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Commentary

Should standardized susceptibility testing for microbial biofilms be introduced in clinical practice?

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Biofilms are communities of microbial cells that are attached to each other and/or a surface, and are embedded in an extracellular matrix produced by the bacteria and/or derived from the host. Treatment of biofilm-related infections is difficult, as sessile microorganisms show reduced antimicrobial susceptibility as a result of various resistance and tolerance mechanisms [1].

In the medical biofilm field, standardization or guidance is important for diagnosis, determining the efficacy of antibiotics and the ability to prevent or reduce biofilm formation on indwelling devices. Guidelines for diagnosing biofilm infections [2] and standardized methods to assess the efficacy of surface disinfection are available [3]. However, parameters that predict the therapeutic success of antibiotics are determined using planktonic bacteria [4] and therefore fail to take into account important local pharmacokinetic and pharmacodynamic factors that modulate antibiotic activity in biofilms. The minimum biofilm inhibitory concentration (MBIC) and the minimum biofilm eradication concentration (MBEC) are utilized to determine antibiotic efficacy against biofilms. MBIC and MBEC values are typically much higher than the minimum inhibitory and minimum bactericidal concentrations, respectively [5]. It has been suggested that treatment decisions should be based on MBIC or MBEC values [6]. However, the relationship between intrinsic activity towards planktonic and sessile cells is complex, and currently there is insufficient evidence to recommend choosing antibiotics on the basis of biofilm susceptibility testing [7]. For example, MBIC values did not predict clinical success for the treatment of catheter-related bloodstream infections due to enterococci [8], and superiority of treatment based on biofilm susceptibility testing over conventional susceptibility testing could not be demonstrated in two clinical trials addressing treatment of *Pseudomonas aeruginosa* respiratory tract infections in cystic fibrosis (CF) patients [7].

Here we discuss the issue whether standard methods for antibiotic susceptibility testing of microbial biofilms should be introduced in clinical practice to guide decisions about treatment, and we discuss their value in the regulatory pathway of approval of antibiofilm products or devices, i.e. products or devices that prevent or reduce biofilm formation and/or allow (partial) eradication of established biofilms (Fig. 1).

Most of our knowledge regarding biofilm tolerance is derived from *in vitro* assays. An important question is whether these *in vitro* tests are predictive of the *in vivo* situation and can be used to help guide clinical therapy and to evaluate novel antimicrobial compounds. *In vitro,* biofilm formation occurs in a sequence of distinct events (attachment—maturation—dispersal), while *in vivo,* this sequence of events might not apply. Most *in vitro* models are poorly representative of an infection site: *in vivo* biofilms are not exposed to a continuous flow of fresh media, and they are not attached to an artificial surface but are embedded in tissues, in the thick mucus of the CF lung or between the implant and the tissue [2]. *In vivo* biofilms occur typically as small aggregates (5–200 μm in

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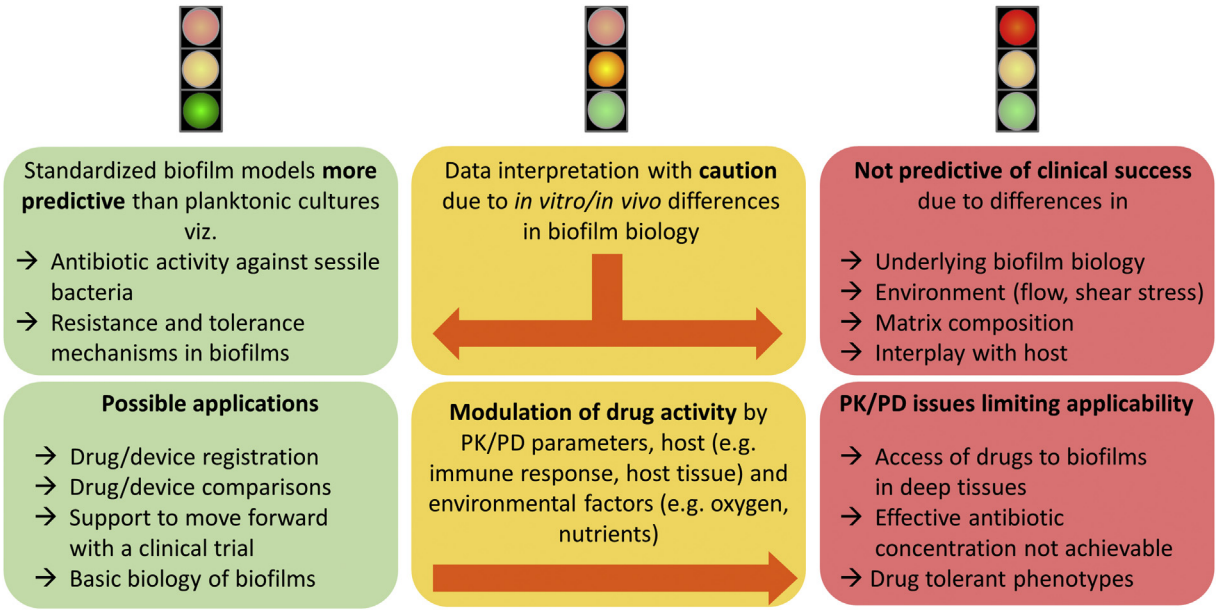

Fig. 1. Pros and cons of *in vitro* standardized biofilm models.

diameter) embedded in host material with a secondary matrix on top of or intermixed with the bacterial matrix, making treatment aimed at dispersal and/or killing even more problematic. Sessile bacteria are often not exposed to flow *in vivo*, and despite their low metabolic activity, they still elicit an inflammatory response [9].

In principle, the susceptibility of an *in vivo* biofilm does not differ from that of an *in vitro* biofilm, but the access of the antibiotics to the biofilm is different: while an antibiotic can be added directly to the biofilm *in vitro*, *in vivo* the antibiotic must first reach the biofilm. These biofilms are often localized in deep tissues, with profound differences in drug access between different types of infections (e.g. chronic wound vs. implant). A second issue is that bacteria in biofilms are extremely tolerant to antibiotics, and higher doses and longer exposure times are required to kill biofilms than planktonic cells [5,10,11]. The antibiotic concentration required to achieve inhibition and/or eradication of biofilm-associated bacteria is often beyond what is achievable *in vivo* if administered systemically (although effective concentrations can sometimes be achieved locally, e.g. using inhaled antibiotics in CF patients or when using antibiotic-coated beads in orthopaedic infections) [11]. Consequently, testing bacterial isolates from chronic infections for biofilm susceptibility towards antibiotics *in vitro* is not particularly helpful to guide clinical decisions because the necessary concentration cannot be achieved in the patient.

Progress has been made in developing laboratory biofilm models that are more representative of the situation in a patient (e.g. in the context of chronic wound infections [12] and CF [13]), but studies establishing the clinical validity of these models are still lacking. However, even in relevant biofilm models, activity of antibiotics is often only observed at concentrations that cannot be reached *in vivo*. The implementation of such models is technically demanding and may not be within the reach of every clinical microbiology laboratory. Despite its limitations, susceptibility testing on planktonic isolates (minimum inhibitory concentration determination) is useful for measuring antibiotic resistance, and once resistance is identified, the use of these antibiotics should be avoided for the treatment of that particular infection. This ensures that the antibiotics used are likely to have an effect on the actively growing (less dormant) bacteria in the infection and suppress their spread from the site of infection.

When it comes to regulatory approval of antibiofilm devices and treatments, standardized biofilm testing is essential [3]. A company that wants to bring a new device or drug to market must register it with the regulatory agencies. These agencies issue guidelines that describe the pathway that must be followed to take the product through the validation process. Many guidelines require *in vitro* data that demonstrate the drug and/or device's effectiveness, and a well-designed *in vitro* standard method is a critical and necessary part of this regulatory pathway. Data collected using a standard method allow regulators to assess a new device and/or drug and compare its performance to existing technology on the market. A standard method allows companies to demonstrate that the new drug or device provides statistically equivalent results to approved technologies and provides motivation for moving forwards with a costly clinical trial, where the question whether the device and/or drug is clinically useful is definitively answered.

In vitro methods are often criticized for not correctly modeling the *in vivo* infection because of the simplifying assumptions made. However, standard methods contain a 'significance and use' section that provides guidance on how the test results should be interpreted. A standard microbiologic method should not be used for an application that the method was never designed to test (e.g. a zone of inhibition test does not provide much information on how an antimicrobial catheter will perform *in vivo*, but rather provides an indication if the active compound diffuses into agar at a concentration that prevents microbial growth). It is thus not surprising that the use of a product 'verified' with a standard method that was designed for a different application results in clinical data that are not in line with the *in vitro* test results. In addition, a standard method [14] does not need to address every parameter, just the most influential ones, as too much complexity results in the potential for greater variability in the data. Besides their role in the process of regulatory approval, standardized methods can play an important role in increasing our understanding of the basic biology of biofilms and the mechanisms involved in biofilm tolerance. Indeed, comparison between different studies is often hampered by a lack of standardization, and standardized methods are an important tool to screen large libraries for potential compounds with antibiofilm activity.

In conclusion, regardless of standardization, *in vitro* biofilm susceptibility tests will frequently yield results that are poorly representative of the activity of the antibiotic against the biofilm *in vivo* because of profound differences between the two types of biofilms. There is currently no evidence that introducing standardized biofilm susceptibility testing in clinical practice would improve patient outcome. However, we are convinced that standardized methods are valuable for research purposes and in the process of regulatory approval (Fig. 1). Whether the development and implementation of more relevant, peer-reviewed and statistically validated methods will lead to better approaches to treating biofilm infections should be the subject of future research.

We hope this commentary will stimulate efforts in elucidating factors affecting antibiotic activity against biofilms *in vivo,* thereby helping the development of *in vitro* assays for testing antibiofilm drugs that more appropriately mimic the *in vivo* biofilm.

Transparency declaration

All authors report no conflicts of interest relevant to this commentary.

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