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

María Blanca Sánchez, José Luis Martínez

Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología, CSIC, Cantoblanco, Madrid, Spain

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 Sm*qnr* (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 (Δ*smeE*), 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 for trimethoprim (SERVA Electrophoresis GmbH, Germany), sulfamethoxazole (Fluka, Sigma-Aldrich Co., USA), or co-trimoxazole (Soltrim; 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

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Address correspondence to José Luis Martínez, jlmtnez@cnb.csic.es.

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

Strain	Phenotype	Etest (2-fold dilution) MIC (μg/ml) of[a]: 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 with no SmeE expression	6 (64)	24 (16)	0.190 (0.5)

[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 *adeIJK* 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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REFERENCES

1. **Crossman LC, Gould VC, Dow JM, Vernikos GS, Okazaki A, Sebaihia M, Saunders D, Arrowsmith C, Carver T, Peters N, Adlem E, Kerhornou A, Lord A, Murphy L, Seeger K, Squares R, Rutter S, Quail MA, Rajandream MA, Harris D, Churcher C, Bentley SD, Parkhill J, Thomson NR, Avison MB.** 2008. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. Genome Biol **9:**R74. http://dx.doi.org/10.1186/gb-2008-9-4-r74.
2. **Sanchez MB, Hernandez A, Martinez JL.** 2009. *Stenotrophomonas maltophilia* drug resistance. Future Microbiol **4:**655–660. http://dx.doi.org/10.2217/fmb.09.45.
3. **García-León G, Salgado F, Oliveros JC, Sanchez MB, Martinez JL.** 2014. Interplay between intrinsic and acquired resistance to quinolones in *Stenotrophomonas maltophilia*. Environ Microbiol **16:**1282–1296. http://dx.doi.org/10.1111/1462-2920.12408.
4. **Sánchez P, Alonso A, Martinez JL.** 2002. Cloning and characterization of SmeT, a repressor of the *Stenotrophomonas maltophilia* multidrug efflux pump SmeDEF. Antimicrob Agents Chemother **46:**3386–3393. http://dx.doi.org/10.1128/AAC.46.11.3386-3393.2002.
5. **Chang L-L, Chen H-F, Chang C-Y, Lee T-M, Wu W-J.** 2004. Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*. J Antimicrob Chemother **53:**518–521. http://dx.doi.org/10.1093/jac/dkh094.
6. **Sader HS, Jones RN, Dowzicky MJ, Fritsche TR.** 2005. Antimicrobial activity of tigecycline tested against nosocomial bacterial pathogens from patients hospitalized in the intensive care unit. Diagn Microbiol Infect Dis **52:**203–208. http://dx.doi.org/10.1016/j.diagmicrobio.2005.05.002.
7. **Wang CH, Lin JC, Lin HA, Chang FY, Wang NC, Chiu SK, Lin TY, Yang YS, Kan LP, Yang CH, Chan MC, Yeh KM.** 28 July 2014. Comparisons between patients with trimethoprim-sulfamethoxazole-susceptible and trimethoprim-sulfamethoxazole-resistant *Stenotrophomonas maltophilia* monomicrobial bacteremia: a 10-year retrospective study. J Microbiol Immunol Infect. http://dx.doi.org/10.1016/j.jmii.2014.06.005.
8. **Martinez JL, Baquero F.** 2000. Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother **44:**1771–1777. http://dx.doi.org/10.1128/AAC.44.7.1771-1777.2000.
9. **Köhler T, Kok M, Michea-Hamzehpour M, Plesiat P, Gotoh N, Nishino T, Curty LK, Pechere JC.** 1996. Multidrug efflux in intrinsic resistance to trimethoprim and sulfamethoxazole in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother **40:**2288–2290.
10. **Liaw SJ, Lee YL, Hsueh PR.** 2010. Multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*: roles of integrons, efflux pumps, phosphoglucomutase (SpgM), and melanin and biofilm formation. Int J Antimicrob Agents **35:**126–130. http://dx.doi.org/10.1016/j.ijantimicag.2009.09.015.
11. **Li XZ, Zhang L, Poole K.** 2002. SmeC, an outer membrane multidrug efflux protein of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother **46:**333–343. http://dx.doi.org/10.1128/AAC.46.2.333-343.2002.
12. **Alonso A, Martinez JL.** 2000. Cloning and characterization of SmeDEF, a novel multidrug efflux pump from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother **44:**3079–3086. http://dx.doi.org/10.1128/AAC.44.11.3079-3086.2000.
13. **Gould VC, Okazaki A, Howe RA, Avison MB.** 2004. Analysis of sequence variation among *smeDEF* multi drug efflux pump genes and flanking DNA from defined 16S rRNA subgroups of clinical *Stenotrophomonas maltophilia* isolates. J Antimicrob Chemother **54:**348–353. http://dx.doi.org/10.1093/jac/dkh367.
14. **Ribbeck-Busch K, Roder A, Hasse D, de Boer W, Martinez JL, Hagemann M, Berg G.** 2005. A molecular biological protocol to distinguish potentially human pathogenic *Stenotrophomonas maltophilia* from plant-associated *Stenotrophomonas rhizophila*. Environ Microbiol **7:**1853–1858. http://dx.doi.org/10.1111/j.1462-2920.2005.00928.x.
15. **García-León G, Hernandez A, Hernando-Amado S, Alavi P, Berg G, Martinez JL.** 2014. A function of SmeDEF, the major quinolone resistance determinant of *Stenotrophomonas maltophilia*, is the colonization of plant roots. Appl Environ Microbiol **80:**4559–4565. http://dx.doi.org/10.1128/AEM.01058-14.
16. **Alonso A, Martinez JL.** 1997. Multiple antibiotic resistance in *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother **41:**1140–1142.
17. **Nicodemo AC, Araujo MR, Ruiz AS, Gales AC.** 2004. *In vitro* susceptibility of *Stenotrophomonas maltophilia* isolates: comparison of disc diffusion, Etest and agar dilution methods. J Antimicrob Chemother **53:**604–608. http://dx.doi.org/10.1093/jac/dkh128.
18. **Yoon EJ, Nait Chabane Y, Goussard S, Snesrud E, Courvalin P, De E, Grillot-Courvalin C.** 2015. Contribution of resistance-nodulation-cell division efflux systems to antibiotic resistance and biofilm formation in *Acinetobacter baumannii*. mBio **6**(2):e00309-15. http://dx.doi.org/10.1128/mBio.00309-15.
19. **Alonso A, Martinez JL.** 2001. Expression of multidrug efflux pump SmeDEF by clinical isolates of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother **45:**1879–1881. http://dx.doi.org/10.1128/AAC.45.6.1879-1881.2001.