A GREATER THAN EXPECTED VARIABILITY AMONG OXA-48-LIKE CARBAPENEMASES

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ABSTRACT

Background: OXA-48-like carbapenemases represent a major health concern given their difficult detection, their epidemic behavior and their propensity to modify their spectrum of hydrolysis through point mutations.

Objective: To get an extensive view on the current variability among OXA-48-like enzymes, we have retrieved all the sequences available from NCBI (National Center for Biotechnology Information).

Method: We carried out several BLAST (Basic Local Alignment Search Tool) searches in the NCBI's "nr" and "nr_env" databases (downloaded on December 20th, 2016) using known members of OXA-48-like subfamily as query.

Results: While 23 variants have assigned OXA-numbers, 62 novel alleles have been identified. They correspond to novel enzymes with mutations located in some cases within the conserved active site motives. The important number of novel variants identified by this study is of great interest, since it provides a more realistic assessment of OXA-48-like variants.

Conclusion: A large variety of OXA-48-like enzymes has been unraveled through our bioinformatic search for variants. The finding of OXA-48-like enzymes in environmental isolates may reflect the contamination by Enterobactericeae producing OXA-48-like enzymes and/or the presence of *Shewanella spp.* isolates.

Keywords: OXA-48, variability, variants.

REZUMAT

Introducere: Carbapenemazele OXA-48-like reprezintă o problemă majoră pentru sănătate, având în vedere detectarea lor dificilă, comportamentul epidemic și tendința lor de a-și modifica spectrul de hidroliză prin mutații punctiforme.

Obiectiv: Pentru a obține o imagine amplă asupra variabilității actuale a enzimelor OXA-48-like, am recuperat toate secvențele disponibile de la NCBI (National Center for Biotechnology Information).

Metodă: Am efectuat mai multe căutări BLAST (Basic Local Alignment Search Tool) în bazele de date „nr" și „nr_env" ale NCBI (descărcate pe 20 decembrie 2016), utilizând ca interogare membrii cunoscuți ai sub-familiei OXA-48.

Rezultate: În timp ce unui număr de 23 de variante li s-au atribuit numere OXA, au fost identificate 62 de alele noi. Acestea corespund noilor enzime cu mutații localizate în unele cazuri în cadrul motivelor conservate ale site-ului activ. Numărul important de variante noi identificate în acest studiu este de mare interes, deoarece oferă o evaluare mai realistă a variantelor de tip OXA-48-like.

Concluzie: O mare varietate de enzime OXA-48-like a fost descoperită în urma căutării bioinformatice a variantelor. Detectarea enzimelor OXA-48-like în izolate din mediu poate reflecta contaminarea cu Enterobactericeae producătoare de enzime OXA-48-like și/sau prezența de izolate *Shewanella spp.*

Cuvinte-cheie: OXA-48, variabilitate, variante.

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INTRODUCTION

In the last decade, the emergence of carbapenem-resistance in Gram-negatives has been observed worldwide, both in non-fermenters and in *Enterobacteriaceae* [1, 2]. The emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) has become a major public health concern [1, 3]. Among these CPEs, OXA-48-producing *Enterobacteriaceae* have now widely disseminated throughout European countries and are identified on all continents [3, 4]. OXA-48 confers high-level resistance to penicillins, including temocillin, and hydrolyzes carbapenems at a low level, but spares extended-spectrum cephalosporins [5, 6]. OXA-48-like enzymes are Ambler class D enzymes, that belong to active-serine β-lactamases. According to the DBL (class D β-lactamase numbering scheme), oxacillinases possess a serine residue at position DBL 70 and a carbamoylated lysine at position DBL 73 [5, 7, 8]. The YGN (positions 144 to 146) and KTG (positions 216 to 218) motives are mostly conserved in oxacillinase sequences, the YGN motif being replaced by a FGN motif in several cases [5, 9]. The omega loop plays an interesting role in the function of the beta-lactamases: mutations in the "omega loop region" of a beta-lactamase can change its specific function and substrate profile, perhaps due to an important functional role of the correlated dynamics of the region [5]. A sign of the current spread of OXA-48-like enzymes is the identification of point mutant derivatives, differing by few amino acid substitutions or deletions. Most of these differences are located within the β5-β6 loop, which is important in the substrate specificity of OXA-48 [9-11].

Whereas some OXA-48-variants (mostly point mutant derivatives) have similar hydrolytic activities as compared to OXA-48 (OXA-181, OXA-204), others have slightly increased carbapenem-hydrolyzing activities (OXA-162) or slightly reduced carbapenem and temocillin hydrolyzing activities (OXA-232, OXA-244) [11, 12]. In contrast, variants presenting a four amino acid deletion within the β5-β6 loop (Table 1) such as OXA-163, OXA-247, OXA-405 have lost their carbapenem-hydrolytic activity but gained instead the capacity to hydrolyze expanded-spectrum cephalosporins (Fig. 1, Table S1) [11, 13]. A comprehensive list of variants identified in clinical isolates can be found in the Beta-Lactamase DataBase (http://bldb.eu/alignment.php?align=D:OXA-48-like).

Twenty-two OXA-48 variants have been described, most of them from enterobacterial isolates, and have been assigned an OXA-number initially by Lahey Clinic (http://www.lahey.org/Studies/other.asp), and now by the Bacterial Antimicrobial Resistance Reference Gene Database at NCBI (https://www.ncbi.nlm.nih.gov/bioproject/313047). *Shewanella* species have been suggested as the reservoir of bla_{OXA-48} type oxacillinase genes [14]. This is the case for OXA-54 from *S. oneidensis* as it shares significant amino acid sequence identity with OXA-48. *Shewanella xianemensis* has recently been identified as the progenitor of OXA-181, OXA-48, and OXA-204 [15, 16]. However, this is not the case for all *Shewanella* species, since *S. algae* produces OXA-55, which has only 57% sequence identity with OXA-48 [14]. Several natural variants (7/23) have been described in *Shewanella* sp. isolates and have been assigned an OXA number. For these variants, kinetic data are not always available [7].

With the high throughput sequencing of many bacterial genomes, and the tremendous amount of metagenomic sequencing data, the available bacterial DNA sequences increase exponentially in the databases. In many cases, these sequence entries have not been carefully analyzed in respect to β-lactamase gene content. In this study, we have searched for the presence of OXA-48-like enzymes in these genomic and metagenomic sequence databases.

MATERIAL AND METHODS

We carried out several BLAST searches in the NCBI's "nr" and "nr_env" databases (downloaded on December 20th, 2016) using known members of OXA-48-like subfamily as query.

ClustalW was used to align the protein sequences of the identified novel chromosomally- and plasmid-encoded OXA-48-like β-lactamases with those already published, and Dendroscope was used to construct a phylogram [17, 18]. The references of all these enzymes can be found in the Beta-

Table 1. Sequence alignment for the OXA-48-like subfamily of class D β-lactamases. Only the sequences and the positions with mutations or deletions from loop β5-β6 are shown. The complete alignment can be found in the Beta-Lactamase DataBase at http://bldb.eu/alignment.php?align=D:OXA-48-like [7]

DBL[a] AA numbering scheme	219[c]	**220**	**224**	**225**	**226**	**227**	**228**	229	230
OXA-48 AA numbering[b]	211	**212**	**213**	**214**	**215**	**216**	**217**	218	219
OXA-48	Y	**S**	**T**	**R**	**I**	**E**	**P**	K	I
OXA-54								Q	
OXA-162			**A**						
OXA-163, OXA-439		**–[d]**	**–**	**–**	**–**	**D**	**T**		
OXA-232				**S**					
OXA-244, OXA-484				**G**					
OXA-247	S	**–**	**–**	**–**	**–**	**N**	**T**		
OXA-370		**E**							
OXA-405		**–**	**–**	**–**	**–**	**S**			
OXA-436			**V**						
OXA-438		**G**	**Y**	**–**	**–**	**D**	**T**		
OXA-538			**G**						F
OXA-D281, OXA-D282, OXA-D284			**V**						
OXA-D303	C								
OXA-D312		**P**							
OXA-D340					**V**				

[a] Class D β-lactamase numbering scheme [5, 8];
[b] OXA-48 specific numbers. Numbers in bold correspond to residues of the β5-β6 loop ;
[c] Only the sequences and the positions with mutations or deletions from loop β5-β6 are shown. The complete alignment can be found in the Beta-Lactamase DataBase at http://bldb.eu/alignment.php?align=D:OXA-48-like
[d] – indicates a deletion.

Lactamase DataBase at http://bldb.eu/BLDB.php?class=D#OXA [7]; (b) Sequence of OXA-48; (c) Three-dimensional structure of OXA-48 (PDB 3HBR). Active site serine 70 is colored in green, and the residues from loop β5-β6 are colored with different shades of blue.

RESULTS-DISCUSSION

In this way, we could identify, along with the known 23 members of the OXA-48-like subfamily (enzymes with an assigned OXA-name), 62 novel OXA-48-like variants (displaying at least one point mutation to any of the 23 known variants) belonging to the OXA-48-like subfamily, and thus that are not present in the Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/313047).

No OXA-number has been assigned to these variants, since most of them were not isolated in clinical settings and their identification was primarily based on *in silico* analysis. These variants have been added to the BLDB database, as OXA-D type enzymes followed by a number, while waiting for a definitive assignment by NCBI. These novel variants show >90% sequence identity as compared with OXA-48. The nucleotide and protein sequences along with their GenBank accession numbers can be found in the Beta-Lactamase DataBase (http://bldb.eu/BLDB.php?class=D#OXA) [7].

Among the sequences presented in Table S1, 2 were retrieved from dye-degrading bacteria [17, 18], 7 from *Shewanella* sp., and 43 from uncultured bacteria.

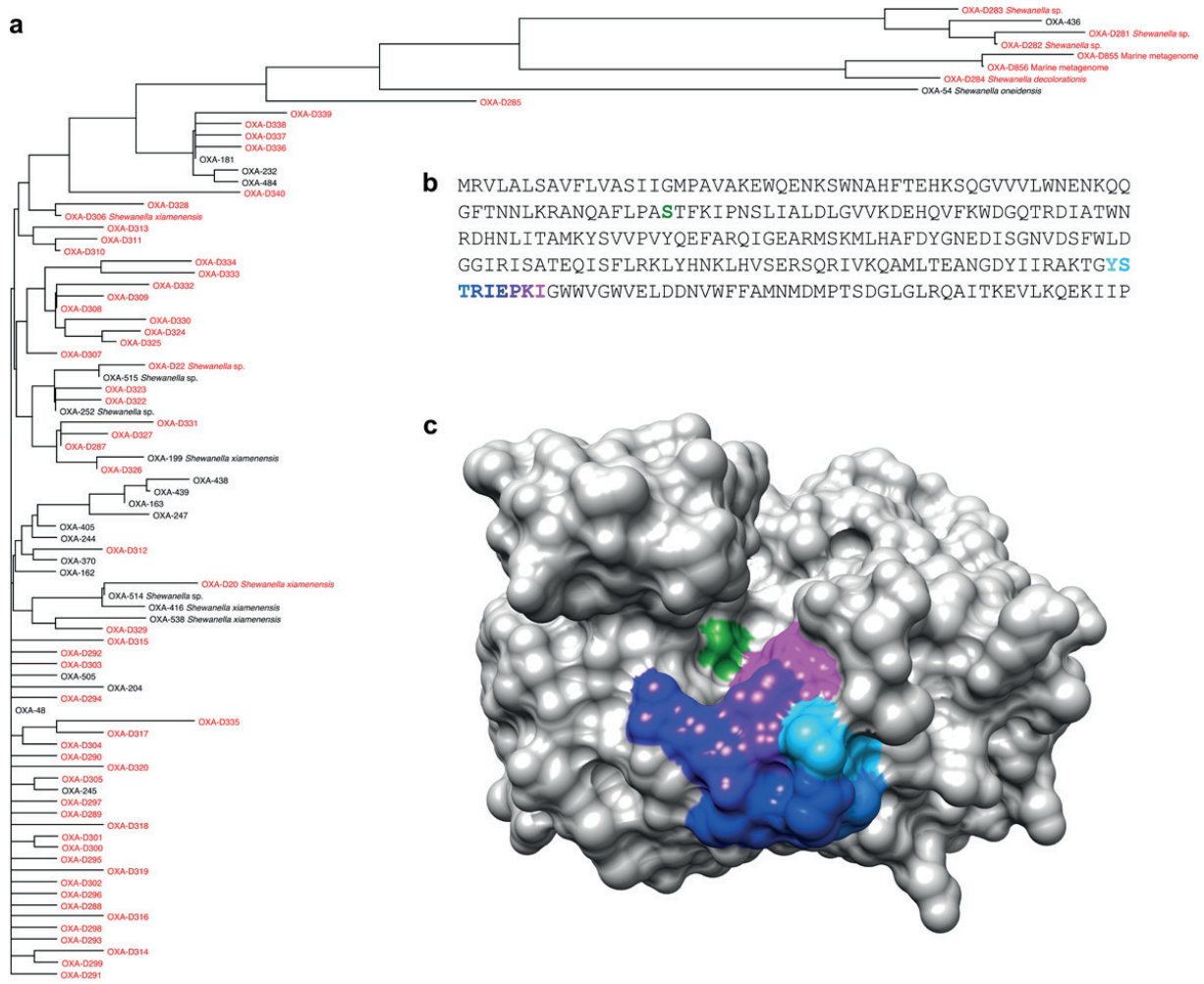

Fig. 1. (a) Phylogeny of the chromosomally- and plasmid-encoded class D β-lactamases belonging to the OXA-48-like subfamily. For naturally-occurring enzymes, the bacterial host name is indicated. The alleles identified in this study are colored in red. The phylogram was constructed with Dendroscope using ClustalW aligned protein sequences [19, 20]. The references of all these enzymes can be found in the Beta-Lactamase DataBase at http://bldb.eu/BLDB.php?class=D#OXA [7]; (b) Sequence of OXA-48; (c) Three-dimensional structure of OXA-48 (PDB 3HBR). Active site serine 70 is colored in green, and the residues from loop β5-β6 are colored with different shades of blue.

Among the 62 novel variants a great variability has been observed differing by one up to 24 amino-acids (Fig. 1). The most distantly related variants are close to OXA-436, a carbapenemase responsible of an outbreak in Denmark. The amino acid changes are scattered all over the sequence, but some are located within the conserved regions of class D enzymes (represented as boxes in the Supplementary Information file), and thus are likely to generate altered phenotypes. For instance, the F72I change in OXA-D320, which is located next to the S70 and the K73 may impact the activity of the enzyme. Similarly, Y144C and N146S, found in OXA-D319 and D338, respectively, are located within the YGN motif. A Y to F change is responsible of NaCl resistance of oxacillinases, as shown for OXA-40 [9].

The T217A change is located within the highly conserved KTG box. This threonine has been replaced by serine in OXA-40 without effect on hydrolysis, but the effect of an alanine in this position has not been addressed [9].

Finally, three novel variants were found with changes in the β5-β6 loop. The T221V as found in OXA-D281, -D282, and -D284 may have increased hydrolysis as shown for OXA-162, even though in the latter the T was replaced by A [11]. The S220P (OXA-D312)

may alter significantly the loop conformation, which would likely induce a drastic effect on the activity of the enzyme. The I226V is likely silent in terms of activity, as we have recently shown that I226A replacement does not alter the activity of the enzyme (T. Naas, personal communication).

CONCLUSION

Genomic and metagenomics data turn to be an inestimable source for discovering novel resistance gene or derivatives of known genes. In most cases, the reasons these genomic or metagenomics data were generated were not linked to antibiotic resistance studies. A large variety of OXA-48-like enzymes has been unraveled through our bioinformatic search for variants. The finding of OXA-48-like enzymes in environmental isolates may reflect the contamination by Enterobactericeae producing OXA-48-like enzymes and/or the presence of *Shewanella spp.* isolates. The important number of novel variants identified by this study is of great interest, since it provides a more realistic assessment of OXA-48-like variants. The finding of some amino-acid changes in the key boxes or the β5-β6 loop, suggests likely changes in hydrolysis profile. Further work will be necessary to address these issues.

Acknowledgements

This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (EA7361), Université Paris Sud, by the Région Ile-de-France (DIM Malinf) and by the Laboratory of Excellence LERMIT supported by a grant from ANR (ANR-10-LABX-33).

Conflict of interests: None to declare.

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