Antimicrobial Resistance: What’s the Big Deal?
Importance of Antimicrobial Resistance to the Equine Practitioner

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Equine veterinary practitioners should be concerned about the issue of antimicrobial resistance for multiple reasons including: 1) the documented occurrence of resistant infections in equine patients, 2) the likelihood that few if any new antimicrobials will come on the market for veterinary use, and 3) potential public health impacts of resistant bacterial infections. Currently, there is no national system to monitor antimicrobial resistance of animal pathogens. Thus, the frequency of treatment failure due to antimicrobial resistance in our equine patients is unknown. Guidelines for judicious use of antimicrobials have been developed by the American Association of Equine Practitioners and have subsequently been endorsed by the American Veterinary Medical Association executive board. Members of the American Association of Equine Practitioners should be aware of the guidelines and practice judicious use of these valuable tools. Author's address: Colorado State University, Dept Clinical Sciences, Ft Collins, CO 80521 (Traub-Dargatz and Morley) USDA:APHIS:VS:CEAH, 2150 Centre Avenue, Bldg B, Fort Collins, CO 80526-811. © 2002 AAEP.

1. Introduction

The use of antimicrobials has increased significantly in human and veterinary medicine. During the second half of the twentieth century the introduction of antimicrobial drugs has had a dramatic impact on improving human and animal health. Antimicrobials are now readily available for use in the treatment of both humans and domestic animals. Antimicrobial resistance is a growing concern within the human and veterinary medical profession. Although there is evidence of multidrug resistant infections in both humans and domestic animals the source of this resistance is not clear. To date, food animal practitioners (poultry, swine, beef, and dairy) have paid greatest attention to the issue of surveillance and control of antimicrobial resistance. This should not be surprising because many of the concerns to date have focused on bacteria that can give rise to food-borne illnesses such as Salmonella spp., Campylobacter spp., and Escherichia coli. However, because equine practitioners use antimicrobials to treat horses both therapeutically and prophylactically, and horse meat is used for human consumption in many parts of the world, it is imperative that practitioners become better informed about the issues and more involved in the monitoring and development of guidelines for the judicious use of antimicrobials.
Equine practitioners should be concerned about this issue for several reasons, which include:

1. Infection with multidrug resistant bacteria in equids makes treatment of affected patients expensive and unfeasible in some situations.
2. All veterinarians need to be well informed on this issue in order to act as good stewards of veterinary medicine when interacting with the general public.
3. Equine multidrug resistant infections have been documented primarily in hospitalized patients and some of these infections appear to have been acquired during hospitalization. When patients acquire infections within veterinary clinics it affects the credibility of the practice and can have financial and emotional impact.
4. The development of new antimicrobials has lagged behind the development of resistance in several bacteria underscoring the need for judicious use of the drugs we currently have available. It is crucial for all who administer or dispense antimicrobials to be informed regarding the issues surrounding antimicrobial resistance.

The American Veterinary Medical Association (AVMA) has published guidelines for the judicious use of antimicrobials for veterinarians. The guidelines are meant to give general direction to the selection of antimicrobials by veterinarians.\(^1\)

The American Association of Equine Practitioners (AAEP) appointed a task force to develop judicious use guidelines for antimicrobials for their membership in 2000. The developed guidelines were approved by the AAEP executive board and the AVMA executive board and have been posted to the AVMA website. The website is www.avma.org/scientist/jtua/equine/jtuaequine.asp.

The guidelines are as follows:

**American Association of Equine Practitioners Prudent Drug Usage Guidelines**

(Approved by the AVMA Executive Board, June 2001)

The health and welfare of horses and their owners is the primary goal of members of the American Association of Equine Practitioners (AAEP). We believe that these guidelines merely reiterate the standard of practice and what is common in equine veterinary medicine. The AAEP provides continuing education for veterinarians that focuses on the appropriate use of antimicrobial drugs. Our members are committed to the practice of preventive immune system management through the use of vaccines, parasiticides, stress reduction, and proper nutritional management. The AAEP recognizes that proper and timely management practices can reduce the incidence of disease and therefore reduce the need for antimicrobials; however, antimicrobials remain a necessary tool to manage infectious disease in horses. In order to reduce animal pain and suffering, prudent use of antimicrobials is encouraged. The following are general guidelines for the prudent therapeutic use of antimicrobials in horses:

1. The veterinarian’s primary responsibility is to aid in the design of management, immunization, housing, and nutrition programs that will reduce the incidence of disease and the need for antimicrobials.
2. Antimicrobials should be used only within the confines of a valid veterinarian-client-patient relationship; this includes both dispensing and issuance of prescriptions.
3. Veterinarians should:
   a. Participate in continuing education programs that include therapeutics and emerging and/or development of antimicrobial resistance.
   b. Avoid antimicrobial use in transient virus associated conditions.
   c. Have clinical evidence of the identification of the pathogen associated with the disease based upon history, clinical signs, laboratory data, and experience.
   d. Select antimicrobials that are appropriate for the target organism and should be administered at a dosage and route that are likely to achieve effective levels in the target organ.
   e. Make product choices and use regimens that are based on available laboratory and package insert information, additional data in the literature, and consideration of the pharmacokinetic and pharmacodynamic aspects of the drug.
   f. Use products that have the narrowest spectrum of activity and known efficacy in vivo and/or in vitro against the pathogen causing the disease problem.
   g. Utilize antimicrobials at a dosage appropriate for the condition treated for as short a period of time as reasonable, i.e., therapy should be discontinued when it is apparent that the immune system can manage the disease, reduce pathogen shedding, and minimize recurrence of clinical disease or development of the carrier state.
   h. Select antimicrobials of lesser importance in human medicine in preference to newer generation drugs that may be in the same class if this can be achieved while protecting the health and safety of the animals.
   i. Utilize antimicrobials labeled for treating the condition diagnosed, and whenever possible, at the labeled dose, route, frequency, and duration if the available scientific information still supports their efficacy.
   j. Utilize antimicrobials on an extra-label basis only within the provisions contained...

k. When appropriate, utilize local therapy over systemic therapy.

l. Be discouraged from using combination antimicrobial therapy unless there is information to show an increase in efficacy or suppression of resistance development for the target organism.

m. Protect integrity through proper handling, storage, and observation of the expiration date.

4. Veterinarians should endeavor to ensure proper on-farm drug use. Prescription or dispensed drug quantities should be appropriate so that stockpiling of antimicrobials on the farm is avoided.

2. Mechanisms of Antimicrobial Resistance

It is important to emphasize that antimicrobial drug use does create resistance among bacteria but can result in selective pressure, thus giving resistant organism competitive advantage. Antimicrobial resistance may be either intrinsic (constitutive) or acquired.1,2 Intrinsic antimicrobial resistance exists when the organism is not affected by the antimicrobial naturally.1 This can occur if the organism lacks target binding sites or structures that the antimicrobial interacts with. Acquired resistance is due to mechanisms developing mechanisms to circumvent the effects of antimicrobials. These include: 1) production of enzymes to inactivate the antimicrobial, 2) decreased cell wall permeability to the drug, 3) active drug export (efflux pumps), 4) alteration of the drug target receptor, and 5) development of alternative biochemical pathways to bypass the effect of the drug.1 Acquired antimicrobial resistance may be the result of natural genetic rearrangement (random mutation) or may be transferable from one organism to another. Transferable resistance may be acquired by any of several possible mechanisms involving transfer of DNA containing resistance genes from one bacterium to another.1,3 These are: 1) transformation, where naked DNA is released from one organism and incorporated into another, 2) transduction, where DNA transfer occurs through a bacteriophage, and 3) conjugation where DNA is transferred via a sex pilus.

Acquired genes that encode for antimicrobial resistance may reside on a plasmid, a free floating piece of extra-chromosomal circular DNA, sometimes called an R factor, or may be incorporated into the chromosome of the organism.1 The genes may be part of a transposon or integron.1 Integrons have been described as “sticky flypaper” for resistance genes.4 That is to say these specialized structures have a propensity to accumulate resistance genes. Because these genes are clustered, they can be transferred as a “resistance cassette.” This provides a means for the development of resistance to several antimicrobials simultaneously. Because they are closely associated, use of any of the antimicrobials subject to resistance can select for the entire set of cassettes of resistance genes.

Acquired and inherent resistance becomes more prevalent when the resistance confers some survival advantage to the organism such as under conditions of exposure to a particular antimicrobial. On the other hand, acquired antimicrobial resistance may be reversed or lost if it does not result in a survival advantage, especially if it is associated with a metabolic cost to the organism.1 Loss of acquired resistance genes occurs more readily if the resistance gene is maintained on a plasmid rather than incorporated into the bacterial chromosome. The rate at which antimicrobial resistance is reversed or lost is often unknown and depends on the specific organism, the antimicrobial(s) in question, the host and the environment. However, it is unlikely that, even with discontinued use, all isolates will in time revert to being sensitive to that drug or chemical.5

An additional effect of exposure to an antimicrobial such as tetracycline, is to stimulate exchange of genetic material among bacteria.6 The exchanged genetic elements may include genes for antimicrobial resistance. Thus, exposure to antimicrobials may not only select for resistant organisms but also stimulate a greater number of organisms to acquire resistance genes.

3. Assessing Antimicrobial Resistance

Antimicrobial resistance is usually assessed by in vitro testing methods.1,2 How well the results of these tests correlate with clinical efficacy is debatable7 and is but one of various factors that the clinician should consider in arriving at a decision about antimicrobial use. In addition to the susceptibility or sensitivity profile of an organism, the pharmacokinetics (fate of a drug in the body over time) and pharmacodynamics (mechanism of action) of each pharmaceutical must be considered.1,8 Other factors that influence the success of antimicrobial treatment include: the drug concentration and duration needed to kill organisms or prevent multiplication; trapping at the site (e.g., pH-induced changes in solubility); secretion into a body compartment or fluid (e.g., urine in the case of urinary tract infections); and presence of organic debris.1

Several methods are used for in vitro susceptibility testing of which the disk diffusion method is the most common. In this method, the putative pathogen is isolated, grown in broth culture, inoculated onto a specialized agar plate to produce a lawn of growth, and small disks impregnated with antimicrobials are placed on the agar. The antimicrobial diffuses from the disk into the agar. In the case of organisms that are sensitive to the antimicrobial being tested, a zone of inhibition of bacterial growth can be seen around the disk. The size of the zone of inhibition depends upon the degree of sensitivity of...
the organism to the antimicrobial as well as the diffusion dynamics of the antimicrobial, the thickness of the agar and other characteristics of the in vitro system. Because the drug concentration progressively decreases the greater the distance from the disk, there is an inverse linear relationship between the log inhibitory concentration and the diameter of the zone of inhibition.\textsuperscript{7} A more accurate method for determination of the MIC is to use serial dilutions of antimicrobials in a broth medium and assess the degree of turbidity after inoculation of each of the culture tubes with the isolate of interest. After incubation, the cultures are checked either manually or using an automated reader for visible signs of growth. The lowest dilution of the antimicrobial that inhibits the growth of the organism is the MIC. Use of an automated reader is preferable because of the relative lack of sensitivity of visually assessing the amount of growth.

A third method of antimicrobial susceptibility testing, the agar dilution method, involves the use of agar plates containing specified dilutions of an antimicrobial. The dilutions are selected to correspond to breakpoints for the antimicrobial being tested. Breakpoints represent concentrations of the same antimicrobials that would be achieved with therapeutic doses in the host. After incubation, plates are checked for growth at each inoculation site. Several individual isolates can be tested on the same plate simultaneously.

Results of susceptibility testing of an isolate are reported qualitatively, i.e., susceptible, intermediate, or resistant to a particular antimicrobial, or semi-quantitatively, based on determination of the MIC. In some cases, susceptibility testing is carried out using a breakpoint configuration. In this testing format, two specified dilutions of the antimicrobial are used. If the organism does not grow in either dilution it is considered “sensitive,” if it grows in both of the dilutions it is considered “resistant,” and if it only grows in the more dilute concentration it is considered “intermediate.” Although breakpoint testing is designed to provide information about the anticipated clinical response of a patient using standard antimicrobial dosages, with only a few exceptions, validated breakpoints for animal pathogens are not available.\textsuperscript{9}

In some cases, the resistance profile for a particular bacterial isolate (i.e., which antimicrobials the isolate is resistant to) is referred to as the antibiogram for the organism. Antibiograms can be helpful in investigating the “relatedness” of isolates.\textsuperscript{10} Although this method is not nearly as precise as molecular typing techniques such as pulsed field gel electrophoresis or ribotyping for making such comparisons, it can provide some general indication of whether isolates might be related.

When antimicrobial susceptibilities (sensitivities) are reported for populations of organisms, such as isolates of a particular bacterial genus and species recovered by a diagnostic laboratory over a period of time, they are often reported in terms of the MIC\textsubscript{50} or the MIC\textsubscript{90} (the concentration of the antimicrobial that inhibits 50% and 90% of the particular organisms in the population, respectively).\textsuperscript{1} Isolates made from diagnostic laboratory submissions often represent the “worst of the worst” in terms of their antimicrobial resistance profiles.\textsuperscript{11} With few exceptions, such isolates come from animals with serious or fatal infections that frequently have been treated with antimicrobials, perhaps extensively.

In a summary of veterinary diagnostic laboratories in which a total of 181 labs were sent a survey in May 2000 there were 86 responses, 22 did not test for bacterial susceptibility to antimicrobials, a total of 64 labs routinely did susceptibility testing on veterinary isolates with over 160,000 isolates tested per year. The majority of isolates (65%) were tested using Kirby-Bauer disk diffusion method. In addition, 84% of labs reported using the National Committee on Clinical Laboratory Standards guidelines. A total of 20,440 equine isolates were tested in a year by a total of 63 labs with ~78% of equine isolates tested by using Kirby-Bauer disk diffusion method.\textsuperscript{12}

4. Antimicrobial Resistance in Equine Pathogens

Antimicrobials are used both therapeutically and prophylactically in equid patients. Prolonged exposure of organisms to antimicrobials within a hospital setting or in the general equine population could encourage the selection of resistant organisms.\textsuperscript{13} Factors that can predispose to resistance problems include use of antimicrobials at inappropriate doses or use for an inappropriate length of time. Comprehensive data on antimicrobial use patterns for equid patients on a regional or national basis are currently lacking.\textsuperscript{a}

Much of the information needed to determine the extent of the antimicrobial resistance problem in equine medicine and how best to minimize it for the future is lacking. Comparative studies of susceptibility patterns of equid isolates are few.\textsuperscript{14} Though there is an extensive amount of antimicrobial susceptibility testing done in veterinary diagnostic laboratories throughout the United States, the results are usually shared only with the veterinary practitioners submitting the samples. In the past it was rare for diagnostic laboratories to collate the information on the isolates they tested into a report. Only recently have some individual diagnostic laboratories elected to publish summaries of susceptibility patterns of selected equine bacterial isolates. The AVMA has funded a pilot effort to collate and uniformly test selected animal pathogens in an effort to monitor for antimicrobial resistance.\textsuperscript{b}

Unless a particular disease occurrence has been severe or extensive enough to be reported in the scientific literature, the antibiogram status of equine isolates has not been well documented. Several reports on susceptibility of single pathogens have been published. Pathogens included in these...
of the 62 strains identified, the most common isolate was S. Typhimurium, others being Salmonella Heidelberg, Salmonella Hadar, Salmonella Thompson, Salmonella Eteritidis, Salmonella Derby, and S. Infantis. Sixteen of the 62 strains were resistant to trimethoprim, sulfonamides, or a combination of these drugs, all of which were isolates of S. Typhimurium.

S. Typhimurium definitive (phage) type 104 (DT104) has caused epidemics in Europe and has been isolated sporadically elsewhere including in the U.S. The epidemic strain of Salmonella DT104 has a characteristic penta-resistance profile that includes ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. The penta-resistant strain of S. Typhimurium DT104 has been isolated from horses in the U.S., but the true extent and distribution of this organism among the equine population is unknown.

The National Animal Health Monitoring System’s (NAHMS) Equine ’98 study estimated that 0.8% of the general horse population shed Salmonella spp. in feces. Fourteen different serotypes from eight different serogroups were identified. There were distinct differences between serotypes identified in the NAHMS study and those reported most frequently from equine clinical cases. The most common serotype identified in the NAHMS Equine ’98 study was S. Muenchen, which was the 8th most common serotype identified from equids in 1997–1998 by the National Veterinary Services Laboratories. In the case of each isolate, the MIC for 16 antimicrobials was determined by using a semi-automated broth microdilution system. Isolates were classified as sensitive, intermediate or resistant using human breakpoints for the antimicrobials evaluated. There were 4.9% of the fecal Salmonella sp isolates in the NAHMS Equine ’98 study were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, or tetracycline, and 2.4% were resistant to kanamycin, gentamicin, or trimethoprim-sulfa. Resistance to amikacin, amoxicillin/clavulanic acid, apramycin, ceftiofur, ceftriaxone, ciprofloxacin, or nalidixic acid was not detected.

A multi-center study was conducted by the State University of Utrecht in the Netherlands to determine the susceptibility patterns of a variety of isolates obtained from the Veterinary Microbiological Diagnostic Centre at the University. Included in the study were 20 isolates of Salmonella spp. (serogroup B), 17 of E. coli, 8 of Klebsiella spp., 7 of Proteus spp., 7 of Pseudomonas aeruginosa, 5 of Actinobacillus equuli, 4 of Rhodococcus equi, 23 of Streptococcus zooepidemicus, and 20 of coagulase-positive Staphylococcus spp. Most of the isolates were from horses hospitalized in the large animal clinic at the University, whereas others were from diagnostic submissions from practitioners or the Regional Animal Health Services. Samples were collected between January 1988 and March 1991. Minimum inhibitory concentrations were deter-
minded for 30 antimicrobial drugs including doxycycline, minocycline, vancomycin, 3 quinolones, and 3 combinations of antimicrobics using the agar dilution method and breakpoint values recommended by the Dutch working party for standardization of susceptibility testing. The susceptibility patterns of isolates of *Streptococcus* spp. were very consistent, with only one isolate resistant to the beta-lactam antimicrobics and a combination of diaminopyrimidines and sulphonamides. The susceptibility of the *Salmonella* spp., *Klebsiella* spp., *Proteus* spp., and coagulase-positive *Staphylococcus* spp. was quite variable. A total of 20 strains of Group B *Salmonella* spp. had high MICs for most of the antimicrobials against which they were tested. Isolates from 10 horses that had severe, sometimes fatal, infections which were *S. Typhimurium* phage type 200, had the highest MIC values. No methicillin-resistant *Staphylococcus aureus* were found. *T. equigenitalia* isolates were susceptible to most of the antimicrobials against which they were tested.

Methicillin resistance among *S. aureus* (MRSA), which is also known as oxacillin-resistant *S. aureus*, is mediated by alterations in membrane-bound enzymes called penicillin-binding proteins. These proteins perform important functions for cell survival and are targets for beta-lactam antimicrobics. The occurrence of hospital acquired MRSA infections has been increasing in humans. Vancomycin remains the antimicrobial of choice for treatment of MRSA infections in humans. *S. aureus* infections in people with intermediate resistance to vancomycin have been identified. Fortunately, in the case of those patients, the organism was susceptible to other antimicrobials. The looming threat of incurable *S. aureus* is a real possibility.

Infection of wounds, surgical incisions, catheter sites or other locations by MRSA has been documented in equine patients at two veterinary teaching hospitals. In both situations, hospital personnel were found to carry the MRSA in the nasal cavity. At one of the veterinary teaching hospitals, the personnel were thought to have acquired the infection from hospitalized large animal patients which, in turn, had become infected with the organism during hospitalization, resulting in a self-perpetuating cycle. In both situations, hospital personnel found to be positive for the organism by nasal swab culture were advised to seek medical care from their physician and to undergo appropriate antimicrobial treatment to clear the organism. Use of barrier clothing and masks was implemented to control spread of the organism among hospitalized large animal patients and personnel.

Although the frequency of vancomycin use in equine patients is unknown, the fact that the equine veterinary community has recognized the impending need to use vancomycin in their patients is evidenced by the publication of a pharmacokinetic study of vancomycin in horses. Several antimicrobial drugs including vancomycin, imipenem, and synergicid should be considered as antimicrobials of last resort and reserved for infections with organisms that are confirmed to be resistant to other available antimicrobials. To maintain the effectiveness of these drugs, they should never be used empirically in humans or animals.

The susceptibility of selected Gram-negative bacteria isolated from horses at the University of Pennsylvania Veterinary Teaching Hospital to gentamicin and amikacin was evaluated from 1983 to 1985. A total of 577 isolates of the five gram negative genera most commonly treated with gentamicin and amikacin (*E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp.) were tested for antimicrobial resistance by using the disk diffusion method. The percentage of isolates resistant to gentamicin varied by genus. *Klebsiella* spp. (50.0%) and *Proteus* spp. (39.1%) were more frequently resistant to gentamicin than *E. coli* (25.0%), *Enterobacter* spp. (20.4%), and *Pseudomonas* spp. (13.0%). Among the isolates examined only two, both *Pseudomonas* spp., were considered resistant to amikacin. The authors of the study concluded that amikacin should be used as a first-line therapy in critically ill patients with suspected Gram-negative bacillus infections in light of the extent of gentamicin resistance detected among the isolates evaluated and the costly nature of a delay in treating a critically ill animal. Furthermore, they predict that bacteria will be slower to develop amikacin resistance than they would to other aminoglycosides, and that intensive amikacin use may result in a decrease in the incidence of gentamicin resistance. In cases where an aminoglycoside is indicated, the use of amikacin as a first-line drug may reverse the trend of aminoglycoside resistance.

### 5. Preserving Antimicrobial Effectiveness

Practitioners have a major responsibility in ensuring the continued effectiveness of antimicrobials, which are vitally important in the treatment of infectious disease in equine patients. The AVMA has developed a set of general guidelines for antimicrobial use.

#### References


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