ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2002, p. 600–601 Vol. 46, No. 2
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.2.600–601.2002

What Constitutes an Extended-Spectrum β-lactamase?

In a recent publication, Madinier et al. describe the cloning and characterization of a Class A β-lactamase, CfxA2, from *Pervotella intermedia* (3). CfxA2 differs by only a single amino acid residue from CfxA, an extended-spectrum β-lactamase in *Bacteroides vulgatus* CLA-341 (3). This is an important finding, as it continues to document the spread of antibiotic resistance determinants among normal flora species that colonize diverse niches fo the human host (oral and intestinal).

Previously, we showed that CfxA can mediate resistance to nearly all β-.actam antibiotics (including cephamycins) but not to carbapenems or related compounds; thus, we concluded that CfxA is an extended-spectrum β-lactamase (5). Since cefoxitin is an important drug in the treatment of *Bacteroides* spp. infections, we felt that the ability of *cfxA* to mediate cefoxitin resistance was a particularly significant aspect of our studies. However, in their publication of CfxA2, Madinier et al. (3) make the claim that "high level of resistance of *B. vulgatus* CLA-341 towards cefoxitin should be attributed to a resistance mechanism other than CfxA production, such as porin mutation." This claim is based on the MIC values obtained for the cloned *cfxA2* gene in a heterologous host (*Escherichia coli*) and in *B. vulgatus* NI-2869. Further, kinetic experiments with purified CfxA2 were used to support their position. In making their claim, Madinier et al. ignore the considerable genetic and biochemical evidence indicating that CfxA is an extended-spectrum β-lactamase responsible for cefoxitin resistance in *Bacteroides* spp. Evidence supporting our position is as follows.

(i) In Table 1 of our original paper (5) and in many subsequent studies we have shown that the cloned *cfxA* gene (containing no other intact genes) can be transferred to any cefoxitin-sensitive *Bacteroides* spp. strain and confer high-level cefoxitin resistance. Controls with vector alone do not become resistant to cefoxitin. In addition, the transmissible Tn*4555* (Genbank accession no. U75371) containing the *cfxA* gene mediates cefoxitin resistance when present in single copy on the chromosome of *Bacteroides* species.

(ii) Similar to Madinier et al. (3), we showed (Table 2 of reference 5) that the β-lactamase activity of CfxA with cefoxitin as a substrate was very low (<0.01% of the activity with cephaloridine), but *Bacteroides* spp. containing the cloned *cfxA* gene could degrade >91% of the cefoxitin in the media after overnight incubation (Table 1 of reference 5). In contrast, *Bacteroides* spp. with the vector alone could not degrade cefoxitin. This indicates that CfxA can slowly degrade cefoxitin even though the rate cannot be readily monitoerd by standard spectrophotometric assays.

(iii) Experiments with *cfxA* in our laboratory were all performed in the natural host, *Bacteroides* (5), where the gene encodes a highly expressed β-lactamase as determined by specific activity of cell extracts (5). Madiner et al. (3) performed some experiements in *E. coli*, where β-lactamase and other resistance genes of *Bacteroides* origin are rarely expressed or are not correctly processed (1, 2, 4–8). Further, experiments performed in *B. vulgatus* NI2869 containing *cfxA2* exhibited extremely low MIC values for amoxicillin (and all β-latams) and no β-lactamase specific activities were reported. These MIC values were even lower than those seen in their *E. coli* work, suggesting that there is a significant problem with expression of their cloned *cfxA2* gene (3). Finally, the authors never directly tested *cfxA* in their *B. vulgatus* NI-2869 host, so there is no way to correct for differences conferred by strain background.

These observations establish that the β-lactamase gene *cfxA* mediates a broad-spectrum β-lactam resistance in *Bacteroides* spp. that is not dependent upon other mechanisms for resistance. This brings us back to the original question: what is an extended-spectrum β-lactamase? Is it an enzyme that rapidly degrades a wide raneg of β-lactam substrates or is it an enzyme that can mediate resistance to a wide range of β-lactams? Clearly, CfxA falls in this latter group and should be considered a broad-spectrum enzyme that mediates resistance to cefoxitin and other β-lactam drugs regardless of the enzymatic rate.

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Authors' Reply

In *Bacteroides* spp., resistance to cefoxitin appeared in the mid-1970s. Attention rapidly focused towards the notions of resistance transfer and variation in resistance profiles, suggesting the combination of different resistance mechanisms. These strains resistant to cefoxitin expressed a broad-spectrum resistance profile and caused clinical problems. Considerable genetic and biochemical evidence led to the cloning and characterization of a class B/group 3 extended-spectrum β-lactamase (*ccrA*/*cfiA* gene). CfiA was responsible for cefoxitin and other β-lactam agents, MIC increases (2, 6). Smith et al. characterized three β-lactamases belonging to a novel class A/group 2e subgroup: CfxA, CepA, and CblA (5, 7, 8). CepA was cloned from a strain of *Bacteroides fragilis* susceptible to cephamycins (7), and CblA was from a cefoxitin-resistant isolate of *Bacte-*

roides uniformis that used a combination of mechanisms for β-lactam resistance (8, 9). CfxA was described as being responsible for cefoxitin resistance in a wild-type donor strain of *Bacteroides vulgatus* and in two recipient strains of *B. fragilis* and *B. uniformis* (5). We recently described the CfxA2 β-lactamase in a cefoxitin-susceptible strain of *Prevotella intermedia* (4). *CfxA2* was cloned and correctly expressed into *Escherichia coli* (amoxicillin MICs of 4 and 1,024 μg/ml, respectively, before and after *cfxA2* transfer). Clavulanate and tazobactam potentiate strongly the activities of most β-lactams with the exception of cefoxitin and imipenem. *CfxA2* shared 98% identity with *cfxA*, and CfxA2 had the characteristics of group 2e β-lactamases. Comparison of CfxA and CfxA2 kinetic parameters showed that the single amino acid substitution (K272E) has no significant influence on their catalytic properties (4).

Phenotypic analysis of CfxA and CfxA2 revealed that (i) there is no cefoxitin-clavulanic acid synergy against the wild-type strains resistant to cefoxitin, and (ii) CfxA and CfxA2 don't hydrolyze cefoxitin in standard spectrophotometric assays (4). The very low degradation rate observed in high-performance liquid chromatography experiments is unexpected compared to the high cefoxitin MIC values (>256 μg/ml) determined in routine MIC determinations (5). We also investigated by PCR the distribution of *CfxA*/*CfxA2* in *Bacteroides* and *Prevotella* species: most of the PCR-positive strains were susceptible to cefoxitin (reference 3 and personal data). These phenotypic and epidemiologic data suggest that CfxA and CfxA2 are probably not a major factor responsible for cefoxitin resistance in *Bacteroides* and *Prevotella* species. Thus, these enzymes belong to Bush's class 2be, as they hydrolyze cefotaxime efficiently, but cefoxitin is not a substrate. As far we know, within class A β-lactamases those able to hydrolyze cefoxitin (with k_{cat} close to 1 s^{-1}) are also hydrolyzing imipenem, such as NMCA, IMI-1, Sme-1, Sme-2, and KPC-1. The resistance spectrum profile extended to a cefoxitin profile expressed by a proportion of strains harboring *cfxA*/*cfxA2* could be attributable to their expression combined with another mechanism, such as permeability alterations. As an example, recently Cao et al. demonstrated to the molecular level that imipenem resistance in *Klebsiella pneumoniae* strain BM 2974 resulted from the combination of these two mechanisms (acquisition of CMY-4 β-lactamase and loss of a 40-kDa outer membrane protein) in order to achieve detectable resistance (1).

These studies suggest that *Bacteroides* spp. and, to a lesser extent, *Prevotella* species can probably become resistant to cefoxitin by the combination of CfxA β-lactamase acquisition and membrane alteration towards cefoxitin, or other problems. Whatever is this yet unknown mechanism, the strains with the proper combination will express an extended-spectrum resistance profile including cefoxitin.

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