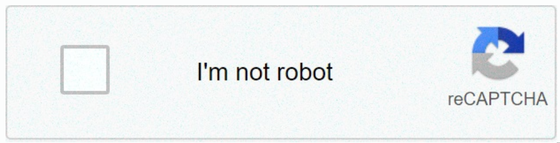
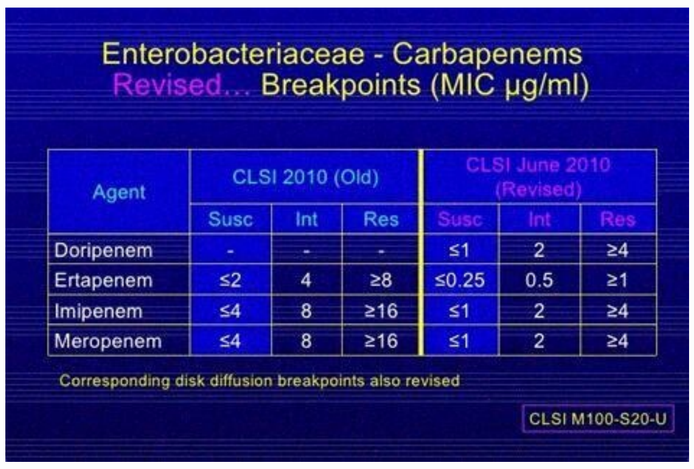


I am not robot!

Clsi guidelines for esbl detection. Antimicrobial susceptibility testing clsi guidelines.

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Köser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen Ø, Woodford N. Ellington MJ, et al. Clin Microbiol Infect. 2017 Jan;23(1):2-22. doi: 10.1016/j.cmi.2016.11.012. Epub 2016 Nov 23. Clin Microbiol Infect. 2017. PMID: 27890457 Review. Antimicrobial resistance (AMR) has emerged as a major threat to public health globally. Accurate and rapid detection of resistance to antimicrobial drugs, and subsequent appropriate antimicrobial treatment, combined with antimicrobial stewardship, are essential for controlling the emergence and spread of AMR. This article reviews common antimicrobial susceptibility testing (AST) methods and relevant issues concerning the advantages and disadvantages of each method. Although accurate, classic technologies used in clinical microbiology to profile antimicrobial susceptibility are time-consuming and relatively expensive. As a result, physicians often prescribe empirical antimicrobial therapies and broad-spectrum antibiotics. Although recently developed AST systems have shown advantages over traditional methods in terms of testing speed and the potential for providing a deeper insight into resistance mechanisms, extensive validation is required to translate these methodologies to clinical practice. With a continuous increase in antimicrobial resistance, additional efforts are needed to develop innovative, rapid, accurate, and portable diagnostic tools for AST. The wide implementation of novel devices would enable the identification of the optimal treatment approaches and the surveillance of antibiotic resistance in health, agriculture, and the environment, allowing monitoring and better tackling the emergence of AMR.Keywords: antimicrobial susceptibility testing, antimicrobial resistance, methodsAntimicrobial resistance (AMR) remains the world's most urgent public health concern [1]. According to the World Health Organization (WHO), Geneva, Switzerland, antibiotic resistance is rising to dangerously high levels in all parts of the world, leading to increased morbidity and mortality [2]. Hence, the six leading mortality-causing pathogens—Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa—were responsible for 929,000 deaths attributable to AMR and 3.57 million deaths associated with AMR in 2019 [3]. This number could rise to 10 million by 2050 according to estimates by the WHO [4]. Furthermore, the SARS-CoV-2 pandemic has exacerbated the existing global crisis of AMR, mostly due to the mis- and over-use of antibiotics, treatments that induce immunosuppression, and prolonged hospitalisation [5]. Besides, during the COVID-19 pandemic, limited ability to work with AMR partnerships, decreases in funding, and reduced availability of nursing, medical, and public health staff affected AMR surveillance, prevention, and control [6]. In addition, increased use of disinfectants, including hand sanitisers and surface cleaners, is anticipated to cause increased rates of antimicrobial resistance in pathogenic microbes in the coming years [7]. Replacement of first-line antibiotics by more expensive medications, a longer duration of illness, and treatment-related to AMR increases healthcare costs as well as the economic burden on patients and societies [2]. The World Bank estimates that drug-resistant infections could cause a global economic crisis, leading to 28 million people who could be pushed into extreme poverty every year by 2050, with an overall cost to the global economy of USD 1 trillion per year [8].Throughout their evolution, bacteria have developed versatile resistance mechanisms to antibiotics. The four main mechanisms of AMR are enzymatic inactivation of antimicrobial compounds, alteration of a drug target, reduced permeability of the outer membrane, and active drug efflux [9]. Hydrolases (e.g., beta-lactamases encoding by bla genes, such as extended-spectrum beta-lactamases, ESBL; cephalosporinases; and carbapenemases), passivation, and modified enzymes are three of the most important drug-inactivating enzymes. An altered target site is a major cause of Gram-positive bacteria's drug resistance (e.g., PBP2a in methicillin-resistant S.



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Antibiotic	Class of antibiotic	Susceptibility Profile (N=124)		
		Resistant N (%)	Intermediate/Decreased susceptibility N (%)	Susceptible N (%)
Penicillin	Penicillins	64 (52 %)	60 (48 %)	0
Tetracycline	Broad-spectrum	69 (56 %)	51 (41 %)	4 (3 %)
Ciprofloxacin	Fluoroquinolones	122 (98.4 %)	1 (0.8 %)	1 (0.8 %)
Spectinomycin	Aminoglycoside	0	0	124 (100 %)
Ceftriaxone	Extended spectrum cephalosporins	0	2 (1.6 %)	122 (98.4 %)
Cefixime	Extended spectrum cephalosporins	0	2 (1.6 %)	122 (98.4 %)
Azithromycin	Macrolides	6 (4.8 %)	0	118 (95.2 %)

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Administration of antibiotics to patients with suspected moderate to severe bacterial infections has been deemed inappropriate in at least half of the cases [11]. Antimicrobial stewardship (AMS) is one of the key strategies for combatting resistance. Implementation of such programs is therefore recommended across the globe [12]. The present review provides an updated overview of the various antimicrobial susceptibility testing (AST) methods that are currently used or potentially applicable in the foreseeable future, as well as their advantages and disadvantages.The choice of the best therapeutic option for the treatment of bacterial infections relies on the results of AST, a part of the routine work of all clinical microbiological laboratories.

Antimicrobial agent [a] [n° of strains]	CLSI susceptibility breakpoint (mg/L)	EUCAST susceptibility breakpoint (mg/L)	CLSI %S	EUCAST %S	Type of discrepancy [b]
Ampicillin-sulbactam (223)	≤2	≤1	98.2	92.4	minor
Amoxicillin-clavulanate (47,030)	≤4	≤2	99.7	98.4	minor
Cefaclor (28,338)	≤8	≤0.5	92.6	3.5	very major
Cefixime (7,403)	≤1	≤0.125	99.9	97.9	minor
Cefpodoxime (20,842)	≤2	≤0.25	99.9	96.7	minor
Cefuroxime axetil (94,671)	≤4	≤0.125	97.9	1.3	very major
Cefuroxime parenteral (94,671)	≤4	≤1	97.9	76.9	major
Cefotaxime (13,655)	≤2	≤0.125	99.6	99.7	-
Ceftriaxone (170)	≤2	≤0.125	100	96.5	minor
Cefepime (396)	≤2	≤0.25	100	91.7	minor
Ceftibuten (444)	≤2	≤1	98.4	97.1	minor
Meropenem non meningitis (6,511)	≤0.5	≤2	99.9	100	-
Imipenem (3,828)	≤4	≤2	98.9	97.4	minor
Ciprofloxacin (12,794)	≤1	≤0.5	99.7	99.6	-
Levofloxacin (22,880)	≤2	≤1	99.9	99.8	-
Moxifloxacin (14,177)	≤1	≤0.5	99.7	99.8	-
Ofloxacin (3,762)	≤2	≤0.5	100	99.9	-
Azithromycin (29,942)	≤4	≤0.125	99.3	1.2	very major
Clarithromycin (27,816)	≤8	≤1	82.9	1.5	very major
Erithromycin (5,382)	≤4	≤0.125	99.0	0.5	very major
Tetracycline (39,928)	≤2	≤1	97.7	96.8	-

‡ CLSI [11] and EUCAST [13].
[a] For Ampicillin, Chloramphenicol, Ertapenem, Rifampin and Trimethoprim/sulfamethoxazole CLSI and EUCAST suggested the same susceptibility breakpoints.
[b] Discrepancy as defined in the materials and methods section.

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Antimicrobial agent [a] (n° of strains)	CLSI susceptibility breakpoint (mg/L)	EUCAST susceptibility breakpoint (mg/L)	CLSI %S	EUCAST %S	Type of discrepancy [b]
Teicoplanin (56,399)	≤8	≤2	99.9	98.4	minor
Gentamicin (45,807)	≤4	≤1	93.6	89.1	minor
Amikacin (6446)	≤16	≤8	97.6	92.6	minor
Tobramycin (3155)	≤4	≤1	94.7	88.2	minor
Azithromycin (7223)	≤2	≤1	58.6	56.2	minor
Clarithromycin (7146)	≤2	≤1	58.8	58.5	-
Erytromycin (36,118)	≤0.5	≤2	74.8	77.4	minor
Tetracycline (1864)	≤4	≤1	74.6	74.3	-
Doxycycline (5037)	≤4	≤1	97.6	88.7	minor
Minocycline (1417)	≤4	≤0.5	99.4	96.9	minor
Clindamycin (25,879)	≤0.5	≤0.25	87.5	87.3	-
Trimethoprim (449)	≤8	≤2	91.5	89.1	minor
Rifampin (1154)	≤1	≤0.064	96.4	95.0	minor

§ CLSI [11] and EUCAST [13].
[a] For Penicillin, Oxacillin, Vancomycin, Daptomycin, Ciprofloxacin, Levofloxacin, Ofloxacin, Moxifloxacin, Chloramphenicol, Trimethoprim-sulfamethoxazole, Quinupristin-dalfopristin and Linezolid CLSI and EUCAST suggested the same susceptibility breakpoints.
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Antibiotic	Disk content	Spectrum of activity	Zone diameter breakpoints nearest whole mm		
			S	I	R
Ciproflox	10 µg	Broad spectrum	≥21	16 - 20	≤15
Gentamicin	10 µg	Broad spectrum	≥15	13–14	≤12
Streptomycin	30 µg	Broad spectrum	≥15	12–14	≤11
Levofloxacin	20 µg	Gram-negative bacteria	-	-	-
Norfloxacin	10 µg	Gram-negative bacteria	≥17	13–16	≤12
Rifampicin	20 µg	Gram-negative bacteria	≥20	17–19	≤16
Erythromycin	30 µg	Gram-negative bacteria	≥23	14–22	≤13
Chloramphenicol	30 µg	Gram-negative bacteria	≥18	13–17	≤12
Ampicillin	30 µg	Gram-negative bacteria	≥17	14–16	≤13

[5]: Zone diameter of inhibition
Key: S: Susceptible, I: Intermediate, R: Resistant

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Evaluation of the effectiveness of prevention and infection control measures relies as well on the results of AST, e.g., monitoring of resistant pathogens such as MRSA (methicillin-resistant Staphylococcus aureus), VRE (vancomycin-resistant enterococci), extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacterales, carbapenem-resistant Acinetobacter baumannii (CRAB), carbapenem-resistant Pseudomonas aeruginosa (CRPA), colistin-resistant bacteria, etc. [15].
Finally, surveillance of antimicrobial resistance is based on routine clinical antimicrobial susceptibility data from microbiological laboratories.
Numerous AMR surveillance systems exist, of which the WHOs Global Antimicrobial Resistance and Use Surveillance System (GLASS), European Antimicrobial Resistance Surveillance Network (EARS-Net), and Antibiotic Resistance Laboratory Network (AR Lab Network) of the Centers for Disease Control and Prevention are the most recognizable networks of national surveillance systems providing information on the actual burden of resistance at the international level. Policymakers and health administrators revise the recommendations for empirical treatment for community or hospital-acquired infections according to the local, national, and international AMR data. In addition, prevention and infection control measures are implemented based on the same data as a part of AMS programs [16,17].
Likewise, continuous monitoring provides early warnings of emerging threats and identifies long-term resistance trends.Although resistance surveillance at the national and international levels is of great benefit to public health, knowledge of the local resistance rates is of even greater practical importance to physicians. An antibiogram represents a convenient and widely available measurement of an institution's pathogens and susceptibilities [18]. Therefore, it is increasingly suggested that there is the necessity to create local (hospital or institutional) antibiograms specific for each hospital and even ward, annually. This principle applies especially to certain hospital departments where resistance rates are high, such as intensive care units. Additionally, this is particularly relevant for secondary and tertiary hospitals that treat chronically ill patients who have already received multiple antibiotic courses and thus increase antimicrobial selective pressure. Klinker et al. provide the rationale for why hospital AMS programs should implement alternative antibiograms, including combination and syndromic antibiograms, in addition to traditional antibiograms [18]. A combination antibiogram is used to determine in vitro rates of susceptibility to potential antibacterial combination regimens consisting of a first-choice antibiotic plus alternatives. A syndromic antibiogram displays the likelihood of adequate coverage for a specific infection syndrome, considering the weighted incidence of pathogens causing that syndrome. It was developed by Hebert et al. [19] as a weighted-incidence syndromic combination antibiogram. While combination antibiograms are useful in determining combined empiric antibiotic regimens for multidrug-resistant pathogens [20], syndromic antibiograms provide effective antibiotic therapy for a specific infectious syndrome, such as hospital- and ventilator-associated pneumonia [21]. The Clinical and Laboratory Standards Institute (CLSI) has developed guidelines (M39-A4) [22] to provide a standardised template for the preparation of institutional antibiograms. In a retrospective study by Puzniak et al. [23], the utility of combination antibiograms in identifying optimal anti-P. aeruginosa drug regimens in US hospitals was evaluated. They found that adding an aminoglycoside to backbone antibiotic, such as extended-spectrum cephalosporin, carbapenem, or piperacillin-tazobactam, resulted in higher susceptibility rates than adding a fluoroquinolone. They concluded that local institutional use of combination antibiograms ensures optimisation and timely administration of appropriate empiric therapy of infections caused by difficult-to-treat pathogens.Clinical laboratories currently employ several AST methods depending on the equipment and laboratory test menu that they provide. Conventional AST based on phenotypic testing examines the bacterial response in the presence of an antimicrobial agent. Classical culture-dependent methods (e.g., a disk diffusion test, gradient diffusion method) are firmly established in the diagnostic routine, and their main limitation is that the results are obtained for most clinically important bacteria within at least 18–24 h or 48 h, including prior bacterial isolation and identification. The turnaround time is prolonged for anaerobes or some slow-growing fastidious bacteria such as the HACEK group (Haemophilus species, Aggregatibacter species, Cardiobacterium hominis, Eikenella corrodens, and Kingella species), Brucella spp.
etc. [24].
For many years, clinical laboratories have been equipped with automated systems based on microdilution trays to provide faster results (6–24 h after initial isolation). However, the time required to obtain the results is similar in comparison with the broth microdilution (BMD) method [25]. Molecular AST is based on the detection of resistance determinants in bacterial isolates or directly in clinical specimens by molecular methods with a turnaround time of approximately 1–6 h [26]. Besides high costs, major drawbacks of molecular methods are detection of the resistance genes targeted only by the known probes and overestimating resistance because the resistance gene is not necessarily associated with the expression of a resistance phenotype.
Because of a significant rise in multi- and pan-drug-resistant infections, there is an urgent need for a more rapid and reliable test to improve infection diagnosis and support evidence-based antimicrobial prescribing [27]. The currently used methods for AST are summarised in Figure 1.Clinical microbiology laboratories are under increasing pressure to provide fast and reliable microbial identification (ID) and AST [86]. Automated and semi-automated devices for bacterial ID and AST are worthy of the task and have significantly improved laboratory efficiency.
Nowadays, automation has been successfully implemented in most clinical microbiological laboratories to reduce turnaround times, increase efficiency, and improve cost-effectiveness [86,87]. Various test systems—such as the VITEK 2 (bioMérieux, France), MicroScan Walkaway (Dade-Behring MicroScan, Deerfield, IL, USA), and Phoenix system (BD Diagnostic Systems, Baltimore, MD, USA)—have been widely used over the last decades. These instruments, using optical systems for measuring subtle changes, determine bacterial growth and antimicrobial susceptibility [88] and can produce results in a shorter time (6–12 h) than conventional manual assessment [36].VITEK 2 Systems—The first generation of VITEK system with a turnaround time of 13 h was developed for enumeration and identification of bacteria and yeasts in 1973. The VITEK 2 System, the next-generation of an instrument, is a BMD-based AST system that uses 64-well plastic cards containing 17–20 antimicrobial agents. If the bacterial isolate is not previously identified, one card is used for bacterial identification (ID card) and the other for antimicrobial susceptibility testing (AST card). Two Vitek 2 instruments are available with test card (ID and AST) capacities of 60 cards (Vitek 2) and 120 cards (Vitek 2 XL). Results are reported in 4–18 h, containing MIC and category of susceptibility, whereas the detection of AMR is facilitated by the Advanced Expert System (AES). The currently available Vitek 2 Compact instruments can use 15, 30, and 60 cards. The main advantage of the Vitek 2 system with computer software is the determination of susceptibility of clinically important resistant pathogens, such as Staphylococcus aureus and Enterococcus faecalis, to an additional four to ten antibiotics [86,89,90].Phoenix System—The Phoenix System is widely accepted and used in clinical microbiology laboratories for identification testing (ID) and antimicrobial susceptibility testing (AST). The principle of determining the susceptibility is based on the use of an oxidation-reduction indicator (resazurin dye or Alamar blue) and the detection of bacterial growth in the presence of various concentrations of the antimicrobial agent. In the Phoenix instrument, a maximum of 100 tests can be performed by using Phoenix ID/AST combination panels (51 for ID and 85 for AST). The instrument performs automatic reading at 20 min intervals during incubation for up to 18 h and provides accurate and rapid susceptibility results with easy workflow for the laboratory worker. In 2014, the new panel for susceptibility of Gram-negative bacteria was introduced for the Phoenix system to be used in combination with the BD Bruker MALDI-TOF [91].MicroScan WalkAway plus System—The MicroScan WalkAway plus System provides accurate and rapid identification and susceptibility results for a wide range of Gram-positive and Gram-negative aerobic bacteria. The instrument utilises three types of panel configurations: combo panels, breakpoint combo panels, and MIC panels. There are two types of system: 40- and 96-panel capacity models.
The panels are manually inoculated, rehydrated by the RENOK inoculator, and read automatically. The results are obtained after 4.5–18 h by reading of rapid panels [91].MicroScan AutoScan 4—The AutoScan 4 is a semiautomated instrument mostly used in smaller laboratories or for the testing of supplemental antimicrobial agents. The instrument provides simplified ID/AST testing in a highly reliable and affordable package. The system uses the off-line incubation of the conventional MicroScan AST panels.
The panels are manually inoculated or with the MicroScan Renok instrument and read automatically [91].MicroScan WalkAway System—The first generation of the MicroScan WalkAway System available on the market is the AutoSCAN-3. The new versions of instruments Auto-ACAN-4 and AutoSCAN-WalkAway are improved and use dry panels that do not need refrigeration.
The AutoSCAN-WalkAway system detects bacterial enzymatic activity and can process 96 panels at the same [86].Each of the above-mentioned systems has inherent advantages and limitations, and the results vary widely by antimicrobial drugs, software versions, and cards used. Hence, some of the systems are not reliable for correct categorisation of susceptibility profiles for certain drugs, leading to wrong classifications of susceptibility categories [92]. It seems that low inoculum size has a major influence on the outcome of these systems, with false susceptibilities being reported. Additionally, software updates and synchronisation of breakpoints according to the current standards are mandatory. Thus, it is incumbent upon the instrument manufacturer to keep pace with the breakpoint updates and make relevant improvements, such as extending the detection limit and verifying the performance of the AST system with the revised breakpoints internally, to avoid the problem of uncategorised results [93]. Panels usually contain only several concentrations of each antimicrobial agent, and the resulting MIC is not always given as an exact value.
In contrast, classical BMD contains a wide range of doubling dilution antimicrobial concentrations for the determination of the MIC, thus obtaining the more precise value. In addition, according to the previously published reports, many of the resistance phenotypes are not easily detected using the automated susceptibility testing methods so prevalent in today's clinical laboratories [94]. Nonetheless, interestingly, the ability of automated systems to detect inducible resistance to clindamycin in 524 isolates of Staphylococcus spp. revealed sensitivity and specificity of 100% and 99.6%, respectively, for Phoenix, and 91.1% and 99.8%, respectively, for Vitek 2 [95]. The multicentre evaluation showed that categorical agreement between the Phoenix system and a BMD reference method for 2013 streptococcal isolates including Streptococcus pneumoniae, viridans group streptococci, and beta-haemolytic Streptococcus groups A, B, C, and G ranged from 92% to 100%, with one exception for viridans streptococci and penicillin, which was 87% [96].
However, according to the results of the evaluation of ASTs obtained using Vitek 2, Phoenix, and MicroScan, caution should be taken for AST of Stenotrophomonas maltophilia, as a high rate of errors may be observed [97].Molecular AST directly detects specific resistance genes, as well as mutations in and expression of these genes. These molecular methods have been developed and tested as an alternative for or complementary to conventional AST and are generally faster than classic culture-based assays, with the test results available within one to a few hours [98] (Figure 3). Most of the molecular AST methods fall into one of the three categories: amplification-based, hybridization-based, or sequence-based. In amplification-based methods, the target gene sequence is amplified to allow detection; in hybridization-based techniques, hybridized nucleic acid probes target gene sequences allowing detection; and in sequence-based approaches, genome sequences are analysed to detect resistance-conferring mutations or resistance genes.The most widely used nucleic acid amplification-based method for the detection of specific resistance genes is polymerase chain reaction (PCR). Both real-time and conventional PCR rely on the amplification of nucleic acid sequences that encode resistance to an antibiotic. New PCR-based methods are being developed for the detection of genetic determinants of resistance to a variety of antibiotics for various bacterial species, as our knowledge about the genetic basis of antibiotic resistance increases [99]. Multiplex assays for simultaneous testing of multiple genetic determinants in various bacterial species have also been developed, i.e., multiplex assays for identifying numerous cephalosporinase- and carbapenemase-encoding genes, such as blaKPC, blaNDM, blaIMP, blaVIM, blaAmpC, blaTEM, blaSHV, and blaOXA, or mecA gene-encoding methicillin resistance in MRSA [100,101]. OpGen, Inc. (Rockville, MD, USA) has recently released the multiplex-based Acuitas® AMR Gene Panel that detects 28 genetic AMR markers, covering select drugs in nine classes of antibiotics, from 26 different pathogens [102]. The advantage of this test in comparison with other commercially available molecular tests is that it also detects non-beta-lactam resistance genes and those for what would be considered "last-resort antibiotics", such as colistin.Real-time PCR (quantitative PCR, qPCR) is one of the most ubiquitous methods found throughout clinical microbiology. Although costlier, qPCR offers several advantages over conventional PCR, including the measurement of data in real-time, greater sensitivity, reduced risk of carryover contamination, and greater amenity to multiplexing. Further advantages are that many systems are partially or even completely automated, such as GeneXpert® Instrument Systems (Cepheid Corp., Sunnyvale, CA, USA) and BD MAX System platform (Becton Dickinson, Franklin Lakes, NJ, USA), which are easily operated and can be used for the detection of carbapenemases, ESBLs, MRSA, VRE, etc. [103,104,105]. The downside is that they are limited to using test assays only from specific manufacturers, with GeneXpert® Instrument Systems requiring GeneXpert assays (Cepheid Corp., Sunnyvale, CA, USA) and BD MAX System using Check-Points® qPCR assays (Wageningen, The Netherlands). Tests based on qPCR can also be used for phenotypic differentiation of resistant and susceptible strains due to its ability to measure genome copy numbers during bacterial growth in the presence of antibiotics [106]. The major disadvantage is that the system cannot provide information about the mechanism of resistance and that it requires the previous culture, meaning that the primary clinical samples cannot be used.DNA-microarrays are used to identify the presence of specific nucleic acid sequences using complementary short oligonucleotides immobilised on a solid surface. Since these oligonucleotides can be assembled onto solid surfaces in close proximity, this method could detect numerous sequences in a single assay, which would allow simultaneous, in parallel detection of different pathogens and detection of vast numbers of different resistance genes, as well as detecting numerous distinct mechanisms of resistance or variants of a single mechanism present in bacterial isolates, as opposed to PCR-based approaches [107]. The Verigene system (Luminex Corporation, Austin, TX, USA) has developed Blood Culture Multiplex Microarray-Based Molecular assays for rapid diagnostics of 12 Gram-positive and 9 Gram-negative bacteria, along with their associated resistance genes (i.e., mecA, vanA, vanB, blaKPC, blaNDM, blaIMP, and blaVIM) [108]. In addition to qPCR-based assays, Check-Points® has also developed the CHECK-MDR CT103 DNA microarray for the detection of the clinically most prevalent ESBLs and carbapenemases, as well as mobile colistin resistance (mcr) genes in Gram-negative bacteria [109]. Both of these microarray tests have shown high sensitivity (94–100%) and specificity (94–98%), and CHECK-MDR CT103 DNA microarray also showed the ability to discriminate between carbapenemase and ESBL variants of GES-type beta-lactamase [108,109].The advantages of currently available molecular-based methods are that they are direct, rapid, highly sensitive, and specific, thus potentially allowing the earlier administration of targeted therapy [98].
Furthermore, for some methods, direct clinical samples can be used. However, it should be noted that the presence of a resistance marker does not always have to correlate with phenotypic resistance. Additionally, the extent and intensity of gene expression are important parameters, as some genes need different expression levels to produce resistance. A potential solution to this issue would be the use of reverse transcription qPCR, which relies on the measurement of gene transcripts (RNA levels) instead of the presence of a gene [110,111]. Another drawback is that these methods can only detect resistances that are searched for, and not novel or uncharacterised mechanisms of resistance, which could lead to false-negative results and inappropriate classification of resistant isolates as susceptible. A final consideration is that these methods are not capable of defining MIC values. As such, these methods have to be validated against phenotypic data to be useful, and extensive resistance marker databases and innovative bioinformatics methodologies are mandatory requirements. Nevertheless, molecular-based AST methods are a safe, efficient, and reliable screening tool in clinical settings. As experience with these tests grows, and as data are gathered on their efficacy and clinical impact, they will likely be more widely adopted.As DNA sequencing technology and bioinformatics pipelines for genome assembly and analysis advance, the possibility of using these techniques for the detection of antibiotic resistance opens. Applying whole-genome sequencing (WGS) would essentially enable the detection of all genes involved in AMR, which would help make comprehensive databases of all species-specific resistance factors (i.e., CARD-Comprehensive Antibiotic Resistance Database— , ResFinder— (accessed on 27 February 2022)) and make in silico AMR detection possible. Recent studies showed high concordance between the resistance profiles obtained using WGS and those obtained using phenotypic susceptibility testing, demonstrating that data obtained from genome sequences can correlate well with phenotypic resistance in some cases [112,113]. In addition to genome-based resistome analyses, RNA-mediated transcriptomic approaches have also been described [114,115]. Despite all of the advantages, WGS is not routinely performed in clinical practice. Considering the turnaround times of WGS, the existence of unknown resistance mechanisms, and the elevated cost compared with traditional and emerging techniques, the use of WGS for AST is not yet part of routine practice in clinical microbiology [116,117].Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was discovered in the 1980s and introduced into the microbiological routine as an effective tool for bacterial and yeast identification about 15 years ago. It has been applied to classify the specific bacterial protein contents and their matching protein biomarkers because of its rapid turnaround time, low sample volume requirements, and per-sample costs [118]. Several MALDI-TOF MS-based methods have been proposed for rapid detection of antimicrobial resistance, including monitoring antibiotic modification by bacterial culture (e.g., beta-lactam hydrolysis [65,119]), acetylation of fluoroquinolones [120], direct detection of proteins involved in specific resistance mechanisms [121,122], and detection of stable isotope labelling that requires expensive, isotopically labelled media [123,124]. The hydrolysis of the target beta-lactam antibiotic, as shown by peak disappearance, is used to detect beta-lactamase-producing bacteria using MALDI-TOF MS. As a result, the assay for detecting carbapenemase production [125] automatically determines sensitivity or resistance depending on the degree of antibiotic hydrolysis. The method had 98% sensitivity and 100% specificity after 30 min of incubation of bacteria with the antibiotic, with both reaching 100% after 60 min of incubation [126,127]. However, beta-lactam resistance is only recognized when it is mediated by beta-lactamases; alternative resistance mechanisms have not been elucidated; therefore, other tests should confirm negative results. Another assay for the detection of carbapenemases is a rapid and novel method using detonation nanodiamonds (DNDs) as a platform for the concentration and extraction of A. baumannii carbapenemase-associated proteins before MALDI-TOF MS analysis [128]. The sensitivity and the specificity of the proposed platform could reach 96% and 73%, as compared with traditional imipenem susceptibility testing, and 100% compared with PCR results. This method may detect the carbapenemases produced by A. baumannii in 90 min and does not require the addition of a carbapenemase substrate, as other mass spectrometric methods do. It is efficient for detecting other carbapenemase-producing bacteria. MALDI Biotyper-Antibiotic Susceptibility Test Rapid Assay (MBT-ASTRA) is an alternative MS-based method for AST which utilises semi-quantitative MALDI-TOF MS to measure the relative growth rates of bacterial isolates exposed to antibiotics compared with untreated controls during a short incubation step. A software tool calculates and compares the area under the curves (AUCs) of spectra of bacteria either exposed or not to an antibiotic [129]. In this method, if the microbial strain is susceptible, the AUC of the bacterial suspension with the antibiotic will be reduced compared with that without antibiotics, whereas with a resistant strain the AUCs with or without antibiotics will be comparable. The main advantage of the MBT-ASTRA is that the assay does not depend on the resistance mechanism and is utilisable with any antibiotic [130]. Moreover, it does not require specialised media or instrumentation, beyond the MALDI-TOF mass spectrometer. However, a drawback of the MBT-ASTRA assay is that the concentration of antibiotics used and the incubation time must be optimised for each species and antibiotic combination [129].MBT-Resist assay, based on the detection of peak shift after stable isotope labelling, is an approach that uses the following principle: bacteria are grown in parallel in two distinct culture mediums, one containing 12C as a carbon component and the other containing 13C [131]. The system compares the mass spectrum of bacteria grown on an isotope-labelled medium with antibiotics to the mass spectrum of the same strain grown on an unlabelled medium without antibiotics.
Resistant strains can thrive in the presence of antibiotics, incorporating 13C into the polypeptide, causing a shift in the peak to a higher m/z in the mass spectrum [131]. Antibiotic resistance by direct-on-target microdroplet growth assay (DOT-MGA) is a novel approach for detecting antimicrobial susceptibility in bacteria treated with breakpoint concentrations of antibiotic on the target plate of MALDI-TOF MS [132]. The best performance was obtained by recovering bacteria from positive blood cultures and after a 4 h incubation of microdroplets with or without meropenem at the breakpoint concentration. Under these conditions, 96.3% validity, 91.7% sensitivity, and 100% specificity were achieved. Recently, a screening panel for the detection of ESBL and AmpC beta-lactamase activity was developed [133]. Compared with the PCR results, positive percentage agreement values for ESBL, AmpC, and ESBL + AmpC resistance were 94.4%, 94.4%, and 100%, and negative percentage agreement values were 100%, 93.7%, and 100%, respectively. The accuracy of the DOT-MGA achieved results incomparable with those of the BMD assay, with a time saving of about 14 h, and higher than combination disk tests.According to Yoon et al., due to the great speed and simple application, MALDI-TOF MS would be the most suitable for endemic AMR clinical strains in specific settings, i.e., MRSA, VRE, CRAB, CRPA, and ESBL-, AmpC-, and carbapenemase-producing Enterobacterales [134].The advantages and disadvantages of the commonly used methods of antimicrobial susceptibility testing were summarised in Table 1.Since there are a large number of antibiotics in use, it would be irrational and pointless to test the susceptibility of isolates to all of them.
Although each laboratory should establish its own panels of antibiotics depending on the bacterial isolate, for which susceptibility is tested, there are some common rules regarding the choice of antibiotics [36]. The number and selection of antimicrobials tested are primarily dependent on the organism isolated, infection site, type of infection (community or hospital-acquired), comorbidities, patient's age, and gender, but also the institution's formulary, physician requests, and the automated panel or other testing methodology used [135].Interpretation of AST results and reporting of bacterial susceptibility categories to antibiotics is based on the breakpoints published by the two most commonly used systems worldwide: CLSI and EUCAST [42,61].
CLSI provides recommendations for agents that are important to test routinely (group A) and those that may be tested or reported selectively based on the institution's formulary (groups B and C) [136]. Accurate identification of bacteria is crucial in the choice of antibiotics [137]. It is well known that resistance mechanisms have not been observed in some bacterial species so far, e.g., continued penicillin susceptibility of Streptococcus pyogenes [26].
It is also known that some bacterial species are intrinsically resistant to particular antibiotics or antibiotic classes. It is systematically presented in the EUCAST document "Intrinsic Resistance and Unusual Phenotypes" [138]. Therefore, it is unnecessary to test certain bacterial species for activity in vitro, but microbiologists should report intrinsic resistance to clinicians. This document also indicates when to avoid the use of antimicrobial agents that are likely to result in treatment failure. Exceptional or unusual resistance phenotypes are also described in the EUCAST document [138], and knowledge of those phenotypes allows microbiologists to further investigate the obtained results.
Examples of such phenotypes are penicillin-resistant group A streptococci or vancomycin-resistant staphylococci. These results should be confirmed and checked before reporting. Organisms with unusual phenotypes could be seen due to the emergence of antibiotic resistance, but it is more likely that an error occurred during the strain identification, antibacterial susceptibility testing, or when the mixed culture was tested [139].Due to the similar in vitro activities of some antimicrobials belonging to the same class, a representative antibiotic should be tested to predict susceptibility to other class members [27]. Thus, for example, in beta-haemolytic streptococci, the susceptibility to penicillins can be inferred from benzylpenicillin susceptibility results [42]. Susceptibility of staphylococci to beta-lactams can be assessed by susceptibility to cefoxitin, while susceptibility of staphylococci and streptococci to macrolides and fluoroquinolones could be predicted using erythromycin and norfloxacin, respectively [135]. In enterococci, ampicillin is used as an indicator of resistance to ampicillin, amoxicillin, and piperacillin with and without beta-lactamase inhibitor. It is not commonly used in Gram-negative bacteria due to their more heterogeneity in resistance.Until recently, both EUCAST and CLSI use "susceptible" (S), "intermediate" (I), and "resistant" (R) categories and also shared their definitions. While categories S and R still do not differ in interpretation in both standards and are easily understood, category I was not clear since it represented few definitions into one: uncertain therapeutic effect, appropriate to use when antibiotic is physiologically concentrated at the site of infection, appropriate to use a high dosage of the drug, etc. [140]. The above-described interpretation of the category I led clinicians to either avoid or misuse certain antibiotics [140]. Consequently, in June 2018 the EUCAST Steering Committee changed the definitions of AST categories to susceptible, standard dosing regimen (S), susceptible, with increased exposure (I), and resistant (R) [141]. This is done to emphasise the close relationship between the susceptibility of the organism and the exposure of the organism to antibiotics at the site of infection [142], encouraging physicians to use antibiotics from the I category at the appropriate dosage. EUCAST breakpoints are based on dose and mode of administration as indicated in rationale documents and files of the recommended AST breakpoints [143]. Tables of recommended doses for antibiotics, standard dose regimen for S, and high doses for the new I category were also published [42]. This relatively new rule still needs to be implemented in healthcare professional training and clinical use [31].Antimicrobial susceptibility categories help clinicians to predict the outcome of antibiotic treatment and provide them information on the recommended dosage. For some infections that require high doses of antibiotics for reaching therapeutic concentrations (e.g., meningitis), EUCAST listed meningitis breakpoints that are different from breakpoints for non-meningeal isolates [144]. Furthermore, for the treatment of infections caused by Pseudomonas spp., an increased antibiotic concentration on the site of infection is required. Therefore, according to the EUCAST recommendations, the majority of isolates belong to the category "Susceptible, increased exposure" when evaluating susceptibility to fluoroquinolones, penicillins, and third and fourth generations of cephalosporins, aztreonam, and imipenem [42].The principle of the automated systems for AST is to interpret the results of MICs to the susceptibility category that corresponds to built-in standards (e.g., EUCAST, CLSI). This allows concurrent reporting of categories of susceptibility, values of MICs, and even some resistance phenotypes, providing valuable information for patient treatment.
It also allows clinicians and pharmacologists to determine the appropriate dosage for each patient, which represents an individual or personalised medicine. Such an approach enables the estimation of the degree of resistance or susceptibility to the tested agent [31]. However, this assumption should not be based on a direct comparison of the MIC values obtained during testing, nor under the assumption that lower MIC values indicate greater sensitivity of tested bacteria in all cases. Additionally, MICs values can indicate lower effectiveness of the antibiotic, despite the susceptibility category. For example, the MIC value of 2 mg/L for vancomycin predicts the risk of treatment failure in tested S. aureus strains, even though it is still in the susceptible category [145]. Although both EUCAST/CLSI standards are constantly being improved and published annually, there are still limitations in reporting. Thus, interpretations of susceptibility to some common pathogens (e.g., Acinetobacter spp.
and Stenotrophomonas maltophilia) and key antibiotics are lacking, resulting in the absence of AST reporting. Overall, the general recommendation is to report susceptibility to narrow-spectrum antibiotics whenever possible and to avoid reporting of broad-spectrum antibiotics that pose higher AMR selective pressure (carbapenems, fluoroquinolones) [146,147]. Selective reporting of AST results is recognized as a useful tool for antibiotic stewardship. It implies performing AST according to usual practices, but the results are reported to the physician only for a few antibiotics recognised as a first-line choice [14].
Therefore, microbiologists play an essential role in AMS [148,149].
As Tebano G et al. [14] propose, selective reporting might achieve the following: firstly, reducing unnecessary antibiotic prescriptions in cases of the detection of contaminants or members of the normal microbiota. Secondly, reducing inappropriate antibiotic prescriptions by persuading clinicians to choose narrow- over broad-spectrum antibiotics.
Both goals could be obtained by reducing the number of reported antibiotics or selective exclusion of certain antibiotics from the reporting where appropriate. Some countries established their National Antimicrobial Committee to address the challenges of AMR [150,151]. These committees adapt the recommendations of EUCAST/CLSI in accordance with the epidemiological specificities of their countries and provide laboratory guidelines for antibiotic selection, testing, and reporting for their national-level use. In conclusion, evidence-based data shows that selective reporting leads to improved antibiotic usage, reducing both unnecessary and inappropriate prescribing. It can be concluded that, although numerous interventions have been proposed to reduce antibiotic consumption and bacterial resistance, much effort is still needed, especially in middle- and low-income countries. Therefore, wider and more consistent support in the implementation of these strategies is highly needed on a global scale [152].Soon after the introduction of AST in clinical laboratories, the need for standardization of the process was recognized. In the past, several of the European national antimicrobial breakpoint Committees developed their own AST standards.
Consequently, the national Committees initiated the standardisation of AST performance and interpretation according to the EUCAST recommendations [153]. A much older and widely used standard is the American CLSI, formerly NCCLS [61]. Although the process of harmonization of these two organizations is ongoing, there are still significant differences.All AST needs to be evaluated in order to provide a certain quality of the results. For conventional methods, manufacturers of antibiotic disks or tablets, gradient tests, and commercial microdilutions plates with a predetermined concentration of antibiotics have to provide a certain quality of the products, as do suppliers of automated or semi-automated tests. In addition, the microbiology laboratories are responsible for the adequate performance of the antimicrobial susceptibility tests. The control of the quality of performance is ensured through internal and external controls. Internal quality assessment should be provided daily or less frequently if the performance is consistent.
External quality assessment tests the overall performance of the laboratory by a collection of bacterial strains of undisclosed susceptibility, usually sent by central international laboratories such as NEQUAS in the UK. After the results are sent back, a detailed evaluation of the participating laboratory performance is provided by the central laboratory [154]. Quality control is responsible only for the analytical phase of susceptibility testing, monitoring only the performance of the used test(s). The troubleshooting algorithm for AST QC is shown in Table 2, adapted from the previously published report [155].Quality assurance (QA), on the other hand, provides the proper performance of all three

phases of testing: pre-analytical, analytical, and post-analytical. Major components of the QA program for susceptibility testing are the following: clinically relevant testing strategies, testing of reference quality control (QC) strains, technical competency, organism antibiogram verification, supervisor review of results, procedure manual, proficiency surveys.

Quality control includes the following: the precision (repeatability) and accuracy of AST procedures, the performance of reagents used in the tests, the performance of persons who carry out the tests and read the results [156].The AST procedures include diffusion susceptibility methods, dilution susceptibility testing, and different automated systems. The results of commercial automated systems are obtained more rapidly and are highly reproducible; the use of the automated system software supports the interpretation according to different standards and expert rules. Unusual resistance phenotype or resistance types that are difficult to detect may represent a challenge [36].As for automated antimicrobial susceptibility systems, which are used more and more frequently in microbiological laboratories, usually, every device has its QC procedure. The most commonly used automated systems for antimicrobial susceptibility are VITEK 2 System (bioMérieux, France), BD Phoenix System (BD Diagnostic Systems, Baltimore, MD, USA), MicroScan WalkAway SI (Siemens Healthcare Diagnostics, West Sacramento, CA, USA), and TREK Sensititre (ARIS 2X, Trek Diagnostic Systems, Cleveland, OH, USA).

Quality controls of such devices are not included in standards such as EUCAST or CLSI [61,156,157]. Agencies that provide the clearance of commercial systems for general use, such as the FDA for the USA, should use reference methods before the clearance for use is given. To evaluate or compare different susceptibility tests, usually these types of errors are considered: very major error, major error, and minor error.

Very major error refers to the characterisation of the resistant isolate as susceptible. A major error is made when a susceptible isolate is characterised as resistant. When an intermediate isolate is characterised as susceptible or resistant, as well as when susceptible or resistant isolate is reported as intermediate, a minor error is made. Errors in antimicrobial susceptibility testing should be monitored by laboratories and carefully analysed. Potential problems either with the identification or susceptibility testing may be revealed [158]. For evaluation of new susceptibility tests or devices, a certain number of susceptible as well as resistant strains should be tested against different antibiotics. Additionally, all types of the above-mentioned errors should be evaluated [36]. For new antimicrobial susceptibility devices, acceptable performance according to FDA considers that major errors should not exceed 3% based on the number of susceptible organisms tested [159]. The acceptable numbers of very major discrepancies rate should be 7.5% or less (for the upper 95% confidence limit) and 1.5% or less (for the lower 95% confidence limit), based on the number of resistant organisms tested. Additionally, growth failure rates in the system have to be less than 10%.The above-mentioned criteria may not be identical for acceptable accuracy compared with the international standards on susceptibility test device evaluation [160].In the last decades, several innovative approaches for AST have been developed. Some of the most promising platforms for the forthcoming period—MALDI-TOF, flow cytometry, and isothermal microcalorimetry—will be briefly described. Application of MALDI-TOF MS as routine rapid AST requires additional validation, such as standardised protocols, test kits, and software [161]. Further research efforts are needed to refine and optimise MALDI-TOF MS-based assays to obtain accurate and reliable results in the shortest possible time. A major focus of future research in this field will be to achieve the standardisation of methods and simultaneous susceptibility testing of microbes to various classes of antimicrobials [162]. So far, only two commercially available kits with software for automated interpretation of spectra have been authorised in Europe to detect carbapenemase activity or resistance toward third-generation cephalosporins in clinical microbiology laboratories.By using flow cytometry, the changes in morphology, physiological and metabolic activity, and survival of microorganisms can be monitored after exposure to antimicrobials. The effects of treatment can be seen within a few hours, suggesting a strong potential for AST. Using nuclear dyes that do not penetrate the cell walls of healthy organisms, the amount of dying and dead cells can be rapidly determined.

In order to determine the emission spectrum, the cells must go through a flow channel and be excited by a laser to release dye [163]. Nevertheless, the future challenge is to improve the ability of a system to distinguish cellular damage caused by -cidal vs. -static antibiotics, as well as to address the issue of autofluorescence of certain species of bacteria.

In addition, an enormous amount of work is required for the verification of the clinical database and the method itself.Isothermal microcalorimetry is a dynamic technique allowing the measurement of heat generated by the metabolism of actively growing cells. This technique is not new; however, it has been successfully adapted for AST of bacteria [164]. Additional advantages of this technique are that it requires a small culture volume, the testing is performed in sealed ampules, and monitoring during testing does not require manual manipulation. The completed analysis can also provide information on the -static vs. -cidal activity of an agent.Up to now, various alternative approaches to combat AMR have been proposed. For instance, epigenetic agents in modulating antibiotic susceptibility [165], agents that inhibit virulence-conferring factors such as multidrug efflux inhibitors [166], or targets' essential processes for bacterial survival, such as cell division inhibitor [167], have been recently developed. If such antibacterial agents become licensed for treatment, antimicrobial susceptibility techniques should be updated along with the new strategies developed to counter AMR.Conceptualisation, I.G.; manuscript writing, all authors; review and editing, I.G., N.O., M.M., D.M.C., A.T. and L.R.; figure creation: I.G., J.K., M.J. and D.K. All authors have read and agreed to the published version of the manuscript.1. Holmes A.H., Moore L.S.P., Sundsfjord A., Steinbakk M., Regmi S., Karkey A., Guerin P.J., Piddock L.J.V. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016;387:176–187. doi: 10.1016/S0140-6736(15)00473-0. [PubMed] [CrossRef] [Google Scholar]2. WHO Antibiotic Resistance. [(accessed on 23 February 2022)]. Available online: . Murray C.J., Ikuta K.S., Sharara F., Swetschinski L., Robles Aguilar G., Gray A., Han C., Bisignano C., Rao P., Wool E., et al. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis.
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Published online 2020 Feb 24. Prepublished online 2020 Jan 8. doi: 10.1128/JCM.01864-19PMCID: PMC7041576aDepartments of Medicine and Pathology & Laboratory Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, New Jersey, USAFind articles by Melvin P. WeinsteinbDepartments of Pharmacy and Infectious Diseases, Oregon Health and Science University, Portland, Oregon, USAFind articles by James S. Lewis, IIColleen Suzanne Kraft, EditorColleen Suzanne Kraft, Emory University;Author information Copyright and License information PMC DisclaimerCopyright © 2020 American Society for Microbiology.The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antimicrobial Susceptibility Testing (AST SC) is a volunteer-led, multidisciplinary consensus body that develops and publishes standards and guidelines (among other products) for antimicrobial susceptibility testing (AST) methods and results interpretation in the United States and internationally. The Subcommittee (SC) meets face-to-face twice yearly, and its working groups (WGs) are active throughout the year via teleconferences.The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antimicrobial Susceptibility Testing (AST SC) is a volunteer-led, multidisciplinary consensus body that develops and publishes standards and guidelines (among other products) for antimicrobial susceptibility testing (AST) methods and results interpretation in the United States and internationally. The Subcommittee (SC) meets face-to-face twice yearly, and its working groups (WGs) are active throughout the year via teleconferences. All meetings are open to the public. Participants include clinical microbiologists, infectious disease (ID) pharmacists, and infectious disease physicians representing the health care professions, government, and industry. Individuals who work for a company with a primary financial dependency on drug sales cannot serve as voting members, and well-defined conflict of interest polices are in place. In addition to developing and updating susceptibility breakpoints, the SC develops and validates new testing methods, provides guidance on how results should be interpreted and applied, sets quality control ranges, and educates users through seminars, symposia, and webinars. Based on its work, the SC publishes print and electronic standards and guidelines, including an annual update, the Performance Standards for Antimicrobial Susceptibility Testing (M100).
This commentary will describe the background, organization, functions, and operational processes of the AST SC.Originally established in 1968 as the National Committee for Clinical Laboratory Standards (NCCLS), the organization renamed itself the Clinical and Laboratory Standards Institute (CLSI) in 2000 to emphasize its expanding Global Health Partnerships and its more global, as opposed to national, reach. The first antimicrobial susceptibility testing (AST) document, Performance Standards for Antimicrobial Disc Susceptibility Tests, was published in 1975.The CLSI Subcommittee on Antimicrobial Susceptibility Testing (AST SC) is a multidisciplinary, consensus body that develops and publishes guidelines and procedures for AST in the United States and internationally. It functions under the umbrella of the larger CLSI organizational structure. The hallmarks of an organization that sets laboratory standards affecting medical practice should be transparency, inclusiveness, and consensus decision-making based on established standards. Indeed, these are the principles espoused by CLSI, a volunteer-led not-for-profit organization with almost 2,000 volunteers. CLSI is a widely recognized laboratory medicine standards development organization (SDO) and is the only fully accredited SDO in the laboratory medicine field.
It adheres to all principles of international standards development required by the World Trade Organization, is the Executive Secretariat for the International Organization for Standardization committee, and is the only laboratory SDO designated a World Health Organization collaborating center. CLSI has set laboratory standards for the past 50 years, producing a library of approximately 240 standards covering the major disciplines of clinical laboratory medicine. Its standards are used in more than 50 countries.Within the subspecialty of AST, CLSI subcommittees (SC) set standards for the scope of bacterial and fungal AST performed in clinical and veterinary practice. Standards for bacteria from human infections are set by the AST SC. This subcommittee consists of volunteers who serve as chairholder, vice-chairholder, voting members, advisors, and reviewers. All volunteers, regardless of their position, can participate in working groups (WGs) of the subcommittee. The AST SC’s work is reviewed by the CLSI Microbiology Expert Panel, and all decisions are approved by the CLSI Consensus Council (see Fig.
1). CLSI salaried staff support the AST SC with project management, meeting logistics, database management, and related non-subject matter support. The AST SC is responsible for setting standard testing methods, updating interpretive criteria (often referred to as breakpoints), establishing data standards for setting breakpoints and quality control ranges, setting guidelines for reporting cumulative susceptibility data (e.g., an antibiogram), and educating health care providers on all aspects of antimicrobial susceptibility testing. The work output of the AST SC includes numerous standards, guidelines, reports, and rationale documents for AST. These include a number of documents that are widely used in clinical microbiology laboratories throughout the United States and the world.
Several current examples are:•Performance Standards for Antimicrobial Disk Susceptibility Tests, 13th ed. CLSI standard M02 (1).•Performance Standards for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th ed. CLSI standard M07 (2).•Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, 9th ed. CLSI standard M11 (3).•Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 3rd ed. CLSI guideline M45 (4).•Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. CLSI supplement M100 (5).•Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters. 5th ed. CLSI standard M23 (6).To accomplish this work, the AST SC works throughout the year through teleconferences for working groups and holds two 3- to 4-day face-to-face meetings per year (January and June). These meetings are open to the public (find future meetings here: , and meeting minutes and presentations are posted on the CLSI website (.
The AST SC meetings typically draw 150 to 200 participants. All meetings are conducted using Robert’s Rules of Order, with documented voting procedures. AST SC deliberations are conducted in the presence of all meeting participants to ensure complete transparency and ample opportunities to contribute to the discussion.Experts in clinical microbiology, infectious disease (ID) pharmacy, and infectious disease medicine work together on the AST SC, and representation from the three disciplines is carefully balanced. In addition, CLSI includes representation from health care professionals, government, and industry. The CLSI consensus process is predicated on the inclusion of the three constituencies. Eliminating any one group means the elimination of a critical knowledge base, and AST SC decisions would suffer as a result. To ensure no undue influence of financial interests on breakpoint decisions, any individual who works for a company with a primary financial dependency on drug sales is not eligible to be a voting member of the AST SC. In addition, conflict-of-interest policies are in place and require full disclosure of financial contributions from industry for all voting members and advisors. The policy of complete transparency is a second-level deterrent against undue financial influence. Because meetings are open, participants’ actions are held accountable by a room full of peers, the AST SC, and CLSI leaders.CLSI standards are used internationally, and, to ensure a global viewpoint, members of the subcommittee include international experts. The chair of the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) serves as an advisor of the AST SC.
In addition to representation from the European Union, the AST SC includes experts from other countries and regions of the world, including Australia, Japan, China, Canada, and Latin America. Official liaisons from professional societies are members of the subcommittee; these societies include the American Society for Microbiology, the Infectious Diseases Society of America, the College of American Pathologists, the Association of Public Health Laboratories, the Society for Healthcare Epidemiology of America, the Society of Infectious Diseases Pharmacists, the Pediatric Infectious Disease Society, and the Susceptibility Testing Manufacturers Association.Most clinical microbiologists, ID physicians, and ID pharmacists know that the AST SC develops and updates interpretive breakpoint criteria, but they may not be familiar with the processes and procedures by which this takes place. Also, it is less well known that the AST SC has a number of other functions, most of which come under the purview of several standing working groups (WGs).The SC’s Breakpoint WG reviews proposed breakpoints for new antimicrobial agents (usually brought to the SC by the drug’s manufacturer), and it also reassesses breakpoints for existing antimicrobial agents and classes thereof when there is evidence of new resistance mechanisms (e.g., extended-spectrum beta-lactamases, carbapenemases) or new scientific data that mandate breakpoint changes (recent examples include pharmacokinetic and clinical data related to daptomycin versus enterococci, ceftaroline versus Staphylococcus aureus, and polymyxin B/colistin versus Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter spp.).When investigators develop a new or innovative method for susceptibility testing or detection of resistance, the Methods Development and Standardization WG assesses the proposed methodology and determines whether the new technique can be added to the repertoire of tests described in the CLSI AST documents (1,–5). Recent examples include the colistin broth disk elution and agar diffusion tests, validation of Mueller-Hinton fastidious agar for Streptococcus pneumoniae, the broth microdilution and agar disk diffusion tests for cefiderocol, and a direct susceptibility test for blood culture isolates (to be finalized in 2020). A third standing WG is the Methods Application and Interpretation WG. This WG provides guidance on how a given new or revised testing method can be applied in the clinical microbiology laboratory and how the results should be interpreted. Several notable examples of this working group’s efforts include the expanded and refined definition of “susceptible dose-dependent” and the addition of the Î breakpoint categories (see [click to use guest access and then select M100]) published in the January 2020 update of M100; the revised and updated table that provides guidance for confirming resistant, intermediate, and nonsusceptible AST results (Appendix A in M100) (5); the Intrinsic Resistance table (Appendix B in M100) (5); and the addition of screening methods for carbapenemases (modified carbapenem inactivation method [mCIM]) and enhanced carbapenem inactivation method ([eCIM] tests) (see Tables 3B and C in M100) (5). The Quality Control (QC) WG reviews and assesses proposed QC ranges for new antimicrobials that are in development, and it reassesses and sometimes revises QC ranges for existing antimicrobials based on feedback from users. Each year, the SC publishes updated susceptibility breakpoint tables in the CLSI M100 document, and the SC’s Text and Tables WG reviews and edits all revisions prior to publication. The Text and Tables WG also oversees and reviews the other AST-related documents, such as M02, M07, and M45, for clarity and accuracy prior to their periodic revisions and publication (usually every 2 to 5 years). Lastly, the SC’s Outreach WG publishes semiannual newsletters and conducts symposia and webinars that inform the clinical microbiology community and other users about the latest updates based on the SC’s deliberations.Decisions about breakpoints are rarely black and white. Making an ethically and scientifically sound decision requires standards for data and the decision-making process. These standards are outlined in CLSI document M23, which is entitled Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters (6). These standards not only provide critical parameters for AST SC action but also a level of predictability for drug sponsors whose antibiotics are under consideration. M23 is also the basis for FDA breakpoint decision making, so data needed for FDA approval are also appropriate for CLSI submission. The following three types of data are analyzed: MIC distribution data, pharmacokinetic and pharmacodynamic data, and clinical trial data. No one data source is sufficient to determine where a breakpoint should be set. Where there is scientific uncertainty, there is debate. In the case of breakpoint setting, scientific and technical debate is not a sign of ignorance or inconsistency. It is a necessary process that leads to the best answer, namely, one that makes sense in the laboratory, in clinical practice, and for patient protection. When making decisions about methods and breakpoints, there is a tension between the time needed for identifying data sources and formal deliberations about those data and the need for timely decisions that can improve patient care.
CLSI continues to review its process to best deal with this tension. On the AST SC, the decision-making process was streamlined in recent years by creating small ad hoc working groups that focus on a single issue and develop a well-researched proposal before the larger group, the AST SC, is asked to make a decision. This change has significantly expedited the time to a decision. In practice, the Breakpoint WG reviews a comprehensive scientific data packet, questions the sponsor or presenter of the data, and accepts or revises the proposed breakpoints. Then, at the face-to-face meeting of the AST SC, the proposal is reviewed by the full SC, and all in attendance have the opportunity to raise questions and comment before there is an open vote.
It should be noted that the meeting agenda materials are provided to all SC members, advisors, reviewers, and guests who have registered for the meeting at least 4 weeks prior to the meeting, so that there is adequate time for review of the materials before the meeting.In the United States, the Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) has statutory authority for setting antimicrobial susceptibility breakpoints. Until recently, FDA breakpoints were published in each antimicrobial agent’s drug label. However, after passage of the 21st Century Cures Act in 2016, FDA breakpoints were moved to the Agency’s Susceptibility Test Interpretative Category (STIC) website (. CLSI applied for and was approved as an FDA-designated standards development organization (SDO).
This has resulted in most, but not all, of the CLSI breakpoints in M100 being listed in the STIC table. A discussion of the reasons why certain CLSI breakpoints are not in the FDA STIC table can be found elsewhere (7).In addition to the FDA and CLSI AST SC, there are organizations in other parts of the world that set interpretive breakpoints, the most well known of which is EUCAST. This organization has established subsidiary committees in other countries, one of which is the United States Committee on AST (USCAST), that have the opportunity to provide additional scientific information to the EUCAST Steering Committee, which is that organization’s voting body. However, the subsidiary committees, while providing input, only have a vote in the final decision on a rotational basis. Key process differences between the CLSI AST SC and EUCAST are shown in Table 1.Key differences between CLSI and EUCAST processesFunctionOrganizationCLSI subcommittee for Antimicrobial Susceptibility TestingEUCASTThe primary decision-making bodyVoting members of the Subcommittee, which consists of individuals, regardless of country of origin, with recognized expertise in a discipline related to antimicrobial susceptibility testing. Individuals working for a company whose revenues are directly or indirectly dependent upon the sales of antimicrobials cannot be a voting member.The Steering Committee, which consists of representatives from selected European Union countries (national delegates) with recognized expertise in a discipline related to antimicrobial susceptibility testing. Committee members are traditionally academics.Additional participants/consultantsSubcommittee advisors and reviewers, which consist of representatives from professions, government, and industry.The General Committee, which includes members of National AST Committees (NAC). Industry is represented on the General Committee, and includes those invited to attend closed-door Steering Committee meetings where breakpoints are determined.Breakpoint decisions meetingsSubcommittee discussions and voting occurs in open, transparent, and inclusive meetings (2 meetings per year).Steering Committee members and 2–4 invited members of the General Committee make decisions behind closed doors (5 meetings per year).Conflict of interestAll Subcommittee voting members and advisors submit conflict of interest statements that are publicly available on the CLSI website and are updated at the beginning of each meeting.Steering Committee members submit a list of commercial interests to the Steering Committee chairperson. These are not publicly available, but can be made available upon request to the committee chairman.
It is not clear if statements are required from General Committee members who participate in Steering Committee meetings.Representation from the other organizationFormal appointment of a EUCAST advisor.No CLSI representation.Meeting minutesMinutes and presentations from all Subcommittee meetings are available on the CLSI website ( ).Minutes from the Steering Committee meetings are on the EUCAST website (.Appeals process for those concerned with an undisclosed conflict of interest or that a consensus process has not been followedYesNoAccredited standards development organizationYesNoEven though breakpoint decisions are rarely clear-cut, it is noteworthy that CLSI and EUCAST breakpoints are so similar.
If “susceptible” breakpoints are compared, most differences are a single MIC doubling dilution—a value that is within the technical error range of an MIC test. One reason breakpoint differences can occur is if different doses of a drug are used, and this is often cited as a theoretical reason for not attempting to harmonize breakpoints. However, differences in drug dose have rarely been the basis for differences between CLSI and EUCAST breakpoints, and they are unlikely to be a factor in the future because the same clinical trials are often used to obtain FDA and European Medicines Agency drug approval. Dosage differences are more likely to be a problem for older drugs and in parts of the world where prescribing practices may differ.More often, differences between CLSI and EUCAST breakpoints reflect different philosophical viewpoints on how to interpret and communicate results. A good example is the use of the “intermediate” interpretive category (8). This category can be applied for multiple reasons, such as to reflect the uncertainty in the MIC result because of technical variability in MIC testing and to account for clinical efficacy if high drug exposure is possible because a higher dose of a drug is used or the drug concentrated at the site of infection.
EUCAST is less likely to include an intermediate range when setting breakpoints and has eliminated technical variability as a reason for setting an intermediate range. Instead, “intermediate” is used when a higher dose of the drug (i.e., a dose higher than the dose used to set the susceptible breakpoint) is available. CLSI has maintained technical variability as a reason to use an intermediate category because MIC testing does have an inherent variability, and failure to include an intermediate range can result in susceptible isolates being falsely reported as resistant, thus removing a potentially lifesaving drug from consideration as a treatment option. In contrast, CLSI has recommended using “susceptible dose-dependent” instead of “intermediate” in some cases where multiple drug dose options are frequently used and both clinical and pharmacokinetic data point to susceptibility at higher MICs if maximum dosing is used.
As a practical matter, a “susceptible dose-dependent” result from CLSI and an “intermediate” result from EUCAST communicate very similar information to the clinician.
Communicating susceptibility results is a challenging problem with no single solution, but it clearly calls for international dialogue and, if possible, alignment.Antimicrobial susceptibility testing methodological differences exist between CLSI and EUCAST. Specifically, EUCAST recommends the use of some disks with different concentrations than those recommended by CLSI. In a recent assessment by CLSI, the EUCAST disks did not result in significant differences in category (e.g., susceptible versus resistant) assignment (summary minutes of the January 2016 AST Subcommittee are available at . As a result, CLSI decided not to convert to the EUCAST disks because the studies needed to meet M23 data requirements would require a significant financial and scientific investment with little to no effect on patient care. Both groups informally agreed to harmonize disk drug content moving forward, and the two organizations have established a joint WG to achieve this goal. Recommendations for media used to test fastidious bacteria also differ. In most cases it is expected that the different medium types are similar enough that breakpoint recommendations do not differ (9). Again, the organizations should agree to harmonize methodology moving forward and identify a process for ensuring that this occurs.Is complete harmonization necessary? Harmonization would mean less confusion for laboratorians and infectious diseases clinicians and would significantly facilitate the implementation of new drugs and new breakpoints on commercial tests systems by making these changes simpler and less expensive. Harmonization would also simplify global surveillance efforts that rely upon data generated as part of routine health care practices. The argument against harmonization is the tremendous amount of work required to reassess breakpoints of older drugs where breakpoint differences are often minor (e.g., within the technical variability of the test) and there is no clinical indication that a breakpoint change is needed. To ensure efficient use of resources, priorities for harmonization should be identified.A big challenge to harmonization is identifying an improved process for working together. Both CLSI and EUCAST intend to stay in the business of revising breakpoints and, optimally, working together would result in synergizing efforts so that each organization can work more efficiently because of the other’s efforts. In such an environment, these two organizations would spend more energy trying to find ways to work together rather than how to set themselves apart. For more than a decade, CLSI has appointed the current Chairperson of EUCAST as an advisor on the AST SC. However, to date, EUCAST has not reciprocated. Both groups are currently engaged in a Transatlantic Task for Antimicrobial Resistance (TATFAR) working group on harmonization and have identified first priorities for harmonization. CLSI will continue to work toward increased harmonization internationally, recognizing that scientific and philosophical disagreements may limit this ideal goal.We thank Jean B. Patel for her valued contributions to the manuscript.M.P.W. and J.S.L. are the chair and vice chair, respectively, of the CLSI Subcommittee on Antimicrobial Susceptibility Testing.The views expressed in this article do not necessarily reflect the views of the journal or of ASM.1. Clinical and Laboratory Standards Institute. 2018. Performance standards for antimicrobial disk susceptibility tests, 13th ed CLSI standard M02 Clinical and Laboratory Standards Institute, Wayne, PA. [Google Scholar]2. Clinical and Laboratory Standards Institute. 2018. Performance standards for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 11th ed CLSI standard M07 Clinical and Laboratory Standards Institute, Wayne, PA. [Google Scholar]3. Clinical and Laboratory Standards Institute. 2018. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 9th ed CLSI standard M11 Clinical and Laboratory Standards Institute, Wayne, PA. [Google Scholar]4. Clinical and Laboratory Standards Institute. 2016. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria, 3rd ed CLSI guideline M45 Clinical and Laboratory Standards Institute, Wayne, PA. [PubMed] [Google Scholar]5. Clinical and Laboratory Standards Institute. 2020.
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