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## Section D

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Supporting information for article:

Structure of ADC-68, a novel carbapenem-hydrolyzing class C extended-spectrum $\beta$-lactamase isolated from Acinetobacter baumannii

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Table S1 Strains, plasmids, and primers used in this study.
Restriction sites appear in bold. The underlined bases indicate the hexahistidine tag site, and italic bases indicate the enterokinase recognition site. r: resistant.

| Strains, plasmids, and primers | Phenotype, genotype and/or characteristics | Source (or reference) |
| :---: | :---: | :---: |
| Strains |  |  |
| Acinetobacter baumannii D015 | Strain with the gene bla $_{\text {ADC-68 }}$ in its chromosome | This study |
| E. coli TOP10 | FmcrA $\Delta$ (mrr-hsdRMS-mcrBC) $\varphi 80$ lacZDM15 $\Delta l a c X 74$ recAl araD139 $\Delta$ (araleu) 7697 galU galK rpsL( $\left.\mathrm{Str}^{\mathrm{R}}\right)$ endA1 nup G | Invitrogen |
| E. coli BL21(DE3) | FompT hsdS $S_{B}\left(\mathrm{r}_{\mathrm{B}} \mathrm{m}_{\mathrm{B}}{ }^{-}\right) \mathrm{gal}$ dcm ( DE 3 ) |  |
| E. coli ATCC25922 | MIC reference strain | ATCC |
| Plasmids |  |  |
| pET28a(+) | Expression vector, kanamycin ${ }^{\text {r }}$ | Novagen |
| pHSG398 | Expression vector, chloramphenicol ${ }^{1}$ | TaKaRa |
| pET-28a(+)/His ${ }_{6}$-bla $_{\text {ADC-68 }}$ | $\mathrm{pET} 28 \mathrm{a}(+)$ containing bla $_{\text {ADC-68 }}$ without signal peptide from A. baumannii D015 | This study |
| pHSG398/bla ${ }_{\text {ADC }-68}$ | pHSG398 containing bla $_{\text {ADC-68 }}$ from A. baumannii D015 | This study |
| Primers |  |  |
| ABAMPC-1 | 5'-ATGCGATTTAAAAAAATTTCTTGT-3' | Bou \& Martinez-Beltran (2000) |


| ABAMPC-2 | 5'-TTATTTCTTTATTGCATTCAG-3' | Bou \& Martinez-Beltran (2000) |
| :--- | :--- | :--- |
| SacI-ADC-68-F | 5'-ATAGAGCTCAATGCGATTTAAAAAAATTTCTTGTCTAC-3' | This study |
| XbaI-ADC-68-R | 5'-GTGTCTAGATTATTTCTTTATTGCATTCAGCACAG-3' | This study |
| NcoI-EK-HIS-ADC-68-F | 5'-ATACCATGGGCCATCATCATCATCATCATGACGACGACGACAAGGG | This study |
| ChoI-ADC-68-R | CAATACACCAAAAGACCAAGA-3' |  |
| 16S rRNA-F | 5'-CAGCTCGAGTTATTTCTTTATTGCATTCAGCACAG-3' | This study |
| 16S rRNA-R | 5'-AGAGTTTGATCHTGGYTYAGA-3' | Yakupogullari et al. (2008) |
| Another AmpC-F | 5'-ACGGYTACCTTGTTACGACTT-3' | Yakupogullari et al. (2008) |
| Another AmpC-R | 5'-GTGAAAATATTTTCGACCAATACCTGT-3' | This study |



Figure S1 Localization of $b l a_{\mathrm{ADC}}-68$ on I-CeuI-generated fragments of Acinetobacter baumannii D015. (a) I-CeuI fragment restriction pattern. (b) Hybridization with a probe that is specific for the 16 S rRNA gene. (c) Hybridization with a probe that is specific for the bla $_{\mathrm{ADC}-68}$ gene. (d) Hybridization with a probe that is specific for another $b l a_{\mathrm{AmpC}}$ gene (GenBank accession number KJ997965). Marker sizes (in kilobases) are indicated on the left. The $b l a_{\mathrm{ADC}}-68$ gene is indicated with an arrow. Lane M, Lambda ladder PFGE marker (Bio-Rad, Hercules, CA, USA); lane 1: I-Ceu I fragment restriction pattern of total DNA from A. baumannii D015 carrying the $b l a_{\mathrm{ADC}-68}$ gene.


Figure S2 The superimposed complex of cefotaxime (PDB entry 3ixh) and ceftazidime (PDB entry 1iel) with ADC-68, ADC-1, and AmpC from E. coli. The $\Omega$-loop of ADC-68, ADC-1, and AmpC are represented as blue, red, and green ribbon diagrams, respectively. Cefotaxime and ceftazidime are represented as hot pink and yellow sticks, respectively. The distances between residue V214 and R1 chains of ligands are shown by black-dashed lines (values are indicated in angstroms). R1 and R2 subsites are indicated as green- and cyan-dashed circles, respectively

