Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** University of Pittsburgh

2. **Reporting Period (start and end date of grant award period):** June 1, 2009-May 31, 2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Debbie Moschetti

4. **Grant Contact Person's Telephone Number:** 412-648-6402

5. **Grant SAP Number:** 4100047864

6. **Project Number and Title of Research Project:** Center of Excellence in Prevention and Control of Antibiotic Resistant Bacterial Infections

7. **Start and End Date of Research Project:** 6/ 1/2009 – 5/31/ 2013

8. **Name of Principal Investigator for the Research Project:** Lee H. Harrison, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $4,724,321

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	Institution	% of Effort on Project	Cost
Afriyie, Abena	Student	Univ of Pittsburgh	100% Yr 1	2,185
Adams, Jennifer	Research IV	Univ of Pittsburgh	100% Yr 1-2	51,301
Adams, Jennifer	Research IV	Univ of Pittsburgh	75% Yr 3-4	56,316
Bacon, Kristina	Student	Univ of Pittsburgh	100% Yr 1	10,026
Bacon, Kristina	Research Assistant	Univ of Pittsburgh	98% Yr 4	41,143
Bailey, Rachel	Research Assistant	Univ of Pittsburgh	10% Yr 2	4,273
Bartsch, Sarah	Research Assistant	Univ of Pittsburgh	25% Yr 1-4	93,608
Brooks, Maria	Associate Professor	Univ of Pittsburgh	20% Yr 1-4	107,378
Brown, Nakita	Trainee	Univ of Pittsburgh	100% Yr 2	25,176
Cakouros, Brigid	Research Assistant	Univ of Pittsburgh	98% Yr 4	40,214
Cashman, Elizabeth	Associate Professor	CMU	5% Yr 1-2	11,449
Cashman, Elizabeth	Associate Professor	CMU	10% Yr 3	12,326
Cashman, Elizabeth	Associate Professor	CMU	15% Yr 4	833
Collier, Naomi	Trainee	Univ of Pittsburgh	100% Yr 2	17,690
Connor, Diana	Student	Univ of Pittsburgh	100% Yr 1	2,980
Connor, Diana	Research Assistant	Univ of Pittsburgh	98% Yr 4	42,659
Curry, Scott	Assistant Professor	Univ of Pittsburgh	78% Yr 1-4	379,231
Daniel, Michael	Student	Univ of Pittsburgh	100% Yr 1	1,000
Doi, Yohei	Assistant Professor	Univ of Pittsburgh	31% Yr 1	36,205
Doi, Yohei	Assistant Professor	Univ of Pittsburgh	18% Yr 2	16,157
Doi, Yohei	Assistant Professor	Univ of Pittsburgh	77% Yr 3	91,161
Doi, Yohei	Assistant Professor	Univ of Pittsburgh	64% Yr 4	75,398
Feura, Jared	Student	Univ of Pittsburgh	100% Yr 1	5,903
Haidari, Leila	Student	Univ of Pittsburgh	100% Yr 4	3,643
Hamilton, Travis	Trainee	Univ of Pittsburgh	100% Yr 2	25,176
Harrison, Lee	Professor	Univ of Pittsburgh	15%	102,136

			Yr 1-3	
Harrison, Lee	Professor	Univ of Pittsburgh	11% Yr 4	27,401
Henderson, Tatianna	Trainee	Univ of Pittsburgh	100% Yr 1	40,327
Johnston, Eleanor	Student	Univ of Pittsburgh	100% Yr 4	3,845
Kitchen, Sarah	Student	Univ of Pittsburgh	100 Yr 2	2,706
Krauland, Mary	Bioinformatician	Univ of Pittsburgh	55% Yr 3	36,111
Krauland, Mary	Bioinformatician	Univ of Pittsburgh	100% Yr 4	68,234
Lave, Lester	Professor	CMU	5% Yr 1-2	24,872
Law, Angela	Administrator	Univ of Pittsburgh	60% Yr 1	25,900
Law, Angela	Administrator	Univ of Pittsburgh	50% Yr 2-3	36,570
Lee, Bruce	Associate Professor	Univ of Pittsburgh	7.5% Yr 1-4	225,081
Maduka, Sobe	Trainee	Univ of Pittsburgh	100% Yr 3	18,997
Marsh, Jane	Research Assistant Professor	Univ of Pittsburgh	20% Yr 1-2	42,841
Marsh, Jane	Research Assistant Professor	Univ of Pittsburgh	40% Yr 3-4	79,174
McGaha, Traci	Research Coordinator	Univ of Pittsburgh	100% Yr 1-4	173,484
Montalmant, Bianca	Trainee	Univ of Pittsburgh	100% Yr 3	17,880
Moore, Deylan	Trainee	Univ of Pittsburgh	100% Yr 4	19,867
Moschetti, Deborah	Administrator	Univ of Pittsburgh	50% Yr 2-3	44,262
Mueller, Leslie	Student	Univ of Pittsburgh	100 Yr 4	3,109
Muto, Carlene	Associate Professor	Univ of Pittsburgh	5% Yr 1-4	13,713
Nelson, Jeremy	Bioinformatics Analyst	Univ of Pittsburgh	100% Yr 4	38,973
O'Hara, Jessica	Research Specialist	Univ of Pittsburgh	85% Yr 3-4	39,853
Ohm, Sara	Student	Univ of Pittsburgh	100% Yr 4	1,211
Onuoha, Obi Ezenwa	Trainee	Univ of Pittsburgh	100% Yr 1	20,510
Pakstis, Diana	Program Director	Univ of Pittsburgh	5% Yr 1-2	10,991

Pakstis, Diana	Program Director	Univ of Pittsburgh	10% Yr 3	11,182
Pakstis, Diana	Program Director	Univ of Pittsburgh	15% Yr 4	18,708
Parker, Bridget	Student	Univ of Pittsburgh	100% Yr 1	1,000
Pasculle, William	Associate Professor	Univ of Pittsburgh	5% Yr 1-4	35,344
Quaye, Borley	Trainee	Univ of Pittsburgh	100% Yr 4	24,840
Ricotta, Emily	Research III	Univ of Pittsburgh	100% Yr 1	37,777
Rivera DeJesus, Jesabel	Student/Trainee	Univ of Pittsburgh	100% Yr 1-3	39,273
Salangsang, Jo-Anne	Instructor	Univ of Pittsburgh	84% Yr 1-2	147,826
Schlackman, Jessica	Research Specialist	Univ of Pittsburgh	100% Yr 1	32,975
Schlackman, Jessica	Research Specialist	Univ of Pittsburgh	76% Yr 2	30,621
Schlackman, Jessica	Research Specialist	Univ of Pittsburgh	25% Yr 3	10,636
Schmitz, Michelle	Research Assistant	Univ of Pittsburgh	98% Yr 4	36,030
Shelton, Micah	Trainee	Univ of Pittsburgh	100% Yr 3	25,560
Shutt, Kathleen	Research Associate	Univ of Pittsburgh	15% Yr 1-2	26,647
Shutt, Kathleen	Research Associate	Univ of Pittsburgh	37% Yr 3	24,975
Shutt, Kathleen	Research Associate	Univ of Pittsburgh	26% Yr 4	25,313
Song, Yeohan	Student	Univ of Pittsburgh	100% Yr 1	6,276
Spychala, Caressa	Research III	Univ of Pittsburgh	100% Yr 3-4	52,667
Stalter, Randy	Student	Univ of Pittsburgh	100% Yr 2	4,000
Syed, Alveena	Research Coordinator	Univ of Pittsburgh	100% Yr 4	32,791
Townsend, Bethany	Trainee	Univ of Pittsburgh	100% Yr 4	23,840
Tsui, Fu Chiang	Assistant Professor	Univ of Pittsburgh	3% Yr 1-4	19,334
Ye, Tian	Student	Univ of Pittsburgh	100% Yr 1	2,288
Willig, Alyssa	Student	Univ of Pittsburgh	100% Yr 1	3,184
Wiringa, Ann	Research Assistant	Univ of Pittsburgh	50% Yr 1	19,191

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if

percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	Institution	% of Effort on Project
None			

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Upright Freezer	Storage of samples until analysis could occur	8,785
Biomerieux EasyMag system	DNA extraction and downstream sequence analysis	51,500
Smartcycler Desktop system	Utilized for MRSA analysis	43,720
Bucket Microplate & adapter	Used in high through put sequencing	8,673
Real Time PCR system	Rapid identification of MRSA and Clostridium gene targets	53,215
Multigene Gradient Thermal Cycler	Optimization of PCR for MRSA and Clostridium gene targets	5,009
Biological Safety Cabinet	Safe handling of infectious agents of MRSA and Clostridium agents and other biological and environmental material	6,625
PourMatic Plate Pourer	High through put for clostridium isolate selection	17,602
Ettan IPGphor3	Utilized for proteomic analysis	8,594

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Optimizing Detection of MRSA Carriage (PI: Y. Doi)	☐NIH X Other federal (specify: AHRQ) ☐ Nonfederal source (specify: _____)	Oct 2011	$100,000	$99,355
Colistin-resistant *A. baumannii* (PI: Y. Doi)	X NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	May 2012	$1,861,161	Fundable score (11 percentile). The status of this grant is pending.
Ecology and evolution of *Clostridium difficile*: Modeling transmission in humans, swine, and the environment (PI: L. Harrison)	☐NIH X Other federal (specify: National Science Foundation) ☐ Nonfederal source (specify: _____)	November 2012	$2,499,702	Pending review of resubmissio n
Novel strategies for control and prevention of *C. difficile* infections (K08, PI: S. Curry)	☐NIH X Other federal (specify: AHRQ) ☐ Nonfederal source (specify: _____)	Aug 2012	$733,030	$ Not funded (to be resubmitted)

Role of asymptomatic carriage in *C. difficile* transmission (R01, PI: L. Harrison)	☐NIH X Other federal (specify:) ☐ Nonfederal source (specify: _____)	October 2013	$1,416,251	Pending Review

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes

If yes, please describe your plans:

This grant has laid the groundwork for additional research in antibiotic-resistant bacterial infections. We plan to submit additional grant applications that involve all of the aims of the project, including MRSA, *C. difficile*, *A. baumannii*, and modeling.

12. Future of Research Project. What are the future plans for this research project?

We anticipate that the work funded by this research project will lead to additional avenues for research over the next 3-5 years.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male	3		4	
Female	9		8	
Unknown				
Total	**12**		**12**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic	3		1	
Non-Hispanic	9		11	
Unknown				
Total	**12**		**12**	

	Undergraduate	Masters	Pre-doc	Post-doc

White	3		1	
Black	9		11	
Asian				
Other				
Unknown				
Total	**12**		**12**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

No.

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes.

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The project allowed us to gain substantial additional experience, most notably in the areas of bacterial genomics, proteomics, and infectious diseases modeling. In addition, the purchase of equipment increased our laboratory infrastructure (9D above).

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes**.**

If **yes**, please describe the collaborations:

John J. Kane Regional Center- Glen Hazel, Division of Nursing Care Facilities in Allegheny County, where we conducted the following aims of the project: *Specific aim 1:* Control of *C. difficile* through identification of asymptomatic carriers and *Sub-aim 1b:* Determining cross-sectional prevalence of asymptomatic colonization at Kane Glen Hazel, a public skilled nursing facility.

16(B) Did the research project result in commercial development of any research products?

No.

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

No.

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The primary goal of the Center of Excellence in Prevention and Control of Antibiotic-Resistant Bacterial Infections is to employ novel strategies to reduce the morbidity and mortality caused by 3 bacterial pathogens that have recently emerged as major threats to vulnerable patients in the healthcare setting: *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), and multidrug-resistant (MDR) *Acinetobacter baumannii.*

The specific aims of the Center are to:

1. Develop, validate, and employ novel molecular detection methods of asymptomatic *C. difficile* carriage and assess an intervention to control this source of *C. difficile* disease

2. Understand characteristics of and risk factors for infection with community-associated MRSA strains that have recently been introduced into the hospital and employ rapid, PCR-based diagnosis of MRSA infection and colonization, to optimize antimicrobial therapy, reduce use of broad spectrum antimicrobials for treatment of *S. aureus* infections, and reduce transmission of MRSA

3. Develop a new, multilocus variable number tandem repeat analysis-based molecular subtyping tool for tracking MDR *A. baumannii* transmission, validate improved methods for detecting MDR *A. baumannii* colonization, and assess an intervention to control the spread of this organism in intensive care units

4. Employ infectious diseases modeling to leverage the findings from specific aims 1-3 and to understand the morbidity, mortality, and economic impact of the strategies we develop

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

Timeline Specific Aim 1 (Sub-aims 1-4)

Period	Milestones
6/1/09-6/30/09	• Submit to IRB • Begin equipment purchase
7/1/09-6/30/10	• Obtain informed consent from Kane Glen Hazel residents (sub-aim 1) • Kane Glen Hazel portion of sub-aim 1 (200 patients) • Pilot studies for sub-aim 3
7/1/10-6/30/11	• Sub-aims 1 and 2 carried out at UPMC-Presbyterian (3,300 patients screened in 6 months) • Data analysis and manuscript preparation for sub-aims 1, 2 • First 2 weeks of field testing for sub-aim 3
7/1/11-6/30/12	• Complete field testing for sub-aim 3 • First 6 months of sub-aim 4 (3,300 patients screened in real time)
7/1/12-5/31/13	• Final 6 months of sub-aim 4 (3,300 patients screened in real time)

Summary of research completed for Specific Aim 1 (Sub-aims 1-4) - *C. difficile*

Sub-Aim 1. Determine 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).

The data below pertain to both sub-aims 1 and 2 which are interdependent aims both conducted in the same prospective study from July – November 2009 and published in Clinical Infectious Diseases (Curry SR et al. *Clin Infect Dis*. 2013;57:1094-102).

Background. Previous studies have suggested that asymptomatic carriers of toxigenic *Clostridium difficile* are a source of hospital-associated (HA) infections. Multilocus variable number of tandem repeats analysis (MLVA) is a highly discriminatory molecular subtyping tool that helps to determine possible transmission sources. We performed MLVA genotyping of *C. difficile* isolates from colonized and infected patients to assess potential of each to HA-CDI and to determine the potential role of active surveillance for *C. difficile* carriage in a hospital with an established infection control program that has been associated with successful control of HA-CDI.

Methods: This study was conducted at the University of Pittsburgh Medical Center Presbyterian–Shadyside (UPMC), a 762-bed (156 intensive care unit [ICU] beds) teaching hospital affiliated with the University of Pittsburgh. Patients undergoing vancomycin-resistant enterococci (VRE) surveillance testing over a 117-day period in July 2009 through November 2009 were also screened for *C. difficile*. VRE surveillance cultures were performed at admission for patients admitted from other healthcare facilities; VRE surveillance was also performed at weekly intervals for all ICU patients and for all other inpatients with length of stay >20 days (>5 days if receiving antibiotics) or who tested positive for MRSA, *C. difficile*, or multidrug-resistant organisms. Patients identified as VRE positive were placed in contact isolation for current and subsequent healthcare and were not rescreened. *Clostridium difficile* reduction interventions did not change throughout the study, including contact isolation for the duration of hospitalization for patients identified as colonized or infected by *C. difficile* stool toxin testing Since 2006, the HA-CDI rate at UPMC has never exceeded our target rate of 8 per 10 000 patient-days. The average HA-CDI rate per year (2006–2012) was 5.6 cases per 10 000 patient-days. In addition to previously reported measures, 5000 ppm sodium hypochlorite was routinely used to disinfect all patient care areas. This study was approved by the University of Pittsburgh Institutional Review Board. Laboratory diagnosis of CDI was done using cell culture cytotoxicity assays (stool toxins) ordered by physicians (Diagnostic Hybrids, Athens, Ohio) of formed and unformed stool specimens. Both perirectal swabs collected for VRE surveillance (screening tests) and positive stool toxins were cultured for *C. difficile* using 48-hour broth enrichment and anaerobic culture. Positive stool toxin Specimens were cultured for C. difficile ±30 days relative to the 117-day study period. Toxigenic *C. difficile* was confirmed by *tcdC* genotyping for both stool toxins and screening tests. The field sensitivity of screening tests for detection of *C. difficile* was calculated as the number of screening tests positive for toxigenic *C. difficile* divided by the total number of screening tests collected patients with positive stool toxins from −1 to 0 days prior to collection

the stool toxin specimen. The results of *C. difficile* screening were not released to clinicians. *Clostridium difficile* isolates were typed using MLVA and *tcdC* genotyping. The *tcdC* genotypes in this study were assigned according to the PubMLST database (http://pubmlst.org/cdifficile) and were used to infer ribotype. Allele designations for *tcdC* conform to those previously described with the exception of genotypes 0, A1, and B, which have been renumbered as 19, 20, and 47 respectively. Minimum-spanning-tree analysis of all isolates recovered in the study was performed using BioNumerics software version 6.6 (Applied Maths, Austin, Texas), using a Manhattan coefficient to calculate the summed tandem-repeat difference (STRD) between isolates. Complexes containing ≥2 isolates whose MLVA genotypes generated an STRD of ≤2 were defined as highly related isolates. Patients with positive stool toxins collected at UPMC during the study period plus a lag period of 10 days (to account for the potential incubation period of CDI) were epidemiologically classified by UPMC infection control according to modified criteria for CDI surveillance. Hospital-associated CDI was defined by toxin positive stool and CDI symptom onset ≥3 days after admission to UPMC or present on readmission with documented UPMC exposure (without exposures to other healthcare facilities) in the preceding 12 weeks. CDIs occurring within 6 months of any previous CDI were classified as recurrent CDI. Patients with positive stool toxins but without signs of CDI on chart review were classified as carriers by infection control personnel. Patients with *C. difficile*–positive screening tests underwent electronic record review of stool toxins sent during the study period ±12 weeks, and patients without any stool toxins sent were defined as carriers and were categorized into 3 groups: (1) persistent carriers: patients with at least 2 screening tests positive for toxigenic *C. difficile* collected ≥7 days apart and no stool toxins ordered by clinicians; (2) transient carriers: patients with only 1 screening test positive for toxigenic *C. difficile* (or >1 test collected within a 7-day period), and negative screening tests collected before and afterward; and (3) indeterminate carriers: patients with single screening cultures (or >1 culture collected within a 7-day period) positive for toxigenic *C. difficile*. Patients who had ≥1 negative stool toxin sent during the study ±12 weeks were defined as discrepant carriers. Chart review to exclude a history of CDI diagnosed at non- UPMC facilities was performed for carriers linked to incident HA-CDI by MLVA. TheraDoc 4.3.0 software (Hospira, Salt Lake City, Utah) was used to determine patient locations for all patients with isolates highly related by MLVA to HA-CDI. These data were used to categorize each HA-CDI case to the most likely transmission route: (1) ward transmission: patients shared a common ward occupancy with a symptomatic CDI patient or carrier within 30 days; (2) non–ward transmission: patient hospitalized concurrently with carrier or CDI patient within 30 days without wards in common; (3) environmental: patient developed CDI after placement in a room previously occupied by a carrier or CDI patient; or (4) indeterminate: patients with no time–place epidemiologic links to patients with highly related isolates. For each patient, the collection date of the presumed source isolate had to precede that of the patient's C. difficile symptom onset date by at least 1 calendar day. To determine whether carriers had room contamination with *C. difficile*, environmental cultures were performed for all patients meeting the following criteria: (1) screening test positive for toxigenic *C. difficile*; (2) not in contact isolation precautions; and (3) inpatient at the time of culture confirmation. Five sites in each room were cultured with selective broth amplification using sterile 2 × 2-inch gauze pads pre-moistened with phosphate-buffered saline. For ICUs, the sites were the patient call bell, bed rails, bedside table, toilet seat, and computer keyboard. For non-ICU rooms, the sites were the same except the patient bedside telephone receiver was substituted for the computer keyboard.

Results: During the screening period, 158 patients had positive stool toxin assays for *C. difficile* ordered by clinicians, of whom 34 (22%) were classified as carriers. Of the remaining 124 asymptomatic patients, 53 (43%), were classified as HA-CDI at UPMC, 13 (10%) as community-acquired, and 57 (46%) as acquired at other facilities or relapses. Of 61 cases of HA-CDI during the screening period plus the 10-day lag period, isolates were obtained from 56 (92%) cases. Of these, 17 cases were unrelated by MLVA to any isolate recovered during the study and 5 were chronologically first within a complex of highly related isolates; these latter were recovered as late as study day 74. Detailed molecular combined with time–place epidemiologic analysis was thus performed for 33 of 61 (54%) HA-CDI cases (Table 1). Carriers identified by screening tests only were identified as the sources for a total of 16 of 56 (29%) HA-CDI cases in the study period; of these, 9 were classified as non–ward transmissions, 2 as ward transmissions, 2 as environmental transmissions, and 2 as indeterminate. The presumed source for 1 HACDI case classified as a non–ward carrier transmission was a patient who was later diagnosed with CDI; this source patient's screening tests were positive 35 days prior to the collection of the first stool toxin assay, and the patient was not in contact isolation at the time of the presumed transmission event. Four of the carriers identified as source patients were already in contact isolation for infections with other organisms (Table 1). One carrier detected by positive stool toxin testing was implicated as the source of a ward transmission (Table 1). No other HA-CDI cases could be traced to a source that was determined to be colonized by stool toxin testing. Transmission events from CDI patients comprised 17 of 56 (30%) cases; of these, 9 were classified as non–ward transmissions, 7 as ward CDI transmissions, and 2 as environmental transmissions (Table 1). The ward transmission events occurred in 7 different units. During the study period, 422 of 4979 (8.5%) screening tests performed on 3006 patients were positive for toxigenic *C. difficile*. Of 3006 patients screened, 314 (10.4%) and 506 (16.8%) were positive for toxigenic *C. difficile* and VRE on at least 1 specimen, respectively. Of the 3006 patients, 1957 (65.1%), 598 (19.9%), 229 (7.6%), 104 (3.5%), 53 (1.8%), and 65 (2.2%) patients had a total of 1, 2, 3, 4, 5, and >5 screening tests performed, respectively. Of 314 patients positive for toxigenic *C. difficile*, 120 (38.2%) patients were also positive for VRE at least once, whereas 194 patients had no positive VRE tests. Positive screening for VRE during the study was associated with increased risk of being positive for toxigenic *C. difficile* on at least 1 specimen (odds ratio [OR], 3.7; 95% confidence interval [CI], 2.9–4.8). Of the 120 patients positive for both C. difficile and VRE, *C. difficile* was identified first in 17 of 120 (14%) patients, on the same day in 95 of 120 patients (80%), and afterward in 6 of 120 (6%) patients. During the 117-day screening period, 417 *C. difficile* isolates were obtained from the positive stool toxins and/or screening cultures of 384 patients. Of these patients, 226 of 384 (58.9%) were detected using screening cultures only. Of these patients, 131 of 226 (58.0%), 58 of 226 (25.7%), 27 of 226 (11.9%), and 10 of 226 (4.4%) were classified as indeterminate-length, discrepant, persistent, and transient carriers, respectively. During the screening period, 3006 of 12 054 (24.9%) admissions were tested for VRE. Five of 6 patients who met the criteria for environmental culturing had recovery of *C. difficile* from at least 1 site. A patient with no recovery of *C. difficile* was classified as a transient carrier and had been moved to a different room by the date of room sampling. All 5 patients with room sites positive for *C. difficile* had isolates highly related by MLVA to isolates from 1 or more screening cultures. Additionally, 3 environmental isolates from 2 patients were unrelated (STRD >10) to any of their screening culture isolates. The most frequently contaminated site was the patient call bell.

Table 1. Epidemiologic analysis of 33 patients with *C. difficile* infections classified as hospital-acquired at UPMC July – November 2009. Patients for whom an isolate was unavailable for genotyping (n=6) and with *C. difficile* having unique MLVA genotypes (n=17) or isolates that were chronologically first within a complex of highly related isolates (n=5) are not depicted.

study day	*tcdC* geno type	same unit?	same bed?	concurrent hospital stay	Dates of exposure to source[a]	Classification of presumed source[b]	# Day between source and patient culture	Classification of most likely transmission Category	STRD (source to case)
9	1	no	no	yes	-6 to 0	**2**	**-6**	NON-WARD CDI	3
10	1	yes	no	yes	-2 to -1	**6**	**-28**	WARD CDI	0
12	1	yes	yes	yes	-7 to -3	**2**	**-9**	ENVIRONMENTAL	1
16	3	yes	no	yes	-5 to 0	**3**	**-8**	WARD CDI	0
19	1	no	no	yes	-7 to 0	**6**	**-9**	NON-WARD CDI	5
27	1	yes	no	yes	-6 to 0	**1**	**-1**	WARD CARRIER	0
33	1	no	no	yes	-11 to -5	**4**	**-11**	NON-WARD CARRIER	0
37[c]	1	yes	no	yes	-6 to -6	**6**	**-12**	WARD CDI	5
44	1	yes	yes	yes	-24 to -22	**2**	**-7**	ENVIRONMENTAL	0
45	1	yes	no	yes	-7 to -4	**2**	**-8**	WARD CDI	0
47	5	no	no	yes	-12 to -11	**5[d]**	**-11**	NON-WARD CARRIER[d]	0
48[c]	1	yes	no	yes	-13 to -1	**1**	**-4**	WARD CARRIER	3
62	1	no	no	yes	-17 to 0	**6**	**-1**	NON-WARD CDI	2
68	20	no	no	yes	-24 to -21	**1**	**-25**	NON-WARD CARRIER[e]	3
71	19	no	no	yes	-34 to -14	**7**	**-14**	NON-WARD CARRIER	0
71[c]	42	no	no	no	unknown	**1**	**-34**	INDETERMINATE[e]	1
75	1	yes	yes	no	-6 to -6	**1**	**-18**	ENVIRONMENTAL	0
90	1	no	no	yes	-16 to 0	**7**	**-10**	NON-WARD CARRIER	1
93[c]	3	no	no	yes	-25 to -8	**1**	**-15**	NON-WARD CARRIER[e]	2
94[c]	1	no	no	yes	-50 to -39	**4**	**-43**	NON-WARD CARRIER[e]	1
94	1	no	no	yes	-55 to -40	**4**	**-44**	NON-WARD CARRIER	1
94	1	no	no	yes	-12 to -12	**4**	**-16**	NON-WARD CARRIER[e]	4
101	1	yes	no	yes	-6 to -1	**2**	**-6**	WARD CDI	0
101	1	no	no	no	unknown	**1**	**-35**	INDETERMINATE	3
114	1	no	no	yes	-4 to 0	**4**	**-4**	NON-WARD CARRIER	0
117	1	no	no	yes	-2 to 0	**2**	**-3**	NON-WARD CDI	0
119	1	yes	no	yes	-3 to 0	**6**	**-8**	WARD CDI	0
120	1	yes	no	yes	-6 to -6	**2**	**-14**	WARD CDI	2
120	1	no	no	yes	-21 to 0	**2**	**-7**	NON-WARD CDI	0
120	1	no	no	yes	-21 to 0	**2**	**-7**	NON-WARD CDI	0
125	1	no	no	yes	-10 to 0	**2**	**-5**	NON-WARD CDI	0
126	1	yes	yes	yes	-12 to -12	**4**	**-15**	ENVIRONMENTAL	3
127	1	no	no	yes	-12 to -4	**2**	**-7**	NON-WARD CDI	1

[a]relative to patient's toxin testing date

[b] 1=discrepant carrier, 2= *C. difficile* infection acquired at University of Pittsburgh Medical Center (UPMC); 3=hospital-acquired colonization with *C. difficile* detected by toxin test ordered by clinicians; 4=indeterminate carrier; 5= other CDI (infection not acquired at UPMC); 6=recurrent CDI; 7= persistent carrier
[c] indicates CDI that was attributable to UPMC after discharge (community-onset, healthcare-facility associated CO-HCFA)
[d] source patient's first positive toxin test occurred on study day 50 after having positive screening cultures on days 15, 22, 36, and 49
[e] source patient was positive other organism requiring isolation prior to screening positive for *C. difficile*
HA-CDI: *C. difficile* infection acquired at UPMC; HA-colonized: hospital-acquired colonization with C. difficile detected by toxin test ordered by clinicians; other-CDI: *C. difficile* infection not acquired at UPMC; STRD: summed tandem repeat difference;

Conclusions: In our hospital with an established infection control program designed to contain transmission from symptomatic CDI patients, asymptomatic carriers appear to have played an important role in transmission. Identification and isolation of carriers may be necessary to further reduce transmission of *C. difficile* in such settings.

Data for sub-aims 1 and 2 were presented as oral presentation at the 2010 Interscience Conference on Antimicrobial Agents and Chemotherapy meeting in Boston (September 14, 2010). The manuscript was also published in *Clinical Infectious Diseases* (Curry SR et al. *Clin Infect Dis*. 2013;57:1094-102) and was accompanied by an editorial by Dr. Clifford McDonald of the Centers for Disease Control and Prevention.

<u>Sub-aim 1b</u>: Prevalence of *C. difficile* carriage and environmental contamination at a publicly funded skilled nursing facility. This sub-aim was part of the original research proposal. The study was conducted over 12 months from October 2010 through October 2011. The full data remain in analysis after completion of the MLVA genotyping for the project in 2013. A manuscript based on these data will be submitted in 2014 to *Infection Control and Hospital Epidemiology*. An abstract of the preliminary results appears below:

Background: Previous cross-sectional estimates of the prevalence of *C. difficile* colonization in long-term-care facility residents have varied widely.

Objective: To determine the prevalence of *C. difficile* colonization over time in long-term care facility associated with a hospital system with low rates of *C. difficile* infections (CDI).

Design: Prospective cohort study.

Setting: A 210-bed public skilled nursing facility in Pittsburgh, PA.

Participants: 78 long-term care residents able to sign informed consent.

Methods: All residents were screened by anaerobic stool or rectal swab culture using broth enrichment culture methods every 3 months for one year for the presence of *C. difficile*. All isolates were typed by MLVA and *tcdC* genotyping. 100 environmental cultures were performed after the last resident sampling

Results: After enrolling 78 of 210 residents, we found toxigenic *C. difficile* in 5/70 (7.1%), 3/58 (5.2%), 4/51 (7.8%), and 3/45 (6.7%) residents during the four samplings of this cohort over 1 year. Only one patient was positive during all four samplings; the room of this patient had the only positive environmental cultures (2/100 or 2%) for toxigenic *C. difficile*.

Conclusions: The residents of this long-term-care facility with effective infection control measures had a relatively low prevalence of *C. difficile* colonization. Most residents who were found to be *C. difficile* positive were colonized transiently.

Specific aim 1, sub-aim 1: The following add-on study was performed in 2010-2011 to further evaluate the extent of environmental contamination of the hospital with *C. difficile.*

Background: Based on our findings that many of our incident *C. difficile* cases were attributable to probable failure to disinfect patient rooms after discharge of a *C. difficile* patient or carrier, we conducted a pilot study of the effectiveness of terminal room disinfection for *C. difficile* patients.

Methods: Fifteen rooms were sampled following 10% bleach disinfection by housekeeping personnel after transfer or discharge of a known CD patient before occupancy by a new patient; housekeepers were unaware of the study. For 14 rooms, 10 high-contact sites (BP cuff, monitor cables, IV pump/pole, bed rails/buttons, call bell, bedside table, patient chair, sink handles, toilet handle, toilet seat) were sampled using sterile gloves and aseptic technique with a single sterile 2x2 inch sterile cotton gauze pad moistened with 2 mL phosphate-buffered saline transferred after sampling to a 50 mL conical tube. For 1 room (room 3), the ten sites were subdivided into 5 groups. Tubes were transported to the lab within 60 minutes of collection where 10 mL CCMB-TAL (Anaerobe Systems, Morgan Hill CA) was added to each tube. Samples were transferred to an anaerobic chamber and incubated for 72 hours at 37 °C. Fermenting samples were sub-cultured to pre-reduced 5% sheep blood agar. *C. difficile* was identified by colony morphology and L-prolineaminopeptidase activity. Isolates were confirmed as toxigenic using RT-PCR for *tcdB* and genotyped by MLVA.

Results: 4/13 (30.8%) rooms sampled were positive for toxigenic *C. difficile*. For the third room sampled, 1/5 culture groups was positive (call bell). Of the CD positive rooms, *C. difficile* stool isolates matched the stool isolate of the prior room occupant by MLVA genotyping for 2/4 patients.

Conclusions: Persistence of room contamination with *C. difficile* was common in our study. As bleach disinfectant is sporicidal, some high contact surfaces, such as the patient call bell which are contacted by patients, RN staff, and physicians for various functions, appear to be either have been missed or were more difficult to disinfect.

The above study was presented as a poster at the Society for Healthcare Epidemiology of America's annual meeting in Dallas, Texas, April 2, 2011, poster #181. The data were not published for peer review but were used as the basis for an ongoing quality assurance initiative for terminal room disinfection within UPMC.

Specific Aim 1, Sub-Aim 3. Develop and validate a real-time (RT) PCR assay for rapid identification of *C. difficile* carriage using peri-rectal swabs

Background: Anaerobic culture of rectal swabs has previously been shown to be as sensitive as stool culture for the detection of *Clostridium difficile*. The development of FDA-approved commercial real-time PCR assays for *C. difficile* has improved the timeliness of diagnosis of *C. difficile* infections (CDI) relative to that with both toxigenic culture and cell culture cytotoxicity assays, but the sensitivity of PCR assays has ranged between 77.3 and 86% compared to

toxigenic culture. We sought to develop a PCR-based test that combines the sensitivity of toxigenic culture with the timeliness of real-time PCR and that could be performed on swab specimens in a clinical microbiology lab.

Methods: All specimens tested were perirectal swabs (BBL CultureSwab Plus Amies medium without charcoal; Becton Dickinson, Sparks, MD) obtained for vancomycin-resistant enterococcus (VRE) surveillance testing at UPMC Presbyterian, a 766-bed tertiary-care teaching facility affiliated with the University of Pittsburgh Schools of the Health Sciences. The criteria for VRE screening were as follows: for admission cultures, VRE testing was performed for patients admitted from other hospitals or from long-term care facilities or patients admitted to rehabilitation and transitional care units; for weekly cultures, patients in contact isolation for methicillin-resistant *Staphylococcus aureus* (MRSA), *C. difficile*, or other multidrug-resistant organisms (MDRO), patients whose length of stay exceeded 20 days, patients whose length of stay exceeded 5 days and who had received antibiotics at any time during hospitalization, or patients in intensive-care units were tested for VRE. UPMC Presbyterian uses cell culture cytotoxicity assays with Hs27 cells and *C. difficile* antitoxin (Diagnostic Hybrids, Athens, OH) for the clinical diagnosis of *C. difficile* in stool. The results of *C. difficile* screening were not released to clinicians. This study was approved by the University of Pittsburgh Institutional Review Board.

Broth pre-amplification and toxigenic culture.
After inoculation of the VRE screening medium, perirectal swabs were reinserted in Amies medium carrier and were then transferred to 5 ml pre-reduced cycloserine cefoxitin mannitol broth with 0.1% taurocholate and 0.5% lysozyme (CCMB-TAL) in 16- by 100-mm glass Hungate tubes (Anaerobe Systems). After 15 s of agitation, tubes were recapped and incubated for as long as 72 h at 37°C. All procedures were performed under aerobic conditions. Fermenting cultures (fermentation was detected as a shift in the medium color from red to yellow) were transferred to an anaerobic chamber (Coy Labs, Grass Lake, MI) containing an atmosphere of 80% N_2, 10% CO_2, and 2 to 5% H_2, and 10 µl of this pre-amplified broth culture was plated onto pre-reduced Trypticase soy agar with 5% sheep blood (TSA II) plates (Becton Dickinson, Franklin Lakes, NJ) and was incubated anaerobically for 48 h at 37°C. Colonies morphologically consistent with *C. difficile* were sub-cultured and incubated for 48 h at 37°C. Identification of *C. difficile* was confirmed by L-proline aminopeptidase activity (PRO Disk; Remel, Lenexa, KS). Isolates were stored in pre-reduced chopped-meat broth (Anaerobe Systems). Toxigenic isolates were identified by *tcdC* genotyping or by the modified Prodesse ProGastro Cd assay (Gen-Probe Prodesse, Waukesha, WI) (described below). Isolates not identified as toxigenic by PCR were confirmed as non-toxigenic using a commercial cell culture cytotoxicity assay according to the manufacturer's instructions (Diagnostic Hybrids, Athens, OH).

Collection of samples.
Perirectal swabs were collected in two phases, one for assay development and the other for validation. For phase I (development), 96 pre-amplified broth cultures collected between July and November 2009 were selected. These included 32 samples with toxigenic *C. difficile*, 4 with non-toxigenic *C. difficile*, and 60 samples negative for *C. difficile*. All pre-amplified broth samples were stored at -80°C until testing.

For phase II (validation), 400 sequential perirectal VRE swabs were collected between September and November 2010 and were processed in real time. Swabs were scored for visible feculence. Investigators were blinded to the results of toxigenic culture.

DNA extraction.
DNA was extracted from *C. difficile* isolates and pre-amplified CCMB-TAL cultures by a NucliSENS easyMAG system (bioMérieux, Durham, NC). For *C. difficile* isolates, 48-h growth from a single tryptone (3%)-yeast extract (2%) agar plate with 50 mM l-arginine (TYR) was inoculated into 2 ml of easyMAG lysis buffer. For pre-amplified CCMB-TAL cultures, 100 µl of the fermented broth was transferred to 2 ml of easyMAG lysis buffer and was lysed for 10 min at room temperature, followed by automated nucleic acid extraction using the off-board lysis protocol with an elution volume of 75 µl. DNA was stored at −20°C.

PCR detection of toxigenic C. difficile.
The ProGastro Cd assay kit for *tcdB* (Gen-Probe Prodesse) was modified to run on the ABI 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA). The total reaction volume was reduced to 12.5 µl. For phase I, all reaction mixtures consisted of 10 µl ProGastro Cd master mix, 1.5 µl genomic DNA eluate, and 1 µl of the internal positive control (IPC) included in the kit. For phase II, 10 µl of the IPC was added to fermented broth cultures during the off-board lysis step according to the manufacturer's instructions. During phase II, all reaction mixtures consisted of 10 µl master mix and 2.5 µl genomic DNA. Samples were run in 100-µl 96-well reaction plates (MicroAmp Fast; Applied Biosystems) on the ABI 7500 Fast instrument using standard ramp speeds and the "Quantitation-Standard Curve" experiment type in the ABI 7500 software, version 2.0.4. Data were collected for two different reporter dyes, 6-carboxyfluorescein (FAM) (for the *tcdB* signal) and Cy5 (for the IPC). Cycling conditions were as follows: 60°C for 1 min, 95°C for 10 min, and 45 cycles consisting of 95°C for 15 s and 60°C for 60 s. The results were analyzed with ABI 7500 software, version 2.0.4, using ΔRn thresholds of 100,000 and 200,000 for the manufacturer's internal positive control and *tcdB*, respectively. IPC-only, positive *C. difficile* DNA, and water controls were run with each 96-well plate tested. Samples were considered positive for *tcdB* if the cycle threshold (C_T) was ≤45, regardless of the IPC result. Samples that did not meet this threshold for *tcdB* were considered negative for *tcdB* if the IPC threshold was met at cycle 45 or any lower cycle. Samples for which neither target met the threshold were considered failed reactions.

Samples in phases I and II that were *tcdB* positive but negative by toxigenic culture were further analyzed using a second PCR target assay, for *tcdR*, the toxin-regulatory gene found only within the 19.6-kb pathogenicity locus of toxigenic strains of *C. difficile*. All reactions were performed in triplicate. Reaction mixtures (20 µl) consisted of 300 nM *tcdR*-F (5′-CATTATGAAGAGGGAGAAACAGATTT-3′) and *tcdR*-R (5′-CTAGACAACTCAAAAGTCTTATTCAG-3′), 1 µl genomic DNA, and 10 µl PerfeCTa SYBR green FastMix, Low ROX (Quanta BioSciences, Gaithersburg, MD) with the following cycling conditions on the ABI 7500 Fast cycler: 95°C for 20 s, followed by 60 cycles of 95°C for 3 s and 60°C for 30 s, with a final melt curve dissociation step. Results were analyzed on ABI 7500 software, version 2.0.4, using a manually entered ΔRn threshold of 0.1. Samples were considered positive for *tcdR* if the C_T was ≤45 for all three reactions, with a standard deviation of <0.5 cycle within replicates and with dissociation melt curves of 72.1°C ± 0.15°C for all three reactions.

Limit-of-detection analysis.
To test the laboratory sensitivity of the modified *tcdB* PCR assay, 25 serial 2-fold dilutions of overnight TYR broth cultures from two genetically distinct *C. difficile* strains were prepared in phosphate-buffered saline under anaerobic conditions. A 100-µl sample of each dilution was plated in triplicate on TYR agar plates. Plates were incubated anaerobically for 72 h at 37°C. The number of CFU/ml was calculated by multiplying the dilution by the number of colonies × 10. In addition, 100 µl of the 10 highest serial 2-fold dilutions (16 through 25) was subjected to either broth pre-amplification followed by DNA extraction or direct DNA extraction without broth pre-amplification. DNA was extracted from 100 µl of pre-amplified broth cultures whether there was evidence of fermentation or not. All DNA extracted was tested using the modified Prodesse PCR assay described above for phase II.

Field sensitivity.
During phase II, the history of *C. difficile* stool toxin testing ± 14 days of the date of collection of each VRE swab was recorded from patients' charts. These results were used to determine the field sensitivity of broth pre-amplification plus PCR for detection of *C. difficile* in patients known to be positive for *C. difficile* by stool tests ordered by clinicians.

Data analysis.
The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the PCR assay C_T values were estimated using toxigenic culture for *C. difficile* as the gold standard. The Clopper-Pearson exact method was used to calculate 95% confidence intervals (CI) for the estimated sensitivity, specificity, PPV, and NPV binomial probabilities using C_Ts of 30 and 45 cycles to define positive reactions in the PCR assay.

Results:
Development phase.
During phase I, 32/32 specimens positive by toxigenic culture were positive by the modified *tcdB* PCR assay, with a C_T range of 16.1 to 26.7 (sensitivity, 100% [95% CI, 89.1 to 100%]). Of the 64 specimens negative by toxigenic culture, 55 (specificity, 85.9% [95% CI, 75.0% to 93.4%]) were negative by the modified *tcdB* PCR assay, with no inhibition events or reaction failures. Nine of 64 (14.1%) toxigenic culture-negative specimens were positive by the modified *tcdB* PCR assay (C_T, ≤45). Of these 9 discrepant samples, 7 were positive for *tcdR*. The modified *tcdB* PCR assay was repeated, and 8 samples had C_T*s* within 1 cycle of the first result, except for 1 sample that was negative upon repeat testing.

Validation phase.
In the validation phase, broth pre-amplification and subsequent *tcdB* PCR, as well as toxigenic culture for *C. difficile*, were performed on 400 sequential VRE surveillance swabs from 370 patients. Of these 370 patients, 39 (10.5%) had *C. difficile* by toxigenic culture. Of the 39 patients with toxigenic *C. difficile*, 12 (30.8% of the patients with positive results; 3.2% of total patients screened) had no history of stool testing for *C. difficile* toxins ordered by clinicians within 14 days of the sample collection date. For the remaining patients from whose swabs *C. difficile* was recovered, 19/39 (48.7%) and 8/39 (20.5%) samples came from patients with histories of positive and negative results, respectively, on tests for *C. difficile* stool toxins ordered by clinicians within 14 days of sample collection.

Visible fermentation was observed in 138/400 (34.5%) pre-amplified broth cultures within 72 h. The remaining 262 (65.5%) pre-amplified broth cultures did not ferment and were not tested further for *C. difficile*. Of the 138 fermenting broth samples, 38 (27.5%) were positive by toxigenic culture. In contrast, 49/138 (35.5%) fermenting broth cultures were positive by *tcdB* PCR within 72 h, with a C_T range of 16.2 to 41.0. Real-time PCR for *tcdR* was performed on the 11 toxigenic-culture-negative broth cultures that were *tcdB* positive. Only 3 of these samples were *tcdR* positive.

Agreement between the PCR assay and toxigenic culture.
Table 2 shows the agreement of the PCR assay and toxigenic culture over eight cycle threshold cutoffs for *tcdB*. A cycle threshold of 30 maximized specificity and sensitivity (Table 2). The sensitivity, specificity, negative predictive value, and positive predictive value obtained by defining the *tcdB* PCR cutoff as a C_T of <30 are shown in Table 3.

Table 2. Comparison of broth pre-amplification plus *tcdB* PCR to toxigenic culture.

Ct for tcdB	No. samples with the following result by toxigenic culture:		No. samples positive / no. tested by PCR for tcdR	Cumulative sensitivity for Ct (%)	Cumulative specificity for Ct (%)
	Positive	Negative			
<20	24	1	1/1	34.3	99.8
20.1-25.0	41	1	1/1	92.9	99.5
25.1-30.0	5	2	2/2	100	99.1
30.1-35.0	0	4	2/4	100	98.1
35.1-40.0	0	7	3/7	100	96.5
40.1-45.0	0	5	1/5	100	95.3
>45	0	144	0/0	100	61.5
No PCR	0	262	0/0	100	0
Total	70	426	10/20		

Table 3. Comparison of broth pre-amplification plus modified *tcdB* PCR assay with toxigenic culture

	Toxigenic culture		Assay performance characteristic (%, [95% CI])			
Broth pre-amplification + tcdB PCR	Pos.	Neg.	Sensitivity	Specificity	PPV	NPV
Positive	70	4	100 (95-100)	99.1 (98-100)	94.6 (87-98)	100.0 (99-100)
Negative	0	422				
Total						

Limit of detection.
The *tcdB* PCR assay was able to detect 21 and 59 CFU for the two different strains tested. However, when a C_T cutoff of <30 was used to define positive results, as above, the limits of detection of the assay were 3.8 and 4.6 $\log_{10}$ CFU. With CCMB-TAL pre-amplification of the same strains, the modified *tcdB* PCR assay is capable of detecting a single organism with a C_T of <20.

Comparison of the modified tcdB assay with clinical C. difficile testing results.
Of the 370 patients from whom 400 VRE swabs were collected during phase II, 30 (8.1%) patients had histories of positive results for *C. difficile* by the stool cytotoxicity assay ± 14 days of VRE swab collection. Based on this sample of 30 patients with concurrent positive results by *C. difficile* testing, we estimate that 17/30 (56.7% [95% CI, 37.4 to 74.5%]), 15/23 (65.2% [95% CI, 42.7 to 83.6%]), 12/18 (66.7% [95% CI, 41.0 to 86.7%]), and 10/10 (100% [95% CI, 69.2 to 100.0%]) patients with positive results by the stool cytotoxicity assay for *C. difficile* had swabs that tested positive for *C. difficile* by the modified *tcdB* PCR assay and were collected within 14, 10, 7, and 3 days of VRE swab collection, respectively. Only one specimen had a *tcdB* C_T of >30 (C_T, 33.0) and was negative by real-time PCR for *tcdR* and toxigenic culture. Only 4/30 swabs were visibly feculent.

Time and cost of the assay.
The time required to extract DNA from 24 specimens was 47 min of machine time plus approximately 45 min of technician time. The PCR run time for a full plate of 96 specimens was 2.8 h plus 45 min of technician time for setup. Specimen inoculation took 30 s per specimen. The time from fermentation to reporting of a PCR result was 4 to 6 h, depending on how many specimens in excess of 24 fermented per 24 h, requiring DNA extraction in two batches prior to PCR. During the validation of the assay, 12/33 (36.4%), 21/80 (26.3%), and 5/25 (20.0%) positive results were finalized at 30, 54, and 78 h from the first processing of the swab, compared to 96 to 168 h for toxigenic anaerobic culture.

The materials cost of the assay, including the swab, is $3.32 + $12.56*x*, where *x* is the fraction of CCMB-TAL specimens that have visible fermentation at 72 h and require DNA extraction and PCR. The cost thus ranges from $3.32 to $15.88 per sample. During phase II of this study, the cost per sample was $7.66.

Conclusions: The performance characteristics of the assay in the present study exceed the published sensitivities and specificities of several published MRSA PCR-based screening tests while the turnaround time is equivalent to that of culture-based MRSA screens that have been associated with reductions in the rate of hospital-acquired MRSA infections. In fact, the addition of selective broth pre-amplification to the PCR assay substantially improves the sensitivity of this assay over the reported sensitivity of 77.3% in the only published study to use anaerobic toxigenic culture on all specimens as the gold standard.

The addition of selective broth amplification dramatically increases the laboratory sensitivity of the PCR assay to 1 CFU, compared to ~4 $\log_{10}$ CFU. Thus, it is not surprising that the sensitivity of the PCR assay, both for the detection of known *C. difficile* patients (n = 10) and for the

detection of *C. difficile* carriers (n = 28), compared to toxigenic culture, is 100%. A correspondingly high specificity (99.3%) is achieved only by lowering the positive C_T value to <30, which lowered the number of false-positive results from 20 to 4 (Table 2). Of these four remaining samples with false-positive results at this C_T cutoff, all were positive for a second target found within the *C. difficile* pathogenicity locus, *tcdR*, suggesting that the PCR assay may be more sensitive than the gold standard of toxigenic culture under some circumstances. These data were published in the *Journal of Clinical Microbiology* (Curry SR et al. Perirectal swab surveillance for *Clostridium difficile* by use of selective broth pre-amplification and real-time PCR detection of tcdB. *J Clin Microbiol*. 2011 Nov;49(11):3788-93, see also Table in section 20).

Specific Aim 1, Sub-Aim 4: Implement enhanced surveillance and control of *C. difficile* at an academic tertiary care hospital using active surveillance testing.

We decided to not move forward with sub-aim 4 in year 3 of the project (2012). The rationale for cancelling this sub-aim is that 1) the rates of *C. difficile* in our institution had become so low that we no longer have the statistical power to show an impact of the intervention and 2) our hospital changed the assay that is performed for diagnosis of *C. difficile* infection in February 2013. This assay is substantially more sensitive than the one that the hospital has been using, which made it impossible to do the planned before and after comparisons of *C. difficile* infection incidence that were required for this sub-aim.

Sub-aim 4 was replaced by three replacement Sub-aims 4a-4c.

Specific Aim 1, Replacement Sub-aim 4a (Study initiated January 2011): Identify the prevalence of food contamination with *C. difficile* in the Pittsburgh area, estimating the potential contribution of the food supply to CDI.

Introduction: The prevalence of *Clostridium difficile* contamination of food products has varied widely, ranging from 0 to 42%. The *C. difficile* strains recovered in these studies have included lineages, such as ribotypes 078 and 027, that are commonly encountered in human outbreaks of *C. difficile* infections (CDI), but no epidemiologic connection between human CDI and the food supply has been made. Recent evidence suggests either that some *C. difficile* strains are widespread in the food supply or that laboratory contamination led to overestimation of the prevalence of *C. difficile* in some studies.

We performed a study of *C. difficile* prevalence in raw retail ground meats in grocery stores in the Pittsburgh, PA, area. Multilocus variable-number tandem-repeat analysis (MLVA), a highly discriminatory genotyping method, was used to evaluate *C. difficil*e isolated from food products and to determine the genetic relatedness between *C. difficile* isolates recovered from food and isolates associated with human CDI.

Methods:

Sampling scheme.

We performed a convenience sampling of 102 raw ground meat products (20 beef, 2 buffalo, 22 chicken, 2 lamb, 41 pork, 10 turkey, and 5 veal) from 3 grocery stores in Pittsburgh, PA. Products in the initial sampling were purchased between 23 February and 28 April 2011. Items purchased included samples of all available deli counter ground meats, all fresh sausages prepared in store, and selected raw, commercially packaged fresh and frozen sausages. Items were kept in their original packaging until the time of sampling. Where available, the USDA establishment number identifying the facility of origin for each product was recorded. Cultures were performed before the sell-by date for each packaged item. Based on the results of the initial sampling, all available brand A pork sausage products were resampled on three separate purchase dates.

Microbiological methods.

Broth enrichment cultures were performed for all samples as follows: 10 g meat was aseptically transferred to 100 ml cycloserine (500 mg/liter)-cefoxitin (15.5 mg/liter)-mannitol broth with 0.1% taurocholate and 0.5% lysozyme (CCMB-TAL) in 120-ml sterile specimen containers (Starplex Scientific, Etobicoke, Ontario, Canada) and incubated anaerobically at 37°C for 5 days in an anaerobic chamber (Coy Labs, Grass Lake, MI). The CCMB-TAL was not pre-reduced prior to use. Lids to the specimen containers were loosened to allow for reduction of the liquid medium by exchange with the chamber atmosphere. Visibly fermented samples were sub-cultured to pre-reduced Trypticase soy agar with 5% sheep blood (SBA; Becton Dickinson, Franklin Lakes, NJ) and further sub-cultured to SBA until pure. Colony morphologies on SBA that were consistent with *C. difficile* were confirmed using l-proline aminopeptidase activity (Pro Disk; Remel, Lenexa, KS). A 10-g meat sample spiked with 10 µl of strain CD41 spore stock ($\sim 10^5$ CFU) served as a positive control for every 8 samples processed. In addition, a CCMB-TAL medium negative control of 100 ml was included for every 8 samples processed. *C. difficile* isolates were stored in chopped meat broth (Anaerobe Systems, Morgan Hill, CA). Genomic DNA was extracted after 48 hours of growth of meat broth stocks on brain heart infusion yeast extract agar with 0.1% taurocholate (BHIYT) using an automated magnetic bead extraction platform (NucliSens easyMag; bioMérieux, Durham, NC).

MLVA and *tcdC* genotyping were performed for all isolates. The *tcdC* genotype for each isolate was used to infer the ribotype. Because two MLVA tandem-repeat loci (CDR4 and CDR5) are absent in inferred ribotype 078 (*tcdC* genotype 20) isolates, the summed tandem-repeat difference (STRD) was calculated using only MLVA loci CDR6, CDR9, CDR48, CDR49, and CDR60.*tcdC* genotypes conform to previously published alleles, with the exception that genotype A/A1 has been renamed genotype 20 to match the *tcdC* genotypes available on the PubMLST website (http://www.pubmlst.org/cdifficile). All isolates with a *tcdC* genotype were presumed to be toxigenic.

The MLVA genotypes recovered were compared to a database of 1,863 isolates collected between 2001 and 2009 from CDI patients and asymptomatic carriers at our institution, including 67 isolates originating from community-acquired, community-onset CDI patients diagnosed between January and June 2011. Food and UPMC clinical isolates were considered highly related if the STRD was ≤ 2 .

Enumeration of C. difficile in positive samples.
Quantitative testing of all positive meat samples was performed by direct plating of a 100-µl aliquot from a 1-g meat sample in 9 ml sterile distilled water on CCMB-TAL agar. Because this method would fail to enumerate samples with a contamination density of <100 spores/g, a most-probable-number (MPN) enumeration for 3 positive samples was performed. For the first dilution, 1 g of each sample was cultured in 10 ml CCMB-TAL in each of 5 tubes. For the second and third dilutions, 1,000 and 100 µl, respectively, of a 1-g suspension of meat in 9 ml CCMB-TAL were cultured in 10 ml CCMB-TAL in each of 5 tubes. Each fermenting tube was evaluated for *C. difficile* as above. Estimates of the spore concentration within the original sample were calculated as outlined in appendix 2 of the Food and Drug Administration's *Bacteriological Analytical Manual*.

Prevention of cross-contamination in food samples.
Samples were kept in their original packaging until laboratory processing and transported in disposable plastic grocery bags.

The following measures were observed to prevent cross-contamination: (i) broth amplification cultures were set up in batches of no more than 25 per day; (ii) samples were set up in a biological safety cabinet in a laboratory separate from the laboratory containing the anaerobic chamber; (iii) all test tube racks, transfer spatulas, and work surfaces were autoclaved on the day of setup; (iv) gloves were changed between each sample; (v) 5,000 ppm sodium hypochlorite was used to disinfect autoclave-intolerant items, including the packages of meat before opening; (vi) the sterile, individually wrapped 120-ml wide-mouthed specimen cups used for broth amplification were kept in sterile overwraps until use; (vii) disposable plastic inoculating loops were used for subcultures; and (viii) meat was kept in a dedicated refrigerator that has never stored *C. difficile* cultures or specimens and is located in a laboratory separate from that used for meat processing and *C. difficile* culture procedures. The selected positive-control strain (CD41) represents a rare MLVA and *tcdC* genotype. The CCMB-TAL negative control was set up after processing every eighth sample to monitor for potential laboratory contamination during processing and culture; in addition, the negative-control specimen cup was used as the liquid measuring device for the preceding 8 samples.

Results:
Prevalence of *C. difficile* in sampled meat products.
Of 102 samples of raw ground meat in the initial sampling (February to April 2011), 2 samples (2.0%; 95% confidence interval [CI], 0.20 to 7.9%) were contaminated with toxigenic *C. difficile*. The two positive samples were brand A pork sausages that were purchased on separate dates and cultured in different batches. One of the positive samples was from sausage patties; the other was a 16-ounce sausage chub. Both products originated from the same processing facility (facility A). One other brand A product processed in facility B was negative for *C. difficile* during the initial sampling. All CCMB-TAL negative controls were culture negative.

Of 34 brand A products resampled on 14 May 2011, 15 August 2011, and 2 January 2012, 4/12 (33%), 6/9 (67%), and 1/10 (10%) were positive for *C. difficile*, respectively. Of 13 samples

positive for *C. difficile* from the entire study, 12 originated from facility A and 1 originated from facility B.

Enumeration of C. difficile spores in positive samples.
Direct plate counts of 13 meat products positive for *C. difficile* were negative for recovery of *C. difficile*. For the three products evaluated by the MPN method, the spore counts were 0.20 (95% CI, 0.01 to 1.0), 0.45 (95% CI, 0.08 to 1.5), and <0.1 (95% CI, <0.18 to 0.68) spores/g.
Genotyping of *C. difficile* isolates.

The 13 samples positive for *C. difficile* yielded 14 isolates with 6 distinct *C. difficile* MLVA genotypes. One food sample (FD113) yielded two isolates with distinct MLVA genotypes; the second was from an isolate recovered during the MPN procedures. All isolates were inferred ribotype 078, *tcdC* genotype 20. Three MLVA genotypes (A, B, and D) were observed on sampling dates 2, 4, and 5 months apart. The isolate from the product originating in facility B had a unique MLVA genotype distantly related to other genotypes (genotype E)
Relationship between *C. difficile* in food and clinical isolates.

Of the MLVA-typed isolates in our database, 24 are *tcdC* genotype 20. One food isolate (FD126) purchased 18 August 2011 was identical by MLVA to the isolate from a CDI case diagnosed 10 years earlier (July 2001). FD27 was highly related (STRD = 2) to 1 other clinical isolate from a patient diagnosed with CDI in 2009. Both patient isolates were categorized as hospital-acquired infections. No patient isolate categorized as community onset or community acquired that was collected between January and June 2011 (n = 67) had a genotype consistent with ribotype 078 (*tcdC* genotype 20).

Conclusions:
In this study, ground pork products from a single brand were shown to be contaminated with *C. difficile*. Of the 13 contaminated products, 12 originated from a single processing facility. This suggests that *C. difficile* contamination of ground pork products occurred at or before the level of the meat processing facility. The genotypes of all of the isolates were consistent with ribotype 078, a lineage common in food animals. Despite MLVA's limited discriminatory power for ribotype 078, 6 unrelated MLVA genotypes were identified among the food isolates. This observation highlights an essential role of MLVA for evaluating the prevalence of *C. difficile* in food. Molecular genotyping methods, such as PCR-ribotyping, do not provide sufficient discrimination to differentiate closely related *C. difficile* isolates. Ribotyping could not discriminate the food isolates in this study and could have led to the erroneous conclusion that identical *C. difficile* strains were present in food products produced in separate processing facilities. MLVA clearly demonstrates the presence of 6 distinct *C. difficile* genotypes in brand A pork products. These data are highly indicative of food-borne *C. difficile* contamination and do not support a laboratory contamination event.

The identification of *C. difficile* with multiple, distinct MLVA genotypes from different ground pork products from a single retail meat processing facility over 5 months suggests probable environmental contamination within that facility. Alternatively, continuous reintroduction of *C. difficile* from various pig farms or contamination of products handled within the facility by workers who carry *C. difficile* may be occurring.

The level of spore contamination was low (<0.18 to 0.45 spores/g), consistent with a previous study (Weese et al 2009. *Appl Environ. Microbiol* 75:5009-5011). Further research is required to determine whether low levels of spore contamination are able to cause human infections. The low prevalence of ribotype 078, *tcdC* genotype 20 *C. difficile* in hospital- and community-acquired CDI cases from our institution, however, suggests that *C. difficile* in the local food supply may result in only a small proportion of human CDI cases.

Our reported prevalence of *C. difficile* in retail meat was low and in accordance with that found in most previous studies. Our approach to avoiding laboratory cross-contamination was rigorous and similar in principle to that used in labs performing PCR using dedicated clean rooms.
In summary, we confirmed that *C. difficile* occurs as a low-level contaminant in some uncooked meats and that this contamination may result from events occurring at or before the processing facility. MLVA genotyping of positive samples and other steps to minimize the chances for laboratory cross-contamination should be considered in future studies of the potential contribution of the food supply to *C. difficile* infections in humans. Additional studies to define the contribution of *C. difficile* in food to human CDI are needed

This work was originally presented in abstract form at the 7th International Conference on the Molecular Biology and Pathogenesis of the Clostridia [ClosPath 2011], Ames, IA, 28 October 2011. These results were published in April 2012 in *Applied and Environmental Microbiology* (Curry SR, et al. *Appl Environ Microbiol*. 2012;78:4183-6).

Specific Aim 1, Replacement Sub-aim 4b (Study initiated December 2012): Genomic studies to identify discriminatory single nucleotide polymorphisms in ribotype 027 *C. difficile* isolates

Objectives: Multi-locus variable number tandem repeat analysis (MLVA) is a highly discriminatory method for tracking transmission of *C. difficile* but may eventually be replaced by whole genome sequencing (WGS). We determined the correlation between *Clostridium difficile* genotypes obtained by whole genome sequencing (WGS) SNP genotyping and MLVA.

***Methods*:** WGS and MLVA were performed on 36 BI/NAP1/027 *C. difficile* isolates that were chosen to include a range of relatedness as determined by MLVA summed tandem repeat differences (STRD) of 0-46. Six pairs of isolates were epidemiologically related; two pairs were identical by MLVA but epidemiologically unrelated. WGS was performed by Illumina with paired ends. R20291 was used as the reference genome for calling single nucleotide polymorphisms (SNPs) using SolSNP. Pairwise comparison and linear regression of SNP and STRD values from all 36 isolates was performed using R.

***Results*:** Number of SNP differences among BI/NAP1/027 isolate pairs ranged from 2-71; linear regression showed a correlation between STRD and SNP differences between pairs (R^2=0.63). For epidemiologically related and un-related pairs, SNPs ranged from 2-19 and 16-20, respectively (Table 4).

***Conclusions*:** MLVA and SNP genotyping correlated well for *C. difficile*. In some instances, SNPs provided additional discrimination. WGS has the potential to replace MLVA for studies of *C. difficile* transmission.

Table 4. MLVA compared to SNP differences for 8 pairs of BI/NAP1/027 *C. difficile* isolates obtained from UPMC Presbyterian 2001-9.

Isolate pair	No. SNPs	STRD	Time interval (days)	Epidemiology
1*	9	0	25	Serial isolates from single patient with recurrent CDI
2*	3	0	92	Serial isolates from single patient with recurrent CDI
3*	10	1	295	Serial isolates from single patient with recurrent CDI
4*	19	6	45	Serial isolates from single patient with recurrent CDI
5*	2	1	9	Same-ward transmission event
6*	9	0	4	Isolates from *C. difficile* carrier and call bell in room
7	20	0	1378	Unrelated
8	16	0	262	Unrelated

*Epidemiologically related pairs

These data were presented as a late-breaking poster exhibition at the European Congress of Clinical Microbiology and Infectious Diseases in Berlin [Curry SR, Krauland MG, Marsh JW, Nelson JS, and Harrison LH. Correlation of Multi-locus Variable Number Tandem Repeat Analysis (MLVA) and Whole Genome Sequence Analysis (WGS) for *Clostridium difficile* Molecular Epidemiology. In: Abstracts of the 23rd European Conference on Clinical Microbiology and Infectious Diseases; Berlin, Germany, April 28, 2013. Late-breaker poster LB2967] These data have not been submitted to peer review.

Specific Aim 1, Replacement Sub-aim 4c (Study initiated February 2012): Identification of asymptomatic *Clostridium difficile* colonization among healthy adults

Background: Thirty percent of *C. difficile* infections (CDI) are estimated to be community-acquired, but the sources of these infections are unclear. Studies conducted outside the U.S. suggest that 7-15% of healthy adults are colonized with *C. difficile* (CD). The aims of this study were to examine the prevalence, genotypic distribution, and duration of CD colonization and to identify risk factors associated with colonization among healthy adults in the U.S.

Methods: Healthy adults were recruited via advertisements. Individuals who reported chronic diarrhea, recent hospitalization, history of CDI, or employment in a health care facility were excluded. Eligible participants provided epidemiologic data via written questionnaire and submitted a stool specimen. Subjects whose stool yielded CD were asked to submit additional specimens. Specimens were cultured for CD using direct plating and broth enrichment. CD isolates underwent *tcdC* genotyping. Genotypic data were compared between study subjects and a historic sample of unique CDI patients from the same geographic region. Epidemiologic data were analyzed to identify characteristics associated with CD colonization. Differences in the prevalence of categorical variables were assessed using the Fisher's exact test; differences in the

prevalence of continuous variables were assessed using the Wilcoxon rank-sum test. Analyses were conducted with SAS® Software (Version 9.2, SAS Institute, Cary, NC, USA).

Results: 106 (81%) of 130 subjects submitted specimens, 7 of whom (6.6%) were colonized with CD. Six subjects submitted additional specimens. Stool from 2 individuals yielded CD on 2 successive occasions 1 month apart. The following *tcdC* genotypic distribution was observed among 9 isolates from colonized individuals: *tcdC* 3, (n=1) *tcdC* 5, (n=1) *tcdC* 14, (n=1) *tcdC* 20, (n=1) tcdC 53, (n=1) *tcdC* 10, (n=2 specimens, 1 individual) *tcdC* 19, (n=2 specimens, 1 individual). The 7 *tcdC* genotypes identified among colonized individuals collectively comprise 33% of infections among a historic sample of 337 patients with CDI at UPMC in 2011. Demographics, diet, health care facility exposure, antibiotic exposure, and exposure to persons with CDI were not significantly associated with CD colonization, although the statistical power of this analysis was limited.

Conclusion: The prevalence of CD colonization in this healthy cohort is concordant with prior estimates in non-U.S. populations. Colonized individuals may be important reservoirs for CDI. A larger sample size is necessary to identify risk factors for CD colonization among healthy individuals.

Data from aims 4b and 4c are being prepared for submission to the *Journal of Clinical Microbiology*.

Specific Aim 1, Replacement Sub-aim 4d (Study not initiated):
Expansion of the original project at Kane Glen Hazel as a quality assurance project to identify and isolate *C. difficile* carriers at two of the four Kane Regional Centers. This study was not begun because we lacked the personnel to carry out the testing required in real time before the end of the grant period.

Original Timeline Specific Aim 2 (Sub-aims 1-2) as presented in Research Proposal

Period	Milestones
6/1/09-6/30/09	• Submit to IRB
7/1/09-6/30/10	• Genotyping of MRSA isolates (collection ongoing) for sub-aim 1 (900 subjects) • Identify pairs of matching nasal and clinical isolates and determine whether clinical isolates represent infection • Data analysis for sub-aim 1, preparation and submission of paper and submission for publication • Validation of BD GeneOhm Assays for blood cultures and nasal specimens
7/1/10-6/30/11	• Sub-aim 2, Group 1, Phases 1 and 2, (1,600 subjects)
7/1/11-6/30/12	• Data analysis for sub-aim 2, Group 1, preparation of paper, and submission for publication • Sub-aim 2, Group 2, Phases 1 and 2, (6,000 subjects) • Data analysis for sub-aim 2, Group 2, preparation of paper, and submission for publication
7/1/12-5/31/13	• Validation of BD GeneOhm StaphSR assay for wound cultures • Sub-aim 2, Group 3, Phases 1 and 2, (1,000 subjects) • Data analysis for sub-aim 2, Group 3, preparation of paper, and submission for publication

<u>Revised</u> Timeline Specific Aim 2 (Sub-aims 1-2) following initiation of 2 new studies (Replacement Sub-aims 2a and 2b)

Period	Milestones
6/1/09-6/30/09	• Submit to IRB
7/1/09-6/30/10	• Genotyping of MRSA isolates for sub-aim 1 (900 subjects) • Identify pairs of matching nasal and clinical isolates and determine whether clinical isolates represent infection • Data analysis for sub-aim 1, preparation of paper • Validation of BD GeneOhm Assays for blood cultures and nasal specimens • Sub-aim 2, Groups 1 and 2 (Nasal and blood cultures), Phases 1
7/1/10-6/30/11	• Sub-aim 2, Group 1, Phases 1, 2, and 3 completed • Group 2 (blood culture s) of Sub-aim 2 study component terminated because of problems with assay • Validation of BD GeneOhm StaphSR assay for wound cultures (failed) • Data analysis for sub-aim 2, Group 1, preparation of paper
3/1/11-5/31/13 7/1/11-5/31/13	• Replacement sub-aim 2a: characterize *S. aureus* blood and wound isolates with incongruent results from original sub-aim 2. • Replacement Sub-aim 2b: perform whole genome sequencing to identify genetic mechanisms of antibiotic resistance and study intra-patient evolution of hVISA.
7/1/11-6/30/12	• Preparation of papers for Sub-aims 1 and 2, and submission for publication
7/1/12-5/31/13	• Preparation of papers for Sub-aims 1 and 2, and submission for publication

<u>Summary of research completed for Specific Aim 2 (original sub-aims 1-2) MRSA</u>

<u>Specific Aim 2, Sub-Aim 1</u>. To compare the incidence of and identify risk factors for development of methicillin-resistant Staphylococcus aureus (MRSA) infection in subjects colonized with traditional healthcare-associated (HA) MRSA strains and the epidemic community-associated (CA) MRSA strain in the hospital setting. All of the information in the following section refers to original sub-aim 1.

Risk of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection in Subjects Colonized with Community-Associated versus Healthcare-Associated MRSA (original sub-aim 1)

Introduction:
In the U.S., 2 CA-MRSA clones are prevalent, designated by pulsed field gel electrophoresis (PFGE) as the more common USA300 and USA400. These patterns are in contrast to HCA-MRSA strains, which are primarily USA100 by PFGE. The clinical consequences of the introduction of CA-MRSA strains into the hospital environment are unclear. We hypothesized that the incidence of MRSA infection is higher in subjects colonized with the CA-MRSA strain compared to those colonized with HCA-MRSA strains. We also aimed to identify risk factors for colonization with CA-MRSA and subsequent development of MRSA infection.

Methods:
A prospective observational cohort study was performed. Subjects were chosen from patients that underwent nasal culture for active MRSA surveillance testing over a 20 month period from February 2008 to September 2009, who had positive cultures and no concurrent MRSA infection. MRSA isolates from both nasal cultures and clinical cultures positive for MRSA on routine testing were collected from the microbiology laboratory from February 2008 to April 2010. Molecular typing methods included SCC*mec* typing and PFGE. The CA-MRSA group included subjects harboring MRSA with SCC*mec* type IV and a USA300 PFGE pattern. The HCA-MRSA group included subjects colonized with MRSA with SCC*mec* type II or III. Demographic data, variables, and clinical data were collected from the UPMC Medical Archival Retrieval System and supplemented with an internal infection control database. Both colonization and the primary outcome were determined by infectious diseases physicians and infection preventionists. The risk of developing MRSA infection in the CA- and HCA- MRSA groups was determined using logistic regression. Baseline characteristics and patient-specific risk factors significantly associated with the occurrence of MRSA infection were included in the analysis as covariates.

Results:
A total of 861 subjects were analyzed, 183 (21.3%) with CA-MRSA and 678 (78.7%) with HCA-MRSA. Black race ($P < .001$), male gender ($P < .001$), and HIV/AIDS (P=.004) were independently associated with colonization with USA300 CA-MRSA. Older age ($P < .001$) and longer length of stay during index hospitalization when MRSA nasal colonization detected (LOS) ($P = .006$) were independently associated with decreased risk of USA300 CA-MRSA colonization (Table 5).

Table 5. Multivariate Model of Factors Associated with Colonization with Community-Associated Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Variable, *n* (%)	**CA-MRSA *n*=180**	**HCA-MRSA *n*=669**	**Adjusted OR (95% CI)**	**Adjusted *P***
Black race	37 (20.6)	54 (8.1)	2.5 (1.52-4.07)	<.001
Male gender	122 (67.8)	347 (51.9)	2.0 (1.39-2.90)	<.001
HIV/AIDS	10 (5.6)	2 (0.3)	10.3 (2.2-49.6)	.004
Age, years, mean +/-SD	52 +/- 17.8	63 +/-17.3	0.97 (0.96-0.98)	<.001
LOS, days, median, IQR	5 (3, 14)	9 (4,20)	0.98 (0.97-1.00)	.006

NOTE. CA, community-associated; HCA, healthcare-associated; OR, odds ratio; CI, confidence interval; SD, standard deviation; IQR, interquartile range; LOS, length of stay during the hospitalization when MRSA colonization was detected.

A total of 86 subjects (10.0%) developed MRSA infection during 6 month follow up. The risk of development of MRSA infection in the CA- versus HCA- MRSA group [15 of 183 subjects

(8.2%) versus 71 of 678 subjects (10.5%); odds ratio 0.76 (95% confidence interval, 0.43-1.37), *P*=.36] and the type of MRSA infection was not significantly different on univariate analysis.

Subsequent MRSA infection was independently associated with underlying immunosuppression from all causes, cerebrovascular disease, solid organ transplant surgery, increased LOS, vancomycin use, and cephalosporin use. Use of trimethoprim-sulfamethoxazole, macrolides and topical mupirocin were associated with decreased risk (Table 6).

Table 6. Multivariate Regression Model of Risk Factors Associated with Development of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection Among Subjects Colonized with Community-Associated MRSA and Healthcare-Associated MRSA, *n*=849

Variable, *n* (%)	**Infection *n*=85**	**No Infection *n*=764**	**Adjusted OR (95% CI)**	**Adjusted *P***
Immunosuppression (all causes)	34 (40.0)	155 (20.3)	2.04 (1.12-3.71)	.02
Cerebrovascular disease	19 (22.4)	108 (14.1)	2.03 (1.08-3.83)	.03
Solid organ transplant surgery	11 (12.9)	27 (3.5)	4.84 (1.22-17.61)	.02
LOS in days (median, IQR)	22 (13, 40)	7 (4,16)	1.02 (1.01-1.03)	<.001
Vancomycin use	71 (83.5)	344 (45.5)	4.92 (2.59-9.34)	<.001
Trimethoprim-sulfamethoxazole use	6 (7.1)	132 (17.5)	0.06 (0.02-0.23)	<.001
Cephalosporin use	66 (77.6)	394 (52.1)	2.26 (1.25-4.08)	.007
Macrolide use	6 (7.1)	104 (13.8)	0.22 (0.09-0.58)	.002
Mupirocin use	3 (3.5)	57 (7.5)	0.16 (0.04-0.64)	.01
CA-MRSA strain*	15 (17.6)	165 (21.6)	1.07 (0.55-2.07)	.85

*not significant, included in the model as an indicator of the main outcome variable of interest

NOTE. OR, odds ratio; LOS, length of stay during the hospitalization when MRSA colonization was detected; CA, community-associated.

Discussion:

This study found that nasal colonization with USA300 CA-MRSA was associated with black race, male gender, HIV/AIDS, younger age, and shorter LOS. These associations may be a reflection of socio-economic status, physical activity, occupational exposure, hygiene, lifestyle, and lack of underlying chronic medical conditions rather than true host predisposition.

Although our sample size was limited, our study also found an association of USA300 nasal colonization with a diagnosis of HIV/AIDS. This is not surprising since HIV-infected patients are known to be at an increased risk of CA-MRSA infection.

We also found a negative association of USA300 nasal colonization with longer LOS. Although we did not track acquisition of MRSA, association of USA300 nasal colonization with shorter LOS may imply acquisition in the community rather than in the hospital setting.

We found no differential risk of developing MRSA infection between subjects colonized with CA- and HCA- MRSA. It was not the strain type but other host-related risk factors and use of

certain antibiotics that influenced development of MRSA infection. Aside from solid organ transplant surgery, there was a notable deficit of medical and surgical procedures associated with development of MRSA infection in our setting. This implies that we should be vigilant about development of MRSA infections in certain populations with underlying risk factors but that medically necessarily procedures need not confer MRSA colonized patients an increased risk of infection. Although it is difficult to interpret the association with certain antibiotics, this finding suggests that antimicrobial management may have a substantial role in hospital infection control or that antibiotics may delay onset of MRSA infection.

Conclusion:
Colonization with USA300 CA-MRSA was associated with black race, male gender, HIV/AIDS, younger age, and shorter LOS, but not a higher risk of infection compared to HCA-MRSA. It is equally important to prevent colonization and subsequent infection with either CA- or HCA-MRSA strains.

Note: All milestones were accomplished for this study (original sub-aim 1), which was funded entirely by our Pennsylvania DOH grant. The manuscript is currently being edited for submission to a journal that has not yet been determined.

Original Specific Aim 2, Sub-aim 2 as presented in the Research Proposal: To evaluate the impact of rapid diagnosis by RT-PCR for detection of S. aureus/MRSA on time to optimal or appropriate antimicrobial therapy for patients with staphylococcal infections and time to initiation of appropriate isolation precautions in patients colonized with MRSA.

Original sub-aim 2 involved 3 groups of patients: Group 1: those with gram-positive cocci/*S. aureus* bacteremia, Group 2: those undergoing nasal screening for MRSA, and Group 3: subjects with gram-positive cocci/*S. aureus* infections. After starting the blood culture and wound components of the study (groups 1 and 3), we observed that a substantial portion of our RT-PCR results were incongruent with the results of bacterial culture and susceptibility testing, both of which we used as the gold standards. Because of concerns about patient safety (i.e., treating patients with true MRSA infection as MSSA or vice versa), we discontinued the portion of sub-aim 2 that involved groups 1 and 3. Sub-Aim 2 was thus revised to: **To evaluate the impact of rapid diagnosis by RT-PCR for detection of MRSA on time to initiation of appropriate isolation precautions in patients colonized with MRSA.** During the conduct of the study, an automated pushed MRSA isolation order was launched by the Infection Control Department as part of routine infection prevention practice so we also aimed to assess its impact on initiation of MRSA contact precautions pre- and post- pushed MRSA isolation order.

Substantial work was performed for groups 1 and 3, sub-aim 2 before we decided to discontinue this portion of the study. The section below refers to this work.

After successful laboratory verification of the StaphSR assay on 10 MRSA, 15 MSSA, and 12 CoNS/streptococci/enterococci blood culture specimens, RT-PCR testing of blood cultures and clinical reporting was initiated on 11/18/2010. However, we found a substantial number of incongruent test results, which was unexpected based on published performance characteristics

of the test. No patient was harmed as a result, but we discontinued reporting the RT-PCR blood culture results on December 17, 2010 and, upon further evaluation, discontinued the component of the study involving evaluation of the time to initiation of optimal or appropriate antimicrobial therapy, on January 10, 2011. The findings that prompted discontinuation of this portion of the study are detailed in this section. Another issue encountered during the conduct of Specific Aim 2, Sub-aim 2 was the failure to validate the RT-PCR assay on wound and skin and soft tissue/surgical specimens identified to have gram-positive cocci/*S. aureus* on routine culture. The results of the laboratory validation are also discussed in brief in this section.

Blood culture component. Using phenotypic antimicrobial susceptibility as the gold standard, of 101 gram positive cocci-positive blood cultures tested, 3 of 101 (3%) had inaccurate RT-PCR results: 2 MSSA isolates wrongly identified as MRSA and 1 MRSA isolate that was wrongly identified as MSSA. In addition, we had 4 other incongruent test results in which the culture result was proven to be correct over the RT-PCR assay. Overall, 6.9% of specimens required additional work-up.

Wound and skin/soft tissue component. A laboratory validation of the BD GeneOhm StaphSR (StaphSR) assay on 264 wound and skin and soft tissue (SST) swab specimens was performed.

The limit of detection (LOD) of StaphSR 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 SR-negative wound broth. Consecutive wound/SST swabs were eluted in TSB; 50ul of wound broth was extracted and StaphSR performed per protocol. For StaphSR results incongruent with culture, repeat culture identification and PCR for *fem*A and *mec*A genes were performed. *fem*A (+) specimens were considered SA (+) and *mec*A (+) SA isolates were considered MRSA. Sensitivity, specificity, and efficiency of StaphSR for detecting MRSA and MSSA were calculated, using the cut-off CT value determined in the LOD studies, with culture as the gold standard and recalculated after resolution 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 routinely cultured specimens after resolution of incongruent results with repeat culture identification and PCR for *fem*A and *mec*A genes were 92.2% (CI, 84.6-96.8%), 97.7 (CI, 94.2-99.4%), and 95.8 (CI, 92.7-97.9) respectively for detecting MRSA alone, and 88.6% (CI, 79.5-94.3%), 94.7% (CI, 93.1-98.8%), and 94.3 (CI, 90.8-96.8) respectively for MSSA alone. Three specimens, culture positive for MSSA, were misidentified by StaphSR as MRSA and were *mec*A negative. Two specimens, culture positive for MRSA, were misidentified as MSSA by StaphSR and were *mec*A positive.

StaphSR identified several MRSA/MSSA positive specimens not detected by routine culture. However, reliability was limited by the high rate of false positive and false negative results. Use of this assay was discontinued because of patient safety concerns.

The following results refer to the analysis of subjects in group 2 of original sub-aim 2, which was successfully completed as originally proposed.

Impact of Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by Real-Time Polymerase Chain Reaction (RT-PCR) and an Automated Pushed MRSA Isolation Order on Initiation of Contact Precautions

Introduction:

Active surveillance testing for methicillin-resistant *Staphylococcus aureus* (MRSA) using nasal swab culture on chromogenic media was being performed at our tertiary care facility, but the impact of rapid screening methods using real-time polymerase chain reaction (RT-PCR) assays was unknown. The MRSA healthcare-associated infection (HAI) rate at UPMC Presbyterian hospital has been less than 1 per 1,000 patient-days since 2004 and under 0.5 per 1,000 patient-days since 2006. Although it is not known if rapid screening methods for MRSA would further reduce MRSA HAI rates or be cost-effective in our setting, this type of study would require a very large sample size. Instead, a prospective observational cohort study, funded entirely by Pennsylvania DOH, was conducted to evaluate the impact of changing active surveillance MRSA detection from chromogenic media to RT-PCR on test result notification time in subjects newly identified as MRSA colonized. During the conduct of the study, an automated pushed MRSA isolation order was launched as part of routine infection prevention practice so we also aimed to assess its impact on initiation of MRSA contact precautions pre- and post- pushed MRSA isolation order.

Methods:

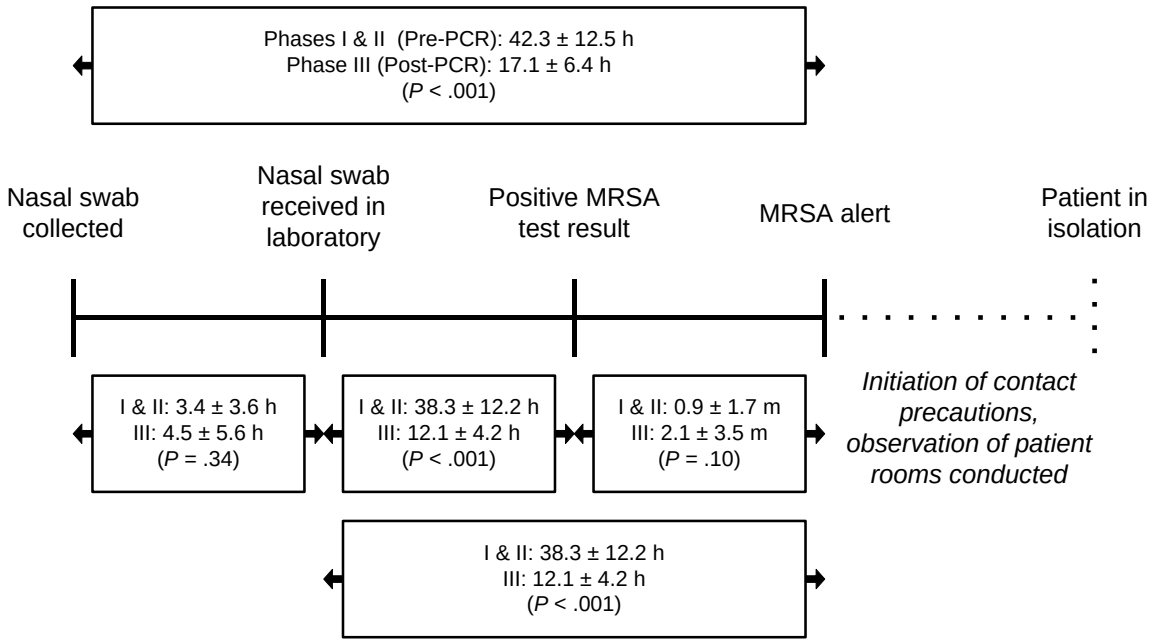
Subjects were hospitalized patients not previously known to be MRSA colonized and who were not already in contact precautions for any indication. The study occurred over a 7 month period from April to October 2010. As part of the UPMC MRSA infection control bundle, patients underwent active surveillance testing for MRSA. Screening cultures were manually ordered at the time of the study. Positive MRSA screens triggered a real-time MRSA alert requesting initiation of contact precautions. The primary outcome was mean time to MRSA alert from time of nasal swab collection and was measured over three phases.

Phase I was Pre-PCR and Pre-Automated Pushed MRSA Isolation Order. Phase II was Pre-PCR and Post-Automated Pushed MRSA Isolation Order. Phase II involved the addition of an automated pushed order to initiate contact precautions for newly identified MRSA colonized patients, generated in the electronic patient chart almost immediately after a real-time MRSA alert fired. Phase III was Post-PCR and Post-Automated Pushed MRSA Isolation Order. The BD GeneOhm MRSA ACP assay (BD GeneOhm, San Diego, CA), a multiplex RT-PCR assay used for direct detection of MRSA nasal colonization was used instead of chromogenic media for Phase III.

The component intervals between time of nasal swab collection, receipt in the microbiology laboratory, positive MRSA test result and MRSA alert were also measured. The secondary outcome variable was the proportion of subjects observed to be in contact precautions within 2 hours of MRSA alert. Mean time from nasal swab collection to MRSA alert was compared before and after application of an RT-PCR assay using t-test. The proportion of subjects observed to be in contact precautions at 2 hours from MRSA alert was compared before and after implementation of an automated pushed MRSA isolation order using Pearson chi-square test.

Results of data generated:
There were 17, 18, and 35 subjects in Phases I, II, and III respectively. Mean time from swab collection to MRSA alert was significantly decreased from 42.3 ± 12.5 hours in Phases I and II (pre-PCR) to 17.1 ± 6.4 hours in Phase III (post-PCR), $P < .001$. The intervals between time of swab collection to receipt in the microbiology laboratory and between MRSA result time to MRSA alert were not significantly different. The intervals between either time of swab collection or receipt in the microbiology laboratory to MRSA result were significantly shorter in Phase III (Figure 1).

Figure 1. Time Intervals Between Nasal Swab Collection and Methicillin-Resistant *Staphylococcus aureus* (MRSA) Alert Among Subjects Newly Identified to be MRSA Colonized



NOTE. PCR, polymerase chain reaction; h, hours; m, minutes.

Nine of 53 enrolled subjects (17.0%) were excluded during the automated pushed MRSA isolated order intervention phases (2 subjects in Phase II and 7 subjects in Phase III) because they were not assessed for implementation of contact precautions, leaving 44 subjects. The proportion of subjects observed to have had contact precautions initiated within 2 hours from MRSA alert significantly increased from 6 of 17 (35.3%) in Phase I (pre-automated pushed MRSA isolation order) to 35 of 44 (79.5%) in Phases II and III (post-automated pushed MRSA isolation order), $P < .001$. Observations were made in an average of 66.6 ± 16.5 and 75.0 ± 21.1 hours for the pre- and post- automated pushed MRSA isolation order phases respectively, $P = .08$.

Discussion:
The UPMC Presbyterian MRSA reduction bundle, which is a routine part of our infection prevention program and was not supported by DOH CURE funds, was first implemented in

2001, and since its implementation, MRSA HAI rates declined from 3.8 HAIs/1,000 patient days in 2000 to 0.36 HAIs/1,000 patient days in 2010. The UPMC Presbyterian microbiology laboratory screens more than 2000 nasal swabs for MRSA monthly. Given the effectiveness of the culture-based MRSA screening method in reducing MRSA HAIs in our setting, and the investment involved in implementing a RT-PCR MRSA screening strategy, it was difficult to justify a change in methodology.

This study showed a 25.2 hour reduction in mean time to MRSA alert with the use of RT-PCR compared to routine culture methods. The reduction can be attributed to a reduction in the turnaround time of the test procedure since the other component time intervals between swab collection to MRSA alert were not significantly different pre- and post- PCR.

The study also showed that implementing an automated pushed isolation order triggered by a positive MRSA test, rather than relying on point of care communication alone, significantly increased the proportion of subjects that were observed to be in contact precautions within 2 hours of the MRSA alert by 44.2%. This shows that whether a test with a rapid turnaround time or traditional culture is used, it is important for institutions to have a reliable system to act upon positive test results.

Since MRSA HAI rates are already very low at our institution, it would take a much larger study to determine the impact of earlier detection of MRSA colonization and reduction in time to institution of contact precautions as it relates to MRSA transmission, MRSA HAI rate, and cost effectiveness. Alternatively, mathematical models may be better suited to address these issues and data generated during this study has potential use in such models.

Conclusion:
Among hospitalized patients undergoing nasal screening for MRSA, a RT-PCR assay and an automated pushed MRSA isolation order had a significant impact on time to initiation of appropriate contact precautions in subjects newly identified as MRSA colonized. The cost-effectiveness and morbidity impact of RT-PCR and an automated pushed MRSA isolation order remains to be determined.

Note: This study was funded entirely by Pennsylvania DOH and all study milestones were completed. A manuscript resulting from this *Specific Aim 2, Sub-aim 2* study is being edited for submission. The target journal has not yet been determined.

<u>Specific Aim 2, Replacement Sub-aim 2a</u> (Study initiated on 3/1/2011):

Rationale behind replacing components of Sub-aim 2 with Sub-aim 2a and 2b
As mentioned in the previous section, after starting the blood culture and wound components of Specific Aim 2, sub-aim 2, we observed that a substantial portion of our RT-PCR results were incongruent with the results of bacterial culture and susceptibility testing, both of which we used as the gold standards. Because of concerns about patient safety, we discontinued this portion of the study (regarding evaluation of the time to initiation of optimal or appropriate antimicrobial therapy) in December 2010.

Replacement sub-aim 2a (molecular characterization of *S. aureus* blood and wound isolates with incongruent results from the original sub-aim 2) was conducted to understand the molecular basis of the incongruent RT-PCR results. Our investigation revealed that the 2 MSSA isolates that were wrongly identified as MRSA did not harbor the *mecA* gene. The 1 MRSA isolate that was incorrectly identified as MSSA did in fact contain the *mecA* gene. During the course of this investigation, there were several publications by other groups that elucidated the molecular mechanisms of erroneous RT-PCR results for identification of MRSA. Therefore, we decided to not pursue publication of this work because it would not have made additional contributions to the literature.

Specific Aim 2, Replacement Sub-aim 2b (Study initiated on 7/1/11):

Background:
S. aureus bacteremia can persist for extended periods despite the use of intravenous antibiotics such as vancomycin and daptomycin. In some cases isolates develop a heteroresistant vancomycin-intermediate S. *aureus* (hVISA) phenotype during the course of treatment. Development of resistance to daptomycin also may occur during treatment. To determine 1) genetic mechanisms of resistance to vancomycin and daptomycin and 2) the intra-patient evolution of hVISA during continued bacteremia, we performed whole genome sequencing and transcriptome analysis, as well as phenotypic analysis on sequential isolates obtained from patients with failed vancomycin therapy.

Methods:
Isolate Selection. Fifteen strains obtained from 4 patients with persistent bacteremia were selected for analysis and are grouped by patient (Table 7). Reference genomes were obtained by combining Roche 454 and Illumina genomic sequence using Celera Assembler v7.0 for a single reference isolate from each isolate set. Single nucleotide polymorphism (SNP) mutations were identified by comparison of raw Illumina reads to 454+ Illumina reference genomes using SolSNP and GATK. The variants detected were annotated using SnpEff. SNPs mutations were verified using polymerase chain reaction and Sanger sequencing.

Table 7. Isolate Description. Isolates were sequenced by Illumina Hi-Seq unless otherwise noted.

Isolate ID	Collection Date[2]	ST[3]	PFGE Profile	SCCmec Type	*spa* Type	Vancomycin	Daptomycin	PAP	PAP	Rifampin
SA2981	0	5	USA100	II	t002	2	0.5	0.703	VSSA	32.0
SA 5211[1]	75					2	0.75	0.742	hVISA	33.0
SA 5286	99					8	3.0[4]	1.118	VISA	28.0
SA 8605	0	5	USA100	II	t002	3	0.5	0.7673	VSSA	32.0
SA 8601[1]	6					3	0.75	0.8157	VSSA	31.0
SA 8581	15					6	2.0[4]	1.06	hVISA	33.0
SA 8578	17					3	0.75	0.737	VSSA	33.0
SA 9080[1]	0	8	USA300	IV	t008	2	0.75	0.7116	VSSA	34.0
SA 9082	29					2	0.5	0.7211	VSSA	33.0
SA 9364	31					2	3.0[4]	0.964	hVISA	33.0
SA 9658	34					3	3.0[4]	0.9399	hVISA	33.0

SA 9743	72					3	1.0	0.831	VSSA	32.0
SA 9908[1]	0	30	Unknown	-	t012	2	0.5	0.706	VSSA	30.0
SA 9904	7					3	2.0[4]	0.9136	hVISA	0.0[4]
SA 9902	10					8	3.0[4]	0.8023	VISA	0.0[4]

[1]Sequenced by 454+Illumina
[2]First isolate is day 0; other days are relative to day 0
[3]Multilocus Sequence Type
[4]Resistant

Transcriptome. Four strains (one sensitive and one resistant each from two patients) underwent transcriptome analysis, in duplicate, by RNA-Seq to identify the changes in RNA caused by the mutations identified in DNA. RNA extractions were performed using RNeasy Protect Mini Kit according to the manufacturer's instructions. RNA samples were subjected to rRNA depletion using the Invitrogen RiboMinus Bacteria module. The resultant rRNA depleted RNA was used to created indexed TruSeq RNA libraries using the Illumina TruSeq RNA Sample Preparation Kit. Tophat-1.4.1 and cuffdiff-2.0.1 were used for determination of gene differential expression.

Growth Curve. Single colonies from overnight growth were inoculated into 10ml BHI and shaken overnight at 37°C. Triplicate 1:100 dilution cultures in BHI were shaken at 37° C, 220rpm. $OD_{600}$was determined at 30 and 60 minutes and every 20 minutes thereafter until stationary phase was reached.

Autolysis. Single colonies were inoculated into 5ml BHI and shaken overnight at 37°C. Duplicate 1:100 dilution cultures in BHI were shaken at 37° C, 220rpm and OD_{600} was monitored. When sample reached OD_{600} ~ 0.7, cells were pelleted at 7500rpm (7232xg), 4°C for 10min. Pellets were washed twice with cold dH2O. Washed pellets were re-suspended with 25ml lysis buffer (50 mM glycine buffer, pH 8.0, containing 0.01% Triton X-100). Samples were shaken at 37°C, 220rpm and OD600 measured every 30 min for 4 hours.

Electron Microscopy
Cell thickness was measured on magnification 10,000 images using MetaMorph (Molecular Devices, LLC, Sunnyvale, California). Four images, 25 measurements per image for total of 100 measurements per isolate were obtained.

Results:
Genome. SNP differences were found in genes involved in regulation of cell wall thickness in the pair 1 resistant isolate SA5268 (Table 8). Pair 2-4 resistant isolates (SA8581, SA9364, SA9904) contained mutations in the gene *mprF*, which encodes phosphatidylglycerol lysyltransferase. Changes in this gene are associated with alterations in the polarity of the cell membrane, leading to antibiotic resistance. All SNPS identified in genomic sequences were verified by Sanger sequencing.

Table 8. SNPs identified in hVISA isolates compared to sensitive paired isolates.

Isolate	Gene Name	Product	Mutation Position in gene	Nucleotide Change	Amino Acid Change
SA5268	*vraR*	Response regulator	29	G->A	V14I
	walK	Sensor protein kinase	1231	C->T	G174E
	greA	Transcription elongation factor	1	T->C	M1T
	-	Conserved hypothetical protein	363	G->A	V122I
	mprF	Phosphatidylglycerol lysyltransferase	1618	G->A	A302V
SA8581		Conserved_hypothetical_protein		G->T	S40*
	mprF	Phosphatidylglycerol_lysyltransferase	1033	C->T	Q340*
SA9364	*mprF*	Phosphatidylglycerol_lysyltransferase	-47	G->A	L826F
SA9904	*mprF*	Phosphatidylglycerol_lysyltransferase	131	ATT->*	EF46D-

*3 nucleotide deletion

Transcriptome

Only three genes were found to be differentially transcribed between pair 1 sensitive and resistant isolates (Table 9). These transcripts encode a secreted toxin, a protein involved in energy production and conversion, and a protein involved in cell wall integrity and virulence. These results are consistent with differences in the cell wall predicted by mutations found by whole genome sequencing.

Transcriptome analysis of the second pair of strains identified 33 genes with differential transcription (see Table 9). These included genes involved in transmembrane transport, antigens, energy production and conversion, metabolism, lipid biogenesis, a toxin, and hypothetical proteins with unknown functions. Overall, the level of differential regulation was modest, with only ten transcripts having differential regulation at $\log_2$ of >2 or < -2. Transcriptome analysis also verified that SNP differences found in the genomic data were confirmed in the RNA.

Table 9. Genes differentially transcribed in set 1 and set 4 isolates.

Gene Symbol	**log2 fold change**	**Gene Name**
Isolate Set 1 (SA5211, SA5286)		
glpD	2.723638	glycerol-3-phosphate dehydrogenase
SAHV_2540	4.502813	hypothetical protein
set15	-4.49552	superantigen-like protein
Isolate Set 3 (SA9008, SA9004)		
uhpT (SAHV_0221)	-3.7508	hexose phosphate transport protein
aldA (SAHV_0166)	-3.57446	aldehyde dehydrogenase homologue
msmX (SAHV_0212)	-3.39503	multiple sugar-binding transport ATP-binding protein

Lip (SAHV_2655)	-3.36409	triacylglycerol lipase precursor
uhpT (SAHV_0220)	-3.29427	hexose phosphate transport protein
SAHV_2422	-2.95299	amino acid ABC transporter
SAHV_1020	-2.89600	hypothetical protein
SAHV_1696	-2.65606	hypothetical protein
SAHV_2414	-2.45535	hypothetical protein
hrcA (SAHV-1569)	-2.42331	heat-inducible transcriptional repressor
SAHV_2587	-2.35309	hypothetical protein
acsA (SAHV_1719)	-2.21021	catalyzes the conversion of acetate and CoA to acetyl-CoA
SAHV_2684	-2.12413	hypothetical protein
SAHV_0951	-1.99649	hypothetical protein
SAHV_1497	-1.88325	hypothetical protein
SAHV_0279	2.22467	hypothetical protein
SAHV_1396	2.26079	hypothetical protein
tyrS (SAHV_1715)	2.26173	tyrosyl-tRNA synthetase
SAHV_1882	2.30579	RNA methyltransferase homolog
SAHV_1615	2.32722	hypothetical protein
glpQ (SAHV_0954)	2.38514	glycerophosphoryl diester phosphodiesterase
SAHV_1397	2.42094	hypothetical protein
SAHV_1928	2.44449	truncated amidase,peptidoglycan hydrolase
sep (SAHV_1934)	2.57255	enterotoxin P
isaA (SAHV_255)	2.63383	immunodominant antigen A
sle1 (SAHV_0462)	2.75657	N-acetylmuramyl-L-alanine amidase
SAHV_0797	2.78419	hypothetical protein
SAHV_1879	2.90188	hypothetical protein
nasF	3.01384	uroporphyrin-III C-methyl transferase
SAHV_2288	3.24472	hypothetical protein
SAHV_2369	3.31283	hypothetical protein
SAHV_1878	3.49887	hypothetical protein
SAHV_1145	4.12405	hypothetical protein

Phenotypic studies

Growth kinetics studies of these isolates demonstrated that only the pair 1 resistant isolate had a growth deficit compared to the sensitive strain. The pair 1 resistant isolate also exhibited decreased autolysis compared to its paired sensitive isolate, consistent with cell wall changes predicted by genomic data. Electron micrographs of the sensitive-resistant pairs showed the pair 1 resistant isolate also had cell walls with increased thickness, again consistent with the development of antibiotic resistance caused by mutations in genes regulating thickness of the cell wall. Pairs 2-4 resistant isolates did not show growth kinetics differences, reduced autolysis, or thicker cell walls.

A collaborative study of biofilm formation differences between sensitive and resistant isolates has been started and will provide information on possible biofilm deficits due to resistance mutations.

Discussion:

S. aureus is highly resistant to many antibiotics, making decreases in sensitivity to additional antibiotics extremely troubling. Vancomycin is frequently used to treat *S. aureus*, particularly MRSA. Although full resistance to vancomycin is rare in *S. aureus*, low levels of resistance to this antibiotic have been detected, particularly in isolates obtained from patients on long term vancomycin therapy and in strains subject to serial passage under antibiotic pressure in the laboratory. The first determination of the mechanism of this decreased sensitivity indicated that changes in the cell wall were responsible and this phenomenon has been documented in several studies. Daptomycin is also frequently used to treat MRSA and resistance to this antibiotic has also been documented. Daptomycin resistance has most frequently been associated alterations in cell membrane charge associated with mutations in the gene *mprF*, which encodes phosphatidylglycerol lysyltransferase. Decreased vancomycin and daptomycin sensitivity was identified in isolates obtained from four patients who remained bacteremic despite continued vancomycin and/or daptomycin therapy. In all four cases, a vancomycin and daptomycin sensitive isolate was obtained from the same patient prior to isolation of the resistant isolate. Serial isolates differing in antibiotic sensitivity such as these are ideal subjects for investigation of the genomic basis of resistance.

Genomic mutations found in resistant strains are consistent with resistance phenotypes. Differential transcription results indicate complex regulatory changes associated with the gene changes. Our results indicate that cell wall changes are not required for expression of the heterogeneous vancomycin intermediate phenotype.

Conclusions:

A simple PCR or protein marker based detection platform for isolates with reduced vancomycin sensitivity would be beneficial for determining optimal treatment strategies for individual patients. The ability to identify isolates that were likely to develop decreased sensitivity would be similarly advantageous. Unfortunately, detection of the signature of reduced vancomycin and daptomycin sensitivity is not simple. These results indicate that a number of mutations in a number of genes are sufficient to cause the same phenotype, making it unlikely that a simple PCR based or protein marker test is feasible.

Timeline Specific Aim 3 (Sub-aims 1-2)

Period	Milestones
6/1/09-6/30/09	• Submit to IRB
7/1/09-6/30/10	• MLVA is developed; optimal screening culture method is established (45 patients)
7/1/10-6/30/11	• MLVA is validated and used for continued molecular surveillance • Active screening is initiated in ICUs • Baseline colonization pressure is determined (1,333 patients) • Manuscript is prepared for the screening method
7/1/11-6/30/12	• Efficacy of decolonization in reducing colonization pressure is determined (1,333 patients) • Manuscript is prepared for MLVA
7/1/12-5/31/13	• Efficacy of enhanced environmental cleaning in reducing colonization pressure is determined (1,333 patients) • Active surveillance protocols are formulated • Manuscript is prepared for the active surveillance program

Summary of research completed for Specific Aim 3 (Sub-aims 1-2) - *A. baumannii*

Specific Aim 3, Sub-Aim 1: *To develop and validate a multilocus variable number tandem repeat analysis (MLVA) assay for molecular typing of A. baumannii.*

Background:
A. baumannii is a gram-negative coccobacillus that has become an infectious agent of importance in hospitals worldwide. In the case of a hospital outbreak, epidemiologic investigation relies on molecular typing to track the source and spread of the organism. A number of molecular typing methods have been developed for *A. baumannii*. Among them, pulsed-field gel electrophoresis (PFGE) is currently considered the gold standard for outbreak investigations. However, it is a time-consuming, labor-intensive technique and interpretation of the banding patterns can be highly subjective.

Multi-locus variable number of tandem repeat analysis (MLVA) has emerged as a faster, more objective, and less expensive molecular typing method. This method capitalizes on the fact that short repeat sequences termed variable number of tandem repeats (VNTRs), have the propensity to change rapidly making them ideal for investigation of short-term evolution.

Two MLVA methods have been reported for *A. baumannii*, one using DNA sequencing of two VNTR loci (Turton JF, et al. Eur J Clin Microbiol Infect Dis 2009;28:499-507), and the other using PCR and fragment sizing by agarose gel electrophoresis of eight VNTR loci (Pourcel C, et al. J Clin Microbiol 2011;49:539-48) Building upon these methods, we aimed to develop and validate a practical and automated MLVA protocol for epidemiologic investigation of *A. baumannii* that can be accomplished by a single PCR reaction and subsequent fragment sizing by capillary electrophoresis without costly DNA sequencing.

Methods:
To better understand the molecular epidemiology of multidrug-resistant *A. baumannii* in U.S. hospitals and build a repository of clinical isolates for the development of MLVA, we first collected carbapenem-non-susceptible *A. baumannii* isolates from hospitals across the U.S. and conducted molecular typing using PFGE and multi-locus sequence typing (MLST). We also collected outbreak-associated clinical isolates from a hospital in Pennsylvania for use in the MLVA development.

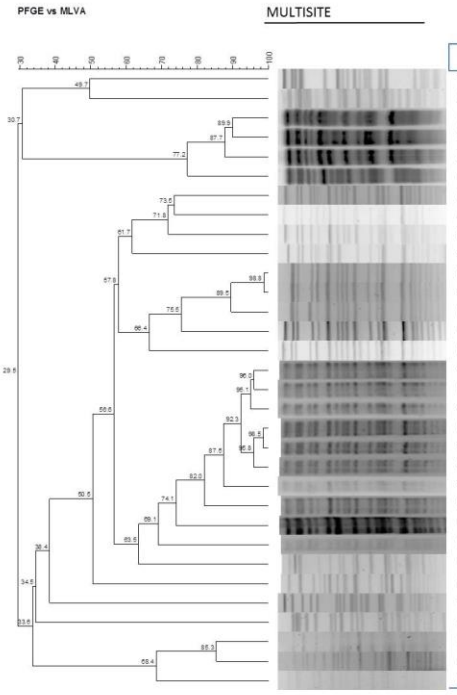
We then searched for candidate VNTRs in publicly available genomes of strains ATCC17978, AYE, SDF and ACICU. VNTR loci that were included in the published MLVA protocols were also considered. After extensive validation of candidate loci, five VNTR loci with the best variability and complete VNTRs were included in the sequencing-based MLVA scheme (MLVA5). Based on the MLVA5, the three most discriminatory loci which produced PCR products with sizes compatible with capillary electrophoresis (CE) were selected for development of the CE-based MLVA scheme (MLVA3). These schemes were tested against a total of 32 clinical isolates collected above.

Analysis of MLVA3 data was performed by minimum spanning tree (MST) analysis using Bionumerics software and the Manhattan coefficient. The sum of the tandem repeat differences (STRD) for all VNTR loci was used to determine the genetic relationships between isolates. For instance, if an isolate has a VNTR combination of 14-10-24 and another has 12-9-20 using MLVA3, the distance between these isolates is defined as 7 (STRD of 2, 1, and 4 at each of the 3 loci). Circle sizes on the MSTs represented the number of isolates with that particular VNTR combination. MLVA3 results were compared with the PFGE results, MLST-defined sequence types (STs)/clonal complexes (CC) and relevant epidemiologic data of the isolate when available (e.g., date collected, location of patients). For each method, the discriminatory index (DI) was calculated utilizing the Simpson's Index of diversity $D = 1 - \frac{1}{N\,(N-1)} \sum_{j}^{S} n_{j^1}\,(n_{j^1} - 1)$, where N is the number of isolates, S is the number of types described and n_j the number of isolates in j^{th} type.

Results:
We collected 65 carbapenem-non-susceptible *A. baumannii* clinical isolates from hospitals in six states across the U.S. While the PFGE patterns tended to cluster within each hospital, MLST sequence types (STs) belonging to the clonal complex 92 (CC92) and the pan-European clonal lineage II (EUII; worldwide clonal lineage 2) were predominant in all hospitals. Of them, ST122 and ST208 were the most common and were found in four of the six hospitals, including the hospital in Pennsylvania. Isolates belonging to the pan-European clonal lineages I and III were identified in one hospital each outside Pennsylvania. Carbapenemase-encoding genes $bla_{\text{OXA-23}}$ and/or IS*Aba1*-$bla_{\text{OXA-51-like}}$ were present among the majority of isolates. These findings suggested that carbapenem-non-susceptible *A. baumannii* isolates found in U.S. hospitals, including Pennsylvania, constituted part of the global epidemic driven by CC92, but have unique STs other than ST92, which may be spreading by means of patient transfer between health care facilities within the United States. This was the first study to describe the contemporary molecular epidemiology of MDR *A. baumannii* in the U.S., and the findings were published in *the Journal of Clinical Microbiology* (Adams-Haduch JM, et al. J Clin Microbiol 2011;3849-54).

This part of the sub-aim was completed by the beginning of Year 2 and provided the diverse strain set for the development of MLVA below.

The above isolates as well as the outbreak isolates collected in Pennsylvania were used to validate the sequence-based MLVA5 and CE-based MLVA3. The development of MLVA took longer than in the proposed timeline due to several technical challenges that were encountered. They included the need for the use of proof-reading DNA polymerase for one of the previously published locus (this locus was ultimately eliminated as unpractical), frequent insertion of an IS element in another locus, and absence of yet another published locus in a minority of isolates. These factors also led to a smaller number of reliably and reproducibly amplified loci that could be adopted in the schemes. The dendrogram based on PFGE patterns and the actual VNTRs of MLVA 5 are shown in Figure 2. Isolates with distinct PFGE patterns always had distinct VNTR combinations, whereas isolates that belonged to the same PFGE pulsotype, as defined by >85% similarity on the dendrogram, often but not always had related VNTR combinations. In the minimum spanning trees (MSTs) based on the *summed tandem-repeat differences* (STRDs), MLVA5 clustered four of the outbreak isolates and four more isolates nearby this cluster, but one isolate was distinct (data not shown). MLVA3 gave similar results, where all but one isolates were included in three neighboring clusters (**Figure 2**). The patient with the outlying outbreak isolate was transferred out of the ICU just before any of the other subsequent cases were identified. While the PFGE pattern was indistinguishable between this outlying isolate and some of the subsequent isolates, the distance on the MSTs (STRD = 8 by MLVA 3) sug*g*ested that this isolate was unlikely to represent the index case of the outbreak. On the other hand, the isolate from a second case, identified a week later in the same ICU, matched closely with the isolates from the other cases on the MSTs (STRD=1). Therefore, it was inferred that this second isolate was more likely to be the index case for the outbreak. In addition, while most of the study isolates belonged to clonal complex (CC) 92 by MLST reflecting the predominant lineage in U.S. hospitals, isolates belonging to non-CC92 lineages (CC113 and European Clone [EU] I) were clearly distinguished from the CC92 isolates by both MLVA3 and MLVA5 (Figure 2).



				Loci				
ST	ID	State	Year	10	4	G	1	3
★ 205	Acat50	FL	2009	12	7	20	6	14
● 122	Acat53	FL	2009	16	8	18	17	16
● 208	Acat27	NV	2009	20	9	15	18	7
● 122	Acat64	PA	2009	20	9	11	7	14
● 122	Acat56	PA	2009	19	9	13	21	6
● 122	Acat61	PA	2009	23	6	11	19	18
● 208	Acat08	MO	2008	22	6	16	16	15
● 122	Acat58	PA	2009	21	6	12	10	17
● 122	Acat62	PA	2009	22	7	13	20	16
● 122	Acat59	PA	2009	21	7	5	12	5
● 122	Acat05	MO	2008	16	8	15	24	16
● 208	Acat06	MO	2008	18	7	13	24	16
● 208	Acat11	MO	2007	18	9	16	17	6
● 122	Acat01	MO	2007	18	8	11	13	15
● 208	2903	PA	2009	9	8	5	17	4
● 208	2880	PA	2011	22	6	15	17	16
● 208	2835	PA	2011	24	6	12	13	16
● 208	2950	PA	2011	23	6	15	17	16
● 208	2843	PA	2011	22	6	14	16	16
● 208	2821	PA	2010	22	6	15	16	16
● 208	2876	PA	2010	22	6	15	16	16
● 208	2859	PA	2010	22	6	11	16	16
● 208	2890	PA	2011	23	6	11	17	18
● 208	2894	PA	2010	18	7	16	21	11
● 208	Acat65	PA	2011	21	6	13	15	18
● 123	Acat17	NY	2009	23	7	5	16	3
● 204	Acat16	NY	2009	17	4	2	20	13
★ 205	Acat07	MO	2008	34	11	16	20	18
♦ 207	Acat42	CA	2009	11	9	15	19	16
● 204	Acat29	NV	2009	17	4	15	19	12
● 122	Acat32	NV	2009	20	7	17	15	15
● 208	Acat48	CA	2009	21	6	13	15	15

Figure 2. PFGE dendrogram and the variable number of tandem repeats (VNTR) copy numbers for MLVA5, which includes MLVA3. For STs, circles, stars and the diamond represent CC92, CC113 and European Clone I, respectively.

These preliminary studies were completed by the end of Year 2 and were presented by a minority trainee at a national conference (Rivera JI, Adams-Haduch JM, Shutt KA, Marsh JW, Harrison LH, Doi Y. Validation of multilocus variable-number tandem-repeat analysis (MLVA) in carbapenem-resistant *Acinetobacter baumannii*. 2011. 51st Intersci Conf Antimicrob Agents Chemother, abstr. K-817).

In Year 3, additional isolates from outbreaks in ICUs at our hospital and an outside hospital were further subjected to MLVA to further test the applicability of the MLVA3 scheme in hospital epidemiology. The MST of MLVA3 correlated well with the clinical epidemiology of the outbreaks (Table 10).

Table 10. Epidemiological data of isolates from patients and their environment at a tertiary teaching hospital.

MST-MLVA3	Isolate	Unit	Source	Date	PFGE Type
Cluster A	8E	TICU	Environmental	8/4/2011	B
Cluster A	6E	TICU	Environmental	8/4/2011	A
Cluster A	7C	TICU	Clinical	8/8/2011	A
Cluster A	7E	TICU	Environmental	8/4/2011	A
Cluster A	4C	TICU	Clinical	8/5/2011	D
Cluster A	4E	TICU	Environmental	8/4/2011	A
Cluster A	5C	TICU	Clinical	8/8/2011	A
Cluster A	5E	TICU	Environmental	8/4/2011	A
Cluster A	2C	TICU	Clinical	8/28/2011	A
Cluster A	2E	TICU	Environmental	8/30/2011	A
Cluster A	3C	TICU	Clinical	10/3/2011	A
Cluster A	3E	TICU	Environmental	9/28/2011	A
Cluster A	1E	TICU	Environmental	8/16/2011	A
Cluster B	11C	SICU-B	Respiratory	10/24/2011	A
Cluster B	14C	SICU-B	Environmental	9/28/2011	A
I1	9C	TICU	Clinical	9/5/2011	C
I2	13C	MICU	Clinical	7/27/2012	A
I3	1C	TICU	Clinical	9/5/2011	A
I4	12C	MICU	Clinical	3/20/2012	A
I5	10C	SICU-B	Rectal	10/10/2011	E
I6	9E	TICU	Environmental	9/28/2011	C

Isolates in MST Cluster A were collected over two months from the ICU. The PFGE dendrogram also clustered these isolates with 95% similarity. Notably, MST for MLVA3 was able to identify a second cluster which originated from a different ICU in a separate building but the PFGE identified as 97% similar. Three isolates that originated outside the ICU were distinguished from the aforementioned clusters in the MST, two of which were also distinguished by PFGE (cluster B and isolates I2, I4 and I5). Finally, three isolates from the ICU were distinguished from the MST clusters whereas they were not distinguished by PFGE (Table 10).

Discussion: In comparison against STs/CCs defined by MLST, both MLVA5 and MLVA3 were able to discriminate the large number of non-outbreak isolates belonging to CC92. The discriminatory indexes were comparable at 88% for MLVA5, 85% for MLVA3 and 89% for PFGE (p = NS with an unpaired *t*-test) for the entire group. For the 10 outbreak isolates, the DI for MLVA3 was 93%, MLVA5 was 98% whereas the discriminatory index for PFGE was 53%, indicating that the superior discriminatory power of MLVA over PFGE is more evident in epidemiologically related isolates, such as in a hospital outbreak.

The operating characteristics of MLVA make it most suitable for use in epidemiological investigation of bacterial isolates related in temporal and geographical contexts. To this end, our results showed that MLVA3 had sufficient discriminatory power that exceeded that of PFGE, and that incremental gains achieved by the use of a larger number of VNTR loci were relatively small while increasing the time and costs required to perform it.

The limitations of the study were that we only tested isolates identified from hospitals in the U.S., which does not fully reflect the global diversity of *A. baumannii*. Also, while DNA sequencing is not needed, the MLVA3 scheme still requires access to an instrument capable of performing capillary electrophoresis.

Conclusion:
MLVA3, which can be performed as a single multiplex PCR reaction followed by capillary electrophoresis without the need for sequencing, was shown to have sufficient discriminatory power that exceeds that of PFGE. Therefore, it is a promising replacement for PFGE in supporting hospital epidemiologic investigation of *A. baumannii*. The final results of the data presented above are currently under review for publication.

Specific Aim 3, Sub-Aim 2:
To implement an active surveillance program at UPMC-Mercy for patients who are colonized with MDR *A. baumannii*. The effectiveness of the subsequent interventions in reducing transmission and overall colonization pressure will be quantified.

Background:
While numerous hospital outbreaks due to MDR *A. baumannii* have been reported in the U.S. and worldwide, knowledge regarding how to prevent their spread in hospital is limited. One key problem is the lack of sensitive method to reliably identify carriers of MDR *A. baumannii*, which is necessary to effectively implement appropriate precautions and interventions. MDR *A. baumannii* primarily resides on the skin and often in the respiratory tract as well. However, screening of these body sites with swabs is known to have suboptimal sensitivity of detection (Marchaim D, et al. J Clin Microbiol 2007;45:1551-5). We hypothesized that, by sampling a large skin area with sponge followed by 1 or 24-hour enrichment and selective cultures for MDR *A. baumannii* the sensitivity of screening could be significantly improved. The aims of this work were to validate this novel screening method in a cohort of patients already known to be colonized with MDR *A. baumannii*, and to implement it at ICUs across UPMC Mercy Hospital in downtown Pittsburgh as a quality improvement initiative, which had high incidence of MDR *A. baumannii* at the time.

Methods: In the first year of the grant, patients admitted to UPMC hospitals and who had previous clinical culture growing MDR *A. baumannii* were recruited to the study. Upon obtaining informed consent, even sites were cultured with separate swabs (the forehead, nostrils, buccal mucosa, antecubital fossa, axilla, groin, and toe web), and three sites were cultured with separate sterile, moist sponges (the forehead, upper arm, and thigh). The specimens were enriched culture overnight and the broth was inoculated to a selective agar medium (modified Leed *Acinetobacter* Medium). Growth suggestive of MDR *A. baumannii* were confirmed as such using standard biochemical methods. The characteristics of the study population were described by using proportions for categorical variables and medians and ranges for continuous variables. Since all of the patients were known to be positive for *A. baumannii*, the sensitivity of each method was calculated as the percentage of tests that were positive. McNemar's test for paired data was used to compare the sensitivities of two methods based on samples from the same patients. Under the assumption that the composite sensitivities of the swab culture method and the sponge culture method were 55% and 80%, respectively, this study was designed to enroll 45 patients in order to achieve 80% power for detecting a difference between the two sensitivities with a two-sided alpha of 0.05. The analyses were performed by using SAS, version 9.2 (SAS Institute Inc., Cary, NC), and a *P* value of 0.05 was considered statistically significant.

In the second year of the grant, the screening method using sponge was implemented to all ICUs at UPMC Mercy Hospital. Patients underwent screening upon admission to an ICU, and every seventh day thereafter. Automatic orders were placed electronically to ensure compliance. Patients newly identified as a carrier of MDR *A. baumannii* were placed under the standard contact precaution. In the third year of the grant, the screening and contact precaution was continued. In addition, the patients underwent daily chlorhexidine bathing unless contraindicated. In the fourth year of the grant, terminal cleaning using bleach was introduced to the ICUs, in addition to the other measures already described. Furthermore, the equipment in the patient rooms (ventilator touch pads, infusion pump touch pads and surface of supply cart) were cultured with a sponge before and after terminal cleaning to determine the effectiveness of the cleaning procedure.

Results:
For the initial validation study, 46 patients were enrolled (54% female and 87% Caucasian). The findings are summarized in Table 11 below.

Table 11. Sensitivity of detecting *A. baumannii* from colonized patients.

Collection method	Site	No. of patients	Selective antimicrobials and enrichment periods					
			ATM			CAZ		
			1 hour	24 hours	*P* value	1 hour	24 hours	*P* value
Sponge	Forehead	46	19.6%	67.4%	<0.0001	21.7%	69.6%	<0.0001
	Upper arm	46	26.1%	76.1%	<0.0001	21.7%	76.1%	<0.0001
	Thigh	46	34.8%	82.6%	<0.0001	19.6%	82.6%	<0.0001
Swab	Forehead	46	4.3%	23.9%	0.01	2.1%	23.9%	0.004
	Nostrils	46	23.9%	32.6%	0.49	26.1%	32.6%	0.66
	Buccal mucosa	46	43.5%	50.0%	0.68	43.5%	52.2%	0.53
	Antecubital fossa	46	0%	19.6%	0.003	0%	21.7%	0.001
	Axilla	46	10.9%	26.1%	0.11	10.9%	26.1%	0.11
	Groin	45	17.8%	48.9%	0.003	15.6%	48.9%	0.001
	Toe web	45	11.1%	22.2%	0.26	6.7%	22.2%	0.07

ATM, aztreonam; CAZ, ceftazidime.

As can be seen, the process of enrichment beyond 1 hour was found to be critical in increasing sensitivity. The best sensitivities were achieved with the sponge cultures, whereas the antimicrobial agent in the selective media (aztreonam [ATM] or ceftazidime [CAZ]) did not make a tangible difference. Table 12 below shows the composite sensitivity of two culture sites for 24-hour enriched cultures.

Table 12. Composite sensitivity of the most sensitive sponge and swab sites.

mLAM	Sponge sites	Swab sites	*P* value
	Upper arm and/or thigh	Nostril and/or buccal mucosa	
ATM	89.1%	56.5%	0.0006
CAZ	89.1%	58.7%	0.001
	Upper arm and/or thigh	Nostril and/or groin	
ATM	88.9%	64.4%	0.002
CAZ	88.9%	66.7%	0.004
	Upper arm and/or thigh	Buccal mucosa and/or groin	
ATM	88.9%	73.3%	0.052
CAZ	88.9%	77.8%	0.13

mLAM, modified Leed *Acinetobacter* Medium; ATM, aztreonam; CAZ, ceftazidime.

The results indicated that sensitivity of close to 89% could be achieved by serially sampling the upper arm and thigh with sponge, significantly better sensitivity than any combination of two swab sites. These findings were published in *the Journal of Clinical Microbiology* (Doi Y, et al. J Clin Microbiol 2011;49:154-8) and it was determined that the serial upper arm and thigh sampling method would be adopted for the subsequent active screening program at UPMC Mercy Hospital.

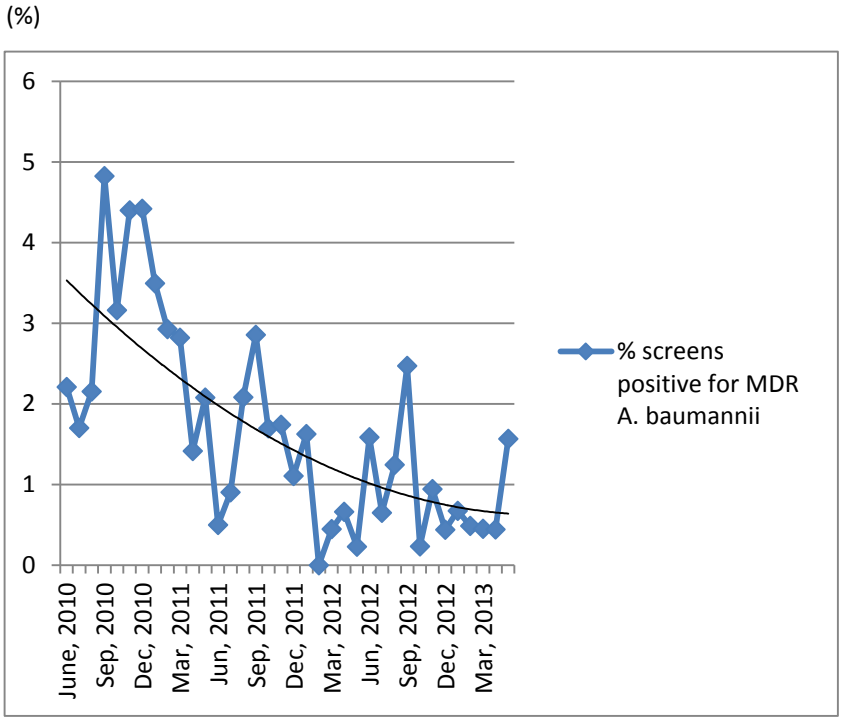


Figure 3. Positivity of the MDR *A. baumannii* active screening program at UPMC Mercy Hospital ICUs. A steady decline was observed over the course of 3 years.

The three-year active surveillance program supported by this grant was completed at the end of May, 2013. A total of 16,115 screens were conducted using the sponge method in the 36-month period, upon admission to any of the ICUs and then every seventh day while in the ICUs. In the first year ending in May, 2011 (screening and targeted isolation), 2.97% of the screens were positive for MDR *A. baumannii* (95% confidence interval [CI], 2.52-3.42). In the second year ending in May, 2012 (screening, targeted isolation and daily chlorhexidine bathing), 1.16% were positive (95% CI, 0.87-1.44). In the third year ending in May, 2013 (screening, targeted isolation, daily chlorhexidine bathing and enhanced terminal cleaning), 0.92% were positive (95% CI, 0.66-1.18). Monthly positivity over the 36 months is shown in Figure 3 where downward trend is observed, suggesting that the stepwise intervention supported by this grant had a beneficial effect in controlling MDR *A. baumannii* in these ICUs.

In the final year of active screening when terminal cleaning using bleach was introduced, patient rooms were cultured for 27 discharges of MDR *A. baumannii*-colonized patients before and after cleaning. Only two pre-cleaning samples grew MDR *A. baumannii* (7.4%). All 27 post-cleaning samples were negative for MDR *A. baumannii*, including the two cases where the pre-cleaning samples were positive, suggesting that, despite the small number of samples, the enhanced terminal cleaning was effective in reducing the burden of MDR *A. baumannii* contamination of ICU rooms.

The preliminary results of clinical implementation from this Sub-Aim have been published in *Infection Control and Hospital Epidemiology* (Doi Y, et al. Infect Control Hosp Epidemiol 2012;33:642-3)and also in abstract form:

Dalton CM, Ferrelli J, Price J, Henry C, Ricci F, Fejka SM, Hariri RS, Yassin MH, Doi Y. Effectiveness of eliminating Acinetobacter baumannii through environmental cleaning. 2013. 40th Assoc Professionals Infect Control Epidemiol, abstr. 2-273

In addition, final analysis of the active screening data is ongoing. Given the enormous success of this program in reducing MDR *A. baumannii* infection, UPMC Mercy Hospital has decided to continue it after the completion of this grant, as part of its routine infection prevention initiatives. Finally, the application of sponge for active screening has now been expanded to methicillin-resistant *Staphylococcus aureus* (MRSA). Validation of this method in detection of MRSA carriage is currently ongoing as an independent project, supported by the Agency for Healthcare Research and Quality.

Discussion:
A. baumannii has become a major hospital pathogen in recent years, disproportionately affecting patients who are immunocompromised as well as those in intensive care units, including at our hospitals in Pennsylvania. However, there had been no highly sensitive screening method to detect carriers of this organism. The present study was designed and conducted with the goal of significantly improving the sensitivity of screening for *A. baumannii* colonization. In this newly developed screening method, the combined sensitivity for the upper arm and leg with a sponge was as high as 89.1%. The method using sterile sponges was easy to perform and achieved excellent sensitivity for the detection of *A. baumannii* colonization.

We therefore implemented this screening method in the three ICUs at UPMC Mercy Hospital in Pittsburgh in Year 2. All newly admitted patients were screened in the arm and leg; those with positive cultures were placed in contact precaution. The screening was repeated every seventh day while the patients remained in the ICUs. In Year 3, daily chlorhexidine bathing was started across the ICUs for patients without contraindication (such as severe burns). In Year 4, enhanced terminal cleaning using bleach was implemented. As can be seen in Figure 3, we saw a steady decrease in the rates of positive screening cultures over the course of 3 years, with the steepest decline observed in Year 2. While the formal analysis of colonization pressure remains ongoing at this time, the data show significant and sustained decrease in the burden of MDR *A. baumannii* at the ICUs in this hospital. The success of the program was well received by the hospital leadership, which decided to continue it beyond the completion of the grant funding using its own resources.

Conclusion:
We successfully implemented a multifaceted infection prevention program using a novel screening approach at ICUs in a community hospital with a high incidence of MDR *A. baumannii* infection. The decrease in the rates of positive screening cultures was significant and sustained. This infection prevention approach will serve as a model for hospitals in Pennsylvania as well as across the U.S. where high incidence rates of MDR *A. baumannii* infection are observed.

<u>Specific Aim 3, Additional Sub-Aim</u> -Study initiated on 6/6/2011: To conduct genomic and proteomic analysis on colistin-resistant *A. baumannii* clinical isolates to elucidate the mechanisms of resistance.

Background:
Rising antimicrobial resistance in *A. baumannii*, in particular to carbapenems, has prompted increased use of colistin, a cationic polypeptide antimicrobial agent, to treat *A. baumannii* infection. However, we are increasingly observing development of colistin resistance in MDR *A. baumannii*. Once resistance develops to colistin, there are no remaining viable treatment options. Resistance to colistin in *A. baumannii* has been associated with modification of lipid A (specifically addition of a phosphoethanolamine moiety), which is caused by substitutions in the PmrAB two-component regulator (Beceiro A, et al. Antimicrob Agents Chemother 2011;55:3370-9, Arroyo LA, et al. Antimicrob Agents Chemother 2011;55:3743-51). However, little work has been performed to characterize how resistance develops in patients on treatment with colistin, and how the potentially impaired fitness is compensated for. At UPMC, we have observed and collected nearly 20 colistin-susceptible and resistant pairs of isolates from the same patients, providing us with an opportunity to examine the dynamics of resistance development combining genetic, genomic and proteomic approaches.

Methods:
Three pairs of colistin-susceptible and -resistant clinical isolates underwent genome sequencing using Ion Torrent (Applied Biosciences) platform to identify variations within each pair. In addition, the colistin-susceptible strains for each of the pairs was sequenced with GS FLX (Roche) and SMRT RS (Pacific Biosciences) to provide well-assembled references. The datasets were analyzed by a repertoire of *de novo* and reference-guided assemblers. For proteomic analysis, bacterial samples were separated into membrane-associated protein fraction and subjected to 1st dimension separation based on isoelectric points followed by 2nd dimension separation based on molecular weights.

The paired isolates were prepared for two dimensional gel electrophoresis (2DE) by broth growth of bacteria under antibiotic selection to maintain their phenotype. Bacterial pellets were suspended in a hypo-osmotic HEPES-NaCl buffer and lysed using a French press. Samples were then separated by ultracentrifugation into soluble and membrane protein fractions. Cell fractions were compared using 1st dimension separation on IPG strips, using pH 3-10 range trips for total protein and pH 6-11 range with cup loading to examine alkaline proteins. For two dimensional gel electrophoresis (2DE) studies, further samples of membrane proteins were labeled with Cy3 and Cy5 dyes. Labeled samples were combined and resolved as noted above, and final gels were scanned in low-absorption glass plates on a Typhoon 9400 fluorescence scanner.
In addition, genetic loci particularly implicated in colistin resistance (*pmr*, *lpx*) will be sequenced using conventional sequencing, and lipid A will be characterized by mass spectrometry to identify modifications commonly associated with colistin resistance.

Results:
The first three sets of colistin-susceptible and -resistant *A. baumannii* isolates from the same patients have been subjected to high-throughput sequencing using the PacBio/GS FLX (for

scaffolding) and Ion PGM (for identification of indels and SNPs). Preliminary identification of the single nucleotide variations within each pair is shown in Table 13.

Table 13. Non-synonymous mutations observed in the three colistin-resistant *A. baumannii* isolates using the earlier colistin-susceptible isolates from the same patients as references.

Non-synonymous mutations in the first colistin-resistant isolate (JA637)				
Reference	**Position**	**Gene**	**Triplet code**	**Amino Acid**
A.baumannii_A2_Chromosome	80762	FIG000988: Predicted permease	CGT->AGT	R->S
A.baumannii_A2_Chromosome	150837	CBS domain protein	TGA->CGA	X->R
A.baumannii_A2_Chromosome	781950	ATP-dependent RNA helicase NGO0650	ACG->GCG	T->A
A.baumannii_A2_Chromosome	915003	Preprotein translocase subunit YajC	GAA->AAA	E->K
A.baumannii_A2_Chromosome	2067931	ATP-dependent Clp protease ATP-binding subunit	ATT->TTT	I->F
A.baumannii_A2_Chromosome	2461958	Adenylate cyclase (EC 4.6.1.1)	TGG->TAG	W->X
Non-synonymous mutations in the second colistin-resistant isolate (JA566)				
scf7180000000196|quiver	48783	conserved hypothetical protein	ATT->TTT	I->F
scf7180000000197|quiver	119	diguanylate cyclase/phosphodiesterase	CAA->CCA	Q->P
scf7180000000200|quiver	7526	Two-component system response regulator PmrAB	ATG->ATA	M->I
scf7180000000248|quiver	1887	Hydrolases of the alpha/beta superfamily	GCC->GGC	A->G
scf7180000000196|quiver	48783	conserved hypothetical protein	ATT->TTT	I->F
Non-synonymous mutations in the third colistin-resistant isolate (JA942)				
scf7180000000160|quiver	35196	Two-component system response regulator PmrAB	CAA->AAA	Q->K
scf7180000000207|quiver	6367	Large repetitive protein	CTT->GTT	L->V

In the first isolate, while there were no substitutions in PmrAB within the pair, PmrB of the colistin-resistant strain contained Q270P substitution consistent with resistance, whereas the susceptible strain had an insertion in the *pmrB* gene, likely leading to its inactivation. In the second and the third isolates, substitutions in PmrA and PmrB consistent with resistance, respectively. These changes were validated by conventional sequencing. We are in the process of validating the other changes. In the lipid A analysis, the three resistant isolates showed three major $[M-H]^-$ ions at *m/z* 1,404, 1,910 (dominant), and 2,034. The most abundant ion at *m/z*

1,910 corresponded to a bis-phosphorylated hepta-acylated lipid A. The ion at *m/z* 2,034 corresponded to the hepta-acylated lipid A (*m/z* 1,910) modified with a phosphoethanolamine at the 4′ position (Δ*m/z* 125), which accounts for colistin resistance and was not observed in the corresponding susceptible isolates (Figure 4).

For proteomic analysis, 2D DIGE (two-dimensional difference in gel electrophoresis) has been conducted on the first set of isolates, which has identified candidate outer membrane proteins which are differentially expressed between the susceptible and resistant isolates (Figure 5). Identification of these proteins using mass spectrometry is ongoing at the time of this final report.

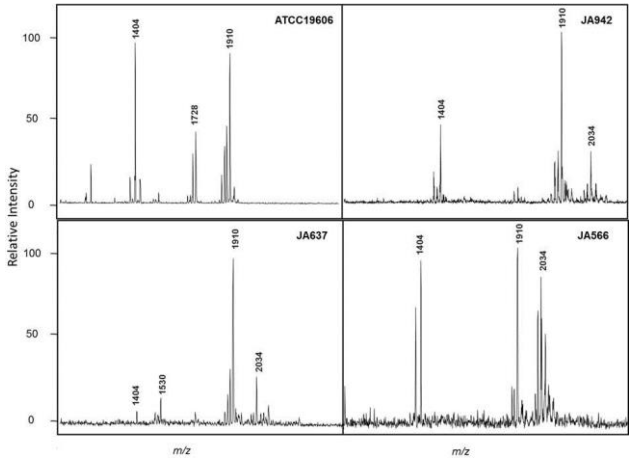


Figure 4. MALDI-TOF negative-ion mode analysis of lipid A isolated from *A. baumannii*. Mass spectrum from the ATCC 19606 strain produced ion peaks at *m/z* 1910, 1728, and 1404, corresponding to a bis-phosphorylated hepta-acylated, hexa-acylated, and tetra-acylated lipid A species, respectively. Mass spectra for lipid A isolated from colistin-resistant strains JA942, JA637, and JA566 produced an ion peak at 2034 *m/z* corresponding to a bis-phosphorylated hepta-acylated lipid A with addition of a phosphoethanolamine group.

Discussion:

A. baumannii has become a major multidrug-resistant hospital pathogen in recent years. As the use of colistimethate (the prodrug of colistin) increased, so has resistance to colistin in *A. baumannii*. Currently, there is no effective therapy against infection due to colistin-resistant *A. baumannii*. Therefore, development of methods to rapidly diagnose colistin resistance and effective therapeutic approaches is of paramount importance. Our preliminary data suggest that certain molecular signatures on the lipid A structure may be used as markers of colistin resistance using mass spectrometry. Furthermore, we have established methodologies to shortlist target genes and proteins implicated in development of colistin resistance using genomic and proteomic approaches. These genes and proteins may serve as

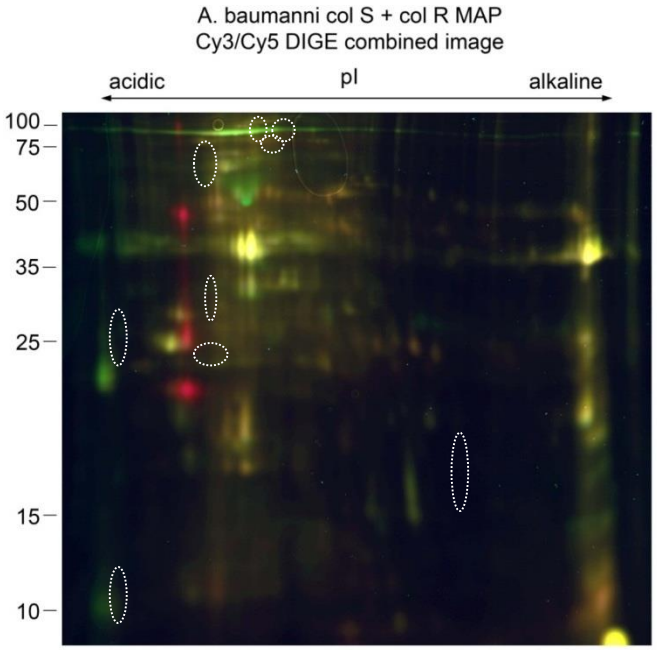


Figure 5. Outer membrane proteomic profile of the paired strains from Pt 1. The Col^S and Col^R strains were directly compared by 2D-DIGE, with an equal quantity of protein loaded per strain. Proteins visualized in yellow have similar expression, while proteins in green and red are differentially expressed by Col^S and Col^R, respectively. Seven proteins were down-regulated from Col^S, seen in green, with no detectable expression in Col^R. Possibly one protein, with various modifications, was up-regulated and solely expressed in the Col^R strain, seen in red. Proteins of interest will be identified by MALDI.

candidate therapeutic targets. The genome sequencing data have provided us with a short list of secondary genomic changes, which we plan to correlate with changes in proteomes and various microbiological properties of the colistin-resistant strains, including fitness and virulence. The preliminary data generated from this additional sub-aim have been incorporated into a grant proposal entitled "Colistin-resistant *Acinetobacter baumannii*" and were instrumental in securing a a competitive score from the NIH. This part of the work has also been published in *Antimiocrobial Agents and Chemotherapy* (O'Hara JA, et al. Antimicrob Agents Chemother 2013;57:2103-8).

Conclusion:
We have successfully established methodologies and collaborations, using a limited number of colistin-susceptible and –resistant *A. baumannii* isolate pairs, to study how to better diagnose and treat these infections. Thus far it appears that addition of a phosphoethanolamine moiety to lipid A, in association with non-synonymous mutations in the *pmrCAB* locus which governs its expression. Leveraging the skills and knowledge base established through these efforts, we plan to continue our work on *A. baumannii* and colistin resistance.

Summary of research completed for Specific Aim 4 (Sub-aims 1-3) – *modeling*

Timeline Specific Aim 4 (Sub-aims 1-3)

Period	Milestones
6/1/09-6/30/09	• Start-up phase
7/1/09 - 6/30/10	• Sub-aim 1: Task 1; Sub-aim 2: Task 1; Sub-Aim 3: Task 1
7/1/10 - 6/30/11	• Sub-aim 1: Task 2; Sub-aim 2: Task 2; Sub-aim 3: Task 2
7/1/11 - 6/30/12	• Sub-aim 1: Task 3; Sub-aim 2: Task 3; Sub-aim 3: Task 3
7/1/12 - 5/31/13	• Sub-aim 1: Task 4; Sub-Aim 2: Task 4

Introduction to specific aim 4, sub-aims 1-3. The following is a description of the work performed for specific aim 4, the modelling component of our project. This component resulted in 6 publications, all of which acknowledged Pennsylvania DOH funding (Section 20). However, completion of this section of the report has been complicated by the fact that the PI for this specific aim left the University of Pittsburgh shortly after the end of the funding period. He has not provided input into this report since leaving the University. What follows is a description of the general methodology that was employed, followed by the results of the specific studies that were conducted for sub-aims 1-3. In addition, copies of the six publications have been provided as reference for additional details.

General Methods for Specific Aim 4, Sub-aims 1-3.
The following methods were common to all 3 pathogens (Sub-aims 1-3):

1. Data collection and abstraction. Table 14 lists the data parameters that were used for our models and their sources. We also used data generated from specific aims 1-3 for some

parameters. Additional data sources included established, well-documented databases and previous studies in the literature at the time that the models were created.

Table 14. Model Parameters and Sources

Parameter	Sources	Aim1	Aim 2	Aim 3
Prevalence of Colonization	• Specific Aims 1-3 • Literature	X	X	X
Probability of Infection, Without Colonization	• Specific Aims 1-3 • Literature	X	X	X
Probability of Infection, With Colonization	• Specific Aims 1-3 • Literature	X	X	X
Probabilities of Clinical Outcomes of Infection	• Specific Aims 1-3 • Literature	X	X	X
Sensitivity and Specificity of Diagnostic Test	• Specific Aims 1-3	X	X	X
Probability of Decolonization Success	• Specific Aims 1-3 • Literature	X	X	X
Probability of Treatment Success	• Specific Aims 1-3 • Literature	X	X	X
Socio-Economic Characteristic Distribution	• Synthetic population • Specific Aims 1-3	X	X	X
Racial and Ethnic Distribution	• Synthetic population • Specific Aims 1-3	X	X	X
Acute and Long-Term Care Facility Characteristics	• Databases: PHC4 • Specific Aims 1-3	X	X	X
Acute and Long-Term Care Facility Patient Characteristics	• Databases: PHC4 • Specific Aims 1-3	X	X	X
Acute and Long-Term Care Facility Admission, Discharge, and Transfer Parameters	• Databases: PHC4 • Specific Aims 1-3	X	X	X
Cost of Medications	• Average wholesale prices	X	X	X
Cost of Hospitalization	• Hospital charges • Databases: Healthcare Utilization Project (HCUP)	X	X	X
Cost of Procedures	• Databases: Center for Medicare and Medicaid Services	X	X	X
Cost of Clinic Visits	• Databases: Center for Medicare and Medicaid Services	X	X	X
Productivity Losses	• Wage Data from Department of Labor	X	X	X
Cost of Diagnostic Tests	• Manufacturers		X	X
Reproductive Number (R_0)	• Literature • Specific Aims 1-3 • County model		X	X
Time-Dependent Morbidity and Mortality	• Literature			X

2: Quantify the morbidity, mortality, and economic burden of C. difficile, MRSA, and MDR A. baumannii in different populations and healthcare facility types. Using the TreeAge Pro Suite (Williamstown, MA) and Microsoft Excel (Redmond, WA), we developed economic burden simulation models. Figure 6 shows the basic structure of the model which feeds into the Infection Outcomes Subtree (Figure 7).

Figure 6. Structure of infection outcomes subtree

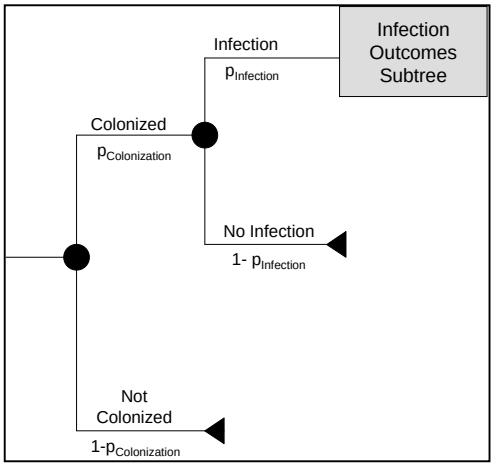
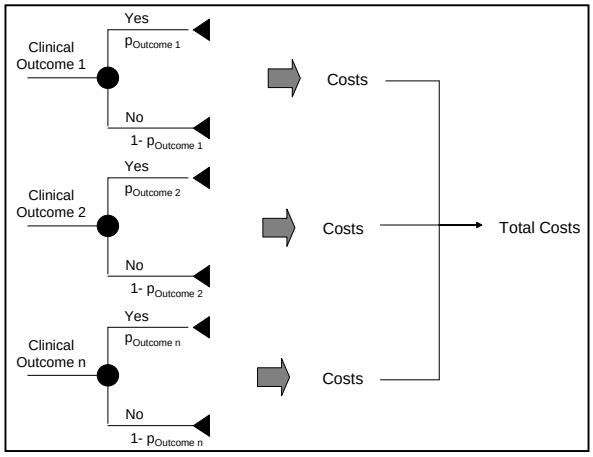


Figure 7. General structure of economic burden model



Using data obtained from our studies and the literature, each colonized person has probability distributions of developing different infections and clinical outcomes in the subtree. Each clinical outcome consumes resources such as medications, hospitalization days, personnel costs, diagnostic tests, and medical procedures. Different iterations of the model take societal, healthcare facility, and third party payer perspectives.

Monte Carlo simulations entailed sending large numbers of simulated patients with different constellations of characteristics (e.g., race/ethnicity, gender, age, socio-economic status, clinical characteristics) through the computer model. Each simulated patient was drawn from the population represented by the model. The value of each parameter for a given patient was drawn from the relevant probability distribution.

Sensitivity analyses were used to identify the important cost drivers for our economic burden models. We varied the input values for different model parameters (e.g., probability of various clinical sequelae, treatment types and costs, patient characteristics, and colonization pressure) to see how the changes affected the overall cost.

3. Determine the morbidity/mortality benefits and cost-effectiveness of performing surveillance for and diagnosis of C. difficile, *MRSA, and MDR* A. baumannii *in various types of healthcare facilities, clinical, socio-demographic, economic, and geographic populations to help target surveillance.*

1. **Building and Attaching Decision Analytic Surveillance Models to the Economic Burden Models**: This process entailed adding a decision prior to the economic burden models.
2. **Building and Incorporating a Transmission Model**: Potential advantages of surveillance for colonization include preventing infection in colonized patients and preventing transmission of the pathogen from the colonized patient to other patients. For the transmission model, the generation number (R_0) was the number of new cases that a colonized patient generated. If, for example, the R_0 is 2, then a colonized, non-isolated patient will create 2 new cases. The 2 new cases then traveled through the economic burden

subtree with similar probabilities of developing infections. The range of possible R_0 was derived from studies in the literature and other available data.

Results, Discussion, and Conclusion for specific Aim 4, Sub-aim 1: Determine the impact of surveillance for and rapid diagnosis of MRSA on transmission of and disease caused by MRSA in the healthcare setting.

The economic value of screening hemodialysis patients for methicillin-resistant *Staphylococcus aureus* in the USA (Lee BY et al Clin Microbiol Infect 2011;17:1717-26).
Background. Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause severe infections in patients undergoing hemodialysis. Routine periodic testing of hemodialysis patients and attempting to decolonize those who test positive may be a strategy to prevent MRSA infections. The economic value of such a strategy has not yet been estimated.
Methods. We constructed a Markov computer simulation model to evaluate the economic value of employing routine testing (agar-based or PCR) at different MRSA prevalence, spontaneous clearance, costs of decolonization and decolonization success rates, performed every 3, 6 or 12 months.
Results. The model showed periodic MRSA surveillance with either test to be cost-effective (incremental cost-effectiveness ratio ≤$50 000/quality-adjusted life-year) for all conditions tested. Agar surveillance was dominant (i.e. less costly and more effective) at an MRSA prevalence ≥10% and a decolonization success rate ≥25% for all decolonization treatment costs tested with no spontaneous clearance. PCR surveillance was dominant when the MRSA prevalence was ≥20% and decolonization success rate was ≥75% with no spontaneous clearance.
Conclusions. Routine periodic testing and decolonization of hemodialysis patients for MRSA may be a cost-effective strategy over a wide range of MRSA prevalences, decolonization success rates, and testing intervals.

The economic effect of screening orthopedic surgery patients preoperatively for methicillin-resistant *Staphylococcus aureus* (Lee BY et al. Infect Control Hosp Epidemiol 2010;31:1130-8).
Background. Patients undergoing orthopedic surgery are susceptible to methicillin-resistant Staphylococcus aureus (MRSA) infections, which can result in increased morbidity, hospital lengths of stay, and medical costs. We sought to estimate the economic value of routine preoperative MRSA screening and decolonization of orthopedic surgery patients.

Methods. A stochastic decision-analytic computer simulation model was used to evaluate the economic value of implementing this strategy (compared with no preoperative screening or decolonization) among orthopedic surgery patients from both the third-party payer and hospital perspectives. Sensitivity analyses explored the effects of varying MRSA colonization prevalence, the cost of screening and decolonization, and the probability of decolonization success.
Results. Preoperative MRSA screening and decolonization was strongly cost-effective (incremental cost-effectiveness ratio less than $6,000 per quality-adjusted life year) from the third-party payer perspective even when MRSA prevalence was as low as 1%, decolonization success was as low as 25%, and decolonization costs were as high as $300 per patient. In most scenarios this strategy was economically dominant (i.e., less costly and more effective than no

screening). From the hospital perspective, preoperative MRSA screening and decolonization was the economically dominant strategy for all scenarios explored.
Conclusions. Routine preoperative screening and decolonization of orthopedic surgery patients may under many circumstances save hospitals and third-party payers money while providing health benefits.

Results, Discussion, and Conclusion for specific Aim 4, Sub-aim 2: Determine the impact of surveillance and control measures for asymptomatic *C. difficile* carriers on transmission of and disease caused by *C. difficile* in the healthcare setting.

The potential economic value of screening hospital admissions for *Clostridium difficile* (Bartsch SM et al Eur J Clin Microbiol Infect Dis 2012;31:3163-71).
Background. Asymptomatic *Clostridium difficile* carriage has a prevalence reported as high as 51-85 %; with up to 84 % of incident hospital-acquired infections linked to carriers. Accurately identifying carriers may limit the spread of *Clostridium difficile*.
Methods. Since new technology adoption depends heavily on its economic value, we developed an analytic simulation model to determine the cost-effectiveness screening hospital admissions for *Clostridium difficile* from the hospital and third party payer perspectives. Isolation precautions were applied to patients testing positive, preventing transmission. Sensitivity analyses varied *Clostridium difficile* colonization rate, infection probability among secondary cases, contact isolation compliance, and screening cost.
Results. Screening was cost-effective (i.e., incremental cost-effectiveness ratio [ICER] ≤$50,000/QALY) for every scenario tested; all ICER values were ≤$256/QALY. Screening was economically dominant (i.e., saved costs and provided health benefits) with a ≥10.3 % colonization rate and ≥5.88 % infection probability when contact isolation compliance was ≥25% (hospital perspective). Under some conditions screening led to cost savings per case averted (range, $53-272).
Conclusions. *Clostridium difficile* screening, coupled with isolation precautions, may be a cost-effective intervention to hospitals and third party payers, based on prevalence. Limiting *Clostridium difficile* transmission can reduce the number of infections, thereby reducing its economic burden to the healthcare system.

The economic burden of *Clostridium difficile* (McGlone SM et al. Clin Microbiol Infect 2012;18:282-9).
Background. Although *Clostridium difficile* is the leading cause of infectious diarrhea in hospitalized patients, the economic burden of this major nosocomial pathogen for hospitals, third-party payers and society remains unclear.
Methods. We developed an economic computer simulation model to determine the costs attributable to healthcare-acquired *C. difficile* infection (CDI) from the hospital, third-party payer and societal perspectives. Sensitivity analyses explored the effects of varying the cost of hospitalization, *C. difficile*-attributable length of stay, and the probability of initial and secondary recurrences.
Results. The median cost of a case ranged from $9179 to $11 456 from the hospital perspective, $8932 to $11 679 from the third-party payer perspective, and $13 310 to $16 464 from the societal perspective. Most of the costs incurred were accrued during a patient's primary CDI

episode. Hospitals with an incidence of 4.1 CDI cases per 100 000 discharges would incur costs ≥$3.2 million (hospital perspective); an incidence of 10.5 would lead to costs ≥$30.6 million. Our model suggests that the annual US economic burden of CDI would be ≥$496 million (hospital perspective), ≥$547 million (third-party payer perspective) and ≥$796 million (societal perspective).
Conclusions. Our results show that *C. difficile* infection is indeed costly, not only to third-party payers and the hospital, but to society as well. These results are consistent with current literature citing *C. difficile* as a costly disease.

Results, Discussion, and Conclusion for Specific Aim 4, Sub-aim 3: Determine the impact of surveillance for MDR *A. baumannii* on transmission of and disease caused by *A. baumannii* in the healthcare setting.

Economic value of *Acinetobacter baumannii* screening in the intensive care unit (Lee BY et al. Clin Microbiol Infect 2011;17:1691-7.)
Background. Although *Acinetobacter baumannii* is an increasingly common nosocomial pathogen that can cause serious infections in the intensive care unit (ICU), most ICUs do not actively screen admissions for this pathogen.
Methods. We developed an economic computer simulation model to determine the potential cost-consequences to the hospital of implementing routine *A. baumannii* screening of ICU admissions and isolating those patients who tested positive, comparing two screening methods, sponge and swab, with each other and no screening. Sensitivity analyses varied the colonization prevalence, percentage of colonized individuals who had active *A. baumannii* infections, *A. baumannii* reproductive rate (R), and contact isolation efficacy.
Results. Both screening methods were cost-effective for almost all scenarios tested, yielding cost-savings ranging from -$1 to -$1563. Sponge screening was not cost-saving when colonization prevalence was ≤1%, probability of infection ≤30%, R ≤ 0.25, and contact isolation efficacy ≤25%. Swab screening was not cost-saving under these same conditions when the probability of infection was ≤40%. Sponge screening tended to be more cost-saving than swab screening (additional savings ranged from $1 to $421).
Conclusions. Routine *A. baumannii* screening of ICU patients may save costs for hospitals.

Timeline Specific Aim 5

Period	Milestones
6/1/09-6/30/09	• Advertise training opportunities
7/1/09-6/30/10	• 4 post-baccalaureate (academic year)
7/1/10-6/30/11	• 4 undergraduates (summer) and 4 post-baccalaureate (academic year)
7/1/11-6/30/12	• 4 undergraduates (summer) and 4 post-baccalaureate (academic year)
7/1/12-5/31/13	• 4 undergraduates (summer)

Summary of research completed for Specific Aim 5 – *Minority training program*

The minority training portion of the project, a seven-week summer research training program and a post-baccalaureate program, were both designed to expose underrepresented and disadvantaged

students to biomedical research in infectious diseases, specifically, MRSA, *C. difficile*, and *A. baumannii*, was highly successful in not only attracting talented trainees, but also in advancing their careers.

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. In 2012, the final summer of the project, 311 submitted basic applications and a record 242 completed their applications.

Program Structure

The comprehensive training curriculum required 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 spent several Saturday mornings learning about test-taking skills for the Medical College Admission Test (MCAT). Finally, each student presented 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 served as the faculty for the admissions skills workshops. A former program participant served as the dormitory monitor/student coordinator. Richard Levitt, MA, UPSOM academic development coordinator, provided the study and reading skills assessment and met individually with each student to discuss his/her approach to learning as it related 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). Listed below are the trainees across the four years of funding and their accomplishments:

Table 15. Summer Trainees

Student	Home Institution	Preceptor	Current Status
2009			
Michael Daniel	University of Pittsburgh	JoAnne Salangsang, MD	Johns Hopkins University School of Medicine, Rising MS2
Bridget Parker	Cheyney University	Yohei Doi, MD/PhD	University of Pittsburgh - Completed Masters degrees in Rehabilitation Science and Public Health
2010			
Jeneana Parks	Lincoln University	Diana Pakstis, RN, BSN	La Salle University - Pediatrics Clinical Speech Pathology/ Audiology
Moises Baltazar Garcia	Florida Atlantic University	Bruce Lee, MD, MBA	Washington University School of Medicine, Rising MS2
Opeyemi Akinbamidele	Lehigh University	Jane Marsh, PhD	Non-Science Career
Mya Stayton	Tuskegee University	Jane Marsh, PhD	Tuskegee University Food & Nutritional Sciences, Masters Completed; applying for medical school.
2011			
Alexandra Aponte-Velez	University of Puerto Rico	JoAnne Salangsang, MD	University of Puerto Rico School of Medicine
Joel James	University of Pittsburgh	Yohei Doi, MD, PhD	Applying to Medical School
Borley Quaye	Spelman College	Scott Curry, MD	Applying to Medical School
Chrystal Miller	Carnegie Mellon University	Scott Curry, MD	Attending premedical Post-baccalaureate program
2012			
Lauretta Ambe	Columbia University	Jane Marsh, PhD	Rising Senior
Isabel Coello	University of New Mexico	Jane Marsh, PhD	Rising Senior

Table 16. Summer Training Outcomes

Student	Home Institution	Research Project/Presentation
2009		
Michael Daniel	University of Pittsburgh	"Antimicrobial Susceptibility Patterns of HCA- vs. CA-MRSA among Blood and Nasal Screening Cultures"
Bridget Parker	Cheyney University	"Multidrug-Resistant *A. baumannii*."
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	"Modeling of *C. difficile* infection"
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	"Molecular characterization of *Staphylococcus aureus* isolates misidentified by the BD GeneOhm StaphSR assay"
Joel James	University of Pittsburgh	"Molecular characterization of *Staphylococcus aureus* isolates misidentified by the BD GeneOhm StaphSR assay"
Borley Quaye	Spelman College	A Comparative Study Assessing Environmental Culturing Methods for *Clostridium difficile* Recovery
Chrystal Miller	Carnegie Mellon University	A Comparative Study Assessing Environmental Culturing Methods for *Clostridium difficile* Recovery
2012		
Lauretta Ambe	Columbia University	"Combination Drug Testing- MDRAB"
Isabel Coello	University of New Mexico	"Improving *Clostridium Difficile* Genotyping"

Post-baccalaureate Training

To meet the goals of the post-baccalaureate training aim, we created the University of Pittsburgh Intramural Research Training Award (UPIRTA) program (www.healthdiversity.pitt.edu/programs/upirta.php), a nine-month to one-year post-baccalaureate 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 began in the fall of each award year.

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

Students were 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 National Conference on Higher Education (formerly 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 were vetted by Ms. Davis and John P. Horn, PhD, associate dean for graduate studies in the School of Medicine. Ms. Davis and Dr. Horn screened applications for each student's interest and potential for success (as judged by grade point average [GPA] and prior research activity).

In addition to their research, the students engaged in a comprehensive schedule of enrichment activities. They participated in all lab meetings and group-wide, comprehensive Pennsylvania Department of Health (PA-DOH) progress meetings. In addition, they participated in the University of Pittsburgh's "Survival Skills and Ethics" program, or CEED, the Career Education and Enhancement for Health Care Research Diversity Program (http://www.icre.pitt.edu/ceed). Both programs designed to teach early career researchers key professional "survival" skills such as making effective presentations, navigating career paths, and obtaining grant funding. CEED

exists specifically to support underrepresented individuals, so UPIRTA students have an opportunity, through this experience, to network with underrepresented role models.

Table 17. Program participants across the four years of the grant:

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
2011-12		
Sobe Maduka	Carnegie Mellon University	Scott Curry, MD
Bianca Montalmont	Lincoln University	Yohei Doi, MD, PhD
Micah Shelton	McDaniel College	Jane Marsh, PhD
2012-13		
Deylan Moore	Cheyney University	Jane Marsh, PhD
Borley Quaye	Spelman University	Scott Curry, MD
Bethany Townsend	St. Mary's University	Yohei Doi, MD, PhD

Table 18. Post baccalaureate Training Outcomes

2009-10		
Tatianna Henderson	Scott Curry, MD	"RT-PCR for Detection of Toxigenic C. difficile from Peri-Rectal Swabs and Stool Specimens"
Esenwa Obi Onuoha	Yohei Doi, MD, PhD	"Improved Detection of Multidrug-Resistant (MDR) A. baumannii Carriers with the use of Sponge"
2010-11		
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"
2011-12		
Sobe Maduka	Scott Curry, MD	"Prevalence of C. Difficile"
Bianca Montalmont	Yohei Doi, MD, PhD	"The MRSA Sponge Study"
Micah Shelton	Jane Marsh, PhD	"Assays of *S. Aureus*"
2012-13		
Deylan Moore	Jane Marsh, PhD	"Development of real time RT-PCR assay to examine differential gene expression in hVISA strains"

Borley Quaye	Scott Curry, MD	”A Twelve-year Retrospective Survey of *Clostridium difficile* Diversity by Multiple Genotyping Methods”
Bethany Townsend	Yohei Doi, MD, PhD	“The effect of desiccation tolerance on survival rates of colistin-resistant *Acinetobacter baumannii* & a study investigating gentamicin-resistant Klebsiella pneumonia”

2009-2010

Ezenwa Obi Onuoha was recruited for the *A. baumannii* component of the grant between 2009 and 2010 (aim 3, sub-aim 2).

Training: The techniques Obi learned include species identification, susceptibility testing, PCR and sequencing analysis, and gene cloning. He was instrumental in processing of the majority of patients enrolled to the study addressing Sub-Aim 2 of Specific Aim 3 during the course of the year. He also devoted a significant effort in building the PFGE/MLST database for Sub-Aim 1 of Specific Aim 3.

Accomplishments: Obi presented his research at the UPIRTA Research Presentation at Pitt and submitted his findings for presentation at the Interscience Conference on Antimicrobial Chemotherapy as the presenting author:

Onuoha EO, Harrison LH, Doi Y. Improved detection of multidrug-resistant *Acinetobacter baumannii* (MDR-AB) carriers with the use of sponge and selective medium. 2010. 50th Intersci Conf Antimicrob Agents Chemother, abstr. K-319.

He also earned co-authorship in two publications:

Doi Y, Onuoha EO, Adams-Haduch JM, Pakstis DL, McGaha TL, Werner CA, Parker BN, Brooks MM, Shutt KA, Pasculle AW, Muto CA, Harrison LH. Screening for *Acinetobacter baumannii* colonization by use of sponges. J Clin Microbiol. 2011;49:154-8
Adams-Haduch JM, Onuoha EO, Bogdanovich T, Tian GB, Marschall J, Urban CM, Spellberg BJ, Rhee D, Halstead DC, Pasculle AW, Doi Y. Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States. J Clin Microbiol. 2011;49:3849-54

Obi currently works as a research technician at the Department of Developmental Biology, University of Pittsburgh School of Medicine.

2010-2011

Nakita Brown and 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* (aim 1, sub-aim 1). 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 aim 1, sub-aim 4. Ms. Brown also learned basic anaerobic microbiology laboratory skills for *C. difficile* and took over the storage and data-basing 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.

Mr. Hamilton is a rising 2nd year medical student at Michigan State University School of Medicine. Ms. Brown is in the process of applying to medical school.

Jesabel Rivera was recruited for the *A. baumannii* component of the grant between 2010 and 2011 (aim 1, sub-aim 2).

Training: The techniques Jesabel learned include species identification, susceptibility testing, PCR and sequencing analysis, and the use of the Bionumerics, a software which is now the standard in molecular epidemiological analysis. She also participated in the laboratory meeting and practiced presentations on several occasions.

Accomplishments: Jesabel presented her research at the intramural UPIRTA Research Presentation. She also presented her findings from Sub-Aim 1 of Specific Aim 3 at the 51st Interscience Conference on Antimicrobial Chemotherapy as the presenting author and also as co-authors on other posters for which she assisted with the work:

1. Rivera JI, Adams-Haduch JM, Shutt KA, Marsh JW, Harrison LH, Doi Y. Validation of multilocus variable-number tandem-repeat analysis (MLVA) in carbapenem-resistant Acinetobacter baumannii. 2011. 51st Intersci Conf Antimicrob Agents Chemother, abstr. K-817
2. Tian GB, Taracila M, Adams-Haduch JM, Rivera JI, Park YS, Wang H, Bonomo RA, Doi Y. Hydrolysis of cefepime by Acinetobacter cephalosporinase ADC-56; an extended-spectrum (ES) AmpC. 2011. 51st Intersci Conf Antimicrob Agents Chemother, abstr. C1-362
3. Park YS, Adams-Haduch JM, Rivera JI, Yarabinec DM 3rd, Johnson LE, Hingwe A, Lewis JS 2nd, Jorgensen JH, Doi Y. Clinical and microbiologic characteristics of cephalosporin-resistant Escherichia coli. 2011. 51st Intersci Conf Antimicrob Agents Chemother, abstr. K-1906.

After the training period, Jesabel was admitted to the master's program in the Department of Infectious Diseases and Microbiology at the University of Pittsburgh Graduate School of Public Health and graduated in 2013. Her curriculum focused on infectious diseases and culminated in the thesis: "InFLUyentes: A Strategy to Improve Flu Vaccination Coverage Among Hispanics in Pittsburgh, PA."

During her studies she continued to work as a student worker in Dr. Doi's laboratory and assisted on many research studies, including those supported by this grant, which has led to co-authorship on the six posters listed below:

1. Doi Y, Park YS, Rivera JI, Adams-Haduch JM, Hingwe A, Kilayko MC, Lewis JS 2nd, Howard WJ, Johnson LE, Sordillo E, Polsky B, Jorgensen JH, Richter SS, Shutt KA, Paterson DL. Community-associated extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* infection in the United States. 2012. 52nd Intersci Conf Antimicrob Agents Chemother, abstr. C2-112.
2. Tian GB, Rivera JI, Park YS, Johnson LE, Hingwe A, Adams-Haduch JM, Doi Y. ST405 *Escherichia coli* producing QepA1, CTX-M-15 and RmtB from Detroit. Antimicrob Agents Chemother 2011;55:3966-7.
3. Park YS, Adams-Haduch JM, Rivera JI, Curry SR, Harrison LH, Doi Y. *Escherichia coli* producing CMY-2 beta-lactamase in retail chicken, Pittsburgh, Pennsylvania, USA. Emerg Infect Dis 2012;18:515-6.
4. Doi Y, Park YS, Rivera JI, Adams-Haduch JM, Hingwe A, Sordillo EM, Lewis JS 2nd, Howard WJ, Johnson LE, Polsky B, Jorgensen JH, Richter SS, Shutt KA, Paterson DL. Community-associated extended-spectrum-beta-lactamase (ESBL)-producing *Escherichia coli* infection in the United States. Clin Infect Dis 2013;56:641-8.
5. Munoz-Price LS, Namias N, Cleary T, Fajardo-Aquino Y, DePascale D, Arheart KL, Rivera JI, Doi Y. *Acinetobacter baumannii*: Association between environmental contamination of patient rooms and occupant status. Infect Control Hosp Epidemiol 2013;34:517-20.
6. Munoz-Price LS, Fajardo-Aquino Y, Arheart KL, Cleary T, DePascale D, Pizzano L, Namias N, Rivera JI, O'Hara JA, Doi Y. Aerosolization of *Acinetobacter baumannii* in a trauma intensive care unit. Crit Care Med 2012; accepted for publication.

2011-2012

Sobie S. Maduka was recruited for the *C. difficile* study with Drs. Scott Curry and Jane Marsh (Aim 1, sub-aim 1). She examined the prevalence of *C. difficile* in nursing homes, and conducted two experiments genotyping the *C. difficile* found in Kane nursing homes. Ms. Maduka is in the process of applying to medical school**.**

Ms. Maduka has just matriculated in the University of Pittsburgh School of Medicine.

Bianca Montalmont was recruited for the *A. baumannii* component of the grant between 2011 and 2012 (Aim 3, sub-aim2).

Training: Bianca was assigned a pilot project to examine the applicability of the sponge-based screening method of *A. baumannii*, which has been developed and validated under this grant, against MRSA (Aim 3, sub-aim 2). The techniques Bianca learned include species identification, susceptibility testing, and PCR analysis. She also participated in laboratory meetings and practiced presentations on several occasions.
Accomplishments: Bianca presented her research at the intramural UPIRTA Research Presentation. She also spent time performing observership under various healthcare providers at UPMC to gain hands-on experience on healthcare-related careers. She is expected to earn co-authorship on the above-mentioned MRSA study when the study is completed and submitted for publication in 2014.

After the training, Bianca was recruited to the Autism arm of the grant to expand on her research experience. Ms. Montalmont completed a second year in UPIRTA working on a study of Autism Spectrum Disorders.

Ms. Montalmont will begin the Master's in Physician Assistant studies at Drexel University in fall, 2013.

Micah Shelton worked with Dr. Jane Marsh on assays of nosocomial *S. aureus*, testing the efficacy of the BD Gene Ohm assay in identifying *S. aureus* (Aim 2, sub-aim 2). The assay generated many false positives. Micah's study found that if the assay detects any number of S. aureus base pairs it returns a resistant *S. aureus* indicator, when the staphylococcus might actually be methicillin sensitive.

Bearing out the primary goal of the UPIRTA program, Mr. Shelton was accepted into the University of Pittsburgh's doctoral program in neuroscience for fall, 2012.

2012-13

Bethany Townsend was recruited for the *A. baumannii* component of the grant between 2012 and 2013 (Aim 3, sub-aim 2).

Training: Bethany was assigned an independent project to study the desiccation tolerance of colistin-susceptible and -resistant clinical isolates. She also assisted in the conduct of the *Galleria mellonella* infection model to determine the efficacy of various antimicrobial combinations against colistin-resistant *A. baumannii*. Finally, she investigated the mechanism of gentamicin resistance among *Klebsiella pneumoniae* clinical isolates which were resistant to carbapenems.

Accomplishments: Bethany found that colistin resistance did not impair desiccation tolerance in *A. baumannii* and presented her data at The Emory University STEM Research and Career Symposium. She is also a co-author on the *A. baumannii* treatment study (aim 3, sub-aim 2) she participated in:

O'Hara JA, Ambe LA, Casella LG, Townsend BM, Pelletier MR, Ernst RK, Shanks RM, Doi Y. Activities of vancomycin-containing regimens against colistin-resistant *Acinetobacter baumannii* clinical strains. Antimicrob Agents Chemother. 2013;57:2103-8

Furthermore, she identified a strong association between the presence of a specific gentamicin resistance gene (aacC4) and resistance to gentamicin, a novel finding. The work remains ongoing in Dr. Doi's laboratory.

Upon completion of the training program, Bethany spent a summer at the NIH in the National Cancer Institute and prepared to enter the Master of Public Health program at Drexel University.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

Yes

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

Yes

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

1 facility and approximately 10 Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

210 Number of subjects originally targeted to be included in the study
78 Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
26 Males
52 Females
0 Unknown

Ethnicity:
0 Latinos or Hispanics
78 Not Latinos or Hispanics

0 Unknown

Race:
0 American Indian or Alaska Native
0 Asian
12 Blacks or African American
0 Native Hawaiian or Other Pacific Islander
66 White
0 Other, specify:_____
0 Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

John J. Kane Regional Center-Glen Hazel located in Allegheny County.

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?

No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic

copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	**Authors:**	**Name of Peer-reviewed Publication:**	**Month and Year Submitted:**	**Publication Status (check appropriate box below):**
Use of MLVA Genotyping to Determine the Role of Asymptomatic Carriers in *C. difficile* Transmission.	Curry SR, Muto CA, Schlackman JL, Pasculle AW, Shutt KA, Marsh JW, Harrison LH	Clin Infect Dis 2013 July 23 [Epub ahead of print]	October 2012	☐Submitted ☐Accepted X Published
The potential economic value of screening hospital admissions for *Clostridium difficile*	Bartsch SM, Curry SR, Harrison LH, Lee BY.	Eur J Clin Microbiol Infect Dis. 2012 Nov;31(11):3163-71.	May 2012	☐Submitted ☐Accepted X Published
Prevalence of *Clostridium difficile* in uncooked ground meat products from Pittsburgh, Pennsylvania.	Curry SR, Marsh JW, Schlackman JL, Harrison LH.	Appl Environ Microbiol. 2012 Jun;78(12):4183-6.	January 2012	☐Submitted ☐Accepted X Published
Perirectal swab surveillance for *Clostridium difficile* by use of selective	Curry SR, Schlackman JL, Hamilton TM, Henderson TK,	J Clin Microbiol. 2011 Nov;49(11):3788-93.	May 2011	☐Submitted ☐Accepted X Published

broth pre-amplification and real-time PCR detection of tcdB.	Brown NT, Marsh JW, Shutt KA, Brooks MM, Pasculle AW, Muto CA, Harrison LH.			
The economic burden of *Clostridium difficile.*	McGlone SM, Bailey RR, Zimmer SM, Popovich MJ, Tian Y, Ufberg P, Muder RR, Lee BY.	Clin Microbiol Infect. 2012 Mar;18(3):282-9.	September 2011	☐Submitted ☐Accepted X Published
The economic value of screening hemodialysis patients for methicillin-resistant *Staphylococcus aureus* in the USA.	Lee BY, Song Y, McGlone SM, Bailey RR, Feura JM, Tai JH, Lewis GJ, Wiringa AE, Smith KJ, Muder RR, Harrison LH, Piraino B.	Clin Microbiol Infect. 2011 Nov;17(11):1717-26.	May 2011	☐Submitted ☐Accepted X Published
The economic effect of screening orthopedic surgery patients preoperatively for methicillin-resistant *Staphylococcus aureus*.	Lee BY, Wiringa AE, Bailey RR, Goyal V, Tsui B, Lewis GJ, Muder RR, Harrison LH.	Infect Control Hosp Epidemiol. 2010 Nov;31(11):1130-8	May 2010	☐Submitted ☐Accepted X Published
Natural history of multidrug-resistant *Acinetobacter baumannii* carriage in intensive care units.	Doi Y, Kandiah S, Hariri RS, Harrison LH.	Infect Control Hosp Epidemiol. 2012 Jun;33(6):642-3.	December 2011	☐Submitted ☐Accepted X Published
Economic value of *Acinetobacter baumannii* screening in the intensive care unit.	Lee BY, McGlone SM, Doi Y, Bailey RR, Harrison LH.	Clin Microbiol Infect. 2011 Nov;17(11):1691-7.	May 2011	☐Submitted ☐Accepted X Published
Screening for *Acinetobacter baumannii* colonization by use of sponges.	Doi Y, Onuoha EO, Adams-Haduch JM, Pakstis DL, McGaha TL, Werner CA, Parker BN, Brooks MM, Shutt KA, Pasculle AW, Muto CA,	J Clin Microbiol. 2011 Jan;49(1):154-8.	June 2010	☐Submitted ☐Accepted X Published

	Harrison LH.			
Economic impact of *Acinetobacter baumannii* infection in the intensive care unit.	Lee BY, McGlone SM, Doi Y, Bailey RR, Harrison LH.	Infect Control Hosp Epidemiol. 2010 Oct;31(10):1087-9.	April 2010	☐Submitted ☐Accepted X Published
Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States	Adams-Haduch JM, Onuoha EO, Bogdanovich T, Tian GB, Marschall J, Urban CM, Spellberg BJ, Rhee D, Halstead DC, Pasculle AW, Doi Y.	J Clin Microbiol. 2011 Nov;49(11):3849-54.	May 2011	☐Submitted ☐Accepted X Published
Activities of vancomycin-containing regimens against colistin-resistant *Acinetobacter baumannii* clinical strains.	O'Hara JA, Ambe LA, Casella LG, Townsend BM, Pelletier MR, Ernst RK, Shanks RMQ, Doi Y.	Antimicrob Agents Chemother 2013;57:2103-8	November 2012	☐Submitted ☐Accepted X Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes

If yes, please describe your plans:

We anticipate that additional publications will result from this project. For example, we are completing analysis of our hVISA/MRSA work and anticipate submission to a peer-reviewed journal.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The rate of positive *A. baumannii* screening culture in the ICUs at UPMC Mercy Hospital declined from an average of 3.0% in the first year to 0.9% in the third year, which we believe is at least in part attributable to the stepwise intervention which was implemented through the grant, consisting of active screening, chlorhexidine body wash and terminal cleaning using bleach.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

A novel high-sensitivity approach was developed for detection of *Acinetobacter baumannii* colonization among hospitalized patients, enabling implementation of appropriate contact precaution. The method has proven highly effective in reducing *A. baumannii* colonization rates at UPMC Mercy Hospital and continues to be employed there as routine clinical care.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant?

No.

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:

Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

No

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Lee H. Harrison, MD (Professor, Division of Infectious Diseases and Department of Epidemiology, Graduate School of Public Health, and Principal Investigator (PI), Public Health Infectious Diseases Laboratory), has been the Center Principal Investigator. Dr. Harrison was trained in clinical epidemiology and molecular epidemiology of antimicrobial-resistant bacterial pathogens as an Epidemic Intelligence Service Officer at the Centers for Disease Control and Prevention (CDC). He has over 15 years of experience in collaborative research in this field. Dr. Harrison is the PI of the CDC-funded Maryland Active Bacterial Core surveillance (ABCs) site, which conducts active laboratory and population-based surveillance and applied research on invasive, antimicrobial-resistant and vaccine preventable bacterial diseases. Dr. Harrison is the recipient of a National Institutes of Health midcareer investigator (K24) award in patient-oriented research entitled *Clinical Research and Mentoring in Epidemiology of Bacterial Diseases*. He recently became a voting member of the CDC Advisory Committee on Immunization Practices.

Yohei Doi, MD, PhD (Assistant Professor) Dr. Doi received an MD degree and PhD in bacteriology from Nagoya University in Aichi, Japan. His main research interests are in the epidemiology, laboratory detection and clinical relevance of new beta-lactamases in gram-

negative bacilli. Dr. Doi's laboratory research focuses on mechanisms of resistance and molecular epidemiology of bacteria resistant to multiple antimicrobials. A current major research focus is epidemiology of antimicrobial resistance genes in *A. baumannii*, and characterization of mechanisms that lead to colistin resistance.

Jo-anne M. Salangsang, MD (Postdoctoral Scholar and Infectious Diseases Physician), conducted her fellowship research with Dr. Harrison on clinical, epidemiologic, and molecular epidemiologic aspects of MRSA infection.

Kathleen A. Shutt, MS (Research Associate), was a Co-Investigator and oversaw all data management and analysis aspects of the project. Ms. Shutt received her MS degree in statistics from the University of South Carolina and spent 4 years working with the CDC as a data analyst before taking her current position at Pitt.

Jane W. Marsh, PhD (Research Assistant Professor and Director, Public Health Infectious Diseases Laboratory) was a Co-Investigator. Dr. Marsh received her PhD from Dartmouth College, investigating the laboratory pathogenesis of *Vibrio cholerae*. Dr. Marsh developed the multilocus variable number tandem repeat analysis (MLVA) assay that has enhanced our ability to detect and control *C. difficile* infection at UPMC.

Bruce Y. Lee, MD, MBA (Assistant Professor of Medicine, Epidemiology, and Biomedical Informatics), was a Co-Investigator and directed the infectious diseases modeling activities outlined in the application. Dr. Lee received his MD degree from Harvard University and MBA degree from Stanford University. Dr. Lee is Co-Investigator on the NIH-funded Models of Infectious Disease Agent Study (MIDAS), a research consortium that develops computational models of the interactions between infectious agents and their hosts, disease spread, prediction systems, and response strategies, and the Vaccine Modeling Initiative (VMI), funded by the Bill and Melinda Gates Foundation. Dr. Lee heads the Public Health and Infectious Diseases Computational and Operational Research (PHICOR) group, which specializes in computer models and simulations to better understand the spread and impact of infectious diseases and operations to control their spread.

Department of Epidemiology, Graduate School of Public Health
Maria M. Brooks, PhD (Associate Professor), received her MA and PhD in statistics from Harvard University and University of North Carolina, Chapel Hill, respectively. She has extensive experience in study design and data analysis.

Paula K. Davis, MA (Assistant Vice Chancellor for Diversity), oversaw the Center program for training of minority students in antimicrobial resistance research. She was involved in the recruitment, selection, placement, and evaluation of trainees selected for our undergraduate and graduate post-baccalaureate programs. Ms. Davis worked closely with Dr. Harrison and other Center investigators to assure that our minority trainees are matched according to their interests with the most appropriate faculty members and type of antimicrobial resistance research. Davis assumed her current position after serving as Assistant Dean in the School of Medicine with responsibility for Admissions and Financial Aid. Prior to her time in Admissions, Davis served as Assistant Dean for Student Affairs and Director of Diversity Programs from 1994-2005.

Recipient of the 2003 Chancellor's Affirmative Action Award, Ms. Davis has spent the past twenty five years working in student support services including admissions, academic advising, alumni relations, financial aid and minority support services. Her experiences include work in pre-college, undergraduate, graduate and medical education. A graduate of the University of Pittsburgh (BA, English; MA, Communications), Davis served as director of admissions for Carnegie Mellon University's H. John Heinz III School of Public Policy and Management before joining Pitt Med in 1994. Davis is serving her second term as director of the northeast region of the National Association of Medical Minority Educators and is an advocate for autism research and education.

A. William Pasculle, ScD (Director of Clinical Microbiology, UPMC-Presbyterian and Associate Professor of Pathology, School of Medicine), has extensive experience in all aspects of microbiology, including all of the bacterial pathogens relevant to this application. He discovered *Legionella micdadei* as a cause of human infection and his group was among the first to recognize *Mycoplasma hominis* as a cause of serious extragenital infections in adults.

Carlene A. Muto, MD, MS (Director of Infection Control, UPMC-Presbyterian and Associate Professor, Division of Infectious Diseases, School of Medicine), received her MD degree from Temple University and completed her infectious diseases fellowship at University of Virginia. As Director of Infection Control, Dr. Muto leads a large team of infection control professionals to control serious bacterial pathogens at UPMC, including the organisms that are the focus of our Center.

UPMC-Mercy Hospital

Scott R. Curry, MD (Clinical Assistant Professor, Division of Infectious Diseases, School of Medicine),received his MD degree from the Medical University of South Carolina and will soon receive his MS degree in clinical epidemiology from the Pitt Graduate School of Public Health. Dr. Curry is currently an attending physician in infectious diseases and acting medical director of infection control at UPMC-Mercy. He conducted his fellowship research with Dr. Harrison on *C. difficile* infection. Dr. C Dr. Curry published a study describing the diversity of the gene encoding the *C. difficile* toxin regulatory protein, *tcdC*.

CARNEGIE MELLON UNIVERSITY

Lester Lave, PhD (Harry B. and James H. Higgins Professor of Economics and University Professor) (deceased) has substantial experience in quantitative risk assessment, risk perception and communication, and assessing the economic value of information in tests. Dr. Lave is a member of the Institute of Medicine of the National Academy of Science and a recipient of the Distinguished Achievement Award of the Society for Risk Analysis.

Elizabeth Casman, PhD (Associate Research Professor, Engineering and Public Policy), specializes in integrated assessment modeling of infectious diseases. She has expertise in risk analysis and the interaction of the environment and infectious diseases, which is important given the influences of local ecosystem characteristics on the impact and spread of antimicrobial-resistant bacteria. Dr. Lave and Dr. Casman are faculty members in the Department of Engineering and Public Policy, a unique department in the College of Engineering at Carnegie Mellon that addresses important problems in science and policy in which the scientific details are

of central importance. The Department has substantial experience and resources to conduct rigorous research in risk analysis and communication and technology policy and management.

KANE REGIONAL CENTERS

Mario Fatigati, MD, is the Chief Medical Officer of the Kane Regional Centers, which are public Allegheny County long-term care facilities.