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1 **A standard numbering scheme for class C  $\beta$ -lactamases**

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60 **Abstract**

61 Unlike classes A and B, a standardized amino acid numbering scheme has not been proposed for  
62 the class C (AmpC)  $\beta$ -lactamases, which complicates communication in the field. Here, we propose a  
63 scheme developed through a collaborative approach that considers both sequence and structure, preserves  
64 traditional numbering of catalytically important residues (Ser<sup>64</sup>, Lys<sup>67</sup>, Tyr<sup>150</sup>, and Lys<sup>315</sup>), is adaptable to  
65 new variants or enzymes yet to be discovered, and includes a variation for genetic and epidemiological  
66 applications.

67

68 An urgent need exists to address current inconsistencies in the numbering of amino acid residues  
69 among class C  $\beta$ -lactamases, both within families and across the class. Established conventions in the  
70 field define three common features shared among the serine-type  $\beta$ -lactamases. In the class C  $\beta$ -  
71 lactamases, also known as AmpC  $\beta$ -lactamases, these features occur at recognizable conserved motifs:  
72 S<sup>64</sup>XXK (where S<sup>64</sup> is the active site serine); Y<sup>150</sup>XN; and K<sup>315</sup>(S/T)G (1–4). These designations align  
73 with the amino acid sequence of the mature form of both the P99 AmpC (NCBI RefSeq Accession  
74 WP\_049134845.1 - originally characterized in an *Enterobacter cloacae* strain – now found to be an *E.*  
75 *hormaechei* strain – in GenBank Accession CAA30257.1), and *Escherichia coli* AmpC (NCBI RefSeq  
76 Accession WP\_001336292.1 – originally characterized in strain K-12 found in GenBank Accession  
77 AAC77110.1). While *E. coli* AmpC has historical significance as both the first  $\beta$ -lactamase reported (5)  
78 and the first class C  $\beta$ -lactamase sequenced, P99 maintains identical numbering of conserved motifs while  
79 the mature form begins with a natural residue one (6). Many other class C  $\beta$  lactamases, however, possess  
80 insertions and deletions that shift the numbering of the conserved residues, significantly complicating  
81 both nomenclature and comparisons between enzymes.

82 For this report, we analyzed 155 unique AmpC structures deposited in the Protein Data Bank  
83 (including 142 supported by 66 publications), and found that 129  $\beta$ -lactamase structures identify the  
84 catalytic serine as Ser<sup>64</sup> (123 naturally and 6 with alignment), 10 number from the beginning of the  
85 precursor form with the signal peptide included, and the remaining 16 number from the beginning of the  
86 mature form, but do not identify the catalytic serine as Ser<sup>64</sup> (of which 8 are not associated with a  
87 publication). Additionally, based on a literature search of PubMed, we found consistency is lacking for  
88 numbering within the various families of class C  $\beta$ -lactamases. As an example, since the term PDC  
89 (*Pseudomonas*-derived cephalosporinase) was coined in 2009 for the chromosomal AmpC of  
90 *Pseudomonas aeruginosa*, three different approaches have been used to number amino acid residues in  
91 this  $\beta$ -lactamase (7). These approaches include: *i*) direct numbering of residues beginning with the N-  
92 terminus of the precursor protein (7); *ii*) direct numbering of residues beginning with the N-terminus of

93 the mature protein (8); and *iii*) alignment-based numbering designed to maintain the conventional  
94 assignment of conserved residues and to simplify numbering for comparisons across families (9).  
95 Unfortunately, it can be unclear to readers which of the various schemes is being used in a given  
96 publication. As a result, authors may sometimes find choosing a numbering scheme and numerically  
97 designating a given residue problematic. Comparing findings from multiple publications may be made  
98 unnecessarily difficult; resolving ambiguity in assignment may be extremely challenging. For example, a  
99 reference to Gly at position 183 in PDC could refer to a site that is described as having a clinically  
100 relevant mutation if numbering begins with Met<sup>1</sup> of the precursor form, but would refer to a different  
101 glycine, 26 residues away, using alignment based numbering (10, 11).

102 To address this growing concern, we propose a numbering scheme to use consistently when  
103 referring to crystallographically equivalent positions in the mature form of any class C  $\beta$ -lactamase. We  
104 suggest the acronym “*SANC*” to name the scheme, for Structural Alignment-based Numbering of class C  
105  $\beta$ -lactamases, or else the simpler term “structural position.” In developing this numbering scheme, we  
106 adapted the approaches used by Ambler et al. for the class A  $\beta$ -lactamases (12) and Galleni et al. for the  
107 class B  $\beta$ -lactamases (13). We conducted an amino acid alignment of 32 AmpC  $\beta$ -lactamases, both  
108 chromosomal and plasmid encoded (**Supplemental Material**) and identified characteristic differences  
109 from P99 for each enzyme (**Table 1** Sequences were obtained from the National Center for  
110 Biotechnology Information Protein Database (14) and signal peptide cleavage sites were determined using  
111 Uniprot (or SignalP 5.0 for entries not present in Uniprot) (15, 16). Mature protein sequences were  
112 aligned using the MUSCLE algorithm (17) with default settings.

113 Consensus secondary structure (defined as a majority of structures in agreement for a given  
114 amino acid position) was determined based on comparisons of a representative structure of each of the ten  
115 AmpC  $\beta$ -lactamases for which one or more structures are available in the Protein Data Bank, specifically:  
116 ACT-1 (PDB: 2ZC7), ADC-7 (PDB: 4U0T), CMY-2 (PDB: 1ZC2), *E. coli* AmpC (PDB: 2BLS), FOX-4  
117 (PDB: 5CGS), MOX-1 (PDB: 3W8K), *Mycobacterium smegmatis* AmpC (PDB: 5E2H), PDC-1 (PDB:

118 4GZB), and TRU-1 (PDB: 6FM6). The consensus agrees with the secondary structure (or lack thereof) of  
119 P99 for just over 90% of residues. This consensus was used to annotate secondary structure, including  
120 stripes to indicate residues with an even split between two secondary structure types, and helix numbers  
121 on the alignment. Finally, a simple literature survey was conducted to determine residues belonging in  
122 either the consensus portion or fullest likely extent of the  $\Omega$ -loop or R2-loop, both of which are also  
123 annotated on the alignment. By including this structural information, we hope to both better correlate the  
124 numbering system with well-known structural features and to provide additional points of reference for  
125 those just beginning to work with AmpC structures.

126 The exact position of one insertion and one deletion within the alignment were manually adjusted  
127 (residue 203a by MUSCLE became 204a by structure to preserve a  $\beta$ -turn and the deletion of residue 247  
128 by MUSCLE became a deletion of residue 245 by structure to preserve an  $\alpha$ -helix) to ensure they  
129 occurred in structurally reasonable areas of both the consensus structure and ten source structures.

130 Amino acid numbering was based on *E. cloacae* complex P99 while preserving the conventional  
131 numbering of the following residues: Ser<sup>64</sup>, Lys<sup>67</sup>, Tyr<sup>150</sup>, and Lys<sup>315</sup>. Insertions relative to P99 were  
132 addressed by appending lowercase letter(s) to the number of the amino acid immediately preceding the  
133 insertion (e.g., 125a in PDC-1). Deletions relative to P99 were skipped, resulting in “ghost residues” (e.g.,  
134 ACC-1 has residues G115 and L117 with a deleted residue at 116). For mature enzymes with more C-  
135 terminal amino acid residues than P99, additional residues are assigned numbers in numerical order  
136 beginning with 362. For mature enzymes with more N-terminal amino acid residues than P99, the first  
137 additional residue is numbered 0 and subsequent residues are numbered by appending a lowercase letter  
138 to zero while moving in an N-terminal direction (e.g., 0 and 0a for BUT-1 and *Edwardsiella* AmpC).  
139 Signal peptide residues are assigned negative numbers, beginning with -1 for the residue adjacent to the  
140 cleavage site and proceeding in the N-terminal direction until all residues are numbered. Multiple  
141 sequence alignments are not considered for the signal peptide regions. **Figure 1** illustrates these principles  
142 with several examples.



143 Amino acid positions should be provided under both a family-specific, precursor-based scheme  
144 (precursor numbering) and the alignment based scheme (*SANC*) at first mention of a given residue in a  
145 publication. Authors are free to choose their favored convention for subsequent mentions, but as a general  
146 suggestion we encourage the use of *SANC* for biochemical and structural publications and precursor  
147 numbering for genetic and epidemiological publications.

148 Providing numbering under both schemes is essential to our proposal. Structural numbering  
149 maintains continuity with the conventional assignment of the catalytic serine as Ser<sup>64</sup> and the majority of  
150 existing literature on class C  $\beta$ -lactamase structure and function while precursor numbering enables direct  
151 gene translation and simplifies interpretation of sequencing results, particularly within a single family.  
152 Utilizing this hybrid approach, an initial description of a typical PDC variant might read “PDC-221  
153 differs from PDC-1 (GenBank AAG07497.1) by a single amino acid substitution, E247K, occurring at  
154 *SANC* position 219.”

155 In the supplementary materials, we provide a table featuring a multiple sequence alignment of 32  
156 class C  $\beta$ -lactamases with column headers indicating the appropriate number to be used at each position.  
157 The spreadsheet also features a text-based alignment of the structures used in determining the consensus  
158 secondary structure. Separately, we provide a protein profile hidden Markov model (HMM) which  
159 implements the *SANC* scheme, built from the multiple sequence alignment using HMMER  
160 (<http://hmmer.org>). Alignments of the HMM to class C  $\beta$ -lactamases can be expected to produce correct  
161 *SANC* assignments when results of the search are examined. We suggest using the HMM, rather than  
162 examinations by eye, to make position assignments under this scheme for novel AmpC enzymes that may  
163 be discovered in the future. Finally, basic instructions for using our HMM with the HMMER software are  
164 also included with the supplementary materials.

165 For the specific case of PDC variants, a database utilizing the three numbering schemes (*SANC*  
166 and both family-specific precursor and mature form numbering) is freely available at  
167 <https://arpbigidisba.com/pseudomonas-aeruginosa-derived-cephalosporinase-pdc-database/>

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<b>Class C <math>\beta</math>-Lactamase</b>	<b>NCBI Accession</b>	<b>Insertions and Deletions Relative to <i>E. cloacae</i> complex P99</b>
ACC-1	WP_032491956.1	-116, +204a, +247a, -289, -290, +362, +363
ACT-1	WP_063857727.1	-361
ADC-7	WP_063857816.1	+0, +204a, -245, -304, -305, -306, +362
ADC-8	WP_004923134.1	+0d, +0c, +0b, +0a, +0, -245, +362, +363, +364, +365, +366, +367

AQU-1	WP_099156042.1	-1, -2, +204a, -243, -245, -301, -302, +362
<i>B. multivorans</i> AmpC1	WP_012218336.1	+204a, -245, +362, +363, +364
BUT-1	WP_104531863.1	+0a, +0
CepH	WP_063843234.1	-1, -2, +204a, -243, -245, +362
CepS	WP_063843235.1	-1, -2, +204a, -243, -245, +362
CFE-1	WP_032490699.1	None
CMA-1	WP_032974004.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362
CMH-1	WP_063859580.1	None
CMY-2	WP_000976514.1	None
CSA-1	WP_007888761.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362
DHA-1	WP_004236386.1	-1, -2, -3, -4, -301
<i>E. coli</i> AmpC	WP_001336292.1	-1, -2, -3
EC-5	WP_001443153.1	-1, -2, -3
<i>Edwardsiella</i> AmpC	WP_041692555.1	+0a, +0
FOX-4	WP_032489727.1	-1, -2, +204a, -243, -245, +362
LHK-1	WP_081666691.1	-1, -2, -3, -4, +204a, -245
LRA-10	WP_099982803.1	-1, -126, +204a, -245, -361
LRA-18	WP_099982801.1	-1, -245, -311, +362, +363, +364, +365
<i>M. smegmatis</i> AmpC	WP_011729443.1	-1, -2, -3, -4, -5, -6, +204a, -245, -305, -306, +362
MIR-1	WP_032489464.1	None
MOX-1	WP_032489888.1	+0, +204a, -243, -245, -301, -302, -303, +362
OCH-1	WP_040129485.1	+0, +204a, -245, +362, +363, +364
PAC-1	WP_034051940.1	-1, -2, -3, -4, -5, -116, +204a, -245, +362

PDC-1	WP_003101289.1	+125a, +204a, -245, +362, +363, +364, +365, +366, +367, +368, +369, +370
SRT-1	WP_063864749.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362, +363
SST-1	WP_063864750.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362, +363
TRU-1	WP_042027926.1	-1, -2, +204a, -243, -245, +362

186 **Table 1:** Insertions and deletions present in the AmpC enzymes examined when compared to *E. cloacae*  
187 complex P99. Minus indicates a deletion and plus indicates an insertion. Appended letters indicate an  
188 insertion follows a given residue number.

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**Figure 1:** Using an alignment to assign SANC-based amino acid residue numbers. Positions corresponding to insertions and deletions are indicated in bold. ADC-7 adds residues 0, 204a, and 262 and deletes residues 245 and 304-306. ADC-8 adds residues 0-0d and 262-267 and deletes residue 245. BUT-1 adds residue 0. PDC-1 adds residues 125a, 204a, and 362-370 and deletes residue 245. For reference, signal sequences are highlighted in yellow, S<sup>64</sup>XXX in green, Y<sup>150</sup>XN in blue, and K<sup>315</sup>(S/T)G in red.

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SANC Position		-20		-10		0		10		20		30		40		50		60		70		80		90		100											
P99	-20	MMRKS	LCCALL	LGISCS	SALA	----	TPVSEK	QLAEV	VANTIT	PLMKA	QSVPG	MAVA	VIYQ	GKPHY	YTFG	KADIA	AANK	PVTP	QTLF	ELGS	ISKTF	TGVL	GGDAI	ARGE	ISLDD	AVTRY	WPQL	TGKQ	WQGI	104							
ADC-7	-23	MRFKK	ISCLLL	SPLF	IFST	SIYA	----	DNTPK	DQEI	IKLL	VQNF	KPLLE	KYDVP	GMAG	VIQNN	KKYE	MYGL	QSVQ	DKKAV	NSNT	IFEL	GSVSK	LFAT	AGGY	AKNG	KISF	DDTP	PKYK	WKLK	NTPI	DQV	104					
ADC-8	-26	MMKD	ILGNL	DVFP	KIMT	GCIAG	LLS	CGTVA	QSTV	QSQS	VDRH	FKPL	MAQY	ISGM	IAVS	INGH	YHYQ	NYGV	ASKQ	TEQN	VSEHT	LFEL	GSVSK	LFNAT	LTYA	QAQ	QQLK	LSDP	APQY	FPEL	KNTA	VNQ	104				
BUT-1	-20	MMKK	LCCAL	LVLS	SASF	SAFA	----	AQTL	SDKQ	LEDV	VNQTL	KPMI	TAQA	IPGM	AVAV	IYQ	GKPHY	TYGV	ADIA	KQP	VTTQ	TFEL	GSVSK	FTG	VLGG	DIVAR	GEVK	LSDP	ATKY	WPEL	TGKQ	WQGI	104				
PDC-1	-26	MRDR	FRPCL	CGIA	ASTL	LFP	AI	----	GEAP	ADRL	KALV	AAVQ	PMK	KAND	IPGL	AV	ISL	GK	EPHY	FSYGL	ASK	EDG	RRV	TPET	LFEI	GSVSK	FTAT	L	AGY	ALTQ	DKM	RLDD	RASQ	HWPAL	QGS	RFDGI	104

  

SANC Position		110		120		125a		130		140		150		160		170		180		190		200		204a		210		220		230																																																																																	
P99	105	RMLD	LATY	TAGG	LPLQ	VP	DEV	-	TDNAS	LLRF	YQNW	QPK	PGT	TRLY	ANAS	IGL	FGAL	AVK	PSG	MPYE	QAM	T	RVLK	PLK	LDHT	IN	VPK	AE	E	AHY	ANG	YR	-	DGK	AVR	VSP	GMLD	AQ	AYG	VK	TN	VQD	MAN	V	MAN	237																																																																	
ADC-7	105	NLLQ	LATY	TSG	NLAL	QF	P	DEV	-	QTDQ	QVLT	FFK	DKW	KPN	PIGE	YRQ	YSNP	SIGL	FGV	VVAL	SMNK	P	FDQ	VLE	KT	IF	PAL	G	LK	HS	V	N	V	PKT	Q	M	Q	N	Y	F	G	Y	N	Q	E	N	Q	P	I	R	V	N	P	G	L	D	A	P	A	Y	G	V	K	S	T	L	P	D	M	L	S	F	I	H	A	N	237																																		
ADC-8	105	TLLN	LGTY	TAGG	FPLQ	F	PEQ	-	KTTQ	DM	TQYF	QHW	QK	AP	RR	I	RE	YS	N	PSI	G	L	M	G	V	T	A	M	K	N	S	N	L	I	E	N	T	L	F	P	A	L	G	M	H	S	V	I	N	V	P	A	A	Q	M	S	N	Y	A	N	G	Y	-	A	D	Q	A	I	R	V	S	P	G	M	F	D	E	A	E	A	G	I	K	S	N	T	A	D	M	L	K	F	L	D	A	Q	237														
BUT-1	105	TLLD	LATY	TAGG	LPLQ	VP	DEV	-	DNQA	LLK	FYQ	NW	Q	PA	AP	G	T	R	R	Q	Y	A	N	S	I	G	L	F	G	A	L	V	K	P	S	G	M	T	F	N	D	A	M	R	Q	R	V	L	Q	P	L	N	L	K	H	T	L	V	P	A	S	E	N	H	A	N	G	Y	R	-	D	G	K	A	M	H	V	G	P	M	L	D	T	E	A	Y	G	V	K	S	T	I	E	D	M	A	S	H	V	Q	Y	N	237								
PDC-1	105	SLLD	LATY	TAGG	LPLQ	F	P	DEV	-	SDV	QDQ	QAQ	I	R	D	Y	R	Q	W	P	T	Y	A	P	G	S	R	L	Y	S	N	P	S	I	G	L	F	G	L	A	A	R	S	L	G	P	F	E	R	L	M	E	Q	V	F	P	P	A	L	G	L	E	Q	T	H	L	D	V	P	E	A	A	L	Q	Y	A	Q	G	Y	G	K	D	R	R	L	R	V	G	P	L	D	A	E	G	Y	G	V	K	T	S	A	A	L	L	R	F	V	D	A	N	237

  

SANC Position		240		245		250		260		270		280		290		300		304		308		310		320		330		340		350		360		370																																																																																													
P99	238	MAPEN	VAD	ASL	KQ	G	I	A	L	A	Q	S	R	Y	W	R	I	G	S	Y	Q	L	G	W	E	L	N	N	P	V	E	A	N	T	V	E	G	S	D	S	K	V	A	L	A	P	L	P	V	A	E	V	N	P	A	P	P	V	K	A	S	W	V	H	K	T	G	S	T	G	F	G	S	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	T	S	Y	P	N	P	A	R	V	E	A	A	H	I	L	E	A	L	Q	-----	361																
ADC-7	238	LNPQ	KYP	-	TDI	Q	R	A	I	N	E	T	H	Q	R	Y	Q	N	T	M	Y	Q	A	L	G	W	E	F	S	P	A	T	L	Q	T	L	L	D	S	N	S	E	Q	I	V	M	K	N	K	V	T	A	I	S	K	---	E	P	S	V	K	M	Y	H	K	T	G	S	T	S	G	F	G	T	Y	V	V	F	I	P	K	E	N	I	G	L	V	M	L	T	N	K	R	I	P	N	E	R	I	K	A	A	Y	V	L	N	A	I	K	-----	362																
ADC-8	238	INPQ	N	L	K	-	P	T	L	R	K	A	I	Q	T	T	M	G	Y	F	R	V	G	M	R	Q	L	G	W	E	Y	T	P	V	S	L	T	T	L	A	G	N	S	A	K	M	A	L	Q	P	V	T	G	I	S	K	P	I	T	A	P	Q	A	L	N	K	T	G	A	T	N	G	F	S	A	Y	V	V	I	P	S	Q	I	G	L	V	M	L	A	N	R	N	F	P	N	D	A	R	V	K	A	A	Y	A	T	L	Q	I	L	N	A	D	I	Q	K	---	367										
BUT-1	238	MNPQ	Q	V	K	Q	P	T	L	Q	G	L	E	L	A	Q	S	R	Y	W	R	S	G	S	Y	Q	L	G	W	E	L	N	N	P	V	A	A	A	T	V	I	N	G	S	D	N	K	V	A	L	A	A	S	P	V	A	I	E	P	P	V	A	P	V	K	A	S	L	V	H	K	T	G	S	T	N	G	F	G	A	Y	V	A	F	I	P	E	K	Q	I	G	I	V	M	L	A	N	K	M	Y	P	N	T	E	R	V	K	A	A	N	T	L	N	T	L	Q	-----	361									
PDC-1	238	LHPER	L	D	-	RP	W	A	Q	A	L	D	A	T	H	R	G	Y	Y	K	V	G	M	T	Q	L	G	W	E	A	Y	D	W	P	I	S	L	K	R	L	Q	A	G	N	S	T	P	M	A	L	Q	P	H	R	I	A	R	L	P	A	P	Q	A	L	E	G	Q	R	L	L	N	K	T	G	S	T	N	G	F	G	A	Y	V	A	F	V	P	G	R	D	L	G	V	I	L	A	N	R	N	Y	P	N	A	E	R	V	K	I	A	Y	A	I	L	S	G	L	E	Q	Q	K	V	P	L	K	R	-----	370