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TITLE: Leveraging the Dose-Dependent Kill of Metronidazole for Targeting Biofilms That Underpin Recalcitrant Infection

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CONTRACTING ORGANIZATION: The University of Utah

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13. SUPPLEMENTARY NOTES

Infection is the greatest mortality risk for Wounded Warriors who survive the first 3 hours from a point of injury. Due to the traumatic nature of battlefield-relevant injuries where bacterial contamination levels can be high, biofilm-related infection is a major concern for military healthcare. We found that the commonly used hypoxia-activated prodrugs metronidazole, has unreported activity against *S. aureus* bacteria in the biofilm phenotype, those underpinning disease. This discovery has likely been overlooked as metronidazole is ineffective against the planktonic phenotypes used in clinical assays. The killing-effect of metronidazole against biofilms is greatly enhanced when paired with certain clinical antibiotics including gentamicin and levofloxacin but not β-lactam antibiotics, although the boundaries of this synergy phenomenon has not been fully elucidated. The rationale of this study is to leverage the dose-dependent biofilm killing-effect of this compound using an experimental drug delivery device under development in our lab. This device creates sustained high-concentration fields of therapeutics in a musculoskeletal site, and is used here as a research tool for studying the potential adjunctive utility of metronidazole for treating experimental *S. aureus* infections in an ovine trauma model of orthopedic infection. This report covers the project results form Year 1 comprising in vitro biofilm assays of combinations of metronidazole and clinical antibiotic compounds from the gamut of antibiotic classes against *S. aureus* (ATCC #6538), and *P. aeruginosa* (ATCC # 27853).

15. SUBJECT TERMS
Infection, Open Fracture, Return to Duty, Biofilm, Drug Delivery, S. aureus, Musculoskeletal

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Infection is the greatest mortality risk for Wounded Warriors who survive the first 3 hours from a point of injury. Due to the traumatic nature of battlefield-relevant injuries where bacterial contamination levels can be high, biofilm-related infection is a major concern for military healthcare. The opportunistic pathogens underpinning these infections grow into complex communities called biofilm; these form cohesive communal aggregates and adhesive attachments to surfaces such as devitalized tissues and orthopedic hardware. Bacteria within the biofilm are in a reduced metabolic state. These metabolically less-active biofilm phenotypes can survive inordinate concentrations of traditional antibiotics as they have downregulated metabolic drug targets. Biofilms serve as a nidus of infection reseeding subsequent infection even after the most aggressive antibiotic regimens. We found that the commonly used hypoxia-activated prodrugs metronidazole, has greater activity against bacteria in the biofilm phenotype than against bacteria in the planktonic phenotype. The poor efficacy of metronidazole against the planktonic phenotypes used in clinical diagnostics, has, in part, shrouded this discovery until now. The killing-effect of this compound against biofilms is greatly enhanced when paired with certain clinical antibiotics including gentamicin and levofloxacin but not β-lactam antibiotics, although the boundaries of this synergy phenomenon has not been fully elucidated. Notably, this compound produces a graded dose-dependent killing effect against bacterial biofilms in contrast with traditional antibiotics that plateau in effect against biofilms. The rationale of this study is to leverage the dose-dependent biofilm killing-effect of this compound using an experimental drug delivery device under development in our lab. This device creates sustained high-concentration fields of therapeutics in a musculoskeletal site; this is a prerequisite for truly leveraging our discovery to treat the most intractable musculoskeletal infections. **The study hypothesis is that adjunctive use of metronidazole with a clinical antibiotic (gentamicin) will reduce site bioburden to a greater degree than the clinical antibiotic alone in an ovine model of traumatic musculoskeletal infection when dosed at high local concentrations by a developmental delivery device**. The first Aim of this project is to test a range of clinical antibiotics in combination with metronidazole against *S. aureus* and *P. aeruginosa* bacterial biofilms to determine if other common clinical antibiotics display synergy. The second project Aim is to assess the adjunctive efficacy of metronidazole alone and in combination with the best candidate low-MIC antibiotic from Aim 1 in an ovine model of traumatic musculoskeletal infection. This study will assess the utility of this compound in combination with a clinical antibiotic to treat biofilm-related musculoskeletal infection using an experimental drug delivery device to achieve high local concentrations of therapeutics. This work will rely on a well-developed sheep model of biofilm-related musculoskeletal infection. Three treatment groups will be tested (n=8/group), one which receives our compound alone, another which receives the clinical antibiotic alone, and the last which receives a combination thereof. Data will be compared to positive and negative controls of infection as well as clinical controls (e.g., gentamicin-loaded Stimulan® beads); these data are available from previous experiments and thus are not included in this proposal. Metronidazole is and FDA approved drug extensively used for other indications orally, topically, and intravenously. In the short-term, the proposed work will guide clinicians and researchers to an alternative clinical compound(s) to fight infections in a verity of indications including those of the musculoskeletal system.

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

Infection, Open Fracture, Return to Duty, Biofilm, Drug Delivery, S. aureus, Musculoskeletal

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.

%Work Complete	Task Name	**Start**	**Projected Finish**	**Actual Finish**
90%	**Specific Aim 1:** Test a range of clinical antibiotics with and without metronidazole against *S. aureus* and *P. aeruginosa* bacterial biofilms to determine if other common low-MIC antibiotics display synergy.	7/1/22	1/1/24	–
100%	**Major Task 1:** Determine synergistic potential of candidate low-MIC antibiotics paired with metronidazole against *S. aureus*.	7/1/22	4/1/23	4/1/23
100%	Subtask 1: Confirm susceptibility of *S. aureus* to candidate antibiotics using a MIC assay Subtask 2: Obtain efficacy data of metronidazole paired with 12 low-MIC antibiotics against *S. aureus* biofilms. • Grow biofilms in CDC reactor, expose to metronidazole + low-MIC antibiotic(s) for 24 h, and determine efficacy (n=5 per test group)	7/1/22	4/1/23	4/1/23
80%	**Major Task 2:** Determine synergistic potential of metronidazole and low-MIC antibiotics against *P. aeruginosa*.	10-18	1/1/24	_
80%	Subtask 1: Confirm susceptibility of *P. aeruginosa* to planned antibiotics using MIC assay Subtask 2: Obtain efficacy data of metronidazole paired with 10 low-MIC antibiotics against *P. aeruginosa* (Grow biofilms in CDC reactor, expose to metronidazole + low-MIC antibiotic(s) for 24 h, and determine efficacy (n=5 per test group))	5/1/23	1/1/24	_

%Work Complete	Task Name	**Start**	**Projected Finish**	**Actual Finish**
10%	**Specific Aim 2:** Assess efficacy of adjunctive metronidazole in combination with the best-performing low-MIC antibiotic from Aim 1 in an ovine model of traumatic musculoskeletal infection.	7/1/23	11/1/23	_
75%	**Major Task 1:** Obtain animal use approvals	7/1/23	11/1/23	_
75%	Subtask 1: Obtain IACUC approvals for sheep work	7/1/23	11/1/23	_
-	Subtask 2: Obtain ACURO approvals for sheep work	7/1/23	11/1/23	_
0%	**Major Task 2:** Test efficacy of metronidazole against *S. aureus* biofilms in sheep model of open fracture-related infection	2/1/24	6/1/2024	_
0%	Subtask 1: Determine ability of metronidazole alone to treat *S. aureus* biofilm implant-related infection via delivery from the Pouch Perform surgeries in n=8 sheep, administer metronidazole for 20 days (via Pouch), collect microbiological and histological data, and compare to other groups	2/1/24	6/1/2024	_
0%	**Major Task 3:** Test efficacy of low-MIC antibiotic against *S. aureus* biofilms in sheep model of open fracture-related infection	7/1/24	11/1/24	_
0%	Subtask 1: Determine ability of low-MIC antibiotic (selected from Aim 1) alone to treat *S. aureus* biofilm implant-related infection via delivery from the Pouch (Perform surgeries in n=8 sheep, administer low-MIC antibiotic for 20 days (via Pouch), collect microbiological and histological data, and compare to other groups)	7/1/24	11/1/24	_
0%	**Major Task 3:** Test efficacy of metronidazole + low-MIC antibiotic pair against *S. aureus* biofilms in sheep model of open fracture-related infection	12/1/24	4/1/2025	_
0%	Subtask 1: Determine ability of metronidazole + low-MIC antibiotic pair (selected from Aim 1) to treat *S. aureus* biofilm implant-related infection via delivery from the Pouch (Perform surgeries in n=8 sheep, administer metronidazole + low-MIC antibiotic for 20 days (via Pouch), collect microbiological and histological data, and compare to other groups)	12/1/24	4/1/2025	_
0%	**Major Task 4:** Complete studies, analyze data, prepare for publication and final report	1/1/25	6/31/2025	_
0%	• Subtask 1: Analyze microbiological and histological outcomes from sheep studies, ensure all studies are complete, include in final report and prepare for publication	1/1/25	6/31/2025	_

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.

During this first annual reporting period, our efforts focused on the benchtop work of specific Aim 1 which precedes Aim 2 animal work. Specific Aim 1 focus on the *in vitro* testing of clinical antibiotics with and without metronidazole against *S. aureus* (ATCC #6538) and *P. aeruginosa* (ATCC #27853) bacterial biofilms to determine if other common low-MIC antibiotics display synergy like that observed in the pilot work motivating this project. This Aim 1 was designed to probe the boundaries of potential adjunctive metronidazole use by choosing companion antibiotics across a range of antibiotic classes thereby represent the gamut of antibiotic mechanisms.

Biofilm testing was preceded in all cases by determining the minimum inhibitory concentration (MIC) for each antibiotic proposed in subsequent biofilm testing. The MIC values were gathered for two reasons. First, to confirm that antibiotic has acceptably low MIC and is therefore acceptable for subsequent biofilm testing (i.e., below breakpoint). Secondly, subsequent biofilm testing was performed at 20X the MIC value for each respective antibiotic as a means of standardizing testing. The original tentative list of antibiotics proposed in testing from the project narrative is shown in Table 1. The Resulting MIC values from our testing with our two isolates are shown in Table 2. The reviewers should note that the P. aeruginosa testing of Aim 1 is not 100% completed.

Table 1: The traditional low-MIC antibiotics that were originally proposed for biofilm testing as companions to metronidazole.

Antibiotic Class	Candidate Compound (S. aureus)	Candidate Compound (P. aeruginosa)
β-lactam	Nafcillin	Cefepime
Aminoglycoside	Gentamicin	Gentamicin
Fluroquinolone	Levofloxacin	Levofloxacin
Glycopeptide	Vancomycin	
Macrolide	Azithromycin	Azithromycin
Lincosamide	Clindamycin	
Oxazolidinone	Linezolid	Linezolid
Polypeptide	Polymyxin E	Polymyxin E
Rifamycin	Rifampin	Rifampin
Sulfonamide	Sulfamethoxazole	Sulfamethoxazole
Tetracycline	Minocycline	Minocycline
phosphonic	Fosfomycin	Fosfomycin

Table 2: The measured MIC values for the antibiotics used subsequently in biofilm testing.

S. aureus, ATCC #6538, MIC (µg/ml)	
Gentamicin	1
Tobramycin	0.5
Levofloxacin	0.125
Vancomycin	1
Azithromycin	0.5
Clindamycin	0.125
Linezolid	0.5
Polymyxin E	32
Rifampin	0.125
Sulfamethoxazole	4
Minocycline	0.5
Fosfomycin	0.5
Nafcillin	0.0625
Metronidazole	>2500

P. aeruginosa, ATCC #27853, MIC (µg/ml)	
Gentamicin	2
Levofloxacin	1
Azithromycin	64
Linezolid	>64
Polymyxin E	1
Rifampin	16
Sulfamethoxazole	>64
Minocycline	8
Fosfomycin	32
Tobramycin	1
Cefepime	2
Metronidzole	>2500

All of the original antibiotics proposed for testing against *S. aureus* ATCC #6538 (Table 1) had acceptably low MIC values to progress towards biofilm testing. Polymyxin E had the highest MIC value at 32 µg/ml. Breakpoints are poorly defined for polymyxin E as it is typically only used topically and not FDA approved for IV administration. On the other hand, several of the antibiotics proposed for testing against *P. aeruginosa* ATCC #27853 were unacceptably high and were not used in subsequent testing: azithromycin, linezolid, and sulfamethoxazole. The MIC for fosfomycin was borderline, but still decided to proceed with subsequent biofilm testing anticipating it would likely not be used for Aim2 in vivo work and would still provide valuable information about antibiofilm mechanisms. We are currently working on identifying replacement compounds for azithromycin, linezolid, and sulfamethoxazole, from the same antibiotic classes to complete this work as part of year 2 efforts.

For biofilm testing of the compounds listed in Table 2, biofilms were grown on polycarbonate coupons in a CDC biofilm reactor using established protocols as outlined in the Project Narrative. Briefly, 500 ml of 100% brain heart infusion broth (BHI) is added to the biofilm reactor, inoculated (either against *S. aureus* ATCC #6538, or *P. aeruginosa* ATCC #27853), and incubated at 34° C for 24 h at 130 RPM stirring speed. After this 24 h batch phase, each reactor was perfused with 10% BHI at 6.4 ml/min for an additional 24 h. Each reactor yielded 24 biofilm-covered coupons. From each reactor 4 coupons were used to obtain baseline bioburden; the remainder (20 coupons) were used for testing antibiotic treatments (4 groups, n=5/group). Each biofilm-covered coupon was submerged separately in 2 ml of antibiotic solution prepared in 100% cation-adjusted Mueller Hinton broth (CAMHB) and exposed to treatments for 24 h at 37° C. The 4 treatment groups comprised, a β-lactam control group (either nafcillin or cefepime for *S. aureus* and *P. aeruginosa* respectively), a group with 20X the MIC of the candidate antibiotics from Table 2, and the other two groups were combinations of the former groups with the addition of 5 mM adjunctive metronidazole (855 µg/ml, below the MIC value Table 2).

The data presented here represents the work from 19 biofilm reactors not including 5 reactors which were used to determine aspects of experimental parameters, and 6 reactors which had to be discarded due to technical failures: unanticipated power interruptions, unexplained poor biofilm growth, and environmental contamination. These data presented in Figures 1-4 represent the outcomes form testing on 456 biofilm-covered coupons.

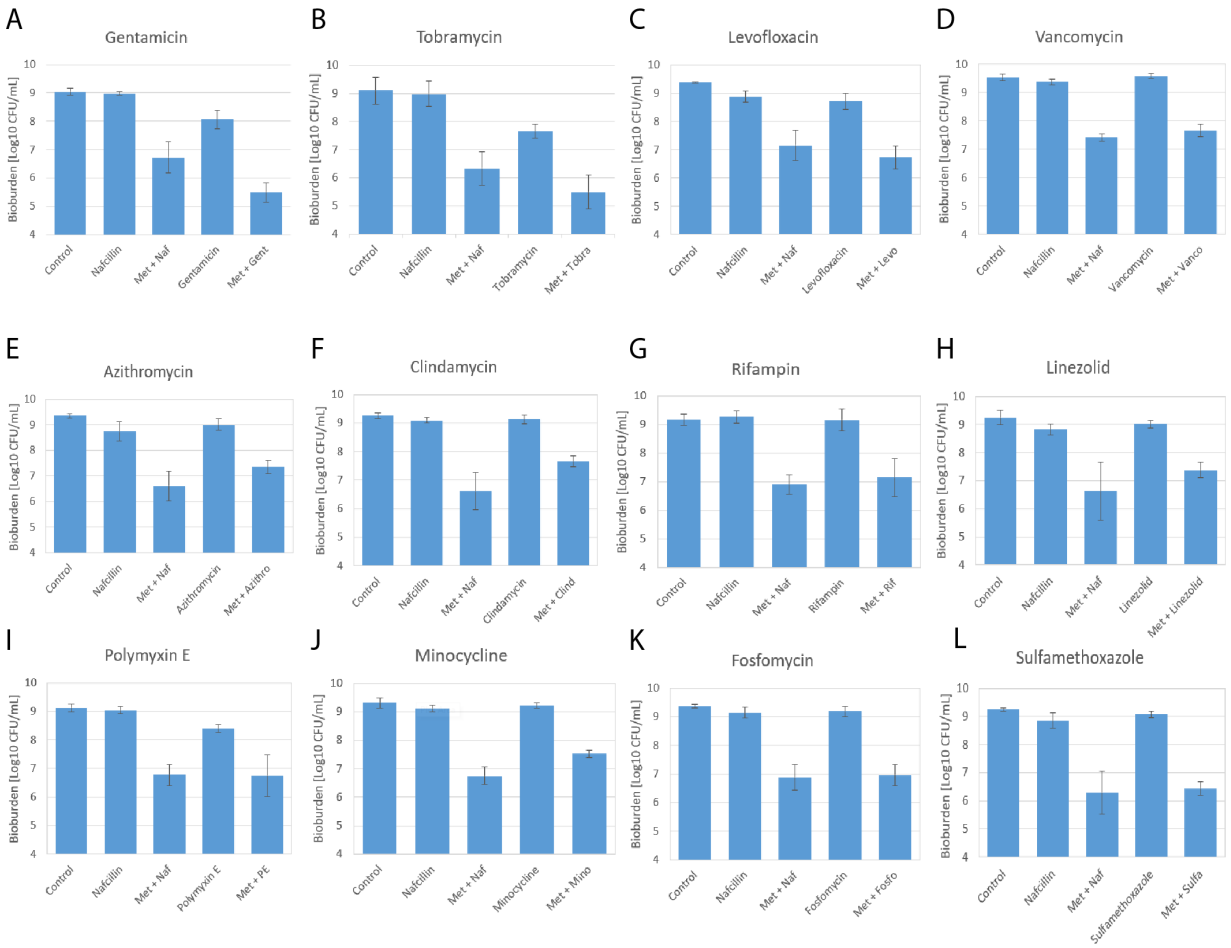

Figure 1: *S. aureus* biofilm testing of low-MIC antibiotics and adjunctive metronidazole. Biofilms grown using a CDC biofilm reactor on polycarbonate coupons with the indicated organism were treated with 2 ml of CAMHB and the indicated antibiotics for 24 h. Treatments were prepared at 20X the MIC value of the antibiotic indicated and 5mM metronidazole. After treatment, the bioburden in the system was quantified using an established method of vertexing, sonicating, pelleting, washing, and serial dilution plating (blue bars). The bars represent average values (n=4 for controls, n=5 for treatment groups). The error bars represent ± S.D.

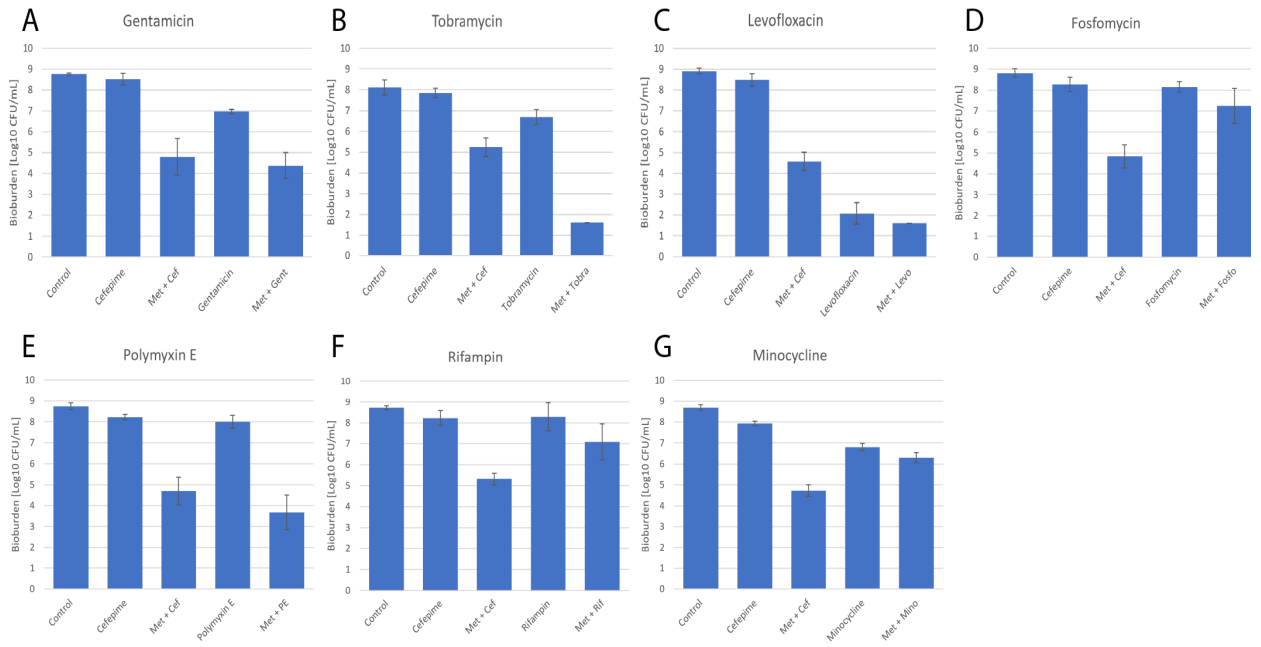

Figure 2: *P. aeruginosa* biofilm testing of low-MIC antibiotics and adjunctive metronidazole. Biofilms grown using a CDC biofilm reactor on polycarbonate coupons with the indicated organism were treated with 2 ml of CAMHB and the indicated antibiotics for 24 h. Treatments were prepared at 20X the MIC value of the antibiotic indicated and 5mM metronidazole. After treatment, the bioburden in the system was quantified using an established method of vertexing, sonicating, pelleting, washing, and serial dilution plating (blue bars). The bars represent average values (n=4 for controls, n=5 for treatment groups). The error bars represent ± S.D.

With every low-MIC antibiotic tested here, (Table 2) there was greater reduction in biofilm bioburden when paired adjunctively with metronidazole even though metronidazole was used below its MIC value (MIC >2500 µg/ml). Cross-group comparison and subsequent interpretation of this dataset is facilitated by $\log_{10}$-reduction plots. Log reduction plots were generated from both the reactor controls and β-lactam controls as baselines. The β-lactam controls were used as an internal adjustment in each reactor to facilitate cross-reactor data comparison. Weaker and less mature biofilms display less tolerance to antibiotics. Thus, the β-lactam controls represent the fraction of biofilm which is most metabolically quiescent, thereby providing a way to appropriately bassline each dataset despite variability in biofilms across reactor runs. These $\log_{10}$-reduciton plots are shown in Figures 3-4 below.

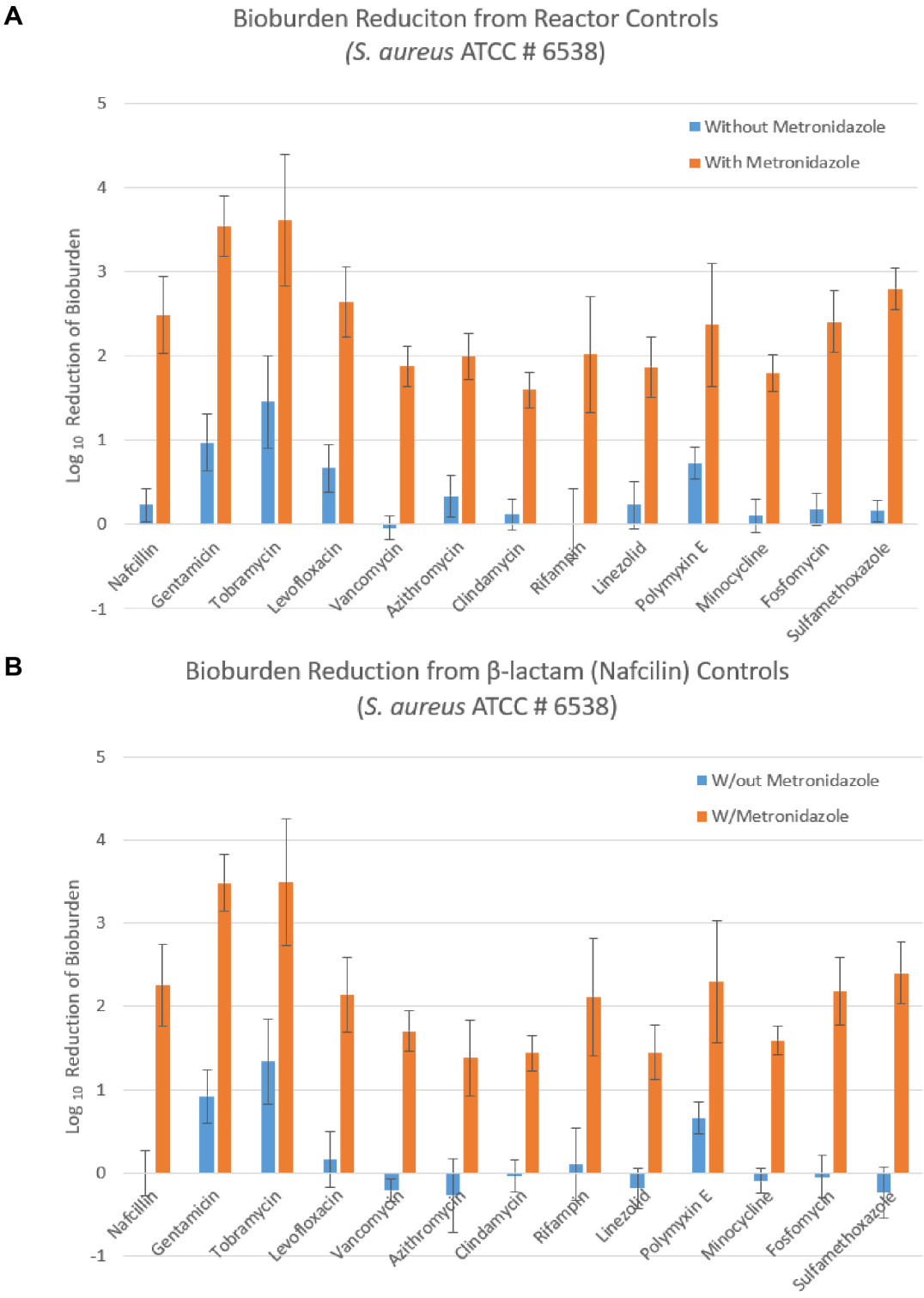

Figure 3: Log_{10} reduction plots of *S. aureus* biofilm tests from figure 1. Biofilms grown using a CDC biofilm reactor on polycarbonate coupons then treated with 2 ml of CAMHB and the indicated antibiotics for 24 h. Treatments were prepared at 20X the MIC value of the antibiotic indicated (blue bars) and with adjunctive 5mM of metronidazole (orange bars). The error bars represent ± S.D. (A) Log_{10}-reductions calculated from reactor controls, and (B) log_{10}-reductions calculated from β-lactam (nafcillin) controls.

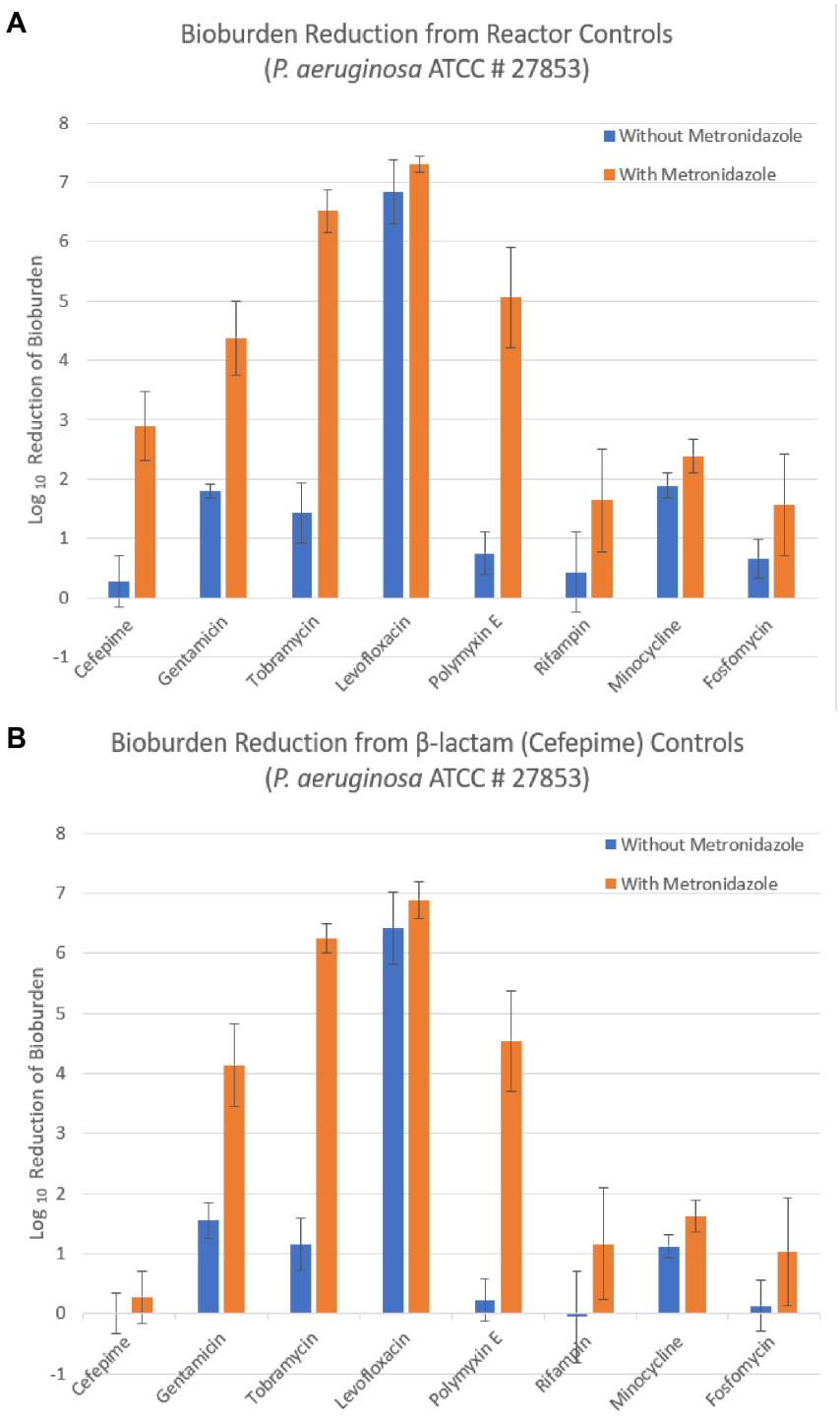

Figure 4: Log_{10} reduction plots of *P. aeruginosa* biofilm tests from figure 2. Biofilms grown using a CDC biofilm reactor on polycarbonate coupons then treated with 2 ml of CAMHB and the indicated antibiotics for 24 h. Treatments were prepared at 20X the MIC value of the antibiotic indicated (blue bars) and with adjunctive 5mM of metronidazole (orange bars). The error bars represent ± S.D. (A) Log_{10}-reductions calculated from reactor controls, and (B) log_{10}-reductions calculated from β-lactam (cefepime) controls.

One of the main purposes of the Aim 1 *in vitro* biofilm testing was to identify the best low-MIC antibiotic for pairing with adjunctive metronidazole in the Aim 2 animal testing portion of this project. Figures 3 and 4 are instrumental in guiding this selection. Although it is emphasized that adjunctive metronidazole increased the bactericidal capacity of all antibiotics tested, there are several combinations standing out in this testing. Of particular note are the aminoglycosides: gentamicin and tobramycin. After extensively considering many tradeoffs with the various antibiotics, we have selected tobramycin for Aim 2 animal testing, delivered through Pouch device described in the Project Narrative (Fig. 5). The rationale for selecting tobramycin is:

1) It displayed exceptional anti-biofilm properties in benchtop testing (Fig.1 &2) with both S. aureus and P. aeruginosa.
2) It shows strong synergy with adjunctive metronidazole (Fig. 1-4).
3) Orthopedic surgeons are familiar with tobramycin.
4) It is a broad-spectrum antibiotic covering most organisms that affect open fractures (S. aureus, Enterobacter species, P. aeruginosa) enabling future work.
5) It is already used in multiple local-therapies in orthopedics, clinic, or under development (e.g., Stimulan®,46 EP Granules,79 OstealTx,80 direct antibiotic powder81).
6) It is IM injectable. Data from years of sheep work in our lab indicate that IM injectable antibiotics are less cytotoxic to host tissues and bone when used locally than non-IM injectables.
7) Tobramycin is a single isomer compound and is easier to analyze chemically than gentamicin, which consists of multiple isomers. This characteristic is beneficial for in vitro and in vivo analyses.
8) It is stable in water. Penicillins, cephalosporins, carbapenems, some glycopeptides, and quinolones rapidly degrade at 37° C in DI-H2O, decreasing by as much as 50% within 7 days.
9) It is highly soluble, up to 94 mg/ml in water.
10) It is reasonably priced, readily available, and sustainable.
10) Its use for local drug delivery layers well with commonplace systemic therapies (e.g., beta lactams) and wouldn't confuse dosing as it is not the same compound type.

We have started building the Purgo Pouch drug delivery devices (Fig. 5) required for animal testing (Aim 2). The iteration of the devices which will go forward to animal testing is shown in figure 5. This device contains a rate controlling membrane (white portion, Fig. 5 A &B) thermally welded to a non-releasing polyurethane pouch. This device comprises a temporarily implanted (<30 days) antimicrobial-filled tubular reservoir with a permeable rate-determining membrane communicating with a percutaneous refilling port. Passive diffusion of antibiotics through the rate-determining membrane and daily refilling, maintain high target concentrations of therapeutics locally at the implant site. As guided by the results presented here, we are finalizing the required IACUC-approved protocol, which is 90% complete, and will be submitting it within the next 30 days for final revision. Once approved, we will subsequently obtain all necessary ACURO approvals before any animal work is commenced.

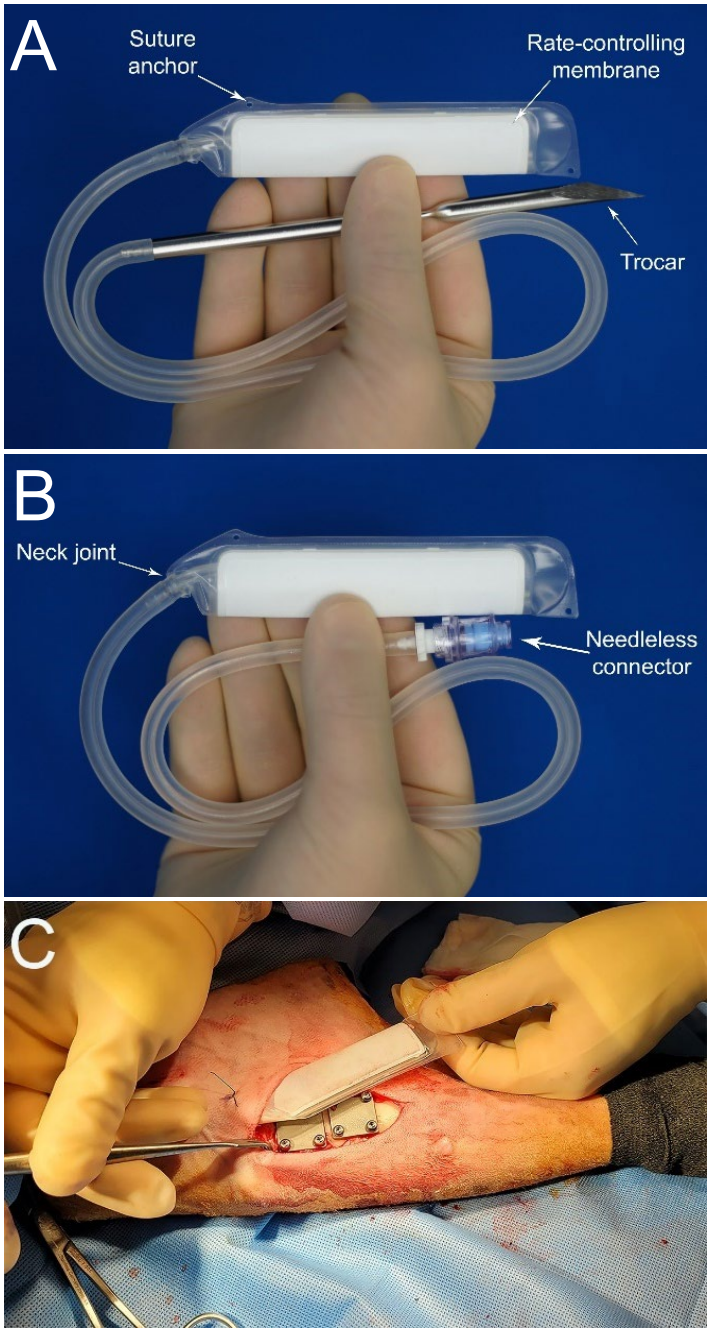

Figure 5: The Purgo Pouch. (A) Purgo Pouch with a trocar attached that facilitates surgical insertion. The Purgo Pouch delivers drug via a rate-controlling membrane (white material) that is incorporated into a refillable reservoir (see Preliminary Data below). (B) Drug delivery is sustained by refilling the reservoir through percutaneous tubing. A needleless connector facilitates refilling. (C) The Purgo Pouch is placed subcutaneously into a surgical site or next to an implanted device(s) where it can deliver drug locally in high concentrations via passive diffusion.

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.

Nothing to Report

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.

We are currently on track with all study tasks and goals. The next reporting period will include accomplishment of the tasks that are listed in the SOW (shown above). This next year we will focus on preparing for animal testing, and starting animal testing with our first set of sheep. Foremost in preparing for this animal testing is sheepherding our animal protocol through the appropriate approval processes with our IACUC and the military's ACURO. Much of our effort over the next several months will be constructing Pouch Device prototypes for this animal work and machining the titanium orthopedic hardware used in our ovine long bone trauma infection model (Fig 5. C). We will also be finishing up the remainder of the Aim 1 biofilm testing with *P. aeruginosa*. This will require us to find alternate antibiotics with acceptably low MIC values (below breakpoints) for the antibiotic classes: macrolide, oxazolidinone, and sulfonamide. Results from these remaining in vitro tests with *P. aeruginosa* will have no influence on which antibiotic we will be selecting for *in vivo* work will only include *S. aureus* infections.

4. **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:*

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).

Nothing to Report

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.

Nothing to Report

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.*

Nothing to Report

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.*

Nothing to Report

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:*

Nothing to Report

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 do not anticipate problems or delays with this work.

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.

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

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).*

  Nothing to Report

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.*

Nothing to report

- **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.

Nothing to Report

- **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.)*

Name: Nicholas Ashton, PhD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-1020-6347
Nearest person month worked: 3
Contribution to Project: Dr. Ashton oversaw all of the work, study design components, and managed day-to-day experimental components.

Name: Dustin Williams, PhD
Project Role: Co-I
Researcher Identifier (e.g. ORCID ID): 0000-0001-5275-1365
Nearest person month worked: 1
Contribution to Project: Dr. Williams supported study design components, antibiotic selection, and data review.

Name: Marissa Badham
Project Role:
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 6
Contribution to Project: Mrs. Badham supported the majority of biofilm growth procedures as well as antimicrobial efficacy testing.

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.

Nothing to Report

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*