Structural basis for PoxtA-mediated resistance to phenicol and oxazolidinone antibiotics

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PoxtA and OptrA are ATP binding cassette (ABC) proteins of the F subtype (ABCF). They confer resistance to oxazolidinone and phenicol antibiotics, such as linezolid and chloramphenicol, which stall translating ribosomes when certain amino acids are present at a defined position in the nascent polypeptide chain. These proteins are often encoded on mobile genetic elements, facilitating their rapid spread amongst Gram-positive bacteria, and are thought to confer resistance by binding to the ribosome and dislodging the bound antibiotic. However, the mechanistic basis of this resistance remains unclear. Here we refine the PoxtA spectrum of action, demonstrate alleviation of linezolid-induced context-dependent translational stalling, and present cryo-electron microscopy structures of PoxtA in complex with the Enterococcus faecalis 70S ribosome. PoxtA perturbs the CCA-end of the P-site tRNA, causing it to shift by ~4 Å out of the ribosome, corresponding to a register shift of approximately one amino acid for an attached nascent polypeptide chain. We postulate that the perturbation of the P-site tRNA by PoxtA thereby alters the conformation of the attached nascent chain to disrupt the drug binding site.
Antibiotic resistance (ARE) is a growing threat to the efficacy of our current arsenal of clinically approved antimicrobial agents. The ATP-binding cassette (ABC) family of proteins are well-known for their role as multidrug resistance transporters, which use the energy of ATP hydrolysis to drive the efflux of antibiotics from the bacterial cytoplasm. In recent years, it has become clear that ARE ABC proteins that belong to subfamily F (ABCF) are not transporters—and thus do not confer resistance via efflux—but rather act via a direct target protection mechanism.

ABCF proteins confer resistance to a diverse range of antibiotics that inhibit protein synthesis by targeting the large subunit (LSU) of the ribosome. Based on the spectrum of antibiotic resistance that they confer, ARE–ABCF proteins fall into three functional groups: (i) those that protect from pleuromutilins, lincosamides and streptogramins A (PLS₅₅), (ii) those that protect from macrolides and streptogramin B (MS₉₉) and, finally, (iii) those that protect from phenicols and oxazolidinones (PhO)⁶⁻¹⁰. These functional groups do not map exactly to the phylogenetic tree of ARE–ABCFs, in which seven subclasses (ARE₁–₇) were originally distinguished, but are rather scattered amongst non-ARE–ABCFs, implying that these resistance factors have arisen multiple times by convergent evolution. Despite the divergence in the spectrum of antibiotic resistance, ARE–ABCFs share a common architecture. ARE–ABCFs are comprised of two ABC nucleotide-binding domains (NBD1 and NBD2) that are separated by a helical linker termed an ARD (antibiotic-resistance domain), and, depending on the species, ARE–ABCFs may also have an additional “Arm” subdomain inserted within NBD1 as well as a C-terminal extension (CTE)⁹. In fact, this architecture is similar to many non-ARE–ABCFs, such as the E. coli housekeeping ABCF ATPase EttA, in which the ARD equivalent is shorter and referred to as a P-site tRNA-interaction motif (PtIM)¹¹,¹², thus making it difficult to judge whether many ABCF proteins are actually resistance determinants or endogenous proteins of mostly unknown function.

Cryo-EM structures of ribosomes in complex with ARE–ABCFs that confer resistance to PLS₅₅ (ARE₁ VgaALC and VgaL, ARE₂ VmrR, ARE₃ LsaA) and MS₉₉ (ARE₁ MsrE) classes of antibiotics have revealed that these proteins bind within the E-site, similar to that reported previously for the housekeeping non-ARE–ABCF EttA.¹¹ However, in contrast to EttA,¹¹,¹² the longer ARD of the ARE–ABCF proteins distorts the P-site tRNA, allowing the factor to access the peptidyl transferase centre (PTC) on the LSU of the ribosome and dislodge the relevant antibiotics from their binding sites.¹³⁻¹⁵ These structural changes revealed that there is often no steric overlap between the ARD of the ARE–ABCF and the drugs and even when there is a steric overlap, the mutational analysis indicated that it is not strictly required for resistance.¹³⁻¹⁵ Collectively, these results support a model where the ARE–ABCFs dislodge the drugs from the PTC by inducing a cascade of conformational changes within the 23S rRNA nucleotides that comprise the drug-binding site.¹⁵ For MsrE, drug release was reported to occur in the presence of a so-called EQ variant of the protein, which is deficient in ATPase activity, suggesting that ATP hydrolysis is not essential for drug release, but is needed for recycling of the factor from the ribosome.

In contrast to the relatively well-studied PLS₅₅- and MS₉₉-protecting ARE–ABCFs, mechanistic insights into how ARE₇ OptrA and ARE₈ PoxA confer resistance to PhO antibiotics are lacking. The first ARE–ABCF from this group to be discovered was OptrA. This factor confers resistance to the phenicols, such as chloramphenicol and florfenicol, as well as to the oxazolidinone linezolid and, to a lesser extent, tedizolid.¹⁶ OptrA was originally found on the conjugative plasmid pE349 from Enterococcus faecalis, but has subsequently been detected, both plasmid and chromosomal-encoded, across many Gram-positive enterococci, staphylococci and streptococci of human and animal origin.¹⁶⁻²² At least 69 variants of the optrA gene have been reported to date, differing by 1–20 aa substitutions, which corresponds to an amino acid identity of 97.1–99.8% compared to the first reported OptrA sequence.²² Moreover, evidence for horizontal transfer of OptrA to Gram-negative bacteria, such as Campylobacter coli, has also recently been described.²³,²⁴ While oxazolidinones are not clinically effective against Gram-negative pathogens, this raises the concern of possible co-selection of antibiotic co-resistance i.e. selection for simultaneous transfer and spread of several antibiotic-resistance genes encoded by one mobile genetic element. In addition to OptrA, a second ARE–ABCF from this group was detected by bioinformatic analysis of the genome of a methicillin-resistant Staphylococcus aureus (MRSA) strain AOU-C-0913 isolated from a cystic fibrosis patient at the Careggi University Hospital in Florence, Italy.²⁵ Expression of the resistance determinant in S. aureus, E. faecalis and E. coli was reported to confer resistance to phenicol-oxazolidinone-tetracycline antibiotics, and was therefore termed PoxA.²⁵ To date, PoxA has been found exclusively in Enterococcus and Staphylococcus species, most frequently in E. faecium isolates of both human and animal origin.²⁵

In the absence of structures of OptrA and PoxA on the ribosome, it has remained unclear how these ARE–ABCFs confer antibiotic resistance. Both chloramphenicol and linezolid bind at the PTC and inhibit the elongation phase of protein synthesis. However, their activity is context-specific such that translation arrest is most efficient when the nascent polypeptide chain on the ribosome carries an alanine residue and, to a lesser extent, serine or threonine in its penultimate position.²⁷⁻²⁹ Although the ARDs of OptrA and PoxA are slightly longer (4–5 aa) than the PtIM of non-ARE–ABCFs such as EttA, they are considerably shorter than the ARDs of ARE–ABCFs from other groups, and at least 20 amino acids shorter than other ARE–ABCFs for which structures have been reported (Fig. 1). Thus, assuming OptrA and PoxA bind similarly to the ribosome as other ARE–ABCFs, the ARDs are unlikely to be able to reach into the PTC to dislodge the drugs from their binding site. Moreover, it is hard to rationalise how PoxA also confers resistance to tetracycline antibiotics, which bind near the decoding site on the small subunit (SSU), which is located far from the PTC on the LSU.²⁶

Here, we systematically characterise the PoxA and OptrA resistance determinants, revealing that both increase the minimum inhibitory concentrations (MICs) to phenicols, such as chloramphenicol, thiamphenicol and florfenicol, as well as to the oxazolidinone linezolid, but not to macrolides, pleuromutilins, lincomycins and streptogramins that bind adjacent to the PTC within the ribosomal exit tunnel. Moreover, we find no evidence for either PoxA or OptrA conferring resistance to the non-PTC binding antibiotic tetracycline. Cryo-EM structures of PoxA on the ribosome reveal that it binds in the E-site and, despite the short ARD, still induces a distortion of the P-site tRNA, leading to retraction of its CCA-end from the PTC. Unlike for other ARE–ABCFs, we observe no conformational rearrangements within the 23S rRNA around the drug-binding sites within the A site of the PTC upon binding of PoxA to the ribosome. This leads us to propose a model whereby the distortion of the P-site tRNA by PoxA (and OptrA) reduces the affinity of the drugs for their binding site by altering the context and therefore interaction of the nascent polypeptide chain with respect to the drugs.

**Results**

PoxA and OptrA confer resistance to phenicols and oxazolidinones, but not tetracyclines. To systematically characterise the
antibiotic-resistance profiles of PoxtA and Optra, representatives of these ARF–ABCf groups were expressed in an E. faecalis TX332 strain where the lsaA gene had been knocked out (△lsaA), and MICS were determined for phenicol (chloramphenicol, tetracycline15,32. By contrast, cCF10-inducible expression of either Optra or PoxtA results in a 2- to 16-fold MIC increase for Pho antibiotics, and does not, as expected, result in resistance against either PLS1 or MSp, as observed in earlier reports16,25. While the earlier study25 reported a minor protective effect of PoxtA against tetracycline (a twofold increase in MIC for E. faecalis and S. aureus), in our hands expression of either of the PoxtA variants resulted in any increase in MIC for this antibiotic. Notably, the lack of effect of PoxtA AOUC-0915 expression on the MIC for the tetracycline antibiotic tigecycline in either E. faecalis or S. aureus25 is consistent with the original E. faecium 9- F-6 strain from which we have isolated PoxtA EF9F6 also being susceptible to tigecycline (Supplementary Table 1). Therefore, we concluded that the antibiotic-resistance spectrum of PoxtA is similar, if not identical, to that of Optra. This similarity appears to be a case of convergent evolution, as there is no phylogenetic support for PoxtA and Optra being more closely related to each other than to any other ABCF subfamily member (Supplementary Fig. 1). Indeed, Optra is more closely related to the vertically inherited and probable housekeeping ABCF YdiF of Firmicutes (84% bootstrap support, Supplementary Fig. 1), while the relationship of PoxtA to other subfamilies is unresolved (bootstrap support below 50%). On these grounds, we conclude that PoxtA-like proteins constitute a separate ARF subfamily which we call ARE8.

**Table 1 Antibiotic-resistance spectra of LsaA, Optra and PoxtA ARE-ABCfs.**

| Antibiotic          | MIC (µg/mL) pCIEspec vector | LsaA | Optra ST16 | Optra 35048 | PoxtA EF9F6 | PoxtA AOUC-0915 |
|---------------------|-----------------------------|------|------------|-------------|-------------|-----------------|
| Chloramphenicol     | 4                           | 4    | 8-16       | 4-8         | 4-8         | 4-8             |
| Thiamphenicol       | 4                           | 4    | 32-64      | 16-32       | 8-16        | 32-64           |
| Florfenicol         | 1-2                         | 1-2  | 16-32      | 8           | 2-4         | 16-32           |
| Lincosamide         | 1                           | 1    | 4-8        | 32          | 4-8         | 32              |
| Erythromycin        | 0.5-1                       | 0.5  | 32-64      | 8-16        | 2-4         | 32-64           |
| Azithromycin        | 0.5                         | 0.5  | 32-64      | 8-16        | 2-4         | 32-64           |
| Leucosamine         | 0.5                         | 0.5  | 32-64      | 8-16        | 2-4         | 32-64           |
| Lincomycin          | 0.125                       | 0.125| 0.125      | 0.125       | 0.0156      | 0.0156          |
| Tetracycline        | 0.025                       | 0.25 | 0.25       | 0.25        | 0.25        | 0.25            |

BHI media supplemented with 2 mg/mL kanamycin (to prevent isA revertants), 0.1 mg/mL spectinomycin (to maintain the pCIEspec plasmid), 100 ng/mL of cCF10 peptide (to induce expression of ABCF proteins) as well as increasing concentrations of antibiotics were inoculated with 5 × 10^7 CFU/mL (OD600 ≈ 0.0005) of E. faecalis lsaA (lca:kan) strain TX332 transformed either with empty pCIEspec plasmid, or with pCIEspec derivatives for expression of ARE-ABCf proteins. After 16-20 h at 37°C, without shaking, the presence or absence of bacterial growth was scored by eye. The MIC values that are higher than the empty vector control are shown in bold. The experiments were performed in triplicates.

In agreement with earlier reports, expression of LsaA confers resistance to PLS1 antibiotics, but not Pho, MSp or tetracycline15,32. By contrast, cCF10-inducible expression of either Optra or PoxtA results in a 2- to 16-fold MIC increase for Pho antibiotics, and does not, as expected, result in resistance against either PLS1 or MSp, as observed in earlier reports16,25.

PoxtA rescues ribosome stalls associated with lincoside treatment. Extensive in vivo and in vitro studies into the mechanism
of action of chloramphenicol and linezolid during E. coli protein synthesis have established that both antibiotics act as context-specific inhibitors of translation elongation. Specifically, efficient ribosomal stalling by linezolid is strongly stimulated by the presence of alanine as the penultimate amino acid in the polypeptide nascent chain (i.e. the −1 position), while the codon for this amino acid occupies the ribosomal E-site. Dependence of chloramphenicol-mediated stalling on alanine in the −1 position of the nascent chain was previously shown in Gram-positive Firmicute species Lactobacillus plantarum and Bacillus subtilis using the recently developed bacterial high-throughput 5P sequencing (5Pseq) method. Analogous to the well-established 5Pseq for eukaryotes, the bacterial version of the technique detects ribosome occupancy by generating in vivo footprints of the 5′-most ribosome on the mRNA molecule through specific sequencing of the 5′ monophosphorylated intermediates of 5′-3′ mRNA degradation.

We used 5Pseq to globally survey the linezolid-induced ribosomal stalls in E. faecalis and directly assess the role of PoxtA in overcoming these stalls. 5Pseq analysis of linezolid-treated E. faecalis robustly detected increased 5Pseq coverage signal −8 nucleotides upstream of the first (5′) nucleotide of the alanine codon (and to lesser extent at serine codons), which is indicative of ribosomal stalling with these codons in the E-site and their respective amino acids in the −1 position of the nascent chain (Fig. 2a and Supplementary Fig. 2). Importantly, all of the alanine iso-codons display near-identical linezolid-induced stalls, which is indicative of the stalling being defined by the nature of the amino acid, not the codon itself (Supplementary Fig 3). There was no particular enrichment of codons in the A- or P-sites (Fig. 2a). The strong stalling at alanine and weaker stalling at serine and threonine is in good agreement with an E. coli ribosome profiling study, indicating that linezolid affects translation similarly in both organisms. PoxtA expression resulted in the reduction of this context-specific stalling signal in response to linezolid (Fig. 2b, c and Supplementary Fig. 2). The rescue effect is highly reproducible between the three biological replicates (Supplementary Fig. 4a–d), and both the linezolid-induced alanine stalling and its rescue by PoxtA expression are statistically significant (Supplementary Fig. 4e).

By contrasting 5Pseq datasets of antibiotic-untreated E. faecalis that do not express PoxtA with that of an untreated PoxtA-overexpressing control, we probed whether sampling of the ribosomes by PoxtA in the absence of antibiotics causes ribosomal stalling. Our analyses revealed slightly increased ribosomal stalling at intrinsically slow initiation and termination steps (Supplementary Fig. 5). Importantly, no stalling signal specific for the −1 nascent chain position (corresponding to the ribosomal E-site) was detectable, consistent with linezolid determining the amino acid specificity of antibiotic-induced stalls.

Collectively, our 5Pseq study confirms the generality of the context-specific mechanism of action of linezolid and indicates that PoxtA alleviates the drug-induced stalling at positions with −1 alanine residues.

Cryo-EM structures of PoxtA–70S complexes. Our attempts to reconstitute OptraA- and PoxtA–70S ribosome complexes in vitro were unsuccessful due to problems obtaining soluble homogenous OptraA and PoxtA proteins. Therefore, we employed an in vivo pull-down approach with strains overexpressing C-terminally FLAG3-tagged OptraA and PoxtA proteins, as used recently to generate other ARE–ABCF–ribosome complexes. We expressed both the wild-type ATPase-competent OptrA and PoxtA and EQ2 ATPase-deficient variants bearing Glu-to-Gln substitutions in both NBD cassettes. Such EQ2 variants have been successfully employed to trap other ABCF proteins on the ribosome because these substitutions allow binding but prevent hydrolysis of ATP by the ABCF11,13,15,33. The OptrA-EQ2 variant was earlier shown to have a compromised in vitro ATPase activity and unable to confer resistance in vivo. Consequently, the addition of chloramphenicol or linezolid to growth media would block CCF10-driven overexpression of ATPase-deficient OptrA/ PoxtA EQ2 variants. Therefore, in vivo ABCF–ribosome complex formation and its subsequent immuno-purification were performed in the absence of the antibiotic. Affinity purification via
the FLAG3 tag was performed in the presence of 0.5 mM ATP from clarified lysates prepared from the E. faecalis ΔlsaA strain expressing the FLAG3-tagged ARE–ABCF, either wild-type or EQ2-variant. Analysis of the elution fractions from the purifications indicated that only the E. faecium PoxtA(EF9F6)-EQ2 factor was bound stably to the ribosome (Supplementary Fig. 6). Furthermore, our attempts with OprA carrying a single individual EQ substitution (E470Q) were equally unsuccessful. Therefore, we focused on the PotxA(EF9F6)-EQ2 sample and subjected it to structural analysis using single-particle cryo-EM.

Using a Titan Krios transmission electron microscope with a K2 direct electron detector, we collected 3640 micrographs which, after 2D classification, yielded 140,310 ribosomal particles (Supplementary Fig. 7). In silico sorting revealed that 80% of these particles contained an additional density for PoxtA and/or tRNAs, which after 3D refinement resulted in a cryo-EM map of E. faecalis 70S ribosome with an average resolution of 2.4 Å (Supplementary Fig. 8a). Subsequent multibody refinement yielded average resolutions of 2.2 Å and 2.5 Å for the LSU and SSU, respectively (Supplementary Table 2). The cryo-EM density for PoxtA in states I–III was generally well-resolved (Supplementary Fig. 10c), enabling a reliable model to be built for NBD1, NBD2 and the ARD (Fig. 3d). By contrast, the Arm domain, which interacts with uL1, appeared flexible (Supplementary Fig. 10c) and could only be modelled as a rigid body fit of the two α-helices (Fig. 3d). The best-resolved region of PoxtA was the ARD, consisting of two α-helices (α1 and α2) and the ARD loop (Fig. 3d and Supplementary Fig. 10c), where the majority of sidechains could

Further subsorting of ribosomal particles using a mask focused on the intersubunit space yielded four defined classes, which we refer to as states I–IV (Supplementary Fig. 7). States I and II contained density for PoxtA bound in the E-site and tRNA in the P site, and had average resolutions of 2.9 and 3.0 Å, respectively (Fig. 3a, Supplementary Fig. 10a, b and Supplementary Table 2). State II (not shown in Fig. 3) differed from the state I by only a slight rotation of the SSU relative to the LSU, and in particular the conformation of PoxtA was nearly identical to that of state I (Supplementary Fig. 11). State III was similar to state II, but additionally contained an A-site tRNA and PoxtA differed only slightly in NBD2, close to the SSU (Fig. 3b and Supplementary Fig. 11), whereas state IV contained P-site tRNA only (Fig. 3c), presumably because PoxtA dissociated during sample preparation. States III and IV were also refined, resulting in final reconstructions with average resolutions of 2.9 Å and 3.1 Å, respectively (Supplementary Fig. 10a, b and Supplementary Table 2). The cryo-EM density for PoxtA in states I–III was well-resolved (Supplementary Fig. 10c), enabling a flexible (Supplementary Fig. 10c) and could only be modelled as a rigid body fit of the two α-helices (Fig. 3d). The best-resolved region of PoxtA was the ARD, consisting of two α-helices (α1 and α2) and the ARD loop (Fig. 3d and Supplementary Fig. 10c), where the majority of sidechains could
be modelled unambiguously (Fig. 3e). Additional density located between NBD1 and NBD2 of PoxtA was attributed to two ATP molecules (ATP-1 and ATP-2) and a likely magnesium ion (Fig. 3f), as expected from the use of the ATPase-deficient PoxtA-EQ2 variant. As observed for other ribosome-bound ARE–ABCF structures,13–15, the NBDs of PoxtA adopt a closed conformation, which is consistent with the inability to hydrolyse ATP. In all states, the anticodon stem loop (ASL) and acceptor arm, including the CCA-end, are well-resolved, whereas the elbow region of the tRNAs exhibits some flexibility (Supplementary Fig. 10d).

In all states, the density for the P-site tRNA is consistent with initiator tRNAfMet, indicating that in the absence of chloramphenicol- or linezolid-stalled ribosomes, the PoxtA-EQ2 variants bind to the vacant E-site of initiation complexes, as observed previously for other ARE–ABCF–EQ2-ribosome complexes prepared by an in vivo pull-down approach. Specific recruitment of PoxtA-EQ2 to initiation complexes is likely due to the availability of the E-site rather than due to the specific affinity to initiating ribosomes as such. In state IV, clear density for the fMet moiety attached to the P-site tRNA is evident, whereas state III appears to be a post-peptide bond formation state with a deacylated P-site tRNA and A-site tRNA bearing a dipeptide. In states I and II, which contain PoxtA, but lack A-site tRNA, some density for the fMet moiety on the distorted P-site tRNA is evident but is poorly resolved.

Interaction of PoxtA with the ribosome and P-site tRNA. In states I–III, PoxtA is located within the ribosomal E-site (Fig. 4a) and generally binds similarly to that observed for other ARE–ABCF proteins, such as VmlR, MsrE, LsaA, VgaL and VgaA–Lc13–15, as well as the non-ARE–ABCF protein EttA11. Unlike these ARE–ABCFs that lack an Arm subdomain (or have a short Arm in the case of LsaA6), the Arm subdomain of PoxtA (and Optra6) is prominent, like that of EttA11,12, and stabilises an open conformation of the L1 stalk via direct interaction with domain II of uL1 (Fig. 4a). Additional contacts to the 23S rRNA helices H77/H78 of the L1 stalk are evident from the NBD1 of PoxtA, as are interactions for NBD1 with H68 and bL33 on the LSU (Fig. 4a). By contrast, NBD2 of PoxtA spans across the intersubunit interface, establishing interactions with uL5 on the LSU as well as uS7 and h41 on the SSU (Fig. 4a). NBD2 also interacts directly with the elbow region of the P-site tRNA, namely, with the G19-C56 basepair that links the D- and T-loops (Fig. 4a, b). Here, Ser430 of PoxtA is within hydrogen bonding distance of the N7 of G19 and the sidechain of Arg426 of PoxtA stacks upon the nucleobase of C56 of the T-loop (Fig. 4b).

However, it is the ARD that makes the most extensive interactions with the P-site tRNA, establishing a complex network of hydrogen bonding interactions with the acceptor arm and CCA-end (Fig. 4a). In particular, two glutamine residues, Gln275 and Gln279, located at the distal end of the α-helix α1 of the ARD insert into the minor groove of the acceptor’s arm where hydrogen bond interactions can form with the C3-G70 basepair (Fig. 4c). Hydrogen bonding is also possible from the ε-amino group of Lys278 and the backbone carbonyl of Gln275 of PoxtA with the phosphate-oxygen of G4 and the ribose-oxygen of C71 of the P-tRNA, respectively. The loop region of the ARD of PoxtA interacts predominantly with the single-stranded CCA-3' end of the P-site tRNA (Fig. 4d). Ser281 is within H-bonding distance to the phosphate-oxygen of A73, whereas the sidechains of Gln282 stacks upon the base of C74 and can interact with the ribose-hydroxyl of A72 (Fig. 4d). C75 of the P-site tRNA is also stabilised by indirect contacts with the backbone carbonyl of Thr283 via a water molecule, as well as a direct H-bond with the sidechain of His285, the first residue of α-helix α2 of the ARD of PoxtA.

Fig. 4 Interactions between PoxtA and the ribosome-P-tRNA complex. a Overview of PoxtA interactions with the 23S rRNA (grey), 16S rRNA h41 (yellow), uL1 (gold), uL5 (pink), bL33 (tan) and the P-tRNA (light blue). The P-tRNA CCA 3’ end, acceptor stem (Acc.), and elbow are indicated. b–e Interactions between the P-tRNA elbow (light blue) and the PoxtA NBD2 (b), the P-tRNA acceptor stem (light blue) and the PoxtA ARD (c), the ARD and the P-tRNA CCA-end, including a modelled water molecule (labelled W) (d, e). f An interaction between the PoxtA ARD α2 and the 23S rRNA. The high-resolution model from the combined 70S volume was used.
PoxtA (Fig. 4e). The ARD is stabilised by multiple contacts between residues within α-helix α2 and rRNA nucleotides located in H74 and H93. For example, the sidechains of Arg294 and Arg297 contact nucleotides A2595–G2598 (E. coli numbering used throughout) located within the loop of H93 of the 23S rRNA, and Glu293 interacts with G2597 directly as well as G2598 via a putative water molecule (Fig. 4f).

PoxtA perturbs the position of the CCA-end of the P-site tRNA at the PTC. Despite the short ARD, binding of PoxtA to the E-site nevertheless causes a distortion of the P-site tRNA when compared to the canonical P-site tRNA binding position, such as that observed in state IV (Fig. 5a, b). While the ASL remains fixed in position on the SSU where it decodes the P-site codon of the mRNA, the elbow region shifts towards the E-site by ~6–7 Å, thus bringing it into contact with NBD2 of PoxtA (Fig. 5b). The shift of the elbow region is very similar to that observed for the distorted P-site tRNAs observed on the ribosome in the presence of the other ARE–ABCFS such as LsaA (Fig. 5c). However, in the PoxtA–70S complex, the distortion is accompanied by a smaller ~4 Å shift of the acceptor arm of the P-site tRNA away from the PTC (Fig. 5d), whereas for other ARE–ABCFS complexes, the CCA-end of the P-site tRNA completely vacates the PTC due to the presence of the longer ARD (as illustrated here for LsaA in Fig. 5c). Although PoxtA contains a shorter ARD than other ARE–ABCFS, the loop of the ARD still contacts the acceptor stem of the P-tRNA, precluding canonical interactions between the tRNA and the ribosome (Fig. 5d). As a consequence, the single-stranded CCA-3′ end of the P-site tRNA becomes contorted in the presence of PoxtA and the canonical interactions of the C75 and C74 of a P-site tRNA with 23S nucleotides G2251 and G2252, respectively, of the P-loop (H180) are disrupted (Fig. 5e, f). This results in a shift in register such that C75 basepairs with G2252 and C74 stacks upon Gln282 of PoxtA and interacts with G2253 and C2254 (Fig. 5f). The register shift is reminiscent of, but distinct to, that observed for the Pint-tRNA in E. coli 70S ribosome complexes formed in the presence of the antimicrobial peptide apidaecin and the termination release factor RF3.

A comparison of the binding position of the ARD of PoxtA with that of the phenicol (chloramphenicol) and oxazolidinone (linezolid) antibiotics reveals that there is no steric overlap between PoxtA and either drug-binding site (Fig. 5b). Indeed, His285 of PoxtA, which comes closest to the drugs, is still located >16 Å away, and furthermore the ARD of PoxtA is partitioned from the drugs by the CCA-3′ end of the P-site tRNA (Fig. 5b).

![Fig. 5 PoxtA modulates the conformation of the P-tRNA.](https://example.com/f5.png)

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inducing conformational changes within the 23S rRNA nucleotides comprising the drug-binding site(s), as proposed for other ARE–ABCFs\textsuperscript{13–15}. To examine this, we compared the conformation of the PTC nucleotides comprising the drug-binding sites in our structures with those in the presence of chloramphenicol and linezolid. We could find no evidence that the presence of PoxtA induces any conformational changes within the drug-binding sites that would lead to dissociation of the drugs (Supplementary Figs. 12 and 13). In fact, we note that the conformation of the A-site pocket where the drugs bind is very similar, if not identical, for states I–IV, regardless of whether PoxtA is present (state I–III) or absent (state IV), or whether the A-site is vacant (state I–II, IV) or occupied (state III) (Supplementary Figs. 12 and 13). Although this suggests that PoxtA does not dislodge the drugs from the ribosome by altering the 23S rRNA in the binding site, we cannot exclude that such conformational changes occur upon ATP hydrolysis or upon dissociation of PoxtA from the ribosome.

An alternative scenario is that the distortion of the P-site tRNA induced by PoxtA binding indirectly reduces the affinity of the drugs for their A-site binding pocket. Both chloramphenicol and linezolid are context-specific inhibitors of translation elongation, such that the highest translation arrest activity occurs when the penultimate amino acid (−1 position) attached to the CCA-end of the P-site tRNA is alanine, and to a lesser extent, serine or threonine\textsuperscript{27–29}. Structures of chloramphenicol on the ribosome with peptidyl-tRNA mimics reveal an intimate interaction between the drug and the nascent polypeptide chain, illustrating how alanine in the −1 position stabilises drug binding via a CH–π interaction (Fig. 5g)\textsuperscript{39}. Since the distortion of the P-site tRNA by PoxtA involves a shift out of the PTC by 4 Å, effectively altering the nascent chain register by one amino acid, this would also result in a shift of the alanine away from chloramphenicol (Fig. 5h) and thereby perturb the interactions and reduce the affinity of the drug for the ribosome.

**Discussion**

Based on the structures of the PoxtA–ribosome complexes determined here, as well as the available literature on the mechanism of action of oxazolidinones and phenicols, we propose a model for how PoxtA can confer antibiotic resistance to these antibiotic classes (Fig. 6). As mentioned, linezolid and chloramphenicol are context-specific inhibitors that stall the ribosome during translation elongation with the peptidyl-tRNA in the P site and the drug bound within the A-site pocket of the PTC (Fig. 6a)\textsuperscript{27–29}. The amino acid in the −1 position of the nascent polypeptide chain influences the strength of the arrest. Specifically, alanine elicited the strongest arrest\textsuperscript{27–29}, apparently due to direct interaction with the ribosome-bound drug, as was recently shown for both chloramphenicol (Fig. 6a)\textsuperscript{39} and the oxazolidinones linezolid and radezolid\textsuperscript{40}.

We propose that PoxtA recognises these drug-arrested ribosomes and binds to the vacant E-site (Fig. 6b). In contrast to other ARE–ABCFs where the CCA-end is completely removed from the PTC\textsuperscript{13–15}, the binding of PoxtA causes the CCA-end to shift by only ~4 Å. Although modest, such a shift would be sufficient to change the register of the nascent polypeptide chain with respect to the drug, such that the drug can no longer interact with the amino acid in the −1 position, thereby decreasing its affinity for the ribosome and leading to its dissociation (Fig. 6b). It is possible that the shift of the nascent chain upon distortion of the P-site tRNA by PoxtA also contributes to “brushing” the drug from the ribosome\textsuperscript{41}, analogous to the mechanism proposed for leader peptides that confer resistance to macrolides\textsuperscript{41}. We also cannot exclude the possibility that conformational changes in PoxtA upon ATP hydrolysis play a role in drug release, in addition to facilitating dissociation of PoxtA from the ribosome (Fig. 6c).

What prevents PhO antibiotics from rebinding following their dissociation by PoxtA? As suggested previously for other ARE–ABCFs\textsuperscript{13,15}, we favour a model where, following PoxtA dissociation, the peptidyl-tRNA can reaccommodate in the P site, thus allowing accommodation of a tRNA in the A site, which, in turn, would occlude the PhO-binding site (Fig. 6c). In this respect, we note that in contrast to previous ARE–ABCF structures\textsuperscript{13,15}, we observe here a subpopulation of PoxtA–70S complexes where the A-site tRNA is fully accommodated at the PTC despite the presence of PoxtA and the distorted P-site tRNA. Thus, once the drug has been released, the A-site tRNA could accommodate at the PTC and following peptide bond formation and translocation, the context of the nascent chain would be shifted by one amino acid i.e. alanine in the −1 position would now be located at the −2 position and therefore disfavour drug rebinding (Fig. 6d). PoxtA and OptrA are often found on the same mobile genetic element as drug efflux pumps\textsuperscript{42}, which may also contribute to preventing drug rebinding by transporting the dissociated drug directly out of the cell.

Our insights into the mechanism of action of PoxtA also provide a possible explanation for why these ARE–ABCFs do not confer resistance to MSβ or PLSα antibiotics. Firstly, the ARD of PoxtA is too short to sterically overlap with these drugs and directly displace them from the ribosome, and secondly, binding of PoxtA does not perturb the tRNA portion of the drug-binding sites and therefore could not induce their dissociation by conformational relays in the 23S rRNA. For PLSα antibiotics, which stall translation after initiation, there is no nascent chain that forms part of the drug-binding site. In the case of MSβ antibiotics, which stall translation elongation but bind deeper in the nascent peptide exit channel, perhaps the “pulling” effect of PoxtA on the nascent chain is mitigated by conformation elasticity of the several amino acids between the P-tRNA and the drug-binding site. Interestingly, ARE–ABCF antibiotics, such as MsrE or LsaA that confer resistance to MSβ and PLSα antibiotics, respectively, do not confer resistance to PhO antibiotics, despite direct overlap between the ARDs and the drug-binding sites. We speculate that in these cases, the peptidyl-tRNA of the PhO-stalled ribosomes are refractory to the action of these ARE–ABCFs to distort the P-site tRNA; however, further investigations will be needed to validate this.

Given the similarity in ARD length and antibiotic spectrum, we believe that the findings and model presented here for PoxtA are likely to also be applicable for OptrA. Indeed, like OptrA, our MIC analysis provides no evidence for PoxtA conferring resistance to tetracycline antibiotics, consistent with the binding site of PoxtA in the E-site being far from the tetracycline-binding site located in the decoding A-site of the small subunit. We also see no evidence for conformational differences in the 16S rRNA nucleotides that comprise the tetracycline-binding site in the absence or presence of PoxtA. For this reason, we suggest reassigning the letters from the PoxtA acronym from phenicol-oxazolidinone tetracycline A to phenicol-oxazolidinone transmissible A, analogous to OptrA.

**Methods**

**Identification of poxtA EF9F6 and characterisation of E. faecium 9-F-6 antibiotic susceptibility.** The *poxtA* EF9F6 gene was identified in the multidrug-resistant *ST872 E. faecium* 9-F-6 isolated in 2012 from faeces of a patient in a Norwegian hospital who had recently also been hospitalised in India\textsuperscript{31}. *ST872* is a single locus variant of ST80 which is a pandemic hospital-adapted genetic lineage. Transferable linezolid resistance (optrA and cfr(D)) has recently been described in blood culture *E. faecium* ST872 strain from Australia\textsuperscript{42}. Species identification was performed by MALDI-TOF (Bruker, Billerica, USA) according to the manufacturer’s instructions and later confirmed by whole-genome sequencing\textsuperscript{31}. 

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Antimicrobial susceptibility testing was performed by broth microdilution using the EUCAST Sensititre plate (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and further by gradient tests for vancomycin, teicoplanin, ampicillin, clindamycin, chloramphenicol and gentamicin (MIC Test strip, Liofilchem, Roseto Degli Abruzzi, Italy). The results (MICS) were interpreted according to EUCAST clinical breakpoints v. 10.2020 and MICs were within the accepted range for quality control strain E. faecalis ATCC 29212. The E. faecium 9-F-6 is resistant to linezolid (MIC = 8 mg/L), ampicillin (>256 mg/L), ciprofloxacin (>16 mg/L), high-level gentamicin (HLGR > 256 mg/L), high-level streptomycin (HLSR > 1024 mg/L) and high-level glycopeptides (vancomycin >256 mg/L and teicoplanin 128 mg/L), but susceptible to quinupristin/dalfopristin (MIC 4 mg/L) and tigecycline (MIC 9 mg/L) respectively, as well as a defunct, functionally inactive lsaA pseudogene.

Strains and plasmids. All bacterial strains and plasmids used in the study are listed in Supplementary Table 1. E. faecalis TX5332 (Rifr Fus+ Kan−; lsaA gene disruption mutant) was obtained from genomic DNA of E. faecalis AOUC-0915 (kindly provided by S. aureus AOUC-0915 (kindly provided by Alberto Antonelli) and inserted in pCEcam plasmid, where the chloramphenicol resistance gene was substituted with spectinomycin, due to intrinsic resistance of PoxtA to phenicol antibiotics. All cloning was performed by the PEP facility (Umeå University). Plasmids encoding OptrA-ST16 (ref. 21) and OptrA-AR were prepared as described previously40. Brie

Bacterial transformation. E. faecalis electrocompetent cells were prepared as described previously40. Briefly, an overnight culture E. faecalis TX5332 grown in the presence of 2 mg/mL of kanamycin was diluted to OD600 of 0.05 in 50 mL of Brain Heart Infusion (BHI) media and grown further to an OD600 of 0.6–0.7 at 37 °C with moderate shaking (160 rpm/10 x g). Cells were collected by centrifugation at 4000 rpm (1200 x g) at 4 °C for 10 min. Cells were resuspended in 0.5 mL of sterile lysozyme buffer (10 mM Tris-HCl pH 8; 50 mM NaCl, 10 mM EDTA, 35 µg/mL lysozyme), transferred to 0.5 mL Eppendorf tube and incubated at 37 °C for 30 min. Cells were pelleted at 10,000 rpm (8000 x g) at 4 °C for 10 min and washed three times with 1.5 mL of ice-cold electroporation buffer (0.5 M sucrose, 10% (w/v) glycerol). After the last wash, the cells were resuspended in 500 µL of ice-cold electroporation buffer and aliquoted and stored at −80 °C. For electroporation, 35 µL of electrocompetent cells were supplemented with 0.5–1 µg of plasmid DNA, transferred to ice-cold 1 mm electroporation cuvette and electroporated at 1.8 kV. Immediately after electroporation 1 mL of ice-cold BHI media was added to the cells, the content of the cuvette was transferred to 1.5-mL Eppendorf tubes and the cells were recovered at 37 °C for 2.5 h and plated to BHI plates containing appropriate antibiotics.

Antibiotic susceptibility testing. E. faecalis cells were grown in BHI media supplemented with 2 mg/mL kanamycin (to prevent lsaA revertants), 0.1 mg/mL spectinomycin (to maintain the pCEcam plasmid), 100 ng/mL of cefclor peptide (to induce expression of proteins of interest) as well as increasing concentrations of antibiotics. The media was inoculated with 5 x 10^6 CFU/mL (OD600 of approximately 0.0005) of E. faecalis ΔlsaA (lsa::kan) strain TX5332 transformed either

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with empty pCIEspec plasmid or with pCIEspec-encoded protein of interest. After 16–20 h at 37 °C without shaking, the presence or absence of bacterial growth was scored by eye.

Preparation of bacterial biomass. For cryo-EM: E. faecalis TX5332 transformed with pCIEspec/pCIEspec-based expression constructs (either empty vector or expressing wild-type/EQ variants of both PoxtA-HTF and Optra-HTF as well as EQ variant of Optra-HTF) were grown overnight in a single colony in BHI media supplemented with appropriate antibiotics (100 μg/mL of spectinomycin for pCIEspec-based constructs, 10 μg/mL chloramphenicol for pCIEspec-based constructs). Overnight cultures were then diluted to a starting OD_{600} of 0.05 in 200 mL of the same media. Cells were grown with intensive shaking at 37 °C till OD_{600} of 0.6 and were induced with 100 ng/mL of CCF10 peptide for 30 min prior harvesting by centrifugation at 10,000 × g for 15 min at 4 °C.

For 5Pseq: E. faecalis TX5332 AkA45-1 was transformed with either the empty vector pCIEspec (VHP426) or a vector expressing poxtA-EPF6e (VHP506). 50 mL cultures were inoculated from an overnight culture to 0.005 OD_{600}, in filtered BHI media containing 100 ng/mL CCF10 peptide (to induce poxtA expression) and the cells were grown at 37 °C with shaking at 160 rpm (10 × g). Once cells reached 0.3 OD_{600}, the cells were treated with endonuclease at 4 °C (final concentration) or corresponding volume of ethanol (for the untreated control) for 10 min, harvested via centrifugation (4000 rpm/20 min) and flash-frozen in liquid nitrogen for storage at −80 °C and subsequent processing.

RNA extraction. RNA was extracted via phenol-chloroform extraction44. Briefly, 300 μL LET-phenol (LET: 25 mM Tris–HCl pH 8.0, 100 mM LiCl, 20 mM EDTA) was added to cell pellets, vortexed and rotated 10 times with 0.1-mm glass beads to lyse cells. In total, 250 μL of water and 250 μL of acidic phenol/chloroform was added before vortexing for a further 2 min. RNA was then extracted from the aqueous phase with an acidic phenol/chloroform step followed by a final chloroform extraction step. RNA was then ethanol precipitated in the presence of NaOAc (pH 5.2). RNA was resuspended by running a 2% agarose gel and quantified by fluorescence (Qubit, Thermo Fisher Scientific).

5Pseq library preparation and data analysis. HT-5Pseq libraries were prepared as described earlier45. Briefly, 4 μg total RNA from E. faecalis was DNase treated (TURBO Dnase kit, Thermo Fisher Scientific), ligated overnight at 16 °C to rRNA-depleted E. faecalis 51S RNA oligo (CrArCrGrArCrGrCrUrCrUrUrCrCrGrAr-rRNA DNA oligo depletion mixes33, following duplex-specific degraded using NaOH. Ribosomal RNA was removed using previously described random hexamer (5Pseq library preparation and data analysis). Adding 1.8 volumes of ethanol (for as the untreated control) for 10 min, and harvested via centrifugation (4000 rpm/20 min), RNA was then resuspended by running a 2% agarose gel and quantified by fluorescence (Qubit, Thermo Fisher Scientific).

Affinity purification on anti-FLAG M2 affinity gel. In all, 100 μL of well-mixed anti-FLAG M2 Affinity Gel aliquots (Sigma) were loaded on columns (Micro Bio-Spin 6; Bio-Rad) and washed with 1 mL of washing buffer with 0.1% TBS with 0.05% Tween 20 by gravity flow. All incubations, washings and elutions were done at 4 °C. The total protein concentration of each individual lysate was separately adjusted to 2 mg/mL with cell opening buffer pH-adjusted with either HEPS pH 7.5 (Optra samples) or glycine pH 9.0 (Porata samples). The pH of the opening buffer was selected through a series of small-scale pull-down experiments to optimize sampling of the pull-down efficiency. 1 mL of each lysate was loaded on columns and incubated for 2 h with end-over-end mixing for binding. The columns were washed five times with 1 mL of cell opening buffer (pH 7.5 in the case of Optra samples, pH 9.0 in the case of PoxtA samples) by gravity flow. For elution of FLAG-tagged proteins and their complexes 100 μL of 0.2 mg/mL FLAG peptide (Sigma) was added to samples, the solutions were incubated at 4 °C for 20 min with end-over-end mixing. Elutions were collected by centrifugation at 2000 × g for 2 min for 4 °C. 20 μL aliquots of collected samples (flow-through, washes and elutions) were mixed with 5 μL of 5× SDS loading buffer and heated up at 95 °C for 15 min. The bands in the column were resuspended in 100 μL of 1× SDS loading buffer. Denatured samples were loaded on 15% SDS-PAGE. SDS-gels were stained by “Blue-Silver” Coomasie Staining46 and destained in water for at least one hour (or overnight) before imaging with LAS6000 (GE Healthcare).

Preparation of cryo-EM grids. Elutions from pull-downs were kept on ice until being applied within 2 h to glow discharged cryo-grids (Quantifoil 2/2 Cu500 coated with 2 nm continuous carbon). In total, 3.5 μL of sample was loaded on grids three times at room temperature and conditions allowed to Lysozyme (1:1,000) to lyse cells. In the case of PoxtA samples) by gravity flow. Denatured samples were loaded on 15% SDS-PAGE. SDS-gels were stained by “Blue-Silver” Coomasie Staining46 and destained in water for at least one hour (or overnight) before imaging with LAS6000 (GE Healthcare).

Single-particle reconstruction. Processing was performed in RELION 3.1 unless otherwise specified48. MotionCor2 with 5 × 5 patches and CTFEFIND4 (uowing power spectra) were used for motion correction and initial CTF estimation19,52. Micrographs with estimated CTF fits beyond 4.5 Å and CTF figure of merits > 0.04 were selected for further processing. Particles were picked with cryoLOU using the general model15. After 2D classification, all ribosome-like classes were selected, placed in a script-picked particle and were re-examined using a 3 Å CTF under conditions of 100% humidity at 4 °C, blotted for 5 s and vitrified by plunge-freezing in liquid ethanol. Samples were imaged on a Titan Krios (FEI) operated at 300 kV at a nominal magnification of ×165,000 (0.86 Å/pixel) with a Gatan K2 Summit camera at an exposure rate of 5.85 electrons/pixel/s with a 4 s exposure and 20 frames using the EPU software.

Molecular modelling. Molecular models were created/adjusted with Coot49 and ISOLDE49, and refined with Phenix51 against unsharpened maps. A previous structure of the E. faecalis 70S ribosome in complex with the ABC-ARE LsaA (PDB 7NKH)15 was used as the starting model for the ribosome, initially into multibody-refined maps from the combined 70S volume. PDB IDs 7K00 (ref.62) and 6Q90 (ref.17) were also used as templates in parts of the 70S, and PDB ID 3ZCP (ref.70) was used as a template for the six resulting classes, labelled states I–IV, were chosen for re-refinement with the original pixel size. For the multibody refinement of the combined 70S class56, volumes corresponding to the LSU core, CP, SSU body, SSU head and E-site were isolated using the volume eraser tool in UCSF Chimera52, and masks created from the densities low-pass-filtered to 30 Å. Boisot was used to estimate local resolution53.
Figure 4. Structures of ABC-F proteins. A) Structural models of the ABC-F proteins Ero, R. F. & Gao, Y. G. Ribosome protection proteins – a key antibiotic resistance mechanism. Nat. Rev. Microbiol., 20, 2182–2190 (2015).

Figure 5. Sequence analysis of ABC-F proteins. B) Structural model of the ABC-F protein Ero, R. F. & Gao, Y. G. Ribosome protection proteins – a key antibiotic resistance mechanism. Nat. Rev. Microbiol., 20, 2182–2190 (2015).

Figure 6. Data availability. C) Cryo-electron microscopy structure of the 70S ribosome from enterococci. Proc. Natl Acad. Sci. USA, 113, 12150–12155 (2016).

Figure 7. Localization of the oxazolidinone resistance gene optrA. D) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 8. Molecular models of the ABC-F proteins Ero, R. F. & Gao, Y. G. Ribosome protection proteins – a key antibiotic resistance mechanism. Nat. Rev. Microbiol., 20, 2182–2190 (2015).

Figure 9. Localization of the oxazolidinone resistance gene optrA. E) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 10. Localization of the oxazolidinone resistance gene optrA. F) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 11. Localization of the oxazolidinone resistance gene optrA. G) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 12. Localization of the oxazolidinone resistance gene optrA. H) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 13. Localization of the oxazolidinone resistance gene optrA. I) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

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Figure 15. Localization of the oxazolidinone resistance gene optrA. K) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 16. Localization of the oxazolidinone resistance gene optrA. L) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 17. Localization of the oxazolidinone resistance gene optrA. M) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 18. Localization of the oxazolidinone resistance gene optrA. N) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 19. Localization of the oxazolidinone resistance gene optrA. O) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 20. Localization of the oxazolidinone resistance gene optrA. P) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 21. Localization of the oxazolidinone resistance gene optrA. Q) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 22. Localization of the oxazolidinone resistance gene optrA. R) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 23. Localization of the oxazolidinone resistance gene optrA. S) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 24. Localization of the oxazolidinone resistance gene optrA. T) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 25. Localization of the oxazolidinone resistance gene optrA. U) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 26. Localization of the oxazolidinone resistance gene optrA. V) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 27. Localization of the oxazolidinone resistance gene optrA. W) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 28. Localization of the oxazolidinone resistance gene optrA. X) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 29. Localization of the oxazolidinone resistance gene optrA. Y) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).

Figure 30. Localization of the oxazolidinone resistance gene optrA. Z) Localization of the oxazolidinone resistance gene optrA. Nat. Struct. Mol. Biol, 29, 1463–1465 (2012).
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**Author contributions**

C.C.M. processed the microscopy data, generated and refined the molecular models and made the structure figures. V.M., H.T. and M.K. designed the ARE constructs, performed genetic manipulations of *E. faecalis*, performed polysomal fractionations and immuno-blotting as well as performed MICs. V.M., K.J.T. and M.K. performed immunoprecipitations. V.M. prepared cryo-EM grids and collected cryo-EM datasets. K.J.T. and H.T. prepared the RNA samples for HT-5Pseq. S.H., L.N. and V.P. performed and analyzed HT-5Pseq data. A.S. and K.H. provided research materials and performed MICs. G.C.A. performed sequence conservation analyses. C.C.M. and D.N.W. declared no competing interests.

**Additional information**

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-29274-9.

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