**Comment**

# Revitalizing antibiotic discovery and development through *in-vitro* modelling of in-patient conditions

Julie Sollier[1], Marek Basler[1], Petr Broz[2], Petra S. Dittrich[3], Knut Drescher[1], Adrian Egli[4], Alexander Harms[5], Andreas Hierlemann[3], Sebastian Hiller[1], Carolyn King[6], John D. McKinney[7], Jacob Moran-Gilad[8], Richard A. Neher[1], Malcolm G.P. Page[9], Sven Panke[3], Alexandre Persat[7], Paola Picotti[10], Katharina M. Rentsch[11], Pablo Rivera-Fuentes[12], Uwe Sauer[10], Daiana Stolz[13,14], Sarah Tschudin-Sutter[15,16], Christian van Delden[17], Erik van Nimwegen[1], Jan-Willem Veening[18], Mattia Zampieri[6], Annelies S. Zinkernagel[19], Nina Khanna[6,15,16], Dirk Bumann[1], Urs Jenal[1], and Christoph Dehio[1,#]

[1] Biozentrum, University of Basel, Basel, Switzerland; [2] Department of Biochemistry, University of Lausanne, Epalinges, Switzerland; [3] Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland; [4] Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland; [5] Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland; [6] Department of Biomedicine, University Basel, Basel, Switzerland; [7] School of Life Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [8] Department of Health Policy and Management, Ben-Gurion University of the Negev, Beer-Sheva, Israel. [9] Malcolm Page GmbH, Basel, Switzerland; [10] Institute of Molecular Systems Biology, Department of Biology, ETH Zurich, Zurich, Switzerland; [11] Laboratory Medicine, University Hospital Basel, Basel, Switzerland; [12] Department of Chemistry, University of Zurich, Zurich, Switzerland; [13] Clinic of Respiratory Medicine and Pulmonary Cell Research, University Hospital Basel, Basel, Switzerland; [14] Department of Pneumology, University Medical Center, Freiburg, Germany; [15] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland; [16] Department of Clinical Research, University Hospital

Basel, Basel, Switzerland; [17] University Hospitals Geneva, Geneva, Switzerland; [18] Department of Fundamental Microbiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland; [19] Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.

[#] Corresponding author: christoph.dehio@unibas.ch

**_In-vitro_ models mimicking within patient conditions have the potential to yield exciting opportunities for antibiotic research and revitalize future antibiotic discovery and development.**

Antibiotics have revolutionized modern medicine. However, their usefulness in treating bacterial infections and as prophylactics accompanying chemotherapy or surgery is under threat by the rise of antibiotic-resistant bacteria. Bacterial infections that could once be effectively treated are resurging as deadly threats[1]. Despite an urgent need, there is a shortage of newly developed antibiotics: the last new antibiotic class approved for treatment was discovered more than three decades ago[2]. The discovery and development process is beset by complex scientific, clinical, regulatory and economic challenges, while the emergence of resistance, need for stringent clinical trial design and limited profit margins have deterred industry and investors from pursuing antibiotic research and development (R&D) programs[3]. Though modification of existing molecules is simpler, less risky and less costly, faster evolution of resistance can weaken their efficacy, often rendering any resulting gains short-lived. Numerous national and international programs are attempting to address this gap in the antibiotic development pipeline through the identification of natural products, improved understanding of molecule permeation into bacteria, creation of improved synthetic compound libraries or the modification of existing drugs[2,4]. We argue that assays that mimic *in-vivo* physiological conditions and are matched to patient biopsies, are an underexplored avenue with potential to boost the development of antimicrobial strategies.

**Limitations of standard laboratory assays used in antibiotic discovery and development**

Antibacterial drug screening has mostly focused on testing compounds for efficacy on bacteria under optimal growth conditions in nutrient-rich culture media (Fig. 1a). Such conditions ensure reproducibility, but inadequately mimic the markedly distinct conditions bacteria actually face during infection within patients. The host environment imposes stresses through numerous antibacterial attack mechanisms, including acidification, antimicrobial peptides and production of reactive oxygen and nitrogen species, as well as limited nutrient availability[5] (Fig. 1b). It is a physically, chemically and biologically complex and varied environment, shaped by tissue-specific arrangements of diverse host cells and spatiotemporal gradients of numerous biomolecules. Bacteria deploy specific physiological adaptation strategies to survive such adverse conditions *in vivo*, which often lead to slow growth. Genetic screens have identified numerous genes that are dispensable in rich culture media but are essential for bacterial fitness in infected tissues[6]. Characterising human physiology and the corresponding strategies through which bacteria adapt during infection, should therefore unveil novel vulnerabilities of these pathogens which are not evident under standard laboratory conditions.

On occasion, our understanding of human physiology has prompted specific adjustments to render standard media more physiologically relevant. Test media used for screening anti-folate compounds are depleted of folic acid and its precursors to mimic the low abundance of such compounds circulating in the human body. Further, iron-depleted media reproducing the strict iron-limitation present in humans are used for screening of siderophore-conjugated compounds such as the antibiotic cefiderocol. However, such occurrences are rare and often only used in secondary assays for characterizing compounds previously selected under more generic conditions. We argue that an emphasis on standard laboratory assays might have overlooked novel classes of antibacterial compounds that are particularly effective under the more challenging and physiologically relevant conditions bacteria encounter within the human body. Nevertheless, these candidates could be in reach through the development of novel assays that more faithfully replicate the *in-vivo* environments formed during infection. Such physiological assays could also offer early toxicity readouts,

complementing toxicity studies in established animal models that are typically performed at later stages of antibiotic development.

Moreover, considering that the spectra of resistance mutations emerging under standard laboratory conditions typically differ from those emerging in clinics, assays mimicking in-patient conditions may proof advantageous for predicting clinically relevant AMR mutations[7,8]. As resistance determinants can be differentially expressed between *in-vitro* and *in-vivo* conditions such models may also prove advantageous over standard antimicrobial susceptibility testing (AST)[7,9].

**Developing *in-vitro* assays mimicking in-patient conditions**

Emerging strategies to mimic in-patient conditions range from supplementing culture media with physiologically relevant inorganic molecules or employing metabolite mixtures that match conditions *in vivo*[10,11], to using more complex tissue-mimetic models such as micro-tissues or organoids[12] (Fig. 1c). Tissue-mimetic models can be rendered immunocompetent by inclusion of immune cells such as macrophages or neutrophils. Advances in bioengineering have also increased model fidelity, for example by including fluid flow and mechanical stretching into bladder chip models of urogenital tract infection[13], or mimicking mucociliary clearance in airway models[14]. High resolution time-lapse microscopy and other fluorescence-based readouts yield detailed insight into the dynamics of the infection process and anti-infective activities. Such micro-physiological systems may provide informative assays for conventional and non-conventional antibacterial strategies including phage therapy, anti-virulence approaches, immunomodulation, microbiota engineering and interventions by live biotherapeutic products.

There are challenges inherent to establishing *in-vitro* models that faithfully replicate conditions within patients. Specialised infrastructure, training, time and expense are required. Compromises between fidelity and throughput may be needed. Simpler, physiologically relevant culture media mimicking body fluids such as synthetic urine or sputum may be better suited for extensive screens of compound libraries, with more complex micro-tissue or organoid models reserved for secondary screening assays

of a limited number of hit compounds. Perhaps the most critical challenge, however, is guaranteeing that these mimetic models accurately reproduce the most relevant aspects of the *in-vivo* context of infection and therefore effectively predict drug efficacy.

**Benchmarking and validation of patient-mimetic models**

We argue that to ensure fidelity of *in vivo*-mimicking models, they must be rigorously benchmarked against patient samples and validated by assessing the efficacy of established antibiotics and advanced-stage drug candidates for which preclinical and clinical datasets are accessible. This requires access to fresh patient samples from common infection sites, including samples from individuals at high risk for treatment failure. This is dependent upon strong interdisciplinary networks of fundamental researchers and clinicians. Patient and model samples should then undergo identical analyses to assess tissue architecture, recruitment of immune cells, bacterial localization and morphology, as well as proteomic, metabolomic and transcriptomic analyses to provide an in-depth picture of host and pathogen physiology. Such methods need extensive optimisation to overcome the inherent challenges associated with scarce bacteria in a preponderance of host components. They also typically provide bulk average data, which make complementary approaches necessary to reveal heterogeneity within an infection as these become more readily available and easier to apply. Extensive variation within patient and bacterial populations also needs to be accounted for by rigorous comparisons across datasets, the development of robust computational methods to analyse these datasets, and the development of standardised approaches[10], to identify the critical common elements of the pathophysiological process as relevant references.

Thorough benchmarking of *in vitro* models should ideally achieve congruent pathogen physiology as observed *in vivo*. Recent studies have shown that *Pseudomonas aeruginosa* grown in artificial CF-sputum medium, or infecting airway organoids derived from CF individuals, reproduces various features of *in-vivo* infection[7,14]. The predictive value of the models needs to be accessed through measurable parameters related to the antibacterial activity as well as the absorption, distribution,

metabolism, and elimination (ADME) properties of antibiotics. This validation approach should be based on a set of clinical isolates representing the genetic diversity, resistance patterns and disease associations of a given pathogen. Pharmacokinetic and pharmacodynamic (PK/PD) parameters should be evaluated for different classes of antibiotics based on their current clinical use, diversity of PK/PD drivers, and the availability of clinical and pre-clinical data. Ideally, model validation includes negative controls, i.e., antibiotics or lead compounds with limited clinical success due to poor tissue penetration, efficacy, or toxicity. Successfully leveraging existing pre-clinical and clinical data will enhance the value of the patient-mimicking *in-vitro* models for predicting PK/PD behaviour of novel compounds.

## Conclusions

Our understanding of host and bacterial physiology during infection and treatment will provide guidance for building and optimizing patient-mimetic *in-vitro* models. The most promising models will then be engineered to yield detailed insight into potential targets for therapeutic development and for screening of existing or new compounds. Innovative engineering approaches to miniaturization and parallelization will optimize throughput and enhance accessibility.

The extent to which the new patient-mimicking *in-vitro* models will surpass traditional methods for developing antimicrobials remains an open question. Nonetheless, proof-of-principle for the early discovery process is provided by a study which found previously unidentified antibacterial compounds using human serum as a bacterial growth medium[15]. Such *in vivo* mimicking approaches are gaining popularity, and are being pursued both by individual research groups and national consortia. This includes PERFECTION, a Danish research consortium studying chronic *Pseudomonas aeruginosa* lung infection, and the Swiss NCCR AntiResist consortium, which develops micro-tissue infection models for bladder, lung, placental tissue and granuloma, as well as body fluid mimetics for four human pathogens. Using these patient-like conditions should reveal both potentials and risks, enabling rapid prioritization of the most promising antibiotic candidates. We believe this will also promote the

development of alternative therapeutic approaches, including phage therapy, virulence inhibitors, immune modulators, or microbiota interventions, which all depend on the host environment. It may also facilitate the development of personalized diagnostics, including more precise and faster determination of antibiotic susceptibilities. Finally, it will advance our mechanistic understanding of host-pathogen interactions, potentially revealing new vulnerabilities of bacterial pathogens, exposing relevant bacterial targets, and consequently aid the development of antibiotics with novel modes of action.

In conclusion, we postulate that an integrated approach which centres on innovative models mimicking in-patient conditions could revitalize antibiotic discovery and development. To achieve this, clinicians, biologists, engineers, chemists and data scientists must collaborate. Bridging the gap between academic research, medicine, bioengineering and industry will be critical to build screening infrastructure, conduct clinical trials, and bring new antibiotic drugs to the market. We believe that this integrated strategy has the potential to help replenish the antibiotic discovery pipeline and to ensure infection control for coming generations.

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**Figure 1: Revitalizing antibiotic discovery through *in-vitro* modelling of in-patient infection conditions**. **a**. Standard laboratory assays used in antibiotic discovery are based on rich media which trigger a homogenous physiological state of robust bacterial growth that is disparate from in-patient conditions, thus limiting opportunities for anti-infective discovery. **b**. Within the infected patient, bacterial pathogens are exposed to multiple stressors, resulting in heterogenous and typically slow growing states. **c**. *In-vitro* modelling of in-patient infection conditions trigger relevant *in-vivo* physiological states and thus expand opportunities for anti-infective discovery. Created with BioRender.com.

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**Competing interest**

The authors declare no competing interests.

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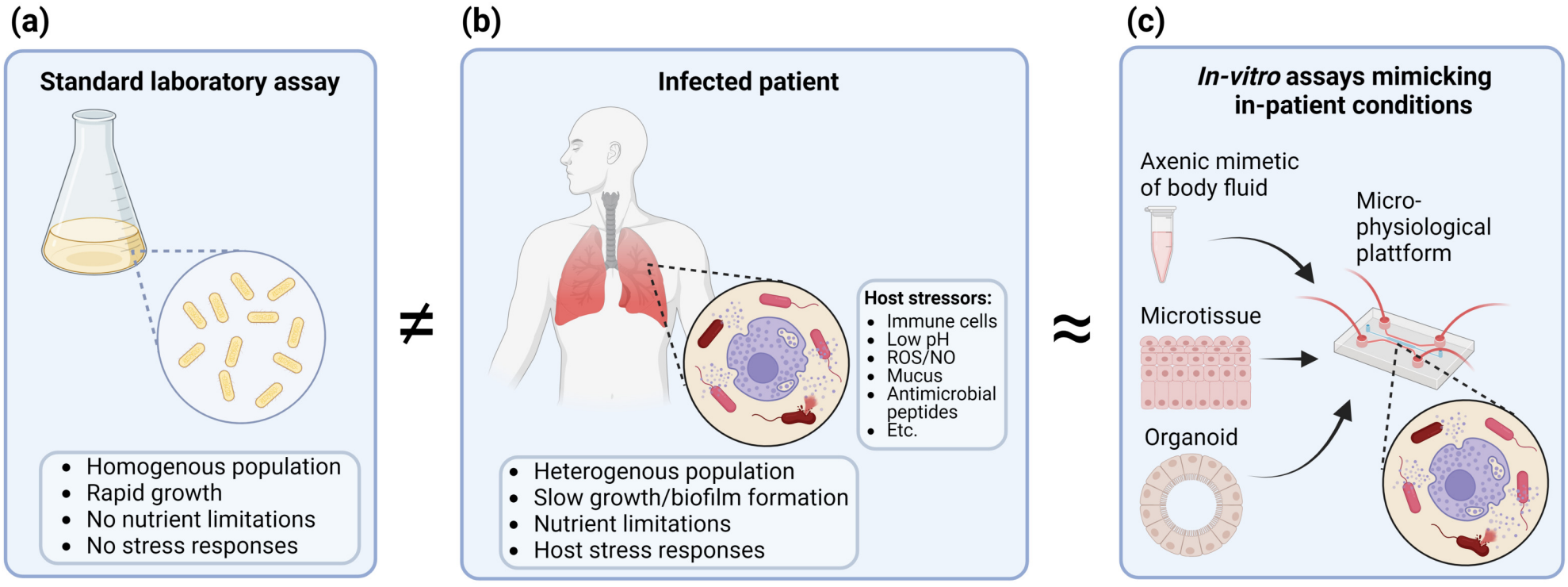
(a)
Standard laboratory assay
• Homogenous population
• Rapid growth
• No nutrient limitations
• No stress responses
≠
(b)
Infected patient
Host stressors:
• Immune cells
• Low pH
• ROS/NO
• Mucus
• Antimicrobial peptides
• Etc.
• Heterogenous population
• Slow growth/biofilm formation
• Nutrient limitations
• Host stress responses
≈
(c)
In-vitro assays mimicking in-patient conditions
Axenic mimetic of body fluid
Micro-physiological plattform
Microtissue
Organoid