Infections Caused by Unusual *Methylobacterium* Species

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We describe six patients with hospital-acquired bacteremia caused by *Methylobacterium* species, including *M. radiotolerans* (n = 2), *M. thiocyanatum* (n = 2), *M. aminovorans* (n = 1), and *M. lusitanum* (n = 1), which were confirmed to species level by 16S rRNA gene sequence analysis. Among these patients, five had catheter-related bacteremia and all had favorable outcomes.

*Methylobacterium* species are fastidious, pink-pigmented, Gram-negative bacilli and are normally distributed in environmental sources, such as soil, sewage, and leaf surfaces (1, 6, 15). The genus was first proposed by Patt et al. in 1976 and consists of more than 20 species, including some previously grouped in the *Pseudomonas* genus (e.g., *P. mesophilica*, *P. radiola*, and *P. rhodos*) (12). *Methylobacterium* species have been reported to cause contamination and colonization and have been isolated from some sterile clinical sites, including blood, bone marrow, sputum, pleural effusion, peritoneal fluid, cerebrospinal fluid, synovium, and skin (2, 5, 7–9, 11, 13, 14, 16). Most *Methylobacterium* infections develop in immunocompromised patients, such as patients with malignancy, organ transplant, HIV infection, renal failure, or alcoholism (5, 8, 11, 14, 17). In the present study, we describe six cases of bacteremia caused by various *Methylobacterium* species, all of which were confirmed by 16S rRNA.

This study was conducted at the National Taiwan University Hospital (NTUH), a 2,500-bed tertiary care center in northern Taiwan. Patients whose blood culture had yielded *Methylobacterium* species were identified from the computerized database of the bacteriology laboratory of the NTUH during the period from January 2000 to December 2010. The diagnosis of bacteremia was based on clinical and bacteriological investigations. Bacteremia was classified as health care-associated infection or community acquired. Catheter-related bloodstream infection was classified as bacteremia with the presence of an intravenous catheter and without other foci. If no primary focus could be identified, the bacteremia was classified as primary.

Blood culture specimens were inoculated into Bactec or Bactec PLUS culture bottles using the Bactec 9240 system (Becton Dickinson, Sparks, MD). Isolates that grew Gram-negative vacuolated rods producing pink pigment which were positive for indophenol oxidase, catalase, and urease and which reduced nitrate were identified as *Methylobacterium* species. All of the isolates were identified using the API 20NE system (bioMerieux Vetek, Marcy l’Etoile, France). These organisms were further confirmed to the species level by 16S rRNA gene sequence analysis using two primers, 8FPL (5′-AAGTTTGATCCTGGCTCAG-3′) and 1492RPL (5′-GGTTACCTTGTTAGACT-3′). The sequences obtained (1,399 bp) were compared to published sequences in the GenBank database using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/blast). The closest matches and GenBank accession numbers were obtained as previously described (10). The susceptibility of all available isolates to 11 agents was determined by the routine disk diffusion method and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) criteria for *Pseudomonas aeruginosa* (4).

During the study period, blood cultures positive for *Methylobacterium* spp. were positively identified by 16S rRNA gene sequencing in 6 patients (Table 1). Overall, there were two isolates of *M. radiotolerans*, two isolates of *M. thiocyanatum*, one isolate of *M. aminovorans*, and one isolate of *M. lusitanum*. The profiles of the six isolates of *Methylobacterium* species generated by the API 20NE are shown in Table 2. All isolates were positive for urease and oxidase, all varied in five reactions, and all were negative for the remainder of 14 reactions.

The clinical features of the six patients with *Methylobacterium* infections are summarized in Table 1. Females comprised most of the patients, and three of the six patients were aged >65 years. Infections were hospital acquired in five patients and community acquired in one patient. All patients had various underlying conditions. End-stage renal disease was the most common disease, followed by leukemia. All of the patients with hospital-acquired bacteremia had catheter-related bloodstream infections. All of the patients had favorable outcomes.

Drug resistance patterns were determined. The *M. lusitanum* isolate was resistant to aztreonam, ceftazidime, and ciprofloxacin. Both of the *M. thiocyanatum* isolates were resistant to aztreonam and ciprofloxacin. *M. radiotolerans* was resistant to aztreonam, ceftazidime, cepfime, and piperacillin-tazobactam. The *M. aminovorans* isolate was resistant to all antimicrobial agents tested.

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TABLE 1. Clinical features of six patients with infections caused by various Methylobacterium species

<table>
<thead>
<tr>
<th>Patient (yr infected)</th>
<th>Underlying disease</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Clinical diagnosis</th>
<th>Site of isolation</th>
<th>Outcome</th>
<th>Antibiotic therapy</th>
<th>Rem. of catheter</th>
<th>API (% identity)</th>
<th>GenBank accession no.</th>
<th>Clinical features of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (2000) 77/M</td>
<td>End-stage renal disease</td>
<td>77</td>
<td>M</td>
<td>Catheter-related bacteremia</td>
<td>Blood (CVC)</td>
<td>Yes</td>
<td>Meropenem</td>
<td>Improved</td>
<td>47.5</td>
<td>AM910532.1 (99)</td>
<td>M. aminovorans</td>
</tr>
<tr>
<td>2 (2004) 49/F</td>
<td>Leukemia, chemotherapy</td>
<td>49</td>
<td>F</td>
<td>Catheter-related bacteremia</td>
<td>Blood (DLC)</td>
<td>Yes</td>
<td>Cefepime</td>
<td>Improved</td>
<td>47.5</td>
<td>GQ895736.1 (100)</td>
<td>M. radiotolerans</td>
</tr>
<tr>
<td>3 (2004) 82/F</td>
<td>Chronic respiratory failure, end-stage renal disease</td>
<td>82</td>
<td>F</td>
<td>Catheter-related bacteremia</td>
<td>Blood (CVC)</td>
<td>Yes</td>
<td>Vancomycin</td>
<td>Improved</td>
<td>99.8</td>
<td>GQ281076.1 (100)</td>
<td>M. lusitanum</td>
</tr>
<tr>
<td>4 (2007) 60/F</td>
<td>Chronic obstructive pulmonary disease</td>
<td>60</td>
<td>F</td>
<td>Catheter-related bacteremia</td>
<td>Blood (DLC)</td>
<td>Yes</td>
<td>Piperacillin-tazobactam</td>
<td>Improved</td>
<td>3.2</td>
<td>AM910534.1 (100)</td>
<td>M. thiocyanatum</td>
</tr>
<tr>
<td>5 (2007) 79/F</td>
<td>Leukemia, febrile neutropenia</td>
<td>79</td>
<td>F</td>
<td>Primary bacteremia</td>
<td>Blood</td>
<td>NA</td>
<td>Vancomycin</td>
<td>NA</td>
<td>94.1</td>
<td>AM910539.1 (100)</td>
<td>M. radiotolerans</td>
</tr>
<tr>
<td>6 (2009) 79/F</td>
<td>Chronic obstructive pulmonary disease</td>
<td>79</td>
<td>F</td>
<td>Catheter-related bacteremia</td>
<td>Blood (CVC)</td>
<td>Yes</td>
<td>Ciprofloxacin</td>
<td>Improved</td>
<td>94.1</td>
<td>AM910535 (99)</td>
<td>M. radiotolerans</td>
</tr>
</tbody>
</table>

**TABLE 2. Profiles of biochemical reactions for the six isolates of Methylobacterium species generated by the API 20NE**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isolate</th>
<th>NO3</th>
<th>URE</th>
<th>ARA</th>
<th>GNT</th>
<th>MLT</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M. aminovorans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>M. radiotolerans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>M. lusitanum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>M. thiocyanatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>M. thiocyanatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>M. radiotolerans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*All isolates were positive for oxidase and negative for other reactions not shown. NO3, reduction of nitrates to nitrites; URE, urease; ARA, arabinose assimilation; GNT, gluconate assimilation; MLT, malate assimilation; PAC, phenyl-acetate assimilation.*

In this study, we have provided evidence of human infections caused by three previously unreported disease-causing species of the genus Methylobacterium, namely, M. thiocyanatum, M. aminovorans, and M. lusitanum. Specifically, M. thiocyanatum caused a catheter-related infection in a patient with uremia (case 4) and primary bacteremia in an immunocompetent patient (case 5). Our findings suggest that this species can cause infection in both immunocompromised and immunocompetent patients. In addition, we present a patient with uremia who developed a double-lumen catheter-related bloodstream infection caused by M. aminovorans. Furthermore, case 3 in this study is the first reported case of catheter-related infection caused by M. lusitanum in an immunocompromised patient, which was successfully managed with antibiotic therapy and removal of the patient’s catheter.

The M. radiotolerans isolate caused a catheter-related infection in a 1-year-old child (case 2) undergoing hemodialysis. In addition, another case (case 6) of M. radiotolerans infection developed in a leukemia patient receiving chemotherapy. Neither of them had fever nor cultures positive for M. radiotolerans after removal of the catheters and receipt of antibiotic therapy. The presentation and favorable outcome in those two patients were similar to those reported previously in a study in Italy (11), which hints at low pathogenicity of this rare pathogen.

Previous in vitro susceptibility testing (3) showed that the majority of Methylobacterium isolates were resistant to beta-lactam agents, with the exception of ceftriaxone and cefotaxime. In addition, most of the isolates were susceptible to ciprofloxacin and trimethoprim-sulfamethoxazole, and aminoglycosides, including gentamicin and amikacin, showed excellent in vitro activity in that study as well as in our study. In this report, the in vitro susceptibility results were not identical for each Methylobacterium species. Despite the difficulty in drawing inferences about optimal antibiotic treatment based on this limited experience, our data suggest that clinical microbiology laboratories have the ability to identify these unusual bacteria to the species level because the different Methylobacterium species have different drug susceptibility patterns.

In conclusion, although Methylobacterium species rarely cause human infections, clinicians should be aware of the possibility of catheter-related infection caused by Methylobacterium spp. In this study, we demonstrated the clinical significance of several unusual Methylobacterium spp., including M.
thiocyanatum, M. aminovorans, and M. lusitanum. Therefore, accurate identification with molecular methods, including 16S rRNA sequencing analysis, is imperative for the understanding and diagnosis of these unusual pathogens.

REFERENCES