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Systemic Infection of an Immunocompromised Patient with *Methylobacterium zatmanii*

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We describe the identification of *Methylobacterium zatmanii* as the causative agent of bacteremia and fever in an immunocompromised patient. The patient, a 60-year-old man, had a 5-month history of acute myeloid leukemia and had been on chemotherapy throughout this period. Seven days after the onset of neutropenia, the patient developed fever. The combination of ciprofloxacin, co-trimoxazole, imipenem, amikacin, and vancomycin led to a complete defervescence. On subculture from six positive blood cultures, the organism grew only on buffered charcoal yeast extract agar and not on standard agars. Identification by universal PCR and subsequent sequence analysis of the amplified 16S rRNA gene segment was achieved. This identification by molecular biology techniques was confirmed by conventional biochemical tests. To our knowledge, this is the first description of *M. zatmanii* isolated from patient material.

Advances in the treatment of leukemia have improved prognosis, but intensive chemotherapy regimens have increased susceptibility to opportunistic infections. We describe the isolation and identification of *Methylobacterium zatmanii* as the causative agent of bacteremia and fever in an immunocompromised patient. The genus *Methylobacterium* is a group of aerobic, gram-negative, rod-shaped bacteria which were isolated previously from various environments.

Case report. A 60-year-old male patient with acute myeloid leukemia and agranulocytosis had been undergoing chemotherapy for 5 months. A central venous catheter (Hickmann) was implanted at the beginning of chemotherapy. After complete remission, the patient was hospitalized and a consolidation chemotherapy was initiated. One week later, the hospitalized patient developed neutropenia (<500 neutrophils/μl), and an antibiotic prophylaxis with ciprofloxacin and co-trimoxazole was initiated. Seven days after the onset of neutropenia, the patient developed fever, which was resistant to additional ceftazidime and teicoplanin treatment. On day 3 of the fever period, the antibiotic therapy was changed to a combination of ciprofloxacin, co-trimoxazole imipenem, amikacin, and vancomycin (Table 1), which led to a complete and durable defervescence.

During the first 3 days of fever, a total of 12 blood specimens of 5 to 10 ml from peripheral blood venipunctures and from the central line were obtained. After 48 h of incubation at 37°C in a BACTEC 9240 system (Becton Dickinson, Heidelberg, Germany), all aerobically incubated blood cultures were positive. In contrast, none of the anaerobic bottles gave a positive signal.

Even though gram-negative vacuolated short rods could be observed by direct Gram staining of the cultures, several attempts to subcultivate the organism on standard solid media, such as sheep blood agar (37°C, aerobic), Mueller-Hinton agar (37°C, aerobic), and chocolate agar (37°C, 5% CO_2), failed. All plates were incubated for 4 days. Media were obtained from Oxoid (Wesel, Germany).

Final plating of the blood cultures on *Legionella*-specific agar (*Legionella* charcoal yeast extract agar supplemented with *Legionella* buffered charcoal yeast extract [BCYE] growth supplement and *Legionella* MWY selective supplement [Oxoid]) revealed faint white-gray colonies after 48 h of incubation at 37°C in 5% CO_2. After a further 48-h incubation, colonies turned slightly red or pink. Interestingly, for unknown reasons, colonies grown on BCYE agar could then be subcultivated on standard media. Serological examinations of the colony material with *Legionella pneumophila* specific antisera against serogroups 1, 4, and 6 (Denka Seiken Ltd., Tokyo, Japan) as well as antisera against *Legionella micdadei* and *Legionella dumoffii* (Denka Seiken Ltd.) were negative. The isolated bacteria were finally identified by sequence determination of the 16S rRNA gene. For this purpose, a universal PCR using primers that hybridize to the 16S rRNA of most eubacteria was performed (10). The PCR fragments generated with primers 16Spro27f (positions 8 to 27 of the 16S rRNA gene, numbering referring to *Escherichia coli* (10) and 16Spro342r (positions 342 to 362) (9) were purified and directly sequenced with primer 16Spro342r (10). Sequence analysis revealed a 98% identity (229 out of 233 nucleotides were identical) to the 16S ribo-

TABLE 1. Antibiotic therapy of the patient

Antibiotic	Therapy[a]		
	Prophylaxis	1st Intervention (beginning of fever)	2nd Intervention (after 3 days of fever)
Ciprofloxacin	x	x	x
Co-trimoxazole	x	x	x
Ceftazidime		x	
Teicoplanin		x	
Imipenem			x
Amikacin			x
Vancomycin			x

[a] An x indicates the administration of the antibiotic at the indicated stage of therapy.

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TABLE 2. Biochemical characteristics of *M. zatmanii* isolated in this study and comparison to those of *Methylobacterium* spp. and *Roseomonas* spp.

Test	Reaction type or result[a]		
	Isolated strain	*Methylobacterium* spp.[b]	*Roseomonas* spp.[b]
Oxidase	+	+	+
Oxidation of methanol	+	+	−
Growth on MacConkey agar	−	±	+
Growth at 42°C	(+)	−	+
Urease	+	+	+
UV absorption of colonies	+	+	−
Gram stain	Gram-negative vacuolated short rods	Gram-negative vacuolated short rods	Gram-negative plump coccoid rods

[a] +, positive reaction within 48 h; −, negative reaction; (+), late reaction (3 to 7 days).
[b] Data taken from references 12 and 14.

somal gene of *M. zatmanii* (7), a bacterium which was first described in 1988 (6).

This result was confirmed by using conventional biochemical methods. The isolated gram-negative vacuolated rods showed pink-pigmented colonies, grew slowly at room temperature, at 30°C, and at 37°C, and showed weak growth at 42°C. The strain was able to oxidize methanol (growth on oxidative-fermentative basal medium [Merck, Darmstadt, Germany]) supplemented with 1% methanol under aerobic conditions), was oxidative, and gave positive catalase, oxidase, and urease reactions (API 20 NE, bioMeriéux, Nürtingen, Germany). Oxidation of methanol is characteristic of members of the genus *Methylobacterium* and allowed differentiation from bacteria belonging to the closely related genus *Roseomonas* (7, 12–14). Further criteria to distinguish the strain isolated in this study from the genus *Roseomonas* were Gram-stain morphology and failure to grow on MacConkey agar (12–14). The biochemical characteristics of diagnostic importance of the isolated strain are summarized in Table 2.

Susceptibility testing by microdilution of the isolated *M. zatmanii* strain was performed with the Sceptor System (Becton Dickinson) at 30°C with a 48-h incubation. The results are shown in Table 3.

In spite of sonographic and radiologic examinations, the focus of this nosocomial bacteremia was not found. The implanted central line remained during the infection episode and was removed 1 month later without further investigations. Microbiological investigations of the hospital environment were not performed, hence the source of the infection is unknown.

Discussion. To our knowledge, this is the first description of *M. zatmanii* isolated from patient material. Members of the genus *Methylobacterium* (nine species identified hitherto) are slow-growing, rod-shaped gram-negative bacteria that form pink colonies (7, 14). They are ubiquitous and are commonly isolated from water and soil (5). Bacteria isolated from man-made water distribution systems were found to be highly resistant to chlorine (7). Some cases of human infections have been reported to date (1, 3, 8, 9, 11) and in all cases, an underlying immunosuppression, e.g., leukemia, tuberculosis, AIDS, or alcohol abuse, existed. The bacteria were isolated from various patient specimens, such as blood, cerebrospinal fluid, sputum, bronchoscopic material, and synovial membrane. Since few cases of infection with *Methylobacterium* species have been described, this may reflect the difficulties in cultivation and identification of the bacteria (13). In general, methylobacteria are opportunistic pathogens of low virulence. They cause mild clinical symptoms, such as fever, which can effectively be treated by appropriate antibiotic therapy. Resistance profiles of a panel of *Methylobacterium* strains have been described previously in a study by Brown et al. (2). In accordance with those results, the *M. zatmanii* strain isolated here was susceptible to amikacin and imipenem but resistant to ciprofloxacin, co-trimoxazole, and ceftazidime. This correlated to the clinical outcome.

TABLE 3. In vitro susceptibilities of the isolated *M. zatmanii* strain as determined by microdilution procedures at 30°C for 48 h

Antimicrobial agent	Result
Ciprofloxacin	Resistant
Co-trimoxazole	Resistant
Ceftazidime	Resistant
Imipenem	Susceptible
Amikacin	Susceptible
Piperacillin	Susceptible
Ceftriaxone	Susceptible

Since methylobacteria have been isolated from tap water in hospital units, and *Methylobacterium mesophilicum* have been isolated from patients who had undergone bronchoscopy (4), it may be useful to survey water distribution systems in hospital units for immunocompromised patients for the occurrence of methylobacteria. In summary, clinicians as well as microbiologists should be aware of *Methylobacterium* species as potentially infectious agents. We would like to emphasize that subcultivation of the *M. zatmanii* strain isolated in this study was successful only after incubation on BCYE agar. Therefore, inclusion of BCYE agar for subcultivation of bacterial organisms from positive indicated blood cultures with gram-negative rods by direct Gram stain appears to be a valuable diagnostic tool. Moreover, universal PCR from the pure culture and subsequent sequence determination of the 16S rRNA gene was found to be a practical approach for the identification of uncommon or fastidious organisms.

REFERENCES

1. **Brown, M. A., J. N. Greene, R. L. Sandin, J. W. Hiemenz, and J. T. Sinnott.** 1996. *Methylobacterium* bacteremia after infusion of contaminated autologous bone marrow. Clin. Infect. Dis. **23:**1191–1192.
2. **Brown, W. J., R. L. Sautter, and A. E. Crist, Jr.** 1992. Susceptibility testing of clinical isolates of *Methylobacterium* species. Antimicrob. Agents Chemother. **36:**1635–1638.
3. **Fernandez, M., Z. Dreyer, M. Hockenberry-Eaton, and C. J. Baker.** 1997. *Methylobacterium mesophilica* as a cause of persistent bacteremia in a child with lymphoma. Pediatr. Infect. Dis. J. **16:**1007–1008.
4. **Flournoy, D. J., R. L. Petrone, and D. W. Voth.** 1992. A pseudo-outbreak of *Methylobacterium mesophilica* isolated from patients undergoing bronchoscopy. Eur. J. Clin. Microbiol. Infect. Dis. **11:**240–243.
5. **Green, P. N.** 1992. The genus *Methylobacterium*, p. 2342–2349. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, Berlin, Germany.
6. **Green, P. N., I. J. Bousfield, and D. Hood.** 1988. Three new *Methylobacterium* species: *M. rhodesianum* sp. nov., *M. zatmanii* sp. nov., and *M. fujisawaense* sp. nov. Int. J. Syst. Bacteriol. **38:**124–127.
7. **Hiraishi, A., K. Furuhata, A. Matsumoto, K. Koike, M. Fukuyama, and K. Tabuchi.** 1995. Phenotypic and genetic diversity of chlorine-resistant *Methy-*

lobacterium strains isolated from various environments. Appl. Environ. Microbiol. **61:**2099–2107.
8. **Kaye, K. M., A. Macone, and P. H. Kazanjian.** 1992. Catheter infection caused by *Methylobacterium* in immunocompromised hosts: report of three cases and review of the literature. Clin. Infect. Dis. **14:**1010–1014.
9. **Korvick, J. A., J. D. Rihs, G. L. Gilardi, and V. L. Yu.** 1989. A pink-pigmented, oxidative, nonmotile bacterium as a cause of opportunistic infections. Arch. Intern. Med. **149:**1449–1451.
10. **Lane, D. J.** 1991. 16S/23S rRNA sequencing, p. 115–175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. Wiley & Sons, Chichester, United Kingdom.
11. **Liu, J. W., J. J. Wu, H. M. Chen, A. H. Huang, W. C. Ko, and Y. C. Chuang.** 1997. *Methylobacterium mesophilicum* synovitis in an alcoholic. Clin. Infect. Dis. **24:**1008–1009.
12. **von Graevenitz, A.** 1995. *Acinetobacter*, *Alcaligenes*, *Moraxella*, and other nonfermentative gram-negative bacteria, p. 520–532. *In* P. R. Murray, et al. (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
13. **Wallace, P. L., D. G. Hollis, R. E. Weaver, and C. W. Moss.** 1990. Biochemical and chemical characterization of pink-pigmented oxidative bacteria. J. Clin. Microbiol. **28:**689–693.
14. **Weyant, R. S., C. W. Moss, R. E. Weaver, D. G. Hollis, J. G. Jordan, E. C. Cook, and M. I. Daneshvar.** 1997. Identification of unusual pathogenic gram-negative aerobic and facultatively anaerobic bacteria, 2nd ed. Williams & Wilkins, Baltimore, Md.