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A lack of drugs for antibiotic-resistant Gram-negative bacteria

Jung Hun Lee, Seok Hoon Jeong, Sun-Shin Cha and Sang Hee Lee

Payne *et al.* recently reported an excellent overview of a target-based approach to new antibacterial development and the lack of new antibacterial drugs in late-stage development several years ago[1]. This observation has also been made by the participants in the recent forum[2] of anti-infective research and development. Also, the Infectious Diseases Society of America recently identified six top-priority dangerous pathogens — extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus* and *Aspergillus* species — for which there are few or no drugs in late-stage development. This could further limit future safe and effective choices for treating these infections[3].

Three of these six pathogens are antibiotic-resistant Gram-negative bacteria. Recently, antibacterial drugs against ESBL-producing Gram-negative bacteria accounted for ~15% (2 out of 13) of all antibacterial drugs undergoing development in Phase II trials or later clinical studies[3]. However, there are no drugs being developed against class C ESBL-producing Gram-negative bacteria. Here, we draw attention to important aspects of urgently needed antibacterial drugs against class C ESBL-producing Gram-negative bacteria, which have been overlooked by these reports. We also suggest that the category of ESBLs should be expanded.

ESBLs are a group of enzymes for which the substrate spectrum has extended to third-generation oxyimino-cephalosporins (for example, cefotaxime and ceftazidime)[4]. Most of the known ESBLs are class A and D β-lactamases, but recently, several class C ESBLs were reported in Gram-negative bacteria: KL[5], HD[6], CMY-10 (REF. 7) and CMY-19 (REF. 8). The hydrolytic efficiency (k_{cat}/K_m) of class C ESBLs for ceftazidime was higher than or similar to that (0.029 $\mu M^{-1}\ s^{-1}$) of SHV-38 (SHV stands for sulphydryl variable)[9], a typical class A ESBL.

Most of the class C β-lactamases have hydrolysing activity against cephamycins (that is, second-generation cephalosporins: cefoxitin and cefotetan), which are not hydrolysed by class A or D ESBLs[4–8]. Cefepime (a fourth-generation oxyimino-cephalosporin) was also inactivated by KL, HD and CMY-19 ESBLs[5,6,8]. Rubinstein and Zhanel have noted that physicians are increasingly being forced to use the carbapenems (for example, imipenem or meropenem) and fluoroquinolones (for example, ciprofloxacin or levofloxacin) as first-line therapy for ESBL-producing Gram-negative bacteria; indeed, the situation will become even more severe as ESBL-producing organisms increasingly become concomitantly resistant to the fluoroquinolones[2].

However, we recently found that the CMY-10 ESBL had higher imipenem-hydrolysing activity than OXA-23, a class D carbapenemase[7,10]. Gram-negative bacteria producing such class C ESBLs could present a major therapeutic challenge, and so new antibacterial drugs against class C ESBL-producing Gram-negative bacteria are urgently needed.

To develop these antibacterial drugs, it is necessary to understand the operative mechanism of class C ESBLs to extend their substrate spectrum. Our kinetic data and crystal structure[7] of a plasmid-encoded class C ESBL (that is, CMY-10) clarify this mechanism. The region responsible for the extended substrate spectrum is the R2-loop (amino-acid residues 289–307)[7]. Our sequence alignment of four class C ESBLs shows that the R2-loop includes all regions responsible for the extended substrate spectrum in all class C ESBLs, compared with P99 (a class C non-ESBL) (FIG. 1):

- three amino-acid deletion (residues 303–305) of CMY-10 (REF. 7);
- four amino-acid deletion (residues 293–296) of HD[6];
- the single amino-acid substitution (L296H) of KL[5];
- the single amino-acid substitution (A292S) of CMY-19 (REF. 8).

These natural mutations in the R2-loop can change the architecture of the active site in class C ESBLs, thereby affecting their hydrolysing activity. Owing to the deletion in CMY-10, for example, the R2-loop in the R2 active site (that is, the region that accommodates the R2 side-chain at C3 of the β-lactam nucleus in oxyimino-cephalosporins) displays noticeable structural alterations. The shortened path of the connection R2-loop between α10 and β11 induces the ~2.5 Å shift of α9 and α10 relative to the adjacent helix α11 in CMY-10 compared with both P99 (REF. 11) and GC1 (REF. 12) β-lactamases, thereby opening the gap between α9–α10 and α11 (REF. 7). Therefore, the bulky R2 side-chain of oxyimino-cephalosporins could fit snugly into the significant widening of the R2 active site in this way.

Clinically available β-lactamase inhibitors (for example, clavulanic acid, sulbactam or tazobactam) co-administered with less

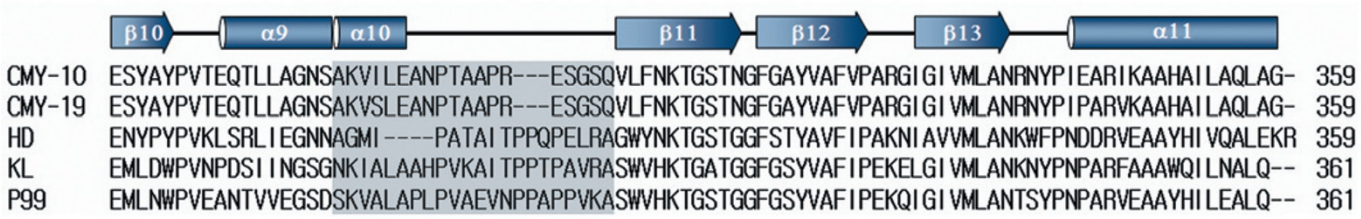

Figure 1 | **A sequence alignment of amino-acid residues near the H-9 (α9) and H-10 (α10) helix of class C β-lactamases with extended substrate spectrum.** Alignment among CMY-10 and P99 β-lactamases for which structures are available is performed based on their superimposed structures. The image above the sequence alignment indicates secondary structure annotation of CMY-10. A partial amino-acid sequence alignment of CMY-10 (*Enterobacter aerogenes* K9911729; GenBank accession no. AF357598; PDB code, 1ZKJ); CMY-19 (*Klebsiella pneumoniae* HKY466; GenBank accession no. AB194410); HD (*Serratia marcescens* HD; GenBank accession no. AY336102), KL (*Escherichia coli* KL; GenBank accession no. AY533244); and P99 (*Enterobacter cloacae* P99; GenBank accession no. X07274; PDB code, 2BLT) is shown. The R2-loop of residues 289–307 is shaded. CMY-10, CMY-19, HD and KL are class C extended-spectrum β-lactamases (ESBLs), whereas P99 is a class C non-ESBL.

effective β-lactams are effective against class A beta-lactamases, but have little or no activity against class C β-lactamases. Because Gram-negative bacteria producing class C ESBLs are becoming an increasingly common cause of nosocomial infections[5–8], there is an urgent need to develop an inhibitor of class C ESBLs or to discover new antibacterial drugs for these class C ESBL-producing clinical isolates. Such efforts could be aided considerably by the structural information on class C ESBLs highlighted above[13]. At present, a few academic research groups (such as our group and Shoichet's laboratory[14]) and small pharmaceutical companies (for example, Novexel[15], which was spun out of Aventis) are seeking such novel β-lactamase inhibitors.

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