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# Identification of Veterinary Pathogens by Use of Commercial Identification Systems and New Trends in Antimicrobial Susceptibility Testing of Veterinary Pathogens

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## INTRODUCTION

For many years, veterinary diagnostic microbiology had been considered a subspecialty within clinical microbiology. This seemed quite logical as many of the pathogens affecting animals are similar to human pathogens and the same basic isolation techniques are employed in both fields. Moreover, the role of veterinary diagnostic microbiologists is similar to that of clinical microbiologists in that their primary responsibility is to provide clinically relevant information to the veterinarian concerning the causative agent of a disease and, when possible, the antimicrobial susceptibility profile of that agent. In recent years, however, it has become apparent that many veterinary pathogens are substantially different from human pathogens and require unique methods for identification and antimicrobial susceptibility testing (12, 82, 85, 103, 106, 107, 115).

The primary difference between clinical microbiology laboratories for human and veterinary pathogens is that the clinical laboratory for humans receives isolates from one host species while the veterinary microbiology laboratory may receive isolates from six major host species and several minor species (12, 35). Generally, the major veterinary species can be divided into companion, food-producing, and exotic animals. The major companion animal species include dogs, cats, and horses, while the major food-producing animals include cattle, swine, sheep, and poultry (chickens and turkeys). Exotic animals include, but are not limited to, reptiles, camelids (llamas and alpacas), ornamental birds (parakeets and cockatiels), ornamental fishes, and zoo animals (land and aquatic). Additionally, aquaculture of fresh- and saltwater fish is rapidly becoming a major economic market and presents a unique set of challenges to the veterinary microbiologist in terms of pathogen identification and interpretation of antimicrobial susceptibility test results.

The type and management of the animal may also impact how laboratory results are utilized. For example, information on the identification and antimicrobial susceptibility of an isolate from companion animals such as dogs will be used to select treatment for that individual animal, whereas the same information for an isolate from a pig might be used to devise treatment (or control measures) for the entire herd. Furthermore, the veterinary microbiologist must also be aware of the consequences of antimicrobial therapy in food-producing animals in terms of efficacy, toxicities, and residue avoidance. Thus, the veterinary microbiologist must be familiar with the clinical and economic significance of pathogens isolated from the various host species and must accurately interpret and report the antimicrobial susceptibilities of those isolates. The purpose of this paper is to review the accuracy of commercial identification systems in identifying bacterial pathogens isolated from various animal species and to consider the problems associated with antimicrobial susceptibility tests (ASTs) in veterinary medicine.

## IDENTIFICATION

Identification of veterinary pathogens by conventional methods is tedious and time-consuming (12, 17). Commercial identification systems are widely used by clinical microbiologists as convenient, cost-effective alternatives to conventional methods. However, many of these systems incorporate a limited number of veterinary strains in their data base which may limit the accuracy of the system for identification of veterinary pathogens (27, 59, 60, 85, 97, 102, 104, 105, 111–113). A review of the commercial systems that have been evaluated with veterinary pathogens follows.

### Staphylococci

In recent years, the number of described species within the genus *Staphylococcus* has increased dramatically (reviewed in references 55 and 106). Many of these new species have been isolated from animals (Table 1) (21, 23, 25, 40–42, 47, 89–91, 100). While host preferences are observed among the various staphylococcal species, isolation of human-associated staphylococcal species from animals is common in close-contact situations (4, 8, 19, 22, 44, 106, 108, 110). Identification of staphylococci from animals may be important in selecting the

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TABLE 1. Animal-associated staphylococcal species and host animals commonly associated with the individual species

| Species | Host(s) | Reference |
|---|---|---|
| *S. aureus* subsp. *anaerobius* | Sheep | 20 |
| *S. intermedius* | Dogs, birds | 38 |
| *S. hyicus* | Cattle, swine | 25 |
| *S. chromogenes* | Cattle | 40 |
| *S. xylosus* | Cattle | 89 |
| *S. sciuri* | Squirrels | 87 |
| *S. lentus* | Goats, sheep | 87 |
| *S. gallinarum* | Turkeys | 22 |
| *S. caprae* | Goats | 22 |
| *S. delphini* | Dolphins | 98 |
| *S. muscae* | Flies, cattle | 39 |
| *S. arlettae* | Horses | 88 |
| *S. equorum* | Horses | 88 |
| *S. kloosii* | Squirrels, opossums | 88 |
| *S. felis* | Cats | 41 |

appropriate therapy as well as in determining the impact of management techniques (4, 8, 19, 22, 44, 108, 110). This task is further complicated for the veterinary microbiologist because *Staphylococcus intermedius*, *Staphylococcus delphini*, and *Staphylococcus hyicus*, in addition to *Staphylococcus aureus*, may be coagulase positive (23, 40, 100). Thus, an identification system used for the identification of staphylococci must be able to differentiate the coagulase-negative staphylococci and the coagulase-positive staphylococci.

The Staph-Ident system (bioMérieux-Vitek, Hazelwood, Mo.) consists of 10 microtubes containing either conventional or chromogenic substrates. This system has the ability to separate *S. intermedius* from *S. aureus* on the basis of β-galactosidase production (56, 57, 113). Kloos and Wolfshohl (56) compared the Staph-Ident system with conventional methods for identification of staphylococci, including 20 *S. intermedius* and 26 *S. hyicus* strains, and found >90.0% agreement between the two systems. Subsequently, Cox et al. (18–20) and Biberstein et al. (8) used the Staph-Ident system successfully to identify staphylococci isolated from a variety of animal sources. The latter studies included primarily isolates from dogs and cats in which the ability of the Staph-Ident system to separate *S. intermedius* from *S. aureus* is important. However, later studies (59, 60, 107, 113) evaluating the Staph-Ident with bovine mammary gland isolates yielded varying results. Langlois et al. (59) determined that the Staph-Ident system identified only 54.0% of staphylococci (41.8% of non-*S. aureus* isolates) isolated from the bovine udder. The primary reason for the poor accuracy was the inability of the system to distinguish *Staphylococcus epidermidis* from *S. hyicus*. Similarly, Jasper et al. (50) found that the Staph-Ident system could identify only 23 of 323 (7.1%) non-*S. aureus* isolates from bovine mammary glands; the misidentification of *S. hyicus* was the primary reason for poor accuracy. Rather et al. (84) determined that the Staph-Ident system identified only 45.2% of staphylococci isolated from bovine milk when compared with Schleifer and Kloos' simplified scheme for identification of human staphylococcal species. Two subsequent evaluations (107, 113) of the Staph-Ident system with staphylococci from the bovine udder determined that the system provided acceptable levels of accuracy if supplemental testing was performed to permit differentiation of *S. hyicus* from *S. epidermidis*.

The Staph-Trac system (bioMérieux-Vitek) consists of 20 microtubes containing dehydrated substrates for 19 biochemical tests and a negative control. Langlois et al. (60) evaluated the Staph-Trac system for identification of staphylococci isolated from bovine mammary glands and determined that the system could identify 91.2% of the isolates tested. The primary advantage of this system was the ability to differentiate *S. hyicus* from *S. epidermidis*. In contrast, Watts and Nickerson (107) determined that the Staph-Trac system could identify only 66.1% of staphylococci isolated from bovine mammary glands. Misidentification of *S. hyicus* as *Staphylococcus simulans* due to negative phosphatase tests was the primary reason for the poor performance of the system. Furthermore, the Staph-Trac system could not differentiate *S. intermedius* from *S. aureus* (107). Recently, the Staph-Trac data base has been updated to permit the differentiation of *S. intermedius* from *S. aureus* on the basis of mannitol and maltose utilization.

The API 20GP system (bioMérieux-Vitek) consists of 20 microcupules containing dehydrated substrates for the identification of staphylococci and group D streptococci (112). The first 10 tests are identical to the Staph-Ident system, while the second 10 tests were selected from the API 20S (bioMérieux-Vitek) streptococcal identification system. This system was evaluated to determine if the second 10 tests improved the accuracy of the Staph-Ident system (112). Although the system correctly identified 90.2% of *S. aureus* strains, overall accuracy was placed at 56.1%. Unfortunately, this system also had difficulty differentiating *S. hyicus* from *S. epidermidis*. Poor performance was attributed to the limited number of veterinary strains in the data base.

The Minitek Gram-Positive Set (BBL Microbiology Systems, Cockeysville, Md.) is a 20-test system based on substrate-impregnated paper disks (111). This system was evaluated with 130 staphylococci isolated from the bovine mammary gland with initial accuracy placed at 79.2%. However, modification of the system to correct for data base deficiencies improved accuracy to 87.7% (111).

The Staph-Zym system (Rosco Diagnostica, Taastrup, Denmark) consists of 10 enzymatic tests in minitubes in a rigid, transparent, plastic cartridge (57, 114). Lammler (57) determined that the Staph-Zym system correctly identified all strains of *S. hyicus* isolated from cattle and swine and *S. intermedius* from dogs. An evaluation (114) of the Staph-Zym system to identify staphylococci isolated from bovine intramammary infections placed overall accuracy of the system at 91.9%. This system correctly identified 100.0, 93.9, and 95.0% of the *S. aureus*, *S. hyicus*, and *S. intermedius* strains tested, respectively, but did have some difficulty distinguishing strains of *S. hominis* from those of *S. warneri*.

The ATB 32 Staph system (API System, Montalieu Vercieu, France) is a new system consisting of a plastic strip containing dehydrated substrates for 26 colorimetric tests (9). In an interlaboratory evaluation (eight laboratories participating), overall accuracy of the system was placed at 95.5%, with interlaboratory reproducibility of >90.0%. Moreover, this system has the ability to identify the more recently described animal-associated staphylococcal species, with the exceptions of *S. delphini* and *S. felis*.

It appears that the majority of the presently available commercial systems for identification of staphylococci have difficulty in identifying important veterinary pathogens. Thus, selection of an identification system must take into consideration the host species from which the majority of isolates will be obtained. For example, a laboratory that receives the majority of isolates from companion animals such as dogs will need to distinguish *S. intermedius* from *S. aureus*. Conversely, a bacteriology laboratory that diagnoses mastitis pathogens will need a system that can distinguish *S. hyicus* from *S. aureus*. However, none of the current systems is capable of identifying

TABLE 2. Examples of streptococci commonly isolated from animals

| Species | Lancefield group | Host(s) | Disease(s) |
|---|---|---|---|
| *S. agalactiae* | B | Cows | Mastitis |
| *S. dysgalactiae* ("*S. equisimilis*") | C | Cows, horses, pigs | Mastitis, strangles, dermatitis |
| *S. equi* subsp. *equi* | C | Horses | Strangles |
| *S. equi* subsp. *zooepidemicus* | C | Horses | Septicemia |
| *S. porcinus* | E | Pigs | Lymphadenitis |
| *S. canis* | G | Dogs, cows | Skin infections, mastitis |
| *S. uberis* | —[a] | Cows | Mastitis |
| *S. suis* | R, S | Pigs | Pneumonia |
| *S. equinus* ("*S. bovis*") | D | Cows | Mastitis |
| *Enterococcus* spp. | D | Cows | Mastitis |

[a] May cross-react with E, P, and U antisera.

all veterinary staphylococci, and many of the newly described species either cannot be identified by currently available systems or will be misidentified. In these situations, the veterinary microbiologist should be familiar with the limitations of the system, which may necessitate the use of conventional test schemes for final identification (26).

## Streptococci

The streptococci are isolated from a variety of animal diseases. *Streptococcus agalactiae* (group B), *Streptococcus dysgalactiae* (includes strains previously designated "*S. equisimilis*") (group C), *Streptococcus equi* subsp. *equi* ("*S. equi*") (group C), *Streptococcus equi* subsp. *zooepidemicus* ("*S. zooepidemicus*") (group C), *Streptococcus porcinus* (group E), and *Streptococcus canis* (group G) are the most frequently encountered beta-hemolytic streptococci (14, 24, 31, 33, 106); group A streptococci (*Streptococcus pyogenes*) are only rarely isolated from animal specimens (106). Viridans streptococci frequently isolated from animal diseases include *Streptococcus equinus* ("*S. bovis*"), *Streptococcus suis*, and *Streptococcus uberis*; alpha-hemolytic strains of *Enterococcus* spp. are also encountered (32, 54, 103, 106). A list of the various disease processes and hosts for the streptococcal and enterococcal species is presented in Table 2. Thus, a system for identification of streptococci isolated from veterinary sources must be able to differentiate the beta-hemolytic streptococci to the species level as well as a diverse group of viridans streptococci.

Commercially available Lancefield grouping tests are useful for rapid, confirmatory identification of beta-hemolytic streptococci. Saxegaard (88) evaluated the Phadebact coagglutination test kit (Pharmacia Diagnostics, Uppsala, Sweden) with 200 streptococci belonging to Lancefield groups B, C, E, G, and L isolated from bovine mastitis and obtained a 95.5% agreement with the Lancefield precipitin test. Poutrel (79) compared both the Phadebact coagglutination test kit and the Streptex latex agglutination test kit (Wellcome Diagnostics, Research Triangle Park, N.C.) with the Lancefield precipitin test with 144 streptococci (groups B, C, and D) isolated from bovine mastitis. This worker found 100.0% agreement with the Phadebact coagglutination test and 98.0% agreement with the Streptex test kit. Unfortunately, these tests are expensive to perform if more than one reagent is tested with each isolate. Moreover, one or more reagents may be of little use (group A with the Phadebact and groups A and F with the Streptex) if the reagents are purchased as a kit rather than as individual components. The Rapid Mastitis Test (Immucell, Portland, Maine) contains a single latex reagent for Lancefield group B to permit identification of *S. agalactiae* isolated from bovine mastitis. An evaluation (109) of this system determined that the Rapid Mastitis Test correctly identified all 84 *S. agalactiae* strains tested; cross-reactions were observed with one *S. dysgalactiae* and three *S. uberis* strains. This system, while less expensive than the Phadebact or Streptex system on a per-isolate basis, requires that non-group B streptococci be identified by other methods.

Of the biochemical test systems available, the Rapid Strep system (bioMérieux-Vitek; also known as the API 20 Strep system in Europe), consisting of 19 enzymatic and biochemical tests for the 4- or 24-h identification of streptococci, has been the system most extensively evaluated with veterinary isolates. Poutrel and Ryniewicz (80) determined that this system identified 71.4% of 84 isolates. However, the system had difficulty identifying strains of *S. uberis* and *S. bovis*, and these workers concluded that an improved data base was needed to optimize identification of mastitis-producing streptococci. A subsequent study (104) determined that the Rapid Strep system correctly identified 88.4% of 199 streptococci isolated from bovine mastitis. Again, the system had difficulty identifying strains of *S. bovis* but correctly identified 96.2% of *S. uberis* strains. These workers also concluded that the Rapid Strep data base needed to be updated to incorporate the latest taxonomic changes as well as additional strains of currently described species. Jayarao et al. (51) evaluated the Rapid Strep system with 144 streptococci isolated from bovine mammary glands and obtained an overall accuracy level of 96.5%, with 95.0% of the *S. uberis* and 100.0% of the *S. bovis* strains correctly identified.

Groothius et al. (38) evaluated the Rapid Strep system with 270 streptococci isolated from human and veterinary sources. Overall, 78.8% of strains were identified in 24 h, but 17.0% could not be identified because the profile numbers were not listed in the data base. In addition, 50.0% of the group B and group C strains could not be identified with the Rapid Strep system and one of ten *S. uberis* strains was misidentified as *S. suis*. An evaluation (5) of the Rapid Strep system for group C streptococci isolated from horses determined that all of the *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* strains were identified by the system. However, serogrouping was required to differentiate *S. equisimilis* from group G and group L streptococci. In comparison, the API 20S (bioMérieux-Vitek) failed to identify any of these equine isolates to the species level, and they required serogrouping for a confirmatory identification. An evaluation of the Rapid Strep system by Hommez et al. (45) with 188 *S. suis* strains isolated from pigs yielded an overall accuracy of 90.0%.

Other systems have been less extensively evaluated with veterinary isolates. The Minitek Gram-positive set (BBL) was evaluated with 127 streptococci isolated from bovine mastitis and yielded an accuracy level of only 34.6% (105). The Minitek correctly identified only 4 of 12 *S. agalactiae* strains and misidentified 95.5% of 44 *S. uberis* strains as *Enterococcus* spp. This poor performance was attributed to a limited number of veterinary strains in the Minitek data base. The Strep-Zym system (Rosco Diagnostica), consisting of 23 enzymatic tests for the 4- or 24-h identification of streptococci, accurately characterized all strains of *S. uberis* (36 strains), *S. agalactiae* (5 strains), and *S. dysgalactiae* (5 strains) (58). Of the automated systems, only the Vitek AMS has been evaluated with veterinary streptococci. Jayarao et al. (51) evaluated the Vitek Gram-Positive Identification card with 144 streptococci iso-

lated from bovine mastitis and obtained an overall accuracy level of 94.4% at an average identification time of 8 h.

In conclusion, only the Rapid Strep system has been found to accurately identify streptococci isolated from a wide range of animal diseases. The remaining systems have been evaluated with streptococci isolated from bovine mastitis but not other animal diseases. Thus, these systems should not be relied upon as a primary method of identifying streptococci from other animal diseases until properly evaluated.

### Gram-Negative Bacilli

The gram-negative bacilli isolated from veterinary sources can generally be categorized into two groups: (i) organisms common to both humans and animals, including the enteric bacilli such as *Escherichia coli*, *Salmonella* spp., and glucose nonfermenters such as *Pseudomonas* spp.; and (ii) veterinary-specific organisms such as *Pasteurella haemolytica*, *Haemophilus somnus*, and *Actinobacillus pleuropneumoniae* (12). Thus, a system with a robust data base may perform well with the former group but fail to provide acceptable accuracy with the latter.

Swanson and Collins (95) compared the API 20E system (bioMérieux-Vitek) with conventional methods for identification of veterinary members of the *Enterobacteriaceae* family and obtained an overall accuracy level of 96.0%. When this system was evaluated with nonenteric isolates (*Pasteurella multocida*, *P. haemolytica*, and *Actinobacillus* spp.), the overall accuracy was reduced to 62.0% (15). In another study (16), the ability of the API 20E, Minitek, and Oxi/Ferm (Roche Diagnostics, Nutley, N.J.) systems to identify *P. multocida* and *P. haemolytica* was determined. None of the systems tested provide acceptable levels of identification; accuracy levels were 64.0, 64.0, and 76.0% for the API 20E, Minitek, and Oxi/Ferm systems, respectively. However, the Oxi/Ferm system provided a category but not a specific identification. More recently, Salmon et al. (85) evaluated the RapID NH system, a 4-h system designed for identification of *Haemophilus* and *Neisseria* spp. isolated from humans, with isolates of *H. somnus*, *P. multocida*, and *P. haemolytica* from bovine respiratory disease and *A. pleuropneumoniae* isolated from swine respiratory disease. These workers (85) determined that minor modification of the data base to include *H. somnus* strains and to include isolate source would permit identification of these important veterinary pathogens.

Only limited evaluations of automated systems with gram-negative veterinary pathogens are available. The Quantum II system (Abbott Laboratories, Irving, Tex.), an automated system that is no longer available, was evaluated for its ability to identify enteric and nonenteric gram-negative veterinary pathogens (53, 97). Jones et al. (53) evaluated this system with 378 isolates of the family *Enterobacteriaceae*, as well as nonfermenters such as *Acinetobacter* spp., *Flavobacterium* spp., *P. multocida*, and *Pseudomonas* spp. Of 378 isolates tested, 333 (88.1%) were correctly identified; 89.9% of the enteric organisms and 81.3% of the nonfermenters were correctly identified, but *P. multocida* was not identified by this system. Teska et al. (97) evaluated the Quantum II to determine the ability of the system to identify gram-negative fish pathogens such as *Aeromonas* spp., *Edwardsiella ictaluri*, and *Yersinia ruckeri*. Most isolates were not identified by the available data base. However, since unique biocodes were obtained for the individual organism groups, the system data base could have been augmented to identify these important fish pathogens.

### Anaerobes

Anaerobic bacteria are isolated from a variety of animal diseases, including bovine mastitis and pneumonia (*Actinomyces pyogenes*), liver and hoof abscesses in cattle (*Fusobacterium necrophorum*), pyelonephritis in sows (*Eubacterium suis*), enterotoxemia and wound infections (*Clostridium* spp.), and swine dysentery (*Serpulina hyodysenteriae*) (3, 12, 93, 102). Walker et al. (102) evaluated four commercially available anaerobe identification systems, the RapID ANA II (Innovative Diagnostics, Atlanta, Ga.), the An-Ident (bioMérieux-Vitek), the API 20A (bioMérieux-Vitek), and the Minitek anaerobe system (BBL), to identify *Eubacterium suis* isolated from swine. Results of this study indicated that the RapID ANA II and the An-Ident were useful adjunct tests to assist in the identification of *E. suis* when used in conjunction with colony morphology and Gram stain reaction. The API 20A and Minitek systems were considered unacceptable due to limited substrate reactivity or variability. In a more extensive evaluation of the RapID ANA system, Adney and Jones (3) determined that this system correctly identified 81.4% of 183 strains representing eight genera to the genus level and 59.6% to the species level. Misidentifications with the RapID ANA resulted from misassignment of veterinary species not in the data base to a human-associated species in the data base.

Two studies (27, 39) have examined the ability of the API Coryne system (bioMérieux-Vitek) to identify strains of *A. pyogenes*. Ding and Lammler (27) determined that 36 of 42 *A. pyogenes* strains were correctly identified with the API Coryne system, with the remaining six strains yielding unlisted profile numbers. These workers concluded that while the system facilitated identification of *A. pyogenes*, an improved data base was needed to enhance accuracy. In contrast, Guerin-Faublee et al. (39) determined that the API Coryne system could identify only 58 of 103 *A. pyogenes* isolates and that the system should be reevaluated before use in veterinary diagnostic laboratories. Morrison and Tillotson (67) determined that strains of *A. pyogenes* yielded unique profile numbers with the Rapid Strep system and that this system could be used to accurately identify this organism. This would be particularly useful in mastitis bacteriology laboratories, because *A. pyogenes* is frequently isolated from bovine mammary glands and the Rapid Strep system is widely employed for identification of streptococci isolated from bovine mastitis.

*Serpulina hyodysenteriae* ("*Treponema hyodysenteriae*"), an anaerobic spirochete, is the causative agent of swine dysentery and can be difficult to differentiate from the nonpathogenic organism, *Serpulina innocens* (2, 93). Achacha and Messier (2) used the RapID ANA II (Innovative Diagnostics) and the ANI card (bioMérieux-Vitek) to identify isolates of *S. hyodysenteriae* and *S. innocens*. With the ANI card, β-galactosidase was the only test that differentiated the two species; with the RapID ANA II system, both the α-galactosidase and the indole tests could be used to differentiate the two organisms. While these workers concluded that both systems could be used to differentiate *S. hyodysenteriae* from *S. innocens*, the RapID ANA II was considered easier to use.

In summary, it appears that the accuracy of commercial identification systems with veterinary pathogens varies widely. The greatest single factor impacting the accuracy of the systems appears to be the number of veterinary strains incorporated into the data base. Additionally, differences in the distribution of bacterial species between various host animals may influence the choice of system. For example, the Staph-Ident system may be preferred for use in diagnostic laboratories receiving predominately canine isolates because this sys-

tem can readily distinguish *S. intermedius* from *S. aureus*, whereas the Staph-Trac is preferred in most mastitis bacteriology laboratories because it accurately identifies *S. hyicus*. Thus, veterinary microbiologists should select systems that have been demonstrated to accurately identify bacterial species likely to be isolated from that host animal.

## ANTIMICROBIAL SUSCEPTIBILITY TESTING OF VETERINARY PATHOGENS

Considerations about antimicrobial usage and the application of ASTs in veterinary medicine should include (i) the type of animal to which an antimicrobial compound is to be administered (i.e., companion or food producing); (ii) residue avoidance in food animals; and (iii) possible extra-label usage of antimicrobial agents. When food-producing animals are the therapeutic target, the last two considerations are especially relevant. A withholding time, the time after therapy before the meat or milk of a food-producing animal can be marketed (necessary for a reduction in the levels of an antimicrobial agent and its metabolites or antimicrobial residues), may be necessary for human safety. Manufacturers of antimicrobial agents are required to establish withholding times for each compound in each host species prior to approval. Extra-label usage of a compound is defined as the use of a compound by any route, dose, or dose regimen for any disease indication or for any age or animal species other than that expressly designated on the Food and Drug Administration-approved label for the antimicrobial agent (36). Veterinarians can use antimicrobial compounds for extra-label purposes if they are willing to accept the responsibility for ensuring that no unsafe residues are incurred. In addition, extra-label usage allows the veterinarian to select an antimicrobial agent approved in one host animal species for use in another species as long as no compound within that class is approved for use in that host animal (36). Extra-label use is sometimes necessary due to the limited number of antimicrobial agents currently approved for use in animals. This is particularly true with exotic or zoo animals. For example, if a veterinarian selects a compound approved for use in horses to treat a disease in zebras, this constitutes extra-label use of the compound as no compounds are approved for use in zebras.

Application of ASTs in companion animals is similar to the use of ASTs in humans in that results obtained with isolates obtained from that animal will be used to select the antimicrobial agent usually used to treat an individual animal. On occasion, AST results for an isolate from an individual animal may be used to treat other animals in a kennel or stable. Due to the relatively small body weight of companion animals, societal taboos against the consumption of dogs and cats, and the permissive regulatory climate toward extra-label use in companion animals (36), the veterinary laboratory may be asked to test compounds commonly used in human medicine.

Administration of antimicrobial agents approved for use in humans but which do not have approved usages in food-producing animals is usually prohibited due to drug cost and the required extended withholding times. For compounds approved for use in food animals, data must be provided about human food safety, residue levels, and host animal safety and efficacy. Given these regulatory requirements for use of antimicrobial agents, only a limited number of compounds are available for therapeutic application in food-producing animals. Examples of compounds representing the different antimicrobial agent classes approved for veterinary use are listed in Table 3. When none of the compounds approved for use in that host animal offers sufficient activity against the pathogen, the veterinarian is allowed to use compounds approved in other species with the caveat that the veterinarian assumes all liability for efficacy and residues. In many situations, AST results are the key consideration used by the veterinarian to select an agent for extra-label use. As a result, many laboratories now include a disclaimer on AST reports indicating that it is the responsibility of the veterinarian to select the most appropriate antimicrobial agent for therapy. The increasing availability of computerized systems for reporting laboratory data may result in veterinary diagnostic laboratories reporting only those antimicrobial agents approved for use in a particular animal.

TABLE 3. Examples of antimicrobial agent classes with compounds approved for therapeutic use in veterinary medicine in the United States

| Antimicrobial class | Antimicrobial agent |
|---|---|
| Penicillin | Penicillin |
| | Ampicillin |
| | Amoxicillin |
| | Hetacillin |
| | Cloxacillin |
| Cephalosporin | Cephapirin |
| | Cefadroxil |
| | Ceftiofur |
| Tetracycline | Oxytetracycline |
| | Chlortetracycline |
| Macrolides | Erythromycin |
| | Tylosin |
| | Tilmicosin |
| Lincosaminides | Lincomycin |
| | Clindamycin |
| | Pirlimycin |
| Aminocyclitols | Spectinomycin |
| | Streptomycin |
| | Gentamicin |
| | Amikacin |
| Fluoroquinolones | Enrofloxacin |
| Other | Sulfonamides |
| | Trimethoprim-sulfadiazine |
| | Novobiocin |

Antimicrobial susceptibility tests are designed to provide reproducible results with a strong correlation to in vivo efficacy (1, 71, 82). While the data necessary to establish these performance characteristics are well defined in human medicine (1, 71), they are less well defined in veterinary medicine. Unlike antimicrobial agents developed for use in humans, AST interpretive criteria and quality control parameters are not required by the Food and Drug Administration as a necessary component of a New Animal Drug Application. Professional groups such as the American Association of Veterinary Laboratory Diagnosticians and the Association of Veterinary Microbiologists initiated the process of developing improved standards during the last decade. More recently, the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Veterinary Antimicrobial Susceptibility Testing has begun to further develop performance standards for antimicrobial susceptibility testing of veterinary pathogens. These performance standards will include test methods, quality control guidelines, and interpretive criteria for veterinary antimicrobial agents.

### Test Methods and Quality Control Parameters

The majority of veterinary diagnostic laboratories rely on NCCLS-recommended testing procedures (12, 28, 61–63, 65, 74, 78) for performing both the agar disk diffusion and the

broth dilution techniques. The NCCLS procedures are well documented and offer high reproducibility and rigorous quality control (69, 70). While current NCCLS guidelines appear to be adequate for the rapidly growing, facultative anaerobes such as the staphylococci, streptococci, and members of the families *Pasteurellaceae* and *Enterobacteriaceae* (12, 29, 61–63, 74, 78), use of these procedures for testing more fastidious organisms such as *H. somnus* and *A. pleuropneumoniae* may be less suitable.

*H. somnus* may be isolated from cattle with bovine respiratory disease (pneumonia) and also causes thromboembolic meningoencephalitis, mastitis, arthritis, and reproductive problems in cattle (46). *H. somnus* is not well defined taxonomically, and Stephens et al. (94) considered *H. somnus*, *Haemophilus agni*, and *Histophilus ovis* to constitute a single taxon. A DNA homology study by Gonzales and Bingham (37) indicated that *H. somnus* could be placed in either the genus *Haemophilus* or the genus *Actinobacillus*. Unlike the other *Haemophilus* spp., *H. somnus* does not require hemin or NAD for growth but does require thiamine monophosphate or pyrophosphate and cysteine for growth (49, 66). Strains of *H. somnus* may require up to 10% $CO_2$ for optimal growth, although some strains may require lesser amounts or no $CO_2$ for growth (47). Indeed, Inzana and Corbiel (49) indicated that oxygen was required for growth and that $CO_2$ may act as a nutritional factor rather than making the environment less aerobic. The variation in the growth requirements of *H. somnus* has made antimicrobial susceptibility testing of this organism problematic.

Various media have been used for antimicrobial susceptibility testing of *H. somnus*. Yancey et al. (117) used brain heart infusion agar supplemented with 2% supplement C and a 5% $CO_2$ atmosphere for agar dilution MIC testing of *H. somnus*. Inzana (48) has indicated that Mueller-Hinton agar supplemented with blood and incubated in a $CO_2$ atmosphere may be adequate for disk diffusion tests of some strains. Inzana (48) has also found that Columbia broth supplemented with 0.1% Trizma base and 0.01% thiamine monophosphate supports excellent growth of *H. somnus* and may be useful for broth dilution MIC testing. Tanner and Hargis (96) evaluated a basal broth medium supplemented with 5% defined equine serum and 1% thiamine monophosphate to determine the reproducibility of microdilution MIC testing of *H. somnus* in dehydrated commercial MIC panels. This medium yielded MICs within ±1 dilution for the majority of antimicrobial agents tested. However, only 11 strains of *H. somnus* were included in the study, and the medium is not commercially available and requires serum supplementation. A multilaboratory study is needed to evaluate the reproducibility of the various media and to establish quality control strains for susceptibility testing of *H. somnus*.

*A. pleuropneumoniae*, previously classified as *Haemophilus pleuropneumoniae*, is a causative agent of pleuropneumonia in swine (77, 92). This organism requires media supplemented with NADH for growth (77, 92). Past studies on the in vitro susceptibility of *A. pleuropneumoniae* have used Mueller-Hinton agar (34), Mueller-Hinton broth (61), Iso-Sensitest agar (65), Iso-Sensitest broth (28), tryptone yeast extract broth (34), and brain heart infusion broth (29). All media in these studies were supplemented with NADH in concentrations ranging from 0.001 to 0.05%. Additionally, many veterinary diagnostic laboratories now use the *Haemophilus* Test Medium recommended by NCCLS for susceptibility testing of *Haemophilus influenzae* (69, 70) for testing *A. pleuropneumoniae* (101). It appears that additional studies are needed to determine which medium and NADH supplementation level provide acceptable performance characteristics for susceptibility testing of *A. pleuropneumoniae*. Additionally, a quality control strain of *A. pleuropneumoniae* needs to be identified for routine testing.

*Staphylococcus hyicus* is frequently isolated from bovine mammary glands and is the etiological agent of exudative dermatitis in pigs (23, 59, 60, 106, 115). Recently, Wegener et al. (115) determined that strains of *S. hyicus* yielded high MICs (>64.0 μg/ml) of sulfadiazine-trimethoprim when tested in Mueller-Hinton broth but were susceptible when tested by the agar disk diffusion method on Mueller-Hinton agar. Even though the *Enterococcus faecalis* quality control strain yielded MICs within stated parameters, indicating that excessive thymidine levels were not present in the test medium, addition of thymidine phosphorylase to the test medium reduced MICs for *S. hyicus* to 0.06 μg/ml. The authors concluded that *S. hyicus* is very sensitive to thymidine levels and recommended that *S. hyicus* ATCC 11249 be included as a quality control strain when *S. hyicus* strains are tested with sulfonamides. In conclusion, it seems likely that as future performance standards are developed for veterinary pathogens, methodology and quality control guidelines will differ from those used for human pathogens.

A limited number of commercially available and/or automated systems have been evaluated for antimicrobial susceptibility testing of veterinary pathogens. In a recent study (75), the Sceptor System (Becton Dickinson) was compared with the agar dilution method for testing 136 gram-positive bacterial isolates and 75 fastidious gram-negative isolates. While complete agreement between the methods was 95.7% with the 10 antimicrobial agents tested against the gram-positive isolates, complete agreement was only 88.3% between methods for 8 agents tested against the fastidious gram-negative isolates. Newman et al. (73) compared a commercially available microdilution panel with the disk diffusion method, using 11 antimicrobial agents and 254 veterinary clinical isolates of staphylococci and streptococci. They found a 92.3% agreement between the systems and concluded that the commercial system (Micro-Media Systems, San Jose, Calif.) was a reliable method for testing gram-positive animal isolates. While several other laboratories have used commercially prepared customized microtiter dilution panels, rigorous validation of these systems has not been reported.

## Interpretive Criteria

Antimicrobial susceptibility tests should provide results that the clinician can use to increase the potential for a successful therapeutic outcome. Interpretive criteria for categorizing isolates as susceptible or resistant are based on a data base consisting of the MIC of the drug for a bacterial population (under quality-controlled conditions), the pharmacokinetics of the antimicrobial agent in the host species, and the reasonable correlation with the clinical outcome (71, 76, 81, 82). It is important for the diagnostician to realize that an isolate cannot be categorized as susceptible or resistant unless all of these data for setting the breakpoint have been established. The bacterial population for which MIC data should be obtained should consist of at least 300 clinically relevant isolates (71). The pharmacokinetic data most relevant to establishing the interpretive criteria are the concentrations of antibacterially active material at the sites of infection. However, the guideline provided by Barry (6), i.e., that the organism is susceptible if inhibited by one-half the mean blood level or one-fourth the peak blood level, is still used with success for setting the MIC breakpoint. Finally, in vitro susceptibility should be correlated

with therapeutic outcome, with experience suggesting that the correlation should exceed 85% (98).

In general, these rigorous interpretive criteria have not have been established for all antimicrobial agents used in veterinary medicine. The most frequently used interpretive criteria for categorizing veterinary isolates as susceptible or resistant are those recommended by the NCCLS (12, 62, 63, 74, 78, 82) on the basis of isolates and pharmacokinetic data from humans. Their use for predicting the antimicrobial susceptibility of veterinary pathogens has been questioned (52, 64, 116). In this regard, the use of AST reports by the veterinary clinician has been characterized, perhaps not altogether facetiously, as "simply a case of the blind leading the blind" (116).

The paucity of veterinary-specific guidelines has resulted in various recommendations for either performance or interpretation of AST. Burrows (11) has recommended a MIC breakpoint of ≥2.0 μg/ml for erythromycin resistance when *P. haemolytica*, *P. multocida*, or *H. somnus* isolates from bovine respiratory disease are tested. In contrast, current NCCLS guidelines (69) for erythromycin recommend a breakpoint of ≤0.5 or ≥8.0 μg/ml for categorizing human pathogens as susceptible or resistant, respectively. While it is not surprising that the breakpoint for cattle would be different from that for humans, the recommendation of Burrows (11) was based on the pharmacokinetic data obtained with erythromycin administered at an extra-label dose of 15 mg/kg rather than the approved dose in cattle of 2.2 to 4.4 mg/kg. Libal (61) and Post et al. (78) have suggested that MIC testing is preferable to agar disk diffusion tests to determine the antimicrobial susceptibilities of veterinary pathogens because of the qualitative nature of the latter test method and lack of zone size interpretive criteria available for veterinary pathogens in the different animal species. However, this recommendation suggests that the veterinarian or veterinary diagnostician has access to host-specific pharmacokinetic information upon which to calculate the appropriate breakpoint. Also, differences in the pharmacokinetics of an antimicrobial agent may necessitate the development of different interpretive criteria for various animal species (10, 68, 118). For example, at a dose of 3 mg/kg administered intramuscularly in both species, the peak concentrations of the cephalosporin antibiotic, ceftiofur, in serum were 16.7 and 2.5 μg/ml for pigs and day-old chicks, respectively (68, 118). Conversely, a single breakpoint for two different animal species may sometimes be appropriate because the label-indicated dose of ceftiofur for treatment of bovine respiratory disease in cattle (2.2 mg/kg) results in peak concentrations in serum approximately the same (16 μg/ml) as in swine. When AST interpretive criteria for veterinary pathogens are set, it cannot be assumed that the breakpoints for isolates from different animals are the same. Also, breakpoints may differ when a compound is used for treating different diseases in the same animal species, particularly when different dosages or dose regimens are indicated.

Current NCCLS interpretive guidelines (69, 70) categorize those isolates producing infections that should respond to therapy with the antimicrobial agent administered at the indicated dose as susceptible, whereas those isolates causing infections that would not respond are classified as resistant. For in vitro testing by the disk method, a "buffer zone" category of intermediate is used to prevent technical variation causing false-susceptible or false-resistant errors (1, 69, 70). A fourth category, moderately susceptible, has been used for those situations in which high concentrations of antimicrobial agents can be achieved at the site of infection, such as with β-lactam antibiotics in treatment of urinary tract infections (1, 69, 70). Many times, veterinary laboratories report isolates as moderately susceptible rather than intermediate (29, 78) or consider isolates falling into the intermediate category as susceptible or resistant (13, 30, 61, 83). These practices could have important consequences by over- or under-estimating resistance and could impact treatment efficacy. More importantly, categorizing isolates as moderately susceptible rather than intermediate could increase the risk of antimicrobial residues because this category might be interpreted by the veterinarian as an edict to increase dosages or to dose more frequently; i.e., this category might encourage extra-label use of the agent. Recently, the NCCLS (72) has combined the intermediate and moderately susceptible categories and included pharmacotoxicity as an additional consideration for distinguishing intermediate from moderately susceptible isolates. In effect, this change places the responsibility on the physician to decide whether appropriate antimicrobial concentrations can be safely achieved. This approach appears to be very appropriate for veterinary use because the veterinarian, not the veterinary microbiologist, should be more aware of the pharmacotoxicity and pharmacokinetics of individual antimicrobial agents. Thus, it would be prudent for veterinary diagnostic laboratories to consolidate the intermediate and moderately susceptible categories for reporting purposes in accordance with NCCLS guidelines.

While MIC information and pharmacokinetic data allow selection of an initial MIC breakpoint, the ultimate criterion for selecting in vitro test interpretive criteria must be based on clinical response with the approved dose (81). In human diseases, to establish an in vitro-in vivo correlation, isolates must be obtained from the patient's tissues before treatment, the course of antimicrobial therapy must then be administered, and the patient must be recultured for presence of the organism (1, 71). A cure is usually defined as an appropriate clinical response and eradication of the pathogen, with an acceptable correlation between in vitro and in vivo data placed at ≥85.0%. Interpretive criteria for susceptibility tests for companion animals can probably be developed using the same criteria as those used for antimicrobial agents for humans. The clinical investigator usually has ready access to the animal patient, and the course of therapy generally can be closely followed in clinical trials. Indeed, Ling et al. (62) determined that AST results correctly predicted clinical outcome in 92.5 and 84.0% of canine urinary tract infections treated with ampicillin and trimethoprim-sulfadiazine, respectively.

Establishing in vitro-in vivo correlations for AST with pathogens for food-producing animals may require modification of current guidelines. For example, *P. haemolytica* and *P. multocida* are normal flora of the respiratory tract of cattle, and previous studies have suggested that isolates obtained from the nasal passages may not be representative of the isolates causing lower respiratory tract infections (52, 64). To obtain isolates from the lower respiratory tract of cattle would require a bronchial washing or lavage. These procedures are not only difficult to perform but also may impose additional stress on the animals which could affect the therapeutic outcome. Other parameters such as clinical response, mortality, or relapse rates may be useful for defining the in vivo correlation. Previous studies (7, 52, 64) have reported that clinical response and mortality rates following therapy of bovine respiratory disease have ranged from 79.4 to 91.5% and 0.8 to 10.0%, respectively, for the various antimicrobial agents (Table 4). However, trial-to-trial variation may be a problem, as clinical response rates were 79.4, 83.3, and 91.5% for trimethoprim-sulfadiazine in these three studies and mortality rates were 0.8 and 8.0% for oxytetracycline in two studies (Table 4). Since clinical response and mortality data are normally collected in the field efficacy

TABLE 4. In vivo response of bovine respiratory disease to various antimicrobial agents

| Study | Antimicrobial agent | No. of animals | Dose of agent (per kg) | Temp (°C)[a] | Clinical response (%)[b] | Mortality (%) | Reference |
|---|---|---|---|---|---|---|---|
| 1 | Trimethoprim-sulfadiazine | 267 | 16 mg | ≥40.3 | 79.4 | 7.1 | 52 |
| | Ceftiofur | 263 | 1.1 mg | ≥40.3 | 91.3 | 5.3 | |
| 2 | Oxytetracycline | 123 | 10 mg | ≥39.5 | 86.2 | 0.8 | 7 |
| | Penicillin | 114 | 45,000 IU | ≥39.5 | 87.8 | 0.9 | |
| | Trimethoprim-sulfadiazine | 126 | 16 mg | ≥39.5 | 83.3 | 2.4 | |
| 3 | Oxytetracycline | 50 | 20 mg | ≥40.5 | 90.0 | 8.0 | 64 |
| | Penicillin | 50 | 65,000 IU | ≥40.5 | 86.0 | 10.0 | |
| | Trimethoprim-sulfadiazine | 59 | 16 mg | ≥40.5 | 91.5 | 3.0 | |

[a] Rectal temperature necessary for enrollment in the study.
[b] Defined as a body temperature of <40°C on day 3 of therapy for studies 1 and 3 and a body temperature of <39.5°C for study 2.

trials necessary for drug approval, in vivo correlations with MIC breakpoints could be easily incorporated into the protocols.

Antimicrobial agents used for therapy of bovine mastitis usually are administered by infusion of the antimicrobial agent directly into the mammary gland via the teat canal. The antimicrobial agent must then exert its effect on the pathogen in milk (74, 86, 87). Milk contains high levels of protein, fat, and cations, all of which may affect the activity of an antibiotic (86). The interaction of milk with the antimicrobial agent has prompted several workers to question the validity of standard agar disk diffusion or MIC methods for susceptibility testing of mastitis pathogens (74, 86, 87). Owens and Watts (74) compared the effect of milk on various antimicrobial agents and determined that milk reduced the activities of novobiocin, streptomycin, gentamicin, tetracycline, and vancomycin. Sandholm et al. (86) compared the activities of various antimicrobial agents against *S. aureus*, using Iso-Sensitest broth and milk, and determined that the activities of ampicillin, spiramycin, and erythromycin were reduced in milk. Saperstein (87) reported that a commercial system in which milk from infected mammary glands was directly inoculated into a microtiter plate containing dehydrated antimicrobial agents was more appropriate for susceptibility testing of mastitis pathogens than conventional AST methods. However, large-scale inter- and intralaboratory reproducibility studies and quality control guidelines have not been published for this system. All of these authors used current NCCLS guidelines as the basis for their conclusions because these are the only interpretive criteria available. Again, the validity of using these guidelines for mastitis pathogens has not been established, and in one publication the authors (74) suggested that these guidelines be used to compare the results obtained with the two medium types (broth and milk) rather than to predict in vivo efficacy.

In order to develop accurate interpretive guidelines for mastitis therapeutics, MIC breakpoints need to be developed on the basis of MIC data for mastitis pathogens and the concentrations of antimicrobial agent achieved in the mammary gland. Once a MIC breakpoint has been established, in vivo correlations can probably be developed for organisms that are confined to milk, such as *S. agalactiae* and *S. dysgalactiae*. Determining the in vivo correlations will be more problematic for *S. aureus* because these organisms may be sequestered in polymorphonuclear leukocytes or behind scar tissue barriers (74, 86), which reduces treatment efficacy. By using MIC data, antimicrobial agent levels in milk and in vivo correlations to develop interpretive criteria, factors that affect AST accuracy, can be taken into consideration and appropriate adjustments can be made to the interpretive criteria. Recently, Thornsberry et al. (99) reported the development of MIC breakpoints and agar disk diffusion interpretive criteria for pirlimycin, a new lincosaminide antimicrobial agent, by using MIC data obtained with mastitis pathogens and the concentration of the antimicrobial agent achieved in the mammary gland.

## CONCLUSIONS

Veterinary diagnostic laboratories are under increasing demand to provide rapid, accurate identification of veterinary pathogens with diminishing resources. Additionally, the current regulatory climate to reduce extra-label antimicrobial agent use and avoid antimicrobial residues in food animals has emphasized the need for well-controlled, clinically relevant antimicrobial susceptibility tests for veterinary pathogens. Currently marketed identification systems offer convenience and rapid identification of veterinary pathogens, but the accuracy of the systems varies widely, particularly between pathogens from different host species. In many instances, simply increasing the number of strains and species of veterinary pathogens in the system data base would dramatically improve system accuracy. Ultimately, it is the responsibility of the veterinary microbiologist to determine if a commercial identification system provides acceptable accuracy levels for a given group of organisms. The development of improved performance standards and AST interpretive criteria in veterinary microbiology will take several years. The NCCLS Veterinary Antimicrobial Susceptibility Testing Subcommittee, in cooperation with other professional and regulatory groups, has begun the process of formalizing these standards. As improved standards become available, the veterinary diagnostic microbiologist will then have the tools to provide the veterinarian with more accurate information from which to make informed decisions on the appropriate course of therapy.

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