Synthetic oxepanoprolinamide iboxamycin is active against *Listeria monocytogenes* despite the intrinsic resistance mediated by VgaL/Lmo0919 ABCF ATPase

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**Background:** Listeriosis is a food-borne disease caused by the Gram-positive Bacillota (Firmicute) bacterium *Listeria monocytogenes*. Clinical *L. monocytogenes* isolates are often resistant to clinically used lincosamide clindamycin, thus excluding clindamycin as a viable treatment option.

**Objectives:** We have established newly developed lincosamide iboxamycin as a potential novel antilisterial agent.

**Methods:** We determined MICs of the lincosamides lincomycin, clindamycin and iboxamycin for *L. monocytogenes*, *Enterococcus faecalis* and *Bacillus subtilis* strains expressing synergetic antibiotic resistance determinants: ABCF ATPases that directly displace antibiotics from the ribosome and Cfr, a 23S rRNA methyltransferase that compromises antibiotic binding. For *L. monocytogenes* strains, either expressing VgaL/Lmo0919 or lacking the resistance factor, we performed time-kill kinetics and post-antibiotic effect assays.

**Results:** We show that the synthetic lincosamide iboxamycin is highly active against *L. monocytogenes* and can overcome the intrinsic lincosamide resistance mediated by VgaL/Lmo0919 ABCF ATPase. While iboxamycin is not bactericidal against *L. monocytogenes*, it displays a pronounced post-antibiotic effect, which is a valuable pharmacokinetic feature. We demonstrate that VmlR ABCF of *B. subtilis* grants significant (33-fold increase in MIC) protection from iboxamycin, while LsaA ABCF of *E. faecalis* grants an 8-fold protective effect. Furthermore, the VmlR-mediated iboxamycin resistance is cooperative with that mediated by the Cfr, resulting in up to a 512-fold increase in MIC.

**Conclusions:** While iboxamycin is a promising new antilisterial agent, our findings suggest that emergence and spread of ABCF ARE variants capable of defeating next-generation lincosamides in the clinic is possible and should be closely monitored.

**Introduction**

Lincosamides constitute an important class of antibiotics used both in veterinary and human medicine. The compounds inhibit protein synthesis by binding to and compromising the enzymatic activity of the peptidyl transferase centre (PTC) of the ribosome, resulting in bacteriostasis. Representatives of this antibiotic class share a common architecture and are typically comprised of a 4′-substituted L-proline residue connected via an amide bond to a unique S-glycosidic amino sugar moiety (Figure 1a and b). The first lincosamide to be discovered, lincomycin (Figure 1a), is a natural product produced by *Streptomyces lincolnensis* subsp. *lincolnensis* and is active against streptococcal, pneumococcal and staphylococcal infections. Its semi-synthetic derivative, clindamycin (Figure 1b), can be produced via a one-step stereoinverting deoxychlorination of lincomycin. Clindamycin is more potent than lincomycin and is currently the lincosamide of choice for human medicine. Like lincomycin,
clindamycin is mostly active against Gram-positive but not Gram-negative bacteria, which restricts the spectrum of its applications.\(^{11,12}\) A cis-4-ethyl-L-pipeolic acid amide of clindamycin, pir- limycin, has a similar spectrum of antibacterial activity\(^{11,12}\) and is approved for veterinary applications in the United States and European Union. Finally, a recently developed semi-synthetic derivative of lincomycin (‘compound A’) was shown to be able to overcome clindamycin resistance in Staphylococcus aureus mediated by ribosomal RNA (rRNA) methylation by ErmA and ErmB antibiotic resistance determinants.\(^{13}\)

Iboxamycin (Figure 1c) is a newly developed lincosamide with an exceptionally broad spectrum of antibacterial activity.\(^{14}\) Featuring a fully synthetic, bicyclic oxepanolinamide aminoacyl fragment, iboxamycin improves upon previous lincosamides in its activity against both Gram-positive and Gram-negative pathogens.\(^{14}\) Iboxamycin was found to be more potent than clindamycin against Gram-positive pathogens and overcomes lincosamide resistance mediated by RNA modification by Erm and Cfr 23S rRNA methyltransferases, both of which are highly clinically important and widespread antibiotic resistance determinants.\(^{15-18}\) While the presence of Cfr renders clinical isolates of S. aureus and Staphylococcus epidermidis virtually nonsusceptible to clindamycin (MIC >128 mg/L), the resistance to iboxamycin conferred by Cfr (MIC of 2–8 mg/L compared with 0.06 mg/L for cfr−strains) is not sufficient to render the drug fully inactive in the context of infection treatment.\(^{14}\)

Importantly, iboxamycin is also highly active against Enterococcus faecalis (MIC 0.06 mg/L as compared with 16 mg/L for clindamycin)—a species that is intrinsically resistant to ‘classical’ lincosamides as it encodes the LsaA antibiotic resistance (ARE) factor in its chromosomal genome,\(^{19}\) a member of the ABCF ATPase protein family that includes multiple resistance factors.\(^{20-22}\)

LsaA provides resistance to pleuromutilin, lincosamide and streptogramin A (PLSA) antibiotics by displacing the drug from the ribosome,\(^{23}\) acting similarly to other ARE ABCFs.\(^{24-27}\) As evident from the 96- to 256-fold higher susceptibility to clindamycin and lincomycin in a ΔlsaA E. faecalis strain as compared with E. faecalis ectopically expressing LsaA,\(^{28}\) LsaA is a potent lincosamide resistance determinant. The high susceptibility of E. faecalis to iboxamycin suggests that this compound has the potential to overcome resistance mediated by other ARE ABCFs as well.

Listeriosis is a dangerous food-borne bacterial disease caused by the Gram-positive Bacilliota (formerly Firmicute) bacterium Listeria monocytogenes, which infects people through contaminated meat, fish and dairy products.\(^{28,29}\) While it is a relatively rare infection that mainly affects people with weakened immune systems, or who are pregnant,\(^{30}\) the majority of listeriosis cases require hospitalization and mortality rates can be as high as 20%–30% even with antibiotic treatment.\(^{31,32}\) Antibiotic treatment options for L. monocytogenes infections include cell wall synthesis disruptors ampicillin and vancomycin, folic acid synthesis inhibitors sulfa-methoxazole and trimethoprim, and protein synthesis inhibitors, such as gentamicin and azithromycin.\(^{33}\) L. monocytogenes strains reported in recent years are often resistant to clindamycin, with the resistant fraction ranging from 29% to 76%, depending on the collection,\(^{34-37}\) thus excluding clindamycin as a viable option for treatment of L. monocytogenes infections. Importantly, just as E. faecalis encodes the ABCF ATPase LsaA, L. monocytogenes encodes the ARE ABCF PLSA resistance factor Vgal/Lmo0919 in its core genome.\(^{38}\) As with LsaA, Vgal operates on the ribosome,\(^{23}\) and loss of Vgal results in increased susceptibility to lincosamides, with the Lmo0919 L. monocytogenes strain being 8- to 16-fold more susceptible to lincomycin as compared with the isogenic wild type (WT).\(^{39}\) Finally, a model Bacilliota, Bacillus subtilis, also encodes an ARE ABCF PLSa resistance factor—VmlR.\(^{27,39}\)

In this report, using lincomycin and clindamycin as reference compounds, we (i) characterized the efficacy of iboxamycin against L. monocytogenes; (ii) probed its ability to specifically counter resistance mediated by ARE ABCF Lmo0919 in L. monocytogenes, LsaA in E. faecalis and VmlR in B. subtilis; (iii) characterized its bactericidal/bacteriostatic mechanism of action; and, finally, (iv) assessed the strength of its post-antibiotic effect (PAE).

**Materials and methods**

**Synthesis of iboxamycin**

Iboxamycin was prepared according to the method reported by Mason and colleagues.\(^{40}\)

**Strains and media**

WT L. monocytogenes 10403S was provided by Daniel A. Portnoy, WT L. monocytogenes EGD-e was provided by Jörgen Johansson,
construction of L. monocytogenes EDG-e Δlmo0919 was described earlier.\textsuperscript{23}

E. faecalis ΔlsaA (lsa:Kon) strain TX332\textsuperscript{19} was provided by Barbara E. Murray, E. faecalis ΔlsaA pCIEspec and E. faecalis ΔlsaA pCIEspec LsaA were described earlier.\textsuperscript{23} WT 168 trpC B. subtilis (laboratory stock) was used. B. subtilis strains trpC ΔavrIR (VHB5) and ΔavrIR thrC::P\textsubscript{hy-spank}'vmlIR (VHB44) were described earlier.\textsuperscript{24} To construct B. subtilis thrC::P\textsubscript{hy-spank}'cfr (VHB138) and ΔavrIR thrC::P\textsubscript{hy-spank}'cfr (VHB139), a PCR product encoding Staphylococcus sciuri cfr gene optimized to Escherichia coli codon usage\textsuperscript{25} was PCR-amplified from the pBRcfr plasmid using primers VHT725 (5′-CGGATAACAATTAAGCTTAGTCGACTTAAGGAGGTGTGTCTCATGAACTTTAAC-AACAAAAACCAAATAC-3′) and VHT726 (5′-GTTTCCACGGAATGGCTGAGCTGCTACTGGGGAGTTCTGATAGTTACCATACA-3′). The second PCR fragment encoding a kanamycin-resistance marker, a polylinker downstream of the P\textsubscript{hy-spank} promoter and the lac repressor ORF—all inserted in the middle of the thrC gene—was PCR-amplified from pH1009 plasmid using primers pH1002_F (5′-GTCGACTAAGCTTAAATGTTATCCGCTCACAATTACACATTATGCC-3′) and pH1002_R (5′-GTCGACTAAGCTTAAATGTTATCCGCTCACAATTACACATTATGCC-3′) and pH1002_F (5′-GTCGACTAAGCTTAAATGTTATCCGCTCACAATTACACATTATGCC-3′) and pH1002_R (5′-GTCGACTAAGCTTAAATGTTATCCGCTCACAATTACACATTATGCC-3′). The two fragments were ligated using the NEBuilder HiFi DNA Assembly master mix (New England BioLabs, Ipswich, MA, USA) yielding the pH1009-cfr plasmid (VHBp439), which was used to transform either WT 168 trpC or ΔavrIR (VHB5) strain. Selection for kanamycin resistance yielded the desired VHB138 and VHB139 strain.

Liquid growth assays

L. monocytogenes was pre-grown on BHI agar plates at 37°C for 48 h. Individual fresh colonies were used to inoculate 2 mL of MH-F broth in 15 mL round bottom tubes, which were then incubated overnight at 37°C with shaking at 180 rpm. The overnight cultures were diluted then with MH-F broth to final OD\textsubscript{600} of 0.005 and incubated for 8 h in a water bath shaker (Eppendorf\textsuperscript{TM} Inova\textsuperscript{TM} 3100 High-Temperature) at 37°C with shaking at 160 rpm. bacterial growth was monitored by OD\textsubscript{600} measurements every 30 min.

Antibiotic susceptibility testing

The MIC antibiotic susceptibility testing was performed according to EUCAST guidelines (http://www.eucast.org/ast_of_bacteria/mic_determination), as described earlier.\textsuperscript{26}

L. monocytogenes strains were grown in MH-F broth inoculated with 5 × 10\textsuperscript{5} cfu/mL (OD\textsubscript{600} of approximately 0.0015) with increasing concentrations of antibiotics. After 24–48 h of incubation at 37°C without shaking, the presence or absence of bacterial growth was scored by eye.

E. faecalis strains were grown in BHI media supplemented with 2 mg/mL kanamycin (to prevent Lsa revertants), 0.1 mg/mL spectinomycin (to maintain the pCIEspec plasmid), 100 mg/mL of cef10 peptide (to induce expression of LsaA protein) as well as increasing concentrations of antibiotics, was inoculated with 5 × 10\textsuperscript{5} cfu/mL (OD\textsubscript{600} of approximately 0.0005) of E. faecalis ΔlsaA (lsa:Kon) strain TX332 transformed either with empty pCIEspec plasmid, or with pCIEspec encoding LsaA. After 16–20 h at 37°C without shaking, the presence or absence of bacterial growth was scored by eye.

B. subtilis strains were grown in LB medium supplemented with increasing concentrations of antibiotics. The cultures were inoculated with 5 × 10\textsuperscript{5} cfu/mL (OD\textsubscript{600} of approximately 0.0005), and after 16–20 h at 37°C without shaking the presence or absence of bacterial growth was scored by eye.

Time-kill kinetics assay

The protocol was based on CLSI\textsuperscript{62} and Svetlo\textsuperscript{2} Exponential L. monocytogenes cultures in MH-F broth (OD\textsubscript{600}=0.3) were diluted to 10\textsuperscript{6} cfu/mL (OD\textsubscript{600}=0.001) in 10 mL of MH-F broth either supplemented with appropriate antibiotic at 4-fold MIC concentration or without antibiotics (positive growth control), and the resultant cultures were incubated at 37°C without shaking. Aliquots (1 mL) were taken at incremental incubation times (0, 2, 4, 6, 8 and 10 h), spun down at 4000 rpm for 5 min at room temperature and cell pellets were gently washed twice with 900 μL of 1 × PBS. Cell pellets were resuspended in 100 μL of 1 × PBS, 10-fold serial dilutions were prepared in 96-well plates (10\textsuperscript{−1}–10\textsuperscript{−8}), and 10 μL, result-ant 10-fold serial dilutions were spotted on BHI agar plates. Colony forming units were scored after 24–48 h incubation at 37°C.

PAE assay

Exponential cultures of L. monocytogenes strains in MH-F blood broth media (OD\textsubscript{600}=0.3) were diluted to 10\textsuperscript{5} cfu/mL (≈OD\textsubscript{600} of 0.001) in 5 mL of MH-F media either supplemented with appropriate antibiotic at 4-fold MIC concentration or without antibiotics (positive growth control) and incubated at 37°C without shaking for 2 h. After the 2 h pretreatment, antibiotics were removed by 1:100 dilution of 100 μL into 10 mL of fresh prewarming MH-F blood broth media. At incremental timepoints (0, 2, 4, 6, 8 and 10 h), 1 mL of the 100 × diluted cell culture was harvested, centrifuged for 5 min at 4000 rpm, 900 μL of the medium was removed, and the pellets were resuspended in the remaining 100 μL. The volume was adjusted to 1 mL with 1 × PBS. Control cultures without antibiotics were handled similarly. Cell solutions were then serially diluted 10-fold to 10\textsuperscript{−8}, and 10 μL was spotted on BHI agar plates. Plates for individual timepoints were incubated at room temperature until the last set of plates were spotted (10 h timepoint), and then incubated at 37°C. The plates were scored after 24 and 48 h incubation at 37°C and imaged using ImageQuant LAS 4000 (GE Healthcare). The last timepoint (24 h) was processed separately analogously to 0–10 h timepoints (see above).

Results

L. monocytogenes is highly susceptible to iboxamycin despite VgaL/Lmo0919 ABCF resistance factor

To test the lincosamide susceptibility of L. monocytogenes we used two widely used WT strains, both belonging to serovar 1/2a: EGD-e\textsuperscript{46} and 10403S, a streptomycin-resistant variant of 10403.\textsuperscript{65} The two WTs are genotypically distinct, e.g. the virulence master-regulator PrfA is overexpressed in EGD-e and the pro- phage content differs between the two strains.\textsuperscript{66} In addition to the two WTs, we also tested an L. monocytogenes EDG-e derivative that was genomically modified to abrogate the expression of VgaL/Lmo0919 PLSR resistance factor (EDG-e Δlmo0919).\textsuperscript{43}

Both WT L. monocytogenes strains are much more susceptible to iboxamycin (MIC of 0.125–0.5 mg/L) as compared with clindamycin (MIC of 1 mg/L) and lincomycin (MIC of 2–8 mg/L) (Table 1). In agreement with the higher susceptibility of Δlmo0919 EDG-e to lincomycin,\textsuperscript{43} this strain is 2–8-fold more susceptible to iboxamycin.
Iboxamycin to the target were to be decreased by, for instance, rRNA cin and lincomycin; 32 susceptibility to all lincosamides tested for ectopic inducible expression of ARE ABCFs: granted by Lmo0919 and VmlR, respectively. Therefore, the difference in iboxamycin susceptibility between WT and Δlmo0919 EDG-e strains reflects both the ability of Lmo0919 to protect the ribosome from the antibiotic as well as the efficiency of iboxamycin-mediated induction of Lmo0919. To deconvolute these two effects, in the following experiments we used engineered E. faecalis and B. subtilis strains that allow for ectopic inducible expression of ARE ABCFs: E. faecalis LsaA and B. subtilis VmlR, respectively.

E. faecalis ABCF LsaA grants a moderate protective effect against iboxamycin

To test the ability of other ABCF PLSA resistance factors to confer resistance to iboxamycin, we compared a pair of E. faecalis strains: one lacking the chromosomally encoded LsaA (ΔlsaA pCIEspec) and the other allowing cCF10-peptide-inducible expression of LsaA (ΔlsaA pCIEspec LsaA).32 Using this experimental set up, we could specifically assess the ability of LsaA to protect the strain from lincosamides. While expression of LsaA dramatically increases resistance to clindamycin and lincomycin (96- to 256-fold, respectively), it results in a mere 8-fold protective effect against iboxamycin (MIC of 0.0625 and 0.5 mg/L, respectively) (Table 1), demonstrating that iboxamycin can also largely overcome LsaA-mediated resistance. Our current results are in agreement with our earlier MIC measurements for WT E. faecalis LsaA+ OG1RF strain (lincomycin 32 mg/L, clindamycin 16–32 mg/L)23 and WT E. faecalis ATCC 29212 LsaA+ strain (clindamycin 16 mg/L and iboxamycin 0.6 mg/L).14

B. subtilis ABCF VmlR acts cooperatively with rRNA methyltransferase Cfr to grant significant protection against iboxamycin

Next we tested a set of B. subtilis strains: WT 168 ΔvmlR (VHBS) as well as a ΔvmlR strain in which VmlR is expressed under the control of IPTG-inducible Phy-spank promoter (VHB44)47 (Table 1). Disruption of vmlR results in a 33-fold increase in iboxamycin susceptibility (MIC of 2 and 0.06 mg/L, respectively), and resistance is restored upon ectopic expression of VmlR (MIC of 4 mg/L, 2-fold increase over the WT levels). The iboxamycin susceptibility of Δlmo0919 L. monocytogenes EDG-e and ΔvmlR B. subtilis is near-identical, indicating that the 16-/4-fold difference in iboxamycin susceptibility between WT L. monocytogenes and B. subtilis is due to the different efficiency of resistance granted by Lmo0919 and VmlR, respectively. Importantly, VmlR loss results in the same relative increase in susceptibility to all lincosamides tested—iboxamycin, clindamycin and lincomycin; 32–33-fold—regardless of the potency of the lincosamide (Table 1). This suggests that if the affinity of iboxamycin to the target were to be decreased by, for instance, rRNA

In the case of L. monocytogenes strains, MIC testing was carried out in MH-F broth and growth inhibition was scored after 48 h incubation at 37°C. E. faecalis MIC testing was carried out in BHI broth supplemented with 2 mg/mL kanamycin (to prevent lsa revertants), 0.1 mg/mL spectinomycin (to maintain the pCIEspec plasmid) and 100 ng/mL of cCF10 peptide (to induce expression of LsaA protein). B. subtilis MIC testing was carried out in either LB medium or LB supplemented with 1 mM IPTG to induce expression of either VmlR or Cfr protein, and growth inhibition was scored after 16–20 h at 37°C. The MIC experiments were performed as described above. The experiment was performed at least three times (L. monocytogenes strains) or two (B. subtilis and E. faecalis strains) biological replicates.

Iboxamycin is bacteriostatic against L. monocytogenes and displays a strong PAE

Macrolide antibiotics that tightly bind the ribosome and dissociate slowly are bactericidal, while macrolides that dissociate rapidly are bacteriostatic.43 As with lincomycin and clindamycin, iboxamycin was shown to be bacteriostatic against a panel of bacterial species.14 However, since effects on L. monocytogenes

Table 1. Broth microdilution MIC testing of lincosamide antibiotics against L. monocytogenes, E. faecalis and B. subtilis strains

| Species/strain | Antibiotic MIC, mg/L |
|----------------|---------------------|
|                | lincomycin | clindamycin | iboxamycin |
| L. monocytogenes 10403S | 4–8 | 2 | 0.125–0.25 |
| L. monocytogenes EDG-e | 8 | 1–2 | 0.125–0.5 |
| L. monocytogenes EDG-e Δlmo0919 | 0.25–1 | 0.125–0.5 | 0.0625 |
| E. faecalis ΔlsaA pCIEspec | 0.125 | 0.125 | 0.0625 |
| E. faecalis ΔlsaA pCIEspec LsaA | 16–32 | 16 | 0.5 |
| B. subtilis WT 168 | 80 | 4 | 2 |
| B. subtilis ΔvmlR | 2.5 | 0.125 | 0.06 |
| B. subtilis ΔvmlR thrC::Ppy-spank-vmlR (IPTG: 1 mM) | 160 | 8 | 4 |
| B. subtilis ΔvmlR thrC::Ppy-spank-cfr (IPTG: 1 mM) | >640 | 640 | 16–32 |
| B. subtilis ΔvmlR thrC::Ppy-spank-cfr (IPTG: 1 mM) | >640 | 320 | 2 |

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were not assessed in the original report—and the species is highly susceptible to iboxamycin—we tested for potential bactericidal effects of iboxamycin against this pathogen. The three *L. monocytogenes* strains that we used for the MIC measurements—WT 10403 and EGD-e as well as ABCF-deficient EDG-e Δlmo0919—were treated with 4 × MIC concentration of either iboxamycin, clindamycin and lincomycin for increasing periods of time (from 2 to 24 h), washed, and then plated on BHI agar plates that contained no antibiotic. The bacterial growth expressed in cfu was scored after either 24 or 48 h incubation of plates at 37°C. When the colony counting was performed after 24 h, we observed potentially bactericidal behaviour of iboxamycin, with almost a two log10 drop in cfu after the 10 h treatment with the antibiotic (Figure 2a–c). Importantly, no similar cfu decrease was observed for either clindamycin or lincomycin (Figure 2a–c). However, this apparent cfu drop effect of iboxamycin disappeared after 48 h of incubation (Figure 2d–f), suggesting slow regrowth rather than cidality, indicative of the so-called PAE.48,49

PAE is characterized by the time after antibiotic removal where no growth of the treated bacteria is observed. This prolonged action of iboxamycin has been previously noted for *S. aureus* and *Enterococcus faecium*.14 Therefore, we next performed PAE experiments in *L. monocytogenes*, demonstrating that, indeed, iboxamycin displays pronounced PAE, suppressing the growth of the WT 10403S and WT EGD-e for 6 and 8 h, respectively (Figure 3b and c). Clindamycin demonstrates a weaker PAE against EGD-e (2 h) and similar PAE against 10403S. No clear PAE is detectible for lincomycin. Compared with the isogenic WT, EDG-e Δlmo0919 displays similar PAE in the case of clindamycin, and, possibly, somewhat more pronounced PAE in the case of iboxamycin.

Discussion

In this report we have evaluated the efficiency of the oxepanoprolinamide iboxamycin against *L. monocytogenes*. The antibiotic can largely overcome the intrinsic PLSA resistance of this species that is mediated by the ribosome-associated ATPase VgaL/Lmo0919, and can similarly counteract the intrinsic resistance mediated by ARE ABCF LsaA in *E. faecalis*. ARE ABCF PLSA resistance factors are broadly distributed among bacterial pathogens,20,22,50,51 and therefore the ability of iboxamycin to largely counteract the ABCF-mediated resistance is a valuable feature.

Figure 2. Iboxamycin is bacteriostatic against *L. monocytogenes*. Exponentially growing *L. monocytogenes* type strains; 10403S (a and d), EDG-e (b and e) or VgaA-deficient EDG-e Δlmo0919 (c and e) were treated with 4 × MIC of either iboxamycin, clindamycin or lincomycin or no antibiotic as control. Cells were harvested at given timepoints and washed before plating. After 24 (a–c) or 48 h (d–f) of incubation, colonies were counted to determine cfu/mL. All experiments were carried out in MH-F broth at 37°C without shaking, data points are from three biological replicates and standard deviation is indicated with error bars.
of the new antibiotic. However, given that B. subtilis VmIR does confer significant levels of iboxamycin resistance (33-fold increase in MIC) and is cooperative with the Cfr rRNA methyltransferase resistance determinant, emergence and spread of ABCF ARE variants capable of defeating next-generation lincosamides in the clinic is possible and should be closely monitored.

Furthermore, we demonstrate that iboxamycin displays a strong PAE against L. monocytogenes, compromising bacterial regrowth for many hours post-antibiotic removal. In clinical settings the longer PAE would allow the design of dosing regiments with larger dosing intervals, resulting in fewer daily administrations of the drug. The PAE is considerably stronger than that of clindamycin while lincomycin displays no PAE. It is possible that the strength of the PAE reflects how tightly the antibiotic binds to the target, the ribosome—and how slowly it dissociates from it. The pronounced PAE suggests that development of even more tight-binding lincosamides could produce effectively bactericidal drugs in the context of infection. Further biochemical studies are necessary to substantiate this hypothesis. Experiments in L. monocytogenes infection models are necessary to further assess the potential of iboxamycin as a novel drug for the treatment of listeriosis.

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Transparency declarations

A.G.M. is an inventor in a provisional patent application submitted by the President and Fellows of Harvard College covering oxepanoprolinamide antibiotics described in this work. A.G.M. has filed the following international patent applications: WO/2019/032936 ‘Lincosamide Antibiotics and Uses Thereof’ and WO/2019/032956 ‘Lincosamide Antibiotics and Uses Thereof’. All other authors: none to declare.

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