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Teaser Enhancing antibiotic activity offers an opportunity for innovative therapeutic strategies. This review examines the effects of non-antibiotic drugs on bacteria or antibiotic activity as a basis for future drug development.

Modulating antibiotic activity towards respiratory bacterial pathogens by co-medications: a multi-target approach

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Non-antibiotic drugs can modulate bacterial physiology and/or antibiotic activity, opening perspectives for innovative therapeutic strategies. Focusing on respiratory pathogens and considering *in vitro*, *in vivo*, and clinical data, here we examine the effect of these drugs on the expression of resistance mechanisms, biofilm formation, and intracellular survival, as well as their influence on the activity of antibiotics on bacteria. Beyond the description of the effects observed, we also comment on concentrations that are active and discuss the mechanisms of drug-drug or drug-target interactions. This discussion should be helpful in defining useful targets for adjuvant therapy and establishing the corresponding pharmacophores for further drug fine-tuning.

Introduction

The misuse and overprescription of antibiotics constitute a major health problem, by contributing to the emergence or selection of bacterial resistance [1]. Moreover, therapeutic options to act upon these multiresistant organisms become limited, because the number of novel molecules coming to market has decreased significantly over the past decade. Yet, several studies have demonstrated that non-antibiotic drugs[2] can also display antibacterial properties towards a range bacterial species via modes of action that are usually unrelated to their main pharmacological activity. In a nutshell, these molecules can inhibit bacterial resistance mechanisms (such as efflux), affect specific bacterial life modes (biofilm or intracellular persistence), and modulate tissue colonization or infection recurrence. They can also directly inhibit the expression and/or activity of several proteins vital for bacterial metabolism but that are not targeted by current antibiotics. Thus, these drugs can show synergistic effects with antibiotics or be themselves

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[2] For the sake of clarity, 'antibiotics' refer to drugs for which the primary approved indication(s) (as set in the corresponding label) are for treatment of infections through a static or a cidal effect on the offending bacteria. Conversely, 'non-antibiotics' refers to drugs for which the primary approved indication(s) are for non-infectious diseases (disregarding the effects they might exert directly or indirectly on bacteria, as discussed in this article).

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bactericidal. Therefore, understanding their antimicrobial properties could help rationalize their beneficial effects and, in a broader context, define novel targets for antibacterial therapy.

In this review, we focus on the modulation of the antibiotic activity by non-antibiotic drugs against bacterial species causing respiratory infections. These infections account for approximately 3/4 of antibiotic prescriptions [2], among which many are unjustified [3]. Moreover, some of these infections can be recurrent or persistent [4], requiring repeated administrations of antibiotics, thereby favoring the risk of emergence of bacterial resistance. Here, we focus on Gram-positive (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis*) and selected Gram-negative (*Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Chlamydiophila pneumoniae*, and *Escherichia coli*) bacterial species, discussing the different mechanisms (summarized in Figs. 1 and 2) by which non-antibiotic drugs might affect antibiotic activity *in vitro*, in experimental animals, or in the clinic.

Description and mechanisms of the antibacterial effect of non-antibiotic drugs

Inhibition of bacterial efflux pumps

Efflux pumps are ubiquitous transmembrane protein transporters present in all prokaryotic and eukaryotic cells. They exert physiological and protective roles by extruding out of the cell endogenous toxic compounds that are produced by cellular metabolism, or harmful exogenous compounds that have entered the cell, such as chemotherapeutic agents. Antibiotics represent typical opportunistic substrates for efflux transporters in bacteria [5].

According to their phylogeny, source of energy, number of transmembrane spanning regions, and substrate specificity, bacterial efflux pumps belong to one of five superfamilies: the resistance-nodulation-division (RND) family; the major facilitator superfamily (MFS); the ATP (adenosine triphosphate)-binding cassette (ABC) superfamily; the small multidrug resistance (SMR) family; and the multidrug and toxic compound extrusion (MATE) family [6]. By reducing the antibiotic concentration within the bacteria, active efflux can not only result in minimal inhibitory concentrations (MICs) that are higher than the clinical susceptibility breakpoint [5], but also select for mutations in genes encoding antibiotic targets (as commonly observed for fluoroquinolones), making them less able to bind the drug, often leading to high-level resistance [6].

In vitro, the expression of antibiotic efflux pumps is often inducible after exposure to subinhibitory concentrations of pump substrates [7–9]. Moreover, some non-antibiotic drugs, such as salicylates or benzodiazepines, have been shown to induce efflux pump expression in Gram-positive and Gram-negative bacteria by downregulating their repressors [10–12]. Conversely, several non-antibiotic drugs have shown an ability to act as inhibitors of efflux pumps *in vitro* (Table 1; see also [13] for a review on the main classes of efflux pump inhibitors, including those deriving from non-antibiotic drugs).

Thus, the antihypertensive calcium channel blocker verapamil inhibits the efflux of fluoroquinolones in *S. pneumoniae* and of bedaquiline and clofazime in *M. tuberculosis* planktonic cultures, causing a reduction in their MICs [14,15]. Verapamil also prevents the selection of mutations in the quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, and *parE* encoding the fluoroquinolone target enzymes DNA gyrase (*gyrA*/*gyrB*) and topoisomerase IV (*parC*/*parE*) in pneumococci, especially in efflux-positive isolates [14]. *In vivo*, verapamil reduces lung colonization by *M. tuberculosis* when combined with antibiotics [16].

All other efflux inhibitors belong to drugs acting on the central nervous system. Among these, phenothiazine [e.g., chlorpromazine, thioridazine, and prochlorperazine] and thioxanthene (e.g., *trans*-chlorprothixene and flupentixol) antipsychotics, tricyclic antidepressants (amitriptyline), and some serotonin selective reuptake inhibitor (SSRI) antidepressants (fluoxetine and paroxetine) inhibit efflux transporters present in different bacterial species (*S. aureus*, *S. pyogenes*, *P. aeruginosa*, and *K. pneumoniae*) and show synergistic activity when combined with antibiotics. The mechanisms responsible for this inhibition appear to be multifactorial. For example, it has been suggested that the ability of these drugs to inhibit the MFS transporter NorA, responsible for fluoroquinolone resistance in *S. aureus*, relies on a direct interaction with the pump as well as on a reduction in transmembrane potential [17]. In other cases, inhibition of efflux has been suggested to explain synergism with antibiotics, although this has yet to be experimentally documented and is largely improbable (see, for example, the synergy between chlorpromazine or amitriptyline with β-lactams against Gram-positive organisms suggested to occur by efflux inhibition [18,19] even though the β-lactam target is exposed at the bacterial surface and, thus, is readily accessible).

Most of these molecules have already been described as inhibitors of efflux pumps in eukaryotic cells, even though transporters expressed by eukaryotic cells belong to other phylogenic families [13]. This suggests that there are common features associated with the recognition of substrates and/or inhibitors between prokaryotic and eukaryotic transporters. It might also limit the use of these molecules *in vivo*, because of the risk of adverse effects associated with an unspecific inhibition of efflux. Moreover, in most cases, inhibitory concentrations are above those that can be reached with therapeutics (Table 1), preventing the use of these drugs as adjuvants in antibiotic treatment. However, further work might lead to the discovery of structural analogs in which the intrinsic pharmacological activity and the efflux inhibitory potency could be dissociated, with the latter being obtained at lower, clinically achievable concentrations.

Antibiofilm effects

Biofilms are 3D communities of sessile microorganisms adhering to a surface or interface and embedded in a matrix called the extracellular polymeric substance (EPS), most often hydrated and containing polysaccharides, proteins, extracellular DNA, and signaling molecules [20]. Biofilm formation is a multistep process, involving successively bacterial adhesion to a support (artificial implanted device or tissue), intensive matrix production, and release of bacterial cells, allowing for colonization of other surfaces. It is estimated that 60% of bacterial infections and up to 80% of chronic infections imply bacterial growth within biofilms [20].

In biofilms, bacteria are highly resistant to unfavorable living conditions, host defenses, and antibiotics. Specifically, a combination of factors contributes to the loss of antibiotic activity in biofilms [21]. First, matrix constituents, such as exopolysaccharides or extracellular DNA, can trap several antibiotics and,

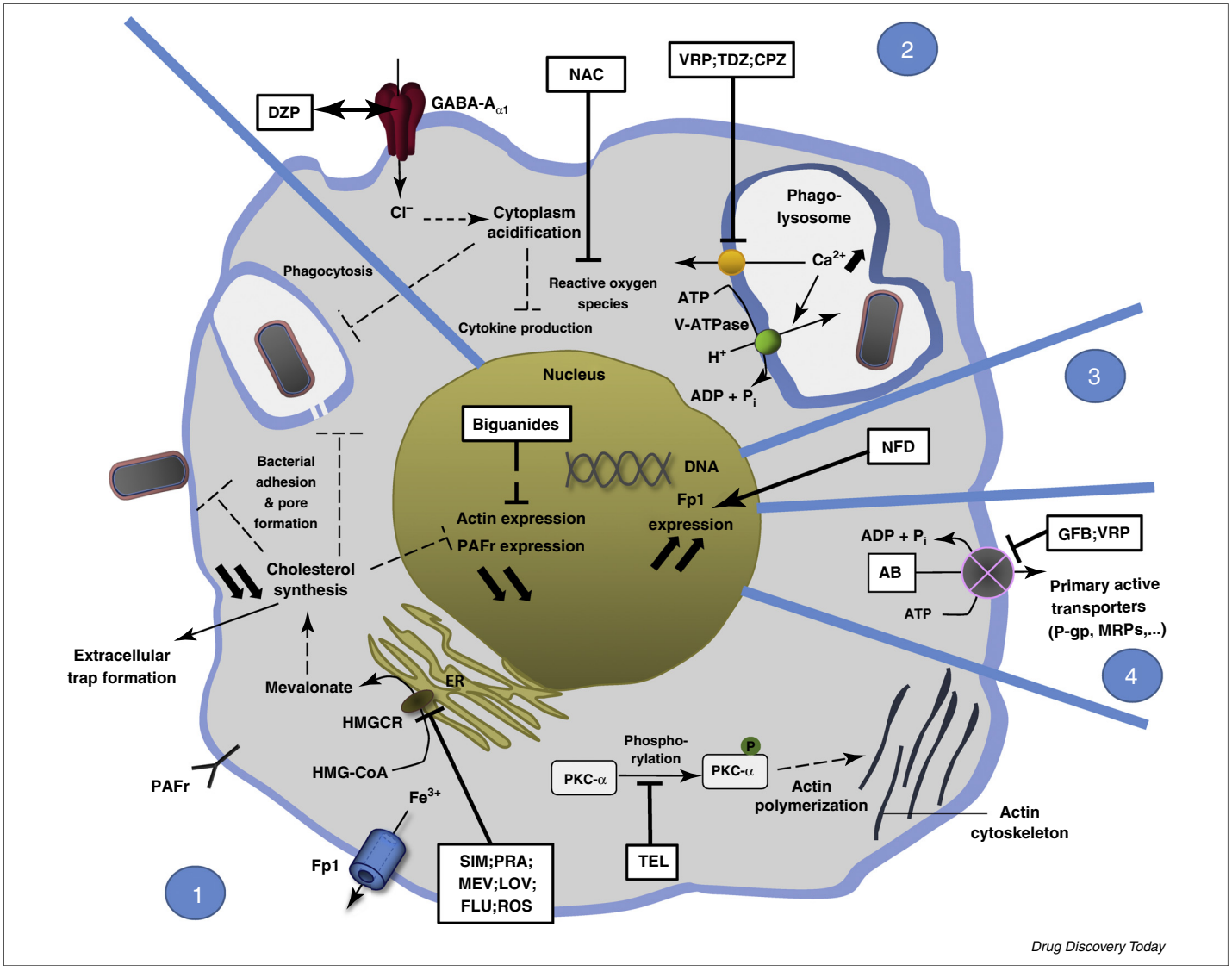

FIGURE 1

Modulation of the macrophage response to infections caused by respiratory bacterial pathogens and of antibiotic (AB) activity by non-antibiotic drugs. Thick lines refer to pathways that are increased or inhibited by non-antibiotic drugs represented by their acronyms (see hereunder and Tables 1–5). Plain lines show direct effects, and dotted lines show multi-step processes. The effects are grouped by numbers (1–4) according to each of the following main type of process(es) modified by the drugs. (1) Modulation of adherence and internalization. Telmisartan (TEL) impairs actin cytoskeleton formation by inhibiting Protein kinase C alpha (PKC-α) phosphorylation; statins [simvastatin (SIM), pravastatin (PRA), mevastatin (MEV)], lovastatin (LOV), fluvastatin (FLU), and rosuvastatin (ROS)] inhibit the eukaryotic hydroxymethylglutaryl-coenzyme A reductase (HMGCR) present at the membrane surface of the endoplasmic reticulum (ER), leading to a decrease in mevalonate levels required for cholesterol synthesis [cholesterol is important for bacterial adherence and internalization, activity of some bacterial pore-forming toxins, or the expression of genes encoding actin and platelet-activating factor receptors (PAFr)]; derivatives of biguanides reduce actin expression. (2) Modulation of cell defense mechanisms: through their ability to concentrate in phagolysosomes, the antihypertensive drug verapamil (VRP), and two antipsychotics [thioridazine (TDZ) and chlorpromazine (CPZ)] enhance the bactericidal effects of macrophages by inhibiting calcium (Ca^{2+}) efflux from phagolysosomes, which activates Ca^{2+}-dependent V-ATPases and leads to bacterial death through the acidification and activation of hydrolytic enzymes in phagolysosomes; conversely, diazepam (DZP) binding to the α1 subunit of GABA-A chloride (Cl^-) channel causes cytoplasmic acidification, which reduces intracellular bacterial killing and cytokine production; the antioxidant *N*-acetylcysteine (NAC) reduces intracellular bacterial killing mediated by oxygen reactive species. (3) Modulation of iron supply: nifedipine (NFD) enhances iron (Fe^{3+}) extrusion out of the cytoplasm by inducing the expression of ferroportin 1 (Fp1), thereby reducing tissue colonization and mortality during *in vivo* infections. (4) Modulation of antibiotic concentration: VRP and gemfibrozil (GFB) inhibit different types of eukaryotic primary active transporter, such as P-glycoprotein (P-gp) and multidrug resistance proteins (MRPs) responsible for the extrusion of some antibiotics, causing them to accumulate at increased levels in the eukaryotic cells.

therefore, limit their penetration within the biofilm. Second, the limited concentration of oxygen or nutrients inside biofilms reduces bacterial growth rate and slows their metabolism, making them insensitive to antibiotics acting on dividing bacteria. More specifically, subpopulations of persisters (i.e., bacterial cells characterized by a slow growth rate and tolerance to antibiotics) constitute a reservoir that can reactivate the infection once the antibiotic stress is no longer present. Third, the biofilm environment is favorable to horizontal gene transfer among bacteria or increases in the expression of specific mechanisms of resistance, such as efflux, making bacteria more resistant than in planktonic cultures.

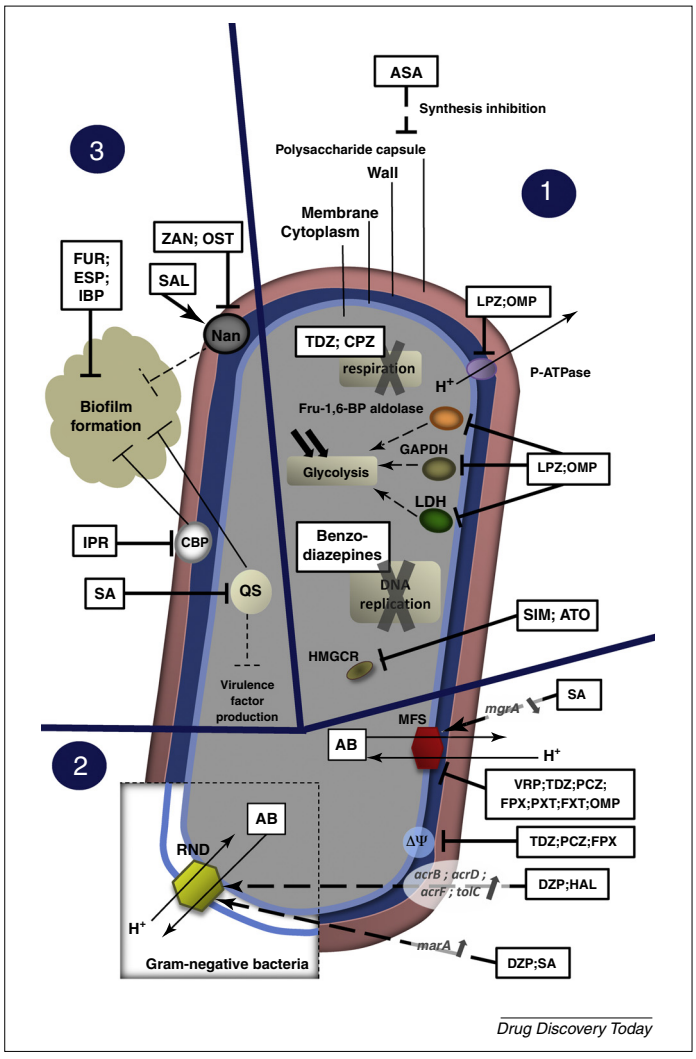

FIGURE 2

Influence of non-antibiotic drugs on bacterial survival, metabolism, expression of mechanisms of resistance to antibiotics, and ability to form biofilms. Thick lines refer to pathways that are increased or inhibited by non-antibiotic drugs represented by their acronyms (see hereunder and Tables 1–5). Plain lines show direct effects, and dotted lines show multi-step processes. The effects are grouped by numbers (1–3) according to each of the following main type of process(es) modified by the drugs. (1) Modulation of metabolic processes: two proton pump inhibitors, lansoprazole (LAN) and omeprazole (OMP) irreversibly inhibit the activity of fructose-1,6-biphosphate aldolase (Fru-1,6-BP), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and lactate dehydrogenase (LDH) through the formation of disulfide bridges; they also inhibit P-ATPases involved in proton extrusion, causing cytosol acidification and bacterial killing; statins [e.g., simvastatin (SIM) or atorvastatin (ATO)] inhibit bacterial hydroxymethyl-glutaryl-coenzyme A reductase (HMGCR) involved in bacterial isoprene synthesis; new benzodiazepine dimers inhibit DNA replication, whereas antipsychotics agents [thioridazine (TDZ) and chlorpromazine (CPZ)] inhibit bacterial respiration; acetylsalicylic acid (ASA) shows bactericidal properties against *S. pneumoniae* by interfering with the synthesis of bacterial capsular polysaccharide (CPS). (2) Modulation of active efflux: several antipsychotics (TDZ and CPZ), flupentixol [FPX]); antidepressants (paroxetine [PXT] and femoxetine [FXT]), calcium channel blockers [e.g., verapamil (VRP)], and proton pump inhibitors (e.g., OMP) are direct inhibitors of Major Facilitator Superfamily (MFS) transporters in *S. aureus*. TDZ, PCZ, and FPX also indirectly inhibit these transporters by interfering with the transmembrane electrical potential ($\Delta\psi$); conversely, diazepam (DZP), haloperidol (HPD), and salicylic acid (SA) increase the expression of Resistance Nodulation Division superfamily (RND) transporters in *Escherichia coli*, causing a reduction in the activity of antibiotic substrates; SA also induces the expression of some MFS pumps. (3) Modulation of biofilm formation: SA inhibits specific quorum sensing (QS) pathways, reducing the production of biofilm and virulence factors; the anticholinergic drug ipratropium (IPR), by its interaction with choline-binding proteins (CBPs), impairs the bacterial adherence needed for biofilm formation, as shown in *S. pneumoniae* models; the antiviral drugs zanamivir (ZAN) and oseltamivir (OST) reduce bacterial counts in the matrix of pneumococcal young biofilms by inhibiting the bacterial neuraminidase A (Nan A); conversely, the β2-agonist bronchodilator salbutamol (SAL) increases Nan A activity, resulting in a decrease in matrix cohesion and antibiotic activity towards pneumococcal biofilms; antibiofilm effect(s) have also been described for furosemide (FUR), esomeprazole (ESP), and ibuprofen (IBP), but the underlying mechanisms have not yet been well described.

Non-antibiotic drugs have been reported as offering new prophylactic or therapeutic strategies against biofilms *in vitro* as well as *in vivo*. Their effects include: (i) inhibition of the enzymatic activity of bacterial proteins involved in adhesion; (ii) decrease in matrix production; (iii) interference with quorum-sensing (QS) signaling; (iv) bactericidal effects within the biofilm matrix; or (v) a destabilizing and disassembling effect leading to a loss of mature biofilm adherence and thickness (Table 2).

Different non-antibiotic drugs have been reported as being able to prevent bacterial attachment to a support by the inhibition of adhesins. This is the case for ipratropium when added to a culture medium of pneumococcal biofilms, but only at supratherapeutic concentrations [22]. Through a structural analogy with choline, this anticholinergic compound can inhibit different choline-binding proteins, namely LytA amidase, LytC lysozyme, and Pce phosphoryl cholinesterase. These enzymes, anchored at the bacterial surface, display a modular organization with a highly conserved choline-binding module that allows the binding of phosphoryl choline residues [22]. They are involved in pneumococcal attachment to eukaryotic membranes and abiotic surfaces and, therefore, support cellular infection and biofilm formation [23]. Their inhibition by ipratropium is accompanied by a loss of pneumococcal adherence and growth and even of viability within biofilms at supratherapeutic concentrations [22]. The nonsteroidal anti-inflammatory drug (NSAID) ibuprofen also causes a loss of pneumococcal adherence *in vitro* at clinically relevant concentrations [24], although the underlying mechanism has yet to be elucidated. In *E. coli*, salicylate has also been shown to prevent its adherence, including at the surface of cells, by impairing the expression of fimbriae [25], an effect that is likely to be more relevant in the context of urinary tract infections.

In vivo studies have also shown that viral neuraminidase inhibitors used in the treatment of infections by Influenza viruses (i.e., oseltamivir and zanamivir, two sialic acid analogs), decrease nasopharynx colonization by pneumococcal biofilms [26]. The underlying mechanism has been elucidated *in vitro* and is directly related to their mechanism of action. By cleaving sialic acid residues, pneumococcal neuraminidase A (Nan A) induces the adherence of bacteria to epithelia, thus having an important role in biofilm matrix production, 3D structure, and cohesion [26,27]. Inhibition of this bacterial enzyme by sialic acid analogs results in a decrease in bacterial counts within the biofilm [26]. Conversely, it was recently shown that the short-acting β_2-agonist salbutamol increases NanA activity at clinically relevant concentrations, improving antibiotic *in vitro*-killing activity towards pneumococcal biofilms [28].

TABLE 1

Bacterial efflux pump inhibition.

Drug class	Modulatory molecules	Human plasma levels	Modulatory dose/ concentrations	Study model	Bacterial species	Main results[g]	Refs
Antihypertensive calcium channel blocker (phenylalkylamine)	Verapamil (VRP)	0.05–0.2 mg/l[a]	50 mg/l	*In vitro*	*S. pneumoniae*	Inhibition of efflux pump PmrA → ↑ intrabacterial ciprofloxacin accumulation → ↓ resistance and *pmrA* mutation rate	[14]
			50 mg/l	*In vitro*	*M. tuberculosis*	Inhibition of bedaquiline and clofazimine efflux → ↑ antibacterial activity	[15]
			9.4 mg/kg	*In vivo*	*M. tuberculosis*	Synergy with antibiotics → ↓ lung colonization	[16]
Antipsychotics (phenothiazines and thioxanthenes)	Chlorpromazine (CPZ), *trans*-chlorprothixene (t-CPT)	CPZ: 0.05–0.3 mg/l[a] t-CPT: 0.35–0.5 mg/l[b]; TDZ: 0.5–1 mg/l[c]; PCZ: 0.3 mg/l[d]; FPX: 0.002 mg/l[e]	3–300 mg/l	*In vitro*	*S. aureus*; *S. pneumoniae*; *P. aeruginosa*; *K. pneumoniae*, etc.	Synergy with aminoglycosides and β-lactams by inhibiting their efflux	[19]
	Chlorpromazine (CPZ), thioridazine (TDZ)		8–12 mg/l	*In vitro*	*S. aureus*; *S. pyogenes*	Synergy with oxacillin or erythromycin through efflux inhibition	[18]
	thioridazine (TDZ), prochlorperazine (PCZ), flupentixol (FPX)		4–22 mg/l	*In vitro*	*S. aureus*	Direct inhibition of efflux pump NorA and of transmembrane electrical potential →↓ proton motive force → inhibition of MFS efflux pumps (including NorA)	[17]
Tricyclic antidepressant	Amitriptyline	0.1–0.25 mg/l[a]	25–100 mg/l	*In vitro*	*S. aureus*; *P. aeruginosa*; *K. pneumoniae*, etc.	Synergy with aminoglycosides and β-lactams through efflux inhibition	[19]
SSRI antidepressants	Paroxetine, femoxetine	0.002–0.02 mg/l[b]	3–30 mg/l	*In vitro*	*S. aureus*	Direct inhibition of efflux pump NorA → synergy with norfloxacin	[82]
Proton pump inhibitors (PPIs) (benzimidazoles)	Omeprazole (OMP)	0.7 mg/l[f]	100 mg/l	*In vitro*	*S. aureus*	Direct inhibition of efflux pump NorA → synergy with norfloxacin and ciprofloxacin	[83]
			128 mg/l			Direct inhibition of efflux pump NorA through interaction between benzimidazole nucleus of omeprazole and NorA→ synergy with norfloxacin	[84]

[a] [85].
[b] [86].
[c] [87].
[d] [88].
[e] [89].
[f] [90].
[g] Symbols: ↓, reduces; ↑, increases; →, leads to.

Lastly, the mucolytic agent *N*-acetylcysteine decreases the synthesis of matrix polysaccharides by *K. pneumoniae*, reduces bacterial adherence, and modifies biofilm texture *in vitro* [29]. It also impairs the adhesion of *S. pneumoniae* and *H. influenzae* to human oropharyngeal epithelial cells *in vitro* [30] and to tonsils in humans [31]. However, the mechanism responsible for these effects has not yet been elucidated.

In *P. aeruginosa* biofilms, modulators of biofilm production mainly interfere with QS. Adherence of *P. aeruginosa* is reduced by approximately 50% on polymers coated with salicylic acid, the major *in vivo* metabolite of acetylsalicylic acid (aspirin). This effect is accompanied by inhibition of the *las* QS system, a major positive regulator of biofilm production [32]. Other studies confirmed the inhibitory effect of salicylic acid on *Pseudomonas* biofilm production, by demonstrating the decreased expression of *Pseudomonas* quinolone signal (PQS) and of LuxRI-type LasR QS systems-related genes, as well as a reduction in bacterial counts within the biofilms [33,34]. Likewise, aspirin also displays multiple effects on *P. aeruginosa* biofilms. At supratherapeutic concentrations (6 mg/ml), aspirin reduces virulence and QS signaling in *P. aeruginosa*,

TABLE 2

Antibiofilm effects.

Drug class	Modulatory molecules	Human plasma levels	Modulatory doses	Study model	Bacterial species	Main results[e,f]	Refs
Proton pump inhibitors (PPIs) (benzimidazoles)	Esomeprazole (ESP)	1.3–2.3 mg/l[a]	86 mg/l	*In vitro*	*S. aureus*; *P. aeruginosa*	Bactericidal effect and ↓ biofilm thickness and ↑ vancomycin (*S. aureus*) and meropenem (*P. aeruginosa*) killing activities	[36]
Neuraminidase inhibitor antiviral drug	Zanamivir (ZAN), oseltamivir (OST)	0.054–1.3 mg/l[b] (OST)	10–250 mg/l	*In vitro*	*S. pneumoniae*	Neuraminidase A inhibition → ↓ bacterial counts within biofilms	[26]
Neuraminidase inhibitor antiviral drug and β2-agonist bronchodilator	Zanamivir (ZAN), oseltamivir (OST)	0.054–1.3 mg/l[b] (OST) NA	1 mg/mouse	*In vivo*	*S. pneumoniae*	↓ nasopharynx biofilm colonization	[26]
			250 mg/l	*In vitro*	*S. pneumoniae*	Neuraminidase A inhibition → ↓ biofilm thickness	[28]
	Salbutamol (SAL)	7.25 mg/l in ELF after inhalation[c]	7.25 mg/l	*In vitro*	*S. pneumoniae*	Neuraminidase A activation → ↓ matrix cohesion → ↑ bactericidal effect on sessile cells	[22,28]
Anticholinergic bronchodilator	Ipratropium (IPR)	1.45 mg/l in ELF after inhalation[c]	822–8220 mg/l	*In vitro*	*S. pneumoniae*	CBP inhibition → ↓ pneumococcal adherence, growth and viability	
Anticholinergic bronchodilator NSAIDs	Ipratropium (IPR), ibuprofen (IBP)	1.45 mg/l in ELF after inhalation (IPR); 10–200 mg/l (IBP)[d]	1.45 mg/l	*In vitro*	*S. pneumoniae*	Biofilm disassembly → ↑ antibiotic activity	[28]
			128 mg/l	*In vitro*	*S. pneumoniae*	↓ pneumococcal adherence and, thus, biofilm formation	[24]
NSAID mucolytic drugs	Salicylic acid (SA)	100–400 mg/l (aspirin)[d]	8 mg/l	*In vitro*	*P. aeruginosa*, *E. coli*, etc.	↓ bacterial counts in planktonic cultures and within biofilms	[34]
			6 mg/ml	*In vitro*	*P. aeruginosa*	↓ QS signaling; ↓ gene expression (*lasI, lasR, rhlI, rhlR, pqsA* and *pqsR, exoS, exoY*), ↓ elastase and proteases expression, ↓ pyocyanin production, ↓ biofilm production and motility	[35]
			140 mg/l	*In vitro*	*E. coli*	↓ expression of fimbriae→ ↓ bacterial adherence	[25]
	Salicylic acid (SA), *N*-acetylcysteine (NAC)	100–400 mg/l (aspirin)[d] NA	300 mg/l	*In vitro*	*P. aeruginosa*	↓ bacterial adherence on SA-coated material; inhibition of QS (Las) → ↓ biofilm formation	[32]
			1600 mg/l	*In vitro*	*P. aeruginosa*	Inhibition of QS → ↓ pyoverdine and biofilm production	[33]
			250–500 mg/l	*In vitro*	*K. pneumoniae* and other bacterial species	↓ matrix polysaccharides synthesis, ↓ planktonic and sessile cells adherence + modification of biofilm texture → ↓ biofilm production	[29]

[a] [91].
[b] [92].
[c] [28].
[d] [85].
[e] *Abbreviations*: NA, not applicable (only concentrations in lower airways are relevant in these cases); CBPs, choline-binding proteins; Fru-1,6-BP, fructose-1,6-biphosphate; Pqs, *Pseudomonas* quinolone signal.
[f] Symbols, ↓, reduces; ↑, increases; →, leads to.

decreasing the expression of the *lasI, lasR, rhlI, rhlR, pqsA*, and *pqsR* genes (assessed by RT-PCR), *exoS* and *exoY* toxins, elastase and total proteases, and pyocyanin. Aspirin also impairs bacterial motility and biofilm production without affecting bacterial viability [35]. Molecular modeling suggests that QS inhibition results from the capacity of the aryl group of aspirin to interact with Tyr-88 of the LasR receptor by strong π–π stacking interactions, inducing a conformational change of the receptor–aspirin complex [35].

Antibiofilm properties and intrinsic bactericidal effect have also been described for the benzimidazole proton pump inhibitors (PPIs), which exert inhibitory effects on biomass and viability within biofilms formed by *S. aureus* and *P. aeruginosa* [36].

A possible mechanism for this effect has been demonstrated for the nonrespiratory pathogen *S. mutans* when exposed to acidic pH (pH ≤5). Under these conditions, PPIs inhibit bacterial P-ATPases (but not F-ATPases) as well as enzymes involved in glycolysis (i.e., aldolase, 3-P-glyceraldehyde dehydrogenase, and lactate dehydrogenase), by forming disulfide bridges with these enzymes after activation in their sulfenamide form [37,38]. Yet, in *P. aeruginosa*, benzimidazole effects on biofilms could also be mediated by their capacity to inhibit active efflux systems, which extrude *N*-acyl-homoserine lactones (AHL; auto-inducers of the QS) from the bacteria [39,40].

Nevertheless, the relevance of these observations in the clinic remains unclear, because the effects observed on biofilms were obtained at higher concentrations than those that can be reached at infection sites or in the blood upon administration of conventional dosages. However, this is not the case when considering the matrix disassembly effect exerted by the muscarinic antagonist ipratropium on *S. pneumoniae* biofilms, which is obtained at concentrations mimicking those found in the epithelial lining fluid after administration of a single dose by inhalation. Notably, this effect was also accompanied by a marked improvement in the activity of the fluoroquinolone moxifloxacin as well as the fluoroketolide solithromycin [28].

Effects on intracellular bacteria and cells of the immune system

A variety of bacterial species are capable of infecting and surviving within eukaryotic cells. Among the respiratory pathogens, these include *C. pneumoniae*, *Legionella pneumophila*, *L. monocytogenes*, *P. aeruginosa*, *M. tuberculosis*, *S. aureus*, *S. pneumoniae*, and *S. pyogenes* [41,42]. This strategy enables them to escape immune defenses and antibiotics, and, when specifically adapted to the intracellular medium, to proliferate using the eukaryotic cellular machinery. Thus, intracellular bacteria constitute a pathogenic reservoir and are implicated in infection recurrence and dissemination [43].

Several *in vitro* and *in vivo* studies (Table 3) have shown that non-antibiotic drugs are able to modulate bacterial internalization or survival within eukaryotic professional and nonprofessional phagocytes. These effects proceed from four independent mechanisms: (i) inhibition of bacterial adherence to host cells and invasion; (ii) inhibition of antibiotic efflux from macrophages, enhancing their cellular accumulation; (iii) alteration of the host cell microenvironment; and (iv) direct intracellular bactericidal activity of these drugs via diverse mechanisms.

In terms of bacterial adherence, telmisartan, an antihypertensive agent acting as an angiotensin II antagonist, inhibits *E. coli* adherence and internalization in human brain microvascular endothelial cells (HBMECs). This effect is related to the inhibition by telmisartan of protein kinase C-α phosphorylation, which resulted in the reduced polymerization of the cytoskeleton protein β-actin. This beneficial effect translated *in vivo* as the capacity of telmisartan to prevent *E. coli* meningitis in newborn mice [44]. Along the same lines, experimental derivatives of hypoglycemic biguanides impaired the infection of HeLa cells by *P. aeruginosa* and *S. aureus* by reducing β-actin expression [45]. A reduction in the invasion of HBMECs by *S. pneumoniae* was also observed in the presence of simvastatin, which downregulates platelet activating factor receptor (PAFr) expression [46]. A protective effect of statins was also described in murine models of pneumonia caused by several microorganisms. Lower mortality in statin-treated animals was attributed to a combination of antibacterial and anti-inflammatory effects, resulting in a reduction in lung and systemic bacterial colonization and lung histopathological damage together with a decrease in the expression of inflammatory mediators, such as tumor necrosis factor (TNF)-α, and interleukin (IL)-1β or IL-6, and in neutrophil infiltration [47–50]. These effects could be partly explained by the mechanism of action of these drugs, which, by reducing cholesterol levels, modulate a variety of cell functions. First, by inhibiting cholesterol synthesis in immune cells, statins enhanced the formation of extracellular traps by neutrophils and macrophages both *in vitro* and *in vivo* in a model of *S. aureus* murine pneumonia [51]. These traps comprise networks of extracellular fibers, primarily composed of DNA, histones, antimicrobial peptides, and proteases, which can retain pathogens and kill them via their enzymatic arsenal [51]. Second, by their depleting effect on cell cholesterol, statins can impair the activity of membrane-disrupting toxins, such as listeriolysin O of *L. monocytogenes* and the pneumococcal pneumolysin [46,52]. The latter effects were confirmed in mice, in which statin administration was shown to reduce tissue colonization and damage and prolong survival [46,52]. Finally, specific effects towards *M. tuberculosis* have also been described for statins. These drugs enhanced the function of immune cells (including in the absence of infection) and resistance to intracellular infections *in vitro* and decreased tissue colonization *in vivo* [50] by interfering with two main mechanisms: (i) an increase in autophagy though the induction of the expression of the gene encoding the light chain 3-II protein (LC-3-II), leading to increased autophagosome–lysosome fusion;, and (ii) promotion of phagosomal maturation by favoring the recruitment of the early endosomal antigen 1 (EEA-1) and the lysosome-associated membrane protein 3 (LAMP-3) [50]. Of interest, some of these effects were observed at concentrations relevant to those found in the plasma of patients treated for hypercholesterolemia [47]. Accordingly, clinical data (Table 4) suggest that the rate of mortality associated with community-acquired pneumonia is lower in patients receiving statins [53], but this protective effect remains a matter of debate [54] and was not confirmed in a recent meta-analysis [55].

Among drugs interacting with eukaryotic efflux pumps, the cholesterol-lowering drug gemfibrozil and the antihypertensive calcium channel blocker verapamil are well known as inhibitors of eukaryotic multidrug resistance proteins (MRP) and of P-glycoprotein (P-gp), respectively. Accordingly, they have been shown to increase the intracellular activity of antibiotic substrates for these efflux transporters, namely fluoroquinolones (for MRP) and macrolides or daptomycin (for P-gp), in models of macrophages infected by *L. monocytogenes or S. aureus* [56,57].

Iron is one of the environmental factors important for bacterial thriving, including intracellular proliferation. In this context, the calcium channel blocker nifedipine decreases the intramacrophage iron content *in vitro* and *in vivo* by upregulating the iron export protein Fpn1. This causes a slowdown of *C. pneumoniae* proliferation within macrophages *in vitro* as well as in the spleen or liver of infected animals [58]. Of note, these *in vitro* effects were observed at concentrations that were the same order of magnitude as those reached in the serum of patients receiving conventional doses of nifedipine (i.e., as needed for control of their hypertension).

TABLE 3

Intracellular antibacterial effects and activation of immune system cells.

Drug class	Modulatory molecules	Human plasma levels	Modulatory dose/ concentrations	Study model	Bacterial species	Main results[h,i]	Refs
Antihypertensive angiotensin II receptor antagonists	Telmisartan	0.045–1.2 mg/l[a]	20 mg/l	*In vitro*	*E. coli*	↓ PKC-α phosphorylation → inhibition of bacterial adherence and internalization in HBMECs	[44]
			5 mg/kg	*In vivo*	*E. coli*	Inhibition of meningitis, brain tissue-related lesions (cortex and meninges) and neutrophil infiltration	
Biguanides	Different derivatives under development	ND	128–1024 mg/l	*In vitro*	*S. aureus*; *P. aeruginosa*	Modulation of β-actin expression → ↓ bacterial adherence and internalization in HeLa cells	[45]
Sulfonylureas	Derivatives under development	ND	50 mg/l	*In vitro*	*M. tuberculosis*	Bactericidal effect within THP-1 macrophages	[59]
Fibrates	Gemfibrozil	15–25 mg/l[b]	62 mg/l	*In vitro*	*L. monocytogenes*	Inhibition of efflux by MRPs in macrophages → ↑ intracellular ciprofloxacin accumulation and activity	[56]
Antihypertensive calcium channel blockers	Verapamil (VRP) (phenylalkylamine)	0.05–0.2 mg/l[c]	9.1 mg/l	*In vitro*	*S. aureus*; *L. monocytogenes*	Inhibition of efflux by P-gp in macrophages → ↑ intracellular azithromycin accumulation and activity	[56]
			45.5 mg/l	*In vitro*	*S. aureus*	Inhibition of efflux by P-gps in macrophages and MDCK epithelial cells → ↑ intracellular potency of daptomycin	[57]
			80 mg/l	*In vitro*	*M. tuberculosis*	Inhibition of Ca^{2+} extrusion from phagolysosomes in HPBMDM → activation of V-ATPases → massive H^+ entry in phagolysosomes → activation of hydrolytic enzymes → bactericidal effect	[60]
	Nifedipine (NFD) (dihydropyridine)	0.10–0.13 mg/l[d]	0.09–34.6 mg/l	*In vitro*	*C. pneumoniae*	Induction of ferroportin 1 expression in RAW264.7 murine macrophage-like cells → ↓ cytoplasmic iron amount available for intracellular bacteria → ↓ growth and proliferation	[58]
			5 mg/kg	*In vivo*	*C. pneumoniae*	↓ serum and splenic iron amounts → ↓ liver and spleen colonization and mouse death	[58]
Antipsychotics (phenothiazines)	Thioridazine (TDZ) and derivatives	0.5–1 mg/l[e] (TDZ) 0.05–0.3 mg/l[b] (CPZ)	0.1 mg/l	*In vitro*	*M. tuberculosis*	Phenothiazines accumulation in phagolysosomes of HPBMDM → cell acidosis and bacterial killing	[60]
	Thioridazine (TDZ)		32 mg/kg	*In vivo*	*M. tuberculosis*	Intracellular accumulation of TDZ → alteration of bacterial membrane integrity and interference with Ca^{2+} transport → bactericidal effect + ↓ pulmonary tissue colonization	[61]
	Thioridazine (TDZ) and chlorpromazine (CPZ)		0.1 mg/l	*In vitro*	*S. aureus*	Phenothiazine accumulation in lysosomes of THP-1 macrophages and HPBMDMs → alteration and blebbing of phagocytosed *S. aureus* cell wall → bactericidal effect	[62]
	Chlorpromazine (CPZ)		0.1 mg/l	*In vitro*	*S. aureus*	CPZ accumulation in lysosomes of THP-1 macrophages and HPBMDMs → bactericidal effect occurring upon fusion of infected phagosomes with lysosomes	[63]

TABLE 3 (*Continued*)

Drug class	Modulatory molecules	Human plasma levels	Modulatory dose/ concentrations	Study model	Bacterial species	Main results[h,i]	Refs
Statins	Simvastatin (SIM), mevastatin (MEV), lovastatin (LOV), fluvastatin (FLU)	0.01–0.03 mg/l[f] (SIM) 0.007–0.011 mg/l[g] (MEV); 0.01–0.02 mg/l[f] (LOV); 0.45 mg/l[f] (FLU) 0.03–0.04 mg/l [f] (ROS); 0.045–0.055 mg/l [f] (PRA)	4–20 mg/l	*In vitro*	*S. aureus*,; *S. pneumoniae*; *S. agalactiae*; *S. typhimurium*	↑ extracellular trap formation by phagocytic cells dependent on eukaryotic HMG-CoA reductase inhibition → bactericidal effect	[51]
	Simvastatin (SIM)		500 mg/kg	*In vivo*	*S. aureus*	↓ lung tissue colonization and bactericidal effect	[51]
			21 mg/l	*In vitro*	*M. tuberculosis*	Inhibition of eukaryotic HMG-CoA reductase → ↓ cholesterol synthesis → ↑ autophagy and phagosome maturation → ↓ infection in PBMCs and MDMs	[50]
			42 mg/l	*In vitro*	*L. monocytogenes*	(i) ↓ actin tails formation + ↓ cholesterol synthesis → ↓ pore formation by listeriolysin O → ↓ bacterial escape from vacuole to eukaryotic host cell cytoplasm → ↓intracellular proliferation in BMDMs and RAW264.7 murine macrophage-like cells; (ii) ↑ TNF-α and IL-12p40 production by macrophages → proinflammatory effect	[52]
			0.42 mg/l	*In vitro*	*S. pneumoniae*	(i) ↓ bacterial adherence to HBMECs cells and ↓ pore formation by pneumolysin; (ii) PAFr expression involved in cell invasion	[46]
			1 mg/kg	*In vivo*	*S. pneumoniae*	↓ pneumococcal adherence and PAFr expression → ↓ lung tissue and blood colonization and damage; modulation of cytokine production → ↑ mouse survival	[46]
			0.5 mg/kg	*In vivo*	*C. pneumoniae*	↓ lung tissue colonization and anti-inflammatory effect	[49]
			0.25 mg/kg	*In vivo*	*S. aureus*	↓ lung tissue colonization and anti-inflammatory effect → ↑ mouse survival	[47]
			120 mg/kg = 10 mg/kg/day	*In vivo*	*S. pneumoniae*	↓ blood and lung tissue colonization and anti-inflammatory effect → ↑ mouse survival	[48]
	Simvastatin (SIM), rosuvastatin (ROS)		20 mg/kg/day	*In vivo*	*M. tuberculosis*	↓ tissue (lung, spleen, liver) colonization	[50]
	Simvastatin (SIM), pravastatin (PRA)		10 and 20 mg/kg/day (1); 2 & 10 mg/kg/day (6)	*In vivo*	*L. monocytogenes*	↓ serum cholesterol in infected mice and ↓ tissue (spleen, liver) colonization	[52]
	Pravastatin (PRA)		50 and 100 mg/kg	*In vivo*	*S. pneumoniae*	↓ lung tissue colonization + ↑ mice survival	[93]
	MEV		2 mg/l	*In vitro*		↓ intracellular (murine J774 A1 macrophages) proliferation	[93]
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Acetylsalicylic acid (ASA)	100–400 mg/l (aspirin)[c]	300 mg/l	*In vitro*	*K. pneumoniae*	↓ CSP production → direct bactericidal effect and facilitation of leucocyte phagocytosis → ↓ bacterial proliferation	[65]

[a] [94].
[b] [95].
[c] [85].
[d] [96].
[e] [87].
[f] [97].
[g] [98].
[h] *Abbreviations*: ND, not determined (no current clinical use: compounds under development); MDCK: Madin-Darby Canine Kidney cells; PKC-α, protein kinase C-α; HPBMDM, human peripheral blood monocyte-derived macrophages; PBMCs, peripheral blood mononuclear cells; MDMs, monocyte-derived macrophages; BMDMs, bone marrow-derived macrophages; CSP, capsular polysaccharide.
[i] Symbols: ↓, reduces; ↑, increases; →, leads to.

TABLE 4

Antibacterial effects reported in clinical studies.

Drug class	Modulatory molecules	Human plasma levels	Modulatory dose/ concentrations	Study type	Infection	Main results[d]	Refs
Statins	Unspecified	–	Unspecified	Retrospective clinical study	Community-acquired pneumonia	↓ infection-associated mortality and pleural effusion, independently on activity of co-administered antibiotics	[53]
	Unspecified	–		Prospective cohort clinical study	Pneumonia	↓ bacteremia, independently on statin anti-inflammatory properties and activity of co-administered antibiotics	[54]
	SIM, atorvastatin (ATO)	0.01–0.03 mg/l[a] (SIM) 0.03–0.07 mg/l[a] (ATV)		Meta-analysis clinical study	Sepsis	No relation between statin intake and sepsis-related mortality	[55]
Antipsychotics (phenothiazines)	Thioridazine (TDZ)	0.5–1 mg/l[b]	Initial daily dose: 25 mg/day for 2 weeks. Thereafter, doses increased by 25 mg weekly until 200 mg/day	Retrospective clinical study	*M. tuberculosis*	Negative cultures after co-administration with linezolid, moxifloxacin and other anti-TB agents	[76]
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Acetylsalicylic acid (ASA)	100–400 mg/l[c]	300 mg/l	Prospective interventional cohort clinical study	*K. pneumoniae*	↓ risk of invasive syndrome	[65]

[a] [97].
[b] [87].
[c] [85].
[d] Symbol: ↓: reduces.

In terms of the direct bactericidal effects of non-antibiotic drugs, derivatives of antidiabetic sulfonylureas inhibited the intracellular multiplication of drug-resistant *M. tuberculosis* in a model of activated human THP-1 macrophages by impairing the activity of the acetohydroxyacid synthase, an enzyme catalyzing the first step in the biosynthesis pathway of branched-chain amino acids [59]. As well as its effect on efflux pumps, verapamil also kills intraphagocytic *M. tuberculosis*. This effect is possibly the consequence of an inhibition of Ca^{2+} (and/or K^{+}) transport, causing subsequent vacuolar acidification and activation of Ca^{2+}-dependent V-ATPases and hydrolytic enzymes [60]. The same mechanism is evoked to explain the activity of phenothiazines such as thioridazine (a structural analog of verapamil) against mycobacteria in the same model or *in vivo* [60,61] or against intracellular *S. aureus* [62,63]. Another study suggested that phenothiazines also inhibit *M. tuberculosis* respiration [64].

Lastly, human therapeutic anti-inflammatory concentrations of acetylsalicylic acid killed *K. pneumoniae* in leucocytes [65]. This effect was mediated by the inhibition of the bacterial capsular polysaccharide (CPS) biosynthesis, which is involved in adherence to host cells and internalization, which might facilitate the phagocytosis process. It could also decrease the risk of *K. pneumoniae* invasive syndrome in patients receiving this drug [65].

Intrinsic antibacterial effect and synergy with antibiotics

In addition to these specific mechanisms, some drugs are reported as displaying direct toxic effects for bacteria or synergism with antibiotics (Table 5).

As well as their indirect effects on biofilms, intracellular infection, and colonization described in the previous sections, statins can exert antibacterial effects against specific bacteria but at non-clinically relevant concentrations. In staphylococci and *L. monocytogenes*, simvastatin, fluvastatin, atorvastatin, and rosuvastatin inhibit the bacterial hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase, causing a reduction in isoprene biosynthesis, leading to a reduction in the bacterial growth and bacterial death [66–68]. In pneumococci, the observed bactericidal effect of statins has not been ascribed to an impairment of lipid synthesis but rather to a detergent-like activity on bacterial membranes related to their hydrophobic character [69]. However, synergic *in vivo* effects have already been demonstrated for simvastatin, in combination with the first-line regimen (isoniazid/rifampicin/pyrazinamide) in mice infected with *M. tuberculosis* [70].

Among antihypertensive dihydropyridines, lacidipine proved bactericidal against planktonic cultures of different bacterial species (*S. aureus, E. coli, K. pneumoniae*, and *P. aeruginosa*) [71]. Two other analogs, nifedipine and amlodipine, were synergic *in vitro* with antibiotics from different classes (β-lactams, macrolides, and aminoglycosides) against *E. coli* and *S. aureus*, respectively [72–74]. Although no specific mechanism has been proposed to explain this effect, the presence of aromatic cycles (and of an halogen atom on amlodipine) is thought to have a role in the effects observed [74].

Among the antipsychotic drugs, promethazine and clomipramine proved synergistic *in vitro* with ampicillin, tetracycline, and erythromycin against *E. coli* [75]. More relevantly, a retrospective

TABLE 5

Intrinsic antibacterial effect and synergy or antagonism with antibiotics.

Drug class	Modulatory molecules	Human plasma levels	Modulatory dose/ concentrations	Study model	Pathogen(s)	Main results[j,k]	Refs
Intrinsic antibacterial effect and synergy							
Antihypertensive calcium channel blocker	Lacidipine (LCD) (dihydropyridine)	0.002–0.02 mg/l[a]	10–200 mg/l	*In vitro*	*S. aureus*; *E. coli*; *K. pneumoniae*; *P. aeruginosa*	Bactericidal effect	[71]
	Nifedipine (NFD) (dihydropyridine);	0.10–0.13 mg/l[b]	5 mg/l	*In vitro*	*E. coli*	Synergy with antibiotics	[72]
	Amlodipine (AML) (dihydropyridine)	0.003–0.005 mg/l[c]	10–200 mg/l	*In vitro*	*S. aureus*	Bactericidal effect	[73]
			6.25–200 mg/l	*In vitro*	*S. aureus*	antibacterial effects and ↑ streptomycin activity	[74]]
Antipsychotics (phenothiazines)	Promethazine (PMZ), clomipramine (CLI)	0.01–0.02 mg/l[d] (PMZ), 0.033–0.063 mg/l[e] (CLI), 0.5–1 mg/l[f] (TDZ), 0.05–0.3 mg/l[g] (CPZ)	8–32 mg/l	*In vitro*	*E. coli*; *S. epidermidis*	Synergy with antibiotics	[75]
	Thioridazine (TDZ), chlorpromazine (CPZ)		4–32 mg/l	*In vitro*	*M. tuberculosis*	Inhibition of bacterial respiration → bactericidal effect.	[64]
Benzodiazepines	Pyrrolobenzodiazepine dimers under development	ND	0.025–0.455 μM	*In vitro*	*S. aureus*	Covalent binding to bacterial DNA → inhibition of DNA replication → bactericidal effect	[99]
Statins	Simvastatin (SIM)	0.01–0.03 mg/l[h] (SIM), 0.03–0.07 mg/l[h] (ATO)	60 mg/kg	*In vivo*	*M. tuberculosis*	Synergy with isoniazid/rifampicin/ pyrazinamide	[70]
			15 mg/l	*In vitro*	*S. pneumoniae*; *M. catarrhalis*	Bactericidal effect, independently on bacterial HMG-CoA reductase inhibition	[69]
			29–75 mg/l	*In vitro*	*S. aureus*; (MRSA, MSSA)	Antibacterial activity at 29–75 mg/l (MICs), possibly mediated by inhibition of bacterial HMG-CoA reductase implicated in isoprene biosynthesis	[66]
	Simvastatin (SIM), atorvastatin (ATO)		15–74 mg/l	*In vitro*	*S. aureus* (MRSA, MSSA), *S. epidermidis*, etc.		[67]
	Rosuvastatin (ROS)	0.03–0.04 mg/l[h] (ROS)	500–2000 mg/l	*In vitro*	*S. aureus*; *L. monocytogenes*	Antibacterial activity at 500–2000 mg/l mediated by inhibition of bacterial HMG-CoA reductase implicated in mevalonate and isoprene biosynthesis. Antibacterial activity of ROS abolished through addition of exogenous mevalonate in culture medium	[68]
Antagonism							
Antihypertensive calcium channel blocker	Verapamil (VRP) (phenylalkylamine)	0.05–0.2 mg/l[e]	1250 mg/l	*In vitro*	*E. coli*	↓ ampicillin activity towards planktonic cultures	[75]

Mucolytic drug	*N*-acetylcysteine (NAC)	0.35–4 mg/l[h]; no data on pulmonary concentration after administration by nebulization	1.6 mg/l	*In vitro*	*P. aeruginosa, K. pneumoniae, E. coli*	↓ activity of many antibiotics towards planktonic cultures	[78]
			4 mg/l	*In vitro*	*S. aureus*	antioxidant effect → ↓ production of ROS by phagocytes → ↓ antibiotic killing activity	[77]
NSAID	Salicylic acid (SA)	100–400 mg/l (aspirin)[e]	320 mg/l	*In vitro*	*S. aureus*	Downregulation of *mgrA* and *sarR* → increase in NorA and NorB efflux pump expression and intrinsic bacterial resistance → ↓ activity of ciprofloxacin and fusidic acid	[10]
			14–690 mg/l	*In vitro*	*E. coli*	Induction of Mar phenotype (AcrAB-TolC efflux pump induction + porin loss) → ↓ intrabacterial antibiotic concentration → resistance	[25]
			800–3200 mg/l	*In vitro*	*K. pneumoniae, E. coli*	Induction of Mar phenotype (AcrAB-TolC efflux pump induction + porin loss) → ↓ intrabacterial antibiotic concentration → resistance	[11]
			80 mg/l	*In vitro*	*M. tuberculosis*	↓ killing activity of many antibiotics towards planktonic cultures	[79]
			320 mg/l	*In vitro*	*S. aureus*	↓ ciprofloxacin and fusidic acid activities towards planktonic cultures, independently of NorA activity	[100]
Benzodiazepines	Diazepam (DZP)	0.2–1.5 mg/l[e]	34–142 mg/l	*In vitro*	*K. pneumoniae, E. coli*	Induction of Mar phenotype (AcrAB-TolC efflux pump induction and porin loss) → ↓ intrabacterial antibiotic concentration → resistance	[11]
			71.2 mg/l	*In vitro*	*E. coli*	Induction of a MDR phenotype by induction of RND efflux pumps → resistance to fluoroquinolones	[80]
			2 mg/kg	*In vivo*	*S. aureus*	↑ α1-$GABA_A$ signaling → macrophages acidosis → ↓ cytokine production, phagocytosis and killing of bacteria	[81]
Antipsychotics (phenothiazines)	Haloperidol	0.005–0.02 mg/l[e]	150 mg/l	*In vitro*	*E. coli*	Induction of MDR phenotype by induction of RND efflux pumps → resistance to fluoroquinolones	[80]

[a] [101].
[b] [96].
[c] [102].
[d] [103].
[e] [85].
[f] [87].
[g] [85].
[h] [97]. [i] [104].
[j] *Abbreviation*: ND: not determined (no current clinical use: compounds under development).
[k] Symbols: ↓, reduces; ↑, increases; →, leads to.

clinical study [76] pointed to the efficacy of thioridazine combined with linezolid and other antibiotics in patients with MDR TB (Table 5). In this study, the authors described a population of 17 individuals treated with linezolid for their *M. tuberculosis* infection. Among them, 14 received co-administration of thioridazine with or without additional antibiotics (moxifloxacin, ethambutol, 4-aminosalicylic acid, or cycloserine). The initial thioridazine daily dose was 25 mg for 2 weeks, thereafter increased to 200 mg/day under strict cardiac monitoring to detect possible adverse events. The patients remained hospitalized for at least 2 months or until a negative bacterial sputum culture, which was achieved in 15 patients. Thioridazine had to be discontinued in 2 patients. Although the evidence for an anti-TB effect of thioridazine is weak, this study was the first to report the administration of this antipsychotic drug to humans for anti-infectious purposes.

Antagonism with antibiotic activity

As opposed to the beneficial effects described above, a decrease in activity can also be observed when antibiotics are combined with non-antibiotic drugs (Table 5). Intriguingly, most of the drugs showing deleterious effects belong to the same class or are even the same molecules as those demonstrating favorable effects, probably pointing to the importance of the model used.

In some cases, no mechanism has been proposed to explain this antagonism. This is the case for verapamil, which, at high concentrations, reduces *E. coli* susceptibility to ampicillin in planktonic cultures [75]. In other cases, negative modulation of host cell defense mechanisms and/or induction of efflux-mediated resistance have been demonstrated. Thus, for instance, the mucolytic agent *N*-acetylcysteine is well known for its antioxidant properties. Therefore, it is not surprising that it can reduce the amount of reactive oxygen species (ROS) produced by macrophages, which complements the activity of antibiotics to obtain the fast and complete eradication of the bacteria. Accordingly, a decrease in intracellular potency has been observed for gentamicin and moxifloxacin when combined with *N*-acetylcysteine against intracellular *S. aureus* [77]. In the same line, *N*-acetylcysteine has also been shown to reduce the activity of aminoglycosides and fluoroquinolones against *P. aeruginosa, K. pneumoniae*, and *E. coli* in broth [78].

As opposed to gemfibrozil or verapamil, which act as inhibitors of efflux transporters, salicylate has been described as an inducer of these transporters, causing resistance in *S. aureus* by downregulating the expression of *mgrA*, a negative regulator of the genes encoding the efflux pumps NorA, NorB, NorC, and Tet38 [10]. In addition, salicylate also downregulates the expression of *sarR*, a repressor of the expression of *sarA*, which itself enhances intrinsic antimicrobial resistance in *S. aureus* [10]. Similarly, salicylate also induces a multiresistant phenotype in *K. pneumoniae* and *E. coli* by inducing the *mar* operon regulating the expression of active efflux systems and porins [11]. It confers resistance to a series of anti-TB agents, such as isoniazid, rifampin, ethambutol, streptomycin, and p-amino salicylate, by an unknown mechanism [79]. These effects were observed for salicylate concentrations relevant to those found in the serum of patients treated with acetylsalicylic acid for its fever-, pain-, and inflammation-limiting properties. Among other drugs inducing efflux, the antipsychotic haloperidol and the benzodiazepine diazepam reduce susceptibility to fluoroquinolones in *K. pneumoniae* and *E. coli* [11,80].

Finally, a recent *in vivo* study in a model of murine pneumonia demonstrated deleterious effects of the benzodiazepine diazepam on bacterial load and mortality. These were attributed to the binding of diazepam to the (α1-γ2) $GABA_A$ receptors present at the surface of macrophages and monocytes. The subsequent increase in $GABA_A$ signaling acidifies the intracellular milieu, leading to impaired cytokine production, bacterial phagocytosis, and killing [81]. Of note, this effect seems to be specific to diazepam because other benzodiazepines that do not bind to the α1-$GABA_A$ subunit of the receptor do not show this effect [81].

Concluding remarks

Non-antibiotic drugs exert a plethora of beneficial effects by acting against respiratory tract pathogens or by stimulating the host response to the infection, which also results in the increased activity of antibiotics. Although not systematically explained at the molecular level, these useful collateral effects probably result from three main distinct features of these drugs. Some of the described effects are directly or indirectly related to the pharmacological action of these drugs. This is clearly exemplified by statins, which can block isoprene synthesis in bacteria; calcium channel blockers, which modulate Ca^{2+} levels inside bacteria; or inhibitors of viral neuraminidase, which can also impair the activity of the corresponding enzyme in pneumococci. Likewise, PPIs impair the activity of specific bacterial enzymes by binding to their active site through the formation of disulfide bridges at acidic pH (i.e., a mechanism similar to that responsible for their pharmacological effect on gastric proton pumps). Conversely, by acting at the level of the host, *N*-acetylcysteine counteracts the oxidative burst via its antioxidant properties and, therefore, is detrimental. Other effects can be explained by the similarity of structure between the drugs and specific substrates for bacterial enzymes or sensor proteins. Thus, non-antibiotic drugs appear in these cases to be opportunistic binders and/or stimulators of the expression of bacterial proteins. As examples, (i) the muscarinic antagonist ipratropium destructures biofilms because it can interact with choline-binding proteins, and (ii) aspirin, salicylic acid, or NSAID induce the expression of efflux pumps by modulating the corresponding regulatory cascades. Lastly, the general physicochemical properties of the drugs rather than their specific chemical features can also explain positive as well as negative collateral effects on host cells or on bacteria. Weak basic compounds, such as agonists or antagonists of central nervous system receptors, accumulate in lysosomes, which increases the local pH and, therefore, interferes with killing mechanisms. The same molecules often harbor aromatic rings and a globally amphiphilic character, which explains why they are not only substrates, but also inhibitors of efflux transporters expressed by both bacterial and eukaryotic cells.

Perspectives for future research

Moving forward into the new millennium, the control of bacterial infectious diseases is becoming an alarming challenge. Resistance is increasing at a quicker rate than the laborious process from discovery to development, registration, and, ultimately delivery of new drugs to the clinic, can achieve. In this context, alternative strategies, such as the combination therapy of existing antibiotics

with other compounds that can restore or increase their activity, could fill a worrying gap.

Many researchers have considered that looking for the potentiation of antibacterial effects by registered non-antibiotic drugs would offer the advantage, in cases of demonstrated synergy, of accelerating and/or even skipping the long process needed to bring a drug to market. Positioning an existing drug in a new indication would nevertheless impose the appropriate preclinical and clinical development requested by registration authorities to document their efficacy and safety at the effective dose. In this context, we have stressed here that, in most cases, these modulatory effects were observed at concentrations above those reached in the conditions of use of these drugs in approved clinical use. This is not surprising, given that the discovery of the antibacterial effect is in general fortuitously made by screenings in *in vitro* experimental settings. Thus, we appear to be far away from the direct exploitation in the clinic of the benefits evidenced in these studies. Nevertheless, such studies provide an impressive amount of encouraging data suggesting that modulating antibiotic activity or bacterial pathogenicity without killing bacteria is feasible. These pioneer studies clearly support interest in specific targets for adjuvant therapies and stimulate research into more specific and potent modulators thereof. Moreover, careful examination of the chemical structure of active molecules could serve as a basis for delineating pharmacophores and, therefore, constitutes a starting point for investigations of more active molecules devoid of their initial pharmacological activity with the help of the powerful tools of modern pharmacochemistry. Reversing the initial assumption that using already registered drugs would enable the rapid implementation of their use in antibacterial chemotherapy, we suggest that dissociating antibacterial and other pharmacological effects is in fact mandatory to limit the risk of adverse effects directly related to interactions with other targets. A dual action would be acceptable only for non-antibiotic drugs that are indicated for the treatment of the symptoms associated with the infection.

In a few specific cases, antibacterial effects were observed at clinically relevant concentrations and, thus, might have direct clinical implications. As stated above, this is of immediate interest when the co-administered drug has an indication for controlling a pathology observed in infected patients. This is clearly the case for bronchodilators, which are synergistic with antibiotics against pneumococcal biofilms, because these drugs are currently recommended for the control of bronchial constriction in patients with chronic obstructive pulmonary disease experiencing infectious exacerbations. The impact of NSAIDs that are also often administered to infected patients as fever relievers is more difficult to define. Indeed, at clinically achievable concentrations, these drugs prevent biofilm formation *in vitro* [24,32–34] and improve the outcome of *K. pneumoniae* invasive syndrome in patients [65], but, conversely, can also induce the expression of antibiotic efflux transporters [10,11]. This underlines the necessity of conducting well-designed animal and/or clinical studies to further document their potential added value for infected patients in addition to their main pharmacological activity.

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