

University of Pennsylvania

Annual Progress Report: 2008 Nonformula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Nonformula Grant Overview

The University of Pennsylvania received \$5,531,053 in nonformula funds for the grant award period June 1, 2009 through May 31, 2013. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Epidemiology and Prevention of MRSA Transmission in the Community - The purpose of this study is to understand the reasons for recent dramatic increases in infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) in the community. Through a broad collaboration with partners in Eastern and Central Pennsylvania, we will study why patients with MRSA infections frequently have recurrent infections despite appropriate treatment. We will also study how often and why household members of such patients develop new MRSA infections. We will determine how often MRSA spreads between household members and how long individuals harbor MRSA over time. We will also test whether treatment to eliminate MRSA colonization prevents MRSA infections in the household. Finally, we will establish a program to develop a pipeline of new scientists and clinicians among underrepresented minorities in the Commonwealth.

Anticipated Duration of Project

6/1/2009 - 5/31/2013

Project Overview

This study will elucidate the longitudinal dynamics of MRSA colonization and infection and test an intervention to prevent MRSA transmission. To achieve this objective, we propose three scientific objectives:

- 1) to identify host, microbiological and environmental risk factors for prolonged MRSA colonization, MRSA transmission, and MRSA infection among patients with MRSA skin or soft tissue infections (SSTIs) and their household contacts and to use stochastic agent-based modeling methods to quantify secondary spread of CO-MRSA in households
- 2) to evaluate the impact of a decolonization intervention on MRSA infections in the household.
- 3) to identify immunological mechanisms underlying the ability of *S. pneumoniae* colonization to inhibit MRSA colonization, transmission and infection.

In conjunction with these scientific goals, we also propose two educational and organizational objectives:

- 1) to foster multi-disciplinary and cross-institutional collaborations and develop the infrastructure for a Center of Excellence focused on antimicrobial drug resistance research.
- 2) to enhance opportunities for basic and clinical research training for undergraduate and graduate students, particularly from underrepresented minorities, to increase the pipeline of future scientists.

To achieve the study aims, we propose a multicenter prospective cohort study of outpatients with newly diagnosed MRSA SSTIs. The source population for this study will be all adults and children receiving care in the emergency departments (EDs) and outpatient practices of the Hospital of the University of Pennsylvania (HUP), Penn Presbyterian Medical Center (PPMC), the Children's Hospital of Philadelphia (CHOP), and Hershey Medical Center (HMC). These subjects and their household members will undergo regular sampling for MRSA colonization over time. Subsequently, we will conduct a randomized controlled trial to assess the impact of two decolonization interventions on MRSA infections in the household. The proposed novel approach to sampling of cases and their household contacts over time represents a unique opportunity to elucidate the longitudinal transmission dynamics of MRSA in the community. The inclusion of adults and children from a geographically, racially, and ethnically diverse population will greatly strengthen the generalizability of the results and maximize the public health impact for all Pennsylvanians.

Principal Investigator

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Other Participating Researchers

Joshua P. Metlay, MD, PhD, Jeffrey N. Weiser, MD, Warren Bilker, PhD, Irving Nachamkin, DrPH, MPH, Paul H. Edelstein, MD, Laurence Gavin, MD, Judd E. Hollander, MD, Darren R. Linkin, MD, MSCE, David J. Margolis MD PhD, Gary Smith, PhD, Neil O. Fishman, MD, Harvey Rubin, MD, Patrick J. Brennan, MD, Brian L. Strom, MD, MPH, Richard Shannon, MD - employed by University of Pennsylvania.

Rakesh D. Mistry, MD, MS, Susan Coffin, MD, MPH, Theoklis Zaoutis, MD, MSCE - employed by Children's Hospital of Philadelphia

Kathleen Julian, MD, Lawrence E. Kass, MD - employed by Penn State Hershey Medical Center

David F. Royer, PhD - employed by Lincoln University

Jonathan Finkelstein, MD - employed by Harvard University

Barry N. Kreiswirth, PhD - employed by New York University

Loren G. Miller, MD, MPH - employed by UCLA Medical Center

Expected Research Outcomes and Benefits

Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) among both adults and children in the community have increased markedly in recent years. These infections are associated with significant morbidity, mortality and cost. Our study will identify why patients with skin and soft tissue infections due to MRSA often have subsequent MRSA infections despite appropriate treatment for the initial infection. Identifying why these subsequent MRSA infections occur will help identify strategies to prevent these infections. We will identify how often, and why, household members of patients with MRSA infections also develop new MRSA infections. Identifying such factors will be critical in targeting approaches to prevent MRSA infections in household members as well. We will also investigate the impact of colonization with *Streptococcus pneumoniae* on MRSA infection. In particular, by elucidating the immunological mechanism underlying the ability of *S. pneumoniae* to inhibit MRSA colonization, we hope to identify immunological targets for future vaccine strategies. While our primary focus will be on MRSA infections, we will also study MRSA colonization (i.e., when an individual harbors the MRSA organism but is not infected). This is important because individuals are typically colonized before they are infected. Thus, preventing colonization will help to prevent infection. We will also identify how MRSA spreads in the household and investigate ways to prevent spread. Indeed, a major focus of our study will be to specifically test two decolonization interventions to prevent MRSA infections in households. This will provide critical evidence to support interventions to halt the further spread and impact of MRSA in the community which can be broadly applied across the commonwealth of Pennsylvania and beyond. In addition to research outcomes, this proposal contains a significant focus on teaching and education in order to produce a durable result for years to come. We will reach out to undergraduate and graduate students at Lincoln University to provide valuable opportunities and role models for aspiring under-represented minority students.

Summary of Research Completed

The milestones listed in our original timeline for this initial time period were: 1) finalize staff recruitment; 2) train staff; 3) finalize microbiological methods for MRSA and *S. pneumoniae* identification and testing; 4) finalize data collection protocols; 5) hold first Scientific Advisory Committee meeting; 6) recruitment of subjects into cohort study to begin 3/1/10; 7) enroll 50 households by end of period; 8) identify specific cross-reactive targets in *S. aureus* and antigens in *S. pneumoniae*; 9) train at least one undergraduate and one graduate under-represented minority student. In the paragraphs below, we address progress to date on these and other components of the study.

1) Finalize staff recruitment

All research staff have been hired for this project. Pam Tolomeo, MPH, serves as the overall study project manager. Ms. Tolomeo's experience, as well as her nearly 10-year track record of working closely with Dr. Lautenbach is invaluable in ensuring the successful conduct of this study. Kateri Leckerman, MPH has been recruited to serve as the project manager for CHOP. Ms. Leckerman also has considerable experience and a long track record of work with Dr. Zaoutis (Co-PI, CHOP Site PI). Research coordinators for each site are also in place as follows:

Jackie Wise (Penn), Grace Ndicu (CHOP), and Brian Stevenson (HMC). We have also recruited Baofeng Hu, MD to serve as the lead laboratory technician for this study. Dr. Hu has considerable experience in identification of bacterial colonization, susceptibility testing, and characterization of resistance phenotypes and genotypes. Finally, she has an established track record of working with the PI and co-investigators. Research space for the project managers and research coordinators at the various sites are in close proximity (i.e., on the same floor) to the offices of the supervising faculty investigators. This greatly facilitates frequent interaction and ability to meet both formally and informally to discuss issues regarding the study.

2) Train staff

All hired staff (noted above) have been trained and are actively working on the project.

3) Finalize microbiological methods for MRSA and *S. pneumoniae* identification and testing

The protocols for the microbiological evaluation of MRSA and *S. pneumoniae* have been finalized. These include swab specimen transport, identification of MRSA, MRSA susceptibility testing, MRSA SCCmec typing, pulsed-field gel electrophoresis (PFGE), MRSA spa typing, *S. pneumoniae* swab specimen transport, *S. pneumoniae* serological typing, and *S. pneumoniae* identification. Indeed, many of the MRSA microbiological techniques were validated as part of recent work supported by this grant (see Table 1, Publication #1, Presentation #1). A total of 502 swabs have thus far been worked up.

4) Finalize data collection protocols

These protocols were finalized in November 2009 and included protocols for collection of clinical data from initial subject recruitment, initial household visits, and ongoing telephone interviews of household members throughout the longitudinal follow up of subjects. Protocols for collection of microbiological data from ongoing swab sampling were also completed. Development and refinement of the study database has also been completed. Concurrently, protocols for identification of eligible patients and tracking the collection of clinical data and microbiological samples from enrolled subjects has also been completed. In the first three months of subject recruitment, these protocols for subject recruitment have been revised and optimized based on early experience of the investigators in conducting this study.

5) Hold first Scientific Advisory Committee meeting

Monthly meetings of PI, Co-PIs, co-investigators, and study staff occurred since the start of the grant. These meetings address study protocol issues, subject recruitment, enrollment and follow-up, microbiology laboratory updates, IRB applications, and recruitment of undergraduate and graduate students. These monthly meetings typically include participation of numerous members of the Scientific Advisory Committee. Due to scheduling issues, the first formal meeting of the Scientific Advisory Committee will take place in September, 2010. However, input from members of this committee has been solicited and incorporated in an ongoing fashion.

6) Recruitment of subjects into cohort study to begin 3/1/10

We exceeded expectations in reaching this milestone. Identification of eligible subjects began at HUP study site on 1/14/10, with the first household enrolled at HUP on 01/30/2010.

7) Enroll 50 households

By June 30, 2010, a total of 31 households had been enrolled. While this total is slightly below the milestone for this period, enrollment rates have increased steadily since the start of recruitment. Household enrollment rates by month are as follows: January=1; February=2; March=2; April=6; May=8; June=12. Based on this trend, we have every expectation that enrollment rates will continue to increase such that we will achieve the enrollment goals for the next reporting period. Indeed, 8 new households are scheduled to be enrolled as of 7/7/10. The 31 enrolled households are distributed as follows: HUP=14; PPMC=5; HMC=5; CHOP=7. These households constitute 108 study subjects. There are 4 one-person households; 5 two-person households; 10 three-person households; and 12 \geq 4-person households.

There are several reasons for the lower than expected number of households enrolled during this time period. While recruitment at HUP and PPMC started in January, recruitment of subjects at HMC and CHOP began on 3/24/10 and 4/26/10, respectively. The first households were enrolled at HMC and CHOP on 4/20/10 and 5/13/10, respectively. The primary reason for these later starts was a delay in obtaining final IRB approval at these sites. In addition, our original intent was to limit recruitment to subjects seen in the EDs of the study sites. This was based on preliminary data suggesting the vast majority of patients who have an SSTI drained, have this done in an ED. However, recent data from the study sites revealed that approximately 20% of SSTI cultures revealing MRSA are obtained in outpatient practices of the study sites. As such, we have modified the study protocol to permit inclusion of patients seen in outpatient practices, provided they meet all other eligibility criteria. This change was very recently approved by all relevant IRBs. This modification to the study protocol will not only expand the eligible study population, but will make the results of the study more generalizable as a broader representation of subjects with SSTIs due to MRSA will now be eligible.

8) Identify specific cross-reactive targets in *S. aureus* and antigens in *S. pneumoniae*

The laboratory based research project has made substantial progress. On-going efforts of two full-time members of Dr. Weiser's lab have identified two specific antigens from *S. pneumoniae* that generate a cross-reactive antibody response to *S. aureus* following model murine colonization. Two candidate pneumococcal antigens have been cloned, expressed and used to raise antibody to confirm this cross-reactivity prior to testing in protection assays against MRSA. These antibodies were primarily directed against two conserved dehydrogenases on *S. aureus*, P5CDH and DLDH. We also identified their respective homologs in *S. pneumoniae*, putative dehydrogenases SP_1119 and SP_1161. These were confirmed as surface exposed antigens highly conserved across the species. Having achieved the milestone of identifying potential cross-protective antigens, current work focuses on testing the protective effects of each of these antigens individually in animal models of *S. aureus* colonization and disease.

9) Train at least one undergraduate and one graduate under-represented minority (URM) student

We are delighted to report that we have far exceeded this milestone. Seven URM students have already worked or are working on this grant. The first was Manuel Bramble, an African-American undergraduate student at the University of Pennsylvania. Mr. Bramble participated in the Summer Undergraduate Minority Research (SUMR) at Penn in the summer of 2009. Mr. Bramble worked on various aspects of the study including drafting and revising informed consent forms, drafting data collection forms and study questionnaires, and drafting of protocol

summaries for IRB correspondence. Furthermore, under the guidance of Dr. Lautenbach, Mr. Bramble conducted a narrative review on a topic closely related to the current grant. This project focused on the potential role of pet animals on household transmission of MRSA (see Publication #2, below). Also in the summer of 2009, Gloria Williams, an African-American undergraduate student at the University of the Sciences in Philadelphia, participated in the Summer Undergraduate Internship Program (SUIP) for students interested in biomedical related research careers. In addition to the regular meetings of this program, Ms. Williams participated in the laboratory based research component of the project in the Weiser group. In 2009-2010, Ehimare Akhabue, an African-American medical student at Penn also worked on the project assisting in finalization of consent forms and data collection and interview forms. To gain more experience in epidemiologic research, he also headed up two projects, both of which resulted in scientific manuscripts (see Table 1, Publications #3 and #4). For his work, Mr. Akhabue received the Moskowitz Award for medical student research.

Other activities

New Grant application

Based in part on the review led by Manuel Bramble (see above), we submitted an NIH R01 application to investigate the impact of MRSA-colonized pet animals in MRSA household transmission (Table 2). This grant proposes to use the ongoing CURE grant as a foundation on which to build this new work to answer important novel questions regarding the role of pets.

Center for Antimicrobial Resistance Research

On 9/25/09, a retreat was held to lay the groundwork for the creation of a new center focused on antimicrobial drug resistance research. The proposal and business plan for this center is currently being finalized by Drs Lautenbach and Zaoutis who will serve as Director and Associate Director of the Center, respectively. There continues to be great institutional enthusiasm for the creation of this center.

Table 1. Publications and Presentations from CURE Grant

Manuscript/ Presentation Number	Authors	Title	Journal / Conference
Manuscripts			
1	Lautenbach E, Tolomeo P, Nachamkin I, Hu B, Zaoutis TE.	The impact of household transmission on duration of outpatient colonization with methicillin-resistant <i>Staphylococcus aureus</i>	<u>Epidemiology and Infection</u> 2010;138:683-5
2	Bramble M, Morris D, Tolomeo P, Lautenbach E.	Potential Role of Pet Animals in Household Transmission of <i>Methicillin-Resistant Staphylococcus aureus</i> : A Narrative Review	Vector-Borne and Zoonotic Diseases (in press)
3	Akhabue E, Synnestvedt M, Weiner MG, Bilker WB, Lautenbach E.	Cefepime Resistance in <i>Pseudomonas Aeruginosa</i> : Risk Factors and Clinical Outcomes	Submitted for publication
4	Akhabue E, Lautenbach E.	“Equal” Contributions and Credit: An Emerging Trend in the Characterization of Authorship	Submitted for publication
Presentations			
1	Hu B, Tolomeo P, Lautenbach E, Nachamkin I.	Assessment of MRSA Survival Using the Copan ESwab Collection and Transport System	110 th Annual Meeting of the American Society for Microbiology, San Diego, CA May 23-2, 2010

Table 2. Abstract of New R01 Application Building on the CURE Grant

Grant Title	Grant abstract
The Role of Pet Animals in Household Transmission of MRSA	<p>Infections due to community-onset methicillin-resistant <i>Staphylococcus aureus</i> (CO-MRSA) have increased significantly. Patients with a CO-MRSA infection (usually a skin or soft tissue infection (SSTI)), often develop repeated infections despite appropriate therapy. Efforts to address this problem have been unsuccessful due to an incomplete understanding of the epidemiology of MRSA household transmission. Increases in MRSA infections in companion animals have been reported in recent years. Also, numerous studies have described transmission of MRSA from pet animals to humans (and vice versa). Finally, several reports describe recurrent MRSA infections in human family members that could not be quelled until the family’s colonized pet was identified as an MRSA carrier and decolonized. Numerous publications have highlighted the urgent need to elucidate the role of domestic animals in MRSA transmission. However, the impact of MRSA colonization of pet animals on human colonization and infection with MRSA in households has not been studied.</p> <p>This application will build substantially on work being conducted as part of a 4-yr, \$5.5 million Pennsylvania Department of Health (PADOH) grant (PI: Dr. Lautenbach). This is a multicenter prospective cohort study of outpatients with newly diagnosed MRSA SSTIs. These subjects and their household members will undergo sampling for MRSA colonization every two weeks for a 6-month follow-up period. While this study will provide valuable information on risk factors for prolonged CO-MRSA colonization and new CO-MRSA infection, the role of pet animals is not addressed. The current application will build on the unique foundation of the PADOH study to assess the impact of MRSA-colonized pets on human MRSA colonization and infection in households</p> <p>The primary aims of this study are:</p> <ol style="list-style-type: none"> 1) to characterize the association between pet animal MRSA colonization and prolonged CO-MRSA colonization in patients with a CO-MRSA SSTI; and 2) to elucidate the association between pet animal MRSA colonization and new CO-MRSA infection among patients with a prior CO-MRSA SSTI <p>The PADOH study offers a novel approach to studying cases and their household contacts over time. It also provides a unique opportunity to elucidate, with great efficiency, the impact of pet animals on human CO-MRSA colonization and infection in households. The participating institutions ensure inclusion of a diverse adult and pediatric study population from urban, suburban, and rural settings. Utilizing this multicenter cohort study, this application will provide novel findings that may then be translated into innovative approaches to limit human colonization and infection with CO-MRSA in households. These novel interventions could include enhanced surveillance for CO-MRSA among pets of humans with recurrent CO-MRSA infection as well as decolonization of pets with MRSA carriage.</p>