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Antimicrobial Stewardship in Laboratory Animal Facilities

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Bacterial resistance to antimicrobial drugs has become a global health crisis. Physicians and veterinarians are embracing the concept of 'antimicrobial stewardship' (AMS) to preserve the efficacy of antibiotics for future generations. Antimicrobials are used in laboratory animals to treat clinical disease, to protect populations that may be vulnerable to infection, and in research projects including studies of the microbiome and to influence expression in genetically engineered animals. This overview provides a critical look at the use of antimicrobials in contemporary vivaria, with special attention to rodents because they are the most commonly used species in research and because antimicrobial use in rodents is not straightforward. Improvements in antibiotic use are encouraged with the goal of decreasing bacterial resistance while continuing to provide quality clinical care and research support. Suggestions are framed by using the 5 Rs: Reduce, Refine, Replace, and Review antimicrobial use in the vivarium, and take Responsibility for the judicious use of antibiotics in research animals.

Abbreviations: AMS, antimicrobial stewardship; MIC, minimum inhibitory concentration

The resistance of bacteria to antimicrobial drugs is a monumental public health problem.[1,17] Bacterial resistance to antibiotics over time is inevitable.[17] Because microbes will mutate or transfer resistance genes to evade the drugs used to treat them, all antibiotic use should be undertaken cautiously.[17] Decades of misuse of antibiotics in human and veterinary medicine and in agriculture have contributed to today's landscape, in which resistant bacteria cause widespread disease and mortality.[1,17] Strictly speaking, *antibiotics* are substances produced by microorganisms that kill or inhibit the growth of other microorganisms, whereas *antimicrobials* include any substance (including synthetic substances) that kills or inhibits microorganisms;[4] however antibiotic and antimicrobial are used interchangeably in this overview. Although resistant bacteria are not necessarily increased in virulence, delays in infection control due to ineffective initial treatment and extensive testing to find appropriate therapy threaten patients' health and dramatically escalate the cost of medical care.[17,36] In some cases, few antibiotics are available to combat infection.[1,17] Unfortunately the pipeline for new antimicrobial drugs is slow and unproductive, owing to stringent preapproval regulatory requirements and relatively low financial returns for antibiotics compared with other types of drugs.[1,17] Antibiotics are used for relatively short durations compared with drugs for other conditions, such as neuromuscular disease and cancer, and they are one of a few drug classes that becomes less effective with use.[1,17] Because new antibiotics are not forthcoming, it is very important to use existing antimicrobials judiciously.

Antimicrobial stewardship (AMS) is an ethic that dictates a comprehensive approach to the selection and use of antibiotics, with the goal of protecting and sustaining their efficacy over time.[1,10,17,26,27] Formal guidelines for AMS programs in human hospitals were developed in 2007 by the Infectious Diseases Society of America and the Society of Healthcare Epidemiology of America.[17] These programs focus on education and include modifying antibiotic prescriptions, where appropriate.[17,27] Multidisciplinary teams including pharmacists, microbiologists, epidemiologists, and infectious disease specialists review antibiotic use in the population they support.[17,27] AMS teams may modify antibiotic dosages, restrict the prescription of overused or valuable antimicrobials, and recommend alternatives.[17,27] Antibiograms provide epidemiologic data to inform antibiotic use.[27] Cumulative antibiograms are lists of clinically significant bacterial isolates that are obtained from a specified patient population over a defined time period and the antibiotic susceptibilities of the local bacteria.[27] AMS is a relatively new concept in veterinary medicine.[10,26,36] The use of antimicrobials as growth promoters in food animals has been scrutinized for contributing to the colonization and spread of resistant bacteria.[1,17] Recent articles encourage companion animal practitioners to adopt AMS practices, including formalized programs in veterinary hospitals.[10,26] Studies have shown that education about bacterial resistance and AMS is effective in reducing the frequency and duration of antibiotic prescriptions and in curtailing inappropriate use, regardless of whether formalized AMS programs are implemented.[10,17,27]

Antimicrobial use in laboratory animals was reviewed 2 decades ago.[24] That review focused on the interference of antimicrobials with research studies, the complexities of dose extrapolation to small laboratory animals such as mice, and adverse side effects of antibiotics such as enterocolitis in rabbits and some rodent species (for example hamsters).[24] Many specific examples of drug interactions and research effects were provided, and questionable uses of antibiotics in research animals, including empirical and unnecessary prophylactic use, were addressed.[24] Despite these words of caution, antimicrobial use in both rodents[7,11,14,20,22,30] and large animal species (for example dogs,[13] pigs,[33] and NHP[15,32]) is commonplace today. For example, genetically engineered mice have been created in which gene expression is manipulated through antibiotic administration.[30] Immunocompromised and perioperative animals are treated prophylactically with antibiotics.[7,11,13] And

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antibiotics are used extensively for the clinical treatment of infectious disease, and spontaneous and research-related conditions in research animals.[7,11,,13-15,20,22,30,32,33] In contrast to other species, antimicrobial use in rodents is not routine or straightforward. Two studies have cast doubt regarding the efficacy of antibiotics to treat bacterial infection when administered enterally (in the food or water) to mice.[20,22] Importantly, antibiotic-resistant bacteria have been recovered recently from pigs and NHP used in research.[9,15,16,33,35]

The '3 Rs' of Replace, Reduce, and Refine—a mnemonic frequently espoused in the laboratory animal field in regard to the use of animals in research—was recently used to support the principles of AMS in the veterinary literature.[10,26] A '5 Rs' approach to AMS was advocated, adding Review and Responsibility to the better known Rs.[10,26] The current overview explores how to reduce unnecessary antibiotic use in laboratory animals, particularly rodents (the species most commonly used in research). Refinements in antibiotic selection, dose, route of administration, and duration of therapy are discussed. The replacement of antimicrobials with nonantibiotic drugs and other interventions is explored. The need for continual review of antibiotic use in each patient and in the population at large is emphasized. Laboratory animal personnel must take responsibility for the judicious use of antibiotics in research animals to protect vivarium personnel, reduce resistant bacteria in the environment, preserve antibiotic efficacy for research use, and treat laboratory animals with bacterial infections that warrant antibiotics.

Reduce Antibiotic Use in Research Animals

One of the biggest contributors to antimicrobial resistance is the use of antibiotics when they are not warranted.[1,17,27] In human medicine, antibiotics are frequently prescribed for respiratory disease caused by viruses, especially in children.[1,17,27] Clinical disease from viruses is rare in research animals today.[11,19] And some antibiotic use in research animals is for diagnosed bacterial infections.[13-15,33] However, antibiotics also are administered empirically to research animals.[11,20,22,32] AMS dictates that diagnostics should be performed when considering antibiotic use in laboratory animals.[1,10,17,26,27] The diagnosis of bacterial infections in research animals is outside the scope of the current overview, but generally speaking the presence, identification, and antibiotic susceptibility of bacteria should be investigated—and ideally confirmed—before antibiotics are prescribed. Diagnostic laboratory personnel should be seen as partners in AMS and should provide recommendations on sampling techniques to help with diagnosis.[10,36] Skin lesions are common in rodents and NHP, and because pathogenic isolates can be difficult to distinguish from commensal flora, dermatologic conditions may be treated empirically.[11,23,32] But small animal veterinary dermatologists encourage cytology to diagnose skin infections and culture and bacterial susceptibility testing for deep lesions or infections that are refractory to treatment.[2,3] Diagnostic labs processing samples from laboratory animals should follow standards used in human diagnostic labs: commensals or clinically irrelevant results that could lead to inappropriate antibiotic treatment should not be reported.[10,36] Unfortunately, recent reports indicate that diagnostic laboratories servicing the laboratory animal field may fall short of human diagnostic laboratories in providing accurate susceptibility information.[6]

Although time-honored microbiologic techniques such as disk diffusion and broth microdilution are still in widespread use, numerous new applications in antimicrobial susceptibility testing are available.[34] Techniques such as mass spectrometry and flow cytometry may ameliorate the need for large numbers of viable organisms and the time-consuming set-up and delayed results inherent in traditional testing methods.[34] Molecular diagnostics, such as PCR analysis, are becoming more common in research animal health surveillance and have the potential to inform antimicrobial use.[1,2,17,34] Genes conferring resistance to bacteria are being identified, and their amplification during molecular testing provides insight into antibiotic susceptibility.[1,34] Procalcitonin is a biomarker that reflects bacterial replication and has shown promise regarding the detection of bacterial infection necessitating antibiotics at presentation. In addition, procalcitonin can be quantified during treatment to check whether the prescribed antibiotic is controlling the infection and benefitting the patient.[1,12,17] Clearly there is a critical need for point-of-care diagnostics to inform antimicrobial use.[1] Access to new testing modalities and accurate interpretation of test results requires a close working relationship with a diagnostic laboratory.

Unlike physicians and companion animal practitioners, laboratory animal veterinarians are unlikely to face undue pressure from their clients (research scientists) to use antibiotics in their animals. In fact, as members of the scientific and medical community, scientists may increasingly question the clinical use of antibiotics as empirical antimicrobial use is scrutinized more closely in human and companion animal veterinary medicine. In addition, new research directions may influence antimicrobial use in lab animals. The contribution of the microbiome to the phenotype of disease is increasingly recognized as important.[4,5] The microbiome of the gut, for example, is now thought to modulate the development of obesity, diabetes, and cardiovascular disease: chronic conditions that are increasingly prevalent.[4,5] The endogenous flora of mammalian research animals is influenced by how they are derived, by housing and husbandry, and by exposure to xenobiotics (foreign chemical substances) including antimicrobial drugs.[4,5] Because of the interaction and competition between bacterial populations, changes in bacterial levels in antibiotic-treated animals are not limited to those that are susceptible to the administered agent.[17] Treatment with antibiotics leads to transient and sustained shifts in host immunity and physiology.[4,5,17] Although engineered mice with genes altered by antibiotics (such as tetracycline-inducible lines) continue to be propagated, researchers today are turning to more sophisticated mechanisms of induction.[30] Gene expression can be manipulated by using nonantibiotic drugs, such as tamoxifen, or by incorporating drug-free genome-editing technologies.[30] Whereas antibiotics might be used less frequently in genetically engineered animals, investigators might begin to incorporate antibiotics into their studies to modify and study the microbiome and its contribution to our "modern plagues,"[4] or they may simply become sensitized to the profound effect antibiotics might have on their research.[4,5] The clinical use of antibiotics in research animals may come to be seen as an unacceptable variable in research.

Refine the Use of Antibiotics in Research Animals

When antibiotics are used, how they are used is important. Historically antibiotic dose has been based on the minimum inhibitory concentration (MIC),[31] which is the lowest concentration of antibiotic that is expected to effectively inhibit bacterial growth.[31] But the MIC for any given antibiotic reflects carefully controlled conditions and a steady-state drug concentration and does not account for the selection for resistant bacteria that

might occur before steady-state concentrations are achieved.[18] In this era of resistant 'bugs,' the MIC may underestimate the drug dose needed to control an infection, especially at sequestered sites such as the lung and CNS.[18] In addition, the MIC for most antibiotics is based on studies in humans; the high metabolic rates of small lab animals such as rodents might make achieving the MIC for many drugs challenging.[20,24] Efforts should be made to treat infections as soon as possible and to maximize the antibiotic dose and efficacy for the initial bacterial kill, either to eradicate the bacteria at the site of infection or to decrease the bacteria to levels that the animal's immune system can fight off.[18] Mice and rats treated with antibiotics typically show few noticeable toxic effects, possibly because they are relatively resistant to the effects of the antibiotic-associated clostridial overgrowth that is so devastating to other rodents and rabbits.[24] But whenever antibiotics are used, animals should be monitored closely for signs of toxicity and adverse drug effects.[24,27]

The specific characteristics of the antibiotic should inform how it is administered (see reference 18 for a list of antibiotic classes and their pharmacodynamic characteristics).[18,20,31] Although some antibiotics (like sulfonamides) are time-dependent, with their efficacy dependent on the duration that the plasma levels of the drug exceed the MIC for the organism being treated, concentration-dependent antibiotics (such as fluoroquinolones) rely on the peak plasma concentration of the antibiotic for efficacy.[18,20,22,31] Whereas enteral dosing in the feed and water may be effective for time-dependent antibiotics, parenteral injectable (bolus) dosing is more appropriate for concentration-dependent antibiotics.[20] Two research groups investigated the enteral administration of commonly used antibiotics (both time and concentration-dependent) in mice and both found that the plasma antibiotic levels from water[20] and feed[22] do not achieve the MIC for any but the most sensitive of bacteria. Delivery in the food or water is an attractive mode of administration for research rodents because handling for topical or parenteral therapy is inefficient, stressful for the animals, and may disrupt research.[20,22] But for administration through the water or food to be effective, the drug has to be soluble in the water (which may be treated or processed) and evenly distributed throughout the food.[20,22] Whereas sick rodents may drink or eat less than their healthy counterparts, animals treated enterally have to drink or eat sufficient amounts to attain plasma levels of the drug that will combat infection.[20,22] In addition, a rodent medicated through the water or feed either has to be singly housed (a practice that has been shown to negatively affect immunity and wound healing),[28] or cage-mates without clinical disease will ingest antibiotics unnecessarily in the water or feed provided to the cage. If the MIC is not achieved, then infection will not be controlled, and animals potentially will be treated for an extended period of time with antibiotics that exert selective pressure on bacterial populations, favoring resistance.[18,20,22,31]

In veterinary medicine, antibiotics are commonly used in surgery and intensive care, internal medicine, and dermatology.[2,10] Skin conditions (dermatitis and traumatic wounds from fighting) account for a significant proportion of disease in mice and NHP.[11,19,23,32] In fact, dermatitis is the most common clinical condition in some mouse colonies.[19,23] Guidance from small animal veterinary practice encourages topical, rather than systemic, antibiotics for focal surface lesions like the majority of skin lesions (dermatitis and fight wounds) in research rodents.[2,3] Ocular lesions rank just behind skin lesions in incidence in some mouse colonies.[19] Eye infections should likewise be treated with topical antimicrobials, given that the eye is difficult to reach with systemic medication.[11] Localized infections (for example, abscesses) should be treated by using incision and drainage; concurrent antibiotics are usually unnecessary.[11,24] If surgeries are performed aseptically, but antibiotic coverage is desired, then topical antibiotic ointment can be mixed with a local anesthetic and applied to surgical incisions for the first few days after surgery (see the Replacement section for further discussion of prophylactic perioperative antibiotics).[8] Large lab animals, such as NHP and dogs, are usually treated with injectable antibiotics; a long-acting, single-dose injectable antibiotic formulation has recently been advocated.[13,32] Extensive traumatic lesions and generalized infections, especially those in immunocompromised animals, may warrant systemic antibiotics.[13,32]

But if systemic antibiotics are administered, then they should be provided for the shortest time that is clinically appropriate; there is no minimum duration of treatment necessary for antibiotics.[1,12,18,27,36] The patient's total exposure to the antibiotic should be limited, both to minimize perturbations to normal flora and physiology and to decrease the risk of selecting for resistant bacteria.[1,4,18,31] Recent research has shown that short courses of antibiotics are as effective as longer courses for infections in several organ systems.[1,12,17,27,36] Some research has advocated reevaluation of the patient (ideally with repeat diagnostics) on day 3 of antibiotic treatment.[18] Professional judgement of the status of the patient and the clinical consequences of the infection should be paramount rather than compliance with standard antibiotic regimens.[18,36] Further study of antibiotic treatment duration is needed, especially in animals in which prolonged antibiotic courses are often prescribed.[10,36] Systemic administration of antimicrobials to research animals should be infrequent and reserved for systemic or severe disease, preferably with diagnosed susceptible bacteria.

Replace Antibiotics in Research Animals

Even when diagnostic test results support antibiotic administration, there are good reasons to be circumspect about the use of even topical antibiotics. Bacterial resistance to topical antibiotics used in human wound care has been documented,[8] and dermatologists are increasingly recommending nonantibiotic topical treatments, such as white petrolatum, povidone–iodine, gentian violet, silver compounds, and botanicals, for superficial wounds and skin infections in people.[8] The use of topical antiseptics (with or without antibiotics) is a mainstay of small animal veterinary dermatology,[2] and antiseptics (such as chlorhexidine and Dakin solution) have been recommended for skin lesions in rodents.[11,23] Nonpharmacologic interventions should be considered also. Nail trims may lessen skin and soft tissue damage from scratching at pruritic lesions.[11] Environmental manipulations, such as cage enrichment to distract from scratching, may be helpful.[11] Transferring a small amount of bedding material from the dirty cage to the clean cage at cage change may decrease aggression within a cage by carrying over the hierarchy established by scent marking; modifying cage enrichment also may decrease fighting in mice.[11] Underlying causes are important as well. Dermatitis is frequently a problem of aged mice;[23] unless aging is integral to the study, improving colony management by euthanizing mice earlier in life may decrease incidence. In addition, dermatitis in mice may have a behavioral component,[11] and tracking dermatitis lesions on the cage cards of individual animals can alert personnel to when treatment is unlikely to achieve a sustained cure in specific animals.

The best replacement for antibiotic use is avoiding bacterial infections altogether. Required training of investigators in aseptic surgical technique and the provision of assistance, space, equipment, and supplies to support it likely will stave

off the need for prophylactic antibiotics in the vast majority of surgeries.[24] Nondisposable instruments should be cleaned and sterilized after use; clipper blades should be autoclaved.[3] Supplies and implants should be sterilized before surgery.[3,24] In addition, advances in caging may be advantageous for infection control in rodents.[29] In an effort to reduce the exposure of employees to allergens, ventilated cages with tighter gaskets and less permissive filters are being refined and marketed by some of the large caging vendors.[29] Running the air pressure of containment caging positive to that in the room may increase personnel allergen exposure, but vulnerable rodents such as immunodeficient lines are better protected from bacterial infection, potentially reducing the need for prophylactic antibiotics.[7,29] Using microisolation technique for cage changes and other animal manipulations and handling vulnerable animals first (early in the work day) may also decrease the incidence of infection.

Review Antibiotic Use in Research Animals

Antibiotic use should be reviewed in individual research animals and in the population at large. If antibiotic therapy is started before a diagnosis is made, then it should be refined (or de-escalated) according to test results.[27,36] Epidemiologic information should inform antibiotic choice.[10,27,36] Diagnostic laboratories should generate annual cumulative antibiograms at the institutional or regional level;[10] guidelines for the formulation of antibiograms have been published by the Clinical and Laboratory Standards Institute.[10] Quantification of clinical antibiotic use, such as antimicrobial days of therapy, is used in human hospitals to identify patterns and to assess the efficacy of AMS practices and programs.[27] Computerized medical records can be tremendously helpful in quantifying and analyzing antibiotic treatment.[27] Computer-based decision support has been useful to educate antibiotic prescribers in human medicine, and online resources are available for veterinarians.[10] Discussion and assessment of antimicrobial use among veterinarians, veterinary technicians, and diagnostic lab personnel at research institutions might be a first step in establishing AMS practices. Ultimately the lab animal community should follow other veterinary specialty organizations[36] and develop guidelines for responsible antimicrobial use in research animals.

Take *Responsibility* for the Judicious Use of Antibiotics in Research Animals

The management of the animal program and vivarium has implications for antimicrobial use as well as for the development and spread of resistant bacteria and zoonoses. These factors, in turn, affect the health and safety of vivarium personnel, researchers who use animals, and the general public. Antibiotics used in the vivarium (for example, in rodent drinking water) should be discarded as chemical waste; they should not be poured down the drain, where they might contaminate ground water and increase resistant bacteria in the environment.[25] Municipal wastewater discharge permits have strict limits for antibiotics.[25] Some improvements in AMS can be administered, whereas others require a cultural shift and the assumption of responsibility for AMS by individual personnel.

A primary tenet of AMS is the prevention of infections,[3,36] and handwashing is one of the most important infection-control activities.[3,17,21] The need for handwashing is not obviated by wearing gloves, because gloves are permeable and can be breached, and contamination can occur as they are removed.[17,21,36] The Centers for Disease Control recommends alcohol-based hand rubs between patients and handwashing with liquid soap and water when hands become visibly soiled.[21] Alcohol-based hand rubs should be readily available in the vivarium. Small bottles of hand sanitizer should be provided in animal holding areas for use by veterinary technicians, veterinarians, and investigators handling animals (Table 1 in reference 21 is a useful catalog of hand hygiene products).

Resistant bacteria are frequently recovered from hand-touch sites, which are often over-looked in favor of cleaning general surfaces, such as floors.[3] Surfaces in the animal facility that are handled frequently such as door handles and panels and microscope knobs should be sanitized with noncorrosive disinfectants at least once daily.[3] Washable computer keyboards should be used in clinical areas, and they should be disinfected frequently.[3] The need for personnel hygiene and zealous sanitation cannot be overstated, because humans are sources of resistant and potentially dangerous bacteria including methicillin-resistant *Staphylococcus aureus*.[35]

Infections with multidrug-resistant bacteria in laboratory animals are probably underreported, but conditions in most vivaria are ripe for colonization and spread.[22,35] Laboratory animals are frequently mixed and have close contact with humans; they may have varying degrees of immunodeficiency; invasive devices and implants may be used; and antibiotics may be used (or overused).[22,35] There have been several reports of methicillin-resistant *S. aureus* and other drug-resistant bacteria in large lab animals (pigs and NHP) in the last decade,[9,15,16,33,35] and reports in rodents cannot be far behind. Methicillin-resistant *S. pseudointermedius* and *E. coli* producing extended-spectrum β-lactamase are important pathogens in dogs and cats.[10] The presence of multidrug-resistant bacteria is concerning both from the standpoint of interspecies transmission and disease and because these infections may impel veterinarians to prescribe antimicrobials that are important in human medicine.[10,36] For example, a recent report on methicillin-resistant *S. aureus* in NHP relates isolate susceptibility to rifampin and vancomycin, valuable antibiotics in humans that should not be overused.[9] Unfortunately, many antibiotics that are deemed 'critically important' for human use are used routinely in veterinary medicine.[10,36] An extended-spectrum third-generation cephalosporin was recently proposed for use in NHP and dogs used in research.[13,32] Another study reports the resistance of *Shigella flexneri*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Campylobacter jejuni* (all zoonotic bacteria isolated from NHP) to several antibiotics but not yet to the fluoroquinolones used most commonly to treat them.[15] Fluoroquinolones are popular veterinary drugs, and their use has been linked to increased incidence of methicillin-resistant *S. aureus*.[10,17] Efforts should be made to categorize antimicrobials used in animals to guide veterinary prescribers so that antibiotics important in human medicine are not overused .[10,36]

Aside from restrictions on the use of some antibiotics in food-producing animals, the censorship of antimicrobial prescription has not been undertaken in veterinary medicine in the United States.[10,36] In contrast, other countries have taken initiatives to control antibiotic use in animals that are not raised for food.[10] For example, Sweden restricts the use of both third-generation cephalosporins and fluoroquinolones to situations in which bacteria are resistant to all other veterinary-licensed antimicrobials.[10]

Conclusion

Personnel caring for laboratory animals face competing obligations relative to antibiotic use. On the one hand, veterinary standards of care and concern for animal patients fosters the

overuse of antibiotics. But this use must, on the other hand, be balanced by a commitment to the public health to minimize the development of resistant bacteria and to preserve the efficacy of antibiotics. As in other clinical disciplines, the use of antibiotics in laboratory animal medicine should be modified to reflect AMS practices. The 5 Rs provide a useful guide to the principles of AMS. *Reduce* antibiotic use: use antibiotics only for animals on permissive research projects with diagnosed bacterial infections. *Refine* dose and route of administration according to the species treated, the features of the infection, and the characteristics of the prescribed drug; decrease the duration of antibiotic treatment. *Replace* antimicrobials with nonantibiotic drugs and other interventions where possible; improve hygiene and sanitation practices to prevent infection so that antibiotics are not necessary. *Review* the need for antibiotics in each patient and in the population at large. Finally, and most importantly, assume *Responsibility* for the judicious use of antimicrobials in laboratory animals.

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