We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

4,000
Open access books available

116,000
International authors and editors

120M
Downloads

154
Countries delivered to

Our authors are among the
TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 19

Modulation of Biofilm Growth by Sub-Inhibitory Amounts of Antibacterial Substances

Stoyanka R. Stoitsova,
Tsvetelina S. Paunova-Krasteva and
Dayana B. Borisova

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62939

Abstract

It is generally accepted that bacteria in biofilm are more resistant to antibacterials than their planktonic counterparts. For numerous antibiotics, it has been shown that minimal inhibitory concentrations (MICs) for bacteria grown in broth are much lower than the minimal biofilm inhibition concentrations. While sub-inhibitory concentrations, that is, amounts of antibacterials below the MIC, do not either influence or suppress to some extent or other the bacterial growth in liquid media, these same amounts of drugs, natural substances, etc., may have diverse effects on bacterial biofilms, ranging from suppression to stimulation of the sessile growth and varying with regard to the bacterial species and strains. This is a source of additional risks for both biofilm infection of host tissues and contamination indwelling devices. When considering the data for biofilm modulation, differences in experimental protocols should be taken into account, as well as the strain-specific mechanisms of biofilm formation.

Keywords: biofilm, sub-MIC, antibiotics, bacteriocins, antimicrobial peptides, plant metabolites

1. Introduction

While the development of antibiotics during the twentieth century resulted in remarkable advances in the fight against infectious microorganisms, it was unfortunately paralleled with the highly increasing risks for the development of antibiotic resistance. These risks are a consequence of the extensive use of antibacterial preparations in both human medicine and agriculture. Resistance has become a threat to human and animal health worldwide, and it

INTECH
open science | open minds

© 2016 The Author(s). Licensee InTech. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

necessitates the development of key measures. Among these, the identification of critical points of control, the development of surveillance measures, and the prevention of environmental contamination are in focus [1].

In the aquatic and terrestrial environments, the contaminated sites (wastewater systems, pharmaceutical factories effluents, animal husbandry facilities, etc.) are characterized by the presence of subtherapeutic concentrations of antibiotics [1–3]. Thus, bacteria present in the environment are often subjected to drug amounts lower than the minimal inhibitory concentrations (MICs) [4]. Antimicrobial sub-MICs are encountered in the human body as well, during treatment, which can occur irregularly at intervals at the site of infection [5] or in cases of low-dose antibiotic prophylaxis [6]. When microorganisms grow in the presence of sub-MICs, the antibiotics can potentially alter the physicochemical characteristics of microbial cells, their functions, and the expression of some virulence genes [7]. While sub-MICs generally do not interfere with bacterial growth dynamics, the microorganisms are subjected to stress. As a way to counter stress, microbes would often form biofilms both in external environments and on indwelling medical devices [3, 8, 9].

It is noteworthy that, when MICs or sub-MICs are considered, this concerns values obtained with bacteria grown in liquid media, that is, as plankton. Such will be the use of the term also in the present review. In biofilms, the inhibitory doses exceed 10 to even 1000 times these of plankton [1, 9, 10]. Interestingly, when plankton and bacteria dispersed from biofilm have been examined, they were shown to have similar antibiotic susceptibility [11]. Hence, increased resistance is likely associated with characteristics that are a consequence of the structure of the sessile microbial communities. They themselves represent heterologous microenvironments in which gradients of physical and chemical parameters exist [3]. The advantages of these structured bacterial communities comprise limited antibiotic diffusion, enhanced transmission of resistance genes, expression of efflux pumps, drug adsorption by extracellular matrix, as well as the presence of metabolically inactive persister cells [12].

Provided the growing concern about the wide spread and the role of environments containing subinhibitory amounts of antibacterials, the present review will focus on the interplay of sub-MICs with biofilm growth and/or detachment. In a previous review, the antibiotic-induced biofilm formation has been discussed [13]. However, the sub-MIC of antibiotics, but also other antibacterials (e.g. antibacterial peptides, natural and synthetic substances, etc.), dependent on the combination drug-bacterial strain or species, may have diverse effects on biofilm, from suppression through no effect to promotion. This determined the aim of the present review: to summarize current data and concepts about the modulation of biofilm growth by sub-MICs of antibacterial substances.

2. Sub-MIC of antibiotics and biofilms

While it was initially believed that antibiotics in nature have the role for fighting against competitors, and that therefore also sub-MICs would reduce virulence, recent evidence reveals a more complicated picture, showing the capacity of some antibiotics at low dose to act as chemical signals to modulate metabolic processes [14] or regulate gene (including virulence gene) expression [15].

The idea on the effects of antibiotic sub-MICs on biofilms is getting more and more complicated with the accumulation of experimental data. This puts forward the question of methodology. The conventional approaches to antibiotic sensitivity do not apply to biofilm-grown bacteria [9]. Due to the potentially very high intrinsic biofilm resistance, the focus has mainly been put on their prevention [16]. Probably for this reason, most results have been obtained while applying the drug during the sessile growth, with only a few studies testing the agent's effects on pre-formed biofilms [17–19]. The routinely applied methodology is to test biofilm biomass on 96-well plates by the crystal violet assay, with only a few other studies that explore cell viability as well, for example, the viable cell counts [20] or live-dead staining for fluorescence microscopy.

We have summarized the available experimental data on the action of sub-MICs of antibiotics on biofilms in **Table 1**–**5**. We could find no strict pattern with regard to the effects of the separate groups of antibiotics. All groups were shown to influence some biofilms positively, and others, negatively. An important observation is the bacterial species and strain specificity of the response to the sub-MICs. Thus, sub-MICs of ampicillin increased biofilm growth of *Staphylococcus saprophyticus* [6], reduced it in *Escherichia coli* K-12 [21], and had no effect on *E. coli* UTI8 and *Mycobacterium avium* [6, 22] (**Table 1**). Sub-MICs of ciprofloxacin promoted biofilms of *S. saprophyticus* [6] and *E. coli* UTI8 [6], but reduction was registered in *Streptococcus suis*, *Salmonella enterica* serovar Typhimurium clinical strains, *Stenotrophomonas maltophilia*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, and *Proteus mirabilis* [17, 18, 20, 23–25] (**Table 2**). Diverse effects have been illustrated for sub-MICs of erythromycin on biofilms of *S. suis*, *Corynebacterium diphtheriae*, and *S. epidermidis* [25–27] (**Table 3**); for gentamycin on *S. enterica* serovar Typhimurium, *S. saprophyticus*, *E. coli*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (**Table 4**); and for tetracyclin on *E. coli*, *Staphylococcus lugdunensis*, *M. avium*, *P. aeruginosa*, and *S. epidermidis* [9, 15, 21, 22, 28] (**Table 5**).

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
		Penicillins		
Dicloxacillin	1/2 MIC	*S. epidermidis* strains 9142, IE186, and M187	32–60% BF inhibition	[29]
	8 μg/ml	*S. epidermidis* M187 *S. haemolyticus* M176	BF biomass reduction; decreased synthesis of the EPS, poly-*N*-acetyl-glucosamine	[16]
Penicillin	1/16–1/2 MIC	*S. suis* NJ-3	Dose-dependent BF suppression	[25]
	1/8 MIC	*C. diphtheriae* subsp. mitis strains	No effect	[26]
Methicillin	1/3–1/8 MIC	*S. aureus* Newman	Denser BF formed by the strain and its small-colony variants	[30]
Nafcillin	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from BF-related infections (endocarditis, prosthetic joint infections, etc.)	Increase in 93% of the tested strains, no effect in 7%	[9]
	1/3–1/8 MIC	*S. aureus* Newman	No effect on BF	[30]
		Cephalosporins		
Cefazolin	1/2 MIC	*S. epidermidis* strains 9142, IE186, and M187	32–55% BF inhibition	[29]
	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from BF-related infections (endocarditis, prosthetic joint infections, etc.)	Increase in 13% of the tested strains, no effect in 80%, and decrease in 7%	[9]
	0.5 MIC	*S. epidermidis* strains SE5 and RP62A	BF decrease	[31]

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
Cefalotin	1/3–1/8 MIC	*S. aureus* Newman	Three- to fourfold denser BF formed by the strain and its small-colony variants	[30]
Cefoperazone	1/3–1/8 MIC	*S. aureus* Newman	No effect on BF	[30]
Cefotaxime	1/2–1/16 MIC	*Salmonella enterica* serovar Typhimurium clinical isolates 75 strains	At 1/2 MIC—significantly increased production of BF and EPS	[5]
Ampicillin	0.005–500 μg/ml	*E. coli* MG1655 wt and MG1655 (pBR322)	BF reduction	[21]
	0.1–1.1 μg/ml	*S. saprophyticus* 15305	Sub-MIC (0.3–0.7 μg/ml) stimulate BF formation	[6]
	1/2–1/1024 MIC	*E. coli* UTI89	BF stimulation at MIC, no effect of sub-MIC	[6]
	10 μg/l	*M. avium* strains 104; 101, A5; 3362-33 and 3362-34)	No effect on BF	[22]
			Carbapenems	
Imipenem	2–4 μg/ml	*P. aeruginosa* PA01	BF induction, changes in BF morphology, upregulation of *ampC* and genes for alginate biosynthesis	[32]
	0.03 and 0.125 μg/ml	73 isolates *A. baumannii*	BF stimulation	[33]
Ceftazidime	1/2–1/8 MIC	5 clinical isolates strains *S. maltophilia*	BF inhibition and reduction of viable cell counts	[20]
	0.125–1.0 MIC	5 strains *P. mirabilis*	BF inhibition	[24]
	2; 8; 32 mg/l	6 clinical isolates of *P. aeruginosa*	Synergistic effect with polymorphonuclears against developed 48 h BF	[34]

Abbreviations: BF, biofilm; EPS, exopolysaccharide; MIC, minimal inhibitory concentration.

Table 1. Effects of sub-MIC of β-lactam antibiotics on biofilms.

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
Ciprofloxacin	1/16–1/2 MIC	*S. suis* NJ-3	Dose-dependent BF suppression	[25]
	1/2–1/16MIC	*Salmonella enterica* serovar *Typhimurium* clinical isolates 75 strains	Inhibition of BF formation and EPS synthesis	[5]
	0.1–1.1 μg/ml	*S. saprophyticus* 15305	BF stimulation by sub-MIC (0.4–0.9 μg/ml)	[6]
	1/2–1/1024 MIC	*E. coli* UTI89	Statistically significant BF increase and upregulation of BF-associated genes at 1/4 MIC	[6]
	1/2–1/8MIC	5 clinical isolates *S. maltophilia*	BF inhibition and reduction of viable cell counts	[20]
	1/2–1/64 MIC	*S. pyogenes* isolates (M56; st38;M89; M65; M100; M74)	Dose-dependent BF inhibition	[23]
	0.5 × MIC	*E. coli* strains (8; 9; 10; 31 ;1583)	Reduction of BF formation and survival of the BF bacteria	[18]
	1/2–1/8 MIC	*Staphylococcus epidermidis*—20 clinical isolates	Reduces BF growth and pre-formed BF	[17]
	0.125–1.0 MIC	5 strains *P. mirabilis*	BF inhibition	[24]
	2; 8; 32 mg/l MIC	*P. aeruginosa*—6 clinical isolates	Synergistic effect with polymorphonuclears against developed 48-h BF	[32]

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
	0.001–100 mg/l	*P. aeruginosa*	BF reduction	[15]
Norfloxacin	1–10,000 mg/l	*S. aureus* ATCC 25923	BF stimulation by 1 mg/l	[8]
	1–10,000 mg/l	*P. aeruginosa* NNRL-B3509	BF stimulation by 1 mg/l	[8]
	1/2–1/8 MIC	*S. maltophilia*—5 clinical isolates strains	BF inhibition and reduction of viable cell counts	[20]
	1/2–1/64 MIC	*S. pyogenes* isolates (M56; st38;M89; M65; M100; M74)	Dose-dependent BF inhibition	[23]
	1/2–1/8 MIC	*Staphylococcus epidermidis*—20 clinical isolates	Suppression of BF growth and reduction of pre-formed BF	[17]
	1/16–1/2 MIC	*S. suis* NJ-3	Dose-dependent biofilm suppression	[25]
Ofloxacin	1/2–1/8MIC	*S. maltophilia*—5 clinical isolates strains	BF inhibition and reduction of viable cell counts	[20]
	1/2–1/64 MIC	*S. pyogenes* isolates (M56; st38;M89; M65; M100; M74)	Dose-dependent BF inhibition	[23]
	0.5 × MIC	*S. epidermidis* strains (SE5; RP62A)	No effect on BF formation	[31]
	1/2–1/8 MIC	*Staphylococcus epidermidis*—20 clinical isolates	Suppression of BF growth and reduction of pre-formed BF	[17]
	1/2–1/8 MIC	*S. maltophilia*—5 clinical isolates strains	Biofilm inhibition and reduction of viable cell counts	[20]
Levofloxacin	1/2–1/64 MIC	*S. pyogenes* isolates (M56; st38; M89; M65; M100; M74)	Dose-dependent BF inhibition	[23]
	0.0625– 0.5 MIC	*S. lugdunensis*—15 isolates from biofilm-related infections (endocarditis, prosthetic joint infections, etc.)	Increase in 20% of the tested strains, in 47% no effect, and in 40% decrease	[9]
Moxifloxacin	1 μg/l	*M. avium* strains (104; 101; A5; 3362-33; 3362-34)	No effect on BF	[22]
	1/2–1/8 MIC	*S. maltophilia*—5 clinical isolates strains	BF inhibition and reduction of viable cell counts	[20]
	0.03–0.06 MIC	*S. maltophilia* strains Sm 132 and Sm 144	Decrease in adhesion and BF formation	[7]
	2; 10; 50; 100 x MIC	*S. aureus*—6 strains of coagulase negative	No effect on BF	[35]
	1 μg/ml	*M. avium* strains (101, 104, 109, and A5)	BF inhibition	[36]
	1/2–1/8MIC	*S. maltophilia* —5 clinical isolates strains	BF inhibition and reduction of viable cell counts	[20]
Grepafloxacin	1/2–1/8MIC	*S. maltophilia*—5 clinical isolates strains	BF inhibition and reduction of viable cell counts	[20]
Pefloxacin	1/2–1/8 MIC	*S. epidermidis*—20 clinical isolates	Reduces BF growth and pre-formed BF	[17]

Table 2. Effects of sub-MIC of fluoroquinolones on biofilms.

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
Erythromycin	1/16–1/2 MIC	*S. suis* NJ-3	Dose-dependent BF suppression	[25]
	1/8 MIC	*C. diphtheriae* subsp. mitis strains	BF increase	[26]

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
	0.25 MIC	*S. epidermidis*—96 clinical isolates	BF inhibition in 4 strains; BF enhancement in 20, other strains—unaffected	[27]
Azithromycin	1/2–1/16 MIC	*S. suis* NJ-3	BF inhibition by 1/4 and 1/2 MIC	[25]
	2.5–10 mg/ml	*S. aureus* strains (B1 487; B1 493; B1 412; B 391; B1 468; B1 483; B1 379; B1 472)	BF reduction	[37]
	0.125 μg/ml	*H. influenzae* NTHi2019	Decreased BF formation, reduction of established BF	[19]
	8 μg/m	*M. avium* strains (101, 104, 109, and A5)	BF inhibition	[36]
	sub-MICs	*P. aeruginosa*—35 clinical isolates	Dose-dependent BF reduction	[38]
Clarithromycin	1 μg/ml	*M. avium* strains (101, 104, 109, and A5)	BF inhibition	[36]
	MIC b/n 50–550 mg/ml	*P. aeruginosa*	Sub-MIC result in altered structure and architecture of BF	[39]

Table 3. Effects of sub-MIC of macrolides on biofilms.

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
Gentamycin	1/2–1/16	*Salmonella enterica* serovar *Typhimurium* clinical isolates 75 strains	Inhibition of BF formation and EPS synthesis	[25]
	0.1–1.1 μg/ml	*S. saprophyticus* 15305	Statistically significant BF increase by 0.6—0.7 μg/ml	[6]
	1/2–1/1024 MIC	*E. coli* UTI89	Statistically significant BF increase by 1/32 MIC	[6]
	8 μg/ml	*H. influenzae* NTHi2019	No effect on BF	[19]
	From sub-MIC up to 100× MIC	*S. aureus* strains (RN6390 ATCC 25923)	BF increase	[11]
	0.1–1.5 μg/ml MIC	*P. aeruginosa* PAK and isogenic mutants	BF increase	[40]
Streptomycin	0.5–2 μg/ml	*M. avium* strains (104; 101; A5; 3362-33; 3362-34; 8G12; 5G4; 6H9)	BF increase, induction of BF-associated genes	[22]
Tobramycin	0.05–2 μg/ml	*P. aeruginosa* PAK and isogenic mutants	BF increase	[40]
	0.3; 0.5; 1.0 μg/ml	*Xylella fastidiosa* ATCC 700964 and 3 isogenic mutants	BF reduction	[41]
	0.001–100 μg/l	*P. aeruginosa*	BF reduction	[15]
Amikacin	0.5× MIC	*E. coli* strains (8; 9; 10; 31; 1583)	Reduction of BF formation and survival of the BF-bacteria	[18]
	2; 8; 32 mg/l	*P. aeruginosa*—6 clinical isolates	Synergistic effect with polymorphonuclears for developed 48-h BF	[32]

Antibiotics	Amount	Bacteria/strains	Effect on biofilm	Ref.
Kanamycin	10–110 μg/ml	*P. aeruginosa* PAK and isogenic mutants	BF increase	[40]

Table 4. Effects of sub-MIC of aminoglycosides on biofilms.

Antibiotics	Amount	Bacteria/ strains	Effect on biofilm	Ref.
Streptogramins Quinupristin-dalfopristin	0.5 μg/ml	*S. epidermidis* strains 567 and *S. epidermidis* 561	Enhancement of *ica*ADBC operon expression and EPS synthesis	[28]
	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from biofilm-related infections (endocarditis, prosthetic joint infections, etc.)	Increase in 20% of the tested strains; in 47% no effect and in 33% decrease	[9]
Glycopeptides Vancomycin	1/2 MIC	*S. epidermidis* strains 9142, IE186 and M187	8–24% BF inhibition	[29]
	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from biofilm-related infections (endocarditis, prosthetic joint infections, etc.)	Increase in 27% of the tested strains; in 47% no effect and in 27% decrease	[9]
	0.5× MIC	*S. epidermidis* strains SE5 and RP62A	Decrease in BF	[31]
Tetracyclins Tetracyclin	0.005–500 μg/ml	*E. coli* MG1655 wt and MG1655 (pBR322)	Significant BF increase in the presence of (pBR322)	[21]
	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from biofilm-related infections (endocarditis, prosthetic joint infections, etc.)	Increase 7% of tested strains; decrease 93%	[9]
	0.5–2 μg/ml	*M. avium* strains (104; 101; A5; 3362-33; 3362-34)	BF increase	[22]
	0.01–100 mg/l	*P. aeruginosa*	BF reduction	[15]
	0.5 μg/ml	*S. epidermidis* strains 567 and *S. epidermidis* 561	Enhancement of *ica*ADBC operon expression and EPS synthesis	[28]
DHFR inhibitors Trimethroprim-sulfamethoxazole	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from biofilm-related infections (endocarditis, prosthetic joint infections, etc.)	Increase in 20% of the tested strains, in 40% no effect, and in 40% decrease	[9]
	1/2–1/8 MIC	5 clinical isolates strains *S. maltophilia*	BF inhibition and reduction of viable cell counts	[20]
Oxazolidonones Linezolid	0.0625–0.5 MIC	*S. lugdunensis*—15 isolates from biofilm-related infections (endocarditis, prosthetic joint infections, etc.)	In 80% no effect, in 20% decrease	[9]

Table 5. Effects of sub-MIC of streptogramins, glycopeptides, tetracyclins, dihydrofolate reductase (DHFR) inhibitors and oxazolidonones on biofilms.

Antibiotics with identical mechanisms of antibacterial action, for example, gentamicin and erythromycin, may have different effects on biofilm [19, 29]. In addition, the sub-MICs of a given antibiotic may have diverse effects on different strains of one species of microorganism. For example, such is the case with the effects of cefazolin and levofloxacin on 15 isolates of *S. lugdunensis* [9] (**Table 1**), the effects of erythromycin on 69 clinical isolates of *S. epidermidis* [27] (**Table 3**), of vancomycin on 3 strains of *S. epidermidis* and 15 isolates of *S. lugdunensis* [9, 30] (**Table 5**), and of trimethoprim-sulfamethoxazole on 15 isolates of *S. lugdunensis* [9] (**Table 5**). Obviously, individual strains use different response mechanisms to oppose the action of sub-MICs [9].

Sub-MICs of antibiotics have the potential to affect the structure of individual bacterial cells. Changes of morphology have been registered in several studies. For instance, sub-MIC of

penicillin induced filamentation of cells of *C. diphtheriae*, while erythromycin reduced cell size of this microorganism [26]. The sub-MIC of the drug combination piperacillin/tazobactam induced the occurrence of filamentous forms in *P. aeruginosa*, while subinhibitory amounts of imipenem resulted in the formation of roundish forms (coccobacilli) of this bacterium [43]. Sub-MICs of ciprofloxacin caused the occurrence of filamentous cells when applied to *E. coli* isolated from urinary tract infections. This was accompanied by alterations in the morphology of the outer membrane cardiolipin domains of the strain [44]. The cell-surface physicochemical characteristics of the bacteria would also be affected. Several studies focus on cell-surface hydrophobicity. For example, sub-MICs of penicillin and streptomycin which enhanced biofilm formation in *C. diphtheriae* also rendered the cell surface more hydrophobic [26]. The combination piperacillin/tazobactam applied as sub-MIC that suppressed biofilm growth also reduced cell-surface hydrophobicity of *P. aeruginosa* [45]. While these examples appear to show a likely positive correlation between cell-surface hydrophobicity (which is also related with cell adhesion) and the effects on biofilm growth, this may not be the rule throughout. Thus, sub-MICs of moxifloxacin that reduced biofilm growth had no influence on the cell-surface hydrophobicity of *S. maltophilia* [7]. Changes in zeta potential [8], flagellum-mediated swimming [45] and type IV fimbria-related twitching motility [39, 45] have been registered as well. When examined, the overall morphology of the biofilm, its thickness, substratum coverage, and roughness would change as well [16, 39].

The extracellular biofilm matrix is an important component of these structured microbial consortia. It has both structural and protective functions. In the interplay with the antibiotics, its barrier role against drug penetration should be underlined [46]. For the time being, available publications show a strict correlation between the effects of sub-MICs on the biofilm and on the extracellular matrix components. More data are available on the extracellular polysaccharide (EPS). In cases of biofilm biomass reduction (e.g. by gentamicin and ciprofloxacin on *S*. Typhimurium, by fluoroquinolones on *P. aeruginosa*, and by dicloxacillin on *S. epidermidis*) this was accompanied by reduced release of EPS [5, 16, 47]. In cases of biofilm biomass increase (by sub-MIC of erythromycin on *S. epidermidis*, of cefotaxime on *S. enterica* serovar Typhimurium, and of azithromycin on representatives of several bacterial genera), this coincided with EPS increase [5, 48, 49]. While less studied, such correlation might also characterize another component of the extracellular matrix, the extracellular DNA. It was registered with increased amounts in *S. epidermidis* biofilms treated with sub-MICs of vancomycin [50, 51].

Sub-MICs of antibiotics can interact with the bacterial-host interactions. Together with their capacity to affect phenotypes, they can influence bacterial sensitivity to oxidative stress [45], suppress host proinflammatory responses [6], and cooperate with host polymorphonuclear leucocytes to destroy biofilms [34].

There is evidence that in nature, antibiotics at non-inhibitory concentrations can have the role of signalling molecules that can interfere with quorum sensing [4, 42]. It was shown that sub-MICs of antibiotics influence quorum-sensing-related phenotypes of *Chromobacterium violaceum*, like the production of the pigment violacein, of acyl-homoserine lactones, and of chitinase [52]. Sub-MICs of tobramycin inhibited the Rhl/R system of *P. aeruginosa* thus

reducing the production of C4-homoserine lactone [53]. Azithromycin also antagonized quorum sensing in *P. aeruginosa* [4]. Cephalotin and cephalotaxime suppressed the *agr* quorum-sensing system in *S. aureus* [54].

Sub-MICs of antibiotics can interact with bacterial regulation mechanisms and gene expression. Transcriptomic studies indicated that the expression of approximately 5% of bacterial promoters may be affected [13]. Genes related with antibiotic resistance should be mentioned in the first place. There was a correlation between the transcription of the *ermC* gene and the biofilm formation of erythromycin-treated *S. epidermidis* [27]. In *P. aeruginosa*, among the 34 genes influenced by imipenem, the most strongly induced gene was *ampC* coding for chromosomal β-lactamase [54]. Genes related with the synthesis of EPS or bacterial capsules may be affected, like the genes from the *ica*ADBC operon in *S. epidermidis* [28, 49]. Other genes related with adhesion and biofilm development that should be mentioned are *comD*, *gtfC*, *luxS*, *gtfB*, and *atlA* of *Streptococcus mutans* upregulated by sub-MIC of triclosan [55] and *guaB2* and *gtf* in *M. avium*, upregulated under the action of sub-MICs of streptomycin and tetracycline [22]. The biofilm growth and detachment have been related with the intracellular levels of the second messenger cyclic-di-GMP [40]. The *eal* gene participating in the pathways for its synthesis was upregulated by sub-MIC of tobramycin [41]. Definitely, the effects of sub-MICs on gene expression are not confined to these related to biofilm, many other genes can be influenced as well [40].

3. Antibiofilm bacterial metabolites

The capacity of released metabolites of bacterial species and strains to modulate biofilm growth of other bacteria is continuously in focus because of the potential for the isolation of novel biofilm modulating substances. As an initial screening step, the action of cell-free supernatants (CFSs) is tested. The activities may vary from stimulation [56, 57] to suppression [58, 59]. Noteworthy, the effects of CFSs on bacterial growth in liquid media do not predict the effects on sessile growth. Thus, subinhibitory amounts of 10^{-2} diluted CFSs from two bacteriocinogenic strains of *Lactobacillus plantarum* slowed down the growth of laboratory and uropathogenic strains of *E. coli*, but stimulated significantly the biofilm development [59]. The active substances in CFSs may be proteins/peptides, carbohydrates, low molecular weight metabolites, etc. [see comments by 57] and for some of them the nature, structure, and mode of action have been explored. However, we shall restrict our review to molecules which have inhibitory activity on bacterial growth, and for which there is data on the effects of subinhibitory amounts on biofilms.

Bacteriocins are proteins/peptides produced by prokaryotes which are active against other bacterial species or strains. For example, colicins are produced by some strains of *E. coli*. One of them, colicin M, is a phosphatase that hydrolyses the peptidoglycan lipid II intermediate, thus interfering with peptidoglycan synthesis and causing cell lysis. In subinhibitory amounts, it upregulated in *P. aeruginosa* PAO1 the *ydeH* gene related with the synthesis of cyclic-di-GMP, as well as several biofilm-related genes, *ycfJ*, *rprA*, *omrA*, and *omrB*. However, no biofilm

stimulation was confirmed by the crystal violet assay [60]. RIP is an RNAIII-inhibiting heptapeptide originally isolated from CFS of *Staphylococcus xylosus.* It inhibits staphylococcal pathogenesis. In sub-MICs, it suppressed biofilm formation by interfering with the quorum-sensing mechanisms [61]. Nisin is a polycyclic antibacterial peptide produced by *Lactococcus lactis*. At growth inhibitory concentrations, it suppressed sessile growth of *S. aureus*; however, sub-MICs had no effect on biofilm [62].

Mupirocin is an antibacterial substance of the monoxycarbolic acid class that was originally isolated from *Pseudomonas fluorescens*. At sub-MIC, it can reduce both biofilm formation and glycocalyx production by *P. aeruginosa* [63]. Phenyl lactic acid is a metabolite of *Lactobacillus* probiotic strains. At subinhibitory amounts, it attenuated the virulence and pathogenicity of *P. aeruginosa* and *S. aureus*, including biofilm formation, by interacting with quorum sensing [64]. The antifungal and antibacterial molecule, 2,4-diacetylphloroglucinol, was isolated from the CFS of *Pseudomonas protegens*. At subinhibitory amounts, it reduced pellicle and biofilm formation, and sporulation of *B. subtilis* [65].

4. Antimicrobial peptides and biofilm modulation

The host-bacterial interactions are also explored with the aim of identifying of novel molecules that would help overcoming the bacterial resistance mechanisms and combating infections. One important group of substances is that of the antibacterial peptides, an important part of the innate immune system.

Colistin is a cationic antimicrobial peptide which is gaining importance in the fight against *P. aeruginosa* cystic fibrosis infections. Subinhibitory concentrations altered the expression of 30 genes of the bacterium. Genes related with quorum sensing, lipopolysaccharides (LPSs) modifications, quinolone biosynthesis, and biofilm formation were upregulated while genes involved with motility and osmotolerance were downregulated. However, biofilm biomass remained unaffected [66].

The major human host defence peptide LL-37 is found in mucosal surfaces, the granules of phagocytes, as well as in bodily fluids. At very low concentrations, far below those that kill or inhibit the growth of *P. aeruginosa*, LL-37 prevented the *in vitro* biofilm formation [67]. It interfered with biofilm growth in at least three ways: by reduction of initial attachment, promotion of twitching motility, and downregulation of key components of the Las and Rhl quorum-sensing systems [67].

The synthetic antimicrobial peptide 1018, derived from the bovine neutrophil defence peptide bactenecin, has recently been identified as biofilm inhibitory compound. While not reflecting on bacterial growth, it could prevent the biofilm growth of *P. aeruginosa*, *E. coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *S. enterica*, *Burkholderia cenocepacia*, and methicillin-resistant *S. aureus*. This co-related with degradation of ppGpp [68]. In addition, peptide 1018 acted in synergism with conventional antibiotics, like ceftazidime, ciprofloxacin, imipenem, or tobramycin, to both prevent development and disperse existing biofilms [69].

http://dx.doi.org/10.5772/62939

Invertebrate antibacterial responses are also explored. Thus, thanatin is an insect antimicrobial peptide on the basis of which a shorter synthetic derivative, R-thanatin, was synthesized. When applied in sub-MIC amounts to *S. epidermidis* (including methicillin-resistant staphylococcus epidermidis, MRSE), *Staphylococcus haemolyticus*, and *Staphylococcus hominis*, it inhibited biofilm formation. Parallelly, MRSE underwent serious morphological alterations like swelling and abnormal divisions [70].

5. Subinhibitory amounts of plant substances

The application of plants for treatment of illness dates back to the very early moments of mankind history, and has laid the basis of modern phytotherapy. The studies on the antibacterial activities of medicinal plant products have a long tradition, more recently expanded to biofilm research. Studies include tests on essential oils and plant extracts, partially purified enriched fractions, as well as isolated pure substances. Also, plant products may be used as a basis for chemical modifications aiming improved antibiofilm activity.

Among the plant products, essential oils are most popular for their wide use in ethnomedicine. Some of them that have antibacterial action proved successful against bacterial biofilms as well. Among the essential oils that at subinhibitory amounts could suppress sessile growth are, for example, these from *Satureja hortensis* L. (active against *Prevotella nigrescens* biofilm) [71], from *Thymus vulgaris* (active against *P. aeruginosa* and *E. coli* biofilms) [72], and from *Mentha piperita* (active against biofilms of *P. aeruginosa* and *A. hydrophylla*) [73]. In addition, peppermint oil suppressed EPS production [73].

Methanol and aqueous branch extracts of five *Juniperus* sp. were examined for their activities against two *S. aureus* strains. The extracts had minimal activity on planktonic growth of *S. aureus* ATCC 3538P but suppressed biofilm formation, while the other strain, *S. aureus* 810, was not affected in either mode of growth [74]. The extract from *Leonurus cardiaca* L. suppressed the adherence of *S. aureus* to both abiotic surfaces and surfaces covered with fibrinogen, fibronectin, or collagen [75]. Sub-MICs (1/2 to 1/32 MIC) of extracts from *Boesenbergia pandurata* (Roxb.) Schltr. and *Eleutherine americana* Merr. significantly prevented biofilm formation. Together with this, the extract from *E. americana* also suppressed quorum sensing in *C. violaceum* test system [76].

In a study on 14 fractions from plant extracts, the total extract and the phenyl propanoid-containing fraction from *Rhodiola rosea*, and the total extract and the sesquiterpene lactone-containing fraction (Am2) from *Arnica montana*, were shown to have no antibacterial effects. However, they suppressed the biofilm growth in *E. coli* urinary tract infection isolates. These same extracts had the opposite effect—biofilm stimulation, on a multidrug-resistant *E. coli* strain isolated from asymptomatic bacteriuria [77]. Noteworthy, the sesquiterpene lactone-containing fraction Am2 also suppressed the quorum-sensing-controlled bioluminescence in *Vibrio harveyi* bioreporter strains (ATCC1116 and ATCC1117) [78, 79].

Carvacol is an antimicrobial monotherpenic phenol with antibacterial potential that is present in many essential oils. In subinhibitory doses, it suppressed sessile growth of a number of

Gram-positive and Gram-negative bacteria [80, 81]. Polyphenols from muscadine grapes with antioxidant and antibacterial activity, at 0.5 MIC, inhibited biofilm growth of *S. aureus* [82]. Similar was the effect of the essential oil components eugenol and citral on *S. aureus* and *Listeria monocytogenes* [83], epigallocatechin-3-gallate from green tea on *S. maltophilia* [84], ursolic acid, genistein, cranberry extract, *p*-hydroxybenzoic acid, and resveratol on *S. aureus* [75, 85]. Menthol, together with biofilm suppression, was shown to inhibit both the *las*- and *pqs*-related quorum sensing [73].

Fenchone is a substance that is present in many essential oils. It had neither antibacterial nor antibiofilm effects on a panel of Gram-positive and Gram-negative strains. This molecule was used to synthesize its chemical derivatives. While the substitutions did not improve the antibacterial properties against *E. coli* ATCC 25922 and six *E. coli* K-12 strains, some of the derivatives showed biofilm modulation potential [86]. Chalcones are a group of flavonoids with antibacterial potential, found in many plants. Synthetic chalcones are applied as well. The effects of sub-MICs of three newly synthesized chalcones on methicillin-resistant *S. aureus* were examined. Both biofilm formation and adherence to human fibronectin were reduced, as well as the release of EPS [87].

6. Other compounds with biofilm-modulating potential

Other substances have also proved a good anti-biofilm potential when applied in sub-MICs. For example, sodium ascorbate, together with suppressing *P. aeruginosa* virulence factors (elastase, protease and haemolysin activities, pyocyanin production, and quorum sensing) also reduced biofilm formation [88]. Biofilm growth was inhibited by subinhibitory amounts of thiourea derivatives [89], thiazolinediones [90], and certain anthraquinones [91]. Organic complexes of metals are also elaborated as antibacterial and/or antibiofilm substances. Newly synthesized dimethylguanin-copper complexes [92], the organo-tellurium compound AS101 [93], and bismuth thiols [94] have shown anti-biofilm activity at sub-MICs. The latter substances are considered as possible coating agents for indwelling devices. For prevention of medical devices from bacterial contamination, other substances may prove useful as coating material, like ovotransferrin, protamine sulfate, ethylenediaminetetraacetic acid (EDTA) [95], cerium nitrate, chitosan, hamamelitannin [96], polyvinyl pyrrilidine [97], etc.

As an opposite effect, the biocides used in food processing facilities, trisodium phosphate, sodium nitrite, and sodium hypochlorite, when applied in sub-MICs, enhanced the capacity of *E. coli* to form biofilms. This was accompanied by a reduction of the antibiotic susceptibility [98].

7. Some final considerations

Presently, there is growing concern about the relationship between the rise of widespread antibiotic resistance and the role of environments containing subinhibitory amounts of

antibacterials [1]. As a major risk for human health, biofilm communities provide the bacteria with prerequisites for rapid resistance development [99]. Among the other more direct risks that biofilms may cause to human health, should be mentioned the possibility for enhanced colonization of indwelling medical devices in the presence of subinhibitory amounts of antibacterial substances, and the contamination of surfaces in medical or food-processing environments. Depending on the aim of a given antibiofilm strategy, different effects may be in the focus. Disinfection of outer surfaces in hospitals and in food industry requires that the used agents have the capacity to detach established biofilms. On the opposite, if biofilms on indwelling devices are concerned, once established, their detachment is hazardous. It may be accompanied with dissemination of the bacteria to other sites in the human body, and there are risks of sepsis [69]. Therefore, the development of medical materials should be directed to biofilm prevention. However, when the effects of a given substance are estimated, the biology of the biofilm as a whole is better to be addressed, starting from the attachment and establishment of the sessile community, and going as far as its detachment. The methodologies used by the predominant amount of the present-day studies search for the effects of sub-MICs by applying the tested agents during biofilm growth. It can be recommended that in the future a more standardized methodology is applied which includes as well tests for dispersion of established biofilms and for microbial vitality. The present review showed several critical points in the effects of sub-MICs of antibacterial substances as biofilm modulators. Among these, the strain- and species-specific responses of the bacteria in their biofilm development, the expression of virulence factors and quorum sensing should necessarily be taken into account when novel antibacterials are tested.

Author details

Stoyanka R. Stoitsova*, Tsvetelina S. Paunova-Krasteva and Dayana B. Borisova

*Address all correspondence to: stoitsova_microbiobas@yahoo.com

Department of General Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

References

[1] Berendonk T, Manaia C, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Bürgmann H, Sørum H, Norström M, Pons M, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F, Martinez J. Tackling antibiotic resistance: the environmental framework. Nat. Rev. 2015;13:310–317. DOI: 10.1038/nrmicro3439

[2] Bernier S, Surette M. Concentration-dependent activity of antibiotics in natural environments. Front. Microbiol. 2013;4:1–14. DOI: 10.3389/fmicb.2013.00020

[3] Laureti L, Matic I, Gutierrez A. Bacterial responses and genome instability induced by subinhibitory concentrations of antibiotics. Antibiotics. 2013;2:100–114. DOI: 10.3390/antibiotics2010100

[4] Andersson D, Hughes D. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 2014;12:465–478. DOI: 10.1038/nrmicro3270

[5] Majtán J, Majtánová L, Xu M, Majtán V. In vitro effect of subinhibitory concentrations of antibiotics on biofilm formation by clinical strains of *Salmonella enterica* serovar Typhimurium isolated in Slovakia. J. Appl. Microbiol. 2008;104:1294–1301. DOI: 10.1111/j.1365-2672.2007.03653.x

[6] Goneau L, Hannan T, MacPhee R, Schwartz D, Macklaim J, Gloor G, Razvi H, Reid G, Hultgren S, Burton J. Subinhibitory antibiotic therapy alters recurrent urinary tract infection pathogenesis through modulation of bacterial virulence and host immunity. MBio. 2015;6:e00356-15. DOI: 10.1128/mBio.00356-15

[7] Pompilio A, Catavitello C, Picciani C, Confalone P, Piccolomini R, Savini V, Fiscarelli E, D'Antonio D, Di Bonaventura G. Subinhibitory concentrations of moxifloxacin decrease adhesion and biofilm formation of *Stenotrophomonas maltophilia* from cystic fibrosis. J. Med. Microbiol. 2010;59:76–81. DOI: 10.1099/jmm.0.011981-0

[8] Kumar A, Ting Y. Effects of sub-inhibitory antibacterial stress on bacterial surface properties and biofilm formation. Colloids Surf. B Biointerfaces. 2013;111:747–754. DOI: 10.1016/j.colsurfb.2013.07.011

[9] Frank K, Reichert E, Piper K, Patel R. In vitro effects of antimicrobial agents on planktonic and biofilm forms of *Staphylococcus lugdunensis* clinical isolates. Antimicrob. Agents Chemother. 2007;51:888–895. DOI: 10.1128/AAC.01052-06

[10] Maestre J, Aguilar L, Mateo M, Giménez M, Méndez M, Alou L, Granizo J, Prieto J. In vitro interference of tigecycline at subinhibitory concentrations on biofilm development by *Enterococcus faecalis*. J. Antimicrob. Chemother. 2012;67:1155–1158. DOI: 10.1093/jac/dks014

[11] Hess D, Henry-Stanley M, Wells C. Gentamicin promotes *Staphylococcus aureus* biofilms on silk suture. J. Surg. Res. 2011;170:302–308. DOI: 10.1016/j.jss.2011.06.011

[12] Soto S. Importance of biofilms in urinary tract infections: new therapeutic approaches. Adv. Biol. 2014;2014:1–13. DOI: 10.1155/2014/543974

[13] Kaplan J. Antibiotic-induced biofilm formation. Int. J. Artif. Organs. 2011;34:737–751. DOI: 10.5301/ijao.5000027

[14] Goh E, Yim G, Tsui W, McClure J, Surette M, Davies J. Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. Proc. Natl. Acad. Sci. U S A. 2002;99:17025–17030. DOI: 10.1073/pnas.252607699

[15] Linares J, Gustafsson I, Baquero F, Martinez J. Antibiotics as intermicrobial signaling agents instead of weapons. Proc. Natl. Acad. Sci. U S A. 2006;103:19484–19489. DOI: 10.1073/pnas.0608949103

[16] Cerca N, Martins S, Sillankorva S, Jefferson K, Pier G, Oliveira R, Azeredo J. Effects of growth in the presence of subinhibitory concentrations of dicloxacillin on *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* biofilms. Appl. Environ. Microbiol. 2005;71:8677–8682. DOI: 10.1128/AEM.71.12.8677-8682.2005

[17] Yassien M, Khardori N. Interaction between biofilms formed by *Staphylococcus epidermidis* and quinolones. Diagn. Microbiol. Infect. Dis. 2001;40:79–89. DOI: 10.1016/S0732-8893(01)00253-X

[18] Wojnicz D, Tichaczek-Goska D. Effect of sub-minimum inhibitory concentrations of ciprofloxacin, amikacin and colistin on biofilm formation and virulence factors of *Escherichia coli* planktonic and biofilm forms isolated from human urine. Braz. J. Microbiol. 2013;44:259–265. DOI: 10.1590/S1517-83822013000100037

[19] Starner T, Shrout J, Parsek M, Appelbaum P, Kim G. Subinhibitory concentrations of azithromycin decrease nontypeable *Haemophilus influenzae* biofilm formation and diminish established biofilms. Antimicrob. Agents Chemother. 2008;52:137–145. DOI: 10.1128/AAC.00607-07

[20] Di Bonaventura G, Spedicato I, D'Antonio D, Robuffo I, Piccolomini R. Biofilm formation by *Stenotrophomonas maltophilia*: modulation by quinolones, trimethoprim-sulfamethoxazole, and ceftazidime. Antimicrob. Agents Chemother. 2004;48:151–160. DOI: 10.1128/AAC.48.1.151-160.2004

[21] May T, Ito A, Okabe S. Induction of multidrug resistance mechanism in *Escherichia coli* biofilms by interplay between tetracycline and ampicillin resistance genes. Antimicrob. Agents Chemother. 2009;53:4628–4639. DOI: 10.1128/AAC.00454-09

[22] McNabe M, Tennant R, Danelishvili L, Young L, Bermudez L. *Mycobacterium avium* ssp. hominissuis biofilm is composed of distinct phenotypes and influenced by the presence of antimicrobials. Clin. Microbiol. Infect. 2011;17:697–703. DOI: 10.1111/j.1469-0691.2010.03307.x

[23] Balaji K, Thenmozhi R, Pandian S. Effect of subinhibitory concentrations of fluoroquinolones on biofilm production by clinical isolates of *Streptococcus pyogenes*. Indian J. Med. Res. 2013;137:963–971.

[24] Kwiecińska-Piróg J, Skowron K, Zniszczol K, Gospodarek E. The assessment of *Proteus mirabilis* susceptibility to ceftazidime and ciprofloxacin and the impact of these antibiotics at subinhibitory concentrations on *Proteus mirabilis* biofilms. Biomed. Res. Int. 2013;2013:1–8. DOI: 10.1155/2013/930876

[25] Dawei G, Liping W, Chengping L. In vitro biofilm forming potential of *Streptococcus suis* isolated from human and swine in China. Braz. J. Microbiol. 2012;43:993–1004. DOI: 10.1590/S1517-83822012000300002I

[26] Gomes D, Peixoto R, Barbosa E, Napoleão F, Sabbadini P, dos Santos K, Mattos-Guaraldi A, Hirata R Jr. SubMICs of penicillin and erythromycin enhance biofilm formation and hydrophobicity of *Corynebacterium diphtheriae* strains. J. Med. Microbiol. 2013;62:754–760. DOI: 10.1099/jmm.0.052373-0

[27] He H, Sun F, Wang Q, Liu Y, Xiong L, Xia P. Erythromycin resistance features and biofilm formation affected by subinhibitory erythromycin in clinical isolates of *Staphylococcus epidermidis*. J. Microbiol. Immunol. Infect. 2014;49:33–40. DOI: 10.1016/j.jmii.2014.03.001

[28] Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrob Agents Chemother. 2000;44:3357–3363. DOI: 10.1128/AAC.44.12.3357-3363.2000

[29] Henriques M, Cerca N, Azeredo J, Oliveira R. Influence of sub-inhibitory concentration of antimicrobial agents on biofilm formation in indwelling medical devices. Int. Artif. Organs. 2005;11:1181–1185.

[30] Subrt N, Mesak L, Davies J. Modulation of virulence gene expression by cell wall active antibiotics in *Staphylococcus aureus*. J. Antimicrob. Chemother. 2011;66:979–984. DOI: 10.1093/jac/dkr043

[31] Rupp M, Hamer K. Effect of subinhibitory concentrations of vancomycin, cefazolin, ofloxacin, L-ofloxacin and D-ofloxacin on adherence to intravascular catheters and biofilm formation by *Staphylococcus epidermidis*. J. Antimicrob. Chemother. 1998;41:155–161. DOI: 10.1093/jac/41.2.155

[32] Bagge N, Schuster M, Hentzer M, Ciofu O, Givskov M, Greenberg EP, Høiby N. *Pseudomonas aeruginosa* biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. Antimicrob. Agents Chemother. 2004;48:1175–1187. DOI: 10.1128/AAC.48.4.1175-1187.2004

[33] Nucleo E, Steffanoni L, Fugazza G, Migliavacca R, Giacobone E, Navarra A, Pagani L, Landini P. Growth in glucose-based medium and exposure to subinhibitory concentrations of imipenem induce biofilm formation in a multidrug-resistant clinical isolate of *Acinetobacter baumannii*. BMC Microbiol. 2009;9:1–14. DOI: 10.1186/1471-2180-9-270

[34] Chatzimoschou A, Simitsopoulou M, Antachopoulos C, Walsh T, Roilides E. Antipseudomonal agents exhibit differential pharmacodynamic interactions with human polymorphonuclear leukocytes against established biofilms of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 2015;59:2198–2205. DOI: 10.1128/AAC.04934-14

[35] Pérez-Giraldo C, Gonzalez-Velasco C, Sánchez-Silos R, Hurtado C, Blanco M, Gómez-García A. Moxifloxacin and biofilm production by coagulase-negative staphylococci. Chemotherapy. 2004;50:101–104. DOI: 10.1159/000077811

[36] Carter G, Young L, Bermudez L. A subinhibitory concentration of clarithromycin inhibits *Mycobacterium avium* biofilm formation. Antimicrob. Agents Chemother. 2004;48:4907–4910. DOI: 10.1128/AAC.48.12.4907-4910.2004

[37] Wu E, Kowalski R, Romanowski E, Mah F, Gordon Y, Shanks R. AzaSite® inhibits *Staphylococcus aureus* and coagulase-negative Staphylococcus biofilm formation in vitro. Ocul. Pharmacol. Ther. 2010;26:557–562. DOI: 10.1089/jop.2010.0097

[38] Vranes J. Effect of subminimal inhibitory concentrations of azithromycin on adherence of *Pseudomonas aeruginosa* to polystyrene. Chemother. 2000;12:280–285. DOI: 10.1179/joc.2000.12.4.280

[39] Wozniak D, Keyser R. Effects of subinhibitory concentrations of macrolide antibiotics on *Pseudomonas aeruginosa*. Chest. 2004;125:62S-69S.

[40] Jones C, Allsopp L, Horlick J, Kulasekara H, Filloux A. Subinhibitory concentration of kanamycin induces the *Pseudomonas aeruginosa* type VI secretion system. PLoS One. 2013;8:e81132. DOI: 10.1371/journal.pone.0081132

[41] de Souza A, Ionescu M, Baccari C, da Silva A, Lindow S. Phenotype overlap in *Xylella fastidiosa* is controlled by the cyclic di-GMP phosphodiesterase Eal in response to antibiotic exposure and diffusible signal factor-mediated cell-cell signalling. Appl. Environ. Microbiol. 2013;79:3444–3454. DOI: 10.1128/AEM.03834-12

[42] Yim G, Wang H, Davies J. Antibiotics as signalling molecules. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 2007;362:1195–1200. DOI: 10.1098/rstb.2007.2044

[43] Fonseca A, Sousa J. Effect of antibiotic-induced morphological changes on surface properties, motility and adhesion of nosocomial *Pseudomonas aeruginosa* strains under different physiological states. J. Appl. Microbiol. 2007;103:1828–1837. DOI: 10.1111/j.1365-2672.2007.03422.x

[44] Kicia M, Janeczko N, Lewicka J, Hendrich A. Comparison of the effects of subinhibitory concentrations of ciprofloxacin and colistin on the morphology of cardiolipin domains in *Escherichia coli* membranes. J. Med. Microbiol. 2012;61:520–524. DOI: 10.1099/jmm.0.037788-0

[45] Fonseca A, Extremina C, Fonseca A, Sousa J. Effect of subinhibitory concentration of piperacillin/tazobactam on *Pseudomonas aeruginosa*. J. Med. Microbiol. 2004;53:903–910. DOI: 10.1099/jmm.0.45637-0

[46] Penesyan A, Gillings M, Paulsen I. Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. Molecules. 2015;20:5286–5298. DOI: 10.3390/molecules20045286

[47] Yassien M, Khardori N, Ahmedy A, Toama M. Modulation of biofilms of *Pseudomonas aeruginosa* by quinolones. Antimicrob. Agents Chemother. 1995;39:2262–2268. DOI: 10.1128/AAC.39.10.2262

[48] Mart'ianov S, Zhurina M, Él'-Registan G, Plakunov V. Activation of formation of bacterial biofilms by azithromycin and prevention of this effect. Mikrobiologiia. 2015;84:27–36.

[49] Wang Q, Sun F, Liu Y, Xiong L, Xie L, Xia P. Enhancement of biofilm formation by subinhibitory concentrations of macrolides in icaADBC-positive and -negative clinical isolates of *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. 2010;54:2707–2711. DOI: 10.1128/AAC.01565-09

[50] Doroshenko N. The biofilm matrix at sub-inhibitory concentrations of vancomycin. Thesis, University of Southampton; 2014.

[51] Doroshenko N, Tseng B, Howlin R, Deacon J, Wharton J, Thurner P, Gilmore B, Parsek M, Stoodley P. Extracellular DNA impedes the transport of vancomycin in *Staphylococcus epidermidis* biofilms preexposed to subinhibitory concentrations of vancomycin. Antimicrob. Agents Chemother. 2014;58:7273–7282. DOI: 10.1128/AAC.03132-14

[52] Liu Z, Wang W, Zhu Y, Gong Q, Yu W, Lu X. Antibiotics at subinhibitory concentrations improve the quorum sensing behavior of *Chromobacterium violaceum*. FEMS Microbiol. Lett. 2013;341:37–44. DOI: 10.1111/1574-6968.12086

[53] Babić F, Venturi V, Maravić-Vlahovicek G. Tobramycin at subinhibitory concentration inhibits the RhlI/R quorum sensing system in a *Pseudomonas aeruginosa* environmental isolate. BMC Infect. Dis. 2010;10:148. DOI: 10.1186/1471-2334-10-148

[54] Nielsen L, Roggenbuck M, Haaber J, Ifrah D, Ingmer H. Diverse modulation of spa transcription by cell wall active antibiotics in *Staphylococcus aureus*. BMC Res. Notes. 2012;5:457. DOI: 10.1186/1756-0500-5-457

[55] Bedran T, Grignon L, Spolidorio D, Grenier D. Subinhibitory concentrations of triclosan promote *Streptococcus mutans* biofilm formation and adherence to oral epithelial cells. PLoS One. 2014;9:e89059. DOI: 10.1371/journal.pone.0089059

[56] Stoitsova S, Ivanova R, Paunova T. Biofilm formation by reference strains of *Escherichia coli*. Comptes rendus del'Academie bulgare des Sciences. 2007;60:71–76.

[57] Vacheva A, Ivanova R, Paunova-Krasteva T, Stoitsova S. Released products of pathogenic bacteria stimulate biofilm formation by *Escherichia coli* K-12 strains. Antonie Van Leeuwenhoek. 2012;102:105–119. DOI: 10.1007/s10482-012-9718-y

[58] Nithya C, Begum M, Pandian S. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of *Pseudomonas aeruginosa* PAO1. Appl. Microbiol. Biotechnol. 2010;88:341–358. DOI: 10.1007/s00253-010-2777-y

[59] Vacheva A, Georgieva R, Danova S, Mihova R, Marhova M, Kostadinova S, Vasileva K, Bivolarska M, Stoitsova S. Modulation of *Escherichia coli* biofilm growth by cell-free

spent cultures from lactobacilli. Centr. Europ. J. Biol. 2012;7:219–229. DOI: 10.2478/s11535-012-0004-9

[60] Kamenšek S, Žgur-Bertok D. Global transcriptional responses to the bacteriocin colicin M in *Escherichia coli*. BMC Microbiol. 2013;13:42. DOI: 10.1186/1471-2180-13-42

[61] Domenico P, Gurzenda E, Giacometti A, Cirioni O, Ghiselli R, Orlando F, Korem M, Saba V, Scalise G, Balaban N. BisEDT and RIP act in synergy to prevent graft infections by resistant staphylococci. Peptides. 2004;25:2047–2053. DOI: 10.1016/j.peptides.2004.08.005

[62] Sudagidan M, Yemenicioğlu A. Effects of nisin and lysozyme on growth inhibition and biofilm formation capacity of *Staphylococcus aureus* strains isolated from raw milk and cheese samples. J. Food Prot. 2012;75:1627–1633. DOI: 10.4315/0362-028X.JFP-12-001

[63] Ishikawa J, Horii T. Effects of mupirocin at subinhibitory concentrations on biofilm formation in *Pseudomonas aeruginosa*. Chemother. 2005;51:361–362. DOI: 10.1159/000088962

[64] Chifiriuc M, Ditu L, Banu O, Bleotu C, Drăcea O, Bucur M, Larion C, Israil A, Lazăr V. Subinhibitory concentrations of phenyl lactic acid interfere with the expression of virulence factors in *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical strains. Roum. Arch. Microbiol. Immunol. 2009;68:27–33.

[65] Powers M, Sanabria-Valentín E, Bowers A, Shank E. Inhibition of cell differentiation in *Bacillus subtilis* by *Pseudomonas protegens*. J. Bacteriol. 2015;197:2129–2138. DOI: 10.1128/JB.02535-14

[66] Cummins J, Reen F, Baysse C, Mooij M, O'Gara F. Subinhibitory concentrations of the cationic antimicrobial peptide colistin induce the pseudomonas quinolone signal in *Pseudomonas aeruginosa*. Microbiol. 2009;155:2826–2837. DOI: 10.1099/mic.0.025643-0

[67] Overhage J, Campisano A, Bains M, Torfs E, Rehm B, Hancock R. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect. Immun. 2008;76:4176–4182. DOI: 10.1128/IAI.00318-08

[68] de la Fuente-Núñez C, Reffuveille F, Haney E, Straus S, Hancock R. Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathog. 2014;10:e1004152. DOI: 10.1371/journal.ppat.1004152

[69] Reffuveille F, de la Fuente-Núñez C, Mansour S, Hancock R. A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. Antimicrob. Agents Chemother. 2014;58;5363–5371. DOI: 10.1128/AAC.03163-14

[70] Hou Z, Da F, Liu B, Xue X, Xu X, Zhou Y, Li M, Li Z, Ma X, Meng J, Jia M, Wang Y, Luo X. R-thanatin inhibits growth and biofilm formation of methicillin-resistant *Staphylococcus epidermidis* in vivo and in vitro. Antimicrob. Agents Chemother. 2013;57:5045–5052. DOI: 10.1128/AAC.00504-13

[71] Gursoy U, Gursoy M, Gursoy O, Cakmakci L, Könönen E, Uitto V. Anti-biofilm properties of *Satureja hortensis* L. essential oil against periodontal pathogens. Anaerobe. 2009;15:164–167. DOI: 10.1016/j.anaerobe.2009.02.004

[72] Al-Shuneigat J, Al-Sarayreh S, Al-Saraireh Y, Al-Qudah M, Al-Tarawneh I. Effects of wild *Thymus vulgaris* essential oil on clinical isolates biofilm-forming bacteria. IOSR J. Dent. Med. Sci. 2014;13:62–66.

[73] Husain F, Ahmad I, Khan M, Ahmad E, Tahseen Q, Khan M, Alshabib N. Sub-MICs of *Mentha piperita* essential oil and menthol inhibits AHL mediated quorum sensing and biofilm of Gram-negative bacteria. Front. Microbiol. 2015;6:1–12. DOI: 10.3389/fmicb. 2015.00420

[74] Marino A, Bellinghieri V, Nostro A, Miceli N, Taviano M, Güvenç A, Bisignano G. In vitro effect of branch extracts of *Juniperus species* from Turkey on *Staphylococcus aureus* biofilm. FEMS Immunol. Med. Microbiol. 2010;59:l470–476. DOI: 10.1111/j.1574-695X. 2010.00705.x

[75] Micota B, Sadowska B, Podsędek A, Redzynia M, Różalska B. *Leonurus cardiaca* L. herb —a derived extract and an ursolic acid as the factors affecting the adhesion capacity of *Staphylococcus aureus* in the context of infective endocarditis. Acta Biochim. Pol. 2014;61:385–388.

[76] Limsuwan S, Voravuthikunchai S. *Boesenbergia pandurata* (Roxb.) Schltr., *Eleutherine americana* Merr. and *Rhodomyrtus tomentosa* (Aiton) Hassk. as antibiofilm producing and antiquorum sensing in *Streptococcus pyogenes*. FEMS Immunol. Med. Microbiol. 2008;53:429–436. DOI: 10.1111/j.1574-695X.2008.00445.x

[77] Vacheva A, Mustafa B, Staneva J, Marhova M, Kostadinova S, Todorova M, Ivanova R, Stoitsova S. Effects of extracts from medicinal plants on biofilm formation by *Escherichia coli* urinary tract isolates. Biotech. Biotech. Equip. 2011;25:92–97. DOI: 10.5504/BBEQ. 2011.0111

[78] Vacheva A, Trendafilova-Slavkova A, Todorova M, Ivanova R, Stoitsova S. Lactone/ furanone-containing fractions from *Arnica montana* inhibit AI-2-based quorum sensing; Youth Scientific Conference; 22–23 November 2011; Sofia, Bulgaria. 2011. p. 5–7.

[79] Stoitsova S, Vacheva A, Paunova-Krasteva T, Ivanova R, Danova S, Manasiev J. The multicellular behavior of *Escherichia coli*: a target for intferences. In: Najdenski H., Angelova M., Stoitsova S., editors. New Trends in Microbiology. Sofia. 2012. p. 147–158.

[80] Nostro A, Cellini L, Zimbalatti V, Blanco A, Marino A, Pizzimenti F, Giulio M, Bisignano G. Enhanced activity of carvacrol against biofilm of *Staphylococcus aureus* and *Staphylococcus epidermidis* in an acidic environment. APMIS. 2012;120:967–973. DOI: 10.1111/j.1600-0463.2012.02928.x

[81] Burt S, Ojo-Fakunle V, Woertman J, Veldhuizen E. The natural antimicrobial carvacrol inhibits quorum sensing in *Chromobacterium violaceum* and reduces bacterial biofilm

formation at sub-lethal concentrations. PLoS One. 2014;9:e93414. DOI: 10.1371/journal.pone.0093414

[82] Xu C, Yagiz Y, Hsu W, Simonne A, Lu J, Marshall M. Antioxidant, antibacterial, and antibiofilm properties of polyphenols from muscadine grape (*Vitis rotundifolia* Michx.) pomace against selected foodborne pathogens. J. Agric. Food Chem. 2014;62:6640–6649. DOI: 10.1021/jf501073q

[83] Apolónio J, Faleiro M, Miguel M, Neto L. No induction of antimicrobial resistance in *Staphylococcus aureus* and *Listeria monocytogenes* during continuous exposure to eugenol and citral. FEMS Microbiol. Lett. 2014;354:92–101. DOI: 10.1111/1574-6968.12440

[84] Vidigal P, Müsken M, Becker K, Häussler S, Wingender J, Steinmann E, Kehrmann J, Gulbins E, Buer J, Rath P, Steinmann J. Effects of green tea compound epigallocatechin-3-gallate against *Stenotrophomonas maltophilia* infection and biofilm. PLoS One. 2014;9:e92876. DOI: 10.1371/journal.pone.0092876

[85] Morán A, Gutiérrez S, Martínez-Blanco H, Ferrero M, Monteagudo-Mera A, Rodríguez-Aparicio L. Non-toxic plant metabolites regulate *Staphylococcus viability* and biofilm formation: a natural therapeutic strategy useful in the treatment and prevention of skin infections. Biofouling. 2014;30:1175–1182. DOI: 10.1080/08927014.2014.976207

[86] Jordanova V, Borisova D, Paunova-Krasteva TS, Dobrikov G, Nikolova Y, Stoitsova S. Anti-biofilm activity of (-)-fenchone and synthetic derivatives. 2nd International Conference on Natural Products Utilization: From Plants to Pharmacy Shelf; 14–17 October 2015; Plovdiv, Bulgaria. 2015. p. 122.

[87] Bozic D, Milenkovic M, Ivkovic B, Cirkovic I. Newly-synthesized chalcones-inhibition of adherence and biofilm formation of methicillin-resistant *Staphylococcus aureus*. Braz. J. Microbiol. 2014;45:263–270.

[88] El-Mowafy S, Shaaban M, Abd El Galil K. Sodium ascorbate as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. J. Appl. Microbiol. 2014;117:1388–1399. DOI: 10.1111/jam.12631

[89] Mărutescu L, Nitulescu M, Bucur M, Ditu L, Mihăescu G, Lazăr V, Sesan T. Antimicrobial and anti-pathogenic activity of some thioureides derivatives against *Erwinia amylovora* phytopathogenic strains. Roum. Arch. Microbiol. Immunol. 2011;70:49–53.

[90] Brackman G, Forier K, Al Quntar A, De Canck E, Enk C, Srebnik M, Braeckmans K, Coenye T. Thiazolidinedione derivatives as novel agents against *Propionibacterium acnes* biofilms. J. Appl. Microbiol. 2014;116:492–501. DOI: 10.1111/jam.12378

[91] Coenye T, Honraet K, Rigole P, Nadal Jimenez P, Nelis H. In vitro inhibition of *Streptococcus mutans* biofilm formation on hydroxyapatite by subinhibitory concentrations of anthraquinones. Antimicrob. Agents Chemother. 2007;51:1541–1544. DOI: 10.1128/AAC.00999-06

[92] Mitache M, Chifiriuc M, Badea A, Geana O, Bucur M, Olar R, Badea M, Panus E, Rosoiu N, Paul I, Sesan T, Lazăr V. Novel antipathogenic strategies against adherent enterobacterial strains isolated from the hospital environment. Roum. Arch. Microbiol. Immunol. 2008;67:43–48.

[93] Daniel-Hoffmann M, Sredni B, Nitzan Y. Bactericidal activity of the organo-tellurium compound AS101 against *Enterobacter cloacae*. J. Antimicrob. Chemother. 2012;67:2165–2172. DOI: 10.1093/jac/dks185

[94] Domenico P, Baldassarri L, Schoch P, Kaehler K, Sasatsu M, Cunha B. Activities of bismuth thiols against staphylococci and staphylococcal biofilms. Antimicrob. Agents Chemother. 2001;45:1417–1421. DOI: 10.1128/AAC.45.5.1417-1421.2001

[95] Yakandawala N, Gawande P, Lovetri K, Madhyastha S. Effect of ovotransferrin, protamine sulfate and EDTA combination on biofilm formation by catheter-associated bacteria. J. Appl. Microbiol. 2007;102:722–727. DOI: 10.1111/j.1365-2672.2006.03129.x

[96] Cobrado L, Silva-Dias A, Azevedo M, Pina-Vaz C, Rodrigues A. In vivo antibiofilm effect of cerium, chitosan and hamamelitannin against usual agents of catheter-related bloodstream infections. J. Antimicrob. Chemother. 2013;68:126–130. DOI: 10.1093/jac/dks376

[97] Oduwole K, Glynn A, Molony D, Murray D, Rowe S, Holland L, McCormack D, O'Gara J. Anti-biofilm activity of sub-inhibitory povidone-iodine concentrations against *Staphylococcus epidermidis* and *Staphylococcus aureus*. J. Orthop. Res. 2010;28:1252–1256. DOI: 10.1002/jor.21110

[98] Capita R, Riesco-Peláez F, Alonso-Hernando A, Alonso-Calleja C. Exposure of *Escherichia coli* ATCC 12806 to sublethal concentrations of food-grade biocides influences its ability to form biofilm, resistance to antimicrobials, and ultrastructure. Appl. Environ. Microbiol. 2014;80:1268–1280. DOI: 10.1128/AEM.02283-13

[99] Balcázar J, Subirats J, Borrego C. The role of biofilms as environmental reservoirs of antibiotic resistance. Front. Microbiol. 2015;6:1–9. DOI: 10.3389/fmicb.2015.01216

IntechOpen
IntechOpen