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SHORT REPORT
Achromobacter xylosoxidans is the predominant *Achromobacter* species isolated from diverse non-respiratory samples

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SUMMARY

Achromobacter spp. are emerging opportunistic Gram-negative rods responsible for diverse nosocomial or community-acquired infections. We describe, for the first time, the distribution of *Achromobacter* spp., defined by *nrdA* gene sequencing, and their antimicrobial susceptibility in a variety of non-respiratory samples recovered from hospitalized patients from 2010 to 2015. Of the 63 isolates studied, *A. xylosoxidans* was the most prevalent (41 isolates), and with the exception of *A. insuavis* (four isolates), the remaining 10 species identified were represented by one or two isolates only. All isolates were uniformly susceptible to piperacillin and piperacillin-tazobactam and 97% to meropenem, but 76% showed resistance to ciprofloxacin. This study confirms the diversity of *Achromobacter* spp. in non-cystic fibrosis (CF) isolates and the predominance of *A. xylosoxidans*, as previously reported for CF sputum isolates. There was no apparent link between the clinical site of infection and the species of *Achromobacter*.

Key words: *Achromobacter*, *Achromobacter xylosoxidans*, identification, *nrdA*, opportunistic pathogen, resistance.

Achromobacter spp. are non-fermenting Gram-negative rods which are increasingly isolated from sputum samples of cystic fibrosis (CF) patients but are on occasion recovered from hospital or community-acquired infections [1–4]. Conventional biochemical identification tests and 16S rRNA gene sequencing are inadequate for accurate discrimination between the species [5] and these methods generally identify most isolates as *A. xylosoxidans* [6]. In recent years molecular sequence-based studies of housekeeping genes have revealed the complexity of the genus and to date 14 species have been defined [7]. Specific sequencing of the housekeeping gene *nrdA* has proved to be a highly discriminatory tool for species-level identification, highlighting not only the predominance of *A. xylosoxidans*, but also the diversity of species in isolates from CF patients [8]. However, to date, the species diversity as defined by *nrdA* sequencing of isolates from infections in non-CF patients is unknown.

Achromobacter spp. are constitutively resistant to all cephalosporins with the exception of ceftazidime, linked to the presence of the AxyABM efflux system [9] and some species harbour the efflux pump AxyXY-OprZ conferring resistance to aminoglycosides [6].

We set out to describe the species distribution of *Achromobacter* isolates recovered from extrapulmonary clinical samples from non-CF patients attending our hospital to detect possible associations of different

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species with specimen type and clinical presentation, and to determine their antimicrobial susceptibility.

From January 2010 to December 2015 we collected all isolates identified as *Achromobacter* spp. from non-CF hospitalized or outpatients attending our university hospital (1700 beds, Dijon, France). Only clinically significant isolates from non-respiratory sites (excluding sputum, sinus, lung, nasopharyngeal, tracheal, pleural or bronchoalveolar aspirates) were included and a single isolate from each patient was entered into the study. Clinical data (age, sex, underlying disease) were collected retrospectively for each patient.

Isolates were identified as *Achromobacter* spp. by conventional methods: API 20NE (bioMérieux, France) and MALDI-TOF (Bruker biotyper; Bruker Daltonique, France). We used 16S rRNA gene sequencing if these techniques failed. Species-level identification was performed by *nrdA* sequencing using primers nrdA-P1 and nrdA-P2 as described previously [8]. The sequences were compared to the pubMLST database (http://pubmlst/Achromobacter/) and new alleles were submitted to the database for confirmation of species identification.

Susceptibility testing was performed by a disk diffusion method and interpreted according to zone diameter breakpoints for *Pseudomonas aeruginosa* according to the Clinical Laboratory Standards Institute (M100-S25, January 2015) as no specific data are, as yet, provided for *Achromobacter* spp. for the agents tested. The antibiotics were ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, imipenem, meropenem, doripenem, ciprofloxacin, tobramycin, amikacin, gentamicin and netilmicin. Reference control strains were *P. aeruginosa* CIP 76110 and *Escherichia coli* CIP 7624.

A total of 63 patients presented positive cultures of isolates belonging to the genus *Achromobacter* during the study period, 16 of whom were outpatients. Hospitalized and outpatients were distributed throughout several departments, the most predominant being Surgery (8 and 2 patients, respectively), Otorhinolarynology (2 and 6), Haematology (6 and 2) and Intensive Care (8 inpatients). The median age was 59 years and 44 (69·8%) were males. Blood (13) and wound cultures (12) were the most prevalent sites of isolation followed by ear (8), bone (6) and contact lens or lens cleaning solution (4) samples (Table 1). Five of seven patients with positive ear swab cultures were diagnosed with cholesteatoma or chronic otitis and all ophthalmic isolates were from patients with corneal abscesses. Diabetes (15) and cancer (12) were the most common underlying diseases and all, but two, of 13 positive blood cultures were from patients with indwelling catheters.

Table 1 lists the 12 species isolated from the 63 study patients. (Full results are available in Supplementary Table S1.) *A. xylosoxidans* accounted for 41 (65%) of all isolates and *A. insuavis* for four (6·3%). Four species were each represented by two isolates and five species by single isolates. An unnamed novel species was identified from five specimens, two of which were placenta samples from patients in a maternity ward in the same year. This species had previously been identified in two patients and closely matched genogroup 19 [6, 10].

All isolates were fully susceptible to piperacillin and piperacillin-tazobactam and only one isolate was not susceptible to ticarcillin and ticarcillin-clavulanic acid. Resistance to imipenem, doripenem, ceftazidime, and meropenem was exhibited by 17%, 11%, 8% and 3% of the isolates, respectively. The frequency of resistance to ciprofloxacin was 76%. Resistance to aminoglycosides was observed only in isolates belonging to intrinsically resistant species [6].

Since the description of *Achromobacter* species-level identification by *nrdA*-sequencing, only one study has reported the identification of a limited number of non-CF isolates by this method [11]. In the present study we took advantage of our large collection of *Achromobacter* isolates systematically recovered from non-respiratory samples from non-CF patients over a 6-year period. Interestingly, *Achromobacter* spp. was isolated from patients attending a wide range of clinical and surgical departments and also from the maternity ward and outpatient units, suggesting nosocomial or community acquisition. Moreover, the organisms were recovered from deep or superficial anatomical sites with blood and wounds being the most frequent. It is noteworthy that 11/13 patients with positive blood cultures had indwelling catheters and that catheters from four other patients were also culture positive. Cancer and/or diabetes have previously been identified as underlying risk factors for *Achromobacter* infections [1] and represented 41% of our study patients. The organisms were also associated with significant ear infections and corneal abscesses. We observed a diversity of species with the predominance of *A. xylosoxidans* which is consistent with previous studies in CF patients [8, 10, 11]. Our results did not identify any apparent association between the site of sampling, clinical context, and

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Table 1. *Distribution of* Achromobacter *spp. according to clinical site of isolation*

	Blood	Bone	Ophthalmic device	Ear	Wound	Other	Total
A. aegrifaciens						Stool (1)	1
A. animicus	1					Catheter (1)	2
A. denitrificans		1					1
A. dolens						Skin (1)	1
A. genogroup 9	1						1
A. insolitus			1	1			2
A. insuavis	1		1			Dialysis catheter (2)	4
A. marplatensis					1	Throat (1)	2
A. mucicolens	1						1
A. spanius					2		2
A. xylosoxidans	9	5	2	7	9	Abcess (1), bile fluid (1), biliary prosthese (1), catheter (1), skin (1), stool (1), throat (1), tissue (2)	41
Novel species 1						Ascitic fluid (1), gastric aspirate (1), placenta (2), urine (1)	5
Total	13	6	4	8	12	20	63

the species of *Achromobacter* and this warrants further study of a larger number of isolates. Indeed, the wide distribution of *A. xylosoxidans* across infection sites argues against a specific clinical tropism for this species.

Resistance to aminoglycosides was observed only in the innately resistant species *A. xylosoxidans, A. dolens, A. insuavis, A. aegrifaciens, A. denitrificans* and *A. insolitus* which have been shown to harbour the AxyXY-OprZ efflux system [6], and notably these species represented 90% of all isolates tested here. The extended spectrum penicillins and meropenem proved to be the most active of the antimicrobials tested which is consistent with the literature [4]. Likewise the high frequency of ciprofloxacin resistance (76%) has been noted among environmental and CF primary colonizing isolates [12].

Finally, the predominance of *A. xylosoxidans* in all types of samples might be explained by either (i) the species is more abundant than other *Achromobacter* in the natural, hospital and domestic environments or (ii) there are selective factors that influence its relatively high frequency in clinical samples. Regarding the latter, we suggest that intrinsic resistance to disinfectants particularly quaternary ammonium compounds (QACs) which have been incriminated in various healthcare-associated infections and pseudobacteraemia with *A. xylosoxidans*, may promote its survival in the hospital setting [2, 13]. Given the wide use of QACs in our hospital for environmental cleaning and disinfection, and their presence as preservatives in contact lens fluids and eardrops, it would be of interest to investigate the susceptibility of the isolates to QACs and other biocides to determine whether their use contributes to the survival and proliferation of the species. Further studies are therefore necessary to test this hypothesis in order to elaborate prevention measures.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268816001564.

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