

Chapter 15

Antimicrobial Drug Efflux Pumps in *Stenotrophomonas maltophilia*

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Abstract *Stenotrophomonas maltophilia* is an emerging opportunistic pathogen with an environmental origin. One of the most cumbersome characteristics of *S. maltophilia* is its natural low susceptibility to different antimicrobial agents that are currently in use in the clinical practice. Because of that, this species is considered as a prototype of intrinsically resistant microorganism. Part of its capability to resist the action of antimicrobials resides in a number of chromosomally encoded efflux pumps. Notably, overexpression of some of these efflux pumps can confer clinically relevant resistance to quinolones. This is likely the reason why, in contrast to other pathogens, no *S. maltophilia*-resistant isolates have been found presenting mutations in the genes encoding bacterial topoisomerases. Along this chapter, we describe different efflux pumps that have been so far reported in *S. maltophilia* as well as the mechanisms that allow their regulation. The clinical relevance these efflux pumps may have for the success of *S. maltophilia* in producing infections in patients is also discussed. Finally, we focus on the function that efflux pumps may have in the adaptation of *S. maltophilia* to nonclinical ecosystems, such as rhizosphere, where antimicrobial selective pressure is likely low.

Keywords *Stenotrophomonas maltophilia* • Antimicrobial resistance • Drug efflux pumps • SmeABC • SmeDEF • SmeIJK • SmeYZ • SmeT

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15.1 Introduction

Stenotrophomonas maltophilia is an opportunistic pathogen with an environmental origin, which is considered to be a prototype of a bacterium intrinsically resistant to antimicrobial agents [1, 2]. Before the wide use of antimicrobial agents, most opportunistic pathogens were species belonging to the commensal microbiota capable of colonizing previously debilitated patients. Whereas commensals are still responsible for a large fraction of nosocomial infections, bacteria with an environmental origin, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, or *S. maltophilia*, also constitute a problem in habitats, such as hospitals hosting immunosuppressed patients [3], patients with a basal disease or needing care after a surgery, and generally speaking, debilitated patients. This situation, in which the host response to infection is too weak to avoid a disease, allows different organisms to colonize a human host that otherwise will not be infected. In the presence of immune system deficiency, the most important line of defense consists of the use of antimicrobials, and, in this context of high antimicrobial load, only antimicrobial-resistant organisms are able to produce an infection, despite their reduced virulence. One of these types of microorganisms is *S. maltophilia*, which is not highly virulent but is an increased cause of infections in hospitals and in people suffering from cystic fibrosis [4]. The reason behind is likely its low susceptibility to antimicrobial agents currently in clinical use. Indeed, one of the risk factors for being infected by *S. maltophilia* is a previous treatment of a patient with antimicrobial drugs.

Reasons for the reduced susceptibility to different antimicrobials are multifaceted. *S. maltophilia*, as other Gram-negative non-fermenting bacteria, presents a reduced membrane permeability, which precludes the fast and easy entrance of antimicrobial agents. In addition, the chromosome of *S. maltophilia* contains several genes coding for proteins that contribute to its resistance to several drugs. Among them, it is important to highlight two β -lactamases (including a metalloenzyme) that are capable of inactivating penicillins, cephalosporins, and/or carbapenems [5, 6]; two aminoglycoside-modifying enzyme genes, *aph(3')-IIc* encoding an aminoglycoside phosphotransferase [7] and *aac(6')-Iz* coding for an *N*-aminoglycoside acetyltransferase [8, 9], which can inactivate most aminoglycosides; one quinolone-resistance protein (SmQnr) [10–12]; and several efflux pumps, belonging to different families of transporters [13], which altogether constitute one of the most important mechanisms of antimicrobial resistance in *S. maltophilia* [1].

Since *S. maltophilia* is not a producer of antibiotics used in clinics for treating human infections, such as β -lactams or aminoglycosides, it is intriguing to know the reasons why this bacterial species presents such a wide range of antimicrobial resistance determinants. Two possibilities (not necessarily mutually exclusive another) may explain this situation: (i) given that *S. maltophilia* colonizes the rhizosphere, it can encounter there a dense bacterial population (including antibiotic producers) and needs to present elements for avoiding the inhibitory activity of competitors; (ii) as proposed for different antimicrobial resistance determinants, their function in nature, an environment where the antimicrobial load is not high, is likely not to

provide resistance to the inhibitory action of antimicrobials [14–17]. As described below, this can be the situation of the SmeDEF efflux pump which seems to be involved in the colonization of the roots of the plants [18]. In the current chapter, we discuss the relevance of *S. maltophilia* efflux pumps for the acquisition of resistance to clinically relevant antimicrobial drugs, as well as the role these efflux pumps may have in other bacterial processes.

15.2 Characterization of Efflux Pumps in *S. maltophilia*

The efflux pump of *S. maltophilia*, SmeDEF, was described in 2000 [19]. Later on, in 2002, the efflux pump SmeABC was also described [20], and in 2008 the sequencing of complete genome of *S. maltophilia* K279a strain [13] allowed the identification of genes encoding several multidrug transporters belonging to three different superfamilies of transporters: the chromosome of *S. maltophilia* contains genes coding for eight multidrug resistance (MDR) efflux pumps belonging to resistance-nodulation-cell division (RND) superfamily, three efflux pumps belonging to the major facilitator superfamily (MFS), and two ATP-binding cassette (ABC) superfamily pumps [13]. After their first identification, different studies aimed to characterize them in detail, in particular their role in antimicrobial resistance, but also their involvement in resistance to biocides as well as their role in other cellular processes, including the colonization of plant hosts and the response to stress.

15.2.1 RND Efflux Pumps

The structure of *S. maltophilia* RND efflux pumps is the same as for other members of the superfamily. These efflux pumps form a tripartite complex composed of three proteins: an inner membrane protein, an RND transporter that binds and translocates substrates, an outer membrane protein (OMP), and a membrane fusion protein (MFP), which links the outer and inner membrane proteins in the periplasmic space. In general, the genes encoding the three efflux pump proteins are located in the same operon, with some exceptions in which the operon does not contain the OMP (Fig. 15.1). In other Gram-negative bacteria, when the OMP is not encoded in the same operon, the substrates can be extruded to the periplasmic space and leave the bacteria through an OMP encoded elsewhere on the chromosome, which is recruited to form a tripartite pump. For instance, the *P. aeruginosa* MexXY efflux pump can accommodate OprM [21, 22], whose gene is located in the operon encoding MexAB-OprM [23]. Usually, located upstream and divergently transcribed from the efflux pump operon are gene(s) encoding for a transcriptional regulator protein [24] or a two-component regulatory system [20]. Expression of these efflux pumps is tightly downregulated by such regulators, and high expression levels can be achieved either in the presence of an inducer or upon mutations of the regulators.

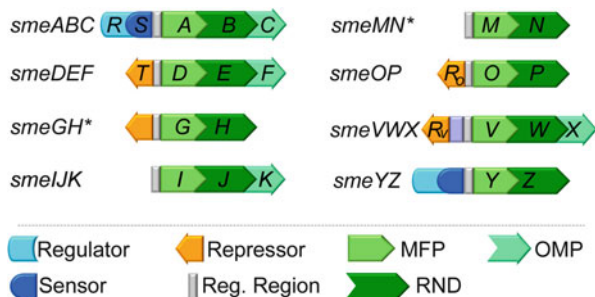


Fig. 15.1 RND operons in *S. maltophilia*. Operons containing RND genes and the genes for their local regulators are represented. The same figure is used for genes codifying for proteins with the same function, as detailed in the figure. RND genes are *smeB*, *smeE*, *smeH*, *smeJ*, *smeN*, *smeP*, *smeW*, and *smeZ*. Adjacent membrane fusion protein (MFP) genes and outer membrane protein (OMP) genes are shown. Genes encoding local regulatory proteins, as well as their DNA regulatory regions (Reg. Region) containing promoters and operator regions, are shown. Asterisks (*) indicate operons whose role in antimicrobial resistance has not been proven yet

Six of the eight RND efflux pumps identified in the *S. maltophilia* chromosomal sequence have been functionally characterized, SmeABC, SmeDEF, SmeIJK, SmeOP, SmeVWX, and SmeYZ (Table 15.1), while the putative role of SmeGH and SmeMN in antimicrobial resistance remains unknown. Among them, SmeDEF, SmeIJK, SmeOP, and SmeYZ, as well as the ABC efflux pump MacABCsm (see below), have shown to have a role in intrinsic resistance to different compounds (Table 15.1). The overexpression of SmeABC provides resistance to β -lactams, aminoglycosides, and quinolones, although only the deletion of the OMP of the system, SmeC, has an effect in the susceptibility to several antimicrobial drugs [20]. This may suggest that the observed effect is not due to the efflux pump itself but of another efflux pump that can make use of SmeC for its activity in extruding antibiotics. SmeDEF has an important role in susceptibility to chloramphenicol, tetracycline, quinolones, and cotrimoxazole, as well as to biocides such as triclosan [28, 35–38]. When overexpressed, the efflux pump SmeVWX is mainly involved in acquired resistance to chloramphenicol and quinolones [28, 29]. The efflux pump SmeOP confers low susceptibility to aminoglycosides, nalidixic acid, doxycycline, and macrolides, as well as to some nonantibiotic compounds, such as carbonyl cyanide *n*-chlorophenylhydrazone (CCCP), crystal violet, sodium dodecyl sulfate, and tetrachlorosalicylanilide. However its overexpression only alters the susceptibility to toxic compounds not used in therapy such as CCCP and tetrachlorosalicylanilide [27]. Other two efflux pumps, SmeIJK and SmeYZ, provide levofloxacin resistance when they are overexpressed [26]. Nevertheless, their substrate profile is different. While efflux pump SmeIJK overexpression affects susceptibility to aminoglycosides, ciprofloxacin, and tetracycline, SmeYZ overproduction only changes aminoglycosides' susceptibility [13].

The overexpression or the inactivation of efflux pumps affects antimicrobial susceptibility in different ways. The fact that the deletion of OMPs, such as SmeC and

Table 15.1 Functionally characterized *S. maltophilia* efflux pumps

Efflux pumps	Superfamily	Regulator (family)	Intrinsic resistance	Antimicrobial resistance	Reference
SmeABC	RND	SmeSR (two-component system)	No	Aminoglycosides, β -lactams, and quinolones	[20]
SmeDEF	RND	SmeT (TetR)	Yes	Chloramphenicol, quinolones, and tetracyclines	[19, 25]
SmeIJK	RND	Unknown	Yes	Aminoglycosides, ciprofloxacin, and tetracycline	[13, 26]
SmeOP	RND	SmeRo (TetR)	Yes	Aminoglycosides, doxycycline, macrolides, and nalidixic acid	[27]
SmeVWX	RND	SmeRv (LysR)	No	Chloramphenicol and quinolones	[28, 29]
SmeYZ	RND	Smlt2199-2130 (two-component system)	Yes	Aminoglycosides and trimethoprim-sulfamethoxazole	[13, 26, 30]
EmrCABsm	MFS	EmrRsm (MarR)	No	CCCP and nalidixic acid	[31]
SmtcrA	MFS	SmqnrR (DeoR)	No	Tetracycline	[32]
SmrA	ABC	Unknown	Unknown	Doxorubicin, fluoroquinolones, and tetracycline	[33]
MacABCsm	ABC	Unknown	Yes	Aminoglycosides, macrolides, and polymyxins	[34]

TolCsm, alters the susceptibility to antimicrobial agents, together with the absence of OMP-encoding genes in the operons encoding some efflux pumps such as SmeIJK, SmeOP, and SmeYZ, suggests that such OMPs are not efflux pump specific and several pumps could use the same OMP. Actually, the deletion of *smeC*, encoded in the *smeABC* operon, affects susceptibility to several antimicrobials, whereas the deletion of the gene encoding the actual inner membrane efflux pump *smeB* does not have any relevant effect on antimicrobial susceptibility [20]. In a similar way, the *tolCsm* gene encodes an OMP and is located upstream *smeOP* in another operon, *smeRo-pcm-tolC*, which suggests that it might form part of the SmeOP efflux pump [27, 39]. Nevertheless the deletion of *tolCsm* increases the susceptibility to several compounds, without showing any correlation with the phenotype observed in a mutant lacking *smeOP* [39].

It has been recently shown that the use of antibodies targeting efflux pumps might increase *S. maltophilia* susceptibility to antimicrobials [40]. These OMPs that are shared by different efflux pumps might be good targets for such antibodies since their inactivation will potentially inhibit simultaneously the action of different efflux pumps.

15.2.2 MFS Efflux Pumps

The study of efflux pumps belonging to the other families already found in *S. maltophilia*, namely, MFS and ABC, has received less attention than in the case of RND pumps. Four MFS efflux pumps have been so far identified in the genome of this bacterial species [13, 41]. One of these, EmrCABsm, is encoded in an operon of four genes that encodes the three efflux pump components and the transcriptional regulator of the expression of the pump *emrRsm* (MarR type) transcribed in the same direction. The deletion of *emrRsm* causes the overexpression of the efflux pump, which reduces *S. maltophilia* susceptibility to nalidixic acid and CCCP [31]. This indicates that *emrRsm* encodes a repressor of *emrCABsm*. The second MFS-type efflux pump (Smlt0032) has been identified only by bioinformatics analysis, and information on its role in antimicrobial resistance remains to be confirmed [13]. The third MFS-type efflux pump, MfsA, has been associated with methyl viologen resistance. Its regulator, SoxR, is a sensor of superoxide-generating agents [41]. However, whether or not this efflux pump has a role in the resistance of *S. maltophilia* against REDOX compounds remains to be established. *SmtcrA* is another MFS efflux pump, which is associated to tetracycline resistance. It is localized near another antimicrobial resistance gene, *Smqnr*. Previous work shows that both genes seem to be regulated by the same regulator SmQnrR [32], although a more recent study indicated that the effect of SmQnrR on *Smqnr* regulation is strain specific and that this repressor regulates *SmtcrA* expression in the two *S. maltophilia* strains studied so far [42].

15.2.3 ABC Efflux Pumps

Two ABC efflux pumps, SmrA and MacABCsm, are present in *S. maltophilia* [33, 34]. The activity of the MacABCsm efflux pump is associated with reduced susceptibility to aminoglycosides, macrolides, and polymyxins. Interestingly, a mutant presenting the deletion of the OMP, *macCsm*, has a different phenotype than mutants lacking *macAB*. These data suggest that the MacABCsm efflux pump can use an alternative, still-unidentified, OMP [34], as it was reported for some of the RND-type efflux pumps (see above). The second ABC efflux pump, SmrA, has been studied only in the heterologous host *E. coli*, in which it provides resistance to fluoroquinolones, tetracycline, doxorubicin, and multiple dyes. Whether or not this efflux pump is involved in resistance to these compounds in *S. maltophilia* remains to be established [33].

15.3 Regulation of the Expression of *S. maltophilia* Efflux Pumps

Expression of efflux pumps is in general tightly controlled by both local and general regulators. A possible reason why they are expressed at low levels unless an effector is present is that their expression can entail fitness costs. This cost can be due to the waste of energy required for the synthesis and activity of these macromolecular complexes and also because efflux pumps may extrude molecules that are important for microbial physiology such as quorum-sensing signals or virulence determinants among other compounds [43–45]. As shown in Table 15.1, hypothetical regulators, belonging to different structural families, have been identified for five RND efflux pumps. Most studies are based on the *in vitro* inactivation of the regulator. In two cases [42], the efflux pumps *smeDEF* and *smeVWX*, mutations inactivating their regulators, SmeT (TetR type) and SmeRv (LysR type) that lead to the overexpression of SmeDEF and SmeVWX, respectively [28, 46], were characterized both *in vitro* and *in vivo* [28, 38, 47–52]. Another TetR-type protein (SmeRo) regulates the efflux pump SmeOP [27]. In the case of SmeABC, a two-component system seems to be responsible of its regulation [20], a situation also observed for the efflux pump SmeYZ [13].

The molecular and structural basis of the regulation of *smeDEF* has been studied in detail. Expression of this efflux pump is downregulated by the transcriptional repressor SmeT, which is encoded upstream of *smeDEF*, in its complementary DNA strand [46]. In addition to repressing *smeDEF* transcription, SmeT also downregulates *smeT* transcription [46]. Crystal structure and biochemical analyses have shown that, like other members of the TetR family of transcriptional repressors, SmeT behaves as a dimer. A pair of dimers binds to a pseudopalindromic 28 bp region that overlaps both *smeT* and *smeD* promoters [53]. Binding of SmeT to this operator region precludes the transcription of *smeT* and *smeDEF*, likely by steric hindrance impeding RNA polymerase binding to DNA (Fig. 15.2). It is to be noticed

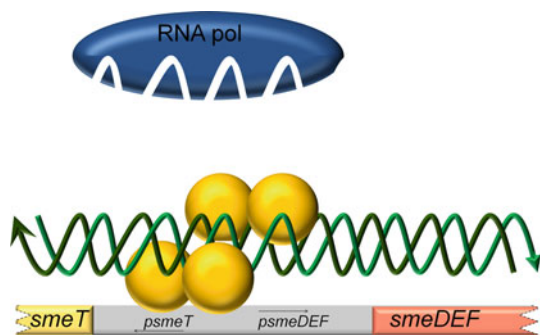
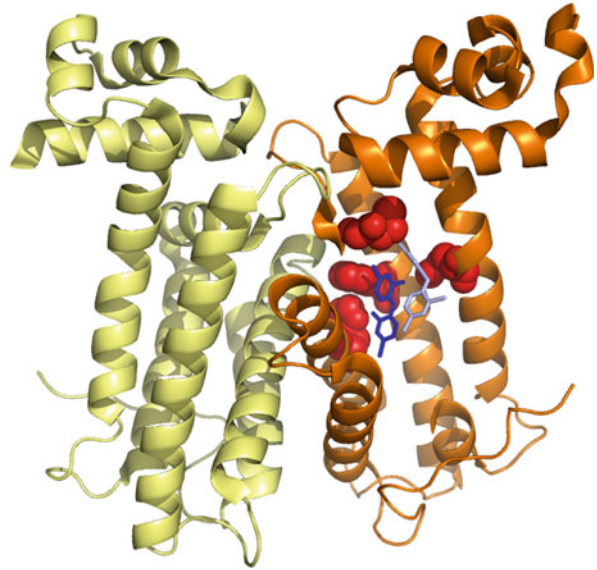


Fig. 15.2 Transcriptional repression of *smeDEF* by SmeT. Representation of a pair of dimers of the transcriptional repressor SmeT (yellow spheres) bound to its DNA operator region (green double helix). The binding of the protein impedes the *smeDEF* pump transcription since the *smeD* promoter overlaps with the repressor's operator, making impossible the RNA polymerase-DNA initiation complex. A bar indicating the order and position of the *smeT* and *smeDEF* genes (yellow and pink, respectively), as well as their promoters (*psmeT* and *psmeDEF*), is included

Fig. 15.3 Crystal structure of the SmeT-triclosan complex. Shown is a cartoon representation of a dimer of the transcriptional regulator SmeT (one monomer in *pale yellow* and the second one in *orange*) accommodating two molecules of the biocide triclosan (*blue* and *light blue* sticks). The residues responsible for the key interactions protein-biocide (His-67, Met-113, Phe-133, and His-167) are represented as *red spheres*



that while most studied clinical isolates overexpressing SmeDEF present mutations in *smeT*, one *smeDEF*-overexpressing clinical isolate did not present relevant changes neither in *smeT* nor in its operator sequence [47]. This result strongly suggests that other proteins besides SmeT may participate in the regulation of *smeDEF* expression.

High-level expression of SmeDEF can be achieved by mutating SmeT or by releasing its binding from its operator in the presence of a SmeT effector. It has been shown that, while antibiotics that are good substrates of the efflux pumps are not effectors of SmeT, other compounds such as biocides or flavonoids present in plant exudates can be accommodated by the SmeT binding pocket (Fig. 15.3), releasing the regulator from its operator and, consequently, allowing the induction of SmeDEF expression [18, 37, 54]. The fact that flavonoids, and not antibiotics, are good SmeT effectors suggests that the original function of SmeDEF is not necessarily the extrusion of antibiotics (see below).

15.4 Clinical Relevance of *S. maltophilia* Efflux Pumps

Most works on the substrate range and regulation of *S. maltophilia* efflux pumps have been performed *in vitro*. From these studies, we can state which efflux pumps are involved in intrinsic *S. maltophilia* resistance to antimicrobial agents (Table 15.1) and predict which ones may contribute to the acquired resistance upon their overexpression. Nevertheless there are few detailed epidemiological analyses describing mutants overexpressing these efflux pumps in clinical isolates. These studies are

hampered because, in contrast to the situation in other Gram-negative bacteria in which efflux can be inferred by determining the minimal inhibitory concentrations (MICs) of the antimicrobials of choice in the presence and in the absence of the efflux pump inhibitor phenylalanine-arginine- β -naphthylamide, this compound does not inhibit the activity of *S. maltophilia* efflux pumps [55]. Despite this situation, functional and molecular assays have shown that overexpression of SmeDEF is common among clinical *S. maltophilia* isolates [47, 48, 56]. In addition, clinical isolates overexpressing SmeVWX have been also found, some of them presenting the same mutation in *smeRv*, which encodes the transcriptional regulator of this efflux pump that has been described for *in vitro* selected *smeVWX* overexpressing mutants [38, 50–52]. These data support the idea that *in vitro* evolution studies may help in predicting mutations appearing *in vivo* during the treatment of patients [57–60]. This could help in improving therapeutic regimens in order to avoid the emergence of antimicrobial resistance, particularly MDR, because selection by a single antimicrobial of mutants that overexpress an efflux pump will render resistance to all the antimicrobials that are substrates of such an efflux pump.

One aspect in which *S. maltophilia* efflux pumps' activity is particularly cumbersome concerns acquired resistance to quinolones. Quinolones are synthetic antimicrobials with a broad spectrum of activity. They were discovered in the 1960s while studying chloroquine for malaria treatment [61]. Afterward, it was proven that a fluoride group added to the central ring of the molecule increases the efficiency of the antimicrobial, creating the fluoroquinolones. Since there are no quinolone producers in nature, it was expected that resistance to this family of drugs would appear just through mutations in the genes encoding for their targets, the bacterial topoisomerases [62]. In fact, mutations in these genes, especially in the quinolone resistance-determining regions (QRDRs), are the main cause of high-level quinolone resistance in all studied microorganisms.

The exception to this rule is *S. maltophilia*. Different works have shown that, in sharp contrast with what happens for other bacterial species, such mutations are not found in quinolone-resistant *S. maltophilia* strains, neither in mutants selected *in vitro* nor in clinical isolates [28, 63–65]. The reason behind this situation is likely that, whereas usually overexpression of efflux pumps confers low-level resistance to quinolones in all bacterial pathogens so far studied, the overexpression of efflux pumps may confer the clinically relevant resistance in the case of *S. maltophilia*. Indeed, it has been shown that the overexpression of SmeDEF correlates with the quinolone resistance of *in vitro* selected mutants [28] and several clinical isolates overexpress this system [38, 47, 52, 56, 66]. Further, even when this efflux pump is inactivated, quinolone-resistant mutants do not present changes in the QRDR of the topoisomerases, but resistance is caused by the overexpression of yet another different efflux pump, SmeVWX [28]. It is important to notice that SmeVWX-overexpressing clinical strains presenting the same mutations in its regulator as the *in vitro* selected mutants have been reported [28, 52].

One intriguing finding is that although SmeDEF overexpression is found more frequently in both the *in vitro* obtained mutants and the clinical isolates, SmeVWX

overexpression leads to higher quinolone MICs in *S. maltophilia* [28, 52]. It is possible that, once a clinically relevant resistance is achieved, the only factor affecting the selection of one mutation over another consists of a fitness cost associated with the acquisition of each of such mutations [59, 60, 67, 68]. In this regard, it has been described that SmeDEF overexpression impairs *S. maltophilia* fitness and virulence [66]. Whether or not the effect of SmeVWX overexpression in *S. maltophilia* is similar (or eventually more drastic) remains to be established.

While efflux pumps seem to be major players in the acquisition of quinolone resistance by *S. maltophilia*, this bacterial species also contains in its genome a gene encoding a quinolone resistance protein SmQnr, which also might contribute to this resistance [11, 12]. Although the *in vitro* studies show that the SmQnr overexpression increases MICs of quinolones [11, 12], the SmQnr overexpression has not been found as the cause of resistance neither in *in vitro* selected quinolone-resistant mutants nor in clinical isolates. A reason for such situation is that in contrast to the overexpression of *S. maltophilia* efflux pumps, the SmQnr overexpression renders only a low-level quinolone resistance [28, 52]. We can then conclude that the overexpression of either SmeDEF or SmeVWX is the only known mechanism of quinolone resistance in *S. maltophilia*, which is present after the *in vitro* selection of quinolone-resistant mutants, and has been described in clinical isolates of this bacterial species [28, 52, 69].

15.5 *S. maltophilia* Efflux Pumps: More Than Antimicrobial Resistance Determinants

Even though the majority of studies related to efflux pumps explore their role in antimicrobial resistance, it is known that functions of MDR pumps are very diverse [14]. It has been shown that, besides antibiotics, efflux pumps can extrude a huge variety of compounds like heavy metals [70], organic solvents [71], dyes [72], detergents [25], bile salts [73], biocides [36], quorum-sensing signals [43–45], or plant-produced compounds [74–77]. However, little is known about functions that *S. maltophilia* efflux pumps have in dealing with the behavior of this microorganism in its natural habitat.

Some recent works begin to address this issue; SmeDEF, for example, is involved in the colonization of plant roots by *S. maltophilia* [28]. Plant-produced flavonoids can bind to SmeT, derepressing *smeDEF* expression. They are bona fide effectors regulating *smeDEF* expression, since they specifically induce this pump and not other *S. maltophilia* efflux pumps. Consistent with these findings, the deletion of *smeE* impairs *S. maltophilia* colonization of plant roots. These results show that one original function of SmeDEF is the colonization of plant roots, and only in clinical settings the quinolone resistance is manifested as a function of this efflux pump, being a good example of exaptation, an evolutionary process in which a

change of function is achieved by an environmental shift, not by a genetic change [17, 78].

Not only has SmeDEF a role in the interaction of *S. maltophilia* with plants, but SmeVWX might also be somehow related. The diffusible signal factor (DSF) is a quorum-sensing molecule of fatty acid nature that was first detected in *Xanthomonas campestris* [79]. This molecule is also present in *S. maltophilia* and is involved in the regulation of the expression of numerous genes directly or indirectly involved in plant growth promotion and biocontrol, including antimicrobial resistance genes such as SmeW [80]. When the gene encoding the DSF synthase, *rpfF*, is deleted, the *smeW* expression is downregulated, indicating that SmeW is an efflux pump that may also be somehow involved in colonization of plants by *S. maltophilia*, although further studies are needed to address this hypothesis. DSF also regulates chaperone biosynthetic genes and therefore the stress resistance [80], establishing an indirect link between SmeVWX efflux pump and stress response.

SmeIJK operon contains two RND-type transporters (SmeJ and SmeK) and is constitutively expressed in the absence of antimicrobial pressure, indicating that antimicrobial resistance is not likely the main function of this pump. A recent study showed a direct relationship among the SmeIJK efflux pump, the cell envelope integrity, and the envelope stress response mediated by the sigma factor RpoE [81]. When *smeIJK* is deleted, *S. maltophilia* is more susceptible to hyposmolarity and to membrane-damaging agents (MDAs) such as sodium dodecyl sulfate or Triton X-100. In addition, the loss of SmeIJK activates the *rpoE* regulon. This regulon can also be activated in the presence of MDAs, and this activation further upregulates SmeIJK expression. In the presence of antimicrobials that are known substrates of SmeIJK efflux pump, *smeIJK* expression is not increased, further confirming the main function of SmeIJK to be a part of the *S. maltophilia* envelope stress responses via RpoE [81]. Another pump with a known function in *S. maltophilia* envelope stress responses is the ABC-type efflux pump, MacABCsm [34]. This pump is also constitutively expressed, and besides envelope stress responses, it also has a role in oxidative tolerance and biofilm formation.

S. maltophilia is an opportunistic pathogen with increasing relevance in nosocomial infections [4]; however, its natural habitats are water and soil, where this microorganism can colonize all parts of plants, including the whole endosphere [82]. As stated previously, little is known about the role of efflux pumps of *S. maltophilia* in the natural environment, but with this natural origin, it is predicted that the majority of efflux pumps should display different functions in these habitats. The interest in knowing the original functions of efflux pumps is increasing, so it is expected that, in the next years, more natural functions for these antimicrobial resistance determinants will be deciphered.

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