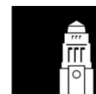

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1 **The native ABC-F proteins of *Staphylococcus aureus* do not contribute to**

2 **intrinsic resistance against ribosome-targeting antibacterial drugs**

3

4

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21

22 Antibiotic resistance ATP-binding cassette F (ARE ABC-F) proteins are a major cause of
23 acquired resistance to antibacterial drugs that target protein synthesis.[1] These proteins bind
24 to the ribosome to drive antibiotic release, a mechanism known as target protection,[2] and
25 thereby mediate resistance to diverse drug classes that act on the 50S subunit (lincosamides,
26 macrolides, oxazolidinones, phenicols, pleuromutilins, and streptogramins).[1]

27

28 In addition to their role in acquired resistance, it is increasingly apparent that ARE ABC-Fs are
29 an important and common source of *intrinsic* resistance to ribosome-targeting antibiotics in
30 many bacterial species, including pathogens. It has long been known that the intrinsic
31 lincosamide/ streptogramin resistance of *Enterococcus faecalis* and *Bacillus subtilis* is
32 attributable to native ABC-F proteins (Lsa(A)[3] and VmlR,[4] respectively), and recent years have
33 seen a dramatic accumulation of additional examples of ABC-F-mediated intrinsic resistance
34 that include the Sal proteins in non-aureus staphylococci,[5] VgaL (Lmo0919) in *Listeria*
35 *monocytogenes,*[6] MAB_2355c in *Mycobacterium abscessus,*[7] and CplR in Clostridia.[8] In
36 addition to providing an explanation for the differing levels of intrinsic susceptibility to
37 ribosome-targeting antibiotics observed across common bacterial species, understanding
38 such intrinsic ARE ABC-Fs may inform improved approaches to deployment or discovery of
39 antibacterial drugs.

40

41 Here, we examined whether the native ABC-F proteins of *Staphylococcus aureus* contribute
42 to the intrinsic background level of resistance to ribosome-targeting antibiotics. This
43 pathogen is the prime exponent of acquired antibiotic resistance mediated by ARE ABC-F
44 proteins,[1] and as indicated above, other members of the same genus are known to harbour
45 native ARE ABC-Fs;[5] consequently, it seemed entirely possible that native ABC-Fs participate
46 in intrinsic antibiotic resistance in *S. aureus*.

47

48 To define the complement of native ABC-F proteins in *S. aureus*, we searched the predicted
49 proteome (GCA_002085525.1) of MRSA strain JE2 downloaded from the NCBI genome
50 database with ABC-F subfamily-specific hidden Markov models[9] using HMMER v.
51 3.3.2 hmmscan[10] and an e-value threshold of $1e^{-70}$. This analysis returned three ABC-F
52 proteins: Uup [ARG45262.1] (previously referred to as EttA[11]), YbiT [ARG45891.1], and YdiF
53 [ARG46606.1]. Whether these proteins provide any degree of intrinsic resistance to

54 ribosome-targeting antibacterial drug classes is unknown; whilst an earlier report assessed
55 the antibiotic susceptibility of a strain in which ARG45262.1 was putatively inactivated,[11] that
56 study did not test the majority of drug classes that fall within the typical spectrum of
57 resistance for ARE ABC-F proteins. We therefore sought to examine susceptibility to relevant
58 drug classes of strains in which these ABC-F genes had been independently inactivated by
59 transposon (Tn) insertion mutagenesis. The corresponding Tn mutants (NE770, NE293 and
60 NE790, respectively) were sourced from the Nebraska Transposon Mutant Library
61 (https://www.unmc.edu/pathology/csr/research/library.html), and modified by allelic
62 exchange to replace the selectable marker on the Tn (*ermB*) with the kanamycin resistance
63 determinant, *aphA-3*; the rationale for this was that *ermB* itself confers resistance to drug
64 classes that we intended to test. Susceptibility testing by CLSI broth microdilution found no
65 differences between the Tn-inactivation strains and the JE2 parent for lincosamides
66 (clindamycin), macrolides (erythromycin), oxazolidinones (linezolid), phenicols
67 (chloramphenicol), pleuromutilins (tiamulin) and streptogramins (virginiamycin M1/ S).
68
69 To corroborate this result and exclude the possibility that Tn insertions had not completely
70 inactivated gene function, we generated independent, markerless deletions of the three ABC-
71 F genes in JE2 by allelic replacement using plasmid pIMAYZ.[12] Again, no difference in antibiotic
72 susceptibility was seen for these strains, even when using concentration increments
73 substantially smaller than those ordinarily employed in susceptibility testing. Failure to detect
74 a change in susceptibility in individual ABC-F deletion mutants could potentially reflect
75 functional redundancy between the encoded proteins; consequently, we employed the same
76 pIMAYZ constructs to sequentially delete all three ABC-F genes in a single strain of JE2. The
77 resultant strain also showed no change in antibiotic susceptibility.
78
79 Having established that deletion of native ABC-F genes of *S. aureus* – alone or in combination
80 – has no apparent effect on susceptibility to ribosome-targeting drugs, we took an orthogonal
81 approach to examine whether the encoded proteins could potentially contribute to antibiotic
82 resistance by assessing whether they impact susceptibility under conditions of increased
83 expression. Since the expression of ARE ABC-F genes is often under the control of antibiotic-
84 responsive regulatory elements,[4,8] we first examined whether challenging *S. aureus* JE2 with
85 a subinhibitory concentration (1/4 MIC) of a ribosome-targeting antibiotic - with a view to

inducing ABC-F expression - would serve to reduce susceptibility to that same agent in a subsequent MIC determination. No change in susceptibility to any of the antibiotics was observed under these conditions. We subsequently generated independent artificial overexpression constructs for each of the three ABC-F genes using the strong, tetracycline-inducible expression system on plasmid pRMC2.[13] Under conditions of maximal induction, no change in antibiotic susceptibility was seen in any case.

Thus, we conclude that in contrast to the situation seen for other medically-important Gram-positive bacteria – including other members of the same genus – the native ABC-F proteins of *S. aureus* do not contribute to intrinsic resistance to ribosome-targeting antibacterial drugs.

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102 **Transparency declarations**

103 None to declare.

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