

Characterization of the first OXA-10 natural variant with significant carbapenemase activity

Stathis D. Kotsakis^{a,b}, Carl-Fredrik Flach^{a,b}, Mohammad Razavi^{a,b}, D.G. Joakim Larsson^{a,b}

^aDepartment of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

^bCentre for Antibiotic Resistance Research (CARE) at University of Gothenburg, Gothenburg, Sweden

Supplemental material S1

Table S1. Primers used in this study

Primer	Sequence (5'-3')	Target; nts	Reference
VIM-F	GTTTGGTCGCATATCGCAAC	blaVIM; 157-176	Monteiro et al, 2012
VIM-R	AATGCGCAGCACCAGGATAG	blaVIM; 538-519	Monteiro et al, 2012
NDM-F	TTGGCCTTGCTGTCCTTG	blaNDM; 647-630	Monteiro et al, 2012
NDM-R	ACACCAGTGACAATATCACCG	blaNDM; 566-586	Monteiro et al, 2012
IMP-F	GAGTGGCTTAATTCTCRATC	blaIMP; 313-332	Monteiro et al, 2012
IMP-R	AACTARCCAATARYTAAC	blaIMP; 415-432	This study
KPC-F	TCGCTAAACTCGAACAGG	blaKPC; 98-115	Monteiro et al, 2012
KPC-R	TTACTGCCCGTTGACGCCCAATCC	blaKPC; 882-859	Monteiro et al, 2012
GES-F	CTATTACTGGCAGGGATCG	blaGES; 19-37	Monteiro et al, 2012
GES-R	CCTCTCAATGGTGTGGGT	blaGES; 612-595	Monteiro et al, 2012
OXA-10-F	CAGTAAATCCTGCGCTAC	blaOXA-10; 141-158	This study
OXA-10-R	GGCGAAAAAGTAAACCTC	blaOXA-10; 708-691	This study
M-SHV-F	AGCCGCTTGAGCAAATTAAC	blaSHV; 71-91	Dallene et al, 2010
M-SHV-R	ATCCCGCAGATAAATCACCCAC	blaSHV; 783-763	Dallene et al, 2010
TEM-A	GAGTATTCAACATTTCCGTGTC	blaTEM; 3-24	Drieux et al, 2009
TEM-B	TAATCAGTGAGGCACCTATCTC	blaTEM; 850-829	Drieux et al, 2009
M-OXA-1-F	GGCACCAGATTCAACTTTCAAG	blaOXA-1; 201-222	Dallene et al, 2010
M-OXA-1-R	GACCCCAAGTTTCCTGTAAGTG	blaOXA-1; 764-743	Dallene et al, 2010
repB_pEA3-F	GATGAGTTGGGCGCAGGG	pEA3; 1216-1199	This study
repB_pEA3-R	GTCGCCCCGGACAACAGC	pEA3; 617-634	This study
mobC-F	GCAGCAAGAGCGCAAGAAC	pQGU13; 184-166	This study
tnpA-Tn3x-F	CAGTACCAAGGTCGGCGC	pQGU13; 13415-13432	This study
tnpR-Tn3x-F	GCGATTTGTGTGTGGAAC	pQGU13; 9850-9833	This study
Int11F	CGTTCCATACAGAAGCTG	pQGU13; 8909-8892	This study
IS4321-F	CTGTGTAGGAAAAGCAACT	pQGU13; 14454-14472	This study
IS4321-R	AGAAGGGTACTCAGTCGGC	pQGU13; 14044-14026	This study

Table S2. Acquired non-beta-lactam resistance determinants detected in WGS data of *E. cloacae* WW13 and *E. coli* WW16.

Antibiotic class	<i>E. cloacae</i> WW13	<i>E. coli</i> WW16
Aminoglycosides	<i>strA</i>	<i>strA, aac(3)-IIa, aadA1, aacA4, aac(6')Ib-cr</i>
Sulfonamides	<i>sul2</i>	<i>sul1, sul2</i>
Fluoroquinolones	none	<i>aac(6')Ib-cr</i>
Tetracyclines	none	none
Phenicol	none	<i>catA2, catB2, catB3</i>
Trimethoprim	<i>none</i>	<i>dfrA12</i>

Table S3. Replicase genes and replicons detected by PlasmidFinder in contigs of *E. cloacae* WW13 and *E. coli* WW16.

Rep.	% ID	Query/HSP length	Contig	Acc. number
<i>E. cloacae</i> WW13				
IncFII	84.85	264 / 258	NODE_16	AF401292
IncQ1	100	450 / 450	NODE_32	HE654726
ColRNAI	88.3	94 / 130	NODE_63	DQ298019
ColRNAI	89.36	94 / 130	NODE_54	DQ298019
ColRNAI	89.36	94 / 130	NODE_61	DQ298019
IncFIB(K)	98.75	560 / 560	NODE_34	JN233704
IncFII(Y)	87.95	224 / 227	NODE_25	CP001049
IncFIB(pECLA)	100	560 / 560	NODE_31	CP001919
ColRNAI	91.49	94 / 130	NODE_38	DQ298019
IncFIA(HI1)	98.97	387 / 388	NODE_55	AF250878
<i>E. coli</i> WW16				
IncHI1B(R27)	100	540/540	NODE_52	AF250878
IncFIA(HI1)	98.45	387/388	NODE_116	AF250878
IncHI1A	99.76	420/420	NODE_52	AF250878
IncFII(K)	98.65	148/148	NODE_29	CP000648
ColRNAI	86.17	94/130	NODE_96	DQ298019
IncQ1	100	450/450	NODE_68	HE654726

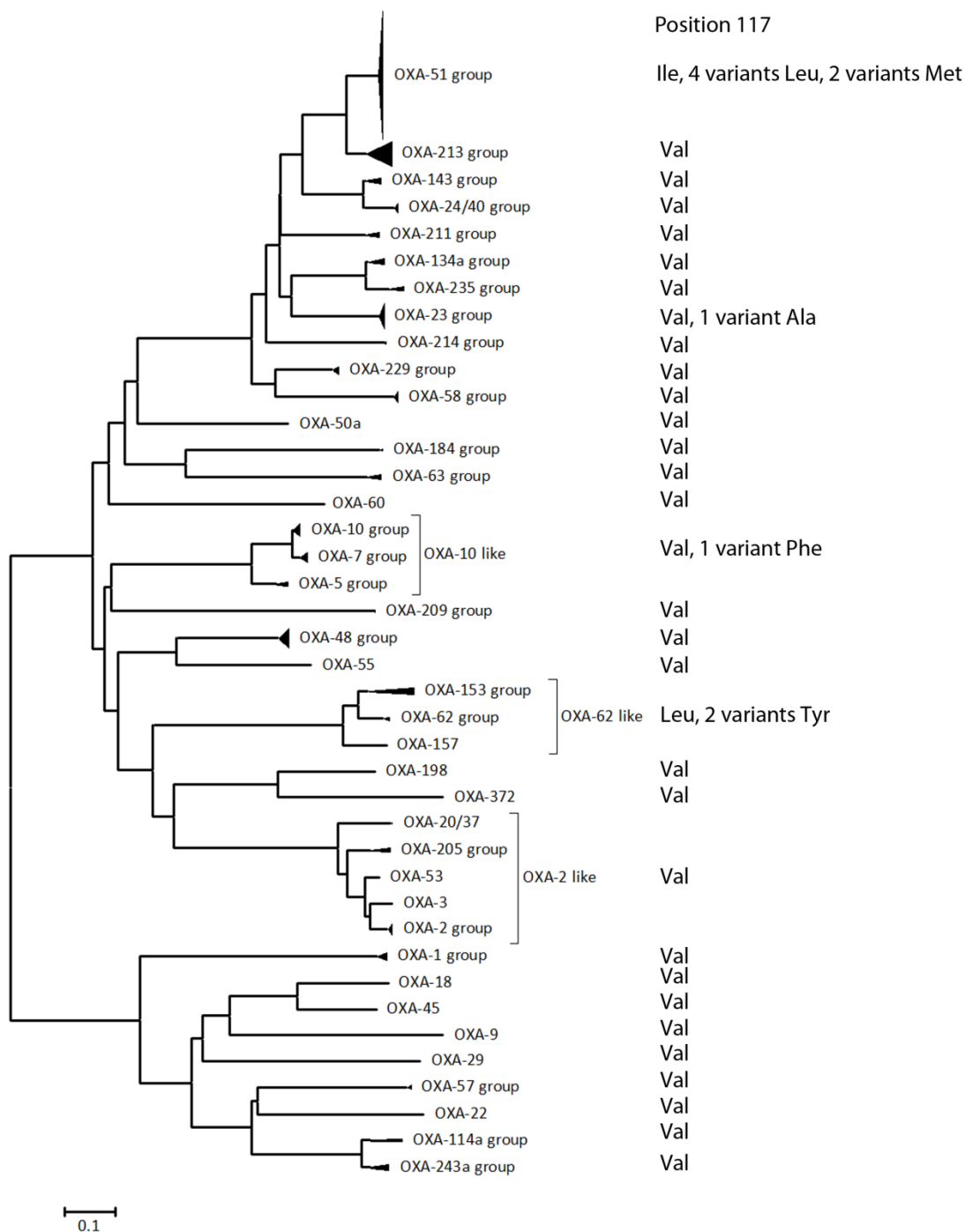


Figure S1. Primary structure relationships among OXA β -lactamases and residue at position 117.

The tree was constructed by the Neighbor-Joining method using available amino-acids sequences (n=320) of the so far published OXA enzymes in the Lahey Clinic Bla database (<https://www.lahey.org/Studies/>). The optimal tree with the sum of branch length = 13.15838779 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7. Amino acids occupying position 117 in the various OXA groups are indicated in the right column as well as the substitutions that have been observed in natural variants.

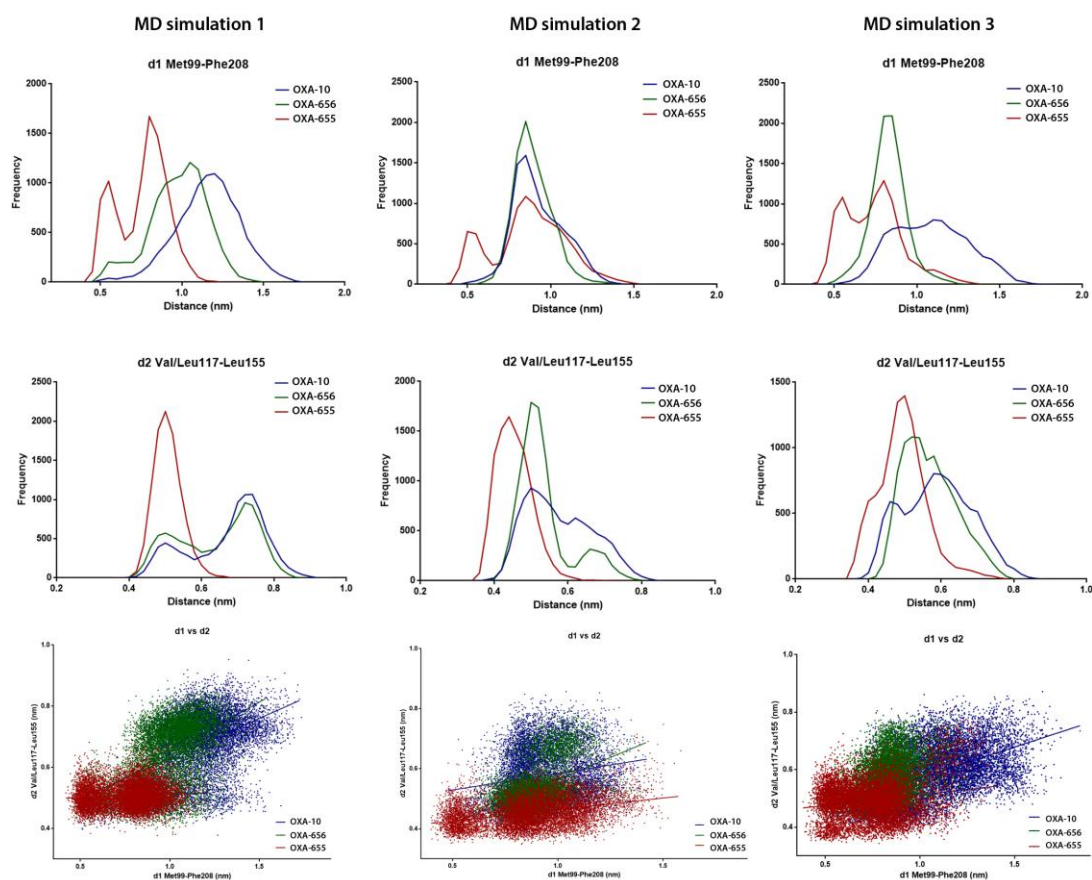


Figure S2. Structural differences between OXA-10, OXA-655 and OXA-656 observed during 3 separate 10 ns MD simulations.

Distribution of distances between the C ϵ atom of Met99 side chain and the center of mass of Phe208 ring carbon atoms (d_1 ; upper plots) and between the methyl groups of Val/Leu117 and Leu155 (d_2 ; medium plots). Frequency denotes actual number of structures. The relation of d_1 and d_2 is depicted in the lower plots.

References

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- Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. 2010;65(3):490-5
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