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Fimbrial Types among Respiratory Isolates Belonging to the Family *Enterobacteriaceae*

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Bacterial attachment is believed to be an early step in gram-negative nosocomial pneumonia. The frequency of fimbria-associated adhesins among respiratory pathogens has not been studied in detail. In this study isolates belonging to the family *Enterobacteriaceae*, prospectively obtained from intensive care unit patients who were suspected of having nosocomial pneumonia, were examined for fimbria-associated adhesins. Type 3, P, type 1, and other fimbrial phenotypes were identified by specific hemagglutination and electron microscopy. The *Klebsiella* type 3 fimbrial phenotype was further characterized by using a monoclonal antibody. Also, both type 3 and *Escherichia coli* P fimbrial genotypes were detected by using DNA colony blot assays. The frequencies of genera or species isolated were as follows: *Enterobacter* (38.6%), *Klebsiella* (26.8%), *Serratia* (17.7%), *E. coli* (13%), and *Proteus* (5.2%). Isolates of *Klebsiella oxytoca*, *K. pneumoniae*, and *Enterobacter cloacae* most commonly possessed the type 3 fimbrial phenotype and genotype. The phenotype and genotype for *E. coli* P fimbriae (46.2 and 50%, respectively), a known pathogenic determinant in the urinary tract, were detected more frequently than expected. In addition, a previously unspecified hemagglutinin that was specific for porcine erythrocytes was almost uniformly expressed among isolates of *Enterobacter aerogenes*. Finally, the expression of the type 1 fimbrial phenotype was widely detected among the isolates tested but notably absent among *K. oxytoca* and *Proteus mirabilis* isolates. The frequency of the various fimbrial types identified suggests a role for these bacterial organelles in adherence to respiratory epithelia.

The National Nosocomial Infection Surveillance (NNIS) report showed that, from January 1985 to August 1988, members of the family *Enterobacteriaceae* were the pathogens responsible for nosocomial pneumonia in approximately one-third of cases (19). Prior investigators have suggested that colonization of the upper respiratory tract is the initial step in the pathogenesis of gram-negative nosocomial pneumonia (18, 22). To colonize the respiratory tract, these bacteria must overcome mucociliary clearance by adhering to the epithelium (4, 24). Investigations of urinary and gastrointestinal pathogens have shown that many members of the *Enterobacteriaceae* may express adhesive proteins, referred to as adhesins. Adhesin proteins interact with specific epithelial receptors, which not only abrogate mechanical clearance by urine flow or peristalsis but also facilitate successful interactions between the bacteria and the host epithelium (e.g., by optimizing delivery of exotoxins to target tissue and access to rich nutrient sources) (10, 18, 22, 29). Although adherence to respiratory epithelium by some isolates of *Enterobacteriaceae* has been examined (8, 11, 25), this study represents the first systematic study of adhesins produced by respiratory tract isolates of *Enterobacteriaceae*.

Bacterial adhesins are frequently associated with fimbriae, which are commonly detected in vitro by hemagglutination (HA) (7, 24). Organisms expressing type 1 fimbriae characteristically display mannose-inhibitable agglutination of guinea pig erythrocytes (i.e., mannose-sensitive [MS] HA), and most members of the family *Enterobacteriaceae* express this fimbrial type (1, 7). Mannose-containing glycoproteins are believed to be the receptors for this adhesin (13, 25). Other hemagglutinins expressed by *Enterobacteriaceae* are a heterogenous group, broadly classified as mannose-resistant (MR) adhesins. For example, *Escherichia coli* producing either P, S, Dr, or F fimbrial adhesins exhibit MR HA, and these adhesins facilitate attachment at extraintestinal sites by pathogenic strains (3, 10, 23, 29).

Although *E. coli* are also associated with nosocomial colonization and infection of the respiratory tract, *Enterobacter* spp. and *Klebsiella* spp. are isolated more frequently (19). These two closely related genera frequently express type 3 fimbriae. These fimbriae mediate agglutination of tannic acid-treated erythrocytes, which is referred to as MR *Klebsiella*-like (MR/K) HA (7). Also, MR/K HA is usually inhibitable by spermidine (15). The role of type 3 fimbriae as a colonization factor has not been established, but recent studies suggest that these appendages may facilitate attachment in vitro to the basement membrane, specifically to type V collagen (31).

Epidemiologic surveys assessing fimbrial phenotypes of clinical isolates based on HA have provided the initial evidence that a particular fimbrial type may play a role in disease pathogenesis, particularly if the fimbrial phenotype is found more commonly associated with the disease state than among indigenous flora (29). In the study described herein, we prospectively obtained isolates of *Enterobacteriaceae* from tracheal aspirates of intubated intensive care unit patients, screened the isolates for fimbrial types that may not have been previously characterized, and determined the distribution of several well-characterized fimbrial types (type 1, type 3, and P fimbriae) among the respiratory isolates.

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MATERIALS AND METHODS

Bacterial strains. Clinical isolates of *Enterobacteriaceae* were cultured from tracheal aspirates of intubated patients from January through December 1989. The cultures were ordered by the supervising physician because of suspicion of pulmonary infection based on the following standard criteria (22): elevated leukocyte count, fever, infiltrate on chest roentgenogram, and positive Gram stain of a tracheal aspirate. Isolates came from patients in either the medical, surgical, or cardiovascular intensive care units within the University of Iowa Hospitals and Clinics. Isolates of *Enterobacteriaceae* were identified by using the Vitec automated system and then prospectively selected for this study if they represented greater than 50% of the total growth on a blood agar plate and the patient had not had a previous isolate entered into the study. Isolates were subcultured once before the fimbrial type was determined, and isolates were saved in the Special Microbiology Laboratory on soft agar slants.

Agglutination assays. Mannose-inhibitable agglutination of guinea pig erythrocytes is uniquely characteristic of the common type 1 fimbriae. This test alone was used to identify isolates expressing this fimbrial type (7). Since isolates of *Enterobacteriaceae* may express several fimbrial types simultaneously, all isolates were also tested for MR HA by using human (type A), sheep, guinea pig, or porcine erythrocytes to identify other fimbria-associated hemagglutinins.

Bacteria were subcultured at 37°C on either Luria (L) agar, or minimal agar supplemented with 1% glycerol and 0.3% Casamino Acids (G-CAA medium). A dense bacterial suspension (10^8 to 10^9 bacteria per ml) in sterile phosphate-buffered saline (PBS) was mixed with erythrocytes in wells on porcelain plates (rock tile method). In each case, erythrocyte agglutinination in the presence of 3% (wt/vol) α-*methyl*-D-mannoside (a nonmetabolizable form of D-mannose) was compared to a control without α-*methyl*-D-mannoside. Isolates that exhibited MS HA with a 3% (wt/vol) fresh guinea pig erythrocyte suspension were recorded as producing type 1 fimbriae.

For isolates that exhibited MR HA with 3% human (type A), guinea pig, sheep, and/or porcine erythrocyte suspensions, the fimbrial type was more specifically identified as follows. Isolates that demonstrated MR/K HA with tannin-treated human (type A) erythrocytes were recorded as producing type 3 fimbriae. Inhibition of MR/K HA by spermidine (final concentration of 40 mM) was determined for all organisms that exhibited MR/K HA (15). Tannin treatment of human erythrocytes was performed by standard techniques (9). Randomly selected isolates that exhibited MR HA of human erythrocytes and all *E. coli* isolates were further tested for P-fimbrial expression. Organisms that agglutinated a galabiose-coated latex bead suspension (Chembiomed, Canada) after 5 min of gentle rotation at 4°C were recorded as producing P fimbriae possessing the galabiose receptor-binding specificity. Isolates that demonstrated MR HA and that could not be further characterized were designated as producing MR *Proteus*-like (MR/P) HA.

Immunoblots and DNA blots. For immunoblot assays, the isolates were cultured on nitrocellulose discs over L agar and then killed in $CHCl_3$ vapor. The membrane was blocked with 0.25% gelatin–PBS and then washed with PBS–0.05% Tween 20. The membrane was incubated with anti-fimbrial serum; after a wash, a secondary biotinylated anti-rabbit immunoglobulin G serum was added. The reaction was developed with a Streptavidin-peroxidase system (Amersham) with 2,2′-azino-di-(3-ethylbenzthiazolinesulfonic acid) as the substrate as previously described (14).

By using growth from membranes incubated as above, DNA colony blots were performed by standard techniques (16). The DNA probes, derived from the cloned *Klebsiella pneumoniae* type 3 and *E. coli* P fimbrial gene clusters, were radiolabeled by the random priming method (12). The DNA probe used to detect the type 3 fimbrial gene cluster was derived from the recombinant plasmid pFK40 (14) and consisted solely of nucleotide sequence from the major fimbrial structural gene, *mrkA* (Fig. 1; 500-bp *Taq*I-*Taq*I fragment). Prior studies had indicated no differences between the *mrkA* probe and two other *mrk* gene fragments in detecting the type 3 fimbrial gene cluster (16). The two DNA fragments used to detect P-fimbrial genes were derived from the recombinant plasmid pDC1 (6) and consisted of the entire 12-kb *pap* gene cluster and the major structural subunit gene, *papA* (Fig. 1; 400-bp *Cla*I-*Hin*dIII fragment). Hybridization under high-stringency conditions was performed as previously described (15).

Transmission electron microscopy. Electron microscopy was used to verify phenotypic expression of fimbriae. One drop of bacterial suspension was placed on a carbon-coated copper grid, and after 1 min the bacteria were negatively stained with 2% phosphotungstic acid (16).

Fimbrial purification. Fimbriae from representative isolates were purified as previously described (16). Briefly, isolates were grown under conditions to optimize fimbrial production, harvested, centrifuged (10,000 × *g* at 4°C for 30 min), resuspended in 5.0 mM Tris-HCl–1 M NaCl buffer (pH 7.5), and then homogenized at 4°C. The bacteria were removed by centrifugation (12,000 × *g* for 30 min). After a second homogenization-centrifugation step, solid ammonium sulfate (10%, wt/vol) was added to the supernatant; after 30 min at the ambient temperature, the precipitate was collected by centrifugation (25,000 × *g* for 15 min) and discarded. Solid ammonium sulfate (30%, wt/vol) was added to the supernatant, and this was allowed to stand at 4°C for at least 2 h. The precipitate was collected by centrifugation (25,000 × *g* for 15 min) and resuspended in sterile distilled H_2O. Cesium chloride (42% wt/vol) was added, and the protein solution was centrifuged in a vertical-angle rotor (Beckman Vti 65) for 7 h at 250,000 × *g*. The fimbriae, present as a dense white band, were collected, diluted 1:5 in sterile distilled H_2O, and pelleted in a fixed-angle rotor at 250,000 × *g* for 2 h. The gelatinous fimbrial pellet was resuspended in sterile distilled H_2O and stored at 4°C. Protein concentrations were determined with a Micro BCA protein assay kit (Pierce, Rockford, Ill.). Purity and molecular weight were assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electroporesis (12% acrylamide, 0.3% bisacrylamide).

RESULTS

Isolates of *Enterobacteriaceae* were obtained from 191 tracheal aspirates of patients with or at high risk for nosocomial pneumonia. The majority (65.4%) of the isolates were members of the genus *Enterobacter* (29.7% *E. cloacae*; 8.9% *E. aerogenes*) or the closely related genus *Klebsiella* (15.8% *K. pneumoniae*; 11.0% *K. oxytoca*). Less frequently, strains of *E. coli* (13%), *Serratia* spp. (17.7%), or *Proteus* spp. (5.2%) were isolated. The fimbrial phenotypes and genotypes of these strains are described below and in Table 1.

Type 3 fimbriae and MR/K HA. MR/K HA was inhibited by 40 mM spermidine in all cases. The type 3 fimbrial

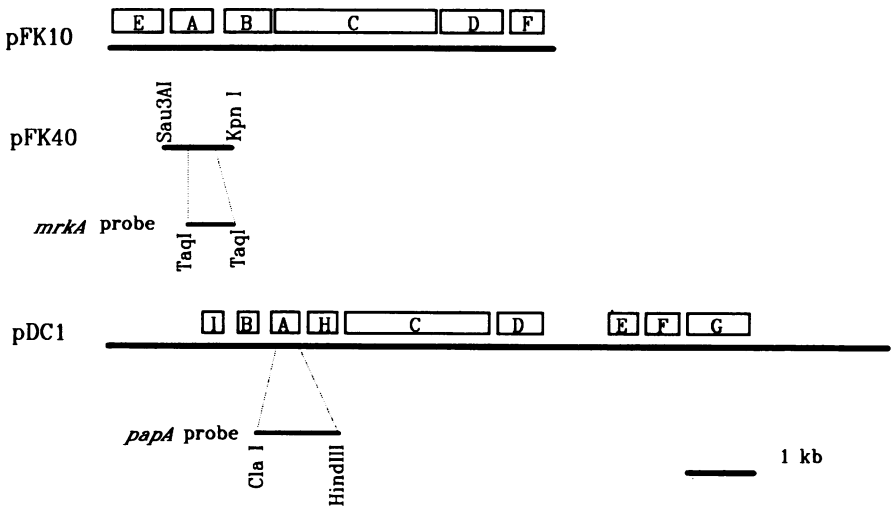

FIG. 1. Physical maps of recombinant plasmids pFK10, pFK40, and pDC1. The solid bars indicate the recombinant plasmid inserts or restriction fragments used as probes. The approximate locations of the associated genes are shown by the open boxes. The *K. pneumoniae* type 3 fimbrial probe used is a *Taq*I-*Taq*I fragment isolated from pFK40 and is derived solely from sequences within the *mrkA* gene, which encodes the type 3 major fimbrial subunit. One of the *E. coli* P-fimbrial probes used was the complete insert of pDC1, which encodes the P-fimbrial F11 serotype. The other P-fimbrial probe consisted of a *Cla*I-*Hin*dIII fragment isolated from pDC1, which consists solely of sequences within the *papA* gene, which encodes the P-fimbrial subunit.

adhesin was found most frequently in *Klebsiella* strains (Table 1). Most respiratory isolates of *K. pneumoniae* (22 of 30) and all but one isolate of *K. oxytoca* (20 of 21) were capable of expressing this fimbrial adhesin. As previously reported by our group, growth on G-CAA agar rather than L agar or nutrient agar enhanced the phenotypic expression of type 3 fimbriae (14). Also, the majority of *Klebsiella* strains expressing MR/K HA activity shared a common type 3 fimbrial antigen that was detected by a monoclonal antibody preparation raised against fimbriae purified from a strain of *K. pneumoniae* (15). Similarly, a gene probe comprising nucleotide sequences from the major fimbrial subunit gene (*mrkA*) detected homologous sequences in the genomic DNA from most of the *Klebsiella* isolates (Table 1).

Five of the 17 strains of *E. aerogenes* exhibited MR/K HA activity. Only one of these strains possessed both the *Klebsiella* type 3 fimbrial antigen and nucleotide sequences similar to that of the *mrkA* probe. Two strains of *E. aerogenes* that possessed the *Klebsiella* type 3 fimbrial antigen and nucleotide sequences similar to the *mrkA* probe did not exhibit MR/K HA activity. The frequency of phenotypic expression of the type 3 fimbrial adhesin was 21% among *E. cloacae* strains and 38% among *S. marcescens* strains. Most of these fimbriae were not recognized by the anti-type 3 fimbrial antibody, confirming a previous observation that genus-specific antigens are associated with the type 3 fimbriae (15). However, 42% of both the *E. cloacae* and the *S. marcescens* strains shared nucleotide sequences common to the *mrkA* determinant. Consequently, even some strains that did not express type 3 fimbriae on their surface did possess sequences similar to the *mrk* gene cluster.

Type 3 fimbrial expression was observed in only one strain of *Proteus mirabilis*. Also, one *E. coli* strain reacted weakly with the type 3 fimbrial DNA probe, but this organism did not exhibit MR/K HA activity, nor did it react with the antifimbrial monoclonal antibody.

TABLE 1. Fimbrial phenotype and genotype among 191 respiratory isolates of *Enterobacteriaceae*[a]

Bacteria	No. of isolates	No. of isolates exhibiting the following									
		Type 3 fimbriae			P fimbriae			Type 1 fimbriae	Unspecified MR/P HA of RBCs		
		MR/K HA[b]	Reactivity with MAb	Hybridization to *mrkA* DNA	MR HA[c]	P particle agglutination	Hybridization to *pap* DNA	MS HA	Porcine	Sheep	Other[d]
E. cloacae	57	12	3	24	0	0	0	38	10	3	4
E. aerogenes	17	5	3	3	0	0	0	4	16	0	2
K. pneumoniae	30	22	21	25	0	0	0	15	1	2	1
K. oxytoca	21	20	20	21	0	0	0	1	0	1	3
S. marcescens	24	9	2	10	0	0	0	15	1	0	1
Serratia spp.	6	2	0	2	0	0	0	2	0	0	1
P. mirabilis	10	1			0	0		0	0	0	1
E. coli	26	0	0	1	14	12	13	17	15	15[e]	0

[a] MAb, monoclonal antibody; RBCs, erythrocytes.
[b] Inhibitable by spermidine (40 mM).
[c] Human (type A) erythrocytes were used; only data for *E. coli* are reported in this column, since P fimbriae not expressed by other species.
[d] Guinea pig and human (type A) erythrocytes were used except for *E. coli*.
[e] Associated with *E. coli* F fimbriae.

P fimbriae associated with MR HA activity. The P fimbriae frequently found on uropathogenic isolates of *E. coli* are characterized by their ability to adhere to galabiose-containing glycoconjugates (29). A total of 14 *E. coli* isolates (54%) exhibited MR HA activity with the human erythrocytes used in these studies. Of these 14 isolates, 12 also agglutinated latex beads coated with galabiose and therefore by definition express P fimbriae. In addition to demonstrating typical P fimbrial agglutination of human erythrocytes, these 14 isolates and another *E. coli* isolate demonstrated evidence for F-fimbrial expression by agglutinating sheep erythrocytes. The F-fimbrial phenotype is closely related to the P-fimbrial phenotype, and the two are often coexpressed in uropathogenic *E. coli* isolates (3).

Two DNA probes derived from the recombinant plasmid pDC1 encoding the F11 serotype of P fimbriae confirmed the P-fimbrial agglutination data. Thirteen MR HA-positive *E. coli* isolates demonstrated sequence homology with both probes. One MR HA-positive isolate that did not agglutinate the galabiose-coated latex beads also reacted with both probes. The remaining MR HA-positive *E. coli* strain did not share common nucleotide sequences with the probes.

The ability to both recognize the galabiose receptor and hybridize with the P-fimbrial DNA probes was restricted to *E. coli* isolates. No other members of the *Enterobacteriaceae* demonstrated this property.

Type 1 fimbriae and MS HA. Previous studies have shown that many isolates of the *Enterobacteriaceae* obtained from the urinary tract and gastrointestinal tract express type 1 fimbriae (1, 7). Many of the strains isolated from the respiratory tract also possessed the ability to agglutinate guinea pig erythrocytes only in the absence of mannose and, therefore, exhibited the characteristic type 1 fimbrial adhesin activity (Table 1). However, the frequency of type 1 fimbrial expression was notably low in some species. For example, none of the 10 tracheal *Proteus* isolates exhibited MS HA activity. In addition, the frequency of type 1 fimbrial expression in *K. oxytoca* (1 of 21) and *E. aerogenes* (4 of 17) was low.

Uncharacterized hemagglutinins. Some tracheal isolates demonstrated MR HA activity that could not be further characterized. Such hemagglutinins may best be classified in the MR/P group of adhesins, which were originally described in strains of *Proteus* species but were subsequently detected on other genera of *Enterobactericeae* (27). In most cases, these MR/P adhesins were exhibited rarely (Table 1). However, isolates of *E. aerogenes* that recognized a receptor found on porcine erythrocytes were the exception. All but one tracheal isolate of *E. aerogenes* produced an MR/P hemagglutinin. Isolates that expressed the MR/P hemagglutinin alone exhibited fimbriae that appeared to be organized into discrete bundles when observed by transmission electron microscopy (Fig. 2). These isolates also demonstrated the most intense HA when grown on G-CAA agar rather than L agar, where HA activity was weak or absent. When HA activity was not observed, the organism was demonstrated to be nonfimbriate by transmission electron microscopy. Bacteria grown at 18 to 20°C retained HA activity, differentiating the phenotypic expression of this adhesin from that associated with the *E. coli* P fimbriae (17). Figure 2 also shows the morphology of fimbriae purified from an *E. aerogenes* isolate demonstrating only MR/P HA activity and compares the size of P fimbriae with those of type 3 and type 1 fimbriae. The purified fimbriae were approximately 6 to 7 nm in width and 300 to 800 nm in length and did not possess HA activity. The fimbrial subunits produced a single band with a molecular weight of 21,600 after SDS-polyacrylamide gel electrophoresis.

DISCUSSION

Previous studies have indicated that specific adhesins of *Enterobacteriaceae* are colonization antigens mediating attachment to the urinary and gastrointestinal epithelia as well as to vascular endothelial surfaces (10, 29). The role of fimbrial adhesins in the pathogenesis of nosocomial pneumonia has not been extensively explored. This study was designed to determine whether *Enterobacteriaceae* that colonize or infect intubated patients produce several different adhesins. Only bacteria isolated from the tracheae of intubated patients were studied, so that the isolates were most representative of lower respiratory tract colonization. Although aspirates were taken from patients who were clinically suspected of having nosocomial pneumonia, this study could not distinguish between organisms associated with colonization and those associated with pneumonia. Currently, substantial controversy exists over whether this distinction can be made in intensive care unit patients based on standard clinical criteria (21, 22). Many prior studies of nosocomial pneumonia, including the NNIS report, established the diagnosis based on these clinical criteria. The frequency of specific genera identified in the NNIS report closely correlated with the frequency of bacteria identified in the study reported herein. A number of the organisms identified by the NNIS and this report may indeed be only colonizing bacteria. Many investigators accept, however, that patients who are colonized by gram-negative bacteria carry a substantial risk for development of nosocomial pneumonia. Therefore the isolates identified in this report most likely represent organisms that either were responsible for the clinical pneumonia or were potential pathogens.

Members of the *Enterobacter-Klebsiella* group were identified in the highest numbers, which is consistent with the NNIS report (19). Most strains of *E. cloacae* produced type 1 fimbriae, and fewer numbers also expressed type 3 fimbriae and/or an MR/P-like adhesin. These strains also may have produced other distinct adhesins that were not detected by the methods used in this study. The role of any particular *E. cloacae* adhesin in facilitating colonization of the respiratory tract has yet to be determined.

Most of the *Klebsiella* spp. were characterized by their ability to produce type 3 fimbriae, and a larger proportion of isolates produced this fimbrial type rather than the more commonly investigated type 1 fimbriae. This observation was particularly evident among *K. oxytoca* isolates, where all but one strain produced type 3 fimbriae. Furthermore, a common type 3 fimbrial antigen could be demonstrated in a majority of *Klebsiella* isolates by using a specific monoclonal antibody preparation. This antigen appeared to be largely genus specific, since *Enterobacter* strains exhibiting MR/K HA activity reacted less frequently with this antibody.

Prior studies have demonstrated that nonclinical *Klebsiella* isolates frequently produce type 3 fimbriae (15). Therefore, specific tropism for respiratory tissue, mediated by type 3 fimbriae, probably does not account for the high prevalence of this adhesin among the isolates studied. The explanation more likely will involve several bacterial factors such as the production of capsule or exoproteins and/or resistance to phagocytosis as well as host factors such as impairment of mucociliary clearance, the type of underlying disease(s), and/or severity of illness.

Most strains of *E. aerogenes* produced a MR/P-like hem-

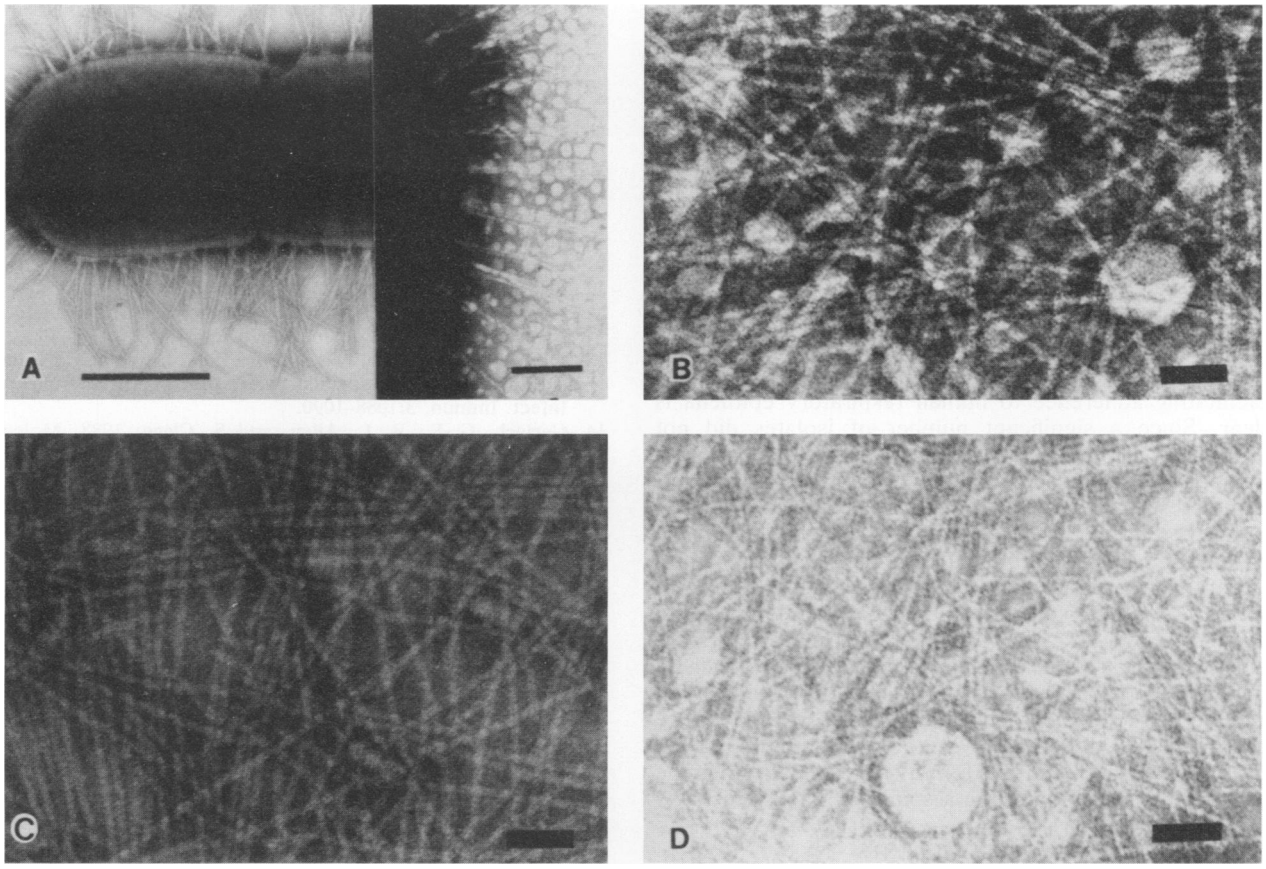

FIG. 2. Transmission electron micrographs comparing morphologic features of purified *Klebsiella* type 3 and type 1 fimbriae with *E. aerogenes* UIR005 fimbriae, which are associated with MR/P HA. (A) *E. aerogenes* UIR005, grown on G-CAA agar, optimally expressing MR/P HA and exhibiting no other HA phenotype. The fimbriae typically vary in length from 300 to 800 nm. The inset photograph demonstrates the organization of these fimbriae into discrete bundles. (B) Purified *E. aerogenes* UIR005 fimbriae. The fimbrial shafts uniformly measure 6 to 7 nm in diameter. (C) Purified *K. pneumoniae* type 1 fimbriae, demonstrating a morphology similar to that shown in panel B. The approximate diameter for these fimbriae is somewhat larger: 7 to 8 nm. (D) Purified *K. pneumoniae* type 3 fimbriae, which are significantly thinner than those shown in panels B and C and measure 3 to 4 nm in diameter. Bars, 0.5 μm in panel A (0.1 μm in the inset photo) and 50 nm in panels B, C, and D.

agglutinin that was specific for porcine erythrocytes. Adegbola and Old (2) have described a similar adhesin on strains of *E. aerogenes*; however, the source of these isolates was undefined, and the adhesin agglutinated primarily guinea pig erythrocytes and did not agglutinate porcine erythrocytes. Figure 2 shows the characteristic morphology of fimbriae associated with the MR/P adhesin reported herein. Although purified fimbriae did not agglutinate erythrocytes, the adhesin was most likely associated with a fimbrial appendage since nonhemagglutinating bacteria were nonfimbriate. In addition, passive HA could be demonstrated by adsorption of the cell-free fimbriae to porcine erythrocytes and subsequent addition of specific antiserum. Serum raised against another MR hemagglutinin (P fimbriae) did not cause this passive HA reaction. Further investigations will be required to determine whether this MR/P-like adhesin facilitates adherence to respiratory epithelial cells.

E. coli can express well-characterized fimbrial types that are believed to be virulence determinants in ascending urinary tract infections of women and children. For example, uropathogenic *E. coli* may produce P, F, or Dr fimbriae with corresponding adhesins that mediate attachment to specific receptors on the upper urinary tract epithelium (3, 29). Some of the support for this virulence concept has come from studies that compare the fimbrial phenotypes and genotypes among *E. coli* isolates from patients with pyelonephritis with the fimbrial phenotypes and genotypes among isolates from the feces of normal individuals. For example, the P phenotype can be demonstrated in 77 to 100% of *E. coli* isolates from patients with pyelonephritis but in only 14 to 16% of isolates from feces (3, 26, 30). Similarly, the P-fimbrial genotype can be identified in almost all isolates from patients with pyelonephritis (3, 26). Our results indicated that approximately 54% of *E. coli* respiratory isolates expressed a MR HA phenotype and that all but one of these isolates contained the P-fimbrial gene cluster. In addition, all but two of these isolates also expressed a receptor binding specificity identical to that of P fimbriae (Table 1). Since the frequency of P-fimbriate *E. coli* isolated from tracheal aspirates is higher than the frequency observed in fecal isolates, it is possible that the expression of this adhesin may be advantageous in the respiratory tract, as has been demonstrated by investigators studying *E. coli* isolates from the urinary tract. A study that effectively distinguishes colonizing bacteria from pathogens responsible for pneumonia may further clarify the question.

Type 1 fimbriae are frequently produced by many species of *Enterobacteriaceae* (7). However, significant serologic variability exists between type 1 fimbrial subunits among the different genera of *Enterobacteriaceae* (28), and significant heterogeneity has also been demonstrated within the DNA sequences encoding type 1 fimbriae (5). Therefore, MS HA alone was used to screen for the type 1 fimbrial phenotype among the spectrum of genera represented. Not surprisingly, type 1 fimbriae were produced by most species of bacteria, with the exception of *P. mirabilis* and *K. oxytoca*. In preliminary studies we demonstrated that these appendages in *K. pneumoniae* mediate attachment to human buccal and trachea epithelial cells but that the magnitude of this attachment is significantly less than the attachment mediated by organisms expressing type 3 fimbriae (20). The role for type 1 fimbriae in facilitating adherence to human respiratory epithelia is unclear. Since a significant number of isolates did not produce type 1 fimbriae, however, this adhesin may not be absolutely required for colonization of the respiratory tract.

In the present communication, we have described a number of adherence factors produced by *Enterobacteriaceae* implicated in nosocomial infections of the respiratory tract. Most strains possessed the ability to produce a variety of adhesins. Additional studies will be necessary, however, to determine which of several possible adherence factors are produced by strains clearly identified as the pathogen in bacterial nosocomial pneumonias. Also, future studies will require both in vitro and in vivo adherence assays that utilize isogenic mutants, differing only in the expression of a given adhesin, to determine the role for any specific adhesin in the colonization or infection of the respiratory tract.

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