Class C β-Lactamases: Molecular Characteristics

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SUMMARY

Class C β-lactamases or cephalosporinases can be classified into two functional groups (1, 1e) with considerable molecular variability (≤ 20 % sequence identity). These enzymes are mostly encoded by chromosomal and inducible genes and are widespread among bacteria, including *Proteobacteria* in particular. Molecular identification is based principally on three catalytic motifs (^{64}SXSK, ^{150}YXN, ^{315}KTG), but more than 70 conserved amino-acid residues (≥ 90%) have been identified, many close to these catalytic motifs. Nevertheless, the identification of a tiny, phylogenetically distant cluster (including enzymes from the genera *Legionella*, *Bradyrhizobium*, and *Parachlamydia*) has raised questions about the possible existence of a C2 subclass of β-lactamases, previously identified as serine hydrolases. In a context of the clinical emergence of extended-spectrum AmpC β-lactamases (ESACs), the genetic modifications observed *in vivo* and *in vitro* (point mutations, insertions or deletions) during the evolution of these enzymes have mostly involved the Ω- and H-10/R2-loops, which vary considerably between genera, and, in some cases, the conserved triplet ^{150}YXN. Furthermore, the conserved deletion of several amino-acid residues in opportunistic pathogenic species of *Acinetobacter*, such as *A. baumannii*, *A. calcoaceticus*, *A. pittii* and *A. nosocomialis* (deletion of residues 304-306), and in *Hafnia alvei* and *H. paralvei* (deletion of residues 289-290), provides support for the notion of natural ESACs. The emergence of higher levels of resistance to β-lactams, including carbapenems, and to inhibitors such as avibactam is a reality, as the enzymes responsible are subject to complex regulation encompassing several other genes (*amp*R, *amp*D, *amp*G, etc.). Combinations of resistance mechanisms may therefore be at work, including

overproduction or change in permeability, with the loss of porins and/or activation of efflux systems.

KEYWORDS

AmpC β-lactamases, cephalosporinases, ESAC, extended-spectrum, phylogeny, primary structure

INTRODUCTION

β-lactamases remain an important natural or acquired mechanism of resistance to β-lactam antibiotics. New enzymes of this type, belonging to the molecular classes defined by Ambler and then completed by several authors (1-3), have been regularly discovered since the 1980s. The class C β-lactamases (BLCs), also known as AmpC or cephalosporinases, have a long history marked by the gradual loss of efficacy for the treatment of many bacterial infections, due initially to their large inactivation spectrum, including penicillins, the first cephalosporins (e.g. cephalothin), and cephamycins (e.g. cefoxitin), together with the general absence of an inhibitory effect of clavulanic acid, sulbactam and tazobactam (4-6). The next problem encountered was the emergence of constitutive or overproduced mutants, eventually overcome by the development of oxyiminocephalosporins, such as cefotaxime and ceftazidime (7). However, plasmid-borne cephalosporinases were subsequently discovered, particularly in species without chromosomal *amp*C genes (e.g. *Klebsiella pneumoniae, Salmonella enterica,* and *Proteus mirabilis*) and in *Escherichia coli,* which possesses an intrinsic *amp*C gene usually not expressed. These enzymes are derived from chromosomally encoded enzymes specific to other species, such as

Enterobacter cloacae and *Citrobacter freundii*, and their discovery raised new fears, allayed by the discovery of cefepime and cefpirome (4,8). However, a new step in resistance development was then detected, with the discovery of extended-spectrum β-lactamases AmpC (ESAC), mutants or variants with an extended inactivation spectrum for oxyiminocephalosporins (ceftazidime, cefotaxime, cefepime and cefpirome) due to the mutation of certain sites, in the R2-loop (9,10), for example, by substitution, deletion or insertion. Carbapenem resistance mediated by a combination of mechanisms, including a constitutive species-specific cephalosporinase and porin loss, has emerged more recently (11,12). Finally, the recent development of novel enzyme inhibitors, such as avibactam, has elicited considerable medical interest due to its ability to inhibit serine β-lactamases (classes A, C and D). However, this has already led to the selection of clinical mutants resistant to ceftazidime-avibactam combinations, most of them bearing deletions of various sizes in the Ω-loop region of AmpC (13,14), similarly to β-lactamases from other classes (e.g. KPC-3).

Various methods for detecting resistant clinical isolates have been proposed, particularly for resistance to oxyiminocephalosporins and carbapenems. These methods include phenotypic tests, enzymatic methods based on hydrolysis, immunochromatographic assays, and molecular tests designed to test for the presence of particular genes, encoding class C β-lactamases, for example (15). Whole-genome sequencing (WGS) is a very useful approach for the precise identification of mechanisms of resistance to β-lactams, and has provided a large body of sequence data. Nevertheless, genotype-to-phenotype extrapolations are not straightforward, due to the variability of gene expression and polymorphisms linked to silent mutations at diverse sites, depending on the β-lactamase

considered (16). Improvements in our knowledge should make it possible to improve analyses of the resistance mechanisms detected, particularly against β-lactams, in the future. These mechanisms are numerous and differ considerably between species. The current classification of β-lactamases into four molecular classes is based on motifs involved in binding and hydrolysis, such as ^{70}SXXK, ^{130}SDN and ^{234}KTG for class A (17), and on diverse residues involved in determining affinity, either increasing the inactivation spectrum (e.g. ESBLs) or decreasing it (e.g. IRT) (18-20). A more detailed comparative analysis of the primary structure of a large number of proteins would facilitate the classification of enzymes into groups of clusters displaying common structural features, as for the enzymes of class A (21). BLCs seem to have a lower level of structural diversity, but the abundance of data now available in databases [e.g. Beta-Lactamase DataBase (BLDB, http://bldb.eu/) (22), Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/313047) (23), CARD (https://card.mcmaster.ca/) (24)] suggests that a more detailed analytical approach, based on several thousand sequences, would now be justified.

When we began this analysis in 2019, several numbering schemes had been proposed for class C β-lactamases, which were unified in a standardized structure-based numbering scheme published in 2020 (25). Accurate comparisons of protein sequences have improved structural classification, by providing a clearer identification of polymorphisms by species, a more precise identification of the bacterium updated in line with the continual changes in taxonomy, a better understanding of the residues or zones involved in the possible extension of the inactivation spectrum by species or bacterial group, and with respect to both substrates and enzyme inhibitors, such as avibactam. The ACC-type β-lactamases

appeared to be plasmid-encoded enzymes originating from *Hafnia alvei* with an unusual susceptibility pattern characterized by resistance to expanded-spectrum cephalosporins such as ceftazidime, cefotaxime, and, sometimes, cefpirome, and by susceptibility to cefepime and inhibition by cefoxitin (see the section on "natural ESACs" for more details).

PHYLOGENETIC COMPARISON

The serine β-lactamases have been divided into three classes (A, C and D) based on sequence similarity (1,2,26). A protein structure-based phylogeny clearly distinguished between these classes (27-29). The amino acid-based phylogenetic tree based on 3943 class C sequences reveals large differences between the major groups (*Acinetobacter*, Aeromonadales, Burkholderiales, Enterobacterales, Pseudomonadales, Rhizobiales), each of which displays at least 24 % amino-acid sequence identity (Fig. 1).

Figure 1 here

Highly conserved and major clusters, including many variants displaying >75% sequence identity with each other, were found for the following genera: *Escherichia/Shigella*, *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Serratia.* Other clusters (*Acinetobacter*, *Aeromonas*, *Burkholderia*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Yersinia*, etc.) are less conserved, with a percent amino-acid identity between sequences of less than 50%. These findings highlight the need for more accurate taxonomic approaches in the future. Sequences from the *Proteobacteria* were particularly prevalent, but BLCs were widely distributed between bacterial groups, with the exception of Gram-positive bacteria,

in which they were rare (Table 1) (28,29). Finally, a separate cluster including several genera (e.g. *Bradyrhizobium, Legionella, Parachlamydia*) was identified and found to contain generic serine hydrolases and some carboxylesterases VIII with weak β-lactamase activity (Fig. 1).

Table 1 here

Table 2 here

PRIMARY STRUCTURE/SEQUENCE ANALYSIS

Highly conserved motifs and residues

A standardized amino-acid numbering scheme was recently developed, through collaboration, for class C β-lactamases (25). This numbering scheme has greatly facilitated molecular comparisons between class C enzymes. Indeed, "SANC" (structural alignment-based numbering of class C β-lactamases) preserves the usual numbering of the major catalytic residues (^{64}S, ^{67}K, ^{150}Y, and ^{315}K). Three highly conserved motifs are currently used to characterize this molecular class (^{64}SXSK, ^{150}YXN and ^{315}KTG) (17,28), but other conserved residues have been characterized and can be used for the more accurate identification of AmpC enzymes (Tables 2 and 3). They include 37 residues strictly conserved (100%) and 33 residues highly conserved (90-97%) in a dataset of 32 representative class C β-lactamases examined in a previous study (25). Proline (P) and aromatic (F, W, Y) residues are overrepresented at these positions compared to class A β-lactamases. Indeed, prolines are present at positions 18, 26, 94, 118, 122, 140, 192, 213,

277, 330, 345 for class C β-lactamases, but only at positions 107, 183 and 226 for class A β-lactamases. Similarly, strictly conserved aromatic residues are present at positions 60, 138, 150, 199, 203, 221, 271, 322, 325, and 328 for class C β-lactamases, but only at positions 66, 210 and 229 for class A β-lactamases. If aromatic amino acids (F, Y and W) are considered together, as a single category, additional conserved positions emerge for BLCs: 43, 69, 134, 135, 170, 188, 233, 260, 276 and 344 (Table 3).

Table 3 here

In a representative set of 50 typical AmpC (see Table 2), the total number of amino acids in these enzymes ranged from 358 (*P. fluorescens* TAE4) to 397 (PDC-1) with a mean value of 383 $\pm$ 5.2, and the mean of molecular mass was estimated at 41.64 $\pm$ 0.58 kDa. The number of amino acids may be much higher for enzymes with fused domains (29,64). AmpC enzymes typically have alkaline isoelectric points (pI), with a mean pI of 8.48 $\pm$ 1.22 for this representative set. Nevertheless, low predicted pI values were obtained for some enzymes (4.37 for CHR-1, 5.27 for BlaE, 4.59 for RHO-1 and 5.59 for IDC-1) (4,58).

Enterobacterales

The Enterobacterales (EB) is one of the major groups emerging from the phylogram in Fig. 1. This group was clearly separated into two major clusters based on the analysis of 413 protein sequences: EB1, including *Buttiauxella, Cedecea, Citrobacter, Edwardsiella, Erwinia, Escherichia, Enterobacter, Klebsiella aerogenes, Lelliottia, Morganella, Pluralibacter, Serratia fonticola, Yersinia,* and *Xenorhabdus,* and EB2, including

Budvicia, Cronobacter, Erwinia, Hafnia, Pantoea, Photorhabdus, Pragia, Providencia, Regiella, Rouxiella, Serratia, and *Siccibacter* (84). Most species generally cluster together in the same genus or cluster, but, surprisingly, several species (e.g. *Erwinia teleogrylli, S. fonticola* and *Y. ruckeri*) were located at some distance from their main clusters. The following conserved residues distinguished between the EB1 and EB2 groups: D47K, V65L, G71A, V72T, G74A, W101F, Y112H, Q120F, S154G, F158L, W201Q, A255T, R258G, G270M, and H314N. A deletion in position 116 and an insertion in position 204a were also identified in EB2 (25,85). These groups displayed a high degree of diversity (e.g. 55-100% sequence identity for the whole EB1 group). Consensus sequences (CS) were therefore determined for the main species.

For *Escherichia* species (*E. coli/Shigella*, *E. albertii,* and *E. fergusonii*), sequence identity ranged from 88 to 100% and 11 consensus sequences were obtained from 367 protein sequences. The *E. coli sensu stricto* population is now considered to have a strong phylogenetic structure, with the identification of at least 12 phylogroups (A, B1, B2, C, D, E, F, G, H, clade 1, clade III and clade IV) (86).

Several conserved residues were found to be specific for phylogroups A, A1 and B1 (^{235}R, ^{238}M, ^{239}N, ^{241}R, ^{245}N), whereas others were specific for phylogroups B2 and D (^{235}Q, ^{238}L, ^{239}K, ^{241}L, ^{245}N/T) (16). All four clusters (A-D) carry RMNRE residues in the 235-245 region (85), and this key feature was observed in clinical isolates able to generate extended-spectrum AmpC β-lactamases (ESACs), as described above. The ^{287}SGN triplet is present in the sequence of such enzymes (87). Phylogroup B2 contains the specific conserved residues ^{175}K, ^{193}S, ^{282}I, ^{288}D, ^{296}R and ^{300}P, and is separated from phylogroup D by the residues ^{185}T and ^{244}T, in particular.

Finally, 78 protein sequences from uncultured bacteria obtained by metagenomic analyses on human fecal and environmental samples, originally found in the NCBI database as class C CMY-LAT-MOX-ACT-MIR-FOX β-lactamases, belong to this major cluster. They have been assigned to *E. coli*, *Shigella*, *E. fergusonii*, or *E. albertii*, and are characterized by a typical triplet (^{6}HSE) (88-90).

One particular feature of interest is that all the protein sequences obtained from the genus *Hafnia* (*H. alvei*, *H. paralvei*) carry a two-residue deletion (residues 289-290) in H-10 and the R2-loop. The plasmid-encoded enzymes ACC-1 and ACC-4 are more than 99.4% identical to the AmpC of *H. paralvei*, a newly identified bacterial species (91,92).

Aeromonas

The second major group identified included 318 protein sequences from *Aeromonas*, a genus for which many new species have been discovered over the last two decades. This is also the group in which the first inducible cephalosporinases (AsbA1, CAV-1, CepH, CepS) were detected, in *A. hydophila* (43), *A. jandei* (93), *A. sobria* (94) and *A. caviae* (41). The group is highly diverse, with a sequence identity ranging from 50 to 100%. Thirty-six species from this group have been identified, including about twenty species pathogenic to humans, such as *A. caviae*, *A. dhakensis*, *A. veronii*, and *A. hydrophila* (95). Phylogenetic analysis identified two CS for *A. caviae*, *A. dhakensis*, *A. hydrophila*, and *A. salmonicida*, with sequence identities of 80 to 100%, suggesting that new species may be discovered in the future (96,97). Some of these species displayed a particular primary structure modification: the deletion of two or three residues (positions 301 to 303) in the R2-loop, as in *A. caviae* and in *A. dhakensis* (25).

Acinetobacter

Genus *Acinetobacter* has a long history of taxonomic changes, but is dominated by *A. baumannii*, the principal genomic species, which plays a major role in nosocomial infections, particularly in intensive care units (98,99). This major group is heterogeneous, with 500 protein sequences and 26 species, and with sequence identities ranging from 42 to 100 % (100). The denomination ADC for "*Acinetobacter*-derived cephalosporinase", followed by a number to distinguish between individual enzymes, was proposed because of the large numbers of *A. baumannii* variants or members of the Abc complex (101) (http://bldb.eu/BLDB.php?prot=C#ADC). In a previous analysis of 103 genomes, the *bla*$_{\text{ADC}}$-like gene was found to be present in 13 validly named species, eight genomic species and six taxa (102). Three major clusters were identified: one cluster including the *A. baumannii-A. calcoaceticus* or Abc complex and a number of other species: *A. lactucae* (formerly known as *A. dijkshoorniae*), *A. nosocomialis*, *A. oleivorans*, *A. pittii*, and *A. seifertii*, a second cluster containing nine species: *A. apis*, *A. albensis*, *A. bereziniae*, *A. bohemicus*, *A. celticus*, *A. guillouiae*, *A. johnsonii*, *A. kyonggiensis*, and *A. rudis*, and a third cluster including a number of other species: *A. baylyi*, *A. beijerinckii*, *A. gyllenbergii*, *A. haemolyticus*, *A. junii*, *A. proteolyticus*, *A. parvus*, *A. soli*, *A. ursingii*, *A. tjernbergiae*, and *A. venetianus* (100). A large number of protein sequences were initially misidentified, for two reasons. The first was the substantial taxonomic modifications that had taken place and the second was the preferential use of standard biochemical methods and automated systems or devices widely used in clinical bacteriology laboratories, such as API 20NE, VITEK 2, Phoenix, Biolog, and MicroScan WalkAway, potentially leading to incorrect

identification. MALDI-TOF can facilitate correct identification of the members of the Abc complex to species level, provided that an accurate database is used (15,99). Accurate identification of the clinically important members of the Abc complex (*A. baumannii*, *A. pittii*, *A. nosocomialis*, *A. seifertti* and *A. dijkshoorniae*) and *A. calcoaceticus*, which is considered pathogenic, is possible only by molecular methods such as DNA-DNA hybridization (gold standard method) and DNA sequence-based analysis on various types of DNA sequences (16S rRNA, *rpoB*, 16S-23S intergenic spacer) (15). The 16S rRNA sequencing method is highly reliable at genus level, but discriminates poorly between species. By contrast, *rpoB* genes are highly variable and considered appropriate for *Acinetobacter* species identification. The multiplex PCR based on species-specific genes, such as *gyrB*, is simple, rapid (results obtained within 2 hours), and reproducible, but limited to major species: *A. baumannii*, *A. nosocomialis*, *A. pittii* and *A. calcoaceticus*.

One structural feature distinguishing these clusters was a conserved deletion of three residues in the R2-loop (residues 304-306) for the first two clusters (103,104). The third cluster, including *A. baylyi* producing ADC-8 with a low susceptibility pattern, particularly for cephalosporins, did not display this deletion (104,105). ADC-1 and ADC-68, naturally produced by *A. baumannii*, carry this deletion of three residues in the R2-loop (residues 304-306) and were considered to be extended-spectrum AmpC (ESACs). Such enzymes typically hydrolyze penicillins and narrow- and expanded-spectrum cephalosporins and aztreonam, but not cefepime. Acquired resistance to cefepime or cefpirome has been reported to be associated with amino-acid substitutions — R148Q (close to YXN), V211A (within the Ω-loop), and N287S (in helix H-10) — also conferring higher levels of resistance (4 to 64 times higher) to ceftazidime and cefotaxime (106,107). Ceftazidime

resistance resulted from overproduction of the ADC β-lactamase and the provision of strong promoter sequences by the insertion sequence IS*Aba*1 (106,108).

In conclusion, the complexity of this bacterial group suggests that other species are likely to be identified, particularly given the very small size of some of the clusters identified.

Pseudomonas

A phylogenetic analysis of 582 class C protein sequences from *Pseudomonas* yielded a broad distribution, with at least 36 clusters. Sequence identity varied considerably, from 42 to 100 %, between the 33 species producing class C β-lactamases from this group. Heterogeneity levels were particularly high for some species, such as *P. putida*, for which at least 11 clusters were observed, and for *P. fluorescens*, suggesting misidentification, as reported by subsequent comparative studies of genomes (109-114). Indeed, the *Pseudomonas* group is among the most diverse in terms of the species it contains, but, with the exception of *P. aeruginosa*, for which 430 sequences have been analyzed, its taxonomy is still under revision.

P. aeruginosa is one of the principal pathogens isolated from immunosuppressed patients and cases of hospital-acquired infections associated with multiresistance (115). It also causes chronic lung infections in patients with cystic fibrosis (116). The effective treatment of *P. aeruginosa* infections is challenging, because several mechanisms of resistance to antibiotics, including β-lactamases and efflux pump overexpression, have evolved in this species (117). An inducible chromosomal AmpC-type enzyme was identified, with a wild-type inactivation spectrum including various β-lactams, such as aminopenicillins, the oldest cephalosporins, and cephamycins. Following its overproduction due to mutations

altering the peptidoglycan recycling process, this cephalosporinase is a major source of resistance to ticarcillin, piperacillin, ceftazidime, and aztreonam (118,119). However, additional missense mutations have extended its inactivation spectrum to cefepime and cefpirome. Many variants, also observed as natural polymorphisms, have been named according to a nomenclature specifically developed for *P. aeruginosa*, as PDCs (*Pseudomonas*-derived cephalosporinases) (118,119). A phylogenetic analysis of 430 sequences displaying 87 to 100 % identity yielded two clusters (C1, C2). The main cluster (C1), containing 416 protein sequences, had a consensus sequence with several variants (120). The second cluster (C2) was very small, comprising only 14 sequences. The polymorphism observed in cluster C1 had already been described in 44 antibiotic-susceptible strains with amino-acid substitutions at positions 53, 71, 79, 149, 178, and 365 (119). However, other variants were observed, in particular in positions 121, 153, 174, 178, 213, 216, 219, and 293, which served as mutation hotspots, thus extending the substrate specificity of PDC β-lactamases (see the section on ESACs).

Plasmid-encoded enzymes

Chromosomal class C β-lactamases predominate, but plasmid-mediated enzymes are nevertheless important, and the protein sequences of 256 such enzymes were examined. Since their emergence in 1989, several types of plasmid-encoded AmpCs and their variants have been identified: ACC, ACT, CFE, CMY-2/BIL/LAT, DHA, FOX, MIR and MOX/CMY-1 (4,8,22). CMY-2 is the most common plasmid-encoded enzyme family, detected worldwide in isolates that do not naturally produce a cephalosporinase (*P. mirabilis*, *K. pneumoniae* and *S. enterica*, in particular), and also in *E. coli*, which has a

weak expression of the natural cephalosporinase. This family displays little variability, with only about 8% of amino acids varying in a comparison of 92 sequences, including BIL-1 and LAT-1. Other families varied in terms of the number of protein sequences and percent identity: ACC (2 and 99 %), ACT (10 and 86 %), CFE (2 and 92 %), DHA (15 and 97 %), FOX (13 and 94 %), MIR (5 and 99 %), MOX/CMY-1 (10 and 75 %). Since the 1990s, many modifications have been made to bacterial taxonomy, with the definition of new bacterial species, particularly in the genera *Citrobacter*, *Enterobacter*, and *Aeromonas*. Finally, phylogenetic analysis confirmed several changes in the identification of the progenitors for chromosomal and plasmid-encoded cephalosporinases (25,121-124) (Table 4). The rare class C β-lactamases SLC-1 and PAC-1, encoded by a gene in a chromosome-inserted Tn*1721*-like transposon, probably originate from an enterobacterium, but the origins of other enzymes (integron-encoded IDC-types, Alaska soil metagenomes LRA10-1, LRA13-1, LRA18-1) remain unknown (58,64,74,75).

Table 4 here

Serine hydrolase, a class C β-lactamase or a carboxylesterase VIII ?

BLCs are usually genus-specific and chromosomally encoded, found mostly in Gram-negative bacteria, and more specifically in α-, β- and ɣ-*Proteobacteria*. However, several clusters (e.g. *Legionella*, *Bradyrhizobium*, *Parachlamydia*) are clearly divergent, with low levels of sequence identity (between 10 and 20 %) (28) (Fig. 1). This early divergence is related to a limited number of highly conserved motifs or residues, most reported in Table 3: ^{43}Y/G, ^{54}V/T, ^{58}T, ^{60}F/E, ^{63}A/G, ^{64}SXXK, ^{94}P, ^{96}L, ^{109}L, ^{111}T, ^{138}W, ^{140}P, ^{145}G, ^{148}R/Y,

^{150}YXN/H, ^{187}T, ^{202}G/Y, ^{229}D, ^{271}W, ^{315}KXG, ^{330}P, ^{335}G, ^{337}V, ^{339}L, ^{341}N, ^{357}L and ^{360}L. Several bacteria from *Bacteroidetes* and *Firmicutes* (e.g. *Pelosinus fermentans*, *Sediminibacterium salmoneum*) also carry enzymes with some of these highly conserved residues and motifs: ^{27}G, ^{44}G, ^{58}T, ^{60}F, ^{61}E/F, ^{63}G, ^{64}SXXK, ^{67}K, ^{94}P, ^{115}G, ^{144}G, ^{150}YXN, ^{200}A, ^{202}G, ^{220}A/S, ^{222}G/S, ^{229}D, ^{315}K/HT/SG, ^{319}T, ^{321}GF, ^{337}V and ^{341}N. These enzymes are generally identified as serine hydrolases in databases, but with no additional bacteriological and enzymatic details that could be used to determine whether or not they should be considered as class C β-lactamases.

One family of enzymes (microbial carboxylesterases VIII, EC 3.1.1.1) mostly identified in metagenomic libraries from various environmental samples, including EstC, EstM-N1/N2, Est22, EstU1 and EstSRT1, appears to be phylogenetically and structurally related to BLCs (Fig. 1). These enzymes generally hydrolyze nitrocefin (EstC), cephalothin (Est22), cephaloridine, cefazolin (EstU1), and oxyiminocephalosporins (EstSRT1) (131-137). The EstB and Est22 enzymes selectively deacetylate cephalosporin-based substrates leaving the amide bond of the β-lactam ring intact (136,137), while for EstU1 it was not clear from the HPLC data if the observed pattern against cephalosporin substrates was due to deacetylation or amide bond hydrolysis of the β-lactam ring (131). The catalytic efficiencies of EstSRT1 for cephalothin, cefotaxime, and cefepime are similar to that of EstU1 for cefazolin (131). These enzymes bear the catalytic nucleophilic motif ^{100}S, ^{103}K and ^{218}Y (^{64}S, ^{67}K and ^{150}Y according to SANC numbering), as well as other residues highly conserved in BLCs (Table 3). They have an overall structure consisting of a mixed α/β domain and a small helical domain, similar to that of class C β-lactamases (134,138-140).

The classification of these carboxylesterases as BLCs does not appear to be appropriate, given the total absence of β-lactamase activity for some of these enzymes, their low levels of *in vitro* antibacterial activity against β-lactams, including cephalosporins, their primary structure and their low levels of sequence identity to genuine BLCs. Nevertheless, these findings suggest that the promiscuous β-lactamase activity of some of these family VIII esterases may have evolved from BLCs or *vice versa*, and that some may have closer evolutionary relationships to BLCs (133).

SECONDARY AND TERTIARY STRUCTURES

The first crystallographic structure of an AmpC was reported in 1994, for the covalent complex between a phosphonate transition-state analog and the *E. hormaechei* P99 (formerly known as *E. cloacae* P99) cephalosporinase, which belongs to the ACT family (141). Many structures from different families of class C β-lactamases have since been published, including ACC (142,143), ACT (141,144-156), ADC (103,157-162), CMH (163), CMY (154,164-168), EC (165,169-206), FOX (207-209), MOX (210,211), PDC (212-221) and TRU (222) enzymes, together with the halophilic β-lactamase (HaBLA) from *Chromohalobacter* sp. 560 (47) and the class C β-lactamase from a psychrophilic organism, *Pseudomonas fluorescens* (223). More than 200 structures of class C β-lactamases have been described, and an updated list of these structures can be found in the Beta-Lactamase DataBase (BLDB, http://bldb.eu/S-BLDB.php) (22).

The typical three-dimensional structure of class C β-lactamase contains two mixed α/β domains: one composed of nine antiparallel β-sheets and three α-helices (H1, H10 and H11) on one side, and the other, composed of three small antiparallel β-sheets and eight α-

helices (H2 to H9) on the other side (Fig. 2). Two additional structural elements important for the interaction with substrates, Ω-loop and R2-loop, are located between H6 and H8 (residues 189-225), and close to H10 (residues 288-309), respectively (see ref. (25) for a detailed analysis of residues involved in these regions). Structurally, class C β-lactamases belong to the *Beta-lactamase* PFAM family (PF00144) and share the same overall fold with class A and class D β-lactamases, penicillin-binding proteins (PBPs) and family VIII carboxylesterases.

Figure 2 here

REGULATION AND EXPRESSION OF CLASS C β-LACTAMASES

In some Enterobacterales (other than *E. coli*) and *P. aeruginosa*, *ampC* gene expression is weak and inducible. This induction involves several proteins (AmpR, AmpD and AmpG) and two muropeptides (4,224-227). Nevertheless, the molecular mechanisms mediating AmpC overproduction in *P. aeruginosa* appear to be more complex than initially thought, as other proteins, such as AmpDh2, AmpDh3, and DacB (PBP4), have since been identified as involved in these mechanisms (228-230).

Genetic context and regulation

The *ampR* and *ampC* genes are linked. AmpR is encoded by a gene located immediately upstream from *amp*C and acts as a transcriptional activator, binding to the intercistronic region upstream from the *amp*C gene promoter. The *ampD* gene encodes an *N*-acetyl-anhydromuramyl-L-alanine amidase involved in recycling the products of peptidoglycan catabolism, including, in particular, the 1,6-anhydro-*N*-acetylmuramyl-tripeptide

(anhNAM-tripeptide). AmpD releases the tripeptide, which is then recycled for synthesis of the UDP-NAM pentapeptide (uridine 5'-pyrophosphoryl-*N*-acetylmuramic acid-pentapeptide), for integration into the neo-peptidoglycan. There is therefore a permanent balance between these two components in the cytoplasm.

Under normal conditions, AmpR associates with the UDM-NAM pentapeptide, and the resulting complex binds to the intercistronic region, thereby preventing transcription from both the *amp*R and *amp*C promoters. However, the presence of excess anhNAM-tripeptide alters this binding and promotes an increase in *ampC* transcription. Two clinical situations can result in an excessive increase in anhNAM-tripeptide levels: substantial degradation of the peptidoglycan due to the presence of β-lactams in the periplasmic space, and changes in the AmpD amidase preventing the recycling of this component.

In *E. cloacae* complex and *C. freundii*, the main cause of *ampC* overexpression is represented by amino-acid substitutions in AmpD, resulting in the constitutive production of large amounts of AmpC and increasing resistance to cefotaxime and ceftazidime. Several resistant clinical isolates or *in vitro* mutants displaying AmpC overproduction have been identified in the Enterobacterales, including *C. freundii* and *E. cloacae* with various amino-acid substitutions (e.g. W7G, H34A, S37R, Y63F, R80H, G82C, A94P/V, Y102, E116A, L117R, R108 D121G, D127G, N150I, H154N, A158D, K162H/Q, D164E/A, W171, A172L) capable of triggering constitutive β-lactamase production (231-235). In some cases, the *amp*D gene is truncated by a premature stop codon or an IS1 insertion, or an IS*4321* element may be inserted into the promoter (231,236,237).

In *P. aeruginosa*, acquired resistance to amino- and ureido-penicillins, cephamycins, and, to a lesser extent, to oxyiminocephalosporins (ceftazidime, cefotaxime, ceftriaxone) and

464 aztreonam, is very often related to overproduction of the PDC β-lactamase, which is also
465 linked to virulence (238). Several variants with *ampD* mutations displaying resistance *in*
466 *vivo* and *in vitro* have been reported (239-242). As in the Enterobacterales, the principal
467 genetic mechanisms involved missense mutations creating amino-acid substitutions
468 (A84G, D61Y, G148A, A136V, G148A, S175L) or insertion sequences (IS*1669*).
469 The LysR-type transcriptional regulator AmpR was initially identified as involved in
470 regulating the inducible class C β-lactamases produced by various Gram-negative rods,
471 such as *C. freundii, E. cloacae, S. marcescens* and *P. aeruginosa*. However, recent results
472 indicate that in *P. aeruginosa* AmpR regulates the expression of many genes involved in
473 other pathways, such as quinolone resistance, quorum sensing and associated virulence
474 phenotypes (243). Phylogenetic analyses revealed the presence of AmpR homologs in
475 many α-, β-, and γ-*Proteobacteria*, and several highly conserved residues were found in *C.*
476 *freundii, E. cloacae,* and *P. aeruginosa* (243,244). AmpR loss leads to susceptibility to β-
477 lactams, whereas constitutively high levels of *ampC* expression were reported for clinical
478 isolates and *in vitro* strains with mutations resulting in AmpR amino-acid substitutions: *C.*
479 *freundii* (S35F, G102D/E, D135A,Y264N) (4,245,246), *E. cloacae* (T84I , R86C,
480 D135N/V, E274K) (231,247), and *P. aeruginosa* (A12R, D135N, G154R, G237A)
481 (239,248-250). Transposon mutagenesis of *P. aeruginosa* strain PAO-1 and
482 complementation experiments have generated two mutants with stronger β-lactam
483 resistance due to transposition into two new genes (*mpl*, *nuoN*) (251).
484 AmpG, an intrinsic membrane protein, displays permease activity mediating the transport
485 of muropeptides from the periplasm to the cytoplasm, such transport being essential for the
486 induction of class C β-lactamases (252-254). Deletion of the gene encoding this protein in

P. aeruginosa results in a lower level of bacterial resistance to ampicillin. AmpG proteins are widespread and highly variable in Gram-negative bacteria (e.g. Enterobacterales, *A. baumannii*, *P. aeruginosa*). Genetic experiments showed that *amp*G genes from *E. coli* and *A. baumannii* can complement AmpG function in *P. aeruginosa* (253). In this species, the site-directed mutagenesis of some highly conserved AmpG residues (G29A, G29V, A129V, A197S and A197D mutants) results in a loss of resistance to ampicillin; *amp*G mRNA levels were found to be normal for two other mutants (A129T and A129D), but the proteins encoded had much lower levels of activity (253).

AmpC overproduction in *E. coli*

In *E. coli*, in the absence of the *amp*R gene, the expression of *amp*C is usually weak, under the control of a naturally non-efficient promoter (4). In this species, acquired resistance to old or narrow-spectrum cephalosporins, cephamycins (e.g. cefoxitin), or even oxyiminocephalosporins, is related to *ampC* overexpression due to the selection of a stronger promoter by mutation, or, less often, by insertion (255,256). The most important factors responsible for strengthening the *amp*C promoter are mutations creating a consensus -35 box (TTGACA) by T/A transversion at position -32 or C/T transition at position -42, and base-pair insertions increasing the distance between the -35 and -10 boxes to 17 or 18 bp (4,256,257). Mutations have also been identified within the attenuator region and the -10 box, but such mutations have little effect on *amp*C expression. Similar promoter mutations have been reported in strains from livestock, such as pigs or calves (258,259).

AmpC overproduction in *A. baumannii*

Overexpression of the chromosomal *ampC* gene in *A. baumannii* was observed following the acquisition of an IS element, mostly IS*Aba1*, resulting in a strong promoter and

resistance to expanded-spectrum cephalosporins (cefotaxime, ceftazidime) and aztreonam (260).

Transferable β-lactamases

Most plasmid-encoded class C β-lactamases mobilized by transposons and insertion sequence elements, with the exception of the ACT-1, CFE-1, and DHA-types, are in a "derepressed" state (45,58,261,262). The genes encoding the ACC-1 and CMY-2 types are transcribed under the control of a strong promoter in the mobile element (IS*Ecp1* or *IS26*) (263,264). The high level of $bla_{\text{MIR-1}}$ gene expression is due to the presence of a more efficient hybrid promoter upstream from the natural promoter (265). All these genes are often associated with integrons inserted in close proximity and forming composite structures with other transposable elements, including a gene cassette in a class 1 integrons characterized by rapid spread (58). Gene cassettes are under the control of a constitutive promoter specific for the gene cassette array. Their expression level is determined not only by promoter strength, but also by distance from the promoter (266). The removal of *ampC* gene cassettes from the integron may constitute a less costly control mechanism than the continuous overproduction of many plasmid-borne class C enzymes (267). However, three plasmid-encoded enzyme types with an *amp*R gene (ACT-1, CFE-1, DHA-1) and a genetic organization identical to those of chromosomal enzymes rarely cause resistance to oxyiminocephalosporins despite constitutively high levels of AmpC activity due to an amino-acid substitution (D135A) (243,246).

In conclusion, given the complexity of the regulation of *ampC* expression, the variability of these genes in databases and the small number of clinical examples of acquired resistance with the identification of hot spots for at least *amp*R, *amp*D and *amp*G, it is not

currently appropriate to analyze such mutants by genomic procedures, particularly in the absence of the initial susceptible clinical isolate. Classical methodologies are preferable for the detection of AmpC β-lactamase overproduction. These methods include phenotypic approaches assessing synergy between a cephamycin or an oxyiminocephalosporin and an inhibitor, such as cloxacillin or boronic acid (4). Nevertheless, the determination of β-lactamase activity with or without imipenem or cefoxitin induction, together with determinations of the mRNA levels for these genes by real-time RT-PCR, is probably much more accurate (119,253,268-270).

ACQUIRED EXTENDED-SPECTRUM CEPHALOSPORINASES (ESACs)

In Gram-negative bacteria, such as the Enterobacterales, various genetic events may increase the level of resistance, particularly to oxyiminocephalosporins, such as ceftazidime, cefotaxime, and, to a lesser extent, cefepime, and cefpirome. The evolution of such resistance led to a new denomination: ESAC, for extended spectrum AmpC β-lactamases (271). The first ESAC was identified in an *E. cloacae* isolate (GC1) in Japan in 1992, based on the duplication of three amino acids at positions 208-210 (Ω-loop) and constitutive production (10).

In *E. coli*, AmpC is constitutively produced in small amounts in the absence of the *amp*R gene, under the control of a weak promoter (4). Within this species, acquired resistance to old or narrow-spectrum cephalosporins (e.g. cefoxitin) is related to the overproduction of AmpC due to the selection of a strong promoter generated by mutation, or, less often, by insertion (255,256). A second step in the development of resistance involved the acquisition of plasmid-encoded AmpCs with a higher level of resistance to ceftazidime and

aztreonam, for example (8). Finally, an even higher level of resistance (e.g. to cefepime or cefpirome) was developed following the emergence of an ESAC at low prevalence ($\leq$ 1 %) in human clinical isolates and even isolates from cattle (16,272-274).

The genetic determinism of ESACs, leading to an increase in the catalytic efficiency of these enzymes against extended-spectrum cephalosporins, is based principally on missense mutations resulting in amino-acid substitutions, with structural modifications to the R1 (Ω-loop between residues 189 and 225) or R2 (H10 helix between residues 280 and 292 and/or R2-loop between residues 286 and 310) binding sites. Insertions or duplications in H10 or the R2 loop were also observed (Table 5).

Table 5 here

Most ESACs belong to phylogenetic group A, but others belong to phylogenetic group B1 and are characterized by the presence of the following conserved residues: ^{235}R, ^{238}M, ^{239}N, ^{241}R, ^{245}D, ^{281}S, ^{288}G and ^{296}H (16,87,273,276). Nevertheless, two isolates with different residues in these positions have been reported (281). A single *E. coli* strain (HKY28) isolated from urine in Japan was found to produce an ESAC with a tripeptide deletion (^{286}GS^{289}D) altering binding to extended-spectrum cephalosporins, but also to sulbactam and tazobactam (277) (Table 5). Reversion of the deletion through a nine-base insertion restored the typical inhibitor-resistant phenotype of class C enzymes and decreased the level of resistance to cefepime and cefpirome. Finally, other positions affecting the ESAC phenotype were identified by the selection of mutants *in vitro* or by site-directed

mutagenesis, resulting in a greater structural flexibility or affinity for extended-spectrum cephalosporins (195,320).

Many BLCs (e.g. those of *Pseudomonas, Citrobacter, Enterobacter, Morganella,* and *Serratia*) are inducible, opening up possibilities for higher levels of production, resulting in stronger resistance to aminopenicillins, old cephalosporins and ceftazidime or cefotaxime, and even to cefepime if the mutated enzyme is overproduced (321). Additionally, a significant proportion of strains has constitutive overproduction of class C enzymes, often after selection with β-lactam antibiotics. However, the risk of transitions between susceptibility and intermediate resistance (S/I) and between susceptibility and resistance (S/R) to cefepime is species-dependent. It is particularly high for the *E. cloacae* complex (66.3%), non-negligible for *H. alvei* (36.4%), moderate for *Citrobacter* and *Proteus* (18.1-21.9%) and *S. marcescens* (12.3%), low for *K. aerogenes* (1.1%) and inexistent for *M. morganii* (0%) (322). As summarized in Table 5, expansion of the inactivation spectrum results from missense mutations at several mutation hotspots (around triplet ^{150}YAN, Ω-loop, H10-helix and R2-loop), from duplications or deletions of 2-4 amino acids in the H10 helix (position 293), or insertions of amino acids into the Ω-loop. In some cases, two such events may be combined (Table 5). Various examples of increased catalytic efficiency (k_{cat}/K_m) of these enzymes, particularly against extended-spectrum cephalosporins (ceftazidime, cefotaxime, cefepime), are illustrated in Table 6.

Table 6 here

In *P. aeruginosa*, acquired resistance to amino- and ureidopenicillins, cephamycins, and, at low levels, to oxyiminocephalosporins (ceftazidime, cefotaxime, ceftriaxone) and monobactams (aztreonam), is frequently related to overproduction of the AmpC β-lactamase (14,118,238,240,319). ESACs emerged in *P. aeruginosa* several years after Enterobacterales, and such enzymes were identified mainly from clinical sources, but also from *in vitro* studies. They differ from the wild-type AmpC of *P. aeruginosa* by various amino-acid substitutions, deletions or insertions in four regions in the vicinity of the active site: the Ω-loop, the H10-helix, the H2-helix, and the C-terminal end of the protein (119,318,319) (Table 5).

Genus *Aeromonas* may have been the origin of several chromosomal (e.g. MOX-3, MOX-10) or plasmid-encoded β-lactamases (e.g. MOX-1, MOX-2, CMY-1, CMY-8, CMY-19) identified on several occasions from clinical isolates, mostly in Enterobacterales (e.g. *K. pneumoniae* and *E. coli*). The first plasmid-encoded enzyme, MOX-1, was identified in a *K. pneumoniae* isolate with a high level of resistance to various broad-spectrum β-lactams, including moxalactam, flomoxef, ceftizoxime, cefotaxime, and ceftazidime (323). According to its kinetic parameters, cephalothin was its ideal substrate, and it had good activity against benzylpenicillin, but poor activity against cloxacillin and piperacillin. Moxalactam and cefoxitin were also hydrolyzed, but ceftazidime and cefepime were poor substrates, with very high K_m values (Table 6). Finally, aztreonam was found to inhibit MOX-1 (69). The X-ray crystallographic structure of MOX-1 suggested that residues 303-306 show a significant structural flexibility, possibly underlying the unique substrate profile of this enzyme, which can hydrolyze penicillins, cephalothin, expanded-spectrum cephalosporins, cefepime and moxalactam (210). The position of H10-helix in both MOX-

623 1 and CMY-10 is shifted further away from the catalytic serine residue compared with the
624 AmpCs from *E. coli*, *E. hormaechei* P99 (formerly known as *E. cloacae* P99) and *E.*
625 *cloacae* GC1, whereas that of the AmpC from *P. aeruginosa* occupies an intermediate
626 position. Such structural features may underlie the extended substrate profile of MOX-1
627 and CMY-10, which are also considered to be ESBLs, but have never been called ESACs
628 (164,210). Finally, the susceptibility profile of these enzymes is characterized by a high
629 level of hydrolysis for cephamycins, oxacephems (e.g. cefoxitin) and moxalactam,
630 increased resistance to cefotaxime compared with ceftazidime and aztreonam, and
631 resistance to cefpirome and/or cefepime (50,129,293,313,316,324,325). The deletion of
632 three residues in the R2-loop (positions 301-303) appears to be responsible for the
633 expanded spectrum activity of CMY-10, and further mutation around this deletion in the
634 P99 enzyme extended its substrate spectrum by widening the R2 region (Table 5) (164).
635 Globally, all chromosomal (e.g. MOX-3, MOX-10, MOX-11) or plasmid-encoded (e.g.
636 MOX-1, MOX-2, MOX-14, CMY-1, CMY-8, CMY-8b, CMY-9, CMY-10, CMY-11,
637 CMY-19) β-lactamases from this family feature the sequence
638 ^{289}AKVILEANPTAAΔΔΔPRESG^{309}S in the conserved R2 region (25). An increase in
639 resistance to cefepime was also achieved by an additional amino-acid substitution (I292S)
640 in the H10-helix region, as observed in CMY-11 and CMY-19 relative to CMY-9 (313)
641 (Table 5).
642 The other enzymes from *Aeromonas* include a cluster of plasmid-encoded FOX-type
643 enzymes without the amino-acid deletion at positions 301-303. These enzymes have a
644 susceptibility profile characterized by a higher degree of resistance to cefoxitin (the origin
645 of the family name, FOX) and to ceftazidime compared with cefotaxime (280,328,333-

335). No ESACs have been identified among FOX variants (22). A tripeptide deletion (^{286}GN^{288}S) in the R2-loop of *E. coli* HKY28 led to an extended hydrolysis spectrum, but the same deletion by site-directed mutagenesis in FOX-4 did not increase catalytic efficiency for ceftazidime, cefotaxime, or cefepime, despite large differences in K_m and k_{cat} values (Table 6). A decrease in the MIC for cefoxitin was obtained in both *E. coli* HKY28 and FOX-4 Δ286-288, together with a slight increase in susceptibility to clavulanate, sulbactam and tazobactam (329).

Structural studies have confirmed the importance of the Ω- and R2-loops for modulating the catalytic activity of class C β-lactamases. In CMY-10, a three-amino acid deletion in the R2-loop appears to underlie the extended spectrum activity (164), whereas a two-amino acid deletion in the R2-loop of CMH-family AmpC enzymes from *E. cloacae* Ent385 leads to reduced susceptibility to ceftazidime-avibactam and cefiderocol (163). Similarly, the Y221H mutation in CMY-136 induces an important change in the confirmation of the Ω-loop, widening the active site cavity and conferring resistance to many β-lactams and combinations, including ceftolozane/tazobactam (167).

NATURAL ESACs

The term "naturally occurring ESAC" has been proposed for the enzymes in *E. coli* isolates with increased hydrolysis of oxyiminocephalosporins, including cefepime and cefpirome (MICs greater than or equal to 16 μg/ml and 0.5 μg/ml for ceftazidime and cefepime, respectively, without a positive synergy test with clavulanic acid), and resistance to amoxicillin and to amoxicillin/clavulanic acid (16). As for class A ESBLs, which are sometimes encoded by chromosomal genes (e.g. OXY-types for *K. oxytoca*) (336), both

chromosomal and natural ESACs have been identified in *A. baumannii* (103), in which these β-lactamases are the principal source of resistance (99). The genes encoding ADC-type β-lactamases are non-inducible and generally expressed at low levels, resulting in resistance to penicillins and narrow-spectrum cephalosporins (33,101). However, overexpression may be observed following the acquisition of an IS element, principally IS*Aba*1, providing a strong promoter, and this may lead to resistance to expanded-spectrum cephalosporins (cefotaxime, ceftazidime) and aztreonam (260). All ADC-type β-lactamases (about 450 protein sequences examined) are characterized by a deletion of three residues (positions 304-306) in the R2-loop that enhances their catalytic efficiency against these clinically important drugs compared with enzymes without this deletion (103,104,162). Clinical variants with higher levels of resistance (4 to 64-fold increase) have been detected, with V211A (Ω-loop) or N287S (H10-helix) substitutions (106) (Table 5). Moreover, acquired resistance to cefepime or cefpirome and increased hydrolysis efficiency (ESAC) were induced by the P210R substitution and duplication of A215 in the Ω-loop (ADC-33) or by the substitution R148Q in the P2-loop (ADC-56) (107,317). ADC-68 has seven amino-acid substitutions compared with ADC-1, one of which (^{321}G) is located in the C-loop and other two (^{192}A and ^{217}D) in the Ω-loop. The overall structures of ADC-68 and ADC-1 are conserved, but there are marked structural differences in the Ω-loop and the C-loop. In particular, residues ^{217}D and ^{321}G in ADC-68 make a major contribution to the structural differences between these two ADC-type β-lactamases (162). There is a two-amino acid deletion (residues 301-302) in the R2-loop of AmpC from two species, *A. dhakensis* and *A. caviae* (25), but an equivalent deletion in AQU-1 does not seem to affect the usual β-lactam resistance phenotype. However, cefotaxime monotherapy

692 should be used with caution for severe *A. dhakensis* infections, because such treatment
693 could lead to the selection of variants with constitutively high levels of β-lactamase
694 production. Nevertheless, cefepime susceptibility was conserved in all cases studied (34).
695 A specific structural alteration to the R2-loop (deletion of two amino acids in positions
696 289-290) was observed in all *Hafnia* protein sequences, and this feature is unique among
697 Enterobacterales (25,85,142) (Table 5). Furthermore, ACC-1 confers an unusual and
698 unique pattern of susceptibility to β-lactams, with higher MICs for ceftazidime and
699 cefotaxime than for cefoxitin (4,280). In addition to the two-amino acid deletion in
700 positions 289-290 mentioned above, ACC-1 features two other remarkable structural
701 alterations, one in the Ω-loop (^{213}ME instead of ^{213}PG) and another along the active-site
702 rim (^{120}F instead of ^{120}Q) (25,142). Moreover, ACC-2, a cephalosporinase encoded by an
703 inducible chromosomal gene, displays marked hydrolysis activity against cefpirome in
704 particular (309), in opposition to the widely accepted view that cefpirome is resistant to
705 hydrolysis by class C enzymes, even when they are overproduced (337). ACC-2 is also
706 strongly inhibited by cefoxitin (309).
707 In conclusion, these natural ESACs constitute a threat because of their resistance to
708 cefepime and/or cefpirome, or even to carbapenems if they are overproduced, combined
709 with porin loss and/or efflux pump activation.
710

711 **CARBAPENEM RESISTANCE**

712 Carbapenem resistance has become a serious threat to public health (338). It has spread
713 worldwide in Gram-negative bacteria, and is continuing to increase due to the production
714 of plasmid-encoded carbapenemases by Enterobacterales and by nonfermenting organisms

715 (mainly *A. baumannii* and *P. aeruginosa*) (339,340). The main mechanism underlying
716 carbapenem resistance is the production of highly transmissible carbapenemases, such as
717 KPC-types (class A), IMP-, NDM- or VIM-types (class B) and OXA-types (class D).
718 Carbapenems, which diffuse efficiently across the native outer membrane of Gram-
719 negative bacteria, are poor substrates for class C β-lactamases (4). However, they may act
720 as inhibitors in some cases, due to the high affinity of AmpCs for these antibiotics,
721 suggesting that resistance may be acquired by a mechanism known and sometimes
722 described as "trapping" (341), in which the cephalosporinase has a good affinity for the
723 substrate combined with a very slow hydrolysis (342-345).
724 Nevertheless, several clinical failures and *in vitro* studies have been published concerning
725 chromosomally encoded and transmissible class C β-lactamases with weak carbapenem
726 hydrolysis activity, suggesting poor carbapenemase activity (287,339,346). Such resistance
727 was always obtained from the combination of a high production of β-lactamase (a
728 hyperproducing mutant of a chromosomally-encoded enzyme or a plasmid-mediated
729 enzyme) with another mutation-driven mechanism, such as porin loss (OmpC, OmpF,
730 OmpG, OmpK35, OmpK36) or efflux overexpression, which was essential for carbapenem
731 resistance, preferentially against ertapenem, then imipenem and finally meropenem
732 (280,347) (Table 7). The prevalence of such non-enzymatic mechanisms is low and differs
733 between bacteria, with relatively few occurrences among Enterobacterales (mostly in *K.*
734 *pneumoniae*, but also in *E. coli*, *P. mirabilis*, and *S. enterica*).
735 Within the genus *Enterobacter*, carbapenem resistance is primarily due to the
736 hyperproduction of chromosomal *ampC* associated with defective penetration of the
737 carbapenemases in the bacterium (348). However, *E. kobei* from the *E. cloacae* complex

738 group naturally produces an ACT-28-type AmpC β-lactamase that hydrolyzes impenem
739 more efficiently (349) and several plasmid-encoded AmpCs show intrinsically low
740 carbapenemase activity (CMY-10, and to a lesser extent, CMY-2 and ACT-1) (280,346).
741 Although in *A. baumannii* carbapenem resistance is mainly related to class B (VIM-, IMP-
742 and NDM-type) and class D (OXA-type) carbapenemases, the combination of
743 overproduced class C β-lactamase and porin loss was also proposed for this species, which
744 produces a natural ESAC with a deletion in the R2-loop and a low binding affinity for
745 imipenem (103,162,350-352). No carbapenemase activity was detected in crude extracts,
746 for example, so it was assumed that porin loss (CarO, Omp22-33, Omp33-36, Omp37,
747 Omp43, Omp44, and Omp47) was the most likely mechanism (339,350,353-355).
748 Carbapenems (e.g. imipenem and meropenem) are important drugs to treat *P. aeruginosa*
749 infections. Carbapenem resistance is mediated by various mechanisms, including the
750 production of a carbapenemase (mostly class B enzymes), the overproduction of efflux
751 pumps (mostly MexA-MexB), the overproduction of class C enzymes (e.g. PDC-type β-
752 lactamases) and decreases in porin expression (mostly OprD), with combinations of these
753 mechanisms observed in many cases (119,268,356,357). In *P. aeruginosa*, transmissible
754 class B carbapenemases or MBLs are considered to be the most clinically relevant source
755 of resistance. Class D (oxacillinases) enzymes are also frequently encountered, and
756 sometimes even class C β-lactamases, which are not real carbapenemases and have a low
757 carbapenem hydrolysis capacity. The combination of PDC overproduction with a decrease
758 in outer membrane permeability (OprD) and/or the overexpression of efflux systems
759 (MexA-MexB-OprM) has been shown to lead to carbapenem resistance (119,268,356,358-
760 360) (Table 7). Various studies on clinical isolates have highlighted the difficulty of clearly

defining the contribution of each mechanism, as resistance is generally a multifactorial phenomenon. However, most imipenem- and meropenem-resistant isolates display high levels of PDC-type enzymes and increased efflux pump expression (268).

Table 7 here

RESISTANCE TO INHIBITORS

Early inhibitors

The first inhibitors to be developed (e.g. clavulanate, sulbactam, tazobactam) were inactive against a majority of class C β-lactamases, but some older combinations have been revamped (e.g. ceftolozane-tazobactam) because ceftolozane has a reduced affinity for PDC enzymes and is not, therefore, hydrolyzed (371). Ceftolozane also has a high potency against *P. aeruginosa* when used alone. Resistance may be acquired during treatment and is mediated principally by transmissible β-lactamases (e.g. ESBLs and serine carbapenemases, such as KPCs). Several missense mutations or deletions in PDC genes have also been reported in *P. aeruginosa* isolates displaying overexpression of these genes and in studies performed *in vitro*, but the underlying mechanisms of resistance (such as combination with porin deficiency and overexpression of various efflux pumps) remain poorly defined (119,318,319,366,372-376).

Avibactam

Novel non-β-lactam (diazabicyclooctane or DBO) β-lactamase inhibitors, such as avibactam, with a high affinity for a wide range of Ambler class A (e.g. ESBLs and KPC-types), class C and some D β-lactamases (e.g. OXA-48) have been developed and showed

784 encouraging results in clinical trials (371,377). Avibactam is currently available in
785 combination with ceftazidime, and is being used to combat multidrug-resistant Gram-
786 negative bacterial infections, which are on the rise worldwide, including carbapenem-
787 resistant Enterobacterales in particular. These inhibitors are not active against class B
788 metallo-β-lactamases (e.g. NDM, VIM) and resistance to the ceftazidime-avibactam
789 combination or combinations of avibactam with other antibiotics have been reported in
790 patients treated since 2015 and in screens *in vitro* (377-383). This type of resistance has
791 yet to be fully elucidated, but several possible underlying mechanisms have been reported.
792 The most frequently encountered resistance mechanism is enzymatic, due to mutations
793 identified mostly in transmissible class A (e.g. KPC and ESBLs) and some class D (e.g.
794 OXA-48) β-lactamases. Other combined mechanisms have been reported, including
795 membrane impermeability and efflux (366,384), and there have been several reports of
796 PBP3 mutations leading to ceftazidime-avibactam resistance (385-387). Finally, the
797 overexpression of chromosomally- or plasmid-encoded AmpCs, with or without mutations
798 and even in combination with porin deficiency appears to make only a small contribution
799 to resistance (13,14,214). Avibactam inhibits the *E. cloacae* CHE ESAC less efficiently
800 than other *E. cloacae* AmpC proteins, due to a subtle rearrangement of the binding site
801 (13). *In vitro* studies with *P. aeruginosa* showed that high-level resistance to combinations
802 of avibactam with ceftazidime or aztreonam occurs with low frequency. In all cases, the
803 mutant displayed alterations to the chromosomal *amp*C gene, mostly deletions of various
804 sizes (5 to 19 residues) in the Ω-loop region of AmpC (14). Eight highly conserved residues
805 made key contributions to binding interactions with avibactam (^{64}S, ^{67}K, ^{120}Q, ^{150}Y, ^{152}N,
806 ^{315}K, ^{316}T and ^{346}N). The PAC-1 enzyme, recently identified in four *P. aeruginosa* isolates

and encoded by a gene on a chromosome-inserted Tn*1721*-like transposon, mediates very high levels of resistance to β-lactams, such as ceftazidime, cefepime, and ceftolozane alone or in combination with avibactam or tazobactam (74). Resistance to the ceftazidime-avibactam combination has been attributed to a deletion of two amino acids in the R2 loop of the AmpC β-lactamase produced independently by *Enterobacter*, which simultaneously causes resistance to cefepime and carbapenems and reduced susceptibility to cefiderocol, a novel siderophore cephalosporin (299).

Six carbapenem-resistant clinical isolates of *K. pneumoniae* in China were recently shown to produce KPC-2, a class A carbapenemase, and CMY-172, a new CMY-2-like class C β-lactamase (291). CMY-172 mediates high levels of resistance to β-lactams, including ceftazidime, cefotaxime, and cefepime, and to the ceftazidime-avibactam combination. This resistance results from a major modification (^{290}KVA deletion) to the R2-loop (ESAC) and an amino-acid substitution, N346I (Table 5). The variant retains all the highly conserved residues for binding to avibactam (^{64}S, ^{67}K, ^{120}Q, ^{150}Y, ^{152}N, ^{315}K, ^{316}T) except for ^{346}N (216). In addition, the residue in position 346, which is well conserved among AmpC-type enzymes, modulates the hydrolysis spectrum of cephalosporinases (327). More generally, *in vitro* resistance to ceftazidime-avibactam and aztreonam-avibactam was examined among Enterobacterales, showing that resistance associated with changes to β-lactamases was seen only for mutants of AmpC. The mutants R148H, G156R/D, N346Y or small deletions at positions 289-294 were obtained with ceftazidime-avibactam, whereas with aztreonam-avibactam the Y150C or N346Y substitutions were observed (388).

Relebactam

The newer imipenem-relebactam combination recently approved by the FDA has antimicrobial activity against Enterobacterales and *P. aeruginosa* strains producing class A and C β-lactamases, through the strong inhibition of KPC and AmpC enzymes (371,389). Isolates resistant to this combination, due to porin loss, have been reported in *Enterobacteriaceae* (390,391). There are *in vivo* and *in vitro* examples of resistance to this combination in *P. aeruginosa* mediated by class C enzymes, but resistance was again multifactorial, dependent on OprD loss, cephalosporinase overproduction, and mutations of the genes encoding the MexAB-OprM efflux system pump and the peptidoglycan recycling machinery (371,392,393).

Vaborbactam

Meropenem-vaborbactam is a fixed-dose combination of a carbapenem antibiotic and a novel boronic acid-based β-lactamase inhibitor. It has *in vitro* activity against Enterobacterales (e.g. *K. pneumoniae*) producing class A (e.g. ESBL, KPC), class C (mostly plasmid-encoded enzymes), and also some class D enzymes (157,159,175,394). The impact of vaborbactam on class C enzymes appears to be limited, but resistance may nevertheless result from combinations of mechanisms, including the overexpression of ESAC mutants after cefepime treatment, associated with OmpK35 and OmpK36 porin deficiencies due to the insertion of an IS*903*-like element, as recently reported in a clinical isolate of *E. hormaechei* (299). This isolate was multidrug-resistant, with resistance even against ceftazidime-avibactam, meropenem-vaborbactam and imipenem-relebactam combinations. This resistance should serve as an important warning that cephalosporins and carbapenems should be used in a judicious manner, whether used alone or in combination with these novel inhibitors.

NEW CEPHALOSPORINS

Cefiderocol

Cefiderocol is an advanced injectable siderophore cephalosporin active against MDR and XDR Gram-negative rods, including producers of various β-lactamases, including members of Enterobacterales, *P. aeruginosa, A. baumannii, Stenotrophomonas maltophila, B. cepacia* and *B. pseudomallei* (395-398). Through its catechol siderophore moiety, cefiderocol exploits iron-transport systems *via* a Trojan-horse strategy, navigating the bacterial periplasm and evading various β-lactamases, including ESBLs, KPC (class A), NDM, IMP, VIM (class B) and various class C and D enzymes (OXA-23, OXA-24, OXA-48 and OXA-51), as well as other mechanisms of resistance (399-401). Resistance to cefiderocol may be due to a two-amino acid deletion in the R2 loop of the AmpC of *E. cloacae* complex (163,299), but can also be acquired through the loss of energy-transducing proteins or catecholate receptors, such as PirA, PiuA, PiuD and TonB in Enterobacterales, *P. aeruginosa* and *A. baumannii* (400,402-404).

CONCLUSIONS

BLCs have a highly variable primary structure, with minimum sequence identity levels as low as 20% (28). However, the three principal catalytic motifs that are characteristic for this molecular class (^{64}SXSK, ^{150}YXN and ^{315}KTG) are highly conserved, together with at least 70 other residues displaying >90% conservation. Some of these other residues, including ^{60}F, ^{61}E, ^{63}G, ^{145}G, ^{148}R, ^{318}T, ^{321}G, ^{322}F, ^{325}Y and ^{328}F, are located around the conserved catalytic motifs. Generally, with the exception of a few sequences corresponding

to serine hydrolases often identified by BLAST analysis, structural classification should be based on the notion of "genus". Genera contain highly variable numbers of species, and future taxonomic reorganizations are possible, as already observed for the genus *Erwinia*, for example, with the discovery of *E. teleogrylli*, or for the genus *Pseudomonas*, with definition of the species *P. putida* and *P. fluorescens*.

The considerable increase in the number of protein sequences present in databases for several species has made it possible to confirm the existence of a certain degree of polymorphism, the large number of clusters observed (more than 10 for *E. coli*/*Shigella*) making it possible to define consensus sequences for each cluster or phylogroup (16). By contrast, enzymes of the DHA- and FOX-types, encoded by plasmid-borne genes, and the species from which they originate, *M. morganii* and *A. allosaccharophila*, respectively, display little polymorphism.

With the development of high-throughput sequencing, this notion of *amp*C gene polymorphism complicates the detection of variants responsible for a significant increase in the MICs of β-lactams, particularly for oxyiminocephalosporins (e.g. cefotaxime, ceftazidime, cefepime, and cefpirome), monobactams (e.g. aztreonam), and carbapenems. However, most infections are identified in hospital inpatients, from whom several isolates of the same species can be obtained, therefore facilitating the detection of such variants. Nevertheless, in the absence of an isolate obtained at the start of treatment, certain variants, such as insertions, duplications or deletions, appear to be easy to detect, whereas others, such as the substitution of a single residue in a hotspot, may be much harder to identify. The diverse examples of ESAC-producing strains observed in clinical practice demonstrate that genetic modifications similar to those described above essentially affect three regions

898 (around the ^{150}YXN motif, the Ω-loop and the H10-helix/R2-loop). Surprisingly, these
899 regions vary to different extents in different species, with a limited number of conserved
900 residues. The notion of a "consensus sequence" should therefore favor the possible
901 detection of ESACs in the framework of increasingly frequent genotype/phenotype
902 analyses (Fig. 3). Such diversity at the heart of species, particularly for the H10-helix/R2-
903 loop region, could account for the considerable variation of MICs between substrates, or
904 the diversity of the β-lactam resistance and kinetic constants reported. Wild-type bacterial
905 species have variable MICs for β-lactams (https://mic.eucast.org/), suggesting that
906 combinations of other, non-enzymatic resistance mechanisms may be at work.
907
908 Figure 3 here
909
910 It should not be forgotten that the chromosomal *amp*C gene is subject to regulation, and
911 thus is dependent on other genes, particularly as it is inducible in most of the bacterial
912 species producing the corresponding enzyme. Diverse genetic events can, therefore, occur
913 in the intercistronic space, generating a strong promoter, or in other genes, such as *amp*R,
914 *amp*D, and *amp*G genes, to generate mutants displaying derepression or overexpression.
915 The analysis would not really be complete without an examination of several systems
916 involved in the transfer of β-lactams across the cell wall, such as porins (e.g. OmpC, OmpF,
917 OmpK15, OmpK36), and efflux systems (e.g. AcrAB-TolC, MexAB-OprM) (387,405-
918 409).
919 For medical biologists, the identification of a β-lactamase from one of the four known
920 molecular classes is based on the presence of at least three motifs (17). The accumulation

of a large number of protein sequences in current databases, with the development of WGS in particular, has made it possible to compare almost 4000 protein sequences from class C β-lactamases, facilitating classification within clusters or taxa. This class is as diverse as class A, with about 20% identity between sequences, but its genetic footprint may be enriched in fewer than 80 highly conserved residues, justifying the exclusion of certain carboxylesterases with β-lactamase activity. In addition, the existence of major taxa in bacterial genera, such as *Citrobacter, Enterobacter, Acinetobacter* and *Aeromonas,* and of recent and more long-standing taxonomic reorganizations provides an indispensable aid to diagnosis at species level, particularly in light of the potential failings of the phenotypic methods currently used in laboratories. This type of analysis, based on primary structure, makes it possible to propose one or several consensus sequences for a species, potentially improving the characterization of possible polymorphisms of the β-lactamase. In the absence of the susceptible strain of the bacterium at the start of infection, prior knowledge of the diverse genetic modifications likely to lead to an extended spectrum of inactivation, which vary between bacterial species, should make it possible to detect these modifications (substitutions, duplications, insertions, deletions). The recent adaptation of a numbering system for this molecular class of enzymes has led to comparisons of protein sequences and emergence of the notion of a "natural ESAC". Finally, medical biologists should not ignore possible interactions with other genes likely to lead to hyperproduction, which are particularly important for this molecular class.

ACKNOWLEDGMENTS

This work was supported, in part, by grants from the Laboratory of Excellence in Research on Medication and Innovative Therapeutics (LERMIT, ANR-10-LABX-33), the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR, ANR-14-JAMR-0002) and the PPR Antibioresistance (ANR-20-PAMR-0010). A.P., G.A., R.L., and B.I.I. declare no potential conflicts of interest.

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AUTHOR BIOS

Alain Philippon is a veterinarian (Ecole Nationale Vétérinaire d'Alfort, 1963) graduated in biochemistry (University of Orsay 1965) and bacteriology-immunology (Institut Pasteur, 1966) who prepared his Ph.D. thesis at the Commissariat à l'Energie Atomique (CEA). Initially trained on *Brucella* and experimental bovine brucellosis at Nouzilly (Institut National de la Recherche Agronomique/INRA) between 1966 and 1970, he started to analyze bacterial resistance to antibiotics, mostly to β-lactams (susceptibility patterns, ESBL, and plasmid-encoded AmpC…..) at CHU Cochin, Paris, France, and in collaboration with François Le Goffic and Roger Labia (ENS Ulm) in 1972. As Professor Emeritus of Microbiology at the University of Paris Descartes and previous Head of the Bacteriology Laboratory at Hopital Cochin, he published more than 200 scientific papers and mentored 20 doctoral and postdoctoral researchers. He was also codirector of a course on medical bacteriology at Institut Pasteur of Paris for a decade.

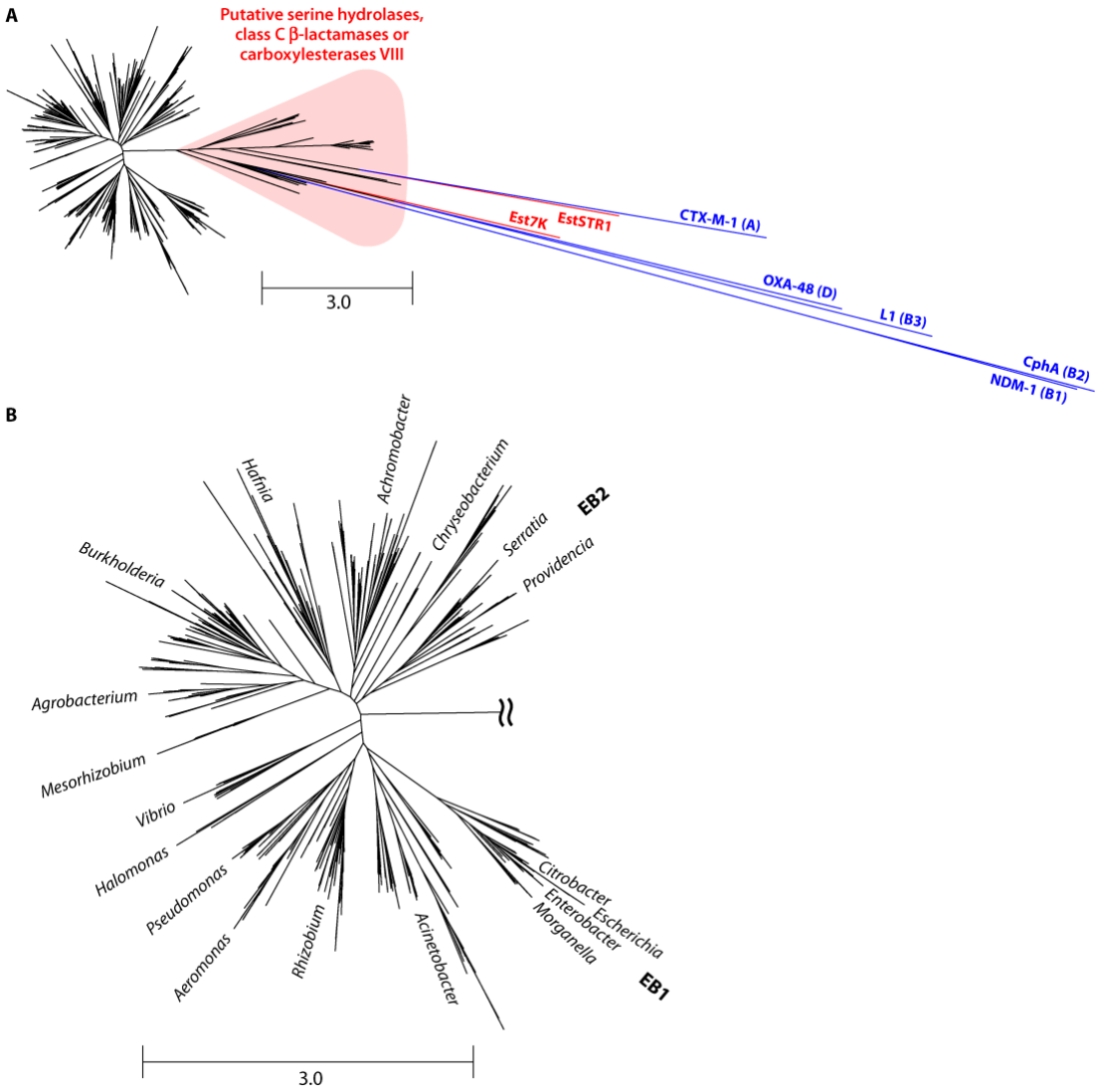
Guillaume Arlet is a doctor (René Descartes University, Paris, 1979), graduate in Medical Biology in 1984, in Biochemistry and Molecular Biology in 1986 (Denis Diderot University, Paris) and Bacteriology (Institut Pasteur, 1986). He did his Ph.D (University Paris-Sud, 1989-1992) under the supervision of Alain Philippon. Associate Professor at

Saint-Louis Hospital and Denis Diderot University (1991-1999), he became Professor at Pierre & Marie Curie University (now Sorbonne University), Head of the medical bacteriology department, first at Tenon Hospital (1999-2012) and then within the Eastern Parisian Hospitals Group (2012-2018). He headed the teaching department of clinical Bacteriology (2007-2018). He joined in 2014 the Center for Immunology and Infectious Diseases (INSERM U1135) at Sorbonne Université. He supervised ten Ph.D students and published more than 210 scientific papers mainly on β-lactamases (ESBL, AmpC β-lactamases, carbapenemases), their genetic supports, and in relation to virulence of their bacterial hosts. He is currently Professor Emeritus.

Roger Labia graduated from the world-renowned Ecole Polytechnique in Paris, France (1962 to 1964), with specialization in mathematics, physics, and chemistry. In relation with his high and early interest in chemical and biological problems, he joined scientific research, first at the Pasteur Institute (starting in 1965). In 1969, he received a Ph.D., studying the organic synthesis of natural compounds. Subsequently, he spent one postdoctoral year at Ottawa University, Canada (1969 to 1970), where he began studying

biochemistry and bacteriology. Back in France, he started a research program on antibiotics, including their mode of action and mechanisms of resistance. This allowed him to develop multiple collaborations with chemists and bacteriologists from France and other countries. He has published more than 320 scientific papers, mostly in high-impact international journals. He has been involved in teaching and directed about 50 theses.

Bogdan I. Iorga received in 2001 a Ph.D in molecular chemistry from the Ecole Polytechnique, France. He is currently CNRS Research Director and heads the Molecular Modeling and Structural Crystallography team at the Institut de Chimie des Substances Naturelles (CNRS, Université Paris-Saclay, Gif-sur-Yvette, France). He is author of 1 book, 6 book chapters, 4 patents and more than 100 publications. He is the developer of the Beta-Lactamase DataBase (BLDB, http://bldb.eu) and is involved in several French and European projects related to the antimicrobial resistance. His main research interests include the design of biologically active compounds, the study of structure-function relationships in different classes of β-lactamases and the development of innovative protocols in molecular modeling. His recent work focuses on the development of tools for *in silico* prediction of antibiotic susceptibility from genomic data using machine learning and deep learning approaches.



2375

2376 **FIG 1** (**a**) Phylogram for representative and putative class C β-lactamases, compared with

2377 β-lactamases from classes A, B and D. (**b**) Focused view on the phylogram of class C β-

2378 lactamases. The protein sequences of representative enzymes are listed in (85,97,104,114).

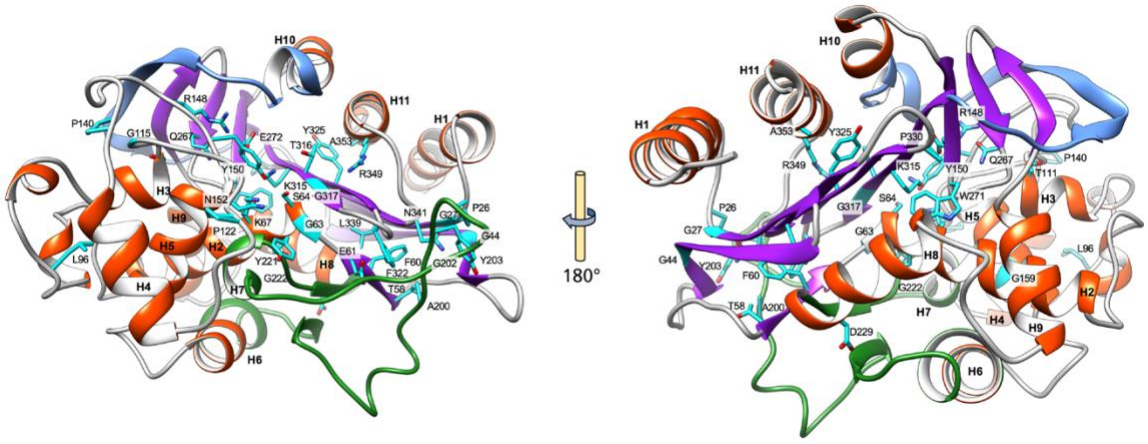
2379 The sequences were filtered using CD-HIT (https://github.com/weizhongli/cdhit) at 90 %

2380 sequence identity, then aligned with Clustal Omega (410). The tree was constructed using

2381 RAxML (411) and the phylogram generated using FigTree (version 1.4.3). The tree was

2382 unrooted.

2383



2384

2385 **FIG 2** Representative three-dimensional structure for class C β-lactamases. The structure
2386 of *E. hormaechei* P99 (formerly known as *E. cloacae* P99) (PDB code 1BLS) (141) is
2387 colored in orange (α-helixes) and purple (β-strands). The Ω- and R2-loops are colored in
2388 green and blue, respectively. The most conserved residues (see Table 3) are represented as
2389 sticks and colored in cyan. The numbering of residues follows the SANC nomenclature
2390 (25).

2391

2392

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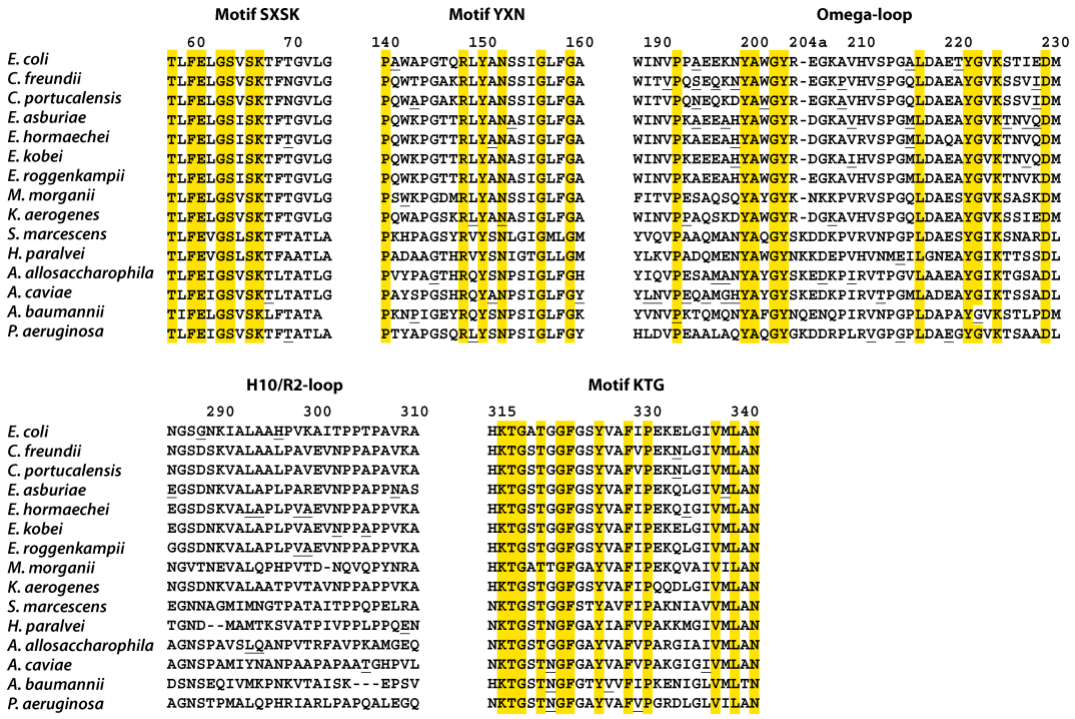

FIG 3 Consensus partial protein sequences of species or their progenitors susceptible to expand their spectrum of inactivation. For *E. coli*, the consensus sequence was calculated from protein sequences of clusters A, B, C and D (84,96,100,113). Residue boxed in gray indicates 100 % conserved. Underlined positions indicate at least two different residues (polymorphism).

2403

2404 **Table 1.** Overview of bacteria producing class C β-lactamases

Class	Order	Genus
Alphaproteobacteria	Rhizobiales	*Agrobacterium, Bosea, Bradyrhizobium, Inorhizobium, Mesorhizobium, Methylobacterium, Microvirga, Ochrobactrum, Phyllobacterium, Pseudorhodoplanes, Rhizobium*
	Rhodobacterales	*Rhodobacter, Ruegeria, Silicibacter, Sulfitobacter*
	Rhodospirillales	*Dongia*
Betaproteobacteria	Burkholderiales	*Achromobacter, Bordetella, Burkholderia, Caballeronia, Collimonas, Cupriavidus, Herbaspirillum, Janthinobacterium, Massilia, Noviherbaspirillum, Pandoraea, Paraburkholderia*
	Neisseriales	*Chromobacterium, Laribacter, Snodgrassella*
	Rhodocyclales	*Thauera*
Gammaproteobacteria	Aeromonadales	*Aeromonas*
	Alteromonadales	*Shewanella*
	Cellvibrionales	*Microbulbifer*
	Enterobacterales	*Budvicia, Buttiauxella, Cedecea, Citrobacter, Cronobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia:Shigella, Ewingella, Hafnia, Klebsiella, Lelliottia, Morganella, Pantoea, Photorhabdus, Pluralibacter, Pragia, Providencia, Regiella, Rouxiella, Serratia, Siccibacter, Xenorhabdus, Yersinia*
	Legionellales	*Legionella*
	Oceanospirillales	*Aidingimonas, Chromohalobacter, Halomonas, Salinicola*
	Pseudomonadales	*Acinetobacter, Pseudomonas, Psychrobacter*
	Vibrionales	*Vibrio*
	Xanthomonadales	*Dyella, Lysobacter, Xanthomonas*
Deltaproteobacteria	Myxococcales	*Myxococcus*
Terrabacteria	Actinobacteria	*Mycobacterium*
	Negativicutes	*Pelosinus*
FCB group	Chitinophagales	*Sediminibacterium*
	Cytophagales	*Dyadobacter, Emticicia, Siphonobacter*

	Flavobacteriales	*Chryseobacterium*
	Sphingobacteriales	*Sphingobacterium*
PVC group	Chlamydiales	*Chlamydia*
	Parachlamydiales	*Parachlamydia*
Unclassified		*Dependentiae*

2405

2406

2407 **Table 2.** Representative class C β-lactamases

Bla	Origin of name	Accession number	Genomic localisation[a]	Organism	Number of residues	References
ACC-1	Ambler Class C-1	AJ133121	P	*Klebsiella pneumoniae*	386	(30)
ACT-1	AmpC Type	U58495	P	*Klebsiella pneumoniae*	381	(31)
ABA-1 (ADC-2)	*Acinetobacter baumannii*	AY177427	IS	*Oligella urethralis*	383	(32)
ABAC-1 (ADC-3)	*Acinetobacter baumannii* Class C	AY178995	Chr	*Acinetobacter baumannii*	383	(32)
ADC-1	*Acinetobacter*-derived cephalosporinase	AJ009979	Chr	*Acinetobacter baumannii*	383	(33)
AQU-1	*Aeromonas aquariorum*	AB765393	Chr	*Aeromonas dhakensis*	380	(34)
AsbA1	*Aeromonas sobria*	U10250	In	*Aeromonas jandaei*	381	(35)
BIL-1 (CMY-2-like)	Name of patient (Bilal)	X74512	P	*Eschericha coli*	383	(36,37)
BlaE	Gene name	AY442183	Chr	*Mycobacterium smegmatis*	380	(38,39)
BUT-1	*Buttiauxella* sp.	AJ415568	Chr	*Buttiauxella sp.*	383	(40)
CAV-1	*Aeromonas caviae*	AF462690	Chr	*Aeromonas caviae*	382	(41)
CDA-1	*Cedecea davisae*	KJ650399	Chr	*Cedecea davisae*	382	(42)
CepH	Cephalosporinase *hydrophila*	AJ276030	Chr	*Aeromonas hydrophila*	382	(43)
CepS	Cephalosporinase *sobria*	X80277	Chr	*Aeromonas sobria*	382	(44)
CFE-1	*Citrobacter freundii*	AB107899.	P	*Escherichia coli*	381	(45)
CHR-1	*Chromohalobacter* sp.	AB070219	Chr	*Chromohalobacter sp.*	396	(46,47)
CMA-1	*Cronobacter malonaticus*	KF640251	Chr	*Cronobacter malonaticus*	375	(48)
CMH-1	Chi Mei Hospital	JQ673557	P	*Enterobacter cloacae*	381	(49)
CMY-1	Active on cephamycins	X92508	P	*Klebsiella pneumoniae*	382	(50)

CMY-2	Active on cephamycins	X91840	P	*Klebsiella pneumoniae*	381	(51)
CSA-1	*Cronobacter sakazakii*	KF623543	Chr	*Cronobacter sakazakii*	375	(48)
DHA-1	Dhahran (Saudi Arabia)	Y16410	P	*Salmonella enteritidis*	379	(52,53)
Ear-1	*Enterobacter aerogenes*	AJ544162	Chr	*Enterobacter aerogenes*	381	(54)
EDC-1	*Edwarsiella* AmpC	EF467366	Chr	*Edwarsiella tarda*	386	–
ENT-1	*Buttiauxella agrestis* CF01Ent1	AJ489827	Chr	*Buttiauxella agrestis*	390	(55)
ERH-1	*Erwinia rhapontici*	AY288518	Chr	*Erwinia rhapontici*	379	(56)
FOX-1	Active on cefoxitin	X77455	P	*Klebsiella pneumoniae*	382	(57)
IDC-1	Integron derived cephalosporinase	MN985649	In	sediment metagenome	395	(58)
K12 (EC-1)	*Escherichia coli* K12	J01611	Chr	*Escherichia coli*	377	(59,60)
LAT-1	Active on latamoxef	X78117	P	*Klebsiella pneumoniae*	381	(61,62)
LHK-1	*Laribacter hongkongii*	AY632070	Chr	*Laribacter hongkongii*	388	(63)
LRA10-1	β-lactam resistance from Alaska	EU408357	?	uncultured bacteria (soil)	375	(64)
LRA13-1[b]	β-lactam resistance from Alaska	EU408352	?	uncultured bacteria (soil)	609*	(64)
LRA18-1	β-lactam resistance from Alaska	EU408355	?	uncultured bacteria (soil)	386	(64)
LYL-1	*Lysobacter lactamgenus*	X56660	Chr	*Lysobacter lactamgenus*	385	(65)
MIR-1	Miriam hospital	M37839	P	*Klebsiella pneumoniae*	381	(66,67)
MOX-1	Active on moxalactam	D13304	P	*Klebsiella pneumoniae*	382	(68,69)
OCH-1	*Ochrobactrum anthropi*	AJ401618	Chr	*Ochrobactrum anthropi*	390	(70)
P99 (ACT-89)	*Enterobacter hormaechei* P99	X07274	Chr	*Enterobacter hormaechei*	397	(71)
PAO-1 (PDC-1)	*Pseudomonas aeruginosa* (*Pseudomonas*-derived cephalosporinase)	AY083595	Chr	*Pseudomonas aeruginosa*	397	(72,73)
PAC-1	*Pseudomonas aeruginosa* Class C	KY285014	Tn	*Pseudomonas aeruginosa*	381	(74)

SLC-1	*Serratia liquefaciens* Class C	DQ022079	–	Uncultured bacteria (soil)	379	(75)
PSI-1	*Psychrobacter immobilis*	X83586	Chr	*Psychrobacter immobilis*	401	(76,77)
RHO-1	*Rhodobacter sphaeroides*	CP000144	Chr	*Rhodobacter sphaeroides*	380	(78)
SR50 (SRT-1-like)	*Serratia marcescens* SR50, Serratia resistant	X52964	Chr	*Serratia marcescens*	376	(79)
SST-1	Susceptible strain	AB008455	Chr	*Serratia marcescens*	378	(80)
TRU-1	Formerly *Aeromonas tructi*	EU046614	Chr	*Aeromonas enteropelogenes*	382	(81)
YEC-1	*Yersinia enterocolitica* cephalosporinase	X63149	Chr	*Yersinia enterocolitica*	388	(82)
YRC-1	*Yersinia ruckeri* cephalosporinase	DQ185144	Chr	*Yersinia ruckeri*	383	(83)

[a] Chr, chromosome; In, integron; P, plasmid; Tn, transposon; IS, insertion sequence.

[b] fusion between two β-lactamases (class C and class D)

2411

2412 **Table 3.** Conserved residues in class C β-lactamases

Position [a]	Residue (> 90 % conserved)	Secondary structure [b]	% conserved [b]
18	P	H1	94
26	P	S	97
27	G	E	100
29	A	E	97
36	G	T	97
43	F/Y/W	E	100
44	G	E	100
54	V		91
58	T		100
60	F	E	100
61	E	E	100
63	G	G	100
64	**S**	G	100
66	**S**	H2	100
67	K	H2	100
71	G/A	H2	100
73	L	H2	94
77	A	H2	91
94	P	G	94
96	L	G	100
109	L	H3	97
110	A/G	H3	100
111	T	T	100
113	T/S		100
115	G	S	100
116	G		96
118	P		94
119	L	S	97
122	P		100
123	D/E	T	100
134	F/Y/W	H4	100
135	Y/F	H4	97
138	W		97
140	P		100
145	G	T	97
148	R	E	100
150	**Y**		100
152	**N**	H5	100
155	I	H5	91
156	G	H5	97

159	G	H5	100
170	F/Y	H6	100
187	T/S	Ω	100
188	Y/W/F	Ω	100
191	V	Ω	97
192	P	Ω	97
199	Y	Ω	97
200	A	Ω	100
202	G	Ω	100
203	Y	Ω	100
210	R/H	Ω	100
211	V	Ω	91
213	P	Ω	94
214	G	Ω	94
221	Y	Ω H7	100
222	G	Ω	100
224	K	Ω	94
229	D	H8	100
260	Y/W/F	E	97
267	Q	E	100
269	L	S	97
271	W		100
272	E	E	100
277	P	S	97
286	G	H10 R2	97
315	**K**	E	100
316	**T**	E	100
317	**G**	E	100
319	T		97
321	G	S	97
322	F		100
325	Y	E	100
328	F	E	97
330	P	E	100
335	G/A	E	100
337	V	E	91
339	L	E	100
340	A	E	97
341	N	S	100
345	P		97
349	R	H11	100
353	A	H11	100

2413

[a] According to the "SANC" class C β-lactamases numbering scheme described in (25). Residues in boldface type are involved in the catalytic mechanism and/or in substrate binding.

[b] From the alignment of 32 representative class C β-lactamases examined in (25). H: α-Helix; S/T: Bend or Turn; E/B: β-Strand or β-Bridge; G: 3_{10}-Helix; Ω: Ω-loop; R2: R2-loop.

2422

2423 **Table 4.** Class C β-lactamases and species-specific progenitors

Enzyme/Strain [a]	First identification	Updated identification	% [b]	References
ACC-1*[c]	*Hafnia alvei*	*Hafnia paralvei*	99.7	(30), this review
ACT-1*[c]	*Enterobacter cloacae*	*Enterobacter asburiae*	98.4	(31), this review
Aer-1	*Enterobacter aerogenes*	*Klebsiella aerogenes*	98.9	(54,125)
AQU-1	*Aeromonas aquariorum*	*Aeromonas dhakensis*	99.2	(34,126)
AsbA1	*Aeromonas sobria*	*Aeromonas jandaei*	94.5	(35), this review
BIL-1	*Citrobacter freundii*	*Citrobacter portucalensis*	96.8	(37), this review
BUT-1	*Buttiauxella* sp.	*Scandinavium goteoborgense*	99.0	(40,127)
BUT-2	*Buttiauxella agrestis*	*Scandinavium goteoborgense*	99.5	(55,127)
CAV-1	*Aeromonas caviae*	*Aeromonas allosaccharophila*	97.1	(41,121)
CFE-1*	*Citrobacter freundii*	*Citrobacter europaeus*	99.2	(45), this review
CFE-2*	*Citrobacter freundii*	*Citrobacter werkmanii*	95.0	(128), this review
CMY-1*[c]	*Aeromonas hydrophila*	*Aeromonas sanarellii*	95.3	(50), this review
CMY-2*	*Citrobacter freundii*	*Citrobacter portucalensis*	98.4	(51), this review
EDC-1	*Edwardsiella tarda*	*Edwardsiella piscicida*	99.1	–, this review
FOX-1*	*Aeromonas caviae*	*Aeromonas allosaccharophila*	94-98	(57,121)
LAT-1*	*Citrobacter freundii*	*Citrobacter portucalensis*	97.4	(62), this review
MIR-1*[c]	*Enterobacter cloacae*	*Enterobacter roggenkampii*	99.7	(25,71)
MOX-1*	*Aeromonas hydrophila*	*Aeromonas sanarellii*	94.5	(69,121)
MOX-2*	*Aeromonas sp.*	*Aeromonas caviae*	98.9	(121,129)
MOX-9*	*Aeromonas caviae*	*Aeromonas media*	98.0	(121,130)
P99 (ACT-89)	*Enterobacter cloacae*	*Enterobacter hormaechei*	97.9	(25,71), this review
TRU-1	*Aeromonas tructi*	*Aeromonas enteropelogenes*	97.1	(81), this review

2424 [a] Plasmid-encoded enzymes are labelled by an asterisk

2425 [b] Percentage of identity with consensus sequence

2426 [c] Other plasmid-encoded types: ACC-4 (99.5 % for *H. paralvei*), ACT-3, ACT-6, ACT-8,

2427 and ACT-10 (≥97.6 % for *E. asburiae*); CMY-17, CMY-55, CMY-132, and CMY-161 (≥

98.1 % for *A. sanarellii*); MIR-4 (99.4 % for *E. roggenkampii*). Their respective phylogenies can be found in (84,96,100,113) and (123,124).

Table 5. Localization of ESAC-associated mutations in chromosomal or plasmid-encoded class C β-lactamases, the exact position of each mutation and the specific regions associated with each cluster

Bla or strain [a]	Location	First numbering	Updated numbering [b]	References
E. coli				
MEV	H10-helix	S282 duplication	idem	(275)
ECB33	H10-helix	I283 duplication	I284	(276)
HKY28	H10-helix	286GSD deletion	idem	(277)
EC16	H10-helix	S287C	idem	(16)
EC13	H10-helix	S287N	idem	(16,87)
8009162	H10-helix, R2-loop	A292V	idem	(273)
EC80	H10-helix, R2-loop	L293P	idem	(278)
BER	R2-loop	293AA insertion	idem	(279)
7014517	R2-loop	295ALA insertion	idem	(273)
EC15	R2-loop	H296P	idem	(16,280)
EC14	R2-loop	V298L	idem	(16,280)
KL	H11-helix	V350F	idem	(281)
Citrobacter				
CHA	close to YSN	R148H	idem + Q196H	(87,282)
CMY-107	Ω-loop	Y199C	idem	(283)
CMY-27	Ω-loop	W221C	W201C	(284)
CMY-30	Ω-loop	V231G	V211G	(285)
CMY-42	Ω-loop	V231S	V211S	(286,287)
CMY-95	Ω-loop	V211A	idem	(278)
CMY-32	Ω-loop	G214E	idem	(288)
CMY-54	Ω-loop	217EL insertion	216aEL insertion	(289)
GN346	Ω-loop	E219K	idem	(290)
CMY-136	Ω-loop	Y221H	idem	(167)

CMY-172	R2-loop	290KVA deletion+ N346I	idem	(291)
CMY-2	R2-loop	A292P or L293P	idem	(292)
CMY-33	R2-loop	293LA deletion	idem	(293,294)
CMY-44	R2-loop	293LAAL deletion	idem	(293)
CMY-69	R2-loop	A295P	A294P	(295)
CMY-99	R2-loop	P306T	idem	(296)
CMY-37	R2-loop	L316I	L296I	(297)
Enterobacter				
LN04004SS1	Ω-loop	213K-226G deletion	idem	(298)
GC1	Ω-loop	208AVR duplication	idem	(10,151)
CHE	R2-loop	289SKVALA294 deletion	idem	(9)
Ent630	R2-loop	292-293 AL deletion	idem	(299)
P99 (ACT-89)	R2-loop	L293P	idem	(300)
MHN	R2-loop	V298E	idem	(301)
LN04004SS1	H11-helix	N366H	N346H	(298)
K. aerogenes				
EA6/13/17/20	H3-helix	Q90H, W101C, L107Y	idem	(302)
Ea595	H10-helix, R2-loop	V291G	idem	(303)
Ear2	R2-loop	L293P	idem	(54)
S. marcescens				
520R	H2-helix	T64I	T70I	(304)
SRT-1	Ω-loop	E213K	E219K	(80)
ES46, ES71	Ω-loop	E235K	E219K	(305)
SMSA	Ω-loop	S220Y	idem	(306)
HD	R2-loop	287MNGT deletion	293MNGT deletion	(307)
Hafnia				
ACC-4*	Ω-loop	V211G + 289-290 deletion	idem	(308), this review
ACC-1*	R2-loop	289-290 deletion	idem	(25,309), this review

ACC-2	R2-loop	289-290 deletion [c]	idem	(309), this review
Aeromonas				
CMY-9	R2-loop	E85D + 299-301 deletion	E61D + 301-303 deletion	(310-312)
CMY-19	R2-loop	I292S + 299-301 deletion	I292S + 301-303 deletion	(311-313)
CMY-1	R2-loop	299-301 deletion	301-303 deletion [d]	(164,313,314)
MOX-1	R2-loop	303-305 deletion	301-303 deletion [d]	(210)
MOX-2	R2-loop	303-305 deletion	301-303 deletion [e]	(129), this review
MOX-13	R2-loop	303-305 deletion + N346I	301-303 deletion [e]	(315), this review
CMY-10	R2-loop, H11-helix	E85D + 299-301 deletion	E61D + 301-303 deletion	(164,311,314,316)
A. baumannii				
ADC-56	H5-helix	R148Q	idem	(107)
ADC-53	Ω-loop	V208A	V211A	(106)
ADC-33	Ω-loop	P210R + 215A duplication	P213R + 218aA duplication	(317)
ADC-51	R2-loop	N283S	N287S	(106)
ADC-1	R2-loop	304-306 deletion	idem deletion [f]	(103)
ADC-68	Ω-loop	G220D + R320G	G217D + R321G	(162)
P. aeruginosa				
PDC-222	H2-helix	T96I	T70I	(318)
PDC-78	H2-H3-loop, Ω-loop	R100H + G216R	R100H + R215G	(119)
PDC-82	H3-H4-loop	F121L + M175L	F121L + M174L	(119,319)
PDC-73	H5-helix	P154L	P153L	(119)
PDC-82	H6-helix	M175L	M174L	(119)
PDC-50	Ω-loop	V213A	V211A	(119)
PDC-74	Ω-loop	G216R	G215R	(119)
PDC-86	Ω-loop, H7-helix	E221K	E219K	(119)
PDC-80	Ω-loop, H7-helix	E221G	E219G	(119)

PDC-85	Ω-loop, H7-helix	Y223H	Y221H	(119)
PDC-223	Ω-loop	229G-247E deletion	202G-219E deletion	(318)
PDC-221	Ω-loop	E247K	E219K	(119)
PDC-88	H10-helix, R2-loop	290TP deletion	289TP deletion	(119)
PDC-89	H10-helix, R2-loop	290TPM deletion	289TPM deletion	(119)
PDC-91	H10-helix, R2-loop	290TPMA deletion	289TPMA deletion	(119)
PDC-44	R2-loop	L294P	L293P	(119)
PDC-92	R2-loop	294LQ deletion	293LQ deletion	(119)
PDC-76	H11-helix	N347I	N346I	(119)

[a] The strain names are underlined

[b] According the Standard Numbering Scheme (25)

[c] All AmpC sequences of *H. paralvei* and *H. alvei* examined have this deletion

[d] This cluster includes CMY-1, MOX-1, CMY-8, CMY-10, CMY-11, CMY-19, and MOX-14

[e] These enzymes were located in different clusters (122)

[f] This deletion was observed for all *A. baumannii* sequences, and particularly for ADC-7 (101).

* Plasmid-encoded

2446 **Table 6.** ESAC and effects on kinetic constants for groups of enzymes with different phenotypes[a]

Bla or strain[b]	Mutation	Ceftazidime (CAZ)			Cefotaxime (CTX)			Cefepime (FEP)			References
		k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (μM · s^{-1})	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (μM · s^{-1})	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (μM · s^{-1})	
CMY-2	–	0.004	0.5	0.008[c]	0.007	0.001	7	–	–	–	(282)
CMY-2	R148H	0.67	0.6	1.12[c]	NM	0.003	NM	–	–	–	(282)
CMY-2	–	0.01	0.02	0.5	<0.01	0.005	<2	–	–	–	(283) (326)
CMY-107	–	0.14	0.15	0.9	0.8	0.075	10.7	–	–	–	(283)
CMY-30	–	0.4	0.14	2.9	1.7	0.3	5.7	–	–	–	(326)
CMY-42	–	0.5	0.3	1.7	0.2	0.08	2.5[c]	–	–	–	(286)
CMY-2	–	0.005	0.15	0.033	0.007	0.001	7	0.37	412	$9 \cdot 10^{-4}$	(167,282,327)
CMY-136	–	6.26	2360	0.003	4.71	20	0.24	1.79	3588	$5 \cdot 10^{-4}$	(167)
CMY-2	–	–	–	–	NM	1.8	NM	NM	108.1	NM	(288)
CMY-32	–	–	–	–	0.9	4.05	0.22	NM	988.9	NM	(288)
ACC-2	–	0.03	5.2	0.006[c]	0.02	19	0.001	3.6	147	0.024	(309)
ACC-4	–	1.5	15	0.1	2.7	9.4	0.29	0.14	73	0.002	(308)
Ser^S	–	<0.5	50[d]	<0.01	2	4	0.5	<0.5	100[d]	<0.005[c]	(306)

Ser^R	S220Y	520	570	0.9	800	980	0.8	330	1000	0.33	(306)
FOX-3	–	0.273	1.18	0.231	0.081	0.076	1.06	ND	ND	$3.32 \cdot 10^{-3}$	(328)
FOX-8	–	$2.6 \cdot 10^{-3}$	0.382	$6.8 \cdot 10^{-3}$	$18.2 \cdot 10^{-3}$	$10.95 \cdot 10^{-3}$	1.66[c]	ND	ND	$1.36 \cdot 10^{-3}$	(328)
FOX-4	–	1.33	13	10	1.33	0.23	5.69	11.01	1071	0.01	(329)
FOX-4	306GNSΔ	0.84	6.44	0.13	0.24	0.087	2.75	2.5	103	0.02	(329)
CMY-9	–	1.8	560	$3.2 \cdot 10^{-3}$	0.27	0.28	0.96	NM	950	ND	(313)
CMY-19	–	0.085	3.7	0.023	0.33	31	0.011	1.8	630	$2.9 \cdot 10^{-3}$	(313)
CMY-8	–	0.091	48	$1.9 \cdot 10^{-3}$	0.36	2.3	0.16	–	–	–	(310)
CMY-9	–	0.53	120	$4.4 \cdot 10^{-3}$	0.48	3.4	0.14	–	–	–	(310)
MOX-1	–	ND	311	ND	–	–	–	ND	211	ND	(69)
CMY-1	–	–	–	–	0.01	0.015	0.67	–	–	–	(330)
CMY-10	–	5	33.9	0.15	–	–	–	–	–	–	(164)
ACT-89 (P99)	–	$6.1 \cdot 10^{-3}$	18.4	$3.2 \cdot 10^{-4}$	–	–	–	–	–	–	(164)
ACT-89 (P99)	–	<1	20[d]	ND	0.5	0.5[d]	1	1	15[d]	0.067	(9)
CHE	289-294Δ	<1	1[d]	ND	0.5	0.05[d]	10	2	3[d]	0.67	(9)

ACT-89 (P99)	–	0.065	28	$2.3 \cdot 10^{-3}$	–	–	–	–	–	–	(331)
ACT-89 (P99)	L293C	0.041	7	$5.9 \cdot 10^{-3}$	–	–	–	–	–	–	(331)
ACT-89 (P99)	–	0.013	15	$8.7 \cdot 10^{-4}$	–	–	–	0.5	100	$4.7 \cdot 10^{-3}$	(300)
ACT-89 (P99)	L293P	0.10	10	0.01	–	–	–	3.1	24	0.13	(300)
Ear	–	ND	16[d]	ND	0.15	>500	ND	0.4	126	0.003	(54)
Ear2	–	ND	9.8[d]	ND	0.15	10[d]	0.015	0.4	9.1	0.044	(54)
ADC-1	–	0.7	16.0	0.044	0.16	0.5	0.32	–	–	–	(103) (162)
ADC-68	–	1.66	147.7	0.01	18.5	117.5	0.16	–	–	–	(162)
ADC-11	–	0.01	10	0.001	0.2	2.5[d]	0.1	1	1800	$5.5 \cdot 10^{-4}$	(317)
ADC-33	–	4	30	0.13	1	0.5[d]	2	10	1300	$7.7 \cdot 10^{-3}$	(317)
ADC-1	–	1.255	265	$4.7 \cdot 10^{-3}$	–	–	–	–	–	–	(260)
ADC-5	–	0.011	232	$4.7 \cdot 10^{-5}$	–	–	–	–	–	–	(260)
ADC-5	P167S	$2.5 \cdot 10^{-3}$	120	$2.1 \cdot 10^{-5}$	–	–	–	–	–	–	(260)

ADC-5	P167S/D242G/ Q163K/G342R	1.235	90	0.014	–	–	–	–	–	–	(260)
ADC-30	–	0.05	1.39	0.04	0.18	0.51	0.32	–	–	–	(107)
ADC-56	–	0.1	1.42	0.07	0.27	1	0.27	0.2	17.17	0.011	(107)
PDC-1	–	0.004	20	$2 \cdot 10^{-4}$	0.02	6	$3.3 \cdot 10^{-3}$	0.08	800	$1 \cdot 10^{-4}$	(118)
PDC-2	–	0.01	20	$5 \cdot 10^{-4}$	0.15	5	0.03	2	850	$2.4 \cdot 10^{-3}$	(118)
PDC-3	–	0.02	35	$5.7 \cdot 10^{-4}$	0.15	8	0.019	2	1300	$1.5 \cdot 10^{-3}$	(118)
PDC-5	–	0.015	30	$5 \cdot 10^{-4}$	0.1	5	0.02	2.5	1700	$1.5 \cdot 10^{-3}$	(118)
PDC-5	–	0.01	7.3	$1.3 \cdot 10^{-3}$	0.07	0.14	0.5	>0.15	>250	$6 \cdot 10^{-4}$	(332)
PDC-5	N346Y	0.06	19	$3.2 \cdot 10^{-3}$	0.2	1.2	0.17	>0.17	>300	$5.5 \cdot 10^{-4}$	(332)

[a] NM, k_{cat} not measurable; ND, not determined

[b] Strain names are underlined

[c] Value computed from k_{cat} and K_m, which is different from the value reported in the original paper

[d] K_i values (μM) were determined instead of K_m values, using cefalothin as a reporter substrate

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2454 **Table 7.** Chromosomal or plasmid-encoded class C β-lactamases: mechanisms of acquired resistance to carbapenems

Bla or strain	**Species**	**Location[a]**	**Mechanisms**	**Fold-increase MICs[b]**					**Reference**
				CAZ	**FEP**	**IMP**	**ERT**	**MER**	
MEV	*E. coli*	Chr	ESAC + OmpC decrease + OmpF loss	>128	>16	8	>16	8	(275)
ACC-1	*E. coli*	P	+ OmpC/OmpF loss	>64	64	4	>128	4	(280)
ACT-1	*E. coli*	P	+ OmpC/OmpF loss	>256	>16	128	>512	32	(280)
CMY-2	*E. coli*	P	+ OmpC/OmpF loss	>256	>16	256	>512	256	(280)
DHA-1	*E. coli*	P	+ OmpC/OmpF loss	>512	64	16	256	8	(280)
FOX-1	*E. coli*	P	+ OmpC/OmpF loss	>512	>128	4	>256	16	(280)
CMY-2	*E. coli*	P	Overproduction + OmpC/OmpF loss	>256	>16	>64	>256	>32	(361)
CMY-2	*E. coli*	P	Overproduction + OmpC insertion IS1			>64		>256	(287)
CMY-2	*E. coli*	P	Overproduction + OmpC/OmpF loss	>256	>128	>256		>512	(344)
EC14	*E. coli*	Chr	ESAC + OmpC/OmpF loss	>256	>128	16	>32	1	(362)
CMY-4	*S. enterica*	P	+ OmpF loss	>512	>64	>16			(363)
ACT-1	*K. pneumoniae*	P	+ Omp42-kDa loss	>128	8-32	8->16			(31)
ACT-1	*K. pneumoniae*	P	+ OmpK35/36 insertion + PhoE decrease	64-256		128		256	(364)
DHA-1	*K. pneumoniae*	P	+ OmpK36 loss		>128	>32		>32	(365)
DHA-1	*K. pneumoniae*	P	+ OmpK35/36 loss + AcrAB/OqxAB			32	>32		(366)
ACC-1	*K. pneumoniae*	P	+ OmpK35/36 loss	>128	16	8	32	16	(347)
FOX-1	*K. pneumoniae*	P	+ OmpK35/36 loss	>512	32	64	128	64	(347)
MOX-1	*K. pneumoniae*	P	+ OmpK35/36 loss	64	8	32	32	32	(347)

EA-Z	*K. aerogenes*	Chr	+ Omp40-kDa loss			16			(367)
E15	*K. aerogenes*	Chr	+ OmpK35/36 loss	>256		>16	>16		(368)
E11	*E. cloacae*	Chr	Overproduction + OmpK35/36 loss	>256		>16	>16		(368)
144	*E. cloacae*	Chr	Overproduction + porin loss	64		16		>128	(369)
213	*P. rettgeri*	Chr	Overproduction + porin loss	4		8		>128	(369)
ACT-28	*E. kobei*	Chr	Overproduction + OmpC-like protein	>256	>64	>128	>512	>512	(349)
A-1	*A. baumannii*	Chr	Overproduction + Omp33/36 kDa decrease	32		>64			(350)
A(4) or B(4)	*A. baumannii*	Chr	Overproduction + Omp37/44/47 kDa decrease	>32	>32	8-16		>16	(370)
Paeβ-04	*P. aeruginosa*	Chr	Overproduction ESAC + *mex*B increase	32	16	16		16	(360)
3-D8	*P. aeruginosa*	Chr	Overproduction + *mex*B increase + OprD loss + *dac*B	128	64	32		>256	(358)
AM339	*P. aeruginosa*	Chr	Overproduction + *mex*A and *mex*C and *mex*X increase + OprD loss	>32	>128	>16	>16	>16	(268)

[a] Chr, chromosome; P, plasmid.

[b] CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; ERT, ertapenem; MER, meropenem

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