PATTERNS OF ANTIBIOTIC RESISTANCE IN BACTERIA ISOLATED FROM MARINE TURTLES

An internship report submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

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Abstract:

Patterns of Antibiotic Resistance in Bacteria Isolated from Marine Turtles, by Lana Piñera-Pasquino: Sea turtles face many natural and human-induced threats to their survival. This has prompted several sea turtle rehabilitation facilities to open in order to treat and release these animals. Treatment of these rehabilitated sea turtles has led to the discovery that some of their bacterial infections do not adequately respond to antibiotic treatment (Tom Sheridan, 2006, personal communication). This has led to questions as to where the sea turtles are acquiring these antibiotic-resistant bacteria. Widespread use of antibiotics in humans, domesticated animals, aquaculture and agriculture has led to their increased presence in the environment, and has created the selective pressure necessary for some bacteria to develop antibiotic resistance (Levy, 2001). Many studies have been done to determine the effects of antibiotic release on terrestrial ecosystems (Haapapuro, *et al*., 1997; Sayah, *et al*., 2005). However, very little research has been done on its effects in aquatic ecosystems (Depaola, *et al*., 1995; Goni-Urriza, *et al*., 2000; Kolpin, *et al*., 2002), and even less has been done to determine its effects on marine ecosystems (Kelly, *et al*., 2006). To determine the possible effects of antibiotic release in the environment on sea turtles, an internship was conducted at the South Carolina Aquarium's Sea Turtle Rescue Program. During this internship, sick and injured sea turtles were rehabilitated and released back into the wild. In addition, the occurrence of antibiotic-resistant bacteria found in wild Loggerhead sea turtles (*Caretta caretta*) was analyzed using the Kirby-Bauer method and a tube-dilution method with a 96-well suspension plate, and a preset panel of antibiotics designed by Dade Behring specifically for the National Oceanic and Atmospheric Administration (NOAA) (Bauer, *et al*., 1966; NCCLS, 2003).

Twenty-one gram negative bacterial strains were isolated from *C. caretta* cloacal samples and analyzed for their resistance to specific antibiotics and also for the minimal inhibitory concentration (MIC) of each antibiotic. Resistance to multiple antibiotics was detected in all of the isolates, with the most common resistances being to lincomycin, clindamycin, erythromycin, penicillin, triple sulfa, cephalexin, and cephalothin. Determining possible patterns of antibiotic resistance in microbes from marine animals is vital in order to establish the significance of antibiotic release into marine environments.

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Chapter One: The South Carolina Aquarium Sea Turtle Rescue Program

Introduction: Natural threats for *Caretta caretta* include erosion of nesting beaches, nest depredation, nest loss due to erosion or inundation, and shark depredation. Human-induced threats to *C. caretta* populations include beach armoring, artificial beachfront lighting, recreational beach equipment (which act as obstacles for nesting females and hatchlings), poaching, destruction of resting and foraging grounds through dredging, longline fisheries, trawl fisheries, boat collisions, pollution, and incidental ingestion of trash (National Marine Fisheries Service and U.S. Fish and Wildlife Service, 1999). Because *C. caretta*, along with many other sea turtle species, face so many threats to their survival, the formation of sea turtle rehabilitation facilities has become more and more prevalent throughout the United States. One such facility is located at the South Carolina Aquarium, where one portion of the internship was performed. At the South Carolina Aquarium Sea Turtle Rescue Program, work was completed to rehabilitate sick and injured sea turtles with the goal of returning them to the wild. In addition to rehabilitating the sea turtles, their specific ailments were documented in order to identify any particular trends that may provide insight into any new threats to the *C. caretta* populations.

Methods: Sea Turtle Rehabilitation: The South Carolina Aquarium Sea Turtle Rescue Program accepts injured or sick sea turtles which have been found along the southeastern coastline. Once an injured sea turtle was delivered to the South Carolina Aquarium, its health was first determined through visual assessment and blood extraction. Measurements were taken, and included the turtle's weight, its straight and curved carapace width and length, and the concavity of its plastron. The amount of

barnacles and other marine organisms which were growing on the turtle's carapace, plastron, head and flippers was also observed. Once the measurements and blood had been taken, the turtle was placed in a tank of shallow freshwater. The freshwater allowed the turtle to re-hydrate itself, while killing the majority of the marine organisms which were attached to the turtle's body. Daily, the freshwater tank was drained, which allowed access to the turtle. While the turtle was out of water, the loose barnacles and other marine growths were removed, and any wounds were sprayed and gently brushed with a disinfectant. The disinfectant remained on the turtle's skin for approximately ten minutes, after which the turtle was sprayed clean with freshwater. This procedure was continued until about 90% of the marine organisms were removed, and the turtle was strong enough to be placed in a full tank of freshwater. The percentage of seawater in the tank was gradually increased during each water change until the tank contained 100% seawater.

Medical rounds, which were completed under the supervision of the South Carolina Aquarium's veterinarian, were performed weekly on all of the sea turtles in the facility. Once a week, each turtle was removed from its tank in order for its health to be assessed. Each turtle was weighed and measured to quantify its growth and weight gain. The amount of food and medication being administered to each individual turtle was occasionally modified as the turtle gained weight. When necessary, each turtle was also debrided with a brush to remove any loose, flaking skin, or any remaining barnacles. Blood was extracted from each turtle and analyzed every week until the turtle's health stabilized. Blood was extracted from the dorsal cervical sinus and analyzed to determine the turtle's packed cell volume (PCV), total protein (TP), and glucose levels.

Sea Turtle Maintenance: In addition to performing the medical rounds each week, routine husbandry tasks were performed daily. These tasks included cutting and weighing out the food being fed to each turtle, feeding each turtle and observing their eating behavior, administering sub-cutaneous and oral medications and vitamins, and recording the eating activities of each individual turtle, including how much they ate, how actively they ate, and what medications they were given with their food. Standard cleaning tasks necessary to maintain a sanitary facility were also performed on a regular basis and included mopping the floor, cleaning counter tops, cleaning containers used to hold turtles during medical inspections, and disinfecting any instruments used to clean the turtles or turtle tanks.

Release of Sea Turtles: A turtle was considered healthy enough for release when it had regained a healthy appetite, had increased both its weight and strength, and had a PCV in the high twenties. Rehabilitated sea turtles were released from the same area in which they were found whenever possible. However, due to complications with the tides, predators, water temperature, or the stress which is placed on the sea turtle during transportation to the release site, that was not always possible. In those cases, the sea turtles were released from areas which were approved by U.S. Fish and Wildlife and the National Oceanic and Atmospheric Administration (NOAA). In every case, the primary concern when releasing a rehabilitated sea turtle was its welfare.

Results: During the time frame of this internship, a total of ten *C. caretta* were admitted to the South Carolina Aquarium Sea Turtle Rescue Program. Five *C. caretta* died before or during treatment, four were successfully rehabilitated and released, and one currently remains at the facility and is scheduled to be released this summer. All ten

C. caretta were diagnosed with having Debilitated Turtle Syndrome (DTS). A sea turtle is determined to have DTS when it is emaciated and has a significant amount of barnacle and epibiotic coverage on its exterior. DTS can occur as the result of a sea turtle becoming weakened by a variety of reasons, from becoming cold-stunned to illness due to exposure to pollutants or ingestion of trash. One study found DTS to occur more frequently in sub adult, female sea turtles, with *C. caretta* being the most commonly afflicted species in the southeastern United States (Norton, *et al*., 2004). Detailed information about DTS is still sparse; thus research needs to continue on the subject to gain greater insight into its possible causative factors.

Chapter 2: Laboratory Work

Introduction: Over the last couple of decades, the study of the environmental impacts of chemical pollution has focused primarily on what are considered "priority pollutants" (*i.e.* potent toxic or carcinogenic chemicals). Little attention has been paid to the effects that "less potent" pharmaceuticals may have on the environment and its inhabiting wildlife, and even smaller attention has been paid to its effects on marine life (Daughton and Ternes, 1999). Antibiotics have been used extensively in both human and animal life since their introduction into medicine in the 1940's and 1950's (Virella, 1997). Antibiotics and antibiotic-resistant bacteria are released in varying amounts into the environment due to the increased and sometimes haphazard use of antibiotics in the medical, veterinary, aquacultural, and agricultural fields (Goni-Urriza, *et al*., 2000). The extensive use of antibiotics in both humans and animals has led to the development of antibiotic resistance in some bacterial strains. Some of the proposed sources through which antibiotics are being introduced to marine creatures are animal agriculture and the

improperly treated wastes of humans and animals (Chee-Sanford, *et al*., 2001; Daughton and Ternes, 1999). Because of the serious implications of antibiotic release in the environment, research was conducted at the South Carolina Aquarium Sea Turtle Rescue Program. In addition to caring for the sick and injured sea turtles contained within the facility, cloacal samples were obtained from several of the turtles upon entry to the South Carolina Aquarium. Cloacal samples were also obtained from the sea turtles which were captured by a South Carolina Department of Natural Resources (SCDNR) research team for an unrelated study. The resistance of the sea turtles' microbiota to antibiotics was studied, utilizing laboratory equipment from both NOAA and the Clemson Veterinary Diagnostic Center (CVDC). This was accomplished by culturing cloacal samples extracted from these rescued sea turtles, isolating and identifying dominant gram negative bacterial strains, and testing them for resistance to antibiotics. Studying the extent to which antibiotic resistance is present in marine animals has far-reaching implications for both marine animal and human health. It is important to determine if the occurrence of antibiotic resistance found in marine animals represents a particular pattern. If a pattern can be established, then sources of the factors leading to the development of antibiotic resistance in marine animals may be able to be determined. The results of this study may serve to guide future research on this topic. Further research could be conducted to locate the origins of antibiotic release into marine environments. This may lead to greater care in the use of antibiotics, and stricter regulations on the release of antibiotics into the surrounding environment.

Methods: Cloacal samples came from two sources. One source was the sea turtles in the South Carolina Aquarium Sea Turtle Rescue Program. The second source

came from sea turtles caught by an SCDNR research team doing an unrelated sea turtle study. The sea turtles which were caught by SCDNR were retained briefly for measurements and sampling, and subsequently released. In all cases, samples were taken from *C. caretta* populations located off the southeastern coast of the United States.

Cloacal samples were acquired by inserting a sterile culturette swab into the cloaca of a sea turtle, and preserving it in a sterile media tube. The samples were stored in different manners, depending upon the circumstances in which they were taken. Samples taken at the South Carolina Aquarium were refrigerated until they could be properly stored in the laboratory at NOAA. In most cases, the samples were refrigerated for less than one hour before storage at NOAA. However, due to the unpredictable nature in which the sea turtles were admitted to the South Carolina Aquarium, samples were sometimes refrigerated for a day before proper storage. In one case, a sample was refrigerated for five days before storage at NOAA. The samples obtained by the individuals from SCDNR were stored in another manner, as the sampling boat (the *Lady Lisa*) remained out to sea for a week before returning to land. To preserve these samples until they could be stored at NOAA, the culturette tubes were either placed in a -80° C freezer, or frozen in liquid nitrogen and stored in a Dewar flask (Mark Mitchell, 2005, personal communication). The samples were stored on the *Lady Lisa* in this manner for seven to ten days.

Once the samples arrived at NOAA, the tips of the culturette swabs were cut off using sterilized scissors and dropped into 2 ml storage vials, containing 1 ml of 80% bacto tryptic soy broth (TSB) with 20% glycerol (Dade Behring, California). After the tip was placed in the vial, the vial was vortexed for approximately ten to fifteen seconds

to reduce clumping of the bacteria, labeled, and placed in the -80° C freezer located on the NOAA facilities. The samples which were stored in liquid nitrogen were the only exception to this procedure, as they were directly placed in the -80° C freezer. It was necessary to store the samples at NOAA, as budgetary issues required the cloacal samples to be shipped in bulk to CVDC for analysis. The samples were wrapped in bubble wrap, and placed on dry ice for transport to CVDC. At CVDC, the dominant gram negative bacterial strains were isolated and identified, and an antibiotic resistance analysis (ARA) was performed using the Kirby-Bauer method (Bauer, *et al.*, 1966). The ARA results, along with the isolated and identified bacteria, were returned to NOAA. An ARA was also performed in the laboratory at NOAA, and the minimal inhibitory concentration (MIC) of the isolates was determined using a tube-dilution method, involving a 96-well suspension plate, and a preset panel of antibiotics (Table 7). Because NOAA was awaiting the shipment of more ARA suspension plates, the isolates were stored again until the arrival of these plates. A small amount of the isolate was transferred to the 2 ml vials containing 1 ml of TSB and glycerol using a sterilized loop. The vial was then vortexed, and frozen in the -80° C freezer.

When the plates arrived, the isolates were removed from the freezer for transfer onto tryptic soy agar (TSA) plates (Dade Behring, California). The vials containing the isolates were placed on ice until they could be transferred to the TSA plates to minimize thawing. Ice flakes from the frozen isolates were streaked onto the plates using a sterilized loop. The inoculated TSA plates were placed in a 37° C incubator for 21 hours before being removed in order to transfer the bacterial colonies to the ARA suspension plates (Dade Behring, California). The isolates were prepared for ARA using the

following method. A small amount of the isolate was removed from the TSA plate using a disposable, sterile wooden rod, and placed into sterile, nutrient-free, inoculum water. After the inoculum water was inoculated, it was vortexed, and its optical density (OD) was measured using a Dade Behring MicroScan Turbidity Meter (Dade Behring, California) (Figure 1). The inoculum water was inoculated with the isolate until it reached an OD between 0.08 and 0.10. Once the inoculum water reached the proper OD, 0.1 ml of the broth was transferred to a tube containing 25 ml of a cation-adjusted Mueller-Hinton broth (CAMHB) using a Gilson Micropipette (Dade Behring, California). The inoculated CAMHB tube was gently shaken back and forth several times before being poured into a disposable, plastic inoculator-D set (Dade Behring, California) (Figures 2 and 3). The inoculator-D set, which is custom made by Dade Behring to accompany the Dade Behring MicroScan Renok Pipette (Figure 4), is used to transfer inoculated broth from a tube to the ARA suspension plates. The inoculator-D set is comprised of two halves. The bottom half functions as a tray, which holds the inoculated broth once it is poured into the inoculator-D set. The top half consists of 96 small holes which correspond to the 96 wells contained within the ARA plates. The inoculated broth was first poured into the bottom half of the inoculator-D set, after which the top half was placed on top of it. The Dade Behring MicroScan Renok Pipette (Dade Behring, California) was next placed on top of the inoculator-D set, where it locked on to the top portion of the set, and siphoned up the broth through the 96 small holes. The MicroScan Renok Pipette, still attached to the top half of the inoculator-D set, was next placed on top of the ARA suspension plate (Figure 5), where it dispensed 115±10 µL of inoculated broth into each of the 96 wells simultaneously. The plates were labeled and incubated at

37° C for approximately 21 hours. The ARA plates were also inoculated with five control strains (*Staphylococcus aureus*, 2 *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*) using the same method. After incubation, the ARA plates were read using a Dade Behring MicroScan Touch Scan and the Max Flex Custom Panel System computer program (Dade Behring, 2001). On the Dade Behring MicroScan Touch Scan (Dade Behring, California), each of the 96 wells was observed for growth of bacteria. The lowest concentration of antibiotic to contain no bacterial growth in its well was recorded to determine the MIC.

Results: A total of 21 gram negative bacterial strains were isolated from the cloacal swabs. Antibiotic resistance was detected in all of the isolates. Of the 17 antibiotics which were tested by CVDC, at least 50% of the isolated bacteria displayed resistance to seven of them. The most frequent resistances displayed by the isolates were to lincomycin (100% of the isolates), clindamycin (95.2%), erythromycin (95.2%), penicillin (95.2%), and triple sulfa (95.2%). Little to no resistance was observed in the isolates to gentamicin (9.5%), amikacin (0%), enrofloxacin (0%), and neomycin (0%) (Table 1). The isolates showing resistance to the greatest amount of antibiotics tested were the *Pseudomonas* strains, which ranged from 47.1% to 70.6% resistance. Also showing significant levels of antibiotic resistance were *Stenotrophomonas maltophilia* (64.7% of the antibiotics tested), *Morganella morganii* (52.9%), *Citrobacter freundii* (52.9%), and several of the *Escherichia coli* strains (Table 2). Of the 26 antibiotics tested at NOAA, eight antibiotics had at least 50% of the isolated bacteria displaying resistances to them. The highest levels of resistances displayed by the isolates were to erythromycin (100% of the isolates), cephalexin (80%), cephalothin (80%), and penicillin (75%). Very

little resistance was noted in the isolates to amikacin (5%), apramycin (5%), ciprofloxacin (5%), gentamicin (5%), imipenem (5%), meropenem (5%), and sulfathiozole (5%). No resistance was observed to moxifloxacin or ofloxacin (Table 3). The isolates which displayed resistance to the greatest number of antibiotics tested were *Stenotrophomonas maltophilia* (69.2% of the antibiotics tested) and *Pseudomonas aeruginosa* (57.7 and 61.5%) (Table 4).

Discussion: Similar patterns of resistance were found within the two separate antibiotic resistance analyses run by CVDC and NOAA. In both tests, the isolates containing the largest variety of antibiotic resistance were *Stenotrophomonas maltophilia*, and the *Pseudomonas* strains. Additionally, both analyses found erythromycin resistance and penicillin resistance to be the most prevalent resistances displayed by the isolates, while amikacin resistance and gentamicin resistance were the least commonly observed. The results from the ARA's run at both CVDC and NOAA found the greatest percentage of resistance displayed by the isolates to be to the beta-lactam, lincosamide, macrolide, and sulfonamide (trimethoprim-sulfadiazine and triple sulfa) classes of antibiotics. The lowest percentage of resistance displayed by the isolates was to the carbapenem group of the beta-lactam class, and to the aminoglycoside, quinolone, and sulfonamide (trimethoprim-sulfamethoxazole and sulfathiazole) classes of antibiotics (Tables 5 and 6) (Beers, *et al*., 2003; Mims, *et al*., 1993).

Although the results of this project indicate that there may be a serious problem involving the release of antibiotics into the ocean, it is important to note when considering these data that some of the antibiotics used in the ARA panels do not selectively target gram negative bacteria. Antibiotics found in the beta-lactam,

lincosamide, and macrolide classes of antibacterials target either both gram negative and gram positive bacteria, or more selectively target gram positive bacterial strains (Mims, *et al*., 1993). For example, while many of the isolates displayed resistance to antibiotics such as erythromycin and penicillin, this may have occurred due to the fact that erythromycin and penicillin are both designed to target gram positive bacteria, and are less effective against gram negative bacterial strains.

An valuable lesson learned from this project was the importance of preserving samples properly. Freezing the cloacal swabs slowly in a -80° C freezer caused the bacterial cells to lyse, resulting in the death of the majority of the bacteria from the samples. When the culturette swabs were stored in this way, only four out of the 21 samples collected yielded bacteria. Bacteria from the samples fared better when frozen in a broth containing glycerol, which prevented cell lysis, or when frozen quickly in liquid nitrogen. This project was greatly hindered by the inability to perform the laboratory work on the cloacal swabs immediately after collecting. The necessity of sending the samples in two large shipments also prevented any immediate feedback on how the storage of the samples affected the amount of bacteria harvested from each cloacal swab.

The widespread use of antibiotics has already been found to present many dangers to both human and animal health. Although many studies have already been performed on terrestrial and freshwater ecosystems, very little has been discovered on how antibiotic release affects marine ecosystems. More research needs to be done on antibiotic resistance displayed by bacteria present in marine organisms. Another possible avenue for future research would be to sample sea turtles upon their entrance to a rehabilitation facility, and taking another sample just prior to release, after they have received treatment

(Tom Sheridan, 2006, personal communication). This would allow a comparison to be made between the types of antibiotics administered to a sea turtle during treatment, and the types of resistances present in the bacteria isolated from the sea turtle after treatment. It is imperative that the relationship between antibiotic release into the ocean and the development of antibiotic resistance in bacteria found in marine organisms continues to be studied, in order to gain a better understanding of its possible impacts on marine life.

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Appendix I
(Tables and Figures)

Table 1. Results of the ARA completed using the Kirby-Bauer method with the antibiotic panel used by CVDC. Results are based solely on isolates surviving the sampling conditions and storing process.

CVDC Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Amikacin	0	0
Ampicillin	13	61.9
Augmentin	8	38.1
Ceftiofur	6	28.6
Cephalothin	13	61.9
Chloramphenicol	8	38.1
Clindamycin	20	95.2
Enrofloxacin	0	0
Erythromycin	20	95.2
Gentamicin	2	9.5
Lincomycin	21	100
Neomycin	0	0
Orbifloxacin	3	14.3
Penicillin	20	95.2
Tetracycline	6	28.6
Trimethoprim-Sulfadiazine	9	42.9
Triple Sulfa	20	95.2

Table 2. The percentage of antibiotic resistance displayed by each isolate as determined by the antibiotic panel used by CVDC. A total of 17 antibiotics were tested at CVDC. Results are based solely on isolates surviving the sampling conditions and storing process.

CVDC Results

Isolate	**Number of Antibiotics to Which the Isolate is Resistant**	**Percentage of Resistance**
Citrobacter braakii (SCA#8)	6	35.3
Citrobacter freundii (SCA#2)	9	52.9
Escherichia coli (CC0348)	7	41.2
Escherichia coli (CC0360)	9	52.9
Escherichia coli (CC0378)	9	52.9
Escherichia coli (SCA#2)	5	29.4
Escherichia coli (SCA#3)	10	58.8
Escherichia coli (SCA#5)	5	29.4
Escherichia coli (SCA#6)	5	29.4
Escherichia coli (SCA#7)	4	23.5
Morganella morganii (CC0382)	9	52.9
Proteus vulgaris (CC0356)	7	41.2
Pseudomonas aeruginosa (CC0380)	12	70.6
Pseudomonas aeruginosa (SCA#3)	11	64.7
Pseudomonas spp. (CC0382)	8	47.1
Pseudomonas spp. (SCA#4)	10	58.8
Pseudomonas stutzeri (SCA#7)	12	70.6
Psuedomonas stutzeri (CC0364)	10	58.8
Salmonella spp., Poly D (CC0380)	4	23.5
Shewanella algae/putrefaciens (CC0384)	6	35.3
Stenotrophomonas maltophilia (SCA#7)	11	64.7

Table 3. Results of the ARA completed using the tube dilution method with the antibiotic panel utilized by NOAA. Results are based solely on isolates surviving the sampling conditions and storing process.

NOAA Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Amikacin	1	5
Amoxicillin	12	60
Ampicillin	11	55
Apramycin	1	5
Azithromycin	10	50
Cefoxitin	10	50
Ceftriaxone	3	15
Cephalexin	16	80
Cephalothin	16	80
Chloramphenicol	4	20
Ciprofloxacin	1	5
Erythromycin	20	100
Gentamicin	1	5
Imipenem	1	5
Meropenem	1	5
Moxifloxacin	0	0
Nalidixic Acid	3	15
Nitrofurantoin	9	45
Ofloxacin	0	0
Oxytetracycline	8	40
Penicillin	15	75
Streptomycin	3	15
Sulfathiazole	1	5
Tetracycline	4	20
Trimethoprim	8	40
Trimethoprim/Sulfamethoxazole	3	15

Table 4. The percentage of antibiotic resistance displayed by each isolate as determined by the antibiotic panel used by NOAA. Twenty-six different antibiotics were tested using the antibiotic panel designed by Dade Behring for NOAA. Results are based solely on isolates surviving the sampling conditions and storing process.

NOAA Results

Isolate	**Number of Antibiotics to Which the Isolate is Resistant**	**Percentage of Resistance**
Citrobacter braakii (SCA#8)	5	19.2
Citrobacter freundii (SCA#2)	11	42.3
Escherichia coli (CC0348)	6	23.1
Escherichia coli (CC0360)	8	30.8
Escherichia coli (CC0378)	9	34.6
Escherichia coli (SCA#2)	1	3.8
Escherichia coli (SCA#3)	9	34.6
Escherichia coli (SCA#5)	3	11.5
Escherichia coli (SCA#6)	6	23.1
Escherichia coli (SCA#7)	2	7.7
Morganella morganii (CC0382)	7	26.9
Proteus vulgaris (CC0356)	10	38.5
Pseudomonas aeruginosa (CC0380)	16	61.5
Pseudomonas aeruginosa (SCA#3)	15	57.7
Pseudomonas spp. (CC0382)	9	34.6
Pseudomonas spp. (SCA#4)	7	26.9
Pseudomonas stutzeri (SCA#7)	10	38.5
Salmonella spp., Poly D (CC0380)	1	3.8
Shewanella algae/putrefaciens (CC0384)	9	34.6
Stenotrophomonas maltophilia (SCA#7)	18	69.2

Table 5. Results of the ARA completed using the Kirby-Bauer method with the antibiotic panel used by CVDC. Antibiotics are grouped into classes. Results are based solely on isolates surviving the sampling conditions and storing process.

CVDC Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Aminoglycosides		
Amikacin	0	0
Gentamicin	2	9.5
Neomycin	0	0
Beta-lactams		
Cephalosporins		
Ceftiofur	6	28.6
Cephalothin	13	61.9
Penicillins		
Ampicillin	13	61.9
Augmentin	8	38.1
Penicillin	20	95.2
Chloramphenicols		
Chloramphenicol	8	38.1
Lincosamides		
Clindamycin	20	95.2
Lincomycin	21	100
Macrolides		
Erythromycin	20	95.2
Quinolones		
Enrofloxacin	0	0
Orbifloxacin	3	14.3
Sulfonamides		
Trimethoprim-Sulfadiazine	9	42.9
Triple Sulfa	20	95.2
Tetracyclines		
Tetracycline	6	28.6

Table 6. Results of the ARA completed using the tube dilution method with the antibiotic panel utilized by NOAA. The antibiotics are grouped into their respective classes. Results are based solely on isolates surviving the sampling conditions and storing process.

NOAA Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Aminoglycosides		
Amikacin	1	5
Apramycin	1	5
Gentamicin	1	5
Streptomycin	3	15
Beta-lactams		
Carbapenems		
Imipenem	1	5
Meropenem	1	5
Cephalosporins		
Cefoxitin	10	50
Ceftriaxone	3	15
Cephalexin	16	80
Cephalothin	16	80
Penicillins		
Amoxicillin	12	60
Ampicillin	11	55
Penicillin	15	75
Chloramphenicols		
Chloramphenicol	4	20
Macrolides		
Azithromycin	10	50
Erythromycin	20	100
Nitrofurantoin		
Nitrofurantoin	9	45
Quinolones		
Ciprofloxacin	1	5
Moxifloxacin	0	0
Nalidixic Acid	3	15
Ofloxacin	0	0
Sulfonamides		

Sulfathiazole	1	5
Trimethoprim/Sulfamethoxazole	3	15
Tetracyclines		
Oxytetracycline	8	40
Tetracycline	4	20
Trimethoprim		
Trimethoprim	8	40

Table 7. The minimal inhibitory concentration (MIC) for the isolates as determined by the ARA panel used at NOAA. The "R" or "S" next to the MIC denotes whether the isolate is resistant (R) or susceptible (S) to each antibiotic. Isolate numbers GSTP12, 27853, and 29212 (*Esceherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, respectively) were used as positive controls, and isolates 29213 and 25922 (*Staphylococcus aureus* and *Escherichia coli*, respectively) were used as negative controls. GSTP12/*Escherichia coli* is a positive control used specifically by NOAA, and was isolated from the Greenwood Sewage Treatment Plant. The antibiotic dilution units are in *u*g/mL. All panels were considered "NOAA Custom Panel".

Test Date	Isolate	Organism	Amikacin	Ampicillin	Amoxicillin	Apramycin	Azithromycin	Chloramphenicol	Ceftriaxone	Cephalexin	Cephalothin	Cefoxitin
12/14/2005	CC0348	*Escherichia coli*	<=8 (S)	16 (S)	32 (R)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	64 (R)	32 (R)	>32 (R)
12/14/2005	SCA #3	*Escherichia coli*	<=8 (S)	32 (R)	>32 (R)	<=8 (S)	4 (S)	<=8 (S)	64 (R)	64 (R)	>32 (R)	<=1 (S)
12/14/2005	SCA #8	*Citrobacter braakii*	<=8 (S)	<=4 (S)	8 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	32 (R)	32 (R)	16 (S)
12/14/2005	CC0382	*Pseudomonas spp.*	<=8 (S)	32 (R)	16 (S)	<=8 (S)	>8 (R)	16 (S)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #7	*Pseudomonas stutzeri*	<=8 (S)	>32 (R)	32 (R)	<=8 (S)	4 (S)	32 (R)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	CC0384	*Shewanella algae/putrefaciens*	<=8 (S)	>32 (R)	>32 (R)	16 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	<=8 (S)
12/14/2005	SCA #5	*Escherichia coli*	<=8 (S)	<=4 (S)	8 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	32 (R)
12/14/2005	SCA #7	*Stenotrophomonas maltophilia*	64 (R)	>32 (R)	>32 (R)	>32 (R)	8 (R)	<=8 (S)	>64 (R)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #6	*Escherichia coli*	<=8 (S)	16 (S)	32 (R)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	128 (R)	32 (R)	>32 (R)
12/14/2005	CC0380	*Salmonella spp.*	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/14/2005	CC0380	*Pseudomonas aeruginosa*	<=8 (S)	>32 (R)	>32 (R)	16 (S)	>8 (R)	>32 (R)	64 (R)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #3	*Pseudomonas aeruginosa*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	>32 (R)	16 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #4	*Pseudomonas spp.*	<=8 (S)	16 (S)	8 (S)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #2	*Escherichia coli*	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/14/2005	SCA #7	*Escherichia coli*	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	<=16 (S)	32 (R)	16 (S)
12/14/2005	SCA #2	*Citrobacter freundii*	<=8 (S)	16 (S)	16 (S)	<=8 (S)	4 (S)	>32 (R)	<=8 (S)	32 (R)	<=16 (S)	16 (S)
12/14/2005	CC0382	*Morganella morganii*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	<=8 (S)
12/14/2005	CC0356	*Proteus vulgaris*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	<=8 (S)
12/14/2005	CC0360	*Escherichia coli*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	8 (R)	<=8 (S)	<=8 (S)	>128 (R)	64 (R)	>32 (R)
12/15/2005	CC0378	*Escherichia coli*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	32 (R)	64 (R)	<=8 (S)
12/15/2005	29213	CONTROL-*S. aureus*	<=8 (S)	<=4 (S)	<=4 (S)	16 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/15/2005	GSTP12	CONTROL-GSTP-12/*E. coli*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	>32 (R)	<=8 (S)	<=16 (S)	64 (R)	<=8 (S)
12/15/2005	27853	CONTROL-*P. aeruginosa*	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	>32 (R)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/15/2005	25922	CONTROL-*E. coli*	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/15/2005	29212	CONTROL-*E. faecalis*	64 (R)	<=4 (S)	<=4 (S)	>32 (R)	4 (S)	<=8 (S)	>64 (R)	128 (R)	32 (R)	>32 (R)

Table 7 cont'd

Test Date	Isolate	Organism	Ciprofloxacin	Erythromycin	Nitrofurantoin	Gentamicin	Imipenem	Meropenem	Moxifloxacin	Nalidixic Acid	Ofloxacin
12/14/2005	CC0348	*Escherichia coli*	<=1 (S)	64 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #3	*Escherichia coli*	64 (R)	32 (R)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)	<=4 (S)
12/14/2005	SCA #8	*Citrobacter braakii*	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0382	*Pseudomonas spp.*	<=1 (S)	64 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	0.5 (S)	8 (S)	<=1 (S)
12/14/2005	SCA #7	*Pseudomonas stutzeri*	<=1 (S)	64 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	1 (S)	16 (S)	<=1 (S)
12/14/2005	CC0384	*Shewanella algae/putrefaciens*	<=1 (S)	128 (R)	64 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #5	*Escherichia coli*	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #7	*Stenotrophomonas maltophilia*	<=1 (S)	64 (R)	>128 (R)	>16 (R)	>16 (R)	>16 (R)	<=0.25 (S)	16 (S)	<=1 (S)
12/14/2005	SCA #6	*Escherichia coli*	<=1 (S)	64 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	8 (S)	<=1 (S)
12/14/2005	CC0380	*Salmonella spp.*	<=1 (S)	64 (R)	32 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0380	*Pseudomonas aeruginosa*	<=1 (S)	128 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	1 (S)	>32 (R)	<=1 (S)
12/14/2005	SCA #3	*Pseudomonas aeruginosa*	<=1 (S)	128 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	1 (S)	>32 (R)	<=1 (S)
12/14/2005	SCA #4	*Pseudomonas spp.*	<=1 (S)	32 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	0.5 (S)	8 (S)	<=1 (S)
12/14/2005	SCA #2	*Escherichia coli*	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #7	*Escherichia coli*	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #2	*Citrobacter freundii*	2 (S)	64 (R)	128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	4 (S)	>32 (R)	4 (S)
12/14/2005	CC0382	*Morganella morganii*	<=1 (S)	128 (R)	64 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0356	*Proteus vulgaris*	<=1 (S)	128 (R)	128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0360	*Escherichia coli*	<=1 (S)	64 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/15/2005	CC0378	*Escherichia coli*	<=1 (S)	>128 (R)	128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/15/2005	29213	CONTROL-*S. aureus*	<=1 (S)	<=16 (S)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	32 (R)	<=1 (S)
12/15/2005	GSTP12	CONTROL-GSTP-12/*E. coli*	>4 (R)	>128 (R)	<=16 (S)	8 (S)	<=2 (S)	<=2 (S)	>4 (S)	>32 (R)	>8 (R)
12/15/2005	27853	CONTROL-*P. aeruginosa*	<=1 (S)	128 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	2 (S)	>32 (R)	2 (S)
12/15/2005	25922	CONTROL-*E. coli*	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/15/2005	29212	CONTROL-*E. faecalis*	<=1 (S)	<=16 (S)	<=16 (S)	4 (S)	<=2 (S)	8 (S)	<=0.25 (S)	>32 (R)	<=1 (S)

Table 7 cont'd

Test Date	Isolate	Organism	Oxytetracycline	Penicillin	Streptomycin	Sulfathiazole	Trimethoprim	Trimethoprim/ Sulfamethoxazole	Tetracycline
12/14/2005	CC0348	*Escherichia coli*	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #3	*Escherichia coli*	>128 (R)	<=16 (S)	<=250 (R)	<=2 (S)	<=4 (S)	<=2/38 (S)	
12/14/2005	SCA #8	*Citrobacter braakii*	<=4 (S)	128 (R)	64 (R)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0382	*Pseudomonas spp.*	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #7	*Pseudomonas stutzeri*	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0384	*Shewanella algae/putrefaciens*	16 (R)	>128 (R)	<=16 (S)	<=250 (S)	16 (R)	<=2/38 (S)	8 (S)
12/14/2005	SCA #5	*Escherichia coli*	<=4 (S)	64 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #7	*Stenotrophomonas maltophilia*	16 (R)	>128 (R)	64 (R)	<=250 (S)	16 (R)	<=2/38 (S)	8 (S)
12/14/2005	SCA #6	*Escherichia coli*	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0380	*Salmonella spp.*	<=4 (S)	<=16 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0380	*Pseudomonas aeruginosa*	16 (R)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	>4/76 (R)	>32 (R)
12/14/2005	SCA #3	*Pseudomonas aeruginosa*	32 (R)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	>4/76 (R)	>32 (R)
12/14/2005	SCA #4	*Pseudomonas spp.*	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	16 (R)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #2	*Escherichia coli*	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #7	*Escherichia coli*	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #2	*Citrobacter freundii*	>32 (R)	64 (R)	<=16 (S)	>500 (R)	>16 (R)	>4/76 (R)	>32 (R)
12/14/2005	CC0382	*Morganella morganii*	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0356	*Proteus vulgaris*	>32 (R)	>128 (R)	<=16 (S)	<=250 (S)	8 (S)	<=2/38 (S)	16 (R)
12/14/2005	CC0360	*Escherichia coli*	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/15/2005	CC0378	*Escherichia coli*	16 (R)	64 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	8 (S)
12/15/2005	29213	CONTROL-*S. aureus*	<=4 (S)	<=16 (S)	<=16 (S)	500 (R)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/15/2005	GSTP12	CONTROL-GSTP-12/*E. coli*	>32 (R)	>128 (R)	128 (R)	>500 (R)	>16 (R)	>4/76 (R)	>32 (R)
12/15/2005	27853	CONTROL-*P. aeruginosa*	16 (R)	>128 (R)	<=16 (S)	>500 (R)	>16 (R)	>4/76 (R)	32 (R)
12/15/2005	25922	CONTROL-*E. coli*	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/15/2005	29212	CONTROL-*E. faecalis*	16 (R)	<=16 (S)	32 (S)	>500 (R)	<=2 (S)	<=2/38 (S)	16 (R)

Figure 1. Dade Behring MicroScan Turbidity Meter (Dade Behring, California).

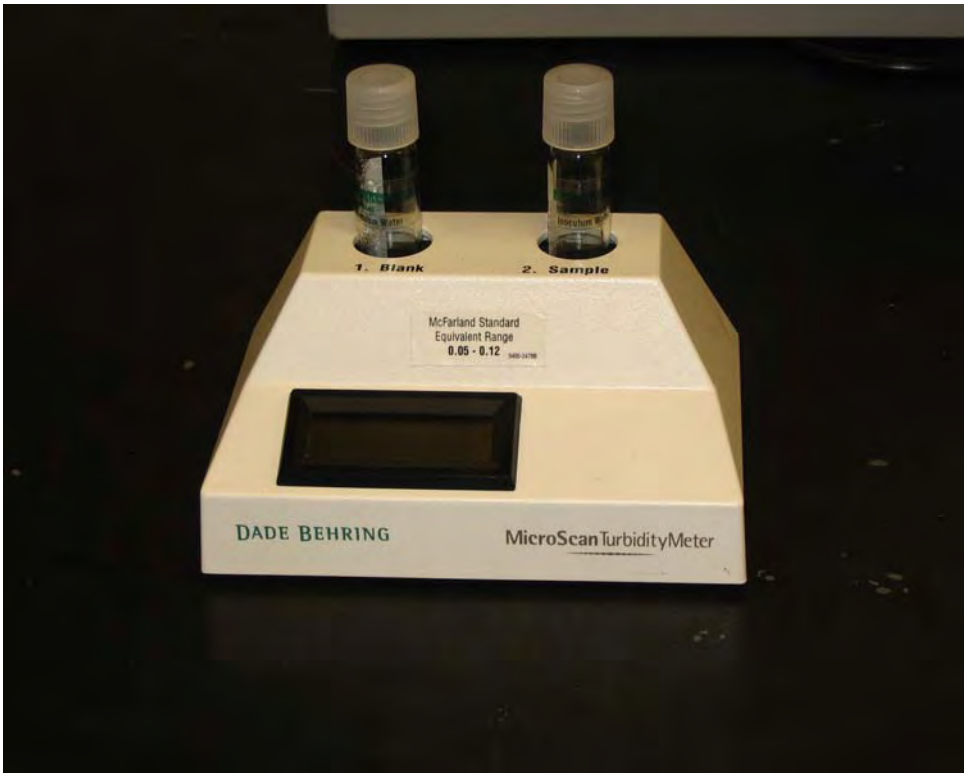


Figure 2. Dade Behring Inoculator D Set with the lid on (Dade Behring, California).

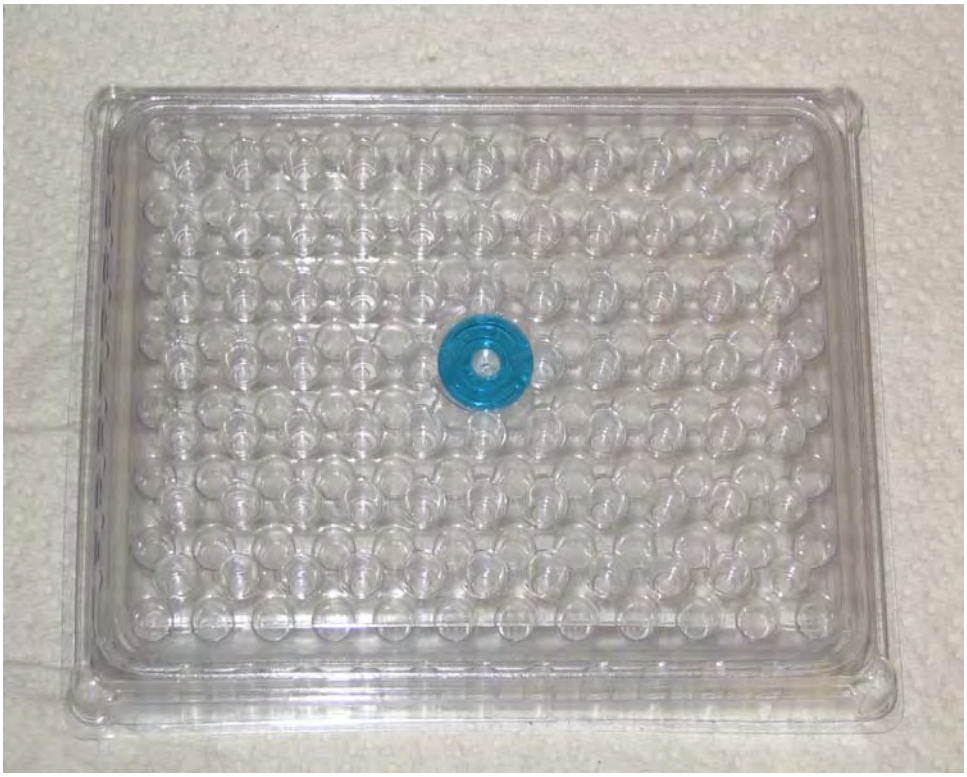


Figure 3. Both halves of the Dade Behring Inoculator D Set (Dade Behring, California).

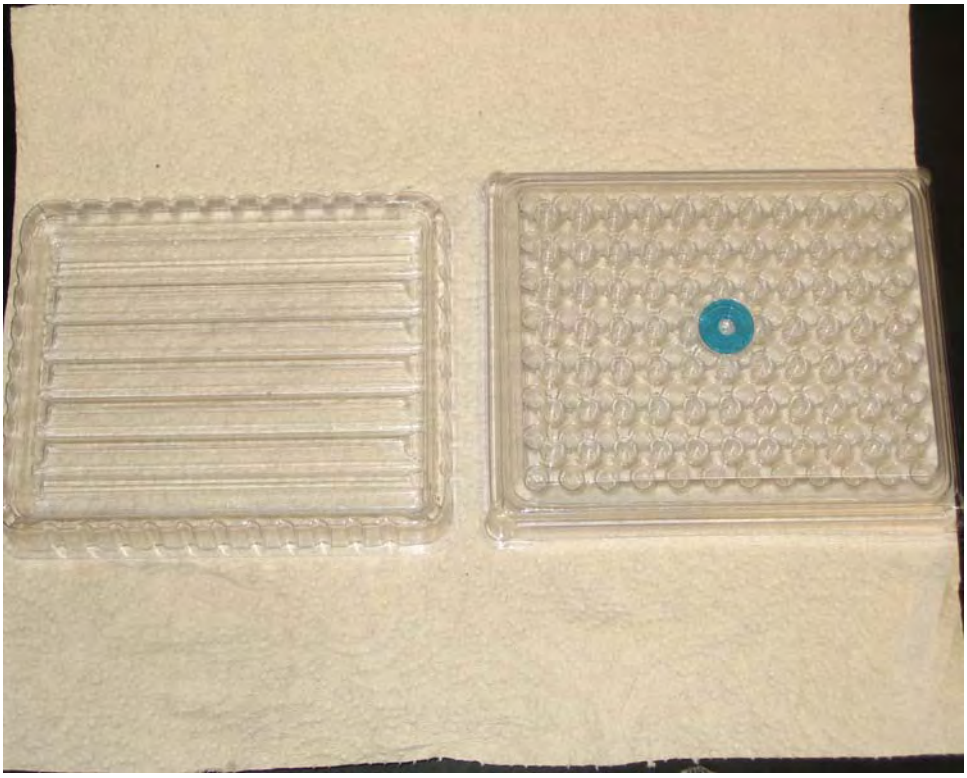


Figure 4. Dade Behring MicroScan Renok Pipette (Dade Behring, California).

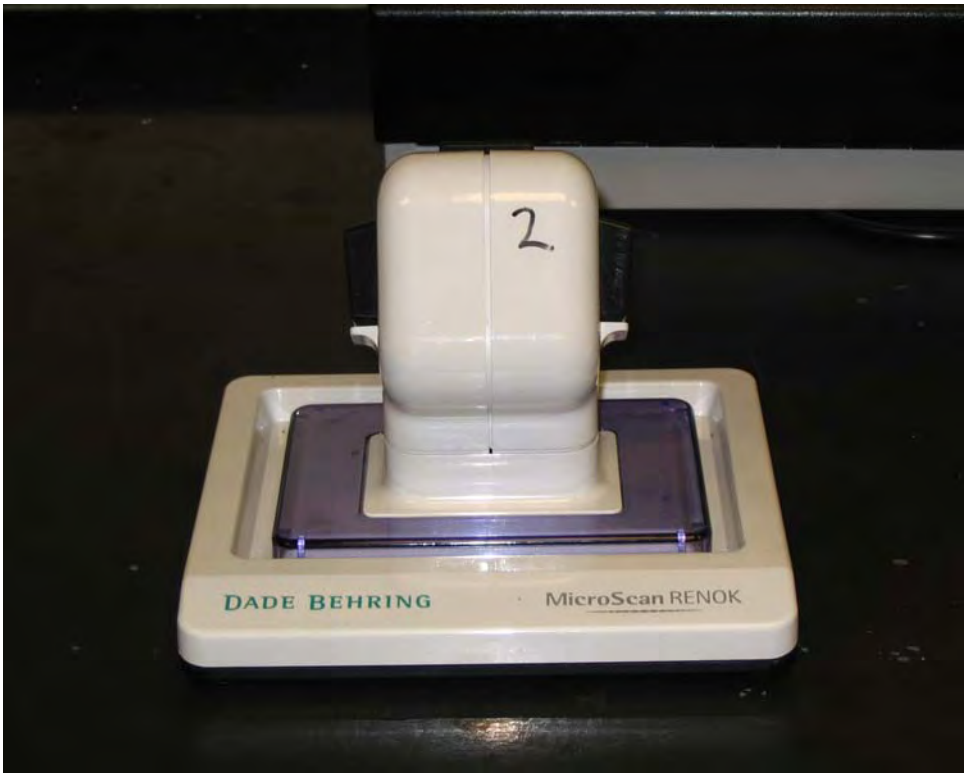
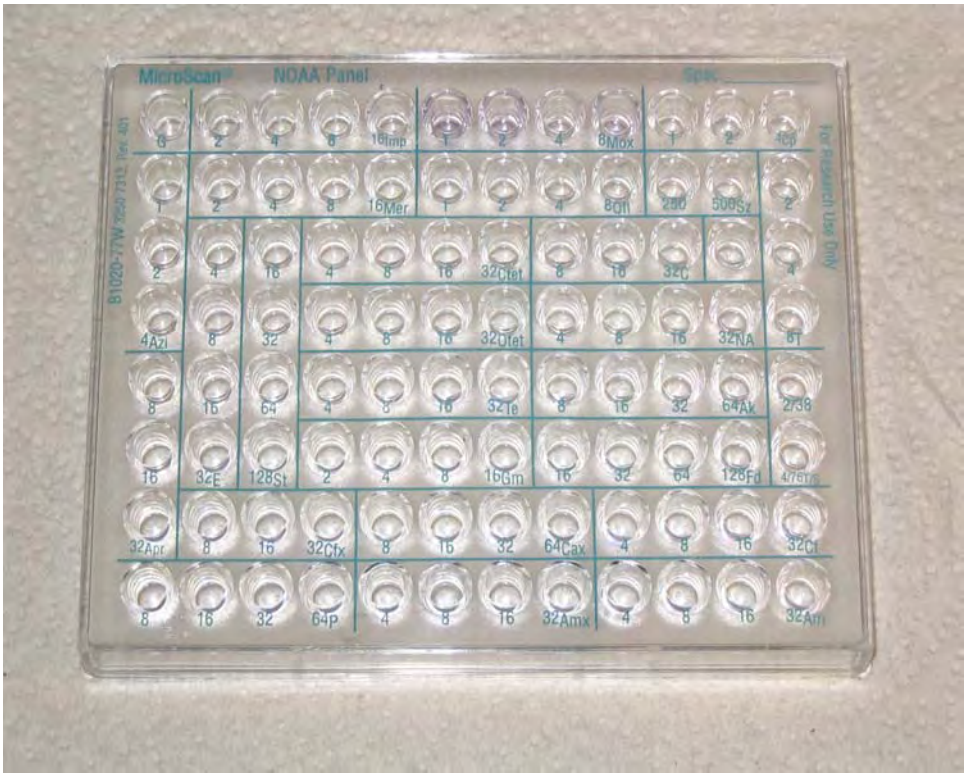


Figure 5. The 96-well suspension plate, containing a preset panel of desiccated antibiotics, designed by Dade Behring specifically for the National Oceanic and Atmospheric Administration (NOAA) (Dade Behring, California).



Appendix II
(List of Acronyms)

Acronym	Definition
ARA	Antibiotic Resistance Analysis
CAMHB	Cation-Adjusted Mueller-Hinton Broth
CVDC	Clemson Veterinary Diagnostic Center
DTS	Debilitated Turtle Syndrome
MIC	Minimal Inhibitory Concentration
NOAA	National Oceanic and Atmospheric Administration
OD	Optical Density
PCV	Packed Cell Volume
SCDNR	South Carolina Department of Natural Resources
TP	Total Protein
TSA	Tryptic Soy Agar
TSB	Bacto Tryptic Soy Broth

Appendix III
(Hours Spent at the South Carolina Aquarium Sea Turtle Rescue Program)

A total of 487.5 hours were accumulated while working with the South Carolina Aquarium Sea Turtle Rescue Program. In addition to the tasks that were mentioned in the body of the report, I also performed several other duties, which I will now list.

- Participated in the "Head-Start" Program- The South Carolina Aquarium is permitted to receive a specified number of hatchlings each year to raise in the "Head-Start Program." The hatchlings are maintained at the South Carolina Aquarium for approximately four years, before they are released into the open ocean. This program benefits sea turtle populations by releasing the turtles when they are large enough in size to preclude their being prey to many marine species, and thus theoretically increase their survivability. I assisted in this program by feeding and maintaining some of the juveniles currently involved in this program.
- Transferred sea turtles from the interior of the South Carolina Aquarium to the outside environment in large plastic buckets. This allowed the sea turtles to receive some exposure to sunlight to assist them in re-calcifying their weakened carapaces.
- Force-fed sea turtles that were uninterested in eating-When sea turtles were uninterested in eating, or too weak to eat, we had to devise an alternative method for getting them to ingest oral medications. First, we pulverized the medications into a powder and mixed them with some mineral oil (to assist in absorption) and Boost (Novartis Nutrition Corporation, 2005). We then placed the turtle on an incline to reduce regurgitation. Next, we pried its mouth open and placed a small piece of a PVC pipe in its mouth to keep it opened. We then fed a lubricated tube

down its esophagus and into its stomach. Once the tube was in place, we used a syringe to shoot approximately 40-50 cc's of the mixture through the tube and into the turtle's stomach. In most cases, the turtle regurgitated approximately half of this mixture. Generally, the turtles that were sick enough to be force fed were too far gone, and did not survive.

- ❖ Attended some of the necropsies performed on the turtles that had died while undergoing treatment at the South Carolina Aquarium, which were used to determine the possible causes of the turtles' demise.
- ❖ Under proper supervision, extracted blood samples from the dorsal cervical sinus of the sea turtles and analyzed it to monitor the turtles' packed cell volume, total protein, and glucose levels. We used this data to ascertain how each turtle was responding to their respective treatments, and to approximate when each turtle would be ready for release.
- ❖ Administered medications intramuscularly.

The following chart lists all of the sea turtles that were admitted to the South Carolina Aquarium Sea Turtle Rescue Program:

Sea Turtles Admitted to the South Carolina Aquarium Sea Turtle Rescue Program

Number	Name	Date Admitted	Location Found	Diagnosis	Outcome
SCA # 1	"Jetty"	5-19-05	Charleston, SC	DTS	Released 8-19-05 on
					Seabrook Island
SCA # 2	"Gardner"	6-2-05	Garden City, SC	DTS	Released 8-19-05 on
					Seabrook Island
	"Myrtle"	6-9-05	North Myrtle Beach, SC	DTS	Not involved in study; died during treatment
					at SCA (6-16-05)
SCA # 3	"Hunter"	6-10-05	Huntington Beach State Park, SC	DTS	Died during treatment at
					SCA (7-11-05)
SCA # 4	"Surfside"	6-24-05	Surfside Beach	DTS	Released 8-30-05 on the
					Isle of Palms
SCA # 5	"Sullivan"	6-30-05	Sullivan's Island, SC	DTS	Died during treatment at
					SCA (7-6-05)
SCA # 6	"Horry"	7-7-05	Myrtle Beach State	DTS	Died during treatment at
			Park, SC		SCA (7-10-05)
SCA # 7	"Little Cumberland"	7-20-05	Little Cumberland	DTS	Died during treatment at
			Island, GA		SCA (7-26-05)
SCA # 8	"Dewees"	8-6-05	Dewees Island, SC	DTS	Released 11-17-05 on
					Dewees Island
	"St. Simons"	8-12-05	St. Simon's Island, SC	DTS	Set to be released this
					summer; not involved in
					study because received
					antibiotics 3 days before a
					sample could be obtained

In addition to the above mentioned sea turtles, I also assisted in caring for seven hatchlings and juveniles, all of which were involved in the “Head Start” Program.

Appendix IV
(Hours Spent at the National Oceanic and Atmospheric Administration-NOAA)

A total of 59.5 hours were accumulated while performing the laboratory work necessary to complete this project at NOAA. In addition to storing my bacterial samples and preparing my samples for ARA and to determine the MIC of each isolate, I also assisted in making media and cleaning and sterilizing laboratory equipment.

Individuals with whom I interacted to complete this project include: Dr. Dave Owens, Dr. Tom Sheridan, Dr. Susan Morrison, Dr. Kem Fronabarger, Dr. Al Segars, Mike Arendt, Brian Thompson, Kelly Thorvalson, Dr. Jan Gooch, Dr. Pamela Parnell, Dr. Craig Harms, and Dr. Terry Norton.