF040 harbored two high-molecular-weight plasmids of 110 and 180 kb, strain CF062 possessed three plasmids of 85, 110, and 180 kb, and strain CF088 had two plasmids of 60 and 180 kb (Fig. 1). Two E. coli transconjugants, CF062Tr and CF088Tr, corresponding to the wild-type CF062 and CF088 strains, respectively, were obtained. The former transconjugant was resistant to gentamicin, streptomycin, and tetracycline, and CF088Tr was resistant to streptomycin and tetracycline. Plasmid DNA analysis revealed the presence of an 85-kb plasmid in transconjugant CF062Tr and a 60-kb plasmid in transconjugant CF088Tr (Fig. 1). The wild-type strains K. pneumoniae CF062 and CF088 adhered to Caco-2 cells with adhesion indices of 8.10 and 2.32, respectively. The corresponding E. coli transconjugants CF062Tr and CF088Tr did not adhere (adhesion indices, 0.08 and 0.05, respectively).

Two different patterns of high-molecular-weight plasmids were observed for the wild-type strains producing an ESBL (Fig. 1). All except three of the strains producing TEM-3, TEM-8, TEM-24, or TEM-16 β-lactamase harbored three plasmids of 85, 110, and 180 kb; the exceptions were two TEM-3-producing strains (strains CF104-1 and CF314) and one TEM-24-producing strain (strain CF110-1), which harbored only two plasmids of 85 and 110 kb. All the corresponding E. coli transconjugants producing either the TEM-3, TEM-8, TEM-24, or TEM-16 β-lactamase harbored an 85-kb plasmid encoding resistance to amikacin, kanamycin, netilmicin, sulfonamide, tetracycline, and trimethoprim. None of these E. coli transconjugants adhered to Caco-2 cells (adhesion index range, 0.01 to 0.26), while the corresponding wild-type strains did (adhesion index range, 2.01 to 8.45) (Table 1).

The involvement of the 85-kb plasmid harboring all the antibiotic resistance genes, including the ESBL-encoding gene, in K. pneumoniae adhesiveness could result in differences in plasmid DNA harbored by adherent and nonadherent strains. We compared the 85-kb R plasmid harbored by adherent strain K. pneumoniae CF124 with that of nonadherent strain CF144, 5% of the 35 strains had similar antibiotic resistance patterns. To generate discriminate endonuclease restriction patterns, the 85-kb R plasmids harbored by the corresponding E. coli transconjugants CF124Tr and CF144Tr were digested with EcoRI-HindIII or PstI enzymes (Fig. 2). The restriction patterns of the two 85-kb R plasmids were indistinguishable.
Thus, there was no difference between an 85-kb R plasmid harbored by an adherent strain and one harbored by a nonadherent strain.

Because none of the E. coli transconjugants harboring an R plasmid coding for β-lactam resistance adhered to Caco-2 cells, we did curing experiments to find whether these plasmids had a role in the adhesion of K. pneumoniae strains to Caco-2 cells. We determined the antibiotic resistance patterns, pla...
**E. coli** TEM-24, respectively; 2 and 4, corresponding transconjugants *K. pneumoniae* CF114 and CF1104 producing SHV-1 plus TEM-1. Lanes: 1 and 2, SHV-1-producing *K. pneumoniae* CF055 and CF057, respectively; 3, 4, and 5, SHV-1- plus TEM-1-producing *K. pneumoniae* CF040, CF062, and CF088, respectively; 6, 7, and 8, corresponding transconjugants *E. coli* CF055Tr, CF062Tr, and CF088Tr, respectively. (B) High-molecular-weight plasmids of extended-spectrum β-lactamase-producing strains. Lanes: 1 and 3, *K. pneumoniae* CF114 and CF1104 producing SHV-1 plus TEM-3 and SHV-1 plus TEM-24, respectively; 2 and 4, corresponding transconjugants *E. coli* CF114Tr and CF1104Tr, respectively. L, linear DNA.

The results are presented in Table 2. The absence of the 85-kb R plasmid encoding TEM-3, TEM-8, TEM-24, or TEM-16 in the prototype strains CF104-1, CF714, CF1104-1, and CF1304, respectively, did not modify the adhesion to Caco-2 cells of the cured *K. pneumoniae* derivatives. Their adhesion indices ranged from 2.32 to 5.93, and adhesion indices of the corresponding wild-type strains ranged from 2.16 to 6.32.

### DISCUSSION

*K. pneumoniae* is one of the more common species involved in nosocomial infections (15). Clinical isolates resistant to multiple antibiotics have been shown to be widespread in French hospitals (2, 17). Many of them produce plasmid-encoded ESBLs and are also resistant to aminoglycosides (7, 14, 28). The colonization of the host's gastrointestinal tract by multidrug-resistant *K. pneumoniae* strains is thought to be an essential step in nosocomial Klebsiella infections (6, 25). In this study, we looked for a link between antibiotic resistance genes and colonization factor genes. We studied 61 clinical *K. pneumoniae* isolates by analyzing bacterial adhesion to the human colon carcinoma cell line Caco-2, which exhibits structural and functional differentiation patterns characteristic of mature enterocytes (12, 22). *K. pneumoniae* adhesion to Caco-2 cells was expressed in 42.6% of clinical isolates. None of the strains produced the TEM-5 or SHV-4 β-lactamase or expressed any of the previously described adhesive factors, CF29K or KPF-28, confirming that the expression of these factors is restricted to TEM-5- or SHV-4-producing *K. pneumoniae* strains (5, 9).

Almost all adherent strains were resistant to several antibiotics. Of the 26 *K. pneumoniae* strains producing only the chromosomally encoded SHV-1 β-lactamase, only 2 (7.7%) adhered to Caco-2 cells. In contrast, 24 (68.5%) of the 35 strains producing a plasmid-encoded β-lactamase (TEM-1, TEM-3, TEM-8, TEM-24, or TEM-16) adhered to Caco-2 cells. The two adherent strains producing only the SHV-1 enzyme harbored an R plasmid encoding resistance to aminoglycosides and tetracycline. None of the strains that did not harbor any R plasmid was adherent. These results suggest an association between resistance to aminoglycosides and/or β-lactams and adhesion to human intestinal epithelial cells for almost all of the clinical *K. pneumoniae* isolates. Thus, an association between antibiotic resistance and adhesiveness is not restricted to the previously studied TEM-5- and SHV-4-producing *K. pneumoniae* strains (5, 9). The existence of a correlation between these two properties is in good agreement with the high level of occurrence of resistant *K. pneumoniae* strains in nosocomial infections. Adhesion to intestinal cells is essential for Klebsiella emergence and persistence in the human gut. Under antibiotic pressure, *K. pneumoniae* strains adhering to epithelial cells which do not harbor any R plasmid will be eliminated. In contrast, the adherent strains harboring R plasmids will survive and colonize the gastrointestinal tract. Moreover, these strains will persist in the human gut even in the absence of antibiotic selection pressure.

We previously described a genetic link between *K. pneumoniae* adherence to Caco-2 cells and antibiotic resistance among TEM-5- and SHV-4-producing strains (5, 9). A 185-kb conjugative R plasmid harboring the TEM-5-encoding gene has been shown to code also for the CF29K adhesive factor (5). The structural gene encoding the KPF-28 fimbrial major subunit has been located on a >185-kb conjugative R plasmid encoding the SHV-4 enzyme (9). We looked for such a genetic link among *K. pneumoniae* strains producing TEM-1 or TEM-derived ESBLs. Among the strains producing the same transmissible β-lactamase, there was no difference in antibiotic resistance phenotypes and high-molecular-weight plasmid patterns between adherent and nonadherent strains. The TEM-3, TEM-8, TEM-16, and TEM-24 enzymes are known to be encoded by closely related 85-kb R plasmids (3). The conjugative transfer of the 85-kb R plasmids in an *E. coli* recipient strain did not promote bacterial adherence to Caco-2 cells. Theendonuclease restriction patterns of the 85-kb R plasmids harbored by some adherent or nonadherent strains were identical, and curing of these plasmids did not modify *K. pneumoniae* adhesiveness. Thus, despite the high level of occurrence of adherent strains among these TEM-derived enzyme-producing

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**FIG. 1.** Agarose gel electrophoresis of plasmid DNA from wild-type *K. pneumoniae* strains and corresponding *E. coli* transconjugants. (A) High-molecular-weight plasmids of strains producing SHV-1 and SHV-1 plus TEM-1. Lanes: 1 and 2, SHV-1-producing *K. pneumoniae* CF055 and CF057, respectively; 3, 4, and 5, SHV-1- plus TEM-1-producing *K. pneumoniae* CF040, CF062, and CF088, respectively; 6, 7, and 8, corresponding transconjugants *E. coli* CF055Tr, CF062Tr, and CF088Tr, respectively. (B) High-molecular-weight plasmids of extended-spectrum β-lactamase-producing strains. Lanes: 1 and 3, *K. pneumoniae* CF114 and CF1104 producing SHV-1 plus TEM-3 and SHV-1 plus TEM-24, respectively; 2 and 4, corresponding transconjugants *E. coli* CF114Tr and CF1104Tr, respectively. L, linear DNA.

**FIG. 2.** Agarose gel electrophoresis of EcoRI-HindIII-digested (A) or PstI-digested (B) 85-kb plasmids. Lanes: 1 and 2, 85-kb R plasmid of transconjugants *E. coli* CF124Tr and CF144Tr, respectively; MW, molecular size standards (1-kb ladder; Gibco BRL SARL, Cergy Pontoise, France).
K. pneumoniae strains, their 85-kb R plasmids are not involved in bacterial adhesion to the Caco-2 cell line.

K. pneumoniae expresses two essential phenotypes, antibiotic resistance and adhesion to intestinal epithelial cells, that can account for the emergence and occurrence of this bacterial species as a nosocomial pathogen. Fuller knowledge of the adhesion mechanisms is essential to better understand and prevent bacterial colonization of the human gut and, therefore, nosocomial Klebsiella infections. We are now working on characterization of the adhesive factor(s) mediating adhesion of K. pneumoniae strains producing transmissible β-lactamases other than TEM-5 and SHV-4, since we showed that these strains did not produce the previously described CF29K or KPF-28 adhesive factors mediating K. pneumoniae adhesion to intestinal epithelial cells.

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