One Health Advances

REVIEW

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Antimicrobial susceptibility testing in veterinary medicine: performance, interpretation of results, best practices and pitfalls

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Abstract

The performance of antimicrobial susceptibility testing (AST) of bacteria and the interpretation of AST results for bacteria isolated from animals are complex tasks which must be performed using standard published methodology and overseen by experts in clinical microbiology and in consultation with clinical pharmacologists. Otherwise, AST has significant potential for errors and mistakes. In this review, we provide guidance on how to correctly perform AST of bacteria isolated from animals and interpret the AST results. Particular emphasis is placed on the various approved or published methodologies for the different bacteria as well as the application of interpretive criteria, including clinical breakpoints and epidemiological cut-off values (ECVs/ECOFFs). Application of approved interpretive criteria and definitions of susceptible, susceptible dose-dependent, nonsusceptible, intermediate, and resistant for clinical breakpoints as well as wild-type and non-wildtype for ECVs, are explained and the difficulties resulting from the lack of approved clinical breakpoints for other bacteria, indications, and animal species is discussed. The requirement of quality controls in any AST approach is also emphasized. In addition, important parameters, often used in monitoring and surveillance studies, such as MIC_{50}, MIC_{90}, and testing range, are explained and criteria for the classification of bacteria as multidrug-resistant, extensively drug-resistant or pandrug-resistant are provided. Common mistakes are presented and the means to avoid them are described. To provide the most accurate AST, one must strictly adhere to approved standards or validated methodologies, like those of the Clinical and Laboratory Standards Institute or other internationally accepted AST documents and the detailed information provided therein.

Keywords Antimicrobial susceptibility testing, Quality controls, Clinical breakpoints, Epidemiological cut-off values, Multidrug resistance

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Introduction

Several organizations have developed and published methods for performing antimicrobial susceptibility testing (AST) on bacteria isolated from humans, including the Clinical and Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the British Society for Antimicrobial Chemotherapy (BSAC) and the Commité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) among others. In veterinary medicine, fewer organizations have taken on this task. Of those previously mentioned, CLSI has led the effort in developing methods for AST of bacteria isolated from animals. Founded in 1993, the Veterinary Subcommittee on Antimicrobial Susceptibility Testing (VAST) of CLSI issued its first document, M31A as tentative standard in 1997 and as approved standard in 1999 [1]. Since the first standards were published, the CLSI-VAST has developed breakpoints for more than 260 antimicrobial agent-bacteria combinations. Currently available veterinary-based documents from CLSI include methods and interpretive criteria for bacteria isolated from animals (VET01/VET01S) [2, 3], guidance and interpretive criteria for bacteria isolated from aquatic animals (VET03/VET04) [4, 5] and guidance and interpretive criteria for infrequently isolated and fastidious organisms (VET06) [6]. Other documents include how to develop breakpoints and quality control ranges (VET02) [7], best practices for antibiogram production (VET05) [8], and understanding AST data geared toward the practicing veterinarian (VET09) [9]. VET01, VET01S and VET04 are standards whereas VET02, VET03, and VET06 are guidelines. While a standard defines essential and specific requirements for methods, practices, and materials that must not be modified, a guideline describes criteria for general operating practices, procedures or materials for voluntary use [10]. The remaining CLSI documents VET05 and VET09 are educational reports to offer guidance on how to use veterinary AST data appropriately and to promote the understanding of veterinary AST data. The VET01S, containing the clinical breakpoints, is updated regularly, whereas the other documents are updated on a less regular schedule as needed.

The following sections describe the AST and interpretation of AST data, as well as the most frequent sources of errors and mistakes, and how to avoid them.

AST methodology

Several methods for phenotypic AST are currently available, producing data in the form of a minimal inhibitory concentration (MIC) value (in µg/mL or mg/L) or zone of inhibition diameter (in mm) of an antimicrobial agent for specific bacteria. These methods include agar disk diffusion, E-test, broth microdilution, broth macrodilution, and agar dilution. For the agar disk diffusion assay, an antimicrobial impregnated paper disk containing a defined amount of the antimicrobial agent is placed on an agar plate inoculated with a set concentration of bacteria. The antimicrobial agent diffuses into the surrounding medium and as a result, a concentration gradient develops around the disk with the highest concentration of the antimicrobial closest to the disk and decreasing concentrations in the periphery. After incubation of the inoculated bacteria, zones of growth inhibition become visible, the diameters of which are measured and compared with reference values, i.e., clinical breakpoints or epidemiological cut-off values (abbreviated either as ECVs or ECOFFs) (Fig. 1a). The E-test is also an agar diffusion test, but instead of a disk with a defined antimicrobial content, a strip is used that contains a concentration gradient of the antimicrobial agent to be tested. After the incubation period, an ellipsoid zone of growth inhibition develops around the strip and the MIC value will be read at the position where the visible bacterial growth hits the E-test strip (Fig. 1b). Serial dilution tests can be conducted using either liquid media, i.e., broth dilution, or solid media, i.e., agar dilution. Broth dilution tests are differentiated with regard to the volume of medium used. Thus, broth microdilution is performed with volumes of 50—100 µl in microtitre plates (Fig. 1c), while broth macrodilution uses volumes of 2—5 mL and is performed in test tubes (Fig. 1d). In agar dilution, agar plates that contain the respective amounts of the antimicrobial agent are used (Fig. 1e). In all dilution tests, a two-fold dilution series, including a concentration of 1 µg/mL [7, 10], of an antimicrobial agent is prepared in a test medium and a defined amount of bacteria is added and incubation for a given time under defined conditions is conducted. Thereafter, the MIC is read as the lowest concentration of the antimicrobial agent that prevents visible bacterial growth. Disk diffusion measurements are typically used only to categorize bacteria (i.e., susceptible) and the mm measurements are not reported. MICs determined by dilution methods may be used for categorization or for a more nuanced understanding of resistance and are usually reported.

Routinely in veterinary microbiological laboratories, AST is conducted either by commercial systems, such as VITEK® 2 (bioMérieux, La Balme les Grottes, France), BD Phoenix™ (BD Diagnostic Systems, Sparks, MD, USA), and Sensititre™ (Thermo Fisher Scientific, Waltham, MA, USA), or by conventional methods, such as agar disk diffusion or broth microdilution. Conventional methods may be recorded by automated instruments, such as the Sensititre™ Optiread™ System (Thermo Fischer Scientific, Waltham, MA, USA) or BIOMIC® V3 (Giles Scientific USA, Santa Barbara, CA, USA). A survey of AST methods used by veterinary diagnostic laboratories in the United States found Sensititre broth microdilution panels was the most widely used

commercial method [11]. Performance of AST by commercially available methods should follow the manufacturers' instructions, which should be validated against a standard method. Detailed information on how to perform AST of bacteria from animals by agar disk diffusion or broth microdilution is given in VET01 and can also be used if commercially available AST is not available or appropriate [2]. This document also contains detailed information on how to perform AST via broth macrodilution and agar dilution, two methods more often used in research and development. Other methods, such as the E-test, are used less commonly in routine veterinary diagnostics and have not been widely adopted.

Performance of any AST requires the user to follow the procedures exactly as described, either from the manufacturer, or from a standard method, like the VET01 [2]. In VET01S [3] as well as other documents, such as VET04 [5] and VET06 [6], summary boxes contain testing conditions for each bacterial order (e.g., Enterobacterales), genus (e.g., *Staphylococcus*) or species (e.g., *Pasteurella multocida*). The method summary lists the required (i) medium, (ii) inoculum preparation method, and (iii) incubation conditions. Examples of the testing conditions as adopted from VET01S are shown in Table 1.

The required medium may be different for different methods, for example disk diffusion requires Mueller–Hinton agar whereas broth dilution methods require cation-adjusted Mueller–Hinton broth. Certain bacteria, such as *Streptococcus* spp., may require the supplementation of the testing medium with sheep blood (5% v/v) or lysed horse blood (2.5–5% v/v). Other more fastidious bacteria, such as *Actinobacillus pleuropneumoniae* or *Histophilus somni*, require specific media that contain ingredients to support their growth, such as chocolate Mueller–Hinton agar or Mueller–Hinton fastidious broth medium with yeast extract (MHF-Y) [3]. Specific media are also required for AST of various fastidious bacteria from animals as outlined in the VET06 document [8]. The use of alternate media or medium supplements (e.g., serum from the target species) for testing, as recommended by some groups [12, 13] is not a standardized method and not acceptable for susceptibility testing, as changes to the medium may alter the in vitro activity of the antimicrobial agent(s) due to changes in pH or ion concentrations. Thus, use of interpretive criteria based on the standardized method is not appropriate, unless it is validated against the standard method. Use of these alternative media, without validation, also reduces the repeatability of the results, leading to results that cannot be compared across laboratories.

The inoculum describes the amount of bacteria used in the test system. The inoculum preparation method may

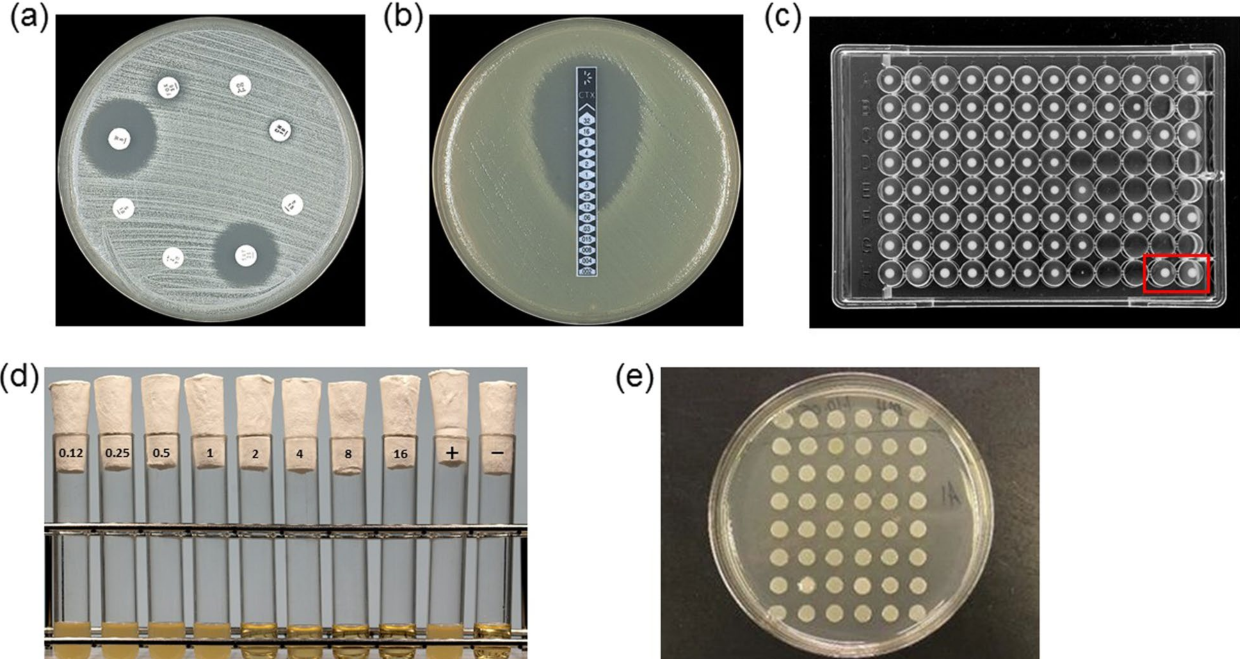


Fig. 1 Results obtained with the different AST methods: **a** agar disk diffusion. **b** E-test. **c** broth microdilution (twofold increasing antimicrobial concentrations from left to right in lines A–H; the boxed wells in the lower right-hand corner represent the growth controls). **d** broth macrodilution (twofold increasing antimicrobial concentrations from left to right in the tubes; the tubes marked as + and – represent the growth control and the sterility control, respectively). **e** agar dilution

Table 1 Testing conditions for Enterobacterales and *Actinobacillus* spp. according to the CLSI document VET01S [3]

Bacteria	Test parameters	Details
Enterobacterales	Medium	Disk diffusion: Mueller–Hinton agar
		Broth dilution: cation-adjusted Mueller–Hinton broth
		Agar dilution: Mueller–Hinton agar
	Inoculum	Broth culture method or colony suspension method, equivalent to a 0.5 McFarland standard
	Incubation	35 °C ± 2 °C; ambient air
		Disk diffusion: 16 – 18 h
		Broth dilution: 16 – 20 h
		Agar dilution: 16 – 20 h
Actinobacillus spp.	Medium	Disk diffusion: chocolate Mueller–Hinton agar
		Broth dilution: MHF-Y broth
		Agar dilution: chocolate Mueller–Hinton agar
	Inoculum	Colony suspension method, equivalent to a 0.5 McFarland standard using colonies from an overnight (18–24 h) culture on a chocolate agar plate incubated in 5% CO_2
	Incubation	35 °C ± 2 °C; 5% CO_2
		Disk diffusion: 20 – 24 h
		Broth dilution: 20 – 24 h
		Agar dilution: 20 – 24 h

be either the broth culture method or the direct colony suspension method. Usually, an inoculum density equivalent to a 0.5 McFarland turbidity standard, resulting in a colony count of $1-2\times10^8$ colony forming units (CFU)/mL for *E. coli* ATCC® 25922 [2], is used. For broth microdilution, this inoculum must be further diluted so that each well of the microtitre plate contains approximately 5×10^5 CFU/mL (range, $2-8\times10^5$ CFU/mL). For specific bacteria, such as *Trueperella pyogenes*, a slightly different inoculum of $1-9\times10^5$ CFU/mL has been recommended [6]. Appropriate inoculum preparation methods may vary depending on the bacterial species and, therefore, must be reviewed for each bacterium of interest. For example, *Staphylococcus* spp. should only be prepared by direct colony suspension method [14].

The incubation information comprises the incubation temperature, atmosphere and time. The incubation temperature is commonly at 35 °C ± 2 °C, but can also be higher at 42 °C for *Campylobacter jejuni*, *Campylobacter coli* and *Brachyspira hyodysenteriae* or lower at 28 °C – 30 °C for rapidly growing mycobacteria [6]. Bacteria causing diseases in fish usually require even lower incubation temperatures of 22 °C ± 2 °C as indicated for *Aeromonas salmonicida* or 18 °C ± 2 °C for *Flavobacterium psychrophilum* [5]. The incubation atmosphere can vary between ambient air (aerobic conditions) and 5% CO_2 (microaerophilic conditions). Certain bacteria, such as *C. jejuni* and *C. coli*, require a specific microaerophilic atmosphere equivalent to 10% CO_2, 5% O_2, and 85% N_2 [6]. Anaerobic incubation conditions must be used for obligate anaerobes, such as *Bacteroides* spp., *Fusobacterium* spp., *Clostridioides* spp., and *Clostridium* spp. The incubation time usually ranges between 16 – 24 h for most fast-growing bacteria, but may be extended to up to 48 h for *C. jejuni*, *C. coli*, and anaerobic bacteria, 96 h for *B. hyodysenteriae*, or 72–100 h for rapidly growing mycobacteria [6]. In some cases, specific combinations of antimicrobial agents and bacteria require a longer incubation time. For example, most antimicrobial agents tested for *Staphylococcus* spp. are read after 16–20 h; however, 24 h incubation periods are required before reading results for oxacillin and vancomycin by dilution methods. [3]. In the case of *Corynebacterium* spp. and coryneforms, 48 h of incubation is required for β-lactams in case the results after 24 h classify the isolates as susceptible [6]. As with other aspects of the standard method (medium, inoculum, etc.), the interpretive criteria for a specific bacterial species–antimicrobial agent combination were developed based on specific incubation conditions and any alterations to the standard method would result in interpretive criteria that are no longer applicable.

Most commonly observed errors and deviations from standards in terms of AST methodology are (i) the use of altered inoculum sizes, incubation times, incubation conditions, (ii) the use of media other than those required by standards or manufacturer protocols, (iii) lack of cation supplementation of the Mueller–Hinton broth, (iv) the use of growth supplements not recommended, (v) the use of a method for bacteria for which the respective method has not been approved, and (vi) the use of a self-made, non-approved method.

Because standard methods may change, it is important that researchers define which methods were used when publishing the findings of an AST study. Simply citing the use of a standard method document is not sufficient, particularly when testing a bacterial species for which no current breakpoints are established. Specifying the precise testing conditions allows for objective assessment of the study findings.

It is important to understand that different bacteria may need different AST methods and that even the same method (e.g., broth microdilution) applied to different bacteria may require different testing conditions. Thus, precisely following the standard methods, for example as described in the respective CLSI documents or manufacturer recommendations, is the easiest way to avoid errors and mistakes in the AST methodology and, therefore, in AST results.

Quality controls

Every diagnostic test method requires the use of quality controls to ensure the validity of the test. This also applies to AST. In AST, defined QC strains, which have proved to be extraordinarily stable, are used side-by-side with the test strains as a method control. These QCs take into account the variations between different media lots and laboratories and are, therefore, developed in interlaboratory trials including at least seven laboratories with at least one veterinary laboratory and ten independent tests per laboratory [4]. For QC purposes, only approved QC strains can be used. They cannot be replaced by laboratory-specific strains [7, 10]. Moreover, the QC strains must fit to the test strains with regard to the respective AST conditions (e.g., test type, test media, atmosphere, temperature). For example, when testing streptococci, the QC strain *Streptococcus pneumoniae* ATCC® 49619 must be used, as it requires the same media with blood supplementation. In cases, where no QC strain of the same genus as the tested bacteria is available, QC strains that show similar growth characteristics should be used, e.g., *Escherichia coli* ATCC® 25922 or *Staphylococcus aureus* ATCC® 25923 (disk diffusion) and *S. aureus* ATCC® 29213 (MIC) should be used for *Bordetella bronchiseptica*. For anaerobes, only anaerobic QC strains should be used [3]. In some cases, specific QC strains are used for the detection of specific resistance mechanisms [3].

Currently approved QC strains

A number of strains of the bacteria, such as *A. pleuropneumoniae*, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Clostridioides difficile*, *Enterococcus faecalis*, *E. coli*, *Eggerthella lenta* (formerly *Eubacterium lentum*), *H. somni*, *Klebsiella pneumoniae*, *Mannheimia haemolytica*, *Pseudomonas aeruginosa*, *S. aureus*, and *S. pneumoniae* are currently approved as QC strains by CLSI and other organizations and are listed in the respective CLSI and other methods documents [3, 5]. As these QC strains are available from different culture collections and every culture collection assigns its own strain number to a particular strain, conveniently, CLSI lists them by organism and associated strain number in the respective strain collections. The strain collections included are the American Type Culture Collection (ATCC®); the Culture Collection University of Gothenburg (CCUG); the Collection de l'Institut Pasteur (CIP); the Centre de Ressources Biologiques de l'Institut Pasteur (CRBIP); the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ); the Japan Collection of Microorganisms (JCM), and the National Collection of Type Cultures (NCTC). As such, the same *S. aureus* QC strain is listed in ATCC as 29213, in DSMZ as 2569, and in JCM as 2874. It should be noted that not all QC strains are available from all of the aforementioned strain collections and that at least some of these QC strains may be available from commercial sources. These QC strains must be used when applying AST methods described in the CLSI documents VET01 [2], VET03 [4], VET06 [6], and other methods documents. When commercial test systems are used for AST, it is necessary to refer to the manufacturer's instructions for QC test recommendations and QC ranges [3]. The same is true when performing AST by E-test as the QC ranges in VET01S are not approved for E-test applications. Routine QC recommendations for each of the bacteria are listed in CLSI document VET01S [3].

CLSI-approved QC ranges

The approved QC ranges for the aforementioned QC strains are also listed in separate tables, differentiated by zone diameter QC ranges for either nonfastidious or fastidious bacteria (Tables 4a and 4b in VET01S), and by MIC QC ranges for nonfastidious and fastidious bacteria (Tables 5a and 5b in VET01S) [3]. MIC QC ranges for anaerobes are separated by the AST method applied, i.e., agar dilution (Table 5c) and broth microdilution (Table 5d) [3]. Zone diameter and MIC QC ranges for testing aquatic bacteria are separated by test conditions and the AST method applied (Tables 4a–4d in VET04) [5].

It is noteworthy that, in contrast to EUCAST, which uses *S. aureus* ATCC® 29213 for both approaches, CLSI requires the use of different *S. aureus* QC strains for disk diffusion (*S. aureus* ATCC® 25923) and broth dilution methods (*S. aureus* ATCC® 29213). Moreover, the approved zone diameter QC ranges are only valid when antimicrobial disks with the approved content are used.

Thus, Tables 4a and 4b in VET01S [3] have one column each that provides the approved disk content for each antimicrobial agent. The approved zone diameter QC ranges vary between 3 mm (e.g., rifampin 5 µg disk and *E. coli* ATCC® 25922, 8–10 mm) and 11 mm (e.g., gamithromycin 15 µg disk and *H. somni* ATCC® 700025, 18–29 mm) [3]. The MIC QC ranges commonly include three or four consecutive dilution steps in a two-fold dilution series. In rare cases, the QC range is indicated as equal to or smaller than a certain value (e.g., $\leq$0.5/9.5 µg/mL trimethoprim/sulfamethoxazole and *S. aureus* ATCC® 29213) or equal to or larger than a certain value (e.g., $\geq$256 µg/mL spectinomycin and *P. aeruginosa* ATCC® 27853).

The most commonly made mistakes in terms of QC are (i) lack of QC data, (ii) replacement of approved QC strains by laboratory-specific strains, (iii) mistaken use of a QC strain for dilution methods rather than the QC strain for disk diffusion (and vice versa), e.g., *S. aureus* ATCC® 25923 (disk diffusion) and *S. aureus* ATCC® 29213 (MIC determination by dilution methods), (iv) use of QC ranges (approved for MIC determination by dilution methods) for E-test applications, (v) use of test concentrations that do not cover the acceptable QC range of the reference strains, (vi) testing of antimicrobial agents for which no acceptable QC ranges of the reference strains are available, and (vii) use of QC strains that are not related to the test strains (e.g., *P. aeruginosa* ATCC® 27853 for staphylococci or *S. aureus* ATCC® 29213 for anaerobes). By following the specifications given above, these mistakes can easily be avoided.

Interpretive criteria

AST performance standards usually contain interpretive criteria. These can be clinical breakpoints or ECVs. It is important to understand that an AST document and the interpretive criteria mentioned therein represent an entity. As the details provided in the different AST performance standards, e.g., CLSI and EUCAST, differ from each other, it is important that there will be no "mixing and matching" of test conditions and interpretive criteria. That means if AST is performed according to CLSI specifications, only CLSI-approved clinical breakpoints can be used. In cases where CLSI-approved testing conditions were used, but no CLSI-approved clinical breakpoints are available, e.g., a MIC clinical breakpoint for streptomycin, using the corresponding MIC clinical breakpoint from EUCAST would not be acceptable.

The purpose of the AST dictates whether clinical breakpoints or ECVs should be applied. If the goal is to determine which antimicrobial agents are most likely to lead to therapeutic success, clinical breakpoints should be used to interpret the MICs. This includes routine diagnostics, in which AST results will provide guidance to the veterinarians concerning the choice of the most suitable antimicrobial agents. When bacteria of animal origin are tested using CLSI or CLSI-validated commercial methods, veterinary-specific clinical breakpoints as listed in the documents VET01S [3] and VET04 [5] should be applied. In contrast, ECVs should be used when describing MIC or zone diameter distributions of bacteria without a clinical context (i.e., AMR monitoring or surveillance). The ECV is defined by CLSI [2] as the MIC or zone diameter value that separates microbial populations into those with and without acquired and/or mutational resistance based on their phenotypes (wild-type or non-wild-type, respectively). The ECV defines the highest MIC or smallest zone diameter for the wild-type population not expressing phenotypic resistance [2].

Requirements for setting clinical breakpoints and ECVs

As the utility of clinical breakpoints and ECVs (clinical vs. non-clinical uses) is different, so are the approaches to developing these different interpretive criteria. The determination of clinical breakpoints is a complex process which is outlined in detail in the document VET02 for CLSI-approved breakpoints [8]. Numerous sources of data play relevant roles in the determination of clinical breakpoints. They include (i) the dosage regimen, (ii) the route of administration, (iii) the antimicrobial agent's pharmacokinetic and pharmacodynamic parameters to evaluate the probability of target attainment to reach the desired index of those parameters, (iv) the results of clinical efficacy studies, and (v) the MIC values/zone diameters of the target bacterial pathogens. In this regard, transparency of data, especially the dosing regimen used, is critical. Appendix D in VET01S contains information on the dosing regimen in the target animal species on the basis of which each veterinary-specific clinical breakpoint was determined. For the determination of ECVs, the aforementioned clinical parameters are not included in the analysis. Instead, the determination of ECVs uses mathematical models to identify subpopulations based on the MIC distribution for that bacterial species–antimicrobial agent combination [15–17]. For this, EUCAST provides a freely available ECOFF (ECV) finder program (https://www.eucast.org/mic_and_zone_distributions_and_ecoffs). EUCAST clearly states that if this software is being used, all rules and conditions described in EUCAST SOP 10.2 should be followed [18]. To set ECVs, at least five data sets obtained by the same methodology from five different laboratories and comprising at least 100 individual measurements of either MICs or zone diameters are necessary [19–21].

Interpretive categories associated with clinical breakpoints and ECVs

There are also differences in the nomenclature of the interpretive categories associated with clinical breakpoints and ECVs. Clinical breakpoints classify bacteria as susceptible (S), susceptible dose-dependent (SDD), nonsusceptible (NS), intermediate (I), or resistant (R). The SDD category for veterinary pathogens was only established recently in 2023 for CLSI and will be included in the 7th edition of VET01S. These categories convey the likelihood of therapeutic success to the prescribing clinician. According to the CLSI documents VET01 [2] and M100 [Performance Standards for Antimicrobial Susceptibility Testing, 33rd Edition (2023)] [22], the definitions of the different categories are as follows:

> *"susceptible (S) – a category defined by a breakpoint that implies that isolates with a MIC at or below or zone diameters at or above the susceptible breakpoint are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used, resulting in likely clinical efficacy."*

> *"susceptible-dose dependent (SDD) – a category defined by a breakpoint that implies that susceptibility of an isolate depends on the dosing regimen that is used in the patient. To achieve levels that are likely to be clinically effective against isolates for which the susceptibility testing results (either MICs or zone diameters) are in the SDD category, it is necessary to use a dosing regimen (i.e., higher doses, more frequent doses, or both) that results in higher drug exposure than that achieved with the dose that was used to establish the susceptible breakpoint."*

> *"nonsusceptible (NS) – a category used for isolates for which only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains at the time when interpretive criteria are developed. Isolates for which the antimicrobial agent MICs are above or zone diameters are below the value indicated for the susceptible breakpoint should be reported as nonsusceptible. Here it is necessary to understand that when an isolate is classified as nonsusceptible, it does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution after the time the susceptible-only breakpoint was set. Moreover, the term "nonsusceptible" should not be used as a summary of the categories "intermediate" and "resistant". When summarizing populations of isolates that are in the combined categories of "intermediate" or "resistant", they should be called "not susceptible" rather than "nonsusceptible"."*

> *"intermediate (I) – a category defined by a breakpoint that includes isolates with MICs or zone diameters within the intermediate range that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. Here it is important to understand that the intermediate category implies clinical efficacy in body sites for which the drugs are physiologically concentrated or when a higher-than-normal dosage of a drug can be used. This category also serves as a buffer zone to prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins."*

> *"resistant (R) – a category defined by a breakpoint that implies that isolates with MICs at or above or zone diameters at or below the resistant breakpoint are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range in which specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in isolates with similar phenotypes."*

In contrast, the categories associated with ECVs are "wild-type" and "non-wild-type". According to the document VET01, these categories are defined as follows:

> *"wild-type (WT) – an ECV interpretive category defined by an ECV that describes isolates with no phenotypically detectable mechanisms of acquired resistance or reduced susceptibility for the antimicrobial agent being evaluated."*

> *"non-wild-type (NWT) – an ECV interpretive category defined by an ECV that describes isolates with presumed or known mechanisms of acquired resistance and reduced susceptibility for the antimicrobial agent being evaluated."*

Wild-type and non-wild-type subpopulations are usually characterized by bimodal (i.e., with two peaks)—or depending on the prevalent resistance mechanisms—sometimes also trimodal (i.e., with three peaks) or multimodal (i.e., with multiple peaks) distributions of MIC or zone diameter values. In bimodal distributions, the subpopulation with lower MICs or higher zone diameters

represents the wild-type subpopulation while the subpopulation with higher MICs or smaller zone diameters represents the non-wild-type population. If there are only unimodal MIC or zone diameter distributions, usually the respective bacteria represent the wild-type subpopulation. However, specific combinations of bacteria and antimicrobial agents show only unimodal MIC or zone diameter distributions, e.g., *B. bronchiseptica* and ampicillin, but virtually all isolates exhibit MICs or zone diameters that classify them as "intermediate" or "resistant" when clinical breakpoints are applied [23]. Reduced membrane permeability of *B. bronchiseptica* but also the presence of a species-specific β-lactamase (BOR-1) result in this unimodal distribution of ampicillin MICs and zone diameters for non-wild-type *B. bronchiseptica* isolates [24].

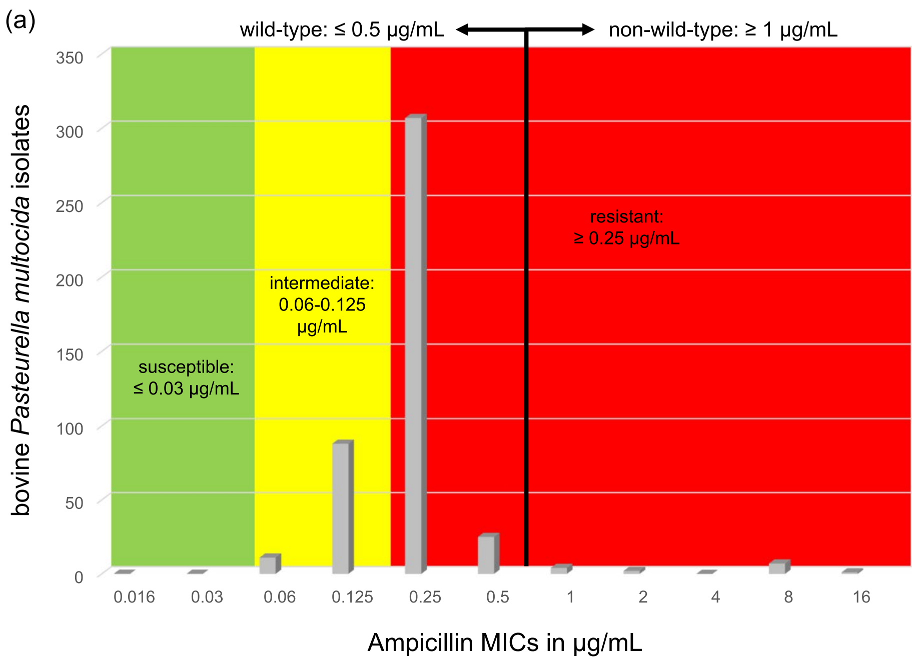
ECVs and clinical breakpoints may be different as shown for ampicillin and bovine *P. multocida* (Fig. 2a). On the other hand, ECVs and clinical breakpoints can be very similar or even the same as shown for tetracycline and bovine *P. multocida* (Fig. 2b). Ideally, clinical breakpoints and ECVs should be very similar to avoid confusion about significant acquired resistance vs. poor pharmacokinetics. The VAST subcommittee of CLSI makes every attempt to avoid splitting the wild-type distribution when setting clinical breakpoints. However, the committee has accepted multiple clinical breakpoints within the wild-type distribution. It is not uncommon for a clinical breakpoint to fall below the wild-type distribution, thus rendering most bacteria in the population resistant to the agent tested. In contrast to human clinical breakpoints, which would not accept this type of breakpoint, CLSI-VAST has accepted clinical breakpoints that will designate all isolates resistant as a negative clinical use recommendation. It is debatable whether or not this should be interpreted as "intrinsic resistance" because the wild-type bacterial population may still be inhibited at concentrations higher than the clinical breakpoint in other animal species or humans with more advantageous pharmacokinetics. CLSI publishes a separate table of intrinsic resistance among bacteria in their documents, and intrinsic resistance is described later. These types of clinical breakpoints must be carefully used when determining a bacterium's potential for significant multidrug resistance in infection control. Even if isolates in the non-wild-type category are likely to harbor either resistance genes or resistance-mediating mutations, it is important to understand that "non-wild-type" and "resistant" are not equivalent or interchangeable interpretive categories; the same applies to "wild-type" and "susceptible". As a consequence, it is a mistake to apply ECVs and then classify the isolates as resistant or susceptible. The terms "resistant" and "susceptible" should be reserved for interpretation using clinical breakpoints.

Additional relevant information about interpretive criteria

Interpretive criteria are not immutable. Clinical breakpoints and ECVs can be subjected to changes over time, and consequently, it is important to use the latest interpretive criteria and currently approved methods, like the latest editions of the CLSI standards. For example, changes in clinical breakpoints for fluoroquinolones used in dogs will be published in the 7th edition of VET01S. This update will show that some bacteria previously interpreted as "susceptible" to enrofloxacin and marbofloxacin will then be classified as "resistant". As a rule for presenting AST data, the range of concentrations tested, and MIC and zone diameter values measured should be presented rather than simply a percentage of isolates within each interpretive category. Researchers, diagnosticians and others reporting AST results are encouraged to retain MIC values or zone diameters to allow for later re-evaluation if clinical breakpoints or ECVs change over time. A meaningful comparison of percentages of resistance from different studies or different time periods requires that the same methodology and the same interpretive criteria have been used, or have undergone validation to the new standard.

It is also imperative to understand that every clinical breakpoint associated with a zone diameter was determined with a defined disk content. For example, the clinical breakpoints for erythromycin are only appropriate for a 15 µg disk. However, there are also commercially available erythromycin disks that contain 5 µg, 10 µg, or 30 µg. Results obtained with any other disk concentration cannot be interpreted using the approved clinical breakpoints. As the relationship between disk concentration and zone diameters is not necessarily linear, it is not possible to convert zone diameters obtained with 5 µg, 10 µg or 30 µg disks to zone diameters obtained with the approved 15 µg disk. Thus, AST using disk diffusion requires assuring the correct disks are being used, whether for susceptibility determination or quality control purposes.

The most frequently made mistakes in applying interpretive criteria are (i) use of outdated clinical breakpoints, (ii) use of clinical breakpoints not approved for the respective combination of bacterium/animal species/disease condition, (iii) use of clinical breakpoints from previously published literature and not from approved performance standards, (iv) use of clinical breakpoints for humans to interpret results from testing antimicrobial agents and bacteria in animals when veterinary-specific clinical breakpoints are available, (v) "mix and match" of clinical breakpoints and ECVs from different AST documents (e.g., CLSI, EUCAST, BSAC, CASFM) or even different



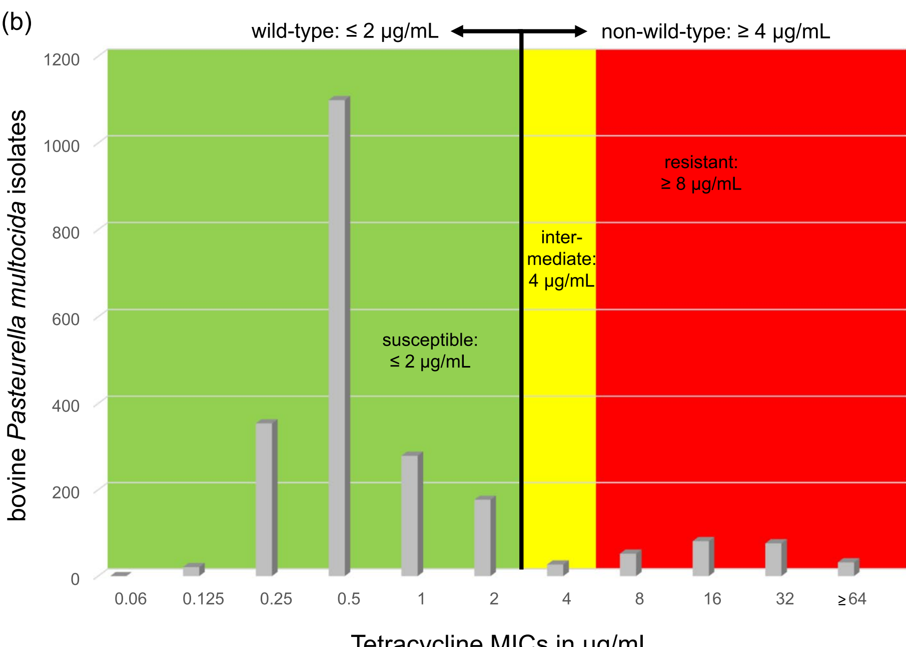


Fig. 2 Comparison of the categories susceptible, intermediate, and resistant (clinical breakpoints) as well as wild-type and non-wild-type (ECVs) using the combinations **a** ampicillin/bovine *P. multocida* and **b** tetracycline/bovine *P. multocida*

versions of AST documents, (vi) use of self-defined breakpoints, (vii) use of ECVs to interpret the bacteria as susceptible or resistant, (viii) use of antimicrobial disks with inappropriate disk concentrations, and (ix) mathematical conversion of zone diameters into MICs and vice versa. For the latter, it is not acceptable to measure a zone diameter and then try to convert it into a MIC value or vice versa. Although there is a certain association between zone diameters and MICs, this is not a linear association and cannot, therefore, be determined mathematically by a "rule of proportion" approach. The other mistakes can be prevented by carefully taking into account the information presented in the previous subsections.

Range of validity and extrapolation of clinical breakpoints

Clinical breakpoints are approved and valid for a specific combination of antimicrobial agent, dosing regimen, bacterium, affected organ system and animal species [3, 10]. Many veterinary-specific clinical breakpoints are listed in Tables 2A–M in the VET01S document [3]. CLSI-VAST has approved over 260 clinical breakpoints for antimicrobial agent–bacteria combinations which are available for common pathogens isolated from companion and livestock animals. Some antimicrobial agents must not be used clinically in a particular animal species or production class, but testing is important for public health considerations; in such cases, human clinical breakpoints may be appropriate for AMR surveillance purposes.

With regard to bacteria, the same clinical breakpoints can generally apply to all bacteria assigned to the same order (e.g., enrofloxacin clinical breakpoints for Enterobacterales in cats), to bacteria of a specific genus (e.g., tetracycline clinical breakpoints for *Staphylococcus* spp. from dogs), to bacteria of different species within the same genus (e.g., cephalexin clinical breakpoints for *Staphylococcus pseudintermedius* and *S. aureus* from dogs), to bacteria of different species of different genera within the same order (e.g., cefovecin clinical breakpoints for *E. coli* and *Proteus mirabilis* from urinary tract infections of dogs), or to bacteria of one specific species (e.g., spectinomycin clinical breakpoints for *P. multocida* from respiratory tract infections of cattle). There are exceptions to these rules. For example, although *P. mirabilis* is within the Enterobacterales order, it is intrinsically resistant to tetracyclines and should always be considered resistant to that class of antimicrobial agents, regardless of the AST result. Additional resources must always be consulted to determine whether exceptions to the rule exist for a particular organism/antimicrobial combination.

With regard to the affected organ system, approved clinical breakpoints are generally appropriate for systemic sites, but some are only appropriate for selected organs or systems (e.g., tilmicosin clinical breakpoints for *M. haemolytica* from respiratory tract infections of cattle, ampicillin clinical breakpoints for Enterobacterales from the lower urinary tract of dogs). It must be noted that protected sites, like the central nervous system, eye and prostate, may complicate breakpoint interpretation as the drug may not reach the site of infection despite the in vitro susceptibility of the bacteria. Clinical breakpoints may occasionally also be used for different organ systems (and different bacteria) within the same animal species (e.g., pradofloxacin clinical breakpoints for *S. pseudintermedius*, *S. aureus*, and *Staphylococcus felis* from respiratory tract or skin infections of cats).

With regard to the animal species, approved clinical breakpoints are commonly provided for a specific combination of bacterial and animal species (e.g., tiamulin clinical breakpoints for *A. pleuropneumoniae* from respiratory tract infections of swine). In rare cases, the same clinical breakpoints, although approved separately for each bacterial and animal species, may also apply to different bacteria affecting the same organ systems in different animal species (e.g., tulathromycin clinical breakpoints for *B. bronchiseptica* and *P. multocida* from respiratory tract infections of swine as well as *P. multocida* and *M. haemolytica* from respiratory tract infections of cattle).

CLSI generally sets clinical breakpoints only for antimicrobial agents that are approved for specific target bacteria and target organ systems in defined target animal species. Thus, the clinical breakpoint is tied to the approved dosage of a particular agent. There may be instances where the label dose is not utilized as at the time when it was approved because it was later determined to not be appropriate for treatment of certain infections. For instance, the label dose for penicillin G is 3000 U/lb (6,600 U/kg) in cattle, but the CLSI clinical breakpoint was determined using a higher dose of 22,000 U/kg for treatment of *P. multocida* respiratory infections. There are some antimicrobial agents, preferentially used in companion animals, that are not approved in all countries, but may be legal to use according to extralabel use guidelines or where no drug sponsor is available to present clinical breakpoints to the CLSI-VAST. These examples and the rationale for their use has been described [25]. In these instances, the CLSI-VAST has a working group (Generic Drug Working Group) dedicated to considering these older and extralabel antimicrobial agents for which clinical breakpoints have not been developed. Over 60% of the clinical breakpoints published by CLSI-VAST since 2015 were proposed by this working group using the guidelines provided in VET02 [8]. Examples (listed in reference [25]) include cefazolin injection in dogs and horses, doxycycline and minocycline in dogs and horses, ceftazidime injection in dogs, piperacillin-tazobactam in dogs, amikacin in dogs and horses, and levofloxacin in dogs.

In monitoring and surveillance studies, there can be more flexibility depending on the purpose of the study. Bacteria other than those listed on the approved label and for which clinical breakpoints are not approved may be evaluated for their susceptibility against a defined panel of antimicrobial agents, including those that have clinical and public health significance, such as vancomycin or linezolid in *Staphylococcus* and *Enterococcus* spp. For example, the German national resistance monitoring program GE*RM*-Vet has a defined set of microtitre plates that are

applied for Gram-positive and Gram-negative bacteria. The antimicrobial agents included in these microtitre plates also comprise some for which no approved clinical breakpoints are available. As a consequence, isolates cannot be assigned to the categories S-I-R. Further examples for this can be seen in two studies published by Feßler and co-workers [26, 27]. This situation also applies to other monitoring or surveillance studies, as that of the China antimicrobial resistance surveillance network for pets (CARPet) [28].

Extrapolation of clinical breakpoints approved for one bacterial species may be possible for other related bacterial species. For this, the VET09 document (currently in revision for the 2nd edition) contains a number of chapters with examples of which extrapolations may and may not be recommended for clinical breakpoints approved in different animal species [9]. For example, canine-specific clinical breakpoints approved for *E. coli* may be extrapolated to other Enterobacterales (except when intrinsic resistance is present) and canine-specific clinical breakpoints approved for *P. aeruginosa* may be extrapolated to other *Pseudomonas* spp., but not to other nonfermenting Gram-negative bacterial species [9].

The application of animal-specific clinical breakpoints to other infection sites in the same animal species is problematic when there are barriers to antimicrobial drug diffusion, such as in the brain or the prostate. Clinical breakpoints and interpretive categories derived for skin and soft-tissue infections in a defined animal species can generally be extrapolated and applied to infections in other body sites in that animal species. That is, a clinical breakpoint listed for "skin-soft tissue" can usually be applied to other tissues in the body in which the free drug concentration freely communicates with extracellular fluid (the site of infection). Exceptions include sites with tissue barriers, such as the brain, prostate, eye, bovine udder, and epithelial lining of the respiratory tract. Because drugs excreted in the urine are physiologically concentrated in this fluid, CLSI has separate and generally higher clinical breakpoints for some antimicrobial agents used to treat urinary tract infections. These include for example the clinical breakpoints for aminopenicillins (ampicillin, amoxicillin) and cephalosporins applicable to urinary tract infections in dogs and cats. In general, clinical breakpoints applicable to urinary tract infections or cases of mastitis should not be extrapolated to other organ systems [9].

CLSI-VAST generally sets clinical breakpoints only for antimicrobial agents that are approved for specific target bacteria and target organ systems in defined target animal species. Thus, the clinical breakpoint is tied to the approved dosage of a particular agent. The application of animal-specific clinical breakpoints to other doses, routes of administration, frequencies, or durations of therapy is problematic. Dose, route, frequency, and duration of therapy are integral components of clinical breakpoint determination, because these factors determine the concentrations of the antimicrobial agent in the animal species. Thus, it is not appropriate to indiscriminately apply an approved clinical breakpoint for an antimicrobial agent to any other dose, route, frequency, or duration [9]. For some specific antimicrobial agents, CLSI-VAST has most recently approved clinical breakpoints in the SDD category to allow for higher doses at these MIC values. For some agents, the intermediate interpretive category can imply that a higher dose can be considered for some infections [29].

The application of human or other species' clinical breakpoints for interpreting AST results of a defined animal species, for which no animal-specific clinical breakpoints are available, should always be done with caution and preferably in consultation with persons with expertise in clinical pharmacology or clinical microbiology [9].

Intrinsic and acquired resistance, multidrug resistance, and other commonly used terms in monitoring/surveillance studies

Two basic types of resistance need to be distinguished: intrinsic resistance and acquired resistance.

Intrinsic resistance is a property shared by all members or strains of a bacterial genus or a bacterial species, respectively, often due to either the absence or the inaccessibility of the target structures for the different antimicrobial agents in the respective bacteria. Examples for these types of intrinsic resistance are (i) the resistance of cell wall-free bacteria, such as *Mycoplasma* spp., to antimicrobial agents which interfere with cell wall synthesis, such as β-lactams, (ii) cephalosporin resistance in *B. bronchiseptica* due to the inability of cephalosporins to penetrate the outer membrane, and (iii) colistin resistance in *P. mirabilis* and *Serratia marcescens* due to alterations of the outer membrane that modify the charge of the LPS so that colistin cannot bind [24, 30, 31]. In addition, intrinsic resistance can also be based on efflux systems, such as the AcrAB-TolC system, which are inherent to certain bacteria and can pump specific or multiple antimicrobial agents out of the bacterial cell [30, 32]. Furthermore, intrinsic resistance can be due to the production of species-specific drug-inactivating enzymes, such as the BOR-1 β-lactamase in *B. bronchiseptica* or the AmpC β-lactamase in *E. coli* [30, 33]. Finally, certain bacteria, such as enterococci, can use exogenous folates and as a consequence do not need to have their own functional folate synthesis pathway. Thus, enterococci are considered intrinsically resistant to folate pathway

inhibitors, such as sulfonamides and trimethoprim [34]. In the VET01S document, Appendix B provides examples of intrinsic resistance known to occur in Gram-positive and Gram-negative bacteria of importance in veterinary medicine [3].

Acquired resistance is a strain-specific property which may be based on resistance-mediating mutations or resistance genes that specify numerous resistance mechanisms that result in a previously effective antimicrobial agent being no longer effective [35]. In contrast to intrinsic resistance, acquired resistance is often transferable across strain, species and sometimes even genus boundaries and, thereby, contributes to the development of antimicrobial resistance or multidrug resistance. When considering multidrug resistance, it should only refer to acquired resistance [10, 36].

Lastly, there is a type of resistance where the clinical breakpoint will categorize all bacteria as resistant regardless of the presence of acquired resistance. The basis of the "resistance" is the poor pharmacologic parameters of the antimicrobial agent in a specific animal species. These clinical breakpoints are used to communicate to veterinarians that these antimicrobial agents should not be used to treat infections. An example includes the clinical breakpoint for amoxicillin-clavuanate for Enterobacterales for skin and soft tissue infections in cats.

Prior to 2018, there was no proposed universal veterinary-specific definition for multidrug resistance of bacteria from animals leading to inconsistency and confusion for clinicians, researchers and surveillance efforts [10]. In 2018, Sweeney and co-workers published definitions for multidrug resistance (MDR), extensive drug resistance (XDR) and pandrug resistance (PDR) applicable to clinically significant livestock and companion animal bacterial pathogens [37]. The basic requirement for assigning an isolate of animal origin to any of these categories is (i) only inclusion of acquired resistance (ii) the availability of a veterinary-approved AST method for the bacterium and the antimicrobial agent in question, and (iii) species-specific clinical breakpoints [37]. For the latter, it should be noted that distinctly more species-specific clinical breakpoints are available for dilution methods than for agar disk diffusion [3]. An isolate is classified as multidrug-resistant when it is not susceptible to at least one antimicrobial agent in three or more antimicrobial classes while an isolate is classified as extensive drug-resistant when it is not susceptible to at least one antimicrobial agent in all but one or two antimicrobial classes. When an isolate is not susceptible to all antimicrobial agents in all antimicrobial classes, it is classified as pandrug-resistant.

Unfortunately, gaps in veterinary-specific clinical breakpoints continue to challenge our ability to apply these recommendations to many animal species/antimicrobial agent/bacteria combinations, and alternative criteria may need to be used. However, any criteria must be clearly, and transparently reported regardless of approach. Currently, CLSI clinical breakpoints for sufficient numbers of antimicrobial classes are available for the following disease/pathogen combinations: (i) bovine respiratory disease due to *M. haemolytica*, *P. multocida*, and *H. somni* (seven classes each), (ii) swine respiratory disease due to *A. pleuropneumoniae* (seven classes), *P. multocida* (six classes), and *Streptococcus suis* (five classes), (iii) canine skin and soft tissue infections due to *Staphylococcus* spp. (six classes), *Streptococcus* spp. (five classes), and *E. coli* (five classes), (iv) canine urinary tract infections due to *E. coli* (five classes), as well as (v) equine respiratory tract infections due to *Streptococcus equi* subsp. *zooepidemicus*, *Streptococcus equi* subsp. *equi*, and *E. coli* (five classes each) (Table 2). It must also be recommended to review the relationship of the clinical breakpoint to the ECV of the bacteria of interest to avoid over- or under-estimation of resistance. For example, ampicillin clinical breakpoints for canine skin and soft tissue infections are designed to categorize all *E. coli* strains as resistant due to poor pharmacologic parameters and the clinical breakpoint falls below the MIC distribution of the wild-type population. This breakpoint would not be appropriate to use for MDR definitions. Conversely, the ceftiofur clinical breakpoint for *P. multocida* is 2 µg/mL, but the wild-type ECV is ≤ 0.06 µg/mL, therefore, potentially underestimating resistance development which may yet not be clinically impactful but of concern.

The assignment of isolates to the MDR, XDR, or PDR categories is commonly based on the results of phenotypic AST results, mainly on those obtained by broth microdilution. However, molecular methods, including whole genome sequencing, are becoming more commonplace and affordable and genotypic approaches must also be considered. There are numerous databases that can be used to investigate whole genome sequences for the presence of resistance-mediating mutations, such as PointFinder [38], or resistance genes, such as ResFinder [39], CARD [40], AMRFinder [41], ARG-ANNOT [42] or MEGARes [43]. The definitions of MDR, XDR and PDR must be based on the number of classes of antimicrobial agents to which an isolate shows resistance, regardless of the number of resistance-mediating mutations and/or resistance genes present in that isolate [7, 10, 36]. This is of particular importance as genes that confer resistance to members of three or more antimicrobial classes have not only been described in bacteria of human origin, but also in those from animal sources. Examples for such "multiresistance genes" include the *erm* genes which

Table 2 Animal diseases and bacteria involved for which species-specific clinical breakpoints are available so that MDR, XDR and PDR definitions can be applied

Animal disease and bacterial species	Antimicrobial classes for which clinical MIC breakpoints are available	Multidrug-resistance (MDR)	Extensive drug-resistance (XDR)	Pandrug-resistance (PDR)
BRD: *M. haemolytica*, *P. multocida*, *H. somni*	aminocyclitols, penicillins, cephalosporins, fluoroquinolones, macrolides, phenicols, and tetracyclines	Not susceptible to at least one agent in ≥ three antimicrobial classes	Not susceptible to at least one agent in all but one or two antimicrobial classes	Not susceptible to all agents in all antimicrobial classes
SRD: *A. pleuropneumoniae*	penicillins, cephalosporins, fluoroquinolones, macrolides, phenicols, pleuromutilins and tetracyclines			
SRD: *P. multocida*	penicillins, cephalosporins, fluoroquinolones, macrolides, phenicols, and tetracyclines			
SRD: *Streptococcus suis*	penicillins, cephalosporins, fluoroquinolones, phenicols, and tetracyclines			
Canine SSTI: *Staphylococcus* spp.	aminoglycosides, penicillins, cephalosporins, fluoroquinolones, tetracyclines, and lincosamides			
Canine SSTI: *Streptococcus* spp.	aminoglycosides, penicillins, cephalosporins, fluoroquinolones, and lincosamides			
Canine SSTI: *E. coli*	aminoglycosides, penicillins, cephalosporins, fluoroquinolones, and tetracyclines			
Canine UTI: *E. coli*	aminoglycosides, penicillins, cephalosporins, fluoroquinolones, and tetracyclines			
Horse RTI: *Streptococcus equi* subsp. *zooepidemicus*; *Streptococcus equi* subsp. *equi*	aminoglycosides, penicillins, cephalosporins, fluoroquinolones, and tetracyclines			
Horse RTI: *E. coli*	aminoglycosides, penicillins, cephalosporins, fluoroquinolones, and tetracyclines			

BRD Bovine respiratory disease, *SRD* Swine respiratory disease, *RTI* Respiratory tract infection, *SSTI* Skin and soft tissue infection

confer resistance to macrolides, lincosamides and streptogramin B antibiotics, the *vga*, *lsa* and *sal* genes which confer resistance to lincosamide, pleuromutilin and streptogramin A antibiotics and *cfr* genes which confer resistance to phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A antibiotics [44, 45].

Other important parameters, commonly used in monitoring and surveillance studies are the MIC range, MIC_{50}, and MIC_{90}. The range represents the area between the lowest and the highest measured MIC values [7, 10]. The MIC_{50} and MIC_{90} values describe the MICs at which at least 50% or 90%, respectively, of a given bacterial population are inhibited in their growth or killed by a defined antimicrobial agent or combination of antimicrobial agents. These values can be determined by simple enumeration or by mathematical calculation. For this, the MIC_{50} value is calculated as $(n \times 0.5)$ and the MIC_{90} is calculated as $(n \times 0.9)$. If these values do not represent an integer, the values must be rounded up to the next integer and the MIC at this position represents the MIC_{50} or MIC_{90}, respectively [7]. It is necessary to understand that the significance of the MIC_{50} and MIC_{90} values increases with the size of the test population. In small test populations ($n < 30$), single isolates with high MIC values may have a disproportionately high influence, especially on the MIC_{90} value [7, 10], so the reporting of MIC ranges or frequency of isolates at individual MICs is highly encouraged.

Last but not least, there is a linguistic issue that needs attention. In various publications and lectures, the authors speak of "resistant genes", "resistant phenotypes" or "resistant plasmids". This is not correct since resistant is an adjective that characterizes a property of the subsequent noun and neither the genes nor the phenotypes or plasmids are resistant. The correct wording, which should be used in publications and presentations, is "resistance genes", "resistance phenotypes" or "resistance plasmids".

Concluding remarks

AST of bacteria from animal sources and subsequent interpretation of the results can be challenging and subject to multiple errors and mistakes. The correct performance of approved AST methods and the use of the recommended quality controls is required. When the obtained results are classified into interpretive categories, it is important to use the correct interpretive criteria (clinical breakpoints or ECVs) and the appropriate categories associated with them based on the purpose of the testing. It is critical that approved standards for antimicrobial agents and bacteria from animals are used and followed precisely. CLSI-VAST is currently the leading standard-setting organization in approving breakpoints for AST of bacteria from animals. In addition, the assignment of bacterial isolates as MDR, XDR, or PDR as well as the calculation of MIC_{50} and MIC_{90} values must be used appropriately as they can be sources of misinterpretation. The information included in this review is intended to provide guidance to researchers and diagnosticians in the correct performance of AST and the correct interpretation of the results and, thereby, help providing the most accurate AST data for clinical, public health and research use.

Acknowledgements
We are grateful to Lori Selden for her support.

Disclosure
Many of the authors are voluntary (unpaid) members of the CLSI-VAST subcommittee. The views expressed in this article do not necessarily reflect those of X.-Z.L.'s affiliation, Health Canada. This article reflects the views of the authors and should not be construed to represent FDA's views or policies.

Authors' contributions
A.T.F., Y.W., J.S. and S.S. wrote the first draft. All authors reviewed, revised, and approved the final report.

Funding
This study did not receive external funding.

Availability of data and materials
All the data supporting the conclusions of this article is included within the article.

Declarations

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no conflicts of interest. Authors Yang Wang, Jianzhong Shen and Stefan Schwarz are Editors-in-Chief of One Health Advances; they were not involved in the journal's review and decisions related to this manuscript.

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Received: 26 July 2023 Revised: 4 September 2023 Accepted: 7 September 2023
Published online: 06 November 2023

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