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Effect of ciprofloxacin on adhesive properties of non-P mannose-resistant uropathogenic *Escherichia coli* isolates

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The influence of sub-MIC of ciprofloxacin on the surface properties of 25 non-P mannose-resistant uropathogenic *Escherichia coli* (UPEC) strains was studied. Thirteen isolates responded to antibiotic treatment with an increase in haemagglutination titre and/or surface hydrophobicity, which correlated with a higher expression of surface proteins. Only UPEC strains with ciprofloxacin-enhanced hydrophobicity increased their adhesiveness to urinary catheters that correlated, in one analysed case, with a dramatic increase in the number of fimbriae peripherally located. The overall results indicate that sub-MICs of ciprofloxacin could increase the adhesiveness, and hence the risk of colonization by UPEC strains expressing mannose-resistant adhesins different from type P.

Introduction

Adhesins of uropathogenic *Escherichia coli* (UPEC) are among the best-studied bacterial adhesins, and the importance of UPEC adherence in uncomplicated urinary tract infections is well-documented.[1] Type P fimbriae are the prototypal adhesins responsible for mannose-resistant haemagglutination, and their expression plus a high hydrophobicity are characteristics of *E. coli* pyelonephritogenic strains.[1] Furthermore, during the last few years, the number of well-characterized adhesins has increased and it is now known that they are also involved in the adhesiveness of pathogenic *E. coli* strains.[2] Thus, a decrease in bacterial adhesiveness could have obvious benefits in the prophylaxis of urinary tract infection bearing in mind the attractive concept of preventing infection with sub-MICs of antibiotics.[3] However, most of these studies are restricted to *E. coli* strains expressing type P fimbriae with a lack of information on how antibiotics might affect the adhesiveness of clinical isolates expressing mannose-resistant adhesins different from type P. We studied the effect of sub-MICs of the fluoroquinolone ciprofloxacin, commonly used to treat urinary infections, on the surface properties of UPEC strains expressing mannose-resistant adhesins different from type P.

Materials and methods

Strains

E. coli strains were isolated from hospitalized patients with urinary infection at the Hospital Provincial del Centenario (Rosario, Argentina). During this screening, only ciprofloxacin-susceptible (*cip*^{s}) strains were considered to avoid the possibility that ciprofloxacin resistance mechanisms interfered with the interpretation of the results. A total of 100 *cip*^{s} *E. coli* strains were recovered, and 25 of them were able to haemagglutinate human group A erythrocytes in the presence of mannose but failed to agglutinate guinea pig erythrocytes coated with the specific P fimbriae receptor. These 25 isolates constituted the pool of strains selected for the present study.

Determination of MICs

Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) was used to determine MICs of ciprofloxacin by the tube dilution method.[4] MICs of ciprofloxacin were in the range 0.03–0.06 mg/L, and for the following experiments we describe essentially the effects of a dose of one quarter the MIC because this concentration was the highest tested that did

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not produce measurable alterations of the cultures (data not shown).

Haemagglutination (HA)

Cultures were grown in colonization factor antigen (CFA) broth containing casein hydrolysate (Merck, Darmstadt, Germany) at 37°C for 18 h. Mannose-sensitive and -resistant haemagglutination (MSH and MRH, respectively) were characterized using fresh guinea pig and human type A erythrocytes in the absence or presence of 1% mannose.[5] Characterization of MRH was made with guinea pig erythrocytes coated with a specific globoside harbouring the receptor for P fimbriae.[5]

Hydrophobicity (PP), surface proteins and adherence assay

Cellular PP was measured with the solvent/water partition test of Chapman & Georgeopapadakou.[6] Extraction and quantification of surface proteins were carried out following an adaptation of the method of Hoschutzky *et al.*[7] For the bacterial adherence assay, cultures were grown on CFA agar plates with and without ciprofloxacin at 37°C for 18 h. Each culture was resuspended in HEPES-buffered Hanks solution to an optical density of 0.8 at 540 nm. Each bacterial suspension was fractionated into tubes and contacted with a segment of latex-silicone urinary catheter (1 mm thick) and incubated at 37°C for 2 h. The catheters were stained with Methylene Blue, washed, and the bacteria adhering to the internal surface of the catheter were counted by light microscopy.

Electron microscopy

Bacterial suspensions were prepared after overnight growth on CFA agar plates with and without ciprofloxacin. Electron microscopy was carried out on copper grids coated with collodion-carbon membrane. Preparations were negatively stained with phosphotungstic acid (PTA) for 1 min.

Results and discussion

Effect of ciprofloxacin on HA and PP

The effect of one quarter the MIC of ciprofloxacin on HA and PP was variable from strain to strain. Whereas 12 isolates did not show any statistically significant modification of the analysed properties (data not shown), 13 strains showed a noticeable enhancement of HA and/or PP after antibiotic treatment (Figure 1a). We interpreted that this increase in adhesive properties could be due to a higher expression of surface proteins, which include appendages such as fimbriae or fibrils. To examine this idea, we selected seven isolates with increased HA and/or PP, and in agreement with our hypothesis all of them showed a significant elevation in the levels of surface proteins when grown in the presence of antibiotic (Figure 1b).

Electron microscopy of ciprofloxacin-treated cells

The ciprofloxacin-induced modifications of surface properties must correlate with changes at a structural level. This presumption prompted us to analyse by electron microscopy individual bacterial cells of two representative strains (RM9015 and RM10387) after overnight exposure to sub-MIC ciprofloxacin (Figure 1c). Cultures of RM9015 grown in the absence of ciprofloxacin showed essentially cells with no fimbriae. In contrast, when the culture was exposed to the antibiotic, it was possible to observe numerous peripherally located fimbriae for most of the cells. These fimbriae were thin and irregularly shaped, with the majority being <1 μm in length but some >2 μm. On the other hand, after several attempts for strain RM10387, it was not possible to observe the presence of defined fimbriae under any condition. This result could indicate the presence of other types of adhesin without a regular shape and morphology (afimbrial adhesins), which could impair their visualization by electron microscopy.[1,2]

Effect of ciprofloxacin on the adherence to urinary catheters and the importance of PP

In order to shed some light on the clinical significance of the positive variation of the studied surface-related properties that should render microbial cells more adhesive, we evaluated whether sub-MIC of ciprofloxacin might affect the ability of these strains to adhere to urinary catheters. We chose this medical device because of its common use in hospitalized patients and the reported risk of urinary tract infections initiated by catheter infiltration.[8] The study with ciprofloxacin-treated strains was carried out in parallel with non-treated and trimethroprim-treated cultures as controls. The inclusion of trimethroprim as a control is based on a previous report indicating that this antibiotic does not increase bacterial adherence.[9] In Table 1, we summarize the values of UPEC cells adhering to the inner surface of urinary catheters of the same seven strains shown in Figure 1b after 18 h of exposure to ciprofloxacin. There was a significant stimulation by ciprofloxacin of the number of cells adhering to the urinary catheter for strains of groups I and II. These strains share in common an increase in average hydrophobicity after antibiotic treatment (Figure 1a), which suggested the importance of this property for the attachment under the experimental conditions. This conclusion was reinforced with the result obtained with strains of group III (increased HA, but unaffected PP after ciprofloxacin treatment). Strains of this group did not show any significant variation of their adhesiveness to the catheter, with or without ciprofloxacin treatment

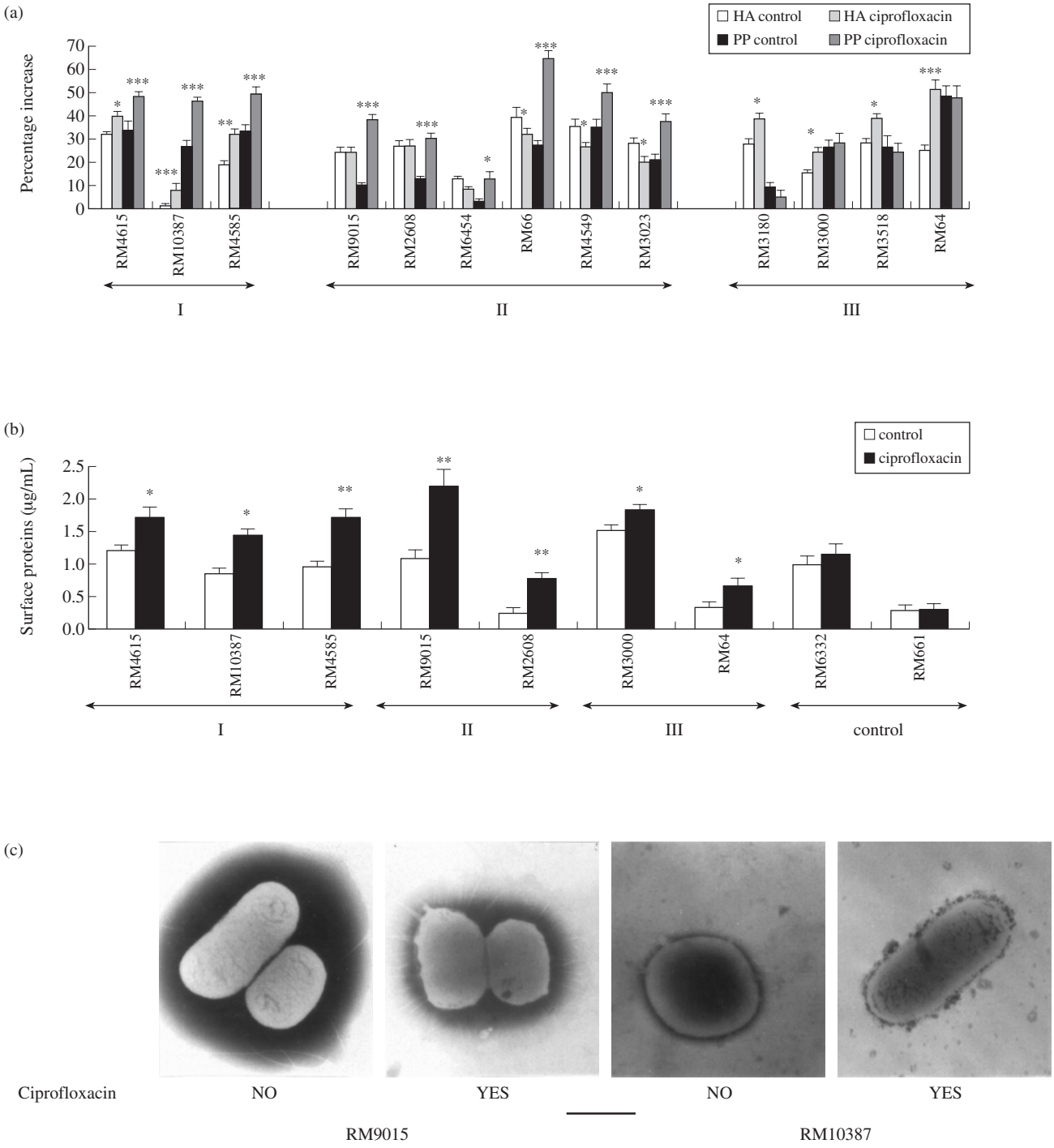

Figure 1. Effect of one quarter the MIC of ciprofloxacin on surface properties of non-P UPEC isolates. (a) Effect on haemagglutination (HA) and hydrophobicity (PP). Strains were divided into three different groups as a function of the response of HA and PP to ciprofloxacin treatment; I, both properties increased; II, only PP increased; III, only HA increased. The data and those shown in (b) and Table 1 are presented as the mean ± S.D. of five independent experiments. Statistical differences were assessed by one-way analysis of variance (ANOVA) followed by Tukey–Kramermultiple comparisons test. Levels of significance were considered statistically significant when $P \leq 0.05$; $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$. (b) Effect on protein surface expression. Representative UPEC strains from groups I, II and III were grown overnight in the presence or abs ence of ciprofloxacin. Strains RM6332 and RM661 (HA and PP not affected by ciprofloxacin treatment) were used as control strains. The graph indicates the average surface protein concentration (expressed in μg/mL) for untreated and ciprofloxacin-treated cultures; $^{**}P < 0.01$ and $^{*}P < 0.05$. (c) Electron micrographs of individual cells from cultures of RM9015 and RM10387 after overnight growth with or without ciprofloxacin. Note for the ciprofloxacin-treated culture of RM9015 that the whole cells are surrounded by fimbriae. Also note that in the case of the ciprofloxacin-treated culture of RM10387 there is a tendency for the phosphotungstic acid stain (PTA) to form precipitates along the periphery of the bacterial cell. Bar, 1 μm.

Table 1. Effect of ciprofloxacin on the adherence of UPEC strains to urinary catheters

Group[b]	Selected strain	No. of cells adhering[a] to internal surface in non-treated culture	ciprofloxacin-treated culture	trimethroprim-treated culture
I	RM10387	160 ± 51	294 ± 56***	126 ± 58
I	RM4615	266 ± 41	385 ± 60*	230 ± 39
I	RM4585	62 ± 8	87 ± 8**	45 ± 9*
II	RM2608	130 ± 13	161 ± 15*	74 ± 17*
II	RM9015	389 ± 36	476 ± 41*	252 ± 37*
III	RM64	159 ± 43	160 ± 51	111 ± 14
III	RM3000	24 ± 9	26 ± 4	25 ± 8

[a]Adherence is expressed as the mean number of bacteria attached ± S.D. from five independent experiments. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared with controls (ANOVA, $n = 5$).
[b]Group number defined as in Figure 1.

(Table 1). These results indicate that the increase in PP, due to the antibiotic, was the cause for the enhanced binding ability of the bacteria to the catheter. Moreover, it was previously reported that urinary catheters are susceptible to colonization by UPEC strains without using fimbriae but exhibiting high PP.[10] Accordingly, strain RM10387, which has a significant increase in its binding to the catheter (Table 1) without using fimbriae (Figure 1c), also showed a tendency of the PTA stain to precipitate along the periphery of the cell during the electron microscopy preparation, something previously observed for cells with elevated PP.[11]

In toto, this work shows that some non-P mannose-resistant UPEC isolates are able to respond to sub-MIC ciprofloxacin treatment with an enhancement of their adhesive properties; this indicates the risk of colonization by UPEC when this fluoroquinolone is used or decays to subinhibitory levels in hospitalized patients.

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