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Original Article

Epidemiology and resistance of *Achromobacter xylosoxidans* from cystic fibrosis patients in Dijon, Burgundy: First French data

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Abstract

Background: Achromobacter xylosoxidans is an emerging pathogen in cystic fibrosis (CF) patients recognised as causal agent of inflammation. The prevalence of infection or colonisation is variable among CF centres. We report here the first epidemiological data about *A. xylosoxidans* in a French CF centre: Dijon, Burgundy.
Methods: All isolates recovered from the patients affiliated with our centre in 2010 since their first visit were included. Antimicrobial susceptibility was determined by disk diffusion method and E-test. Molecular epidemiology was performed by Pulsed Field Gel Electrophoresis (PFGE) and compared with repetitive sequence-based PCR (rep-PCR, DiversiLab®). We also sequenced the constitutive *bla*-$_{\text{oxa-114}}$ gene.
Results: Out of 120 patients, 21 (17.5%) had at least one positive culture with *A. xylosoxidans* since they started to receive routine care in our CF centre (447 isolates). Median age at first positive culture was 16 years (range 3–34 years). Most patients were colonised by their own strain, cross-contamination was very rare. We observed two cases of intra-family spread. DiversiLab® is a useful tool as efficient as PFGE to compare isolates recovered simultaneously from different patients when an outbreak is suspected. However, PFGE remains the reference method for long-term survey of chronically colonised patients. We detected new OXA-114 variants and the new oxacillinase OXA-243 (88% amino acid identity with OXA-114). Acquired resistance to ciprofloxacin, ceftazidime and carbapenems was frequent. In 2010, 7 patients harboured strains resistant to ceftazidime, 6 patients strains with decreased susceptibility to carbapenems (especially meropenem) and 12 patients strains resistant to ciprofloxacin.
Conclusions: In our centre, the high prevalence of colonisation is not due to cross-contamination. Our main concern is the high rate of antimicrobial resistance.

Keywords: Achromobacter xylosoxidans; Cystic fibrosis; Genotyping; DiversiLab; OXA-114; OXA-243; Resistance

1. Introduction

Methicillin-susceptible *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa* remain the most common pathogens isolated during airway infection in cystic fibrosis (CF) patients [1]. Nevertheless since the 2000s additional organisms have been increasingly reported: methicillin-resistant *S. aureus*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* complex and *Achromobacter xylosoxidans* [2–5]. The impact of *A. xylosoxidans* on lung disease is unclear but the recent study by Hansen suggests that it can cause inflammation in chronically infected CF patients [6].

A. xylosoxidans is an aerobic, non-fermentative, Gram-negative rod. It is frequently misidentified as *P. aeruginosa*, and therefore its prevalence is likely to be underestimated in lung colonisation/infection in CF patients [7,8].

The rate of colonisation/infection is very different among the studies, ranging from 2% in the 2000s [9] to 21% in 2011 [10].

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Table 1
Characteristics of the patients and isolates. C: chronically colonised patient; CIP: ciprofloxacin; CAZ: ceftazidime; IPM: imipenem; MEM: meropenem.

Patient	Age at first positive culture	PFGE pulsotype (no. of subtypes)	No. of positive cultures (no. of strains)	OXA variant	Resistance at first positive culture (year)	Resistance at last positive culture (year)
P1[C]	12	AXX42	$12_{(13)}$	114m	CIP (2004)	CIP, CAZ, MEM (2010)
P2[C]	12	AXX42	$12_{(13)}$	114m	CIP(2004)	CIP, CAZ, MEM (2010)
P3	8	AXX61 (2)	$2_{(2)}$	243d	None (2001)	CIP (2010)
		AXX0	$1_{(1)}$	/	None (2002)	/
P4	9	AXX0	$1_{(1)}$	243c	CIP, CAZ (2010)	/
P5[C]	16	AXX1 (3)	$12_{(18)}$	243b	CIP, CAZ (2003)	CAZ (2007)
P6[C]	4	AXX1 (6)	$56_{(78)}$	243b	None (1997)	CIP, CAZ, IPM, MEM (2010)
P7	18	AXX53 (2)	$5_{(6)}$	114c	None (2007)	CIP (2008)
		AXX0	$1_{(1)}$	/	None (2007)	/
		AXX0	$1_{(1)}$	/	None (2008)	/
		AXX0	$1_{(1)}$	/	None (2010)	/
P8[C]	18	AXX2 (6)	$79_{(86)}$	114h	CIP (1997)	CIP, CAZ, MEM (2010)
P9[C]	16	AXX62 (3)	$5_{(9)}$	114e	None (2009)	CIP, CAZ (2010)
P10	7	AXX65	$2_{(2)}$	114i	CIP (2010)	CIP (2010)
P11	25	AXX56	$4_{(4)}$	114 l	CIP (2006)	CIP, MEM (2010)
P12	8	AXX46 (3)	$9_{(11)}$	114g	CIP, CAZ,IPM (2005)	CIP (2010)
P13[C]	22	AXX50	$17_{(23)}$	114a	CIP (2006)	CIP (2010)
		AXX55	$4_{(4)}$	114f	CIP (2008)	CIP(2008)
P14	34	AXX0	$1_{(1)}$	114k	CIP (2005)	/
		AXX47	$2_{(2)}$	/	CIP (2005)	CIP (2005)
P15	13	AXX43	$15_{(18)}$	114i	None (2004)	CIP (2006)
P16	17	AXX57	$2_{(2)}$	114f	None (2008)	None (2008)
P17	25	AXX59 (2)	$8_{(9)}$	243d	CIP, CAZ (2009)	CIP, CAZ (2010)
		AXX63	$2_{(2)}$	114c	CIP (2010)	CIP (2010)
P18	16	AXX58 (2)	$3_{(3)}$	114c	None (2007)	CIP (2008)
P19[C]	25	AXX3 (8)	$80_{(134)}$	114h	CIP (1995)	CIP, CAZ, MEM (2010)
P20	4	AXX43	$1_{(1)}$	114i	CIP, CAZ (2005)	/
P21	3	AXX0	$1_{(1)}$	114a	CIP (2010)	/

Whatever the rate, most of the authors report an increase in prevalence of *A. xylosoxidans* in the CF population [11,12]. The reservoir of the isolates remains unknown. Most patients harbour their own strain but cross-contamination has also been described in some centres [10,13,14]. As previously described, this species is naturally resistant to cefalotin, cefoxitin, cefotaxime, aztreonam, and aminoglycosides [15,16]. OXA-114 has been described as a constitutive beta-lactamase in *A. xylosoxidans* [17]. Subsequently it has been proposed as an identification tool of this species by Turton who reported several variants of this enzyme [18].

A good knowledge of local epidemiological data is necessary to detect cross-contamination or common-source outbreak. It will be also a help to progress in the understanding of the phenomenon leading to the worldwide emergence of this pathogen.

This study is a retrospective analysis of the 120 CF patients affiliated with our centre in 2010, from their first visit to December 2010. Among them, 111 visited the centre in 2010 and sputum analysis was obtained for 108 of them.

The aim of this study was

i. To perform the genotypic analysis of all isolates of *A. xylosoxidans* recovered from the patients from their first visit in our centre until the 31st of December 2010 by Pulsed Field Gel Electrophoresis (PFGE) and to compare the performance of the typing provided by the Diversilab® technology to that of PFGE.
ii. To evaluate the evolution of the antimicrobial susceptibility of the isolates.
iii. To detect and sequence the $bla_{\text{OXA-114}}$ gene.

2. Materials and methods

2.1. Patients

One hundred and twenty patients receive care in the CF Centre of the Paediatric Department of Dijon's Hospital: the CRCM (Centre de Ressources et de Compétences pour la Mucoviscidose). Most patients attend regularly the centre: every three months or every month for the youngest, 111 in 2010. All bacteriological analyses are performed in our Laboratory of Bacteriology. In this study, we will use the term "colonisation" referring to a positive culture and not "infection" because we did not include any clinical data concerning the lung function of the patients. Patients were considered chronically colonised according to the criteria defined by Pereira: "when at least three positive cultures in 1 year were obtained, with a minimum 1-month interval between them, for at least 2 years" [10]. The definition was applied retrospectively to each of the colonised patients listed in Table 1.

2.2. Processing of sputum samples

The sputum are pre-treated (V/V) with Digest'EUR (Eurobio) for fluidification and then mixed vigorously to obtain

a homogenous sample. The following plates are inoculated: Cetrimide agar and *Burkholderia cepacia* selective agar (not diluted), Trypticase Soy agar with 5% sheep blood (aerobic and anaerobic conditions), Chocolate agar and Drigalski agar (after dilution).

2.3. Identification

A. xylosoxidans colonies are lactose-negative, oxidase-positive and non-pigmented on Muller–Hinton agar.

Isolates were identified as *A. xylosoxidans* by conventional methods including API 20NE (bioMérieux, Marcy l'Etoile, France) and were submitted to further identification when these conventional methods failed or in case of unusual phenotype of resistance. For this purpose we used Matrix-Assisted Laser-Desorption/Ionization Time-of-Flight mass spectrometry (MALDI Biotyper, Bruker) and sequencing of the whole *rrs* gene with primers P8: (5′-AGAGTTTGATCCTGGCTCAG-3′) and P1525: (5′-AAGGAGGTGATCCAGCCGCA-3′) [19].

2.4. Detection and sequencing of $bla_{OXA\text{-}114\text{-}like}$ genes

The $bla_{OXA\text{-}114}$ gene was sequenced in at least one isolate from each PFGE pulsotype recovered more than twice and in four isolates recovered only once (total of 41 strains). We used either the primer pair OXA-114A: (5′-ACGCCTGAACCCTTTTATCC-3′) and OXA-114B: (5′-ATCGACAGGCCGGGCAGT-3′) [17] or AXXAF: (5′-TGTCCAAGACCGGCAACTC-3′) and AXXAR (5′-ACCAGCAGGATCGACAGTC-3′) designed from the whole genome shotgun sequence of *A. xylosoxidans* isolate AXX-A (Genbank accession number AFRQ01000000). Positive PCR products were purified with a Millipore centrifugal filter unit (Amicon Microcon PCR kit, Millipore). Double strand sequencing was then performed using BigDye v1.1 Terminator chemistry and a 3130XL Genetic Analyzer (Applied Biosystems).

2.5. Reference strains

Reference strains *A. xylosoxidans* CIP 7132T, CIP 102062, CIP 102236, CIP 101902 and strain AXX-A were used as positive controls and *Achromobacter ruhlandii* CIP 7726T, *Achromobacter denitrificans* CIP 7715T, *Achromobacter spanius* CIP 108199T, *Achromobacter piechaudii* CIP 6075T, *Bordetella petrii* CIP 107267T, *Bordetella bronchiseptica* CIP 55110T, *Alcaligenes faecalis* CIP 6080 T were used for negative controls for PCR $bla_{OXA\text{-}114}$.

2.6. Susceptibility testing

Susceptibility testing of the isolates was performed by the disk diffusion method according to recommendations of the French Society for Microbiology (http://www.sfm.asso.fr/). Minimal inhibitory concentrations (MICs) of meropenem and imipenem were determined by E-test (bioMérieux, Marcy l'Etoile, France). For each patient, we compared the susceptibility of the first isolate with the susceptibility of the last one.

2.7. Pulsed Field Gel Electrophoresis (PFGE)

All the strains identified as *A. xylosoxidans* in the respiratory samples of the CF patients since their first visit were submitted to genotypic analysis by PFGE as already described [20]. Isolates were analysed using restriction enzyme *XbaI*. Electrophoresis was carried out for 20 h at 5.4 V/cm, with pulse-times ranging from 5 s to 35 s using the CHEF-DR® II system (Bio-Rad). Restriction patterns were interpreted according to Tenover's criteria [21], and the isolates have been classified into different pulsotypes. The pulsotypes have been numbered (e.g. AXX1 for *A. xylosoxidans* pulsotype 1). Pulsotypes that differed by two or three fragments were considered to be subtypes (e.g. AXX1A, AXX1B, AXX1C...) according to Tenover's criteria [21]. "AXX0" was attributed to pulsotypes recovered only once and different from all those already identified to date in our hospital. Therefore all "AXX0" pulsotypes are distinct and unique.

2.8. Automated repetitive-PCR DNA fingerprinting

A total of 42 strains isolated from 19 patients were analysed by repetitive sequence-based PCR (rep-PCR; DiversiLab®) for comparison with PFGE results.

Total bacterial DNA was extracted with the UltraClean™ Microbial DNA Isolation Kit (MO Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. The yield of extracted total DNA was estimated by NanoDrop® ND-1000 (LabTech.) and adjusted to approximately 25 to 50 ng/μL. Rep-PCR was performed using the Diversilab® Bacterial Kit (bioMérieux SA, Marcy l'Etoile, France) and AmpliTaq® DNA Polymerase with GeneAmp® 10× PCR Buffer (Applied Biosystems, Foster City, CA) in a final volume of 25 μL following the manufacturer's instructions. Rep-PCR profiles were obtained using Diversilab® LabChip Devices (bioMérieux SA, Marcy l'Etoile, France) and an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Fingerprinting profiles were compared by the DiversiLab® (version 3.4) software using the Pearson correlation coefficient. The isolates were considered to belong to the same pattern when similarity was above 95% and profiles showed two or less peak changes. The isolates were classified into different patterns if similarity was less than 95% or if profiles showed more than two peak changes. One number was attributed to each pattern.

3. Results

3.1. Patients and isolates

The results are summarised in Table 1.

A total of 447 isolates identified as *A. xylosoxidans* in the respiratory samples of the CF patients were recovered. Sometimes, on culture media, different morphotypes of *A. xylosoxidans* displaying different antimicrobial susceptibility were encountered. Therefore the number of isolates (447) is higher than the number of positive cultures (339). Twenty-one out of the 120 patients affiliated with the centre in 2010 had at least one positive culture

(17.5%) since their first visit. In 2010 positive culture was obtained from 15 patients out of the 108 from whom sputum analysis was performed (13.9%).

The number of isolates per patient ranged from one (3 patients: P4, P20 and P21) to 134 over 15 years (P19). Eight patients were chronically colonised according to the criteria described above (6.7%).

A. xylosoxidans was mostly associated with other pathogens in the specimens, except for 2 patients (P13 and P19) from whom *A. xylosoxidans* was isolated alone in 9 and 11 sputum samples respectively. Associated pathogenic bacteria were mostly *S. aureus*, *H. influenzae*, *Enterobacteriaceae*, *P. aeruginosa*, or *S. maltophilia* (data not shown).

Median age at first positive culture was 16 years (range: 3–34).

3.2. Genotyping analysis by PFGE

The 447 isolates recovered from the 21 patients have been submitted to genotypic analysis by PFGE. The 447 isolates belonged to 25 distinct genotypes. We have detected three cases of cross-contamination. It was an intra-familial one for patients P1 and P2 as well as for patients P5 and P6. The patients P15 and P20 who shared the genotype AXX43 were unrelated. Strains isolated successively from the same patient had the same genotype. The strains are consistent over time, even over very long periods: 13 years for P6 and P8 to 15 years for P19. Five patients harboured isolates of different genotypes: either once sporadically (patients P3, P7 and P14), or for a longer period (patients P13 and P17). Among them it is noteworthy that the newly acquired strain never replaced the initial one.

3.3. Genotyping by Diversilab® technology

Genotyping of strains of *A. xylosoxidans* by rep-PCR was performed for 42 strains isolated from 19 patients (1 to 4 isolates per patient) (Table 2).

The 42 strains were classified into 27 genotypes by Diversilab® technology and only into 19 genotypes by PFGE. All strains that belonged to different PFGE genotypes also belonged to different genotypes as determined by Diversilab®. Nevertheless strains that belonged to the same PFGE genotype were classified into different genotypes by Diversilab® for 8 patients (i.e. patients P1, P2, P3, P7, P8, P11, P13 and P19). Indeed, minor differences in the profiles determined by PFGE led to the classification of the isolates in the same pattern whereas minor variations in the profiles determined by rep-PCR led to the classification of the isolates in different patterns.

Table 2
Comparision of genotyping by Diversilab® technology with PFGE on strains isolated from 19 patients.

Patient	Date of isolation	PFGE pulsotype	Diversilab pattern
P1	16/05/2007	AXX42	1
	10/11/2010	AXX42	2
P2	25/07/2007	AXX42	1
	26/10/2010	AXX42	2
P3	11/07/2001	AXX61A	3
	26/01/2010	AXX61B	4
P4	17/03/2010	AXX0	5
P5	23/06/2004	AXX1A	6
P6	17/06/2005	AXX1A	6
	23/07/2009	AXX1C	7
	06/12/2010	AXX1C	8
P7	21/07/2007	AXX53A	9
	27/09/2007	AXX53A	9
	15/05/2008	AXX53B	10
P8	11/09/2007	AXX2C	11
	22/11/2007	AXX2A	11
	04/06/2009	AXX2C	12
	07/09/2010	AXX2A	12
P9	03/02/2010	AXX62A	13
	27/10/2010	AXX62A	13
P10	14/09/2010	AXX65	14
	07/12/2010	AXX65	14
P11	06/03/2008	AXX56	15
	25/02/2010	AXX56	16
	06/05/2010	AXX56	16
P12	01/07/2009	AXX46A	17
	03/03/2010	AXX46B	17
P13	15/11/2007	AXX50A	18
	17/07/2008	AXX55	19
	10/11/2010	AXX50A	18
P14	04/08/2005	AXX0	20
P15	04/04/2005	AXX43	21
	30/08/2006	AXX43	21
P16	25/05/2008	AXX57	22
P17	13/01/2010	AXX63	23
	13/01/2010	AXX59A	24
	18/03/2010	AXX59A	24
	18/03/2010	AXX63	23
P19	09/01/2002	AXX3B	25
	30/11/2006	AXX3A	25
	29/04/2010	AXX3B	26
P21	27/01/2010	AXX0	27

3.4. Antibiotic susceptibility

The 447 isolates of *A. xylosoxidans* included in the study displayed the innate resistance of the species to aztreonam, cefalotin, cefoxitin, cefotaxime, kanamycin, amikacin, tobramycin, gentamicin, netilmicin, nalidixic acid and ofloxacin. We report here for each patient the susceptibility of the first isolate in comparison with the susceptibility of the last one to the following molecules: ceftazidime, piperacillin/tazobactam, imipenem, meropenem and ciprofloxacin (year of isolation indicated in Table 1). At first positive culture, the isolate was susceptible to these 5 antibiotics for 7 patients, resistant to ceftazidime for 5 patients, resistant to imipenem for 1 patient and resistant to ciprofloxacin for 16 patients. At last positive culture, isolates resistant to ceftazidime have been recovered from 7 patients. The evolution of carbapenem resistance is important mainly among strains recovered from chronically colonised patients: in 2010, 6 patients harboured strains with decreased susceptibility to meropenem (Table 3). For 3 isolates, MIC of imipenem was much lower than MIC of meropenem. Piperacillin/tazobactam was the most active molecule: no resistant strain has been detected in 2010.

3.5. Sequencing of $bla_{OXA\text{-}114\text{-}like}$ gene

The entire $bla_{OXA\text{-}114like}$ gene from 41 strains (20 different PFGE patterns) was sequenced.

Analysis of the sequences revealed polymorphisms. We have detected five variants ($bla_{OXA\text{-}114\ a,\ c,\ e,\ f,\ g}$) already described [17,18] and five new variants ($bla_{OXA\text{-}114\ h,\ i,\ k,\ l,\ m}$). (Genbank accession numbers JX206446; JX206447; JX206449; JX206450; JX206451). Moreover we have found a new oxacillinase differing from OXA-114a by 34 amino acid substitutions (88% identity). This enzyme was assigned the number OXA-243 (http://www.lahey.org/Studies/). A total of four variants of this enzyme were characterised in four isolates as well as in the reference strain AXX-A ($bla_{OXA\text{-}243\ a,\ b,\ c,\ d}$). Genbank accession numbers JX206453; JX206454; JX206455; JX206456). All isolates that belonged to the same genotype harboured the same bla_{OXA} gene.

4. Discussion

This is the first epidemiological study about *A. xylosoxidans* in a French CF centre. Among the 120 affiliated patients, 21 had at least one positive culture since their first visit. In 2010, positive(s) culture(s) were obtained for 13.9% of the 108 patients for whom sputum analysis was performed. This percentage is much higher than the 4.4% reported in the French global data in 2010 (http://www.vaincrelamuco.org/e_upload/pdf/rapport_registre_2010.pdf). This difference might be due to misidentification in some centres as already reported [8,22]. According to our experience, many isolates of *A. xylosoxidans* share common properties with non-pigmented isolates of *P. aeruginosa*: aerobic, oxidase positive, growth at 41 °C but not at 4 °C, growth on cetrimide medium. The difficulty of identification is enhanced in case of co-culture of both species.

Such a high prevalence has already been reported in some other European centres: 17.6% (among 300 patients) in Italy [23] or 17.9% in a Belgian CF centre (among 140 patients) [22]. Nevertheless the median age at first positive culture was 16 years in our centre which is younger compared to the Belgian centre (median: 20 years). Eight of our patients (6.7%) were chronically colonised with *A. xylosoxidans*. It is a long-term colonisation, these patients being still colonised in 2011 and 2012 (except patient P5, who did not visit the centre since 2007). This rate is somewhat higher than in the Belgian centre (5.6%). Using the same criteria as De Baets (at least 3 positive cultures over at least nine months) the number of chronically colonised patients in our centre would be of 10% instead of 6.7%. To date, the highest rate of chronically colonised patients has been reported in a Brazilian centre: 12.8% [10].

Table 3
MICs (mg/L) of imipenem and meropenem of isolates recovered in 2010 with decreased susceptibility to imipenem and/or meropenem. (E-test method).

Patient	Imipenem	Meropenem
P1	3	4
P2	16	3
P6	4	4
P8	3	>32
P11	4	>32
P19	2	>32

The genotypic analysis by PFGE results proved that patients harboured their own strain. Thanks to the analysis of the strains isolated successively from the patients we have demonstrated that this long-term colonisation lasted over 15 years for one patient. We did not observe strain displacement. As PFGE analysis is time consuming we compared this method with the Diversilab® technology. This is the first study of this typing method for *A. xylosoxidans* strains. In all cases, isolates that were classified as unrelated by PFGE were also classified as different by Diversilab ®. Nevertheless, some isolates belonging to the same PFGE pattern were classified as different by Diversilab®. Such discrepancies were already described for other bacterial species such as *Pseudomonas aeruginosa* [24]. It happened when isolates were recovered with more than 8 months interval. It seems therefore that Diversilab® is more sensitive than PFGE to minor variations of the strains over time. We conclude that PFGE remains the reference method for the survey of long-term colonisation. We propose the Diversilab® as a useful and rapid typing tool to compare isolates recovered simultaneously when an outbreak is suspected or for the comparison of clinical isolates with environmental isolates as already suggested for other pathogens [25].

The cross-contamination between patients was very uncommon in our centre except in 2 cystic fibrosis families. A cross-contamination occurred also between two unrelated patients, one colonised and one non- colonised patient visiting the centre the same day. Nevertheless, it is important to note that the non-colonised patient never happened to have *A. xylosoxidans* again in his subsequent expectorations.

Some patients were colonised with a second strain, genotypically distinct. This co-colonisation was sporadic and lasted for a short time (mostly less than one year).

This diversity of genotypes suggests that the majority of *A. xylosoxidans* isolates from CF patients probably originate from domestic environmental sources. A study of environmental samples might be a help to identify the potential source(s).

Acquired resistance to antibiotics was frequent. The first isolates recovered from our patients were mostly resistant to ciprofloxacin and the mechanism leading to this resistance has to be explored. Acquired resistance to ceftazidime and carbapenems was high among patients attending our centre, and concerned mainly chronically colonised patients.

Repeated administration of antibiotics to treat infections due to common pathogens, especially *P. aeruginosa*, might explain this selection of resistant *A. xylosoxidans*.

It is also important to note that for the first time we report an unusual phenotype in the strains resistant to carbapenems, some of them being much more resistant to meropenem than to imipenem. We recently described the efflux system AxyXY-oprZ in *A. xylosoxidans* which can extrude meropenem (submitted manuscript). The production level of this system will be explored in the clinical isolates to assess its potential contribution to meropenem resistance. Our results are not in accord with Jacquier who recently reported that meropenem displays an interesting antimicrobial activity against *A. xylosoxidans* as compared with

imipenem [26]. Another study conducted in Naples shows that the activity of meropenem is comparable to that of imipenem on 53 isolates [23]. The recommendations for the treatment of *A. xylosoxidans* infection in CF patients are to use intravenous combination of antibiotics including either imipenem or meropenem [27]. Therefore more data are needed to propose the most active carbapenem.

OXA-114 has been described as constitutive in *A. xylosoxidans* [17]. We have detected a wide diversity of $bla_{\text{OXA-114}}$ genes as reported elsewhere [18] as well as five new variants of this enzyme. Nevertheless the primers proposed by Turton did not allow the detection of $bla_{\text{OXA-114}}$ in all our isolates. This highlights the limits of the method. More interestingly we also detected the new oxacillinase OXA-243 with 3 variants. The identification of the isolates harbouring $bla_{\text{OXA-243}}$ was confirmed by sequencing house-keeping genes (data not shown). This method proved to be a very discriminant tool for identification of *A. xylosoxidans*. A similar method has been published very recently [28]. We have detected the different variants of OXA-114 and OXA-243 among strains harbouring various phenotypes of antimicrobial resistance. Therefore these enzymes do not account for acquired resistance to ceftazidime or carbapenems. The diversity of oxacillinases produced by clinical strains might only reflect the diversity of *A. xylosoxidans* natural reservoirs.

5. Conclusion

This study reports for the first time the survey of colonisation of 21 patients by *A. xylosoxidans* since it was first isolated. The prevalence of *A. xylosoxidans* is high in our CF centre. Till now we observed very rare cross-contamination between patients. There is a wide diversity of the isolates and the length of colonisation is sometimes very long. Our main concern is the acquired antimicrobial resistance of the isolates, especially in chronically colonised patients. These first French data including a very large number of isolates should be compared with those of other CF centres from France.

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