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Small World Initiative
(Microbiology)

Small World Initiative
crowdsourcing antibiotic discovery

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The Gaston Gazette

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Metagenomic Analysis of Soil Bacteria from Rhodes Clay Hole in Lincoln County

Chandra Carswell, Patricia Jones, Ashley Hagler M.A.T., M.S.
Arts and Science Division
Gaston College

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Abstract

The main purpose of this research was to conduct a survey of bacterial species in local soil samples and to examine a claim that a local clay deposit was antimicrobial. Microbial biodiversity in local soil samples was examined using 16S rRNA sequencing. 16S rRNA sequences are relatively short sequences found as part of the bacterial ribosome that exhibit both interspecies diversity and intraspecies similarity. This method was selected based on its ability to detect organisms that are unculturable under normal lab conditions.

Six of the examined fifteen samples from the local clay deposit were successful for 16S amplification using the 27F and 1492R primer set. Samples were sequenced using Sanger sequencing methods at the DNA Analysis Facility at Yale University. After analyzing resulting sequences using BLAST, MEGA5.0 and ClustalW, it was found that the samples matched to various uncultured environmental bacteria. In addition, bacteria was definitively shown to be present in the "antimicrobial" soil.

Hypothesis

Since soil is known to be a preferred environment for many bacterial species, it is expected that various species of bacteria will be found in all soil samples, including clay samples from an undisclosed location, which is touted to be antimicrobial. It is also expected that many of the species of bacteria isolated will be "uncultured".

Methods

Soil samples were collected by the owner of the property and numbered before being brought into the lab to prevent bias by researchers. Researchers examined 15 soil samples from various locations on the owner's property in addition to other soil samples from locations in Lincoln and Gaston Counties.

DNA was isolated using the MoBio PowerSoil Isolation Kit (MoBio) following the included instructions. The 16S region for each sample was amplified using the 27F and 1492 primers (see below for sequences) using the following conditions (95°C Denaturing, 46°C Annealing, 72°C Extension; 35 cycles). Primers were ordered from Eurofins MWG and used for the first time on this project at a 100uM concentration. Resulting samples were analyzed using gel electrophoresis (IXTAE, 45min@120V), stained using SybrSafe DNA Gel Stain (Life Technologies). Samples showing a positive amplification result were gel purified and used in TOPO-TA cloning (Life Technologies) in order to isolate individual sequences.

Sanger sequencing was conducted on the isolated sequences using the universal M13 sequencing primer at the DNA Analysis Facility at Yale University. Resulting sequences were analyzed using MEGA5.0, BLAST, and ClustalW.

5' —> 3' Sequence	Primer Name	Position	*Tm* (°C) (50 mM KCl)
GGT TAC CTT GTT ACG ACT T	16S_1492R	1492-1510	49
AGA GTT TGA TCM TGG CTC AG	16S_27F	8-27	56

Acknowledgements

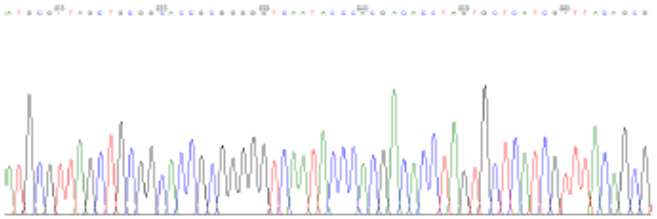
I would like to thank Patricia Jones who contributed to this work as well as the landowner who provided the samples of the soil from the undisclosed site in Lincolnton, NC. I would also like to thank CCURI for providing materials and Gaston College for allowing me the opportunity to participate in this experience.

Results

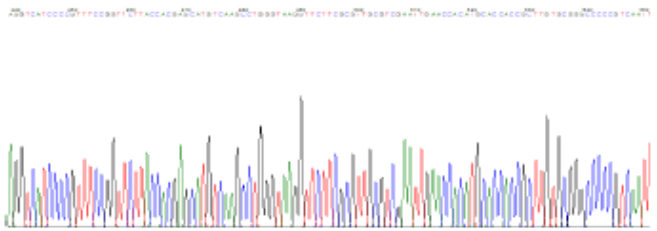
Sample Number	Final DNA concentration ng/µl	A 260/280
1	30.5	1.77
2	39.1	1.79
3	56.3	1.82
4	61.1	1.79
5	33.8	1.77
6	22.7	1.72

DNA Concentration and A260/280 ratio results from NanoDrop Lite.

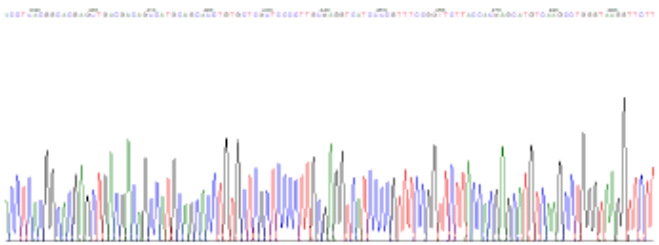
The 260/280 ratios indicate DNA purity, pure DNA should be between 1.7 and 1.8. Lower than 1.7 shows protein contamination.



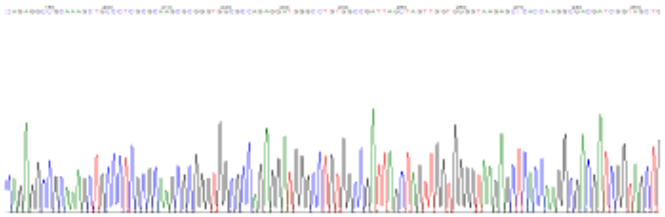
Trimmed Chromatogram for Forward Sequence Sample 2 (MEGA5.0)



Trimmed Chromatogram for Forward Sequence Sample 4 (MEGA5.0)



Trimmed Chromatogram for Forward Sequence Sample 5 (MEGA5.0)

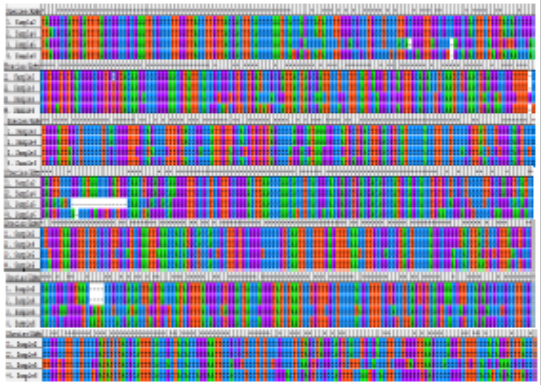


Trimmed Chromatogram for Forward Sequence Sample 6 (MEGA5.0)

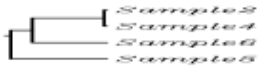
Identification and Phylogeny

Sample #	Sequence #	Primer	Match Identity	E Value	Identities Value
2	3	M13F	Uncultured bacterium clone BacB_057 16S ribosomal RNA gene, partial sequence	0	95%
	4	M13R	Uncultured bacterium clone BacB_057 16S ribosomal RNA gene, partial sequence	0	99%
3	5	M13F	Cloning vector PJAC96, complete sequence	0	99%
	6	M13R	Expression vector pPV472, complete sequence	0	99%
4	7	M13F	Uncultured Nitrospira sp. clone E361 16S ribosomal RNA gene, partial sequence	0	99%
	8	M13R	Uncultured Nitrospira sp. clone E361 16S ribosomal RNA gene, partial sequence	0	99%
5	9	M13F	Uncultured Nitrospira sp. clone E361 16S ribosomal RNA gene, partial sequence	0	99%
	10	M13R	Uncultured Nitrospira sp. clone E361 16S ribosomal RNA gene, partial sequence	0	99%
6	11	M13F	Uncultured bacterium clone 85-20 16S ribosomal RNA gene, partial sequence	0	95%
	12	M13R	Uncultured bacterium clone TIBW1008 16S ribosomal RNA gene, partial sequence	0	92%

Sample 1 did not provide any usable sequence data. Sample 3 is indicative of the sequencing of a chimera.



Alignment of Samples 2,4,5,and 6 using MEGA 5.0, positions 1 - 825. * exact nucleotide match.



Phylogenetic tree constructed using ClustalW. Samples 2 and 4 are most closely related.

Discussion

Six of the examined fifteen samples from the local clay deposit were successful for 16S amplification using the 27F and 1492R primer set. Four valid sequences were obtained after Sanger Sequencing and elimination of chimera data. After analyzing the resulting sequences using BLAST, MEGA5.0 and ClustalW, it was found that the samples matched to various uncultured environmental bacteria. The analyzed 16S sequences showed high levels of conservation, which was expected and samples 2 and 4 appear to be most closely related. This supports the claim that the biodiversity of soil contains bacteria that are not culturable.

In addition, our data shows that bacteria is present in the soil that the landowner is currently claiming to be anti-microbial.

ANALYSIS OF SOIL ISOLATES FROM ANTIBIOTIC PRODUCING BACTERIA

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Arts and Science Division , Gaston College

SPARC³ · Gaston College Opportunities for Life · Small World Initiative · CCURI · NSF

ABSTRACT

The **Small World Initiative (SWI)** incorporates the search for antibiotic producing microbes from soil in the undergraduate biology curriculum. Gaston College has integrated the SWI in Introductory Microbiology (BIO 275). The rising threat of antibiotic resistant bacteria is the rationale behind the SWI. ESKAPE pathogens are responsible for a large percentage of nosocomial infections and represent the majority of antibiotic resistant isolates. Secondary metabolites from the genera *Pseudomonas* and *Bacillus* have more than antibiotic activity. Sporulation, quorum sensing and biofilm formation may also be affected by these secondary metabolites (Chen, et al.). Gramicidin and pumulin are examples of secondary metabolites yielding antibiotic activity (Abdulkadir). Soil samples from various locations around Gaston County were plated. Techniques used included spread/patch against safe relative and a combination of biochemical and genetic techniques. The media used was LB agar with cycloheximide to decrease fungal growth.

BACKGROUND

ESKAPE Pathogens	ESKAPE Safe Relative	Gram Staining
Staphylococcus aureus	*Staphylococcus cohnii*	Gram positive Cocci
Enterobacter species	*Enterobacter aerogenes*	Gram negative Rods
Klebsiella pneumoniae	*Escherichia coli*	Gram negative Rods
Pseudomonas aeruginosa	*Pseudomonas putida*	Gram negative Rods
Enterococcus faecium	*Enterococcus raffinosus*	Gram positive Cocci (chain)
	Bacillus subtilis	Gram positive Rods

Antibiotics are molecules that are produced by bacteria or fungi or synthesized in the laboratory. Antibiotics have the ability to kill or inhibit other microbes. The modern discovery and subsequent development of antibiotics into pharmaceutical products from the 1930's until present have saved millions of lives worldwide. In the late 1930s, the ability of bacteria to overcome the effects of the antibiotic was observed (Aminov; Davies and Davies). Antibiotic resistance may arise via random mutations or the exposure to temperature or pH changes. The main cause of resistance development is due to medical personnel overprescribing antibiotics with failure to provide adequate patient instructions. The lack of patient compliance with antibiotics has also contributed to the public health crisis. (Lee, et al.; WHO). The meat industry also plays a large role in the development of resistance. Antibiotics, including those used for human disease, have been routinely administered by farmers to food animals at low doses for long periods of time as growth promoting agents and preservatives (WHO). Antibiotic resistance in pathogens has become a serious threat to global public health, with some bacteria acquiring multiple resistance mechanisms, leaving few or even no antibiotics that can effectively cure the infection. There are now estimated to be more than 1600 known different types of resistance genes (McArthur, et. al.). Concurrent with the rise of antibiotic resistance bacteria is the decline in the development of new antibiotics. Since the 1960s only 2 new major classes of antibiotics have made it to the market (Lee, et al.). The high cost of drug development has lead many pharmaceutical companies to focus more on profitable drugs classes (Krans).

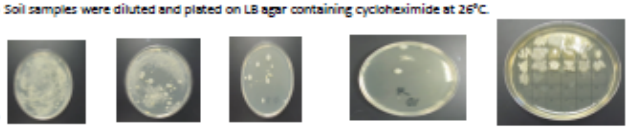
ACKNOWLEDGEMENTS

This project was supported by funding from the Gaston College Science Department. The SWI is supported by the Howard Hughes Medical Institute, the Leona and Harry B. Helmsley Charitable Trust, the Davis Educational Foundation, and the Yale Center for Scientific Teaching.

Yale University · Davis Marvin · HHMI Howard Hughes Medical Institute · The Leona M. and Harry B. Helmsley Charitable Trust

METHODS

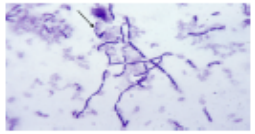
- Soil samples were diluted and plated on LB agar containing cycloheximide at 26°C.



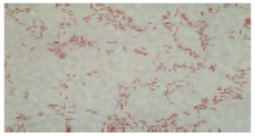
- Spread/patch technique was used to test for antibiotic activity against ESKAPE safe relatives.

- Isolates with antibiotic activity were further characterized by morphological and genetic (16S rRNA) tests. The 16S sequences were amplified using primers 27F and 149R and puRE Taq Ready-To-Go PCR beads. Sequencing was done by Yale University. Data evaluation by BLAST.

RESULTS



Bacillus (rod) with spores
Gram + (CK1 & Ck19)



Bacillus (rod) No spores
Gram—(CR2 & CR7)

Four isolates: CR2, CR17, Ck1 and Ck19 inhibited the growth of ESKAPE relatives.

Characteristic of the 4 isolates

	CR2	CR7	CK1	CK19
	PS	PS	BS	BS
Pigmentation	Yellow	Yellow	Opaque	Opaque
Cell Morphology	G- Rods	G- Rod	G+ Rod	G+ Rod
Antibiotic Producer	Yes	Yes	Yes	Yes
Motility	Yes	Yes	Yes	Yes
Amylase	Yes	Yes	Yes	Yes

The bacteria found in soil is influenced by geographical location such as temperature, soil type, soil pH, organic matter, cultivation, aeration and moisture content (Abdulkadir).

Data Analysis

DNA Blast Analysis for *Pseudomonas*

cgagctgacaactgccatgcagcacctgtctcaatgctcccgaagggccaatcccctctct
cgaaagtcattggatgtcaaggcctggtaggttcttctcgttgcttcaattgaaccaatg
ctccaccgcttgtgcgggcccccgtaaattcaattgagttttaaccttgtgggcgtactc
cccaagcggtcaacttcatgcgtgagctgcgccagtaagagctccaggctctccacgagc
tagatgacactcttttacagcgtggaactacccggggatctaatcctgtgtggttcccc
cactttcgcgtccactgtcagactcagtcccgggggtcgccttcctccctggtgttcctt
cctatatgtcgcagc

DNA Blast Analysis for *Bacillus*

Taatgcagtcgagcgatcgatgggagcttgctccctgagattagcggcggac-
gggttaacacgtgggcaacctgcctataagactggaaacttcgggaaaccggagctaataccggatacgttctt
ttctcgcatgagagaagatggaaagacggttttcgctgtcacttatagatgggcccgcggcgcattagctagttgg
tgaggtaatggctcacccaaggcgacaatgcgtagccaacctgagagggtgatcggccacactgggactgaaac
acggcccaaactcctacgggaggcagcagtagggaatcttccgcaatggacaaaagtctgacggagcaacgc
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cgttgtccggaattattgggcgtaaagcgcgcgcaggtggttcttttaagtctgatgtgaaagcccacggctcaacc
gtggagggtcattggaaactgggaacttgagtgcagaagaggaaagtggaattccaagtgtagcggtgaaat
gcgtagagatgggaggacccggttggtcaaagcggtttttcccgcccccgtaacgcg

DISCUSSION/FUTURE RESEARCH

The SWI provides a clear and precise method for screening and identification of antibiotic producing microbes. Two *Bacillus* soil isolates and two *Pseudomonas* soil isolates with antibiotic activity against ESKAPE safe relatives were characterized by 16S rRNA sequencing and various other traditional methods including Gram staining, KOH testing, colony morphology amylase production and motility. Genetic similarities made it difficult to classify species.

Future plans include incorporation of more chemical analysis and change from the PuReTaq™ Ready-to-Go™ PCR beads. Future students will also use an expanded variety of agars for plating isolates.

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THE EFFECTS OF UVB AND UVA RADIATION ON KERATINOCYTES AND FIBROBLAST

Madison Staves
Instructor: Anat Lev
Science Division
Gaston College

ABSTRACT

This experiment focused on UVA (320-400nm) and UVB (290-320nm) radiation on cultured skin cells. The cells were exposed to the UV radiations to ultimately test the effect of cell death, cytotoxicity, and viability. The cells were plated on flat bottom 24-well cell culture plates. The media used for plating the cells is DMEM based, supplemented with 10% FBS, 50U Penicillin, 50 mg/mL Streptomycin, and 45 pg/mL amphotericin. The purpose of this study is to see what wavelength results in the most cell damage. The cells were placed under the lamps at 30cm for a total of 15 minutes per day. This experiment was repeated over the course of six days. Within this experiment much data was gained to show a change in cell viability. With the use of PrestoBlue™ Cell Viability Reagent it was possible to read viability. Pictures taken with an inverted microscope shows the change of the cells formation and obvious cell death. This experiment shows valuable data that could result in finding what wavelength ultimately harms human skin most.

BACKGROUND

UVA and UVB radiation are found within most light sources including the sun, lamps and other sources of light. UVA is considered the longest wavelength between UVA and UVB. UVA is 320-400nm. The Skin Cancer Foundation states that, "UVA rays account for up to 95 percent of the UV radiation reaching the Earth's surface. Although they are less intense than UVB, UVA rays are 30 to 50 times more prevalent" (Epstein, and Wang par. 5).

The shorter Ultra-Violent wavelength is UVB which is measured from 290-320nm. UVB radiation is known to have more damaging effects on the upper level of the epidermis.

Over the past years research has been done on keratinocytes in the basil layer of the skin. Keratinocytes are a predominate cell type within the epidermis. Keratinocytes function as a barrier against damage that occur within the environment, such as UV radiation. Research that was constructed over the past decades has tested the UVA damage that occurs on the basal level.

What I researched was the effect on keratinocytes and fibroblast at a constant distance of 30cm over the span of five days. This study shows what the individual rays do at a close range to fibroblast and keratinocytes. This study is useful by showing how the rays affect these cells. By understanding how the rays manipulate the cells viability, cell count and cell health further research can be done to see how to rejuvenate damaged cells.

ACKNOWLEDGEMENTS

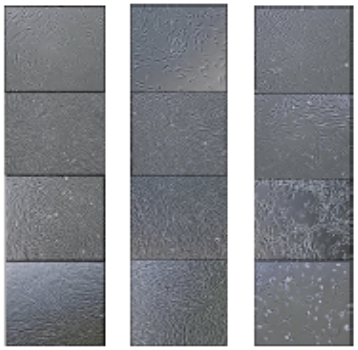
Active Concepts Laboratory Lincolnton, Gastonia.
Meghan Darley
Erica Segura
Doctor Anat Lev
Thank you for all the time and effort put into this experiment.

METHODS

On the first day, the cells were plated on flat bottom 24-well cell culture plates. The media used for plating the cells is DMEM based, supplemented with 10% FBS, 50U Penicillin, 50 mg/mL Streptomycin, and 45 pg/mL amphotericin. The two cell lines that will be used are, Normal Human Dermal Fibroblasts (NHDF) – primary human cells; purchased from Lonza, product #CC-2511 and HaCaT Keratinocytes – human cell line; purchased from AddexBio, product # T0020001.

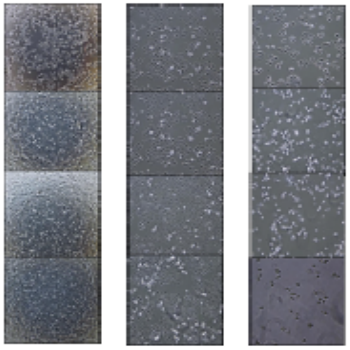
Each day the cells were tested at a distance of 30cm from the UV light source. The fibroblast were tested first then proceeding were the keratinocytes. After each plate had been exposed, pictures were taken then, a viability reading was constructed followed by a cell count.

RESULTS



CONTROL FIBROBLAST | UVA FIBROBLAST | UVB FIBROBLAST

The photo sequence shown is the fibroblast control, UVA exposed, and UVB exposed. These photos show the cells after each day of exposure. It is easily seen that cell count diminishes as the exposure time increases. The actual cell count data is found in data analysis.



CONTROL KERATINOCYTES | UVA KERATINOCYTES | UVB KERATINOCYTES

The photo sequence shown is the keratinocytes control, UVA exposed, and UVB exposed. These photos show the cells after each day of exposure. It is easily seen that cell count diminishes as the exposure time increases. The actual cell count

DATA ANALYSIS

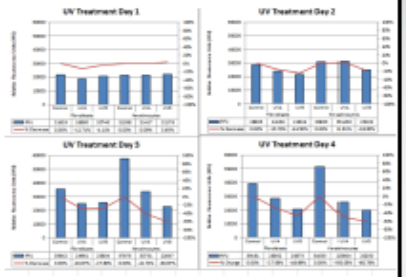
Experiment conducted in 6 well tissue culture plate
Plating Density – approximately 100,000 cell per well

Date	Cell Type	Treatment	Cytometer Cell Count	Cytometer Trypan Stained	Total Cell Count	Total Trypan Stained	Percent Dead Cells
1-Tuesday	NHDF	Control	300	0	3000000	0	100.00%
		UVA	204	0	2040000	0	100.00%
		UVB	124.5	0	1245000	0	100.00%
	HaCaT	Control	112.5	0	2250000	0	100.00%
		UVA	186.5	4	3730000	8000	99.78%
		UVB	84	9.5	1680000	19000	98.87%
2-Wednesday	NHDF	Control	90.5	0.5	1810000	10000	99.45%
		UVA	195.5	9	3910000	180000	95.40%
		UVB	84.5	3.5	1690000	70000	97.93%
	HaCaT	Control	118.5	2	2370000	40000	98.58%
		UVA	164.5	13	3290000	260000	92.59%
		UVB	25	58	500000	1160000	69.90%
3-Thursday	NHDF	Control	195.5	8.5	3910000	170000	97.14%
		UVA	190	2.5	3800000	50000	98.68%
		UVB	82	10	1640000	200000	91.71%
	HaCaT	Control	414	6.5	8280000	130000	98.43%
		UVA	22.5	13.5	450000	270000	95.21%
		UVB	25	22	250000	220000	89.89%
4-Friday	NHDF	Control	225	3.5	4500000	70000	98.44%
		UVA	320	5	3200000	50000	98.44%
		UVB	81	8.5	1620000	170000	89.51%
	HaCaT	Control	394	38.5	7880000	770000	90.26%
		UVA	336	98.5	6720000	1970000	66.42%
		UVB	21.5	25	110000	420000	20.64%

This data is the supporting evidence of the cell count. NHDF cell type is fibroblasts and HaCaT is keratinocytes. The data shows that by the end of the experiment the HaCaT had the lowest cell count resulting in the most cell death. The UVB is seen to cause the most cell death in both NHDF and HaCaT cells.

This data analysis shows the viability results as the experiment proceeded. Viability is the ability of the cell to maintain or recover itself. On the "Y" axis, the table displays relative fluorescence units (RFU). This is a measurement used in an electrophoresis method for the cell analysis. The RFU is a unit of measurement gathered from fluorescence detection.

It is seen as the treatment days pass the UVB for keratinocytes declines. The controls within this data shows great potential to rejuvenate cell health with no exposure. Overall this data shows the decline in cell rejuvenation from being treated



DISCUSSION/CONCLUSION/FUTURE RESEARCH PLANS

Concluding that within this experiment it is seen that UVB treatment had the most effect on both keratinocytes and fibroblast. The reason I hypothesize this is because the UVB ray being shorter penetrates the cells more intensely which results in a more rapid cell death. From this I can draw the conclusion that UVB causes the most cellular damage within the cells.

Preceding this experiment I plan on researching ways to rejuvenate damaged cells from UV radiation that would also not cause abnormal cell growth.

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THE EFFECTS OF TEMPERATURE CHANGE ON THE MANGROVE JELLYFISH

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CCURI

NSF

ABSTRACT

Jellyfish are not really fish at all, they are free floating , see through, gelatinous plankton. Their bodies are 90% water, to put that in prospective humans are about 70% water. The Population of jellyfish have exploded over the past 10 years. Scientist believe this is due to global warming.

Figure 1: Mangrove Jellyfish

BACKGROUND

The *Cassiopeaxamachana*is the type of jellyfish we will be observing for this study. It is known by such names as the mangrove jellyfish, *c.xamachana* the upside down jellyfish. It is often called the upside down jellyfish because it spends much of its time upside down on the seafloor, appearing to be a flower. They can swim, but spend most of their time on the bottom of the sea (Fleck & Fitt, 1999). They spend most of their lives in the medusa stage. The turtle is there natural enemy. The taxonomy of the mangrove jellyfish Phylum: Cnidaria Class: Scyphoza Order: Rhizostomae Family:Cassiopeidae.

Jellyfish best survive in nutrient rich, oxygen poor water. Most jellyfish love warm waters, but there are species that can live in cooler waters (Fitt & Castley 1998).
The increasing population is believed to be because of global warming. Jellyfish tend to flourish when there is something is out of balance in nature, if it is from global warming, to overfishing, or even from toxic chemicals and trash being introduced into the ocean (Muller &Leitz 2002).

METHODS

Two tanks will be set up using the exact same water, tank, lighting, and heaters. Each tank will be fitted with ten hand crafted cages so to keep each jellyfish separate. Tank A will be the control tank and kept at a constant temperature of 76 degrees. Tank B will be the test tank and the temperature of that tank will be lowered each week by 5 degrees to see if any change can be measured.

Figure 2: Mesh separation cages to allow for individual sampling.

RESULTS

Over an eight week period of time the size of the jellyfish decreased in both tanks due to them being in captivity. The control tank A, the jellyfish were much smaller than the tank that the temperature was reduced.

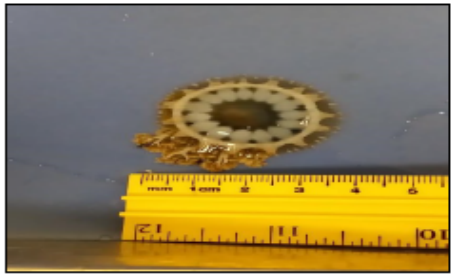

Figure 3: Diameter of Mangrove Jellyfish measured in centimeters.

DATA

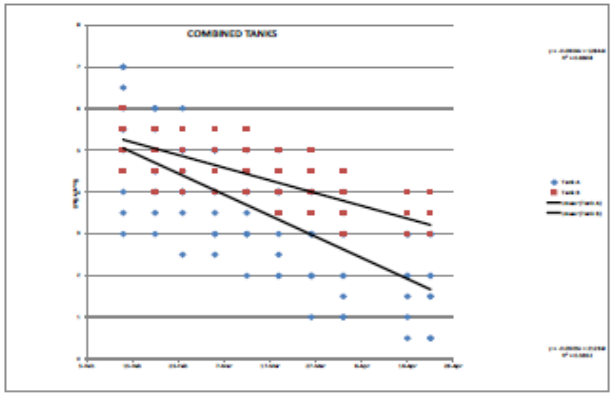

Figure 4: Decline in diameter of Mangrove Jellyfish over 2-month period.
Control Tank A compared to Experimental Tank B.

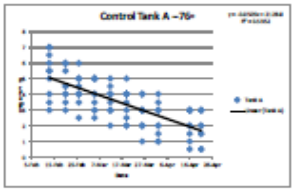

Figure 5: Decline in diameter of Mangrove Jellyfish over 2 month period.
Control Tank A only

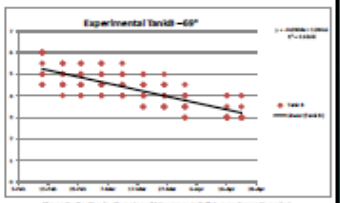

Figure 6: Decline in diameter of Mangrove Jellyfish over 2 month period.
Experimental Tank B only

DISCUSSION

In accordance with the research on the mangrove jellyfish they are happiest in warm environments. They bread, feed and flourish in warmer temperatures. My studies showed just the opposite. The jellyfish in the cooler tank were larger and healthier than the jellyfish in the warmer tank.

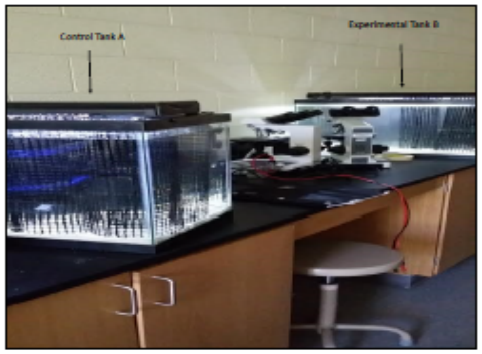

Figure 7: Control Tank A and Experimental Tank B in Lab Environment.

ACKNOWLEDGEMENTS

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Joe Issa, Lab Manager Gaston College

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ANTIBIOTIC RESISTANCE IN WASTEWATER EFFLUENT

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NSF#1118679

ABSTRACT

Antimicrobial compounds do not completely metabolize in humans, causing large amounts of them to enter wastewater treatment plants (Nagulapally, 2007). The wastewater treatment facilities in Gaston County, North Carolina currently do not test for, or practice the removal of, any known antibiotic compounds in wastewater effluent. This study focused on an area Wastewater Treatment Plant found in North Carolina. We hypothesized the following. If antibiotic compounds make it through the wastewater treatment process and are discharged into the local stream, then there will be an increase in antibiotic resistance within environmental bacteria found near the effluent discharge site. We compared environmental soil and water bacteria samples collected immediately downstream of the effluent discharge site with environmental soil and water bacteria samples collected from Crowders Creek, which runs parallel to the discharge site, prior to the convergence of both streams. Both soil and water samples were taken from each site and were plated on LB broth agar plates treated with cycloheximide. Master spread patch plates were created for all soil and water samples. The 16s region of Ribosomal DNA from individual colonies was extracted using Amplictac gold enzymes, then 1492R and 27F primers were added and samples were amplified using PCR. All samples were sent to Yale University's DNA analysis facility for Sanger sequencing. Identified samples were then streaked onto plates containing the following antibacterial discs: Ciprofloxacin, Streptomycin, Erythromycin, Tetracycline, and Ampicillin. Zones of inhibition were measured and noted for each bacterial species. Statistical comparisons were made between both sites to determine any significant difference

GOAL

The goal of this project is to test for antibiotic resistant populations of water bacteria found in both wastewater effluent discharge and Crowders Creek stream waters. A comparison was then made between both local aquatic environments to see if there was a significant difference in antibiotic resistance between the bacteria populations. This study is intended to determine if antibiotic resistance is prevalent in stream ecosystems associated with local wastewater treatment plants.

METHODS

Eight samples were pulled from both the effluent water and Crowders Creek Proper. The samples from the effluent water were taken starting at the initial flow of the water and were taken in increments of 10 feet stopping at 70 feet. The Crowders Creek Proper samples were taken in the same manner starting at a point parallel to the effluent water initial flow. Samples were plated within 6 hours onto plates of L.B. Agar with cycloheximide. 3 drops of water were placed on each plate and were spread evenly across the agar. The plates were incubated at 28 degrees Celsius for up to 48 hours. After plates had grown sufficient amount of bacteria, individual colonies were plucked from the original plates and patched onto master plates with 32 sectioned grids. These plates were incubated at 28 degrees Celsius for 24-48 hours depending on the amount of growth needed. Each individual colony was then plucked from the master plate and a broth tube was made from the bacteria. The 16s region of Ribosomal DNA from individual colonies was extracted using Amplictac gold enzymes, then1492R and 27F primers were added and samples were amplified using PCR. Returned samples were run through a blast program and identified to the genus level. Identified samples were compared to cultured plates containing the following antibacterial discs: Ciprofloxacin, Streptomycin, Erythromycin, Tetracycline, and Ampicillin. Zones of inhibition were

RESULTS

Data For Crowders Creek WWTP Effluent Winter 2015

Antibiotic Tested	Total Number of Plates Cultured	Total Number of Plates Showing Resistance	% of Cultures Showing Resistance
Ciprofloxacin	79	7	8.86%
Erythromycin	79	46	58.23%
Streptomycin	79	17	21.51%
Tetracycline	79	16	20.24%
Ampicillin	79	42	53.16%

Data for Crowders Creek Stream Effluent

Antibiotic Tested	Total Number of Plates Cultured	Total Number of Plates Showing Resistance	% of Cultures Showing Resistance
Ciprofloxacin	76	0	0%
Erythromycin	76	42	55.26%
Streptomycin	76	6	7.89%
Tetracycline	76	2	2.63%
Ampicillin	76	33	43.42%

For each antibiotic tested the percentage of resistant bacteria was higher coming out of the WWTP effluent when compared with Crowders Creek Stream. To determine if the percentage of cultures showing resistance was a significant amount a two sample proportional test was used. A two sample proportional test is to determine whether the proportion of one independent sample is significant when compared to the proportion of another independent sample. The equation is:

$$Z_0 = \frac{(\hat{p}_1 - \hat{p}_2)}{\sqrt{\hat{p}(1-\hat{p})(\frac{1}{n_1}+\frac{1}{n_2})}}$$

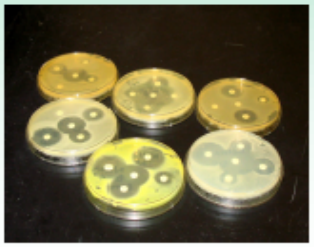
Where, $\hat{p} = (x_1 + x_2)/(n_1 + n_2)$

In this equation you need a critical value to indicate whether the difference was significant for each item. The critical valued used in this experiment was ($\alpha = 0.05$) 1.96 meaning that if the test statistic after running this equation was higher than 1.96, there was a significant difference in the proportion of antibiotic resistant bacteria coming out of the effluent of the Waste Water Treatment Plant than there was running in Crowders Creek Stream.

Results were as follows:

- Ciprofloxacin Z_0=265
- Erythromycin Z_0= 37.27
- Streptomycin Z_0= 132.26
- Tetracycline Z_0= 342
- Ampicillin Z_0 = 121

These numbers indicate there is a significantly higher proportion of antibiotic resistant bacteria coming out of the waste water treatment plant than there is in



DISCUSSION

If antibiotic compounds make it through the wastewater treatment process and are discharged into the local stream, then there will be an increase in antibiotic resistance within environmental bacteria found near the effluent discharge site. In the present study, statistical analysis concludes that an increase in antibiotic resistance exists in the waste water run-off in comparison to the natural creek parallel to the discharge site. The five antibiotics tested in the discharge samples resulted in the following percentages of resistance from lowest to highest: Erythromycin 58.23%, Ampicillin 53.16%, Streptomycin 21.51%, Tetracycline 20.24%, Ciprofloxacin 8.86%. The same five antibiotics were tested for Crowders Creek and resulted in the following percentages of resistance from lowest to highest: Ciprofloxacin 0%, Tetracycline 2.63%, Streptomycin 7.89%, Ampicillin 43.42%. The two sample proportional test shows that in comparison of the discharge site and the creek, the discharge site had a significant difference of antibiotic resistance as follows: Ciprofloxacin 265, Erythromycin 37.27, Streptomycin 132.26, Tetracycline 342, Ampicillin 121. The critical value of the two sample proportional test is $\alpha = 0.05$ and the mean is 1.96 with anything measuring over the value mean shows a significant difference. Our results support the hypothesis that antibiotic resistance is more frequent in bacteria found in the discharge site than found in Crowders Creek.

RECOMMENDATIONS

Although a significant difference in the antibiotic resistance between the Wastewater Effluent and Crowders Creek Stream was found for all antibiotics tested, further cultures are necessary to help confirm these results. More cultures are needed to ensure the statistical accuracy of the data presented on this poster.

SOURCES

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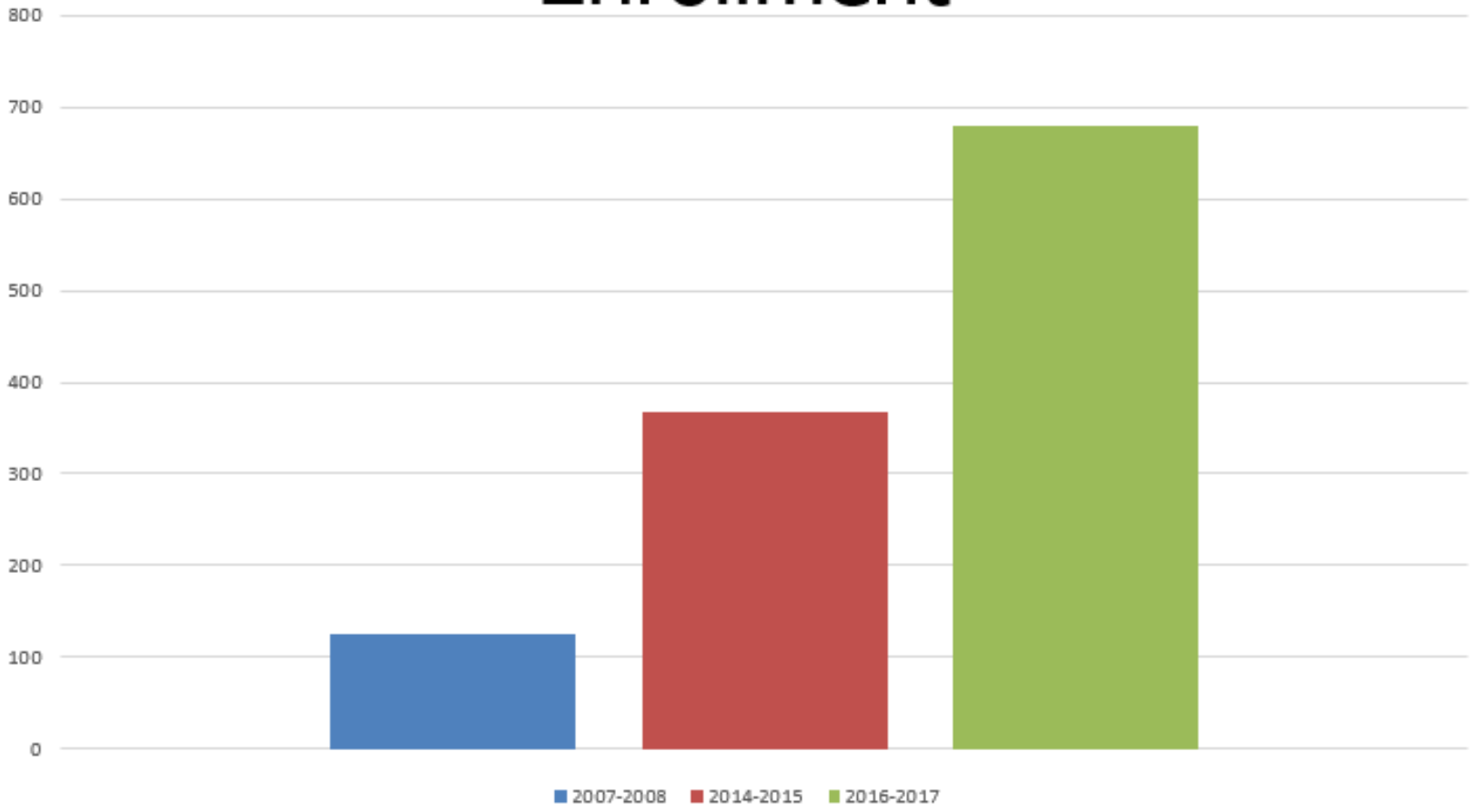

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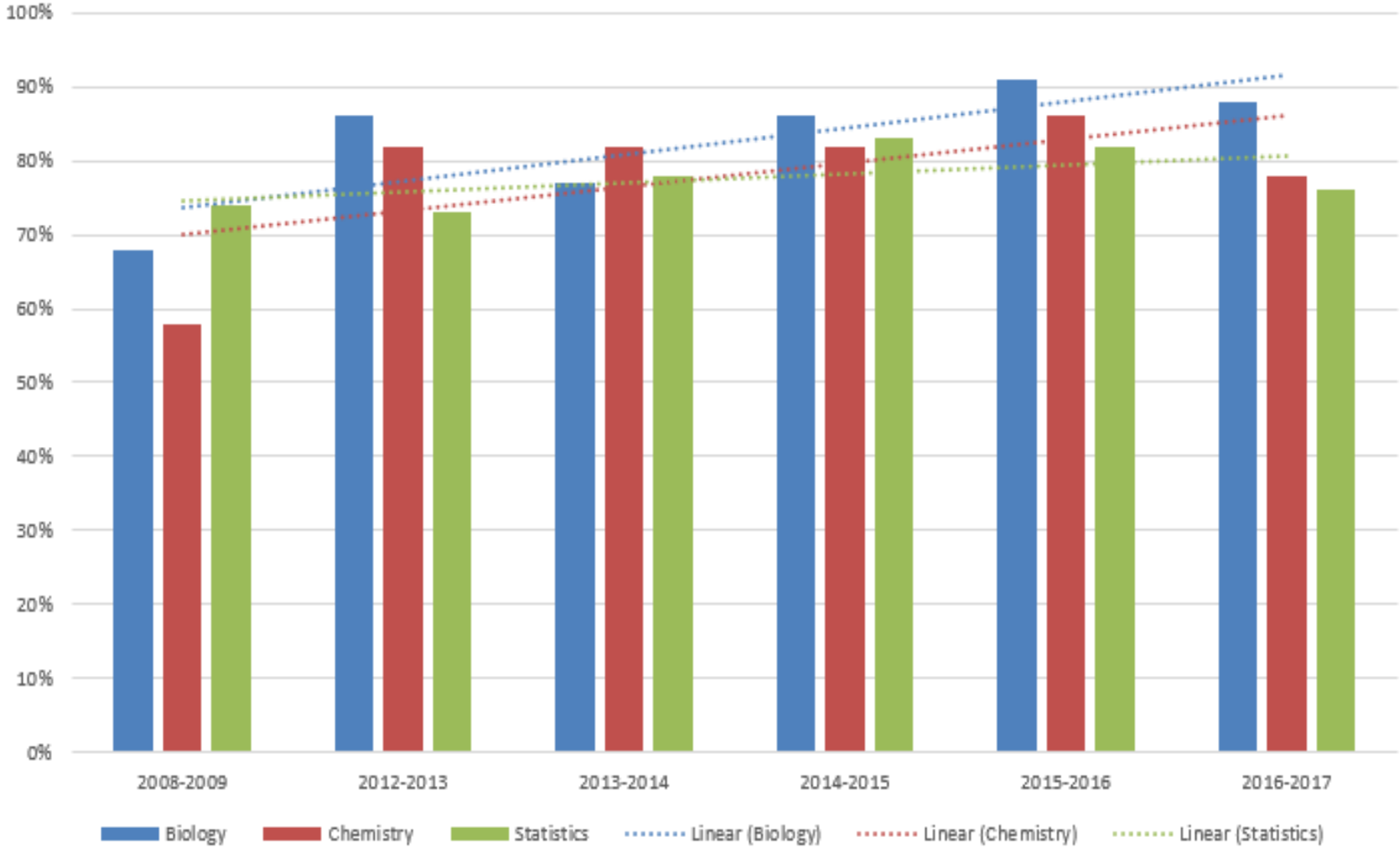

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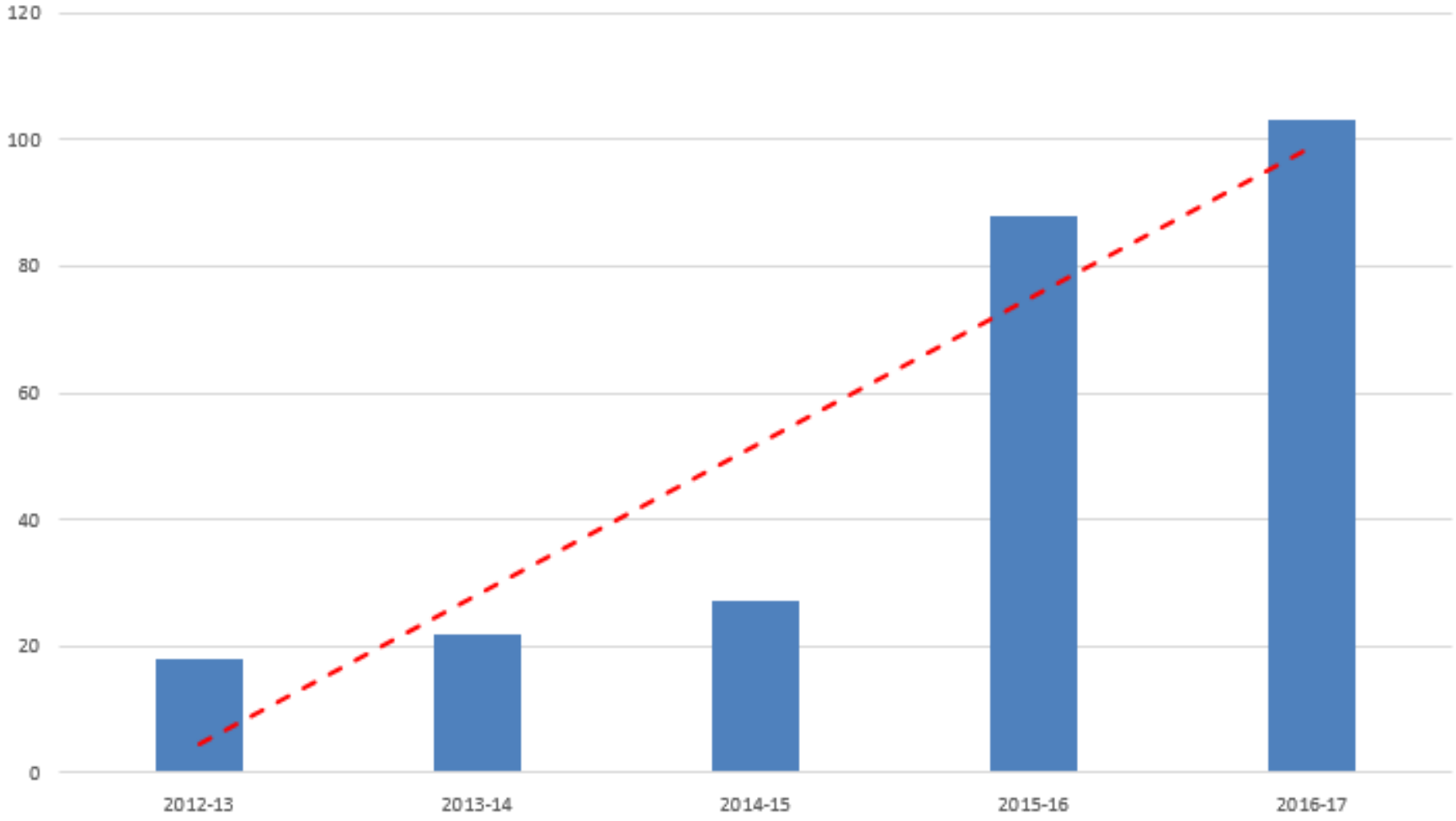

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