The diazabicyclooctane serine β-lactamase inhibitors have recently been introduced to help fight the increasingly severe threat of antibacterial drug resistance by Gram negative bacterial pathogens.1,2 Avibactam (Figure 1a) was the first member of the class to obtain regulatory approval. It is used in combination with ceftazidime for intravenous therapy of complicated urinary tract infections (cUTI), hospital-acquired bacterial pneumonia; and ventilator-associated bacterial pneumonia; and in combination with ceftazidime and metronidazole for intravenous therapy of complicated intra-abdominal infections (cIAI).3,4 Another diazabicyclooctane, relebactam, in combination with imipenem/cilastatin, was recently approved for treatment of cUTI and cIAI.3,4 Durlobactam (ETX2514)5 (Figure 1d) is in Phase 3 testing in combination with sulbactam for treatment of carbapenem-resistant Acinetobacter baumannii pneumonia.

ETX0282 (Figure 1b) was rationally designed to be orally bioavailable.7 It is currently in early clinical trials in combination with the β-lactam cefpodoxime proxetil, also an orally bioavailable antibacterial prodrug. The target pathogens for the combination therapy are the Enterobacteria causing urinary tract infections, including Escherichia coli and Klebsiella pneumoniae. The β-lactamase inhibitor protects the β-lactam from degradation by a range of serine β-lactamases commonly found in these bacteria. These include Ambler class A enzymes such as members of the TEM, CTX-M, and KPC families; class C cephalosporinas; and the class D oxacillinase OXA-48.8

ETX1317 (Figure 1c) is the active product of ETX0282 isopropyl ester hydrolysis, which occurs in the liver after absorption from the intestine.7 We investigated the interaction of ETX1317 with several β-lactamases representing those that are prevalent in relevant bacterial pathogens. We measured the second-order rate constants \( k_{\text{inact}}/K_i \) for inactivation of the enzymes, or the dissociation constant \( K_i \) for the combination therapy are the Enterobacters causing urinary tract infections, including Escherichia coli and Klebsiella pneumoniae. The β-lactamase inhibitor protects the β-lactam
enzymes by ETX1317 by measuring the rate constants for dissociation of the inhibitor. Finally, we examined the ability of ETX1317 to dissociate from β-lactamases in its original form by recycling, a phenomenon that has been observed for avibactam and durlobactam.

**RESULTS AND DISCUSSION**

**β-Lactamase Inhibition Kinetics.** Table 1 shows the values of $k_{\text{on}}/{K}_i$ or $K_i$ for ETX1317, durlobactam, and avibactam measured for several Ambler class A, C, and D β-lactamases. The inhibitory potency of ETX1317 was comparable to, or somewhat lower than, that of durlobactam and higher than that of avibactam. The potency of ETX1317 was lowest for the class D enzymes OXA-10, −23, and −24. These enzymes are common in A. baumannii, the target pathogen for the carbapenem– β-lactamase ETX1317 to dissociate from β-lactamases. Finally, we examined the ability of ETX1317 (c), and durlobactam (d).

**Figure 1.** Chemical structures of avibactam (a), ETX0282 (b), ETX1317 (c), and durlobactam (d).

### Table 1. Inhibition of β-Lactamases by ETX1317

| β-lactamase | ETX1317 $k_{\text{on}}/{K}_i$ (M$^{-1}$ s$^{-1}$) or $K_i$ (µM) | durlobactam $k_{\text{on}}/{K}_i$ (M$^{-1}$ s$^{-1}$) | avibactam $k_{\text{on}}/{K}_i$ (M$^{-1}$ s$^{-1}$) |
|-------------|---------------------------------------------------------------|-------------------------------------------------|-----------------------------------|
| CTX-M-15    | 4.8 (±0.4) $\times$ 10$^6$                                    | 7 (±2) $\times$ 10$^6$                          | 8 $\times$ 10$^4$                |
| KPC-2       | 4.9 (±0.2) $\times$ 10$^4$                                    | 9.3 (±0.6) $\times$ 10$^5$                      | 6 $\times$ 10$^4$               |
| SHV-5       | 3.2 ± (0.1) $\times$ 10$^6$                                   | 6.4 (±0.5) $\times$ 10$^6$                      | 1 $\times$ 10$^4$               |
| TEM-1       | $K_i = 3.4 \ (±0.4) \times 10^{-4}$                           | 1.4 (±0.6) $\times$ 10$^7$                      | 4 $\times$ 10$^3$               |
| *P. aeruginosa* AmpC | 1.20 (±0.04) $\times$ 10$^4$                                   | 9 (±5) $\times$ 10$^5$                          | 3 $\times$ 10$^1$               |
| *E. cloacae* P99 | 3.9 (±0.2) $\times$ 10$^4$                                   | 2.3 (±0.4) $\times$ 10$^4$                      | 8 $\times$ 10$^3$               |
| OXA-10      | 6.8 ± (0.3) $\times$ 10$^2$                                   | 9 (±2) $\times$ 10$^3$                          | 70                               |
| OXA-23      | 1.54 ± (0.06) $\times$ 10$^3$                                  | 5.1 (±0.2) $\times$ 10$^3$                      | 100                              |
| OXA-24      | 4.6 (±0.2) $\times$ 10$^3$                                    | 9 (±2) $\times$ 10$^3$                          | 80                               |
| OXA-48      | 5.3 (±0.2) $\times$ 10$^4$                                    | 8 (±2) $\times$ 10$^5$                          | 5 $\times$ 10$^3$               |

*Values shown are the averages ± standard deviations of two or three measurements for ETX1317 and durlobactam, or a single measurement for avibactam. Data are reproduced from refs 6 and 7.*
observed was +273 Da, corresponding to the mass of ETX1317. This result contrasts with the results observed for avibactam and durlobactam, for which adducts with masses less than that of entire inhibitor were observed with some enzymes due, it is thought, to loss of an SO₃ or SO₄ moiety. Even after 17–19 h, no change in the mass of the ETX1317 adduct or loss of the entire adduct was observed for any of the enzymes (Figure 6), (Figure 3).

This result is consistent with the observation of measurable rates of dissociation (see above), because the protein concentrations in the jump dilution experiment are several orders of magnitude lower than in the mass spectrometry experiment. In the jump dilution experiment, if the inhibitor recylizes and dissociates from the enzyme, the enzyme and inhibitor concentrations are so low that reformation of the covalent complex is very slow. In contrast, in the mass spectrometry experiment, the enzyme and inhibitor concentrations are so high that reformation of the covalent complex is essentially instantaneous.

This explanation presupposes that ETX1317 is capable of recylizing and dissociating from the enzyme in its original state. To further test this concept, we employed the acylation exchange experiment. The covalent complex of ETX1317 with a donor β-lactamase was dialyzed to remove unbound ETX1317 and then mixed with an acceptor β-lactamase. The loss of ETX1317 from the donor enzyme and appearance of a covalent ETX1317–acceptor enzyme complex demonstrates recylization and dissociation of intact ETX1317. This phenomenon was observed for donor enzymes *Pseudomonas aeruginosa* AmpC (Figure 4a), KPC-2 (Figure 5), CTX-M-15 (Figure S1), *Enterobacter cloacae* P99 (Figure S2a), SHV-5 (Figure S3), and TEM-1 (Figure S4a), but not with OXA-48 (Figure 6). The lack of acylation exchange from OXA-48 is consistent with the immeasurably low $k_{\text{off}}$ for ETX1317 with OXA-48 (Table 2). An additional factor may have been the lack of added bicarbonate ion in the acylation exchange experiment, since OXA family β-lactamases are activated by bicarbonate. In contrast, the time-dependent exchanges of ETX1317 from other donor enzymes are shown for AmpC (Figure 4b), P99 (Figure S2b), and TEM-1 (Figure S4b). Since dissociated ETX1317 partitions between the donor and acceptor enzymes in the acylation exchange experiment, the rate of exchange to the acceptor enzyme is slower than the $k_{\text{off}}$ measured by jump dilution.

Figure 2. Partition ratio measurements for inhibition by ETX1317 of several β-lactamases. The partition ratios were 1.1 for KPC-2, 0.7 for CTX-M-15, 1.1 for OXA-48, 1.0 for AmpC, and 0.9 for SHV-5. Incubations of enzyme and inhibitor were for 3 h with KPC-2 and 1 h with the other enzymes.
To create a diazabicyclooctane β-lactamase inhibitor with high oral bioavailability, the sulfate moiety of all previous members of the class was replaced with a fluoroacetate moiety, allowing an ester prodrug to be prepared.7 Interestingly, a side effect of replacement of the sulfate moiety is that the covalent complex of the inhibitor with certain β-lactamases, especially

![Figure 3](https://dx.doi.org/10.1021/acsinfecdis.0c00656)

**Figure 3.** Dissociation kinetics of ETX1317 from several β-lactamases. Progress curves are shown for enzymes preincubated without (blue) and with (red) ETX1317 before jump dilution. Dissociation rate constants are reported in Table 2.

| β-lactamase      | ETX1317 $k_{\text{eff}}$ (s$^{-1}$) | Durlobactam $k_{\text{eff}}$ (s$^{-1}$)$^{10}$ | Avibactam $k_{\text{eff}}$ (s$^{-1}$)$^{11}$ |
|------------------|-------------------------------------|-----------------------------------------------|---------------------------------------------|
| CTX-M-15         | 1.3 (±0.1) × 10$^{-4}$              | 2.2 (±0.5) × 10$^{-4}$                          | 3 (±1) × 10$^{-4}$                          |
| KPC-2            | 5.2 (±0.6) × 10$^{-4}$              | 1.0 (±0.1) × 10$^{-3}$                          | 1.4 (±0.1) × 10$^{-4}$                      |
| SHV-5            | 2.0 (±0.7) × 10$^{-3}$              | 5.5 (±0.3) × 10$^{-4}$                          | ND                                          |
| TEM-1            | 8 (±5) × 10$^{-3}$                  | 1.4 (±0.2) × 10$^{-3}$                          | 8 (±4) × 10$^{-4}$                          |
| P. aeruginosa AmpC | 6 (±1) × 10$^{-4}$                  | 4 (±1) × 10$^{-3}$                              | 1.9 (±0.6) × 10$^{-3}$                      |
| E. cloacae P99   | 9.2 (±0.8) × 10$^{-3}$              | 3.4 (±0.5) × 10$^{-4}$                          | 3.8 (±0.2) × 10$^{-3}$                      |
| OXA-48           | ~0                                  | 2.5 (±0.3) × 10$^{-5}$                          | 1.2 (±0.4) × 10$^{-5}$                      |

$^{12}$Dissociation of ETX1317 from OXA-48 was too slow to measure. ND, not done.
KPC-2, is no longer subject to loss of the anionic group that was observed with both durlobactam and avibactam. In the case of KPC-2, desulfated durlobactam and avibactam were gradually lost from the enzyme. Since the desulfated compounds were no longer inhibitory, this resulted in gradual loss of inhibition, demonstrated by a time-dependent increase in the partition ratio of durlobactam. ETX1317 was impervious to this effect.

As with the related DBO-class β-lactamase inhibitors avibactam and durlobactam, the rate of recovery of activity of the class D enzyme OXA-48 due to recyclization and dissociation of the inhibitor was much slower than for class A and C enzymes. Based on X-ray crystallography, Lahiri et al. hypothesized that this results from the requirement for a carboxylated lysine residue in the catalytic mechanism of class D enzymes and that binding of the inhibitor (avibactam in this case) changes the charge distribution in the vicinity of the carboxy-Lys residue, causing its decarboxylation. Since this carboxy-Lys residue participates in the recyclization of the inhibitor, it was proposed that recyclization is inhibited. Lysine decarboxylation of avibactam-acylated OXA-48 at neutral pH was also observed by King et al. Lohans et al. found that lysine carboxylation of OXA-48 was disfavored but not ablated by avibactam in solution using 13C NMR. Using a novel 19F

Figure 4. Intact protein mass spectrometry for exchange of covalently bound ETX1317 (273 Da) from P. aeruginosa AmpC (40 679 Da) to OXA-48 (28 279 Da) after 17 h. (a) Mass spectra of untreated AmpC (black), the AmpC–ETX1317 covalent complex (blue; 40 952 Da), the mixture of untreated AmpC and OXA-48 (green), and the mixture of the AmpC–ETX1317 covalent complex with OXA-48 (red). The mass of the OXA-48–ETX1317 covalent complex is 28 552 Da. (b) Time-dependence of exchange of ETX1317 from AmpC to OXA-48 at 1, 2, and 17 h of incubation.
NMR technique that did not require the presence of bicarbonate, van Groessen et al. later observed that avibactam derivatization of OXA-48 more substantially disfavored lysine carboxylation. Their suggested explanation for this effect was that a hydrogen bond from the nucleophilic

Figure 5. Intact protein mass spectrometry for exchange of covalently bound ETX1317 (273 Da) from KPC-2 (28 720 Da) to OXA-48 (28 280 Da) after 19 h. Shown are the mass spectra of untreated KPC-2 (black), the KPC-2-ETX1317 covalent complex (blue; 28 993 Da), the mixture of untreated KPC-2 and OXA-48 (green), and the mixture of KPC-2-ETX1317 covalent complex with OXA-48 (red). The mass of the OXA-48-ETX1317 covalent complex is 28 553 Da.

Figure 6. Intact protein mass spectrometry showing lack of exchange of covalently bound ETX1317 (273 Da) from OXA-48-ETX1317 covalent complex (28 553 Da) to CTX-M-15 (28 110 Da) after 19 h of incubation. Shown are the mass spectra of untreated OXA-48 (black; 28 280 Da), the OXA-48-ETX1317 covalent complex (blue), the mixture of untreated OXA-48 and CTX-M-15 (green), and the mixture of OXA-48-ETX1317 covalent complex with CTX-M-15 (red). The mass of a CTX-M-15-ETX1317 covalent complex would be 28 383 Da.
serine, which is acylated by avibactam, stabilizes the carbamoyl lysine.

Observations with avibactam and durlobactam, which have a sulfate moiety instead of the fluoroacetate moiety in ETX1317, showed a loss of mass from the acyl−enzyme complexes with some β-lactamases, such as KPC-2.10,12 This is thought to be due to loss of the sulfate moiety, with additional chemical changes possible subsequently. In contrast, we observed no loss of mass from any of the ETX1317-enzyme complexes we studied. The bond strength and electronics between the core of the inhibitor and the 2-fluoroacetic acid activating group are different from those between the core and the sulfate activating group in avibactam and durlobactam. The proposed mechanism for the desulfation of avibactam12 cannot occur with ETX1317 due to the different chemical nature of the side chain. The chemical differences between the sulfate-containing and fluoroacetate-containing inhibitors likely also explain the inability of KPC-2 to gradually degrade ETX1317, as shown by the lack of a time-dependent increase in the partition ratio.

Avibactam was the first β-lactamase inhibitor of the DBO class introduced to clinical practice. Its spectrum of β-lactamase inhibition is largely limited to class A and C enzymes, except that it is also able to inhibit certain class D enzymes, particularly OXA-48, which is prevalent among the bacterial species commonly responsible for urinary tract infections. To expand the spectrum of DBOs to include a broader range of class D enzymes, such as those prevalent in the pathogen Acinetobacter baumannii, durlobactam was developed. Two factors are responsible for the enhanced inhibitory potency and class D spectrum of inhibition of durlobactam relative to avibactam.6 First, the double bond in the ring system increases the ring strain, making the compound more reactive. Second, the methyl substitution on the ring provides a hydrophobic interaction with the hydrophobic bridge found in the active site of most class D enzymes, but not in OXA-48. Both of these features were retained in ETX1317. The result is that ETX1317 has intermediate potency between avibactam and durlobactam, despite having a less-than-optimal anionic substituent needed for preparation of the oral produg, and it retains the broad spectrum of class D β-lactamase inhibition of durlobactam.

ETX1317 has been co-crystallized with only one β-lactamase to date, CTX-M-14.7 The potency of CTX-M-14 inhibition by ETX1317 is the same as that of durlobactam and about 10-fold higher than that of avibactam.8 Comparing the CTX-M-14 crystal structures with covalently bound ETX1317 and avibactam ring-opened products showed slight differences that could account for the difference in potencies.5 Further elucidation of the structural correlates of inhibitory potency and dissociation rates will await solution of additional acyl-enzyme structures. It should be noted, however, that these structures are unable to reveal the structure of the encounter complex prior to the acylation reaction.

**CONCLUSION**

ETX1317, the active component of the orally bioavailable β-lactamase inhibitor ETX0282, broadly inhibited Ambler class A, C, and D serine β-lactamases. The partition ratio was approximately 1 in each case tested (CTX-M-15, KPC-2, SHV-5, P. aeruginosa AmpC and OXA-48), indicating that there was no detectable hydrolysis of the inhibitor by the enzymes. The covalent inhibitor−enzyme complex consisted of the full mass of ETX1317 in every case. In most cases, the covalent complexes were reversible due to recyclization of the inhibitor and dissociation. For OXA-48, dissociation of ETX1317 was too slow to measure. The qualities of the interactions of ETX1317 with these serine β-lactamases support the usefulness of ETX0282 in combination with cefpodoxime proxetil for treatment of complicated urinary tract infections.

**METHODS**

**Chemicals.** ETX1317,7 avibactam,14 and durlobactam6 were prepared by Entasis Therapeutics according to established methods. Purity was measured by HPLC at 95%, 90%, and 97.4%, respectively.

**β-Lactamase Inhibition **$k_{\text{inact}}/K_i$ Measurements. β-Lactamases were prepared as described previously.6,12,13 Measurements of $k_{\text{inact}}/K_i$ or $K_i$, where appropriate, were made as described previously.6 The assay buffer (Buffer A) consisted of 100 mM sodium phosphate (pH 7.0), 10 mM sodium bicarbonate, and 0.005% Triton X-100 (ThermoFisher Scientific, Waltham, MA). The assay volume was 45 μL. Assays were performed at ambient temperature in clear polystyrene 384-well plates (Greiner Bio-one, Monroe, NC). Inhibitors were freshly dissolved in assay buffer. Fourteen 3-fold serial dilutions of inhibitors were prepared in assay buffer, starting from 300 μM and ending at 188 pM, and 15 μL of each was added to the assay plate. Two additional wells received 15 μL of assay buffer. To all wells was then added 15 μL of 300 μM nitrocefin substrate (custom-synthesized by Syngene, Bangalore, India). Reactions were initiated by addition of 15 μL of 3× β-lactamase enzyme diluted in assay buffer, except that one well without inhibitor received buffer to act as a blank. The final enzyme concentrations were as follows: 15 pM AmpC, 10 pM CTX-M-15, 55 pM KPC-2, 20 pM OXA-10, 10 pM OXA-23, 10 pM OXA-24, 10 pM OXA-48, 8 pM P99, 64 pM SHV-5 and 4 pM TEM-1. Absorbance at 490 nm was monitored at 30 s intervals for 1 h with a Spectramax Plus 384 plate reader (Molecular Devices, San Jose, CA).

The progress curve of the blank was subtracted from the progress curves of the enzyme-containing wells. The resulting 15 progress curves were then fit globally to the kinetic model below, using Global Kinetic Explorer (Kintek, Snow Shoe, PA). The units used were μM and s.

$$E + S \xrightleftharpoons {k_{-1}} {k_1} \text{ES} \xrightleftharpoons {k_{-2}} {k_2} \text{E} + \text{P} \quad \text{and} \quad \text{E} + \text{I} \xrightleftharpoons {k_{-j}} {k_j} \text{EI}$$

where E, S, ES, P, I, and EI represent, respectively, the enzyme, the nitrocefin substrate, the enzyme−substrate complex, the product of nitrocefin hydrolysis, the inhibitor, and the enzyme−inhibitor complex. The premeasured $K_M$ (nitrocefin) values, where $K_M = (k_{-1} + k_{-2})/k_1$, were entered as $k_{-1} = 1$ and $k_{-2} = K_M - 1$. The value of $k_{-2}$ was allowed to float in the fitting. The values of $K_i$(nitrocefin) for the enzymes were 130 μM for AmpC, 8 μM for CTX-M-15, 64 μM for KPC-2, 6 μM for OXA-10, 160 μM for OXA-23, 30 μM for OXA-24, 16 μM for OXA-48, 110 μM for P99, 3 μM for SHV-5, and 20 μM for TEM-1. The value of $k_{-j}$ corresponds to $k_{\text{inact}}/K_i$ and the value of $k_{-j}$ corresponds to $k_{\text{off}}$. The measurement of $k_{\text{off}}$ does not distinguish between reversible binding and hydrolysis of the inhibitor.

**Partition Ratio Measurements.** Partition ratios were measured as described previously15 at ambient temperature in Buffer A. ETX1317 was freshly dissolved in assay buffer and an arithmetic dilution series was prepared from 0 to 12 μM in 1.2
µM steps. Each of these was mixed with an equal volume of 6 µM β-lactamase. At each time point, the mixtures were diluted in buffer to 2-fold the final reaction concentration of enzyme. Then, 22.5 µL of enzyme + inhibitor solutions were mixed with 22.5 µL of 200 µM nitrocefin substrate in a clear polystyrene 384-well assay plate. The absorbance at 490 nm was monitored at 10-s intervals for 10 min with a Spectramax Plus 384 plate reader. The slopes of the progress curves (ΔA_{490}/s) were calculated. The percent of the control value for each inhibitor concentration was plotted versus the inhibitor:enzyme molar ratio. A best-fit line was calculated with a fixed point at 100% on the y-axis.

**β-Lactamase Inhibition k_{eff} Measurements.** Dissociation rate constants (k_{eff}) were measured by the jump-dilution method at ambient temperature as described previously. Enzymes at 500,000-fold the final β-lactamase assay concentration (see above) were incubated for 15–20 min with 3-fold molar excess of freshly dissolved ETX1317 at ambient temperature in Buffer A. The mixtures were then diluted 250,000-fold with Buffer A, and 22.5 µL of the dilutions was mixed with 22.5 µL of 200 µM nitrocefin in Buffer A. The absorbance at 490 nm was monitored at 30 s intervals for 2 h at ambient temperature with a Spectramax Plus 384 plate reader. Progress curves were fit by nonlinear regression using XLfit (ID Business Solutions, Boston, MA) to the following equation:

\[
P = C + V_0t + (V_f - V_0)(1 - e^{-kt})/k
\]

where P is A_{490}, C is the initial A_{490}, V_0 is the initial rate of absorbance increase, V_f is the final rate of absorbance increase, t is time in seconds, and k is the first order rate constant for enzyme reactivation due to inhibitor dissociation, k_{eff}.

**β-Lactamase Acylation Exchange.** β-Lactamase acylation exchange experiments and intact protein mass spectrometry were performed as described previously. OXA-48 was used as the acceptor enzyme in most cases because of the stability of the ETX1317–OXA-48 complex. When OXA-48 was the donor enzyme, CTX-M-15 was used as the acceptor.

Each β-lactamase (5 µM) was incubated with or without 25 µM ETX1317 in a 60 µL volume of 50 mM HEPES (pH 7.0) buffer for 1 h at ambient temperature. The samples were diluted to 400 µL with ice-cold buffer and then concentrated to ~40 µL with Microcon YM-10 centrifugal ultrafilters (Millipore-Sigma, Burlington, MA) at 14,000g for 45 min at 4 °C. The retentates were rediluted to 400 µL with ice-cold buffer and recentrifuged as before. This was repeated a further time, leaving no significant amount of unbound ETX1317. The sample volumes were restored to 60 µL with ice-cold buffer and the samples were divided into two equal aliquots of 30 µL. Buffer (6 µL) was added to one aliquot, and 6 µL of acceptor enzyme was added to the other. The final acceptor enzyme concentrations were 5 µM. Samples were incubated at ambient temperature for the times indicated and then stored at −80 °C until analysis. LC-MS analysis was performed on a Triple TOFS6000+ (AB Sciex, Redwood City, CA) equipped with a Duospray Ion Source and a Shimadzu LC 20-AD HPLC system (Shimadzu Scientific Instruments, Marlborough, MA). A 2 µL portion of each sample was injected onto a Poroshell 300SB-C8 75 x 2.1 mm, 5 µm column (Agilent) at 30 °C with a gradient of acetonitrile (5–95%) in 0.1% formic acid for 3 min followed by a 1 min hold at 95% before returning to 5% acetonitrile at a flow rate of 0.4 mL/min. The mass spectrometer was operated in positive ion and intact protein mode with source temperature of 450 °C. LC-MS data were acquired in the TOF MS mode for m/z+ from 600 to 2000. Nebulizer gas (GS1), heater gas (GS2), and curtain gas were set at 60, 70, and 30 psi, respectively. Ion spray voltage was 5500 V. Declustering voltage was 100 V, and collision energy was 10 V. Peak masses for protein species were determined following spectrum deconvolution using PeakView version 2.2. Mass accuracy of intact proteins is ±1 Da.

**ASSOCIATED CONTENT**

* Supporting Information
  The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00656.

Intact protein mass spectrometry for exchange of covalently bound ETX1317 from CTX-M-15, P99, SHV-5 and TEM-1 to OXA-48 (PDF)

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**Author Contributions**

A.B.S performed biochemical experiments and prepared the manuscript. N.G. performed mass spectrometry.

**Notes**

The authors declare the following competing financial interest(s): A.B.S. is an employee of Entasis Therapeutics and may possess stock and/or stock options.

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**ABBREVIATIONS**

cUTI, complicated urinary tract infection; cIAI, complicated intraabdominal infection

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