Curr Microbiol (2013) 67:596–600
DOI 10.1007/s00284-013-0407-5

# Distribution of Allelic Variants of the Chromosomal Gene *bla*$_{\text{OXA-114-like}}$ in *Achromobacter xylosoxidans* Clinical Isolates

**German Matías Traglia · Marisa Almuzara · Andrea Karina Merkier · Mariana Papalia · Laura Galanternik · Marcela Radice · Carlos Vay · Daniela Centrón · María Soledad Ramírez**

Received: 18 March 2013 / Accepted: 12 May 2013 / Published online: 15 June 2013


**Abstract** *Achromobacter xylosoxidans* is increasingly being documented in cystic fibrosis patients. The *bla*$_{\text{OXA-114}}$ gene has been recognized as a naturally occurring chromosomal gene, exhibiting different allelic variants. In the population under study, the *bla*$_{\text{OXA-114-like}}$ gene was found in 19/19 non-epidemiological-related clinical isolates of *A. xylosoxidans* with ten different alleles including 1 novel OXA-114 variant.

G. M. Traglia · A. K. Merkier · D. Centrón · M. S. Ramírez
Instituto de Microbiología y Parasitología Médica, Universidad de Buenos Aires—Consejo Nacional de Investigaciones Científicas y Tecnológicas (IMPaM, UBACONICET), Buenos Aires, Argentina

M. Almuzara · C. Vay
Laboratorio de Bacteriología Clínica, Departamento de Bioquímica Clínica, Instituto de Fisiopatología y Bioquímica Clínica, Hospital de Clínicas José de San Martín, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

M. Papalia · M. Radice
Laboratorio de Resistencia Bacteriana, Cátedra de Microbiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

L. Galanternik
Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina

M. S. Ramírez (✉)
Depto. de Microbiología Parasitología e Inmunología, Investigador Asistente CONICET, Facultad de Medicina—UBA, Paraguay 2155 Piso 12, CP 1121 Buenos Aires, Argentina
e-mail: ramirez.mariasoledad@gmail.com

## Introduction

Infections due to *Achromobacter xylosoxidans*, the most frequent species among *Achromobacter* spp. isolates, have been increasingly documented in immunocompromised and cystic fibrosis (CF) patients [1, 8, 10, 13]. Since accurate identification of *A. xylosoxidans* is difficult, in 2011, Turton et al. [15] proposed the amplification of the *bla*$_{\text{OXA-114}}$ gene as a PCR based method for specific identification of this species.

Previous studies have investigated the presence of *bla*$_{\text{OXA-114}}$ among *A. xylosoxidans* clinical isolates, finding 12 variants of this gene among the analyzed isolates [1, 5, 15].

The aim of the present study was to determine the presence of this gene and also identify the variants that are present among clinical isolates of *A. xylosoxidans* recovered in our region.

## Materials and Methods

### Bacterial Strains

Nineteen non-epidemiological-related clinical isolates of *A. xylosoxidans,* belonging to 15 different clones (data not shown) were used. Standard biochemical tests, API 20NE (bioMérieux), PCR amplification and sequencing of the 16S rRNA gene, and a MLST approach were conducted to arrive to a correct identification of the species [14, 18]. The isolates exhibited the typical multiresistance profile previously described for this species, being the third and fourth-generation cephalosporins, fluorquinolones, and aminoglycosides inactive against *Achromobacter* spp. [17].

**Table 1** Features of the $bla_{OXA-114}$ genes found in the *A. xylosoxidans* isolates

| Strain | Sequence comparison subject sequence (AN) nucleotide identity/ amino acid identity (%)[a] | Nucleotide differences/ synonymous mutations/ non-synonymous mutations in each allelic variant against the closest sequence | Nucleotide differences/ synonymous mutations/ non-synonymous mutations in each allelic variant against the $bla_{OXA-114a}$ | β-Lactamase allelic variants |
|---|---|---|---|---|
| AX11 | HM368376 100/100 | 0/0/0 | 8/4/4 | Oxa-114 $_g$ |
| AX22 | JX206450 99/100 | 2/2/0 | 8/5/3 | Oxa-114$_i$ |
| AX44 | HM368375 100/100 | 0/0/0 | 2/1/1 | Oxa-114$_f$ |
| AX56 | HM368376 100/100 | 0/0/0 | 8/4/4 | Oxa-114 $_g$ |
| AX69 | JX206453 100/100 | 0/0/0 | 3/2/1 | Oxa-114 $_l$ |
| AX72 | HM368375 100/100 | 0/0/0 | 2/1/1 | Oxa-114$_f$ |
| AX77 | HM056041 99/100 | 2/2/0 | 3/1/2 | Oxa-114$_c$ |
| AX81 | HM368375 100/100 | 0/0/0 | 2/1/1 | Oxa-114$_f$ |
| AX82 | JX206453 99/100 | 2/2/0 | 3/1/2 | Oxa-114 $_l$ |
| AX90 | HM368375 100/100 | 0/0/0 | 2/1/1 | Oxa-114$_f$ |
| AX91 | HM368375 100/100 | 0/0/0 | 2/1/1 | Oxa-114$_f$ |
| AX92 | HM104634 99/100 | 3/3/0 | 5/3/2 | Oxa-114$_e$ |
| AX93 | JX206453 99/100 | 2/2/0 | 3/2/1 | Oxa-114 $_l$ |
| AX126 | HM056041 98/100 | 10/10/0 | 13/11/2 | Oxa-114$_c$ |
| AX144 | HM056041 99/100 | 3/3/0 | 4/2/2 | Oxa-114$_c$ |
| AX210 | HM368375 100/100 | 0/0/0 | 2/1/1 | Oxa-114$_f$ |
| AX247 | EU188842 99/100 | 1/1/0 | 1/1/0 | Oxa-114$_a$ |
| AX281 | JX206450 99/100 | 4/4/0 | 8/5/3 | Oxa-114$_i$ |
| AX304 | JX206450 99/99 | 6/5/1 | 10/6/4 | Oxa-114$_o$ |

*AN* GenBank accession number

[a] The percentage of identity in each allelic variant was defined against the most closed sequence in every case

## DNA Techniques

Total DNA was extracted using the master pure DNA purification kit following manufacturer's instructions (Epicentre, Madison, WI, USA). The specific primers proposed by Turton et al. were used to analyze the presence of $bla_{OXA-114-like}$ genes. PCR amplifications were carried out in 50 µl volumes containing 10 ng of DNA, 10 µl of 5× PCR buffer, 0.5 µl of 10× deoxynucleoside triphosphate mix (2 mM each dATP, dCTP, dGTP, and dTTP), 2 µl of each primer stock solution (2.5 pmol of each primer per µl), and sterile distilled water. Taq DNA polymerase

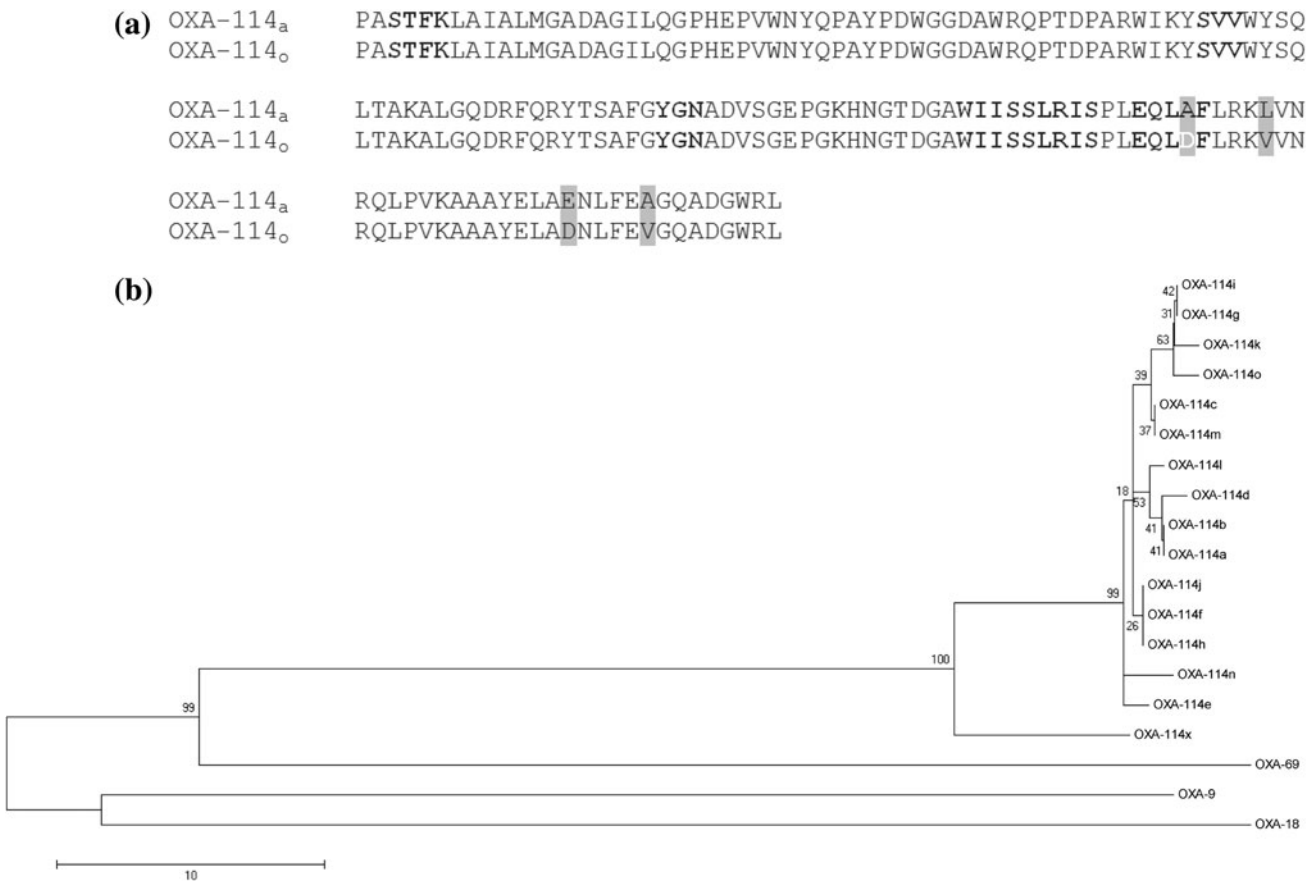


**Fig. 1** **a** Amino acid sequence comparison of the OXA-114 new allelic variants described in this study. *In bold* it was represented the oxacillinase conserved amino acid motifs (S-T-F-K, Y-G-N, S-V-V, E-Q-L-A-F, and W-I-IS- S-L-R-I-S). The comparison was performed using the OXA-114a as reference. The *highlight letters* showed changes in the amino acid sequence compared to OXA-114a. *In white letters*, it was represented the amino acid substitutions inside the conserved motifs. **b** Tree representing the OXA-114 variants. The dendrogram was inferred using the Neighbor-Joining method for OXA-114. The distances were computed using the number of differences method proposed for Nei 2001. The alignment used for tree calculation was performed with ClustalX

according to manufacturer's instructions (Promega, Madison, USA) was added. The thermocycler used was from Perkin–Elmer Cetus, Emeryville, CA, and a three-step profile was utilized. The obtained products were detected by agarose gel electrophoresis.

### Sequence Analysis

Sequencing was performed on both DNA strands using an ABIPrism 3100 BioAnalyzer and Taq FS Terminator Chemistry (Taq FS, Perkin–Elmer). Sequences were examined and assembled with Sequencher 4.7 software (Gene Codes Corp.) and BLAST (version 2.0) software (http://www.ncbi.nlm.nih.gov/BLAST/).

## Results and Discussion

Positive results were obtained for the amplification of $bla_{\text{OXA-114}}$ in all *A. xylosoxidans* isolates included in this study. We also tested one *A. denitrificans* and one *A. piechaudii* isolates obtaining negative amplification as was expected. Nucleotide sequence analysis of the obtained amplicons revealed the presence of 10 new $bla_{\text{OXA-114}}$ alleles, being 9 of them synonymous mutations (Table 1). In this work, we defined as variant when nucleotide differences are related to an amino acid change, and as alleles when nucleotide differences are synonymous or non-synonymous mutations.

In our isolates, we found the previously described variants a, c, e, f, g, i, and, l [1, 5, 15], being the f variant the most widespread ($n = 6$). In addition to the already described alleles, we identified 1 novel variant. The analysis at protein level, exposed the presence of one new OXA-114 variant, designated as OXA-114o (Fig. 1a, b). The partial sequence of the new $bla_{\text{OXA-114o}}$ variant has been deposited in GenBank under accession number JX306686.1.

Taking into account the described motifs for class D β-lactamases, we decided to determine the presence of

amino acid substitutions in the OXA-114o variant [4, 5]. No substitutions were found in the S-T-F-K, Y-G-N, and S-V-V motifs. In the novel variant identified in this work, an amino acid substitution A169D was observed in the E-Q-L-A-F motif of the OXA-114o variant (Fig. 1a; Table 1). Comparing the amino acid sequences of the novel variant against the OXA-114f, the most widespread variant, we observed 99 % of identity with 10 nucleotides differences.

Eight OXA-114 variants were found among the isolates included in this study, and no correlation was observed between the presence of one variant and one specific clone, since we observed the distribution of a particular variant in many different clones (Table 1). This could be explained in part due to the polyclonal behavior exhibited by *A. xylosoxidans*, being this result in accordance with those previously reported by Turton et al. [15].

As was previously described, several naturally harbored $bla_{\text{OXA}}$ genes are associated upstream with an insertion sequence, which could provide a potential promoter that enhances their expression [6–8, 11, 16]. Searching in the GenBank database only in the *A.* genogroup 2b strain AXX-A, we could identify in the neighboring sequences the presence of an anti-repressor regulating drug resistance gene and an acyltransferase-3 gene, respectively. In order to determine if $bla_{\text{OXA114-like}}$ genes found in the studied isolates had the same genetic context, as the one described in AXX-A strain, total DNA was used to carry out PCR reactions with specific primers annealing in the mention surroundings genes (EntF: CTGGGACATCCGCCTGTC and EntR: CTCAAGCCGTTGACGCT). In parallel PCR reactions to amplify the 16S RNA gene were performed, giving positive results in all of the tested samples. When we tested the primers combinations EntF/oxa114R2 and EntR/oxa114F2, negative results were obtained in all samples, suggesting a different genetic context for this gene.

Moreover, searching neighboring sequences of $bla_{\text{OXA}}$ in the AXX-A strain, we did not found any insertion sequences near the $bla_{\text{OXA}}$ gene. But the fact that this event is highly frequent in other species and genus [6, 7, 12, 16] suggests that the same event could occur in *A. xylosoxidans* not only modifying the OXA-114 expression if not also allowing its dispersion.

As described for the naturally occurring oxacillinase OXA-51/69 from *A. baumannii* [2, 3, 9, 16], in the present work numerous different variants were found. These results are in accordance and clearly exposed a great intrinsic plasticity for the $bla_{\text{OXA}}$ genes.

The importance of studying the different allelic variants of this gene, contributes in the knowledge of the evolution and variability of $bla_{\text{OXA}}$ genes. The intrinsic plasticity observed could evolve in the development of different levels of activity to β-lactams enlarging its antibiotic resistance profile, placing this gene as a reservoir of allelic variants. The increasing frequency of *A. xylosoxidans* isolation in cystic fibrosis patients and also from hospitalized patients point out the fact that this species should be considered as an important emerging pathogen that could provide a good reservoir of resistance genes.

**Acknowledgments** M.S.R., D.C, and M.R are members of the career investigator of CONICET, Argentina. G.M.T has a Doctoral Fellowship from CONICET. This study was supported by Grant UBACyT 2011–2012 and 2011–2014 to MSR, DC, and CV, respectively, Buenos Aires, Argentina.

## References

1. Amoureux L, Bador J, Siebor E, Taillefumier N, Fanton A, Neuwirth C (2012) Epidemiology and resistance of *Achromobacter xylosoxidans* from cystic fibrosis patients in Dijon. First French data J Cyst Fibros, Burgundy
2. Brown S, Amyes SG (2005) The sequences of seven class D beta-lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents. Clin Microbiol Infect 11:326–329
3. Brown S, Young HK, Amyes SG (2005) Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin Microbiol Infect 11:15–23
4. Couture F, Lachapelle J, Levesque RC (1992) Phylogeny of LCR-1 and OXA-5 with class A and class D beta-lactamases. Mol Microbiol 6:1693–1705
5. Doi Y, Poirel L, Paterson DL, Nordmann P (2008) Characterization of a naturally occurring class D beta-lactamase from *Achromobacter xylosoxidans*. Antimicrob Agents Chemother 52:1952–1956
6. Evans BA, Brown S, Hamouda A, Findlay J, Amyes SG (2007) Eleven novel OXA-51-like enzymes from clinical isolates of *Acinetobacter baumannii*. Clin Microbiol Infect 13:1137–1138
7. Figueiredo S, Poirel L, Papa A, Koulourida V, Nordmann P (2009) Overexpression of the naturally occurring *bla*OXA-51 gene in *Acinetobacter baumannii* mediated by novel insertion sequence IS*Aba9*. Antimicrob Agents Chemother 53:4045–4047
8. Hansen CR, Pressler T, Nielsen KG, Jensen PO, Bjarnsholt T, Hoiby N (2010) Inflammation in *Achromobacter xylosoxidans* infected cystic fibrosis patients. J Cyst Fibros 9:51–58
9. Heritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P (2005) Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother 49:4174–4179
10. Magni A, Trancassini M, Varesi P, Iebba V, Curci A, Pecoraro C, Cimino G, Schippa S, Quattrucci S (2010) *Achromobacter xylosoxidans* genomic characterization and correlation of randomly amplified polymorphic DNA profiles with relevant clinical features [corrected] of cystic fibrosis patients. J Clin Microbiol 48:1035–1039
11. Merkier AK, Centrón D (2006) *bla*(OXA-51)-type beta-lactamase genes are ubiquitous and vary within a strain in *Acinetobacter baumannii*. Int J Antimicrob Agents 28:110–113
12. Ravasi P, Limansky AS, Rodriguez RE, Viale AM, Mussi MA (2011) IS*Aba825*, a functional insertion sequence modulating genomic plasticity and *bla*(OXA-58) expression in *Acinetobacter baumannii*. Antimicrob Agents Chemother 55:917–920

13. Spilker T, Vandamme P, Lipuma JJ (2012) Identification and distribution of *Achromobacter* species in cystic fibrosis. J Cyst Fibros 12(3):206–215
14. Spilker T, Vandamme P, Lipuma JJ (2012) A multilocus sequence typing scheme implies population structure and reveals several putative novel *Achromobacter* species. J Clin Microbiol 50:3010–3015
15. Turton JF, Mustafa N, Shah J, Hampton CV, Pike R, Kenna DT (2011) Identification of *Achromobacter xylosoxidans* by detection of the *bla*(OXA-114-like) gene intrinsic in this species. Diagn Microbiol Infect Dis 70:408–411
16. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, Pitt TL (2006) The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett 258:72–77
17. Vay CA, Almuzara MN, Rodriguez CH, Pugliese ML, Lorenzo Barba F, Mattera JC, Famiglietti AM (2005) 'In vitro' activity of different antimicrobial agents on Gram-negative nonfermentative bacilli, excluding *Pseudomonas aeruginosa* and *Acinetobacter* spp. Rev Argent Microbiol 37:34–45
18. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703