JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2008, p. 3165–3168 Vol. 46, No. 9
0095-1137/08/$08.00+0 doi:10.1128/JCM.00855-08
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Misidentification of *Pandoraea sputorum* Isolated from Sputum of a Patient with Cystic Fibrosis and Review of *Pandoraea* Species Infections in Transplant Patients[▽]

Jason D. Pimentel* and Colin MacLeod

Department of Microbiology and Infectious Diseases, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Received 5 May 2008/Returned for modification 30 June 2008/Accepted 9 July 2008

***Pandoraea* species are considered emerging pathogens in cystic fibrosis (CF) patients, but few data exist regarding outcomes of patients colonized with these organisms. We report a case of *Pandoraea sputorum* colonization in a CF patient under consideration for lung transplantation and review five cases of lung transplantation involving *Pandoraea* species.**

CASE REPORT

In October 2007, a 32-year-old man with end stage cystic fibrosis (CF) was admitted to the Royal Prince Alfred Hospital (RPAH), Sydney, Australia, due to an exacerbation of his respiratory illness with increased shortness of breath and the following blood gas values: pO_2, 53 mm Hg; pCO_2, 50 mm Hg. Cultures from sputum grew *Pseudomonas aeruginosa* and *Pandoraea sputorum* profusely. In addition to respiratory complications, he had also developed diabetes mellitus and pancreatic exocrine insufficiency and required a percutaneous gastrostomy tube for supplemental feeding. As an inpatient, he received 2 weeks of intravenous antimicrobials (tobramycin and ticarcillin-clavulanate) and chest physiotherapy. At discharge spirometry values were as follows: forced expiratory volume in 1 s (FEV1), 0.35 liter (10% predicted); forced vital capacity, 1.25 liters (36% predicted). He returned home on oxygen therapy, nocturnal noninvasive ventilation, and nebulized colistin. During the admission, he had continued a work-up for lung transplantation and was referred to a transplant center. Though he had never been colonized with any members of the *Burkholderia cepacia* complex, his medical record indicated that, in addition to *Pseudomonas aeruginosa*, several different species of multiresistant gram-negative bacilli from his sputum had been reported during the previous 2 years. Subsequently, he was not considered to be a good transplant candidate, due to the number of different multiresistant organisms isolated from his sputum.

As part of an unrelated study, stored DNA from three of this patient's isolates was reexamined. The absence of the *B. cepacia* complex *recA* gene confirmed that none of these three isolates were members of the *B. cepacia* complex. However, the identities of two of the isolates, as determined by the 16S rRNA gene sequence, did not match the identities previously reported on the basis of biochemical tests. One isolate had been reported in July 2005 as *Ralstonia pickettii*. The second was reported in November 2005 as *Achromobacter xylosoxidans*. In March 2006, the identity of the third isolate had been reported by an external laboratory as *Pandoraea sputorum*, susceptible only to piperacillin and piperacillin-tazobactam by the calibrated dichotomous sensitivity disk diffusion method (4). The 16S rRNA gene sequence data that we generated indicated that all of these organisms were, in fact, *Pandoraea* species. In addition, a review of laboratory records indicated that a number of isolates from this patient had been presumptively identified as various organisms, such as CDC group IVc-2, an *Alcaligenes* sp., *Alcaligenes faecalis*, a *Pandoraea* sp., and *Ralstonia pickettii*, but until July 2005 had been reported only as "nonfermentative gram-negative bacillus, not *Burkholderia cepacia*." All these isolates had similar biochemical profiles (notably lack of growth at 42°C) and susceptibility patterns identical to those of the isolates in question.

Subsequently, in January 2008, the two reports from 30 months and 26 months earlier were corrected and reissued. These new results were sent directly to the RPAH CF unit, which then notified the transplant unit. Initially, the transplant unit still decided not to list the patient for a transplant due to concerns about the *Pandoraea* genus. However, the literature review that follows prompted further consideration of this patient's case. While this patient remains culture positive for *P. sputorum*, his condition remains stable on home oxygen, with an FEV1 that is 20% predicted.

Microbiology. The study isolates had been cultured from respiratory samples obtained from this patient and processed according to the laboratory's protocol for handling sputum from CF patients. Specimens were inoculated onto a *B. cepacia* complex-selective agar plate (BCA) (Oxoid, Thebarton, SA, Australia) and incubated at 37°C in 5% CO_2. The BCA plates were examined daily for up to 7 days, and identification proceeded with organisms that grew on the BCA medium. Laboratory records do not clearly state how the identification of *R. pickettii* was derived but indicated that the RapID NF Plus kit (code 510211; Remel, Lenexa, KS) and the replicator method described by Lennox and Ackerman (12) were both used. The second isolate from 2005 was identified as *A. xylosoxidans* by the RapID NF Plus kit (code 610206). The isolate from 2006 had been referred to an external laboratory after the RapID NF Plus kit failed to provide any identification. The three study

* Corresponding author. Present address: Department of Pathology and Laboratory Medicine, Henry Ford Hospital, 2799 W. Grand Blvd., Detroit, MI 48202-2608. Phone: (313) 916-7389. Fax: (313) 916-2385. E-mail: jdpimentel@member.rcpa.edu.au.

▽ Published ahead of print on 23 July 2008.

isolates had also been referred for conventional *recA* gene PCR (13) to rule out the possibility of the *B. cepacia* complex. DNA extraction was performed using the QIAamp DNA mini-kit spin column method (Qiagen, Hilden, Germany) according to the manufacturer's instructions. An aliquot of the DNA was used for the PCR, and the remainder was stored at −80°C. In December 2006, a multiplex, real-time PCR to detect both the 16S rRNA gene and the *recA* gene was performed on the stored DNA (15). The real-time PCR product was used to generate a 16S rRNA gene sequence, as previously described (14), which was later used to identify these organisms by a nucleotide BLAST search (2).

The BLAST match of the 500-bp partial 16S rRNA gene sequences of our isolates indicated a high degree of similarity not only to *P. sputorum* LMG 18819 but also to *Pandoraea* genomospecies 2 (GenBank accession no. AF247693), *Pandoraea* genomospecies 3 (AF247697), and a single isolate of *Pandoraea apista* (AF247699, but none of the other *P. apista* isolates in GenBank). However, DNA-DNA hybridization studies have previously established that these four isolates, with similar 16S rRNA gene sequences, are in fact located in different species-level hybridization groups (9). After a review of our records and those provided by the external laboratory, the biochemical profile of our isolates was found to be most consistent with characteristics reported for *P. sputorum* isolates described in previous studies (6, 9): no growth at 42°C, growth on cetrimide agar, negative nitrate reduction, positive urease activity, and a negative oxidase reaction. While these studies have demonstrated that the phenotypic characteristics may vary between isolates of the same species, absence of growth at 42°C appears to be a characteristic only of *P. sputorum*, *Pandoraea norimbergensis*, and *Pandoraea* genomospecies 1 (the last two of which were not in question, due to other differing characteristics).

Discussion. The genus classification *Pandoraea* was created in 2000 to accommodate organisms in *Pseudomonas* rRNA homology group II that had previously been tentatively assigned to either the genus *Burkholderia* or *Ralstonia* (6). Differentiation of *Pandoraea* species from *Burkholderia* or *Ralstonia* can be difficult, and molecular methods of differentiation have been recommended (8, 16). The genus comprises motile, non-spore-forming, nonfermentative, gram-negative bacilli that have been isolated from both environmental and human clinical samples (6, 9). Descriptions of additional phenotypic, biochemical, and genotypic features have been published previously (6, 9). A few distinguishing features of *P. sputorum* have been mentioned above. The limitations of the 16S rRNA gene for differentiating the *Pandoraea* species have been noted previously (7, 8, 9) and are particularly relevant for *P. sputorum*. The *P. sputorum*-specific 16S rRNA gene-based primers described in another study were reported to be cross-reactive with *Pandoraea* genomospecies 2 and 3 (8). Even the *gyrB* gene sequences of *P. sputorum* and *Pandoraea* genomospecies 2 have been found to share a high degree of similarity (7). Due to these limitations a polyphasic approach to identification is recommended (7).

Though the *Pandoraea* species are considered to be emerging pathogens in CF patients, there are still little clinical data regarding the course and outcomes for patients colonized with these organisms (5, 11). In particular, there are scant data reported for *P. sputorum*. This is no doubt partly due to the difficulty in correctly identifying and differentiating the species of this genus (6). Due to the concerns that had been expressed regarding our patient's transplant candidacy, we searched for other transplant cases involving colonization with *Pandoraea* species. In our review of the literature, we found no cases of transplantation in the setting of *P. sputorum* colonization and only five other cases of transplantation in patients colonized or infected with other *Pandoraea* species (Table 1).

Stryjewski et al. identified *Pandoraea pnomenusa* from the blood of a 30-year-old male patient with end stage sarcoidosis after a bilateral lung transplant (BLT) and from bronchoalveolar lavage fluid on postoperative day 10 (17). The patient died on postoperative day 17 from shock and multiple organ failure. Gram-negative rods had not been isolated from respiratory samples from the patient or the donor in the preoperative period. Jørgensen et al. described a 21-year-old patient cocolonized with *Pseudomas aeruginosa* and *Pandoraea apista* who survived a BLT in 1998 and then became culture negative for *P. apista* (11). Atkinson et al. reported two cases in a single report (3). One patient was a 30-year-old female with CF, cocolonized with *P. aeruginosa* and *P. apista*, who received a BLT in July 2004. A pleural effusion 6 weeks postoperation that was culture positive for *P. aeruginosa* and *P. apista* responded to antimicrobial therapy. Respiratory cultures remained negative for *P. apista* by April 2005. The other patient was a 36-year-old male with CF, also cocolonized with *P. aeruginosa* and *P. apista*, who received a BLT in September 2003. While the first posttransplant bronchial washing was culture positive for *P. apista*, all subsequent cultures remained negative. The fifth patient, mentioned briefly in a report by Caraher et al. (5), had been cocolonized with *P. aeruginosa* and *Pandoraea pulmonicola* and was doing well postoperatively at the time of the report.

The patients with reported survival beyond 12 months were all CF patients and previously colonized with a *Pandoraea* species. The patient who died soon after transplantation did not have CF and was not previously colonized with *Pandoraea*, but death was attributed to *P. pnomenusa* infection. There was one report of posttransplant infection among the CF patients. It is also notable that all the CF patients were also cocolonized with *P. aeruginosa*. The reported sensitivity profiles among these isolates varied. The *P. apista* isolates were all susceptible to trimethoprim-sulfamethoxazole, two were resistant to imipenem, and one was intermediately sensitive to meropenem. The *P. pnomenusa* isolate was resistant to trimethoprim-sulfamethoxazole and sensitive to imipenem.

Pandoraea species have been isolated from a variety of specimens from CF and non-CF patients including sputum, blood, the upper airways, lung tissue, urine, and a wound (9). While clinical evidence of invasive potential has been demonstrated by nine reported cases of *Pandoraea* bacteremia (6, 9, 10, 17, 18) (Table 2), only one of these cases was reported to be a CF patient (who survived the episode of bacteremia) (10). The one reported fatality is mentioned above. Case reports have also indicated that many CF patients experience a decline in lung function after chronic *Pandoraea* colonization is established (3,

TABLE 2. Nine reports of bacteremia secondary to *Pandoraea* species[a]

Patient	Location	Age (yr)	Sex	Dx	Organism	Reference
1	BEL	NR	NR	NR	*P. norimbergensis*	6
2	CA	66	F	COPD	*P. apista*	9
3	TX	46	M	NR	*P. pnomenusa*	9
4	HI	76	M	MVR	*P. pnomenusa*	9
5	LA	49	M	NR	*P. pnomenusa*	9
6	NC	30	M	Sar	*P. pnomenusa*	17
7	WA	16	M	CF	*P. apista*	10
8	NC	NR	NR	NR	*Pandoraea* sp.	18
9	NC	NR	NR	NR	*Pandoraea* sp.	18

[a] BEL, Belgium; CA, California; COPD, chronic obstructive pulmonary disease; Dx, underlying condition; HI, Hawaii; LA, Louisiana; MVR, mitral valve replacement; NC, North Carolina; NR, not reported; Sar, sarcoidosis; TX, Texas; WA, Washington State.

11). This may be due to a *Pandoraea*-induced increase in interleukin-6 and interleukin-8 production, as suggested by recent in vitro data (5). It has also been demonstrated that colonized individuals develop anti-*Pandoraea* antibodies (11), which may partially account for the fact that three of the colonized patients were noted to have survived beyond 12 months, while the acutely infected patient rapidly succumbed. In addition to lung transplantation and aggressive, long-term combination antimicrobial therapy, clearance of *Pandoraea* colonization has even occurred after replacement by colonization with *Burkholderia multivorans* (11). Due to a report of person-to-person spread (11), some centers have introduced segregation of patients colonized with *Pandoraea* species just as is carried out for those colonized with the *B. cepacia* complex (3, 11). None of the other patients included in a recent molecular survey of the patients in the RPAH CF unit were found to harbor *P. sputorum* or any of the other *Pandoraea* species (15).

In conclusion, the first step to establishing or disproving the clinical significance of *Pandoraea* species is consistent and accurate identification. We hope this report will encourage additional reports of the experience of others with these organisms. Secondly, we note that, for accurate identification of this genus, the sole use of biochemical tests appears inadequate. Moreover, molecular identification may need to go beyond the oft relied upon 16S rRNA gene sequence for purposes of species level identification. While the data are currently limited, in time speciation may prove important if the evidence indicates that transplant outcomes differ significantly depending on the colonizing species, as they do with the *B. cepacia* complex (1). Because antimicrobial susceptibility appears difficult to predict, therapy should be tailored (preferably prior to transplant) for each isolate. Finally, the role of the genus *Pandoraea* in the course and prognosis of CF merits further study due to the demonstrated invasive potential and reports of a decline in lung function. However, the cases reviewed above indicate that successful transplant outcomes are possible in spite of *Pandoraea* colonization.

Nucleotide sequence accession number. The partial 16S rRNA gene sequences from the case isolates are deposited in GenBank under accession numbers EF427778, EF427768, and EF427725.

TABLE 1. Five reports of transplant patients infected or colonized with *Pandoraea* species[a]

Pt	Location	Yr of report	Age at TP (yr)	Sex	Dx	FEV1 at TP (% predicted)	*Pandoraea* sp. isolated	Previously colonized	Other pathogen[b]	Drug(s) to which organism was:		Method[c]	Survival time post-TP[d]	Cleared *Pandoraea*	Ref
										Sensitive or intermediate[e]	Resistant				
1	NC	2003	30	M	Sar	NR	*P. pnomenusa*	No	*Nocardia* sp.	IPM	AG, CAZ, CIP, MEM, TZP, SXT	DD	17 days	NA	17
2	DNK	2003	21	F	CF	20	*P. apista*	Yes	*P. aeruginosa*	SXT, sulf, TET, (CAZ, CRO, MEM, thien)	AMP, AG, CHL, CIP, CLR, MAC, OFX, TMP	DD	4.5 yr[f]	Yes	11
3	MO	2006	30	F	CF	22	*P. apista*	Yes	*P. aeruginosa*	CRO, SXT	AMP, ATM, colistin, FEP, GEN, IPM, TOB, TZP	DD	1.5 yr[f]	Yes	3
4	MO	2006	36	M	CF	19	*P. apista*	Yes	*P. aeruginosa*	CRO, FEP, SXT, TZP	AMK, AMP, ATM, colistin, GEN, IPM, TOB	DD	2.5 yr[f]	Yes	3
5	IRL	2008	NR	nr	CF	55	*P. pulmonicola*	Yes	*P. aeruginosa*	NR	NR	NR	NR[f]	NR	5

[a] AG, aminoglycosides; AMK, amikacin; AMP, ampicillin; ATM, aztreonam; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; CLX, clinafloxacin; CRO, ceftriaxone; DD, disk diffusion; DNK, Denmark; Dx, underlying diagnosis; FEP, cefepime; GEN, gentamicin; IPM, imipenem; IRL, Ireland; MAC, macrolides; MEM, meropenem; MO, Missouri; NA, not applicable; NC, North Carolina; NR, not reported; OFX, ofloxacin; PIP, piperacillin; Pt, patient; Ref, reference; Sar, sarcoidosis; sulf, sulfamethoxazole; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; thien, thienamycin; TMP, trimethoprim; TOB, tobramycin; TP, transplant; TZP, piperacillin-tazobactam.

[b] Other pathogens present in sputum.

[c] Method of antimicrobial susceptibility testing.

[d] At the time of original report.

[e] Parentheses indicate intermediate susceptibility.

[f] Alive at the time of the original report.

We are grateful for the assistance of Peter Bye and Carmel Moriarty of the Royal Prince Alfred Hospital Cystic Fibrosis Unit, Camperdown, NSW, Australia, and Glenn Hawkins, Department of Microbiology, Bacteriology Laboratory, Sydney Eastern Area Laboratory Service, Randwick, NSW, Australia.

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