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Influence of Fluoroquinolones on Expression and Function of P Fimbriae in Uropathogenic *Escherichia coli*

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P fimbriae are the major adhesins mediating attachment of pyelonephritogenic *Escherichia coli* to urinary tract tissues, and they therefore constitute a recognized virulence factor. In this work, the effect of fluoroquinolones on P fimbria expression and function in *E. coli* SS142 and C1212 was assessed. Ciprofloxacin, fleroxacin, and norfloxacin were compared with their precursor nalidixic acid and with trimethoprim in sublethal concentrations ranging from 1/32 to 1/4 of the MIC. Fimbria function was assessed in a standard hemagglutination assay and in a parallel hemagglutination inhibition assay in which the titer of antifimbrial antiserum necessary to inhibit hemagglutination by SS142 was determined. Adhesion of antibiotic-exposed bacteria to human uroma T24 cells in suspension was also measured. Fimbria production was quantitated in an inhibition enzyme-linked immunosorbent assay. Trimethoprim produced a dose-dependent decrease of three to four hemagglutination titers for both strains and a decline in the antiserum titer from 1:16 (control) to 1:128 (1/4 MIC) for *E. coli* SS142. Adherence exhibited similar decrements from 130 ± 28 (control) to 16 ± 3 (1/4 MIC) and from 83 ± 19 (control) to 30 ± 11 (1/4 MIC) *E. coli* cells per uroepithelial cell (mean ± standard error) for SS142 and C1212, respectively ($P < 0.015$). By enzyme-linked immunosorbent assay, the inhibition following exposure decreased in a dose-dependent manner from 31% (control) to 8% (1/4 MIC). By contrast, none of the quinolones produced significant changes in the parameters assessed above. At sublethal concentrations, trimethoprim decreased fimbria production. Following exposure to fluoroquinolones, however, *E. coli* expressed morphologically and functionally intact P fimbriae.

Among the common attributes of pathogenic *Escherichia coli* causing urinary tract infections is the expression of P fimbriae (6, 14). These proteinaceous filaments projecting from the surface of many pyelonephritogenic *E. coli* isolates mediate attachment to Galα1-Galβ-containing receptors on uroepithelial cells (7). Inasmuch as bacterial attachment precedes colonization and infection, the ability of *E. coli* to produce P fimbriae constitutes a recognized virulence factor (4, 9, 15). Research in the prevention of urinary tract infections has therefore been directed at interfering with the production of fimbrial ligands and with ligand-receptor interactions.

Antibiotic exposure may alter fimbria production, with consequences for bacterial attachment (18, 19). The newly introduced broad-spectrum antimicrobial agents of the fluoroquinolone class have been shown to be effective in the treatment of urinary tract infections (11); however, their impact on bacterial adherence has received minimal attention (2, 8). In the present study, three quinolones were compared with their precursor nalidixic acid and with trimethoprim, an agent known to alter fimbriation. The attachment of *E. coli* SS142 and C1212 was assessed by hemagglutination and by adherence to human uroma cell line T24. Purified SS142 fimbriae and antifimbrial antiserum were used to quantitate fimbria production in an inhibition enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Strains, culture conditions, and viable counts. *E. coli* SS142 serotype O2:K− (from K. Vosbeck, CIBA Geigy, Basel, Switzerland) and *E. coli* C1212 serotype O6:K2:H1:F7 were used. Both strains were isolated from the urine of pyelonephritis patients and are well-characterized P-fimbriated strains (10, 13). Stock cultures of these bacteria were maintained on deep agar slants and subcultured every 2 weeks on blood agar plates. Mueller-Hinton broth (10 ml; Difco Laboratories, Detroit, Mich.) was inoculated with 5×10^5 bacterial cells per ml, and the cells were grown statically for 18 h at 37°C. They were subsequently centrifuged and suspended in phosphate-buffered saline to the desired optical density spectrophotometrically. Viability was determined from these solutions by preparing 10-fold serial dilutions in physiologic saline. Aliquots were plated on blood agar plates and incubated overnight at 37°C.

Antibiotics. The following antibiotics were used: trimethoprim, ciprofloxacin (Bayer AG, Leverkusen, Federal Republic of Germany), fleroxacin (Hoffmann-La Roche, Basel, Switzerland), norfloxacin, and nalidixic acid.

MIC determination. The MIC of each antibiotic for the strains tested was determined under conditions identical to those used for bacterial culture with serial twofold dilutions of antibiotic.

Hemagglutination and hemagglutination inhibition assays. Hemagglutination was performed by the method of Mett et al. (10), with freshly obtained human group A erythrocytes and guinea pig erythrocytes. Hemagglutination was read after incubation at 4°C for 2 h. Units of hemagglutination were expressed as the reciprocal titer 2^{n-1}, where n is the number of wells exhibiting visible agglutination. For hemagglutination inhibition tests of strain SS142, twofold serial dilutions of antifimbrial antiserum (50 μl) were prepared in microdilution wells to which were added in sequence a constant number of bacteria and erythrocytes sufficient to give a hemagglutination reaction. The lowest titer of antiserum needed to inhibit hemagglutination was recorded for each antibiotic treatment group.

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Bacterial adherence assay. The method of Svanborg Eden et al. (20) was performed, with slight modifications. The human uroma T24 cell line (kindly provided by R. Bolhuis, Organization for Applied Scientific Research, Leiden, The Netherlands) was used as the cellular support. Cells were dissociated with 3 mM EDTA (E. Merck AG, Darmstadt, Federal Republic of Germany) in phosphate-buffered saline. Adhesion was expressed as the mean number of bacteria attached to 40 cells.

Purification of fimbriae from *E. coli* SS142 and production of antifimbrial antiserum. Fimbriae of strain SS142 were isolated and purified by the method of Mett et al. (10). Rabbits were immunized with an aqueous solution of purified fimbriae (1 mg of protein per ml) in Freund complete adjuvant (Difco) (1:1, vol/vol). The first dose (of 0.2 ml) was injected subcutaneously at multiple sites. The second and third doses (of 0.5 ml) were injected intraperitoneally on days 14 and 41. Blood was collected 7 days after the final injection.

Enzyme-linked immunosorbant assay. An inhibition ELISA modified from that of Schifferli et al. (17) was performed with *E. coli* SS142.

RESULTS

MIC, morphology, and viability. MICs of each antibiotic against *E. coli* SS142 and C1212, respectively, were as follows (in micrograms per milliliter): nalidixic acid, 3.0 and 3.0; ciprofloxacin, 0.008 and 0.013; fleroxacin, 0.06 and 0.06; norfloxacin, 0.05 and 0.06; and trimethoprim, 0.12 and 0.19. After overnight exposure to sublethal concentrations of quinolones, bacteria exhibited only slight morphologic changes as assessed by light microscopy. Slight thickening was occasionally noted. Bacterial elongation (defined as a ≥threefold lengthening with respect to the control) generally occurred with a frequency of less than 5%. Following exposure to nalidixic acid at 1/8 and 1/4 MIC, however, 10 to 20% of *E. coli* cells showed elongation. Because morphological changes can interfere with concentration adjustments by spectrophotometry and because nonviable bacteria lose the ability to adhere to epithelial cells (20), viable counts were also performed for control and treated bacteria. Differences in viability of 0.5 log unit or less from control growth were considered acceptable. Because of a 1-log unit or greater decrease in the viability of bacteria exposed to 1/2 MIC of antibiotics, only concentrations of 1/4 MIC or less could be tested.

Hemagglutination. Both *E. coli* strains agglutinated human erythrocytes of all ABO blood groups, but not guinea pig erythrocytes. Agglutination patterns were not altered in the presence of mannose, demonstrating that these were P-fimbriated strains (10, 13). Hemagglutination by strain SS142 was inhibited by the addition of antifimbrial antiserum (5 μl per well), whereas purified fimbriae alone (6 μg per well or more) gave hemagglutination patterns similar to those of intact bacteria. Reciprocal titers for strain C1212 were 8, which is lower than for equal amounts of SS142, for which the reciprocal titer was 128. Following exposure to trimethoprim, a dose-dependent decrease of three to four hemagglutination titers occurred. This decrease was most evident at 1/16 MIC and above and confirms the observations of other investigators (22, 24). The quinolones and nalidixic acid produced, at most, a one- or two-hemagglutination-titer change, but in the majority of cases, no effect was seen relative to the control agglutination.

Hemagglutination inhibition. The lowest titer of antifimbrial antiserum necessary to inhibit hemagglutination by antibiotic-exposed SS142 exhibited a dose-dependent decrease following trimethoprim treatment: 1:16 (control), 1:32 (1/32 and 1/16 MIC), 1:64 (1/8 MIC), and 1:128 (1/4 MIC). Antiserum titers necessary to inhibit the hemagglutination of quinolone- or nalidixic acid-exposed SS142 were identical to the control titer over the entire range of sublethal concentrations (1/32 to 1/4 MIC) tested (data not shown).

TABLE 1. Adherence of antibiotic-exposed *E. coli* isolates to human uroepithelial cells in vitro

E. coli strain and drug concn	Adherence[a] after incubation with:				
	Trimethoprim	Ciprofloxacin	Fleroxacin	Norfloxacin	Nalidixic acid
SS142					
Control	130 ± 28	111 ± 15	102 ± 12	125 ± 6	130 ± 21
1/8 MIC	23 ± 8[b]	92 ± 21	120 ± 28	122 ± 13	90 ± 14
1/4 MIC	16 ± 3[b]	88 ± 4	80 ± 22	94 ± 28	–[c]
C1212					
Control	83 ± 19	71 ± 10	74 ± 32	72 ± 16	59 ± 15
1/8 MIC	61 ± 21	71 ± 3	68 ± 16	79 ± 14	72 ± 11
1/4 MIC	30 ± 11[b]	54 ± 7	56 ± 32	59 ± 18	44 ± 21

[a] Adherence is expressed as the mean number of bacteria attached to 40 uroepithelial cells ± standard error of the mean from three independent experiments.

[b] $P < 0.015$ by the Student t test for paired data. All other comparisons between treated bacteria and untreated controls were insignificant ($P > 0.05$).

[c] –, No data; greater than 1-log unit decrease in viability following an 18-h exposure of SS142 to 1/4 MIC of nalidixic acid.

Adherence assay. Both strains of *E. coli* adhered well to uroepithelial cells in suspension. At equal concentrations, strain C1212 adhered in smaller numbers than did strain SS142 (control values in Table 1). This is in agreement with the lower hemagglutination titers observed for C1212 than for SS142. Preincubation of SS142 with 10% antifimbrial antiserum prior to the adhesion assay blocked adhesion completely. Exposure to trimethoprim produced a significant decrease in adherence for both SS142 and C1212 (Table 1), but such an effect was not produced by the quinolones or nalidixic acid. Despite the elongation of SS142 after exposure to 1/8 MIC of nalidixic acid, no significant change in adherence was detected: of attached bacteria, 24% were elongated, which was comparable to the percentage of elongated bacteria (20%) seen in the preassay bacterial suspension.

Inhibition ELISA. The inhibition ELISA of Schifferli et al. (17) was applied to P-fimbriated strain SS142. In previous methods (3, 17), whole bacteria and antiserum were added directly to fimbria-coated wells. We found, however, that preincubating serial dilutions of whole *E. coli* cells with antiserum, centrifuging the mixture to remove bacteria, and transferring aliquots of the supernatant to the coated wells for the ELISA produced more consistent and reproducible results. Whole fimbriated *E. coli* SS142 cells and purified fimbriae alone inhibited the binding of antifimbrial antiserum to isolated fimbriae in a dose-dependent manner. Figure 1A shows the dose-dependent inhibition produced by trimethoprim-treated strain SS142, and Fig. 1B depicts a representative plot following quinolone exposure. The maximal inhibition for 2×10^8 CFU/ml in our assay was approximately 60%. For *E. coli* cells unexposed to antibiotics, 30% inhibition was produced by approximately 1×10^7 CFU/ml. Accordingly, Fig. 2 shows the percent inhibition produced by 10^7 CFU of *E. coli* per ml in each of the treatment groups. Fimbria production following quinolone and nalidixic acid treatment remained unchanged, despite exposure to antibi-

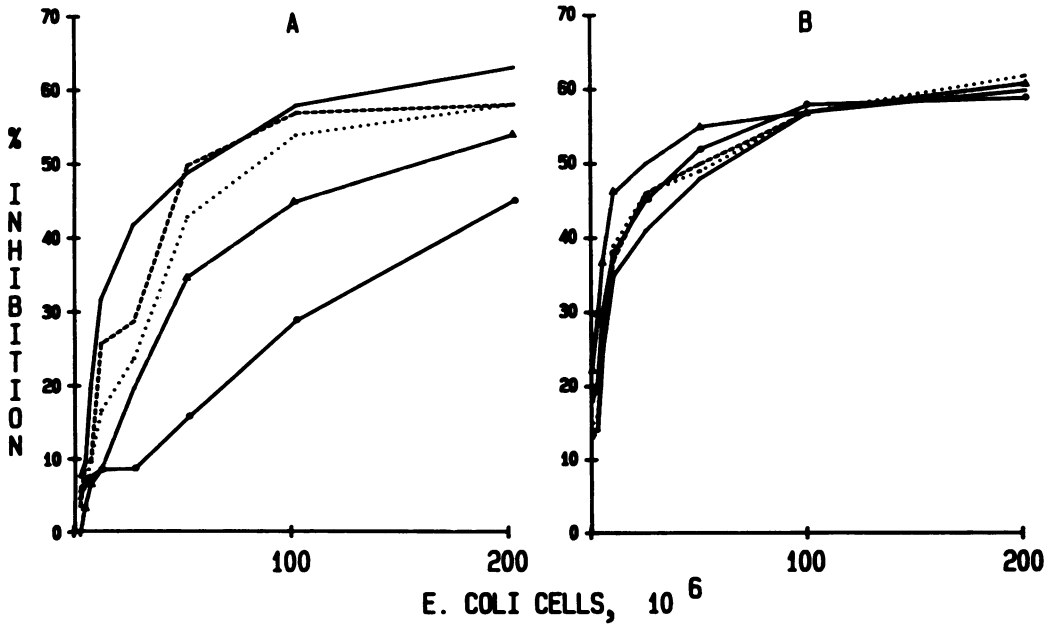

FIG. 1. Inhibition ELISA. The inhibition assay was performed by incubating serial twofold dilutions of whole, fimbriated *E. coli* SS142 cells with antifimbrial antiserum. After centrifugation of bacteria, aliquots of supernatant were transferred to microdilution wells coated with SS142 fimbriae. Bacteria were grown for 18 h in Mueller-Hinton broth without drugs (——) or with trimethoprim (A) or ciprofloxacin (B) at 1/32 MIC (– – –), 1/16 MIC (· · ·), 1/8 MIC (▲), or 1/4 MIC (●).

otic concentrations as high as 1/4 MIC. Trimethoprim, by contrast, caused a sharp decline (from 31 to 8%) in the ability of *E. coli* SS142 to inhibit the assay. By comparing the inhibition obtained by using intact bacteria with that obtained by using isolated fimbriae (3), it was estimated that the amount of fimbriae on *E. coli* SS142 decreased 80%, from 2.5 ng/10^7 untreated *E. coli* cells to 0.5 ng/10^7 *E. coli* cells treated with 1/4 MIC trimethoprim.

DISCUSSION

It is generally accepted that most bacterial infections are initiated by the adhesion of pathogenic microorganisms to host epithelial surfaces. The ability to adhere confers several advantages upon the bacterium, including resistance to the cleansing action of body fluids and provision of a niche from which colonization and tissue infection can proceed (5). Indeed, the capacity for in vitro adherence to epithelial cells has been directly related to the severity of urinary tract infections caused by *E. coli* strains isolated from the urine of patients (21). Adherence of pyelonephritogenic *E. coli* is mediated by P fimbriae, which recognize carbohydrate receptors on virtually all tissues of the urinary tract (23). Preventing ligand-receptor interactions can have obvious benefits in the prophylaxis and therapy of urinary tract infections; this has been the impetus behind investigations into the influence of antibiotics on bacterial adhesion and fimbria expression (1, 12, 16). The recently introduced fluoroquinolones have found a place in the armamentarium used in the prophylaxis and treatment of urinary tract infections (11, 25); however, their effect on adherence has not been adequately explored.

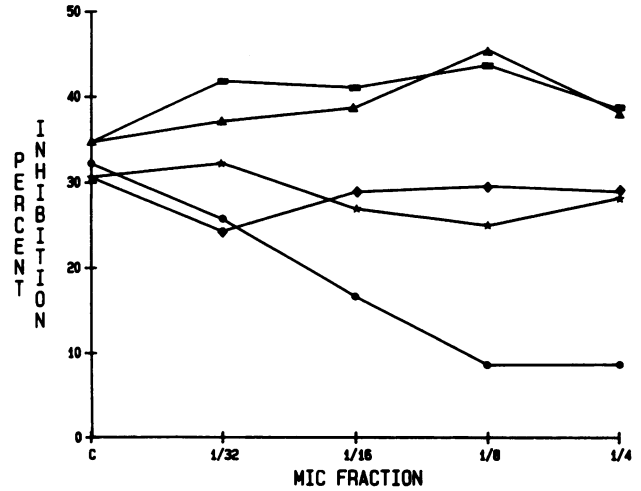

FIG. 2. Percent inhibition of an ELISA by 10^7 *E. coli* SS142 cells exposed to sublethal concentrations of antibiotics. Symbols: ●, trimethoprim; ▲, ciprofloxacin; ◆, fleroxacin; ■, norfloxacin; ★, nalidixic acid.

Hemagglutination was used as a simple in vitro adherence assay and as a way of characterizing the type of fimbriae present. For *E. coli* exposed to concentrations of ciprofloxacin, fleroxacin, norfloxacin, and nalidixic acid below the MIC, no change in hemagglutination titer was observed relative to that produced by untreated *E. coli*. Trimethoprim, which is known to decrease fimbriation, produced a dose-dependent decrease in titer. To eliminate interference from nonfimbrial binding factors, we performed a hemagglutination inhibition assay with antifimbrial antiserum to SS142. The fact that quinolone-treated SS142 caused no alteration in the antiserum titer necessary to inhibit hemagglutination but that a dose-dependent decrease in titer was clearly evident for trimethoprim-exposed SS142 confirmed the hemagglutination assay results.

In vitro adherence tests involving the use of uroepithelial cells provide a more physiologically relevant assay to assess

the effect of antibiotics on attachment. Quinolone and nalidixic acid treatment did not significantly affect the adherence of *E. coli* to uroepithelial cells at 1/8 or 1/4 MIC, whereas a significant decline in adherence occurred for trimethoprim-exposed *E. coli*.

Only a limited number of studies have assessed the influence of 4-quinolones on bacterial adherence. Vosbeck et al. (24) noted an increase in attachment of radiolabeled *E. coli* SS142 cells to an intestinal cell monolayer after treatment with 1/4 MIC of nalidixic acid. However, all bacteria were filamentous, and the increase in cell-associated radioactivity detected may reflect greater label uptake by elongated bacteria rather than increased attachment. Desnottes et al. (2) assessed the effect of sublethal concentrations of pefloxacin on *E. coli* adherence to uroepithelial cells. Of the 10 strains tested, 8 produced filaments in the presence of pefloxacin at 1/4 and 1/2 MIC and 9 showed reduced adherence. The viability of bacteria following pefloxacin exposure, however, was not assessed; this may obscure the interpretation of the results. It is imperative to assess the viability, because nonviable bacteria lose the ability to adhere to epithelial cells. For the quinolones we tested, significant decrements in viability were measured after exposure to 1/2 MIC and occasionally after exposure to other concentrations. Results of experiments were included only when equal numbers of viable *E. coli* cells were obtained from the adhesion assay suspensions containing antibiotic-treated bacteria and from those containing control bacteria. Finally, Karam et al. (8) observed that the urine of patients receiving nitroxoline inhibited the adhesion of *E. coli* to a HeLa 229 cell monolayer. Nitroxoline, however, was present only during the 1-h incubation of *E. coli* with the monolayer. Altered adhesion under these conditions may reflect a direct effect of quinolones on the physicochemical properties of bacterial or epithelial cell surfaces. Because of methodology differences, a direct comparison between these and the present experiments is difficult to make.

To investigate the effects of quinolones on fimbriae per se, we raised antiserum to isolated *E. coli* fimbriae. The antiserum was then used in an inhibition ELISA in which the ability of whole, fimbriated *E. coli* SS142 cells to inhibit the binding of antifimbrial antiserum to isolated SS142 fimbriae was tested. Following exposure to increasing concentrations of trimethoprim, *E. coli* SS142 exhibited a dose-dependent decrease in inhibition. This reflects a decreased production of fimbriae, which occurs after trimethoprim treatment. No change in the inhibition ability was observed for quinolone- or nalidixic acid-treated *E. coli* SS142 cells. These results complement and substantiate the findings from the hemagglutination and adherence assays. Hence, the results of the present study indicate that after exposure to 4-quinolones, *E. coli* cells produce morphologically and functionally intact P fimbriae.

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