Innovative and rapid antimicrobial susceptibility testing systems

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Abstract | Antimicrobial resistance (AMR) is a major threat to human health worldwide, and the rapid detection and quantification of resistance, combined with antimicrobial stewardship, are key interventions to combat the spread and emergence of AMR. Antimicrobial susceptibility testing (AST) systems are the collective set of diagnostic processes that facilitate the phenotypic and genotypic assessment of AMR and antibiotic susceptibility. Over the past 30 years, only a few high-throughput AST methods have been developed and widely implemented. By contrast, several studies have established proof of principle for various innovative AST methods, including both molecular-based and genome-based methods, which await clinical trials and regulatory review. In this Review, we discuss the current state of AST systems in the broadest technical, translational and implementation-related scope.

Over the past few decades, the emergence of antimicrobial resistance (AMR) has outpaced the development and market entry of new antimicrobial agents[1], and we need to encourage interventions to preserve the currently available antimicrobials[2]. Antimicrobial susceptibility testing (AST) systems can play a major role in achieving this goal. These diagnostic tests inform about drug susceptibility for a particular pathogen and detect possible drug resistance, thus guiding the appropriate use of antimicrobials. The main problem with current AST methods is speed, for in most cases conducting AST requires overnight incubation, and it may require 48–72 h to complete, depending on the drug–organism combination. There are many opinions as to what constitutes 'rapid AST', but most clinical microbiologists define rapid testing as being feasible during a single working shift — that is, within 8 h or less. Rapid AST provides antibiotic susceptibility profiles that can support and facilitate antimicrobial stewardship as well as epidemiological surveillance[3]. How best to manage infectious diseases by combining stewardship, optimized rapid AST and AMR data collection is an important objective for investigation[4].

For the purpose of this Review, we define phenotypic AST as a microbiological procedure whereby a pure culture of a single organism is grown (in solid or liquid media) in the presence or absence of an antimicrobial agent. Microbial growth or inhibition is observed longitudinally. Phenotypic AST methods provide a direct indication of the susceptibility of a given microorganism to an antimicrobial agent at defined concentrations, and in some cases such methods provide a quantitative assessment of the minimal inhibitory concentration (MIC) of the antibiotic. By contrast, the term 'AMR detection methods' is used to describe methods that detect (pre-existing) proteomic or genomic signatures that predict antimicrobial resistance (FIG. 1).

Although most commercially available and frequently used AST methods have been shown to be reliable and reproducible[5], the systems are complex and have several limitations, and one of the main limitations is speed, as we mentioned above. The early stage of microbial growth (lag phase) is one time-limiting factor[6]. Monitoring cell density in suspension is different from (visually) analysing cell division and requires different technological approaches. Density can be monitored by relatively simple spectral means, whereas visualization of cells requires sophisticated microscopy. Moreover, growth rate (net cellular doubling time), the concentration of growth markers to be detected, intrinsic natural genetic mutability, fitness decrease following the acquisition of antibiotic resistance, biofilm formation and the ability to transduce, transform or conjugate all need to be taken into account when assessing antimicrobial susceptibility or resistance by AST methods[7]. Antimicrobial susceptibility may differ between individual cells, leading to heterogeneous populations; the adequate detection of heterogeneity is clinically important but is difficult with current AST methods[8]. Some bacterial species exhibit mechanisms that drive the emergence of de novo resistance or gene mutations, enabling the rapid adaptation to antibiotics, which may not be detected with current methods[9]. AST usually identifies individual cells that remain alive or in a state of suspended animation in the presence of

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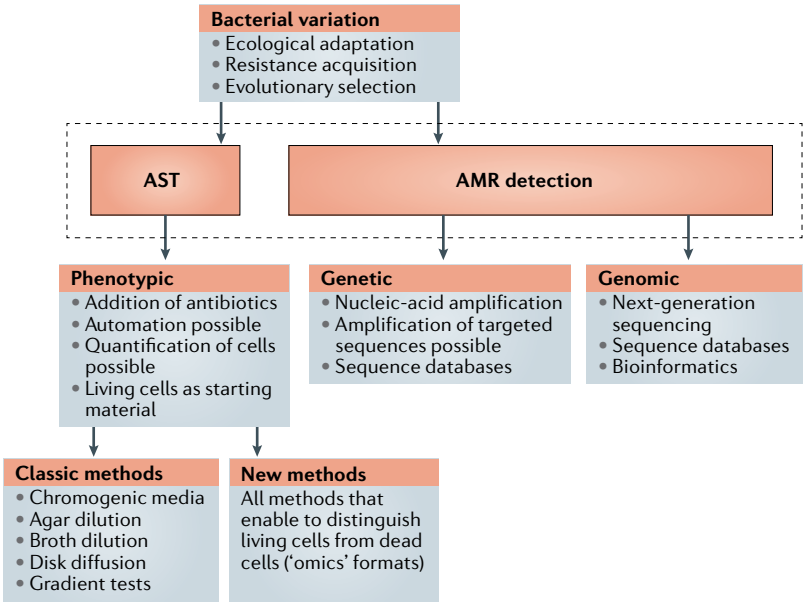

Fig. 1 | **Schematic overview of antimicrobial susceptibility testing and antimicrobial resistance detection methods.** Antimicrobial susceptibility testing (AST) and antimicrobial resistance (AMR) detection methods detect basic features of bacterial variation that result from ecological adaptation, resistance acquisition and evolutionary selection. Phenotypic AST, including the use of selective chromogenic culture media, mostly detects the arrest of growth in the presence of different concentrations of different antimicrobial agents[157]. There is a need for quantifying the cells, however, as inoculum effects may substantially affect determination of the minimal inhibitory concentration[158]. Rapid evolution of phenotypes may occur, and the new methods especially are focused towards circumventing such possible changes, by providing a more definite live–dead distinction[159]. Molecular AMR detection attempts to specify resistance genes, as well as mutations in and expression of these genes or their genomic signature, using molecular-based (both DNA and RNA) methods. This is surrogate AST and has to be validated against phenotypic data to be useful[101,106]. For this method, extensive resistance marker databases and innovative bioinformatics methodologies are mandatory requirements.

Minimal inhibitory concentration
(MIC). The lowest concentration of an antimicrobial agent that prevents visible growth of a bacterium species or isolate. The MIC is defined by combined activities of the microorganism, the affected patient and the antimicrobial agent itself.

Lag phase
The temporary period in which microorganisms are adapting to a new environment, avoiding threats and metabolizing, and increasing in cell size but not yet actively dividing and multiplying. During this period, cells are synthesizing enzymes and other factors needed for actual cell division under the new environmental conditions.

antimicrobials (tolerance)[10,11]. Distinguishing reversibly growth-inhibited as opposed to dead microbial cells may be more difficult but is relevant, as reversal of the inhibition may increase infection risk once more. Although sensitivity is often limited, some protocols that reliably distinguish growth-inhibited from dead cells have been reported[12]. In addition, AMR is also increasing in anaerobes[13], and specialized infrastructure and laboratory equipment is required in order to conduct AST in the absence of oxygen, which might not be generally available[14]. A number of the compromising aspects of phenotypic tests are unlikely to be improved, as these are of an intrinsic biological nature, such as the duration of the lag or growth phase. The development of new AST methods is slow, costly, logistically complex and riddled with regulatory issues[15]. Current key issues include the needs for improved speed, cost control and platforms that cover as many microorganism–drug combinations as possible. In addition, to enable routine use in clinical microbiology laboratories, the system platform should have a small footprint — since laboratory space is very costly — have low maintenance needs and provide high throughput.

Newer AST methods might not directly measure phenotypic cell viability, but rather detect surrogates of viability, such as the modulation of proteins, metabolites, DNA and/or mRNA, or changes in proteomes, metabolomes, genomes and/or transcriptomes, to provide indirect evidence of antibiotic susceptibility or resistance[16]. Such tests include proteomic, lipidomic or genotypic methods, which characterize proteins, lipids and fatty acids, or nucleic acids, respectively. A notable limitation is that the correlation between these newer, marker-directed methods and classic phenotypic methods may vary across specific combinations of organisms and antimicrobial agents[17,18]. For these newer methods to be used in clinical decision making, they need to be calibrated and referenced back to standard phenotypic methods that have been used for many decades. Moreover, phenotypic methods are generally known to predict susceptibility or resistance more accurately. They can also generate MICs of various antimicrobial agents, even though this usually requires extended incubation and a large inoculum (10^4–10^6 cells per unit volume) (BOX 1). In light of the many reviews on AST that have been published recently[19–22], in this Review we aim to assess new technologies in terms of developmental phase, regulatory review and commercialization. First we briefly discuss the routinely used AST methods, and then we explore current efforts to improve phenotypic AST systems, including new emerging technologies, as well as genomic and gene-based AMR detection methods. We conclude by highlighting the challenges and opportunities for new rapid AST systems at both the academic and company levels.

Automated AST methods

Routinely used traditional and automated methods. 'Traditional' AST methods include disc diffusion and broth dilution assays, which are used to compare the visible growth of microorganisms in the presence of specific concentrations of antimicrobials under defined test conditions (Supplementary Fig. 1). Analysis is based either on decreased or no visible growth within the zone of inhibition on solid agar, or on the lack of visible turbidity in broth after appropriate incubation. Traditional methods help define the MICs, which are the current reference standard in measuring antibiotic activity. These two key AST formats have been automated: during disc diffusion assays, inhibition zones can be read automatically[23], and several formats of automated broth dilution assays are commercially available[24].

The first automated AST instrument was the Autobac-1, which was presented as a prototype in 1971 (REF.[25]). Most of the current automated systems enable high throughput and extensive testing of many microorganism–antibiotic combinations, but they vary in terms of accuracy or time to result. Four automated instruments are currently cleared by the FDA for in vitro diagnostics (IVD): VITEK2 (bioMérieux), MicroScan WalkAway (Siemens Healthcare Diagnostics), BD Phoenix (BD Diagnostics) and Sensititre ARIS 2X (Trek Diagnostic Systems). Three of these systems generate rapid (3.5–16 h) results, whereas the fourth (Sensititre ARIS 2X) takes longer on average to report end points. However, it must be noted that even the so-called rapid methods require a standardized microbial inoculum, which entails culturing the specimen for 24–48 h

Tolerance
The microbial ability to resist being killed by antimicrobials. This ability is distinct from (multi)drug resistance and is not caused by mutant microorganisms, but rather by cells existing in a dormant, non-dividing state.

Zone of inhibition
If bacteria are grown as layers on solid growth media and an antibiotic stops the bacteria from growing or kills them, there will be an area around the place where the antibiotic has been positioned (usually in a well or on a paper disc) where the bacteria have not grown enough to be visible. The radius of such a region of growth inhibition is correlated with the level of antibiotic susceptibility of the strain being tested.

(sometimes longer) prior to inoculation into the AST system. All of the automated methods require dense suspensions of bacteria as the primary inoculum. The MicroScan WalkAway is a large incubator and reader that utilizes microdilution trays that are inoculated manually, incubated and examined for growth. Susceptibility test panels for Gram-negative bacteria contain fluorogenic substrates and can be read in 3.5–7 h. Separate panels for Gram-positive and Gram-negative bacteria read turbidimetric end points and are completed in 4.5–18 h. The BD Phoenix Automated Microbiology System has a large incubator with a capacity to process 99 test panels containing doubling dilutions of individual antibiotics. The system monitors each panel every 20 min using turbidimetric and colorimetric detection. The VITEK2 system uses compact plastic reagent cards that contain small quantities of antimicrobial agents and test media in a 64-well format and is based on turbidimetric monitoring of growth. The instrument can perform up to 240 simultaneous tests, and this number will increase when VITEK2 cards with a larger capacity become available. This example shows that a simple redesign of the disposables used in classic AST methods may already lead to improvements. The Sensititre ARIS 2X is an automated incubation and reading system with a 64-panel capacity. The test panels are standard 96-well microdilution plates that can be inoculated manually or automatically. Growth is determined by fluorescence measurement after 18–24 h of incubation.

These systems are linked to software used to interpret the AST results, including 'expert systems' for the detection of atypical patterns and unusual resistance phenotypes[26]. It remains important to be aware of the risk of overestimating susceptibility, as was recently emphasized for nontuberculous mycobacteria[27]. For more technical detail, additional reviews are available[21,28].

New automated AST systems. More recently, new entrants have penetrated the automated AST market. Two methods, both of which are real-time microscopy-based, are the most likely techniques to reduce turnaround and hands-on time and are close to launch or have been commercially launched. These technologies, developed by USA-based Accelerate Diagnostics[29,30] and Korean Quantamatrix[31,32], have undergone clinical evaluation, but whether current claims for rapid turnaround time (TAT) will translate into favourable patient outcomes has yet to be determined. Some of the tests developed by Accelerate Diagnostics have been cleared by the FDA[30]. Although the initial test array for new commercial systems is likely to be small, the use of various technologies may potentially lead to broad-based testing covering many drug–organism combinations. Still, none of the aforementioned technologies fulfils all the routine clinical needs for rapid AST. The major limitations of the methods described are test completeness, number of different offerings and cost.

Box 1 | **Quantitative versus qualitative antimicrobial susceptibility testing**

There is a need for quantitative susceptibility testing systems, and the question is how this relates to genomics[35]. With a diminishing number of fully active, broad-spectrum antimicrobial agents, it is becoming increasingly important to use the variability in phenotypic resistance levels detected by phenotypic tests. There is a difference between successful meropenem therapy for isolates with a minimal inhibitory concentration (MIC) of 16 mg/l and isolates with a MIC of 128 mg/l. Both are resistant isolates according to all available breakpoints, but many would consider it reasonable to try meropenem in the first but not the second case. Dosing would be adjusted, and meropenem would be given in combination with another agent, but differences in the expression of meropenem resistance markers are worth considering, as the number of effective antimicrobials available for resistant bacteria decreases. Such important phenotypic differences may not be reliably detectable using current molecular methods, as the relationship between molecular antimicrobial susceptibility testing systems, quantitative differences in gene copy numbers and the differential expression of genes has not yet been defined in much detail.

Improving phenotypic AST systems

Automating result interpretation for traditional phenotypic analyses. Recently, laboratory automation has become increasingly prominent, and automation is being eagerly adopted in the clinical–diagnostic setting. Information technology is an important aspect of all automation-related activities. With Total Laboratory Automation (TLA), instrumentation is utilized to barcode and inoculate culture media, streak culture plates and move the plates to specialized incubators with high-resolution cameras that are then used to image the culture plates during the incubation process[33]. The next phases of microbiology laboratory automation include enhancements such as automated disc dispensing and interpretation of disc zone sizes. With the consistent incubation temperature and atmosphere that is provided by the automation system, there is more rapid growth of microorganisms and recovery of fastidious organisms[34]. Thus, it is likely that disc diffusion testing will be able to be read and interpreted earlier, and thus the reference method of disc diffusion will likely be able to provide results more rapidly than current methods when used in the context of laboratory automation[23]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has already developed standardized rapid lecture protocol cultures for inhibition zones, permitting disc diffusion testing in 4, 6 and 8 h directly from positive blood.

There have been interesting developments in phenotypic methods (TABLE 1), such as the use of imaging and software tools to interrogate resistance levels. Still, these methods are rarely incorporated into routine laboratory use. Analytical methods have been developed to assist clinicians with the interpretation of AST data. AntibiogramJ is a user-friendly, free, open-source, platform-independent tool that uses images of disc diffusion tests for more reliable interpretation of the size of the inhibition zone[36]. The data are 87% concordant with those generated by an experienced microbiologist. Mobile phone photos can be used for data sharing and independent verification at a distance. There are many options for expanding this format, and successful application of mobile technology 'in the field' has already been achieved[37].

Emerging methods. Simple detection of microbial resistance is the mainstay of selective chromogenic culture media (FIG. 1). Resistant organisms can easily be recognized by their coloured colonies while overgrowth

Table 1 | **New offers in qualitative and quantitative AST systems**

Companies[a]	Technologies used	Approach and system	Country of origin	Status
Abacus Diagnostica	PCR platform; portfolio includes methicillin-resistant *Staphylococcus aureus* and *Clostridioides difficile*	Molecular; Genomera CDX	Finland	Developed in 2012
Affinity Biosensors	Microorganism mass measurement	Phenotypic; LifeScale AST	USA	Sold since 2017 but unavailable in the USA
Arc Bio	Identification and AST based on shotgun sequencing	Genomic; Galileo pathogen solution	USA	Launched in 2018
ARCDIA International Oy Ltd	mariAST measures species-specific bacterial growth in real time and combines in-well culture and high-accuracy detection	Phenotypic; mariPOC	Finland	In approval process
ARES Genetics	Development of AST database and currently developing an associated wet laboratory approach	Genomic; bioinformatics; GEAR database	Austria	Established in 2017
Ascenion GmBH	Combination of a BacLight viability staining with automated confocal laser scanning microscopy and detailed image analysis	Phenotypic; autofocus microscopy	Germany	Patent registered in 2010; the technology is offered for in-licensing or co-development of a screening platform
AUS Diagnostics	Multiplexed tandem PCR	Molecular; Mini- and UltraPlex	Australia	Sold since 2018, European conformity marking announced
BacterioScan	Laser light-scattering instrument	Phenotypic; 216 Dx urinary tract infection (UTI) system	USA	In 2018 the FDA issued a 510K premarket notification clearance for the 216Dx UTI detection system
BioFire	Multiplexed, syndrome-oriented PCR	Molecular; FilmArray	USA	Various tests cleared for sale by the FDA
Biotrack Diagnostics	Solid-state cytometry or fluorescence in situ hybridization and specific antibodies for fluorescent micro-agglutination are used for specific detection of molecules	Phenotypic; biochemical; AquaScope and AquaPrep	The Netherlands	Not known
Click Diagnostics	Cartridge-based, hand-held thermocyclers configured to move a fluid between distinct chambers and visually read colorimetric results	Molecular; company in stealth mode	USA	Not known
Dayzero Diagnostics	High-throughput bacterial DNA sequencing and proprietary machine-learning algorithms to rapidly predict pathogen species and drug resistance profiles	Genomic; bioinformatics; epiXact	USA	In development since March 2018
EliTech Group	Triplex PCR assay	Molecular; URIFAST	France	Sold since 2018
FASTinov	Cell sorting	Cellular detection, FACS	Portugal	Patent available since 2012, no products to date
Firebird	Molecularly targeted nucleic acid testing	Molecular; next-generation DNA sequencing	US	Not known
First Light Biosciences	Offers sensitive detection of diagnostic markers directly in complex sample matrices	Molecular; MultiPath platform	USA	Presented at the American Society for Microbiology conference in 2019
GeneCapture	Non-amplified RNA detection	Molecular; CAPTURE platform	USA	Early development; POC platform estimated date of launch, 2025
GeneFluidics	Molecular-based, PCR-less identification of species-specific phenotypic markers of resistance and susceptibility, as they are clinically relevant end products of many genetic pathways	Molecular; UTImax	USA	Sold for research use only since December 2017
GFC Diagnostics Ltd	DNA hybridization technology and have developed a rapid, POC test for detection of MRSA	Molecular; SafeTube IsoScreen	UK	Product announced in October 2017
Gradientech AB	Proprietary microfluidic technology solution to create stable substance gradients for AST of positive blood culture samples in 2 h	Phenotypic; QuickMIC and CellDirector	Sweden	Product estimated date of launch, 2023

Table 1 (cont.) | **New offers in qualitative and quantitative AST systems**

Companies[a]	Technologies used	Approach and system	Country of origin	Status
Klaris Diagnostics (Pattern)	Uses deep neural networks to recognize the unique 'biometric fingerprint' produced by different bacterial species encapsulated within microfluidic droplets	Phenotypic; bioinformatic; single-cell biometric analysis	US	Not known
MicrobeDx Inc	Hybridization and capture of target ribosomal RNA, thus leveraging its natural amplification and negating the need for PCR	Molecular; UMD SelectNA	Germany	Product estimated date of launch, 2024
Nanopore Diagnostics LLC	iNDxer, which is a nanopore sensor for counting dilute amounts of nucleic acid biomarkers (for example, DNA, RNA) directly in minimally processed samples	Molecular; iNDxer	USA	Product estimated date of launch, 2024
Nexogen Inc	Programmable enrichment and real-time selective sequencing method for the rapid diagnostics for AMR	Molecular; not defined	USA	Prototype phase
Next Gen Diagnostics	Overnight whole-genome sequencing with bioinformatics assessment	Genomic; bioinformatics; proprietary bio-info dashboard	UK and USA	Not known
Orbital diagnostics	Scattered light integrated collector	Phenotypic; scattered light integration collector	UK	Not known
Phase Genomics	High-throughput chromatin conformation proximity ligation technology	Molecular; next-generation DNA sequencing	USA	Proof of concept published in 2017; product launch currently not known
PhAST Diagnostics	Single-cell imaging	Phenotypic system	USA	Not known
QSM Diagnostics, Inc.	Quantitative electrochemical measurement of bacterial colonization levels	Phenotypic system	USA	Not known
Resistell	Nanomotion detection-based antibiotic susceptibility testing	Phenotypic; atomic force microscopy and cantilevers	Switzerland	Not known
Selux Diagnostics	Europium-cryptate-diamine chelate used to universally label the bacterial surface; cryptates and cryptands form 3D structures that function as ion cages	Phenotypic; biochemical; bacterial surface area chemistry, next-generation phenotyping	USA	Product estimated date of launch, 2023
Seraph Biosciences Inc.	Field portable ultra-high sensitive Raman system	Phenotypic; SeraSpec	USA	Not known
Specific Diagnostics LLC	Small molecule sensor array responds to metabolic by-products and detects volatile organic compounds	Biochemical; reveal system	USA	Product estimated date of launch, 2022
Spectromics	Spectrometric monitoring of phenotypic changes that occur in reactions between the sample and a panel of candidate antibiotics	Phenotypic; 10-min POC test at the general practitioner	UK	Not known
Spindiag GmBH	Ultrafast and highly sensitive PCR for drug-resistant pneumonia and sepsis	Molecular; SpindiagONE	Germany	Product estimated date of launch, 2024
Symcel Sverige AB	Label-free multichannel assay that measures the specific metabolic phenotype of cells and pathogens in real time	Phenotypic; calScreener	Sweden	Product estimated date of launch, end of 2020
Talis Biomedical Corp.	Combination of SlipChips, fluorescence and bright field real-time imaging that enables unlimited test formats in a compact platform	Phenotypic; LAMP technology	USA	Not known
TheoremDx Inc	Simultaneous protein and DNA and/or RNA assays; high-tech graphene chips combining all methods; cheap, reliable, cloud-based artificial intelligence	Proteomic; molecular; graphene rapid identification platform	USA	Product estimated date of launch, 2025

This is a highly dynamic area of research and development, with companies being launched and disappearing; hence, this table provides a snapshot rather than a comprehensive list. The companies listed are mostly small or medium sized, with a focus on the translation of academic proof-of-concept or proof-of-principle findings into clinical applications, including dedicated instruments and case-specific assays for as many organism–drug combinations as possible. The diversity shown in the table reflects the fact that the gold standard genomic technology (next-generation sequencing in combination with the bioinformatics pipeline) has certainly not been defined yet, and that it is quite unlikely that this gap will be resolved within the next 5 years. A brief description of efforts to develop genomic tests, often in combination with classic PCR-based methods, is provided. AMR, antimicrobial resistance; AST, antimicrobial susceptibility testing; FACS, fluorescence-activated cell sorting; POC, point of care. [a]Listed in alphabetical order.

of non-relevant and susceptible microbiota is suppressed[38]. To enhance the sensitivity of chromogenic media, fluorescent substrates can be added to the media to facilitate the detection of microcolonies after a short incubation[39]. In addition, methods that enable the identification of microscopic changes in cell morphology[40] have improved the detection of drug susceptibility, including cumbersome resistance traits such as, for example, polymyxin resistance[41]. These procedures can be directly implemented in microbiology laboratories, without the requirement of new equipment or increased workload. Recently, it was shown that surface labelling of bacteria substantially shortened the microscopic analysis of AST results in 96-well formats using only a standard microplate reader[42]. To improve the current systems, various prototype AST methods with different approaches have been reported, including the use of mobile phones[37], laser scatter technology[43], sensing of bacterial vibrations using phase-noise measurements on resonant piezo-electric substrates[44], protein-adsorbed magnetic nanoparticle-mediated protocols[45], field-effect enzymatic detection[46], microfluidics[47–49], glucose metabolization[50], optical screening and diffusometry[51], nanometre-scale bacterial deformation measurement[52], mass spectrometry[53], nanowire sensors[54], electrochemical sensing[55], real-time laser scattering[56], atomic force microscopy[57], fluorescence-activated cell sorting (FACS)[58], Raman and infrared spectroscopy at the single-cell level[59,60], nanotube-assisted microwave electroporation[61], hydrodynamic trapping[62] and video microscopy[63]. In this Review, we do not describe these methods in detail, due to the vast differences in the protocols. FIGURE 2 summarizes some of the newer test principles. It is noteworthy that these methods all require upscaling and transition from experimental to diagnostic laboratories. Furthermore, extensive clinical and outcome studies will be needed to prove their value in the clinical decision-making process.

One emerging method that is more advanced uses matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS), a technique that is already being used in clinical laboratories for bacterial identification. MALDI-TOF-MS is a relatively new technology, and implementation of the technique for the rapid identification of causative bacterial organisms has been shown to be effective. For positive blood cultures, MALDI-TOF-MS can quickly identify bacterial growth, which accelerates the overall process of AST reporting[64]. Note that the MS applications mentioned above do not change the AST strategy as such. Very recently, a microdroplet-based MALDI-TOF-MS approach that enables rapid AST was presented[65]. However, clinical trials that can demonstrate direct detection of resistance factors via MALDI-TOF-MS are still lacking[66].

Phenotypic AST for mixed bacterial populations. Some AST methods have been developed that can identify resistance in complex mixtures of bacterial species[67,68]. Recently it was shown that the dynamics of kanamycin resistance could be monitored using Raman spectroscopy in artificial mixtures of different microbial strains[69]. The fraction of cells that exhibit changes in their individual Raman spectrum increases with increasing antibiotic concentrations[59]. This type of AST, sometimes referred to as 'phenotypic resistomics', would potentially enable many resistance markers in complex microbial populations to be catalogued. Currently, few software programs can translate phenotypic databases into clinically actionable advice for clinicians, but these programs are being developed for clinical use[70].

Molecular AMR detection methods

Detection of protein markers of resistance. Not all molecular methods for the detection of resistance are nucleic-acid-based. Several AST methods that involve the direct detection of protein markers of resistance (such as β-lactamases) have been described — for example, those using specific protein arrays[71]. These AST methods often involve using antibodies to capture and enrich proteins. Either the antibodies or the proteins can be labelled with fluorescent tags for visualization. The interaction between the antibodies and resistance enzymes requires optimization, but several lateral flow assays (LFAs) have been developed for the detection of β-lactamases[72]. An LFA specific to New Delhi metallo-β-lactamase (NDM) 1 was 100% sensitive and specific in a collection of 350

Microbiological parameters

- One versus many cells
- One versus more species
- Heterogeneous AMR
- Cell permeability
- Metabolic status
- Rapid versus slow growth
- Induction of resistance
- Low-level resistance
- New resistance mechanism

Toolbox	Read-out	Suited for single cells
Microfluidics	Viability, growth	✓
Droplet test	Viability, growth	
Cytometry	Viability, growth	✓
Microscopy	Morphology	✓
Mass spectrometry	Spectral change	
Light scattering	Spectral change	
Electrochemistry	Conductivity	
Cantilevers	Viability, growth	✓
NMR	Spectral change	
Microsound	Movement	
Phages	Viability, growth	
Calorimetrics	Viability, growth	
Transcriptomics	Viability, growth	✓

Fig. 2 | **New phenotypic methods: microbial characteristics along with restricted survey and description of the mechanisms of new phenotypic methods to help overcome the limitations of current methods.** The box on the left defines the microbiological features that pose a challenge to classic antimicrobial susceptibility testing methods. The box on the right summarizes some of the new technologies, the phenotypic data types they generate and whether or not the tools are suited to the analysis of single cells. The latter question is of importance for the direct analysis of clinical specimens, where the number of bacteria is a limiting factor to classic technologies. AMR, antimicrobial resistance; NMR, nuclear magnetic resonance.

isolates from Myanmar[73]. Similar performance was established for a combined LFA that detected OXA-48, IMP, NDM and VIM enzymes[74]. Moreover, an LFA for mobilized colistin resistance 1 (MCR1) also exhibited 100% sensitivity and 98% specificity[75], and an LFA that targeted OXA-48 and/or OXA-163 was 100% sensitive and specific[76].

Whereas MALDI-TOF-MS enables overall protein profiling of microorganisms for their identification, more advanced methods can detect individual proteins by peptide mapping in the selected reaction-monitoring (SRM) mode[77]. Similar approaches also work for lipids[78]. In conclusion, methods have been developed that can efficiently detect and identify resistance-associated macromolecules. However, none of these methods is fully comprehensive, the test formats are technically diverse and the applications are usually very specific for a resistance mechanism.

Assessing AMR via multiplex gene detection. Various non-phenotypic methods have recently been reported, which include niche applications of nucleic acid probe array-mediated detection of specific resistance genes, for example[79], and PCR tests for similar targets[80]. Very recently, LAMP, an isothermal molecular amplification method, was used for the detection of AMR-defining genes or mutations, leading to detection of AMR in *Escherichia coli* directly from urine[81]. Molecular detection of resistance genes can also benefit from high-resolution melting analyses of amplicons after classic PCR. A recent study described a method that combines species identification and AST based on melting curves and machine learning[82]. Despite the technological innovation and rapidity of these methods, more data are needed to evaluate the value they add, and although the first data are being presented, formal publications of the results are still largely missing. The assays described above usually detect a single gene target, or at the most a few targets. These include the methicillin resistance markers *mecA* and *mecC*, the vancomycin resistance markers *vanA* and *vanB*, and the genes encoding extended spectrum β-lactamases (ESBLs). Many resistance gene-targeting tests, usually PCR-based, have entered the market. It would be beyond the scope of this Review to summarize all currently available tests, and the reader is referred to the existing literature[83]. Several of these diagnostic tests enable the multiplexed detection of various genes, including genetic polymorphisms, in a single rapid assay[84].

Molecular AST assays based on nucleic-acid-mediated amplification of specific resistance markers have the added the potential for fast TATs to hasten the administration of empiric patient treatment or to allow early adjustment of targeted therapy[85] (FIG. 3). Here we consider all those methods that detect resistance genes to be molecular AMR detection tests, and we note that these methods are essentially indirect surrogates for classic AST methods. The molecular methods currently available have minimal hands-on time, often use a 'sample-to-answer' approach and are rapid. One of the first systems on the market, the IDI-MRSA assay (Infectio Diagnostic), used in combination with the Smart Cycler II rapid DNA amplification system (Cepheid), was used for methicillin-resistant *Staphylococcus aureus* (MRSA) screening[86]. Aside from screening for MRSA in nasal swabs[87], tests for the most common vancomycin resistance markers, *vanA* and *vanB*[88], and a screening method for the carbapenem resistance genes bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA48} and bla_{IMP} in rectal swabs have been released[89], and more such tests will be released in the future. However, most of these assays were intended for surveillance rather than for guiding treatment in the setting of infection. The positive predictive value (PPV) and negative predictive value (NPV) of a specific test will vary depending on local resistance prevalence. New multiplex amplification assays have recently become available for 'syndromic infectious disease' testing, whereby, in addition to organism identification, the detection of resistance genes can be considered another advantage. The BioFire FilmArray BCID panel (TAT ≈ 1 h) identifies 24 common causes of bacteraemia and 3 resistance genes — *mecA, vanA/B* and bla_{KPC} — from blood cultures[90]. The Curetis Unyvero system can be used for the diagnosis of pneumonia, implant and tissue infection, blood culture infection and intra-abdominal infection, and includes more comprehensive resistance panels with 19, 17, 16 and 22 resistance markers per test, respectively[91,92]. The Nanosphere VERIGENE system (recently acquired by Luminex) is a blood culture identification system with two panels: Gram-positive bacteria and Gram-negative bacteria. This system also includes a few resistance markers (*mecA*, *vanA*, *vanB*, bla_{CTX-M}, bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{OXA} and bla_{VIM})[93]. DiagCORE by STAT-Dx (recently acquired by Qiagen and cleared by the FDA) is intended to quickly diagnose respiratory infections. The molecular approach to AMR detection is becoming more popular, given its rapidity and broad coverage. Molecular detection of AMR will go beyond PCR testing once isothermal nucleic amplification becomes more available and is shown to be cost-effective[94].

Implementing molecular AMR detection. The molecular assays described above are the first truly rapid diagnostic methods that compete with classic phenotypic AST methods. It is likely that one method could eventually combine proteomic, immunological and nucleic acid-mediated detection[95]. One of the most important barriers preventing universal acceptance of molecular tests as compared with growth-based methods is cost. In addition, the absence of a certain target resistance marker often does not correlate with phenotypic susceptibility. Current PCR tests are not able to monitor all resistance factors comprehensively for all bacterial species in a single test. There is also a need for expertise regarding the interpretation of molecular AMR detection and for an understanding that the detection of a resistance gene or mutation does not necessarily correlate with resistance. Also, most resistance marker assays cannot assign the marker to a specific organism in a polymicrobial sample. In addition, new mutations or non-characterized resistance mechanisms will remain elusive when researchers turn exclusively to molecular AMR detection. However,

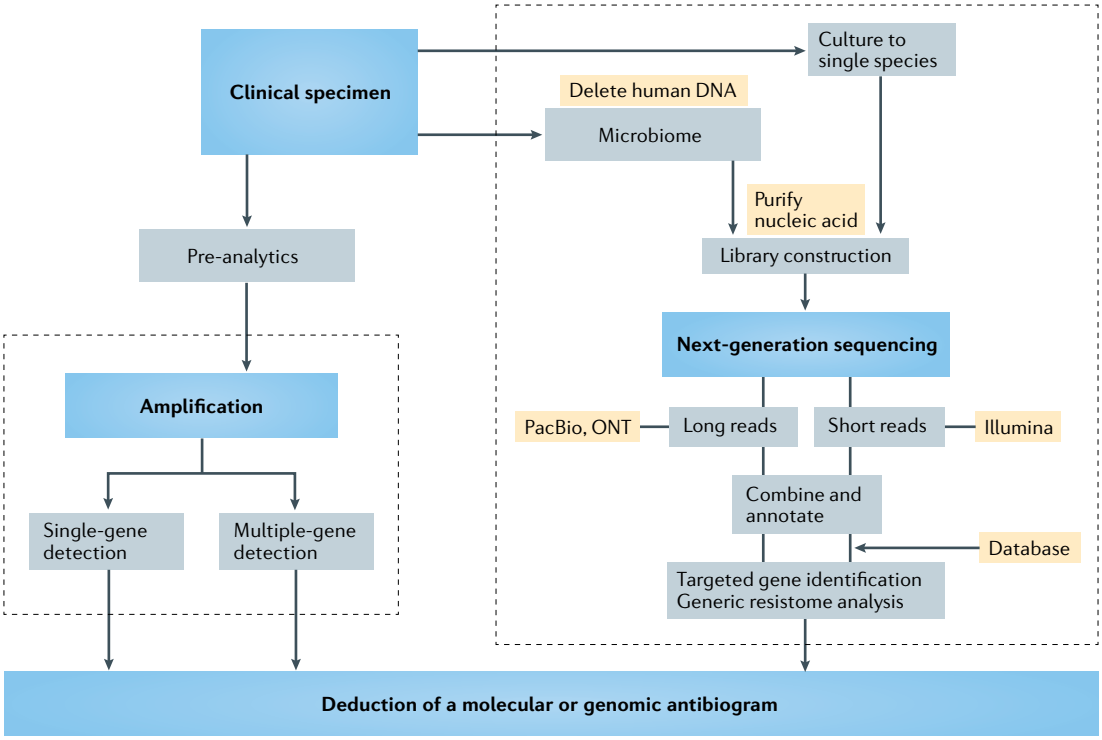

Fig. 3 | **Molecular antimicrobial susceptibility testing assays.** Concepts are shown that relate to new, mostly nucleic-acid amplification-based molecular and next-generation sequencing-based genomic methods for antibiotic susceptibility testing. The route from a clinical specimen to a diagnostic result is indicated by arrows. For the molecular test (left dashed box) this suggests a mostly three-step procedure: rapid sample preparation, amplification and amplicon characterization. The sequencing protocol (right dashed box) is more complicated, owing to the diversity of technologies available, their sequence output (long versus short reads) and the still non-standardized bioinformatic pipelines, new versions of which are continually being presented in this new diagnostic domain. ONT, Oxford Nanopore Technologies.

as experience with these tests grows, and as data are gathered on their efficacy and clinical impact, it is likely that they will be more widely adopted.

Broad-spectrum genomic AST

A trend towards the use of genomic rather than gene-based methods for the combined identification of bacterial species and antibiotic resistance is emerging in the diagnostic market. Applying whole-genome sequencing (WGS) enables essentially all genes involved in AMR to be traced. In principle, this would facilitate comprehensive genomic cataloguing of all resistance factors present in a given bacterial cell. This technique is not yet without pitfalls (see below). The switch towards WGS is being fuelled by the need for more accurate and rapid infection prevention measures[96]. In fact, once an organism has been isolated by culture, bacterial identification and antibiotic resistance gene detection via bioinformatic analysis (for example, using the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder) are surprisingly easy to perform. In many cases, these pipelines have the potential to be fully automated[97]. Still, currently there is no convincing evidence that WGS outperforms multiplex PCR, despite the limitation on the number of genes that can be screened by PCR.

The most impactful and timely diagnostic innovations developed recently have used 'omics' approaches[98], and the current third-generation or fourth-generation protocols and methods may soon be available to the diagnostic community, with the first clinically validated and FDA-approved genomic tests for cystic fibrosis already being used[99]. EUCAST has created a subcommittee on AST by WGS. The WGS strategy requires databases that include sequences for all resistance genes and resistance-associated mutations, as well as sequencing instruments that are currently being sold for less than US$20,000. When a complete bacterial genome is subsequently analysed for overlap with the entries in such databases, an isolate-specific 'resistome' can be mapped[100]. Clinical microbiologists should not use these software packages without understanding how gene sequence matching occurs. There is a danger in relying on default values and presuming that all antibiotic resistance genes within a sample will be found using these. A review of the software packages and databases that are available can be found in BOX 2.

Some studies have reported an approach that is partly species independent, is compatible with MIC determination[101] and details resistance traits against families of antimicrobials[102]. In addition to genome-based resistome analyses, RNA-mediated transcriptomic approaches have been described[103,104]. Deep sequencing of amplified DNA still adds distinct value in this field, as well[105]. The omics-oriented approach is promising, but its use for AST still needs validation[106].

Box 2 | **The importance of genomic databases**

Well-known resistance factor databases include ResistoMap (also suited to microbiome research[129]), the Canadian Antibiotic Resistance Database (CARD)[130], Antibiotic Resistance Gene Annotation (ARG-ANNOT)[131], ResFinder[132], GEAR-base[133], NanoARG[134] and others, most of which are proprietary and industry-owned and industry-curated. There is a need for target-specific databases; those describing overall heterogeneity among β-lactamases[135] and all antimicrobial resistance-associated mutations in *Mycobacterium tuberculosis* (MUBII-TB-DB)[136] are just two examples. A recent review described the close correlation between such databases and the intricacies of the sequencing strategies and methods used[137]. Each of these databases is continuously updated and curated, especially when new resistance markers or mechanisms are being discovered. The databases are combined with specific software packages to facilitate searches for resistance markers; these packages all have their specific advantages and disadvantages[138,139]. Finally, clinical validation studies have been performed for large collections of bacterial strains that belong to clinically relevant species. These species include, among many others, *M. tuberculosis*[18,140,141], *Staphylococcus aureus*[142], *Klebsiella pneumoniae*[143,144], *Neisseria gonorrhoeae*[35,145], *Escherichia coli*[146], *Shigella sonnei*[147] and *Pseudomonas aeruginosa*[148–150].

Note that the use of different bioinformatic pipelines in combination with different databases may result in different outcomes[107]. The biggest barriers to routine application of species-independent approaches are the need for cheaper sequencing platforms and the urgent need for user-friendly 'sample in, data out' bioinformatic solutions. One technology that is generating a lot of interest is nanopore sequencing (for example, Oxford Nanopore Technologies (ONT)). This technique is relatively easy to use, and the bioinformatic interface has progressed substantially[108]. The sequences generated by this technology are long, enable easier assembly of complete genomes and can identify plasmids and large-scale genomic re-arrangements. However, the accuracy of ONT sequencing is still lagging behind that of other sequencing technologies. New mathematical methods (for example, machine learning) may correct these deficiencies. It has already been shown that WGS substantially enhances the quality of genotype-to-phenotype transitions[109,110]. This will require continuous phenotypic and genotypic screening for new resistance mechanisms and markers, database maturation and the development of smart software and visualization tools. BOX 3 exemplifies how these methods can be used to identify resistance genes in complex mixtures of bacterial species.

The implementation of WGS in a clinical microbiology setting is being seriously considered[111–114] (TABLE 1). Its integration into routine use will require access to clinical sites for method evaluation, strain collection for the expansion of databases, increasingly mature next-generation sequencing platforms, reduced cost and studies demonstrating its impact on patient management.

Challenges for new AST methods

The major issues restricting the successful implementation of new AST methods are the need for substantial biomass, prolonged time to result, competition in the market and complexities of the developmental process. All current high-throughput AST methods require large microbial inocula. Practical approaches towards innovation that lower the inoculum density are rare. A means of reducing the input while maintaining the TAT has not yet been identified. What has surfaced over the past decade are methods that enable the concentration and purification of microbial cells directly from clinical specimens or positive blood cultures. Direct AST of cell pellets derived from urine samples has been shown to be successful[115]. A number of protocols for the identification of microorganisms from positive blood cultures have been reported, consisting of combinations of host cell lysis with the centrifugation and/or filtration of bacterial cells, and these methods have been shown to be useful for direct AST[116]. However, the ability to purify low numbers of microorganisms from large volumes of biological samples (bacteraemia) remains a challenge in diagnostic microbiology[117].

In addition, time and cost pose further challenges to the development and implementation of a new AST method. Fortunately, a pipeline is emerging for the new methods that have been described[118–120]. Typically, the innovation process involves a novel scientific discovery (that is, defining the methodology that will be used to distinguish living from dead bacterial cells) and a verification period to improve the technological aspects of the assay, usually by limiting trials to few bacterial species and one or two key antimicrobial

Box 3 | **Clinical metagenomics and resistance gene detection**

Clinical metagenomics is the study of genetic material recovered directly from clinical samples using whole-genome sequencing technologies[151]. This so-called deep sequencing (or shotgun sequencing) produces a diverse profile for detecting, characterizing and (semi-)quantifying all species present in a sample. This approach has revealed that new resistance genes are easily identifiable[152]. This implies that clinical metagenomics can be considered an additional and complete approach for the detection of all antimicrobial resistance marker genes in a single specimen[153,154]. This is frequently referred to as 'resistome analysis' or 'resistomics'.

Most data have been generated using faecal specimens, and studies on the recovery of the gastrointestinal microbiota after antibiotic treatment have been a productive area of research[155]. Faecal resistome analysis has helped quantify the dynamics of the microbiota before, during and after antibiotic treatment and has shown that the microbiota of young adults restores well after antibiotic use. The use of machine learning has already shown that in certain cases dynamic changes of the microbiota of the gastrointestinal tract in the presence of antibiotics can be predicted. Thus, metagenomics can help in the development of personalized antibiotic treatment regimens[156]. Generating complete inventories of resistance genes in clinical specimens will be helpful in defining which antibiotics will have no effect. Longitudinal follow-up studies of patients can lead to the early detection of emerging resistance during treatment and thus to changes in treatment protocols. However, one remaining issue is the accurate association of a resistance gene with a particular species (phasing).

agents (usually of the bactericidal variety). If the proof of concept is successful, a scientific publication ensues, and potential patents are secured. At this point, one big barrier is finding a corporate partner capable of developing the test and that is willing to invest substantial resources for further improvements. The decision of whether to invest is usually based on due diligence assessment, business development discussions and the perceived competitiveness of the new AST method[15]. It is relatively easy to get to the proof-of-principle stage, but a full-blown validation of new AST technology for all possible combinations of bacterial species and antibiotics is daunting and beyond the financial capabilities of start-up companies. Defining the microbial panel width and global robustness of a new test requires huge investments. Many new methods fail to mature, since getting validation projects financed without a guarantee of success is problematic[121].

When discussing the performance of various AST technologies, it is mandatory that a non-biased approach be developed. This is not an easy task, owing to the diversity of the reported protocols, of the antibiotics to be analysed and of the species of microorganisms to be evaluated. In this context, important criteria for comparing different methods include microbiological and diagnostic value (that is, resistance mechanism versus phenotypic testing), as well as TAT[15]. Current TATs range from 20 min, for rapid PCR-based gene profiling, to days, for cell-division-based assays and genome sequencing[122,123], although the TAT for genome sequencing is likely to become shorter in the future. A second comparative approach evaluates the commercial landscape, to provide a sense of how new diagnostics will fit in the market and what competition already exists in that space. This approach is limited, since it tends to magnify the shortcomings rather than the strengths of new technologies. These two approaches can be used in combination, which is probably more realistic for predicting success. There is a tendency to overestimate the capabilities of regulatory-approved methods and to undervalue the use of new technologies.

Concluding remarks

AST is constantly evolving, with many alternative methods being ready for implementation either now or in the near future[120]. In this context, it is important to spend less time on small, incremental improvements to existing technology, but rather to strive for substantial advances so that new tests with superior performance characteristics can be approved and marketed as soon as possible. This will require the community of AST developers, manufacturers and end-users to recognize and leave the limitations of traditional AST behind. For example, debate is still ongoing regarding the actual number of bacterial colonies that should be tested for phenotypic or genotypic resistance[124]. New AST technologies may identify all of the resistance phenotypes and/or genotypes present within a clinical sample, but without isolation and species identification, the significance of the identified resistance mechanisms may not be obvious or important[125]. Perhaps it can be concluded that many options for AST could be 'mixed and matched' in a clinical laboratory, depending on the diversity of the patient population or workflow, but it seems impossible to recommend which could be the best combination or combinations. It is unlikely that a single method will completely replace the existing automated high-throughput methods. It is more likely that new methods will be supplemental, for the time being. For instance, most new AST formats test positive blood cultures — which means that a method should be capable of identifying species or be closely coupled to classic or MALDI-TOF-MS-mediated identification. Obviously, the need to identify a clinical isolate at the species level slows the diagnostic process. Additionally, EUCAST, the Clinical and Laboratory Standards Institute (CLSI) and the FDA should be integral parts of the development process and should recommend clinical MIC breakpoints to be used in diagnostic laboratories[126]. New technologies will also require optimizations of both pre-analytics (clinical sample and strain handling) and post-analytic follow-up at the clinical level. Finally, patient health management and cost are of pivotal importance. Costly tests or tests for which the clinical and financial return on investment has not been made clear will fail to be implemented[127]. Bringing the issues surrounding AMR and AST to broader public attention is a must. This will require promoting a better understanding of antimicrobial usage (stewardship) and clear explanations to the general public about drug selection and the development of multidrug resistance from overuse of antimicrobial agents[128]. The One Health principle, which integrates microbiology, the environment and human and animal interactions in one continuum, should be used to explain the scope of the problems associated with AMR. Approaches that prioritize and maximize infection prevention and antibiotic stewardship should become the standard of care. Clearly, the research agenda for AMR and AST should include translational funding for bridging the current gap between established and new technologies and for speeding the application of new technologies following regulatory approval. In this context, it is of interest to mention the new EU regulation (Regulation (EU) 2017/746 of the European Parliament and Council, published 5 April 2017, on in vitro diagnostic medical devices, repealing Directive 98/79/EC and Commission Decision 2010/227/EU), which should facilitate the rapid implementation of ground-breaking diagnostic tools. The EU and the Innovative Medicines Initiative (IMI) also fund various projects aimed at improving routine diagnostics (for example, New Diagnostics for Infectious Diseases (ND4ID), the Viral and Bacterial Adhesion Training Network (ViBrANT), Combatting Bacterial Resistance in Europe (COMBACTE), Value Dx and other projects).

As we have outlined in this Review, several promising new AST systems and protocols are being developed and implemented in the clinical setting, and such improved AST methods will help manage AMR.

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1. Theuretzbacher, U. et al. Analysis of the clinical antibacterial and antituberculosis pipeline. *Lancet Infect. Dis.* **19**, e40–e50 (2019). **Excellent analysis of the state of the art in antibiotic discovery and codification, with shortcomings in development routes succinctly described**.
2. Bartlett, J. G., Gilbert, D. N. & Spellberg, B. Seven ways to preserve the miracle of antibiotics. *Clin. Infect. Dis.* **56**, 1445–1450 (2013).
3. Nathwani, D. et al. Value of hospital antimicrobial stewardship programs [ASPs]: a systematic review. *Antimicrob. Resist. Infect. Control* **8**, 35 (2019).
4. Okeke, I. N. et al. Diagnostics as essential tools for containing antibacterial resistance. *Drug Resist. Updat.* **14**, 95–106 (2011).
5. Kjeldgaard, J. S. Results from the MIC Survey 2017 (DTU Food, National Food Institute, Denmark, 2018).
6. Bertrand, R. L. Lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division. *J. Bacteriol.* **201**, e00697–18 (2019).
7. Sommer, M. O. A., Munck, C., Toft-Kehler, R. V. & Andersson, D. I. Prediction of antibiotic resistance: time for a new preclinical paradigm? *Nat. Rev. Microbiol.* **15**, 689–696 (2017).
8. Nicoloff, H., Hjort, K., Levin, B. R. & Andersson, D. I. The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification. *Nat. Microbiol.* **4**, 504–514 (2019).
9. Knopp, M. et al. De novo emergence of peptides that confer antibiotic resistance. *mBio* **10**, e00837–19 (2019).
10. Brauner, A., Fridman, O., Gefen, O. & Balaban, N. Q. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat. Rev. Microbiol.* **14**, 320–330 (2016).
11. Brauner, A., Shoresh, N., Fridman, O. & Balaban, N. Q. An experimental framework for quantifying bacterial tolerance. *Biophys. J.* **112**, 2664–2671 (2017).
12. Bakshi, S. et al. Nonperturbative imaging of nucleoid morphology in live bacterial cells during an antimicrobial peptide attack. *Appl. Environ. Microbiol.* **80**, 4977–4986 (2016).
13. Veloo, A. C. M., Baas, W. H., Haan, F. J., Coco, J. & Rossen, J. W. Prevalence of antimicrobial resistance genes in *Bacteroides* spp. and *Prevotella* spp. Dutch clinical isolates. *Clin. Microbiol. Infect.* **25**, S1198–S1743 (2019).
14. Veloo, A. C. M., Chlebowicz, M., Winter, H. L. J., Bathoorn, D. & Rossen, J. W. A. Three metronidazole-resistant *Prevotella bivia* strains harbour a mobile element, encoding a novel *nim* gene, *nimK*, and an efflux small MDR transporter. *J. Antimicrob. Chemother.* **73**, 2687–2690 (2018).
15. Van Belkum, A. et al. Developmental roadmap for antimicrobial susceptibility testing systems. *Nat. Rev. Microbiol.* **17**, 51–62 (2019).
16. Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O. & Piddock, L. J. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **13**, 42–51 (2015).
17. Mason, A. et al. Accuracy of different bioinformatics methods in detecting antibiotic resistance and virulence factors from *Staphylococcus aureus* whole-genome sequences. *J. Clin. Microbiol.* **56**, 1815–1817 (2018).
18. Heyckendorf, J. et al. What is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant tuberculosis. *Antimicrob. Agents Chemother.* **62**, e01550–17 (2018). **This report clearly describes the discrepancies between phenotypic and genotypic assessments of antibiotic susceptibility and suggests useful solutions to some of these discrepancies**.
19. Schumacher, A., Vranken, T., Malhotra, A., Arts, J. J. C. & Habibovic, P. In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models. *Eur. J. Clin. Microbiol. Infect. Dis.* **37**, 187–208 (2018).
20. Shin, D. J., Andini, N., Hsieh, K., Yang, S. & Wang, T. H. Emerging analytical techniques for rapid pathogen identification and susceptibility testing. *Annu. Rev. Anal. Chem.* **12**, 41–67 (2019).
21. Leonard, H., Colodner, R., Halachmi, S. & Segal, E. Recent advances in the race to design a rapid diagnostic test for antimicrobial resistance. *ACS Sens.* **3**, 2202–2217 (2018).
22. Idelevich, E. A. & Becker, K. How to accelerate antimicrobial susceptibility testing. *Clin. Microbiol. Infect.* **25**, 1347–1355 (2019).
23. Hombach, M. et al. Rapid detection of ESBL, carbapenemases, MRSA and other important resistance phenotypes within 6–8 h by automated disc diffusion antibiotic susceptibility testing. *J. Antimicrob. Chemother.* **72**, 3063–3069 (2017). **This study shows that simple add-ons to classic AST systems can still lead to substantial gains in turn-around time**.
24. Junkins, A. D. et al. BD Phoenix and Vitek 2 detection of *mecA*-mediated resistance in *Staphylococcus aureus* with cefoxitin. *J. Clin. Microbiol.* **47**, 2879–2882 (2009).
25. Isenberg, H. D., Reichler, A. & Wiseman, D. Prototype of a fully automated device for determination of bacterial antibiotic susceptibility in the clinical laboratory. *Appl. Microbiol.* **22**, 980–986 (1971).
26. Cantón, R. et al. Validation of the VITEK2 and the advance expert system with a collection of Enterobacteriaceae harboring extended spectrum or inhibitor resistant β-lactamases. *Diagn. Microbiol. Infect. Dis.* **41**, 65–70 (2001).
27. Rockland, M. et al. Implementation of semiautomated antimicrobial susceptibility interpretation hardware for nontuberculous mycobacteria may overestimate susceptibility. *J. Clin. Microbiol.* **57**, e01756–18 (2019).
28. Reller, L. B., Weinstein, M., Jorgensen, J. H. & Ferraro, M. J. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin. Infect. Dis.* **49**, 1749–1755 (2009).
29. Marschal, M. et al. 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 (2017).
30. Pancholi, P. et al. Multicenter evaluation of the accelerate phenotest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. *J. Clin. Microbiol.* **56**, e01329–17 (2018).
31. Wang, H. Y., Uh, Y., Kim, S., Shim, T. S. & Lee, H. Evaluation of the Quantamatrix Multiplexed Assay Platform system for simultaneous detection of *Mycobacterium tuberculosis* and the rifampicin resistance gene using culture-positive mycobacteria. *Int. J. Infect. Dis.* **61**, 107–113 (2017).
32. Kim, J. H. et al. Prospective evaluation of a rapid antimicrobial susceptibility test (QMAC-dRAST) for selecting optimal targeted antibiotics in positive blood culture. *J. Antimicrob. Chemother.* **74**, 2255–2260 (2019).
33. Bailey, A. L., Ledeboer, N. & Burnham, C. D. Clinical microbiology is growing up: the total laboratory automation revolution. *Clin. Chem.* **65**, 634–643 (2019).
34. Lainhart, W. & Burnham, C. A. Enhanced recovery of fastidious organisms from urine culture in the setting of total laboratory automation. *J. Clin. Microbiol.* **56**, e00546–18 (2018).
35. Eyre, D. W. et al. WGS to predict antibiotic MICs for *Neisseria gonorrhoeae*. *J. Antimicrob. Chemother.* **72**, 1937–1947 (2017).
36. Alonso, C. A. et al. Antibiogramj: a tool for analysing images from disk diffusion tests. *Comput. Methods Prog. Biomed.* **143**, 159–169 (2017).
37. Wood, C. S. et al. Taking connected mobile-health diagnostics of infectious diseases to the field. *Nature* **566**, 467–474 (2019).
38. Perry, J. D. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. *Clin. Microbiol. Rev.* **30**, 449–479 (2017). **Perry et al. provide an excellent overview of combined classic microbial identification and targeted AST in the format of the oldest (growth-based) type of diagnostics in clinical bacteriology**.
39. Park, M. J. et al. Performance of a novel fluorogenic chimeric analog for the detection of third-generation cephalosporin resistant bacteria. *J. Microbiol. Methods* **131**, 161–165 (2016).
40. Smith, K. P., Richmond, D. L., Brennan-Krohn, T., Elliott, H. L. & Kirby, J. E. Development of MAST: a microscopy-based antimicrobial susceptibility testing platform. *SLAS Technol.* **22**, 662–674 (2017).
41. Nordmann, P., Jayol, A. & Poirel, L. Rapid detection of polymyxin resistance in Enterobacteriaceae. *Emerg. Infect. Dis.* **22**, 1038–1043 (2016).
42. Flentie, K. et al. Microplate-based surface area assay for rapid phenotypic antibiotic susceptibility testing. *Sci. Rep.* **9**, 237 (2019).
43. Hayden, R. T. et al. Rapid antimicrobial susceptibility testing using forward laser light scatter technology. *J. Clin. Microbiol.* **54**, 2701–2706 (2016).
44. Johnson, W. L., France, D. C., Rentz, N. S., Cordell, W. T. & Walls, F. L. Sensing bacterial vibrations and early response to antibiotics with phase noise of a resonant crystal. *Sci. Rep.* **7**, 12138 (2017).
45. Cowger, T. A. et al. Protein-adsorbed magnetic-nanoparticle-mediated assay for rapid detection of bacterial antibiotic resistance. *Bioconjug. Chem.* **28**, 890–896 (2017).
46. Shi, X., Kadiyala, U., VanEpps, J. S. & Yau, S. T. Culture-free bacterial detection and identification from blood with rapid, phenotypic, antibiotic susceptibility testing. *Sci. Rep.* **8**, 3416 (2018).
47. Malmberg, C. et al. A novel microfluidic assay for rapid phenotypic antibiotic susceptibility testing of bacteria detected in clinical blood cultures. *PLoS One* **11**, e0167356 (2016).
48. Baltekin, Ö., Boucharin, A., Tano, E., Andersson, D. I. & Elf, J. Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging. *Proc. Natl Acad. Sci. USA* **114**, 9170–9175 (2017).
49. Li, H. et al. Adaptable microfluidic system for single-cell pathogen classification and antimicrobial susceptibility testing. *Proc. Natl Acad. Sci. USA* **116**, 201819569 (2019).
50. Kittel, M. et al. Rapid susceptibility testing of multi-drug resistant *Escherichia coli* and *Klebsiella* by glucose metabolization monitoring. *Clin. Chem. Lab. Med.* **57**, 1271–1279 (2019).
51. Chung, C. Y., Wang, J. C. & Chuang, H. S. Rapid bead-based antimicrobial susceptibility testing by optical diffusometry. *PLoS One* **11**, e0148864 (2016).
52. Iriya, R. et al. Real-time detection of antibiotic activity by measuring nanometer-scale bacterial deformation. *J. Biomed. Opt.* **22**, 1–9 (2017).
53. Sparbier, K., Schubert, S. & Kostrzewa, M. MBT-ASTRA: A suitable tool for fast antibiotic susceptibility testing? *Methods* **104**, 48–54 (2016).
54. Ibarlucea, B. et al. Nanowire sensors monitor bacterial growth kinetics and response to antibiotics. *Lab Chip* **17**, 4283–4293 (2017).
55. Kuss, S. et al. Versatile electrochemical sensing platform for bacteria. *Anal. Chem.* **91**, 4317–4322 (2019).
56. Idelevich, E. A. et al. Rapid phenotypic detection of microbial resistance in Gram-positive bacteria by a real-time laser scattering method. *Front. Microbiol.* **8**, 1064 (2017).
57. Stupar, P. et al. Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections. *Clin. Microbiol. Infect.* **23**, 400–405 (2017).
58. Fonseca, E. et al. Evaluation of rapid colistin susceptibility directly from positive blood cultures using a flow cytometry assay. *Int. J. Antimicrob. Agents* **54**, 820–823 (2019).
59. Novelli-Rousseau, A. et al. Culture-free antibiotic-susceptibility determination from single-bacterium Raman spectra. *Sci. Rep.* **8**, 3957 (2018).
60. Tannert, A., Grohs, R., Popp, J. & Neugebauer, U. Phenotypic antibiotic susceptibility testing of pathogenic bacteria using photonic readout methods: recent achievements and impact. *Appl. Microbiol. Biotechnol.* **103**, 549–566 (2019).
61. Gao, J. et al. Nanotube assisted microwave electroporation for single cell pathogen identification and antimicrobial susceptibility testing. *Nanomedicine* **17**, 246–253 (2019).
62. Pitruzzello, G. et al. Multiparameter antibiotic resistance detection based on hydrodynamic trapping of individual *E. coli*. *Lab Chip* **19**, 1417–1426 (2019).
63. Yu, H. et al. Phenotypic antimicrobial susceptibility testing with deep learning video microscopy. *Anal. Chem.* **90**, 6314–6322 (2018).
64. Machen, A., Drake, T. & Wang, Y. F. Same day identification and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method with MALDI-TOF VITEK mass spectrometry and the VITEK2 system. *PLoS One* **9**, e87870 (2014).
65. Correa-Martínez, C. L., Idelevich, E. A., Sparbier, K., Kostrzewa, M. & Becker, K. rapid detection of extended-spectrum β-lactamases (ESBL) and *AmpC* β-lactamases in Enterobacterales: development of a screening panel using the MALDI-TOF MS-based direct-on-target microdroplet growth assay. *Front. Microbiol.* **10**, 13 (2019).
66. Köck, R. et al. Implementation of short incubation MALDI-TOF MS identification from positive blood cultures in routine diagnostics and effects on empiric

antimicrobial therapy. *Antimicrob. Resist. Infect. Control* **6**, 12 (2017).
67. Ruppé, E. et al. Prediction of the intestinal resistome by a three-dimensional structure-based method. *Nat. Microbiol.* **4**, 112–123 (2019).
This study describes a novel software-mediated approach for resistomics, showing that resistance traits can be reliably defined without prior (selective) culture.
68. Hendriksen, R. S. et al. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat. Commun.* **10**, 1124 (2019).
This analysis of resistance markers found in a global collection of sewage specimens underscores the depth of environmental penetration of antibiotic resistance.
69. Jin, N., Paraskevaidi, M., Semple, K. T., Martin, F. L. & Zhang, D. Infrared spectroscopy coupled with a dispersion model for quantifying the real-time dynamics of kanamycin resistance in artificial microbiota. *Anal. Chem.* **89**, 9814–9821 (2017).
70. Fowler, P. W. et al. Robust prediction of resistance to trimethoprim in *Staphylococcus aureus*. *Cell. Chem. Biol.* **25**, 339–349 (2018).
71. Chen, B. A. et al. Detection of multidrug-resistance proteins with protein array chips [Chinese]. *Zhonghua Zhong Liu Za Zhi* **27**, 528–530 (2005).
72. Mertins, S. et al. Generation and selection of antibodies for a novel immunochromatographic lateral flow test to rapidly identify OXA-23-like-mediated carbapenem resistance in *Acinetobacter baumannii*. *J. Med. Microbiol.* **68**, 1021–1032 (2019).
73. Tada, T. et al. Assessment of a newly developed immunochromatographic assay for NDM-type metallo-β-lactamase producing Gram-negative pathogens in Myanmar. *BMC Infect. Dis.* **28**, 565 (2019).
74. Rösner, S. et al. Evaluation of a novel immunochromatographic lateral flow assay for rapid detection of OXA-48, NDM, KPC and VIM carbapenemases in multidrug-resistant Enterobacteriaceae. *J. Med. Microbiol.* **68**, 379–381 (2019).
75. Volland, H. et al. Development and multicentric validation of a lateral flow immunoassay for rapid detection of MCR-1-producing Enterobacteriaceae. *J. Clin. Microbiol.* **57**, e01454–18 (2019).
76. Pasteran, F. et al. Rapid identification of OXA-48 and OXA-163 subfamilies in carbapenem-resistant Gram-negative bacilli with a novel immunochromatographic lateral flow assay. *J. Clin. Microbiol.* **54**, 2832–2836 (2016).
77. Charretier, Y. et al. Rapid bacterial identification, resistance, virulence and type profiling using selected reaction monitoring mass spectrometry. *Sci. Rep.* **5**, 13944 (2015).
78. Liang, T. et al. Rapid microbial identification and antibiotic resistance detection by mass spectrometric analysis of membrane lipids. *Anal. Chem.* **91**, 1286–1294 (2019).
79. Schulze, H., Rubtsova, M. & Bachmann, T. T. in *Modern Techniques for Pathogen Detection* (eds Popp, J. & Bauer, M.) 113–220 (Wiley, 2015).
80. Liu, Y. Y. et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* **16**, 161–168 (2016).
81. Schoepp, N. G. et al. Rapid pathogen-specific phenotypic antibiotic susceptibility testing using digital LAMP quantification in clinical samples. *Sci. Transl Med.* **9**, eaal3693 (2017).
82. Athamanolap, P., Hsieh, K., Chen, L., Yang, S. & Wang, T. H. Integrated bacterial identification and antimicrobial susceptibility testing using PCR and high-resolution melt. *Anal. Chem.* **89**, 11529–11536 (2017).
83. Dunne, W. M. Jr, Jaillard, M., Rochas, O. & Van Belkum, A. Microbial genomics and antimicrobial susceptibility testing. *Expert Rev. Mol. Diagn.* **17**, 257–269 (2017).
84. Igarashi, Y. et al. Laboratory evaluation of the Anyplex™ II MTB/MDR and MTB/XDR tests based on multiplex real-time PCR and melting-temperature analysis to identify *Mycobacterium tuberculosis* and drug resistance. *Diagn. Microbiol. Infect. Dis.* **89**, 276–281 (2017).
85. Özenci, V. & Rossolini, G. M. Rapid microbial identification and antimicrobial susceptibility testing to drive better patient care: an evolving scenario. *J. Antimicrob. Chemother.* **74**, i2–i5 (2019).
86. Bishop, E. J. et al. Concurrent analysis of nose and groin swab specimens by the IDI-MRSA PCR assay is comparable to analysis by individual-specimen PCR and routine culture assays for detection of colonization by methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* **44**, 2904–2908 (2006).
87. Jacqmin, H., Schuermans, A., Desmet, S., Verhaegen, J. & Saegeman, V. Performance of three generations of Xpert MRSA in routine practice: approaching the aim? *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 1363–1365 (2017).
88. Holzknecht, B. J., Hansen, D. S., Nielsen, L., Kailow, A. & Jarløv, J. O. Screening for vancomycin-resistant enterococci with Xpert® *vanA/vanB*: diagnostic accuracy and impact on infection control decision making. *New Microbes New Infect.* **2**, 54–59 (2017).
89. Cortegiani, A. et al. Use of Cepheid Xpert Carba-R® for rapid detection of carbapenemase-producing bacteria in abdominal septic patients admitted to intensive care unit. *PLoS One* **11**, e0160643 (2016).
90. MacVane, S. H. & Nolte, F. S. Benefits of adding a rapid PCR-based blood culture identification panel to an established antimicrobial stewardship program. *J. Clin. Microbiol.* **54**, 2455–2463 (2016).
91. Gadsby, N. J. et al. Comparison of Unyvero P55 pneumonia cartridge, in-house PCR and culture for the identification of respiratory pathogens and antibiotic resistance in bronchoalveolar lavage fluids in the critical care setting. *Eur. J. Clin. Microbiol. Infect. Dis.* **38**, 1171–1178 (2019).
92. Hischebeth, G. T. et al. Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. *J. Microbiol. Methods* **121**, 27–32 (2016).
93. Felsenstein, S. et al. Impact of a rapid blood culture assay for Gram-positive identification and detection of resistance markers in a pediatric hospital. *Arch. Pathol. Lab. Med.* **140**, 267–275 (2016).
94. Zboromyrska, Y. et al. Rapid detection of β-lactamases directly from positive blood cultures using a loop-mediated isothermal amplification (LAMP)-based assay. *Int. J. Antimicrob. Agents* **46**, 355–356 (2015).
95. Schumacher, S. et al. Highly-integrated lab-on-chip system for point-of-care multiparameter analysis. *Lab Chip* **12**, 464–473 (2012).
96. Leopold, S. R., Goering, R. V., Witten, A., Harmsen, D. & Mellmann, A. Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. *J. Clin. Microbiol.* **52**, 2365–2370 (2014).
97. Khaledi, A. et al. Transcriptome profiling of antimicrobial resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **60**, 4722–4733 (2016).
98. Shendure, J. et al. DNA sequencing at 40: past, present and future. *Nature* **550**, 345–353 (2017).
99. Hughes, E. E. et al. Clinical sensitivity of Cystic Fibrosis mutation panels in a diverse population. *Hum. Mutat.* **37**, 201–208 (2016).
100. Su, M., Satola, S. W. & Read, T. D. Genome-based prediction of bacterial antibiotic resistance. *J. Clin. Microbiol.* **57**, e01405–e01418 (2019).
101. Nguyen, M. et al. Developing an in silico minimum inhibitory concentration panel test for *Klebsiella pneumoniae*. *Sci. Rep.* **8**, 421 (2018).
102. Bertrand, D. et al. Hybrid metagenomic assembly enables high-resolution analysis of resistance determinants and mobile elements in human microbiomes. *Nat. Biotechnol.* **37**, 937–944 (2019).
103. Khazaei, T., Barlow, J. T., Schoepp, N. G. & Ismagilov, R. F. RNA markers enable phenotypic test of antibiotic susceptibility in *Neisseria gonorrhoeae* after 10 minutes of ciprofloxacin exposure. *Sci. Rep.* **8**, 11606 (2018).
104. Bhattacharyya, R. P. et al. Simultaneous detection of genotype and phenotype enables rapid and accurate antibiotic susceptibility determination. *Nat. Med.* **25**, 1858–1864 (2019).
Bhattacharyya et al. have published one of the few studies in which phenotypic and genotypic analyses of the resistome are presented and in which the current gold standard methods are seriously challenged.
105. Guérillot, R. et al. Comprehensive antibiotic-linked mutation assessment by resistance mutation sequencing (RM-seq). *Genome Med.* **10**, 63 (2018).
106. Ellington, M. J. et al. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. *Clin. Microbiol. Infect.* **23**, 2–22 (2017).
107. Couto, N. et al. Critical steps in clinical shotgun metagenomics for the concomitant detection and typing of microbial pathogens. *Sci. Rep.* **8**, 13767 (2018).
108. Lemon, J. K., Khil, P. P., Frank, K. M. & Dekker, J. P. Rapid nanopore sequencing of plasmids and resistance gene detection in clinical isolates. *J. Clin. Microbiol.* **55**, 3530–3543 (2017).
109. Yang, Y. et al. Machine learning for classifying tuberculosis drug-resistance from DNA sequencing data. *Bioinformatics* **34**, 1666–1671 (2018).
110. Golparian, D. et al. Antimicrobial resistance prediction and phylogenetic analysis of *Neisseria gonorrhoeae* isolates using the Oxford Nanopore MinION sequencer. *Sci. Rep.* **8**, 17596 (2018).
111. Rossen, J. W. A., Friedrich, A. W. & Moran-Gilad, J. ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD): practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology. *Clin. Microbiol. Infect.* **24**, 355–360 (2018).
112. Westblade, L. F. et al. Role of clinicogenomics in infectious disease diagnostics and public health microbiology. *J. Clin. Microbiol.* **54**, 1686–1693 (2016).
113. Quainoo, S. et al. Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. *Clin. Microbiol. Rev.* **30**, 1015–1063 (2017).
114. Blauwkamp, T. A. et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nat. Microbiol.* **4**, 663–674 (2019).
115. Angaali, N., Vemu, L., Padmasri, C., Mamidi, N. & Teja, V. D. Direct identification and susceptibility testing of Gram-negative bacilli from turbid urine samples using VITEK2. *J. Lab. Physicians* **10**, 299–303 (2018).
116. Campigotto, A., Goneau, L. & Matukas, L. M. Direct identification and antimicrobial susceptibility testing of microorganisms from positive blood cultures following isolation by lysis-centrifugation. *Diagn. Microbiol. Infect. Dis.* **92**, 189–193 (2018).
117. Kang, J. H. et al. An extracorporeal blood-cleansing device for sepsis therapy. *Nat. Med.* **20**, 1211–1216 (2015).
118. Van Belkum, A. & Dunne, W. M. Jr. Next-generation antimicrobial susceptibility testing. *J. Clin. Microbiol.* **51**, 2018–2024 (2013).
119. Pulido, M. R., García-Quintanilla, M., Martín-Peña, R., Cisneros, J. M. & McConnell, M. J. Progress on the development of rapid methods for antimicrobial susceptibility testing. *J. Antimicrob. Chemother.* **68**, 2710–2717 (2013).
120. Behera, B. et al. Emerging technologies for antibiotic susceptibility testing. *Biosens. Bioelectron.* **142**, 111552 (2019).
This study provides excellent graphical illustrations of emerging technologies for AST.
121. Hays, J. P. et al. The successful uptake and sustainability of rapid infectious disease and antimicrobial resistance point-of-care testing requires a complex 'mix-and-match' implementation package. *Eur. J. Clin. Microbiol. Infect. Dis.* **38**, 1015–1022 (2019).
122. Warhurst, G. et al. Rapid detection of health-care-associated bloodstream infection in critical care using multipathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review. *Health Technol. Assess.* **19**, 1–142 (2015).
123. Van Belkum, A. & Rochas, O. Laboratory-based and point-of-care testing for MSSA/MRSA detection in the age of whole genome sequencing. *Front. Microbiol.* **9**, 1437 (2018).
124. Peretz, A., Pastukh, N. & Nitzan, O. Is one colony enough? *J. Clin. Microbiol.* **54**, 925 (2016).
Peretz et al. develop a critical assessment of a long-lasting practical problem with potentially substantial diagnostic and financial consequences.
125. Boulund, F. et al. Computational discovery and functional validation of novel fluoroquinolone resistance genes in public metagenomic data sets. *BMC Genomics* **18**, 682 (2017).
126. Humphries, R. M., Abbott, A. N. & Hindler, J. A. Understanding and addressing CLSI breakpoint revisions: a primer for clinical laboratories. *J. Clin. Microbiol.* **57**, e00203–e00219 (2019).
Breakpoints are subject to continuous discussion and evolution, with definitions being modified; the authors clearly describe the rationales for breakpoint management.

127. Roope, L. S. J. et al. The challenge of antimicrobial resistance: what economics can contribute. *Science* **364**, eaau4679 (2019).
128. Legenza, L. et al. Geographic mapping of *Escherichia coli* susceptibility to develop a novel clinical decision support tool. *Antimicrob. Agents Chemother.* **63**, e00048–19 (2019).
129. Yarygin, K. S. et al. ResistoMap — online visualization of human gut microbiota antibiotic resistome. *Bioinformatics* **33**, 2205–2206 (2017).
130. McArthur, A. G. et al. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* **57**, 3348–3357 (2013).
131. Gupta, S. K. et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob. Agents Chemother.* **58**, 212–220 (2014).
132. Kleinheinz, K. A., Joensen, K. G. & Larsen, M. V. Applying the ResFinder and VirulenceFinder web-services for easy identification of acquired antibiotic resistance and *E. coli* virulence genes in bacteriophage and prophage nucleotide sequences. *Bacteriophage* **4**, e27943 (2014).
133. Galata, V. et al. Integrating culture-based antibiotic resistance profiles with whole-genome sequencing data for 11,087 clinical isolates. *Genomics Proteomics Bioinformatics* **17**, 169–182 (2019).
134. Arango-Argoty, G. et al. DeepARG: a deep learning approach for predicting antibiotic resistance genes from metagenomic data. *Microbiome* **6**, 23 (2018).
135. Keshri, V. et al. An integrative database of β-lactamase enzymes: sequences, structures, functions, and phylogenetic trees. *Antimicrob. Agents Chemother.* **63**, e02319–18 (2019).
136. Flandrois, J. P., Lina, G. & Dumitrescu, O. MUBII-TB-DB: a database of mutations associated with antibiotic resistance in *Mycobacterium tuberculosis*. *BMC Bioinformatics* **15**, 107 (2014).
137. Boolchandani, M., D'Souza, A. W. & Dantas, G. Sequencing-based methods and resources to study antimicrobial resistance. *Nat. Rev. Genet.* **20**, 356–370 (2019).
138. Clausen, P. T., Zankari, E., Aarestrup, F. M. & Lund, O. Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data. *J. Antimicrob. Chemother.* **71**, 2484–2488 (2016).
This study underscores that bioinformatic pipelines are intrinsically different, with different systems sometimes predicting conflicting genotypes and hence phenotypes.
139. Xavier, B. B. et al. Consolidating and exploring antibiotic resistance gene data resources. *J. Clin. Microbiol.* **54**, 851–859 (2016).
140. The CRyPTIC Consortium and the 100,000 Genomes Project. Prediction of susceptibility to first-line tuberculosis drugs by DNA sequencing. *N. Engl. J. Med.* **379**, 1403–1415 (2018).
141. Gygli, S. M. et al. Whole-genome sequencing for drug resistance profile prediction in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **63**, e02175–18 (2019).
142. Fowler, P. W. et al. Automated detection of bacterial growth on 96-well plates for high-throughput drug susceptibility testing of *Mycobacterium tuberculosis*. *Microbiology* **164**, 1522–1530 (2018).
143. Holt, K. E. et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc. Natl Acad. Sci. USA* **112**, E3574–E3581 (2015).
144. Tamma, P. D. et al. Applying rapid whole-genome sequencing to predict phenotypic antimicrobial susceptibility testing results among carbapenem-resistant *Klebsiella pneumoniae* clinical isolates. *Antimicrob. Agents Chemother.* **63**, e01923–18 (2018).
145. Peng, J. P. et al. A whole-genome sequencing analysis of *Neisseria gonorrhoeae* isolates in China: an observational study. *EClinicalmedicine* **7**, P47–P54 (2019).
146. Do Nascimento, V. et al. Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of enteroaggregative *Escherichia coli* isolated from cases of diarrhoeal disease in England, 2015–16. *J. Antimicrob. Chemother.* **72**, 3288–3297 (2017).
147. Sadouki, Z. et al. Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of *Shigella sonnei* isolated from cases of diarrhoeal disease in England and Wales, 2015. *J. Antimicrob. Chemother.* **72**, 2496–2502 (2017).
148. Kos, V. N. et al. The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. *Antimicrob. Agents Chemother.* **59**, 427–436 (2015).
149. Van Belkum, A. et al. Phylogenetic distribution of CRISPR–Cas systems in antibiotic-resistant *Pseudomonas aeruginosa*. *MBio* **6**, e01796–15 (2015).
150. Jaillard, M. et al. Correlation between phenotypic antibiotic susceptibility and the resistome in *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents* **50**, 210–218 (2017).
151. Chiu, C. Y. & Miller, S. A. Clinical metagenomics. *Nat. Rev. Genet.* **20**, 341–355 (2019).
152. Asante, J. & Osei Sekyere, J. Understanding antimicrobial discovery and resistance from a metagenomic and metatranscriptomic perspective: advances and applications. *Environ. Microbiol. Rep.* **11**, 62–86 (2019).
153. Yan, Q. et al. Evaluation of the CosmosID bioinformatics platform for prosthetic joint-associated sonicate fluid shotgun metagenomic data analysis. *J. Clin. Microbiol.* **57**, e01182–18 (2019).
154. Lakin, S. M. et al. MEGARes: an antimicrobial resistance database for high throughput sequencing. *Nucleic Acids Res.* **45**, D574–D580 (2017).
155. Palleja, A. et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat. Microbiol.* **3**, 1255–1265 (2018).
156. Rahman, S. F., Olm, M. R., Morowitz, M. J. & Banfield, J. F. Machine learning leveraging genomes from metagenomes identifies influential antibiotic resistance genes in the infant gut microbiome. *mSystems* **3**, e00123–17 (2018).
157. Cangelosi, G. A. & Meschke, J. S. Dead or alive: molecular assessment of microbial viability. *Appl. Environ. Microbiol.* **80**, 5884–5891 (2014).
158. Smith, K. P. & Kirby, J. E. The inoculum effect in the era of multidrug resistance: minor differences in inoculum have dramatic effect on MIC determination. *Antimicrob. Agents Chemother.* **62**, e00433–18 (2018).
159. van Dorp, L. et al. Rapid phenotypic evolution in multidrug-resistant *Klebsiella pneumoniae* hospital outbreak strains. *Microb. Genom.* **5**, 263 (2019).

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Author contributions
A.v.B., O.R. and W.M.D. researched data for the article. A.v.B., C.-A.D.B., F.M., O.R. and W.M.D. wrote the article. A.v.B., C.-A.D.B., J.W.A.R., F.M. and W.M.D. substantially contributed to discussion of the content. A.v.B, C.-A.D.B., J.W.A.R. and W.M.D. reviewed and edited the manuscript before submission.

Competing interests
A.v.B., F.M. and O.R. are employees at bioMérieux, a company that designs, markets and sells antimicrobial susceptibility testing tools and systems. C.A.B. has received research support from bioMérieux, BioFire, Cepheid, Accelerate Diagnostics, Luminex, Bio-Rad Laboratories, Thermo Fisher and SeLux.

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