The efflux pump SmeDEF contributes to trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia*

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Abstract

Trimethoprim/sulfamethoxazole (cotrimoxazole) 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 cotrimoxazole resistance in *S. maltophilia*. Since SmeDEF can extrude a variety of antibiotics, selection with such antimicrobials, including quinolones, might also select for *S. maltophilia* cotrimoxazole 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. 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, antibiotic-modifying enzymes and the quinolone resistance gene Smqnr (1, 2). Acquired resistance is mediated by acquisition of mobile genetic elements containing antibiotic resistance genes, as well as to overexpression of chromosomally-encoded resistance genes, as 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 (cotrimoxazole), is one of the choices for S. maltophilia treatment. In 2005 S. maltophilia presented only a 4.7% of cotrimoxazole resistant isolates (6), although this percentage varies geographically and has increased in last years. While from 1998 to 2008, only 14.6% of isolates in Taiwan were cotrimoxazole resistant, nowadays this number has increased until 31.1% (7). The rationality behind the use of antimicrobial combinations is that the frequency of resistant mutants will be lower than 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 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 cotrimoxazole resistance and overexpression of the MDR efflux pumps SmeDEF and SmeABC (10). The overexpression of the efflux pump SmeABC reduces
the susceptibility to aminoglycosides, β-lactams, and fluoroquinolones, but only the deletion of \textit{smeC} gene (outer membrane protein) has a direct effect in 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 \textit{smeE} makes \textit{S. maltophilia} more susceptible to such antibiotics (13). Some works have shown that SmeDEF is ubiquitously present in \textit{S. maltophilia} strains from different origins (14, 15) and that, despite being a relevant quinolone resistance determinant in this bacterial pathogen, it is involved in \textit{S. maltophilia} colonization of the roots of the plants (16).

To precisely define the role of the efflux pump SmeDEF in cotrimoxazole resistance, we used the clinical \textit{S. maltophilia} strain D457 (17) and two isogenic mutants; D457R overexpressing the efflux pump SmeDEF (12), and MBS411 in which the \textit{smeE} gene has been deleted (13). The Minimal Inhibitory Concentrations (MICs) of trimethoprim, sulfamethoxazole and the combination of both, cotrimoxazole, was determined by E-test (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 cotrimoxazole than the wild type strain D457. In addition, the mutant without the efflux pump, MBS411 (Δ\textit{smeE}), was more susceptible to trimethoprim and cotrimoxazole than D457, the wild type strain, although it displays the same susceptibility to sulfamethoxazole. We could not establish the MIC trimethoprim of the SmeDEF overexpressing mutant using this technique because the value was above the detection limits of the E-test 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 for trimethoprim (SERVA Electrophoresis GmbH,
Germany), sulfamethoxazole (Fluka, Sigma-Aldrich Co, United States) or cotrimoxazole (Soltrim, laboratorios Almofarma, S.L., Barcelona, Spain) in 96-well microtiter plates in Mueller Hinton (Pronadisa) by twofold dilution. Data were recorded after 24 h of incubation at 37°C in at least three independent assays and are shown in Table 1. It has been reported that E-test and double dilution methods do not always present a good correlation in S. maltophilia for all analyzed strains (18). 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 E-test. 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 just contribute to intrinsic resistance to trimethoprim and cotrimoxazole, but not for 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 adeIJK increases Acinetobacter baumannii MICs for ticarcillin, aztreonam, cephalothin and ceftriaxone among other antibiotics, whereas deletion of adeJ just decreases the MICs for ticarcillin and aztreonam, without changing the values for cephalothin and ceftriaxone (19). 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 for trimethoprim; a phenotype for sulfamethoxazole would only be detected when 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, 20), quinolone treatment, or any other treatment that selects mutants leading to SmeDEF overexpression, will also reduce *S. maltophilia* to cotrimoxazole.

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References


Table 1. Effect of SmeDEF on the susceptibility to antibiotics of *S. maltophilia* strains.

| Strain   | Phenotype                  | MIC (µg/ml)
|----------|---------------------------|---------------------|
|          |                           | TM     | SMX    | SXT
| D457     | Wild type                 | >32(128) | 24(16) | 0.25(1)
| D457R    | D457 overexpressing SmeDEF| >32(512) | 64(32) | 2(8)
| MBS411   | D457 no SmeE expression   | 6(64)   | 24(16) | 0.190 (0.5) |
a TM, trimethoprim; SMX, sulfamethoxazole; SXT, trimethoprim/sulfamethoxazole. First data correspond to E-test assay. Data between brackets correspond to twofold dilution assay.