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Resistance of *Pseudomonas aeruginosa* to Imipenem Induced by Eluates from Siliconized Latex Urinary Catheters Is Related to Outer Membrane Protein Alterations

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The activity of imipenem against *Pseudomonas aeruginosa* HUS-3 decreased by 16 times in the presence of substances eluted from siliconized latex urinary catheters (SLUCs). SLUCs did not inactivate imipenem or increase β-lactamase activity. The outer membrane of *P. aeruginosa* HUS-3 grown in the presence of eluate lacked an OprD-like protein and expressed a new 50-kDa protein. The decreased activity of imipenem against *P. aeruginosa* in the presence of SLUCs is related to the loss of an OprD-like protein and the expression of a new outer membrane protein.

Pseudomonas aeruginosa is an important cause of urinary tract infection in patients with urinary catheters (17). The organism is able to colonize the surface of the catheter, forming biofilms that interfere with the activity of antimicrobial agents (14). It has been shown that *P. aeruginosa* adheres in vitro more efficaciously to siliconized latex urinary catheters (SLUCs) than to other plastic biomaterials (7–9). SLUCs elute substances that can be used by *P. aeruginosa* to grow (8) but are toxic for *Escherichia coli* or human polymorphonuclear leukocytes (7). It was reported previously that MICs of meropenem (MPM) against *P. aeruginosa* increased by 8 to 16 times in the presence of SLUC segments (15). It was postulated that the decreased activity of MPM could be related to the elution of substances from SLUCs but other mechanisms were also considered.

Resistance to imipenem (IMP) in *P. aeruginosa* has been shown to be related to the loss of the outer membrane protein OprD (23) coupled with the production of chromosomal β-lactamase (6, 22). MPM is more active than IMP against this microorganism, which could be related to its greater stability in the presence of the chromosomal β-lactamase (22). More recently, it has been reported that resistance of *P. aeruginosa* to these and other agents could be also related to the elimination of drugs by efflux systems (4, 12, 18–20).

This study was undertaken to evaluate the role of outer membrane protein alterations and/or production of β-lactamase in the decreased activity of IMP against *P. aeruginosa* in the presence of eluates from SLUCs.

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P. aeruginosa HUS-3 is a previously described clinical isolate (15). *P. aeruginosa* PAO1 and an OprD mutant were kindly provided by J. Trías (Microcide Pharmaceuticals Inc., Mountain View, Calif.). IMP-resistant mutants from *P. aeruginosa* HUS-3 were obtained on plates of Mueller-Hinton agar containing 8 μg of IMP/ml. One of these mutants was retained and was designated *P. aeruginosa* HUS-3/MUT2. Segments (eight segments 0.5 cm in length/ml of media) of SLUCs (pediatric siliconized latex Foley catheters; Euromedical, Kuala Lumpur, Malaysia) were incubated in sterile cation-adjusted Mueller-Hinton broth (MHB) at 37°C for 24 h. Catheter segments were removed, and the resulting broth containing the substances eluted from SLUCs (eluate) was used immediately. MICs of IMP, ceftazidime, cefepime, cefpirome, trimethoprim, tetracycline, and chloramphenicol were determined by a microdilution assay according to National Committee for Clinical Laboratory Standards guidelines (13) using both MHB and eluate as media. Eluate diluted in MHB in different proportions (1:2, 1:4, 1:8, and 1:16) was also used to determine the activities of IMP against *P. aeruginosa* HUS-3 and MUT2. To evaluate the possible inactivation of IMP by eluate, IMP at a concentration of 5,120 μg/ml was incubated at 37°C for 24 h in MHB and in eluate. MICs of IMP, preincubated in either MHB or eluate, for *P. aeruginosa* HUS-3 were determined in both MHB and eluate and compared with MICs of freshly prepared IMP determined in the same conditions. In another set of experiments, *P. aeruginosa* HUS-3 was grown in MHB and in eluate. The activity of IMP against bacteria grown in either medium was again determined in MHB and in eluate. Outer membrane proteins of *P. aeruginosa* HUS-3 grown in MHB or eluate were prepared as previously described (4), separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using Laemmli's buffers (5), and stained with Coomassie blue. β-Lactamase activity was determined spectrophotometrically using the crude supernatants obtained after sonication of *P. aeruginosa* grown either in HMB or in eluate and cephaloridine as substrate. One unit of activity was defined as the amount of enzyme that hydrolyzed 1 μmol of substrate per minute at 37°C at 295 nm.

In a previous report, we showed that SLUCs interfere in vitro with the inhibitory activity of MPM against *P. aeruginosa* (15). In the present report, it is shown that SLUCs also affect the activity of IMP. It was previously postulated that SLUCs are degraded when immersed in a liquid phase independently of the presence of microorganisms and that the silicone layer covering urinary catheters may dissolve in vivo, thus exposing the inner latex layer to the environment, the latex being responsible for tissue toxicity (21). The results of this study suggest that the eluate from SLUCs contains some substance(s) which inhibits IMP activity against *P. aeruginosa* HUS-3. The

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TABLE 1. Susceptibility of *P. aeruginosa* strains to antimicrobial agents in MHB or MHB plus eluate from SLUCs

Strain	MIC (μg/ml)													
	Imipenem		Ceftazidime		Cefepime		Cefpirome		Trimethoprim		Tetracycline		Chloramphenicol	
	MHB	E[a]	MHB	E	MHB	E	MHB	E	MHB	E	MHB	E	MHB	E
HUS-3	1	16	32	32–64	8	32	64	128	128	256	16	16	128	256
PAO1	1	8–16	1	2	1	2	2	4	128	128	16	16	32	64
PAO1 OprD(−)	16	16	1	4	1	2	2	4	128	128	16	16	32	64

[a] E, MHB plus eluate from SLUCs.

activity of IMP against *P. aeruginosa* HUS-3 decreased by 16 times when it was measured in eluate from SLUCs (Table 1). This effect progressively disappeared when the eluate was diluted in MHB. Eluate kept at 4°C for up to 4 weeks maintained its activity against IMP (data not shown). MICs of IMP against *P. aeruginosa* HUS-3 were 8, 4, 1, and 1 μg/ml when eluate was diluted in MHB 1:2, 1:4, 1:8, and 1:16, respectively. MICs of IMP against MUT2 were the same (32 μg/ml) in both MHB and eluate (either pure or diluted 1:2 to 1:16 in MHB). Similarly, the MIC of IMP against *P. aeruginosa* PAO1 was 8 to 16 times higher than the corresponding value against the organism grown in MHB, while against the OprD deficient mutant both MICs were the same (16 μg/ml). Preincubation of IMP in eluate did not result in inactivation of the drug as determined by the bioassay with *P. aeruginosa* HUS-3. MICs of eluate-preincubated IMP were 2 μg/ml in MHB and 32 μg/ml in eluate, exactly the same values as obtained with IMP preincubated in MHB, and only one dilution step higher than those obtained with freshly prepared IMP. The MIC of IMP against *P. aeruginosa* HUS-3 grown in eluate was 16 μg/ml when performed in eluate and 1 μg/ml when performed in MHB; the corresponding values, determined in a parallel experiment with the organism grown in MHB were the same, as previously observed.

P. aeruginosa HUS-3 grown in eluate lost an outer membrane protein comigrating with OprD of *P. aeruginosa* PAO1 and expressed a new outer membrane protein of about 50 kDa (Fig. 1). Mutant MUT2 did not express OprD in MHB and, like its parental strain, expressed a new protein of ca. 50 kDa when grown in eluate. The outer membrane protein profile of *P. aeruginosa* PAO1 was similar to that of HUS-3, while that of the PAO1 OprD-deficient mutant was similar to that of MUT2 (data not shown). The expression of new proteins of around 50 kDa in the outer membrane of *P. aeruginosa* has been recently related to the expression of efflux systems (11). The pattern of outer membrane proteins of *P. aeruginosa* HUS-3 grown in the presence of eluate is similar to that of *nfxC*-type mutants (12). Köhler et al. (4) have recently reported that *nfxC*-type mutants express the MexE-MexF-OprN efflux system along with decreased OprD expression. Unfortunately, the position of the 50-kDa band cannot be used to distinguish between the three outer membrane proteins (OprM, OprJ, or OprN) associated with efflux systems of *P. aeruginosa* because of their similar mobilities during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It has been reported that MexE-MexF-OprN increases resistance to chloramphenicol and trimethoprim but not to tetracycline or cephalosporins (4). In order to evaluate the possible activation of MexE-MexF-OprN by eluates, the MICs of six antimicrobial agents (Table 1) against strains HUS-3, PAO1, and OprD-deficient PAO1 were determined in both MHB and eluate. The results showed in the table, however, are not conclusive. At this moment, it is not possible to establish that the new 50-kDa protein is any of the known outer membrane proteins associated with efflux systems or even a totally different one. New experiments with *P. aeruginosa* strains carrying mutations in the MexA-MexB-OprM, MexC-MexD-OprJ, and MexE-MexF-OprN systems would contribute to evaluation of their role, if any, in the resistance of *P. aeruginosa* induced by SLUCs.

It is difficult to assess the relative importance of OprD deficiency and of the 50-kDa protein expression induced by eluate in the resistance of *P. aeruginosa* HUS-3. In the case of IMP, it may be hypothesized that the loss of OprD is more important than the expression of the 50-kDa protein, as MICs of IMP against mutants MUT2 and OprD-deficient PAO1 are the same in both MHB and eluate, although when both mutants grow in eluate they also express the 50-kDa protein. Outer membrane protein changes and resistance to IMP reverted when organisms grown in the presence of eluate were subsequently cultured in MHB, which indicates that eluate regulates, by an unknown mechanism, the physiology of *P. aeruginosa* rather than selects for rare mutants.

β-Lactamase activities in *P. aeruginosa* HUS-3 and MUT-2 grown in eluate (707 and 825 mU/mg of protein, respectively) were similar to those observed after growing the organism in MHB (809 and 832 mU/mg of protein, respectively). The high level of enzyme produced by *P. aeruginosa* HUS-3 may contribute to the observed resistance to IMP in the presence of eluate. It is well known that the expression of chromosomal β-lactamase in OprD-deficient strains determines resistance to IMP in *P. aeruginosa* (6, 22).

The exact chemical nature of the materials used for making SLUCs is not known. Preliminary chromatographic characterization of the eluate resulted in the identification of several major peaks, including *N*,*N*-dibutylformamide; 1,1,3-

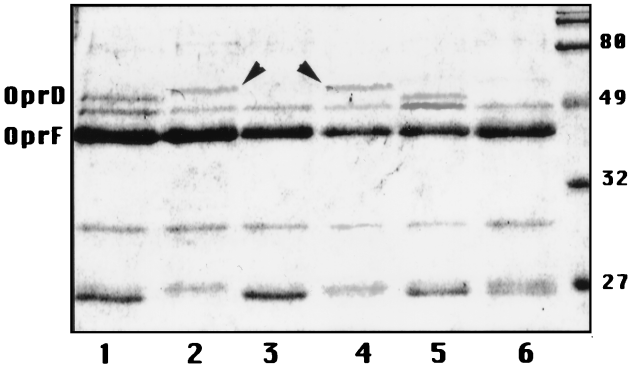

FIG. 1. Outer membrane proteins of *P. aeruginosa* grown in MHB (lanes 1, 3, 5, and 6) or in eluate (lanes 2 and 4). Lanes: 1 and 2, strain HUS-3; 3 and 4, mutant MUT2; 5, strain PAO1; 6, OprD-deficient mutant of PAO1. Molecular markers are shown at right. The ca. 50-kDa new protein expressed in eluate is marked with arrowheads.

trimethyl-3-phenylindane; ethane-1,1-2-di-3,4-xylil; and phthalate derivatives. The activity of IMP against *P. aeruginosa* HUS-3 remained unaltered in the presence of *N*,*N*-dimethylformamide; dimethyl-phthalate; or diethyl-phthalate in concentrations ranging between 0.5 and 40 μg/ml (data not shown). New experiments to evaluate the role of other SLUC components as a cause of resistance of *P. aeruginosa* HUS-3 to IMP are planned.

The clinical importance of these findings is unknown. Assuming that the concentrations of substances eluted from SLUCs could be high in the microenvironment of bacterial biofilm, we may speculate that this is an advantageous situation for *P. aeruginosa* attached to SLUCs because of its ability to grow using the eluate as a nutrient and to evade the activity of some antimicrobial agents.

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