Misidentification of *Pandoraea sputorum* Isolated from Sputum of a Patient with Cystic Fibrosis and Review of *Pandoraea* Species Infections in Transplant Patients

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**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: pO₂, 53 mm Hg; pCO₂, 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 his 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 *Achromobacter xylosidans*. 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% CO₂. 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. xylosidans* 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 strains were reported in November 2005 as *Achromobacter xylosidans*. 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.

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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 norimbergenensis, 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* 16S 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 emerg-
TABLE 2. Nine reports of bacteremia secondary to *Pandoraea* species

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism</th>
<th>Patient sex</th>
<th>Organism reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td><em>P. norimbergensis</em></td>
<td>NR</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td><em>P. promenusa</em></td>
<td>NR</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td><em>P. promenusa</em></td>
<td>NR</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td><em>P. apista</em></td>
<td>M</td>
<td>18</td>
</tr>
<tr>
<td>18</td>
<td><em>Pandoraea</em> sp.</td>
<td>NR</td>
<td>18</td>
</tr>
<tr>
<td>18</td>
<td><em>Pandoraea</em> sp.</td>
<td>NR</td>
<td>18</td>
</tr>
</tbody>
</table>

* 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.

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 genus level identification. The partial 16S rRNA gene sequences from the case isolates are deposited in GenBank under accession numbers EF427778, EF427768, and EF427725.
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REFERENCES