The Veterinary Journal 203 (2015) 149–154

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

Antimicrobial susceptibility testing for bovine respiratory disease: Getting more from diagnostic results

Brian V. Lubbers [a,*], John Turnidge [b,c,d]

[a] Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States
[b] SA Pathology, Women's and Children's Hospital, North Adelaide, South Australia, Australia
[c] Paediatrics and Molecular and Biomedical Sciences, University of Adelaide, Adelaide, South Australia, Australia
[d] Senior Medical Advisor, Australian Commission on Safety and Quality in Health Care, Sydney, NSW, Australia

ARTICLE INFO

Article history:
Accepted 9 December 2014

Keywords:
Antimicrobial susceptibility testing
Bovine respiratory disease

ABSTRACT

Bovine respiratory disease (BRD) is one of the most common diseases of cattle worldwide. Given the significant bacterial component of this disease, antimicrobial agents remain one of the mainstays of therapy. However, the potential welfare and economic impact resulting from the selection of inappropriate antimicrobial therapy for BRD poses significant risks to both animal and animal owner. To determine the 'best' antimicrobial agent for a specific case, the decision-making process needs to incorporate all available evidence, often including the results of bacterial culture and antimicrobial susceptibility testing. While antimicrobial susceptibility testing can be a valuable diagnostic tool, integrating the test results into the clinical decision making process can be a challenging experience. This review details the process by which interpretive criteria for susceptibility tests are developed. Principles for how to best integrate antimicrobial susceptibility testing, both at the individual animal test and aggregate test levels, into the clinical decision making process are discussed. Non-traditional testing methodologies and how they may improve susceptibility testing in the future are also reviewed.

Introduction

Antimicrobial susceptibility testing (AST) is one of the most used, but often confusing, diagnostic tests performed in veterinary medicine. In the 50 years since the first standardised in vitro method was published (Bauer et al., 1966), the place of AST in clinical practice has been the source of much debate. Proponents have discussed the utility and reproducibility of AST (Watts and Yancey, 1994a; Stratton, 2006), with government, professional and industry groups around the world issuing statements touting AST as one component of 'responsible antimicrobial use' (Teale and Moulin, 2012)[1], while critics, both veterinary (McClary et al., 2011) and human (Greenwood, 1981; Doern and Brecher, 2011), have questioned its clinical predictive value (or lack thereof). Seemingly, the veterinary practitioner is left with two options: either they will use AST despite its limitations or they do not use AST because it has no perceived clinical value.

This review is designed to provide a foundational understanding of AST and how results can be incorporated into the selection process to determine antimicrobial treatment of bovine respiratory disease (BRD). For purposes of clarity, this review will focus on diagnostic testing and the application of test results to selection of antimicrobial therapy. Expansive reviews on individual topics, such as laboratory methods, antimicrobial resistance and pharmacotherapy of BRD, have been published previously (McManus, 1997; Walsh, 2000; Cusack et al., 2003; Apley, 2006; Jenkins and Schuetz, 2012; O'Connor et al., 2013).

The primary focus of this article will be 'best practice' for using antimicrobial susceptibility testing and how to apply results, both for individual animal and cumulative data, with special emphasis on *Mannheimia haemolytica*, as the main bacterial pathogen associated with BRD. This review will conclude with a discussion of several non-traditional methodologies that hold promise for use in the future for susceptibility testing of pathogens associated with BRD.

Impact and pathogenesis of bovine respiratory disease

BRD is the most prevalent disease of feedlot cattle, veal calves, weaned dairy heifers and weaned/unweaned beef calves (Pardon et al., 2013)[2,3,4] worldwide. The pathogenesis of BRD has been

* Corresponding author. Tel.: +1 785 5324012.
E-mail address: blubbers@vet.k-state.edu (B.V. Lubbers).

[1] See: https://www.avma.org/KB/Policies/Pages/AABP-Prudent-Drug-Usage-Guidelines-for-Cattle.aspx (accessed 17 April 2014).

[2] See: http://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_dr_PartIV.pdf (accessed 17 April 2014).

[3] See: http://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairyheifer11/HeiferRaiser.pdf (accessed 17 April 2014).

[4] See: http://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_ir_Antimicrobial.pdf (accessed 17 April 2014).

http://dx.doi.org/10.1016/j.tvjl.2014.12.009
1090-0233/

discussed in depth elsewhere, with the consensus viewpoint that disease results from a complex interaction between the host, environment and pathogen(s) (Duff and Galyean, 2006; Hodgson et al., 2012).

The most significant bacterial pathogens of BRD are *M. haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* (Gagea et al., 2006; Arcangioli et al., 2008). Normally commensals of the upper respiratory tract, these bacteria become opportunistic pathogens causing bovine pneumonia under conditions of viral infection and host immune suppression (Griffin, 2010). Although vaccination and other preventative measures have been widely adopted, current BRD management practices rely heavily on the use of antimicrobial therapy to treat and control infections caused by these bacteria (Taylor et al., 2010).

Antimicrobial susceptibility testing: Terminology

(Clinical) breakpoint/interpretive criteria

Minimal inhibitory concentrations (MICs) or zone diameter values are used to indicate isolates as susceptible (S), intermediate (I) or resistant (R) (Table 1). The modifier 'clinical' is often added to the term 'breakpoint' to distinguish it from epidemiological cut-off values defined below.

Epidemiological cut-off values

Epidemiological cut-off values (ECOFFs or ECVs) are MIC values that distinguish 'wild-type' isolates (those without acquired resistance) from 'non-wild-type' isolates (isolates with resistance elements). ECOFFs are especially valuable for monitoring development of resistance, but are not meant to be used for guiding therapy in the individual animal (Kahlmeter et al., 2003). For this reason, further discussion in this review will be limited only to (clinical) breakpoints, not ECOFFs.

Broth dilution susceptibility testing

Broth dilution susceptibility testing is an AST method that exposes the bacterial pathogen of interest to a 2-fold dilution series of an antimicrobial agent within a liquid culture media (broth). The measured result of this test method is the MIC, which can be compared to defined MIC breakpoints. The MIC is the lowest concentration of antimicrobial agent that inhibits visible growth of a microorganism in a broth dilution susceptibility test.

Disc diffusion susceptibility testing

The disc diffusion method, also referred to as the (modified) Kirby-Bauer method, uses antimicrobial impregnated paper discs on a solid agar medium to determine antimicrobial susceptibility of a bacterial pathogen. The measured result of this test method is a zone of inhibition (ZOI), which can be compared to defined zone diameter breakpoints.

Table 1
Antimicrobial susceptibility testing interpretive criteria definitions (CLSI, 2013).

Interpretative criteria		Interpretive criteria definition
S	Susceptible	Category implies an infection that may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and infecting (bacterial) species
I	Intermediate	Category implies an infection that may be appropriately treated in body sites where the drugs are physiologically concentrated, or when a high dosage of drug can be used
R	Resistant	Strains (in this category) are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or fall in the range (of MICs) where specific microbial resistance mechanisms are likely and clinical outcome has not been predictable in effectiveness studies

Antimicrobial susceptibility testing: Principles of testing methods

Using standardised test methods is of paramount importance. Minor alterations in test conditions, such as pH, bacterial inoculum and testing media type, can have profound impacts on AST results. For this reason, non-standard testing practices, such as direct testing of clinical specimens or testing mixed cultures, leads to unreliable results (Shahidi and Ellner, 1969). It is important for practitioners to adhere to these standards when performing in-house testing and to verify that their diagnostic laboratory follows these standards as well (Apley, 2003).

Quality control testing is performed using the same methods, equipment and test conditions as is used with diagnostic isolates, but with bacterial reference strains of known antimicrobial susceptibility. The use of routine quality control testing provides the testing laboratory (and end user) with the assurance that the procedures and media have performed within acceptable limits, provided that the reference strains are suitable for the antimicrobial agents tested, the range of concentrations is able to detect non-conforming results and the disc contains the amount of specified antimicrobial agent for testing quality control strains (Schwarz et al., 2010).

Development of interpretive criteria (breakpoints)

Interpretive criteria represent the mechanism by which an in vitro laboratory result (MIC for broth dilution testing or ZOI for disc diffusion testing) is translated to an expected clinical outcome. Broth microdilution is the reference method for determining the activity of an antimicrobial agent against a strain of a bacterial species. This modern adaptation of an old technique yields an MIC value, which unfortunately cannot be directly applied or compared to concentrations achieved in vivo, and is therefore of limited clinical value. Instead, the medical and veterinary sciences have agreed that categorical reporting (S, I or R) (Table 1) offers more practical advice to the veterinarian in managing diseased animals.

At present, the Clinical and Laboratory Standards Institute (CLSI) Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee is the primary entity responsible for establishing veterinary interpretive criteria used worldwide. The VAST subcommittee, similar to their human medical counterparts, uses the following three types of data: (1) MIC distribution data, (2) pharmacokinetic/pharmacodynamics (PK/PD) data; and (3) clinical outcome data. These data individually generate cut-off values which are then evaluated together to define the most robust MIC values at which S, I and R can be reported to the veterinary clinician.

The MIC distribution data are a frequency distribution of field isolates for the bacterial species being evaluated. This information allows for an initial delineation between wild-type and non-wild-type bacterial subpopulations. Non-wild-type subpopulations are those with acquired resistance mechanisms (not to be confused with R as it appears on reports). As an example, the frequency distribution in Fig. 1 demonstrates two distinct populations of *M. haemolytica* when tested for susceptibility to penicillin: (1) a sub-population to the left with MIC values of 0.12, 0.25 and 0.5 μg/mL; and (2) a sub-population to the right with MIC values ≥8 μg/mL. As the first step in the breakpoint development process, S would likely include the strains with low MIC values (wild-type isolates) and the R breakpoint should be set to encompass the strains with high MIC values (non-wild-type). These preliminary classifications would then be compared and adjusted, if required, with PK/PD data.