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Isolating Microbial Compounds from the Invasive Lion Fish (*Pterois volitans*). A Potential New Method for the Control of MRSA Strains

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Abstract

The invasive species of *Pterois volitans* or commonly known as the lionfish, is spread throughout the sub-tropical and tropical Western Atlantic. The lionfish species have begun to rise as a very successful intrusive predator. Their invasion of coral reef ecosystems is a major conservation concern. Many studies have shown that this top coral reef predator is able to reproduce quickly, it's able to survive better in foreign waters than in its native location, and it lacks significant predators when fully matured. These fish are commonly found in shallow waters with rocks or reefs, and are easily recognizable by their elegant plume-like fins.

The defensive mechanism of the lionfish proves to be quite successful due to its venomous spines projecting from its body. The spines produce a combination of protein, a neuromuscular toxin and a neurotransmitter called acetylcholine. To humans the venomous sting can cause extreme pain, sweating, respiratory problems, and sometimes paralysis if stung severely. However, these invasive species could provide an alternate medicine or use with their venom to control MRSA.

Methicillin-resistant Staphylococcus aureus (MRSA) a resistant microbe that can withstand first-line antibiotics. MRSA infections account for many of the "staph" infections around the world and can also be fatal as well if not treated quickly. MRSA infections are resistant to most antibiotics though it can be treated with the powerful antibiotics, vancomycin and teicoplanin. Because the oral absorption of these drugs is low, these agents must be administered intravenously to control systemic infections. Though, the properties of the lionfish venom may prove to be an alternative to control the growth of MRSA.

This experiment is designed to test the effectiveness of the venom extracted from the lionfish as an alternative medicine to controlling or hindering the growth of the bacterium MRSA when applied to the infected area. The efficiency of the isolated venom was evaluated by looking at the growth rates of the different colonies of MRSA were treated with the venom. The venom proved to not hinder the growth of the MRSA bacteria a significant amount.

Keywords: Methicillin-resistant Staphylococcus aureus; *Pterois volitans;* Neurotransmission

Literature Review

Background

A MRSA bacterium is mostly resistant to most present antibiotics, except for sulfonamide drugs and vancomycin [1-4]. It has become a major problem for skin and wound infections. The medical community is running out of options with regards to its treatment, and once resistance to sulfonamide drugs and vancomycin is encountered, MRSA would be untreatable. We need to find new alternative sources similar to that of sulfonamide drugs or any new drugs altogether.

Abilities of lionfish and their venom

The lionfish's immunity to parasites and diseases is still unknown. The origin of this immunity could be because of the lionfish's venom or the biochemical makeup of its body tissues. The venom inside the lionfish's protruding spines goes all the way down to the base of the spines. Which at the base of the spines is the venom gland. The venom is made up of a neuromuscular toxin and a neurotransmitter called acetylcholine. The acetylcholine acts as a neurotransmitter [5] in both the peripheral nervous system (PNS) and central nervous system (CNS) [6]. The venom is able to induce in humans and animals, arterial hypotension and respiratory changes. With larger doses of the venom it can cause respiratory problems, partial or complete paralysis of the legs, and muscular weakness. On the surface, wounds swell, blister, and change to a bruise type color.

Method

Opportunity

The chance arose for me to study the venom was when two of the teacher's aquarium lionfish died due to the lack of heat in the building during the weekends. This allowed me to come up with the idea of extracting the venom from the deceased lionfish to test against methicillin-resistant Staphylococcus aureus (MRSA).

Design

Based on literature review, it was decided that *Pterois volitan's* [7] venom was to be used for the experiment due to its inclusion in the body tissue system of the lionfish.

Safety precautions

Safety was a big part in the handling of these lionfish even though they were deceased. The spines are still able to inject venom even if the lionfish isn't alive. To prevent danger, gloves and safety glasses were

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worn at all times even when handled by the teacher, the veterinarian and myself.

Experimentation of *Pterois volitan's* venom in inhibiting MRSA

This experiment was to test the capabilities and effectiveness of *Pterois Volitan's* venom in preventing the growth of MRSA. The bacterial gram-positive cocci MRSA was obtained from hospital patient wounds at Lake Regional Hospital (Figure 1). Then, using an electronic turbidity meter, the bacteria were scrapped off an MRSA cultured agar plate with a plastic loop and put into a sterile water vial (Figure 2).

The turbidity meter then measured the bacteria in the vial to around 0.05-0.07 (Figure 3). After the correct measuring of the bacteria, a sterile cotton swab was used to swab the substance in the vial and then plated onto two new blood agar plates (Figure 4). Once plated with the MRSA bacteria, the plates were put into the incubator and incubated for 9 hours at a temperature of 37°C. The venom extract from the lionfish [8] was obtained through two recently deceased aquarium lionfish. The lionfish died of natural causes due to temperature fluctuations in the tank. The two lionfish were roughly 3.5 inches in length. The dead lionfish were scooped up very carefully due to their venomous spines and placed into a clear petri dish (Figure 5). The petri dish was then put into a cooler with ice to keep them frozen. My research advisor (Mr. Chris Reeves) then transported the lionfish to the Linn Creek Animal Hospital in the small cooler (Figure 6). There at the animal hospital, Veterinarian James Wilsman, proceeded to extract the venom. The lionfish were place onto a blue mat where Vet. Wilsman proceeded to extract the venom with 1cc syringes (Figure 7). Vet. Wilsman had gloves on for safety and a bright light to see the structure of the lionfish. He used magnifying lenses to precisely extract the venom from the venom glands located at the base of each of the lionfish's dorsal spines. Once the venom was extracted he then took a milliliter of distilled

Figure 1: Original MRSA plate, which was obtained from a Lake Regional Hospital patient.

Figure 2: The scraping of the original MRSA.

Figure 3: The measuring of the bacteria in the sterile water vial to 0.07.

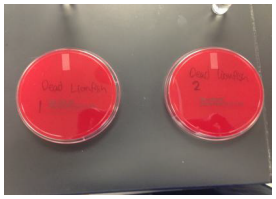

Figure 4: Newly plated dead lionfish 1 and 2 plates.

Figure 5: The dead lionfish in the petri dish.

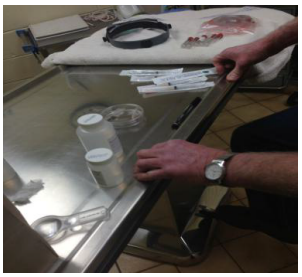

Figure 6: Cooler and workstation of Veterinarian Wilsman.

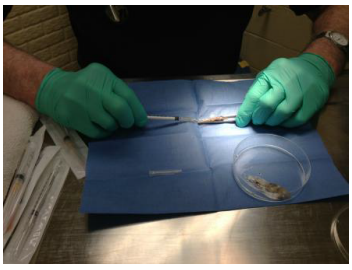

Figure 7: Extraction of venom with 1cc syringe.

water into the syringe and injected the whole solution into a sterile vial (Figure 8). Vet. Wilsman proceeded to do the exact same procedure with the second dead lionfish. Once both vials were occupied with little venom they were put into the cooler to maintain the proteins in the venom. The lionfish were also put back into the cooler as well.

The next step in conducting this experiment was to try and conduct research on the effects of lionfish venom on gram-positive cocci MRSA. After 24 hours, I returned to the lab at the hospital with the vials of lionfish venom. The newly plated MRSA plates were grown and so we knew that it was ready to be plated again with the sterile sensitivity disks containing the lionfish venom. I proceeded to plate two new plates of MRSA with supervision from the microbiology lab supervisor. I used the procedures as stated in the beginning of the method. Before adding the two new plates into the incubator to grow, I added two sterile blank sensitivity disks onto the two blood agar plates (Figure 9). I then got a sterile plastic pipette and put two drops of the first dead lionfish solution onto each of plate 1's sensitivity disks (Figure 10). For the second lionfish solution, I added two drops to each of plate 2's two sensitivity disks as well. Once both of the plate's sensitivity disks were soaked with venom (Figure 11), I covered them up and put them into the incubator to grow for about nine hours.

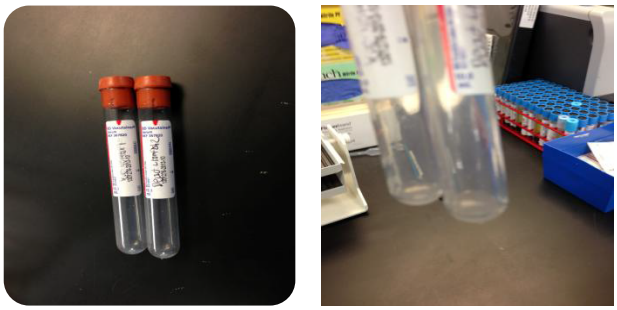

Figure 8: Sterile vials with venom solution.

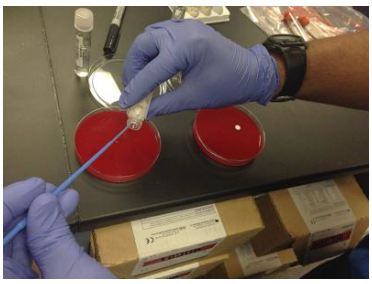

Figure 9: The adding of the sterile sensitivity disks.

Figure 10: Adding the venom drops to the sensitivity disks with the plastic pipette.

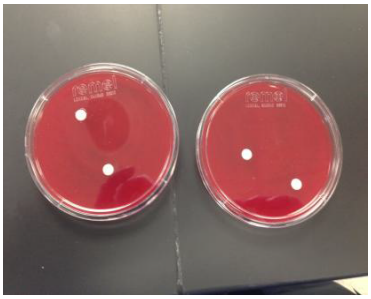

Figure 11: Finished MRSA plates, before adding them to the incubator to be incubated.

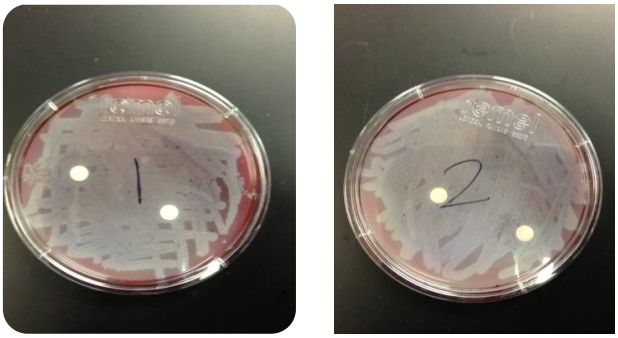

Figure 12: Results of study. Plates 1 and 2.

Discussion of Results

Summary

Literature data suggested that lionfish venom or tissue might have anti-bacterial properties that would possibly inhibit the growth of MRSA: the extracts being acetylcholine and a neurotoxin.

A positive result of the study was at least a one-millimeter zone of inhibition around the individual sensitivity disks of the two plates. This inhibition means the extract inhibited the growth of MRSA to some extent. A negative result would portray that no zone of inhibition was seen around the sensitivity disks. In this study, no zone of inhibition was seen in the cultured MRSA plates.

Conclusion

After reviewing the results of this study, the extracts did not inhibit the growth of the MRSA, as no zone of inhibition was seen around the sensitivity discs (Figure 12). This may illustrate the infectivity of the extracts in inhibiting the growth of the MRSA. The possibility being that the venom degraded before the extraction process or because there was no correct measurement of the exact amount of venom in the extract. Nevertheless, further testing should be done with regards to obtaining higher concentrations of the venom or maintaining the venom from degrading.

Future Studies

Future studies could attempt to:

1. Find a more concentrated solution of the venom that would inhibit the growth of MRSA.
2. Determine whether or not other types of bacteria would be susceptible to these solutions from the lionfish.

3. Determine how much acetylcholine and neurotoxins are actually present in the solutions.

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- Supervised me and taught me the safety procedures in handling and culturing the MRSA Drs. Paul and Maria Bernabe, Parents
- Continually pushing me to do my best, giving me information about the MRSA bacteria and the vaccines they normally use to treat the bacteria, and also providing the syringes, distilled water, and sterile vials.

References

1. National Oceanic and Atmospheric Administration (2011) Lionfish Biology Fact Sheet. Retrieved January 4, 2013, from National Oceanic and Atmospheric Administration.
2. National Geographic (2013) National Geographic Lionfish. Retrieved January 5, 2013, from National Geographic.
3. Vone Research (2010) Danger Surrounding the Venemous Lionfish Sting. (T. Beckel, Editor) Retrieved January 4, 2013, from lionfishhunters.org.
4. Methicillin-resistant Staphylococcus aureus; Hospital-acquired MRSA (HA-MRSA) (2012) PubMed Health.
5. Boeree DC (2009) Neurotransmitters. Retrieved January 6, 2013, from General Psychology.
6. Waymire CJ (2000) Chapter 11: Acetylcholine Neurotransmission. Retrieved January 6, 2013, from Neuroscience.
7. James A. Morris J (2008) Biology and Ecology of the Invasive Lionfishes, Pterois miles and Pterois Volitans. 61st Gulf and Caribbean Fisheries Institute.
8. Sri Balasubashini SK (2006) *In vivo* and *In vitro* Characterization of the Biochemical and Pathological Changes Induced By Lionfish (Pterois Volitans) Venom in Mice. Department of Biochemistry, Annamalai University, Annamalai Nagar, 608 002, India 16: 525-531.