Enterococcus cecorum INFECTION IN TWO CRITICALLY ILL CHILDREN AND IN TWO ADULT SEPTIC PATIENTS

David Stubljar*, Miha Skvarc
Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
*Corresponding author, E-mail: d.stubljar@gmail.com

Summary: Enterococcus cecorum is mostly found as normal gut flora in farm animals, especially pigs and poultry. However, sometimes it can cause extended infections and disease in those animals, as was recently reported in Canada where it caused arthritis and osteomyelitis in chickens. Until now, only a few reports have been published on Enterococcus cecorum as a potential pathogen in humans. We have reported 4 cases of infection with this rare human pathogen. The organism was proven in 2 blood samples from adult patients with sepsis and in 2 cerebrospinal fluid (CSF) samples taken from children with external ventricular drainage (EVD) and diagnosed ventriculitis. In two cases (one child and one adult), other bacterial pathogens were also detected. The organism could not be cultivated and could only be identified with analysis of 16S rRNA gene PCR. The following molecular biomarkers were used to confirm the infection, and exclude sample contamination: white blood cell count, neutrophils, C-reactive protein (CRP), procalcitonin (PCT) and presepsin (sCD14-ST). Enterococcus cecorum was identified as a pathogen with 16S rRNA gene PCR and could have caused the infection in all patients. We also suspected the first possible human-to-human transmission of bacteria from a mother to a newborn child.

Key words: Enterococcus cecorum; broad-range 16S rRNA gene PCR; infection in children and adults; human-to-human transmission; presepsin sCD14-ST

Introduction

Enterococcus cecorum has previously been classified as Streptococcus cecorum and was renamed Enterococcus cecorum after sequencing of its 16S rRNA gene. Phenotypically it is often mistakenly described as Streptococcus group D. In addition to conventional cultivating tests, it is possible to prove infection with proteins, analysis of fatty acids from culture or 16S rRNA gene sequencing [1]. Enterococcus cecorum is a normal inhabitant of the intestines of birds and other vertebrates. It recently emerged in Canada and some other countries as an important cause of arthritis and osteomyelitis in chickens [2], isolated from the intestines of poultry, pigs, cattle, cats and dogs as part of normal gut flora [3, 4]. Human infections were diagnosed in rare cases. The first human case was reported in 1997 when E. cecorum was isolated from the blood of a patient with severe septicemia by conventional biochemical tests. Its identity as E. cecorum was confirmed by SDS-PAGE analysis of whole cell proteins [1]. Furthermore, bacteria were isolated as an etiologic agent of infection from a patient on continuous ambulatory peritoneal dialysis with episodes of peritonitis [5], later from a 60-year-
old man with liver cirrhosis and hepatocellular carcinoma who then developed peritonitis [6], from a man with empyema thoracis successfully treated with cefotaxime [7] and from a 44-year-old man with infectious aortic valve endocarditis [8]. In the last case, E. cecorum was mistakenly identified as Streptococcus salivarius from blood culture and was later confirmed as E. cecorum from a valve tissue sample with 16S rRNA gene PCR.

In this article, we described 4 cases of E. cecorum as a cause of (co)infection. We discovered E. cecorum as a potential cause of sepsis in 2 adult immunocompromised patients and in 2 children with ventriculitis, who had increased intracranial pressure (ICP) and needed external ventricular drainage (EVD). In all the cases, we used 16S rRNA gene sequence analysis to prove the presence of E. cecorum as a pathogen.

Case reports

A 63-year-old Caucasian woman on hemodialysis due to kidney failure and type II diabetes was brought to a general hospital with a short history of increasing breath shortness associated with fever and rigors in May 2011. The woman was accepted to the general hospital’s intensive care unit with suspected sepsis. Four years before admittance, she had a massive ischemic stroke and was admitted to a care unit for the elderly and disabled. No history of animal contact and travelling abroad was reported. At presentation, the patient was pyrexial (39.1 °C), hypotensive, with signs of septic shock, her heart rate was > 150 beats per minute and her respiratory rate was > 20 breaths per minute. She had ulcers on her left thigh and sacral region. The patient was subsequently commenced on empirical cefazolin and then switched after 5 days of hospitalization to piperacillin/tazobactam. After 20 days of hospitalization at the intensive care unit, she got better, her vital signs improved, the values of biomarkers lowered and she was discharged.

A 66-year-old male patient was admitted to the intensive care unit of a general hospital because of acute respiratory distress syndrome (ARDS) and septic shock. He has been treated in the rheumatology ward for severe knee arthritis and leukemia since 2003. It transformed to myelodysplastic syndrome in 2010.

A 9-month-old male child with raised ICP due to an operation of a cystic lesion in the brains and installed EVD was treated at an intensive care unit of a tertiary clinic. The child had 3 episodes of fever with epileptic attacks and vomiting afterwards, which developed due to bacterial ventriculitis/meningitis. The last episode was 6 weeks after the operation. The child was still on meropenem and the treatment was switched to gentamycin and vancomycin after the pathogens were identified.

A newborn male with raised ICP due to subdural hemorrhage and installed EVD was treated in June 2012. The mother was healthy and the baby was born on term with an Apgar score of 9/10. The baby was transported from the general hospital to a tertiary intensive care unit because of apneic attacks 3 days after birth. The baby was still in the hospital with the mother and they were both transferred to another hospital where the facilities to treat such newborns were possible. Subdural hematoma was discovered and it was decided that the baby needed EVD due to raised ICP. The catheter for monitoring cranial pressure was removed after 5 days and on the 8th day pus was discovered around the drainage device. The biomarkers of infection were also taken, but the analysis of CSF was inconclusive.

Methods and results

All the patients were admitted to intensive care units between May 2011 and June 2012. The blood to set the values of infection biomarkers and for blood cultures was collected. The observed markers of infection were: white blood cell count, percentage of neutrophils, C-reactive protein (CRP, Siemens, Germany), procalcitonin (PCT, Brahms, Germany) and sCD14-ST (presepsin) in CSF of two children (Mitsubishi Chemical Europe, Germany) (Table 1). Respiratory tract samples and urine were taken for cultures. The blood cultures were negative in 3 cases. We isolated S. pneumonia in the case of a 66-year-old man (Table 1). In the case of a 9-month-old child with raised ICP, Serratia marcescens from CSF was isolated. Because of clinical suspicion of bacterial infection, broad-range 16S rRNA gene PCR to prove the causing pathogen was performed in duplicates. Bacterial DNA was extracted from a whole blood sample or CSF using SepsiTst™ (Molzym, Germany). Isolation and PCR amplification were performed following the
manufacturer’s instructions. Sequences of the PCR product were compared with GenBank 16S rRNA gene sequences available at the National Centre for Biotechnology Information and Ribosomal Database Project using BLAST algorithm and with Sepsiblast program (Molzym, Germany) [9, 10]. In all 4 cases, the sequence score for \textit{E. cecorum} was 100% and the sequence coverage with the available ones was not less than 99%.

**Discussion**

Few cases of infection with \textit{E. cecorum} have recently been reported in English literature and all patients had underlying diseases [8]. We reported the first 2 cases of presence of \textit{E. cecorum} in children and 2 cases of possible sepsis in adult immunocompromised patients, like in the study from Tan et al. [11]. The patients’ data is presented in Table 1. In our cases, conventional methods could not identify \textit{E. cecorum} from stool samples because standard routine microbiological methods are not able to differentiate \textit{E. cecorum} from other enterococcal species [11]. All our patients were on broad-spectrum antibiotics for 24 hours before the samples for cultures were taken, so the therapy could explain why we could not identify the cause of infection with conventional methods.

In 2 patients, bacteria other than \textit{E. cecorum} were also identified with broad-range 16S rRNA PCR. In one child, \textit{S. marcescens} was cultured from CSF, which definitely contributed to the severity of the child’s infection, and \textit{E. cecorum} was the only (co)pathogen which might have contributed to the severity of infection. Furthermore, in the case of a septic adult, \textit{S. pneumoniae} was identified with 16S rRNA gene PCR as the cause of infection and most probably resulted in disease severity and pneumonia. The biomarkers which were used to prove bacterial infection were elevated and excluded other potential causes of infection like viruses and fungi or sample contamination by potential normal bacterial flora from the skin. They were above the limit of positivity for bacterial infection in all cases (Table 1). However, the use of conventional infection biomarkers did not help in the case of 2 children. We found normal levels of leukocytes, CRP and PCT in whole blood in a newborn and in a 9-month-old child with ventriculitis [12]. In a recent article, authors described the use of sCD14-ST to identify septic patients. The concentration of sCD14-ST was significantly higher in the sepsis group than in the healthy group [13]. We used the same analogy for CSF and assumed that a value of sCD14-ST above 1000 pg/mL was a good marker to confirm ventriculitis. In the case of the newborn, all conventional biomarkers failed to prove the infection. Only the use of sCD14-ST revealed that the newborn could have bacterial ventriculitis. In the case of the 9-month-old child with bacterial ventriculitis/meningitis, the cells and proteins in CSF were increased, the glucose levels were lower than normal and sCD14-ST was also raised. We have not been able to measure the sCD14-ST in blood from children since we could not obtain the samples.

**Conclusion**

It was recently reported that poultry in Canada and Europe got infected with \textit{E. cecorum}. It was never proven that animal-human transfer had happened [2, 4, 14]. All our patients came from rural areas where small family farms raise a great variety of farm animals including pigs and poultry. We assumed that farm animals were a possible source of \textit{E. cecorum} but could not prove our thesis, since prevalence in humans is only 0.1% [11]. We also assumed that people could also carry \textit{E. cecorum}, as is the case in \textit{E. faecalis}, in their gut or on their skin. We had a case of a newborn with ventriculitis, possibly caused by \textit{E. cecorum}, the only organism proven. We assumed that only his mother, who lived in a rural hilly area where they raise farm animals and come into indirect contact with them, could be the source of bacteria. We lacked strong epidemiological data that human-to-human transmission had happened. The newborn was transferred from one hospital to another and had never come in contact with animals during the stays. In our opinion, human-to-human transmission was thus the only explanation of how \textit{E. cecorum} could come into CSF through EVD and cause bacterial ventriculitis. In the case of isolation of \textit{E. cecorum} in humans from sterile places, contamination of samples from the environment is possible. In such cases, we recommend the use of bacterial infection biomarkers such as sCD14-ST that show an activating immune system response to distinguish between contaminations and
**Table 1: The patients’ data**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Contact with animals</th>
<th>Admittance diagnosis</th>
<th>Co-morbidity</th>
<th>Cultures</th>
<th>16S rRNA gene analysis from whole blood or CSF</th>
<th>Sequence score/coverage for E. cecorum</th>
<th>Biomarkers in blood</th>
<th>Biomarkers in CSF (leuco, proteins, glucose, sCD14-ST)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>63 years</td>
<td>Lived in a rural area on a farm. Lived in a care unit for the elderly and disabled during the last years of her life.</td>
<td>Sepsis</td>
<td>End kidney failure on hemodialysis</td>
<td>Blood cultures negative</td>
<td>Enterococcus cecorum from blood</td>
<td>100% 302bp of 302 (100%)</td>
<td>36.2 × 10^9/L</td>
<td>EVD</td>
<td>Died of uncontrolled diabetes and kidney failure two months after discharge</td>
</tr>
<tr>
<td>Male</td>
<td>66 years</td>
<td>Lives in a village with many farms, has a flock of chickens, possible animal-to-human transfer.</td>
<td>Septic shock</td>
<td>Leukemia with transformation to myelodysplastic syndrome</td>
<td>Blood cultures negative</td>
<td>S. pneumoniae and Enterococcus cecorum from blood</td>
<td>100% 410bp of 412 (99%)</td>
<td>8.6 × 10^9/L</td>
<td>EVD</td>
<td>Good and discharged from the hospital</td>
</tr>
<tr>
<td>Male</td>
<td>9 months</td>
<td>Lives in a rural area, contact with farm animals possible.</td>
<td>Bacterial ventriculitis/meningitis</td>
<td>Subdural hematoma with raised ICP and EVD</td>
<td>Serratia marcescens from CSF</td>
<td>Enterococcus cecorum from CSF</td>
<td>100% 413bp of 417 (99%)</td>
<td>5.3 × 10^9/L</td>
<td>EVD</td>
<td>Good and discharged from the hospital</td>
</tr>
<tr>
<td>Newborn male</td>
<td>3 days</td>
<td>Mother from a village with many farms. Newborn transferred directly from a regional general hospital to a university clinical center.</td>
<td>Bacterial ventriculitis</td>
<td>Cystic lesion in the brain and EVD</td>
<td>Negative CSF</td>
<td>Enterococcus cecorum from CSF</td>
<td>100% 413bp of 417 (99%)</td>
<td>5.3 × 10^9/L</td>
<td>EVD</td>
<td>Good and discharged from the hospital</td>
</tr>
</tbody>
</table>

Leuco-leucocytes, neutro-neutrophils, CRP-C-reactive protein (normal value less than 5mg/L), PCT-procalcitonin (normal values less than 0.5 µg/L), CSF-cerebrospinal fluid, presepsin sCD14-ST (blood value above 1000pg/mL is typical of septic shock)
Enterococcus cecorum infection in two critically ill children and in two adult septic patients

corrected molecular method of 16S rRNA broad-range PCR is used.

Conflict of interest statement

The authors declare that there is no conflict of interest.

References


OKUŽBA Z Enterococcus cecorum PRI DVEH KRITIČNO BOLNIH OTROCIH IN DVEH ODRASLIH PACIENTIH S SEPTIKEMIJO

David Štubljar, Miha Skvarč

Povzetek: Enterococcus cecorum večinoma najdemo kot del normalne črevesne flore domačih živali, zlasti prašičev in perutnine. Kljub temu pa mikroorganizem lahko povzroči okužbo in razvoj bolezni kot pomemben vzrok artritisa in osteomielitisa pri piščancih, kot je bil zabeležen primer v Kanadi. Do sedaj je bilo objavljenih le nekaj poročil o Enterococcus cecorum kot potencialnem patogenu človeka. Tukaj poročamo o štirih primerih redke okužbe s človeškim patogenom Enterococcus cecorum. Organizem smo dokazali v dveh krvnih vzorcih odraslih bolnikov s sepso in v dveh vzorcih cefalospinalne tekočine, odzvete pri otrocih z zunanj ventrikularno drenažo in diagnosticiranim ventrikulitism. Pri enem otroku in enem odraslem bolniku so bili prisotni tudi drugi bakterijski patogeni. Organizem smo lahko določili le z analizo gena 16S rRNA. Za potrditev okužbe smo določili molekularne biološke označevalce, kot so C-reaktivni protein (CRP), prokalcitonin (PCT) in presepsin (sCD14-ST), in s tem izključili možnost kontaminacije vzorca. Domnevamo, da je prišlo do mogočeega prenosa bakterije od matere na novorojenčka, kar bi predstavljalo prvo poročilo o prenosu okužbe s človeka na človeka.

Ključne besede: Enterococcus cecorum; širokospektralni PCR; gena 16S rRNA; okužba pri otrocih in odraslih; prenos okužbe s človeka na človeka, presepsin sCD14-ST