The Efflux Pump SmeDEF Contributes to Trimethoprim-Sulfamethoxazole Resistance in Stenotrophomonas maltophilia

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Trimethoprim-sulfamethoxazole (co-trimoxazole) is one of the antimicrobials of choice for the treatment of Stenotrophomonas maltophilia infections. The analysis of mutants either lacking or overexpressing the efflux pump SmeDEF shows that this efflux pump contributes to intrinsic and acquired co-trimoxazole resistance in S. maltophilia. Since SmeDEF can extrude a variety of antibiotics, selection with such antimicrobials, including quinolones, might also select for S. maltophilia co-trimoxazole resistance.

The treatment of Stenotrophomonas maltophilia, an opportunistic pathogen involved in different nosocomial infections, is difficult, owing to its low level of susceptibility to several antibiotics and its capability to acquire further resistance during clinical treatment. The intrinsic resistance of this pathogen is mainly due to the presence in its genome of genes encoding different resistance determinants, as several multidrug resistance (MDR) efflux pumps and antibiotic-modifying enzymes, and the quinolone resistance gene Smqpr (1, 2). Acquired resistance is mediated by the acquisition of mobile genetic elements containing antibiotic resistance genes and by the overexpression of chromosomal resistance genes encoding efflux pumps due to mutations in genes encoding the local regulators of these determinants (3–5).

The combination of antibiotics that inhibit enzymes of the folate biosynthesis pathway, trimethoprim and sulfamethoxazole (co-trimoxazole), is one of the choices for S. maltophilia treatment. In 2005, S. maltophilia represented only 4.7% of co-trimoxazole-resistant isolates (6), although this percentage varies geographically and has increased in the last few years. While from 1998 to 2008, only 14.6% of isolates in Taiwan were co-trimoxazole resistant, nowadays, this number has increased to 31.1% (7). The rationale behind the use of antimicrobial combinations is that the frequency of resistant mutants will be lower than that for single-target drugs, since mutations at the genes encoding both targets will be required in the case of combined drugs (8). We reasoned that bacteria could overcome this situation if one efflux pump is able to extrude both antimicrobials, since a single mutation will lead to the overexpression of the efflux pump and confer resistance. This possibility was suggested in Pseudomonas aeruginosa, because strains overexpressing oprM, the gene encoding the outer membrane protein of the mexAB efflux pump, were less susceptible to sulfamethoxazole and trimethoprim (9). In addition, the study of clinical S. maltophilia isolates has shown a weak correlation between co-trimoxazole resistance and overexpression of the MDR efflux pumps SmeDEF and SmeABC (10). The overexpression of the efflux pump SmeABC reduces susceptibility to aminoglycosides, β-lactams, and fluoroquinolones, but only the deletion of the smeC gene (outer membrane protein gene) has a direct effect on intrinsic resistance (11). The efflux pump SmeDEF is responsible for intrinsic and acquired resistance to tetracycline, chloramphenicol, macrolides, and fluoroquinolones. Its overexpression, usually due to mutations in the regulator SmeT, reduces its susceptibility to several antibiotics (12), whereas the deletion of smeE makes S. maltophilia more susceptible to such antibiotics (3). Some works have shown that SmeDEF is ubiquitously present in S. maltophilia strains from different origins (13, 14) and that despite being a relevant quinolone resistance determinant in this bacterial pathogen, it is involved in S. maltophilia colonization of the roots of plants (15).

To precisely define the role of the efflux pump SmeDEF in co-trimoxazole resistance, we used the clinical S. maltophilia strain D457 (16) and two isogenic mutants: D457R, overexpressing the efflux pump SmeDEF (12), and MBS411, in which the smeE gene has been deleted (3). The MICs of trimethoprim, sulfamethoxazole, and the combination of both, co-trimoxazole, were determined by Etest (bioMérieux, Marcy l’Étoile, France) (Table 1). As shown in Table 1, the strain overexpressing the efflux pump SmeDEF (D457R) was less susceptible to sulfamethoxazole and co-trimoxazole than the wild-type strain D457. In addition, the mutant without the efflux pump, MBS411 (ΔsmeE), was more susceptible to trimethoprim and co-trimoxazole than D457, the wild-type strain, although it displays the same susceptibility to sulfamethoxazole. We could not establish the trimethoprim MIC of the SmeDEF-overexpressing mutant using this technique, because the value was above the detection limits of the Etest strip. To confirm these data and further study the role of the efflux pump SmeDEF in acquired resistance to trimethoprim, we analyzed the antibiotic susceptibility of trimethoprim (SERVA Electrophoresis GmbH, Germany), sulfamethoxazole (Fluka, Sigma-Aldrich Co., USA), or co-trimoxazole (Solvit; Laboratorios Almofarma, S.L., Barcelona, Spain) in 96-well microtiter plates in Mueller-Hinton medium (Pronadisa) by 2-fold dilution. The data were recorded after 24 h of incubation at 37°C in at least three independent assays and are shown in Table 1. Is has been reported that...
TABLE 1 Effect of SmeDEF on susceptibility of S. maltophilia strains to antibiotics

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>MIC (μg/ml) of a:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Etest (2-fold dilution)</td>
<td>SMX</td>
</tr>
<tr>
<td>D457 Wild type</td>
<td>&gt;32 (128)</td>
<td>24 (16)</td>
</tr>
<tr>
<td>D457R</td>
<td>&gt;32 (512)</td>
<td>64 (32)</td>
</tr>
<tr>
<td>MBS411</td>
<td>6 (64)</td>
<td>24 (16)</td>
</tr>
</tbody>
</table>

a: TM, trimethoprim; SMX, sulfamethoxazole; SXT, trimethoprim-sulfamethoxazole.

Etest and double-dilution methods do not always present a good correlation in S. maltophilia for all analyzed strains (17). Because of this, we preferred to include information of the MICs obtained using both methodologies. As shown in Table 1, although the obtained values are different, the observed trends (the mutant overproducing smeDEF is more resistant, and the mutant lacking smeE is more susceptible than the wild-type strain) are the same and clarify previous data for trimethoprim susceptibility, which could not be fully deciphered by Etest. Altogether, our results show that overexpression of the efflux pump SmeDEF reduces the susceptibility of trimethoprim, sulfamethoxazole, and the combination of both, whereas its presence contributes to intrinsic resistance to trimethoprim and co-trimoxazole only but not sulfamethoxazole. A differential effect of deleting or overexpressing efflux pumps on the susceptibility to antimicrobials has been reported in other cases. For instance, overexpression of the efflux pump genes adeJK increases Acinetobacter baumannii MICs for ticarcillin, aztreonam, cephalothin, and ceftriaxone, among other antibiotics, whereas the deletion of adeJ decreases the MICs for ticarcillin and aztreonam only without changing the MICs for cephalothin and ceftriaxone (18). We believe this could be due to the different affinities of the efflux pumps for their substrates. In the case described in the current work, SmeDEF would present a lower affinity (and extrusion capability) for sulfamethoxazole than that for trimethoprim; a phenotype for sulfamethoxazole would be detected only when the expression of SmeDEF reaches a given threshold, in this case, in the D457R mutant.

Since SmeDEF is a major determinant of quinolone resistance in S. maltophilia (3,19), quinolone treatment, or any other treatment that selects mutants leading to SmeDEF overexpression, will also reduce S. maltophilia susceptibility to co-trimoxazole.

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