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Antibiotic Resistance to Oxytetracycline HCL in Kansas Department of Wildlife Fish Hatchery of Pratt, KS

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ABSTRACT

The overuse of antibiotics in today's society has caused reduced susceptibility in many species of bacteria. Antibiotics are used to treat humans, as well as animals including domestic, livestock and aquatic animals. Antibiotics are administered to aquatic animals by food mixture. There are two FDA approved antibiotics used in treatment of aquatic animals, oxytetracycline HCL and sulphadimethoxine. The objective of this study was to determine if there were bacteria with reduced susceptibility to oxytetracycline HCL in Kansas Department of Wildlife Fish Hatchery in Pratt, KS where oxytetracycline has been used. Samples were taken from channel catfish (Ictalurus punctatus), soil, and water that was exposed to oxytetracycline HCL in the summer of 2008. This study found evidence of three individual species resistant to oxytetracycline HCL. Bacteria were isolated by use of nutrient agar plates amended with oxytetracycline HCL in the recommended concentration of 105mg/1L. Three species of bacteria were isolated. Bacteria with reduced susceptibility isolated from (Ictalurus punctatus) were found to be *Stenotrophomonas maltophilia*.

Keywords: *Aquatic Animals*, *Antibiotic Resistance, Channel Catfish (Ictalurus punctatus), Fish Hatchery, Stenotrophomonas maltophilia*

INTRODUCTION

Antibiotics have been used to treat humans as well as animals such as domestic animals, livestock, and aquatic animals such as fish and shrimp for many years to prevent or cure infections among the population. Recent epidemics of pathogenic diseases and infections have shown that the overuse of antibiotics may be harmful and may need to be regulated (Dietze, 2005). Microorganisms also have the potential to be resistant to many types of antibiotics (Akinbowale, 2007). Pathogenic bacteria in shrimp have been linked to bacteria in humans and it is believed that there should be a concern for safety of human consumption (Boinapally and Jiang, 2007). Microorganisms can be found in feces, gills, epidermal slime layer, as well as in the water and sediments from areas they inhabit (Schmidt 2000). The recent study performed by Boinapally and Jiang (2007) showed that there was a bacterium with reduced susceptibility that was being used for human consumption.

In hatcheries, aquatic animals such as the channel catfish (*Ictalurus punctatus*), receive doses of antibiotics orally by mixture with their food (McPhearson, 1991). The approved antibiotics for channel catfish include sulphadimethoxine, and oxytetracycline HCL (Dietze, 2005). Dietze did a study from 2001-2003 sampling water from thirteen fish hatcheries including Kansas Department of Wildlife Hatcheries that were tested for traces of antibiotics. This study was done to help determine if antibiotic residue was prominent in these waters and to help monitor the usage of antibiotics. Of the samples taken, 31% contained antibiotic residue which was relatively low, however there is the potential that antibiotics could be transported beyond the fish hatchery (Dietze, 2005).

The Kansas Department of Wildlife and Parks fish hatchery in Pratt, Kansas has been actively feeding oxytetracycline HCL (Terramycin) to their channel catfish since 2007 and the summer of 2008. Oxytetracycline HCL has been used in previous years although no records were made of when antibiotics were first administered. This study is to determine if bacterial resistance to oxytetracycline HCL occurs in the Kansas Department of Wildlife and Parks fish hatchery in Pratt, Kansas.

MATERIALS AND METHODS

Bacteria samples were taken from the Kansas Department of Wildlife and Parks fish hatchery in Pratt, KS. Samples were gathered from a pond in which antibiotics were used on two previous occasions earlier in the summer of 2008. Bacteria were sampled from the water, soil, and the fish that were treated with the antibiotic. Samples were also obtained from a solar pond where antibiotics have never been used before. Water and soil samples were taken from the solar pond as fish have never inhabited this pond. A spatula disinfected with 70% isopropyl alcohol was used to take soil samples. A 1000 µL pipette and autoclaved pipette tips were used to obtain water samples. To acquire fish samples, cotton swabs that were placed in test tubes and autoclaved were used. Screw-top vials containing nutrient broth were autoclaved and all samples were transported to these vials using standard sterile technique.

All soil samples were taken in the same way. A spatula was sterilized with 70% isopropyl alcohol. A sample was collected by digging into the soil, transported directly to a previously labeled vial and capped immediately. Twenty samples were taken from each pond.

Water samples from both ponds were obtained by using a sterile pipette tip and micro pipette at a depth of 1cm and were transported directly to a previously labeled vial and capped immediately (Akinbowale, 2006). Twenty samples were taken from each body of water.

Samples from *Ictalurus punctatus* were obtained by swabbing a 1cm² area on the lateral aspect of the fish using a sterile cotton swab. Swabs were immediately transported to previously labeled vials and capped immediately (Schmidt, 2000). One sample was taken from 20 fish for a total of 20 samples. All samples were immediately taken to the lab and incubated at 37 C for 48 hours.

Oxytetracycline HCL was the antibiotic that the bacteria were tested against in this experiment. This was chosen because this is the specific antibiotic used in the fish feed at the hatchery. A concentration of 105mg/1L was used as this is the concentration recommended by Pfizer for aquatic animal treatment. Nutrient agar was prepared and autoclaved. Nutrient agar was allowed to cool to 50 C before antibiotics were added in order not to degrade the antibiotic. Culture plates were prepared using standard sterile techniques. All bacterial samples previously obtained were plated onto culture plates using a 1000 µL pipette and autoclaved pipette tips. Bacteria were spread on plates using sterile spread sticks. Cultures were incubated at 37 C for 48 hours. After 48 hour bacteria had grown throughout the plate and individual colonies could not be identified. Bacteria were replated using a sterile loop and streaked onto antibiotic prepared culture plates to separate colonies. Separated bacteria were streaked onto nutrient agar slant tubes to be sent to Aperio Scientific of Overland Park, KS for identification.

RESULTS

Bacteria resistant to oxytetracycline HCL were found in cultures taken from the soil in the control pond, soil from the test pond, and cultures taken from the fish as well. When plated on nutrient agar treated with oxytetracycline HCL, bacteria of a few different species spread throughout the plate, not in observable colonies. Bacteria were then separated, coming up with one species of bacteria from each sample group.

After 48 hours, colonies that formed from the control soil group were approximately 5mm in diameter and were irregular in form. Colonies had a flat elevation and undulate margin. They were white in color and had a scaly appearance on the surface but also had a mucous looking texture under the surface. When viewed under a microscope, it was evident that the bacteria were gram positive baccilli.

When allowed to colonize on nutrient agar, bacteria from the soil of the test pond appeared in colonies of about 5mm and were circular in form. They also had a flat elevation and an entire margin Colonies were cream in appearance with a smooth appearance. Colonies showed signs of motility. When viewed under a microscope, all bacteria were small cocci. When gram stained most cocci were gram negative however, there were a few gram positive as well. These gram positive cells may be present for a variety of reasons. The isolated culture may be impure and a few foreign bacteria may still exist. Crystal Violet stain used in the first step of gram staining may have not been washed from some cells with decolorizer. Another possibility is that older gram negative bacteria cultures can stain gram positive.

Colonies taken from fish samples appeared in small colonies about 1mm in diameter and circular in form. They had a flat elevation and an entire margin. Colonies were cream in color and had a smooth texture. When these bacteria were viewed under a light microscope, bacteria were small baccilli. After gram staining the bacteria it was found that most of the bacteria were gram negative, however there were also few bacteria present that were gram negative. This could be due to the same possibilities discussed previously. Aperio Scientific identified this bacteria as *Stenotrophomonas maltophilia.*

S. maltophilia is a non fermenting gram negative baccilli pathogen that is known to have nosocomial potential. It is a threat due to its resistance to a number of antimicrobial agents. It is a particular threat to becoming resistant to Trimethoprim/Sulfamethoxazole (Toleman, 2007). Studies have shown that S. maltophilia is the third leading cause of gram negative infections. It effects patients with cystic fibrosis and the immunocompromised the most (Crossman, 2008). It has many pili/fimbriae that allow for formation of biofilms.(Crossman, 2008). It has been isolated in clinical and soil environments (Crossman, 2008). It has also been isolated in rivers, wells, lakes, and sewage. *S. maltophilia* likes moist areas, studies found that *S. maltophilia* has rarely been found on hospital floors or skin of clinical workers. It has been isolated from water drains and orophranygeal swabs (Denton, 1998).

DISCUSSION

The use of antibiotics in human and animal environments has been evident for many years. Antibiotic resistance is now becoming a problem in not only human environments, but also animal environments. This study has shown that there is at least three species of bacteria showing resistance to oxytetracycline in Kansas Department of Wildlife and

Parks fish Hatchery in Pratt, Ks. Resistance was found in the supply pond sediment where there have never been fish or antibiotic usage. Further research could be done to explain resistance in the solar pond. Resistance was also found on fish and the water and soil they inhabit. It has determined that at least one species of bacteria, *S. maltophilia,* is pathogenic.

Alderman, et al. (1998) stated that bacteria in waters of temperate regions are rarely human pathogens. However, evidence of a pathogen was shown in this study. The results of finding resistant bacteria may show that this strain of bacteria has survived and evolved. Natural selection may have lead this strain of bacteria to be able to survive and reproduce with antibiotics present over time. that there is an increase in resistance to oxytetracycline HCL over time. If the resistant microbials found pathogenic are transferable to humans, workers at hatcheries would be threatened most with the constant exposure they have. With resistance to antibiotics evident and more frequent use of antibiotics, there may be means to create a safer environment As found by McPhearson, et al. (1991) rivers with free flowing water have fewer antibiotic resistant microbials than do waters from ponds. A possible environment change would be to have a closed system of mechanically pumped water to replicate a river environment. Filtration of recycled water is also a way to reduce microbial populations. Recycling water would not allow for microorganisms to reach other aquatic environments thus reducing threat to humans using other aquatic environments. Further study could be done to show evidence of if and how *S. maltophilia* is transferred to humans. Studies could also be done to account for resistance in the supply pond where there has been no antibiotic usage.

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