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CTX-M as the predominant extended-spectrum β-lactamases among Enterobacteriaceae in Manila, Philippines

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Sir,

The Philippines, an archipelago in the Western Pacific region with a population of ∼89 million, has a tradition of robust emigration and thus large traffic in international travel with countries such as China, India and the USA. However, data on the epidemiology of antimicrobial resistance genes among Enterobacteriaceae in the Philippines are limited. Here we report an analysis of plasmid-mediated antimicrobial resistance determinants present among extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae identified at a tertiary hospital in Manila, Philippines.

Three hundred non-duplicate Enterobacteriaceae that were identified from clinical specimens at the Central Microbiology Laboratory of the Philippine General Hospital were randomly collected between September and December 2007. Clinical isolates that were suspected to produce ESBL based on the disc diffusion method[1] were subjected to Etest ESBL (bioMérieux, Durham, NC, USA) for confirmatory testing. A reduction in the MIC of ceftazidime of at least three dilutions in the presence of clavulanate was interpreted as a positive test. As a result, a total of 39 ESBL-producing isolates were identified. The species were then further verified with the API20E system (bioMérieux). They included 15 *Escherichia coli*, 15 *Klebsiella pneumoniae*, 5 *Enterobacter cloacae*, 3 *Citrobacter freundii* and 1 *Proteus mirabilis*. PFGE was performed for all *K. pneumoniae* and *E. coli* isolates using XbaI as the restriction enzyme (New England Biolabs, Ipswich, MA, USA). For *E. coli*, phylogenetic typing and PCR analysis for identification of the sequence type (ST) 131 international epidemic clone were also performed.[2,3] PCR analyses were performed to identify various resistance genes in all study isolates. They included: β-lactamase genes bla_{TEM}, bla_{SHV} and bla_{CTX-M} (including $bla_{CTX-M-1}$, $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ groups);[4] plasmid-mediated AmpC β-lactamase genes bla_{CMY-1}, bla_{CMY-2}, bla_{DHA}, bla_{ACC}, bla_{ACT} and bla_{FOX};[5] 16S rRNA methylase genes *armA*, *rmtB* and *rmtC*;[6] pentapeptide repeat protein genes *qnrA*, *qnrB*, *qnrC* and *qnrS*; the fluoroquinolone-modifying aminoglycoside acetyltransferase gene *aac(6′)-Ib-cr*; and the plasmid-mediated fluoroquinolone efflux pump gene *qepA*.[7] PCR products were sequenced on both strands using an ABI 3100 instrument (Applied Biosystems, Foster City, CA, USA).

Of the 39 study isolates, 30 (77%), 39 (100%), 19 (49%) and 34 (87%) were non-susceptible to ceftazidime, cefotaxime, cefepime and aztreonam, respectively. All were susceptible to ertapenem. As with non-β-lactam agents, non-susceptibility to ciprofloxacin and gentamicin was very common [37 isolates (95%) for both], whereas susceptibility to amikacin was better maintained [7 isolates (18%) non-susceptible].

No or little clonal relationship was detected among the 15 *E. coli* and 15 *K. pneumoniae* isolates. Of the *E. coli* isolates, three, five, one and six belonged to phylogenetic groups A, B1, B2 and D, respectively. The only isolate belonging to phylogenetic group B2 was identified as ST131, which represents the international epidemic clone.

Table 1 summarizes various resistance genes present in the study isolates. An ESBL gene was identified in all 39 isolates. Thirty-seven (95%) possessed bla_{CTX-M}. Of them, the $bla_{CTX-M-1}$ group was the most common, followed by the $bla_{CTX-M-9}$ group.

Table 1. Distribution of antimicrobial resistance genes among the Enterobacteriaceae clinical isolates

		No. (%) of strains positive for the genes																			
		β-lactamase resistance genes											quinolone and aminoglycoside resistance genes								
								AmpC						16S rRNA methylase			Qnr				
Species	No. of strains	$bla_{\text{CTX-M-1 group}}$[a]	$bla_{\text{CTX-M-2 group}}$	$bla_{\text{CTX-M-9 group}}$[b]	bla_{SHV}[c]	bla_{TEM}	$bla_{\text{CMY-1}}$	$bla_{\text{CMY-2}}$	bla_{DHA}	bla_{ACC}	bla_{ACT}	bla_{FOX}	*aac(6′)-Ib-cr*	*armA*	*rmtB*	*rmtC*	*qepA*	*qnrA*	*qnrB*	*qnrC*	*qnrS*
E. coli	15	12	0	3	1	13	0	0	2	0	0	0	3	0	0	0	0	0	2	0	2
K. pneumoniae	15	14	0	0	13	14	0	0	2	0	0	0	9	3	0	0	0	0	5	0	9
E. cloacae	5	4	0	0	4	5	0	0	0	0	0	0	3	2	0	0	0	0	2	0	4
C. freundii	3	3	0	0	1	2	0	0	0	0	0	0	2	0	0	0	0	0	3	0	0
P. mirabilis	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Total	39	34 (87)	0 (0)	3 (8)	19 (49)	35 (90)	0 (0)	0 (0)	4 (10)	0 (0)	0 (0)	0 (0)	18 (46)	5 (13)	0 (0)	0 (0)	0 (0)	0 (0)	12 (31)	0 (0)	16[d] (41)

[a]Twenty-four isolates with $bla_{\text{CTX-M-15}}$, six isolates with $bla_{\text{CTX-M-3}}$ and four isolates with $bla_{\text{CTX-M-55}}$.
[b]Two isolates with $bla_{\text{CTX-M-14}}$ and one isolate with $bla_{\text{CTX-M-24}}$.
[c]Six isolates with $bla_{\text{SHV-12}}$, one isolate each with $bla_{\text{SHV-28}}$, $bla_{\text{SHV-32}}$ and $bla_{\text{SHV-11}}$, respectively, and 10 isolates with $bla_{\text{SHV-1}}$.
[d]Four isolates also positive for *qnrB*.

The $bla_{\text{CTX-M-2}}$ group was not identified. The two $bla_{\text{CTX-M}}$-negative isolates had $bla_{\text{SHV-12}}$. In addition, six isolates had bla_{SHV} known to exhibit the ESBL phenotype in addition to $bla_{\text{CTX-M}}$ (Table 1). All bla_{TEM} were identified as $bla_{\text{TEM-1}}$ encoding a non-ESBL. Four isolates carried $bla_{\text{DHA-1}}$, a plasmid-mediated AmpC β-lactamase gene, in addition to $bla_{\text{CTX-M-15}}$ and were resistant to cefoxitin.

Eight and 12 isolates carried the *qnrB* and *qnrS* alleles, respectively. Four isolates possessed both. Three of the eight *qnrB* were *qnrB4*, which occurred in the presence of $bla_{\text{DHA-1}}$. Two of the *qnrS*-positive isolates were susceptible to ciprofloxacin, whereas all *qnrB*-positive isolates were non-susceptible to this agent. None of the isolates gave positive PCR results for *qnrA*, *qnrC* or *qepA*. Twenty-six isolates were positive for *aac(6′)-Ib*. The deduced amino acid sequences for 18 of them were consistent with AAC(6′)-Ib-cr, which is a variant of aminoglycoside acetyltransferase AAC(6′)-Ib and has been implicated in low-level resistance to fluoroquinolones.[8] Of six isolates that were resistant to amikacin, five isolates possessed *armA* encoding 16S rRNA methylase.

To the best of our knowledge, the only previous report which studied ESBL-producing Enterobacteriaceae in the Philippines was conducted at the same hospital between 2001 and 2002 and found $bla_{\text{SHV-12}}$ to be the sole ESBL gene across species.[9] No $bla_{\text{CTX-M}}$ was found in that study. Our results indicate that the ESBL gene contents of Enterobacteriaceae have shifted dramatically from $bla_{\text{SHV-12}}$ to $bla_{\text{CTX-M-15}}$ in less than a decade in the same hospital in the Philippines, as has been observed in many other parts of the world.[10] They further underscore the urgent need for effective infection control measures and antimicrobial stewardship especially in countries such as the Philippines, where the costs of medical care related to infections due to multidrug-resistant organisms often need to be paid for by the patients themselves.

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AmpC induction by ceftaroline

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Sir,

Ceftaroline is a novel, parenteral, broad-spectrum oxyimino cephalosporin active against staphylococci, including methicillin-resistant strains, and pneumococci, including penicillin- and cefotaxime-resistant strains.[1] It also has anti-Enterobacteriaceae activity, but this is constrained by lability to β-lactamases, including AmpC, extended-spectrum, KPC and metallo types.[2] It has recently completed Phase III trials in complicated skin and skin structure infections and in moderate to severe community-acquired pneumonia, with better outcomes than seen with ceftriaxone in the latter setting.[3]

Like available oxyimino cephalosporins,[4] ceftaroline has near-equal activity against AmpC-inducible Enterobacteriaceae and their AmpC-basal mutants, and the potential for these enzymes to cause resistance is revealed only when they are manufactured copiously without induction, as in derepressed mutants, or by strains with plasmid-determined expression.[3] This behaviour, along with a propensity to select AmpC-derepressed mutants from AmpC-inducible populations *in vitro*,[3] implies that ceftaroline, like other oxyimino cephalosporins, is a weak inducer of AmpC enzymes at sub-MIC concentrations. We attempted to confirm or refute this inference by direct enzyme assays.

The test strains (two per species) comprised the β-lactamase-inducible parent organisms from the AmpC inducibility mutant series previously used for MIC comparisons.[3] All except the *Proteus vulgaris* strains have AmpC-type enzymes; *P. vulgaris* has a class A (or 2e) chromosomal β-lactamase, which is inhibited by clavulanate. We also examined pre- and post-therapy *Enterobacter cloacae* isolates (designated 615 and 616, respectively) from the sole patient in the Phase III clinical trials in whom resistant variants were selected during ceftaroline therapy. This patient had a skin structure infection. MICs were determined by CLSI agar dilution, and AmpC induction was measured using logarithmic-phase cells exposed to MIC multiples of ceftaroline, cefotaxime, ceftriaxone and cefoxitin for 2 h, with shaking, in nutrient broth at 37°C. β-Lactamase activity was assayed versus 0.1 mM nitrocefin in 0.1 M phosphate buffer pH 7.0 by spectrophotometry at 482 nm and 37°C, and standardized against protein concentration, measured by the Bradford method (Bio-Rad Protein Assay, Bio-Rad, Oxford, UK). β-Lactamase profiles of *E. cloacae* 615 and 616 were examined by isoelectric focusing (IEF) and their relatedness was assessed by PFGE of XbaI-restricted DNA.

All the reference strains had minimal β-lactamase activity in the absence of inducers but showed strong induction by cefoxitin at 1× the MIC, with induction ratios (β-lactamase specific activity with inducer/specific activity without inducer) averaging 116-fold, and ranging up to 457-fold (Table 1). In contrast, ceftaroline, cefotaxime and ceftriaxone induced weakly at 1× the MIC, with geometric mean induction ratios for all tested strains of 1.81-, 1.78- and 2.95-fold, respectively, and with no ratio exceeding 5-fold. Stronger induction was seen at 4× to 16× the MIC but, even then, the induction ratios remained lower than for cefoxitin.

IEF showed that *E. cloacae* 615 and 616 lacked other β-lactamases besides AmpC, which gave a cloxacillin-inhibited band at a pI $>$ 8.0, above any of the reference standards. Both isolates had identical PFGE profiles, confirming that isolate 616 was a cephalosporin-resistant mutant of 615, not a case of superinfection. Neither organism showed total derepression of AmpC; nevertheless isolate 616 had an ~5-fold higher uninduced β-lactamase specific activity compared with isolate 615 and, with ceftaroline at 1× the MIC, had 12.8-fold greater specific activity, implying stronger inducibility.

The present data confirm that ceftaroline resembles other oxyimino cephalosporins in being a weak inducer of AmpC β-lactamases at or below the MIC, thus agreeing both with the relative MIC data for AmpC-inducible, AmpC-basal and AmpC-derepressed Enterobacteriaceae and with the propensity to select AmpC-derepressed or hyperinducible mutants.[3] Such selection is a significant concern with oxyimino cephalosporins in some settings, occurring in 20%–30% of *Enterobacter* bacteraemias treated with these agents and leading to increased mortality, length of stay and cost.[5–7] Nevertheless, *in vivo* selection by ceftaroline should be limited by two factors: first,