INFECTION AND IMMUNITY, Jan. 1989, p. 219–224
0019-9567/89/010219-06$02.00/0

Vol. 57, No. 1

Type 3 Fimbriae among Enterobacteria and the Ability of Spermidine To Inhibit MR/K Hemagglutination

GERALD-F. GERLACH, BRADLEY L. ALLEN, AND STEVEN CLEGG*

Department of Microbiology, College of Medicine, The University of Iowa, Iowa City, Iowa 52242

Received 5 August 1988/Accepted 22 September 1988

The distribution of the gene cluster encoding type 3 fimbriae among various isolates of the family *Enterobacteriaceae* was investigated by using 112 clinical and nonclinical isolates. Closely related DNA sequences were detected in all *Klebsiella* strains, in most *Enterobacter* isolates, in a smaller number of *Escherichia coli* and *Salmonella* spp., and in a single isolate each of *Yersinia enterocolitica* and *Serratia liquefaciens* but not in isolates of *Morganella* or *Providencia* species or *Serratia marcescens*. Except for *E. coli* and *Salmonella* strains, the presence of gene sequences was correlated with the phenotypic expression of either the MR/K hemagglutinin or fimbriae that reacted with specific antibodies. In one isolate of *Y. enterocolitica* the expression of type 3 fimbriae was plasmid determined. The polyamine spermidine was identified as an inhibitor of MR/K hemagglutinating activity, exhibiting an MIC of 1.2 mM. Spermidine inhibited the hemagglutination of 37 MR/K-positive clinical isolates from various genera. However, one clinical isolate of *Enterobacter cloacae* and most (four of five) nonclinical *Klebsiella* isolates were not completely inhibited.

Type 3 fimbriae are thin, filamenteous appendages mediating the agglutination of tannic acid-treated erythrocytes from a wide variety of species in the presence and absence of D-mannose (7). These fimbriae were originally described in *Klebsiella* species and were shown to mediate the mannose-resistant *Klebsiella* (MR/K) hemagglutination phenotype (7). Type 3 fimbriae are produced by most *Klebsiella* isolates (26, 28, 29) and represent, within this genus, an antigenically homogeneous group (22, 24, 26). Serologically related fimbriae are also found on *Enterobacter, Serratia, Salmonella,* and *Yersinia* strains (24). Morphologically indistinguishable but serologically distinct filaments have been described in *Morganella, Proteus, Providencia,* and *Serratia* species (23, 25). It has been suggested that the type 3 fimbriae of nonclinical strains of *Klebsiella pneumoniae* facilitate adherence to plant roots (16), whereas the MR/K adhesion of clinical isolates of *Providencia stuartii* has been implicated in mediating adherence to urinary catheters (19). Using DNA probes derived from a recombinant molecule encoding the *K. pneumoniae* type 3 fimbriae (12), we found gene sequences that encode these fimbriae among isolates of enteric bacteria. In addition, using both monoclonal and polyclonal antibodies, we examine the serologic specificity of type 3 fimbrial antigens among the family *Enterobacteriaceae*. Finally, we present data demonstrating the ability of the polyamine spermidine to specifically inhibit the hemagglutinating activity of type 3 fimbriae in clinical isolates.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. All clinical isolates except *Yersinia* species were obtained from the Special Microbiology Laboratory of the University of Iowa Hospitals and Clinics, Iowa City. Nonclinical *Klebsiella* isolates and all *Yersinia* strains were obtained from the State Hygienic Laboratory of the University of Iowa. The isolates used in these studies are shown in Table 1. Isolates were maintained on blood agar, and *Escherichia coli* HB101 possessing recombinant plasmids was grown on Luria agar supplemented with the appropriate antibiotics (18). The recombinant plasmid pFK12, encoding the expression of *K. pneumoniae* type 3 fimbriae, has been described elsewhere (12). The construction of recombinant molecules encoding the expression of *K. pneumoniae* type 1 fimbriae (13) and the P pili of uropathogenic *E. coli* has been previously described (4).

DNA colony blot. Bacterial isolates were subcultured and grown overnight on L-agar plates covered with a nitrocellulose disk (Schleicher & Schuell, Inc., Keene, N.H.). The bacteria were lysed and the DNA colony blot was performed by standard procedures (18). DNA probes derived from different regions of the type 3 fimbrial gene cluster (Fig. 1) were radiolabeled by random priming (9). Hybridization washes were performed in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)– 0.05% sodium pyrophosphate–0.1% sodium dodecyl sulfate at 65°C, and then the membranes were exposed to X-ray film for 2 to 12 h.

DNA dot blot and Southern hybridization. Genomic DNA was prepared as previously described (32). Plasmid DNA was prepared by cesium chloride-ethidium bromide density gradient centrifugation (14). For the DNA dot blot assay, approximately 3 μg of DNA was applied directly onto GeneScreen Plus membranes (Dupont, NEN Research Products, Boston, Mass.); in the Southern hybridization analyses, 3 μg of *Eco*RI-restricted genomic DNA or 1 μg of plasmid DNA was used. The *Eco*RI restriction enzyme was used because we have previously shown that two enzyme sites are located within but close to the boundaries of the *K. pneumoniae* type 3 fimbrial gene cluster (Fig. 1) (11). After electrophoresis through agarose, the DNA was denatured with 1 M NaOH and transferred to GeneScreen membranes by capillary action. Hybridization to radiolabeled DNA probes under high-stringency conditions was performed according to the manufacturer's instructions.

Immunological assays. The immunoblots were performed with fimbria-specific monoclonal antibodies as previously described (11). Also, a competitive enzyme-linked immunosorbent assay was used to detect type 3 fimbrial antigens on the bacterial surface (13). The bacterial suspensions to be tested (10^9 organisms per ml) were preincubated with appropriate dilutions of antiserum and subsequently added to

* Corresponding author.

TABLE 1. Genetic, serologic, and functional relatedness of type 3 fimbriae among members of the family *Enterobacteriaceae*

Isolate and source	No. of isolates	No. of isolates exhibiting:				
		MR/K HA$^+$	MR/P HA$^+$	Hybridization to PI[a] DNA	Reactivity with MAb[b]	HAI by spermidine[c]
Klebsiella pneumoniae						
Respiratory tract	6	6	0	6	6	6
Blood	3	3	0	3	3	3
Tissue	3	2	0	3	1	2
Urine	4	3	0	4	3	3
Water	4	2	0	4	2	0
Klebsiella oxytoca						
Respiratory tract	5	4	0	5	4	4
Water	6	3	0	6	6	1
Enterobacter cloacae						
Respiratory tract	10	7	3[d]	7	3	2
Escherichia coli						
Respiratory tract	9	0	0	3	0	–[e]
Blood	5	2	2[f]	0	0	–
Tissue	5	1	1	0	0	–
Skin	1	1	1	0	0	–
Salmonella typhimurium						
Blood	4	0	0	0	0	–
Feces	5	0	0	2	0	–
Serratia marcescens						
Respiratory tract	9	3	0	0	0	3
Serratia liquefaciens						
Respiratory tract	2	1	0	1	1	1
Morganella morganii						
Respiratory tract	2	2	0	0	0	2
Blood	4	3	0	0	0	3
Tissue	5	5	0	0	0	5
Proteus rettgeri						
Tissue	2	0	0	0	0	–
Urine	4	0	0	0	0	–
Proteus stuartii						
Wound	1	1	0	0	0	1
Urine	3	3	0	0	0	3
Yersinia spp.[g]						
Feces	10	1	0	1	1	1

[a] PI, DNA probe shown in Fig. 1.
[b] MAb, Monoclonal antibody.
[c] Spermidine was used at a final concentration of 40 mM.
[d] MR/P HA$^+$ strains cannot be differentiated from MR/K HA$^+$ strains by hemagglutination.
[e] –, Not tested.
[f] *E. coli* strains possessing P pili.
[g] Eight strains of *Y. enterocolitica* and one each of *Y. intermedia* and *Y. frederiksenii*.

microdilution wells coated with purified type 3 fimbriae as the solid-phase antigen.

Hemagglutination assays. Bacteria were grown overnight on L agar or for 72 h in Luria broth at 37°C. The hemagglutinating activity (HA$^+$ phenotype) was determined by using fresh guinea pig and human erythrocytes in the presence and absence of D-mannose. In addition, tanned human and guinea pig erythrocytes were used to detect type 3 fimbrial adhesins. Bacteria exhibiting the MR/K HA$^+$ phenotype were subsequently used to detect inhibitory compounds. The hemagglutination inhibition assay was performed as previously described (5), except the reaction mixtures were simultaneously mixed and shaken at ambient temperature in the depression of a porcelain tile. The MIC was defined as the lowest concentration of reagent that completely inhibited the MR/K hemagglutinating activity.

RESULTS

Distribution of type 3 fimbrial gene sequences. Table 1 indicates the bacteria possessing nucleotide sequences sim-

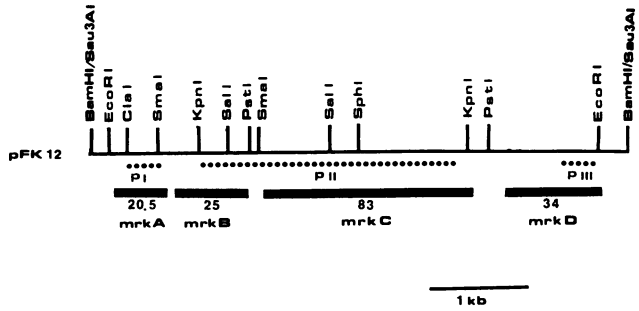

FIG. 1. Physical maps of recombinant plasmid pFK12. The genes that have been shown to be necessary for phenotypic expression of type 3 fimbriae are designated *mrA*, *B*, *C*, and *D*, and the locations of these genes are indicated by the solid boxes. The sizes (kilodaltons) of the gene products are as illustrated, and a complete description of the gene cluster can be found in reference 11. The dotted lines indicate the positions of the DNA fragments used as probes (PI, PII, and PIII) for the colony blot assay.

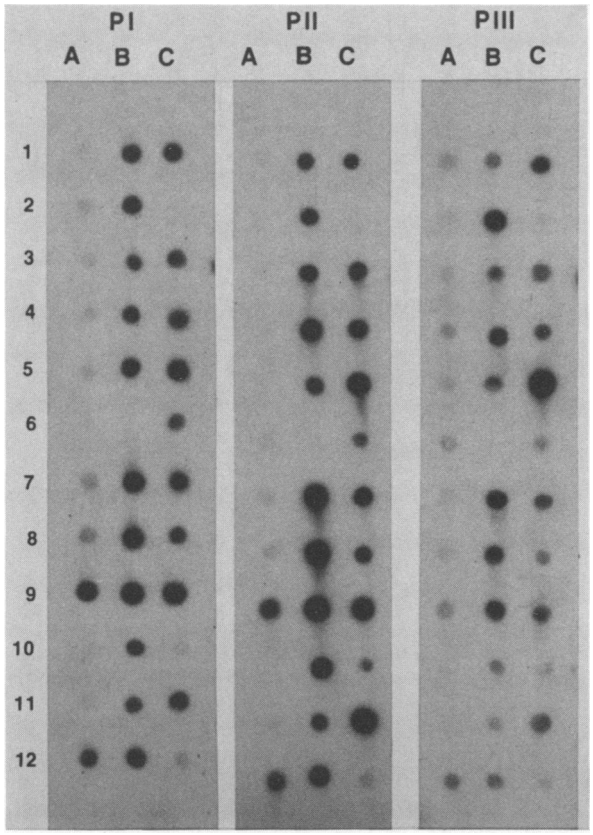

FIG. 2. DNA dot blot analysis of clinical isolates. For individual DNA preparations, when hybridization was observed, hybridization to all three probes occurred. Hybridization of genomic DNA from representative strains to the DNA probes PI, PII, and PIII is shown. Key: A1 and A2, *S. typhimurium*; A3 through A5, *E. coli* A6 through A12, *E. cloacae*; B1 through B5, *K. pneumoniae;* B6, *E. coli* HB101; B7 through B10, *K. oxytoca*; B11, *Y. enterocolitica*; B12, *S. liquefaciens*; C1, *K. pneumoniae* IA565; C2, *E. coli* ORN103; C3 through C12, *K. pneumoniae*. Genomic DNA from two isolates of *K. pneumoniae* (C10 and C12) exhibited weak hybridization to the DNA probes. *K. pneumoniae* IA565 (C1) is the isolate from which the type 3 fimbrial gene cluster was cloned. Genomic DNA from the laboratory-derived strains *E. coli* HB101 (B6), ORN103 (C2), and an *Enterobacter* isolate (A10) were used as negative controls.

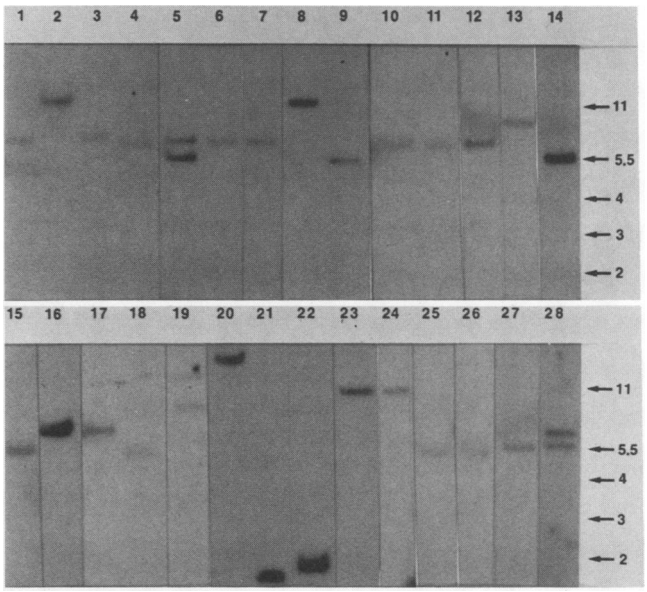

FIG. 3. Southern hybridization analysis of total cellular DNA from clinical isolates. The genomic DNA was restricted with *Eco*RI, and the probe PI was used in all assays. Genomic DNA was prepared from the following strains (lanes): 1 through 13, *K. pneumoniae*; 14, recombinant plasmid pFK12; 15, *S. liquefaciens*; 16, *Y. enterocolitica*; 17 through 19, *E. cloacae*; 20 through 24, *K. oxytoca*; 25 through 26, *E. coli*; 27, *S. typhimurium*; 28, *K. pneumoniae* IA565. *K. pneumoniae* IA565 is the isolate from which the type 3 fimbrial gene cluster was cloned. pFK12 is the recombinant plasmid encoding type 3 fimbriae.

ilar to those of the DNA probes used in the colony blot assay. Hybridization to DNA probe PI was associated with hybridization to the other two probes (Fig. 2). Therefore all 31 *Klebsiella* strains and most (7 of 10) *Enterobacter* strains, 1 *Serratia* isolate, and 1 *Yersinia* strain possessed similar sequences to all three DNA probes used. A weak hybridization signal was seen with three *E. coli* and two *Salmonella* strains. The hybridization of the genomic DNA from representative strains with the three DNA is shown in Fig. 2.

Southern hybridization profiles. *Eco*RI-restricted genomic DNA from representative strains is shown in Fig. 3. The probe used in these analyses was composed of nucleotides from the gene (*mrkA*) encoding the major fimbrial subunit (PI in Fig. 1). Ten isolates of *K. pneumoniae* (Fig. 3, lanes 1, 3 through 7, 10 through 12, and 28), one isolate of *Enterobacter cloacae* (lane 17), and a single strain of *Yersinia enterocolitica* (lane 16) possessed the fimbrial subunit gene on a 6-kilobase-pair (kb) *Eco*RI DNA fragment. In addition, two *K. pneumoniae* isolates (Fig. 3, lanes 5 and 28) possessed an additional 5.5-kb *Eco*RI DNA fragment that hybridized to the PI DNA probe. One of these strains (Fig. 3, lane 28) is the source of *Klebsiella*-derived DNA in the recombinant molecule pFK12 (Fig. 3, lane 14). A single 5.5-kb fragment was detected in DNA from one strain of *K. pneumoniae* (lane 9) , *E. cloacae* (lane 18), *Salmonella typhimurium* (lane 27), and *Serratia liquefaciens* (lane 15). Plasmid DNA was prepared from various clinical isolates, but the fimbrial subunit gene hybridized to only that from a single strain of *Y. enterocolitica* (lane 16). No hybridization to total genomic DNA was observed in the remaining nine *Yersinia* strains.

Immunological detection of type 3 fimbriae. As seen in Table 1, 15 of 16 MR/K HA^+ strains of *K. pneumoniae* (including both MR/K HA^+ nonclinical isolates) and all MR/K HA^+ *Klebsiella oxytoca* isolates reacted with antifimbrial monoclonal antibodies. In addition, three of four MR/K HA^+ isolates of *E. cloacae*, which did not produce the mannose-resistant and *Proteus*-like (MR/P) hemagglutinin, and one isolate each of *S. liquefaciens* and *Y. enterocolitica* reacted with the monoclonal antibody preparation. No *E. coli* or *S. typhimurium* strain were positive in this assay; in addition, all the MR/K HA^+ isolates of *Morganella* and *Providencia* strains were negative.

Five MR/K HA^+ strains (four of *E. cloacae*, one of *K. pneumoniae*) which possessed nucleotide sequences that hybridize to the DNA probe but were not recognized by the monoclonal antibody were tested in the competitive enzyme-linked immunosorbent assay with a polyclonal serum. A significant (at least 50%) inhibition was observed only with the one *K. pneumoniae* isolate.

Type 3 fimbrial expression of *Y. enterocolitica* is plasmid mediated. As indicated above, plasmid DNA from a single MR/K Ha^+ *Yersinia* isolate hybridized to the fimbrial gene probe. Therefore, *E. coli* HB101 was transformed with this

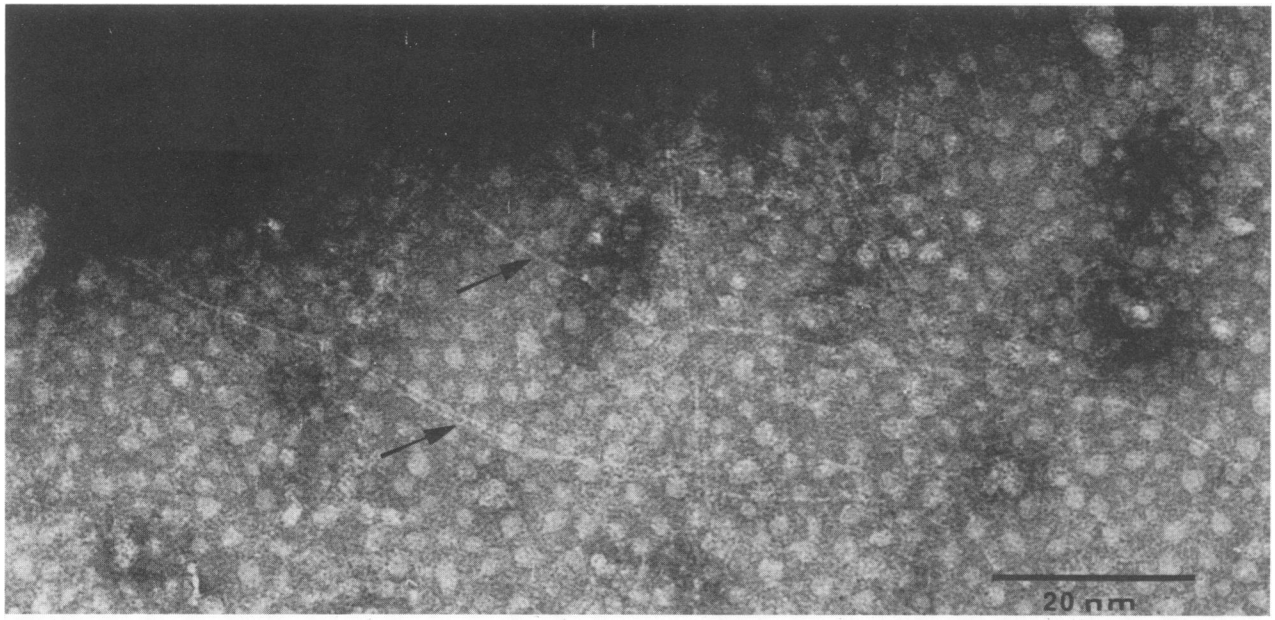

FIG. 4. Electron micrograph of *E. coli* HB101 possessing pBR322 and the *Y. enterocolitica*-derived plasmid. The thin type 3 fimbriae are indicated by arrows.

plasmid along with pBR322 by the method of Kretschmer et al. (17) to obtain double transformants. Ampicillin-resistant transformants were tested for type 3 fimbrial expression by the immunoblot assay. Of 1,500 ampicillin-resistant transformants, 3 were reactive; each possessed two distinct plasmid molecules corresponding in size to pBR322 and the *Yersinia*-derived plasmid. All three double transformants expressed type 3 fimbriae (Fig. 4) and exhibited an MR/K HA^+ phenotype.

Inhibition of MR/K hemagglutination activity. The compounds shown in Table 2 were tested for their ability to inhibit the MR/K hemagglutinating activity. Spermidine, putrescine, and agmatine sulfate inhibited hemagglutination of *E. coli* possessing the plasmid pFK12 at concentrations of 1.2, 15, and 7.5 mM respectively, with tanned human erythrocytes (Table 2). In addition, spermidine at concentrations of 300 mM could partially reverse the hemagglutination caused by MR/K HA^+ cultures. Furthermore, it could be demonstrated that a 1% (40 mM) solution of spermidine completely inhibited hemagglutinating activity of 39 MR/K HA^+ MR/P HA^- clinical isolates (Table 1). One clinical isolate of *Enterobacter* sp. and four of five MR/K HA^+ nonclinical isolates of *K. pneumoniae* were not completely inhibited by the 40 mM spermidine solution. However, the hemagglutinating activity of these strains was significantly decreased in the presence of spermidine. The hemagglutination mediated by type 1 and P pili was not affected by spermidine (Table 2).

DISCUSSION

The early work of Przondo-Hessek and Pulverer (29) demonstrated that 85% of *Klebsiella* clinical isolates possess the MR/K hemagglutinin, and Old and co-workers (24, 26) showed a close serological relationship among all *Klebsiella* type 3 fimbriae. The hybridization of the DNA probes under high-stringency conditions to most type 3 fimbriate strains of enterobacteria confirms the relatedness of type 3 fimbriae in

TABLE 2. MICs of compounds affecting the MR/K HA phenotype[a]

E. coli strain[b]	RBC source[c]	MIC[d] (mM)			
		Spermidine	Putrescine	Agmatine sulfate	α-Methyl-D-mannoside
HB101(pFK12)	Human	1.2	15	7.5	–
	Guinea pig	1.2	7.5	30	–
HB101(pGG101)	Human	0	0	0	0
	Guinea pig	–	–	–	10
HB101(pDC1)	Human	–	–	–	–
	Guinea pig	0	0	0	0

[a] Compounds tested which did not inhibit MR/K HA activity were fibronectin, chondroitin, chitin, heparin, galactose, lactose, spermine, arginine, histidine, lysine, glutamine, putrescine dihydrochloride, spermidine trihydrochloride, and ornithine hydrochloride.
[b] *E. coli* HB101(pFK12) produces type 3 fimbriae, *E. coli* HB101(pGG101) produces type 1 fimbriae, and *E. coli* HB101(pDC1) produces P pili.
[c] All erythrocyte (RBC) suspensions were treated with tannic acid.
[d] –, No inhibition; 0, no hemagglutination.

these strains (24). Furthermore, Alecsić and Alecsić (1) and Old and co-workers (26) have shown a close similarity in the amino acid composition of type 3 fimbriae from *Klebsiella*, *Yersinia*, and *Salmonella* isolates. Thus, the hybridization of *Klebsiella*-derived DNA probes to *Salmonella* and *Yersinia* isolates is to be expected. The reactivity of the DNA probes with three *E. coli* isolates, however, is interesting because this species has never been reported to express type 3 fimbriae. Correspondingly, we were unable to establish conditions under which these hybridization-positive strains produced type 3 fimbriae detectable by MR/K hemagglutinating activity or serology. Therefore, it is possible that these *E. coli* isolates possess regions of the type 3 fimbrial gene cluster that may have been conserved due to selection of essential flanking genes. This conclusion would be consistent with the relatively weak hybridization between the DNA probe and the genomic DNA of these isolates. Alternatively, these three *E. coli* isolates lack regions of DNA necessary for type 3 fimbrial expression but retain most of the nucleotide sequences from this system. In this case, the reasons for maintaining the possession of these DNA sequences in the three *E. coli* isolates are unknown.

The Southern hybridization analysis confirmed the high degree of genetic conservation among fimbriate strains. Many strains of *K. pneumoniae* possess a single 6-kb *Eco*RI DNA fragment carrying the fimbrial genes. Two *Klebsiella* isolates were found to possess two distinct DNA fragments carrying the *mrkA* gene (Fig. 3, lanes 5 and 28), and this may represent two copies of the type 3 fimbrial subunit gene on the chromosome of these strains. Similarly, multiple gene copies of the P pili gene have been reported for pathogenic *E. coli* (30). The *mrkA* gene was located on 5- to 6-kb *Eco*RI DNA fragments of *Enterobacter*, *E. coli*, *Salmonella*, *Serratia*, and *Yersinia* isolates. In strains of *K. oxytoca*, the *mrkA* gene was carried on *Eco*RI-restricted fragments of various sizes (Fig. 3, lanes 20 to 24). In two isolates of *K. oxytoca*, the *mrkA* gene probe hybridized to DNA fragments that were smaller than the type 3 gene cluster contained within pFK12 (Fig. 1). These data indicate that those strains possess additional *Eco*RI restriction enzyme sites within the gene cluster and that minor variations of DNA sequence exist between strains.

The lack of reactivity in the immunoblot assay with a monoclonal antibody by one MR/K HA^+ isolate of *K. pneumoniae* and its reactivity in the competitive enzyme-linked immunosorbent assay with a polyclonal serum indicates variability in the antigenicity of type 3 fimbriae of this strain. Similar serological differences have been observed for the P pili of *E. coli* (27) and for type 1 fimbriae of *E. coli* (15) and *K. pneumoniae* (13).

The expression by a single isolate of *Y. enterocolitica* of a fimbrial appendage that was both serologically and genetically related to that of *Klebsiella* fimbriae is interesting since both genera are distantly related within the family *Enterobacteriaceae* (2). The data indicate that the fimbrial gene in this isolate is plasmid encoded. The *Yersinia* plasmid possessing the type 3 fimbrial gene cluster was approximately 35 kb in size as determined by gel electrophoresis and therefore is considerably smaller than the known virulence plasmids of this species (21). Previous reports have indicated that expression of type 3 fimbriae among *Yersinia* strains occurs at a relatively low frequency (1). However, because only one of the isolates described in this study exhibited the MR/K HA^+ phenotype, it is not possible to state definitively that type 3 fimbriation is invariably plasmid encoded in this genus. Such a conclusion must await further characterization of larger numbers of hemagglutinating *Yersinia* strains.

Although many *Providencia* and *Morganella* isolates possess the MR/K hemagglutinin, none of the strains tested by us possessed nucleotide sequences similar to those of the three DNA probes. In addition, neither polyclonal nor monoclonal fimbria-specific antibodies prepared against the *Klebsiella* fimbriae reacted with these strains. Previous studies have indicated that the *Morganella* and *Providencia* type 3 fimbriae comprise a serologically distinct group (24), and our results confirm such observations. In addition, lack of reactivity with the DNA probes suggests that the gene cluster encoding type 3 fimbriae within these two genera represents a more distantly related system than those present in other members of the family *Enterobacteriaceae*. However, fimbrial receptor specificity may be conserved, as evidenced by hemagglutinating activity, indicating a strong selection pressure to maintain adhesiveness.

Because MR/K hemagglutination occurs with tannic acid- or heat-treated erythrocytes from a variety of animals, it is unlikely that a membrane-bound component found in fresh untreated erythrocytes is a receptor for the MR/K hemagglutinin. Therefore, among the compounds we examined for inhibitory activity were those that may be exposed on the hemagglutinin. Therefore, among the compounds we examined for inhibitory activity were those that may be exposed on the cell surface after tissue damage. The polyamine spermidine and, to a lesser degree, putrescine and the polyamine analog agmatine sulfate were inhibitory. Furthermore, hemagglutination could be significantly reversed by the addition of spermidine to bacterium-erythrocyte mixtures. Since hemagglutination by bacteria of both tanned human and guinea pig erythrocytes was inhibited, the activity of spermidine was independent of the erythrocyte origin. Furthermore, the mannose-sensitive hemagglutination mediated by type 1 fimbriae with both tanned and untanned guinea pig erythrocytes was not inhibited by this compound; also, the reactivity of P pili with human erythrocytes was not affected. These results suggest that spermidine is a competitive inhibitor of the MR/K hemagglutinin. Considering that the concentration of spermidine in erythrocytes is approximately 12 μM in healthy individuals (31), the MIC of spermidine (1.2 mM) in vitro is significantly higher. However, the MIC in vitro of methyl α-D-mannoside, a competitive inhibitor of the type 1 fimbrial adhesin, is 0.5 to 3 mM, which also may be considerably higher than concentrations found in vivo (10). Therefore, it is not surprising that relatively high concentrations of spermidine are required to inhibit hemagglutination in vitro; these concentrations may be higher than those affecting adherence in vivo.

Spermidine at a concentration of 40 mM (1% [wt/vol] aqueous solution) inhibited hemagglutination by most isolates exhibiting only MR/K HA^+ activity, including *Morganella* and *Providencia* isolates, which express genetically and serologically distinct type 3 fimbriae. It was of course not possible to test all MR/K HA^+ isolates for spermidine sensitivity, since some strains also express an MR/P hemagglutinating activity, binding both tanned and fresh erythrocytes. This adhesive activity is not blocked by spermidine; therefore, MR/K HA^+ and MR/P HA^+ isolates will demonstrate spermidine insensitivity due to the MR/P adhesin. The incomplete inhibition of one isolate of *E. cloacae* and four nonclinical *Klebsiella* isolates may indicate receptor variability among serologically and genetically related adhesins. Receptor variability has also been observed for the type 1

fimbria-associated mannose sensitive adhesins of different members of the family *Enterobacteriaceae* (10).

The number of clinical and nonclinical isolates used in this study is too small to make a decisive statement concerning the significance of the differing sensitivity to spermidine in these two groups. However, it is interesting to note that elevated spermidine levels are found in patients suffering from extensive tissue damage or neoplastic disease (3, 8, 20). Likewise, these patients are among those showing an increased susceptibility to enterobacterial infections. Similarly recent reports by Mobley and co-workers (19) have suggested that the MR/K adhesin of *P. stuartii* mediates adhesion to urinary catheters. Since such catheters may be rapidly covered by biofilms after insertion (6), it is possible that the type 3 fimbriae are responsible for colonization by enteric bacteria of the surfaces of indwelling devices.

ACKNOWLEDGMENTS

G.-F.G. is a fellow of the postdoctoral program of the Federal Republic of Germany. B.L.A. is supported, in part, by Public Health Service Medical Scientist Training Program grant GM07337 from the National Institutes of Health.

LITERATURE CITED

1. **Aleksić, S., and V. Aleksić.** 1979. Purification and physicochemical analysis of the fimbrial antigen in two different genera of *Enterobacteriaceae*: *Salmonella enteritidis* and *Yersinia enterocolitica*. Zentralbl. Bakteriol. Parasitenkd. Infektionstr. Hyg. Abt. 1 Orig. Reihe A **243**:177–196.
2. **Brenner, D. J.** 1984. Family I. *Enterobacteriaceae* Rahn 1937, p. 408–423. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
3. **Chatel, M., F. Darcel, V. Quemener, H. Hercouet, and J.-P. Moulinoux.** 1987. Red blood cell polyamines as biochemical markers of supratentorial malignant gliomas. Anticancer Res. **7**: 33–38.
4. **Clegg, S.** 1982. Cloning of genes determining the production of mannose-resistant fimbriae in a uropathogenic strain of *Escherichia coli* belonging to serogroup O6. Infect. Immun. **38**:739–744.
5. **Clegg, S., S. Hull, R. Hull, and J. Pruckler.** 1985. Construction and comparison of recombinant plasmids encoding type 1 fimbriae of members of the family *Enterobacteriaceae*. Infect. Immun. **48**:275–270.
6. **Costerton, J. W.** 1984. Mechanisms in microbial adhesion to surfaces, p. 115–123. *In* M. J. Klug and C. A. Reddy (ed.), Current perspectives in microbial ecology. American Society for Microbiology, Washington, D.C.
7. **Duguid, J. P.** 1959. Fimbriae and adhesive properties in *Klebsiella* strains. J. Gen. Microbiol. **21**:271–286.
8. **Elsayed, N. M.** 1987. Influence of vitamine E on polyamine metabolism in ozone-exposed rat lungs. Arch. Biochem. Biophys. **255**:392–399.
9. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
10. **Firon, N., I. Ofek, and N. Sharon.** 1983. Carbohydrate specificity of the surface lectins of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. Carbohydr. Res. **120**:235–249.
11. **Gerlach, G.-F., B. L. Allen, and S. Clegg.** 1988. Molecular characterization of the type 3 (MR/K) fimbriae of *Klebsiella pneumoniae*. J. Bacteriol. **170**:3547–3553.
12. **Gerlach, G.-F., and S. Clegg.** 1988. Cloning and characterisation of the gene cluster encoding type 3 (MR/K) fimbriae of *Klebsiella pneumoniae*. FEMS Microbiol. Lett. **49**:377–383.
13. **Gerlach, G.-F., and S. Clegg.** 1988. Characterization of two genes encoding antigenically distinct type-1 fimbriae of *Klebsiella pneumoniae*. Gene **64**:231–240.
14. **Guerry, P., D. J. Leblanc, and S. Falkow.** 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. **116**:1064–1066.
15. **Klemm, P., I. Ørskov, and F. Ørskov.** 1982. F7 and type 1-like fimbriae from three *Escherichia coli* strains isolated from urinary tract infections: protein, chemical, and immunological aspects. Infect. Immun. **36**:462–468.
16. **Korhonen, T. K., E. Tarkka, H. Ranta, and K. Haahtela.** 1983. Type 3 fimbriae of *Klebsiella* sp.: molecular characterization and role in bacterial adherence to plant roots. J. Bacteriol. **155**: 860–865.
17. **Kretschmer, P. J., A. C. Y. Chang, and S. N. Cohen.** 1975. Indirect selection of bacterial plasmids lacking identifiable phenotypic properties. J. Bacteriol. **124**:225–231.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Mobley, H. L. T., G. R. Chippendale, J. H. Tenney, A. R. Mayrer, L. J. Crisp, J. L. Penner, and J. W. Warren.** 1988. MR/K hemagglutination of *Providencia stuartii* correlates with adherence to catheters and with persistence in catheter-associated bacteriuria. J. Infect. Dis. **157**:264–271.
20. **Moulinoux, J.-P., V. Quemener, and Y. Chambon.** 1987. Evolution of red blood cell polyamine levels in partially hepatectomized rat. Eur. J. Cancer Clin. Oncol. **23**:237–244.
21. **Nesbakken, T., G. Kapperud, H. Sorum, and K. Dommarsnes.** 1987. Structural variability of 40-50 MDal virulence plasmids from *Yersinia enterocolitica*. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **95**:167–173.
22. **Nowotarska, M., and M. Mulczyk.** 1977. Serologic relationship of fimbriae among *Enterobacteriaceae*. Arch. Immunol. Ther. Exp. **25**:7–18.
23. **Old, D. C., and R. A. Adegbola.** 1982. Haemagglutinins and fimbriae of *Morganella*, *Proteus*, and *Providencia*. J. Med. Microbiol. **15**:551–564.
24. **Old, D. C., and R. A. Adegbola.** 1985. Antigenic relationships among type-3 fimbriae of *Enterobacteriaceae* revealed by immunoelectron microscopy. J. Med. Microbiol. **20**:113–121.
25. **Old, D. C., R. A. Adegbola, and S. S. Scott.** 1983. Multiple fimbrial haemagglutinins in Serratia species. Med. Microbiol. Immunol. **172**:107–115.
26. **Old, D. C., A. Tavendale, and B. W. Senior.** 1985. A comparative study of the type-3 fimbriae of *Klebsiella* species. J. Med. Microbiol. **20**:203–214.
27. **Pere, A., V. Väisänen-Rhen, M. Rhen, J. Tenhunen, and T. K. Korhonen.** 1986. Analysis of P fimbriae on *Escherichia coli* O2, O4, and O6 strains by immunoprecipitation. Infect. Immun. **51**: 618–625.
28. **Podschun, R., P. Heineken, and H. G. Sonntag.** 1987. Haemagglutinins and adherence properties to HeLa and intestine 407 cells of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **263**:585–593.
29. **Przondo-Hessek, A., and G. Pulverer.** 1983. Hemagglutinins of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. Zentralbl. Bakteriol. Parasiterkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **255**: 472–478.
30. **Rhen, M., V. Väisänen-Rhen, A. Pere, and T. K. Korhonen.** 1985. Complementation and regulatory interaction between two cloned fimbrial gene clusters of *Escherichia coli* strain KS71. Mol. Gen. Genet. **200**:60–64.
31. **Russel, D. H.** 1983. Clinical relevance of polyamines. Crit. Rev. Clin. Lab. Sci. **18**:261–311.
32. **Stauffer, G. V., M. D. Plamann, and C. T. Stauffer.** 1981. Construction and expression of hybrid plasmids containing the *Escherichia coli glyA* gene. Gene **14**:63–72.