NM WRRI Student Water Research Grant Progress Report Form

Progress Report due October 1, 2020
Draft Final Report due April 30, 2021
Final Report due May 31, 2021

1. Student Researcher: Kasandra D. Velarde
   Faculty Advisor: Linda DeVeaux

2. Project title: *Exploring surface water as the reservoir of CRE infecting patients in SE New Mexico*

3. Description of research problem and research objectives.

Antibiotic resistance is an increasing concern around the world due to exposure in clinical settings and food production, which contribute to the dissemination of antibiotic resistance genes (ARGs) [2, 3]. Human activity can lead to the transfer of ARGs throughout natural reservoirs such as soil, marine water, and surface waters [4]. Surface waters, such as rivers and streams, are an increasing environmental source for research concerning human and animal health concerns due to indirect or direct contact with the water. These aquatic environments can range from a few miles to thousands of miles, which can expose resident bacteria to antibiotic resistant bacteria (ARBs) that could potentially transfer these determinants through horizontal gene transfer. Carbapenem-resistant *Enterobacteriaceae* (CRE) are an emerging group of antibiotic resistant pathogens. Carbapenem-resistant *Enterobacteriaceae* are commonly observed in clinical settings but can be found in the environment. As of 2018, the Centers for Disease Control and Prevention have been tracking organisms carrying the genes for carbapenemases KPC, NDM, VIM, IMP, and OXA48 [5]. New Mexico has had a rapid increase in carbapenem-resistant infections [6], including pathogens carrying the VIM gene, which is less common but an increasing concern.The New Mexico Department of Health discovered four cases of antibiotic resistance infections carrying the VIM gene, but which had no known health-care source; the only commonality between all the patients are their counties of residence (DeBaca, Chaves, and Eddy) which are connected by the Pecos River [1]. The Pecos River in New Mexico runs from Mora County and empties into the Rio Grande and is a potential reservoir of the organisms that carry this gene.

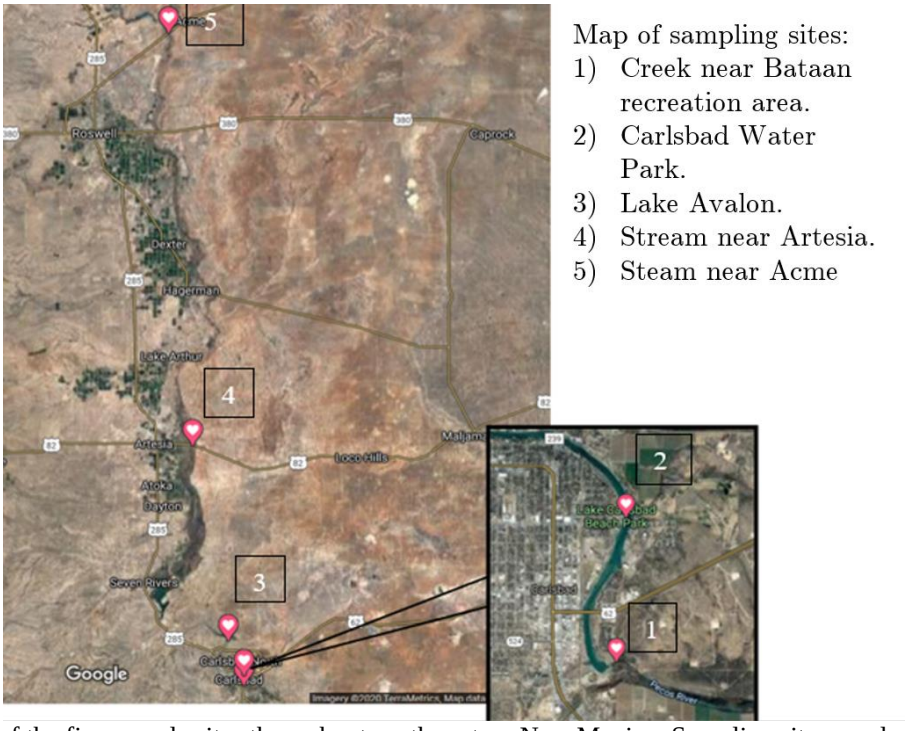
This study aims to provide insight in the dissemination of carbapenem resistance genes in the Pecos River, specifically the VIM gene, which can potentially provide a correlation between clinical and environmental reservoirs. Samples will also be tested for virulence genes (VGs) and the harboring organism will be compared to clinical isolates.

4. Description of methodology employed.

Water samples from the Pecos River were collected twice from two different counties (Chaves and Eddy) at five different locations. The sample locations were a creek near Bataan recreational area (Carlsbad, NM), Carlsbad Water Park (Carlsbad, NM), Lake Avalon (North Carlsbad, NM), a stream (Artesia, NM), and another stream (Acme, NM). The environmental water samples were filtered 24 hours after collection through a 0.45µM filter. Total DNA was extracted using either the Qiagen DNeasy PowerSoil Pro Kit, DNeasy PowerWater Kit, or the ZymoBIOMICS DNA Miniprep Kit according to the manufacturer instructions. Samples were also tested for coliforms using m-ColiBlue24® Broth according to the manufacturer's instructions. DNA from only the ZymoBIOMICS DNA Miniprep Kit was screened by Polymerase Chain Reaction (PCR) for the presence of the 16s rRNA gene, carbapenemase genes, and virulence genes. If genes were amplifiable but were not at the correct base pair length, a PCR cleanup was performed using the Wizard® SV Gel and PCR Clean-Up System according to the manufactures instructions. DNA sequencing was performed by GeneWiz (South Plainfield, NJ). If VIM was present at any of the sites, the individual site was plated on Difco™ McConkey agar then colony-PCR was performed.

5. Description of results; include findings, conclusions, and recommendations for further research.

Samples were collected from five different sites (Fig 1.), which were chosen based on distribution and accessibility within the target area. The samples were collected on two different occasions: July 24, 2020 and again on September 6, 2020.



Map of sampling sites:
1) Creek near Bataan recreation area.
2) Carlsbad Water Park.
3) Lake Avalon.
4) Stream near Artesia.
5) Steam near Acme

Figure 1. Map of the five sample sites throughout south eastern New Mexico. Sampling sites are denoted 1-5 going North and in order of collection.

All sample locations were tested for total and fecal coliforms (Table 1).

Table 1. Coliforms per 100mL

	Total Coliforms	Fecal Coliforms
Sample Site 1	TNTC	125
Sample Site 2	318	17
Sample Site3	TNTC	2
Sample Site 4	395	65
Sample Site 5	1000	15

Each of the sites varied in total coliforms. In general, the number of total coliforms stayed consistent throughout all sample sites, whereas fecal coliforms were most numerous at the most southern collection point. However, more experimental replicates would be needed to draw a definitive conclusion.

Challenges included low concentration yields of DNA from the Qiagen DNeasy PowerSoil Pro Kit and DNeasy PowerWater Kit. However, after testing other extraction methods, the ZymoBIOMICS DNA Miniprep Kit was able to provide high quality DNA for PCR amplification. DNA extracted from the filtered water was screened by PCR using primers to the bacterial 16s rRNA gene to verify that amplifiable bacterial DNA was present. The results are shown in Figure 2, which verifies that all the sites had amplifiable DNA. This is an important control when determining the presence/absence of the specific carbapenemase and virulence genes.

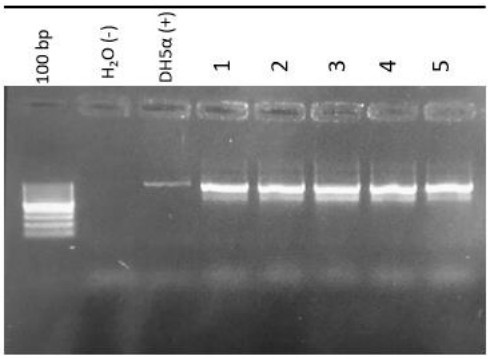


Figure 2. Verification of amplifiable DNA from sample sites. Total DNA was extracted from water samples with ZymoBIOMICS SNA Miniprep Kit and used as a template for PCR with 16s rRNA gene specific primers. PCR products were run on a 1.5% agarose gel. Negative control contained no template (H2O) and positive control was DNA extracted from the laboratory E. coli strain DH5α.

The next step was to screen the DNA for the presence of the VIM gene, other carbapanemase genes, and virulence genes by PCR, using primers specific to each gene. Total extracted DNA was screened first for the presence of the virulence genes *espP*, *stx1*, *stx2*, *exhA*, and *eaeA* [Supplementary Figure C]. Table 2 shows the presence or absence of these virulence genes.

Table 2. Virulence genes DNA extracted from water from sample sites

	Sample Site 1	*Sample Site 2*	*Sample Site 3*	*Sample Site 4*	*Sample Site 5*
espP	-	-	-	-	-
stx1	-	-	-	-	-
stx2	-	-	-	-	-
exhA	+	+	+	+	+
eaeA	-	-	-	-	-

Presence of a gene is indicated by +. Absence of a gene is indicated by a -.

exhA, which encodes the *E. coli* enterhemolysin, was the only virulence gene present in all sample sites. The enterohemolysin gene has been shown to be common among environmental strains of *E. coli* [7].

Next, extracted total DNA was screened for the presence of the following carbapenemase genes: bla_{VIM}, $bla_{OXA\text{-}48}$, $bla_{NDM\text{-}1}$, $bla_{IMP\text{-}8}$, $bla_{KPC\text{-}2}$, and $bla_{GES\text{-}1}$ (Table 3, [Supplementary Figures A and B]).

Table 3. Amplification of Carbapenemase genes from DNA extracted from water from sample sites

	Sample Site 1	*Sample Site 2*	*Sample Site 3*	*Sample Site 4*	*Sample Site 5*
bla_{VIM}	-	-	-	+	-
$bla_{OXA\text{-}48}$	-/+	-	-/+	-	-
$bla_{NDM\text{-}1}$	$+^{\S}$	-	$+^{\S}$	-	$+^{\S}$
$bla_{IMP\text{-}8}$	-	-	-	-	-
$bla_{KPC\text{-}2}$	-	-	-	-	-
$bla_{GES\text{-}1}$	$+^{\S}$	-	-	-	$+^{\S}$

Presence of a gene is indicated by +. Absence of a gene is indicated by a -. Genes that were present beforehand then were not amplifiable after another biological sample are denoted -/+. Genes that were shown to amplify but were not at the correct base pair length are denoted $+^{\S}$.

Sample site 4 was the only site that demonstrated amplification of the bla_{VIM} gene. The $bla_{OXA\text{-}48}$ gene was present inconsistently in Sample Sites 1 and 3; however, it is possible that the inconsistent amplification was due to the concentration of the gene in the sample being close to the detection limit of PCR. Amplified genes from both $bla_{NDM\text{-}1}$ and $bla_{GES\text{-}1}$ were not at the predicted base pair size. To determine what DNA sequence was being amplified by these specific primers, the DNA was purified and sequenced via Sanger sequencing.

The sequences of the amplified products were put into NCBI's BLAST tool. The two amplified product sequences aligned most closely with a HAMP domain-containing protein, diguanylate cyclase, succinate dehydrogenase, a NADH-quinone oxidoreductase subunit, and a NADH dehydrogenase. These results indicate that the amplification was non-specific and/or these genes have a similar sequence but encode a different protein. However, neither amplified product aligned with either $bla_{NDM\text{-}1}$ and $bla_{GES\text{-}1}$ and did not encode carbapenemases.

Finally, because Sample Site 4 tested positive for the VIM gene, individual isolates were selected on MacConkey agar. The 11 isolates that grew on MacConkey agar were tested through colony-PCR for the presence of the VIM gene (Fig 3.).

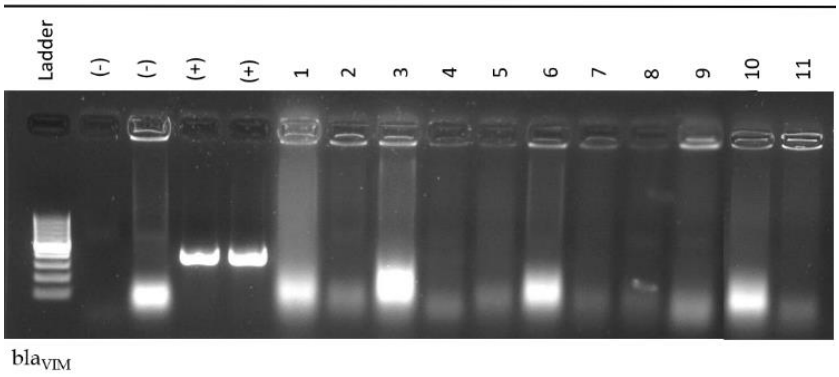


Figure 3. Colony-PCR used to indicate the organism carrying the bla$_{VIM}$ gene. PCR product was run on a 1.5% agarose gel.

The *bla$_{VIM}$* gene was not amplified in any of the selected colonies compared to two negative controls (molecular grade water and *E. coli* DH5α DNA as template) and two positive controls (CDC AR Isolate Bank Strains 239 and 0501 DNA as template). However, since there were very few colonies isolated on MacConkey agar, more biological replicates would be needed to receive a definitive answer on which organism is harboring this specific carbapenem resistance gene.

In summary, the carbapenemase genes that were present throughout several locations in the Pecos River were *bla$_{VIM}$*, *bla$_{NDM-1}$*, *bla$_{GES-1}$*, *bla$_{OXA-48}$*. The virulence gene, *exhA*, was also present and in all sample sites; the results are summarized in Figure 4. The presence of these genes ranged possibly due to the nature of the water, such as flowing or stagnant. Flowing sites included Sample Site 1, Sample Site 4, and Sample Site 5, whereas Sample Site 2 and Sample Site 3 were stagnant.

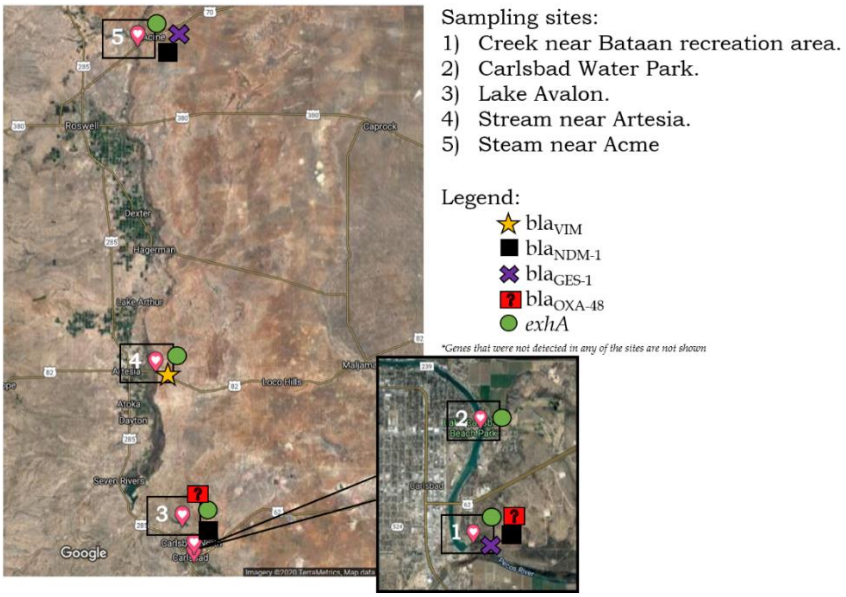


Figure 4. Genes present throughout Southeastern NM within the Pecos River.

6. Provide a paragraph on who will benefit from your research results. Include any water agency that could use your results.

VIM was present in Sample Site 4, which is a stream near Artesia, NM. Therefore, further investigation of the potential source of the bacterial population in and around this site would provide insight into environmental reservoirs of antibiotic resistance. This also potentially eliminates some sites from the Pecos River as a potential source of pathogens carrying the carbapenemase gene that encodes VIM. Because of the rise of clinical cases, the New Mexico Department of Health epidemiologists could identify the source of clinical cases and possibly allow intervention efforts to be implemented. The New Mexico Environment Department, which works with surface waters, could potentially benefit from this research by gaining an understanding about the fate and transport of antibiotic resistant pathogens.

7. Describe how you have spent your grant funds. Also provide your budget balance and how you will use any remaining funds. If you anticipate any funds remaining after May 31, 2021, please contact Carolina Mijares immediately. (575-646-7991; mijares@nmsu.edu)

Grant funds have been spent on supplies, travel, and salary; a break down is shown in the table below.

Table 4. Budget Analysis

	Budget	**Cumulative**	**Balance**
Student Salary	$5,250.00	$5,400.00	$-150.00
Fringe Benefits	$100.00	$176.58	$-76.58
General Supplies	$1,500.00	$605.26	$894.74
Travel	$250.00	$722.29	$-472.29
Total	$7,100.00	$6,904.13	$195.87

8. List presentations you have made related to the project.

Velarde, K., L.C. DeVeaux. (2020, October 27-29). *Exploring surface water as the reservoir of CRE infecting patients in SE New Mexico* [Conference presentation]. 65th NM WRRI Annual New Mexico Water Conference.

Velarde, K., L.C. DeVeaux. (2021, April 8-9). *Exploring surface water as the reservoir of CRE infecting patients in SE New Mexico* [Conference presentation]. Rio Grande Branch of the American Society for Microbiology.

Velarde, K., L.C. DeVeaux. (2021, April 14-16). *Exploring surface water as the reservoir of CRE infecting patients in SE New Mexico* [Conference presentation]. New Mexico Institute of Mining and Technology Student Research Symposium.

9. List publications or reports, if any, that you are preparing. For all publications/reports and posters resulting from this award, please attribute the funding to NM WRRI and the New Mexico State Legislature by including the account number: NMWRRI-SG-2020.

An abstract publication in the journal of *Clinical Infection and Immunity* is being prepared with the Rio Grande ASM chapter.

10. List any other students or faculty members who have assisted you with your project.

Joseph Ulbrich, helped with collecting samples in all five sites.
Katherine Persinger, helped with collecting samples in all five sites.

11. Provide special recognition awards or notable achievements as a result of the research including any publicity such as newspaper articles, or similar.

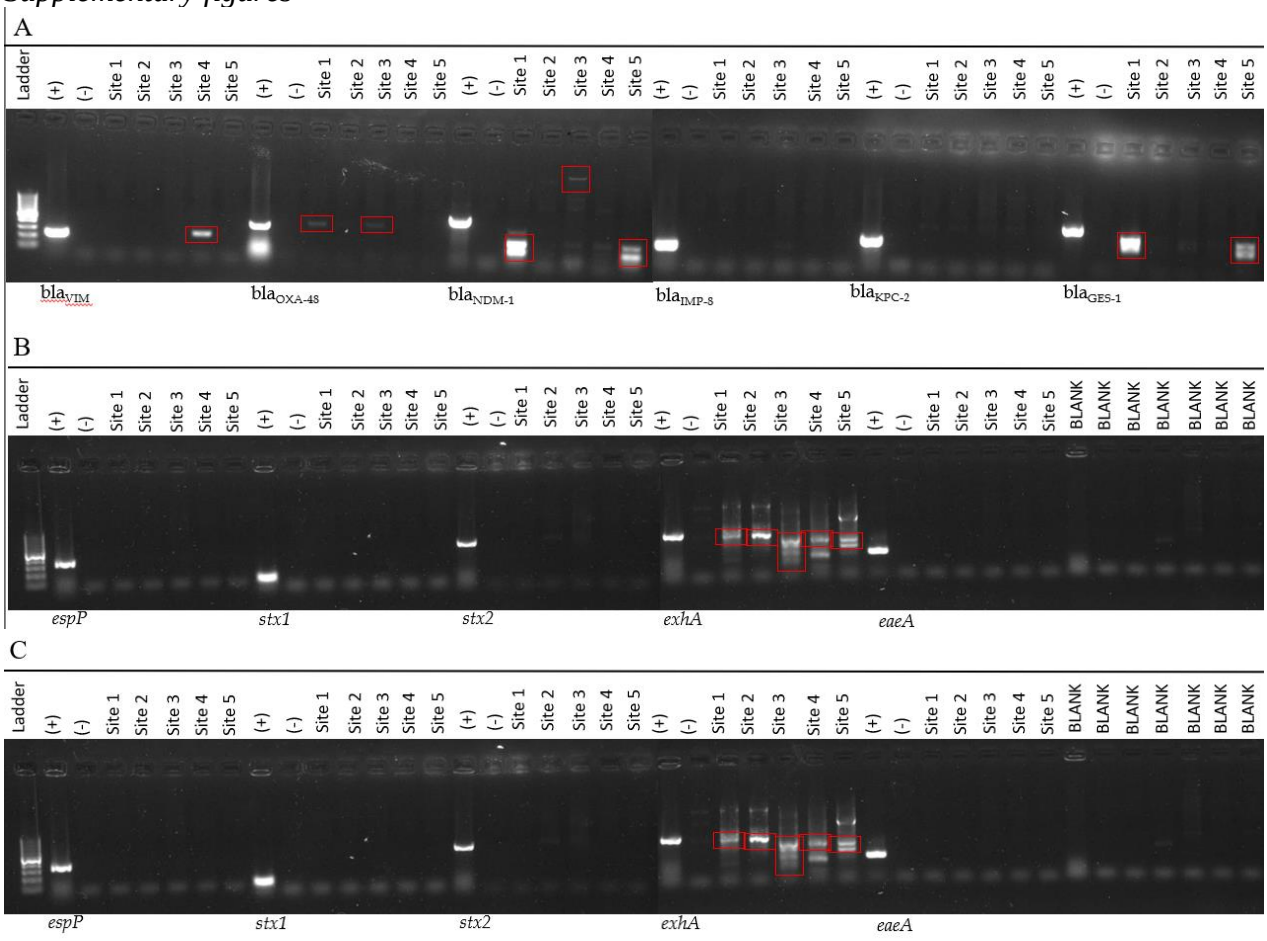
Articles that have been written about my research are exclusive to the NMWRRI; links are below.

https://nmwrri.nmsu.edu/nmt-student-receives-nm-wrri-student-water-research-grant-to-study/
https://us10.campaign-archive.com/?u=f726015b153e91a9b63d89b48&id=a1b97eb2d0

12. Provide information on degree completion and future career plans. Funding for student grants comes from the New Mexico Legislature and legislators are interested in whether recipients of these grants go on to complete academic degrees and work in a water-related field in New Mexico or elsewhere.

I earned my Bachelor of Science in Biology from New Mexico Tech in May 2021and in May 2022I will obtain my Master of Science in Biology, focusing in microbiology, at New Mexico Institute of Mining and Technology. Future career plans include researching for private industry or through a government job in New Mexico.

Supplementary figures



Photos shown below are from sample sites two, four, and five respectively at different sampling dates. The other photo includes the results of the coliform test for one sampling site.

References

1. Sievers, M. and B. Hudspeth, *Personal Communication*. 2019.

2. Allen, H.K., et al., *Call of the wild: antibiotic resistance genes in natural environments.* Nature Reviews Microbiology, 2010. **8**(4): p. 251-259.

3. Li, R., J.A. Jay, and M.K. Stenstrom, *Fate of antibiotic resistance genes and antibiotic-resistant bacteria in water resource recovery facilities.* Water Environment Research, 2019. **91**(1): p. 5-20.

4. Wellington, E.M.H., et al., *The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria.* The Lancet Infectious Diseases, 2013. **13**(2): p. 155-165.

5. Prevention, C.f.D.C.a. *Tracking CRE in the United States*. 2019.

6. Phipps, E.C., E.B. Hancock, and N. Kenslow, *Carbapenem-Resistant Enterobacteriaceae (CRE) in Bernalillo County, New Mexico, 2013-2014.* Open Forum Infectious Diseases, 2015. **2**(suppl_1).

7. Boczek, L.A., et al., *The widespread occurrence of the enterohemolysin gene ehlyA among environmental strains of Escherichia coli.* FEMS Microbiol Lett, 2006. **254**(2): p. 281-4.