What veterinarians need to know about ANTIMICROBIAL SUSCEPTIBILITY TESTING

FOOD FISH

Antimicrobial susceptibility testing (AST) can be a powerful diagnostic tool to guide selection of antimicrobials and to help predict outcomes for bacterial diseases in food fish. The following recommendations on AST use are intended to assist veterinarians when making decisions about antimicrobial therapy for food fish.

Food fish antimicrobials are administered in feed under a Veterinary Feed Directive (VFD), and only three approved antimicrobials are available: florfenicol, oxytetracycline, and sulfadimethoxine/ormetoprim. These antimicrobials can only be used as labeled, although the U.S. Food and Drug Administration (FDA) allows some regulatory discretion.[1]

// LIMITATIONS OF AST IN FOOD FISH //

Unless laboratories are specifically equipped to test fish pathogens, most laboratories will benchmark antimicrobial breakpoints based on those for terrestrial animals because most fish bacterial pathogens lack established breakpoints. Breakpoints for terrestrial animals are likely invalid for several reasons, including differences in these factors:

- **Host physiology.** Most fish are poikilotherms, and their body temperatures fluctuate based on water temperature. The physiology of each fish species works optimally within a certain water temperature range. When fish are in lower-than-optimal water temperatures, they become stressed and more susceptible to diseases. Many fish pathogens cause infections only within a certain temperature range and do not infect terrestrial animals. This is in contrast to terrestrial animals, which maintain a relatively constant body temperature.
- **Optimal bacterial growth conditions.** Fish-adapted bacteria have specific *in vitro* incubation temperatures, incubation times, and nutrient requirements that differ from terrestrial animal bacteria.
- **Treatment effectiveness from environmental conditions.** Substandard water quality can predispose fish to opportunistic infections and reduce antibiotic effectiveness, regardless of AST results. Therefore, optimization of water quality could both alleviate the need for antibiotic therapy and improve antibiotic effectiveness.

// SCENARIOS WHEN CULTURE ALONE MIGHT BE USEFUL //

Bacterial culture in the diagnosis of fish disease is recommended whenever there is significant morbidity or mortality. Culture alone can identify causative agents and assist in the selection of an appropriate antibiotic-medicated feed.

// SCENARIOS WHEN AST IS RECOMMENDED TO IMPROVE OUTCOMES //

Oxytetracycline for *Aeromonas salmonicida* infections is the only FDA-approved antibiotic for which the Clinical and Laboratory Standards Institute (CLSI) has standardized breakpoints approved for food fish.[2,3] Oxolinic acid, for which CLSI-standardized breakpoints also exist, is not legal for use in food fish in the U.S. Therefore, when *A. salmonicida* is suspected or cultured in fish, AST is recommended using the CLSI breakpoints for oxytetracycline and, where legal outside the U.S., oxolinic acid.

The CLSI has standardized epidemiological cutoff values (ECVs) derived from *in vitro* tests that distinguish wild-type isolates of aquatic bacteria (i.e., those with no reduction in susceptibility to the antimicrobial agent being tested) from those with acquired and/or mutational antimicrobial resistance mechanisms. These include *Aeromonas salmonicida, Aeromonas hydrophila, Flavobacterium columnare*, and *Flavobacterium psychrophilum*. Some veterinarians base their treatment decision on whether a bacterial isolate is categorized as wild-type, believing that the bacteria will be responsive to the antibiotic *in vivo*. However, ECVs are not based on clinical response of the surviving population of fish, but, instead, on *in vitro* AST values (i.e., minimal inhibitory concentrations or disk diffusion zones). If a bacterial isolate is categorized as wild-type, that does not ensure that the clinical outcome achieved antibiotic-medicated feed will be successful.

Fish veterinarians are encouraged to communicate with regional fish laboratories. Often established in universities, specialized diagnostic laboratories develop provisional, clinically meaningful breakpoints derived from aggregated, documented treatment responses from local fish farms. It is important that laboratories use provisionally developed

breakpoints specific to the area where the fish are located, as these breakpoints cannot be used interchangeably from one area of the country to another (e.g., from the southeastern to northwestern U.S.) unless the laboratories formally collaborated on a standardization method.

Diagnostic reports should indicate whether CLSI-standardized breakpoints or ECVs vs. regional antibiograms have been used to classify a bacterial isolate's susceptibility to an antibiotic.

// ADDITIONAL RESOURCES //

AVMA Committee on Antimicrobials. What veterinarians need to know about antimicrobial susceptibility testing (avma.org/AntimicrobialTools):

- General overview
- Non-culture-based antimicrobial resistance genetic panels in animals

AVMA. *Judicious therapeutic use of antimicrobials in aquatic animal medicine.* Accessed October 16, 2023. https://www.avma.org/resources-tools/avma-policies/judicious-therapeutic-use-antimicrobials-aquatic-animal-medicine

CLSI*. Methods for Antimicrobial Broth Dilution and Disk Diffusion Susceptibility Testing of Bacteria Isolated From Aquatic Animals.* 2nd ed. CLSI guideline VET03. CLSI; 2020.

// REFERENCES //

1. Compliance Policy Guide Sec. 615.115 *Extralabel Use of Medicated Feeds for Minor Species.* Accessed October 16, 2023. https://www.fda.gov/media/71960/download
2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals.* 3rd ed. CLSI supplement VET04. CLSI; 2020.
3. CLSI. *Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings.* 1st ed. CLSI report VET09. CLSI; 2019.