The Novel CTX-M-116 β -Lactamase Gene Discovered in *Proteus mirabilis* Is Composed of Parts of the CTX-M-22 and CTX-M-23 Genes

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The novel β -lactamase gene *bla*_{CTX-M-116} was identified in a *Proteus mirabilis* nosocomial isolate recovered from the urine of a patient in Moscow in 2005. DNA sequence analysis showed *bla*_{CTX-M-116} to be a hybrid gene consisting of 5' *bla*_{CTX-M-23} (nucleotides 1 to 278) and 3' *bla*_{CTX-M-22} (nucleotides 286 to 876) moieties separated by an intervening putative site of recombination (GTAAAT). A retrospective analysis of available *bla*_{CTX-M} genes in the GenBank database revealed 19 *bla*_{CTX-M} genes that display the same hybrid structure.

CTX-M extended-spectrum β -lactamases (ESBLs) are a major cause of β -lactam resistance among Gram-negative bacteria worldwide (1–3). A total of 130 CTX-M sequence types have been identified (www.lahey.org/studies) grouped into five major clusters (CTX-M-1, -2, -8, -9, and -25) based on their amino acid sequences (4). The CTX-M-1 cluster is the most diversified group and also includes the most prevalent CTX-M variants described to date (5, 6). The family of *bla*_{CTX-M} genes is an excellent model for studying evolution in real time (7–9). It has been shown in natural CTX-M variants and in phylogenetic reconstruction experiments that new CTX-M enzymes arise by point mutation of their genes (10). One clear example of a recombination event that generated a novel *bla*_{CTX-M} variant found in *Shigella sonnei* is *bla*_{CTX-M-64}, which evolved as a result of homologous recombination be-

tween the *bla*_{CTX-M-15}-like gene (N- and C-terminal moieties) and the *bla*_{CTX-M-14}-like gene (central moieties) (11).

In this report, we describe a novel *bla*_{CTX-M-116} gene that was identified in *Proteus mirabilis* strain K-27 (State Collection of Pathogenic Microbes—Obolensk, accession number B-6773) isolated from the urine of a patient in the Urology Unit of the Sech-

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FIG 1 Alignments of the CTX-M-116 nucleotide sequence (A) and amino acid sequence (B) with those of CTX-M-23 and CTX-M-22. Nucleotides in positions bp 50 to 100, bp 550 to 600, and bp 651 to 876 are not shown because of 100% identity. Bold type highlights specific nucleotides and amino acids; the proposed core site at nucleotides 279 to 285 is indicated by gray shading. The amino acids are numbered according to the standard numbering scheme for the class A β -lactamases of Ambler et al. (26). The underlined area represents the active-site omega loop. Bold type in gray boxes shows specific amino acids.

enov Moscow Medical Academy in March 2005 (12). The bacterial strain was resistant to ampicillin (MIC > 256 μ g/ml), ampicillin/sulbactam (MIC = 32 μ g/ml), cefuroxime (MIC > 256 μ g/ml), cefoxitin (MIC = 32 μ g/ml), cefotaxime (MIC = 32 μ g/ml), ceftazidime (MIC = 32 μ g/ml), gentamicin (MIC = 32 μ g/ml), ciprofloxacin (MIC = 256 μ g/ml), doxycycline (MIC > 256 μ g/ml), chloramphenicol (MIC = 128 μ g/ml), and trimethoprim-sulfamethoxazole (MIC = 16/304 μ g/ml); showed intermediate resistance to aztreonam (MIC = 2 μ g/ml); and was sensitive to ceftazidime (MIC = 0.25 μ g/ml), meropenem (MIC = <0.12 μ g/ml), and amikacin (MIC = 0.5 μ g/ml) as determined by broth microdilution in Mueller-Hinton broth (Difco) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (13). Genetic determinants carried by *Proteus mirabilis* K-27 revealed by PCR using specific primers (12, 14–19) included *bla*_{TEM}, *ISEcp1*, *bla*_{CTX-M}, class 1 integrase, and an integron insertion carrying the *aadA1* gene. A specific 45-bp sequence was located between the *ISEcp1* and *bla*_{CTX-M-116} sequences (20). The PCR was carried out in a 25- μ l reaction mixture using GradientPalmCycler (Corbett Research, Australia).

The novel *bla*_{CTX-M-116} gene was first identified as *bla*_{CTX-M-23} using a PCR-restriction fragment length polymorphism (PCR-RFLP) algorithm (21). However, DNA sequence analysis using Vector NTI 9 software (Invitrogen) and nucleotide alignments using the BLAST Web resource (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed that only the 5' moiety (nucleotides 1 to 278, starting from the start site of translation) of *bla*_{CTX-M-116} was identical to the corresponding segment of *bla*_{CTX-M-23} (including specific single nucleotide polymorphisms [SNP] in positions 21, 138, 139, and 239), while the 3' moiety (nucleotides 286 to 876) matched *bla*_{CTX-M-22} (including specific SNP in positions 313, 315, 508, and 609), suggesting that *bla*_{CTX-M-116} originated through a recombination event. The putative 7-bp site of recombination (GTTAAAT) is located between the *bla*_{CTX-M-23}-associated 5' moiety and the *bla*_{CTX-M-22}-associated 3' moiety from nucleotide 279 to nucleotide 285 (Fig. 1A). So we propose that the *bla*_{CTX-M-116} gene originated as a result of recombination between *bla*_{CTX-M-23} and *bla*_{CTX-M-22}.

The hybrid *bla*_{CTX-M-116} gene confers a phenotype of resistance that differs from the phenotypes of both *bla*_{CTX-M-23} and *bla*_{CTX-M-22} genes. The CTX-M-23 β -lactamase contains the amino acid substitution at position 167 (Pro to Thr) associated with ceftazidime-hydrolyzing activity resulting in an elevated MIC of ceftazidime (MIC = 64 to 128 μ g/ml), but a lower MIC of cefotaxime (MIC = 2 to 16 μ g/ml) (22). However, the CTX-M-22 β -lactamase, a variant of CTX-M-1, typically hydrolyzes cefotaxime (MIC = 64 μ g/ml) more efficiently than ceftazidime (MIC = 2 μ g/ml) (23). Consequently, the cephalosporin resistance profile of *P. mirabilis* K-27 (cefotaxime MIC = 32 μ g/ml and ceftazidime MIC = 0.25 μ g/ml) conferred by the hybrid *bla*_{CTX-M-116} gene may be influenced by the amino acid at position 167 (Pro) of the CTX-M-22 enzyme because of just this SNP located at the active-site omega loop (Fig. 1B).

Comparison of available *bla*_{CTX-M} sequences (<http://www.lahey.org/studies/other.asp#table1> and GenBank) showed that three other *bla*_{CTX-M} genes are organized in a similar manner with 5' and 3' moieties matching two different *bla*_{CTX-M} genes: *bla*_{CTX-M-57} includes 5'-*bla*_{CTX-M-52} and 3'-*bla*_{CTX-M-15}; *bla*_{CTX-M-79} includes 5'-*bla*_{CTX-M-52} and 3'-*bla*_{CTX-M-28}; and *bla*_{CTX-M-89} includes 5'-*bla*_{CTX-M-25} and 3'-*bla*_{CTX-M-39} (Table 1). Moreover, 13 *bla*_{CTX-M}

TABLE 1 5' moiety-proposed site of recombination-3' moiety structure of *bla*_{CTX-M} genes^a

<i>bla</i> _{CTX-M} gene (gene no.)	GenBank accession no.	5' moiety (gene no.)	RS (oligonucleotides)	3' moiety (gene no.)
Cluster 1				
1	X92506	1	GTTAAAT	1
10	AF255298	10	GTTAAAT	10
11	AY005110	11	GTTAAAT	11
12	AF305837	12	GTTAAAT	12
15	AY044436	15	GTTAAAT	15
23	AF488377	23	GTTAAAT	23
28	AJ549244	28	GTTAAAT	28
29	AY267213	29	GTTAAAT	29
30	AY292654	30	GTTAAAT	30
33	AY238472	33	GTTAAAT	33
36	AB177384	36	GTTAAAT	36
37	AY649755	37	GTTAAAT	37
52	DQ223685	52	GTTAAAT	52
53	DQ268764	53	GTTAAAT	53
60	AM411407	60	GTTAAAT	60
62	EF219134	62	GTTAAAT	62
66	EF576988	66	GTTAAAT	66
68	EU177100	68	GTTAAAT	68
80	EU202673	80	GTTAAAT	80
82	DQ256091	82	GTTAAAT	82
101	HQ398214	101	GTTAAAT	101
114	GQ351346	114	GTTAAAT	114
3*	Y10278	15	GTTAAAT	3
22*	AY080894	15	GTTAAAT	22
32*	AJ557142	1	GTTAAAT	32
34*	AY515297	10	GTTAAAT	34
42*	DQ061159	15	GTTAAAT	42
54*	DQ303459	15	GTTAAAT	54
58*	EF210159	1	GTTAAAT	58
61*	EF219142	1	GTTAAAT	61
69*	EU402393	52	GTTAAAT	69
71*	FJ815436	15	GTTAAAT	71
72*	AY847148	15	GTTAAAT	72
88*	FJ873739	15	GTTAAAT	88
96*	AJ704396	12	GTTAAAT	96
57**	DQ810789	52	GTTAAAT	15
79**	EF426798	52	GTTAAAT	28
116**	JF966749	23	GTTAAAT	22
Cluster 8/25				
8	AF189721	8	GTTGAGT	8
25	AF518567	25	GTTGAGT	25
26	AY157676	26	GTTGAGT	26
40	AY750914	40	GTTGAGT	40
63	AB205197	63	GTTGAGT	63
91	GQ870432	91	GTTGAGT	91
39*	AY954516	26	GTTGAGT	39
41*	DQ023162	26	GTTGAGT	41
94*	HM167760	26	GTTGAGT	94
89**	FJ971899	25	GTTGAGT	39

^a *, the sequence of the 5' moiety is contributed by another *bla*_{CTX-M} gene, but the sequence of the 3' moiety is unique. **, both the 5' and 3' moieties are contributed by other *bla*_{CTX-M} genes. RS, proposed site of recombination.

genes belonging to cluster 1 and 3 genes from cluster 8/25 display similar basic hybrid structures with some differences. In these instances, while the 5' moiety originated from other *bla*_{CTX-M} genes, the 3' moiety is unique for the particular variant (Table 1). Finally, there are *bla*_{CTX-M} genes from cluster 1 and cluster 8/25 with an entirely unique sequence for the particular variant (Table 1). The

TABLE 2 Prevalence of 5' and 3' moieties among *bla*_{CTX-M} genes

5' moiety of <i>bla</i> _{CTX-M} (gene no.)	No. of genes	3' moiety of <i>bla</i> _{CTX-M} (gene no.)	No. of genes
Cluster 1			
15	8	15	4
1	4	3	3
52	4	28	2
12	2	12	2
23	2	22	2
10	2		
Cluster 8/25			
26	4	39	2
25	2		

contribution of 5' and 3' moieties may be more disseminated for some *bla*_{CTX-M} genes than others. For example, the 5' and 3' moieties associated with the gene encoding the epidemic CTX-M-15 enzyme that is globally disseminated among bacterial pathogens (6) were identified in 8 and 4 genes, respectively (Table 2). The basic hybrid structure of *bla*_{CTX-M} genes was found only in genes belonging to clusters 1 and 8/25, but not in genes in clusters 2 and 9. Proposed sites of recombination are specific for *bla*_{CTX-M} cluster 1 (GTAAAT) and cluster 8/25 (GTTGAGT) (Table 1).

Since the presence of two different genes is needed for the hybrid recombination event, it is interesting that two and three different CTX-M genes in the same strain were described in some studies, namely, in 5.3% (24), 10.4% (25), and 1.0% (12) of CTX-M-positive *Enterobacteriaceae* isolates.

In conclusion, we describe the novel hybrid *bla*_{CTX-M-116} gene and propose that it was derived through the recombination of *bla*_{CTX-M-23} and *bla*_{CTX-M-22}. Thus, recombination is a powerful mechanism driving the evolution of resistance genes encoding CTX-M β-lactamases along with the accumulation of point mutations.

Nucleotide sequence accession number. The nucleotide sequence of the *bla*_{CTX-M-116} gene is available under GenBank accession number [JF966749](#).

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