HOW TO GET THE BEST OUT OF MY CULTURE AND SENSITIVITY REPORT?

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SUBMITTING SAMPLES FOR BACTERIAL CULTURE AND SUSCEPTIBILITY TESTING

Thorough laboratory testing and correct interpretation of results are critical for the management of patients with pyoderma in an era of increasing antimicrobial resistance amongst pathogens. Empirically chosen systemic antibacterial therapy is increasingly hampered by the continuing spread of multidrug-resistance and culture-based prescribing of antimicrobials may become compulsory prior to prescribing antimicrobials to help limit the threat from resistance (1). However, cost and perceived time delay associated with submitting samples for bacterial culture can be challenging. It is therefore important to maximise what we can gain from microbiology results for the benefit of our patients. This can be achieved through:

1. Understanding the principles of microbiology methods used in diagnostic laboratories
2. Careful interpretation of the reported results in the context of the patient's history and clinical signs
3. Communication with laboratory staff for extended testing and clarification where needed
4. Combination of culture results with in-house cytology findings.

A swab sample from skin, even from healthy skin, is likely to yield staphylococci and in the majority of dogs a *Staphylococcus pseudintermedius*. The clinician will need to decide whether this result reflects infection or merely the dog's cutaneous microbiota. In addition, it is the clinician's responsibility to choose a laboratory experienced in working with bacterial pathogens isolated from animals. While semi-automated and automated methods, such as API® and Vitek® will have typically been designed for use in human microbiology laboratories, these can be used for veterinary diagnostics provided results are carefully interpreted by veterinary-trained microbiologists. Similarly, smaller veterinary diagnostic laboratories will need to keep up to date with the rapidly advancing methods and improvements, particularly in resistance testing, while practice in-house microbiology can no longer be recommended at a time when multidrug-resistant zoonotic pathogens have emerged and require accurate identification and careful handling.

BACTERIAL IDENTIFICATION

The use of mass spectrometry in the form of MALDI-TOF technology has substantially improved bacterial identification, reduced the time to reporting and increased specificity of the report compared to phenotypic identification. However, this technology is not yet widely available in all laboratories due to initial investment cost. This session highlights key points of the microbiology laboratory report with regard to pathogen identification (phenotypic vs. molecular speciation) where careful interpretation by the clinician will be necessary. In the laboratory, staphylococci are easy to grow on many different nutrient agars and differentiation from other Gram-positive cocci such as the micrococci (usually by colony colour) and streptococci and enterococci (catalase test) is also straightforward with appropriate biochemical tests. What is difficult though, is accurate species differentiation amongst staphylococci using phenotypic tests. Prior to the emergence of MRSA and MRSP as canine pathogens, species identification of coagulase-positive staphylococci isolated from a pet was of little relevance. Nowadays though, correct discrimination between *S. aureus* and *S. pseudintermedius* is critical in the context of meticillin-resistant isolates as epidemiology and zoonotic risk differ substantially between MRSA and MRSP. Mistaken identities of *S. aureus* and *S. pseudintermedius* based on phenotypic testing occur (2), despite a common belief that the differentiation is straightforward.
ANTIMICROBIAL SUSCEPTIBILITY TESTING

Testing for antimicrobial susceptibility is commonly done by (modified) Kirby-Bauer disc diffusion tests. Alternatively (or in combination), some laboratories offer minimum inhibitory concentrations (MICs) determined through the more labor-intensive agar dilution or broth dilution method or by E-strip testing (paper strips impregnated with increasing concentrations of antimicrobial). Breakpoints are used to categorize microorganisms as clinically susceptible (S), intermediate (I) or resistant (R) dependent on the quantitative antimicrobial susceptibility as indicated by the MIC value determined in a well-defined standard test system and defined procedures. Categories S, I and R should help to distinguish between patients that are likely or unlikely to respond to antimicrobial treatment. Clinical breakpoints, determined by breakpoint committees such as from the Clinical and Laboratory Standards Institute of the United States of America (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST/VetCAST), are nowadays available specifically for bacteria from animals to increase relevance for veterinary treatment but adherence to recommended testing methods is critical for accuracy (3-5).

Knowledge of actual MICs is rarely required for the treatment of skin infections but more relevant for serious infections such as bacteremia involving multidrug-resistant bacteria as increasing the dose of an antimicrobial may be preferable to choosing a more toxic drug. Susceptibility testing for topical therapy is not recommended as no clinical breakpoints are currently available. Surface and superficial pyoderma, otitis externa and superficial wounds can be treated with topical antimicrobial therapy alone and susceptibility test results will be of little clinical value. For example, a Pseudomonas spp. isolated from a dog’s ear swab and reported as resistant to enrofloxacin or marbofloxacin based on breakpoints for systemic therapy is very likely to be sensitive to topical therapy with these agents as concentrations achieved at the site of infection through topical therapy (i.e. ear drop application) are expected to substantially exceed MICs.

METICILLIN-RESISTANCE

MRSP (and to a lesser extent MRSA) can already be suspected from an antimicrobial susceptibility profile due to their extensive drug-resistance. However, testing specifically for meticillin-resistance as a marker for broad β-lactam resistance is indicated to allow correct allocation to the epidemiologically important group of meticillin-resistant staphylococci. Phenotypic testing for meticillin-resistance can be done either on screening agar or by disc testing and the chemically almost identical (but more stable) oxacillin is nowadays used instead of meticillin. For S. aureus in human medical laboratories, cefoxitin disc have replaced oxacillin as a better predictor for the presence of mecA, the gene encoding broad β-lactam resistance. For S. pseudintermedius though, cefoxitin discs are currently not recommended by CLSI. Confirmation of the mecA gene by polymerase-chain reaction (PCR) or of its product (a mutated penicillin-binding protein in the cell wall) by latex agglutination is commonplace in medical laboratories and for research purposes but not in veterinary diagnostic laboratories. An important area where clinicians need to interpret the laboratory report carefully is the broad β-lactam resistance associated with mecA-positive isolates. If meticillin-resistance is reported, resistance should be assumed to all β-lactams, including the first and the veterinary third-generation cephalosporins, irrespective of what is reported.

REFERENCES
