Identification of Novel *Acinetobacter baumannii* Host Fatty Acid Stress Adaptation Strategies

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ABSTRACT Free fatty acids hold important immune-modulatory roles during infection. However, the host’s long-chain polyunsaturated fatty acids, not commonly found in the membranes of bacterial pathogens, also have significant broad-spectrum antibacterial potential. Of these, the omega-6 fatty acid arachidonic acid (AA) and the omega-3 fatty acid decosahexaenoic acid (DHA) are highly abundant; hence, we investigated their effects on the multidrug-resistant human pathogen *Acinetobacter baumannii*. Our analyses reveal that AA and DHA incorporate into the *A. baumannii* bacterial membrane and impact bacterial fitness and membrane integrity, with DHA having a more pronounced effect. Through transcriptional profiling and mutant analyses, we show that the *A. baumannii* /H9252-oxidation pathway plays a protective role against AA and DHA, by limiting their incorporation into the phospholipids of the bacterial membrane. Furthermore, our study identified a second bacterial membrane protection system mediated by the AdeIJK efflux system, which modulates the lipid content of the membrane via direct efflux of lipids other than AA and DHA, thereby providing a novel function for this major efflux system in *A. baumannii*. This is the first study to examine the antimicrobial effects of host fatty acids on *A. baumannii* and highlights the potential of AA and DHA to protect against *A. baumannii* infections.

IMPORTANCE A shift in the Western diet since the industrial revolution has resulted in a dramatic increase in the consumption of omega-6 fatty acids, with a concurrent decrease in the consumption of omega-3 fatty acids. This decrease in omega-3 fatty acid consumption has been associated with significant disease burden, including increased susceptibility to infectious diseases. Here we provide evidence that DHA, an omega-3 fatty acid, has superior antimicrobial effects upon the highly drug-resistant pathogen *Acinetobacter baumannii*, thereby providing insights into one of the potential health benefits of omega-3 fatty acids. The identification and characterization of two novel bacterial membrane protective mechanisms against host fatty acids provide important insights into *A. baumannii* adaptation during disease. Furthermore, we describe a novel role for the major multidrug efflux system AdeIJK in *A. baumannii* membrane maintenance and lipid transport. This core function, beyond drug efflux, increases the appeal of AdeIJK as a therapeutic target.

KEYWORDS AdeIJK, antimicrobial host lipids, RND efflux, β-oxidation, free fatty acids, lipidomics

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Omega-3 and omega-6 fatty acids have distinct anti-Acinetobacter potential. Considering the major disease burden associated with an imbalance in the human omega-3 and -6 fatty acid status (1, 2) and the well-documented antibacterial activity of long-chain polyunsaturated fatty acids (LC-PUFAs) (3, 4), we examined the impact of omega-3 and -6 fatty acids on Acinetobacter baumannii. A. baumannii is a human pathogen that is of primary concern in the hospital environment, where its exceptional capacity for antimicrobial resistance allows it to cause significant morbidity and mortality in susceptible patients (5, 6). Here, we investigated the effect of arachidonic acid (AA; 20:4n-6) or docosahexaenoic acid (DHA; 22:6n-3) at 31.25 to 500 μM upon the growth of A. baumannii strain AB5075_UW. These concentrations and the magnitude of variation are physiologically relevant, considering differences greater than 10-fold in serum LC-PUFAs between individuals can be seen, depending largely on dietary intake (7). We found that both LC-PUFAs induced growth perturbation, but this was more pronounced with DHA compared to AA exposure (Fig. 1A). Interestingly, preexposure to DHA did not affect the cell’s subsequent growth dynamics in the presence of DHA (see Fig. S1A in the supplemental material).
acids revealed that the antimicrobial potential of omega-3 and -6 fatty acids may be conserved because γ-linolenic acid (GLA; omega-6) and eicosapentaenoic acid (EPA; omega-3) have similar impacts on the growth of strain ABS075_UW as AA and DHA, respectively (Fig. S1B and C).

We next examined the relative accumulation of the exogenous fatty acids in *A. baumannii* upon *in vitro* treatment with either 125 μM AA or 125 μM DHA, representing the lowest concentration at which the omega-3 fatty acid shows a significantly greater antimicrobial potential compared to the omega-6 fatty acid (Fig. 1A; Fig S1B and C). This revealed that not only are the exogenous fatty acids incorporated, but *A. baumannii* partly degrades AA and DHA, generating GLA (18:3n-6) and EPA (20:5n-3), respectively (Fig. 1B). Examination of the two major phospholipid species phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) suggested that the exogenous lipids were readily incorporated into phospholipids (28% and 20% of total phospholipids after AA and DHA treatment, respectively) (Fig. 1C). This strongly indicates that the bacterium expresses systems that actively interact with host fatty acids. Based on the level of host fatty acid incorporation into the bacterial membrane, we investigated the impact of AA or DHA on bacterial membrane integrity (Fig. 1D). Treatment with AA or DHA increased the membrane permeability, but this was most significant (3.1-fold) after exposure to DHA (Fig. 1D). Overall, despite AA accumulating in the bacterial cell at higher levels, the longer and more desaturated host fatty acid DHA exerted a greater effect on *A. baumannii* growth and membrane integrity.

Membrane phospholipids play a key role in the defense against antimicrobials, including host fatty acids (8). Hence, we examined the effect of AA or DHA treatment upon the abundance of the endogenous *A. baumannii* fatty acids. The results showed a highly specific depletion (≥50%) of the monounsaturated fatty acids 16:1n-7 and 18:1n-9 (Fig. S1D). This could not be accounted for through the transcriptional dysregulation of the FASI pathway, as seen in Gram-positive species (9). However, the fadAB operon (ABUW_3573-3572) was significantly upregulated upon treatment with AA or DHA (Fig. S1E). The fadAB operon encodes critical components of the fatty acid β-oxidation pathway, indicating fatty acid degradation may provide protection against LC-PUFAs. Indeed, the fitness of fadA- and fadB-inactivated mutants was significantly compromised under AA and DHA stress (Fig. 1E). Interestingly, the accumulation of DHA or EPA was not affected by mutation of fadB, despite its hypersusceptibility to DHA (Fig. S1F). Instead, we observed that of the PE and PG species with exogenous fatty acid incorporated, PE(36:5) and PG(36:6), the levels were significantly higher in the fadB mutant strain upon treatment with DHA (Fig. 1F). This suggests that the β-oxidation pathway is likely to restrict conversion of DHA and its derivatives into phospholipids. Collectively, these data indicate that the β-oxidation pathway contributes to protection against LC-PUFAs, which may impact the success of *A. baumannii* as a human pathogen.

The *AdeUK RND efflux system is involved in LC-PUFA resistance*. The *A. baumannii* resistance nodulation cell division (RND) family of efflux systems has been associated with *in vivo* survival (10). Hence, we first studied their involvement in LC-PUFA stress resistance by examining the transcriptional responsiveness upon AA or DHA supplementation. Interestingly, AA and DHA induced the specific upregulation of adeJ (Fig. 2A). *AdeJ* is part of the *A. baumannii* core genome and, in combination with *adeI* and *adeK*, encodes the complete RND efflux system (AdeUK) that plays an important role in *A. baumannii* multidrug resistance (10, 11). In recent years, several studies have revealed the RND efflux systems of *Acinetobacter* species, including AdeUK, to play roles in virulence and virulence-associated phenotypes (11, 12). Although their exact mode of action remains unknown, it has been postulated to involve lipid homeostasis (13). We examined the effect of *adeJ* inactivation on susceptibility to AA and DHA in both *A. baumannii* strain ABS075_UW (14) and *Acinetobacter baylyi* strain ADP1 (15). Growth delays were observed in both *adeJ* mutants (Fig. 2B), confirming that AdeUK plays a role in protection against AA and DHA and that this function is conserved between at least two different *Acinetobacter* species. Expression of *adeUK* is repressed by AdeN (16);
hence, we analyzed the growth dynamics of an adeN mutation in A. baumannii. Consistent with previous reports (10), the growth rate of the adeN mutant is compromised compared to that of the parental strain (see Fig. S2A in the supplemental material). However, growth rates in the presence of DHA were similar between the adeN mutant and wild type (WT) (Fig. S2A), indicating that DHA-mediated adeJK derepression provides A. baumannii with DHA resistance at a level similar to that when adeN has been inactivated. We then hypothesized that the AdeJK RND efflux system was responsible for the export of the exogenous fatty acid as a protection mechanism. Hence, we examined the accumulation of DHA in the adeJ mutant, but found that the accumulation of DHA and its derivative EPA, as well as the conversion into phospholipids, was significantly lower in the mutant compared to the parental strain (Fig. S2B and C). We then ascertained whether AdeJK may be involved in membrane modulation by removing endogenous fatty acids from the membrane to achieve lipid homeostasis, similar to the EmhABC RND efflux system of Pseudomonas fluorescens (17). Lipid
analyses demonstrated that the concentrations of two endogenous species of PG and two of cardiolipin were affected by adeJ mutation prior to treatment (Fig. 2C), with eight species in total being affected posttreatment with DHA (Fig. S2D). These findings implicate a role for AdeIJK in membrane modulation. Indeed, even without treatment, AdeIJK-mediated changes in the phospholipids affected the membrane integrity, as the permeability of the adeJ mutant was significantly greater than that of the WT (Fig. 2D). We then examined the fatty acids in the media following growth of the WT or adeJ mutant (Fig. 2E). Consistent with a role for AdeJ in lipid efflux, growth of the WT resulted in an increase in fatty acids in the media, whereas growth of the mutant strain resulted in fatty acid depletion (Fig. 2E). Hence, we speculate that the differences seen between the WT and adeJ mutant could most likely be ascribed to AdeIJK-mediated export of fatty acids—a significant observation that indicates a novel role for AdeIJK. We also found that mutation of adeJ leads to an increase in biofilm formation but a decrease in surface motility in A. baumannii (Fig. 2F and G). Thus, our data suggest that the exported lipids may function as a surfactant, which renders the biofilm unstable, but promotes surface migration. Here, we show for the first time, through its identification as a protective mechanism against LC-PUFAs, that the AdeIJK RND efflux system is directly involved in the maintenance of lipid homeostasis in A. baumannii. Furthermore, through its proposed role as a lipid export system, this work has significantly advanced our understanding of the molecular basis behind AdeIJK’s far-reaching impacts on A. baumannii biofilm formation and in vivo fitness. Furthermore, similar to the lipid-mediated sequestration of daptomycin in Staphylococcus aureus (18, 19), the export of lipids may serve as a novel mechanism by which AdeIJK provides protection against amphiphilic or hydrophobic antimicrobials.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.02056-18.

FIG S1, TIF file, 0.4 MB.
FIG S2, TIF file, 0.4 MB.
TABLE S1, DOCX file, 0.1 MB.
TABLE S2, XLSX file, 0.1 MB.

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REFERENCES

1. Anes E, Jordao L. 2008. Trick-or-treat: dietary lipids and host resistance to infectious disease. Mini Rev Med Chem 8:1452–1458.
2. Simopoulos AP. 2011. Evolutionary aspects of diet: the omega-6/omega-3 ratio and the brain. Mol Neurobiol 44:203–215. https://doi.org/10.1007/s12035-010-8162-0.
3. Desbois AP, Smith VJ. 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol 85:1629–1642. https://doi.org/10.1007/s00253-009-2355-3.
4. Churchward CP, Alany RG, Snyder LAS. 2018. Alternative antimicrobials: the properties of fatty acids and monoglycerides.Crit Rev Microbiol 44:561–570. https://doi.org/10.1080/1040041X.2018.1467875.
5. Harding CM, Hennon SW, Feldman MF. 2018. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat Rev Microbiol 16:91–102. https://doi.org/10.1038/nrmicro.2017.148.
6. Dijkshoorn L, Nemec A, Seifert H. 2007. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol 5:939–951. https://doi.org/10.1038/nrmicro1789.
7. Abdelmagid SA, Clarke SE, Nielsen DE, Badawi A, El-Sohemy A, Mutch DM, Ma DWL. 2015. Comprehensive profiling of plasma fatty acid concentrations in young healthy Canadian adults. PLoS One 10:e0116195. https://doi.org/10.1371/journal.pone.0116195.
8. Eijkelkamp BA, Begg SL, Pedericik VG, Trapetti C, Gregory MK, Whittall JJ, Paton JC, McDavitt CA. 2018. Arachidonic acid stress impacts pneumococcal fatty acid homeostasis. Front Microbiol 9:813. https://doi.org/10.3389/fmicb.2018.00813.
9. Yao J, Rock CO. 2017. Exogenous fatty acid metabolism in bacteria. Biochimie 141:30–39. https://doi.org/10.1016/j.bioch.2017.06.015.
10. Yoon E-J, Chabane YN, Goussard S, Snesrud E, Courvalin P, Dé E, Grillot-Courvalin C. 2015. Contribution of resistance-nodulation-cell division efflux systems to antibiotic resistance and biofilm formation.
in Acinetobacter baumannii. mBio 6:e00309-15. https://doi.org/10.1128/mBio.00309-15.

11. Yoon E-J, Balloy V, Fiette L, Chignard M, Courvalin P, Grillot-Courvalin C. 2016. Contribution of the Ade resistance-nodulation-cell division-type efflux pumps to fitness and pathogenesis of Acinetobacter baumannii. mBio 7:e00697-16. https://doi.org/10.1128/mBio.00697-16.

12. Knight DB, Rudin SD, Bonomo RA, Rather PN. 2018. Acinetobacter nosocomialis: defining the role of efflux pumps in resistance to antimicrobial therapy, surface motility, and biofilm formation. Front Microbiol 9:1902. https://doi.org/10.3389/fmicb.2018.01902.

13. Leus IV, Weeks JW, Bonifay V, Smith L, Richardson S, Zgurskaya HI. 2018. Substrate specificities and efflux efficiencies of RND efflux pumps of Acinetobacter baumannii. J Bacteriol 200:e00049-18. https://doi.org/10.1128/JB.00049-18.

14. Gallagher LA, Ramage E, Weiss EJ, Radey M, Hayden HS, Held KG, Huse HK, Zurawski DV, Bittmacher MJ, Manoil C. 2015. Resources for genetic and genomic analysis of emerging pathogen Acinetobacter baumannii. J Bacteriol 197:2027–2035. https://doi.org/10.1128/JB.00131-15.

15. Brzoska AJ, Hassan KA, de Leon EJ, Paulsen IT, Lewis PJ. 2013. Single-step selection of drug resistant Acinetobacter baylyi ADP1 mutants reveals a functional redundancy in the recruitment of multidrug efflux systems. PLoS One 8:e56090. https://doi.org/10.1371/journal.pone.0056090.

16. Rosenfeld N, Bouchier C, Courvalin P, Périchon B. 2012. Expression of the resistance-nodulation-cell division pump AdeIJK in Acinetobacter baumannii is regulated by AdeN, a TetR-type regulator. Antimicrob Agents Chemother 56:2504–2510. https://doi.org/10.1128/AAC.06422-11.

17. Adebusuyi AA, Foyt JM. 2011. An alternative physiological role for the EmhABC efflux pump in Pseudomonas fluorescens cLP6a. BMC Microbiol 11:252. https://doi.org/10.1186/1471-2180-11-252.

18. Sabnis A, Ledger EVK, Pader V, Edwards AM. 2018. Antibiotic interceptors: creating safe spaces for bacteria. PLoS Pathog 14:e1006924. https://doi.org/10.1371/journal.ppat.1006924.

19. Pader V, Hakim S, Painter KL, Wigneshweraraj S, Clarke TB, Edwards AM. 2016. Staphylococcus aureus inactivates daptomycin by releasing membrane phospholipids. Nat Microbiol 2:16194. https://doi.org/10.1038/nmicrobiol.2016.194.

20. Hassan KA, Pederick VG, Elbourne LDH, Paulsen IT, Paton JC, McDevitt CA, Eijkelkamp BA. 2017. Zinc stress induces copper depletion in Acinetobacter baumannii. BMC Microbiol 17:59. https://doi.org/10.1186/s12866-017-0965-y.

21. Botté CY, Yamayo-Botté Y, Rupasinghe TWT, Mullin KA, MacRae JI, Spurck TP, Kalanon M, Shears MJ, Coppel PK, Marechal E, McConville MJ, McFadden GI. 2013. Atypical lipid composition in the purified relict plastid (apicoplast) of malaria parasites. Proc Natl Acad Sci U S A 110:7506–7511. https://doi.org/10.1073/pnas.1301251110.

22. Eijkelkamp BA, Stroehrer UH, Hassan KA, Papadimitrion MS, Paulsen IT, Brown MH. 2011. Adherence and motility characteristics of clinical Acinetobacter baumannii isolates. FEMS Microbiol Lett 323:44–51. https://doi.org/10.1111/j.1574-6968.2011.02362.x.
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