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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Novel methods are urgently needed to support precision wound infection care. A lack of diagnostic information limits the ability to act and intervene in the context of wound infection. The effort described in this report develops and tests a targeted sequencing platform for measuring microbial genomic determinants critical to wound healing. We applied a custom bioinformatics approach to design DNA probes for capture of sample sequence that indicates the species present (for a select set of wound-associated pathogens), in addition to genomic determinants for virulence and antimicrobial resistance. The panel was tested against control reference microbial genomes in samples within simulated human background. Sensitive, specific detection via the targeted sequencing panel was demonstrated in these validation samples for microbial species identity and virulence gene profile. The potential benefits of this developed panel to wound infection care in the Military Health System are numerous and wide ranging, including more timely evaluation of infection properties.

15. SUBJECT TERMS

combat injury; wound infection; antimicrobial resistance; virulence; next-generation sequencing; targeted sequencing; genomics; metagenomics; pathogen detection

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TABLE OF CONTENTS

	Page
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
i. Aim 1	4
ii. Aim 2	7
4. Impact	12
5. Changes/Problems	13
6. Products	13
7. Participants & Other Collaborating Organizations	14
8. Special Reporting Requirements	14
9. Appendices	14

1. INTRODUCTION

Genomics and informatics analyses continue to advance at an accelerating pace and, in the modern age of precision medicine, will prove invaluable for exploring the role of microbial communities in healthy and disease states. These technologies hold potential for tremendous benefit in combat casualty care. The severely invasive nature of combat trauma creates massive regions of injury and infection, requiring specialized diagnostic and aggressive therapeutic approaches. Even in the hands of experienced surgeons, some wounds fail to heal due to a lack of actionable diagnostic information. More timely, informative evaluation of wound infection would reduce morbidity, shorten hospital stays, and improve rehabilitation for combat-wounded service members. Microbial metagenomic sequencing has the potential to provide this information through analysis of genomic factors such as antibiotic resistance; however, whole genome sequencing does not yield the coverage required for sensitively detecting and analyzing individual microbial genes due to background created by human genomic sequence in clinical samples. A new approach is needed to bring the utility of DNA sequencing to management of wound infections. The low limit of detection and the high specificity that could be delivered by an approach employing targeted sequencing would facilitate detection of microbial genomic determinants in clinical samples, even before they result in emergent phenotypes that are detrimental to the patient.

The current effort aims to develop a targeted sequencing panel for capture and sequencing of microbial genomic determinants with relevance to wound colonization and healing. This platform will selectively sequence thousands of microbial genomic regions, thereby facilitating high-confidence detection of critical microbial factors that are otherwise difficult or impossible to assess. These microbial factors will include those associated with pathogen genus/species identity, antimicrobial resistance, and virulence. Profiling and delivering information on these factors could potentially guide and influence subsequent therapeutic interventions.

In support of this effort, the major aims of this project are to 1) Select microbial genomic targets relevant to wound healing and construct a targeted sequencing panel, 2) Validate the sensitivity and specificity of the resultant panel using reference genome controls, and 3) Validate the panel for predicting select clinical parameters in combat injuries.

2. KEYWORDS

combat injury; wound infection; antimicrobial resistance; virulence; next-generation sequencing; targeted sequencing; genomics; metagenomics; pathogen detection; infection diagnostic; polymicrobial infection; bioinformatics; microbial genomics; military medicine; nosocomial pathogen; hospital-acquired infection

3. ACCOMPLISHMENTS

Major goals and associated project accomplishments for Months 1-12:

Aim 1: Select microbial genomic targets and construct targeted sequencing panel
Timeline: Months 1-6; Status: 100% complete

The goal of Aim 1 was to select microbial genes with an anticipated role in wound progression. Identified targets would then be evaluated based on their anticipated impact on the wound healing process and employed for construction of an enrichment panel designed for identifying these factors in samples using targeted sequencing.

Task 1: Identify informative microbial gene targets
Timeline: Months 1-4; Status: 100% complete

In Task 1, the bioinformatics team at LLNL performed identification of microbial genomic targets likely to be relevant to wound infection and corresponding treatment using publicly available databases and other pre-existing data. This included a) microbial species/genus identity, b) virulence factors, and c) genomic signatures associated with antimicrobial resistance (AMR).

a) Species/genus identity targets: A subset of microbial genera/species, known to be relevant to wound healing and hospital-acquired infections, was selected based on pre-existing data and expertise (Table 1). DNA sequences conserved within, and unique to, these species were identified. Previous experience of LLNL team members in development of the Lawrence Livermore Microbial Detection Array platform (for identification of all known, sequenced microorganisms) was leveraged toward this effort.

Bacteria	**Fungi**
Achromobacter sp.	*Absidia sp.*
Acinetobacter baumannii	*Aspergillus sp.*
Enterobacter cloacae	*Bipolaris sp.*
Enterococcus faecalis	*Candida sp.*
Enterococcus faecium	*Cunninghamella sp.*
Escherichia coli	*Fusarium sp.*
Klebsiella pneumoniae	*Lichtheimia sp.*
Proteus sp.	*Mucor sp.*
Pseudomonas aeruginosa	*Pythium sp.*
Pseudomonas putida	*Saksenaea sp.*
Staphylococcus aureus	
Stenotrophomonas maltophila	

Table 1. Wound infection-associated bacterial and fungal species selected for genomic target identification.

b) Virulence targets: Microbial virulence targets likely to play a role in wound infection and the healing process were identified using the Virulence Factor Database (VFDB). VFDB confers several advantages toward this purpose. Namely, it organizes data by genus (facilitating selection of target organisms), provides both protein function and pathway level annotations for each gene, is regularly updated, and provides multiple sequence alignments and fasta files for each gene.
c) AMR targets: Through previous collaborations, members of the team have previously piloted an amplification-based targeted sequencing platform for detection of AMR. A

modified, supplemented list of the genes included in this panel was finalized by the team for subsequent hybridization capture probe design. The targeted AMR content covers >700 resistance-associated genes, including both bacterial and fungal resistance determinants.

Task 1 milestone/outcome: All genomic targets for the targeted sequencing panel were identified. The resultant target database represents a compilation of genomic signatures whose presence or absence may indicate aspects of the wound healing process.

Task 2: Design and computationally optimize target probes
Timeline: Months 4-6; Status: 100% complete

Following selection of microbial genomic targets in Task 1, Task 2 involved design of DNA probes for these determinants for inclusion in the targeted sequencing platform. Distinct approaches were performed in parallel for design of probes intended for detection of microbial a) species/genus, b) virulence genes, and c) AMR gene signatures.

a) Species/genus probe design: Previous experience of the team with array development was applied for extraction of >11,000 candidate array probes for identification of microbial species and >400 for microbial genera (Table 1). For genus-level detection, all probes were included to facilitate detection of fungi (critical for characterization of invasive fungal wound infections), as fungal reference sequences are frequently less well-curated relative to bacterial references. To downselect the species probe pool to a feasible quantity, probes with maximal predicted breadth and sensitivity were identified. Hybridization probabilities were calculated for each probe as an indication of the likelihood that corresponding sequence would capture target content. Approximately 20 probes were selected for each species and screened against reference genomes and type strains to maximize breadth and sensitivity. Probes were then screened against pre-existing data from previous efforts, to confirm potential capture of sequence from known nosocomial strains and other sequences relevant to wound and tissue infections. An additional 20 probes per species were selected based on these analyses. Probes with substantial hybridization potential in human background were replaced.
b) Virulence probe design: A custom bioinformatics approach was designed and scripted for identification of virulence probe regions in VFDB and subsequent probe design. This multi-step process is briefly summarized below.
    i. Multiple sequence alignments were downloaded for virulence genes in all available VFDB genera. A custom script was designed to find conserved regions for each gene such that a minimal number of probes generated from these regions would detect most variants of the gene. This software tool iteratively tests different dominant base frequency thresholds for each gene. The highest frequency threshold that yields at least one region of a predetermined window size that is free of indeterminate bases is used to generate the consensus sequence.
    ii. For genes with high variability within a genus, such that conserved regions cannot be determined, the gene is split into two alignments using a phylogenetic tree, generally leading to two consensus sequences for a gene. If at least one consensus sequence could not be generated, a subset of the original gene entries was used.

iii. Probe generation was performed using a publicly available tool (CATCH: Compact Aggregation of Targets for Comprehensive Hybridization, Broad Institute). Probes with more than three indeterminate bases or predicted capture of human content were omitted. A set of 3,217 probes were generated to target virulence gene targets across all available genera.

c) AMR probe design: Two approaches were used for selection of probes for AMR-associated genes. Specific selected genes require a larger proportion of captured sequence to confidently assign a detection event, due to multiple required function-specific domains. For these genes, probes were selected such that the entirety of the gene would be tiled and captured. For all other genes a minimum of two probes were selected for inclusion.

Task 2 milestone/outcome: All capture enrichment probe design was completed, and probes were prepared for testing. This panel represents a novel tool for evaluation of microbial content in a potential injury, targeting those determinants most likely to play a role in the progression of microbial colonization in that injury.

Aim 2: Validate sensitivity and specificity of targeted sequencing panel using reference genome controls
Timeline: Months 6-12; Status: 100% complete

Probe design was completed as outlined above and the oligonucleotide pool was acquired (Illumina), prepared for optimization and validation testing. The first step in this process was to select reference genomes for testing, which need to be informatically screened to determine which of the genomic loci included in the panel are present in the sequence of each reference genome to be tested. Samples containing genomic DNA from these references were then experimentally tested.

Task 3: Identify presence of genomic targets in ≥2 reference genomes
Timeline: Months 6-7; Status: 100% complete

Appropriate reference genomes relevant to wound infection in combat injuries were identified and screened for genomic content. These were selected to include reference assemblies from *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Future clinical samples are likely to contain polymicrobial content, thus a pre-defined mixed microbial pathogen gDNA sample was also selected, containing 10 human pathogen species (ATCC MSA-4000: *Acinetobacter baumannii, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Neisseria meningitidis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes*). Clinical samples may also contain sequence from non-pathogenic, commensal microbes. To test whether pathogen signals are generated from ubiquitous natural microflora, a mix of gDNA from six bacterial species associated with skin microflora was tested (ATCC MSA-1005: *Acinetobacter johnsonii, Corynebacterium striatum, Micrococcus luteus, Cutibacterium acnes, Staphylococcus epidermidis, Streptococcus mitis*). This test serves as a polymicrobial “negative control,” assessing the detection response elicited from naturally occurring skin microbes.

Reference genomes for indicated species were screened for virulence and AMR genomic targets from the designed panel to create a "ground truth" table, indicating which genes are expected to be present in the strains that will be spiked into validation samples. In rare cases where the sequence for the exact strain to be employed was not available, a closest neighbor strain was used. Since any given microbial gene can exhibit many variations between strains, capture regions/probes were used for mapping; probe target regions should be well conserved and gene-specific. Mapping was performed for the identified strains (Table 2) via BLAST, as regions corresponding to AMR signatures were longer (~275 bp) than the probes themselves (80 bp). Virulence mapping was performed at the probe level via Bowtie, to obtain improved specificity for shorter sequences (80 bp).

Overall, observed AMR content was relatively low across species, with generally increased region detection in pathogens relative to commensal skin microflora. A summary of results from the two titrated pathogen controls and skin microflora are shown in Table 2. As anticipated, genomic content corresponding to virulence-targeted probes was substantially higher in pathogenic species relative to skin microflora, which exhibited very few hits to virulence probes.

Control species	**Category**	**AMR regions in reference**	**Virulence probes in reference**
Acinetobacter baumannii	Pathogen	1-6 regions per gene for 6 AMR genes	1-2 probes per gene for 46 virulence genes
Pseudomonas aeruginosa	Pathogen	1-4 regions per gene for 3 AMR genes	1-2 probes per gene for 126 virulence genes
Acinetobacter johnsonii	Skin microflora	0 regions	0 probes
Corynebacterium striatum	Skin microflora	1 amplicon from 1 AMR gene	1 probe from 1 virulence gene
Micrococcus luteus	Skin microflora	1 amplicon from 1 AMR gene	0 probes
Cutibacterium acnes	Skin microflora	0 regions	0 probes
Staphylococcus epidermidis	Skin microflora	1 to 3 regions per gene for 7 AMR genes	1 probe from 1 virulence gene
Streptococcus mitis	Skin microflora	1 to 2 regions per gene for 2 AMR genes	1 probe from 5 virulence genes

Table 2. Results of mapping AMR and virulence capture regions to reference genomes.

Task 3 milestone/outcome: Anticipated detection levels were characterized for each target in validation reference genomes. This informatics effort provides the project with known targets, present in control references, which will facilitate further validation of the panel.

Task 4: Experimental validation of panel using selected reference genomes
Timeline: Months 7-12; Experimental status: 100% complete; Analysis status: 80% complete

Samples were prepared for experimental validation of the targeted panel. Reference genomes for both *A. baumannii* and *P. aeruginosa* were spiked into human reference control background at 10, 100, 1000, 10,000, and 100,000 genomic copies. Mixed polymicrobial controls containing either pathogen or skin commensal microbe gDNA (species content described above) were similarly spiked into human background at 100,000 total genomic copies. Samples were

subjected to hybridization capture and targeted sequencing. Briefly, mixed genomic samples were prepared according to the Illumina Nextera Flex for Enrichment workflow, target sequences captured using our panel of custom targets, and sequencing performed on the Illumina NextSeq 500. Data analysis was performed in a multi-step process as follows.

a) Sequence data were analyzed via several pipelines. Reads for each sample were aligned to panel probe targets corresponding to either genus/species, virulence, and AMR content using either the capture probe sequence (for genus/species and virulence) or the full gene target sequence (for AMR). This alignment was performed via BLAST for genus/species and virulence targets and via the BWA aligner for AMR gene targets. For virulence and AMR, these results were analyzed directly.
b) For genus/species identity, an additional step was undertaken. Reads were analyzed using the Livermore Metagenomics Analysis Toolkit, a previously developed LLNL pipeline for scalable metagenomic classification of DNA sequence, which assigns reads to appropriate taxonomic identifiers. This analysis evaluated the taxonomic classification of reads aligning to each probe category; results are described below.

Panel preliminary performance part 1: genus and species identification. Targets associated with AMR and virulence content were designed to be general to multiple potential pathogens. However, the precise sequence content of the captured targets may differ slightly for a given gene, dependent on genus of origin. This allows our analysis to leverage sequence content captured by virulence/AMR-directed probes for genus-level taxonomic identification. Species-level detection is then subsequently facilitated by sequence that is captured by probes specifically designed for species identification. This analysis was first applied to the sequence data from titrated samples of *A. baumannii* and *P. aeruginosa*, spiked into a control human background. For both tested microbes, reads were captured, sequenced, and identified, for the correct genus, at the lowest tested concentration of 10 spiked genomic copies or higher (Table 3). For *A. baumannii,* species-specific reads were also identified at 10 copies and above, while species-specific identification for *P. aeruginosa* was made at 1,000 copies and above.

	10 copies		**100 copies**		**1,000 copies**		**10,000 copies**		**100,000 copies**	
	Genus	*Species*	*Genus*	*Species*	*Genus*	*Species*	*Genus*	*Species*	*Genus*	*Species*
A. baumannii	1,345	133	7,210	498	52,572	4,061	82,453	8,046	10,179 M	993,972
P. aeruginosa	1,103	N/A	4,398	N/A	165,149	148	345,388	496	3,968M	4,828

Table 3. Number of reads detected following targeted capture sequencing of samples containing defined genomic copy numbers of *A. baumannii* and *P. aeruginosa* in human reference background. Color indicates whether reads were detected (green) or not detected (red) for the correct genus or species at the corresponding copy number.

Testing of the targeted capture sequencing panel was also performed with a polymicrobial pathogen mix of genomic DNA, with each species present at a range of genomic copy numbers, and a total copy number of 100,000 (Table 4). Similar to the results described above, reads corresponding to the correct genus were detected for all species at all copy numbers. Reads corresponding to the correct species were detected at all copy numbers for all species that were included in the probe design process. *Neisseria* and *Streptococcus* were not included for species probe design. Their genera are detected due to virulence/AMR gene capture from these genera.

	% of Mix	Copy #	# reads Genus	# reads Species
Acinetobacter baumannii	0.1%	100	463	13
Enterococcus faecalis	0.7%	700	710	173
Escherichia coli	1.4%	1,400	16,198	163
Klebsiella pneumoniae	14.4%	14,400	23,891	2366
Neisseria meningitidis	28.9%	28,900	181,684	0*
Pseudomonas aeruginosa	0.3%	300	16,415	70
Staphylococcus aureus	15.1%	15,100	17,716	1437
Streptococcus agalacitae	2.9%	2,900	31,392	0*
Streptococcus pneumoniae	28.9%	28,900		0*
Streptococcus pyogenes	7.2%	7,200		0*

Table 4. Number of reads detected following targeted capture sequencing of samples containing a defined polymicrobial pathogen control genomic DNA mix. Color indicates whether reads were detected (green) or not detected (red) for the correct genus or species at the corresponding copy number. *Indicates that probes for this species (*Neisseria, Streptococcus*) were not included in the designed panel due to their lower relevance to wound healing.

These results indicate that genus-level detection of microbes by the panel is highly sensitive down to 10 genomic copies (or lower) in human background. Species-level detection was also sensitive, observed in this initial test in the range of 10-1,000 genomic copies. This detection limit will likely vary, to a degree, according to species of interest.

Panel preliminary performance part 2: virulence and AMR signature detection. As noted in Task 3, reference control genomes were assessed informatically to determine which panel targets were present in these references. The described validation targeted capture sequence data was analyzed by mapping to virulence probes as described above, and experimental detection of virulence genes was compared to expected detection based on reference analysis (Table 5). For preliminary analysis, detection thresholds were set at ≥ 1 read per probe and ≥ 1 probe per gene; subsequent analyses during the period of performance will tune these thresholds as appropriate. As anticipated, the number of virulence genes that were detected increased with copy number, with 45 out of the 46 expected (98%) being detected for *A. baumannii* and 100 out of the 106 expected (94%) being detected for *P. aeruginosa* at 100K genome copies. False positive detection events were minimal; for *A. baumannii*, only one instance occurred where a probe region was not present in the reference but was detected experimentally (at 1,000 copies). For *P. aeruginosa*, three instances of such detection events were observed at 10K copies and four at 100K copies.

	Virulence probe analysis			
Sample copy number	**# genes expected based on reference**	**# genes detected and present in reference**	**# genes detected but not present in reference**	**# genes undetected but present in reference**
Acinetobacter baumannii				
10	46	14	0	32
100	46	25	0	21

1,000	46	39	1	7
10,000	46	39	0	7
100,000	46	45	0	1

Pseudomonas aeruginosa

10	106	6	0	100
100	106	29	0	77
1,000	106	98	0	8
10,000	106	96	3	10
100,000	106	100	4	6

Table 5. Detection of virulence genes via targeted sequencing capture panel in control reference genomes at varying copy numbers. Comparisons are made to genes indicated as present vs. absent in the reference sequence of the corresponding strain via informatic analysis.

Further assessment of panel specificity was performed by performing the same analysis on targeted capture sequence data from the skin microbiome mix. This mixture contained only human commensals, not virulent pathogens. This sample was, therefore, not expected to generate substantial sequence signal using the current panel, although commensal species such as those belonging to the genera *Staphylococcus* and *Streptococcus* may contain some virulence-related signature content, despite a typically avirulent profile. These expectations were confirmed in our testing at 10K copies of skin commensals, where only two total reads were captured and assigned to one virulence probe. At 100K copies, five total probes exhibited mapped sequence data, each at < 600 reads.

Initial efforts at mapping targeted capture sequence for AMR content focused on mapping to larger target regions and genes; however, this approach resulted in potentially confounding factors, such as instances of mapping to non-overlapping target regions. Instead, a probe-based mapping approach, now already in place for the virulence analysis described above, will be applied and is currently in progress. Analysis of virulence/AMR gene signature detection in the pathogen mix control is also in progress.

Taken together, these results indicate good sensitivity for gene detection (≥ ~80%) at 1,000 genome copies, and excellent sensitivity (>90%) at 100K copies. High specificity, as indicated by low false positive detection rate, was also observed, both in samples containing pathogens and commensal microbiota. The microbial gDNA in these samples was present in a large simulated human background. It is important to note that the desired species and genes were still identified with high sensitivity and specificity, even though the overwhelming quantity of total DNA material corresponded to non-microbial and non-target sequence.

Task 4 milestone/outcome: The completed panel was experimentally tested to assess detection of targeted microbial genomic regions in simulated samples containing known microbial genomic determinants in a simulated human background. Resultant data were analyzed to assess capacity for genus/species detection as well as virulence gene detection, with AMR analysis currently in progress. These analyses demonstrated that the panel achieves detection of microbial genera, exhibits species-level detection resolution, and accomplishes sensitive and specific detection of the intended virulence genes.

Training and professional development: Nothing to report.

Results dissemination: Results relevant to, and impactful for, the military medical scientific community were communicated at the 2019 Military Health System Research Symposium.

Activities in next reporting period: In the following quarter of performance we will complete all AMR and related analyses for experimental data collected from simulated control samples. In this quarter we will also initiate work for Aim 3, Task 5, which will apply the designed and tested targeted sequencing panel to samples from combat injuries. We will prepare genomic DNA from effluent and/or tissue samples. These DNA samples will be applied to the designed targeted capture sequencing panel, subjected to library preparation, and sequencing. Initial steps will be performed for data analysis.

4. IMPACT

Discipline-specific impact for combat wound infection: More comprehensive assessment of wound colonization and bioburden can critically influence decisions that improve outcomes in combat injuries. Advances in metagenomic sequencing and analysis could address this need by expanding the ability to measure and track microbial populations as they relate to clinical outcomes. The effort in this report describes performance of a targeted enrichment panel designed to sequence genomic determinants with the highest degree of relevance to combat wound microbial colonization and subsequent healing, and therefore the highest potential to influence interventions. The described validation study indicates sensitive and comprehensive detection of microbial factors, even in high background simulated samples, which would be highly challenging to accomplish via other currently applied platforms. The ability to detect these microbial determinants at the sensitivity demonstrated in the initial validation studies described here could allow for early detection of clinically impactful factors, facilitating a more precise approach to therapy.

Outside impact in military medicine: The potential impact of these results and developed panel include applications relevant to battlefield injuries and beyond. More timely evaluation of infection properties and profiles could hold utility in many contexts, including infections in other body sites. Corresponding interventions would have the potential to reduce morbidity, shorten hospital stays, and improve rehabilitation for service members with traumatic injuries or other conditions associated with microbial infection. Currently, early evaluation of infectious parameters related to infection identity, virulence, and AMR, is challenging or unavailable, particularly the simultaneous evaluation of multiple such factors. The advancement catalyzed by the effort described in this report has the potential for benefit throughout the military health system, including hospital-acquired and surgical infections as a whole.

Societal impact: Infections resulting from potentially resistant nosocomial pathogens represent a tremendous burden on the U.S. healthcare system. The factors selected for inclusion in the proposed panel are highly relevant to both wound and other infections throughout not only military medicine, but also civilian healthcare, including infections residing in non-healing wounds, such as diabetic ulcers. The initial results described in this report lay groundwork for expansion of the utility of targeted sequencing throughout both military and civilian healthcare

diagnostic infrastructures. The advantages to the targeted sequencing panel developed for this effort are wide ranging, such that they could apply to evaluation of polymicrobial infection in a broad sense, including 1) low limit of detection, 2) enhanced depth for analysis of gene variation, 3) increased coverage of any desired region, and 4) fractional cost and analysis burden. A particularly advantageous impact of the proposed panel could be the enabling of gene- and mutation-level analysis in near-point of care applications. Thus, while it is not described in the current report, any resultant data could potentially allow for downstream analysis of variation within these genes and impact on clinical decision making, particularly important for accurate assessment of antimicrobial susceptibility profiles. Microbial parameters relevant to genus/species identification, resistance, and virulence are critical for clinical application; amplifying the ability to assess these factors has the potential to change the landscape of microbial profiling.

Technology transfer impact: Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach: Nothing to report.

Actual or anticipated delays: As previously reported, one co-Investigator at Uniformed Services University is no longer contributing to the project. This role has been filled by another USU co-Investigator, Dr. Seth Schobel. Accordingly, several tasks needed to be transferred to Dr. Schobel, involving administrative and training requirements. These requirements have now been met; however, their completion, and this transition, resulted in administrative delays.

Other changes: Nothing to report.

6. PRODUCTS

Journal publications: Nothing to report.

Books: Nothing to report.

Conference poster presentations:
Be NA, et al. Composition, function, and relevance of the microbial microenvironment in wounds from combat injuries. Military Health System Research Symposium. August 2019. Abstract # MHSRS-19-01833.

Websites: Nothing to report.

Technologies or techniques: Nothing to report.

Inventions: Nothing to report.

7. PARTICIPANTS AND COLLABORATING ORGANIZATIONS

Individuals working at least one month per year during period:

Name:	Nicholas Be, Ph.D.
Project Role:	PI
Nearest person month:	2
Contribution to Project:	Project management, technical oversight, experimental design

Name:	Nisha Mulakken, M.S.
Project Role:	co-I
Nearest person month:	3
Contribution to Project:	Target identification, probe design, data analysis

Name:	James Thissen, M.S.
Project Role:	co-I
Nearest person month:	3
Contribution to Project:	Workflow design, experimentation/sequencing, data analysis

Change in active other support: The previously reported pending support indicated below is now active. No impact is anticipated on project effort for the subject of this project report.

Sponsor: Defense Threat Reduction Agency
Project title: Development of CNS penetrating oxime countermeasures for nerve agent exposure: A path toward an IND application
Role: co-Investigator
Time commitment: 20%
Overlap: None

Other organizations:

Name: Uniformed Services University/Henry M. Jackson Foundation
Location: Bethesda, MD
Partner's contribution: collaboration, data analysis, and application guidance

8. SPECIAL REPORTING REQUIREMENTS

Quad chart submitted with attachments.

9. APPENDICES

No additional materials for attachment.