Antibiotic Resistance in Fecal Microbiota of Rehabilitated California Sea Lions, *Zalophus californianus*

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Abstract

California sea lions, *Zalophus californianus,* have established a large breeding ground in the Channel Islands, located off of the central coast of California. Due to ocean temperatures increasing, the natural baitfish populations at the Channel Islands have moved farther north, which in turn causes the California sea lions to have to travel farther north in order to feed (1). The animals must travel farther in order to maintain a healthy weight, but their pups become extremely malnourished when the mothers leave for longer periods of time (2). Channel Islands Marine and Wildlife Institute (CIMWI) is a local organization that is committed to rescuing and rehabilitating all marine mammals, but their main patients include emaciated California sea lion pups. Once the pups return to a healthy weight, they are released at the Channel Islands. We are studying antibiotic resistance within the gut microbiota of the California sea lions to see the effects of the overuse and misuse of antibiotics, which is generating a worldwide health concern due to the increase in antibiotic resistant microbes.

This research project will compare the California sea lions' gut microbiota before, during, and after rehabilitation at CIMWI, which includes the administration of antibiotics, probiotics, and a controlled diet. CIMWI staff collected fecal samples from each pup when they entered the facility before receiving any treatment, twenty days after antibiotic administration, and again before each pup was released. We extracted the DNA from each sample and performed PCR using eight different primers in order to amplify the DNA that encodes for antibiotic resistance genes. We then ran agarose gels with the PCR products to find out if the antibiotic resistance genes were found in each sample. It is expected that wild California sea lion gut microbiota will be resistant to amoxicillin, tetracycline, and third generation cephalosporins due to human pollution and waste runoff into the ocean. After receiving treatment, it is expected that the sea lions gut microbiota will have more resistance to antibiotics.

Figure 1. Patient 262 with trash around his neck, before being rescued by CIMWI.

Figure 2. Patient 262 after removing trash from his neck and beginning rehabilitation.

Figure 3. Patient 262 before release.

Figure 4. Patient 255 eating in rehabilitation.

Figure 5. Patient 214 sun bathing in a bath after eating.

Figure 6. Patient 223 shortly after entering rehabilitation.

Methods

Sample collection was done by CIMWI staff. When an abandoned pup entered the rehabilitation center, a fecal sample was taken before any medication was administered. The pup proceeded to receive fluids, antibiotics, probiotics and fish that contained dextrose, to prevent refeeding syndrome. The second sample was taken after 20 days of administered antibiotics. The pup continued to take probiotics and was slowly weaned off of dextrose fish. Once each pup reached a certain weight and exhibited no sign of disease, it was processed for released and the final sample was collected.

1st Fecal Sample → **2nd Fecal Sample** → **3rd Fecal Sample**

Sea lion pup rescued
- **Pup admitted to CIMWI**

Primary Treatment:
- **Fluids and D-glucose**
- **Pobiotics**
- **Antibiotics (~20 days)**

Continuing Treatment:
- **Fluids and D-glucose**
- **Probiotics**
- **Pup at healthy weight**

All the samples were transported from CIMWI to California Lutheran University and stored in the -80°C freezer. The process began with extracting prokaryotic DNA from each fecal sample, using the Mo Bio PowerFecal® DNA Isolation Kit. The DNA concentration of each sample was checked by using the NanoDrop and Bio Rad Versa Fluor Fluorometer. These DNA concentrations were used to calculate how each DNA sample would be diluted for polymerase chain reaction (PCR), where the genes encoding for antibiotic resistance were amplified. Eight different primers were used, that each encoded for a different antibiotic resistance gene. These primers consisted of one for amoxicillin resistance, one for tetracycline resistance, three for third generation cephalosporin resistance, two for vancomycin resistance, and one for methicillin reisistance. After PCR, an agarose gel was run to see if the antibiotic resistance gene was found in each DNA sample.

DNA Extraction
↓
NanoDrop & Flourometer
↓
Dilution Calculations for PCR
↓
Addition of Primers
↓
PCR each sample
↓
Gel Electrophoresis
↓
Analysis of Data

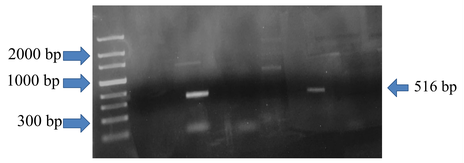

Figure 7. Agarose gel viewed under UV light. Positive control test with five primer sets. Only blaTEM primer set (lanes 4 and 9) bands showed up in the appropriate location.

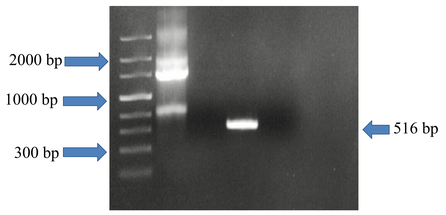

Figure 8. Agarose gel viewed under UV light. Positive control test with the three primer sets for cephalosporin resistance. Only blaTEM primer set (lane 4) showed up.

Results

From the 143 samples collected from CIMWI, 55 were chosen for DNA extraction, PCR, and gel electrophoresis. Of those 55 samples, 45 samples were of rehabilitated sea lions. From the 15 rehabilitated sea lions 11 were males and 4 were females. Ten of the 55 samples were of deceased sea lions, which included 3 males and 2 females. Currently, preliminary data has been obtained and analyzed. This data shows that the sea lions gut microbiota do have resistance to third generation cephalosporins.

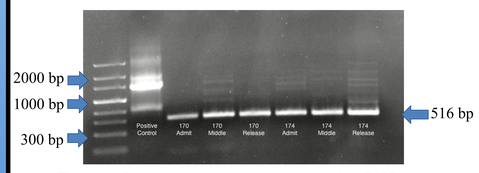

Figure 9. Agarose gel viewed under UV light. Samples 170 (1st, 2nd, 3rd) and 174 (1st, 2nd, 3rd), all positive for blaTEM gene.

Figure 10. Patient 174 (top) rehabilitating well at CIMWI.

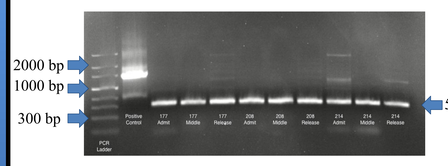

Figure 11. Agarose gel viewed under UV light. All three samples for 177, 208, and 214 positive for blaTEM gene.

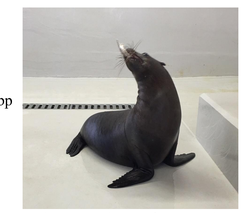

Figure 12. Patient 170 before release.

Conclusion

Because the DNA samples all show resistance to third generation cephalosporins, the next step in understanding how the gut microbiota antibiotic resistance changes throughout rehabilitation would be to use quantitative PCR (qPCR). Rather than giving a yes or no answer on whether antibiotic resistant gut microbiota are there, qPCR would show how much antibiotic resistant gut microbiota are in each sample, and changes in the gut microbiota could be compared throughout rehabilitation. It may also be necessary to use different primers for the other types of antibiotic resistance, because many of the primers in this study would not work with the positive control.

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References

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