Northern Michigan University

Internal Faculty Reassigned Time Award Cover Sheet

Relationship Between Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization and Vaccination with a Pneumococcal Conjugate Vaccine in Adults

A proposal submitted to:
NMU Faculty Grants Committee

By

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14 September 2015

Years at NMU:	3
Total Reassigned Time Requested:	4
Semester Requested:	Winter 2016
Dates of Previous support (indicate if none):	Winter 2014

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Relationship Between Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization and Vaccination With a Pneumococcal Conjugate Vaccine In Adults

Abstract

Childhood pneumoccocal conjugate vaccines were adopted in 2001 following recommendation by the Centers for Disease Control and Prevention. Studies demonstrate children given these vaccines have decreased *Streptococcus pneumoniae* nasal carriage and changes in other bacterial flora. Since *S. pneumoniae* inhibits the growth of *Staphylococcus aureus,* it is hypothesized that this vaccination creates a potential for increased *S. aureus* carriage. The increase in community acquired Methicillin Resistant *S. aureus* (CA-MRSA) infections correlates with the advent of pneumococcal conjugate vaccination. Additionally, the major risk factor for MRSA infection is nasal carriage. This supports a hypothesis that pneumococcal vaccination may increase MRSA carriage. In 2010, a pneumococcal conjugate vaccine was approved for use in adults over the age 50. To date, no studies have investigated if vaccination with pneumoccocal conjugate vaccines alters the nasal flora of adults. This project proposes to examine the prevalence and carriage of *S. pneumoniae*, *S. aureus* and MRSA in vaccinated adults and compare with an unvaccinated control group. Consenting adult volunteers with known vaccine status will submit 2 nasal swabs for analysis. Methods will include bacterial culture, real time quantitative polymerase chain reaction, and a laser light scattering method. The study will provide publishable data on the impact of this vaccine on a major community health threat; MRSA and provide bacterial isolates and an initial data set to pursue external funding. Additional benefits will be a comparative analysis of bacterial culture, molecular, and light scattering based diagnostic techniques, and interdisciplinary educational opportunities for graduate and undergraduate students.

I. Introduction

Streptococcus pneumoniae is a Gram positive diplococci that is a normal bacteria found in the human upper respiratory tract. Carriage of the bacterium is usually asymptomatic, however, in very young, elderly, or otherwise immunocompromised individuals the bacterium can cause significant morbidity with the majority of severe infections occurring in children and older adults (Lynch JP et al. 2010). *S. pneumoniae* causes a diverse range of illnesses, ranging from meningitis, bacteremia, pneumonia, and ear infections and it is the most common source of bacterial pneumonia. The Prevnar 13® vaccine is a pneumococcal vaccine that is effective at generating an immune response capable of preventing infection with 13 of the most common *S. pneumoniae* strains (Miller EV et al. 2008). In 2011 the Centers for Disease Control and Prevention (CDC) recommend the Prevnar 13® vaccine for individuals 6 months to 17 years old and those over 50.

S. aureus is a Gram positive cocci and opportunistic pathogen that takes advantage of damaged cutaneous barriers, causing infections ranging from mild skin and soft tissue infections, to more serious tissue abscesses, bone infections, pneumonias, toxic shock syndrome, and sepsis (Cheng et. al 2011, Pääkkönen and Peltola, 2013). The CDC reports that *S. aureus* infections cause nearly 500,000 hospitalizations per year. Approximately 94,000 of those cases are due to Methicillin Resistant *Staphylococcus aureus* (MRSA) (Klein et al. 2007, Klevens et al. 2007). MRSA strains are multidrug resistant and cannot be easily treated with many of the currently available antibiotics. While *S. aureus* can cause significant disease, it can also colonize human skin or nose and be considered part of the normal human microflora. Approximately 29% of people are asymptomatic carriers of *S. aureus* and 1.5% are carriers of MRSA that show no signs of disease (Kuehnert et al. 2006).

Studies have demonstrated a negative correlation between *S. aureus* and *S. pneumoniae* carriage in several populations (Regev-Yochay et al. 2004, Regev-Yochay et al. 2006, Regev-Yochay et al. 2009). Moreover, cases of community acquired MRSA infections have increased since the introduction of pneumococcal vaccines (Bogut et al. 2004, CDC 2008). This data could suggest a potential adverse effect of pneumococcal vaccination; increased *S. aureus* carriage in the nose. When grown together in laboratory experiments it has been shown that *S. pneumoniae* can antagonize and suppress the growth of *S. aureus* (Regev-Yochay et al. 2006). Moreover, one study also indicated that co-culture of *S. pneumoniae* and *S. aureus* from the nose in children is rare (Regev-Yochay et al. 2004). Currently, there are no published studies that examine the impact of *S. pneumoniae* vaccination on *S. aureus* or MRSA carriage in adults. This is of interest as MRSA carriage rates are correlated with increased age (CDC 2011).

A major aim of this study is to ascertain if the removal of *S. pneumoniae* carriage in the nose of adults due to Prevnar 13® vaccination results in increased *S. aureus* or MRSA colonization of this niche. One prediction of our study is that if Prevnar 13® vaccination significantly reduces *S. pneumoniae* carriage in the nose, then that niche would become available for colonization by *S. aureus/*MRSA with less competition for resources. If Prevnar 13® vaccination does lead to increase MRSA carriage in the nose of adults, this would be a significant finding because MRSA colonization of the nose is a risk factor for MRSA infection in that person and close contacts (Croft et al. 2009, Huang et al. 2006, Lu et al. 2007). Moreover, the results of this study could suggest that the current protocol for Prevnar 13® vaccination may have to be altered.

Because the severity of *S. aureus* infections are often dependent on the which toxins genes are carried by particular strains, a secondary aim of this project is to utilize PCR-based molecular subtyping to elucidate which toxin genes are present in the *S. aureus* isolates in the

Upper Peninsula community. The presence of these toxins can result in severe clinical symptoms. The Enterotoxins cause nausea and vomiting. Panton-Valentine Leukocidin and Leukocidin A/B cause necrosis of white blood cells and infected tissues. Hemolysins lyses red blood cells, Exfoliative toxins cause blistering/skin peeling (Scaled-Skin Syndrome), and Toxic Shock Protein can cause high fever and rapid drops in blood pressure that can be fatal (Renwick et al. 2013, Abdalrahman et al. 2015, Zetola et al. 2005, and Johnson, et al. 1991). Knowledge of the types of *S. aureus* carried by humans in the community could be useful for physicians to determine the best course of treatment for a patient when a *S. aureus* infection is suspected.

Project Status. This project was chosen to be funded as one of first NMU Prime Grants in 2015. The NMU Prime Grant is for a single year of funding. Therefore much work needs to be accomplished in that time frame. I am seeking a reassigned time award to establish and coordinate this new research project and execute the experiments as outlined in the methods section.

Applicant qualifications. In my CV in Appendix 2, I provide evidence that I have been engaged in both teaching medical microbiology and researching infectious diseases for a significant number of years. Those experiences qualify me to conduct this project which is related to these fields. Additionally, because this project utilizes human subjects an Internal Review Board approval for the use of human subjects is included in Appendix 4.

Why this project should be done. It is very exciting to be part of one of the first groups to be awarded a Northern Prime Grant. This project is the first collaborative research effort between the Clinical Life Sciences and Biology Departments. We will be performing research that addresses a real world medical problem and that involves our local community. Moreover, this creates essential training opportunities for our graduate and undergraduate students that will prepare them well for their future careers. Our students will directly engage in multiple aspects of

microbiology, genetics, physics, and infectious disease research – including experimental design, hands-on bench-work, and presentation of results. Data will be generated for poster presentations at public and scientific meetings. Preliminary data generated would substantially strengthen future applications for external funding. Finally, the creation of such research opportunities continues to establish that NMU is committed to training and recruiting high caliber students.

II. Methods (A graphical flow chart for the project methods can be found in Appendix 6)

The equipment required to execute this study already exists in the Biology or CLS Departments.

To acquire volunteers for the study:

1) Educate potential volunteers on Prevnar vaccination, MRSA, and the scope of study and use of results. The initial study aims to recruit 200 volunteers from the UP community.

2) Distribute questionnaire to gather subject data and informed consent (See Appendix 5).

To isolate and identify culturalable bacteria from the volunteer nose:

3) Collect nasal swabs (Self-administered by volunteer with supervision), 2 per subject.

4) One swab will be streaked to the following agars:

    a. Mannitol Salt Agar, MRSA Chrome Agar, Sheep's Blood Agar

    b. Plates will be incubated for 24 hours and examined for growth consistent with *S. pneumonia or S. aureus.*

    c. Suspected *S. pneumoniae* isolates will be tested using API 20 Strep system.

    d. Suspected *S. aureus* isolates will be tested using Staph aurex.

e. Suspected *S. aureus* isolates will be tested on a laser light scattering system for identification.

f. All *S. aureus* strains will be tested for methicillin resistance using Cefoxitin Disk Diffusion Test.

g. Utilizing PCR with specific primers and probes, all *S. aureus* strains will be tested for the presence and expression of toxin genes associated with more serve clinical outcomes. This will provide information on the types of *S. aureus* circulating in our community.

5) A 2nd swab will be placed in enrichment broth and incubated for 6-8 hours.

a. DNA will be extracted from the enrichment broth using a DNA extraction kit.

b. DNA will be tested for the presence of *S. pneumoniae* using sequence specific primers and probes. Positive samples will have a quantitative assessment of DNA copy number that is correlated to colony forming units (CFU), and indication of bacterial burden.

c. DNA will be tested for the presence of *S. aureus* using sequence specific primers and probes. Positive samples will have a quantitative assessment of DNA copy number that is correlated to (CFU), and indication of bacterial burden.

d. DNA that tests positive for *S. aureus* specific DNA sequences will be analyzed for the presence of the mecA gene using sequence specific primers and probes.

e. Enrichment broth will be tested for the presence of *S. aureus* using a laser light scatter device

Data Analysis

6) Following bacterial identification by culture the prevalence rates of *S. pneumoniae*, *S. aureus* and MRSA will be calculated and stratified by age group, vaccination status, and previous infection history using appropriate statistical analysis tools. Carriage patterns will initially be analyzed by individual vaccine status. Demographic factors that differ between groups with similar carriage patterns will be evaluated for potential confounders. Odds ratios and 95% confidence intervals will be calculated to determine statistical significance. Additionally, results from the culture, PCR, and light scattering methods will be compared using regression analysis to evaluate analytical sensitivity, specificity, and limits of detection.

Project Timetable

Date	**Activity**
Jan.-Feb. 2016	● Recruit volunteers for study. Isolate and culture nasal microflora. ● Make freezer stocks of bacteria isolates for further study.
Feb. 1 – Mar. 31 2016	● Identify if initial volunteers carry *S. pneumoniae*, *S. aureus*, both, or neither. ● Determine if *S. aureus* isolates are MRSA. ● Begin PCR based molecular subtyping of *S. aureus* isolates.
Apr. 1 – May 31 2016	● Gather preliminary result on *S. aureus* subtypes found in the UP community. ● Compile preliminary data and design poster for presentation at the American Society for Microbiology General Meeting in Boston MA.
May 2016 – onward	● Begin data analysis to determine if there is a correlation between *S. pneumoniae* and *S. aureus*/MRSA carriage. ● Compose an external funding proposal for the National Institutes of Health R15 grant program.

III. Reassigned Time

I am requesting 4 credits of reassigned time. The amount of time requested is based on the time required to (1) perform and coordinate the above research as indicated in the methods section, (2) mentor 1 graduate student and 2 undergraduate students in that research, and (3) prepare grant applications for external funding as compared to the time required to prepare and teach courses. Thank you for your consideration to support my application for reassigned time.

Appendix 1. Literature Cited

Abdalrahman, L. S. and Fakhr, M. K. (2015). Incidence, antimicrobial susceptibility, and toxin genes possession screening of *Staphylococcus aureus* in retail chicken livers and gizzards. *Foods* 4: 115-129.

Bogut, D., van Belkum, A. Sluijer, M., Luijendijk, A., de Goot, R., Rumke, H. C., Verbruge, H. A., and Hermens, P. W. (2004). Colonization by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet*. 363:1871-1872.

Centers for Disease Control and Prevention. (2008). Progress in the introduction of pneumococcal conjugate vaccine-worldwide, 2000-2008. *MMWR Morb. Mortal. Wkly. Rep*. 50:919-922.

Centers for Disease Control and Prevention (2011). Active Bacterial Core Surveillance (ABCs*) Report Emerging Infections Program Network Methicillin -Resistant Staphylococcus aureus.*

Cheng A.G., DeDent, A.C., Schneewind, O., and Missiakas, D. (2011). A play in four acts: *Staphylococcus aureus* abscess formation. *Trends Microbiol*. 2011(5):225-232.

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Huang, Y.C., Chou, Y.H., Su, L.H., Lien, R.I., and Lin, T.Y. **(**2006). Methicillin-resistant *Staphylococcus aureus* colonization and its association with infection among infants hospitalized in neonatal intensive care units. *Pediatrics*. 118:469-474.

Johnson, W. et al. 1991. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the Polymerase Chain Reaction. *Journal of Clinical Microbiology* 29: 426-430.

Klein, E., Smith, D.L., and Laxminarayan, R. (2007). Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerg Infect Dis*. 13(12):1840-1846.

Klevens, R.M., Morrison M.A., and Nadle, J. (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA*. 298:1763-1771.

Kuehnert M.J., Kruszon-Moran D., and Hill, H.A. (2006). Prevalence of *Staphylococcus aureus* Nasal Colonization in the United States, 2001–2002. *J. Infect. Dis.* 193(2):172-179.

Lu, P.L., Tsai, J.C., Chiu, Y.W., Chang, F.Y., Chen, Y.W., Hsiao, C.F., and Siu, L.K. (2007). Methicillin-resistant *Staphylococcus aureus* carriage, infection and transmission in dialysis patients, healthcare workers and their family members. *Nephrol. Dial. Transplant*. 23:1659-1665.

Lynch. JP 3rd, and Zhanel. GG. (2010). *Streptococcus pneumoniae*: epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. *Curr Opin Pulm Med*. 16(3):217-25.

Pääkkönen, M., and Peltola H. (2013). Bone and joint infections. *Pediatr Clin North Am*. 60(2):425-436.

Regev-Yochay, G., Dagan, R., Raz, M., Carmeli, Y., Shainberg, B., Derazane, E., Rahav, G., and Rubinstein, E. **(**2004). Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children. *JAMA*. 292:716-720.

Regev-Yochay, G., Trzcinski, K., Thompson, C.M., Malley, R., and Lipsitch, M. (2006). Interference between *Streptococcus pneumoniae* and *Staphylococcus aureus*: in vitro hydrogen peroxide-mediated killing by *Streptococcus pneumoniae*. *J. Bacteriol*. 188:4996-5001.

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Renwick, L., Holmes, A., and Templeton, K. (2013). Multiplex real-time PCR assay for the detection of methicillin-resistant *Staphylococcus aureus* and Panton-Valentine Leukocidin from clinical samples. *PCR Detection of Microbial Pathogens* (2nd edition) 105-113.

Zetola, N., Francis, J. S., Nuermberger, E. L., and Bishai, W. R. (2005). Community-acquired methicillin-resistant *Staphylococcus aureus:* an emerging threat. *Lancet Infectious Diseases* 5: 269-270.

Appendix 2. Applicant *Curriculum vitae*

Josh S. Sharp, Ph.D.
jsharp@nmu.edu
210 Northwoods Rd. Apt. 4, Marquette, MI 49855 646-279-5080

EDUCATION

Mount Sinai School of Medicine of New York University, New York, NY 2006
Ph.D. Biological Sciences

Western Michigan University, Kalamazoo, MI 1998
B.Sc. *cum laude* Biomedical Sciences

EXPERIENCE

Assistant Professor of Biology – **Northern Michigan University** 2012-Present
Teaching areas: Medical Microbiology, Microbial Pathogenesis, Virology

Postdoctoral Research Fellow – Laboratory of Simon Dove 2006-2012
Children's Hospital Boston, Division of Infectious Diseases, Boston, MA and
Harvard Medical School, Department of Microbiology and Immunobiology**,** Boston, MA

- Researched the role RNA degrading proteins have on regulating gene expression in the opportunistic pathogen *Pseudomonas aeruginosa*.
- Demonstrated that a novel class of RNAs, nanoRNAs, can prime transcription in bacteria and dramatically impact gene expression.
- Extensive experience with microarray transcriptional profiling, chromatin immunoprecipitation, quantitative real-time PCR, and protein purification.
- Acquired broad knowledge and practical skills in the fields of genetics, molecular biology, and microbiology.
- Established successful collaborations with numerous laboratories.
- Trained graduate students, undergraduates, and technicians.

Laboratory Instructor – Clinical Pathological Microbiological Conferences. 2008, 2010-2011
Harvard Medical School, Boston, MA

- Led laboratory sessions in clinical microbiology for M.D. and Ph.D. students.
- Students learned methods to identify pathogenic microorganisms in clinical specimens.

Graduate Student – Laboratory of David Bechhofer 2001-2006
Mount Sinai School of Medicine, New York, NY

- Characterized essential elements required for the initiation of mRNA degradation in *Bacillus subtilis*.

PROFESSIONAL MEMBERSHIPS, AWARDS, AND HONORS

International Association for Food Protection Member 2015
Association of Analytical Communities Member 2014
Michigan Branch of the American Society for Microbiology Board Member 2012
Sigma Xi, Scientific Honor Society Member 2012
Combined Infectious Diseases Training Grant, Children's Hospital Boston 2009-2010
Ann Weinberg Memorial Research Fellow, Cystic Fibrosis Foundation 2007-2009

Mount Sinai School of Medicine Travel Award	2002-2004
Merrill Wiseman Award in Microbiology, Western Michigan University	1999
Undergraduate Creative Research Award, Western Michigan University	1998
The American Society for Microbiology Member	1995

SELECTED PUBLICATIONS

Vvedenskaya IO, *__Sharp JS__, *Goldman SR, Kanaber PN, Livny J, Dove SL, and Nickels BE (2012) Growth phase-dependent control of transcription start-site selection and gene expression by nanoRNAs. *Genes Dev.* 26:1498-1507. ***Joint second Authors.**

*Goldman SR, *__Sharp JS__, Vvedenskaya IO, Livny J, Dove SL, and Nickels BE. (2011) NanoRNA Prime Transcription Initiation In Vivo. *Mol. Cell.* 42(6):817-25. ***Joint First Authors.**

Yao S, **Sharp JS**, and Bechhofer DH (2009) *Bacillus subtilis* RNase J1 endouclease and 5' exonuclease activities in the turnover of Δ*ermC* mRNA. *RNA*. 15:2331-2339.

*Yuan, AH, *Gregory, BD, **Sharp, JS**, McCleary, KD, Dove, SL, and Hochschild, A. (2008) Rsd family proteins make simultaneous interactions with regions 2 and 4 of the primary sigma factor. *Mol. Microbiol*. 70:1136-1151. ***Joint first authors.**

Vallet-Gely, I, **Sharp, JS**, and Dove, SL. (2007) Local and global regulators linking anaerobiosis to *cupA* fimbrial gene expression in *Pseudomonas aeruginosa*. *J. Bacteriol*. 189:8667-8676.

Sharp JS, and Bechhofer DH (2005) Effect of 5'-proximal elements on decay of a model mRNA in *Bacillus subtilis*. *Mol. Microbiol*. 57:484-495.

Sharp JS, and Bechhofer DH (2003) Effect of translational signals on mRNA decay in *Bacillus subtilis*. *J.Bacteriol*. 185:5372-5379.

SELECTED ABSTRACTS AND PRESENTATIONS

Hollen, KR[Δ], Haavig, DL, and **Sharp JS** (2015) Identification of *Staphylococcus aureus* utilizing laser light scattering. *American Society for Microbiology General Meeting.* New Orleans, LA.

Hollen, KR[Δ], Haavig, DL, and **Sharp JS** (2014) Identification of *Staphylococcus* species utilizing laser light scattering. *AOAC Annual Meeting.* Boca Raton, FL.

Jacob KM[Δ], Purdy A, **Sharp JS** (2013) Gene regulation by a novel two-component system in *Pseudomonas entomophila*. *American Society for Microbiology General Meeting.* Boston, MA.

Sharp JS, and Haavig DL (2013) Laser-based rapid identification of bacterial pathogens. *American Society of Microbiology Michigan Branch Fall Meeting.* Bay City, MI.

Sharp JS, and Dove SL (2011) NanoRNAs: A class of small RNAs that can prime transcription initiation in bacteria. *13th International Conference on Pseudomonas.* Sydney, Australia.

Sharp JS, and Dove SL (2009) RNase E is required for expression of the type III secretion system in *Pseudomonas aeruginosa*. *12th International Conference on Pseudomonas.* Hannover, Germany.

[Δ] Kaylagh Hollen and Kristin Jacob are Graduate Students in the Sharp laboratory at NMU.

Appendix 3. Final Report From Previous Reassigned Time Award

Final Report: Reassigned Time Award

Please note that the original final report was previously submitted to the Faculty Grants Committee, The Biology Department Head, and the Dean of Arts and Sciences before the specified deadline.

Award Recipient: Josh S. Sharp

Reassigned Time Proposal Title: Laser-Based Rapid Identification of *Staphylococcus aureus* and MRSA

Award: Winter Semester 2014

This is my final report for my reassigned time award for the Winter Semester 2014. This report address the following six areas.

I. What was done?

During the award period the following work was done. We tested a *Staphylococcus* species identifier developed by Micro Identification Technologies (MIT) for use with their MIT 1000 rapid bacterial identification system. The goal was to determine if the *Staphylococcus* species identifier could be used to rapidly identify *Staphylococcus* bacteria from various sources. We analyzed 6 different *Staphylococcus aureus* strains, 1 *Staphylococcus intermedius* strain, 5 different *Staphylococcus epidermidis* strains, 3 different *Streptococcus* strains, and 1 *Micrococcus luteus* strain. All of these strains were isolated from different sources to test the robustness of the *Staphylococcus* identifier. The *Streptococcus* strains and *Micrococcus* strain were used as negative controls since they are Gram positive bacteria that are similar in size and shape to *Staphylococcus* bacteria. We tested the accuracy of this identifier to identify these bacteria grown on 5 different solid agar medias (Blood agar, LB agar, TSB agar, BHI agar, and

MSA agar). Additionally we tested the accuracy of this identifier to identify these bacteria grown in 3 different liquid medias (LB broth, TSB broth, and BHI broth).

II. What were the results?

We found that the *Staphylococcus* species identifier developed by MIT was 90-100% accurate at identifying Staphylococcus species grown on Blood agar. The false positive rates, identifying *Streptococcus* strains or *Micrococcus luteus* as *Staphylococcus*, on Blood agar was typically less than 10%. The *Staphylococcus* species identifier was somewhat less accurate at identifying *Staphylococcus* species grown on the other solid medias. The accuracy rate ranged from 70%-90% depend on the media type. The false positive rates were also somewhat higher on other media besides Blood agar.

We were moderately successful at identifying *Staphylococcus* species grown in liquid culture as well, however more testing needs to done in this area. Liquid LB or TSB media gave the best results, while BHI media was found to give highly variable results thus testing in this media was discontinued.

III. What was produced?

The data we acquired from this reassigned time award period was presented by my Graduate Student Kaylagh Hollen, and myself at the General Meeting of the Association of Analytical Communities (AOAC) that was held in September 2014. Kaylagh Hollen also presented an updated version of this data at the 2015 American Society for Microbiology General Meeting. Finally, a manuscript is currently in preparation for submission to the journal of biomedical Optics.

IV. What further research might this lead to?

This work is the first step in developing a more specific identifier. My laboratory in conjunction with MIT are developing a specific *Staphylococcus aureus* identifier. After the *S. aureus* identifier is developed we have plans to develop an identifier that can distinguish *S. aureus* from Methicillin Resistant *S. aureus* (MRSA). We are also working a method to reduce the time it takes to identify *S. aureus*. It would involve using magnetic beads that have antibodies to *S. aureus* attached to them to capture the bacteria in a patient specimen. We could then do a short enrichment of the bacteria in a liquid media then perform the identification test in the MIT 1000 instrument. This could reduce the detection time for *S. aureus* infection from 18-24 hours to 3-5 hours.

V. What sources of external grants did you seek?

In August 2014 MIT and my laboratory submitted a Small Business Technology Transfer (STTR) grant application to the National Institutes of Health (NIH).

VI. Detail the actual budget expenditures.

This is not applicable. This was a reassigned time award, no monetary award was made.

Appendix 4. Letter of IRB Approval

Northern Michigan University

Office of Graduate Education and Research
1401 Presque Isle Avenue
Marquette, MI 49855-5301
906-227-2300
FAX: 906-227-2315
Web site: www.nmu.edu

Memorandum

TO: Paul Mann, Cathy Bammert, Josh Sharp, Yuba Gautam
Clinical Lab Science, Biology

DATE: August 13, 2015

FROM: Brian Cherry, Ph.D. BC
Assistant Provost/IRB Administrator

SUBJECT: **IRB Proposal** HS15-678
IRB Approval Dates: 8/13/2015- 8/13/2016**
Proposed Project Dates: 8/1/2015-8/1/2016
"Relationship between methicillin-resistant Staphylococcus aureas nasal colonization and vaccination with a pneumococcal vaccine in adults"

The Institutional Review Board (IRB) has reviewed your proposal and has given it final approval. To maintain permission from the Federal government to use human subjects in research, certain reporting processes are required.

A. You must include the statement "Approved by IRB: Project # HS15-678" on all research materials you distribute, as well as on any correspondence concerning this project.

B. If a subject suffers an injury during research, or if there is an incident of non-compliance with IRB policies and procedures, you must take immediate action to assist the subject and notify the IRB chair (dereande@nmu.edu) and NMU's IRB administrator (bcherry@nmu.edu) within 48 hours. Additionally, you must complete an Unanticipated Problem or Adverse Event Form for Research Involving Human Subjects

C. Please remember that informed consent is a process beginning with a description of the project and insurance of participant understanding. Informed consent must continue throughout the project via a dialogue between the researcher and research participant.

D. If you find that modifications of methods or procedures are necessary, you must submit a Project Modification Form for Research Involving Human Subjects before collecting data.

E. **If you complete your project within 12 months from the date of your approval notification, you must submit a Project Completion Form for Research Involving Human Subjects. If you do not complete your project within 12 months from the date of your approval notification, you must submit a Project Renewal Form for Research Involving Human Subjects. You may apply for a one-year project renewal up to four times.

NOTE: Failure to submit a Project Completion Form or Project Renewal Form within 12 months from the date of your approval notification will result in a suspension of Human Subjects Research privileges <u>for all investigators listed on the application</u> until the form is submitted and approved.

All forms can be found at the NMU Grants and Research website:
http://www.nmu.edu/grantsandresearch/node/102

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Appendix 5. Volunteer Questionnaire and Informed Consent Statement

Questionnaire to find relationship between MRSA and Prevnar Vaccination among adults:

Q1. Age

- ❍ 18-30 years
- ❍ 31-40 years
- ❍ 41-49 years
- ❍ 50-59 years
- ❍ 60-69 years
- ❍ 75 years and above

Q2. Gender

- ❍ Male
- ❍ Female
- ❍ Other

Q3. Race/Ethnicity

- ❍ White
- ❍ Hispanic or Latino
- ❍ Black or African American
- ❍ Native American or American Indian
- ❍ Asian or Pacific Islander
- ❍ Other

Q4. Have you ever had frequent skin infections?

- ❍ Yes
- ❍ No
- ❍ Not Sure

Q5. Have you ever had frequent eye, nose, and throat infections?

- ❍ Yes
- ❍ No
- ❍ Not Sure

Q6. Have you ever been diagnosed with S. Aureus or MRSA infection?

- ❍ Yes
- ❍ No
- ❍ Not Sure

Q7. Have you ever been diagnosed with S. Pneumoniae infection?

- ❍ Yes
- ❍ No
- ❍ Not Sure

Q8. Do you have history of cancer, bone marrow, organ transplant, or immunosuppressive condition?

- ❍ Yes
- ❍ No
- ❍ Not Sure

Q9. Do you have a history of recent or frequent hospitalization?

- ❍ Yes
- ❍ No
- ❍ Not Sure

Q10. Have you ever been treated with antibiotics in the last 3 months? If yes, what kind of antibiotics?

- ❍ Yes
- ❍ No
- ❍ Not Sure

List antibiotics: _____

Q11. Have you been given the Pneumococcal vaccine? If so, do you know if it was the Prevnar vaccine?

- ❍ Yes
- ❍ No
- ❍ Not Sure
- ❍ Prevnar Yes_____, No _____, Not Sure _____

Q12. I have read the inform consent. I am willing to participate in this study and give consent for Nasal Swab.

Signature of the participant: _____

Informed Consent Statement

The Study
You are invited to be in a research study entitled, *The relationship between methicillin-resistant S. aureus nasal colonization and vaccination with a pneumococcal conjugate vaccine in adults*". The goal of this study is to explore whether vaccines that prevent pneumonia alter nasal flora of adults. This study is being conducted by Dr. Yuba Gautam, Dr. Paul Mann, Dr. Josh Sharp, Catherine Bammert, and graduate students Carol Kessel, Melissa Pierpont and Polly Hockberger from NMU. Up to 100 participants will be asked to be in this study.

Free to Choose
You are free to choose whether or not to be in this study. You may refuse to answer any questions you do not wish to answer. You may withdraw in part or in full from this study at any time without any ill will or penalty.

What to Expect
If you consent to participating in this research, you will be asked to complete a questionnaire which will take about 10 minutes of your time. You will also be asked to collect a swab from each of your nostrils. This process should take no more than 5 minutes.
Because we are an academic and not a certified or accredited, clinical laboratory, we are not legally allowed to share information with you regarding the bacteria we grow from your nasal swab.

Confidentiality
Your answers will remain anonymous. All raw data will be stored away from this consent form in a locked file drawer. Only the principle investigators listed above will have access to the data from this study. If the data is published, your name will not be linked to the data. Additionally, the Northern Michigan University Institutional Review Board and federal reviewers may access our research data to review our research processes, if deemed necessary. In very rare cases, loss of confidentiality may occur if a court orders that research files or information be submitted as evidence in a legal matter.

Risks and Benefits
If you are uncomfortable collecting your nasal swab in an open area, we will make an accommodation to find a private area so that you will be more comfortable. While the risks associated with collecting a nasal swab are minimal, you may experience minor discomfort. Your participation in this study will increase our knowledge regarding the relationship between vaccines that prevent pneumonia and the composition of nasal bacteria.

Questions about the Study

Please let us know now if you have any questions about this study. You may contact the researchers, as follows: Paul Mann (pmann@nmu.edu), Josh Sharp (jsharp@nmu.edu), Yuba Gautum (ygautam@nmu.edu), or Cathy Bammert (cabammer@nmu.edu).

Questions about Your Rights
If you have any questions about your rights as a research subject you may contact the NMU IRB Administrator, Dr. Brian Cherry, Assistant Provost of Graduate Studies and Research at Northern Michigan University 001-906-227-2300 or bcherry@nmu.edu.

I have read or the above informed consent has been read to me. The nature of risks, demands, and benefits of the project were made clear to me. I know that I may ask questions. I know I am free to withdraw from the project at any time without having any ill will or penalty. I also know that this informed consent sheet will be kept away from the project data to maintain confidentiality. Only the principle investigators or an authorized representative will have access to this consent statement. A copy of this consent statement will be given to me.

_____	_____	_____
Please print your name	Please sign your name	Date

I have taken care to explain the nature of the above project to the subject. I hereby confirm that to the best of my knowledge, the subject signing the informed consent form clearly understands the nature, risks, and demands of being a subject in this study. A language barrier, health

problem, or other barriers have not precluded a clear understanding of his or her involvement in this project.

_____ _____

Name Date

Thank you for your participation in this research study!

Appendix 6. Project Methods Flowchart