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Effects of Subinhibitory Concentrations of Nitroxoline on the Surface Properties of *Escherichia coli*

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ABSTRACT. Nitroxoline (5-nitro-8-quinolinol; NIQ) at subinhibitory concentrations (sub-MIC) decreased the adherence of uropathogenic *Escherichia coli* to catheter surface and significantly enhanced cell surface hydrophobicity. The surface hydrophobicity increased in the presence of sub-MIC of NIQ and also in an excess of Mg^{2+}. The effect of NIQ on the cell surface was not related to the bacteriostatic effect of this agent. The increase in nitrogen and decrease in phosphate content in the cell surface was found in the presence of NIQ. NIQ did not inhibit the expression of fimbriae.

The urinary tract is a common site of nosocomial infections (de Jong *et al.* 1991; Buisson 1993). Urinary tract infections (UTI) are very often related to the presence of catheters (Schaffer 1982; Daifuku and Stamm 1986). Bacteriuria was shown to be the result of an ascending bacterial colonization of catheter surfaces (Nickel *et al.* 1985).

Escherichia coli is the most frequent nosocomial pathogen and a major contaminant in catheter-associated urinary tract infections (UTI) (Platt *et al.* 1982). Type 1 fimbriae were found in cells of *E. coli* isolated from catheter-associated UTI (Amundsen *et al.* 1988); they might affect the persistence of this species in the catheterized urinary tract (Mobley and Chippendale 1987).

The adherence of bacteria to uroepithelial cells is mediated by specific adhesins (Gregor and Sobet 1987; Archambaud and Labigne 1989). The adhesion of microorganisms to solid substrates is generally thought to be mediated by a complicated interplay of hydrophobic and charge properties of the interacting surface (James 1991; Van der Mei *et al.* 1991), possibly complemented by a distinct influence of the absence or presence of specific receptor sites and appendages on the microbial cell surface (Busscher and Weerkamp 1987). The hydrophobicity of the microbial cell surface is generally accepted as a major factor in adhesion (Rosenberg and Doyle 1990; Hoštacká 1999; Majtánová and Majtán 1998, 2000).

The ability of uropathogenic *E. coli* and other bacteria to adhere to uroepithelial cells was shown to decrease after an exposure to subinhibitory concentrations (sub-MIC) of a variety of antimicrobial agents (Sandberg *et al.* 1979; Väisänen-Rhen *et al.* 1988; Tawfik *et al.* 1997); this decreased adherence is often related to the inhibition of adhesin synthesis (Väisänen-Rhen *et al.* 1988).

Literature data on the inhibitory activity of sub-MIC of antimicrobial agents on the adherence of microorganisms to catheter surfaces are scarce (Carsenti-Etesse *et al.* 1992); in addition, also the mechanisms of this inhibition have rarely been studied.

The purpose of the present study was to evaluate the inhibitory effect of sub-MIC of NIQ, an agent used in the treatment of acute or recurrent uncomplicated UTI, on the adherence of uropathogenic *E. coli* to siliconized latex catheter and its influence on the surface hydrophobicity of bacterial cells. We also analyzed the surface chemical composition and the expression of fimbriae (filaments) to evaluate the impact of this agent on surface layers.

MATERIALS AND METHODS

Bacteria. Uropathogenic *E. coli* strain 382 was isolated from lower urinary tract infection (C.H.S. Perray-Vaucluse, France). Phenotypic detection and identification of adhesins was performed by hæmagglutination of guinea pig and human erythrocytes in the presence and absence of α-D-mannose. Strain 382 expresses only mannose-sensitive adhesin (type 1). This strain was genotypically negative for *pap* and *sfa* homologous DNA. Polysaccharidic O-antigens, capsular K-antigens and flagellar H-antigens were examined by the *Statens Seruminstitut* (Denmark). The strain synthetizes flagella and it is rough (R-antigen).

Media. For testing the effects of sub-MIC of NIQ, the bacteria were subcultured and grown at 37 °C in minimal medium 1 (MM1) containing (in mmol/L): $Na_2HPO_4 \cdot 2H_2O$ 48, KH_2PO_4 22, NaCl 9, NH_4Cl 19,

$MgSO_4 \cdot 7H_2O$ 0.5, $CaCl_2 \cdot 2H_2O$ 0.07, $FeSO_4 \cdot 7H_2O$ 0.02, D-glucose 28 (pH 7.2); NIQ was added before inoculation. Minimal medium 2 (MM2) was used for testing the influence of Mg^{2+}. It contained (in mmol/L): NH_4Cl 19, NaCl 9, K_2HPO_4 3.7, sodium citrate 2, $FeSO_4 \cdot 7H_2O$ 0.02, D-glucose 28 (pH 7.0) and was supplemented with 50 mmol/L Mg^{2+}. The lowest concentration that completely inhibited growth was considered to be the MIC; it was found to be 8 mg/L in MM1 and in nonsupplemented MM2.

Bacterial adhesion to a siliconized latex urinary catheter was measured according to the modified method of Sugarman *et al.* (1982). The bacteria were radiolabeled by incubation overnight at 37 °C in MM1 into which 8-^{3}H-adenine (185 TBq/L; *Amersham*) was added. After growth with NIQ (1 or 2 mg/L), the cells were collected, washed thrice in phosphate buffer saline (PBS) and suspended in PBS; their concentration was adjusted (checked spectrophotometrically) to 100/nL (*i.e.* 10^8 cells per mL). Strips of catheter material (siliconized latex Foley catheter provided by *Progès*, France) were incubated for 1.5 h in 2 mL of a bacterial suspension. After incubation, the catheters were washed five times in PBS (pH 7.2) and then placed in 2 mL of 0.1 mol/L NaOH for 1 h. The pH of the solution was adjusted to 7 (by 1 mol/L HCl; 0.2 mL); then 10 mL scintillation liquid was added and radioactivity measured in a liquid scintillation counter. The results were expressed as number of cells adhering per mm^2.

Bacterial surface hydrophobicity by partition between two aqueous phases. The effect of sub-MIC of NIQ on the culture was examined by inoculation of cells in MM1 with 1 or 2 mg/L NIQ. After 22 h of incubation, the bacteria were washed twice and suspended in PBS. To test the growth independent effect of NIQ on the surface, cells were grown in MM1 without NIQ, collected at 4, 6, 8 or 22 h after inoculation, centrifuged, washed and suspended in PBS. NIQ (80 mg/L) was added to each suspension and the mixture was incubated for 1 h at room temperature. The bacteria were then washed 4 times and suspended in PBS pH 7.2. The influence of Mg^{2+} ions on the NIQ effect was examined by inoculation of the cells into MM2 containing 50 mmol/L $MgCl_2$. Two hours after inoculation 0.5 mg/L NIQ was added to the culture and the incubation continued for 22 h. The bacteria were washed twice and suspended in PBS (pH 7.2).The surface hydrophobicity of the bacteria was evaluated by measuring their partition between two aqueous phases of different surface tension. The system (Albertsson 1978) consisted of a mixture of 4 % (*W/W*) polyethylene glycol 6000 (PEG), and 5 % (*W/W*) dextran in 23 mmol/L phosphate buffer (pH 6.8) and 123 mmol/L NaCl (Fischer 1981), equilibrated overnight in a separation funnel at 4 °C. The bottom phase (rich in dextran) and the top phase (rich in PEG) were then collected and stored separately.

A portion of 0.5 mL of the bacterial suspension was added to a mixture of 2 mL of the PEG-rich phase and 2 mL of the dextran-rich phase in a test tube; the material was mixed and the phases were allowed to separate for 30 min at 4 °C followed by 30 min at room temperature. After separation, the concentration of bacteria in the top phase (lower surface tension) was estimated turbidimetrically. The hydrophobicity was expressed (in %) as the ratio between the concentration of cells in the top phase (PEG-rich) and the total concentration of cells in the test (Walter 1977).

Assay of elemental surface composition by X-ray photoelectron spectroscopy (XPS). The XPS analysis provided information on the concentration of different elements in the cell surface level; the results were expressed as atomic fraction. The bacteria were cultured with 1 mg/L NIQ, collected by centrifugation, suspended in distilled water and washed twice. The pellet was kept frozen at –80 °C. Samples were lyophilized in a Lyovac GT4 (*Leyobold Heraeus*). The elemental composition of the cell surface was determined by XPS using a SSX 100 Spectrometer (model 206, *Surface Science Instruments*), with Al-anode for X-ray production; experimental details were described previously (Latrache *et al.* 1994).

Detection of fimbriae by negative staining. After growth in the absence or presence of sub-MIC NIQ (1 mg/L), the bacteria were washed with PBS and stained by floating the grids (*Fullam*, France) in 1 % phosphotungstate (pH 7.0). Observations were made under a transmission electron microscope (EM 301 *Philips*) operating at 80 kV.

RESULTS AND DISCUSSION

Physico-chemical properties of the cell surface (Marshall *et al.* 1971) and consequently its overall chemical composition (Rouxhet and Genet 1991) play an important role in adhesion to solid surfaces.

According to Reitherman *et al.* (1973) the interfacial electric potential is very low and the top phase in the surface hydrofobicity assay has lower surface energy (Albertsson 1971), the partition of cells between the two phases probably depends upon surface characteristics other than charge, that might be referred to as cell surface hydrophobicity (Gerson and Akil 1980; Fischer 1981).

Adhesion to catheters and surface hydrophobicity. The adhesion of *E. coli* to the siliconized latex catheter decreased when the bacteria were grown in the presence of sub-MIC of NIQ. The partition coeffi-

cient (which is proportional to the cell concentration in the PEG rich-phase) was taken as a direct index of hydrophobicity. Cultivation of the bacteria in MM1 in the presence of sub-MIC NIQ led to remarkable increase in hydrophobicity (Table I). Medium MM2 supplemented with 50 mmol/L $MgCl_2$ provided more hydrophilic cells than medium MM1, and addition of sub-MIC of NIQ increased again the cell surface hydrophobicity.

Table II presents the growth-independent effect of NIQ on the cells. The partition coefficient changed during culture, but the contact of washed bacteria with NIQ for different periods of time did not influence the surface hydrophobicity.

Table I. Adhesion of cells to a catheter (number of bacteria adhering per mm^2) and partition coefficients between two aqueous phases[a] (% of cells in the PEG phase)

NIQ mg/L	Adhered bacteria	Partition coefficient[b]	
		MM1	MM2 + Mg^{2+}
0	8600 (700)	23 (3.5)	10 (3)
1	7000 (1300)*	54 (5.8)	19 (2)
2	4600 (900)**	63 (4.2)	25 (2)

[a]See *Material and Methods.*
[b]Mean of 3 measurements, standard deviation in parentheses; Student's *t*-test: $*p < 0.001$, $**p < 0.05$.

Table II. The influence of NIQ (mg/L) on cell surface hydrophobicity[a]

Growth time, h	NIQ	
	0	80
4	30 (1.5)	27 (4.5)
6	25 (2.1)	23 (5.0)
8	36 (3.0)	40 (5.0)
22	28 (1.8)	27 (2.2)

[a]After growth without NIQ (4–22 h) the cells were collected, treated by NIQ for 1 h in PBS and washed; mean of 3 measurements; standard deviation in parentheses.

Our data indicate an effect of sub-MIC of NIQ on the cell surface composition and hydrophobicity. The partitioning test showed that cells cultured in the presence of NIQ were more hydrophobic. The catheter used in the work has a hydrophobic surface (Oliviero 1993). The concomitant higher surface hydrophobicity of strain 382 with impaired adhesion is surprising since hydrophilic cells are in general expected to preferentially adhere to hydrophilic substrata, while hydrophobic cells adhere preferentially to hydrophobic ones. Thus, the inhibition of adhesion of *E. coli* to the catheter cannot be explained by a modification of cell surface hydrophobicity. However, the change in this property reflects a global change in cell surface since it is the combination of the heterogeneous molecules of the microbial surface that imparts the characteristic physico-chemical properties. This change was dependent on the presence of NIQ during the cultivation, suggesting that it affects the synthesis and incorporation of surface components, not adsorption of the reagent on cell surface. This was shown by the zero effect of NIQ when added to bacterial suspension under non-growing conditions. The effect of sub-MIC of NIQ does not seem to be related to its bacteriostatic activity since this activity is inhibited by excess Mg^{2+} (increased hydrophobicity is not inhibited; Bourlioux *et al.* 1989).

The difference in the growth medium composition did not influence the sub-MIC NIQ effects but it significantly influenced the cell surface hydrophobicity (Table I). Environmental changes are known to influence the cell surface composition and physico-chemical properties of pathogens (Brown and Williams 1985; Williams 1988). The influence of urine components on pathogen cell surface properties should therefore be tested *in vivo.*

Expression of fimbriae. The effect of sub-MIC NIQ on fimbriae production was assessed by electron microscopy (by negative staining; Fig. 1). After growth with NIQ, the cells produced fimbriae. It had been reported that prior exposure of uropathogenic *E. coli* to sub-MIC of a variety of antimicrobial agents induced the inhibition of fimbriae synthesis (Sandberg *et al.* 1979; Loubeyre *et al.* 1993). Our strain produced fimbriae that extended from the cell periphery, and these cells were not devoid of fimbriae when sub-MIC of NIQ was added to the growth medium. The inhibition of adhesion of *E. coli* 382 therefore could not be explained by inhibition of fimbriae expression.

Elemental composition of cell surface. The presence of sub-MIC of NIQ in cultures of strain 382 in MM1 caused an increase of the surface nitrogen concentration and a decrease of surface phosphorus concentration (Table III). This can be interpreted as an increase of surface proteins and a decrease of surface phosphate concentration (Mozes *et al.* 1988).

The observed decrease of surface phosphate concentration might be a cause for diminution of the cell surface charge. It was shown previously (Latrache *et al.* 1994) for three strains of *E. coli* cultured under various conditions that the electrophoretic mobility of the cells could be accounted for by a change in surface

phosphate concentration: as the phosphate concentration increased, the surface became more negative. Moreover, the phosphate concentration was directly related to the sum of cations (Na^+, K^+, NH_4^+). The predominant role of phosphate in determining the surface charge of microorganisms has been recognized (Amory *et al.* 1988; Mozes *et al.* 1988; Van der Mei *et al.*1989).

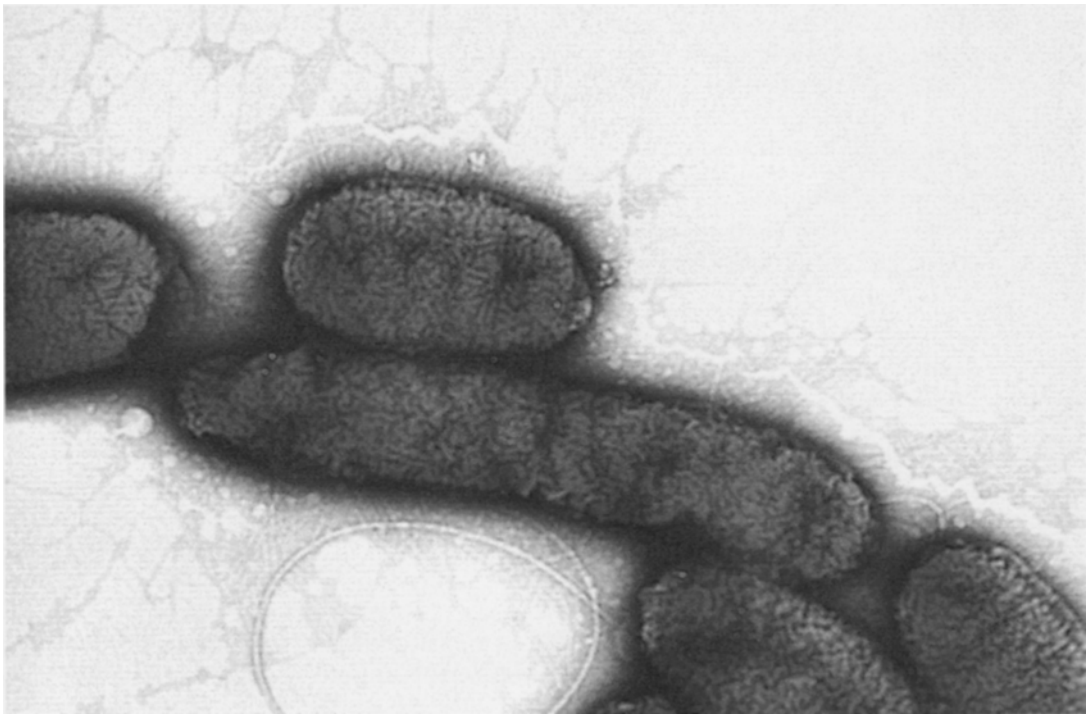

Fig. 1. *E. coli* strain 382 cultured in MM1 in the presence of sub-MIC of NIQ after 22 h; ×30 000.

NIQ decreased the cell surface phosphate concentration and hence its negative charge; such an effect diminished the electrostatic repulsion from the substrate and would therefore produce more adhesive cells. In contrast to this, we observed that NIQ inhibited the adhesion of the bacteria.

Our observations are at variance with expectations concerning the relation between the influence of sub-MIC of NIQ on cell surface physico-chemical properties and on adhesion. The increased hydrophobicity and/or the decreased negative charge of the cell surface was supposed to increase adhesion but in fact it was found to diminish it. Hence the inhibition of adhesion of these cells caused by sub-MIC of NIQ was very probably not due to an inhibition of fimbriae synthesis and did not depend on the modification of hydrophobicity or electrical properties.

Table III. Surface elemental composition[a] of *E. coli* strain 382 cultivated in the absence or presence of sub-MIC of NIQ (mg/L)

NIQ	C	O	N	P
0	63.3 (11.4)	27.7 (1.30)	6.7 (0.04)	1.48 (0.01)
1	62.1 (0.70)	26.9 (1.30)	9.2 (0.60)	0.95 (0.16)

[a]Atomic fractions excluding hydrogen; mean value of 2 independent analyses; standard deviation in parentheses.

It can be presumed that the influence of other surface parameters such as LPS and proteins or a morphological aspect cannot be excluded from the phenomenon of inhibition of cell adhesion. The altered adherence ability of *E. coli* to catheter surface caused by sub-MIC of NIQ may be important in improving antiadherence properties of biomaterial implants and in the prophylaxis of infections.

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