

## *Enterococcus cecorum* Empyema Thoracis Successfully Treated with Cefotaxime

Patrick C. Y. Woo, Dorothy M. W. Tam, Susanna K. P. Lau, Ami M. Y. Fung, and Kwok-Yung Yuen\*

Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong

Received 15 August 2003/Returned for modification 10 October 2003/Accepted 24 October 2003

**We report the first case of *Enterococcus cecorum* empyema thoracis and spontaneous bacterial peritonitis in a 44-year-old man with underlying cirrhosis. The patient responded to cefotaxime (MIC, 0.25 µg/ml) treatment and drainage of the empyema. Susceptibility of *E. cecorum* to expanded-spectrum cephalosporins could be due to its production of types of penicillin-binding proteins similar to those produced by *Streptococcus* species rather than to those produced by *Enterococcus* species (as predicted by phylogenetic analysis of the 16S rRNA gene sequences).**

### CASE REPORT

A 44-year-old Chinese man was admitted to a hospital because of fever, shortness of breath, and generalized abdominal pain for 2 days. He had Wilson's disease complicated by cirrhosis, esophageal varices, portal hypertension, recurrent ascites, recurrent spontaneous bacterial peritonitis, right indirect inguinal hernia, and hemorrhoids. Examination showed an oral temperature of 37.7°C, right pleural effusion, and generalized abdominal tenderness and guarding but minimal ascites. Chest radiograph showed right pleural effusion. The hemoglobin level was 10.6 g/dl, the total white cell count was  $13.7 \times 10^9$ /liter, and the platelet count was  $73 \times 10^9$ /liter. The serum bilirubin level was 76 µmol/liter, the albumin level was 24 g/liter, the alkaline phosphatase level was 249 U/liter, and the alanine aminotransferase level was 40 U/liter. The serum urea and creatinine levels were 15.8 mmol/liter and 111 µmol/liter, respectively. The prothrombin time and activated partial thromboplastin time were 16.6 s (normal range, 11.3 to 13.2 s) and 55.8 s (normal range, 27.6 to 37.6 s), respectively. Blood culture and percutaneous thoracocentesis procedures were performed, and empirical intravenous cefotaxime was administered. Abdominal paracentesis was not performed because of minimal ascites. Examination of the turbid pleural fluid revealed a white cell count of  $765 \times 10^6$ /liter with 82% neutrophils and 18% lymphocytes in the presence of cell clumps, a protein level of 5 g/liter, a lactate dehydrogenase level of 209 U/liter, and a pH of 8.0. Gram smearing of the pleural fluid revealed the presence of numerous polymorphs. Aerobic and anaerobic cultures of the pleural fluid yielded pure growth of a gram-positive coccus. Blood culture results were negative. The

patient responded to cefotaxime treatment and adequate drainage of the empyema and was discharged after 10 days of hospitalization.

*Enterococcus cecorum*, one of the 30 species of the genus *Enterococcus*, was first described in 1983 as *Streptococcus cecorum* (3). Since then, it has been identified as part of the intestinal floras of various animals, including pigs, cattle, chickens, ducks, cats, dogs, and canaries (4). In contrast to the common recovery of *E. cecorum* from animals, human infections associated with *E. cecorum* have been rarely reported, with only three cases described in the English literature (MEDLINE search [1996 to 2003]) (2, 6, 7). Phenotypically, *E. cecorum* is often described as more *Streptococcus*-like than other *Enterococcus* species in that it prefers incubation in atmospheric conditions with CO<sub>2</sub> enrichment, fails to grow on *Enterococcus*-selective medium and in 6.5% NaCl, and grows poorly on bile-esculin agar (10). However, speciation of *E. cecorum* is difficult, especially when commercial kits are used. In the three cases of human infections, the three isolates were identified by whole-cell protein analysis, cellular fatty acid analysis, 16S rRNA gene sequencing, and/or tRNA gene PCR and capillary electrophoresis in addition to the use of conventional phenotypic tests. In this article, we describe the first case of empyema thoracis associated with *E. cecorum*. The epidemiology of *E. cecorum* infections, as well as its susceptibility to cefotaxime in relation to its phylogenetic position as deduced by 16S rRNA gene sequence analysis, are discussed.

**Clinical and microbiological data.** All clinical data were collected prospectively as described in a previous publication by Woo et al. (13). The bacterium was identified by standard conventional biochemical methods (9). Lancefield serogrouping was performed using Streptex (Murex Biotech Ltd., Dartford, United Kingdom). All tests were performed in triplicate with freshly prepared medium on separate occasions. In addition, a Vitek System (GPI) (bioMérieux Vitek, Hazelwood, Mo.), an API system (20 STREP) (bioMérieux Vitek), and an ATB expression system (ID32 STREP) were used for the iden-

\* Corresponding author. Mailing address: Department of Microbiology, The University of Hong Kong, Rm. 423, University Pathology Building, Queen Mary Hospital, Hong Kong. Phone: (852) 2855 4892. Fax: (852) 2855 1241. E-mail: hkumicro@hkucc.hku.hk.

tification of the bacterial isolate. *Enterococcus faecalis* (ATCC 29212) was used as a control. The pleural fluid isolate was a gram-positive, non-spore-forming coccus arranged in chains. It grew on sheep blood agar as nonhemolytic, gray colonies 1 mm in diameter after 24 h of incubation at 37°C in 5% CO<sub>2</sub> and pinpoint colonies after 24 h of incubation at 37°C in ambient air. It also grew well on chocolate and MacConkey agars and in an anaerobic environment but slowly in 40% bile, on bile esculin agar, or in 6.5% NaCl. It was catalase negative and resistant to optochin and bacitracin. It is nongroupable with Lancefield group A, B, C, D, F, or G antisera. It is nonmotile at both 25 and 37°C. The biochemical profile of the isolate is shown in Table 1. Positive results were obtained for a Voges-Proskauer test, alkaline phosphatase, β-glucuronidase, and α-galactosidase and for utilization of trehalose, raffinose, maltose, ribose, cyclodextrin, methyl-β-D-glucopyranoside, melibiose, melezitose, lactose, salicin, sucrose, inulin, cellobiose, D-mannose, starch, and tagatose. It did not produce pyrrolidonylarylamidase. Investigations using a Vitek system (GPI), an API system (20 STREP), and an ATB Expression system (ID32 STREP) yielded no identification.

**Antibiotic susceptibility.** Antimicrobial susceptibility was investigated by E-testing, and the results were interpreted according to National Committee for Clinical Laboratory Standards criteria, with *Staphylococcus aureus* (ATCC 29213) and *Streptococcus pneumoniae* (ATCC 49619) as controls. The MICs of penicillin, cefotaxime, and vancomycin were 0.094, 0.25, and 1.5 μg/ml, respectively.

**16S rRNA gene sequencing and phylogenetic characterization.** PCR amplification and DNA sequencing of the 16S rRNA gene of the isolate were performed as previously described (15, 16). LPW55 (5'-AGTTTGATCCTGGCTCAG-3') and LPW205 (5'-CTTGTTACGACTTCACCC-3') (Gibco BRL, Rockville, Md.) were used as the PCR and sequencing primers. *E. faecalis* (ATCC 29212) was used as a control. The sequences of the PCR products were compared with known GenBank 16S rRNA gene sequences by multiple sequence alignment using the CLUSTAL W program (11). Phylogenetic relationships of the isolate recovered from the patient with other *Enterococcus* species, other representative *Streptococcus* species (*S. pyogenes*, *S. mitis*, *S. agalactiae*, *S. intermedius*, *S. mutans*, and *S. bovis*), *Granulicatella adiacens*, and *Abiotrophia defectiva* were determined using PileUp and the neighbor-joining method with GrowTree (Genetics Computer Group, Inc.). A total of 1,235 nucleotide positions of the 16S rRNA genes were included in the analysis. PCR of the 16S rRNA gene of the pleural fluid isolate showed a band at about 1,460 bp. There were two (0.1%) base differences between the 16S rRNA gene sequence of the isolate and that of *E. cecorum* (GenBank accession no. AF061009), 33 (2.3%) base differences between the 16S rRNA gene sequence of the isolate and that of *E. columbae* (GenBank accession no. Y18275), and 55 (3.8%) base differences between the 16S rRNA gene sequence of the isolate and that of *E. gallinarum* (GenBank accession no. AF277567), indicating that the isolate was a strain of *E. cecorum* (Fig. 1).

We report the first case of empyema thoracis caused by *E. cecorum*. The patient probably had concomitant spontaneous bacterial peritonitis, as he had abdominal pain, generalized abdominal tenderness, and guarding, although abdominal

TABLE 1. Biochemical profile of the pleural fluid isolate by conventional biochemical tests and Vitek GPI, API 20 STREP, and ATB ID32 STREP systems

Biochemical reaction or enzyme or test	Results by indicated test <sup>a</sup>			
	Conventional	Vitek GPI	API 20 STREP	ATB ID32 STREP
Catalase	—	—		
Resistance to:				
Bacitracin	+	+		
Optochin	+	+		
Growth in:				
6% NaCl	+	+		
10% bile	+	+		
40% bile	+	+		
Esculin hydrolysis	+	+	+	
Hippurate hydrolysis			—	—
Arginine hydrolysis		—	—	—
Lysine decarboxylase	—			
Omithine decarboxylase	—			
Urease	—	—		—
Voges-Proskauer test	+		+	+
Tetrazolium reduction				
Novobiocin	—	—		
Polymyxin B	+	+		
Utilization of:				
Hemicellulase		+		
Dextrose		+		
Lactose	+	+	+	+
Mannitol	—	—	—	—
Raffinose	+	+	+	+
Salicin	+	+		
Sorbitol	—	—	—	—
Sucrose	+	+		+
Trehalose	+	+	+	+
Arabinose	—	—	—	—
Pyruvate		—		—
Pullulan		—		—
Inulin	+	+	+	
Melibiose	+	+		+
Melezitose		+		+
Cellobiose	+	+		
Ribose	+	+	+	+
Xylose	—	—		
D-glucose	—			
D-mannose	+			
Maltose	+			
Starch				
Glycogen			—	—
Arabitol				—
Methyl-β-D-glucopyranoside				+
Tagatose				+
Cyclodextrin				+
Pyrrolidonylarylamidase	—		—	—
α-Galactosidase			+	+
β-Glucuronidase			+	+
β-Galactosidase			+	+
Leucine arylamidase			+	
β-Glucosidase				+
Alanine-phenylalanine-proline arylamidase				—
Acetyl-β-glucosaminidase				+
Glycyl-tryptophane arylamidase				+
β-Mannosidase				+
Alkaline phosphatase			+	+

<sup>a</sup> +, positive; —, negative.

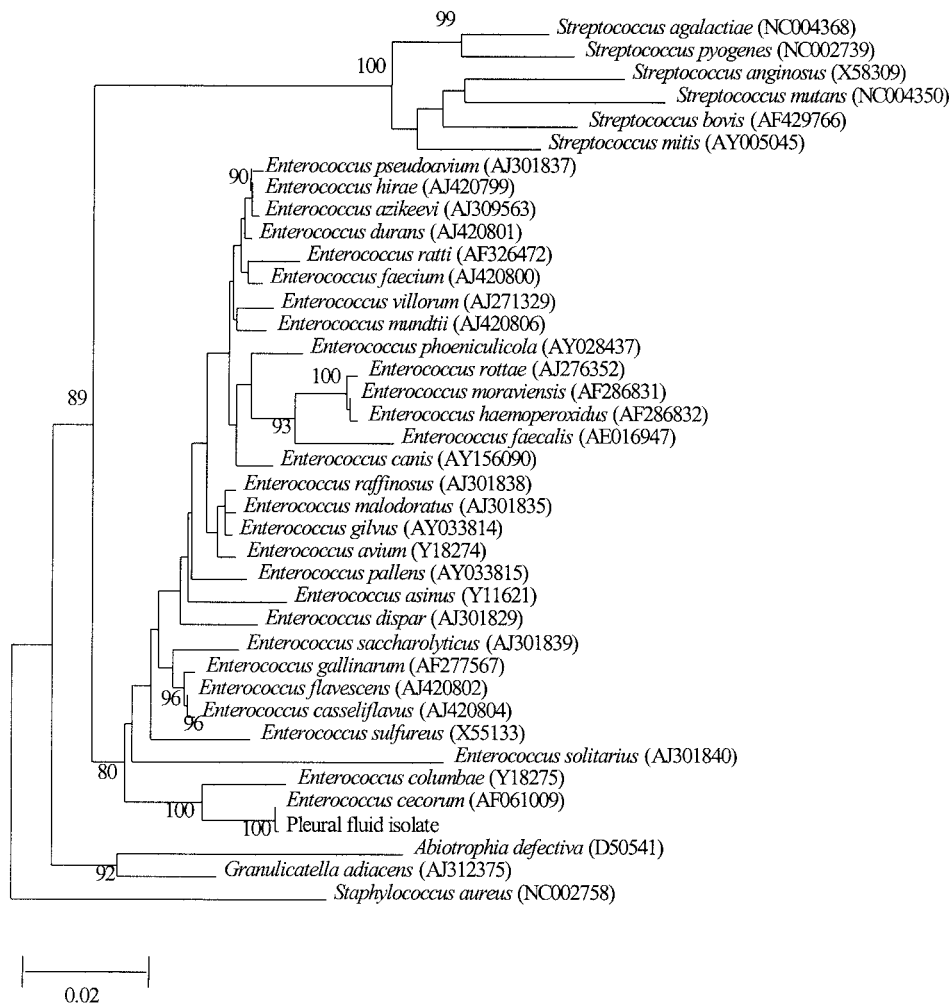


FIG. 1. Phylogenetic tree showing the relationships of the pleural fluid isolate species to other *Enterococcus* species, other representative *Streptococcus* species (*S. pyogenes*, *S. mitis*, *S. agalactiae*, *S. intermedius*, *S. mutans*, and *S. bovis*), *G. adiacens*, and *A. defectiva*. The tree was inferred from 16S rRNA data by the neighbor-joining method and rooted using the 16S rRNA gene sequence of *S. aureus*. Bootstrap values were calculated from 1,000 trees. The scale bar indicates the number of substitutions per 50 bases (estimated using the Jukes-Cantor correction). Names and accession numbers are given as cited in the GenBank database.

paracentesis was not performed because of minimal ascites. Including the present case, four cases of *E. cecorum* infections have been reported in the English literature (2, 6, 7). Three patients were males, and one was female. All four patients had underlying diseases. Three of them had cirrhosis of the liver, which seems to be a major underlying disease predisposing patients to *E. cecorum* infections. Two patients had bacteremia and two had peritonitis. Overall, one of the four patients died.

The portal of entry for *E. cecorum* in the present case, as well as in the cases of the other two patients with *E. cecorum* peritonitis, was probably the gastrointestinal tract. It is well known that decompensated liver cirrhosis with portal hypertension and ascites is associated with the presence of intestinal mucosal edema and local immunosuppression secondary to portal venous congestive vasculopathy. Therefore, the gastrointestinal tracts of patients with liver cirrhosis are often the primary site of infection or the portal of entry for extraintestinal infections. According to one recently published article on spontaneous bacterial empyema in cirrhotic patients, 13 of the

18 patients with positive cultures from pleural fluid had pathogens (*Escherichia coli* in eight patients, *Enterococcus* species in three, and *Klebsiella pneumoniae* in two) that likely originated from the gastrointestinal tract (17). None of these bacteria are conventional pathogens causing pneumonia, bone and joint infections, or skin and soft tissue infections. Interestingly, *Laribacter hongkongensis*, a recently described organism which has been recovered from fecal specimens of patients with gastroenteritis, was also originally discovered in the blood and empyema pus cultures of a patient with alcoholic liver cirrhosis (8, 14, 18).

Sensitivity of *E. cecorum* to expanded-spectrum cephalosporins could be due to its production of types of penicillin-binding proteins (PBPs) different from those produced by other *Enterococcus* species but similar to the PBPs produced by other *Streptococcus* species. Enterococci, as opposed to streptococci, are intrinsically resistant to the cephalosporins. This differential susceptibility to cephalosporins in these two closely related genera is related to the production of different types of PBPs,

of which PBP 5, the major PBP in *E. faecalis* and *E. faecium*, is demonstrated to have a low affinity to cephalosporins (1, 5, 12). However, in the present case and in another case reported in the literature (2), *E. cecorum* has been noted to be susceptible to cefotaxime and ceftriaxone. We speculate that this unexpected finding was due to the presence of types of PBPs in *E. cecorum* different from those found with *E. faecalis* and *E. faecium*. In fact, *E. cecorum* and *E. columbae*, with their 16S rRNA gene sequences making up the first branch in the phylogenetic tree among the *Enterococcus* species (Fig. 1), are probably the ancestors of the other *Enterococcus* species and are also more closely related to the *Streptococcus* species than the other *Enterococcus* species. This is in line with the more *Streptococcus*-like culture characteristics of *E. cecorum* (10). Therefore, it is not surprising that the PBPs of *E. cecorum*, which render *E. cecorum* susceptible to cefotaxime and ceftriaxone, might be closely related to those of the *Streptococcus* species.

**Nucleotide sequence accession number.** The 16S rRNA gene sequence of the isolate has been deposited in GenBank (accession no. AY365054).

#### ACKNOWLEDGMENTS

This work is partly supported by the University Development Fund, University Research Grant Council, and the Committee for Research and Conference Grant, The University of Hong Kong.

#### REFERENCES

- Chen, H. Y., and J. D. Williams. 1987. Penicillin-binding proteins in *Streptococcus faecalis* and *S. faecium*. *J. Med. Microbiol.* **23**:141–147.
- De Baere, T., G. Claeys, G. Verschraegen, L. A. Devriese, M. Baele, B. Van Vlem, R. Vanholder, C. Dequidt, and M. Vanechoutte. 2000. Continuous ambulatory peritoneal dialysis peritonitis due to *Enterococcus cecorum*. *J. Clin. Microbiol.* **38**:3511–3512.
- Devriese, L. A., G. N. Nutta, J. A. E. Farrow, A. van de Kerckhove, and B. A. Phillips. 1983. *Streptococcus cecorum*, a new species isolated from chickens. *Int. J. Syst. Bacteriol.* **33**:772–776.
- Devriese, L. A., K. Ceyssens, and F. Haesebrouck. 1991. Characteristics of *Enterococcus cecorum* strains from the intestines of different animal species. *Lett. Appl. Microbiol.* **12**:137–139.
- Fontana, R., P. Canepari, M. M. Lio, and G. Satta. 1990. Mechanisms of resistance of enterococci to beta-lactam antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* **9**:103–105.
- Greub, G., L. A. Devriese, B. Pot, J. Dominguez, and J. Bille. 1997. *Enterococcus cecorum* septicemia in a malnourished adult patient. *Eur. J. Clin. Microbiol. Infect. Dis.* **16**:594–598.
- Hsueh, P.-R., L.-J. Teng, Y.-C. Chen, P.-C. Yang, S.-W. Ho, and K.-T. Luh. 2000. Recurrent bacteremic peritonitis caused by *Enterococcus cecorum* in a patient with liver cirrhosis. *J. Clin. Microbiol.* **38**:2450–2452.
- Lau, S. K. P., P. C. Y. Woo, W.-T. Hui, M. W. S. Li, J. L. L. Teng, T.-L. Que, R. W. H. Yung, W.-K. Luk, R. W. M. Lai, and K.-Y. Yuen. 2003. Cefoperazone MacConkey agar for selective isolation of *Laribacter hongkongensis*. *J. Clin. Microbiol.* **41**:4839–4841.
- Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.). 1999. *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
- Reuter, G. 1992. Culture media for enterococci and group D-streptococci. *Int. J. Food. Microbiol.* **17**:101–111.
- 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.
- Williamson, R., S. B. Calderwood, R. C. Moellering, Jr., and A. Tomasz. 1983. Studies on the mechanism of intrinsic resistance to beta-lactam antibiotics in group D streptococci. *J. Gen. Microbiol.* **129**:813–822.
- Woo, P. C. Y., S. S. Y. Wong, P. N. L. Lum, W. T. Hui, and K. Y. Yuen. 2001. Cell-wall-deficient bacteria and culture-negative febrile episodes in bone-marrow-transplant recipients. *Lancet* **357**:675–679.
- Woo, P. C. Y., P. Kuhnert, A. P. Burnens, J. L. L. Teng, S. K. P. Lau, T. L. Que, H. H. Yau, and K. Y. Yuen. 2003. *Laribacter hongkongensis*: a potential cause of infectious diarrhea. *Diagn. Microbiol. Infect. Dis.* **47**:551–556.
- Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, S. S. Y. Wong, and K.-Y. Yuen. 2001. Group G beta-hemolytic streptococcal bacteremia characterized by 16S ribosomal RNA gene sequencing. *J. Clin. Microbiol.* **39**:3147–3155.
- 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.
- Xiol, X., J. M. Castellvi, J. Guardiola, E. Sese, J. Castellote, A. Perello, X. Cervantes, and M. J. Iborra. 1996. Spontaneous bacterial empyema in cirrhotic patients: a prospective study. *Hepatology* **23**:719–723.
- 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.