

## **SUPPLEMENTARY MATERIAL**

### **Ceftazidime-avibactam resistance mediated by the N<sup>346</sup>Y substitution in various AmpC $\beta$ -lactamases**

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**Supplementary Table S1. Primers used in this study**

<b>Primers</b>	<b>Nucleotide sequence (5' – 3')<sup>a</sup></b>
<b>Cloning in pTRC99k (phenotypic analysis in <i>E. coli</i> Top10)</b>	
PDC-5_EcoRI_F	AAAGAATTCATGCGCGATACCAGATTCC
PDC-5_XbaI_R	AAATCTAGATCAGCGCTTCAGCGGCACC
DHA-1_EcoRI_F	AAAGAATTCATGAAAAAATCGTTATCTG
DHA-1_SalI_R	AAAGTCGACTTATTCCAGTGC ACTCAA
FOX-3_EcoRI_F	AAAGAATTCATGCAACAACGACGTGCGT
FOX-3_SalI_R	AAAGTCGACTCACTCGGCCAACTGACTC
AmpC <sub>cloacae</sub> _EcoRI_F	AAAGAATTCATGATGAGAAAATCCCTTT
AmpC <sub>cloacae</sub> _SalI_R	AAAGTCGACTTACTGTAGCGCCTCGAGG
<b>Cloning in pET-TEV (protein production in <i>E. coli</i> BL21(DE3))</b>	
PDC-5_NdeI_F	AAACATATGGGCGAGGCCCGGCGGATC
PDC-5_XhoI_R	AAAACCTCGAGTCAGCGCTTCAGCGGCACC
DHA-1_NdeI_F	AACATATGGATAATGTTCGCGGCGGTG
DHA-1_XhoI_R	AACTCGAGTTATTCCAGTGC ACTCAA
FOX-3_NdeI_F	AACATATGCGCGGGGAGGCTCCGCTGAC
FOX-3_XhoI_R	AACTCGAGTCACTCGGCCAACTGACTCA
AmpC <sub>cloacae</sub> _NdeI_F	AAACATATGACGCCAGTGT CAGAAAAC
AmpC <sub>cloacae</sub> _NotI_R	AAGCGGCCGCTTACTGTAGCGCCTCGAGG
<b>Site-directed mutagenesis</b>	
PDC-5_N346Y_R	AAAGTCGACTCAGCGCTTCAGCGGCACCTTGCCCTGCT GCTCCAGGCCGCTGAGGATGGCGTAGGCGATCTTCACC CGCTCGGCGTAGGGATAGTTGCGGTT
DHA-1_N346Y_R	AAAGTCGACTTATTCCAGTGC ACTCAAATAGCCTGTG CAGCTTTGACTCTTTCGGTGTACGGGTAGTTTTTATTC
FOX-3_N346Y_R	AAAGTCGACTCACTCGGCCAACTGACTCAGGATGGCGT GAGCCGCCTTCACCCTGGCCTCGTAGGGATAGTTGC

The restriction sites used for cloning are underlined; F, forward; R, reverse. For mutagenesis, bases introduced into the nucleotide sequence to generate the N<sup>346</sup>Y and I<sup>346</sup>Y substitutions are indicated in bold. Primers used for production of AmpC variants were identical to those used for production of native AmpC cephalosporinases.

**Supplementary Table S2. Wavelength ( $\lambda$ ) and variation in the molecular extinction coefficient ( $\Delta\epsilon$ ) used for kinetic analyses of  $\beta$ -lactam hydrolysis based on spectrophotometry**

$\beta$ -lactam	$\lambda$ (nm)	$\Delta\epsilon$ ( $M^{-1} cm^{-1}$ )
Amoxicillin	244	-1,200
Cephalothin	265	-8,100
Cefamandole	268	-6,000
Cefoxitin	265	-7,400
Cefotaxime	255	-8,400
Ceftazidime	256	-9,800
Aztreonam	318	-400
Cefepime	257	-8,100
Nitrocefin	486	14,600

	1	10	20	30	40	50	60	70	80	90	100	
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<i>E. cloacae</i> AmpC	---MMRKS--	----LCCAL	LLGISCSALA	TPVSEKQLAE	VVANTITPLM	KAQSVPGMAV	AVIYQGKPHY	YTFGKADIAA	NKPVTPQTLF	EL	SISK	TFT
<i>P. aeruginosa</i> AmpC	----MRDTRF	PCLCGIAAST	LLFATTPAIA	GEAPADRLKA	LVDAAVQPVM	KANDIPGLAV	AISLKGEPHY	FSYGLASKED	GRQVTPETLF	EI	SVSK	TFT
<i>E. coli</i> AmpC	----MFKT--	----TLCTL	LITASCSTFA	AP---QQIND	IVHRTITPLI	EQQKIPGMAV	AVIYQGKPHY	FTWGYADIAK	KQPVTQOTLF	EL	SVSK	TFT
<i>C. freundii</i> AmpC	---MMKKS--	----ICCAL	LLTASFSTFA	AAKTEQQIAD	IVNRTITPLM	QEQAIPGMAV	AIYEGKPHY	FTWGKADIAN	NHPVTQOTLF	EL	SVSK	TFN
<i>H. alvei</i> AmpC	MRKKMQNT--	-LKLLSVITC	LAATAQGALA	ANIDESKIKD	TVDDLIQPLM	QKNNIPGMSV	AVTVNGKNIY	YNYGLAAKQP	QQPVTEENTLF	EV	SLSK	TFA
<i>M. organii</i> AmpC	---MKKS--	----LSATL	ISALLAFSAP	GFSAADNVAA	VVDSTIKPLM	AQQDIPGMAV	AVSVKGPYY	FNYGFADVQA	KQPVTENTLF	EL	SVSK	TFT
<i>P. stuartii</i> AmpC	----MDNS--	-MKNIFRQGR	LFIALSLAMT	SISAFALTQQ	EVDDIHKPLM	KQEQIPGMSV	AISVNGKQAI	YHYGVQSKQT	QIPVSDRTLY	EI	SLSK	TFT
<i>S. marcescens</i> AmpC	-----	----MTKMNR	LAAALIAALI	LPTAHAAQQQ	DIDAVIQPLM	KKYGVPGMAI	AVSVDGKQI	YPYGVASKQT	GKPIEQTLF	EV	SLSK	TFT
CMY-1	----MQQRQS	ILWG--AVAT	LMWAGLAHAG	EASPVDPRLP	VVDASIQPLL	KEHRIPGMAV	AVLKDGHKAHY	FNYGVANRES	GAGVSEQTLF	EI	SVSK	TLT
DHA-1	---MKKS--	----LSATL	ISALLAFSAP	GFSAADNVAA	VVDSTIKPLM	AQQDIPGMAV	AVSVKGPYY	FNYGFADIQA	KQPVTENTLF	EL	SVSK	TFT
ACC-1	----MQNT--	-LKLLSVITC	LAATAQGALA	ANIDESKIKD	TVDDLIQPLM	QKNNIPGMSV	AVTVNGKNIY	YNYGLAAKQP	QQPVTEENTLF	EV	SLSK	TFA
ACT-1	--MMTKS--	----LCCAL	LLSTSCSVLA	TPMSEKQLAE	VVERTVTPLM	KAQAIPGMAV	AVIYEGQPHY	FTFGKADVAA	NKPVTPQTLF	EL	SISK	TFT
MIR-1	---MMTKS--	----LSCAL	LLSVASSAFA	APMSEKQLAE	VVERTVTPLM	NAQAIPGMAV	AVIYQGQPHY	FTFGKADVAA	NKPVTPQTLF	EL	SISK	TFT
MOX-1	---MQQRQS	ILWG--AVAT	LMWAGLAHAG	EASPVDPRLP	VVDASIQPLL	KEHRIPGMAV	AVLKDGHKAHY	FNYGVANRES	GASVSEQTLF	EI	SVSK	TLT
BIL-1	---MMKKS--	----LCCAL	LLTASFSTFA	AAKTEQQIAD	IVNRTITPLM	QEQAIPGMAV	AVIYQGKPHY	FTWGKADIAN	NHPVTQOTLF	EL	SVSK	TFN
LAT-1	---MMKKS--	----LCSAL	LLTASFSTFA	AAKTEQQIAD	IVNRTITPLM	QEQAIPGMAV	AVIYQGKPHY	FTWGKADIAN	NHPVTQOTLF	EL	SVSK	TFN
FOX-3	---MQQRRR	FALL--TLGS	LLLAPCTYA-	--SGEAPLTA	TVDGIHQPLM	KEYRIPGIAV	AVLKDGHKAHY	FNYGVANRES	GQRVSEQTLF	EI	SVSK	TLT
CFE-1	---MMKKS--	----ICCAL	LLTASFSTFA	AAKTEQQIAD	IVNRTITPLM	QEQAIPGMAV	AVIYQGKPHY	FTWGKADIAN	NRPVTQOTLF	EL	SVSK	TFN
CMY-2	---MMKKS--	----LCCAL	LLTASFSTFA	AAKTEQQIAD	IVNRTITPLM	QEQAIPGMAV	AVIYQGKPHY	FTWGKADIAN	NHPVTQOTLF	EL	SVSK	TFN

	101	110	120	130	140	150	160	170	180	190	200
	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....	.... ....
<i>E. cloacae</i> AmpC	GVLGGDAIAR	GEISLDDAVT	RYWPQLTGKQ	WQGIRMLDLA	TYTAGGLPLQ	VPDEVTDN-A	SLLRFYQNWQ	PQWKPGTTRL	YANASIGLFG	ALAVKPSGMP	
<i>P. aeruginosa</i> AmpC	ATLAGYALAQ	DKMRLDDRAS	QHWPALQGSR	FDGISLLDLA	TYTAGGLPLQ	FPDSVQKQQA	QIRDYRQWQ	PTYAPGSQRL	YNSNSIGLFG	YLAARSLGQP	
<i>E. coli</i> AmpC	GVLGGDAIAR	GEIKLSDPPT	KYWPELTAKQ	WNGITLLHLA	TYTAGGLPLQ	VPDEVKSS-S	DLLRFYQNWQ	PAWAPGTQRL	YANSSIGLFG	ALAVKPSGLS	
<i>C. freundii</i> AmpC	GVLGGDRIAR	GEIKLSDPVT	KYWPELTGKQ	WRGISLLHLA	TYTAGGLPLQ	IPGDVTDK-A	ELLRFYQNWQ	PQWTPGAKRL	YANSSIGLFG	ALAVKSSGMS	
<i>H. alvei</i> AmpC	ATLASAQQVS	GKLSLDQSVS	HYVPELRGSS	FDHVSVLNVG	THTSG-LQLF	MPEDIKNT-T	QLMAYLKAWK	PADAAGTHR	YNSIGTGLL	MIAAKSLGVS	
<i>M. organii</i> AmpC	GVLGAVSVAK	KEMTLNDPAE	KYQPELALPQ	WKGITLLDLA	TYTAGGLPLQ	VPDAVKSR-A	DLLHFIYQWQ	PSRKPDMRL	YANSSIGLFG	ALTANAAGMP	
<i>P. stuartii</i> AmpC	ATLATYAQIQ	GKLDIFSQVS	HYLPELKGSA	FDNVSMNLA	THTSG-LSLF	VPSDIKTN-D	QLMAYYQKWL	PDNEVGQYRS	YSNLGVGLL	IVTAKQLNDP	
<i>S. marcescens</i> AmpC	ATLAVYAQQQ	GKLSFKDPAS	RYLPELRGSA	FDGVSLLNLA	THTSG-LPLF	VPDDVTDN-A	QLMAYYRAWQ	PKHPAGSYRV	YSNLGIQML	MIAAKSLDQP	
CMY-1	ATLGAYAVVK	GAMQLDDKAS	RHAPWLKGS	FDSITMGELA	TYSAGGLPLQ	FPEEVDSS-E	KMRAYYRQWA	PVYSPGSHRQ	YNSNSIGLFG	HLAASSLKQP	
DHA-1	GVLGAVSVAK	KEMALNDPAA	KYQPELALPQ	WKGITLLDLA	TYTAGGLPLQ	VPDAVKSR-A	DLLHFIYQWQ	PSRKPDMRL	YANSSIGLFG	ALTANAAGMP	
ACC-1	ATLASAQQVS	GKLSLDQSVS	HYVPELRGSS	FDHVSVLNVG	THTSG-LQLF	MPEDIKNT-T	QLMAYLKAWK	PADAAGTHR	YNSIGTGLL	MIAAKSLGVS	
ACT-1	GVLGGDAIAR	GEISLDPVPT	KYWPELTGKQ	WQGIRMLDLA	TYTAGGLPLQ	VPDEVKDN-A	SLLRFYQNWQ	PQWKPGTTRL	YANASIGLFG	ALAVKPSGMS	
MIR-1	GVLGGDAIAR	GEIALGDPVA	KYWPELTGKQ	WQGIRMLDLA	TYTAGGLPLQ	VPDEVTDN-A	SLLRFYQNWQ	PQWKPGTTRL	YANASIGLFG	ALAVKPSGMS	
MOX-1	ATLGAYAVVK	GAMQLDDKAS	RHAPWLKGSV	FDSITMGELA	TYSAGGLPLQ	FPEEVDSS-E	KMRAYYRQWA	PVYSPGSHRQ	YNSNSIGLFG	HLAASSLKQP	
BIL-1	GVLGRDAIAR	GEIKLSDPVT	KYWPELTGKQ	WQGIRLLHLA	TYTAGGLPLQ	IPDDVRDK-A	ALLHFIYQWQ	PQWTPGAKRL	YANSSIGLFG	ALAVKPSGMS	
LAT-1	GVLGGDCIAR	GEIKLSDPVT	KYWPELTGKQ	WQGIRLLHLA	TYTAGGLPLQ	IPDDVRDK-A	ALLHFIYQWQ	PQWTPGAKRL	YANSSIGLFG	ALAVKPSGMS	
FOX-3	ATLGAYAARK	GGFVLDKVS	QHAPWLKGS	LDGVTMAELA	TYSAGGLPLQ	FPDVKVDS-D	KMLTYRQWQ	PVYPAGTHRQ	YNSNSIGLFG	HLAANSLGQP	
CFE-1	GVLGGDAIAR	GEIKLSDPVT	KYWPELTGKQ	WQGIRLLHLA	TYTAGGLPLQ	VPDDVTDK-A	SLLRFYQNWQ	PQWAPGAKRL	YANSSIGLFG	ALAVKPSGMS	

CMY-2 GVLGGDAIAR GEIKLSDPVT KYWPELTGKQ WQGIRLLHLA TYTAGGLPLQ IPDDVRDK-A ALLHFYQNWQ PQWTPGAKRL YANSSIGLFG ALAVKPSGMS

201 210 220 230 240 250 260 270 280 290 300
E. cloacae AmpC YEQAMTTRVL KPLKLDHTWI NVPKAEAAHY AWGY-RDGKA VRVSPGMLDA QAYGVKTNVQ
P. aeruginosa AmpC FERLMEQVVF PALGLEQTHL DVPEAALAAQY AQQYGKDDRP LRVGPGPLDA EYGVKTSAA
E. coli AmpC FEQAMKTRVF QPLKLNHTWI NVPKAEAAHY AWGY-REGKA VHVSPGALDA EAYGVKSTIE
C. freundii AmpC YEEAMTRRVL QPLKLAHTWI TVPQSEQKNY AWGY-LEGKP VHVSPGALDA EAYGVKSSVI
H. alvei AmpC YEDAIEKTLL PQLGMHHSYL KVPADQMENY AWGYNKKDEP VHVNMEILGN EAYGIKTSS
M. morgani AmpC YEQLLTARIL APLGLSHTFI TVPESAQSQY AYGK-KNKKP VRVSPGQALDA ESYGVKSSASK
P. stuartii AmpC FSQAMEKLML PSLGLKHTYI HVPKSEQEKY AQQYKQKQNY VRLNLEILGP EAYGLKSNAR
S. marcescens AmpC FIQAMEQGML PALGMSHTYV QVPAQAMANY AQQYSKDDKP VRVNPGPLDA EAYGVKSNAR
CMY-1 FAPLMEQTLL PGLGMHHTYV NVPKQAMASY AYGSKEDKP IRVNPGLAD EAYGIKTSSA
DHA-1 YEQLLTARIL APLGLSHTFI TVPESAQSQY AYGK-KNKKP VRVSPGQALDA ESYGVKSSASK
ACC-1 YEDAIEKTLL PQLGMHHSYL KVPADQMENY AWGYNKKDEP VHVNMEILGN EAYGIKTSS
ACT-1 YEQAITTRVF KPLKLDHTWI NVPKAEAAHY AWGY-RDGKA VHVSPGALDA EAYGVKTNVQ
MIR-1 YEQAMTTRVF KPLKLDHTWI NVPKAEAAHF AWGY-REGKA VHVSPGMLDA EAYGVKTNVK
MOX-1 FAQLMEQTLL PGLGMHHTYV NVPKQAMASY AYGSKEDKP IRVNPGLAD EAYGIKTSSA
BIL-1 YEEAMTRRVL QPLKLAHTWI TVPQNEQKDY AWAY-REGKP VHVSPGQALDA EAYGVKSSVI
LAT-1 YEEAMTRRVL QPLKLAHTWI TVPQNEQKDY AWGY-REGKP VHVSPGALDA EAYGVKSSVI
FOX-3 FEQLMSQTLL PKLGLHHTYI QVPESAMANY AYGSKEDKP IRVTPGVLA EAYGIKTGSA
CFE-1 YEEAMTTRVL QPLKLAHTWI TVPQSEQKDY ALGY-REGKP VHVSPGQALDA EAYGVKSSV
CMY-2 YEEAMTRRVL QPLKLAHTWI TVPQNEQKDY AWGY-REGKP VHVSPGQALDA EAYGVKSSVI

Ambler positions 315-317 KTG Ambler positions 346
301 310 320 330 340 350 360 370 380 390 400
E. cloacae AmpC GLGWEMLNWP VEANTVVEGS DSKVALAPL VAEVNPPAPP VKAS-WVHKT GSTGGFGSYV AFVPEKQIGI VMLANTSYPN PARVEAAYHI LEALQ-----
P. aeruginosa AmpC GLGWAEYDWP ISLKRLQAGN STPMALQPHR IARLPAPQAL EGQR-LLNKT GSTNGFGAYV AFVPGRDGLG VILANRNPYN AERVKIAYAI LSGLEQQGKV
E. coli AmpC GLGWEMLDWP VNPDIINGS DNKIALAARP VKPITPPTPA VRAS-WVHKT GATGGFGSYV AFVPEKELGI VMLANKNYPN PARVEAAWQI LNALQ-----
C. freundii AmpC GLGWEMLNWP LKADSIINGS DSKVALAALP AVEVNPPAPA VKAS-WVHKT GSTGGFGSYV AFVPEKNLGI VMLANKSYPN PARVEAAWRI LEKLQ-----
H. alvei AmpC DLMWEQLPYP VSLPNLLTGN --DMAMTKSV ATPIVPPLPP QENV-WINKT GSTNGFGAYI AFVPAKMG I VMLANKNYSI DQRTVYAYKI LSSLEGNK--
M. morgani AmpC GLGWEMYDWP QQKDMIINGV TNEVALQPHP VTDN-QVQPY NRAS-WVHKT GATGFGAYV AFVPEKQVAI VILANKNYPN TERVKAQA I LSALE-----
P. stuartii AmpC DMMWESYPWP VLSQLLQGN RDDMALKPQK VELIKPAMAP EVRA-YYNKT GSSNGFATYA IFVPEEKI AI VMLSNKWIPI PQRITATYQL LEKIER----
S. marcescens AmpC DLMWENYPYP VKLSRLVEGN NAGMIMNGTP ATAITPPQ-P ELRAGWYNKT GSTGGFSTYA VFIPAKNIAV VMLANKWFPN DDRVEAAYHI IQALEKH---
CMY-1 GLGWESYAYP VTEQTLLAGN SAKVILEANP T---AAPRES GSQV-LFNKT GSTNGFGAYV AFVPARGIGI VMLANRNPYN EARIKAAHAI LAQLAG----
DHA-1 GLGWEMYDWP QQKDMIINGV TNEVALQPHP VTDN-QVQPY NRAS-WVHKT GATGFGAYV AFVPEKQVAI VILANKNYPN TERVKAQA I LSALE-----
ACC-1 DLMWEQLPYP VSLPNLLTGN --DMAMTKSV ATPIVPPLPP QENV-WINKT GSTNGFGAYI AFVPAKMG I VMLANKNYSI DQRTVYAYKI LSSLEGNK--
ACT-1 GLGWEMLNWP VDAKTVEGS DNKVALAPL AREVNPPAPP VNAS-WVHKT GSTGGFGSYV AFVPEKQLGI VMLANKSYPN PARVEAAYRI LSAL-----
MIR-1 GLGWEMLNWP VDAKTVEGS DNKVALAPL AVEVNPPAPP VKAS-WVHKT GSTGGFGSYV AFVPEKQLGI VMLANKSYPN PARVEAAYRI LDALQ-----
MOX-1 GLGWESYAYP VTEQTLLAGN SAKVILEANP T---AAPRES GSQV-LFNKT GSTNGFGAYV AFVPARGIGI VMLANRNPYN PARVKAHA I LAQLAG----
BIL-1 GLGWEMLNWP LKADSIINGS DTKVALAAVP AVEVNPPAPA VKAS-WVHKT GSTGGFGSYV AFVPEKNLGI VIVANKSYPN PVRVEAAWRI LEKLQ-----
LAT-1 GLGWEMLNWP LKADSIINGS DSKVALAALP AVEVNPPAPA VKAS-WVHKT GSTGGFGSYV AFVPEKNLGI VMLANKSYPN PVRVEAAWRI LEKLQ-----
FOX-3 GLGWESYDYP VTEQVLLAGN SPAVSFQANP VTRFAVPKAM GEQR-LYNKT GSTGGFGAYV AFVPARGIAI VMLANRNPYN EARVKAHA I LSQLAE----
CFE-1 GLGWEMLNWP VKADSIINGS DSKVALAALP AVEVNPPAPA VKAS-WVHKT GSTGGFGSYV AFVPEKNLGI VMLANKSYPN PARVEAAWRI LEKLQ-----
CMY-2 GLGWEMLNWP LKADSIINGS DSKVALAALP AVEVNPPAPA VKAS-WVHKT GSTGGFGSYV AFVPEKNLGI VMLANKSYPN PVRVEAAWRI LEKLQ-----

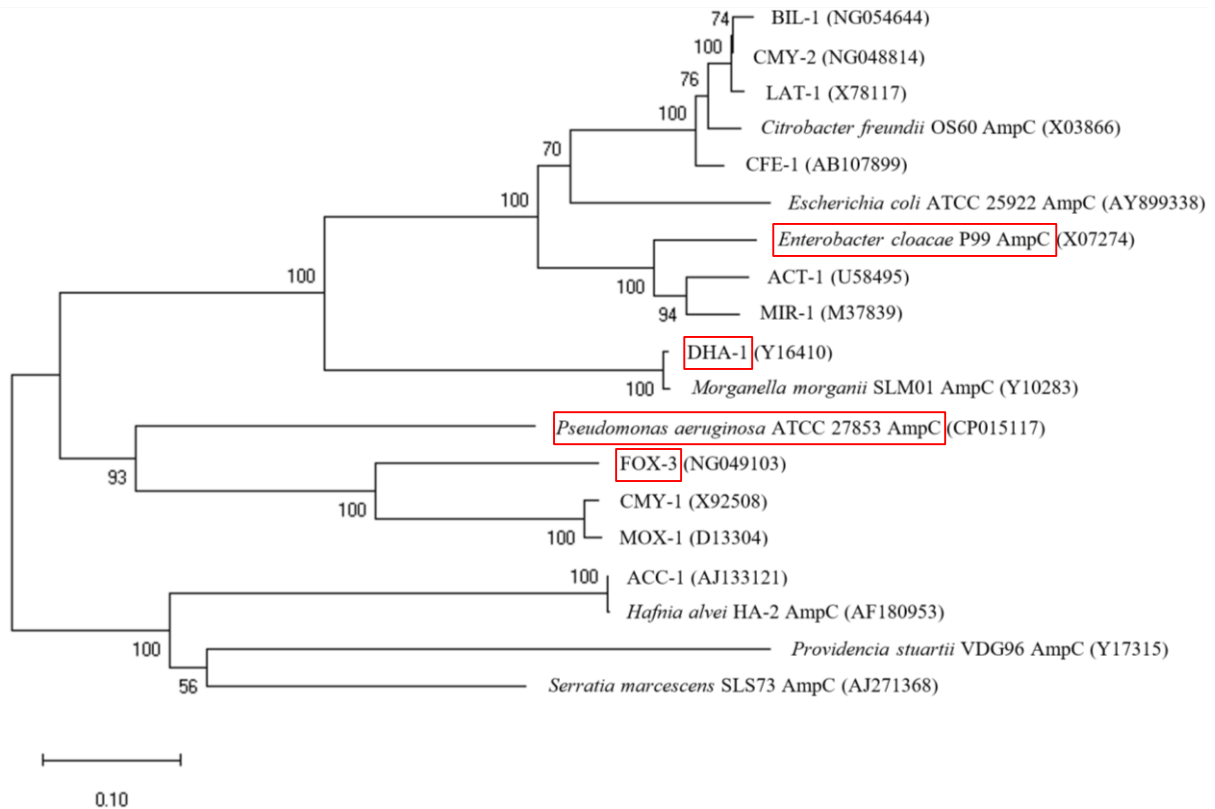
401404

P. aeruginosa AmpC

PLKR

**Supplementary Figure S1. Alignment of the amino acid sequence of representative chromosomal and plasmid-borne AmpC  $\beta$ -lactamases.**

Conserved motifs of are highlighted in red. Additional key residues in interaction with avibactam are shown in green-colored font. The asparagine residue studied here, Asn346 in Ambler numbering scheme, is highlighted in yellow. This residue is substituted by an isoleucine in *H. alvei* AmpC, *P. stuartii* AmpC, ACC-1, MOX-1, and FOX-3. Residues of the  $\Omega$  loop are indicated in orange. The deletion of RVGPGPL in the cAmpC for *P. aeruginosa* (PDC-5), which emerged ,under the selective pressure of ceftazidime combined to avibactam, is highlighted in blue. Residue E, replaced by K in another mutant is also highlighted in blue. Genbank accession numbers of AmpC  $\beta$ -lactamases are as follows: X07274 (*E. cloacae* P99), CP015117 (*P. aeruginosa* ATCC 27853), AY899338 (*E. coli* ATCC 25922), X03866 (*C. freundii* OS60), AF180953 (*H. alvei* HA-2), Y10283 (*M. morgani* SLM01), Y17315 (*P. stuartii* VDG96), AJ271368 (*S. marcescens* SLS73), X92508 (CMY-1), Y16410 (DHA-1), AJ133121 (ACC-1), U58495 (ACT-1), M37839 (MIR-1), D13304 (MOX-1), NG054644 (BIL-1), X78117 (LAT-1), NG049103 (FOX-3), AB107899 (CFE-1), NG048814 (CMY-2).



**Supplementary Figure S2. Unrooted evolutionary tree constructed with a set of 19 sequences representative of chromosomal and plasmid-borne AmpC  $\beta$ -lactamases.** Amino acid sequences are designated by their GenBank accession number. Evolutionary analyses were conducted in MEGA X using the neighbor-joining method (1). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances are in the units of the number of amino acid substitutions per site. Red boxes indicate AmpC cephalosporinases used in our study.

## SUPPLEMENTARY REFERENCES

- 1 Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549.