University of Pittsburgh

Annual Progress Report: 2008 Nonformula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Nonformula Grant Overview

The University of Pittsburgh received $4,708,555 in nonformula funds for the grant award period June 1, 2009 through May 31, 2013. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Center of Excellence in Prevention and Control of Antibiotic Resistant Bacterial Infections – The primary goals of this project are to employ novel strategies to reduce the morbidity and mortality caused by *Acinetobacter baumannii*, *Clostridium difficile*, and methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitalized patients and to develop a research training program for racial minorities that are underrepresented in biomedical, health services, and clinical research. We plan to develop and test new tools that will lead to substantial reductions in disease caused by these organisms

Anticipated Duration of Project

6/1/2009 - 5/31/2013

Project Overview

The specific aims of the Center of Excellence in Prevention and Control of Antibiotic Resistant Bacterial Infections are to:

1. Develop, validate, and employ novel molecular detection of asymptomatic *C. difficile* carriage and assess an intervention to control this source of *C. difficile* disease;
2. Understand the implications of introduction of community-associated MRSA strains into the hospital and employ rapid, polymerase chain reaction (PCR)-based diagnosis of MRSA infection to reduce use of broad spectrum antibiotics;
3. Develop a new, multilocus variable number tandem repeat analysis (MLVA) assay for tracking transmission, validate improved methods for detecting *A. baumannii* colonization, and assess an intervention to control spread in intensive care units;
4. Employ infectious diseases modeling to understand the health and economic impacts of these novel strategies on the prevention and control of serious infections caused by these bacterial pathogens; and

5. Establish a research training program for racial minorities that are underrepresented in biomedical, health services, and clinical research.

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Expected Research Outcomes and Benefits

By the end of this four-year project, we will have developed and tested new tools that will lead to substantial reductions in disease caused by our targeted organisms.

Summary of Research Completed

Specific Aim 1 (C. difficile)
Sub-Aim 1. Determine the prevalence of asymptomatic *C. difficile* carriage and environmental contamination
Sub-Aim 2. Determine genetic relatedness of *C. difficile* isolates from asymptomatic colonized patients to *C. difficile* isolates from the environment and from patients with hospital-acquired *C. difficile* infections (HA-CDI)

Data for sub-aims 1 and 2 were presented as a slide session at the 2010 Interscience Conference on Antimicrobial Agents and Chemotherapy meeting in Boston, Massachusetts (September 14, 2010). These data are being prepared as a manuscript for a summer 2011 submission.

We enrolled 70 of 210 residents of Kane Glen Hazel, a public long-term care facility, for the study of asymptomatic carriage. Cross-sectional culturing began in October 2010, at which time 6/70 (8.6 percent) participants were positive for carriage of toxigenic *C. difficile*. The second sampling took place in February 2011, at which time 64 patients remained in the study and of whom 3 (4.7 percent) were positive for toxigenic *C. difficile*. Two more samplings are planned for this cohort.

Sub-Aim 3. Develop and validate a real-time (RT)-PCR assay for rapid identification of *C. difficile* carriage using perirectal swabs

We tested deoxyribonucleic acid (DNA), obtained from perirectal surveillance specimens preamplified in selective liquid medium for *C. difficile*, using a modified commercial RT-PCR assay (GenProbe Prodesse, Waukesha, WI) for toxin B gene (*tcdB*). All fermenting specimens were subcultured, and isolates were tested for toxigenicity. Culture-positive, toxigenic isolates served as the standard for comparison with the broth preamplification/PCR assay. The limit of detection for the assay was one colony-forming unit. Compared to toxigenic anaerobic culture,

the sensitivity, specificity, and positive and negative predictive values of this assay were 70/70 (100.0 percent), 422/426 (99.1 percent), 70/74 (94.6 percent), and 422/422 (100.0 percent), respectively. Turn-around time was 28-76 hours (median 54 hours) from receipt of the swab. These data demonstrate that selective broth preamplification and RT-PCR of perirectal swab specimens is a practical approach to detect asymptomatic carriage of *C. difficile*. These data were submitted as a manuscript to the *Journal of Clinical Microbiology* in April 2011.

Specific Aim 2 (MRSA)

Sub-Aim 1. To evaluate the effects of rapid RT-PCR detection of *S. aureus*/MRSA on time to optimal or appropriate antimicrobial therapy for patients and time to initiation of appropriate isolation precautions in patients colonized with MRSA

Group 1: Patients with gram-positive cocci/*S. aureus* bacteremia

Phase 1 involved baseline data collection using the standard microbiology techniques for identifying *S. aureus* and antimicrobial susceptibilities (methycillin-sensitive versus methycillin-resistant). As soon as an organism is identified, preliminary results are released to clinicians; daily updates are made; and final results, including antimicrobial susceptibilities, are posted on the patients' electronic medical records. Phase 2 involved the application of the BD GeneOhm StaphSR assay for rapid PCR-detection of *S. aureus*/MRSA in blood cultures. Physicians are notified of the assay results by a clinical infectious diseases pharmacist, who also provides clinical recommendations on specific antimicrobial therapies.

We verified the StaphSR assay on 10 MRSA, 15 methicillin-sensitive *S. aureus* (MSSA), and 12 coagulase-negative staphylococcus (CoNS)/streptococci/enterococci samples. RT-PCR testing of blood cultures and clinical reporting were initiated in November 2010. Using phenotypic antimicrobial susceptibility as the standard, 3 of 101 gram-positive, cocci-positive blood cultures had inaccurate RT-PCR results due to an altered PCR target. We found two isolates that did not harbor the *mecA* gene but were wrongly identified as MRSA. We also found one MRSA isolate that contained the *mecA* gene but was wrongly identified as MSSA. In addition, in four other incongruent test results, the culture result was proven to be correct over the RT-PCR assay result. Overall, 6.9 percent of the specimens required additional testing. No patient was harmed as a result of these inconsistencies. However, in response to this high rate of incongruent results, which was unexpected based on published performance characteristics of the test, we discontinued reporting the RT-PCR results in December 2010 and, upon further evaluation, discontinued this component of the study on January 10, 2011.

Group 2: Patients undergoing nasal screening for MRSA

Participants in this group were hospitalized patients undergoing MRSA nasal screening. Positive MRSA screens triggered a real-time MRSA alert that requested initiation of contact precautions (CP). Mean time from nasal swab collection to MRSA alert was compared before and after application of an RT-PCR assay. Data were analyzed by t-test. The proportion of patients observed to be in CP at two hours from MRSA alert was compared before and after the

implementation of automated isolation orders. Data were analyzed using the Pearson chi-square test.

Mean time from swab collection to MRSA alert significantly decreased from 42.3 ± 12.5 hours pre-PCR to 17.1 ± 6.4 hours with PCR, $p < .001$. The proportion of patients observed to be in CP within two hours from MRSA alert significantly increased with implementation of automated isolation order from 6 of 17 (35.3 percent) to 35 of 44 (79.5 percent), $p < .001$. Among hospitalized patients undergoing MRSA nasal screening, an RT-PCR assay and automated MRSA isolation order had a significant effect on time to initiation of appropriate isolation precautions in patients newly identified as MRSA-colonized.

Group 3: Patients with gram-positive cocci/*S. aureus* wound/skin and soft tissue/surgical site infections

The limit of detection (LOD) of the StaphSR assay for detecting MRSA and *S. aureus* (SA) was determined by testing serial dilutions of MRSA and MSSA strains in trypticase soy broth (TSB) or pooled culture- and spacer region (SR)-negative wound broth. Consecutive wound/SST (skin and soft tissue) swabs were eluted in TSB; 50µl of wound broth was extracted and StaphSR performed per protocol. For StaphSR results incongruent with culture, repeat culture identification and PCR for femA and mecA genes were performed. femA-positive specimens were considered SA-positive, and mecA-positive SA isolates were considered MRSA-positive. Sensitivity, specificity, and efficiency of StaphSR for detecting MRSA and MSSA were calculated using the cut-off cycle threshold (CT) value determined in the LOD studies. Culture was used as the diagnostic standard, and results were recalculated in the case of incongruent results.

Average LOD for MRSA and SA of 250 and 390 CFU/ml, respectively, consistently generated StaphSR CT values ≤39. The sensitivity, specificity, and efficiency of StaphSR in 264 wound/SST specimens after resolution of incongruent results were 92.2 percent (confidence interval [CI], 84.6-96.8 percent), 97.7 (CI, 94.2-99.4 percent), and 95.8 (CI, 92.7-97.9 percent), respectively, for MRSA alone and 88.6 percent (CI, 79.5-94.3 percent), 94.7 percent (CI, 93.1-98.8 percent), and 94.3 (CI, 90.8-96.8), respectively, for MSSA alone. Of 26 incongruent results, 5 (1.9 percent) were misidentified by StaphSR due to altered genetic targets, 14 (5.4 percent) were false StaphSR results not attributable to altered genetic targets, and 7 (2.7 percent) were MRSA/MSSA-positive by StaphSR and confirmatory tests but MRSA/MSSA-negative by routine culture. Three specimens, culture-positive for MSSA, were misidentified by StaphSR as MRSA and were mecA-negative. Two specimens, culture-positive for MRSA, were misidentified as MSSA by StaphSR and were mecA-positive.

The StaphSR assay identified several MRSA/MSSA-positive specimens that were not detected by routine culture. However, StaphSR was not reliable for use on wound/SST specimens due to the high rate of incongruent results and the existence of certain SA clones in which methicillin susceptibility could not be accurately assessed. Use of this assay was discontinued.

Specific Aim 3 (A. baumannii)

Sub-Aim 1. To develop and validate an MLVA assay for molecular typing of *A. baumannii*

Building on our previous findings, we applied MLVA using seven loci to three sets of isolates with different epidemiologic backgrounds:
1. 14 isolates from six U.S. states with different pulse-field gel electrophoresis (PFGE) patterns
2. 10 isolates from the above collection with identical/highly similar PFGE patterns
3. 10 isolates from a recent outbreak at our hospital with highly similar PFGE patterns.

One of the loci we used (locus A) did not provide consistent variable number of tandem repeat (VNTR) numbers, depending on the primer sets or annealing temperatures used. This inconsistency was resolved by determining the accurate repeat numbers using proofreading DNA polymerase and defining the PCR condition that yielded the same results using regular DNA polymerase. We determined that the use of four loci (MLVA4) gave excellent discriminatory power compared with PFGE and multilocus sequence typing (MLST) and provided epidemiologically-relevant grouping information. These findings were submitted as an abstract to the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC; September 2011). In addition, a manuscript on the epidemiological study of carbapenem-resistant *A. baumannii* is being revised for the *Journal of Clinical Microbiology*.

Sub-Aim 2. To implement an active surveillance program at UPMC (University of Pittsburgh Medical Center) Mercy for patients colonized with MDR *A. baumannii* and to determine the effectiveness of this intervention

Building on the results from the first year, in which we evaluated a novel active screening method using sponges, an *A. baumannii* screening program was launched at UPMC Mercy on June 1, 2010. Every admission to the three ICUs is screened upon admission, then every seventh day thereafter.

An average of 450 screening specimens have been processed at the clinical microbiology laboratory of UPMC Mercy monthly. The positivity ranged from 2.1-5.4 percent, depending on the month, but overall remained stable with no clear trends. We plan to systematically implement daily chlorhexidine mouth wash and bathing for patients who are positive for *A. baumannii*. In addition, data from our previous study examining screening using sponges were published in the January 2011 issue of the *Journal of Clinical Microbiology*.

Specific Aim 4 (Modeling)

Sub-aim 1: Determine the effect of surveillance and rapid diagnosis of MRSA on transmission and disease caused by MRSA in the health care setting

Sub-aim 2: Determine the effect of surveillance and control measures for asymptomatic *C. difficile* carriers on transmission and disease caused by *C. difficile* in the health care setting

Sub-aim 3: Determine the effect of surveillance and control measures for multi-drug resistant (MDR) *A. baumannii* on transmission and disease caused by MDR *A. baumannii* in the health care setting

We developed an economic computer simulation model to determine the potential cost consequences of implementing routine *A. baumannii* screening of intensive care unit (ICU) admissions and isolating those patients who tested positive and to compare the cost of two screening methods (sponge versus swab). Sensitivity analyses varied between colonization prevalence, percentage of colonized individuals who had active *A. baumannii* infections, *A. baumannii* reproductive rate (R), and contact isolation efficacy. Both screening methods were cost effective for almost all scenarios tested, yielding cost savings ranging from $1 to $1,563. Sponge screening was not cost-saving when colonization prevalence was ≤1 percent, probability of infection ≤30 percent, R ≤0.25, and contact isolation efficacy ≤25 percent. Swab screening was not cost-saving under these same conditions when the probability of infection was ≤40 percent. Sponge screening tended to be more cost-saving than swab screening (additional savings ranged from $1 to $421). Routinely screening ICU patients for *A. baumannii* may provide economic value to hospitals. These results are in press in *Clinical Microbiology and Infection*.

Specific Aim 5 (Minority training program)

The minority training portion of the project continued to flourish during the past reporting period. A seven-week research training program is designed to expose underrepresented and disadvantaged students to biomedical research in infectious diseases, specifically, MRSA, *C. difficile,* and *A. baumannii.*

Summer Research

Admission Procedures

To support this programmatic aim, students were recruited from the applicant pool of the University of Pittsburgh School of Medicine (UPSOM) Summer Premedical Academic Enrichment Program (SPAEP), Level II. For the summer 2010 program, 271 students submitted the basic application, and 161 completed their applications with all required documentation. For the summer 2011 program, 299 students submitted basic applications, while 178 completed the full application.

Program Structure

The comprehensive training curriculum requires each student to spend 4.5 days each week performing mentored laboratory research and .5 days each week on enrichment activities, such as attending application skills seminars and talks by underrepresented role model physicians and scientists, shadowing physicians and scientists, and completing a mock interview. Students spend several Saturday mornings learning about test-taking skills for the Medical College Admission Test (MCAT). Finally, each student must present her/his research to an audience of peers and mentors, producing a formal research poster and a PowerPoint presentation.

The training coordinator, Paula K. Davis, MA, assistant vice chancellor for health sciences diversity, and staff in UPSOM's Offices of Admissions and Financial Aid and Student Affairs/Diversity Programs serve as the faculty for the admissions skills workshops. A former program participant serves as the dormitory monitor/student coordinator. Richard Levitt, MA, UPSOM academic development coordinator, provides the study and reading skills assessment and meets individually with each student to discuss his/her approach to learning as it relates to their future goals.

Students were selected according to CURE requirements that they belong to an underrepresented group, paying special attention to students attending Pennsylvania's historically black colleges and universities (HBCUs). The trainees during this reporting period were:

Student	Home Institution	Preceptor
2010		
Jeneana Parks	Lincoln University	Diana Pakstis, RN, BSN
Moises Baltazar Garcia	Florida Atlantic University	Bruce Lee, MD, MBA
Opeyemi Akinbamidele	Lehigh University	Jane Marsh, PhD
Mya Stayton	Tuskegee University	Jane Marsh, PhD
2011		
Alexandra Aponte-Velez	University of Puerto Rico	JoAnne Salangsang, MD
Joel James	University of Pittsburgh	Yohei Doi, MD, PhD
Borley Quaye	Spelman College	Scott Curry, MD
Chrystal Miller	Carnegie Mellon University	Scott Curry, MD

The 2010 participants are all in the process of completing their undergraduate degrees. The 2011 cohort comprises three rising seniors (Aponte, James, and Quaye) and one recent graduate (Miller) with aspirations in medicine/biomedical research.

Ms. Opeyemi Akinbamidele was trained in molecular typing and microbiologic techniques for studying MRSA. Ms. Akinbamidele conducted a study to test the accuracy and utility of an algorithm that uses a combination of MRSA molecular typing methods for predicting common PFGE types of MRSA. Specifically, she tested the utility of detecting the toxic shock syndrome toxin gene and testing trimethoprim-sulfamethoxazole susceptibility within the algorithm. Ms. Akinbamidele became proficient in performing molecular typing of MRSA using PCR and antimicrobial susceptibility testing of SA isolates using disk diffusion. In addition, she learned to critically evaluate scientific literature, calculate performance characteristics of a diagnostic test, design a PowerPoint presentation, and present data to a scientific audience. Her study showed that the algorithm had good sensitivity and negative predictive value for common MRSA pulsotypes. Ms. Akinbamidele gave an oral presentation on her study at the University of Pittsburgh at the conclusion of her training.

Ms. Mya Stayton was recruited for the *C. difficile* component of the grant. Ms. Stayton was trained in the operation and maintenance of the Coy anaerobic chamber, isolation of *C. difficile* from specimens, and antimicrobial sensitivity testing for anaerobes. She performed a pilot project demonstrating that tetracycline-resistant *C. difficile* at UPMC remains susceptible *in vitro*

to tigecycline. Ms. Stayton presented a poster of her work at the Professional Agricultural Workers Conference in Tuskeegee, Alabama, and took the top prize in a poster competition.

Mr. Moises Baltazar was trained in computational biology techniques. Mr. Baltazar assisted in constructing MRSA and *C. difficile* computational models, which involved searching for appropriate literature, analyzing the literature, abstracting information and numbers, constructing the models, running simulations, and analyzing results. Mr. Baltazar also gained other practical skills, such as how to critically evaluate scientific literature, design a scientific poster and PowerPoint presentation, and present data to a scientific audience. He also gave an oral presentation at the conclusion of his training.

Ms. Jeneana Cierra Parks learned techniques for molecular typing of MRSA isolates. She was trained in basic laboratory techniques, such as susceptibility testing of *S. aureus* by disk diffusion and basic molecular biology techniques. In addition, she had the opportunity to work with infectious diseases research nurses and learn data collection techniques, consenting processes, and critical thinking skills. Ms. Parks gained practical evaluation and presentation skills and presented her data at the conclusion of her training.

2010-2011 Project Titles

Student	**Home Institution**	**Research Project/Presentation**
	2010	
Jeneana Parks	Lincoln University	"Investigating Better Ways to Catch and Treat Infectious Diseases in Transplant and Non-Transplant Patients"
Moises Baltazar Garcia	Florida Atlantic University	Studied computational models of MRSA and *C. difficile*
Opeyemi Akinbamidele	Lehigh University	"The Utility of a Molecular Typing Algorithm for Inferring Common Pulsotypes of Methicillin-Resistant *Staphylococcus aureus* (MRSA)"
Mya Stayton	Tuskegee University	"Tetracycline and Tigecycline Resistance in *Clostridium difficile"*
	2011	
Alexandra Aponte-Velez	University of Puerto Rico	Title TBA
Joel James	University of Pittsburgh	Title TBA
Borley Quaye	Spelman College	Title TBA
Chrystal Miller	Carnegie Mellon University	Title TBA

Postbaccalaureate Training

To meet the goals of the postbaccalaureate training aim, we created the University of Pittsburgh Intramural Research Training Award (UPIRTA) program (http://www.healthdiversity.pitt.edu/programs/upirta.php), a nine-month to one-year postbaccalaureate experience for underrepresented students who are interested in biomedical graduate training. Students are placed with research preceptors (under the umbrella of Dr.

Harrison's lab) according to their interests and abilities. Experiences begin in the fall of each award year.

As with the summer research program, all students are eligible to apply; however, preference in admission is given to students from Lincoln and Cheyney Universities (Pennsylvania's two HBCUs), University of Pittsburgh students, and Pittsburgh-area and Pennsylvania natives.

Students are recruited at minority pre-health and graduate opportunity fairs (such as the Annual Biomedical Research Conference for Minority Students, the Society for the Advancement of Chicanos and Native Americans in Science, and the Chaka Fattah Graduate Opportunities Conference), through the honors programs of Lincoln and Cheyney Universities, and through the National Association for Advisors of the Health Professions. Applicants are vetted by Ms. Davis and John P. Horn, PhD, associate dean for graduate studies in the School of Medicine. Ms. Davis and Dr. Horn screen applications for each student's interest and potential for success (as judged by grade point average [GPA] and prior research activity).

Program participants during this reporting period were:

Student	Undergraduate Institution	Preceptor
	2009-10	
Tatianna Henderson	Lincoln University	Scott Curry, MD
Esenwa Obi Onuoha	Virginia Union University	Yohei Doi, MD, PhD
2010-11		
Travis Hamilton	University of Pittsburgh	Scott Curry, MD
Jesabel Rivera	University of Puerto Rico	Yohei Doi, MD/PhD
Nakita Brown	Lincoln University	Scott Curry, MD
Naomi Collier (did not complete)	Cheyney University	Bruce Lee, MD

2009-2010
Ms. Tatianna Henderson and Ms. Esenwa Onuoha were both successful in parlaying their UPIRTA time into an additional year of funded research experience. Both are currently preparing to apply to medical school, with the intention of including research as part of their medical careers.

2010-2011
Ms. Nakita Brown and Mr. Travis Hamilton were recruited for the *C. difficile* component of the grant. Mr. Hamilton was trained in the operation and maintenance of the Coy anaerobic chamber, isolation of *C. difficile* from specimens and antimicrobial sensitivity testing for anaerobes, cell culture cytotoxicity testing, routine PCR and gene sequencing, RT-PCR assay development for detection of *C. difficile tcdB* and toxin C (*tcdC*), and in MLVA for genotyping of *C. difficile.* He completed all of the laboratory aims for the typing of *C. difficile* from the long-term care facility sub-aim as well as for the typing of *C. difficile* recovered from the gastroenterology clinic and terminal disinfection pilot projects. Ms. Brown was trained by infection control personnel at UPMC Presbyterian to observe hand hygiene habits of health care workers. She devised a data collection form and database to record observations of contact

isolation practices for *C. difficile* inpatients. Her baseline data will be instrumental in analyzing the results of sub-aim 4. Ms. Brown also learned basic anaerobic microbiology laboratory skills for *C. difficile* and took over the storage and databasing of *C. difficile*-positive stool specimens for the project year. Both students were presenting authors for poster presentations/abstracts at the Society for Healthcare Epidemiology of America conference in Dallas, Texas, in April 2011.

Ms. Jesabel Rivera was recruited for the *A. baumannii* component of the grant. The techniques she learned include species identification; susceptibility testing; PCR and sequencing analysis; and the use of the Bionumerics, a software program that is now the standard in molecular epidemiological analysis. She also participated in laboratory meetings and practiced presentations on several occasions. Ms. Rivera presented her research at the intramural UPIRTA research presentation. She was also selected to present her work and received a travel scholarship to attend the 51st Interscience Conference on Antimicrobial Chemotherapy in Chicago this fall. She wrote this abstract herself with assistance from the co-investigator. Ms. Rivera has since been admitted to the University of Pittsburgh Graduate School of Public Health's Master of Public Health (MPH) program in infectious diseases and microbiology. She plans to continue to work with the co-investigator to prepare manuscript after completion of her training period.

Ms. Naomi Collier was recruited to work on infectious disease modeling of MRSA infection. During her limited time on the MRSA project, she performed literature searches and abstracted data from the relevant literature to help populate and calibrate the MRSA models. Unfortunately, Ms. Collier left the program early, marking the first instance of attrition in the program.

2010-2011 Projects:

Student	**Preceptor**	**Presentation**
Travis Hamilton	Scott Curry, MD	"Hic et Ubique: *Clostridium Difficile* in Nursing Facilities and Hospital Rooms"
Jesabel Rivera	Yohei Doi, MD, PhD	"Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) in *Acinetobacter Baumannii*"
Nakita Brown	Scott Curry, MD	"To Err is Human: *C. Difficile* vs. Health Care Workers at Outpatient Clinics"

In addition to their research, the students engage in a comprehensive schedule of enrichment activities. They participate in all lab meetings and group-wide, comprehensive Pennsylvania Department of Health (PA-DOH) progress meetings. In addition, they participate in the University of Pittsburgh's "Survival Skills and Ethics" program, designed to teach graduate students and early career researchers key professional "survival" skills such as making effective presentations, navigating career paths, and obtaining grant funding.

The admission process for the 2011-12 programs has recently concluded, and the new UPIRTA participants will be:

Student	Undergraduate Institution
2011 - 12	
Sobie Maduka	Carnegie Mellon University
Bianca Montalmont	Lincoln University
Micah Shelton	McDaniel College (Maryland)