# Measuring Antimicrobial Resistance

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Antimicrobial resistance can be measured in the laboratory and can be suggested clinically in bacterial infections that fail to respond to antimicrobial therapy. A discussion of *in vivo* factors contributing to antimicrobial resistance is beyond the scope of this paper; however, a brief review of *in vitro* determination of antimicrobial resistance will be covered.

*In vitro* testing for antimicrobial resistance/susceptibility provides an estimate of an antimicrobial's ability to kill bacteria or inhibit their growth. A variety of laboratory methods can be used to measure the susceptibility of bacteria to antimicrobials. Two of the most common methods are agar disk diffusion and broth or agar dilution.

Most veterinarians are familiar with the first method used to determine susceptibility—the Kirby-Bauer agar disk diffusion method. An antimicrobial disk is placed on the surface of agar inoculated with the bacterial isolate to be tested. Antimicrobial active ingredient from the disk diffuses into the agar. A clear circular space indicating no visible bacterial growth occurs around the antimicrobial disk. The clear circular space, measured in millimeters and called the zone of inhibition (ZI), is proportional to the antimicrobial's activity against the bacteria being tested.

A second method used to determine susceptibility of bacteria is the broth or agar dilution method. The agar or broth medium containing different dilutions of the antimicrobial is inoculated with the bacterial isolate to be tested. The lowest concentration of the antimicrobial agent (measured in mg/ml) that visibly inhibits the growth of the bacterial isolate is called the minimum inhibitory concentration (MIC). The concentration at which growth is visibly inhibited determines whether the isolate is susceptible or resistant to the antimicrobial.

To determine whether a bacterial isolate is susceptible or resistant to a specific antimicrobial, MIC break points and/or zone diameter interpretive criteria must be established. Established through presentation of data to the National Committee for Clinical Laboratory Standards, these are a set of MICs and ZIs, respectively, that define limits of susceptibility and resistance of an organism to an antimicrobial. The MIC break points and zone diameter interpretive criteria are specific for an antimicrobial and pathogen(s) and are determined by correlating pharmacodynamic data (including pharmacokinetic and *in vitro* susceptibility data) with clinical efficacy data. If break points or interpretive criteria have been established for the bacteria, an isolate of that bacteria being tested can be classified as susceptible, intermediate or resistant for a specific antimicrobial. Without established break points or interpretive criteria, an MIC or ZI can be reported for a pathogen, but cannot be interpreted as susceptible or resistant.

Until recently, to the veterinary profession, measuring antimicrobial resistance implied determining the susceptibility/resistance pattern of a specific animal pathogen for 1 or more antimicrobials. The susceptibility profiles of the antimicrobials were used to determine which would serve as the best therapeutic agent for a specific disease process. Traditionally, a letter – "S" for susceptible, "I" for intermediate, and "R" for resistant – was reported for each antimicrobial tested. Usually, the susceptibility profiles were determined using the disk diffusion technique.

In today's world, antimicrobial susceptibility/resistance is still determined for specific disease-causing veterinary pathogens. However, measuring antimicrobial resistance also has taken on a whole new dimension because of the concern by some individuals that treatment of food-producing animals with antimicrobials may select for antimicrobial-resistant subpopulations of such foodborne pathogens as *Salmonella* and *Campylobacter*. Should these resistant foodborne pathogens be transferred to humans, and if systemic illness requiring therapy with the pathogen-resistant antimicrobial was the outcome, response to therapy could be jeopardized. Because of this concern, measuring of antimicrobial resistance still includes specific veterinary pathogens (such as *Pasteurella haemolytica*), but new emphasis is being placed on potential human pathogens (such as *Salmonella* or *Campylobacter* spp.) found in the feces or on the carcasses of animals intended for human consumption.

A variety of programs to measure antimicrobial resistance in animal or potential foodborne pathogens exist in the US. In the last few years, national monitoring programs to track the development and prevalence of antimicrobial resistance of potential foodborne patho-

gens have been implemented. A privately supported and administered national program that monitors numerous pathogens of human origin also is operational. Many pharmaceutical companies maintain programs to monitor the susceptibility profile of 1 or more animal pathogens to a specific antimicrobial and its competitors. Very recently, pharmaceutical companies marketing newly approved antimicrobials (specifically, the fluoroquinolones) have been required to fund and administer monitoring programs involving target animal pathogens and non-target bacteria.

A national program to track development and prevalence of antimicrobial resistance of potential foodborne pathogens was initiated in 1995. The National Antimicrobial Resistance Monitoring System: Enteric Bacteria (NARMS), a collaborative effort between the Food and Drug Administration, Centers for Disease Control and U.S. Department of Agriculture, tracks 2 populations of isolates: veterinary and human.

The veterinary component of NARMS, when first initiated, monitored resistance patterns for *Salmonella* and *E. coli* 0157:H7 (when available). In 1998, resistance monitoring for *Campylobacter* was added to the program. Isolates of veterinary origin are obtained from several sources. There currently are 3 diagnostic laboratory sentinel sites located in California, Washington and New York that supply salmonella isolates from submitted diagnostic/necropsy samples. *Salmonella* and *Campylobacter* isolates obtained at slaughter through the Hazard Analysis Critical Control Points (HACCP) program also are tested. Additionally, *Salmonella* isolates from major food-producing species and companion animals that were sent to the National Veterinary Services Laboratory for serotyping are selected. Finally, isolates obtained from other survey programs such as those conducted by National Animal Health Monitoring System may be included.[1]

The human component of NARMS monitors resistance patterns of these potential foodborne pathogens: *Salmonella* spp, *E. coli* 0157:H7, and *Campylobacter jejuni*. Beginning in 1999, the resistance patterns of *S. typhi* and *Shigella,* both non-foodborne enteric pathogens, also are being monitored. *Salmonella, Shigella,* and *E. coli* 0157:H7 isolates are obtained from 17 public health laboratories distributed throughout the US, representing about one-third of the US population. Eight of the 17 laboratories also submit *Campylobacter* isolates to the program.[2]

Each *Salmonella, Shigella,* and *E. coli* isolate is subjected to antimicrobial susceptibility testing for 17 antimicrobials using the microdilution broth technique. *Campylobacter* isolates are tested against 7 antimicrobials. Results are reported as MICs. One objective of the program is to identify changes in MIC profiles ("MIC shifts") that may indicate the emergence of a sub-population of resistant bacteria. A summary of the most current human and veterinary data will be presented.

A privately supported national monitoring program called the Thornsberry Surveillance Network (TSN) Database is sponsored by MRL Pharmaceutical Services. This program collects susceptibility data on human bacterial isolates from laboratories throughout the US. A summary of the most recent susceptibility data from selected bacteria will be presented.

The approval of fluoroquinolones for use in food-producing animals—for poultry in 1995 and 1996, and for cattle in 1998—was contingent upon the implementation of voluntary, sponsor-supported, post-approval monitoring programs (PAMPs). The current PAMP for both poultry products involves tracking the susceptibility profile for a label-indicated animal pathogen - *E. coli.* However, it is expected that the poultry PAMPs will be modified to include testing of potential foodborne pathogens. The PAMP for the cattle fluoroquinolone involves monitoring the susceptibility profiles of one sentinel or "indicator" organism and one potential foodborne pathogen. A summary of available data from the monitoring programs will be presented.

## References

1. Personal communication: Fedorka-Cray PJ, USDA-ARS-RRC, Athens, GA
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