**Achromobacter xylosoxidans**: An Emerging Pathogen Carrying Different Elements Involved in Horizontal Genetic Transfer

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**Abstract** In the last few years, numerous cases of multi-drug-resistant *Achromobacter xylosoxidans* infections have been documented in immunocompromised and cystic fibrosis patients. To gain insights into the molecular mechanisms and mobile elements related to multidrug resistance in this bacterium, we studied 24 non-epidemiological *A. xylosoxidans* clinical isolates from Argentina. Specific primers for plasmids, transposons, insertion sequences, *bla*ampC, *intI1*, and *intI2* genes were used in PCR reactions. The obtained results showed the presence of wide host range IncP plasmids in ten isolates and a high dispersion of class 1 integrons (*n* = 10) and class 2 integrons (*n* = 3). Four arrays in the variable region (vr) of class 1 integrons were identified carrying different gene cassettes as the aminoglycoside resistance *aac(6’)-Ib* and *aadA1*, the trimethoprim resistance *dfrA1* and *dfrA16*, and the β-lactamase *bla*OXA-2. In only one of the class 2 integrons, a vr was amplified that includes *sat2-aadA1*. The *bla*ampC gene was found in all isolates, confirming its ubiquitous nature. Our results show that *A. xylosoxidans* clinical isolates contain a rich variety of genetic elements commonly associated with resistance genes and their dissemination. This supports the hypothesis that *A. xylosoxidans* is becoming a reservoir of horizontal genetic transfer elements commonly involved in spreading antibiotic resistance.

**Introduction**

*Achromobacter* spp. is a rarely nosocomial and community pathogen, being *Achromobacter xylosoxidans* the most frequent species among *Achromobacter* spp. isolates [6, 8, 18]. Many reports of *A. xylosoxidans* infections are documented in immunocompromised and cystic fibrosis (CF) patients, where its pathogenic role has not yet been properly clarified [7, 8]. In Argentina, the relative frequency of *A. xylosoxidans* among the uncommon non-glucose-fermenting gram-negative bacilli infections has been increasing reaching 66 % of total non-glucose-fermenting gram-negative bacilli infection isolates [18].

Although clinical *A. xylosoxidans* isolates usually show multiple drug resistance, the relative low attention paid to this pathogen resulted in poor understanding of their resistance mechanisms. Little is known about molecular mechanisms and transferable elements contributing to the acquisition and dissemination of antibiotic resistance determinants in *A. xylosoxidans* clinical isolates.

The aim of this study was to explore the occurrence of mobile elements related to antibiotic-resistance determinants among a collection of 24 non-epidemiological-related...
clinical isolates of *A. xylosoxidans* recovered in Argentina from six centers.

**Materials and Methods**

**Bacterial Strains**

Twenty-four non-epidemiological-related clinical isolates of *A. xylosoxidans* recovered in Argentina from six centers were used (Table 1). All isolates were identified using standard biochemical tests and API 20NE (Biomerieux), and the species level was confirmed by sequencing the 16S rRNA gene [19]. Clonal relationships analysis, using the macrorestriction technique, showed the presence of 15 different clones among the isolates included in the study (data not shown). The antibiotic susceptibility was performed by agar dilution method following the general recommendations of the Clinical and Laboratory Standards Institute (CLSI) [4].

**DNA Techniques**

Total DNAs were prepared and used as template for PCR reactions. PCR reactions were carried out using the GoTaq enzyme according to manufacturer’s instructions (Promega, Madison, WI), and the products were detected by agarose gel electrophoresis. To reveal the presence of transferable determinants associated to horizontal gene transfer, specific primers for plasmids (IncP, IncW, IncA/C, IncN, IncFII, *repAci1*), transposons (Tn1331, Tn3, Tn7), insertion sequences (IS) (IS26, IS440), and the *bla*<sub>ampC</sub>, *intI1*, and *intI2* genes were used (Table 2). The selection of the mobile elements was based on their association with antibiotic-resistance determinants and also its distribution in our hospitals [12, 13, 16].

### Table 1  Characteristic and obtained results of the 24 *A. xylosoxidans* isolates used in the study

| Isolate<sup>a</sup> | Hospital | Year | Source<sup>b</sup> | IncP | IS26 | IS440 | *intI1* | vr<sup>c</sup> | *intI2* |
|--------------------|----------|------|---------------------|------|------|-------|---------|---------|---------|
| Ax79               | Center 2 | 2004 | NP                  | +    | –    | +     | +       | dfrA1-aadA1 | +       |
| Ax169              | Center 3 | 2004 | NP                  | +    | –    | +     | +       | dfrA1-aadA1 | +       |
| Ax126              | Center 1 | 2001 | NP                  | +    | +    | –     | +       | dfrA1-aadA1 | +       |
| Ax144              | Center 1 | 2001 | NP                  | +    | –    | +     | –       | NA               | –       |
| Ax69               | Center 2 | 2002 | CF                  | –    | –    | +     | –       | NA               | –       |
| Ax72               | Center 2 | 2007 | CF                  | +    | –    | –     | +       | *aac(6')-lb*  | –       |
| Ax77               | Center 2 | 2007 | CF                  | –    | –    | +     | –       | NA               | –       |
| Ax210              | Center 3 | 2007 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax81               | Center 2 | 2008 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax82               | Center 2 | 2008 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax90               | Center 2 | 2008 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax91               | Center 2 | 2008 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax92               | Center 2 | 2008 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax93               | Center 2 | 2008 | CF                  | –    | +    | –     | –       | NA               | –       |
| Ax97               | Center 2 | 2007 | CF                  | –    | –    | –     | –       | NA               | –       |
| Ax336              | Center 2 | 2010 | CF                  | –    | –    | +     | –       | NA               | –       |
| Ax11               | Center 2 | 2004 | NP                  | –    | –    | –     | +       | *aac(6')-lb*  | –       |
| Ax22               | Center 1 | 1995 | NP                  | –    | –    | –     | –       | NA               | –       |
| Ax44               | Center 1 | 2006 | NP                  | +    | –    | –     | +       | dfrA16            | –       |
| Ax56               | Center 1 | 2003 | NP                  | +    | –    | –     | +       | *aac(6')-lb*  | –       |
| Ax68               | Center 6 | 2010 | NP                  | +    | –    | –     | –       | NA               | –       |
| Ax114              | Center 1 | 2002 | NP                  | +    | –    | –     | +       | dfrA1-aadA1  | –       |
| Ax247              | Center 1 | 2006 | NP                  | –    | –    | +     | –       | NA               | –       |
| Ax304              | Center 4 | 1996 | NP                  | –    | –    | –     | +       | *bla*<sub>OXA-2</sub> | –       |
| Ax2700             | Center 5 | 2006 | NP                  | +    | –    | –     | –       | NA               | –       |

<sup>a</sup> Isolates of the study: Ax for *Achromobacter xylosoxidans*

<sup>b</sup> NP for nosocomial patient’s samples and CF for cystic fibrosis patient’s samples

<sup>c</sup> vr: class 1 integron variable region

NA not applicable
Table 2 Oligonucleotides used in the study

| Target | Oligonucleotide       | Sequence 5′–3′ | References |
|--------|-----------------------|----------------|------------|
| IncW   | TrwAB1                | AGCGTATGAGCCCCGTGAAGGG | [3]        |
|        | TrwAB2                | AAGATTAAGCAGGAGAAGCAGAATAACG | [3]        |
| IncP   | TrfA2 1               | CGAAATTCATATGGGAGAAAGTA | [3]        |
|        | TrfA2 2               | CGTTTGCAATTGCAACCAGAGTC | [3]        |
| IncN   | KikA1                 | ACTTACCTTTTATCAACACATTGGCCG | [3]        |
|        | KikA2                 | CGACTGGTATTTACCACTCTGGCC | [3]        |
| IncF   | REPA                  | GGAGGGATTGGCATTCCG | [3]        |
|        | REPC                  | AAAAGCCTGTGTGAG | [3]        |
| IncA/C | CA1                   | ATGTCGAGACAGAAGAATGC | [3]        |
|        | OR1                   | CTTGCAATTGAAATGTGAATAA | [3]        |
| IS26   | IS26F                 | GCTGCGTGACAGCCGGAG | [9]        |
|        | IS26R                 | ATACCTGTGAGGTGTCG | [9]        |
| IS440  | IS440F                | CTCAGCTCCGTCCGACT | [9]        |
|        | IS440R                | GCCATGCGAGCGCCGG | [9]        |
| Tn1331 | Tn1331NF              | GAATTCCTCGTGAACCGCCTATTT | [15]      |
|        | Tn1331NR              | GGGCCCGGAGATTTTGGGCTGAGC AATT | [15]      |
| Tn3    | Tn3F                  | AAGTTCATGGGCTTCG | [9]        |
|        | 201L                  | ACTACGATACGAGGGGCT | [9]        |
| msA    | TnsAF                 | CTCCATATTCCACTTTGGCT | [5, 14] |
|        | TnsAR                 | GCTAACATGACAGAAGTCC | [5, 14] |
| msB    | TnsBF                 | CATGTTGCTACAGCCAGAATAAG | [5, 14] |
|        | TnsBR                 | GACCAAGGTATTTACAAAAGC | [5, 14] |
| msC    | TnsCF                 | GTTTATGCTGACGAGGGG | [5, 14] |
|        | TnsCR                 | GCTATCCAGCGCTGCGG | [5, 14] |
| msD    | Tn5DF                 | GGGATTTGTTAGCTCTAAGC | [5, 14] |
|        | Tn5DR                 | CCCTTAAATTTGCTATCTC | [5, 14] |
| msE    | TnsEF                 | TTGCTCTCTAAACCCTCT | [5, 14] |
|        | TnsER                 | TCGATTTTGTCTTGTGATG | [5, 14] |
| aac(6′)-Ib | aac(6′)IbF | TGTGACGGGATCTGTCG | [13]  |
|        | aac(6′)IbR           | CAGTGAAGCTGTCTTGCC | [13]  |
| intI1  | Inti1F                | CGAGGCATAGACTGTAC | [12]  |
|        | Inti1R                | TTCGAATGCTGTAACCGC | [12]  |
| intI2  | Inti2F                | GCAAAATGAGTGCACACGC | [12]  |
|        | Inti2R                | ACCCGTTGCAACAGT | [12]  |
| 5′CS   | Sulpro                | GCCTGACAGGCTGAAAAG | [12]  |
| 3′CS   | 3′CS                  | AACGAGACTTGACCTGATAG | [12]  |
| sat    | SatF                  | TGAGCAGTGGGGGCAAAAC | [12]  |
|        | SatR                  | TCATCCCTGCTGCCGAG | [12]  |
| aadA1  | aadA1r                | TCATTGCGCTGCACCACC | [12]  |
|        | aadA1                | TCGATGACGGCAACTAC | [12]  |
| dfrA1  | Dhfr1r                | CCTGAAATCCCGCAGCAAA | [12]  |
|        | dfrA1                | AGCCTGTCACCTTGGGC | [12]  |
| blaOXA-2 | Oxa2F       | GAAGAAACGCCTCTGC | [12]  |
|        | Oxa2R                | TACCCACACCAATCCACAT | [12]  |
| dfrA16 | Dhfr16F              | CAAAGGGGACGACCTTCT | This study |
|        | Dhfr16R              | CACCCCTCATCATCGTA | This study |
DNA Sequencing

PCR products were sequenced after purifying the DNA by using the Wizard SV Gel and PCR clean-up System kit according to the manufacturer’s directions (Promega, USA). Sequencing was performed on both DNA strands, using an ABI Prism 3100 BioAnalyzer equipment. The nucleotide sequences were analyzed using the Blast V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/).

Results and Discussion

The 24 A. xylosoxidans isolates studied exhibited the typical multiresistance profile previously described for this species, being the third and fourth-generation cephalosporins, fluoroquinolones, and aminoglycosides not active against Achromobacter spp. [18]. All isolates were susceptible to tazobactam, imipenem, and meropenem (Table S1 in Supplementary material).

Among the PCR reactions performed for the selected transferable elements, positive results were obtained in ten isolates (42 %) for the IncP plasmids, a wide host range and self-transmissible plasmid important in the dissemination of resistant genes around the world [11] (Table 1). Negative results were obtained for the other Inc groups searched (IncW, IncA/C, IncN, IncFII). Sequence analysis of the amplification products showed 99 % of identity in 200-bp length with the replication gene trfA (AN GU186864). The GC% of the trfA replication gene of IncP plasmid is 60.5 %, which is very similar to the GC% (67 %) of A. xylosoxidans. We also noticed in this study that most isolates containing IncP plasmids corresponded to nosocomial isolates (n = 9). In only one CF patient isolate (Ax72), an IncP plasmid was identified.

Regarding IS and transposons, positive results were obtained for IS26 (n = 2) and IS440 (n = 7) (Table 1), two ISs frequently associated to antimicrobial resistance genes and to classes 1 and 2 integrons [1, 2, 10], obtaining negative results for the transposons Tn1331, Tn3, and Tn7.

In addition, a high dispersion of class 1 integrons was found (42 %). Most of the positive isolates corresponded to nosocomial patient samples (n = 9), being only one positive isolate from a CF patient sample (Ax72). To

Fig. 1 Schematic representation of arrays of class 1 integrons found among the A. xylosoxidans (n = 24) isolates. Thin black vertical closed bar The attI1 site, thin gray vertical closed bar the attC sites of the gene cassettes. Arrows The primers used to identify the class 1 integron vr. Figure is not in scale.
characterize the vr of class 1 integrons, PCR cartography was carried out as previously described [12]. Four vr were identified, being all the arrays different to the previous sulfamethoxazole, CIP.

Moreover, the similar GC% between the trfA replicon of the IncP plasmid and the A. xylosoxidans genome reinforces the argument that A. xylosoxidans could be considered as a reservoir of transferable elements. It is likely that its intrinsic antibiotic multidrug resistant profile that ensures its selectivity under antibiotic pressure, along with its ability to survive in fluids and in the environment [18], makes A. xylosoxidans a reservoir of transferable elements that could contribute to the dissemination and acquisition of antimicrobial resistance mechanisms within the nosocomial environment.

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| Isolate | CAZ | FEP | PIP | IPM | MEM | AMK | GEN | TMP | CIP | vr^2 |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Ax79    | 8   | 32  | 0.25| 1   | 0.125| 128 | 128 | 0.25| 8   | dfrA1-aadA1 |
| Ax169   | 32  | 128 | 0.25| 0.5 | 0.5  | 128 | 128 | 1   | 16  | dfrA1-aadA1 |
| Ax126   | 4   | 32  | 0.5 | 1   | 0.25 | 128 | 128 | 0.125| 16  | dfrA1-aadA1 |
| Ax72    | 4   | 32  | 0.25| 1   | 0.25 | 256 | 256 | 4   | 6   | aac(6^Ib) |
| Ax11    | 32  | 128 | 8   | 4   | 0.24 | 128 | 128 | 64  | 64  | aac(6^Ib) |
| Ax44    | 16  | 32  | 0.5 | 1   | 0.5  | 128 | 128 | 256 | 4   | dfrA16 |
| Ax56    | 8   | 32  | 8   | 2   | 0.06 | 64  | 32  | 0.125| 2   | aac(6^Ib) |
| Ax114   | 16  | 32  | 0.125| 1 | 0.125| 128 | 128 | 16  | 16  | dfrA1-aadA1 |
| Ax304   | 32  | 128 | 8   | 4   | 0.125| 128 | 128 | 32  | 4   | blaOXA-2 |

CAZ ceftazidime, FEP cefepime, PIP piperacillin, IPM imipenem, MEM meropenem, AMK amikacin, GEN gentamicin, TMP trimethoprim, CIP ciprofloxacin

vr^2: class 1 integron variable region found in the Ax isolates

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