Case study

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# NOSOCOMIAL INFECTION DUE TO *ENTEROCOCCUS CECORUM* IDENTIFIED BY MALDI-TOF MS AND VITEK 2 FROM A BLOOD CULTURE OF A SEPTIC PATIENT

**Philipp Warnke[1,*], Thomas Köller[1], Paul Stoll[2] and Andreas Podbielski[1]**

[1]Institute of Medical Microbiology, Virology, and Hygiene, Rostock University Hospital, Rostock, Germany
[2]Department of Pneumology and Critical Care Medicine, Rostock University Hospital, Rostock, Germany

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We report the case of a nosocomial infection due to *Enterococcus cecorum* isolated from a blood culture of a 75-year-old septic male patient. Matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Vitek 2 succeeded in identification of the isolate.

**Keywords:** *Enterococcus cecorum,* nosocomial infection, MALDI-TOF, mass spectrometry, Vitek 2

## Case report

A 75-year-old patient was admitted to the inpatient department for psychiatric disorders for therapy of Parkinson's disease associated dementia. Further comorbidities were hypothyroidism and arterial hypertension. During his five-week stay in the hospital, he developed signs of severe infection and was subsequently transferred to the department of internal medicine. On examination, his central body temperature was 36.8 °C, with a heart rate of 99 beats/min and a blood pressure of 87/57 mmHg. Pathologically altered laboratory parameters are displayed in *Table 1*. Chest X-ray showed no abnormalities, in detail, no signs of pneumonia, lung congestion, or pleural effusion. Electrocardiogram displayed a sinus rhythm, a vertical type, and no signs of ischemia. Antibiotic treatment was initiated with piperacillin–tazobactam.

One pair of two paired blood cultures was positive within 24 h. Subcultivation on Columbia agar supplemented with 5% sheep blood (BD, Heidelberg, Germany) revealed growth of Gram-positive cocci. Identification by matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry (AXIMA Assurance, Shimadzu, Kyoto, Japan) yielded *Enterococcus cecorum*. The isolate was prepared using alpha-cyano-4-hydroxy cinnamic acid (bioMérieux, Marcy l'Etoile, France) as ma-

**Table 1.** Pathologic laboratory parameters

| Test | Value | Reference value |
|---|---|---|
| White blood cells ($10^9$/l) | 29.1 | 4–9 |
| Platelets ($10^9$/l) | 8 | 150–450 |
| C-reactive protein (mg/l) | 107 | <5 |
| Procalcitonin (ng/ml) | 2.47 | <0.064 |
| D-dimers (mg/l FEU) | 12 | <0.5 |
| Creatine kinase MB (ng/ml) | 33.2 | <6.22 |
| Troponin T (ng/ml) | 0.164 | <0.03 |
| Creatinine (μmol/l) | 165 | 57–113 |
| AST (U/l) | 88.9 | <50 |

* Corresponding author: Dr. Philipp Warnke; Institute of Medical Microbiology, Virology and Hygiene, Rostock University Hospital, Schillingallee 70, 18057 Rostock, Germany; E-mail: philipp.warnke@med.uni-rostock.de

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trix. Spectral fingerprint was analyzed by using Vitek MS IVD V2, database MS-CE version CLI 2.0.0 (bioMérieux, Marcy l'Etoile, France). As *E. cecorum* is rarely identified in clinical samples, identification was subsequently confirmed by VITEK 2 GP card (bioMérieux, Marcy l'Etoile, France) as well as by sequencing of the 16S rRNA gene. Identification performed with VITEK 2 GP card confirmed identification of *E. cecorum* with a probability of 97%.

For molecular identification of the isolate, PCR amplification (≈500 bp) of the 16S rRNA gene was performed using the primers 27F (AGAGTTTGATCMTGGCTCAG) and 519R (GWATTACCGCGGCKGCTG) followed by single-strand sequencing (Microsynth/Seqlab, Goettingen, Germany) [1, 2]. This procedure revealed a 468-bp sequence that showed 99% homology with the *E. cecorum* 16S rRNA gene (GenBank accession number: AB932534.1). The 16S rRNA gene sequence from the present case has been deposited in GenBank under accession number: KP100645.

Antimicrobial susceptibility testing was performed using agar diffusion epsilometer testing according to EUCAST guidelines. The isolate was susceptible to ampicillin (MIC = 0.94 mg/l), vancomycin (MIC = 0.75 mg/l), teicoplanin (MIC = 0.094 mg/l), tigecyclin (MIC = 0.047 mg/l), linezolid (MIC = 1.0 mg/l), and imipenem (MIC = 0.064 mg/l).

Microbiological diagnosis of a catheter urine revealed *Escherichia coli* at quantities of 20,000 CFU/ml, with detection of leucocytes but no detection of nitrite. The clinical significance of the first microbiological urine analysis was questioned because of the negative results from a second urine specimen analyzed 4 days after the first specimen.

As susceptibility of *Enterococcus* species to piperacillin–tazobactam could be inferred from susceptibility to ampicillin, antibiotic treatment was unchanged and continued to a total of 10 days. Under this antibiotic treatment, the patient recovered and could be discharged from hospital.

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*E. cecorum* was first described in 1983 as *Streptococcus cecorum* [3]. It is part of the intestinal flora of pigs, cattle, chickens, ducks, cats, dogs, and canaries [4]. Reports on poultry infections caused by *E. cecorum* are frequent, but reports on human infections are extremely rare. Medline database search for *E. cecorum* revealed only five reports on human infections, i.e., a case of thorax empyema [5], septicaemia [6], peritonitis [7, 8], and aortic valve endocarditis [9].

Prior reports have shown difficulties in speciation of *E. cecorum*, especially when commercial kits, like API system (20 Strep), ATB Expression system (ID32 STREP), and Vitek system, were used [5–7, 9]. Therefore, identification of this species was only achieved by using time-consuming and expensive analyses, like sodium dodecyl sulfate–polyacrylamide gel (SDS–PAGE) analysis of whole-cell protein, cellular fatty acid chromatography, 16S rRNA gene sequencing, and/or tRNA gene PCR and capillary electrophoresis [5–9].

In the present case, it could be demonstrated for the first time that *E. cecorum* can clearly be identified with modern – but broadly established – routine diagnostic methods like MALDI-TOF or Vitek 2. The problems of misidentification of *E. cecorum* by traditional identification methods, as recently claimed by Ahmed et al. [9], and their advice to routinely use 16S rRNA PCR to maximize chances of identifying the correct pathogen, seems in our opinion – at least for the identification of *E. cecorum* – no longer necessary.

We doubt that *E. cecorum* is underdiagnosed due to misidentification, as both methods, MALDI-TOF MS and Vitek 2, yielded excellent identification, but think that it is still a very rare pathogen.

Transmission of *E. cecorum* might be facilitated by contact to poultry, broiler breeders or chickens, as this pathogen has recently emerged as an important pathogen within these animals [10–12]. Even domestic animals like cats and dogs are thought to be a possible source for transmission leading to *E. cecorum* septicaemia [6]. As the present patient denied contact to poultry or other livestock animals, declined journey abroads for many years, had no pets, and of note, stayed in the psychiatry ward for 5 weeks prior to the timepoint when signs of infection were noticed, we assume the gastrointestinal tract as the possible endogenous source of the infection.

This report shows a severe systemic infection developed under a nosocomial setting due to so far very rarely isolated species *E. cecorum*. This setting strongly supports the consideration of an endogenous infection source. Identification of this bacterium by MALDI-TOF mass spectrometry and Vitek 2 is reported here for the first time. As these technologies provide the possibility for fast and excellent identification, more reports on infections due to *E. cecorum* can be expected, which will help to identify a potential human habitat and to understand the pathogenic potential of this species.

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