Antimicrobial resistance: standardisation and harmonisation of laboratory methodologies for the detection and quantification of antimicrobial resistance

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Summary
The Ad hoc Group of experts on antimicrobial resistance of the Office International des Epizooties has developed a guideline on the standardisation and harmonisation of laboratory methodologies used for the detection and quantification of antimicrobial resistance. The existing methods (disk diffusion [including concentration gradient strips], agar dilution and broth dilution) are reviewed, including a comparison of their advantages and disadvantages. The definitions of resistance characteristics of bacteria (susceptible, intermediate and resistant) are addressed and the criteria for the establishment of breakpoints are discussed. Due consideration has to be given to these aspects in the interpretation and comparison of resistance monitoring or surveillance data. The use of validated laboratory methods and the establishment of quality assurance (internal and external) for microbiological laboratory work and the reporting of quantitative test results is recommended. Equivalence of different methods and laboratory test results is also recommended to be established by external proficiency testing, which should be achieved by the means of a reference laboratory system. This approach allows the comparison of test results obtained using different methods generated by laboratories in different countries.
Introduction

The objective of this document is to review currently used antimicrobial susceptibility testing methodologies and protocols and to encourage the Member Countries of the Office International des Epizooties (OIE) to initiate standardisation and harmonisation of bacterial antimicrobial susceptibility testing and results. The similarities, differences, advantages and disadvantages of accepted standardised antimicrobial susceptibility testing methods are described. Additionally, the requirements of each antimicrobial susceptibility testing method are discussed (equipment, training, resources and quality assurance). The need for internal quality control and external proficiency testing is emphasised. Standardisation and harmonisation of antimicrobial susceptibility testing methodologies are critical if data is to be compared among the international surveillance/monitoring programmes of OIE Member Countries.

Background

There is increasing international concern regarding both the potential transfer of antimicrobial resistant bacteria between animals and humans and of resistance genes from animal strains of bacteria to human bacterial pathogens. Concern about antimicrobial resistance in relation to animal health is also growing. In response to these concerns, antimicrobial resistance testing initiatives, together with surveillance and monitoring programmes focusing on zoonotic bacterial pathogens and enteric commensals in animals have been initiated in numerous countries throughout the world (3, 5, 21). Data generated from these surveillance and monitoring programmes will eventually play a key role in the development of national, and perhaps international polices for the containment of antimicrobial resistant bacterial pathogens from animals and their immediate environments. The need to compare susceptibility testing data between laboratories in different countries necessitates a re-examination of the standardisation and harmonisation of the antimicrobial susceptibility testing (AST) methods currently in use worldwide (12).

Historically, veterinarians and medical practitioners selected effective antimicrobials based on past clinical experiences. However, with an observed increase in bacterial resistance to regularly used antimicrobials, it has become gradually more difficult for clinicians to empirically select an appropriate antimicrobial agent (13, 24). As a result, laboratory in vitro AST of the relevant bacterial pathogens from properly collected specimens is currently standard procedure (13, 17, 25).

Antimicrobial susceptibility testing was initiated in many countries world-wide soon after the introduction of antimicrobials for treatment of bacterial diseases (12). Rapid bacterial identification systems and subsequent improvements in AST in both human and veterinary clinical laboratories were primarily driven by the need to identify the appropriate antimicrobials for successful clinical use. Additionally, the need for laboratory reproducibility of AST methods arose to ensure that data generated was technically accurate and consistent. This required that AST laboratories adopt quality control measures to guarantee the reporting of reliable and reproducible susceptibility data (9, 17). Although protocols for bacterial identification, AST and data analysis developed very rapidly, standardisation and validation of these three procedures is relatively recent compared to the progress achieved in analytical chemistry. Historically, most laboratories have employed disk diffusion methods for AST. Reported results can be quantitative if zone diameters are recorded, but they are generally reported qualitatively as either susceptible, intermediate, or resistant (9, 14, 17, 25). In the past few years, many laboratories have adopted either broth microdilution or agar dilution methods (9, 11). Results from these assays may be quantitative, in that they provide the minimal concentration of an antimicrobial required to inhibit the growth of the test organism (minimum inhibitory concentration [MIC]), as well as providing a qualitative description (susceptible, intermediate and resistant). Some laboratories have not been as successful in adopting these methods, primarily due to training and financial limitations. Additionally, the need to develop and implement quality assurance programmes for bacterial identification and AST is a fairly new concept which may take time to implement. However, some initiatives have been introduced and are currently underway in an attempt to standardise and/or harmonise AST.

In veterinary and human medicine, antimicrobial resistance data is being shared between a number of laboratories through the creation of antimicrobial resistance surveillance networks. Some of these networks are linked internationally. This has resulted in the standardisation and harmonisation of AST methods between participating laboratories. Participating laboratories adhere to strict standards of AST and quality control monitoring to ensure accuracy and comparability of the data. Examples of international and national surveillance...
systems employing standardised methods include the European Antimicrobial Resistance Surveillance System (EARSS), the Alexander Project for Respiratory Pathogens, Antibiotic Resistance in Bacteria of Animal Origin (ARBAO), SENTRY, the Surveillance Network (TSN), the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP), the World Health Organization Network on Antimicrobial Resistance Monitoring (WHONET), Enter-Net and the National Antimicrobial Resistance Monitoring System (NARMS) (3, 15, 16, 21, 22). The success of these surveillance and monitoring programmes suggests that standardisation and harmonisation of AST methods are both conceivable and progressing globally.

Although in the past few years there has been a move to standardise AST methods within countries, the move to harmonise methods and susceptibility data among countries has not been initiated on a global scale for bacteria originating from animals. One significant obstacle is the fact that there is no international monitoring system for AST that utilises a single methodology with identical quality control organisms. To obtain comparable antimicrobial susceptibility data from different laboratories in the same country, or in different countries, laboratory methodologies need to be standardised and harmonised. This can be best accomplished if the antimicrobial susceptibility data collected is quantitative (i.e. MIC, zone diameters), rather than qualitative for comparison purposes. Data to be used for epidemiological surveillance purposes must be reported quantitatively in order to both detect shifts in antimicrobial susceptibility in bacterial strains and be comparable with other surveillance programmes.

Quantitative in vitro bacterial antimicrobial susceptibility testing is essential for the purpose of monitoring shifts in susceptibility to antimicrobial agents. However, to achieve its aim, testing must be performed according to standardised testing methods. Comparison of the frequency of antimicrobial resistance in bacterial pathogens among the many countries that have surveillance systems in place is difficult for many reasons. Antimicrobial susceptibility testing currently serves two purposes, firstly to provide meaningful results to the clinician and secondly to monitor shifts in susceptibility of targeted bacterial populations (12). Historically, laboratories have been restricted in reporting bacterial AST data as ‘susceptible, intermediate or resistant’. Bacterial antimicrobial susceptibilities reported this way are primarily for the immediate needs of physicians or veterinarians as guidelines for appropriate antimicrobial therapies. Taking into account the different AST protocols and interpretive criteria among the numerous testing methods and guidelines available, it is evident that this type of reporting excludes any possibility for the comparison of susceptibility data. Unfortunately, there is no world-wide consensus on interpretive criteria for susceptibility testing. Additionally, the emphasis of many surveillance programmes is to monitor shifts in antimicrobial susceptibilities in target bacterial pathogens. Since there are no standardised dilution schemes available world-wide, it becomes difficult to compare susceptibility profiles of bacterial pathogens from different countries.

Standardisation and harmonisation of AST methods are needed for meaningful comparisons of quality and accurate susceptibility data between individual OIE Member Countries involved in both national and international surveillance programmes. This will be best accomplished by the use of accurate and reliable standardised AST methods in conjunction with monitoring of AST performance with defined quality control bacterial strains among participating laboratories. If results achieved with different AST methods are to be presented side by side, then comparability of results must be demonstrated and consensus on interpretation achieved. It is essential that AST methods provide reproducible results in day-to-day laboratory use and that the generated data be comparable to those results obtained by an acknowledged ‘gold standard’ reference method. In the absence of standardised methods or reference procedures, susceptibility results from different laboratories cannot be adequately compared with assurance.

Antimicrobial susceptibility testing methodologies

It is essential that the bacteria subjected to AST be isolated in pure culture from the submitted sample. The isolation procedure for that particular bacterium should be standardised so that the subject bacteria are consistently and correctly identified to the genus and/or species level. When possible, bacterial isolates should be stored for future analysis via either lyophilisation or cryogenic preservation at −70°C to −80°C. Once the bacterium has been isolated in pure culture, the inoculum must be standardised to obtain accurate susceptibility results, since variations may substantially affect both the qualitative and quantitative endpoint determinations. Other factors influencing AST methods that require standardisation and harmonisation include the composition of the agar and broth media used (pH, cations, thymidine or thymine, use of supplemented media), content of antimicrobial agent in the carrier (disk, strip, tablet), growth and incubation conditions (time, temperature, oxygen), agar depth and the subsequent interpretive criteria (17, 18, 24). For these reasons, special emphasis needs to be placed on reference procedures and standardised methods, as sufficient reproducibility can be attained only through standardisation.

The decisions regarding which antimicrobials to test can be difficult given the vast numbers of antimicrobials available. Testing all antimicrobial agents is neither necessary (since numerous antimicrobials have similar, if not identical, in vitro activities), nor practical (given the economic restraints faced by laboratories). This is further discussed in Antimicrobial resistance: harmonisation of national antimicrobial resistance
monitoring and surveillance programmes in animals and in animal-derived food, later in this volume).

A wide variety of bacterial AST methodologies are being used by microbiological laboratories around the world. The selection of an AST methodology may be based on numerous factors, such as ease of performance, flexibility, adaptability to automated or semi-automated systems, cost, reproducibility, reliability, accuracy and national preference. However, only three primary methods have been shown to be reproducible and repeatable. These are disk diffusion (including concentration gradient strips), broth dilution and agar dilution.

Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into solid culture media seeded with a standardised bacterial inoculum. The diffusion of the antimicrobial agent into the seeded culture media results in an antimicrobial gradient. When the concentration of the antimicrobial becomes so dilute that it can no longer inhibit the growth of the test bacterium, a zone of inhibition is formed. The edge of this zone of inhibition correlates with the MIC for that particular bacterium/antimicrobial combination. In other words, the zone of inhibition correlates inversely with the MIC of the test bacterium. The larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms. However, the MIC cannot always be easily determined using disk diffusion methods, due to the variation of the tested antimicrobial agent concentration at the edge of the zone of inhibition for each drug-bacterium combination (9, 13). It should be emphasised that disk diffusion tests based solely on the presence or absence of a zone of inhibition without regard to the size of the zone of inhibition are not acceptable. Disk diffusion is technically straightforward to perform, reproducible, and does not require expensive equipment. The main advantages of the disk diffusion method are the low cost and the ease in modifying test formats when needed. Although disk diffusion is the simplest and most cost-effective method for AST, many aspects of this method require standardisation, as mentioned previously. Additionally, manual measurement of zones of inhibition may be time-consuming, making this method impractical for some laboratories (2). However, automated zone reading devices are available which can be integrated with laboratory reporting and data handling systems (2, 13). It is important to remember that no more than twelve disks should be placed on one 150 mm agar plate, and no more than five disks on a 100 mm plate (18). Regardless of the number of disks placed on the agar surface, the disks should be distributed evenly so that they are no closer than 24 mm from centre to centre (18). Additionally, bacterial antimicrobial MICs can be obtained from commercially available gradient strips which diffuse a pre-formed antibiotic concentration (4). However, the use of strips containing antimicrobials at predefined concentrations can be very expensive and MIC discrepancies can be found when compared with agar dilution results (4).

The aim of the broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial that inhibits the growth of the bacterium being tested (MIC, usually expressed in mg/ml or mg/l). However, the MIC does not always represent an absolute value. The ‘true’ MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration (18). Antimicrobial ranges should be utilised that encompass both the interpretive criteria (susceptible, intermediate and resistant) and quality control reference organisms. Additionally, laboratory results should take into consideration the antimicrobial concentrations that are achievable in vivo for a specific bacteria/antibiotic combination. Antimicrobial susceptibility dilution methods appear to be more reproducible and quantitative than agar disk diffusion, although antibiotics are usually tested in doubling dilutions which can produce inexact MIC data (11). Any laboratory that intends to use a dilution method and set up its own reagents and antibiotic dilutions must have the ability to obtain, prepare and maintain appropriate stock solutions of reagent grade antimicrobials and to generate working dilutions on a regular basis. It is then essential that such laboratories utilise quality control organisms to assure accuracy and standardisation of their procedures.

Broth dilution is a technique in which a standardised suspension of bacteria is tested against varying concentrations of an antimicrobial agent (usually doubling dilutions) in a standardised liquid medium. The broth dilution method can be performed either in tubes containing a minimum volume of 2 ml (macrodilution) or in smaller volumes using microtitration plates (microdilution) (18). Numerous microtitre plates containing prediluted antibiotics within the wells are commercially available. The use of these plates with a standardised protocol, including appropriate quality control reference strains, is the most likely choice to achieve standardisation of AST world-wide. Additionally, the use of identical lots of microdilution plates may eliminate potential errors that may arise due to preparation and dilution of the antimicrobials in participating laboratories (18). However, due to the fact that most broth microdilution test panels are prepared commercially, they can be considered less flexible than agar dilution or disk diffusion in adjusting to the changing needs of the surveillance/monitoring programme. Additionally, purchasing the equipment and antimicrobial panels can be quite costly and may not be a choice for laboratories with limited budgets.

Agar dilution involves the incorporation of an antimicrobial agent into an agar medium in a geometrical progression of concentrations, followed by the application of a defined bacterial inoculum to the agar surface of the plate. This results in the accurate determination of a MIC for the test bacterium/antimicrobial combination. Agar dilution methods offer several advantages; these include a greater control of the purity of the test bacterium and the ability to test multiple bacteria on the same set of agar plates and at the same time.
Another attractive benefit of this technique is the potential to improve the identification of MIC endpoints and extend the antibiotic concentration range as far as necessary. Additionally, it is the only recommended standardised antimicrobial susceptibility testing method for many fastidious organisms, such as anaerobes, Helicobacter and Campylobacter species (14).

Agar dilution can also be adapted to semi-automation. Commercially available inoculum-replicators are available and these can transfer between thirty-two and thirty-seven different bacterial inocula to each agar plate (18). Agar dilution is referred to as the ‘gold standard’ of AST; however, the technique requires extensive training of personnel and may be more expensive and labour-intensive than other testing methods.

Routine AST methods are best standardised for aerobic and facultative bacteria and antimicrobial agents that are intended for systemic use. These methods have not been standardised and in some cases are not recommended for uncommon or fastidious bacteria, due to potential inaccurate results. Regardless of the AST method used, the procedures must be standardised to ensure accurate and reproducible results. Additionally, appropriate quality control reference organisms need to be tested every time AST is performed, to ensure accuracy of the data. Clearly, the appropriate AST choice will ultimately depend on the growth characteristics of the bacterium in question, for example, disk diffusion should not be used to test anaerobes, Campylobacter, or other bacteria with considerable strain-to-strain variability in growth rates (14, 25).

Given the many biological and technical variables that may influence AST, standardisation is essential for the correct interpretation of generated results. Lastly, if one of these standardised AST methods is to be adopted by a laboratory of an OIE Member Country where it has not been previously used, programmes should be developed to educate and train the appropriate technical staff.

In special circumstances, novel test methods and assays may be more appropriate for detection of particular resistance phenotypes than the standardised AST methods described above. For example, chromogenic cephalosporin-based tests (e.g. nitrocefin) or equivalent methods may provide more reliable and rapid results for beta-lactamase determination in certain bacteria compared to traditional AST methods (18). Extended-spectrum beta-lactamase (ESBL) activity in certain bacteria can also be detected by using standard disk diffusion susceptibility test methods utilising specific cephalosporins (cefotaxime and ceftazidime) in combination with a beta-lactamase inhibitor (clavulanic acid) and measuring the resulting zones of inhibition (20). Additionally, chloramphenicol resistance attributed to production of chloramphenicol acetyl transferase (CAT) can be detected in some bacteria via rapid tube or filter paper tests within 1 h to 2 h (18).

Interpretation of antimicrobial susceptibility testing results

The objective of in vitro AST is to predict the way in which a bacterial pathogen may respond to the antimicrobial agent in vivo. The results generated by bacterial in vitro antimicrobial susceptibility tests, regardless of whether disk diffusion or dilution methods are used, are generally reported as resistant, susceptible or intermediate to the action of a particular antimicrobial. Resistant implies that the bacterium would not respond to treatment with that particular antimicrobial agent at the usually achievable systemic concentrations and/or possesses a specific resistance mechanism. Susceptible implies that the antimicrobial agent should be successful in treating the bacterial infection with the recommended dosage. Intermediate indicates that the antimicrobial agent may be successful in treating the bacterial infection if high levels of the agent can be achieved at the site of infection. The term intermediate also indicates a buffer zone, which prevents bacterial strains exhibiting borderline susceptibility from being misconstrued as resistant. Similarly, it can serve to indicate that treatment failure may occur, even though the strains exhibit MICs which are below the theoretical treatment levels for a particular antimicrobial. These designations are obtained by determining in vitro breakpoints, those MICs or zones of inhibition at which a bacterium is considered to be susceptible, intermediate or resistant, based on both obtainable serum concentrations of the antimicrobial agent administered at therapeutic doses and through clinical trials (17, 24). A susceptible breakpoint implies that the recommended dosage of the antimicrobial agent will attain serum or tissue concentrations adequate to inhibit the growth of the bacterium in vivo. Intermediate breakpoints represent 'buffer zones', in which unforeseen laboratory technical problems inadvertently categorise a susceptible bacterium as resistant, or vice versa. Resistant breakpoints represent those antimicrobial concentrations that cannot be achieved in the host using normal dosing regimes.

Two primary factors enable a bacterium to be interpreted as susceptible or resistant to an antimicrobial agent. The first factor is the development and establishment of quality control (QC) ranges, using diffusion when possible and dilution testing, for QC micro-organisms. This is essential for validating the specific AST method used. The QC ranges for the QC micro-organisms must be established prior to the development of the second factor, which is the determination of the appropriate interpretive criteria. The determination of the interpretive criteria involves the generation of three distinct pieces of data, population distribution of relevant micro-organisms, pharmacokinetic parameters of the antimicrobial agent, and results of clinical trials and experience (1, 24). Interpretation of the data involves creating a scattergram from the bacterial population distribution (300-600 representative bacterial isolates), by plotting the zone of inhibition against the MIC for
each bacterial pathogen and calculating a linear regression line (19). The selection of breakpoints is then based on multiple factors, including regression line analysis, bacterial population distributions, error rate bounding, pharmacokinetics, and ultimately, clinical verification (1, 18, 24).

Antimicrobial susceptibility breakpoints derived by professional societies or regulatory agencies in various countries are often very similar. However, there can be notable breakpoint differences among different countries for the same antimicrobial agent. These differences may be due to many factors, such as variation in technical AST factors (inoculum density, test media and test method), and the fact that different countries use different dosages or administration intervals for some antimicrobials. Some countries are also more conservative in setting interpretive criteria for specific antimicrobials. Additionally, it is important to remember that interpretive criteria developed for human clinical medicine are not always relevant for veterinary use, as pharmacokinetics, pharmacodynamics, and relevant infectious agents may differ significantly (18). The development of a concept known as ‘microbiological breakpoints’, which is based on the population distributions of the specific bacterial species tested, may be more appropriate for some antimicrobial surveillance programmes. In this case, bacterial isolates that deviate from the normal susceptible population would be designated as resistant, and shifts in susceptibility to the specific antimicrobial/bacterium combination could be monitored.

Standardisation and harmonisation of antimicrobial susceptibility testing methodologies

The most effective approach for the local, national and international surveillance of antimicrobial resistance would be for all participating OIE Member Country laboratories to use a common AST method, including similar quality control reference organisms and ranges. However, since there are several variations in methodologies, techniques and interpretive criteria currently being used, this will not be an easy task. A number of guidelines are currently available for antimicrobial susceptibility testing and subsequent interpretive criteria throughout the world. These include standards and guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS), the Japan Society for Chemotherapy (JSC), the Swedish Reference Group for Antibiotics (SIR), Deutsches Institut für Normung (DIN), Comité de l’Antibiogramme de la Société française de Microbiologie (CASFM), Werkgroep richtlijnen gevoeligheidsbepalingen (WRG system, the Netherlands), the British Society for Antimicrobial Chemotherapy (BSAC) and others (6, 7, 8, 10, 18, 26). Because of the variations in diffusion and dilution AST methods and the differing interpretive criteria among the many countries (i.e. choice of agar medium, inoculum size, growth conditions and susceptibility breakpoints), comparison of susceptibility data from one system to another is difficult. Additionally, as mentioned earlier, the majority of the interpretive criteria was developed by AST of bacteria and antimicrobials relevant to human medical pharmacokinetics and there are few breakpoints for many veterinary antimicrobials that may be included in surveillance and monitoring programmes. These data may also not be directly applicable to veterinary medicine in terms of standardisation of testing of animal bacterial isolates.

It appears that only the NCCLS has developed protocols for susceptibility testing of bacteria of animal origin and determination of interpretive criteria (18, 19). However, protocols and guidelines are available for susceptibility testing for similar bacterial species which cause infections in humans. It is possible that such guidelines can be adopted for susceptibility testing for bacteria of animal origin, but each country must evaluate its own AST standards and guidelines. Additionally, efforts focusing on harmonisation of susceptibility breakpoints on an international scale are progressing. These efforts have primarily focused on the adoption of the standards and guidelines of the NCCLS, which provide laboratories with standardised methods and quality control values enabling comparisons of AST methods and generated data. For those OIE Member Countries that have not standardised AST methods, the adoption of NCCLS guidelines and standards would be an appropriate initial step.

To determine the comparability of results originating from different surveillance systems from OIE Member Countries, antimicrobial susceptibility test results must be reported quantitatively, including information on the methods, quality control organisms and ranges tested. Also essential is agreement upon which micro-organisms are to be susceptibility tested (e.g. Campylobacter species, for which no susceptibility testing methods have currently been published and the choice of antimicrobials to be tested is under discussion). Minimum inhibitory concentration values or zone diameters should be the desired outcome of AST testing to be able to determine shifts in antimicrobial susceptibility among the target bacterial pathogens. This can be achieved by either broth or agar dilution methods, or by statistical transformation of the zone of inhibition diameters obtained by disk diffusion methods to MICs (1). Quantitative data can then be transformed into contingency tables or histograms for comparative purposes and analysis. Regardless of the AST method used, laboratories engaged in antimicrobial susceptibility testing must give high priority to both producing and reporting technically accurate data. Additionally, susceptibility data should be stored electronically in databases, when possible, with additional descriptive information regarding the origin of bacterial strains tested and other appropriate details.
Quality control and quality assurance of antimicrobial susceptibility testing

The implementation of quality control in laboratories that perform AST aims to help to monitor the precision and accuracy of the AST procedure, the performance of the appropriate reagents, and the personnel involved (18). Strict adherence to standardised techniques in conjunction with quality control of media and reagents is necessary for the collection of reliable and reproducible antimicrobial susceptibility data from OIE Member Country laboratories. Records should be kept regarding lot numbers and expiration dates of all appropriate materials and reagents used in AST. The appropriate quality control reference bacteria must also be tested to ensure standardisation regardless of the AST method used. Reference bacterial strains to be used for quality control should be catalogued and characterised with stable defined antimicrobial susceptibility phenotypes (18). These quality control strains should also encompass resistant and susceptible ranges of the antimicrobials to be assayed. Laboratories involved in AST need to use identical or similar quality control reference strains. Reference strains should be kept as stock cultures from which working cultures are derived and should be obtained from national or international culture collections (e.g. American type culture collection [ATCC]). If possible, the preferred method for analysing the overall performance of each laboratory is to test the appropriate quality control bacterial strains on each day that susceptibility tests are performed (18).

Because this may not always be practical or economic, the frequency of such quality control tests may be reduced if the laboratory can demonstrate that the susceptibility testing procedures are reproducible. If a laboratory can document the reproducibility of the susceptibility testing methods used, testing may be performed on a weekly basis (18). If quality control errors emerge, the laboratory has a responsibility to determine the cause(s) and repeat the tests. If the laboratory cannot determine the source of error(s), then quality control testing should be re-initiated on a daily basis (18).

Recognised quality control strains should be tested each time a new batch of medium or plate lot is used and on a regular basis in parallel with the bacterial strains to be assayed. Reference bacterial strains should be stored at designated centralised or regional laboratories. Appropriate biosecurity issues should be addressed in obtaining and dispersing quality control reference strains to participating laboratories. The use of such strains will allow for comparison of antimicrobial susceptibility data among the many surveillance systems in place among OIE Member Countries. OIE Member Country laboratories should ultimately base quality control testing on factors and circumstances specific to their needs and within reason. However, without the appropriate quality control testing, susceptibility data derived from antimicrobial surveillance and monitoring systems will be of limited value.

External proficiency testing (e.g. third party testing) of participating laboratories should be initiated for major bacterial species included in national surveillance systems and should be mandatory. Designated national laboratories should be appointed or established to monitor quality assurance of the participating surveillance laboratories. The responsibilities of the reference laboratory may include the development of a set of reference bacterial strains with varying antimicrobial susceptibilities to be sent to the participating laboratories to ensure the accuracy and precision of the AST methods and results. The participating laboratories will test these strains under their normal AST conditions. Proficiency testing on a regular basis would become one of the foundations of quality assurance for participating laboratories in a surveillance programme and ensure that reported susceptibility data is accurate (21).

Future directions in antimicrobial resistance detection

The most recent and perhaps the state-of-the-art approach for detection of certain bacterial antimicrobial resistance phenotypes is via identification and characterisation of the known genes that encode specific resistance mechanisms. Methods that employ the use of genetic probes, nucleic acid amplification techniques (e.g. polymerase chain reaction [PCR]), and deoxyribonucleic acid (DNA) sequencing offer the promise of increased sensitivity, specificity and speed in the detection of specific known resistance genes (14, 17). These genotypic methods are important supplements to traditional phenotypic methods, e.g. for the verification of methicillin resistance in staphylococci, vancomycin resistance in enterococci, and detection of fluoroquinolone resistance mutations (14, 17, 23). Additionally, recent technological advances may facilitate the ability to probe bacterial species for large numbers of antimicrobial resistance genes rapidly and at low cost, thereby providing additional relevant data for surveillance and monitoring programmes.

Recommendations

To standardise AST methods and achieve comparability of antimicrobial susceptibility test results between OIE Member Countries, the following recommendations are presented:

- standardised antimicrobial susceptibility testing methods and harmonisation of susceptibility data (including interpretive criteria) are essential for national and international surveillance comparisons in OIE Member Countries
standardised AST methods and similar interpretive criteria must be accepted and used by all participating laboratories in surveillance and monitoring programmes

it is essential that all data, regardless of the AST method, be reproducible and reported quantitatively if comparisons are to be drawn on a world-wide scale between surveillance programmes

establishment of national or regional designated laboratories is essential for co-ordination of AST methodologies, interpretations and quality controls

microbiological laboratories must conduct their work under internal quality assurance

it is desirable for laboratories to become accredited, where applicable, and to participate in external proficiency testing programmes

specific bacterial reference/quality control strains, with varying susceptibility ranges (susceptible, intermediate and resistant), are essential for determining intra- and inter-laboratory quality assurance and proficiency testing

interpretive criteria should be determined, developed and internationally agreed upon for commonly encountered bacteria, especially zoonotic pathogens such as Salmonella and Campylobacter

coordination, where appropriate, with other international organisations (Food and Agriculture Organization, World Health Organization) and/or regional organisations (e.g. European Committee on Antimicrobial Susceptibility Testing, NCCLS) may be important in providing support for standardisation and harmonisation of AST methodologies and data among OIE Member Countries.

Résumé
Le Groupe ad hoc d’experts sur l’antibiorésistance créé par l’Office international des épidémiologies a élaboré une ligne directrice sur la standardisation et l’harmonisation des méthodes de laboratoire appliquées à la détection et à la quantification de l’antibiorésistance. Les auteurs analysent les méthodes existantes (diffusion sur disque [avec les bandes de gradients de concentration], dilution en gélose et dilution en bouillon de culture) et comparent leurs avantages et inconvénients respectifs. Ils définissent les caractéristiques correspondant au classement des bactéries du point de vue de leur résistance (sensibles, intermédiaires et résistantes) et discutent les critères relatifs à la détermination des valeurs critiques. Tous ces aspects doivent être pris en compte lors de l’interprétation et de la comparaison des données de suivi ou de surveillance de la résistance. Il est recommandé de recourir à des méthodes de laboratoire validées, d’établir des programmes d’assurance qualité (interne et externe) pour les travaux microbiologiques en laboratoire et de communiquer les résultats des
épreuves quantitatives. Il est également conseillé de faire établir l’équivalence entre les différentes méthodes et résultats de tests de laboratoire par des évaluations externes des performances qui devraient être conduites par un réseau de laboratoires de référence. Cette approche permettrait de comparer les résultats des tests obtenus par différentes méthodes dans des laboratoires de divers pays.

Mots-clés

Resistencia a los antimicrobianos: normalización y armonización de los métodos de laboratorio para detectar y cuantificar la resistencia a los antimicrobianos


Resumen
El Grupo Ad hoc de expertos sobre la resistencia de las bacterias a los productos antimicrobianos, creado por la Oficina Internacional de Epizootias, ha elaborado una directriz sobre la normalización y armonización de los métodos de laboratorio para detectar y cuantificar la resistencia a los productos antimicrobianos. Los autores pasan revista a las técnicas existentes (difusión en disco [incluidas las tiras de gradiente de concentración], dilución en agar y dilución en caldo) y comparan sus respectivas ventajas e inconvenientes. También definen las categorías de bacterias en función de su resistencia (susceptibles, intermedias y resistentes) y examinan los criterios para determinar los valores críticos, aspectos que conviene tener en cuenta a la hora de interpretar y comparar datos procedentes del seguimiento o la vigilancia de las resistencias. Los autores recomiendan utilizar métodos de laboratorio validados y someter a procesos (internos y externos) de garantía de calidad tanto el trabajo microbiológico como los informes sobre resultados de pruebas cuantitativas. Recomiendan asimismo que se establezcan equivalencias entre distintos métodos y resultados de laboratorio mediante pruebas externas de eficiencia, proceso en el que ha de intervenir un sistema de laboratorios de referencia. Esta fórmula serviría para comparar los resultados obtenidos mediante métodos diversos y por laboratorios de países distintos.

Palabras clave
References


