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Bacteremia Due to *Clostridium hathewayi* in a Patient with Acute Appendicitis

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***Clostridium hathewayi* is a newly described *Clostridium* species isolated from the feces of healthy human individuals, but its clinical significance is not known. We describe a case of human infection associated with *C. hathewayi*. The bacterium (strain HKU18) was isolated from the blood culture of a 39-year-old patient with acute gangrenous appendicitis complicated by septic shock. The cells were strictly anaerobic, nonmotile rods that stained gram negative. Conventional phenotypic tests and commercial identification systems failed to identify HKU18 to the species level. 16S rRNA gene analysis showed 1.4% nucleotide difference between the sequence of HKU18 and that of *C. hathewayi*, indicating that HKU18 was a strain of *C. hathewayi*. The patient responded to appendectomy and antibiotic treatment. 16S rRNA gene sequencing would be useful in further characterizing the clinical disease spectrum of *C. hathewayi*.**

CASE REPORT

A 39-year-old Indonesian woman was admitted to the hospital because of fever, chills, and rigor for 1 day. She also complained of lower abdominal pain which radiated to the right loin. There were no urinary or bowel symptoms. Her past medical history was unremarkable. On admission, her oral temperature was 39°C. The physical examination revealed tenderness, guarding, and rebound tenderness over the right loin and suprapubic areas. Bowel sounds were active. Her total leukocyte count was 10.2×10^9/liter (neutrophils, 9.5×10^9/liter; and lymphocytes, 0.6×10^9/liter), a hemoglobin level of 13.7 g/dl, and a platelet count of 272×10^9/liter. Her renal and liver function tests were within normal limits. An abdominal radiograph was unremarkable. Blood culture was performed. She went into septic shock with metabolic acidosis soon after admission. Fluid resuscitation was administered, and empirical intravenous cefuroxime and metronidazole were commenced. Contrast-computed tomography of the abdomen showed a swollen appendix with fecalith and free fluid in the pouch of Douglas. An emergency appendectomy was performed. Intraoperatively, an acutely inflamed and gangrenous appendix pending perforation was found. Culture of the peritoneal swab grew only scanty *Escherichia coli*. The patient recovered uneventfully after the operation and was discharged after 4 days of hospitalization.

Clostridium is a heterogeneous genus that consists of over 150 species. Besides *Clostridium perfringens*, *C. difficile*, *C. tetani*, and *C. botulinum*, of which the epidemiology and clinical disease spectra are better defined, studies of the pathogenic potential and disease association of the other *Clostridium* species have been hampered by difficulties in accurately identifying these bacteria. Since the recognition of the 16S rRNA gene as a new standard for classification and identification of bacteria (12, 13), most *Clostridium* species have been subjected to 16S rRNA gene sequence analysis, with revisions being made in their classifications and with new species being identified (3, 4, 14, 15, 17). Recently, the use of this technique for identifying and defining the clinical significance of anaerobic gram-positive bacilli, including the identification of a strain of *Lactobacillus salivarius* isolated from a patient with cholecystitis (19) and a strain of *Actinomyces odontolyticus* from a patient with pelvic inflammatory disease (20) and the discovery of a novel *Actinomyces* species (21) and two novel *Eggerthella* species (7), has been reported.

Clostridium hathewayi is a newly discovered *Clostridium* species isolated from stool samples of healthy human subjects. Although the phenotypic characteristics of the two strains isolated were consistent with the genus *Clostridium*, their species identification was determined only after 16S rRNA gene sequencing, which showed that they represent a new species within the *Clostridium coccoides* rRNA complex (16). Since this first report, there has been no additional information on the bacterium in the literature and the clinical significance of the bacterium has been unknown. In this article, we describe a case of *C. hathewayi* bacteremia in a patient with acute appendicitis complicated by septic shock.

Clinical and microbiological data. All clinical data were collected prospectively as described previously (8). The isolates were identified by standard conventional biochemical methods (10), the VITEK system (ANI) (bioMérieux Vitek, Hazelwood, Mo.), the API system (20A) (bioMérieux Vitek), and the ATB Expression system (rapid ID32A) (bioMérieux Vitek). Antimicrobial susceptibility was tested by E-test (AB Biodisk, Solna, Sweden) on brucella blood agar plates, and the results were interpreted according to the NCCLS criteria for anaerobic bacteria (11). All tests were performed in triplicate with freshly prepared media on separate occasions.

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On day 2 postincubation, the anaerobic blood culture bottle turned positive, with a straight or slightly curved bacillus which stained gram negative (strain HKU18). It grows on sheep blood agar as nonhemolytic, gray colonies of 1 mm in diameter with a slightly irregular margin after 48 h of incubation at 37°C in an anaerobic environment. It does not grow in ambient air, ambient air supplemented with 5% CO_2, or microaerophilic conditions. Cells form ovoid, terminal, or subterminal endospores. It is nonmotile. The biochemical profile of HKU18 is summarized in Table 1. The Vitek system (ANI) showed that it was "unidentified." The API system (20A) showed that it was 46% *Bifidobacterium* spp., 36% *Actinomyces israelii*, and 12% *Clostridium clostridioforme*, while the ATB Expression system (ID32A) showed that it was 99.9% *C. clostridioforme*. The isolate was sensitive to vancomycin but resistant to colistin. The MICs of penicillin, cefotaxime, vancomycin, and metronidazole were 0.5, >32, 0.5, and 0.23 μg/ml, respectively.

Scanning electron microscopy. Scanning electron microscopy was performed according to previously published protocols (22, 23). Bacterial cells of HKU18 were bacilli which multiplied by longitudinal division.

16S rRNA gene sequencing, G+C content determination, and phylogenetic characterization. PCR amplification and DNA sequencing of the 16S rRNA gene of the isolate were performed according to protocols in previous publications (19, 20). LPW57 (5′-AGTTTGATCCTGGCTCAG-3′) and LPW205 (5′-CTTGTTACGACTTCACCC-3′) (Gibco BRL, Rockville, Md.) were used as the PCR primers, and LPW57, LPW205, LPW284 (5′-GTTTACAACCCGAAGGCC-3′), and LPW306 (5′-TGAGATGTTGGGTTAAGT-3′) were used as the sequencing primers. The sequences of the PCR products were compared with known 16S rRNA gene sequences in GenBank (http://www.ncbi.nlm.nih.gov) by multiple sequence alignment by using the Clustal W program (18), and phylogenetic analysis was done by using Clustal X version 1.81 (6). A total of 1,333 nucleotide positions were included in the analysis. Preparation of genomic DNA for determination of G+C content was performed according to previous published protocols (1, 23), the G+C content was determined by thermal denaturation, and DNA was calculated by the formula $(G+C)\% = 2.44T_m - 169$ (5). PCR of the 16S rRNA gene of HKU18 showed a band at about 1,400 bp. The 16S rRNA gene sequence of HKU18 had 1.4% nucleotide difference from that of *C. hathewayi* (GenBank accession no. AJ311620), 4.8% nucleotide difference from that of *Clostridium indolis* (GenBank accession no. Y18184), 4.9% nucleotide difference from that of *Clostridium aerotolerans* (GenBank accession no. X76163), 5.1% difference from that of *Clostridium celerecrescens* (GenBank accession no. X71848), and 5.1% difference from that of *C. methoxybenzovorans* (GenBank accession no. AF067965), indicating that HKU18 was a strain of *C. hathewayi*. The G+C content of HKU18 was 55.4 ± 1.2 mol%.

The phenotypic characteristics of HKU18 closely resembled those of the two previously described isolates of *C. hathewayi* (Table 1). All three isolates were strictly anaerobic gram-positive rods but stained gram negative. HKU18 and one of the strains previously described were nonmotile, while the other previous strain was motile. However, the two previous isolates fermented melezitose while HKU18 did not. The identification of more strains of *C. hathewayi* would be helpful in delineating its key phenotypic profile to be differentiated from closely related species.

TABLE 1. Phenotypic characteristics of *C. hathewayi* and the blood culture isolate

Phenotypic characteristic	*Clostridium hathewayi* (16)	Blood culture isolate
Gram stain appearance	Gram-negative rods	Gram-negative rods
Spores	Subterminal	Terminal or subterminal
Motility	Variable	−
Catalase	−	−
Esculin hydrolysis	+	+
Gelatin hydrolysis	−	−
Arginine dehydrogenase		−
Alkaline phosphatase		−
Glutamic acid decarboxylase		−
Indole production	−	−
Phosphate choline		−
Urease	−	−
Reduction of nitrate	−	−
Reduction of triphenyl tetrazolium		−
Oxidation/fermentation of:		
Amygdalin	+	
Arabinose	+	+
Cellobiose	+	+
Fructose	+	
Galactose	+	
Glucose	+	+
Glycerol		−
Lactose	+	+
Maltose	+	+
Mannitol		−
Mannose	+	+
Mellbiose	+	
Melezitose	+	−
Raffinose	+	+
Rhamnose	+	+
Ribose	+	
Salicin	+	+
Sorbitol	+	+
Sucrose	+	+
Trehalose	+	+
Xylose	+	+
α-Arabinosidase		+
α-Fucosidase		−
β-Fucosidase		+
α-Galactosidase		+
β-Galactosidase		+
β-Galactosidase-6-phosphate		−
α-Glucosidase		+
β-Glucosidase		+
β-Glucuronidase		+
α-Mannosidase		+
β-Lactosidase		+
β-Xylosidase		+
N-acetyl-glucosaminidase		+
Alanine arylamidase		−
Arginine arylamidase		−
Benzoyl-arginine arylamidase		−
Glutamyl glutamic acid arylamidase		−
Glycine arylamidase		−
Histidine arylamidase		−
Leucine arylamidase		−
Leucyl glycine arylamidase		+
Lysine arylamidase		+
Phenylalanine arylamidase		−
Proline arylamidase		−
Pyroglutamic acid arylamidase		+
Serine arylamidase		−
Tyrosine arylamidase		−

The clinical significance of *C. hathewayi* in the present patient is evident by its pure growth in the blood culture of an

immunocompetent patient before the administration of antibiotics, which was associated with the development of fever, neutrophilia, and septic shock. The source of the bacteremia is most likely the inflamed appendix. Since *C. hathewayi* has been isolated from the stool of healthy individuals, it is likely a normal gut commensal in humans. We speculate that the bacterium may have been in the gut flora of our patient and may have translocated through the inflamed intestinal mucosa to the bloodstream. Although other anaerobic bacteria, such as the *Bacteroides fragilis* group and *C. perfringens*, have been associated with or implicated in the pathogenesis of acute appendicitis (2, 9), the role of *C. hathewayi* in the development of the acute appendicitis in the present patient cannot be determined. The failure to isolate the bacterium from her peritoneal swab could be due to its stringent transport and growth requirements and the overgrowth of the less fastidious *E. coli*. Further studies are required to investigate the pathogenic potential of *C. hathewayi* in humans.

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REFERENCES

1. **Ausubel, F. M., R. Brent, and R. E. Kingston (ed.).** 1998. Current protocols in molecular biology, p. 2.4.1–2.4.2. John Wiley & Sons, Inc., New York, N.Y.
2. **Bennion, R. S., E. J. Baron, J. E. Thompson, J. Downes, P. Summanen, D. A. Talan, and S. M. Finegold.** 1990. The bacteriology of gangrenous and perforated appendicitis—revisited. Ann. Surg. **211:**165–171.
3. **Breitenstein, A., J. Wiegel, C. Haertig, N. Weiss, J. R. Andreesen, and U. Lechner.** 2002. Reclassification of *Clostridium hydroxybenzoicum* as *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., and description of *Sedimentibacter saalensis* sp. nov. Int. J. Syst. Evol. Microbiol. **52:**801–807.
4. **Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. Farrow.** 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol. **44:**812–826.
5. **Goodfellow, M.** 1985. Chemical methods in bacterial systematics, p. 67–93. Academic Press, London, United Kingdom.
6. **Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins, and T. J. Gibson.** 1998. Multiple sequence alignment with ClustalX. Trends Biochem. Sci. **10:**403–405.
7. **Lau, S. K. P., P. C. Y. Woo, G. K. S. Woo, A. M. Y. Fung, K. M. Wong, K. M. Chan, S. F. Tang, and K. Y. Yuen.** 2004. *Eggerthella hongkongensis* sp. nov. and *Eggerthella sinensis* sp. nov., two novel *Eggerthella* species, account for half of the cases of *Eggerthella* bacteremia. Diagn. Microbiol. Infect. Dis. **49:**255–263.
8. **Luk, W. K., S. S. Wong, K. Y. Yuen, P. L. Ho, P. C. Y. Woo, R. A. Lee, and P. Y. Chau.** 1998. Inpatient emergencies encountered by an infectious disease consultative service. Clin. Infect. Dis. **26:**695–701.
9. **Martirosian, G., M. Bulanda, B. Wojcik-Stojek, P. Obuch-Woszczatynski, G. Rouyan, P. Heczko, and F. Meisel-Mikolajczyk.** 2001. Acute appendicitis: the role of enterotoxigenic strains of *Bacteroides fragilis* and *Clostridium difficile*. Med. Sci. Monit. **7:**382–386.
10. **Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.).** 2003. Manual of clinical microbiology, 8th ed. ASM Press, Washington, D.C.
11. **National Committee for Clinical Laboratory Standards.** 2003. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 5th ed. Approved standard M11-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **Relman, D. A., J. S. Loutit, T. M. Schmidt, S. Falkow, and L. S. Tompkins.** 1990. The agent of bacillary angiomatosis. An approach to the identification of uncultured pathogens. N. Engl. J. Med. **323:**1573–1580.
13. **Relman, D. A., T. M. Schmidt, R. P. MacDermott, and S. Falkow.** 1992. Identification of the uncultured bacillus of Whipple's disease. N. Engl. J. Med. **327:**293–301.
14. **Spring, S., B. Merkhoffer, N. Weiss, R. M. Kroppenstedt, H. Hippe, and E. Stackebrandt.** 2003. Characterization of novel psychrophilic clostridia from an Antarctic microbial mat: description of *Clostridium frigoris* sp. nov., *Clostridium lacusfryxellense* sp. nov., *Clostridium bowmanii* sp. nov. and *Clostridium psychrophilum* sp. nov. and reclassification of *Clostridium laramiense* as *Clostridium estertheticum* subsp. *laramiense* subsp. nov. Int. J. Syst. Evol. Microbiol. **53:**1019–1029.
15. **Stackebrandt, E., I. Kramer, J. Swiderski, and H. Hippe.** 1999. Phylogenetic basis for a taxonomic dissection of the genus *Clostridium*. FEMS Immunol. Med. Microbiol. **24:**253–258.
16. **Steer, T., M. D. Collins, G. R. Gibson, H. Hippe, and P. A. Lawson.** 2001. *Clostridium hathewayi* sp. nov., from human faeces. Syst. Appl. Microbiol. **24:**353–357.
17. **Strompl, C., B. J. Tindall, H. Lunsdorf, T. Y. Wong, E. R. Moore, and H. Hippe.** 2000. Reclassification of *Clostridium quercicolum* as *Dendrosporobacter quercicolus* gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol. **50:**101–106.
18. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
19. **Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, and K. Y. Yuen.** 2002. Identification by 16S ribosomal RNA gene sequencing of *Lactobacillus salivarius* bacteremic cholecystitis. J. Clin. Microbiol. **40:**265–267.
20. **Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, G. Y. Wong, and K. Y. Yuen.** 2002. Diagnosis of pelvic actinomycosis by 16S ribosomal RNA gene sequencing and its clinical significance. Diagn. Microbiol. Infect. Dis. **43:**113–118.
21. **Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, J. L. L. Teng, B. H. L. Wong, M. K. M. Wong, E. Hon, G. W. K. Tang, and K. Y. Yuen.** 2003. *Actinomyces hongkongensis* sp. nov. A novel *Actinomyces* species isolated from a patient with pelvic actinomycosis. Syst. Appl. Microbiol. **26:**518–522.
22. **Woo, P. C. Y., D. M. W. Tam, K. W. Leung, S. K. P. Lau, J. L. L. Teng, M. K. M. Wong, and K. Y. Yuen.** 2002. *Streptococcus sinensis* sp. nov., a novel *Streptococcus* species isolated from a patient with infective endocarditis. J. Clin. Microbiol. **40:**805–810.
23. **Yuen, K. Y., P. C. Y. Woo, J. L. L. Teng, K. W. Leung, M. K. M. Wong, and S. K. P. Lau.** 2001. *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. J. Clin. Microbiol. **39:**4227–4232.