Diagnostic microbiology in veterinary dermatology: present and future

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Background – The microbiology laboratory can be perceived as a service provider rather than an integral part of the healthcare team.

Objectives – The aim of this review is to discuss the current challenges of providing a state-of-the-art diagnostic veterinary microbiology service including the identification (ID) and antimicrobial susceptibility testing (AST) of key pathogens in veterinary dermatology.

Methods – The Study Group for Veterinary Microbiology (ESGVM) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) identified scientific, technological, educational and regulatory issues impacting the predictive value of AST and the quality of the service offered by microbiology laboratories.

Results – The advent of mass spectrometry has significantly reduced the time required for ID of key pathogens such as Staphylococcus pseudintermedius. However, the turnaround time for validated AST methods has remained unchanged for many years. Beyond scientific and technological constraints, AST methods are not harmonized and clinical breakpoints for some antimicrobial drugs are either missing or inadequate. Small laboratories, including in-clinic laboratories, are usually not adequately equipped to run up-to-date clinical microbiologic diagnostic tests.

Conclusions and clinical importance – ESGVM recommends the use of laboratories employing mass spectrometry for ID and broth micro-dilution for AST, and offering assistance by expert microbiologists on pre- and post-analytical issues. Setting general standards for veterinary clinical microbiology, promoting antimicrobial stewardship, and the development of new, validated and rapid diagnostic methods, especially for AST, are among the missions of ESGVM.

Introduction

In veterinary medicine, the microbiology laboratory is perceived as a service provider rather than an integral part of the healthcare team, resulting in limited interaction between microbiologists and clinicians. This differs from human medicine, where microbiologists interact with infectious disease specialists to provide advice on antimicrobial therapy, infection control, antimicrobial stewardship practices, antimicrobial resistance trends and compliance with antimicrobial guidelines. The use of diagnostic microbiology is comparatively lower than in human medicine, although differences exist between countries and veterinary practices. This difference is attributable to structural, economic and cultural factors that differentiate the veterinary healthcare system from the human counterpart. The limited utilization of microbiology tests in veterinary practice has negative consequences on the costs, with these being as much as three times higher than the costs of comparable tests in the human healthcare sector. Formal antimicrobial stewardship programmes, which traditionally involve microbiology laboratories in human hospitals, are rarely implemented by veterinary clinics. Antimicrobials are mainly used empirically and the use of antimicrobial susceptibility testing (AST) is generally limited to difficult cases with poor response to initial therapy. This trend is unfortunate given the current concerns regarding antimicrobial use and emergence of multidrug-resistant bacteria in animals, including companion animals. Use of culture and AST to guide antimicrobial choice is recommended.
by numerous guidelines on responsible antimicrobial use developed by governmental, animal health and veterinary organizations, including the European Commission, the World Organization of Animal Health (OIE) and the American Veterinary Medical Association (AVMA). As demonstrated in human medicine, implementation of antimicrobial stewardship at the clinic level has positive consequences on appropriate antimicrobial use, control of antimicrobial resistance and patient care.

Quality and quality control are important in clinical microbiology. International standards and manuals for clinical microbiology are available but their use is, for the most part, voluntary, although some guidelines have been adopted by accrediting organizations as part of their accreditation requirements. Uniform guidelines for best practice are not widely available for veterinary clinical microbiological laboratories; in general, accredited laboratories have implemented the guidelines for human clinical microbiology laboratories. Furthermore, there is an increasing trend for veterinary clinics to perform in-house microbiology. Despite the advantages of reduced turnaround time and costs, there are also disadvantages and risks associated with this practice. The microbiological expertise required to accurately perform and interpret the diagnostic tests, as well as to perform routine quality control and manage the biohazard risks, are lacking in most in-clinic and small diagnostic laboratories.

The aim of the Study Group of Veterinary Microbiology (ESGVM), established within the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), is to promote state-of-the-art veterinary clinical microbiology. This review highlights some of the current challenges in veterinary microbiology and outlines the quality standards required with particular reference to veterinary dermatology.

State-of-the-art methodologies

Microbe identification

Classic culture-based methods have been the mainstay of clinical microbiology for the past century. Automated systems are being implemented, but to date most of these technologies rely on pure culture of the microorganism. Identification (ID) of the micro-organism is an important prerequisite before AST to distinguish between potentially pathogenic micro-organisms and possible contaminants from the commensal microbiota on nonsterile body sites. Microbial ID has traditionally been performed by testing biochemical properties of the micro-organism. A step forward was achieved with the development of standardized commercial test systems (e.g. API®, rapiD®), which have gradually replaced the use of in-house tube tests, enabling diagnostic laboratories to use a validated manual system without expensive hardware. The next step was to offer these tests in more or less automated versions to avoid subjective interpretation (e.g. VITEK® Systems, BD Phoenix™ Automated Systems, TREK Sensititre® Diagnostic Systems). The quality of these systems in veterinary microbiology is strongly dependent on the databases used. Species found commonly in human microbiology, such as Pseudomonas aeruginosa, are well represented within the databases of these ID systems and therefore reliably identified. However, some species of veterinary relevance, including Staphylococcus pseudintermedius and Staphylococcus felis, are very difficult to reliably identify and differentiate from closely related staphylococci. Additionally, as the biochemical activity of a strain depends on growth, micro-organisms that do not grow in these systems cannot be identified (e.g. some members of Pasteurellaceae) and the ID may not be reliable for some micro-organisms (e.g. Malassezia) if the patient is under treatment with antimicrobials at the time of specimen collection.

New technologies have been introduced in recent years to overcome the disadvantages of biochemical ID. One technology that has gained increasing attention in veterinary microbiology is MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) mass spectrometry (MS) (Figure 1). This technique identifies any culturable bacteria within minutes and has low running costs. For most fungi a somewhat more complex sample preparation is necessary, but even dermato phytes can be identified with this method within 2 h. Again, identification depends on database entries, but the ability to discriminate between different bacteria is generally very good for most species. In general, the available databases are much broader than any of the former biochemistry based databases, but still some veterinary specific entries are lacking. The databases are updated...
regularly and each laboratory can add entries to the database. This approach has been shown to be successful for the *Staphylococcus intermedius* group (SIG), which is of special importance in the field of dermatology. Of course, a prerequisite for database expansions are strict protocols for quality control that must be followed to ensure highly reliable entries. In general, confirmation of the respective strains by sequencing before addition to the database is necessary. In human medicine, MALDI-TOF MS is used for direct ID of bacteria in blood cultures. Similar applications for direct ID in veterinary clinical specimens have not yet been developed. The main disadvantage of this technology is the high cost for purchasing and servicing the instrument, which makes it unaffordable by small diagnostic laboratories. However, the actual cost of the test is extremely low and alliance between laboratories may be used to make this technology accessible without every laboratory buying the instrument.

Another technology, DNA sequencing, is widely used as a research tool to investigate bacterial evolution and molecular epidemiology; at the time of writing this is not frequently employed in routine clinical microbiology. Recently, more advanced sequence-based techniques have become available. Isolated and purified microorganisms can be identified by Whole Genome Sequencing (WGS) over 24 h, and publicly available web tools are available for multi-locus sequence typing (MLST) and ID of acquired antimicrobial resistance genes using raw WGS data. Direct sequencing of DNA extracted from clinical specimens enables bacteria ID in polymicrobial samples and reduces diagnostic times to 24 h. DNA sequencing technologies are rapidly evolving and becoming more affordable, but widespread implementation in veterinary microbiology laboratories in the near future probably is limited to larger laboratories.

**Antimicrobial susceptibility testing**

Broth micro-dilution and disk diffusion are the most widely used methods for AST. Broth micro-dilution is the gold standard method for AST and the only method for which an internationally accepted ISO standard exists (ISO 20776-1, 2006). The principle of this method is simple. Broth suspensions containing the test strain are added to wells containing two-fold dilutions of antimicrobials. Upon incubation, the minimum inhibitory concentration (MIC) is read for each antimicrobial as the lowest concentration inhibiting visible bacterial growth, and used for interpretation of susceptibility. The method can be highly automated and is generally performed using commercial panels with a fixed composition of antimicrobials. Disk diffusion, also known as the Kirby–Bauer method, is performed by streaking broth containing the test strain on an agar plate followed by applying antimicrobial-impregnated disks. Upon incubation, inhibited bacterial growth around each disc is measured as a zone diameter and used for interpretation of susceptibility. This method is cheaper and more flexible than broth micro-dilution, as the user can easily change the antimicrobials between tests. It is, however, less robust and reproducible, and semi-quantitative in nature as it only indicates whether the test strain is susceptible (S), intermediate (I) or resistant (R). Laboratories have to select the most appropriate antimicrobials for routine AST based on bacterial species, breakpoint availability, animal species, infection site and available guidelines. The major shortcoming of both methods is turnaround time (approximately 48 h) from culture of the clinical specimen to reporting of the results. Both methods must be performed following quality standards (e.g. inoculum density and size, media, incubation conditions, etc.) that are set by two international committees; namely the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), and various national committees. To date, only CLSI provides clinical breakpoints and interpretive criteria for veterinary pathogens. A veterinary subcommittee of EUCAST (VetCAST) recently has been established with the purpose of harmonizing AST in Europe as well as on a global scale (http://www.euCAST.org/organization/subcommittees/ve-tcast/).

Alternative technologies are currently being evaluated to reduce the turnaround time of AST. Real-time PCR assays have been developed for rapid detection of resistant bacteria of high clinical relevance such as metillin-resistant *Staphylococcus aureus* (MRSA) directly from specimens. MALDI-TOF MS can be employed for rapid detection of extended-spectrum beta-lactamase (ESBL)-producing bacteria in blood cultures through quantification of beta-lactam degradation products. Flow cytometry is a method used for detection of morphological and metabolic changes of cells, for example upon antimicrobial exposure. This method has been tested for rapid AST of various organisms, and one study demonstrated the potential for detecting ESBL in 3 h from pure bacterial cultures.

WGS is not yet as rapid as these two other methods but offers the advantage of enabling screening of all known resistance genes by a single analysis, and it requires little hands-on time. WGS provides information on the presence of resistance genes, allowing prediction of antimicrobial susceptibility. High (99.7%) accordance between pheno- and genotypic resistance was demonstrated between 100 bacterial isolates belonging to four different species, and the same predicted susceptibility profiles have been obtained using direct sequencing on clinical specimens and sequencing of single isolates. The disadvantage of WGS is that it fails to reveal as yet undescribed resistance genotypes, and the actual phenotype may not always be deduced from sequencing data. For example, detection of nonfunctional pseudogenes or repressed efflux systems may lead to false positive (R) results.

**Point-of-Care testing**

Point-of-Care (PoC) tests are diagnostic tests that can be performed with the patient, therefore reducing turnaround time. The tests are based on different technologies, predominantly immunochromatography, agglutination assays and real-time PCR. A rapid immunoassay for PoC detection of urinary tract infection in dogs (RapidBac™ Vet; http://www.rapidbacvet.com/) has a high sensitivity (97.4%) and specificity (98.8%) for identification of clinical bacteriuria. A limited number of commercial PoC tests
are available for on-site AST in veterinary clinics. A simple diagnostic system (Speed-Biogram; https://www.bvt.fr/en/home/diagnostic-solutions/pour-le-veterinaire-praticien/infectious-diseases/main/gamme-speed/speed-biogram-1.html) has become available and can perform simultaneous ID and AST on cutaneous and ear specimens within 24–48 h. The main disadvantage is that the inoculum might be polymicrobial and cannot be standardized, leading to possible false resistance or false susceptibility reporting, which may also arise with disk diffusion testing.

Direct AST of clinical specimens (e.g., urine), without prior isolation of bacterial colonies, has the advantage of making results available earlier but this is controversial because of concerns regarding its accuracy. A human study demonstrated a 93% agreement between direct and conventional AST. The highest percentage of discordance (13%) was observed for β-lactam antimicrobial drugs such as amoxicillin clavulanate and cephalosporins. Similar results have been reported for another PoC test designed for direct ID and AST of uropathogens (Flexicul+ Vet; http://www.ssidiagnostica.dk/da/Produkter/Substrater/Flexicult-Vet-URINKIT). In human medicine, direct AST is recommended only for critically ill patients and does not replace conventional AST, which is additionally performed to confirm the preliminary results obtained by direct AST. Accordingly, ESGVM recommends that PoC tests are sent to accredited laboratories for AST by validated methods. In some countries (e.g., France), PoC tests are not permitted for AST of critical antimicrobial drugs (e.g., fluoroquinolones and higher generation cephalosporins) due to test limitations. Conversely, PoC tests are not permitted for AST of critical antimicrobial drugs (e.g., fluoroquinolones and higher generation cephalosporins) due to test limitations. Conversely, PoC tests may be useful for rapid detection of negative samples and susceptible strains, avoiding the time and the cost of laboratory analysis.

**Current challenges in veterinary diagnostic microbiology**

**Specimen management**

Improper specimen management impacts on both the diagnosis and outcome of therapy. Microbiology laboratories should provide information to ensure the appropriate selection, collection, storage and transportation of clinical specimens. National and international guidelines provide detailed information on the best sample type, sampling technique and transport conditions for bacterial infections. For superficial bacterial folliculitis, pustular contents and papule biopsies are optimum. Swabs of crusts and epidermal collarettes result in a higher risk of contamination with commensal skin surface bacteria. For wound infections, the type of specimen and sampling technique depend on the wound type. In general, biopsy samples obtained after initial debridement and cleansing are the most useful for determining the microbial load and the presence of relevant pathogens. Fluid samples obtained by aseptic needle aspiration may be used for cavity wounds (e.g., pressure sores) and cutaneous abscesses. The value of wound swabs even after cleansing a wound prior to sampling is questionable. Visible contamination, however, should be removed before a sample is collected.

Usually a single lesion is sampled and relatively few colonies are used by the laboratory for both ID and AST. Recent studies have demonstrated, however, that multiple strains with distinct antimicrobial resistance profiles may occur in the same lesion or in different lesions from the same patient. Further evaluation to assess the magnitude and clinical significance of this phenomenon is indicated. In theory, the involvement of multiple strains from canine skin infections is plausible given the frequent carriage of multiple S. pseudintermedius strains in dogs. Primary isolation using commercial selective agar plates may be performed in addition to nonselective isolation on blood agar to facilitate detection of meticillin-resistant staphylococci occurring at low numbers in mixed cultures. Unless anaerobic bacteria are being investigated (e.g., deep wound infections), storage and transportation of dermatological specimens does not present any specific challenges, because the main pathogens involved (Table 1) can survive for several days in transport media. Nevertheless, sample pickup by courier and overnight transport offer the advantage of reducing the overall turnaround time.

**Pathogen identification**

Bacterial species relevant for common disease conditions in veterinary dermatology are listed in Table 1. Staphylococci are the most frequent bacterial pathogens associated with skin and soft tissue infections. Historically,
animal pathogenic staphylococci have been associated with coagulase-positive staphylococci (CoPS), whereas CoNS generally have been regarded as bacteria with low pathogenic potential. Before the description of *S. intermedius* in 1976,36 all CoPS isolated from animals were (mis)identified as *S. aureus*. Subsequently, *S. intermedius* was differentiated into three distinct species: *S. intermedius*, *S. delphini* and *S. pseudintermedius* (referred to as the SIG group).36 The latter species is the normal commensal and opportunistic pathogen of the dog, even though infections also are reported in cats and less frequently in other hosts, including humans.37 *Staphylococcus pseudintermedius* cannot be easily distinguished from the other members of the SIG group by phenotypic methods and its speciation requires PCR-based tests or MALDI-TOF MS, provided that the database has been specifically refined for identification of this species (see above).

CoNS are commensal organisms with a relatively high rate of meticillin-resistance in companion animals.38 CoNS have been regarded as “contaminants” and either not reported or speciated except when isolated in pure culture from hospital-acquired infections associated with surgery or invasive procedures. The recognition of *S. schleiferi*39,40 as a canine pathogen underpins the importance of identifying CoNS species as the coagulase activity of this species and subspecies (subsp. *schleiferi* and *coagulans*) is variable. MALDI-TOF MS is superior to other methods for the identification of this group of staphylococci.41 ESGVM recommends that AST profiles for *S. schleiferi* and other CoNS should only be reported when the organisms are isolated in pure culture from sterile sites or from intact primary skin lesions sampled under strict aseptic conditions.

Polymicrobial cultures are common for otitis and wound infections, and can occur from skin samples. In these cases, the relevance of the culture result and the selection of the isolate for AST need to be determined. The current recommendation for human wound infections is that growth of potential pathogens should be reported, preferably semi-quantitatively.30 AST should be performed when a pathogen is isolated in pure culture or in abundance with minimal involvement of other micro-organisms. Antimicrobial therapy should target the micro-organism with greatest pathogenic potential. Discriminate reporting of AST profiles for micro-organisms of minimal clinical relevance is discouraged to avoid unnecessary use of broad-spectrum antimicrobial drugs to cover the composite AST profile of multiple isolates.

Lack or inadequacy of clinical breakpoints

A clinical breakpoint (CBP) is the critical MIC (or the corresponding interpretive inhibition zone diameter for disk diffusion) selected by ad hoc committees (e.g. CLSI or EUCAST) or national (e.g. US Food and Drug Administration) committees to categorize a bacterial strain as susceptible (S), intermediate (I) or resistant (R). CBPs are typically established on the basis of microbiological, pharmacokinetic (PK), pharmacodynamic (PD) and clinical outcome data.42 The purpose of CBPs is to assist clinicians to select appropriate drugs for therapy. In vitro AST does not, however, consider other factors that affect the outcome of antimicrobial therapy, such as host immune status, co-morbidities, strain virulence and compliance. By definition, a strain is reported susceptible to a drug when the standard dosage regimen is associated with a high likelihood of therapeutic success (approximately 90% according to human studies). The resistant category does not unequivocally predict treatment failure but a reduction of therapeutic success with a cure rate up to 60%. This is referred to as the 90–60% rule in human medicine.43,44 The clinical predictive value of AST is further impacted in veterinary medicine by the lack, or inadequacy, of available breakpoints. For example, breakpoints are unavailable for several antibiotics suitable for the treatment of skin infections in cats (Table 2). In those cases a CBP from dogs would typically be used. For bacteria or infections without any veterinary CBP, a human-derived CBP may be employed. This is the case for sulphonamides/trimethoprim and antibiotics such as chloramphenicol or rifampicin used for treatment of MRSA and meticillin-resistant *S. pseudintermedius* (MRSP) infections (Table 2). Cefovecin is a veterinary drug for which no CBP exist, hence the *in vivo* efficacy of this drug is difficult to predict by AST. Clearly, the predictive value of AST can be severely impacted by the use of inadequate CBPs, because a human CBP reflects the dosage regimen and the PK of the drug in humans, and both dosage regimen and drug disposition exhibit large differences between animal species. Reliable CBPs require animal species-specific determinations and there is an urgent need for animal-specific CBPs.

CBPs are dosage regimen-dependent because they are set by PK/PD analysis according to a specific dosage. Thus, a CBP set for a drug administered twice a day may not be appropriate if the same drug is administered three times a day. For example, amoxicillin clavulanate has a set breakpoint according to a defined dosage regimen (11 mg/kg per os (PO) twice daily)21 even though an increased dose according to label recommendations (12.5–25 mg/kg PO twice daily) can be used and three doses a day are recommended by international guidelines for treatment of urinary tract infections.45 Similarly, for time-dependent drugs such as the β-lactams, CBPs are heavily influenced by drug formulation. For example, a CBP that is valid for oral tablets may not be valid for the same drug administered by a long-acting intravenous formulation, even if the total dose is the same. To overcome this, several CBPs should be determined for a given substance depending on dose and formulation. However, this approach would be very difficult to manage for diagnostic companies and microbiology laboratories, because commercial systems for AST should be implemented and validated for each CBP.

Currently no CBPs are available for topical antimicrobial therapy, which is often used as a sole treatment in veterinary dermatology, especially for management of otitis externa. The relevance of AST for guiding topical antimicrobial therapy is questionable because CBPs are set for systemic therapy, and the drug concentrations achieved in serum by systemic administration are markedly lower than those obtained by the topical route. Such concentrations may exceed the MICs of skin pathogens greater than 100,000 fold (Table 3). These data suggest that infections
Table 2. Bacteria for which host- and infection-specific clinical breakpoints exist in veterinary dermatology according to Clinical Laboratory Standards Committee (CLSI).* † Drugs for which only human-derived breakpoints are available are highlighted in bold.

| Antibiotic | Dogs | Cats |
|------------|------|------|
| Amoxicillin-clavulanic acid | Escherichia coli, Staphylococcus spp. | E. coli, Staphylococcus spp., Streptococcus spp., Pasteurella spp. |
| Ampicillin | E. coli, Streptococcus canis, Staphylococcus pseudintermedius | None* |
| Cefalothin | E. coli, Staphylococcus aureus, S. pseudintermedius, Streptococcus spp. | None* |
| Cefazolin | E. coli, S. aureus, S. pseudintermedius, Pasteurella multocida, Streptococcus spp. | None* |
| Cefovecin | None | None |
| Cefpodoxime | E. coli, S. aureus, S. pseudintermedius, Pasteurella multocida, Proteus mirabilis, Streptococcus spp. | None* |
| Chloramphenicol | None* | None* |
| Clindamycin | Staphylococcus spp., Streptococcus spp. | None* |
| Doxycycline | Enterobacteriaceae, Staphylococcus spp. | None* |
| Gentamicin | None* | None* |
| Marbofloxacin | Enterobacteriaceae, Staphylococcus spp. | None* |
| Ofloxacin | Enterobacteriaceae, Staphylococcus spp. | None* |
| Pradofloxacin | E. coli, S. pseudintermedius | E. coli, S. pseudintermedius, Staphylococcus felis, Staphylococcus aureus, S. canis, Pasteurella spp. |
| Rifampicin | None* | None* |
| Trimethoprim-sulfamethoxazole | None* | None* |
| Tetracycline | Staphylococcus spp. | None* |
| Ticarcillin | None* | None* |

*Breakpoints (BP) from human medicine or another animal species are used instead.
†A generic BP exists for Enterobacteriaceae and Pseudomonas spp. in dogs, but this is not specific to any infection type.
‡A generic BP exists for skin and soft tissue infections in cats, but this is not specific to any bacterial species.

Table 3. Examples of antimicrobial concentrations in veterinary products for topical use and minimum inhibitory concentrations (MICs)

| Active compound | Examples of topical products containing compound | Concentration in commercial product (mg/L)* | Reported MIC ranges (mg/L) | Reported MIC90 (mg/L) | References for MIC ranges |
|----------------|-----------------------------------------------|---------------------------------------------|----------------------------|-----------------------|--------------------------|
| Gentamicin     | OtoMax Vet/EasOtic®                          | 4,119/2,348                                 | Pseudomonas aeruginosa: 0.25–16 | 8                     | 54                       |
| Miconazole     | EasOtic®/Surelon® Vet                       | 13,100/19,970                               | Coagulase-positive staphylococci: 1–8 | NA                   | 55                       |
| Polymyxin B    | Surelon® Vet                                | 654                                         | Coagulase-positive staphylococci: 0.25–64 | NA                   | 55                       |
| Fusidic acid   | Canural®                                     | 4,150                                       | Coagulase-positive staphylococci: 0.06–1,024 | 0.5–4                | 56                       |
| Framycetin     | Canural®                                     | 4,300                                       | Coagulase-positive staphylococci: ≤0.5–64 | NA                   | 55                       |
| Mupirocin      | Mucirin®                                     | 20,000                                      | Staphylococcus pseudintermedius: ≤0.03 to >1,024 | NA                   | 58                       |
| Enrofloxacin   | Baytril® Otic                                | 5,000                                       | P. aeruginosa: 8–1,024           | 32                    | 54                       |
| Florfenicol    | Osurnia®                                     | 10,000                                      | P. aeruginosa: 0.015–32         | 32                    | 59                       |

NA data not available.
*The concentrations stated for Canural® and Mucirin® represent mg/kg instead of mg/L.
†Framycetin is a synonym for neomycin B and MIC data are reported here for neomycin.

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caused by strains categorized as resistant by AST can be treated successfully by topical therapy. However, this hypothesis has not been validated clinically or experimentally and needs to be supported by scientific evidence in order to be translated into guidelines for antimicrobial use.

Detection of meticillin resistance in staphylococci

According to the MRSA expert rule, a *S. aureus* strain found to be meticillin-resistant, as determined by oxacillin, cefoxitin, or detection of *mecA* or its product PBP2a, should be reported as resistant to all β-lactams, except those that have been specifically licensed to treat MRSA infections (e.g. ceftaroline and cefotiboprole, which are not licensed for veterinary use). This rule was established based on clinical and microbiological evidence that MRSA strains display cross-resistance to β-lactams used in clinical practice for treatment of human staphylococcal infections. This rule has been translated to veterinary medicine without any clinical and/or microbiological evidence that MRSP and meticillin-resistant *S. schleiferi* (MRSS) display cross-resistance to the β-lactams used in veterinary dermatology. Various factors suggest that this rule may lead to reporting of false resistance to these β-lactams in strains expressing low-level meticillin resistance. A considerable proportion of MRSP strains display oxacillin MICs (0.5–4 μg/mL) that are significantly (2–8-fold) lower than the resistance breakpoint for MRSA detection (*R* ≥ 4 μg/mL). This is why, similarly to CoNS, the resistance breakpoint set for MRSP detection is considerably lower compared to MRSA (*R* ≥ 0.5 μg/mL). Cefalexin is one of the most active cephalosporins against staphylococci and has been associated with good clinical cure rates (90–100%) for uncomplicated MRSA skin infections in humans.

Studies have demonstrated that cefalexin resistance in CoNS, which display levels of meticillin resistance comparable to those in MRSP, is dependent on the degree of meticillin resistance expressed by the strain. Lastly, amoxicillin and ampicillin have been reported to have relatively good affinity for PBP2a, and older *in vivo* studies claimed anti-MRSA efficacy of high doses of aminopenicillins combined with β-lactamase inhibitors for treatment of skin and soft tissue infections, and urinary tract infections.

Research to provide evidence to support this expert rule in veterinary medicine is indicated. In the interim, the authors recommend that any oxacillin-resistant staphylococci should be reported as resistant to all β-lactams licensed for veterinary use. However, if therapy with amoxicillin clavulanate or cefalexin has been initiated and the causative strain has a low MIC of oxacillin, we recommend evaluating the clinical outcome of therapy before changing antimicrobial prescription. As already mentioned, AST has a limited predictive value for infections caused by strains reported as resistant.

Although the cefoxitin disk test is generally recognized as reliable for MRSA detection, a recent study has shown that cefoxitin may not be a good surrogate for MRSP detection by disk diffusion. In the absence of an internationally recognized cefoxitin breakpoint clearly differentiating *mecA*-positive from *mecA*-negative isolates of *S. pseudintermedius*, we recommend that laboratories use oxacillin disk or MIC tests for detection of meticillin resistance in this and other staphylococcal species, other than *S. aureus*.

Result reporting

Reporting of polymicrobial skin and wound culture results is a challenge, especially when samples derive from contaminated sites. In these cases, the dominant colony type(s) associated with micro-organisms of clinical relevance should be selected or the report should outline that an unspecified mixed growth with limited or no clinical relevance was detected. Samples from ears also tend to be polymicrobial. For these samples, the same principle of reporting the dominant colony type should be used, but additional factors complicate the decision of selection for subculture and AST: (i) relatively few bacterial species (*Proteus* spp. and *Pseudomonas aeruginosa*) are obligate pathogens of canine ears, whereas other species also occur in healthy dogs, hence the latter would only be relevant in case of pure or almost pure culture; (ii) *Corynebacterium auriscanis* should not be selected for AST as it seems clinically irrelevant and there is no CBP for this species.

Clinicians should consider the limited value of AST for topical therapy when sampling ear infections and when interpreting results obtained from diagnostic laboratories that indiscriminately report any type of growth.

Various measures such as selective or cascade reporting of AST results can be used by the microbiology laboratory to guide rational choice of antimicrobials. This approach is used extensively in human hospitals to encourage use of first-line drugs. The practice of not reporting the results for selected agents is regarded as selective reporting. For example, AST data should not be reported for critically important drugs that are not licensed for veterinary use (e.g. imipenem, vancomycin and linezolid), even if these drugs are included in the antimicrobial panel as last-resort agents for surveillance purposes. Cascade reporting is the practice of reporting the AST result for only one drug that tests susceptible within a certain class (e.g. gentamicin within the aminoglycosides) to reduce the use of more expensive and/or broader spectrum drugs of the same class (e.g. amikacin). In the absence of guidelines for selective or cascade reporting, decisions should be made in consultation with an infectious disease specialist. Linking the clinic to the laboratory information management system to enable data exchange and implementation of antimicrobial stewardship programmes would be optimal.

A variety of software programmes are available on the market for effective management of veterinary practices but they are not designed to interact with the laboratory or are difficult to implement. It is desirable for manufacturing companies to improve veterinary practice management software in order to facilitate antimicrobial stewardship.

Conclusions

The microbiology laboratory should play an important role in the diagnosis of infectious diseases by providing key support to various steps of the diagnostic process, from specimen collection and transportation to
interpretation of AST results. The laboratory’s role and responsibilities should extend beyond correct specimen testing and reporting of results, and include guidance in both the pre- and postanalytical phases of the diagnostic process. Furthermore, a good microbiology service is essential for implementation of antimicrobial stewardship programmes in veterinary practice.

The advent of MALDI-TOF MS in clinical microbiology has significantly reduced the time required for bacterial ID and facilitated ID of veterinary pathogens that previously could not be identified. The concomitant developments in genome sequencing technologies are improving our understanding of the taxonomy, ecology and population structure of key pathogens in veterinary dermatology such as *S. pseudintermedius* and *S. schleiferi*. Despite these technological advances, veterinary diagnostic microbiology is still based predominantly on traditional culture methods, and the turnaround time for AST has essentially remained unchanged for many years. Methods for AST are not yet harmonized and clinical breakpoints for important drug–pathogen combinations are either missing or inadequate. Small veterinary microbiology laboratories, including in-clinic laboratories, often neither have the infrastructure nor the expertise required to run up-to-date clinical microbiology, and adequate postgraduate training in veterinary clinical microbiology is not available in most countries.

ESGVM recommends that diagnostic microbiology laboratories are selected by veterinary practitioners taking into consideration the following factors:

- Guidance for optimal specimen management (i.e. selection, collection, storage and transportation of clinical specimens).
- State-of-the-art methods for ID (MALDI-TOF-MS) and AST (MIC determination by broth micro-dilution).
- Implementation of transparent and ongoing quality assurance measures, preferably by accredited laboratories.
- Availability of skilled microbiologists for case-based expert advice and data interpretation.

Other factors include the availability of a courier system for overnight delivery of specimens to the laboratory, and access to data for passive epidemiological surveillance and implementation of antimicrobial stewardship programmes at the clinic level. Certification of veterinary microbiologists at a national or, preferably, international level should be a prerequisite. National accreditation, such as according to ISO standards, should be obtained to ensure minimum quality and safety standards.

ESGVM supports the development of PoC tests that could rationalize antimicrobial use in veterinary practice, provided that (i) the performance of the test has been evaluated scientifically, (ii) clinical staff are adequately trained to interpret the results and (iii) clinics meet the minimal requirements for handling microbiological specimens (biosafety level 1). There is concern about direct AST replacing conventional AST due to the potential for error and the subsequent selection of a drug that is not effective.

ESGVM has a mission to set standards for veterinary clinical microbiology, including methods and training, and the promotion of antimicrobial stewardship and constructive interaction between microbiologists and clinicians. The group promotes diagnostic microbiology in veterinary practice by standardizing procedures and by educating veterinarians about the key role played by microbiology laboratories in antimicrobial stewardship and patient care. ESGVM strongly supports (i) global harmonization of methods and setting of infection-, animal- and bacterial-specific CBPs for AST of veterinary pathogens; (ii) postgraduate education and board certification of specialists in veterinary clinical microbiology and antimicrobial stewardship; (iii) official licensing of veterinary diagnostic microbiology laboratories and quality assurance to guarantee the minimum quality and biosafety standards required to perform veterinary microbiology; and (iv) development of new diagnostic tests providing veterinarians with rapid and reliable results at reasonable cost.

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laboratorios, incluidos los laboratorios dentro de las clínicas, generalmente no están adecuadamente equipados para realizar pruebas de diagnóstico microbiológico clínico actualizadas.

Conclusiones e importancia clínica – ESGVM recomienda el uso de laboratorios que emplean espectrometría de masas para ID y micro-dilución de caldo de cultivo para AST, y que ofrecen asistencia de microbiólogos expertos en cuestiones pre- y post-analíticas. Entre las misiones del ESGVM figuran el establecimiento de normas generales para la microbiología clínica veterinaria, la promoción de la administración antimicrobiana y el desarrollo de métodos de diagnóstico nuevos, validados y rápidos, especialmente para la AST.

Zusammenfassung

Hintergrund – Das Mikrobiologielabor ist eher als Dienstleister und nicht als ein integraler Bestandteil des Gesundheitsteams zu betrachten.

Ziele – Das Ziel dieser Review ist eine Diskussion über die derzeitigen Herausforderungen ein State-of-the-Art diagnostisches veterinär-mikrobiologisches Service zu bieten, welches die Identifizierung (ID) und die antimikrobiellen Empfindlichkeitsmessungen (AST) der Schlüsselpathogene der Veterinärmedizin inkludiert.

Methoden – Die Forschungsgruppe für Veterinär-mikrobiologie (ESGVM) der European Society für klinische Mikrobiologie und Infektionskrankheiten (ESCMID) identifizierte wissenschaftliche, technologische, erzieherische und angeordnete Weglassungen, welche den Vorhersagewert für AST und die Qualität der geleisteten Dienste durch die Mikrobiologielaboratorien beeinflusste.

Ergebnisse – Das Erscheinen der Massenspektrometrie hat die Zeit, die für die ID der Schlüsselpathogene wie Staphylococcus pseudintermedius nötig ist, signifikant reduziert. Nichtsdestotrotz bleibt die Umlaufzeit für validierte AST Methoden seit vielen Jahren unverändert. Neben wissenschaftlichen und technologischen Grenzen sind die AST Methoden nicht harmonisiert und die klinischen Messpunkte für einige antimikrobielle Wirkstoffe fehlen entweder oder sind nicht adäquat. Kleine Laboratorien, zu denen auch die Laboratorien in den Kliniken gehören, sind normalerweise nicht ausreichend ausgerüstet, um klinische mikrobiologische diagnostische Tests auf dem neuesten Stand durchzuführen.

Schlussfolgerungen und klinische Bedeutung – Die ESGVM empfiehlt die Verwendung von Laboratorien, die die Massenspektrometrie zur ID und ein Mikroboilou-Dilutionsverfahren für AST einsetzen und Unterstützung von Mikrobiologienexperten bei Problemen vor sowie nach der Analyse anbieten. Das Etablieren von Allgemeinstandards für die klinische Veterinärmikrobiologie, antimikrobiologische Verantwortung zu fördern und die Entwicklung von neuen, validierten und raschen diagnostischen Methoden, vor allem für AST, sind unter anderem Ziele von ESGVM.
Resumo

Contexto – O laboratório de microbiologia pode ser tido como um prestador de serviços ao invés de uma parte integral da área da saúde.

Objetivos – O objetivo desta revisão é discutir os desafios atuais de fornecer um serviço de diagnóstico em microbiologia veterinária de alto padrão de qualidade, incluindo testes de identificação (ID) e susceptibilidade a antimicrobianos (SAM) de patógenos relevantes em dermatologia veterinária.

Métodos – O Grupo de Estudos em Microbiologia Veterinária (GEMV) da Sociedade Europeia de Microbiologia Clínica e Doenças Infecciosas (SEMCDI) identificou omissões científicas, tecnológicas, educacionais e regulatórias que impactam no valor preditivo de SAM e na qualidade dos serviços oferecidos pelos laboratórios de microbiologia.

Resultados – O advento da espectrometria de massa reduziu significativamente o tempo requerido para ID de patógenos importantes como Staphylococcus pseudintermedius. Entretanto, o tempo de processamento necessário para SAM tem se mantido o sem alterações há anos. Além de limitações tecnológicas e científicas, métodos de SAM não são harmonizados e os intervalos de suscetibilidade e resistência para determinados antibióticos são inadequados ou inexistentes. Laboratórios pequenos, incluindo os internos de clínicas, são geralmente inadequadamente equipados para processar testes microbiológicos atualizados.

Conclusões e importância clínica – SEMCDI recomenda o uso de laboratórios que utilizam espectrometria de massa para ID e microdiluição em caldo para SAM, e ofereçam assistência de microbiologistas para problemas pré e pós-analíticos. Desenvolver padronização para microbiologia veterinária clínica, promover regulação e melhorias no uso de antimicrobianos e o desenvolvimento de novos métodos de diagnóstico rápidos e validados, especialmente para SAM, estão entre as funções de SEMCDI.