

Antimicrobial susceptibility testing plays role in mastitis treatment

By Rubén González

Use antibiotic susceptibility testing data in conjunction with clinical experience, published efficacy data and, where possible, on-farm trials to determine appropriate antibiotics for mastitis treatment.

Since the discovery of antibiotics during the first half of the 20th century, precise bacterial diagnosis has become important as a way to test the susceptibility of pathogens to available antimicrobial drugs. Rational antibiotic treatment is essential: Animal well-being and prolonged usefulness of antibiotics depend upon it.

Antibiotic treatment of mastitis is often started empirically, based on a herd manager's or veterinarian's judgment on the most likely bacterium involved. The outcome of treatment in lactating animals depends on several factors: the cow, bacteria, herd management, antibiotic choice and treatment regimen.

Antibiotic susceptibility testing results, provided by a diagnostic laboratory, may serve two purposes:

- To confirm the efficacy of the antibiotic being administered.
- Or to modify therapy if results demonstrate the antibiotic failed against a pathogen.

Antibiotic susceptibility testing results provided before starting treatment may help dairies institute a more judicious therapy, especially for subclinical mastitis.

Methodology

The disc-diffusion method is the most commonly used procedure to test for a pathogen's antibiotic susceptibility. It was developed about 40 years ago for use on bacterial isolates from human patients. In the United States, the Clinical and Laboratory Standards Institute (CLSI) has established guidelines to standardize the method.

The disc-diffusion method follows these steps:

1. Culture a milk sample obtained aseptically from a mastitic quarter on blood agar. (Figure 1)
2. Standardize the concentration of the inoculum.
3. Spread the pure culture on a specific agar medium (Mueller-Hinton).
4. Apply filter paper discs containing standardized quantities of antimicrobial drug onto the agar sur-

face. (Figure 2)

5. Incubate the culture for approximately 18 hours at 35° C.

The concentration of antibiotic in the disc varies from drug to drug and, with the exception of ceftiofur, pirlamycin and penicillin-novobiocin, is based on levels attained in human serum following treatment.

6. Measure the zone of inhibition – the area where there is an absence of any visible bacterial growth around each disc. (Figure 3)

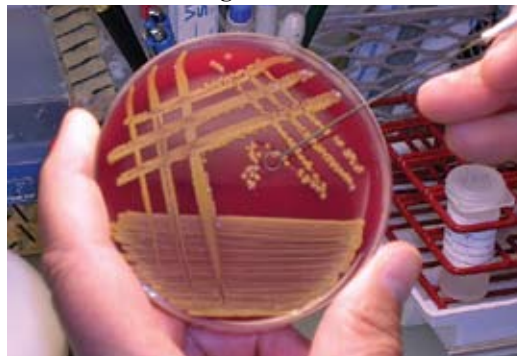
By referring the measurement to a chart, the organism is classified into three categories: susceptible (S), intermediate sensitive (I) or resistant (R) to the antimicrobial drug.

The inhibition zone size is drug-specific due to differing diffusion rates of the antibiotics in the agar. This method is used mainly for tests on rapid-growing bacteria such as *Staphylococcus*, *Streptococcus* and coliform bacteria. It cannot be used for testing slow-growing organisms such as *Arcanobacterium pyogenes*.

Value on-farm

No single antimicrobial drug is appropriate for every mastitis-causing organism. Thus, when selecting

Figure 1. The first step is to select bacteria for antibiogram.



antibiotics for treatment, it's important to understand how the results obtained in the laboratory (*in vitro*) can be used to treat a cow (*in vivo*) with mastitis.

Two measures are used to determine the efficacy of an antibiotic:

1. Its minimum inhibitory concentration. The MIC is the lowest concentration of an antimicrobial required to inhibit visible growth of bacteria *in vitro*.

2. Susceptibility breakpoint. This is the concentration of an antimicrobial slightly greater than that required to kill sensitive bacteria.

At this time for mastitis treatment, three antibiotics – ceftiofur, pirlimycin, penicillin-novobiocin – have veterinary-validated breakpoints by the Veterinary Antimicrobial Susceptibility Testing Subcommittee of the CLSI. These breakpoints can be applied only for the pathogens included on the label.

For other antibiotics, such as amoxicillin, erythromycin, penicillin G, and tetracycline, results are reported based on breakpoints that CLSI has adapted from human medicine. The validity of using antimicrobial susceptibility breakpoints derived from humans to the treatment of mastitis has not been established.

Antimicrobial therapy aims to achieve drug levels in excess of the known *in vitro* MIC for the target organism at the site of infection and maintain an effective

concentration for sufficient time to kill or inhibit the organism. If a laboratory test indicates that an organism is resistant *in vitro* to a particular antibiotic, that drug must not be used to treat the affected cow because it's very likely to fail.

On the other hand, if an organism is susceptible *in vitro* to a particular antibiotic, that drug can be used to treat an affected cow.

However, there's no guarantee the therapy with that drug will be successful. The clinical outcome depends on a wide range of factors: susceptibility of the pathogen, mechanism of action of the drug, distribution in tissues and milk, physiological barriers such as microabscesses or necrotic tissue, the route, frequency of administration and the duration of treatment.

Antibiotic susceptibility testing data should be used in conjunction with clinical experience, published efficacy data and, where possible, on-farm trials to determine appropriate antibiotics for treatment of mastitis in a given herd.

Although more commonly used for clinical mastitis, testing of isolates from subclinical infections due to *Staphylococcus aureus* and some *Streptococcus* species during lactation might help select antimicrobial agents, including those for dry cow therapy, in a mastitis control program.

To be effective, an antibiotic must be present at the site of the infection in sufficient concentration and for sufficient time to kill or inhibit the invading organisms. The final elimination of the infection from the mammary gland depends not only on antibiotic action but also on the activity of the cow's own defenses and the removal of organisms by milking.

Figure 2. Antibiotogram equipment includes filter paper discs containing standardized quantities of antimicrobial drug.

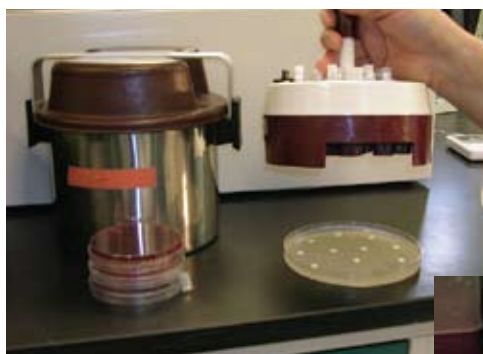


Figure 3. The zone of inhibition around each disc is measured to provide antibiotogram plate results.



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QMPS is a program within the Animal Health Diagnostic Center, a partnership between the New York State Department of Agriculture and Markets and the College of Veterinary Medicine at Cornell.

The QMPS staff of veterinarians, technicians and researchers works with New York dairies to improve milk quality by addressing high somatic cell counts, milking equipment and procedures, and milker training in English and Spanish. QMPS also conducts research and teaching programs.

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Dairy One is an information technology cooperative, providing DHI records services and herd management software to dairies throughout the Northeast and Mid-Atlantic region. A comprehensive laboratory network provides milk quality testing as well as forage, soil, manure and water testing.

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