Bacterial adaptation to antibiotics through regulatory RNAs

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Abstract

The extensive use of antibiotics has resulted in a situation where multidrug-resistant pathogens have become a severe menace to human health worldwide. A deeper understanding of the principles used by pathogens to adapt, respond and resist against antibiotics will pave the road to drugs with novel mechanisms. For bacteria, antibiotics are clinically-relevant stresses that induce protective responses. The recent implication of regulatory RNAs (sRNAs) into antibiotic response and resistance in many bacterial pathogens suggests that they should be considered as innovative drug targets. This review discusses sRNA-mediated mechanisms exploited by bacterial pathogens to fight against antibiotics. A critical discussion of the newest findings in the field is provided, with emphasis on the implication of sRNAs in major mechanisms leading to antibiotic resistance: drug uptake, active drug efflux, drug target modifications, biofilms, cell wall and LPS biosynthesis. Of interest is the lack of knowledge about sRNAs implicated in Gram-positive resistance, compared to Gram-negative bacteria.

Worldwide burden of antimicrobial resistance

Bacterial resistance to antibiotics has become a main challenge for public health worldwide. The World Health Organization has claimed 'antibiotic resistance' as one of the three most important public health threats of the 21st century (1). Most pathogens are becoming multidrug resistant (MDR), with an increased risk of failure of conventional therapies with higher morbidity, mortality, hospitalization lengths and treatment costs (1). Resistant Gram-positive pathogens responsible for health-care associated infections include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and MDR *Streptococcus pneumoniae*. For infections caused by Gram-negative bacteria (such as *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter*), treatment choices are also becoming limited. In the USA, more than 2 million people annually develop infections due to MDR organisms, resulting in more than 23,000 deaths. Deaths attributable to MDR bacteria every year are now 700,000 worldwide and the projected mortality rates by 2050 are 10 million, more than deaths caused by all cancers (2). In the near future, there are serious odds that no treatment options will be available for the "ESKAPE" pathogens, which comprise *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* spp. (3). Therefore, development of novel antibacterial agents is essential to keep up with the constantly evolving resistance in bacteria. However, very few novel classes of antibacterial drugs have been discovered in the last three decades (4).

Antibiotics kill bacteria or inhibit their growth by blocking key cellular pathways. They also allow our natural defenses, including the host immune system, to eliminate the invading microorganisms. Resistance against any antibiotic drug, regardless of its mechanism of action, was reported soon after its clinical use. The unreasonable use of antibiotics in

animals and humans has promoted evolution of resistance. Using natural selection, bacterial pathogens evolve to survive and evade drugs designed to eliminate them, as they have done for millions of years against natural antibiotics produced by competing organisms in their environment (5).

Bacterial adaptation to antibiotics

Bacterial genome plasticity is mandatory to adapt and respond to environmental threats, including antibiotic stress. Antibacterial resistance is ancient, resulting from the interaction among organisms and their environment. Most antibiotics were produced naturally by bacteria and fungi for millions of years (6), and bacteria evolved mechanisms to overcome their action, survive, and spread. As a consequence, many bacteria are naturally resistant to one, several, or even most of antibiotics. Acquired resistance develops with gene mutations or *via* external genetic acquisition from nearby resistant organisms, through horizontal gene transfer (HGT). Non- or slow-growing bacteria can survive most bactericidal antibiotics that require active growth for action, a property called “tolerance”, leading to persistence. Neutral mutations during bacterial genomes evolution can pave the way for the subsequent evolution of resistance (7). Mutations triggering resistance alter antibiotic action by either drug target modifications, reducing drug uptake, stimulating drug efflux or by modifying regulatory networks implicated in general metabolism. Another parameter that influences the emergence and evolution of antibiotic resistance is the existence of antibiotic concentration gradients in the environment, livestock and humans. It implies that pathogens are frequently exposed to non-lethal, subinhibitory drug concentrations (SICs) shaping the evolution of antibiotic resistance (8). The rationale behind dosing for antibiotic treatments is to maintain a concentration higher than the MIC (i.e., the lowest concentration of a chemical

which prevents visible growth of a bacterium) in the relevant body compartments for long enough, to clear the infection. Under MIC antibiotic concentrations, bacterial growth is inhibited but cells are not killed and the infection can resume later. Low antibiotic concentrations in body fluids and tissues can therefore favor resistance development.

Bacteria come across many stresses in their natural habitat. Pathogens fight or adapt to their host's innate or adaptive defenses. Various stresses (including oxidative, acidic, osmotic, temperature, nutrient starvation, and antibiotic) trigger adaptive responses from the pathogen (9). Antibiotic exposure, when not lethal, induces stress responses in bacteria. Since antibiotics are stresses, they often elicit protective responses in bacteria that will reduce antibiotic activity. Conversely, stress can impact antimicrobial susceptibility. As specific example, stress-induced growth arrest impact antibiotic susceptibility since antimicrobials usually act on growing cells. Stresses raise tightly regulated adaptive and protective responses, including gene expression reprogramming by signaling pathways including transcriptional factors (10) and regulatory RNAs (sRNAs) (11).

sRNAs as stress response regulators including antibiotics

sRNAs participate in many regulatory events, from plasmid copy number control in bacteria to X-chromosome inactivation in mammals. Sensing the environment also requires appropriate sRNA-mediated responses to adapt gene expression fast and efficiently. Once an external or internal signal is detected, sRNAs, alone or in cooperation with additional regulators, tune target gene product levels and control expression timing, for optimal adaptation. That regulation is reversible once the signal vanishes, and sRNAs as well as antisense RNAs (asRNAs, transcribed from the opposite DNA strand of their target mRNA) are usually consumed upon their action since they often are co-degraded with targets (12).

As in eukaryotes (13), many sRNAs are expressed and implicated in complex gene regulatory networks in *eubacteria* and *archaea* (14). sRNAs are ~50 to 600 nucleotide-long, usually stable and non-coding (there are exceptions, 15). They act on their own or require associated RNA-binding proteins, such as Hfq (16). They are implicated in physiological responses influenced by signals from their surroundings. sRNAs modulate DNA maintenance and silencing, transcription and/or translation of target genes, protein quality control and secretion mechanisms. They enhance bacterial fitness to many stresses, inducing adaptive metabolic changes. They optimize utilization of available nutrients and improve survival, virulence (17) and persistence (18).

Very few sRNAs are constitutively expressed. The majority are transcriptionally induced under specific conditions (19) such as cold or heat, pH or nutrient changes, iron homeostasis, membrane remodeling, virulence gene expression, motility, biofilm production, virus or plasmid invasions (clustered regularly interspaced short palindromic repeats or CRISPRs, 20), and antibiotic exposure (21). sRNAs, transcription factors (TFs) and small signaling molecules frequently interact with regulatory networks, for gene reprogramming during stress (22). TFs influence gene expression at the transcriptional level, whereas sRNAs intervene essentially post-transcriptionally. We will not cover 5' untranslated regions (UTRs) of antibiotic resistance genes. These sensory RNAs respond to environmental signals by inducing or preventing expression of downstream genes (for recent review (23)).

sRNAs as modulators of antibiotic response and resistance

Antibiotics differ based on the cellular component(s) they affect, in addition to whether they induce cell death (bactericidal) or merely inhibit cell growth (bacteriostatic). Most antibiotics act by perturbing bacterial cell wall synthesis, DNA replication, RNA transcription or protein

synthesis (24). Recent evidence indicates that bacterial sRNAs are important actors during stress responses and for the development of resistance to various antibiotics (21). Several sRNAs are involved in regulatory circuits controlling antibiotic resistance (Table 1), and we anticipate that this only represents the tip of the iceberg. Resistance to antimicrobial agents commonly results from the following mechanisms: 1) enzymatic antibiotic inactivation, 2) decreased affinity of the antibiotic for its target, by target modification or protection, and 3) decreased of intracellular antibiotic concentration due to decreased permeability and/or overexpression of efflux pumps (25). Also, biofilm formation is clinically relevant since bacteria associated with biofilms are resistant/tolerant to many antibiotics (26). As specific examples, sRNAs influence antibiotic resistance by pairing with target mRNAs expressing drug efflux pumps, antibiotic transporters or enzymes involved in drug catabolism.

sRNAs and drug uptake

In Gram-negative bacteria, antibiotics must cross over the outer membrane to reach their intracellular targets through a lipid-mediated pathway (for hydrophobic antibiotics), or *via* water-filled porins (for hydrophilic antibiotics) (27). To become resistant, bacteria can alter permeation of antibiotics through the outer membrane by modifying these uptake pathways. Interestingly, the expression of some of these macromolecules can be regulated by sRNAs, and therefore impacts resistance.

In *Escherichia coli*, GcvB sRNA regulates *sstT*, *oppA* and *dppA* involved in amino acid, dipeptide and oligopeptide transports. GcvB also negatively regulates *cycA* mRNA, which encodes a permease for glycine, D-alanine, D-serine and D-cycloserine transport into the bacteria (28). Note that D-cycloserine is an analogue of D-alanine that interferes with bacterial cell wall synthesis, used in the treatment of multi- and extensively-drug-resistant

tuberculosis (29). Interestingly, a Δ*gcvB* mutant is more susceptible to D-cycloserine than the parental strain, due to increased CycA levels and increased transport of the antibiotic (28). GcvB also negatively regulates the PhoPQ two-component system by translational repression of PhoP and could be involved, through *eptB*, in LPS modifications and resistance to antimicrobial peptides (see below).

Colicin Ia is a pore-forming *E. coli*-specific bacteriocin, which targets outer membrane protein (OMP) CirA. The latter is a TonB-dependent transporter involved in ferric iron uptake. RyhB is a Hfq-dependent sRNA that regulates iron homeostasis (Fur represses *cirA* and *ryhB*, while RyhB activates *cirA*). RyhB is essential for CirA synthesis during iron starvation by pairing to *cirA* mRNA, leading to its translational activation and prevention of degradation by RNase E. Consequently, increased CirA levels render cells more susceptible to colicin Ia bactericidal action (30). An interesting class of sRNAs is those acting as RNA 'sponges' that interact and repress the functions of other base-pairing sRNAs. An example is 3'ETS^{leuZ} RNA that is a 3' external transcribed spacer of the *glyW-cysT-leuZ* polycistronic tRNA produced *via* RNase E-mediated processing (31). This RNA 'sponge' pairs with RyhB and RybB (a sRNA that downregulates CsgD – see below), suppressing transcriptional noise from those sRNAs. Accordingly, a 3'ETS^{leuZ} deletion mutant is killed by colicin Ia, compared to the parental strain.

In *E. coli*, MicF sRNA regulates *ompF* expression by pairing with *ompF* mRNA, inducing translation inhibition and mRNA degradation, in turn reducing permeability to several antibiotics (32). When overexpressed in *E. coli*, MicF increases cephalosporin, norfloxacin, and minocycline MICs while depletion of this sRNA reverses those phenotypes, except for minocycline (33).

Two novel sRNAs, Sr0161 and ErsA, have recently been identified in *P. aeruginosa* using a new method called High-throughput Global sRNA target Identification by Ligation and sequencing (Hi-GRIL-seq) (34). They interact with the *oprD* 5' UTR, which expresses a major porin required for carbapenem uptake. Both sRNAs negatively regulate *oprD* expression leading, when induced, to reducing OprD protein expression and, in turn, increasing carbapenem resistance. Mutant strains lacking Sr0161 or ErsA are therefore more susceptible to carbapenems.

sRNAs and active drug efflux

Categorized into five families, multidrug efflux pumps in bacteria are widely distributed in both Gram-positive and negative bacteria. By expelling a broad range of structurally varied molecules, they lower the intracellular antibiotic concentration, and are involved in intrinsic and acquired bacterial resistance (35). Mostly encoded on the chromosome, efflux pumps are implicated into stress adaptation, detoxification, pathogenesis and bacterial virulence (36). Their expression is subjected to tight regulations in response to environmental and physiological stimuli.

The *yejABEF* operon encoding an ATP-binding cassette (ABC) transporter in several Gram-negative bacterial species confers antimicrobial peptide (AMP) resistance to *Salmonella* (37) and *Brucella melitensis* (38) by stimulating active AMP efflux. AMPs induce *yej* operon expression, allowing bacteria to counteract antibiotic activity by decreasing AMP intracellular concentrations. RydC sRNA pseudoknot, with the aid of the Hfq chaperone, regulates curli synthesis and biofilm formation in enteric bacteria (39). In *Salmonella*, RydC also remodels phospholipid composition of the membrane by controlling the cyclopropane fatty acid (CFA) synthase (40). The *yej* mRNA is degraded when RydC expression is stimulated

(41). Since RydC negatively regulates the expression the *yejABEF* mRNA, this sRNA may be associated with an increase in susceptibility to AMPs.

DsrA sRNA is a key regulator of essential pathways in *E. coli*, including general stress response (σ^S), genome compaction (H-NS), cell wall biosynthesis (MreB), and ribose metabolism (RbsD) (42). DsrA is also involved in antimicrobial resistance by regulating the expression of the MdtEF efflux pump (43). Indeed, when overexpressed in efflux-defective Δ*acrB* mutants, DsrA significantly increases oxacillin (×8), erythromycin (×4), and novobiocin MICs (×4) *via* an RpoS-dependent pathway.

In *E. coli*, while overexpression of RyeB increases susceptibility to quinolones, depletion of this sRNA reverses that phenotype (33). By overexpressing RyeB, there is a decrease in the expression level of *tolC* mRNA, whereas *tolC* mRNA expression is upregulated in a Δ*ryeB* mutant. TolC is an OMP of the 'AcrAB-TolC' efflux system, which has a broad spectrum of substrates including most of lipophilic antibiotics, and is also a component of other efflux transport systems (44). Named SdsR in *Salmonella* spp., RyeB is an abundant and stationary-phase Hfq-dependent sRNA, of whose transcription depends on σ^S (45). SdsR represses *tolC* mRNA levels by pairing with its 5' UTR, 33 nucleotides upstream of target mRNA ribosome binding site (RBS) (46). SdsR overexpression also increases susceptibility to other antibiotics, such as novobiocin and, to a lesser extent, erythromycin and rifampin. SdsR represses biofilm formation independently of pairing with *tolC* mRNA, suggesting additional targets. SdsR is a conserved sRNA from enterobacteria and its role in *tolC* mRNA repression was also found in *Salmonella* (47).

MtrF is an inner membrane protein belonging to the AbgT family described in *Neisseria gonorrhoeae* (48, 49). This membrane protein is required for gonococcal high-level resistance to hydrophobic antimicrobials (e.g. penicillins, erythromycin, rifampin) mediated

by the MtrCDE efflux system (48, 49). MtrF is also by itself a proton-motive-force (PMF)-dependent antibiotic efflux pump that expels sulfonamides from the bacteria (50). Interestingly, *trans*-acting, iron-regulated sRNA NrrF directly controls the MtrF expression by reducing *mtrF* mRNA stability by increasing its turnover (51, 52). Thus, NrrF attenuates MtrF action in antibiotic resistance. MtrF transcripts are also repressed by Fur, MtrR (repressor of *mtrCDE*), and MpeR (repressor of *mtrR*) (52), implying that expression of that inner membrane protein is tightly controlled by several additional regulators.

sRNAs and drug target modifications

Spontaneous or acquired variations in antibiotics target sites preventing drug binding is a widespread resistance mechanism (25). Noteworthy, modest alterations of the targets can induce substantial variations on antibiotic binding affinity. A common mechanism of resistance to AMP in Gram-negative bacteria is LPS modifications (53). While several sRNAs are known to regulate the expression of different proteins involved in LPS metabolism MgrR, a Hfq-dependent sRNA expressed in *E. coli* and other *Enterobacteriaceae*, is part of the PhoPQ regulon, a two-component system (TCS) activated under low Mg^{2+} conditions or by AMPs. PhoPQ has been extensively studied; it consists in the sensor kinase PhoQ and the cognate response regulator PhoP (54). MgrR actually downregulates eptB mRNA, which encodes a phosphoethanolamine transferase involved in LPS modifications (55). EptB modifies the keto-deoxyoctulosonate (KDO) residue (part of the core oligosaccharide of the LPS), which reduces the net anion charges and electrostatic repulsion between LPS molecules, leading to polymyxin resistance. An *mgrR*-deleted mutant is 10 times more resistant to polymyxin B than the parental strain whereas complementation of the *mgrR*

mutation restores polymyxin susceptibility. Noteworthy, the reduction of *eptB* mRNA levels by MgrR was also demonstrated in *Salmonella* (56).

SroC is an RNA sponge that originates from the GcvB-mediated decay of the polycistronic *gltIJKL* mRNA (57). SroC negatively controls GcvB action and activates the GcvB-repressed genes involved in amino acid metabolism. In *Salmonella*, SroC also pairs with the MgrR sRNA to interfere with its action, thus indirectly activating *etpB* expression (56). SroC overexpression increases EtpB expression and a Δ*sroC* mutant is more susceptible to polymyxin B than the parental strain. Finally, Δ*mgrR* and Δ*sroC*Δ*mgrR* mutants exhibit a similar resistance phenotype, suggesting that *mgrR* mutation may be epistatic to the *sroC* mutation.

As previously mentioned, the PhoPQ TCS is induced in response to low Mg^{2+} and Ca^{2+} concentrations and in the presence of AMPs. The PhoPQ regulon includes genes involved in Mg^{2+} transport, LPS modifications, acid resistance, virulence, and resistance to AMPs (54). In *E. coli*, the expression of *phoPQ* is directly repressed by MicA sRNA (also named SraD). MicA transcription is activated by σ^E, which is induced under envelope stresses (58). More precisely, MicA pairs with *phoPQ* mRNA around the *phoP* initiation codon, probably to modulate translation initiation. MicA may influence AMP resistance since it downregulates MgrR, *via* its action on PhoP, that itself represses *eptB* mRNA expression (Figure 1).

Another sRNA involved in *P. aeruginosa* antibiotic resistance is Sr006, which is a positive post-transcriptional regulator of *pagL* mRNA expression that encodes an enzyme responsible for lipid deacylation (34). When overexpressed, Sr006 confers increased polymyxin resistance through PagL-mediated LPS modifications. The upregulation of *pagL* by Sr006 appears Hfq-independent.

sRNAs regulating cell wall biosynthesis

Cell wall synthesis and recycling are critical cellular processes essential for cell growth, elongation and division, and peptidoglycan is the main component of this complex entity (59). Peptidoglycan synthesis involves an array of enzymes across all cellular compartments (cytoplasm, inner membrane, and periplasm) and the expression of some of these enzymes can be regulated by sRNAs.

GlmS catalyzes synthesis of glucosamine-6-phosphate (GlcN6P) from fructose-6-phosphate and glutamine, a key metabolite in cell wall biosynthesis. GlcN6P is further converted by GlmM and GlmU enzymes to UDP-N-acetyl-glucosamine (UDP-GlcNAc), a common precursor for peptidoglycan and LPS synthesis. Bacilysin (tetaine) and Nva-FMDP are dipeptide antibiotics that impair cell envelope synthesis by GlmS inhibition through covalent modification (60). In *E. coli* and presumably in most *Enterobacteriaceae*, *glmS* expression is controlled by GlmY and GlmZ sRNAs (61). GlmZ pairs with and activates *glmS* mRNA translation. Although GlmY is similar to GlmZ sequence and predicted structure, GlmY lacks a complementary region to *glmS* mRNA and does not directly activate *glmS* translation. Instead, GlmY expression inhibits a GlmZ processing event, disallowing *glmS* translation activation. Thus, GlmY functions by titrating an RNA processing factor away from homologous GlmZ sRNA. The GlmY/GlmZ pair provides resistance to bacilysin. Both *E. coli* and *Salmonella* respond to these antibiotics by increasing *glmS* expression to compensate for GlmS activity inhibition (62). GlmS inhibition by antibiotics leads to GlcN6P deprivation, sensed by GlmY sRNA, triggering its accumulation. Cells adjust GlmS expression levels to overcome growth inhibition by the GlmS inhibitor.

sRNAs modulating biofilm formation and antibiotic activity

Bacterial biofilms are multicellular populations, with cells surrounded by self-produced extracellular matrix that can include exopolysaccharides, proteins, amyloid fibers, and DNA. They are typically less susceptible to antimicrobial agents than non-adherent, planktonic cells, because of the poor drug diffusion inside the biofilm structure, and also since they contain metabolically inactive cells. Biofilm formation is tuned by complex regulatory hubs that integrate various environmental signals *via* alternative sigma factors, two-component systems, second messengers, and sRNAs. Many chronic infections are associated to bacterial biofilms, which increase tolerance to antibiotics and biocides as well as resist host cell phagocytosis (63). Conventional antibiotic resistance mechanisms in bacteria also include survival as biofilm communities. Those mechanisms include nutrient gradient (less nutrient availability in the biofilm core), compact exopolysaccharides matrices, extracellular DNAs, stress responses, genetic determinants specifically expressed in biofilms, multidrug efflux pumps, intercellular interactions and persister cells (64). In *Enterobactericeae*, RpoS and CsgD transcription factors control regulons implicated in biofilm formation, and their mRNA levels are controlled by numerous sRNAs (65). At least seven sRNAs (namely GcvB, McaS, OmrA, OmrB, RprA, RybB, and RydC) downregulate CsgD expression by direct binding with the *csgD* mRNA and, in turn, reduce biofilm formation (Figure 1). Thus, these sRNAs, when expressed, are expected to increase antibiotic susceptibility for biofilm-associated bacteria.

sRNA-control of transcription factors involved in antibiotic resistance

Regulatory systems in bacteria (including two-component, transcription, and sigma factors) respond to extracellular signals to modulate gene expression, contributing antimicrobial resistance genes.

SpoVG is a transcription factor (66) contributing to *S. aureus* methicillin and glycopeptide resistances, acting as a DNA-binding protein in *eubacteria* (67). In *S. aureus*, SprX sRNA (*alias* RsaOR), modulates resistance to glycopeptides (68), antibiotics that inhibit cell wall peptidoglycan synthesis and are treatment of choice of MRSA infections (69). Modifying SprX levels influences both vancomycin and teicoplanin susceptibility profiles. SprX negatively regulates SpoVG expression by direct pairings at the SpoVG translation initiation signals. SpoVG is not the unique target of SprX (70, 71), and those other targets could also impact glycopeptide resistance and pathogenicity.

Trans-encoded sRNAs often rely on sRNA-binding proteins for function (72). RNA chaperone Hfq promotes pairings between sRNAs and their mRNA targets to induce post-transcriptional regulations affecting mRNA stability and/or translation (73). Consequently, all the Hfq-binding sRNAs involved in antibiotic response (several in Gram-negative bacteria) will be impacted by the presence/absence of the protein. In *E. coli*, Hfq regulates a multidrug efflux pump at post-transcriptional level, and therefore impacts multidrug resistance (74). Compared to Gram-negative bacteria, the role of Hfq in sRNA functions seems less important in Gram-positive bacteria (75).

tmRNA and antibiotic resistance

Trans-translation, monitored by an hybrid transfer-messenger RNA (the so-called tmRNA [76]) with the SmpB protein, is a conserved quality control in *eubacteria* activated in response to ribosome stalling on truncated or non-stop mRNAs that can arise in cells due to premature transcription termination or mRNA damage (77). Accumulation of stalled ribosomes on those problematic mRNAs is toxic and needs to be rescued, otherwise protein synthesis would come to halt. As a consequence, impairment of *trans*-translation leads to

346 increased sensitivity to antibiotics targeting protein synthesis in several bacterial pathogens
347 (78, 79). Mutations that inactivate tmRNA or SmpB are lethal in some bacteria, including
348 *Neisseria gonorrhoeae* and *Shigella flexneri* (80). In other species, deletion phenotypes
349 include deficiencies in virulence, sporulation, cell cycle progression and antibiotic resistance
350 (81). Apart from ribosome rescue, these phenotypes could be due to the misregulation of
351 specific regulatory proteins in the absence of tmRNA. Also, *trans*-translation may be
352 coordinated with other essential co-translational processes such as protein folding and
353 secretion. In *S. pneumoniae*, the lack of tmRNA protects bacteria against fluoroquinolones
354 (82). In *S. pneumoniae*, deletion of tmRNA prevented chromosome fragmentation associated
355 to levofloxacin treatment. Such protective effect mainly depends on protein synthesis
356 inhibition. The increased susceptibility to translation inhibitors in different bacteria defective
357 in *trans*-translation implies that tmRNA is an attractive target for the development of novel
358 antibacterial agents (83). Indeed, the components of *trans*-translation were detected in
359 every sequenced bacterial genome, and mutations in these components affect viability or
360 virulence in many bacteria, suggesting that *trans*-translation inhibitors could be effective
361 'broad-spectrum' antibiotics.
362
363 **Concluding remarks and perspectives**
364 Antibiotic stress responses usually include sophisticated regulatory networks that were
365 recently investigated by extensive whole genome RNA-seq studies, with and without
366 antibiotics at SIC, in various bacteria (*S. aureus* [84, 85]; *E. faecium* [86]; *P. putida* [87]; and *S.*
367 *enterica* [88]). These global transcriptomic studies revealed that the expression of several
368 sRNAs is induced or repressed as a result of antibiotic SIC exposure, but the roles and

mechanisms connecting those sRNAs with the bacterial antibiotic responses await to be uncovered.

A major challenge with antibiotic use in human and veterinary medicine is bacterial resistance. Pioneering investigations in *E. coli*, *Salmonella*, *P. aeruginosa* and *S. aureus*, indicate that some sRNAs contribute to antibiotic response, susceptibility and resistance. Accumulating evidence indicates that specific sRNAs are essential players in adaptive networks to control key processes (such as drug efflux or uptake, LPS and cell wall syntheses, and biofilm formation) involved in resistance to the major classes of antibiotic drugs. Also, growing evidence suggests that *cis*-acting RNAs also regulate the expression of many resistance genes, sensing the presence of antibiotics and regulating resistance genes accordingly (89). Because there is an international spread of MDR opportunistic organisms including several Gram-positive bacteria, the implication of sRNAs in antibiotic resistance in these pathogens should be investigated thoroughly in the coming years. The development and application of genome-wide transcriptomic approaches will facilitate the identification of the set of riboregulators implicated in antibiotic response and resistance in many bacterial pathogens, a starting point to uncover and analyze the underlying regulation principles. A better understanding of the implication of sRNAs in antibiotic resistance networks will allow the design of new compounds preventing their actions in the future. They could be used with existing drugs to enhance their activities and lower the development of resistance. However, the development of resistances against new drugs targeting sRNA-regulated processes cannot be ruled out. However, since each sRNA usually impacts the expression of several targets (sRNA-associated regulon), it may be more complicated for bacteria to produce resistances against each regulated target. Many challenges remain to be solved, prior to clinical application.

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Legend of the figure

FIG 1 sRNAs regulating antibiotic resistance in bacteria. Mechanisms subjected to sRNA-mediated antibiotic response and resistance were divided into five main sections (dotted color boxes): drug uptake (green), active drug efflux (blue), lipopolysaccharide (LPS) and cell wall impairments (orange), biofilm formation (red), and transcription factors (TF) regulations (purple). The antibiotics subjected to sRNA-induced controls are indicated. sRNA targets involved are presented. Arrows correspond to sRNA-induced target gene expression upregulations; broken line are sRNA-induced target gene downregulations. Riboswitches were excluded. Of interest is the lack of knowledge for Gram-positives relative to Gram-negatives bacteria. AMP, antimicrobial peptides.

TABLE 1 The *trans*-acting regulatory RNAs associated with antibiotic resistance in Gram-negative and positive bacteria.

sRNA	Bacterial species	Mechanism(s) of resistance	Antibiotics[a]	Targets	Regulation	References
Gram-negative bacteria						
DsrA	*E. coli*	Active drug efflux	Oxacillin, erythromycin, novobiocin	*mdtEF*	+	43
ErsA	*P. aeruginosa*	Drug uptake	Meropenem	*oprD*	-	34
GvcB	*E. coli*		D-cycloserine	*cycA*	-	28
GlmY, GlmZ	*E. coli*, *Salmonella*	Cell wall changes	Bacilysin (tetaine), Nva-FMDP	*glmS*	+	62
MicA	*E. coli*	LPS changes	AMP	*phoPQ*	-	58
MicF	*E. coli*, *Salmonella*	Drug uptake	Cephalosporins, norfloxacin	*ompF*	-	33
MgrR	*E. coli*	LPS changes	Polymyxin B	*eptB*	-	55
NrrF	*N. gonorrhoeae*	Active drug efflux	Sulphonamides	*mtrF*	-	50, 52
RydC	*E. coli*, *Salmonella*	Active drug efflux	AMP, Microcin C	*yejABEF*	-	41
RybB	*E. coli*	Biofilm formation	EGCG	*csgD*	-	90
RyhB	*E. coli*	Drug uptake	Colicin Ia	*cirA*	+	30
SdsR (RyeB)	*E. coli*, *Salmonella*	Active drug efflux	Quinolones	*tolC*	-	33, 46, 47
Sr006	*P. aeruginosa*	LPS changes	Polymyxin	*pagL*	+	34
Sr0161	*P. aeruginosa*	Drug uptake	Meropenem	*oprD*	-	34
SroC	*Salmonella*	LPS changes	Polymyxin B	MgrR	-	56
3'ETS^{leuZ}	*E. coli*	Biofilm formation, drug uptake	Colicin Ia	RybB, RyhB	-	31
Gram-positive bacteria						
SprX (RsaOR)	*S. aureus*	Global effect	Glycopeptides	*spoVG*		68

[a]AMP, Antimicrobial peptide; EGCG, Epigallocatechin gallate (catechin from green tea); LPS, Lipopolysaccharide.