Characterization of fluoroquinolone resistance in a clinical isolate of Pseudomonas stutzeri

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Sir,

Pseudomonas stutzeri is a non-fluorescent denitrifying bacterium widely distributed in the environment and rarely isolated as an opportunistic pathogen from humans.1–2 This species accounts for 1%–2% of hospital-acquired Pseudomonas spp. infections.3,4 Pseudomonas stutzeri is susceptible to many more antibiotics than the human pathogen Pseudomonas aeruginosa, its most closely related species.4,5 This higher susceptibility may be explained by its reduced occurrence in the clinical environment and, consequently, its lower exposure to antibiotics.6 However, drug-resistant P. stutzeri clinical isolates have been recovered for almost all antibiotic families, with the noticeable exception of fluoroquinolones.7

We report herein a multidrug-resistant P. stutzeri (strain 13) isolated in 2007 at the VU Medical Center, Amsterdam, The Netherlands. P. stutzeri strain 13 was identified with the API32GN system (bioMérieux, Marcy l’Étoile, France) and PCR sequencing of 16S rDNA.5 Disc diffusion and broth microdilution methods were used to determine its antibiotic susceptibility, and results were interpreted according to the CLSI guidelines.7 P. stutzeri ATCC 17588 was used as a reference strain. P. stutzeri 13 was resistant to ticarcillin, piperacillin, piperacillin/tazobactam and imipenem, had reduced susceptibility to ceftazidime, ceftipime and cefpirome, and was fully susceptible to aztreonam. A preliminary analysis showed that P. stutzeri isolate 13 expressed a metallo-β-lactamase named DIM-1 that was responsible for a high level of resistance to all β-lactams including carbapenems.8 In addition, P. stutzeri 13 was also resistant to gentamicin, tobramycin, rifampicin, chloramphenicol, tetracycline and, surprisingly, fluoroquinolones, remaining susceptible only to amikacin, netilmicin and colistin.

Since resistance to fluoroquinolones had never been described in P. stutzeri so far,4 it was investigated in isolate P. stutzeri 13 showing high-level fluoroquinolone resistance (MIC of ciprofloxacin > 32 mg/L) (Table 1). Since mutations in the DNA gyrase and topoisomerase IV genes are the most common mechanisms of fluoroquinolone resistance in Gram-negative organisms, whole-cell DNA of isolate 13 was extracted and used as a template for PCR amplification using primers able to amplify the corresponding quinolone resistance determining regions (‘QRDRs’) of the gyrA and parC genes of P. stutzeri ATCC 17588 (as control) and of P. stutzeri 13, and subsequently sequenced. The primers used were: GyrA-PstF (5’-CAT GGG CGA ACT GGC CAA AG-3’) and GyrA-PstR (5’-ATG AGC GAA TCC CTC GAC CTG-3’); ParC-PstF (5’-ATG AGC GAA TCC CTC GAC CTG-3’) and ParC-PstR (5’-ATG AGC GAA TCC CTC GAC CTG-3’); and ParC-PstF (5’-ATG AGC GAA TCC CTC GAC CTG-3’) and ParC-PstR (5’-ATG AGC GAA TCC CTC GAC CTG-3’). PCR products were sequenced. The primers used were: GyrA-PstF (5’-CAT GGG CGA ACT GGC CAA AG-3’) and GyrA-PstR (5’-ATG AGC GAA TCC CTC GAC CTG-3’); ParC-PstF (5’-ATG AGC GAA TCC CTC GAC CTG-3’) and ParC-PstR (5’-ATG AGC GAA TCC CTC GAC CTG-3’).

Table 1. MICs of quinolones/fluoroquinolones for P. stutzeri 13 and P. stutzeri ATCC 17588

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MICs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. stutzeri 13</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

aPAβN, phenylalanyl arginyl β-naphthylamide used at 20 mg/L.

In order to check for additional fluoroquinolone resistance mechanisms, plasmid-mediated quinolone resistance qnrA, qnrB, qnrS, qnrVC, qepA and aac(6’)-Ib-cr genes were searched for, but no positive result was obtained. Considering that the overexpression of multidrug efflux systems plays an important role in fluoroquinolone resistance in P. aeruginosa,10 antimicrobial susceptibility testing was performed on Mueller–Hinton agar with or without the antibiotic efflux pump inhibitor phenylalanyl arginyl β-naphthylamide (PAβN) at 20 mg/L.11 The addition of PAβN significantly reduced the MICs of nalidixic acid, ofloxacin, norfloxacin, ciprofloxacin, levofloxacin, moxifloxacin, enrofloxacin and pefloxacin (Table 1), but also those of tetracycline, rifampicin, trimethoprim, chloramphenicol and several β-lactams (including meropenem, data not shown), suggesting that overexpression of efflux pumps may also be involved. No effect was observed for the aminoglycosides tobramycin and gentamicin. This could be related to the P. stutzeri TbtABM efflux pump, which has been

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previously identified as contributing to resistance to n-hexane, nalidixic acid, chloramphenicol and sulfamethoxazole.\(^1\)\(^2\)

This study is the first report of a \(P.\) stutzeri isolate exhibiting fluoroquinolone resistance that was related at least to mutations in gyrA and parC topoisomerase genes.

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**References**


**Variations in colistin susceptibility among different species of the genus \(Acinetobacter\)**

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Sir,

Bacteria of the genus \(Acinetobacter\) with \(Acinetobacter\) \(baumannii\) in particular have emerged as important nosocomial pathogens, especially for patients in intensive care.\(^1\) \(A.\) \(baumannii\) has a remarkable capacity to develop resistance to all clinically relevant antimicrobial agents. Strains of this species resistant to most available agents are encountered in hospitals worldwide. The recent spread of carbapenem resistance has drastically narrowed options for treatment of \(A.\) \(baumannii\) infections and has led to the reintroduction of polymyxins with colistin in particular for the systemic treatment of infections caused by these bacteria.\(^1\)

Even though \(A.\) \(baumannii\) is clinically and epidemiologically the most important species of the genus, other \(Acinetobacter\) species have also been implicated in human infections and strains of some of these species may also be multidrug resistant combined with the ability to spread among hospitalized patients.\(^1\) Currently, 22 species with valid names and at least 11 additional putative species are recognized within the genus with as many as 25 of them having been found in human specimens.\(^2\)\(^3\) However, the identification of \(Acinetobacter\) species in diagnostic laboratories is cumbersome owing to the lack of practical diagnostic systems providing reliable identification of these microorganisms.\(^3\) Consequently, \(Acinetobacter\) identified by routine procedures as belonging to a particular species may actually represent another species, which can result in the incorrect association of an antimicrobial resistance phenotype or genotype with a given species.

As only limited information was available in the literature on the susceptibility of non-\(A.\) \(baumannii\) strains to polymyxins, we decided to determine the in vitro colistin susceptibility of

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