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PR161914

TITLE: The Urinary Fungal Mycobiome and Host Responses in Patients with Interstitial Cystitis

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4. TITLE AND SUBTITLE

The Urinary Fungal Mycobiome and Host Responses in Patients with Interstitial Cystitis

5a. CONTRACT NUMBER W81XWH-17-1-0433

5b. GRANT NUMBER **PR161914**

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Department of Surgery
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have completed recruitment of 200 subjects, have identified distinctive bacterial community types with a relationship to dysbiosis, and developed new techniques for identifying both bacterial and fungal species.

We have successfully extracted both fungal and bacterial DNA from the catheterized urine samples and confirmed this through both quantitative polymerase chain reaction (qPCR) and gel electrophoresis. We have refined classification of bacterial communities found in both controls and individuals diagnosed with Interstitial Cystitis and confirmed the connection between specific community types and pelvic pain/disease. We have developed specific probes that allow us to complete fungal analysis. We have developed a more sophisticated approach to biostatistical analysis and are currently completing, proteomics, analysis and Multiple Reaction Monitoring validation.

15. SUBJECT TERMS

Interstitial Cystitis, Microbiome, Mycobiome, Genomics, catherization, Proteomics, biomarkers

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Using blood samples and catheterized samples of urine from both healthy controls and Interstitial Cystitis patients, we are studying the relationship of changes in the fungal and bacterial communities in the bladder to dysbiosis. In addition, we are looking at related changes to proteins in subjects' blood that may serve as biomarkers of disease.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Interstitial Cystitis, Microbiome, Mycobiome, Genomics, catherization, Proteomics, biomarkers

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1:
Major Task 1: Patient Recruitment and Sample Accrual: Goal=300 by Month 6 – Revised Goal of 200 100% complete
Task 2: Bacterial and Fungal Community Profiling: Goals=*Sample Processing* Month 10 Completed for subjects recruited. *Microbial Community Profiling*: Goal Month 14 – completed for all subjects. *Bioinformatics/Biostatistical Analysis*: Goal Month 16 – in process. *Validation of Community Profiling*: Goal Month 18 – 80% complete; Primer design and validation – 100% complete.
Specific Aim 2:
Major Task 1: *De Novo* Discovery of Novel Protein Biomarkers by Deep Proteomics
Goal=Month 14 in process. *Sample Preparation*: Goal Month 10 - Completed for subjects recruited. *Proteomics*: Goal Month 12 – exploratory data set of 110 subjects completed, balance in process. *Bioinformatics/Biostatistical Analysis*: Goal Month 16 – in process.
Major Task 2: Multiple Reaction Monitoring Validation: Goal Months 14 to 18 – in process.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We have confirmed and tested primer design for independent identification of bacterial and fungal species not characterized by genomics processing. We have defined bacterial communities found in both controls and individuals diagnosed with Interstitial Cystitis and are confirming the connection between specific community types and pelvic pain/disease. These distinct microbial communities function as clinically useful, non-invasive biomarkers that distinguish disease from control subjects and discriminate among disease sub-types. This correlation has been confirmed in both pilot and validation subsets of our subject population. We have created a rapid, PCR based assay to confirm phenotypes that will have prognostic value in a clinical setting. Preliminary data (to be tested in future studies) demonstrate the prognostic utility of this approach in predicting responses to treatment.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The Principal Investigator continues to participate in regular, professional conferences of the American Urology Association (AUA) and the Society for Urogynecology and Female Urology (SUFU). In addition, she has attended the meetings of the Western Section of the AUA. She completed an intensive course in Biostatistics at the Marine Biology Laboratory (Wood's Hole) in the summer of 2018. The PI is developing knowledge of statistical analyses and has gained proficiency with analytical software for microbial analysis within the 'R' environment such as QIIME and Phyloseq. PI has also completed the course, Introduction to Data Science, at UCLA extension and is using her knowledge of PYTHON and SQL in data analysis for this study. She continues to meet regularly with a mentor, 1 on 1, at Cedars-Sinai Medical Center.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Subject recruitment has been terminated at 200 as statistical modeling indicates that a population of 160 will adequately power this study. This will more than allow for the exclusion of a small number of participants, if necessary. Statistical analysis continues. We currently have the final group of samples under analysis at the Genomics Core while we continue proteomics and biostatistical analysis.

We have selected 11 bacterial and fungal taxa with the greatest differences in relative abundance between cases and controls and are analyzing them in all samples using independent aliquots. This allows us to confirm the accuracy of our NGS results and perform technical validations with genus- or species-specific qPCR assays for bacterial or fungal genera that are significantly altered in storage LUTS using Genus- or species-specific primers we have designed. The output of this assay, after normalization to urinary creatinine, provides confirmation of the NGS results and allows comparison of relative amounts of critical species between subjects.

We will complete Proteomics and classification of the last group of subjects and go on to the final biostatistical analysis and multi-omics correlation. Due to the risk of significant batch effects, we have waited to complete recruitment before doing MRM. Dr. Yang will now complete the MRM Assay, multiquanta analysis and correlation of biomarkers.

IMPACT: D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

Nothing to Report currently, but we anticipate that our improved classification systems will provide a foundation to improve care and prediction of treatment outcomes for patients with painful bladder syndrome. In addition, these discoveries hint at the underlying pathophysiology of these conditions, which can be testing in later studies.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We have developed testing for a useful, non-invasive biomarker that could revolutionize diagnosis and management of patients with this debilitating and elusive condition. The process potentially provides a rapid, inexpensive and, most importantly, definitive indicator that appears to correlate with disease. This will require further validation in a clinical setting. Future studies will explore the utility of this biomarker in predicting responses to individual treatments.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The development of this technique dispels significant confusion in the diagnosis of patients with pelvic pain symptoms which will strongly impact neurology, gynecology and gastro-enterology. This work develops a test to identify two subsets in the pelvic pain population. For one group of these patients with significant gynecological symptoms, we can now direct patients into an effective pathway for treatment.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Initiation of a patent application for a simple, point-of-care assay to guide the treatment of pelvic pain.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

The identification of a biologic foundation for a condition that has been considered a diagnosis of exclusion that many MD's consider a functional, not physiologic, disease provides validation to a distressed population which has been historically marginalized.

Having a better understanding of the physiology behind this condition will help both prevention and care by non-specialty physicians, providing them with a tool to direct treatment.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

We have developed quantitative PCR approaches that will identify species that are not fully defined by processing through our Genomics Core. This method will be used alongside the Genomics analysis originally proposed. In the last six months we have confirmed the reliability of these techniques.

A more sophisticated statistical analysis indicated that 160 subjects would adequately power this study. Therefore we have revised our recruitment goal to 200.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We have noted previously issues connected to the reliability of species level identification by NGS. We have confirmed the accuracy of this data by external deep sequencing of an independent ribosomal locus. It is still necessary to overcome the potential variations resulting from any particular approach to NGS. Dr. Ackerman's additional training in microbial bio-informatics and data science has allowed her to understand these limitations and develop a more sophisticated and reliable microbial analysis. For example, we have replaced the use of Operational Taxonomic Units (OTUs) with Amplicon Sequence Variants to resolve variations in sequences.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Not Applicable

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Two papers are in preparation – federal support will be acknowledged - Microbial Phenotypes of Interstitial Cystitis - Normal Fungal Mycobiome of the Genitourinary Tract

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Abstract – presented at the Western Section annual meeting of the American Urological Association

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Patent application in preparation.

- **Other Products**
Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Dr. A. Lenore Ackerman	no change
Dr. Michael Freeman	no change
Dr. Jennifer Anger	no change
Dr. Wei Yang	no change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

NIH/NIDDK K 08 pending.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: ***N/A***