

Resistance Patterns and Genetic Characterization of Carbapenem Resistance in *Enterobacteriaceae* Isolated from Water Samples Collected from the Middle Delaware and Raritan Watersheds

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INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE), a family of intestinal bacteria, are resistant to a class of antibiotics known as carbapenems. An infection caused by *Klebsiella pneumoniae* Carbapenemase (KPC)-producing bacteria has a high mortality rate. Misuse of antibiotics in the medical field and in farms has led to the continuous emergence and spread of multidrug resistant bacterial infections.

To investigate whether KPC-producing bacteria have already spread into the environment, water samples were collected from impaired streams of Edison, New Jersey and from Middle Delaware and Raritan Watersheds.



Mill Brook Stream Edison, NJ

Primary and secondary biochemical tests were performed on the samples. Characterization of *Enterobacteriaceae* was done using API 20E rapid biochemical test strips, and susceptibility patterns for select antibiotics were determined by Kirby-Bauer disk diffusion testing.



MACConkey

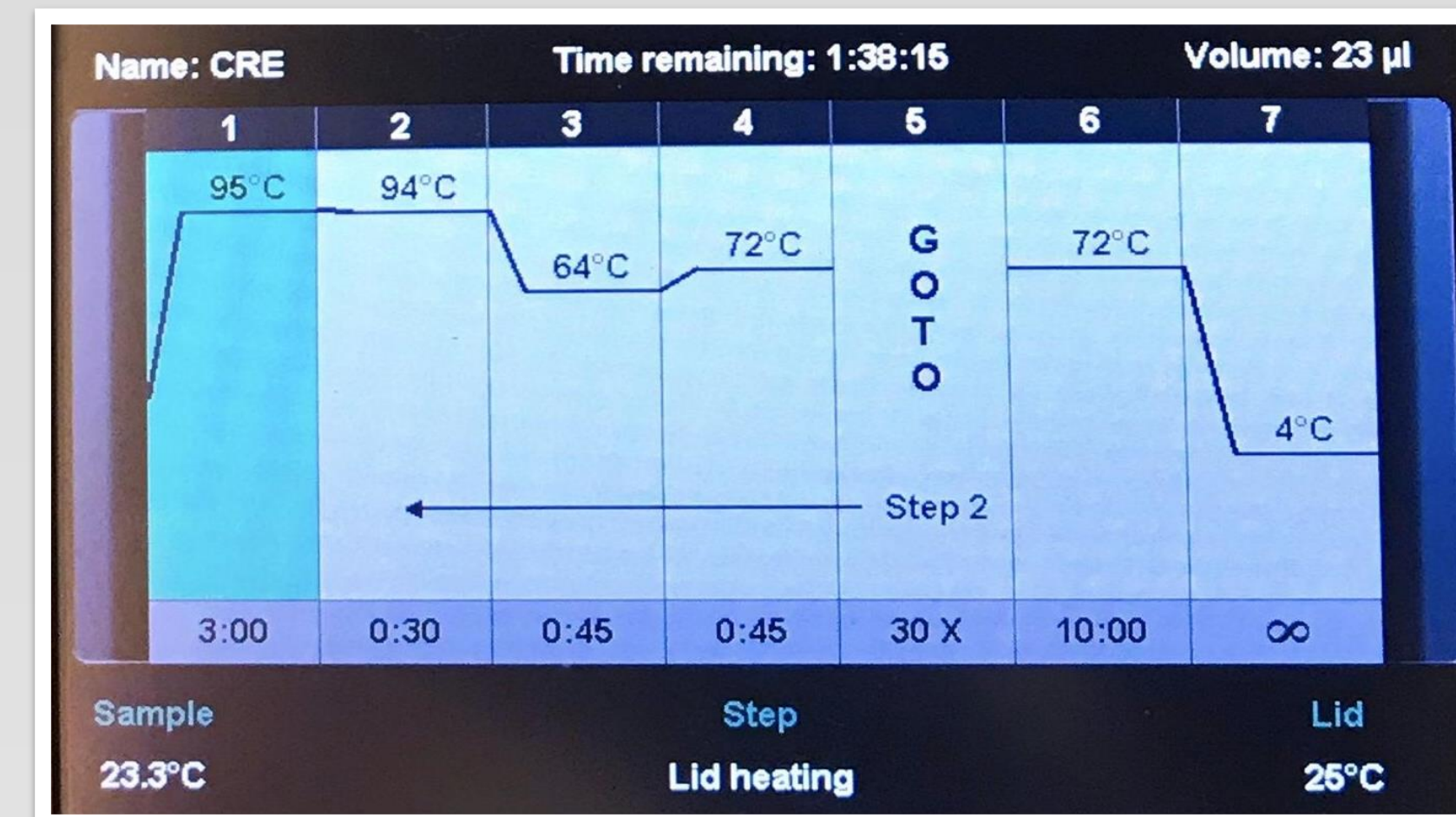
Eosin Methylene Blue

Mueller-Hinton

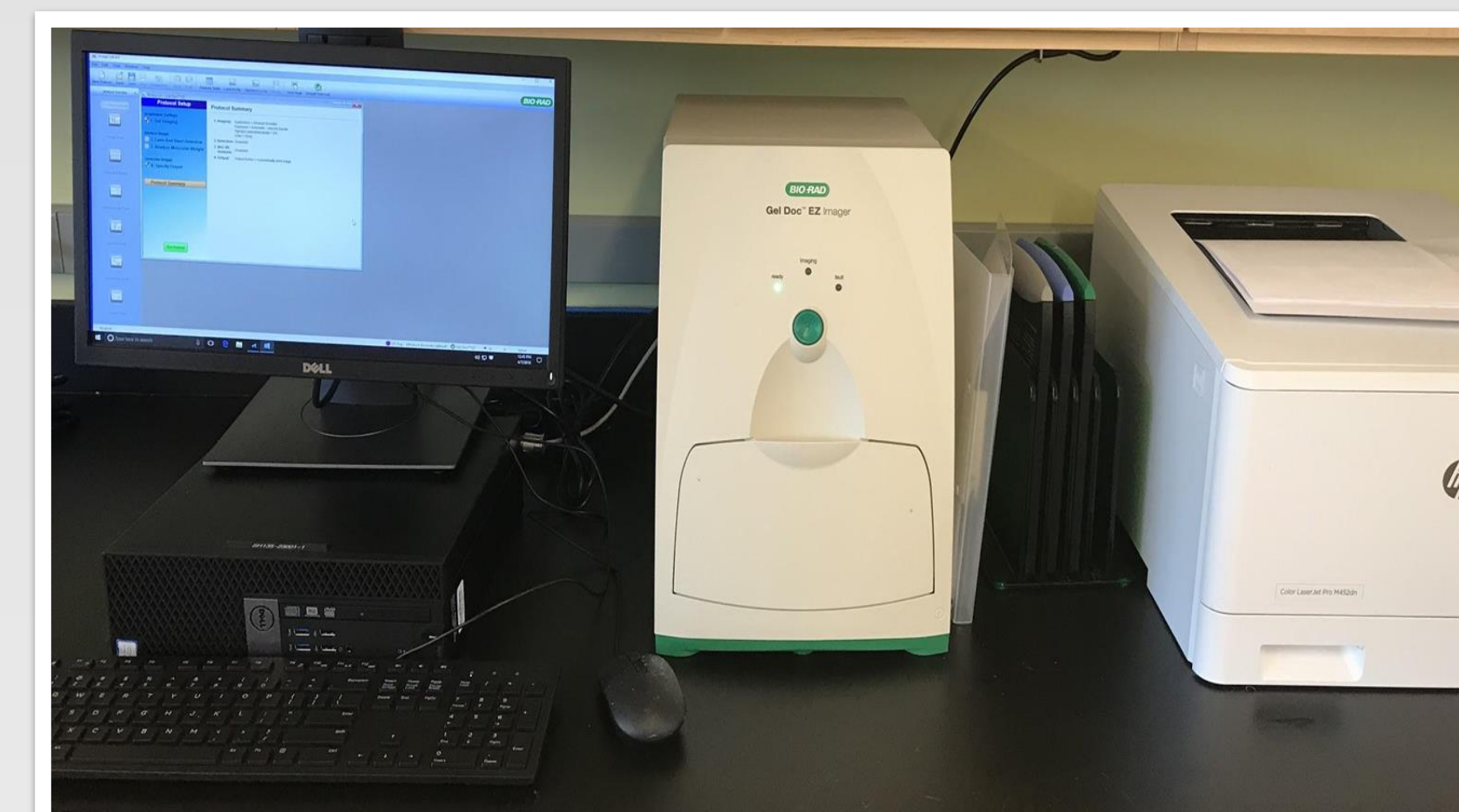


API 20E bioMerieux © SA Marcy l'Etoile-France

Plasmid DNA from select strains was extracted using the Quantum Prep® Plasmid Miniprep system, and PCR, using primers 5'-TGTTGCTGAAGGAGTTGGGC-3' (Forward) and 5'-ACGACGGCATAGTCATTTGC-3' (Reverse) to determine presence or absence of the carbapenem resistance gene *bla_{kpc}*.



BIO-RAD T100 Thermal Cycler



Bio-Rad Gel Doc Ez Viewer

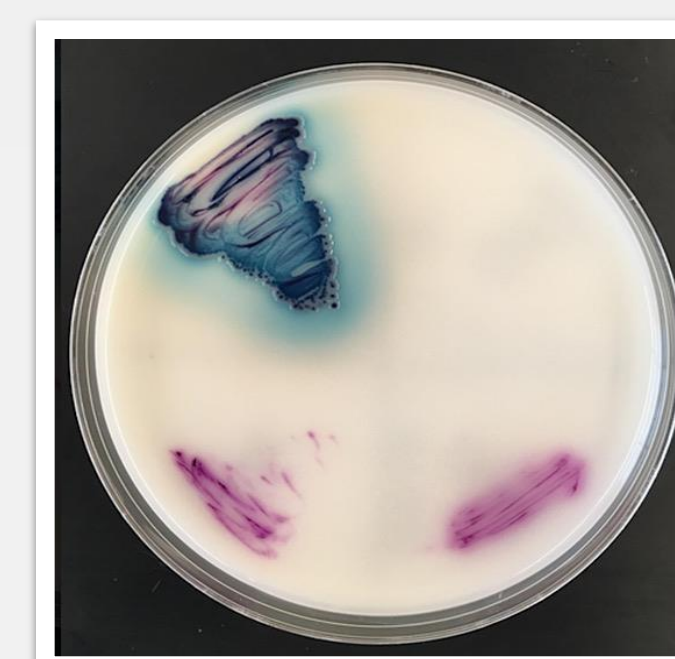
Carriage of carbapenem-resistance elements such as *bla_{kpc}* by bacteria isolated from the natural environment may serve to further the spread of emerging antibiotic resistance among native bacteria in non-clinical settings.

HYPOTHESIS

Isolates that grow on the HardyChrom CRE agar would carry the carbapenem resistance gene *bla_{kpc}*.

PROTOCOL

1. Bacterial Isolation
2. Biochemical Testing using Gram stain, Oxidase, Nitrate test and API 20E rapid identification
3. Antibiotic Susceptibility Testing using Kirby-Bauer Disk Diffusion Method
4. Genetic Testing using PCR and Gel Electrophoresis



Cultures also plated on HardyChrom to detect carbapenem resistant cultures.

RESULTS

Antibiotic Susceptibility Testing of *Enterobacteriaceae* using the Kirby Bauer Disk Diffusion Method

Organism	<i>Pasteurella pneumotropica/Mannheimia haemolytica</i>	<i>Stenotrophomonas maltophilia</i>	<i>Klebsiella pneumoniae</i> ssp (<i>pneumoniae</i> 2)*	<i>Klebsiella pneumoniae</i> ssp (<i>pneumoniae</i> 1)	<i>Klebsiella oxytoca</i>	<i>Pantoea</i> spp 3	<i>Pantoea</i> spp 2*
Cephalothin 30 µg/mL	6mm (S)	0mm (R)	24mm (S)	20mm (S)	20mm (S)	23mm (S)	21mm (S)
Chloramphenicol 30µg/mL	32mm (S)	32mm (S)	38mm (S)	26mm (S)	24mm (S)	35mm (S)	30mm (S)
Sulfamethoxazole 23.75/1.25 µg/mL	28mm (S)	35mm (S)	38mm (S)	23mm (S)	24mm (S)	42mm (S)	36mm (S)
Ampicillin 10 µg/mL	7mm (R)	10mm (R)	5mm (R)	7mm (R)	6mm (R)	23mm (S)	7mm (R)
Imipenem 10 µg/mL	26mm(S)	40mm (S)	39mm (S)	29mm (S)	29mm (S)	38mm (S)	32mm (S)
Meropenem 10 µg/mL	27mm(S)	43mm (S)	40mm (S)	33mm (S)	30mm (S)	41mm (S)	33mm (S)
Ertapenem 10 µg/mL	24mm(S)	39mm (S)	45mm (S)	30mm (S)	30mm (S)	43mm (S)	37mm (S)
Tetracycline 30 µg/mL	28mm(S)	27mm (S)	32mm (S)	23mm (S)	23mm (S)	31mm (S)	27mm (S)
Streptomycin 10 µg/mL	N/A	N/A	-	11mm (R)	10mm (R)	33mm (S)	-
Ciprofloxacin 5 µg/mL	N/A	N/A	-	26mm (S)	30mm (S)	50mm (S)	-
Penicillin 10 µg/mL	N/A	N/A	-	0mm (R)	0mm (R)	18mm (S)	-
Novobiocin 5 µg/mL	N/A	N/A	-	9mm (R)	10mm (R)	0mm (R)	-
Erythromycin 15 µg/mL	N/A	N/A	-	11mm (R)	11mm (R)	15mm (S)	-
Vancomycin 30 µg/mL	N/A	N/A	-	0mm (R)	0mm (R)	0mm (R)	-
Neomycin 30 µg/mL	N/A	N/A	-	17mm (S)	16mm (I)	28mm (S)	-
Bacitracin 10 µg/mL	N/A	N/A	-	0mm (R)	0mm (R)	0mm (R)	-
Gentamicin 10 µg/mL	N/A	N/A	-	20mm (S)	20mm (S)	38mm (S)	-

*These cultures did not grow on MH plate for the second-half of antibiotic testing. N/A: further antibiotic susceptibility testing was not performed on these isolated cultures.

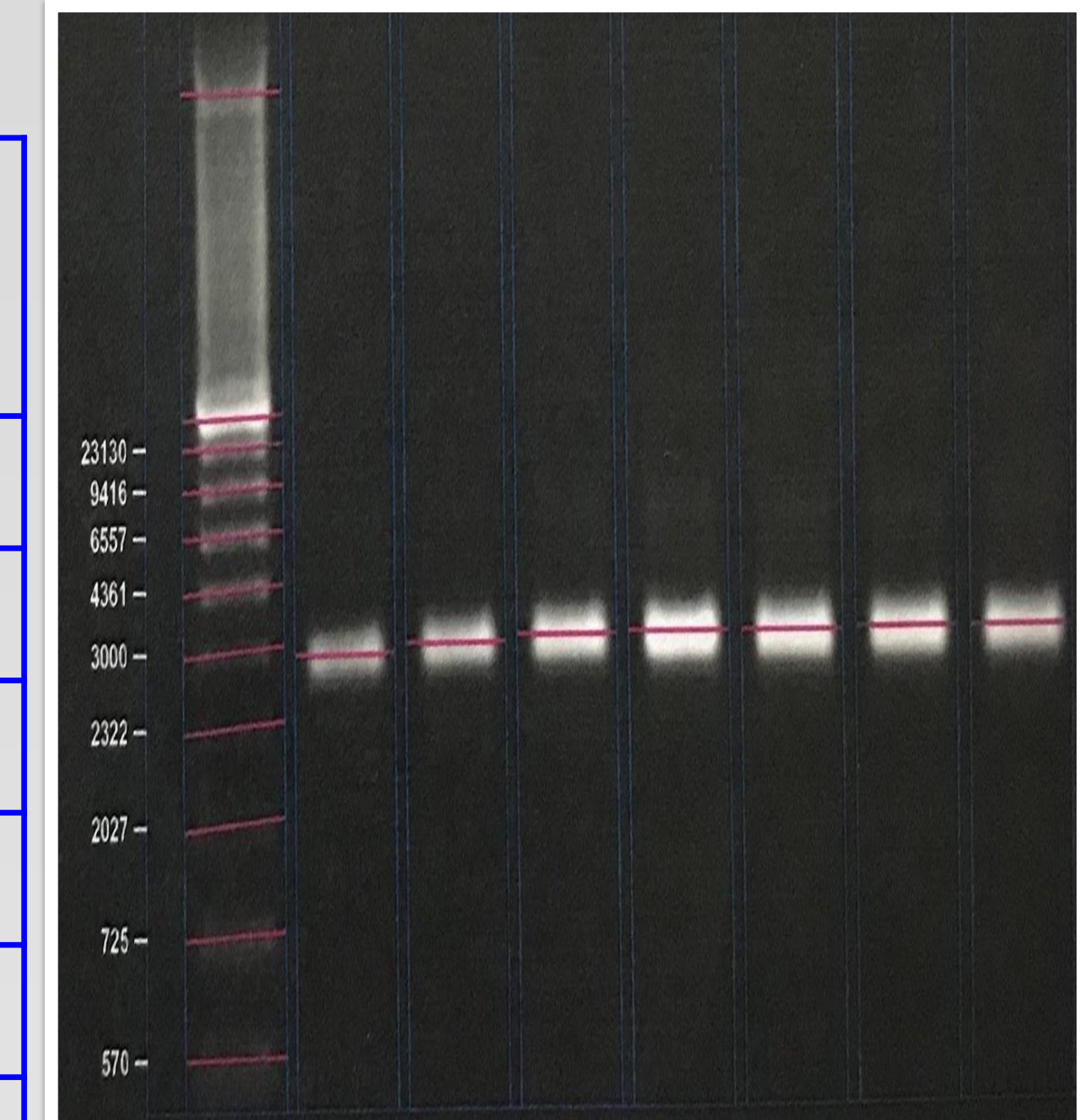
Data from Antibiotic Susceptibility Testing shows that there are no cultures that have acquired resistance to any of the tested antibiotics; rather, the cultures follow their intrinsic resistance patterns.

CONCLUSIONS

Presence of CRE in the aquatic environment would lead to various health complications of which most cases have a high rate of mortality. Although cultures grew on HardyChrom CRE, none of them showed resistance to carbapenems, and results from genetic testing further confirmed that the resistance gene, *bla_{kpc}*, was not present in the cultures. Further genetic testing sampling would need to be performed to verify whether CRE are absent in the aquatic environment.

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CRE Lois.doc(JKC-0.6uM)

Images from the gel reconfirmed that the resistant strains did not possess the gene *bla_{kpc}*.

RESEARCH STUDENTS



Ms. Anusha Gunti and Ms. Lois Twum-Barimah at the Delaware River in Trenton, NJ.