Host polyunsaturated fatty acids potentiate aminoglycoside killing of \textit{Staphylococcus aureus}

William Beavers, Matthew Munneke, Alex Stackhouse, Jeffrey Freiberg, and Eric Skaar

Corresponding Author(s): Eric Skaar, Vanderbilt University Medical Center

Review Timeline:

- Submission Date: January 4, 2022
- Editorial Decision: January 31, 2022
- Revision Received: March 8, 2022
- Accepted: March 15, 2022

Editor: Amanda Oglesby

Reviewer(s): The reviewers have opted to remain anonymous.

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1128/spectrum.02767-21
January 31, 2022

Dr. Eric P Skaar
Vanderbilt University Medical Center
Pathology, Microbiology, and Immunology
1161 21st Avenue South
MCN A5211
Nashville, TN 37232

Re: Spectrum02767-21 (Host polyunsaturated fatty acids potentiate aminoglycoside killing of *Staphylococcus aureus*)

Dear Dr. Eric P Skaar:

Thank you for submitting your manuscript to Microbiology Spectrum. Your manuscript was reviewed by two experts, both of whom were very positive about this study. Reviewer 1 has made some suggestions for improvement that I would like you to consider. The suggested experiments are not critical for acceptance, but they would obviously strengthen the manuscript if they are feasible.

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

Link Not Available

Thank you for the privilege of reviewing your work. Below you will find instructions from the Microbiology Spectrum editorial office and comments generated during the review.

The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick [Author Survey](#).

Sincerely,

Amanda Oglesby
Editor, Microbiology Spectrum
Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #1 (Comments for the Author):

This well written and informative manuscript by Beavers et al. documents the synergistic activity between arachidonic acid and aminoglycosides with *S. aureus*. It was documented using growth analysis that a combination of arachidonic acid and gentamicin is synergistic against *S. aureus*. Further, it was documented this combination was also very active against staphylococcal persisters. I just have a few comments that may improve the manuscript.

1. The authors should use the word susceptible throughout instead of sensitive.

2. The investigators may want to perform some standard MIC assays/synergy assays to document the synergistic activity of the two compounds. It is clear from the growth assays that they are synergistic, but these analyses would make the data more interpretable and relatable to other studies assessing synergy.
3. It would also seem relevant to assess if synergy occurs with an isolate that is resistant to aminoglycosides via expression of an aminoglycoside modifying enzyme. This would be a clinically relevant assessment.

4. Do the investigators believe that arachidonic acid can be utilized as a treatment paradigm with aminoglycosides? Or would they be rapidly metabolized by the human host?

Reviewer #2 (Comments for the Author):

The manuscript by Beavers et al. describes how PUFAs can potentiate aminoglycoside activity against S. aureus. This work builds on previous studies examining how membrane acting agents can induce aminoglycoside uptake independently of the proton-motive force. The ability of PUFAs to similarly potentiate aminoglycosides is important and may influence the development of novel therapeutic approaches. The paper is well written, the results are impressive and they have been appropriately interpreted.

Staff Comments:

Preparing Revision Guidelines

To submit your modified manuscript, log onto the eJP submission site at https://spectrum.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

• Point-by-point responses to the issues raised by the reviewers in a file named “Response to Reviewers,” NOT IN YOUR COVER LETTER.
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• Manuscript: A .DOC version of the revised manuscript
• Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at https://journals.asm.org/journal/Spectrum/submission-review-process. Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript.

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Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

Thank you for submitting your paper to Microbiology Spectrum.
We thank the Reviewers for their thoughtful and thorough review of this manuscript and think that the resulting manuscript is much improved. Below is a point-by-point, detailed description of all of the changes made to the manuscript in response to the following reviewer comments.

Reviewer #1 (Comments for the Author):

This well written and informative manuscript by Beavers et al. documents the synergistic activity between arachidonic acid and aminoglycosides with *S. aureus*. It was documented using growth analysis that a combination of arachidonic acid and gentamicin is synergistic against *S. aureus*. Further, it was documented this combination was also very active against staphylococcal persisters. I just have a few comments that may improve the manuscript.

1. The authors should use the word susceptible throughout instead of sensitive.

   **Susceptible is now used in place of sensitive in the manuscript.**

2. The investigators may want to perform some standard MIC assays/synergy assays to document the synergistic activity of the two compounds. It is clear from the growth assays that they are synergistic, but these analyses would make the data more interpretable and relatable to other studies assessing synergy.

   To address this comment, we determined the gentamicin MIC for *S. aureus* JE2 +/- 50 µM AA. Co-treatment of AA with gentamicin decreases the MIC by greater than 20-fold (Figure S1E).

3. It would also seem relevant to assess if synergy occurs with an isolate that is resistant to aminoglycosides via expression of an aminoglycoside modifying enzyme. This would be a clinically relevant assessment.

   **We obtained two clinical isolates from a collaborator. MRSA 5005 is susceptible to gentamicin, and synergy is observed when AA and gentamicin are co-treated (Figure S2E). MRSA 10554 is resistant to gentamicin through the gene aac(6')-aph(2''), which encodes for a bifunctional gentamicin modifying enzyme. Aac(6')-Aph(2'') modification of gentamicin by acetylation of the 6' amine or phosphorylation of the 2'' hydroxyl, prevents synergy between AA and gentamicin because this resistance mechanism alters the ability of gentamicin to bind the ribosome, while the synergy described in this manuscript alters the ability of gentamicin to enter the *S. aureus* cell (Figure S2F).**

4. Do the investigators believe that arachidonic acid can be utilized as a treatment paradigm with aminoglycosides? Or would they be rapidly metabolized by the human host?

   **A sentence was added to the concluding paragraph acknowledging the difficulties in delivering AA systemically in the host due to uptake and metabolism, and emphasizing that using the combination treatment of AA and gentamicin for skin infections can be attempted in experimental models immediately.**

Reviewer #2 (Comments for the Author):

The manuscript by Beavers et al. describes how PUFAs can potentiate aminoglycoside activity against *S. aureus*. This work builds on previous studies examining how membrane acting agents can induce aminoglycoside uptake independently of the proton-motive force. The ability of PUFAs to similarly potentiate aminoglycosides is important and may influence the development of novel therapeutic approaches. The paper is well written, the results are impressive and they have been appropriately interpreted.
March 15, 2022

Dr. Eric P Skaar  
Vanderbilt University Medical Center  
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Re: Spectrum02767-21R1 (Host polyunsaturated fatty acids potentiate aminoglycoside killing of Staphylococcus aureus)

Dear Dr. Eric P Skaar:

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Sincerely,

Amanda Oglesby  
Editor, Microbiology Spectrum  

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1752 N St., NW  
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E-mail: spectrum@asmusa.org

Supplemental Material: Accept