AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews

Antimicrobial Chemotherapy | Review

Biofilm antimicrobial susceptibility testing: where are we and where could we be going?

Tom Coenye[1]

AUTHOR AFFILIATION See affiliation list on p. 16.

SUMMARY Our knowledge about the fundamental aspects of biofilm biology, including the mechanisms behind the reduced antimicrobial susceptibility of biofilms, has increased drastically over the last decades. However, this knowledge has so far not been translated into major changes in clinical practice. While the biofilm concept is increasingly on the radar of clinical microbiologists, physicians, and healthcare professionals in general, the standardized tools to study biofilms in the clinical microbiology laboratory are still lacking; one area in which this is particularly obvious is that of antimicrobial susceptibility testing (AST). It is generally accepted that the biofilm lifestyle has a tremendous impact on antibiotic susceptibility, yet AST is typically still carried out with planktonic cells. On top of that, the microenvironment at the site of infection is an important driver for microbial physiology and hence susceptibility; but this is poorly

Editor Ferric C. Fang, University of Washington, Seattle, Washington, USA

Address correspondence to Tom Coenye, Tom.Coenye@UGent.be.

The author declares no conflict of interest.

Published 9 October 2023

reflected in current AST methods. The goal of this review is to provide an overview of the state of the art concerning biofilm AST and highlight the knowledge gaps in this area. Subsequently, potential ways to improve biofilm-based AST will be discussed. Finally, bottlenecks currently preventing the use of biofilm AST in clinical practice, as well as the steps needed to get past these bottlenecks, will be discussed.

KEYWORDS biofilm, susceptibility testing

INTRODUCTION

Microbial biofilms are communities of one or more microorganisms (bacteria and/or fungi) embedded in an extracellular polymeric matrix (produced at least partially by the microorganisms themselves); biofilms can be surface attached or occur as suspended aggregates (1–3). Although cells in the surface-attached biofilms and suspended aggregates show the same phenotype (1), the molecular mechanisms underlying their formation are not necessarily identical (4). In line with previous work, microbial aggregates will be defined as biofilms in this text, regardless of whether they are attached to a biotic or abiotic surface (1).

Microbial biofilms are present in virtually every ecological niche on Earth, and it has been estimated that 40–80% of all microbial cells are biofilm associated (5). An estimated 65–80% of all infections are considered to be biofilm-related (6, 7), and although it is not always completely clear what criteria are used to define an infection as biofilm-related, there is no doubt they have a considerable impact on morbidity, mortality, and healthcare-related costs (8). Biofilms can be found in many types of infections, and while typically associated with chronic infections, recent data point to a role for biofilms in acute infections as well (9, 10). Many biofilms are associated with the use of indwelling medical devices, including (but not limited to) cardiovascular implants, intravascular devices, orthopedic implants (mainly knees and hips), urinary catheters, endotracheal tubes, breast implants, contact lenses, dental implants, and intrauterine devices (8, 11–16). Risk factors for developing a chronic-device related infection include immunomodulatory therapy, diabetes, smoking, and renal disease, suggesting that a compromised innate immune response increases the risk for developing these infections (17). However, not all biofilm infections are related to the use of medical devices, and examples of native tissue biofilms include these identified in respiratory tract infections [e.g., in patients with cystic fibrosis (CF) and chronic rhinosinusitis], chronic otitis media, native valve endocarditis, the oral cavity, and chronically infected wounds (14, 18–22).

While our knowledge about fundamental aspects of microbial biofilms (including knowledge concerning the mechanisms behind their reduced antimicrobial susceptibility) has increased tremendously over the past decades (1, 13, 23–26), the translation of this increased knowledge about biofilm biology to clinical practice is lagging behind. That does not mean no progress was made: for example, guidelines for improved diagnosis of biofilm-associated infections have been published (27, 28), and at least for prosthetic joint infections, "biofilm-active" antibiotics (e.g., rifampicin, ciprofloxacin) have been identified (29–31). However, biofilm-based susceptibility testing, i.e., antimicrobial susceptibility testing (AST) using biofilm-grown bacteria to select the antibiotic(s) to treat a biofilm-related infection, has not yet found its way to the clinical microbiology laboratory, although proposed technologies to do so have been around for over two decades (32). In the present review, I outline the state of the art concerning biofilm AST, highlight the knowledge gaps, and propose solutions to improve biofilm-based AST. In addition, I will discuss what will likely be needed for these biofilm AST methods to be implemented in the clinical microbiology laboratory.

CURRENT APPROACHES FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Conventional approaches

In most cases (empirical therapy being the notable exception), the selection of antimicrobial therapy is made based on the susceptibility profile of the infecting organism, as determined using phenotypic tests in which susceptibility is quantified by measuring the effect of the antibiotic on bacterial or fungal growth, using broth microdilution or gradient strip-based methods. Values obtained in these tests (i.e., minimal inhibitory concentrations, MICs) are then compared to breakpoints established for specific dosing regimens by international organizations like EUCAST and CLSI (33, 34): if the MIC is below the breakpoint, the organism is considered susceptible to the antibiotic, and therapy with this antibiotic is predicted to be successful. Alternatively, susceptibility can be assessed using disk diffusion assays in which susceptibility is quantified based on the size of the inhibition zone (35, 36). While there are automated systems for phenotypic susceptibility testing (37), the majority of these also rely on the growth of the bacterium, and as a consequence, it typically takes 1–2 days to complete the test for rapidly growing microorganisms, and even more time is required for fastidious, slow-growing microorganisms.

Genomic detection of resistance mechanisms

A potential solution for the latter problem is to move beyond phenotypic (growth-based) susceptibility testing and to use bacterial whole-genome sequences (WGS) to infer antimicrobial susceptibility (38–42). However, most WGS-based approaches focus on finding known resistance mechanisms, and while they are successful in that, identifying (combinations of) mutations in one or more genes not previously associated with reduced susceptibility, and incorporating these in a prediction algorithm, remains a major challenge (43). In addition, information derived from WGS cannot predict the expression patterns of genes involved in antimicrobial susceptibility in specific conditions (44). Indeed, the specific conditions in a biofilm and at the infection site lead to distinct gene expression profiles that are different from those observed *in vitro* (45–47), complicating the prediction of biofilm susceptibility based on WGS. For example, several biofilm-specific efflux systems have been described (48, 49) as well as the biofilm-specific synthesis of cyclic-β−1,3-glucans that sequester antibiotics (50) and these mechanisms would be difficult to pick up with WGS alone.

Alternative methods for susceptibility testing

An alternative approach potentially yielding faster results relies on mass spectrometry (more specifically on matrix-assisted laser desorption ionization time-of-flight mass spectrometry, MALDI-TOF MS). With MALDI-TOF MS, a spectrum can be obtained from a microbial sample that can be used for rapid and accurate identification to the species level (51, 52) but also to predict antimicrobial susceptibility (53–55). Discrimination between susceptible and resistant isolates can be made based on presence/absence or change in intensity of certain peaks in the MALDI-TOF spectrum (56, 57). More recently, advanced machine learning algorithms have been used to predict antimicrobial susceptibility of various pathogens based on MALDI-TOF profiles (58–60).

Heat is a by-product of the majority of biological processes; the amount produced is directly related to growth, and the heat production rate is related to the metabolic fluxes; using microcalorimetric devices, the energy released during metabolic processes in microorganisms can be measured (61). Microcalorimetry has two major advantages: (i) it is label free and can be applied in virtually all conditions (e.g., also in turbid media containing blood) and (ii) it allows real-time measurements. Microcalorimetry has been used to determine antimicrobial susceptibility in different organisms, and the results obtained so far are overall in agreement with results obtained with conventional susceptibility tests (62–68).

Alternative culture-based approaches for AST are also being developed. An example of such an approach is the AtbFinder system, in which a medium is used that supports the growth of many different bacteria (TGV medium) (69, 70). The system is based on direct plating of clinical specimens on TGV agar, with or without antibiotics added at a concentration that can be achieved at the infection site; the approach claims to also consider polymicrobial interactions influencing antimicrobial susceptibility. Case studies have suggested that this approach leads to the selection of antibiotics with better efficacy for treating nosocomial pneumonia (71) and chronic relapsing urinary tract infections (72). A recently published clinical trial in which the AtbFinder system was used in the context of respiratory tract infections in CF patients (35 patients, of which 33 were chronically colonized with *Pseudomonas aeruginosa*) suggests that antibiotics selected with AtbFinder lead to clearance of *P. aeruginosa*, a decrease in the number of pulmonary exacerbations, and an increase in lung function (73).

Finally, various microscopy-based approaches for AST have been developed (74–77). For example, the Accelerate Pheno system uses tracking of the size, shape, and division rate of growing cells exposed to antibiotics, to estimate the susceptibility (74, 75); in a clinical trial, the use of this system led to faster changes in antibiotic therapy for bloodstream infections caused by Gram-negative bacteria (78).

However, despite the promising results obtained with some of the alternative AST methods discussed above, additional validation will be required prior to their routine clinical use.

Shortcoming of current approaches

There is frequently a poor correlation between results obtained with *in vitro* susceptibility tests and the effect *in vivo*, for example, in respiratory tract infections in patients with CF (79–81). Indeed, both pharmacodynamic parameters (determining the relationship between the concentration of the antibiotic at the site of action and its physiological effects) and pharmacokinetic parameters (determining the relationship between the concentration of the antibiotic in body fluids and tissues and time) are crucial for the activity of antibiotics *in vivo* (82–84). However, the behavior of microorganisms *in vitro* can be very different from that observed *in vivo*. An important factor contributing to the failure of antimicrobial therapy is that *in vivo* microorganisms form biofilms that show reduced susceptibility toward antimicrobial agents (23, 25). Biofilm cells are phenotypically very different from planktonic cells, and the microenvironment in these surface-attached or suspended biofilms (including gradients of O_2, nutrients and waste products) (85, 86) leads to an altered metabolism linked to reduced susceptibility (24). In addition, the spatial heterogeneity of biofilms may support diversification, i.e., the development of subpopulations with varying degrees of susceptibility, within a patient (87–90). The presence of such subpopulations leads to intrasample diversity in antibiotic susceptibility of isolates and raises questions about the validity of sampling procedures and the common practice of performing susceptibility testing on a limited number of isolates (91, 92). It is worth pointing out that this is not only the case for respiratory tract infections in CF patients, as adaptation and diversification (also in terms of antimicrobial susceptibility) are also observed in other diseases, including non-CF bronchiectasis and urinary tract infections (93–96). Finally, interactions between different microorganisms during (chronic) infections (97–102), as well as interactions between pathogens and the host (103, 104), play an important role in antimicrobial susceptibility, but are difficult to mimick *in vitro*.

BIOFILM-BASED ANTIMICROBIAL SUSCEPTIBILITY TESTING

Pharmacodynamic parameters for the assessment of antimicrobial activity in biofilms

While the MIC and minimal bactericidal concentration (MBC, defined as the lowest concentration that kills all planktonic bacteria) are well-established parameters to assess

antimicrobial activity and predict the success of a treatment, no such standardized parameters are available for biofilm susceptibility testing. Several parameters, including minimal biofilm inhibitory concentration (MBIC), biofilm inhibitory concentration (BIC), minimal biofilm eradication concentration (MBEC), biofilm prevention concentration (BPC), minimum biofilm bactericidal concentration (MBBC), minimum antibiotic concentration for killing (MCK), and biofilm tolerance factor (BTF), have been introduced as measures of biofilm susceptibility (105–111). However, their exact definition frequently varies between different studies and may also depend on the method used to quantify biofilms (e.g., plate counts, crystal violet staining, resazurin-based viability staining) (112, 113) (Table 1). On top of this lack of unambiguously defined pharmacodynamic parameters, there is also an overall lack of standardization in biofilm research that makes comparison between different studies difficult (114–116). Finally, no biofilm-specific breakpoints have been defined yet, complicating the interpretation and clinical use of the above-mentioned parameters.

Tools for biofilm-based antimicrobial susceptibility testing

While most studies on biofilm susceptibility use microtiter plate (MTP)-based systems, in principle any biofilm model system can be used to determine biofilm susceptibility (12, 122–126). Nevertheless, specific methods for biofilm susceptibility testing have been developed, and the most well-known in this context is the MBEC Assay Kit, also known as the Calgary Biofilm Device (32, 107). In this MTP-based assay, biofilms are formed on plastic pegs (uncoated or coated) that are attached to the lid of a 96-well MTP and are immersed in a liquid; subsequently, the established biofilms are transferred to a new 96-well plate for AST (127). Examples of recently described advanced model systems for biofilm susceptibility testing include a microfluidic platform with an integrated sensor (the BiofilmChip) (128), an *ex vivo* CF lung model comprised of pig bronchiolar tissue and synthetic CF sputum (129), the BioFlux system (130, 131), and dissolvable alginate hydrogel-based biofilm microreactors (132). Other innovative models for biofilm AST were recently reviewed (133).

An important part of biofilm-based AST is the quantification of the number of (remaining) viable and/or culturable cells in treated and untreated biofilms. Quantification can be done using detached/dispersed cells, either immediately (i.e., plating of detached cells and counting CFUs after a suitably long incubation time) or after a regrowth phase. In the latter case, the presence or absence of growth can be measured

TABLE 1 Proposed key pharmacodynamic parameters that could be used as measures for biofilm susceptibility and their definition[e]

	Parameter	Abbreviation	Proposed definition/comment[a]
Prevention	Biofilm prevention concentration	BPC	Lowest concentration of an antibiotic required to fully prevent formation of a biofilm (including biofilm aggregates) starting from planktonic cells
Inhibition	Minimal biofilm inhibitory concentration	MBIC	Lowest concentration of an antibiotic required to fully prevent the further development of a biofilm
Eradication	Minimal biofilm eradication concentration	MBEC	Lowest concentration of an antibiotic required to fully eradicate an established biofilm (i.e., resulting in a readout below the detection limit)
Killing	Minimum antibiotic concentration for biofilm killing to achieve x-log reduction[b]	MCBK-x	Lowest concentration of an antibiotic required to achieve x-log reduction in an established biofilm[c]
Relative parameters	Biofilm tolerance[d] factor-prevention	BTF-P	The ratio of the BPC and the MIC
	Biofilm tolerance factor-inhibition	BTF-I	The ratio of the MBIC and the MIC
	Biofilm tolerance factor-eradication	BTF-E	The ratio of the MBEC and the MIC
	Biofilm tolerance factor-x	BTF-x	The ratio of the MCBK-x and the MIC

[a]The definitions are proposed in general terms, i.e., independent of a specific quantification method.
[b]The word "biofilm" was added to the definition previously proposed (110) to avoid any confusion.
[c]The MCBK resulting in complete eradication is equal to the MBEC.
[d]For an in-depth discussion and definition of tolerance, see references (25, 117–121).
[e]Information in this table is partially based on (but not necessarily equal to) definitions proposed previously (107, 109–111, 113).

(spectrophotometrically or by plating) or the length of the lag phase can be used to quantify the number of viable cells (134). Alternatively, quantification can be done directly on the biofilm, using, for example, ATP measurements, crystal violet staining, resazurin-based viability staining, microscopy, electrical impedance, or molecular methods (12, 128, 135–139). A detailed description of biofilm quantification approaches is outside the scope of the present review but it is important to reiterate that different quantification approaches often measure very different things (e.g., measuring optical density after regrowth does not allow to determine the log reduction in CFU, crystal violet stains more than only living cells) and that minor modifications to procedures may lead to different outcomes, as documented, for example, with crystal violet staining (115, 140). Crystal violet staining of surface-attached biofilms is argued as the most used technique, but due to its limitations, it is insufficient as the only method to measure biofilm reduction, and it is recommended that the results obtained with crystal violet staining are confirmed using other approaches (e.g., CFU counts, microscopy). In addition, in many studies, important characteristics like repeatability (i.e., the ability to obtain the same results when performing multiple tests in the same laboratory), reproducibility (i.e., the ability to obtain the same results when performing multiple tests across multiple laboratories), and responsiveness (i.e., the ability to differentiate between different concentrations of the treatment) (116, 141) are not investigated. A thorough assessment of these parameters is of course crucial prior to any clinical implementation. Examples of biofilm-based antimicrobial susceptibility test for which this was done include the MBEC biofilm disinfectant efficacy test (142) and several MTP-based approaches (115).

Is there an association between biofilm formation and antimicrobial susceptibility?

If there would be an association between the biofilm formation *in vitro* (i.e., Can an organism form a biofilm in a certain model system? How much biofilm is formed in a certain period of time?) and antimicrobial susceptibility (i.e., the MIC value), the capability and extent of biofilm formation could be used to predict susceptibility. Below I present a selection of the many studies in which this question has been addressed, organized per taxonomic group in order to facilitate comparisons between studies.

Staphylococcus spp.

Biofilm formation was associated with amikacin resistance in a collection of 49 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, but not with susceptibility to 15 other antibiotics (143). In a collection of 300 *S. aureus* isolates, no associations could be detected between methicillin resistance and biofilm formation, while resistance to erythromycin, clindamycin, and rifampin was associated with increased biofilm formation (144). In a collection of 111 staphylococci from prosthetic joint infections, no association was found between MBEC/MIC ratios and biofilm formation for *S. aureus*, while for *S. epidermidis,* increased biofilm resistance (i.e., high MBEC/MIC ratio) to several antibiotics was observed in strong biofilm producers (145). No significant differences were observed between the biofilm-forming capacity of methicillin-susceptible and methicillin-resistant *Staphylococcus* spp. isolates, or between isolates susceptible or resistant to most other tested antibiotics (total of 229 isolates investigated) (146). The exception was rifampicin: on average, rifampicin-resistant strains formed significantly more biofilm than susceptible strains (146) (Fig. 1A). In a collection of 70 staphylococci from prosthetic joint infections, MBEC/MIC ratios for ciprofloxacin (but not for seven other antibiotics tested) were significantly higher for "strong biofilm producers" than for "non/weak producers" (147).

Acinetobacter baumannii

In a collection of 271 *A. baumannii* isolates, non-multidrug-resistant (MDR) *A. baumannii* isolates tended to form stronger biofilms than MDR and extensively drug-resistant (XDR)

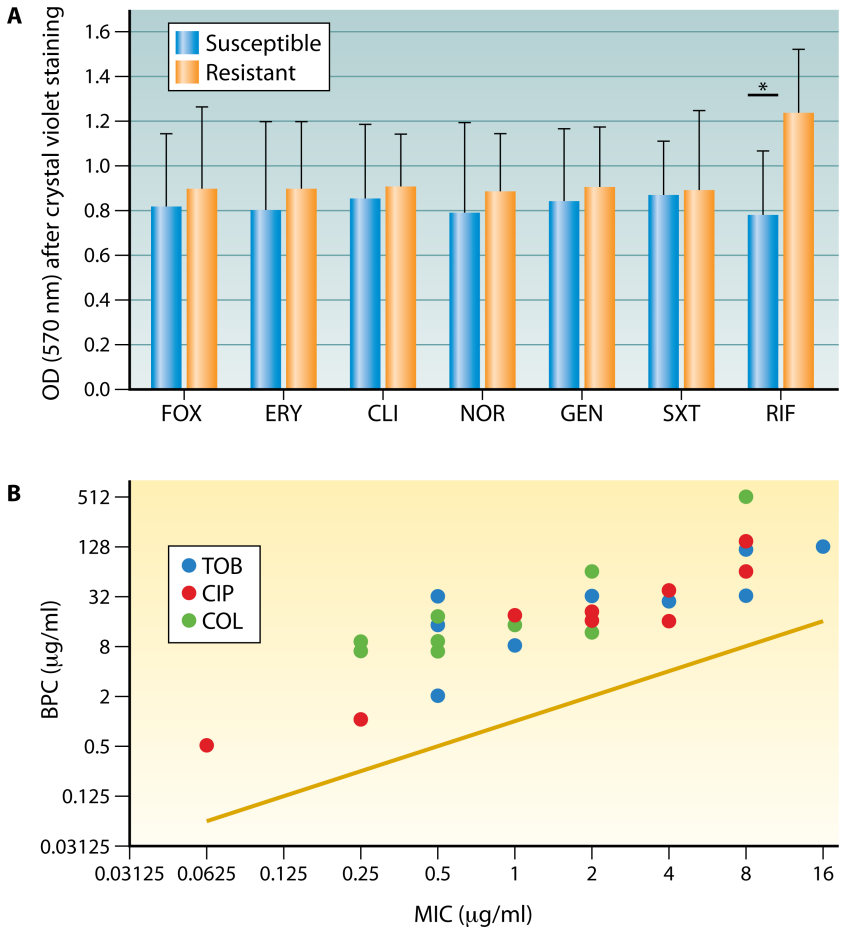

FIG 1 (A) Association between biofilm-forming capacity and resistance to specific antibiotics in a collection of 299 *Staphylococcus* spp. strains; *: $P < 0.05$. Only for rifampicin a significant association between increased biofilm formation (assessed by crystal violet staining) and resistance was observed. Based on data reported in (146). Abbreviations: FOX, cefoxitin; ERY, erythromycin; CLI, clindamycin; NOR, norfloxacin; GEN, gentamicin; SXT, sulfamethoxazole/trimethoprim; TIG, tigecycline; LZD, linezolid; FUS, fusidic acid; RIF, rifampicin; VAN, vancomycin. (B) Association between planktonic (MIC) and biofilm (BPC) susceptibility toward three antibiotics for nine *P. aeruginosa* isolates. The yellow line indicates the situation in which both parameters would be identical. While the BPC is always higher than the MIC, exact BPC values cannot be predicted based on MIC based on the data reported in (148). TOB, tobramycin; CIP, ciprofloxacin; COL, colistin.

strains. For 20/21 antibiotics tested (polymyxin being the exception), susceptible isolates were stronger biofilm formers than intermediate and resistant ones (149). However, in a study with 207 *A. baumannii* isolates, susceptible and less-susceptible strains were found to be equally capable of biofilm formation (150). Likewise, in a collection of 309 *A. baumannii* isolates, no difference was observed between MDR and non-MDR isolates in terms of their biofilm-forming capacity (151).

Escherichia coli and Klebsiella pneumoniae

In a meta-analysis of the link between biofilm formation and antibiotic resistance in uropathogenic *E. coli* (17 studies included), 14 studies showed a positive association between biofilm formation and antibiotic resistance, 2 studies did not show any association, and 1 study reported a negative association between biofilm production and antibiotic resistance (152). Two studies addressed this question in *K. pneumoniae*. In a first study (120 isolates), XDR strains showed a higher ability to form biofilms than

MDR and susceptible strains (153). In a second study with 100 *K. pneumoniae* isolates, ciprofloxacin-susceptible isolates formed stronger biofilms than resistant isolates; such a difference was, however, not observed for other antibiotics (154).

Pseudomonas aeruginosa

Increased biofilm formation (as well as reduced motility) was observed in MDR/XDR high-risk *P. aeruginosa* clones (ST-111, ST-175, and ST-235) (155). However, in a collection of 302 *P. aeruginosa* isolates, the distribution of isolates with different biofilm-forming capacities did not differ among the MDR and non-MDR groups (156). In contrast, in a study with 66 isolates (of which 40 were MDR), an inverse association between resistance and biofilm formation was observed, with more biofilm formation in isolates categorized as non-MDR (157). Finally, a meta-analysis (20 eligible studies published between 2000 and 2019, on isolates recovered in Iran) found that overall biofilm formation was higher in MDR *P. aeruginosa*, although a significant association between biofilm formation and antibiotic resistance was only observed in 10 studies (50%) (158). The above-mentioned studies suggest that the interaction between antimicrobial resistance mechanisms and biofilm formation in *P. aeruginosa* is complex. For example, inactivation of the negative regulator NfxB leads to overexpression of the MexCD-OprJ efflux pump but also to impaired constitutive AmpC overexpression and consequently to decreased periplasmic β-lactamase activity (important for β-lactam resistance). While this leads to increased susceptibility to β-lactam antibiotics in planktonic cells, AmpC secreted by *nfxB* mutants still protects biofilm cells, probably due to the accumulation of AmpC in the biofilm matrix (159).

Discussion

The studies mentioned above clearly indicate that the question whether there is an association between biofilm formation and antimicrobial susceptibility is difficult to answer, with conclusions differing between different studies, even within the same taxonomic group. However, closer inspection reveals that the setup of many studies is suboptimal in terms of including a sufficiently diverse and large collection of isolates, the biofilm model system and quantification approach used, as well as analysis and interpretation of data. In many cases, the biomass of surface-attached biofilms is indirectly quantified (e.g., by using crystal violet), and the values obtained are compared to that of a reference strain and/or arbitrary cut-offs. For example, in one study, biofilms yielding optical density (OD) readouts (at 550 nm, OD_{550nm}) after crystal violet staining that were higher than that of the negative control, but lower than that of a particular reference strain, were designated as "weak biofilm formers," while those with OD_{550nm} values higher than that of the reference strain were considered "strong biofilm formers" (149). In another study, the mean of blank-corrected OD values was used to group isolates into the categories "non-producer" (OD <0.120), "weak producer" (0.120 < OD < 0.240), and "strong producer" (OD > 0.240) (145). While these approaches may work well within a single study, they will likely be difficult to reproduce between different laboratories, and the biological relevance of the (seemingly arbitrary) cut-offs established is unclear. In addition, biofilm susceptibility is often defined based on the MIC of a particular antibiotic for a given isolate, and as discussed in more detail below, using breakpoints established for planktonic cells to categorize biofilms as "susceptible" or "resistant" may lead to misleading results. Finally, the *post hoc ergo propter hoc* assumption (after this, therefore because of this) is frequently made in studies in which a link between biofilm formation and antimicrobial susceptibility is observed, but we need to be careful to accept such an assumption. Biofilm formation and antimicrobial susceptibility (of planktonic and biofilm cells) are influenced by many factors, including stochastic events (e.g., stochastic formation of dormant persister cells) (160), variability in microbial populations (e.g., occurrence of heteroresistance in populations containing subpopulations of cells with lower susceptibility than the majority of the population)

(117, 161), and the microenvironment (*in vitro* as well as *in vivo* at the site of infection, e.g., presence of certain nutrients) (26, 162, 163), and it may very well be that there simply is no mechanistic link between biofilm formation and planktonic susceptibility.

Can biofilm susceptibility be predicted based on the MIC?

The question whether planktonic susceptibility can be used to predict biofilm susceptibility is an important one, because if MIC values, determined according to highly standardized EUCAST or CLSI procedures, would be a good proxy for biofilm susceptibility, dedicated biofilm AST would not be needed. Although planktonic and biofilm susceptibility parameter values for the same strain/antibiotic combinations have been determined in many studies, direct comparisons are again difficult due to differences in methodology and/or the lack of reporting susceptibility data for individual isolates. Below I focus on a selected set of studies that addressed this question for *P. aeruginosa* clinical isolates.

Moskowitz et al. compared the susceptibility of planktonic cultures (MIC, determined according to CLSI guidelines) and biofilms (BIC, using the Calgary Biofilm Device) for 94 *P. aeruginosa* isolates toward 12 antibiotics (105). BICs were substantially higher than MICs for doxycycline and most of the β-lactam antibiotics investigated (aztreonam, ceftazidime, piperacillin-tazobactam, and ticarcillin-clavulanate), while BICs of gentamicin and meropenem were only somewhat higher than the corresponding MICs, and BICs and MICs were fairly similar for amikacin, tobramycin, and ciprofloxacin. Azithromycin showed fairly low BICs, although *P. aeruginosa* is considered as resistant in standard susceptibility testing. In a study with 57 non-mucoid *P. aeruginosa* isolates, planktonic (MIC) and biofilm (BPC, BIC) susceptibilities were determined for levofloxacin, ciprofloxacin, imipenem, ceftazidime, tobramycin, colistin, and azithromycin (106). Some antibiotics showed median BPCs that were in the same range as MICs (fluoroquinolones, tobramycin, colistin), while others (ceftazidime, imipenem) had BPCs that were much higher than MICs. The former antibiotics also had relatively low BICs, indicating they may have activity against established biofilms. In a study with 133 *P. aeruginosa* isolates, marked differences between MIC and "biofilm active score" (BAS) values (the latter determined based on microscopic assessment of the fraction of living cells after treatment) were observed for aztreonam and tobramycin (164). For 19.4% and 30.0% of the isolates that are resistant toward aztreonam and tobramycin, respectively, when grown planktonically, the biofilm biomass (as evaluated microscopically) was reduced with 50–75%. *Vice versa,* 63.6% of the aztreonam-sensitive and 66.2% of the tobramycin-sensitive isolates were non-responsive when grown as a biofilm. Using MIC, minimum antibiotic concentrations for killing (MCK, the concentration that resulted in a certain reduction in number of CFU of biofilm-grown cells) and the biofilm tolerance factor (BTF, the ratio of MCK and the MIC) (Table 1) as parameters for susceptibility to tobramycin, ciprofloxacin and colistin, Thöming & Häussler (110) observed that in a large (n = 352) collection of clinical *P. aeruginosa* isolates, biofilm susceptibility values showed a wide distribution, even among isolates for which MIC values were similar; in addition, among isolates with a similar MCK value, a wide spread in MIC values was observed (110). In a recent study, BPC values of tobramycin, ciprofloxacin, or colistin (obtained with a resazurin-based viability staining on *P. aeruginosa* biofilms formed in a synthetic CF sputum medium) were at least four-fold higher than the MIC values (148) (Fig. 1B). However, BPC/MIC ratios were antibiotic dependent, with BPC/MIC ratios for colistin being significantly higher than those for ciprofloxacin. Overall, a strong and significant rank correlation was observed between the MIC and the BPC for all antibiotics (i.e., strains showing higher MICs also show higher BPCs). Comparison of BPC with the MBC yielded a different picture. BPC values could be higher, equal, or lower than the MBC, and the overall differences between BPC and MBC were smaller than the differences between BPC and MIC. The BPC/MBC ratio was significantly smaller for ciprofloxacin than for colistin or tobramycin, and while strong and significant correlations were observed

between MBC and BPC for tobramycin and ciprofloxacin, this was not the case for colistin (148).

The selected studies discussed above suggest that while there may be an overall positive correlation between planktonic and biofilm susceptibility measurements, in many cases the reduced susceptibility observed in biofilms is independent of resistance in planktonic cultures. In addition, the relation between planktonic and biofilm susceptibility is antibiotic dependent, and the impact of the biofilm model used and the stage in which the biofilms are tested on this relation is likely substantial (165–169). Finally, due to the lack of biofilm-specific antimicrobial susceptibility breakpoints, in many studies BPC, MBIC, or MBEC values that are above the MIC are taken as evidence for "biofilm resistance". Considering the profound differences between planktonic cultures and biofilms, it seems, however, ill-advised to use breakpoints established for planktonic cells to categorize biofilms as "susceptible" or "resistant."

Do the results of biofilm-based susceptibility tests correlate with clinical outcome?

While there are many *in vitro* studies in which planktonic and biofilm susceptibility toward different antibiotics are compared, there are few studies in which these data are linked to the clinical outcome of treatment with these particular antibiotics. Most of these pertain to prosthetic joint infections or respiratory tract infections in CF.

Prosthetic joint infections

In the context of prosthetic joint infections, biofilm-active antibiotics (defined as antibiotics that penetrate into the biofilm and are able to eradicate the bacteria in the biofilm) have been identified; these include rifampicin for staphylococci and ciprofloxacin for Gram-negative bacteria (31). A distinction is frequently made been "difficult-to-treat" infections that are caused by pathogens resistant to these biofilm-active antibiotics and prosthetic joint infections caused by susceptible organisms (29). Using a prospective cohort of patients (n = 163) treated with a two-stage prosthesis exchange according to a standardized algorithm, Akgun et al. investigated whether the outcome of "difficult-to-treat" prosthetic joint infections (n = 30, 18.4%) is worse than that of other prosthetic joint infections (n = 133, 81.6%) (170). While the infection-free survival rate at 2 years did not differ between both groups, hospital stay, prosthesis-free interval, and duration of treatment were significantly longer in the "difficult-to-treat" group than in the other group. This indicates that treatment with antibiotics that have activity against biofilms improves outcome, suggesting that knowing which antibiotic has such an anti-biofilm activity could be clinically relevant. In a prospective cohort study with 131 patients with a prosthetic knee infection, the outcome of the treatment was compared between patients treated with biofilm-active antibiotics (n = 55, 42%) or other antibiotics (n = 76, 58%) (30). The infection-free survival after 1 year and 2 years was significantly higher for patients who received biofilm-active antibiotics, and treatment with biofilm-active antibiotics was associated with lower pain intensity (30). In a group of 93 patients with infected spinal implants, treatment outcome was also compared between patients receiving biofilm-active antibiotics (n = 30, 32%) and those who received no biofilm-active antibiotics (n = 63, 68%). The infection-free survival differed significantly between both groups: for patients who received biofilm-active antibiotics, it was 94% and 84% after 1 year and 2 years, respectively, while it was only 57% and 49% for patients who received no biofilm-active antibiotics. In addition, patients receiving biofilm-active antimicrobial therapy reported lower intensity of postoperative pain (171). In a retrospective, observational, multicenter study involving 203 cases, treatment with biofilm-active antibiotics (rifampicin/fluoroquinolones) had a favorable impact on infections caused by staphylococci and Gram-negative bacteria. For example, the combination fluoroquinolone/rifampicin for staphylococcal infections significantly reduced implant failure (2% compared to 11% in the control group) (172). However, despite these observations, no association between MBEC values (for

oxacillin, daptomycin, levofloxacin, rifampicin, and levofloxacin/rifampicin combinations) and clinical outcome was observed in a study with 88 patients with a *S. aureus* prosthetic joint infection (173). This seems to contradict the evidence that the good *in vitro* anti-biofilm activity of antibiotic combinations containing rifampicin translates into high activity in animal prosthetic joint infection models and in patients suffering from biofilm-associated staphylococcal prosthetic joint infections (147, 174–180). It should be noted that the addition of rifampicin to the standard treatment did not lead to better outcomes in a recent clinical trial (181), although the setup of this trial was later criticized (31, 182). In two recent studies, MBEC/MIC ratios were determined for staphylococci recovered from prosthetic joint infections and linked to clinical outcome (145, 147). In both studies, these ratios were lowest for rifampicin, again suggesting rifampicin has good antibiofilm activity *in vivo*. For 70 strains recovered from 49 patients with a first-time prosthetic joint infection (monomicrobial infection caused by staphylococci or polymicrobial infection caused by two different species of staphylococci), the oxacillin MBEC/MIC ratios were significantly higher in recurrent infections compared to resolved infections; no significant differences between the two patient groups were observed for MBEC/MIC ratios for other antibiotics (147). In a subsequent study (111 staphylococcal strains from 66 patients), the increased oxacillin MBEC/MIC ratios for *S. aureus* from unresolved prosthetic joint infections (median MBEC/MIC ratio of 1,166 for isolates from unresolved infections vs median MBEC/MIC ratio of 808 for isolates from resolved infections) were confirmed (145), suggesting that high relative MBEC values (compared to the MIC) are associated with poorer treatment outcome after a staphylococcal prosthetic joint infection. There are less data on the added value of using biofilm-active fluoroquinolones against prosthetic joint infections caused by Gram-negatives. In a study with 47 patients with acute prosthetic joint infections caused by a Gram-negative organism, treatment with a fluoroquinolone (when all the strains isolated were susceptible to this antibiotic) was associated with a good prognosis (183). In a study on 160 patients with an early prosthetic joint infection, treatment failed in 43 patients (26.9%), and the presence of a Gram-negative infection not treated with fluoroquinolones was identified as an independent predictor of therapy failure (184). Finally, in patients with prosthetic joint infections due to ciprofloxacin-susceptible Gram-negatives, the success rate of treatment was 79% (98/124 patients) in patients receiving ciprofloxacin; this was significantly lower in patients not treated with ciprofloxacin (40%, 6/15 patients) (185).

Respiratory tract infections in CF

In a retrospective study involving 110 CF patients (infected with different microorganisms), patients treated with antibiotics that were found to be active against biofilm-grown bacteria *in vitro* showed a significant reduction in the sputum bacterial density, a significant reduction in the length of hospital stay, and a non-significant decrease in treatment failure (186). However, the only two randomized clinical studies addressing the added value of using antibiotics with activity against biofilms yielded no evidence for choosing antibiotics based on biofilm AST for the treatment of *P. aeruginosa* respiratory tract infections in people with CF (187). In the first study (188), 39 patients were randomized to biofilm and conventional treatment groups, in which antibiotics were selected based on biofilm susceptibility testing with the Calgary biofilm device and broth susceptibility testing, respectively. However, no microbiological or clinical differences were observed between both groups. In the second study (189), the effect of 14 d of intravenous antibiotic treatment for pulmonary exacerbations due to *P. aeruginosa* was compared between patients receiving treatment based on conventional or biofilm antimicrobial susceptibility results. Also in this study, no differences in microbiological (sputum density at day 14 of the treatment and at the 1 mo follow-up visit) or lung function parameters could be observed between both groups.

Potential explanations for the lack of association between biofilm susceptibility and clinical outcome

While large randomized clinical trials about the use of biofilm-active antibiotics in prosthetic joint infections are lacking, the data summarized above seem to indicate an added value of using biofilm-active antibiotics in this context, suggesting that predicting which antibiotics would have activity against biofilms (especially in the context of "difficult-to-treat" infections and/or infections caused by less-frequently encountered pathogens) could lead to an improved outcome (although the apparently conflicting data about biofilm activity of rifampicin remains to be settled). The situation is, however, different in the context of biofilm-related respiratory tract infections in CF, where two randomized clinical trials could not find an added value of biofilm-based susceptibility testing, despite promising data in a retrospective study (186). While it cannot be ruled out that the very different etiology of prosthetic joint infections and respiratory tract infections in CF is behind this apparent discrepancy, it should be noted that in the two clinical trials in CF patients, biofilm susceptibility was determined using the Calgary biofilm device and cation-adjusted Mueller-Hinton broth as growth medium (105, 188, 189). In this model, biofilms will develop as surface-attached communities in a growth medium that is physicochemically very different from CF sputum. However, we know that the microenvironment plays an important role in various aspects of biofilm biology (including metabolism) and likely has a profound impact on antimicrobial susceptibility (13, 26, 148, 190, 191). It should thus maybe not come as a surprise that biofilm susceptibility testing in an *in vitro* model that is poorly representative of the *in vivo* situation yields susceptibility data that are poorly representative of the activity of the antibiotic against *in vivo* biofilms (114, 192); indeed, such tests may not be a better predictor of *in vivo* anti-biofilm activity than planktonic susceptibility tests.

HOW CAN WE IMPROVE BIOFILM SUSCEPTIBILITY TESTING AND MAKE IT MORE RELEVANT FOR CLINICAL PRACTICE?

The importance of standardization and use of appropriate parameters

In order for biofilm AST to find its way to clinical practice, substantial standardization will be required in order to obtain methods that are reproducible and repeatable and yield susceptibility data that are in categorical agreement, regardless of the place where they were obtained (114). Standardization and reproducibility in biofilm research have been receiving increasing attention, especially (but not exclusively) in the context of developing products or devices with anti-biofilm activity (114–116, 125, 142, 192–196). The recent launch of an International Biofilm Standards Task Group (https://www.biofilms.ac.uk/international-standards-task-group/) is in line with this increased attention for standards. The challenge of developing standardized biofilm susceptibility tests should not be underestimated. Biofilm-based assays are inherently more complex than assays based on planktonic cells, and even results from these (technically less-demanding) conventional susceptibility tests are influenced by minor deviations from the published reference methods, again highlighting the need for standardization and adequate quality control (34, 197–200). While many factors influence the outcome of a biofilm experiment, results from several studies suggest that how the biofilm is grown and how the inoculum is prepared are crucial (115, 201–203) and that reproducibility between laboratories improves when a common (standardized) protocol is used (115).

However, prior to standardization, there needs to be a consensus on which pharmacodynamic parameter(s) (Table 1; Fig. 2) is (are) the most important. It could be argued that in line with planktonic susceptibility testing, we first and foremost want to know which antibiotic will affect the development of a biofilm, but whether this pertains to the development starting from a planktonic culture (i.e., *prevention* of biofilm formation, parameter: BPC) or from a young biofilm (i.e., *inhibition* of progression of biofilm formation, parameter: MBIC) is open for discussion. It is currently unclear whether biofilm-associated infections are initiated by the introduction of single cells, aggregates,

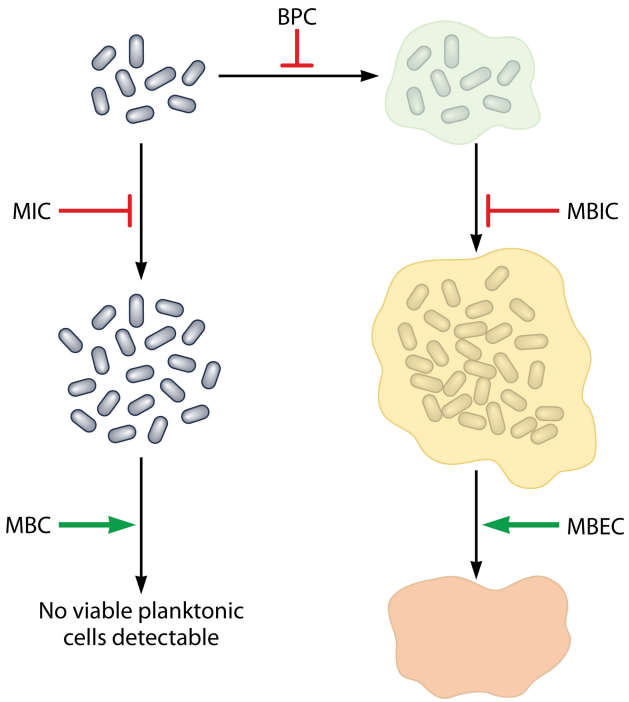

FIG 2 Illustration of key pharmacodynamic parameters that could be used as measures for biofilm susceptibility. MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; BPC, biofilm prevention concentration; MBIC, minimal biofilm inhibitory concentration; MBEC, minimal biofilm eradication concentration.

or both (1), but regardless of this, it seems in most cases unlikely that antibiotic therapy would be started so quickly after the introduction of the organisms that no aggregates would be present at the start of the treatment (even if the infection was initiated by single cells), which would argue for the use of MBIC as parameter. An exception to this would be antibiotic therapy started prior, during, or immediately after surgery in which case the presence of single cells or very small aggregates is more likely. In many cases, antibiotic therapy will only be started after the patient starts showing symptoms, and this means that in most cases, biofilm aggregates will already have formed. This implies that it is also important to know which concentrations of an antibiotic will lead to partial reduction (i.e., a reduction in biofilm, but not complete eradication) or full eradication. For the latter, the MBEC is an appropriate parameter, while the MCK-x (i.e., the concentration required to achieve x-log reduction) can be used for the former. Finally, biofilm tolerance factors (BTF-I, BTF-E, BTF-x; Table 1) could be used to quantify biofilm-related reduced susceptibility in comparison to susceptibility of planktonic cells (110).

The proposed definitions in Table 1 are independent of the analysis method used and are (at least in theory) equally valid for different biofilm quantification approaches. However, in the context of biofilm AST, approaches that directly (e.g., plate counts) or indirectly (e.g., resazurin-based viability staining, ATP measurements) quantify the number of living and/or culturable cells will likely be preferred over methods that only provide crude measurements of biofilm biomass (e.g., biofilm biomass staining with crystal violet).

Setting of biofilm breakpoints

Breakpoints are used to distinguish between "susceptible" organisms ("susceptible" implying that the use of a particular antibiotic for this organism is associated with a high likelihood of therapeutic success) and "resistant" organisms ("resistance" implying that

the use of this particular antibiotic for an infection caused by this organism is typically associated with clinical failure) (33, 204). These breakpoints are set by organizations like EUCAST and CLSI and take into account a wide range of parameters, including data from large-scale clinical studies, wild-type MIC distributions, and PK/PD aspects (33, 35, 36, 205–207). As none of these data are currently available for biofilm infections, setting biofilm breakpoints will be far from trivial, and as already mentioned above, there is no evidence for an added value of using planktonic breakpoints to categorize biofilms as "susceptible" or "resistant." Recently, a potential solution was proposed for the lack of biofilm breakpoints, i.e., determining epidemiological cut-off (ECOFF) values (MBIC-ECOFF and MBEC-ECOFF) to distinguish between strains belonging to the wild-type population and strains belonging to the population possessing acquired mechanisms responsible for reduced antimicrobial susceptibility of biofilms (208). This approach is in line with the EUCAST recommendations for setting breakpoints for the topical use of antimicrobial agents and the use of inhaled antibiotics (209). Of course, establishing such ECOFFs would only be the first step, and biofilm breakpoints should ultimately be based on data from large clinical studies.

Increasing the biological relevance of *in vitro* tests

We know that the nutritional environment can influence the results of conventional AST, and several attempts have been made to increase the biological relevance of *in vitro* AST by re-creating the *in vivo* conditions *in vitro* (104, 163, 210–216). However, in the absence of a thorough validation, it is unclear whether these modified test conditions really are more *in vivo*-like, and it is often also unclear whether microorganisms grown in these systems reflect the *in vivo* biofilm phenotype.

Many different artificial or synthetic sputum media, mimicking the composition of CF sputum, have been developed (217–220), and it is also in this context that the "*in vivo*-likeness" of at least some media has been evaluated to the greatest extent, both in terms of gene expression (45, 47) and in terms of morphological similarity between *in vitro* and *in vivo P. aeruginosa* aggregates (221). Likewise, substantial efforts have been made to develop growth media that better represent the *in vivo* microenvironment of a prosthetic joint infection, mainly based on the addition of human or animal synovial fluid, or the development of synthetic synovial fluid (222–230) (Fig. 3). Most of the work done in these media so far has focused on studying the formation of biofilm aggregates in various staphylococci, but some of the media developed have been used to asses biofilm antimicrobial susceptibility as well (223, 224, 226). Finally, a range of relevant models for the study of infected wounds have been developed that allow to study antimicrobial treatments of these biofilm-related infections under *in vivo* or *in vivo*-like conditions (231–238).

The need for clinical trials to validate the use of biofilm-based susceptibility testing in clinical practice

Even if we manage to develop standardized and physiologically relevant *in vivo*-like biofilm models that can be incorporated in the workflow of a clinical microbiology lab, their success will ultimately depend on whether using them improves the clinical outcome of a treatment.

The added value of biofilm-based AST for treating a specific biofilm-related infection could be determined in a clinical trial in which patients are randomized to a "conventional treatment group" (in which antibiotic treatment is selected based on conventional susceptibility testing) and a "biofilm treatment group" (in which antibiotic treatment is selected based on biofilm-based susceptibility testing), much like was done for CF (188, 189). A protocol of a proposed prospective randomized clinical trial for the selection of antibiotics in periprosthetic joint infections guided by MBEC and MIC determinations was recently published (239). This trial aims to include patients with first-time prosthetic joint (hip or knee) infections (monomicrobial infections with *Staphylococcus* spp.), and its

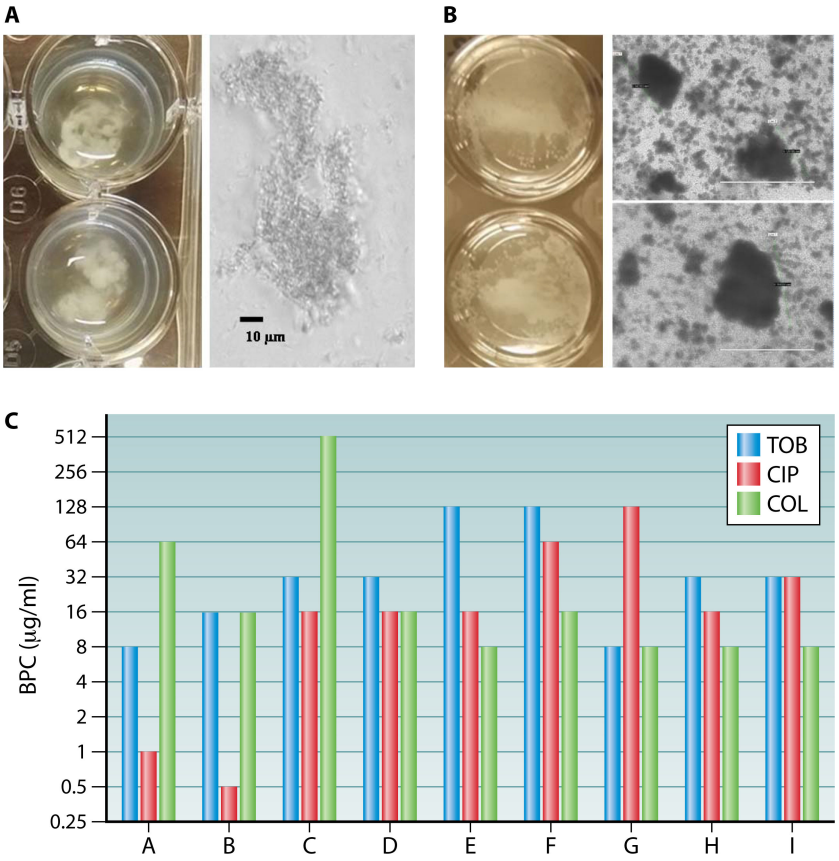

FIG 3 (A) *P. aeruginosa* biofilm aggregate grown in SCFM2 medium. (B) *S. aureus* biofilm aggregate grown in synthetic synovial fluid medium. (C) Biofilm prevention concentration of three antibiotics against nine *P. aeruginosa* biofilms (A–I) determined in SCFM2 [based on data reported in (148)].

primary outcome measurement is the proportion of changes in antimicrobial regimen from first-line treatment. The trial aims to recruit 64 patients who will be randomized to a standard of care arm (choice of antibiotic guided by MIC) or a comparative arm (selection of antibiotics based on MIC and MBEC) (239).

However, setting up such a randomized controlled trial, with a sufficiently high number of patients in each group and clearly defined endpoints, will be challenging. Obtaining ethical approval might also be difficult, either because it is accepted by many that a particular antibiotic is superior to others, e.g., in the case of rifampicin for treating prosthetic joint infections (182), or because of the disappointing outcomes in earlier trials, e.g., in CF (188, 189). Finally, for many biofilm-related infection (including wound infections and prosthetic joint infections), administration of antibiotics is only a part of the treatment; and variations in other interventions (e.g., surgical debridement, one-or two-stage revision surgery) will complicate recruitment, randomization, and interpretation of the outcome (240). Considering these difficulties, a more feasible alternative approach could be envisaged in which the antibiofilm activity of antibiotics is determined in one or more optimized models in order to devise treatment regimens with potential *in vivo* activity against biofilms. In a second step, the clinical outcome of these biofilm-active regimens can then be compared to the outcome observed with conventional therapy (i.e., therapy with antibiotics selected based on conventional AST).

The results obtained such studies will allow to build a knowledge base for further research that could ultimately pave the way for a broader introduction of these approaches in the clinical microbiology laboratory.

Practical aspects

The success of biofilm-based AST in the clinical laboratory will also depend on the development and implementation of affordable, reproducible, and high-throughput tools that yield results that are easy to interpret, as it seems very unlikely that methods based on complex low-throughput biofilm model systems, using expensive advanced approaches for readouts, and/or requiring extensive hands-on time, will find their way to clinical practice. However, the highly successful introduction of MALDI-TOF mass spectrometry for rapid and accurate identification of microorganisms in the clinical microbiology laboratory (241–244) shows that the development and implementation of advanced methodology are possible. While it is at this point difficult to predict what exactly will be needed, it will likely involve the development of validated and standardized premade relevant media to grow biofilms and the development and implementation of automated and high-throughput methods for reading biofilm susceptibility. Regardless of what form biofilm-based AST ultimately will take, the successful implementation will require the collaboration between basic researchers, clinical microbiology laboratories, and (potentially new) companies involved in developing and marketing diagnostic tools.

CONCLUDING REMARKS

The call for bringing biofilm AST to the clinic is not new. Already in 2006, Sandoe et al. wrote that "*Data from large numbers of clinical episodes would be required to define the relationship between MBIC and clinical outcome before any advantages over MIC could be assessed. We hope that this work will stimulate the investigation of susceptibility tests that have more relevance to biofilm infections than current methods*." (245). Our profound knowledge about biofilm formation (1), our insights into mechanisms responsible for reduced susceptibility in biofilms (25, 86), and the realization that the infectious microenvironment plays a crucial role in antimicrobial susceptibility (26) will be essential to develop and validate relevant biofilm-based AST methods that can be used in clinical microbiology laboratories. The crucial next step will be the evaluation of these methods in well-designed clinical trials, with an ultimate goal to improve antibiotic treatment of patients suffering from biofilm-related infections.

ACKNOWLEDGMENTS

I want to thank the Lundbeck Foundation (Denmark) and FWO-Vlaanderen (Belgium) for supporting a stay at the Costerton Biofilm Center (Copenhagen, Denmark), during which most of his review was written.

I also thank Amber De Bleeckere (Laboratory of Pharmaceutical Microbiology, Ghent University) for sharing the unpublished data used in Fig. 3.

AUTHOR AFFILIATION

[1]Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

AUTHOR ORCIDs

Tom Coenye http://orcid.org/0000-0002-6407-0601

AUTHOR CONTRIBUTIONS

Tom Coenye, Conceptualization, Funding acquisition, Investigation, Writing – original draft

REFERENCES

1. Sauer K, Stoodley P, Goeres DM, Hall-Stoodley L, BurmolleM, Stewart PS, Bjarnsholt T. 2022. The biofilm life cycle: expanding the conceptual model of biofilm formation. Nat Rev Microbiol 20:608–620. https://doi.org/10.1038/s41579-022-00767-0

2. Flemming HC, Baveye P, Neu TR, Stoodley P, Szewzyk U, Wingender J, Wuertz S. 2021. Who put the film in biofilm? The migration of a term from wastewater engineering to medicine and beyond. NPJ Biofilms Microbiomes 7:10. https://doi.org/10.1038/s41522-020-00183-3
3. Flemming H-C, van Hullebusch ED, Neu TR, Nielsen PH, Seviour T, Stoodley P, Wingender J, Wuertz S. 2023. The biofilm matrix: multitasking in a shared space. Nat Rev Microbiol 21:70–86. https://doi.org/10.1038/s41579-022-00791-0
4. Staudinger BJ, Muller JF, Halldórsson S, Boles B, Angermeyer A, Nguyen D, Rosen H, Baldursson O, Gottfreðsson M, Guðmundsson GH, Singh PK. 2014. Conditions associated with the cystic fibrosis defect promote chronic *Pseudomonas aeruginosa* infection. Am J Respir Crit Care Med 189:812–824. https://doi.org/10.1164/rccm.201312-2142OC
5. Flemming HC, Wuertz S. 2019. Bacteria and archaea on earth and their abundance in biofilms. Nat Rev Microbiol 17:247–260. https://doi.org/10.1038/s41579-019-0158-9
6. Wolcott RD, Ehrlich GD. 2008. Biofilms and chronic infections. JAMA 299:2682–2684. https://doi.org/10.1001/jama.299.22.2682
7. Lewis K. 2007. Persister cells, dormancy and infectious disease. Nat Rev Microbiol 5:48–56. https://doi.org/10.1038/nrmicro1557
8. Wolcott RD, Rhoads DD, Bennett ME, Wolcott BM, Gogokhia L, Costerton JW, Dowd SE. 2010. Chronic wounds and the medical biofilm paradigm. J Wound Care 19:45–46, https://doi.org/10.12968/jowc.2010.19.2.46966
9. Kolpen M, Jensen PØ, Faurholt-Jepsen D, Bjarnsholt T. 2022. Prevalence of biofilms in acute infections challenges a longstanding paradigm. Biofilm 4:100080. https://doi.org/10.1016/j.bioflm.2022.100080
10. Kolpen M, Kragh KN, Enciso JB, Faurholt-Jepsen D, Lindegaard B, Egelund GB, Jensen AV, Ravn P, Mathiesen IHM, Gheorge AG, Hertz FB, Qvist T, Whiteley M, Jensen PØ, Bjarnsholt T. 2022. Bacterial biofilms predominate in both acute and chronic human lung infections. Thorax 77:1015–1022. https://doi.org/10.1136/thoraxjnl-2021-217576
11. Caldara M, Belgiovine C, Secchi E, Rusconi R. 2022. Environmental, microbiological, and immunological features of bacterial biofilms associated with implanted medical devices. Clin Microbiol Rev 35:e0022120. https://doi.org/10.1128/cmr.00221-20
12. Magana M, Sereti C, Ioannidis A, Mitchell CA, Ball AR, Magiorkinis E, Chatzipanagiotou S, Hamblin MR, Hadjifrangiskou M, Tegos GP. 2018. Options and limitations in clinical investigation of bacterial biofilms. Clin Microbiol Rev 31:e00084-16. https://doi.org/10.1128/CMR.00084-16
13. Bjarnsholt Thomas, Alhede M, Alhede M, Eickhardt-Sørensen SR, Moser C, Kühl M, Jensen PØ, Høiby N. 2013. The *in vivo* biofilm. Trends in Microbiology 21:466–474. https://doi.org/10.1016/j.tim.2013.06.002
14. Bjarnsholt T, Jensen PØ, Moser C, Høiby N. 2011. Biofilm infections. New York, NY. https://doi.org/10.1007/978-1-4419-6084-9
15. Shirtliff M, Leid JG. 2009. The role of biofilms in device-related infections. Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-68119-9
16. Donelli G. 2015. Biofilm-based Healthcare-associated infections. Cham. https://doi.org/10.1007/978-3-319-11038-7
17. Stewart PS, Bjarnsholt T. 2020. Risk factors for chronic biofilm-related infection associated with implanted medical devices. Clin Microbiol Infect 26:1034–1038. https://doi.org/10.1016/j.cmi.2020.02.027
18. Hajishengallis G, Lamont RJ, Koo H. 2023. Oral polymicrobial communities: assembly, function, and impact on diseases. Cell Host Microbe 31:528–538. https://doi.org/10.1016/j.chom.2023.02.009
19. Kouijzer JJP, Noordermeer DJ, van Leeuwen WJ, Verkaik NJ, Lattwein KR. 2022. Native valve, prosthetic valve, and cardiac device-related infective endocarditis: a review and update on current innovative diagnostic and therapeutic strategies. Front Cell Dev Biol 10:995508. https://doi.org/10.3389/fcell.2022.995508
20. Buch PJ, Chai Y, Goluch ED. 2019. Treating polymicrobial infections in chronic diabetic wounds. Clin Microbiol Rev 32:e00091-18. https://doi.org/10.1128/CMR.00091-18
21. Welp AL, Bomberger JM. 2020. Bacterial community interactions during chronic respiratory disease. Front Cell Infect Microbiol 10:213. https://doi.org/10.3389/fcimb.2020.00213
22. Boisvert AA, Cheng MP, Sheppard DC, Nguyen D. 2016. Microbial biofilms in pulmonary and critical care diseases. Ann Am Thorac Soc 13:1615–1623. https://doi.org/10.1513/AnnalsATS.201603-194FR
23. Van Acker H, Van Dijck P, Coenye T. 2014. Molecular mechanisms of antimicrobial tolerance and resistance in bacterial and fungal biofilms. Trends Microbiol. 22:326–333. https://doi.org/10.1016/j.tim.2014.02.001
24. Crabbe A, Jensen PO, Bjarnsholt T, Coenye T. 2019. Antimicrobial tolerance and metabolic adaptations in microbial biofilms. Trends Microbiol. 27:850–863. https://doi.org/10.1016/j.tim.2019.05.003
25. Ciofu O, Moser C, Jensen PØ, Hoiby N. 2022. Tolerance and resistance of microbial biofilms. Nat Rev Microbiol 20:621–635. https://doi.org/10.1038/s41579-022-00682-4
26. Bjarnsholt T, Whiteley M, Rumbaugh KP, Stewart PS, Jensen PØ, Frimodt-Møller N. 2022. The importance of understanding the infectious microenvironment. Lancet Infect Dis 22:e88–e92. https://doi.org/10.1016/S1473-3099(21)00122-5
27. Hall-Stoodley L, Stoodley P, Kathju S, Høiby N, Moser C, Costerton JW, Moter A, Bjarnsholt T. 2012. Towards diagnostic guidelines for biofilm-associated infections. FEMS Immunol Med Microbiol 65:127–145. https://doi.org/10.1111/j.1574-695X.2012.00968.x
28. Høiby N, Bjarnsholt T, Moser C, Bassi GL, Coenye T, Donelli G, Hall-Stoodley L, Holá V, Imbert C, Kirketerp-Møller K, Lebeaux D, Oliver A, Ullmann AJ, Williams C, ESCMID Study Group for Biofilms and Consulting External Expert Werner Zimmerli. 2015. ESCMID guideline for the diagnosis and treatment of Biofilm infections 2014. Clin Microbiol Infect 21 Suppl 1:S1–25. https://doi.org/10.1016/j.cmi.2014.10.024
29. Izakovicova P, Borens O, Trampuz A. 2019. Periprosthetic joint infection: current concepts and outlook. EFORT Open Rev 4:482–494. https://doi.org/10.1302/2058-5241.4.180092
30. Gellert M, Hardt S, Köder K, Renz N, Perka C, Trampuz A. 2020. Biofilm-active antibiotic treatment improves the outcome of knee periprosthetic joint infection: results from a 6-year prospective cohort study. Int J Antimicrob Agents 55:105904. https://doi.org/10.1016/j.ijantimicag.2020.105904
31. Rottier W, Seidelman J, Wouthuyzen-Bakker M. 2023. Antimicrobial treatment of patients with a periprosthetic joint infection: basic principles. Arthroplasty 5:10. https://doi.org/10.1186/s42836-023-00169-4
32. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. 1999. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 37:1771–1776. https://doi.org/10.1128/JCM.37.6.1771-1776.1999
33. Mouton JW, Brown DFJ, Apfalter P, Cantón R, Giske CG, Ivanova M, MacGowan AP, Rodloff A, Soussy C-J, Steinbakk M, Kahlmeter G. 2012. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. Clin Microbiol Infect 18:E37–E45. https://doi.org/10.1111/j.1469-0691.2011.03752.x
34. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, Koeth L, Sei K, Development CM, . 2018. CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. J Clin Microbiol 56:e01934-17. https://doi.org/10.1128/JCM.01934-17
35. Bengtsson S, Bjelkenbrant C, Kahlmeter G. 2014. Validation of EUCAST zone diameter breakpoints against reference broth microdilution. Clin Microbiol Infect 20:353–360. https://doi.org/10.1111/1469-0691.12414
36. Matuschek E, Brown DFJ, Kahlmeter G. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect 20:255–266. https://doi.org/10.1111/1469-0691.12373
37. Jorgensen JH, Ferraro MJ. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis 49:1749–1755. https://doi.org/10.1086/647952
38. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Koser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen O, Woodford N. 2017. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST subcommittee. Clin Microbiol Infect 23:2–22. https://doi.org/10.1016/j.cmi.2016.11.012

39. Su M, Satola SW, Read TD. 2019. Genome-based prediction of bacterial antibiotic resistance. J Clin Microbiol 57:e01405-18. https://doi.org/10.1128/JCM.01405-18
40. Cortes-Lara S, Barrio-Tofiño ED, López-Causapé C, Oliver A, GEMARA-SEIMC/REIPI Pseudomonas study Group. 2021. Predicting *Pseudomonas aeruginosa* susceptibility phenotypes from whole genome sequence resistome analysis. Clin Microbiol Infect 27:1631–1637. https://doi.org/10.1016/j.cmi.2021.05.011
41. Kim JI, Maguire F, Tsang KK, Gouliouris T, Peacock SJ, McAllister TA, McArthur AG, Beiko RG. 2022. Machine learning for antimicrobial resistance prediction: current practice, limitations, and clinical perspective. Clin Microbiol Rev 35:e0017921. https://doi.org/10.1128/cmr.00179-21
42. Biggel M, Johler S, Roloff T, Tschudin-Sutter S, Bassetti S, Siegemund M, Egli A, Stephan R, Seth-Smith HMB. 2023. PorinPredict: *in Silico* identification of OprD loss from WGS data for improved genotype-phenotype predictions of *P. aeruginosa* carbapenem resistance. Microbiol Spectr 11:e0358822. https://doi.org/10.1128/spectrum.03588-22
43. Kavvas ES, Catoiu E, Mih N, Yurkovich JT, Seif Y, Dillon N, Heckmann D, Anand A, Yang L, Nizet V, Monk JM, Palsson BO. 2018. Machine learning and structural analysis of *Mycobacterium tuberculosis* pan-genome identifies genetic signatures of antibiotic resistance. Nature Communications 9:4306.
44. Jeukens J, Kukavica-Ibrulj I, Emond-Rheault JG, Freschi L, Levesque RC. 2017. Comparative genomics of a drug-resistant *Pseudomonas aeruginosa* panel and the challenges of antimicrobial resistance prediction from genomes. FEMS Microbiology Letters 364. https://doi.org/10.1093/femsle/fnx161
45. Cornforth DM, Dees JL, Ibberson CB, Huse HK, Mathiesen IH, Kirketerp-Møller K, Wolcott RD, Rumbaugh KP, Bjarnsholt T, Whiteley M. 2018. *Pseudomonas aeruginosa* transcriptome during human infection. Proc Natl Acad Sci U S A 115:E5125–E5134. https://doi.org/10.1073/pnas.1717525115
46. Ibberson CB, Whiteley M. 2019. The *Staphylococcus aureus* transcriptome during cystic fibrosis lung infection. mBio 10:e02774-19. https://doi.org/10.1128/mBio.02774-19
47. Cornforth DM, Diggle FL, Melvin JA, Bomberger JM, Whiteley M. 2020. Quantitative framework for model evaluation in microbiology research using *Pseudomonas aeruginosa* and cystic fibrosis infection as a test case. mBio 11:e03042-19 https://doi.org/10.1128/mBio.03042-19
48. Zhang L, Mah TF. 2008. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. J Bacteriol 190:4447–4452. https://doi.org/10.1128/JB.01655-07
49. Coenye T, Van Acker H, Peeters E, Sass A, Buroni S, Riccardi G, Mahenthiralingam E. 2011. Molecular mechanisms of chlorhexidine tolerance in *Burkholderia cenocepacia* biofilms. Antimicrob Agents Chemother 55:1912–1919. https://doi.org/10.1128/AAC.01571-10
50. Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. 2003. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. Nature 426:306–310. https://doi.org/10.1038/nature02122
51. Chen XF, Hou X, Xiao M, Zhang L, Cheng JW, Zhou ML, Huang JJ, Zhang JJ, Xu YC, Hsueh PR. 2021. Matrix-assisted laser desorption/Ionization time of flight mass Spectrometry (MALDI-TOF MS) analysis for the identification of pathogenic microorganisms: A review. Microorganisms 9:1536. https://doi.org/10.3390/microorganisms9071536
52. Torres-Sangiao E, Leal Rodriguez C, García-Riestra C. 2021. Application and perspectives of MALDI-TOF mass spectrometry in clinical microbiology laboratories. Microorganisms 9:1539. https://doi.org/10.3390/microorganisms9071539
53. Burckhardt I, Zimmermann S. 2018. Susceptibility testing of bacteria using MALDI-TOF mass spectrometry. Front Microbiol 9:1744. https://doi.org/10.3389/fmicb.2018.01744
54. Idelevich EA, Becker K. 2019. How to accelerate antimicrobial susceptibility testing. Clin Microbiol Infect 25:1347–1355. https://doi.org/10.1016/j.cmi.2019.04.025
55. Yoon EJ, Jeong SH. 2021. MALDI-TOF mass spectrometry technology as a tool for the rapid diagnosis of antimicrobial resistance in bacteria. Antibiotics (Basel) 10:982. https://doi.org/10.3390/antibiotics10080982
56. Maenchantrarath C, Khumdee P, Samosornsuk S, Mungkornkaew N, Samosornsuk W. 2022. Investigation of fluconazole susceptibility to *Candida albicans* by MALDI-TOF MS and real-time PCR for CDR1, CDR2, MDR1 and ERG11. BMC Microbiol. 22:153. https://doi.org/10.1186/s12866-022-02564-4
57. Kim JM, Kim I, Chung SH, Chung Y, Han M, Kim JS. 2019. Rapid discrimination of methicillin-resistant *Staphylococcus aureus* by MALDI-TOF MS. Pathogens 8:214. https://doi.org/10.3390/pathogens8040214
58. Liu X, Su T, Hsu Y-MS, Yu H, Yang HS, Jiang L, Zhao Z. 2021. Rapid identification and discrimination of methicillin-resistant *Staphylococcus aureus* strains via matrix-assisted laser desorption/Ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 35:e8972. https://doi.org/10.1002/rcm.8972
59. Weis C, Cuénod A, Rieck B, Dubuis O, Graf S, Lang C, Oberle M, Brackmann M, Søgaard KK, Osthoff M, Borgwardt K, Egli A. 2022. Direct antimicrobial resistance prediction from clinical MALDI-TOF mass spectra using machine learning. Nat Med 28:164–174. https://doi.org/10.1038/s41591-021-01619-9
60. Yu J, Lin YT, Chen WC, Tseng KH, Lin HH, Tien N, Cho CF, Huang JY, Liang SJ, Ho LC, Hsieh YW, Hsu KC, Ho MW, Hsueh PR, Cho DY. 2023. Direct prediction of carbapenem-resistant, carbapenemase-producing, and colistin-resistant *Klebsiella pneumoniae* isolates from routine MALDI-TOF mass spectra using machine learning and outcome evaluation. Int J Antimicrob 61:106799. https://doi.org/10.1016/j.ijantimicag.2023.106799
61. Braissant O, Wirz D, Göpfert B, Daniels AU. 2010. Use of isothermal microcalorimetry to monitor microbial activities. FEMS Microbiol Lett 303:1–8. https://doi.org/10.1111/j.1574-6968.2009.01819.x
62. Butini ME, Gonzalez Moreno M, Czuban M, Koliszak A, Tkhilaishvili T, Trampuz A, Di Luca M. 2019. Real-time antimicrobial susceptibility assay of planktonic and biofilm bacteria by isothermal microcalorimetry. Adv Exp Med Biol 1214:61–77. https://doi.org/10.1007/5584_2018_291
63. Antonelli A, Coppi M, Tellapragada C, Hasan B, Maruri A, Gijon D, Morecchiato F, de Vogel C, Verbon A, van Wamel W, Kragh KN, Frimodt-Moller N, Canton R, Giske CG, Rossolini GM. 2022. Isothermal microcalorimetry vs checkerboard assay to evaluate *in-vitro* synergism of meropenem-amikacin and meropenem-colistin combinations against multi-drug-resistant gram-negative pathogens. Int J Antimicrob Agents 60:106668. https://doi.org/10.1016/j.ijantimicag.2022.106668
64. Kragh KN, Gijon D, Maruri A, Antonelli A, Coppi M, Kolpen M, Crone S, Tellapragada C, Hasan B, Radmer S, de Vogel C, van Wamel W, Verbon A, Giske CG, Rossolini GM, Canton R, Frimodt-Moller N. 2021. Effective antimicrobial combination *in vivo* treatment predicted with microcalorimetry screening. J Antimicrob Chemother 76:1001–1009. https://doi.org/10.1093/jac/dkaa543
65. Tellapragada C, Hasan B, Antonelli A, Maruri A, de Vogel C, Gijón D, Coppi M, Verbon A, van Wamel W, Rossolini GM, Cantón R, Giske CG. 2020. Isothermal microcalorimetry minimal inhibitory concentration testing in extensively drug resistant gram-negative bacilli: a multicentre study. Clin Microbiol Infect 26:1413. https://doi.org/10.1016/j.cmi.2020.01.026
66. Sultan AR, Tavakol M, Lemmens-den Toom NA, Croughs PD, Verkaik NJ, Verbon A, van Wamel WJB, Sobral RG. 2022. Real time monitoring of *Staphylococcus aureus* biofilm sensitivity towards antibiotics with isothermal microcalorimetry. PLoS One 17:e0260272. https://doi.org/10.1371/journal.pone.0260272
67. Di Luca M, Koliszak A, Karbysheva S, Chowdhary A, Meis JF, Trampuz A. 2019. Thermogenic characterization and antifungal susceptibility of *Candida auris* by microcalorimetry. J Fungi (Basel) 5:103. https://doi.org/10.3390/jof5040103
68. Grütter AE, Lafranca T, Sigg AP, Mariotti M, Bonkat G, Braissant O. 2021. Detection and drug susceptibility testing of *Neisseria gonorrhoeae* using isothermal microcalorimetry. Microorganisms 9:2337. https://doi.org/10.3390/microorganisms9112337
69. Tetz G, Tetz V. 2021. Evaluation of a new culture-based AtbFinder test-system employing a novel nutrient medium for the selection of optimal antibiotics for critically ill patients with polymicrobial infections within 4 h. Microorganisms 9:990. https://doi.org/10.3390/microorganisms9050990
70. Tetz G, Tetz V. 2022. Overcoming antibiotic resistance with novel paradigms of antibiotic selection. Microorganisms 10:2383. https://doi.org/10.3390/microorganisms10122383

71. Tetz GV, Vecherkovskaya M, Kardava K, Tetz V. 2022. Race for life: antibiotic selection in nosocomial pneumonia. Chest 161:A124. https://doi.org/10.1016/j.chest.2021.12.156
72. Tetz GV, Kardava KM, Vecherkovskaya MF, Tsifansky MD, Tetz VV. 2023. Treatment of chronic relapsing urinary tract infection with antibiotics selected by AtbFinder. Urol Case Rep 46:102312. https://doi.org/10.1016/j.eucr.2022.102312
73. Tetz G, Kardava K, Vecherkovskaya M, Hahn A, Tsifansky M, Koumbourlis A, Tetz V. 2023. AtbFinder diagnostic test system improves optimal selection of antibiotic therapy in persons with cystic fibrosis. J Clin Microbiol 61:e0155822. https://doi.org/10.1128/jcm.01558-22
74. Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Willmann M, Peter S. 2017. Evaluation of the Accelerate Pheno system for fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by gram-negative pathogens. J Clin Microbiol 55:2116–2126. https://doi.org/10.1128/JCM.00181-17
75. Cenci E, Paggi R, Socio GVD, Bozza S, Camilloni B, Pietrella D, Mencacci A. 2020. Accelerate Pheno blood culture detection system: a literature review. Future Microbiol 15:1595–1605. https://doi.org/10.2217/fmb-2020-0177
76. Smith KP, Richmond DL, Brennan-Krohn T, Elliott HL, Kirby JE. 2017. Development of MAST: a microscopy-based antimicrobial susceptibility testing platform. SLAS Technol 22:662–674. https://doi.org/10.1177/2472630317727721
77. Yu H, Jing W, Iriya R, Yang Y, Syal K, Mo M, Grys TE, Haydel SE, Wang S, Tao N. 2018. Phenotypic antimicrobial susceptibility testing with deep learning video microscopy. Anal Chem 90:6314–6322. https://doi.org/10.1021/acs.analchem.8b01128
78. Banerjee R, Komarow L, Virk A, Rajapakse N, Schuetz AN, Dylla B, Earley M, Lok J, Kohner P, Ihde S, Cole N, Hines L, Reed K, Garner OB, Chandrasekaran S, de St Maurice A, Kanatani M, Curello J, Arias R, Swearingen W, Doernberg SB, Patel R. 2021. Randomized trial evaluating clinical impact of rapid identification and susceptibility testing for gram-negative bacteremia: RAPIDS-GN. Clin Infect Dis 73:e39–e46. https://doi.org/10.1093/cid/ciaa528
79. Somayaji R, Parkins MD, Shah A, Martiniano SL, Tunney MM, Kahle JS, Waters VJ, Elborn JS, Bell SC, Flume PA, VanDevanter DR, Antimicrobial Resistance in Cystic Fibrosis InternationalWorking Group. 2019. Antimicrobial susceptibility testing (AST) and associated clinical outcomes in individuals with cystic fibrosis: a systematic review. J Cyst Fibros 18:236–243. https://doi.org/10.1016/j.jcf.2019.01.008
80. Waters VJ, Kidd TJ, Canton R, Ekkelenkamp MB, Johansen HK, LiPuma JJ, Bell SC, Elborn JS, Flume PA, VanDevanter DR, Gilligan P, Antimicrobial Resistance International Working Group in Cystic Fibrosis. 2019. Reconciling antimicrobial susceptibility testing and clinical response in antimicrobial treatment of chronic cystic fibrosis lung infections. Clin Infect Dis 69:1812–1816. https://doi.org/10.1093/cid/ciz364
81. LiPuma JJ. 2022. The sense and nonsense of antimicrobial susceptibility testing in cystic fibrosis. J Pediatric Infect Dis Soc 11:S46–S52. https://doi.org/10.1093/jpids/piac040
82. Van Bambeke F, Barcia-Macay M, Lemaire S, Tulkens PM. 2006. Cellular pharmacodynamics and pharmacokinetics of antibiotics: current views and perspectives. Curr Opin Drug Discov Dev 9:218–230.
83. Stratton CW. 2006. *In vitro* susceptibility testing versus *in vivo* effectiveness. Med Clin North Am 90:1077–1088. https://doi.org/10.1016/j.mcna.2006.07.003
84. Rathi C, Lee RE, Meibohm B. 2016. Translational PK/PD of anti-infective therapeutics. Drug Discov Today Technol 21–22:41–49. https://doi.org/10.1016/j.ddtec.2016.08.004
85. Stewart PS, Franklin MJ. 2008. Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199–210. https://doi.org/10.1038/nrmicro1838
86. Stewart PS, White B, Boegli L, Hamerly T, Williamson KS, Franklin MJ, Bothner B, James GA, Fisher S, Vital-Lopez FG, Wallqvist A. 2019. Conceptual model of biofilm antibiotic tolerance that integrates phenomena of diffusion, metabolism, gene expression, and physiology. J Bacteriol 201:e00307-19. https://doi.org/10.1128/JB.00307-19
87. Lieberman TD, Flett KB, Yelin I, Martin TR, McAdam AJ, Priebe GP, Kishony R. 2014. Genetic variation of a bacterial pathogen within individuals with cystic fibrosis provides a record of selective pressures. Nat Genet 46:82–87. https://doi.org/10.1038/ng.2848
88. Diaz Caballero J, Clark ST, Coburn B, Zhang Y, Wang PW, Donaldson SL, Tullis DE, Yau YCW, Waters VJ, Hwang DM, Guttman DS, Hanage B, Pier GB. 2015. Selective sweeps and parallel pathoadaptation drive *Pseudomonas aeruginosa* evolution in the cystic fibrosis lung. mBio 6:e00981-15. https://doi.org/10.1128/mBio.00981-15
89. Jorth P, Staudinger BJ, Wu X, Hisert KB, Hayden H, Garudathri J, Harding CL, Radey MC, Rezayat A, Bautista G, Berrington WR, Goddard AF, Zheng C, Angermeyer A, Brittnacher MJ, Kitzman J, Shendure J, Fligner CL, Mittler J, Aitken ML, Manoil C, Bruce JE, Yahr TL, Singh PK. 2015. Regional isolation drives bacterial diversification within cystic fibrosis lungs. Cell Host Microbe 18:307–319. https://doi.org/10.1016/j.chom.2015.07.006
90. Markussen T, Marvig RL, Gómez-Lozano M, Aanæs K, Burleigh AE, Høiby N, Johansen HK, Molin S, Jelsbak L, Kolter R. 2014. Environmental heterogeneity drives within-host diversification and evolution of *Pseudomonas aeruginosa*. mBio 5:e01592-14. https://doi.org/10.1128/mBio.01592-14
91. Foweraker JE, Laughton CR, Brown DFJ, Bilton D. 2005. Phenotypic variability of *Pseudomonas aeruginosa* in sputa from patients with acute infective exacerbation of cystic fibrosis and its impact on the validity of antimicrobial susceptibility testing. J Antimicrob Chemother 55:921–927. https://doi.org/10.1093/jac/dki146
92. Rojas LJ, Yasmin M, Benjamino J, Marshall SM, DeRonde KJ, Krishnan NP, Perez F, Colin AA, Cardenas M, Martinez O, Pérez-Cardona A, Rhoads DD, Jacobs MR, LiPuma JJ, Konstan MW, Vila AJ, Smania A, Mack AR, Scott JG, Adams MD, Abbo LM, Bonomo RA. 2022. Genomic heterogeneity underlies multidrug resistance in *Pseudomonas aeruginosa*: a population-level analysis beyond susceptibility testing. PLoS One 17:e0265129. https://doi.org/10.1371/journal.pone.0265129
93. Gillham MI, Sundaram S, Laughton CR, Haworth CS, Bilton D, Foweraker JE. 2009. Variable antibiotic susceptibility in populations of *Pseudomonas aeruginosa* infecting patients with bronchiectasis. J Antimicrob Chemother 63:728–732. https://doi.org/10.1093/jac/dkp007
94. Hilliam Y, Moore MP, Lamont IL, Bilton D, Haworth CS, Foweraker J, Walshaw MJ, Williams D, Fothergill JL, De Soyza A, Winstanley C. 2017. *Pseudomonas aeruginosa* adaptation and diversification in the non-cystic fibrosis bronchiectasis lung. Eur Respir J 49:1602108. https://doi.org/10.1183/13993003.02108-2016
95. Köck R, Schuler F, Idelevich EA, Schaumburg F. 2021. Variability of antibiograms: how often do changes in the antimicrobial susceptibility pattern occur in isolates from one patient Clin Microbiol Infect 27:1638–1643. https://doi.org/10.1016/j.cmi.2021.02.012
96. Cottalorda A, Dahyot S, Soares A, Alexandre K, Zorgniotti I, Etienne M, Jumas-Bilak E, Pestel-Caron M. 2022. Phenotypic and genotypic within-host diversity of *Pseudomonas aeruginosa* urinary isolates. Sci Rep 12:5421. https://doi.org/10.1038/s41598-022-09234-5
97. Kart D, Tavernier S, Van Acker H, Nelis HJ, Coenye T. 2014. Activity of disinfectants against multispecies biofilms formed by *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa*. Biofouling 30:377–383. https://doi.org/10.1080/08927014.2013.878333
98. Tavernier S, Crabbé A, Hacioglu M, Stuer L, Henry S, Rigole P, Dhondt I, Coenye T. 2017. Community composition determines activity of antibiotics against multispecies biofilms. Antimicrob Agents Chemother 61:e00302-17. https://doi.org/10.1128/AAC.00302-17
99. Vandeplassche E, Tavernier S, Coenye T, Crabbé A. 2019. Influence of the lung microbiome on antibiotic susceptibility of cystic fibrosis pathogens. Eur Respir Rev 28:190041. https://doi.org/10.1183/16000617.0041-2019
100. Orazi G, O'Toole GA. 2019. It takes a village": mechanisms underlying antimicrobial recalcitrance of polymicrobial biofilms. J Bacteriol 202:e00530-19. https://doi.org/10.1128/JB.00530-19
101. Orazi G, Jean-Pierre F, O'Toole GA. 2020. *Pseudomonas aeruginosa* PA14 enhances the efficacy of norfloxacin against *Staphylococcus aureus* Newman biofilms. J Bacteriol 202:e00159-20. https://doi.org/10.1128/JB.00159-20
102. Ibberson CB, Barraza JP, Holmes AL, Cao P, Whiteley M. 2022. Precise spatial structure impacts antimicrobial susceptibility of *S. aureus* in polymicrobial wound infections. Proc Natl Acad Sci U S A 119:e2212340119. https://doi.org/10.1073/pnas.2212340119
103. Pan X, Dong Y, Fan Z, Liu C, Xia B, Shi J, Bai F, Jin Y, Cheng Z, Jin S, Wu W. 2017. *In vivo* host environment alters *Pseudomonas aeruginosa*

susceptibility to aminoglycoside antibiotics. Front Cell Infect Microbiol 7:83. https://doi.org/10.3389/fcimb.2017.00083
104. Crabbé A, Ostyn L, Staelens S, Rigauts C, Risseeuw M, Dhaenens M, Daled S, Van Acker H, Deforce D, Van Calenbergh S, Coenye T. 2019. Host metabolites stimulate the bacterial proton motive force to enhance the activity of aminoglycoside antibiotics. PLoS Pathog 15:e1007697. https://doi.org/10.1371/journal.ppat.1007697
105. Moskowitz SM, Foster JM, Emerson J, Burns JL. 2004. Clinically feasible biofilm susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J Clin Microbiol 42:1915–1922. https://doi.org/10.1128/JCM.42.5.1915-1922.2004
106. Fernández-Olmos A, García-Castillo M, Maiz L, Lamas A, Baquero F, Cantón R. 2012. *In vitro* prevention of *Pseudomonas aeruginosa* early biofilm formation with antibiotics used in cystic fibrosis patients. Int J Antimicrob Agents 40:173–176. https://doi.org/10.1016/j.ijantimicag.2012.04.006
107. Macià MD, Rojo-Molinero E, Oliver A. 2014. Antimicrobial susceptibility testing in biofilm-growing bacteria. Clin Microbiol Infect 20:981–990. https://doi.org/10.1111/1469-0691.12651
108. Velez Perez AL, Schmidt-Malan SM, Kohner PC, Karau MJ, Greenwood-Quaintance KE, Patel R. 2016. *In vitro* activity of ceftolozane/tazobactam against clinical isolates of *Pseudomonas aeruginosa* in the planktonic and biofilm states. Diagn Microbiol Infect Dis 85:356–359. https://doi.org/10.1016/j.diagmicrobio.2016.02.014
109. Brady AJ, Laverty G, Gilpin DF, Kearney P, Tunney M. 2017. Antibiotic susceptibility of planktonic- and biofilm-grown staphylococci isolated from implant-associated infections: should MBEC and nature of biofilm formation replace MIC? J Med Microbiol 66:461–469. https://doi.org/10.1099/jmm.0.000466
110. Thöming JG, Häussler S. 2022. *Pseudomonas aeruginosa* is more tolerant under biofilm than under planktonic growth conditions: a multi-isolate survey. Front Cell Infect Microbiol 12:851784. https://doi.org/10.3389/fcimb.2022.851784
111. Drevinek P, Canton R, Johansen HK, Hoffman L, Coenye T, Burgel PR, Davies JC. 2022. New concepts in antimicrobial resistance in cystic fibrosis respiratory infections. J Cyst Fibros 21:937–945. https://doi.org/10.1016/j.jcf.2022.10.005
112. Cruz CD, Shah S, Tammela P. 2018. Defining conditions for biofilm inhibition and eradication assays for gram-positive clinical reference strains. BMC Microbiol. 18:173. https://doi.org/10.1186/s12866-018-1321-6
113. Thieme L, Hartung A, Tramm K, Klinger-Strobel M, Jandt KD, Makarewicz O, Pletz MW. 2019. MBEC versus MBIC: the lack of differentiation between biofilm reducing and inhibitory effects as a current problem in biofilm methodology. Biol Proced Online 21. https://doi.org/10.1186/s12575-019-0106-0
114. Malone M, Goeres DM, Gosbell I, Vickery K, Jensen S, Stoodley P. 2017. Approaches to biofilm-associated infections: the need for standardized and relevant biofilm methods for clinical applications. Expert Rev Anti Infect Ther 15:147–156. https://doi.org/10.1080/14787210.2017.1262257
115. Allkja J, van Charante F, Aizawa J, Reigada I, Guarch-Perez C, Vazquez-Rodriguez JA, Cos P, Coenye T, Fallarero A, Zaat SAJ, Felici A, Ferrari L, Azevedo NF, Parker AE, Goeres DM. 2021. Interlaboratory study for the evaluation of three microtiter plate-based biofilm quantification methods. Sci Rep 11:13779. https://doi.org/10.1038/s41598-021-93115-w
116. Azevedo NF, Allkja J, Goeres DM. 2021. Biofilms vs. cities and humans vs. aliens - a tale of reproducibility in biofilms. Trends Microbiol 29:1062–1071. https://doi.org/10.1016/j.tim.2021.05.003
117. Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, Andersson DI, Brynildsen MP, Bumann D, Camilli A, Collins JJ, Dehio C, Fortune S, Ghigo J-M, Hardt W-D, Harms A, Heinemann M, Hung DT, Jenal U, Levin BR, Michiels J, Storz G, Tan M-W, Tenson T, Van Melderen L, Zinkernagel A. 2019. Definitions and guidelines for research on antibiotic persistence. Nat Rev Microbiol 17:441–448. https://doi.org/10.1038/s41579-019-0207-4
118. Lebeaux D, Ghigo JM, Beloin C. 2014. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev 78:510–543. https://doi.org/10.1128/MMBR.00013-14
119. Brauner A, Fridman O, Gefen O, Balaban NQ. 2016. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Microbiol 14:320–330. https://doi.org/10.1038/nrmicro.2016.34
120. Ciofu O, Tolker-Nielsen T. 2019. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents-how *P. aeruginosa* can escape antibiotics. Front Microbiol 10:913. https://doi.org/10.3389/fmicb.2019.00913
121. Coenye T, Bové M, Bjarnsholt T. 2022. Biofilm antimicrobial susceptibility through an experimental evolutionary lens. NPJ Biofilms Microbiomes 8:82. https://doi.org/10.1038/s41522-022-00346-4
122. Coenye T, Nelis HJ. 2010. *In vitro* and *in vivo model* systems to study microbial biofilm formation. J Microbiol Methods 83:89–105. https://doi.org/10.1016/j.mimet.2010.08.018
123. Lebeaux D, Chauhan A, Rendueles O, Beloin C. 2013. From *in vitro* to *in vivo* models of bacterial biofilm-related infections. Pathogens 2:288–356. https://doi.org/10.3390/pathogens2020288
124. Azeredo J, Azevedo NF, Briandet R, Cerca N, Coenye T, Costa AR, Desvaux M, Di Bonaventura G, Hebraud M, Jaglic Z, Kacaniova M, Knochel S, Lourenco A, Mergulhao F, Meyer RL, Nychas G, Simoes M, Tresse O, Sternberg C. 2017. Critical review on biofilm methods. Crit Rev Microbiol 43:313–351. https://doi.org/10.1080/1040841X.2016.1208146
125. Gomes IB, Meireles A, Goncalves AL, Goeres DM, Sjollema J, Simoes LC, Simoes M. 2018. Standardized reactors for the study of medical biofilms: a review of the principles and latest modifications. Critical Reviews in Biotechnology 38:657-670.
126. Vyas HKN, Xia B, Mai-Prochnow A. 2022. Clinically relevant *in vitro* biofilm models: a need to mimic and recapitulate the host environment. Biofilm 4:100069. https://doi.org/10.1016/j.bioflm.2022.100069
127. Harrison JJ, Stremick CA, Turner RJ, Allan ND, Olson ME, Ceri H. 2010. Microtiter susceptibility testing of microbes growing on peg lids: a miniaturized biofilm model for high-throughput screening. Nat Protoc 5:1236–1254. https://doi.org/10.1038/nprot.2010.71
128. Blanco-Cabra N, López-Martínez MJ, Arévalo-Jaimes BV, Martin-Gómez MT, Samitier J, Torrents E. 2021. A new biofilmchip device for testing biofilm formation and antibiotic susceptibility. NPJ Biofilms Microbiomes 7:62. https://doi.org/10.1038/s41522-021-00236-1
129. Harrington NE, Sweeney E, Alav I, Allen F, Moat J, Harrison F. 2021. Antibiotic efficacy testing in an *ex vivo* model of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms in the cystic fibrosis lung. J Vis Exp. https://doi.org/10.3791/62187
130. Pouget C, Pantel A, Dunyach-Remy C, Magnan C, Sotto A, Lavigne JP. 2023. Antimicrobial activity of antibiotics on biofilm formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in an open microfluidic model mimicking the diabetic foot environment. J Antimicrob Chemother 78:540–545. https://doi.org/10.1093/jac/dkac438
131. Díez-Aguilar M, Morosini MI, Köksal E, Oliver A, Ekkelenkamp M, Cantón R. 2018. Use of Calgary and microfluidic BioFlux systems to test the activity of fosfomycin and tobramycin alone and in combination against cystic fibrosis *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 62:e01650-17. https://doi.org/10.1128/AAC.01650-17
132. Pham LHP, Ly KL, Colon-Ascanio M, Ou J, Wang H, Lee SW, Wang Y, Choy JS, Phillips KS, Luo X. 2023. Dissolvable alginate hydrogel-based biofilm microreactors for antibiotic susceptibility assays. Biofilm 5:100103. https://doi.org/10.1016/j.bioflm.2022.100103
133. Di Bonaventura G, Pompilio A. 2022. *In vitro* antimicrobial susceptibility testing of biofilm-growing bacteria: current and emerging methods. Adv Exp Med Biol 1369:33–51. https://doi.org/10.1007/5584_2021_641
134. Thieme L, Hartung A, Tramm K, Graf J, Spott R, Makarewicz O, Pletz MW. 2021. Adaptation of the start-growth-time method for high-throughput biofilm quantification. Front Microbiol 12:631248. https://doi.org/10.3389/fmicb.2021.631248
135. Monzón M, Oteiza C, Leiva J, Lamata M, Amorena B. 2002. Biofilm testing of *Staphylococcus epidermidis* clinical isolates: low performance of vancomycin in relation to other antibiotics. Diagn Microbiol Infect Dis 44:319–324. https://doi.org/10.1016/s0732-8893(02)00464-9
136. Pettit RK, Weber CA, Kean MJ, Hoffmann H, Pettit GR, Tan R, Franks KS, Horton ML. 2005. Microplate alamar blue assay for *Staphylococcus epidermidis* biofilm susceptibility testing. Antimicrob Agents Chemother 49:2612–2617. https://doi.org/10.1128/AAC.49.7.2612-2617.2005
137. Peeters E, Nelis HJ, Coenye T. 2008. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. J Microbiol Methods 72:157–165. https://doi.org/10.1016/j.mimet.2007.11.010
138. Ravi NS, Aslam RF, Veeraraghavan B. 2019. A new method for determination of minimum biofilm eradication concentration for

accurate antimicrobial therapy. Methods Mol Biol 1946:61–67. https://doi.org/10.1007/978-1-4939-9118-1_6
139. Žiemytė M, Rodríguez-Díaz JC, Ventero-Martín MP, Mira A, Ferrer MD. 2023. Real-time monitoring of biofilm growth identifies andrographolide as a potent antifungal compound eradicating *Candida* biofilms. Biofilm 5:100134. https://doi.org/10.1016/j.bioflm.2023.100134
140. Kragh KN, Alhede M, Kvich L, Bjarnsholt T. 2019. Into the well-A close look at the complex structures of a microtiter biofilm and the crystal violet assay. Biofilm 1:100006. https://doi.org/10.1016/j.bioflm.2019.100006
141. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM. 2005. Statistical assessment of a laboratory method for growing biofilms. Microbiology 151:757–762. https://doi.org/10.1099/mic.0.27709-0
142. Parker AE, Walker DK, Goeres DM, Allan N, Olson ME, Omar A. 2014. Ruggedness and reproducibility of the MBEC biofilm disinfectant efficacy test. J Microbiol Methods 102:55–64. https://doi.org/10.1016/j.mimet.2014.04.013
143. Nour El-Din HT, Yassin AS, Ragab YM, Hashem AM. 2021. Phenotype-genotype characterization and antibiotic-resistance correlations among colonizing and infectious methicillin-resistant *Staphylococcus aureus* recovered from intensive care units. Infect Drug Resist 14:1557–1571. https://doi.org/10.2147/IDR.S296000
144. Senobar Tahaei SA, Stájer A, Barrak I, Ostorházi E, Szabó D, Gajdács M. 2021. Correlation between biofilm-formation and the antibiotic resistant phenotype in *Staphylococcus aureus* isolates: a laboratory-based study in Hungary and a review of the literature. Infect Drug Resist 14:1155–1168. https://doi.org/10.2147/IDR.S303992
145. Trobos M, Firdaus R, Svensson Malchau K, Tillander J, Arnellos D, Rolfson O, Thomsen P, Lasa I. 2022. Genomics of *Staphylococcus aureus* and *Staphylococcus* epidermidis from periprosthetic joint infections and correlation to clinical outcome. Microbiol Spectr 10:e0218121. https://doi.org/10.1128/spectrum.02181-21
146. Donadu MG, Ferrari M, Mazzarello V, Zanetti S, Kushkevych I, Rittmann SK-MR, Stájer A, Baráth Z, Szabó D, Urbán E, Gajdács M. 2022. No correlation between Biofilm-forming capacity and antibiotic resistance in environmental *Staphylococcus* spp.: *in vitro* results. Pathogens 11:471. https://doi.org/10.3390/pathogens11040471
147. Svensson Malchau K, Tillander J, Zaborowska M, Hoffman M, Lasa I, Thomsen P, Malchau H, Rolfson O, Trobos M. 2021. Biofilm properties in relation to treatment outcome in patients with first-time periprosthetic hip or knee joint infection. J Orthop Translat 30:31–40. https://doi.org/10.1016/j.jot.2021.05.008
148. De Bleeckere A, Van den Bossche S, De Sutter P-J, Beirens T, Crabbe A, Coenye T. 2023. High throughput determination of the biofilm prevention concentration for *Pseudomonas aeruginosa* biofilms using a synthetic cystic fibrosis sputum medium. Biofilm 5:100106. https://doi.org/10.1016/j.bioflm.2023.100106
149. Qi L, Li H, Zhang C, Liang B, Li J, Wang L, Du X, Liu X, Qiu S, Song H. 2016. Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in *Acinetobacter baumannii*. Front Microbiol 7:483. https://doi.org/10.3389/fmicb.2016.00483
150. Alamri AM, Alsultan AA, Ansari MA, Alnimr AM. 2020. Biofilm-formation in clonally unrelated multidrug-resistant *Acinetobacter baumannii* isolates. Pathogens 9:630. https://doi.org/10.3390/pathogens9080630
151. Donadu MG, Mazzarello V, Cappuccinelli P, Zanetti S, Madléna M, Nagy ÁL, Stájer A, Burián K, Gajdács M. 2021. Relationship between the biofilm-forming capacity and antimicrobial resistance in clinical *Acinetobacter baumannii* isolates. Microorganisms 9:2384. https://doi.org/10.3390/microorganisms9112384
152. Garousi M, Monazami Tabar S, Mirazi H, Asgari P, Sabeghi P, Salehi A, Khaledi A, Ghenaat Pisheh Sanani M, Mirzahosseini HK. 2022. A global systematic review and meta-analysis on correlation between biofilm producers and non-biofilm producers with antibiotic resistance in uropathogenic *Escherichia coli*. Microb Pathog 164:105412. https://doi.org/10.1016/j.micpath.2022.105412
153. Vuotto C, Longo F, Pascolini C, Donelli G, Balice MP, Libori MF, Tiracchia V, Salvia A, Varaldo PE. 2017. Biofilm formation and antibiotic resistance in *Klebsiella pneumoniae* urinary strains. J Appl Microbiol 123:1003–1018. https://doi.org/10.1111/jam.13533
154. Türkel İ, Yıldırım T, Yazgan B, Bilgin M, Başbulut E. 2018. Relationship between antibiotic resistance, efflux pumps, and biofilm formation in extended-spectrum β-lactamase producing *Klebsiella pneumoniae*. J Chemother 30:354–363. https://doi.org/10.1080/1120009X.2018.1521773
155. Mulet X, MoyaB, JuanC, MaciaMD, PerezJL, BlazquezJ, OliverA. 2011. Antagonistic interactions of *Pseudomonas aeruginosa* antibiotic resistance mechanisms in planktonic but not biofilm growth. Antimicrob Agents Chemother 57:4560–4568. https://doi.org/10.1128/AAC.01481-13
156. Gajdács M, Baráth Z, Kárpáti K, Szabó D, Usai D, Zanetti S, Donadu MG. 2021. No correlation between biofilm formation, virulence factors, and antibiotic resistance in *Pseudomonas aeruginosa*: results from a laboratory-based *in vitro* study. Antibiotics (Basel) 10:1134. https://doi.org/10.3390/antibiotics10091134
157. Yamani L, Alamri A, Alsultan A, Alfifi S, Ansari MA, Alnimr A. 2021. Inverse correlation between biofilm production efficiency and antimicrobial resistance in clinical isolates of *Pseudomonas aeruginosa*. Microb Pathog 157:104989. https://doi.org/10.1016/j.micpath.2021.104989
158. Karballaei Mirzahosseini H, Hadadi-Fishani M, Morshedi K, Khaledi A. 2020. Meta-analysis of biofilm formation, antibiotic resistance pattern, and biofilm-related genes in *Pseudomonas aeruginosa* isolated from clinical samples. Microb Drug Resist 26:815–824. https://doi.org/10.1089/mdr.2019.0274
159. Mulet X, Moyá B, Juan C, Macià MD, Pérez JL, Blázquez J, Oliver A. 2011. Antagonistic interactions of *Pseudomonas aeruginosa* antibiotic resistance mechanisms in planktonic but not biofilm growth. Antimicrob Agents Chemother 55:4560–4568. https://doi.org/10.1128/AAC.00519-11
160. Harms A, Maisonneuve E, Gerdes K. 2016. Mechanisms of bacterial persistence during stress and antibiotic exposure. Science 354:354. https://doi.org/10.1126/science.aaf4268
161. Lopes SP, Jorge P, Sousa AM, Pereira MO. 2021. Discerning the role of polymicrobial biofilms in the ascent, prevalence, and extent of heteroresistance in clinical practice. Crit Rev Microbiol 47:162–191. https://doi.org/10.1080/1040841X.2020.1863329
162. Ersoy SC, Heithoff DM, Barnes L, Tripp GK, House JK, Marth JD, Smith JW, Mahan MJ. 2017. Correcting a fundamental flaw in the paradigm for antimicrobial susceptibility testing. EBioMedicine 20:173–181. https://doi.org/10.1016/j.ebiom.2017.05.026
163. Belanger CR, Hancock REW. 2021. Testing physiologically relevant conditions in minimal inhibitory concentration assays. Nat Protoc 16:3761–3774. https://doi.org/10.1038/s41596-021-00572-8
164. Müsken M, Klimmek K, Sauer-Heilborn A, Donnert M, Sedlacek L, Suerbaum S, Häussler S. 2017. Towards individualized diagnostics of biofilm-associated infections: a case study. NPJ Biofilms Microbiomes 3:22. https://doi.org/10.1038/s41522-017-0030-5
165. Stewart PS. 2015. Antimicrobial tolerance in biofilms. Microbiol Spectr 3:MB-0010-2014. https://doi.org/10.1128/microbiolspec.MB-0010-2014
166. Tre-Hardy M, Mace C, El Manssouri N, Vanderbist F, Traore H, Devleeschouwer MJ. 2009. Effect of antibiotic co-administration on young and mature biofilms of cystic fibrosis clinical isolates: the importance of the biofilm model. Int J Antimicrob Agents 33:40–45. https://doi.org/10.1016/j.ijantimicag.2008.07.012
167. Singla S, Harjai K, Chhibber S. 2013. Susceptibility of different phases of biofilm of *Klebsiella pneumoniae* to three different antibiotics. J Antibiot 66:61–66. https://doi.org/10.1038/ja.2012.101
168. Wolcott RD, Rumbaugh KP, James G, Schultz G, Phillips P, Yang Q, Watters C, Stewart PS, Dowd SE. 2010. Biofilm maturity studies indicate sharp debridement opens a time- dependent therapeutic window. J Wound Care 19:320–328. https://doi.org/10.12968/jowc.2010.19.8.77709
169. Swimberghe RCD, Crabbe A, De Moor RJG, Coenye T, Meire MA. 2021. Model system parameters influence the sodium hypochlorite susceptibility of endodontic biofilms. Int Endod J 54:1557–1570. https://doi.org/10.1111/iej.13544
170. Akgün D, Perka C, Trampuz A, Renz N. 2018. Outcome of hip and knee periprosthetic joint infections caused by pathogens resistant to biofilm-active antibiotics: results from a prospective cohort study. Arch Orthop Trauma Surg 138:635–642. https://doi.org/10.1007/s00402-018-2886-0
171. Koder K, Hardt S, Gellert MS, Haupenthal J, Renz N, Putzier M, Perka C, Trampuz A. 2020. Outcome of spinal implant-associated infections treated with or without biofilm-active antibiotics: results from a 10-year cohort study. Infection 48:559–568. https://doi.org/10.1007/s15010-020-01435-2

172. Mancheño-Losa M, Lora-Tamayo J, Fernández-Sampedro M, Rodríguez-Pardo D, Muñoz-Mahamud E, Soldevila L, Palou M, Barbero JM, Del Toro MD, Iribarren JA, Sobrino B, Rico-Nieto A, Guío-Carrión L, Gómez L, Escudero-Sánchez R, García-País MJ, Jover-Sáenz A, Praena J, Baraia-Etxaburu JM, Auñón Á, Múñez-Rubio E, Murillo O, List of study collaborators. 2021. Prognosis of unexpected positive intraoperative cultures in arthroplasty revision: A large multicenter cohort. J Infect 83:542–549. https://doi.org/10.1016/j.jinf.2021.09.001
173. Muñoz-Gallego I, Viedma E, Esteban J, Mancheño-Losa M, García-Cañete J, Blanco-García A, Rico A, García-Perea A, Ruiz Garbajosa P, Escudero-Sánchez R, Sánchez Somolinos M, Marín Arriaza M, Romanyk J, Barbero JM, Arribi Vilela A, González Romo F, Pérez-Jorge C, M Arana D, Monereo A, Domingo D, Cordero J, Sánchez Romero MI, García Viejo MÁ, Lora-Tamayo J, Chaves F, Grupo de Infección Osteoarticular de la Comunidad de Madrid. 2020. Genotypic and phenotypic characteristics of *Staphylococcus aureus* prosthetic joint infections: Insight on the pathogenesis and prognosis of a multicenter prospective cohort. Open Forum Infect Dis 7:faa344. https://doi.org/10.1093/ofid/ofaa344
174. Widmer AF, Frei R, Rajacic Z, Zimmerli W. 1990. Correlation between *in vivo* and *in vitro* efficacy of antimicrobial agents against foreign body infections. J Infect Dis 162:96–102. https://doi.org/10.1093/infdis/162.1.96
175. Zimmerli W, Frei R, Widmer AF, Rajacic Z. 1994. Microbiological tests to predict treatment outcome in experimental device-related infections due to *Staphylococcus aureus*. J Antimicrob Chemother 33:959–967. https://doi.org/10.1093/jac/33.5.959
176. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. 1998. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. JAMA 279:1537–1541. https://doi.org/10.1001/jama.279.19.1537
177. Schierholz JM, Beuth J, König D, Nürnberger A, Pulverer G. 1999. Antimicrobial substances and effects on sessile bacteria. Zentralbl Bakteriol 289:165–177. https://doi.org/10.1016/s0934-8840(99)80101-7
178. König DP, Schierholz JM, Münnich U, Rütt J. 2001. Treatment of staphylococcal implant infection with rifampicin-ciprofloxacin in stable implants. Arch Orthop Trauma Surg 121:297–299. https://doi.org/10.1007/s004020000242
179. Saginur R, StDenis M, Ferris W, Aaron SD, Chan F, Lee C, Ramotar K. 2006. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. Antimicrob Agents Chemother 50:55–61. https://doi.org/10.1128/AAC.50.1.55-61.2006
180. Zimmerli W, Sendi P. 2019. Role of rifampin against staphylococcal biofilm infections *in vitro,* in animal models, and in orthopedic-device-related infections. Antimicrob Agents Chemother 63. https://doi.org/10.1128/AAC.01746-18
181. Karlsen OE, Borgen P, Bragnes B, Figved W, Grogaard B, Rydinge J, Sandberg L, Snorrason F, Wangen H, Witsoe E, Westberg M. 2020. Rifampin combination therapy in staphylococcal prosthetic joint infections: a randomized controlled trial. J Orthop Surg Res 15:365. https://doi.org/10.1186/s13018-020-01877-2
182. Renz N, Trampuz A, Zimmerli W. 2021. Controversy about the role of rifampin in biofilm infections Antibiotics 10:165. https://doi.org/10.3390/antibiotics10020165
183. Martínez-Pastor JC, Muñoz-Mahamud E, Vilchez F, García-Ramiro S, Bori G, Sierra J, Martínez JA, Font L, Mensa J, Soriano A. 2009. Outcome of acute prosthetic joint infections due to gram-negative bacilli treated with open debridement and retention of the prosthesis. Antimicrob Agents Chemother 53:4772–4777. https://doi.org/10.1128/AAC.00188-09
184. Tornero E, Martínez-Pastor JC, Bori G, García-Ramiro S, Morata L, Bosch J, Mensa J, Soriano A. 2014. Risk factors for failure in early prosthetic joint infection treated with debridement. Influence of etiology and antibiotic treatment. J Appl Biomater Funct Mater 12:129–134. https://doi.org/10.5301/jabfm.5000209
185. Rodriguez-Pardo D, Pigrau C, Lora-Tamayo J, Soriano A, del Toro MD, Cobo J, Palomino J, Euba G, Riera M, Sanchez-Somolinos M, Benito N, Fernandez-Sampedro M, Sorli L, Guio L, Iribarren JA, Baraia-Etxaburu JM, Ramos A, Bahamonde A, Flores-Sanchez X, Corona PS, Ariza J, Infection RGftSoP. 2014. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect 20:911–919. https://doi.org/10.1111/1469-0691.12649
186. Keays T, Ferris W, Vandemheen KL, Chan F, Yeung SW, Mah TF, Ramotar K, Saginur R, Aaron SD. 2009. A retrospective analysis of biofilm antibiotic susceptibility testing: a better predictor of clinical response in cystic fibrosis exacerbations. J Cyst Fibros 8:122–127. https://doi.org/10.1016/j.jcf.2008.10.005
187. Smith S, Waters V, Jahnke N, Ratjen F. 2020. Standard versus biofilm antimicrobial susceptibility testing to guide antibiotic therapy in cystic fibrosis. Cochrane Database Syst Rev 6:CD009528. https://doi.org/10.1002/14651858.CD009528.pub5
188. Moskowitz SM, Emerson JC, McNamara S, Shell RD, Orenstein DM, Rosenbluth D, Katz MF, Ahrens R, Hornick D, Joseph PM, Gibson RL, Aitken ML, Benton WW, Burns JL. 2011. Randomized trial of biofilm testing to select antibiotics for cystic fibrosis airway infection. Pediatr Pulmonol 46:184–192. https://doi.org/10.1002/ppul.21350
189. Yau YCW, Ratjen F, Tullis E, Wilcox P, Freitag A, Chilvers M, Grasemann H, Zlosnik J, Speert D, Corey M, Stanojevic S, Matukas L, Leahy TR, Shih S, Waters V. 2015. Randomized controlled trial of biofilm antimicrobial susceptibility testing in cystic fibrosis patients. J Cyst Fibros 14:262–266. https://doi.org/10.1016/j.jcf.2014.09.013
190. Sønderholm M, Bjarnsholt T, Alhede M, Kolpen M, Jensen PØ, Kühl M, Kragh KN. 2017. The consequences of being in an infectious biofilm: microenvironmental conditions governing antibiotic tolerance. Int J Mol Sci 18:2688. https://doi.org/10.3390/ijms18122688
191. Lichtenberg M, Jakobsen TH, Kühl M, Kolpen M, Jensen PØ, Bjarnsholt T. 2022. The structure-function relationship of *Pseudomonas aeruginosa* in infections and its influence on the microenvironment. FEMS Microbiol Rev 46:fuac018. https://doi.org/10.1093/femsre/fuac018
192. Coenye T, Goeres D, Van Bambeke F, Bjarnsholt T. 2018. Should standardized susceptibility testing for microbial biofilms be introduced in clinical practice Clin Microbiol Infect 24:570–572. https://doi.org/10.1016/j.cmi.2018.01.003
193. Lourenco A, Coenye T, Goeres DM, Donelli G, Azevedo AS, Ceri H, Coelho FL, Flemming H-C, Juhna T, Lopes SP, Oliveira R, Oliver A, Shirtliff ME, Sousa AM, Stoodley P, Pereira MO, Azevedo NF. 2014. Minimum information about a biofilm experiment (MIABiE): standards for reporting experiments and data on sessile microbial communities living at interfaces. Pathog Dis 70:250–256. https://doi.org/10.1111/2049-632X.12146
194. Goeres DM, Walker DK, Buckingham-Meyer K, Lorenz L, Summers J, Fritz B, Goveia D, Dickerman G, Schultz J, Parker AE. 2019. Development, standardization, and validation of a biofilm efficacy test: the single tube method. J Microbiol Methods 165:105694. https://doi.org/10.1016/j.mimet.2019.105694
195. Allkja J, Bjarnsholt T, Coenye T, Cos P, Fallarero A, Harrison JJ, Lopes SP, Oliver A, Pereira MO, Ramage G, Shirtliff ME, Stoodley P, Webb JS, Zaat SAJ, Goeres DM, Azevedo NF. 2020. Minimum information guideline for spectrophotometric and fluorometric methods to assess biofilm formation in microplates. Biofilm 2:100010. https://doi.org/10.1016/j.bioflm.2019.100010
196. Goeres DM, Parker AE, Walker DK, Meier K, Lorenz LA, Buckingham-Meyer K. 2020. Drip flow reactor method exhibits excellent reproducibility based on a 10-laboratory collaborative study. J Microbiol Methods 174:105963. https://doi.org/10.1016/j.mimet.2020.105963
197. Åhman J, Matuschek E, Kahlmeter G. 2019. The quality of antimicrobial discs from nine manufacturers-EUCAST evaluations in 2014 and 2017. Clin Microbiol Infect 25:346–352. https://doi.org/10.1016/j.cmi.2018.05.021
198. Åhman J, Matuschek E, Kahlmeter G. 2020. EUCAST evaluation of 21 brands of Mueller-Hinton dehydrated media for disc diffusion testing. Clin Microbiol Infect 26:1412. https://doi.org/10.1016/j.cmi.2020.01.018
199. Åhman J, Matuschek E, Kahlmeter G. 2022. Evaluation of ten brands of pre-poured Mueller-Hinton Agar plates for EUCAST disc diffusion testing. Clin Microbiol Infect 28:1499. https://doi.org/10.1016/j.cmi.2022.05.030
200. Humphries RM, Kircher S, Ferrell A, Krause KM, Malherbe R, Hsiung A, Burnham CA. 2018. The continued value of disk diffusion for assessing antimicrobial susceptibility in clinical laboratories: report from the clinical and laboratory standards institute methods development and standardization working group. J Clin Microbiol 56:e00437-18. https://doi.org/10.1128/JCM.00437-18
201. Seneviratne CJ, Jin LJ, Samaranayake YH, Samaranayake LP. 2008. Cell density and cell aging as factors modulating antifungal resistance of *Candida albicans* biofilms. Antimicrob Agents Chemother 52:3259–3266. https://doi.org/10.1128/AAC.00541-08
202. Obaid NA, Tristram S, Narkowicz CK, Jacobson GA. 2016. Reliability of *Haemophilus influenzae* biofilm measurement via static method, and

determinants of *in vitro* biofilm production. Can J Microbiol 62:1013–1020. https://doi.org/10.1139/cjm-2016-0228
203. Kragh KN, Alhede M, Rybtke M, Stavnsberg C, Jensen PØ, Tolker-Nielsen T, Whiteley M, Bjarnsholt T. 2018. The inoculation method could impact the outcome of microbiological experiments. Appl Environ Microbiol 84:e02264-17. https://doi.org/10.1128/AEM.02264-17
204. Kahlmeter G, Brown DFJ, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, Rodloff A, Steinbakk M, Urbaskova P, Vatopoulos A. 2003. European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. J Antimicrob Chemother 52:145–148. https://doi.org/10.1093/jac/dkg312
205. Kahlmeter G, Turnidge J. 2022. How to: ECOFFs-the why, the how, and the don'ts of EUCAST epidemiological cutoff values. Clin Microbiol Infect 28:952–954. https://doi.org/10.1016/j.cmi.2022.02.024
206. Pierce VM, Mathers AJ. 2022. Setting antimicrobial susceptibility testing breakpoints: a primer for pediatric infectious diseases specialists on the clinical and laboratory standards Institute approach. J Pediatric Infect Dis Soc 11:73–80. https://doi.org/10.1093/jpids/piab106
207. Giske CG, Turnidge J, Cantón R, Kahlmeter G, EUCAST Steering Committee. 2022. Update from the European committee on antimicrobial susceptibility testing (EUCAST). J Clin Microbiol 60:e0027621. https://doi.org/10.1128/JCM.00276-21
208. Díez-Aguilar M, Ekkelenkamp M, Morosini M-I, Huertas N, Del Campo R, Zamora J, Fluit AC, Tunney MM, Obrecht D, Bernardini F, Cantón R. 2021. Anti-biofilm activity of murepavadin against cystic fibrosis *Pseudomonas aeruginosa* isolates. J Antimicrob Chemother 76:2578–2585. https://doi.org/10.1093/jac/dkab222
209. Ekkelenkamp MB, Díez-Aguilar M, Tunney MM, Elborn JS, Fluit AC, Cantón R. 2022. Establishing antimicrobial susceptibility testing methods and clinical breakpoints for inhaled antibiotic therapy. Open Forum Infect Dis 9:ofac082. https://doi.org/10.1093/ofid/ofac082
210. Yeaman MR, Gank KD, Bayer AS, Brass EP. 2002. Synthetic peptides that exert antimicrobial activities in whole blood and blood-derived matrices. Antimicrob Agents Chemother 46:3883–3891. https://doi.org/10.1128/AAC.46.12.3883-3891.2002
211. Colquhoun JM, Wozniak RAF, Dunman PM. 2015. Clinically relevant growth conditions alter *Acinetobacter baumannii* antibiotic susceptibility and promote identification of novel antibacterial agents. PLoS One 10:e0143033. https://doi.org/10.1371/journal.pone.0143033
212. Lin L, Nonejuie P, Munguia J, Hollands A, Olson J, Dam Q, Kumaraswamy M, Rivera H, Corriden R, Rohde M, Hensler ME, Burkart MD, Pogliano J, Sakoulas G, Nizet V. 2015. Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multidrug-resistant gram-negative bacterial pathogens. EBioMedicine 2:690–698. https://doi.org/10.1016/j.ebiom.2015.05.021
213. Belanger CR, Lee A-Y, Pletzer D, Dhillon BK, Falsafi R, Hancock REW. 2020. Identification of novel targets of azithromycin activity against *Pseudomonas aeruginosa* grown in physiologically relevant media. Proc Natl Acad Sci U S A 117:33519–33529. https://doi.org/10.1073/pnas.2007626117
214. Weber BS, De Jong AM, Guo ABY, Dharavath S, French S, Fiebig-Comyn AA, Coombes BK, Magolan J, Brown ED. 2020. Genetic and chemical screening in human blood serum reveals unique antibacterial targets and compounds against *Klebsiella pneumoniae*. Cell Rep 32:107927. https://doi.org/10.1016/j.celrep.2020.107927
215. Tasse J, Dieppois G, Peyrane F, Tesse N. 2021. Improving the ability of antimicrobial susceptibility tests to predict clinical outcome accurately: adding metabolic evasion to the equation. Drug Discov Today 26:2182–2189. https://doi.org/10.1016/j.drudis.2021.05.018
216. Hinnu M, Putrinš M, Kogermann K, Bumann D, Tenson T. 2022. Making antimicrobial susceptibility testing more physiologically relevant with bicarbonate? Antimicrob Agents Chemother 66:e0241221. https://doi.org/10.1128/aac.02412-21
217. Palmer KL, Mashburn LM, Singh PK, Whiteley M. 2005. Cystic fibrosis sputum supports growth and cues key aspects of *Pseudomonas aeruginosa* physiology. J Bacteriol 187:5267–5277. https://doi.org/10.1128/JB.187.15.5267-5277.2005
218. Palmer KL, Aye LM, Whiteley M. 2007. Nutritional cues control *Pseudomonas aeruginosa* multicellular behavior in cystic fibrosis sputum. J Bacteriol 189:8079–8087. https://doi.org/10.1128/JB.01138-07
219. Neve RL, Carrillo BD, Phelan VV. 2021. Impact of artificial sputum medium formulation on *Pseudomonas aeruginosa* secondary metabolite production. J Bacteriol 203:e0025021. https://doi.org/10.1128/JB.00250-21
220. Aiyer A, Manos J. 2022. The use of artificial sputum media to enhance investigation and subsequent treatment of cystic fibrosis bacterial infections. Microorganisms 10:1269. https://doi.org/10.3390/microorganisms10071269
221. Darch SE, Kragh KN, Abbott EA, Bjarnsholt T, Bull JJ, Whiteley M. 2017. Phage inhibit pathogen dissemination by targeting bacterial migrants in a chronic infection model. mBio 8:e00240-17 https://doi.org/10.1128/mBio.00240-17
222. Chen P, Abercrombie JJ, Jeffrey NR, Leung KP. 2012. An improved medium for growing *Staphylococcus aureus* biofilm. J Microbiol Methods 90:115–118. https://doi.org/10.1016/j.mimet.2012.04.009
223. Dastgheyb S, Parvizi J, Shapiro IM, Hickok NJ, Otto M. 2015. Effect of biofilms on recalcitrance of staphylococcal joint infection to antibiotic treatment. J Infect Dis 211:641–650. https://doi.org/10.1093/infdis/jiu514
224. Gilbertie JM, Schnabel LV, Hickok NJ, Jacob ME, Conlon BP, Shapiro IM, Parvizi J, Schaer TP. 2019. Equine or porcine synovial fluid as a novel *ex vivo* model for the study of bacterial free-floating biofilms that form in human joint infections. PLoS One 14:e0221012. https://doi.org/10.1371/journal.pone.0221012
225. Pestrak MJ, Gupta TT, Dusane DH, Guzior DV, Staats A, Harro J, Horswill AR, Stoodley P. 2020. Investigation of synovial fluid induced *Staphylococcus aureus* aggregate development and its impact on surface attachment and biofilm formation. PLoS One 15:e0231791. https://doi.org/10.1371/journal.pone.0231791
226. Gupta TT, Gupta NK, Burback P, Stoodley P. 2021. Free-floating aggregate and single-cell-initiated biofilms of *Staphylococcus aureus* Antibiotics (Basel) 10:889. https://doi.org/10.3390/antibiotics10080889
227. Macias-Valcayo A, Staats A, Aguilera-Correa JJ, Brooks J, Gupta T, Dusane D, Stoodley P, Esteban J. 2021. Synovial fluid mediated aggregation of clinical strains of four enterobacterial species. Adv Exp Med Biol 1323:81–90. https://doi.org/10.1007/5584_2020_573
228. Staats A, Burback PW, Eltobgy M, Parker DM, Amer AO, Wozniak DJ, Wang SH, Stevenson KB, Urish KL, Stoodley P. 2021. Synovial fluid-induced aggregation occurs across *Staphylococcus aureus* clinical isolates and is mechanistically independent of attached biofilm formation. Microbiol Spectr 9:e0026721. https://doi.org/10.1128/Spectrum.00267-21
229. Staats A, Burback PW, Schwieters A, Li D, Sullivan A, Horswill AR, Stoodley P. 2022. Rapid aggregation of *Staphylococcus aureus* in synovial fluid is influenced by synovial fluid concentration, viscosity, and fluid dynamics, with evidence of polymer bridging. mBio 13:e0023622. https://doi.org/10.1128/mbio.00236-22
230. Stamm J, Weißelberg S, Both A, Failla AV, Nordholt G, Büttner H, Linder S, Aepfelbacher M, Rohde H. 2022. Development of an artificial synovial fluid useful for studying *Staphylococcus epidermidis* joint infections. Front Cell Infect Microbiol 12:948151. https://doi.org/10.3389/fcimb.2022.948151
231. Brackman G, Coenye T. 2016. *In vitro* and *in vivo* biofilm wound models and their application. Adv Exp Med Biol 897:15–32. https://doi.org/10.1007/5584_2015_5002
232. Brackman G, Garcia-Fernandez MJ, Lenoir J, De Meyer L, Remon J-P, De Beer T, Concheiro A, Alvarez-Lorenzo C, Coenye T. 2016. Dressings loaded with cyclodextrin-hamamelitannin complexes increase *Staphylococcus aureus* susceptibility toward antibiotics both single as well as in mixed biofilm communities. Macromol Biosci 16:859–869. https://doi.org/10.1002/mabi.201500437
233. Thaarup IC, Bjarnsholt T. 2021. Current *in vitro* biofilm-infected chronic wound models for developing new treatment possibilities. Adv Wound Care (New Rochelle) 10:91–102. https://doi.org/10.1089/wound.2020.1176
234. Kadam S, Madhusoodhanan V, Dhekane R, Bhide D, Ugale R, Tikhole U, Kaushik KS. 2021. Milieu matters: an *in vitro* wound milieu to recapitulate key features of, and probe new insights into, mixed-species bacterial biofilms. Biofilm 3:100047. https://doi.org/10.1016/j.bioflm.2021.100047
235. Trivedi U, Madsen JS, Rumbaugh KP, Wolcott RD, Burmølle M, Sørensen SJ. 2017. A post-planktonic era of *in vitro* infectious models: issues and changes addressed by a clinically relevant wound like media. Crit Rev Microbiol 43:453–465. https://doi.org/10.1080/1040841X.2016.1252312
236. de Breij A, Riool M, Cordfunke RA, Malanovic N, de Boer L, Koning RI, Ravensbergen E, Franken M, van der Heijde T, Boekema BK, Kwakman

PHS, Kamp N, El Ghalbzouri A, Lohner K, Zaat SAJ, Drijfhout JW, Nibbering PH. 2018. The antimicrobial peptide SAAP-148 combats drug-resistant bacteria and biofilms. Sci Transl Med 10:eaan4044. https://doi.org/10.1126/scitranslmed.aan4044
237. Pestrak MJ, Baker P, Dellos-Nolan S, Hill PJ, Passos da Silva D, Silver H, Lacdao I, Raju D, Parsek MR, Wozniak DJ, Howell PL. 2019. Treatment with the *Pseudomonas aeruginosa* glycoside hydrolase PslG combats wound infection by improving antibiotic efficacy and host innate immune activity. Antimicrob Agents Chemother 63:e00234-19. https://doi.org/10.1128/AAC.00234-19
238. Redman WK, Welch GS, Williams AC, Damron AJ, Northcut WO, Rumbaugh KP. 2021. Efficacy and safety of biofilm dispersal by glycoside hydrolases in wounds. Biofilm 3:100061. https://doi.org/10.1016/j.bioflm.2021.100061
239. Tillander JAN, Rilby K, Svensson Malchau K, Skovbjerg S, Lindberg E, Rolfson O, Trobos M. 2022. Treatment of periprosthetic joint infections guided by minimum biofilm eradication concentration (MBEC) in addition to minimum inhibitory concentration (MIC): protocol for a prospective randomised clinical trial. BMJ Open 12:e058168. https://doi.org/10.1136/bmjopen-2021-058168
240. Zimmerli W, Trebse R. 2023. Which trial do we need? Rational therapeutic management of periprosthetic joint infection. Clin Microbiol Infect 29:820–822. https://doi.org/10.1016/j.cmi.2023.03.014
241. Bizzini A, Greub G. 2010. Matrix-assisted laser desorption Ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. Clin Microbiol Infect 16:1614–1619. https://doi.org/10.1111/j.1469-0691.2010.03311.x
242. Croxatto A, Prod'hom G, Greub G. 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev 36:380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x
243. Clark AE, Kaleta EJ, Arora A, Wolk DM. 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 26:547–603. https://doi.org/10.1128/CMR.00072-12
244. Patel R. 2015. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem 61:100–111. https://doi.org/10.1373/clinchem.2014.221770
245. Sandoe JAT, Wysome J, West AP, Heritage J, Wilcox MH. 2006. Measurement of ampicillin, vancomycin, linezolid and gentamicin activity against enterococcal biofilms. J Antimicrob Chemother 57:767–770. https://doi.org/10.1093/jac/dkl013

AUTHOR BIO

Tom Coenye is a Professor of Microbiology at the Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium where he leads the Laboratory of Pharmaceutical Microbiology. He obtained a master's degree (in 1996) and a PhD (in 2000) in Biochemistry from Ghent University (Belgium) and then joined the University of Michigan (United States) for a postdoctoral fellowship (2001-2002). He has been working on microbial biofilms for almost 20 years and his current research is focused on the identification of molecular mechanisms of reduced susceptibility in microbial biofilms and the translation of novel insights in fundamental biofilm biology to innovative approaches for diagnosis, susceptibility testing and treatment (mainly in the context of biofilm-related respiratory tract and prosthetic joint infections). He was vice-chair (2013-2016) and chair (2017-2021) of the European Society for Clinical Microbiology and Infectious Diseases Study Group on Biofilms and is Senior Editor of the journal Biofilm since 2018.