FISH FARMING NEWS

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Antibiotic susceptibility testing matters

FISH HEALTH NOTES

by Rod Getchell

SAN FRANCISCO, CA – In 30 minutes, Dr. Peter Smith convinced me that there is a need for a standard method of measuring antibiotic susceptibility.

This National University of Ireland professor spoke with such passion that, by the time his plenary session at the 5th International Symposium on Aquatic Animal Health had ended, method standardization seemed like a worthy quest to follow. I left the room feeling we should all try to contribute to this mission.

The prudent and rational use of antimicrobials is critical to a successful fish farming operation. Yet, discovering whether a particular bacterial isolate is sensitive or resistant to a particular drug can be a somewhat expensive proposition. In the best case, you lay out some bucks and the treatment is successful. In the worst case, the medicine fails to prevent additional losses and the bacterial pathogens develop antimicrobial resistance mechanisms.

It is in this area that the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing – Aquaculture Working Group (VAST-AWG) plays an important role. In June of 2006, this group, of which Dr. Smith is a member, published two testing guidelines that represent the first standardized antimicrobial susceptibility testing methods for aquatic pathogens.

These guidelines were built on the opinions of those present at the Workshop on MIC Methodologies in Aquaculture that took place in 1998 in Weymouth, UK. MIC stands for minimal inhibitory concentration. The methods described in the CLSI documents were standardized through multi-laboratory and multi-national collaborative studies.

CLSI guidelines

Standard methods provide the first essential part of the international effort that Dr. Smith implored us to join. If labs use the same exact methods, meaningful communication can take place. In the past, the wide variety of different test protocols led to serious confusion and disagreements as to the clinical meaning of the results.

"These documents satisfy a long-time need for standardized susceptibility testing methods in aquaculture. They provide a central core set of testing methods for the field of aquatic animal medicine," says Ron Miller, MS, US Food and Drug Administration (FDA) Center for Veterinary Medicine.

On the CLSI website, Dr. Smith says that this extensive collaboration has resulted in "a standard, universal language in which we can investigate the epidemiology of resistance and discuss therapies for the aquatic environment."

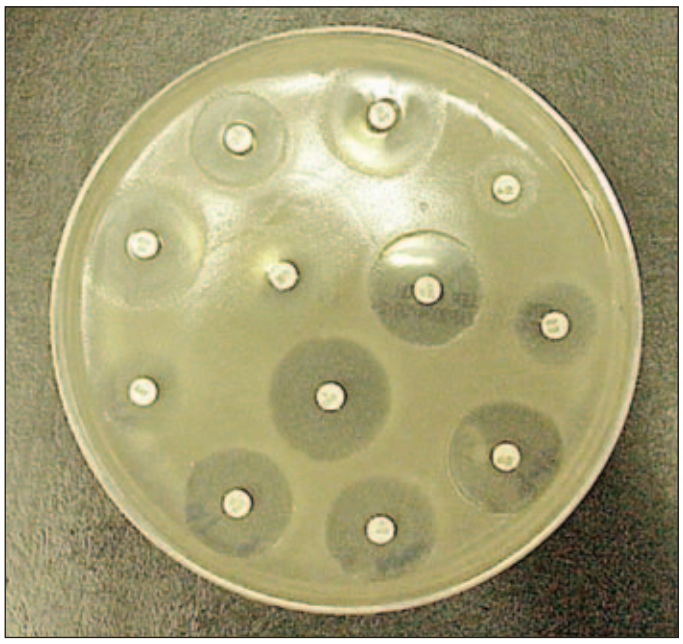
Resistance

Defining antibiotic resistance is not as easy as it sounds. The clinical definition involves how the drug interacts with the fish host and whether the therapy is successful or not. A bacterium must be classified as resistant if the concentration of the antibiotic required to inhibit its ability to contribute to the morbidity of the host is greater than the concentration that can be achieved in that host.

Bacteriologists identify resistance by measuring in-vitro susceptibility. An example of a typical antibiotic susceptibility testing plate appears in Figure 1.

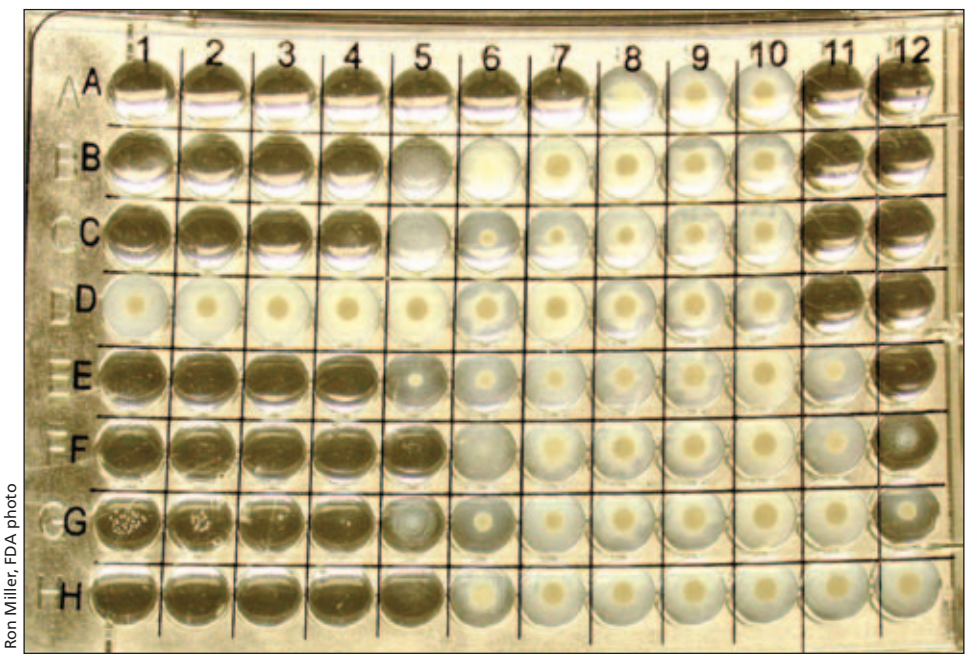
Dr. Smith described in three steps how determining resistance was a combination of methods.

The first step determines resistance and it takes place every day in fish health labs around the world. A bacterial isolate cultured from sick fish is tested against the commercially available antibiotics. The drug-impregnated disks with large zones of inhibition around them indicate the bacteria's susceptibility (Figure 1). The smaller zones shown around the disk in the upper right (arrow) probably indicate the bug may be resistant to this one. We call this an in-vitro test because the assay is not run in a living animal, which would be in-vivo.



NOAA photo

Figure 1. Antibiotic susceptibility testing plate showing zones of inhibition around the antibiotic-impregnated disks.



Ron Miller, FDA photo

Figure 2. Micro-broth dilution testing eliminates the use of agar media as 96-well plates are used. Each well contains a different antibiotic at a particular concentration. When you see a pellet at the bottom, bacterial growth was not inhibited.

Susceptibility

The next step is to gauge susceptibility and it is more complicated. To interpret susceptibility data, you need to know the breakpoint value (zone diameter or minimum inhibitory concentration) for the pathogen you are attempting to control in the animal you are trying to treat. Breakpoint values provide an important basis for the prescription of drug that you believe will lead to a successful outcome.

The veterinarian needs to know, "Can this zone diameter or concentration of drug measured in-vitro be used to help predict the level of effectiveness of treatment in-vivo? Or will the treatment only exacerbate the problem by creating more potentially antibiotic resistant bacteria?"

So how do you accumulate that kind of real-world treatment data to estimate the breakpoint value? Running a few trials in the lab may give you an idea of what concentrations will work, but the environment of the commercial industry is more complex. The biological activity of the antimicrobial agent will be different in fish raised in the lab versus in the field, and some will be different in saltwater versus freshwater. All the physical and chemical parameters of a net-pen, raceway, or pond will have an effect on how a population of fish responds to treatment.

Validation

The third step in determining whether a bacterial species is resistant or sensitive is validating the breakpoint values. In lab trials, we always have a group of untreated control fish to compare with the treated fish results. This is part of the validation process. Most farmers would resist leaving some of their fish untreated during a disease outbreak. So that kind of on-the-farm data is usually not available for validation studies.

Accumulation of enough data from treatments in the field will involve extensive worldwide cooperation, according to Dr. Smith. Teamwork is required from many laboratories working in different geographic regions and servicing different aspects of the fish farming industry. This was a big part of the message that Dr. Smith preached.

What these researchers need is susceptibility distribution data. It's a mouthful, but what it comes down to is accumulating minimum inhibitory concentrations (see Figure 2) and, if possible, zone diameters for any given drug against the pathogen of interest.

What's even more critical in gathering all this valuable data is that every lab involved must use the same standard methods to measure susceptibility. If researchers and clinicians use many different techniques, it will be difficult to analyze the data together and end up with accurate breakpoints.

What I have tried to convey here is

Continued on next page

grounded in the work of many dedicated professionals. Their message is fourfold. Method standardization matters. Accurate data matters. Cooperation matters. Antibiotic susceptibility testing matters.

More info

For more information on the new CLSI protocols, check out the institute's August 2006 press release at <http://enews.clsi.org/clsi/issues/2006-08-01/1.html>.

CLSI document M42-A provides veterinary microbiologists with the most up-to-date techniques for disk diffusion susceptibility testing of aquatic species isolates. M49-A provides methods for determining MICs of aquatic bacteria by both micro- and macrodilution.

As often is the case, the University of Florida extension folks have a great primer on the use of antibiotics for aquacultured species. Check out <http://edis.ifas.ufl.edu/FA084>. The web site provides some good background on the use of antimicrobials.

Thanks for reading Fish Health Notes.

Dr. Rod Getchell works in the Aquatic Animal Health Program at the Cornell University College of Veterinary Medicine.

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—Dr. Peter Smith

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