Escherichia vulneris: an unusual cause of complicated diarrhoea and sepsis in an infant. A case report and review of literature

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Abstract

Escherichia vulneris is an opportunistic human pathogen. It has been primarily reported in adult patients and invasive infections have been observed in immune-suppressed individuals. This is the first report of *E. vulneris* causing complicated diarrhoea and sepsis in an infant.

Two month old sick infant, born full-term, was admitted to the paediatrics department with loose motions and refusal to feed for four days. *E. vulneris* was isolated from blood in pure culture. The isolate was characterized for diarrhoeal virulence markers: heat labile and heat stable toxins (LT, ST) and hemolysin (*hlyA*) by PCR. The presence of LT enterotoxin and hemolysin provides strong evidence of the diarrhoeagenic potential of *E. vulneris*, further leading to the invasive infection triggering sepsis.

As *E. vulneris* can lead to serious complications, an attempt should be made in clinical laboratories to identify and further characterize this new *Escherichia* species.

Keywords: Complicated diarrhoea, *Escherichia vulneris*, hemolysin (*hlyA*), infant, LT toxin, sepsis
Original Submission: 23 January 2016; **Revised Submission:** 21 June 2016; **Accepted:** 1 July 2016
Article published online: 9 July 2016

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Case report

A 2-month-old lethargic and sick female infant presented to the Paediatrics Department with diarrhoea. She had been refusing to breastfeed for 4 days before presentation and had no history of fever, vomiting or seizures. There was no history of maternal fever during the antenatal period. She was born full-term by lower-segment caesarean section and the postnatal period was uneventful. The infant was receiving bottle feeds. Physical examination revealed that the pulse and respiratory rates were 120/min and 51/min, respectively. She was dehydrated and febrile with a temperature of 38.3°C (101°F). The neonatal reflexes were poor along with a reduced muscle tone and low activity. A probable diagnosis of acute gastroenteritis with moderate dehydration was made. The infant was admitted and intravenous fluids were administered. Her stool examination and culture were unremarkable.

On day two of hospitalization, the infant developed high-grade fever and repeated episodes of seizures. Laboratory investigations revealed haemoglobin of 163g/L, total leucocyte count of 18.2×10^9/L and a platelet count of 572×10^9/L. The level of serum calcium was slightly decreased to 1.9 mmol/L. The qualitative C-reactive protein measurement was positive (>10 mg/L). On day 3, blood and cerebrospinal fluid samples were sent for examination, querying the possibility of sepsis. An empirical therapy for sepsis consisting of intravenous ampicillin and amikacin was initiated. Cerebrospinal fluid microscopy and biochemical parameters were unremarkable and culture was negative. Blood culture yielded pure growth of non-lactose fermenting, motile, Gram-negative bacilli after 48 h of incubation. The colonies showed the presence of yellow pigment.

Biochemical tests revealed a positive catalase test and exhibited glucose fermentation with production of gas in a triple sugar iron agar differential medium. The isolate was

http://dx.doi.org/10.1016/j.nmni.2016.07.002

negative for oxidase reaction, indole, urease or H_2S production, citrate utilization and Voges–Proskauer test. After 48 h of incubation, an ortho-nitrophenyl-β-D-galactopyranoside test was positive. The isolate was identified as *Escherichia vulneris* by Vitek 2 Compact using ID GN card (BioMérieux, Marcy l'Etoile, France) and Vitek MS (matrix-assisted laser desorption/ionization time-of flight mass spectrometry; BioMérieux) with a confidence value of 99.4. The identity of the isolate was further confirmed by 16s rRNA gene sequencing. A single isolated colony from an overnight culture was used to perform a colony PCR using universal primers 27F and 1492R. The PCR product was run on gel and the amplicon of around 1400 bp was eluted. The eluted DNA was further sequenced using a Sanger dideoxy sequencing method. The sequence obtained was blasted using NCBI nucleotide blast. The blast results confirmed the isolate as *E. vulneris*. The sequence is submitted to NCBI (Accession No. KX357823).

Antimicrobial susceptibility was performed by Kirby–Bauer's disc diffusion method as per the CLSI guidelines. The isolate was extended spectrum β-lactamase negative and was susceptible to ampicillin, amoxicillin-clavulanate, cephalosporins (Cefuroxime, Cefoxitin, Ceftazidime and Cefotaxime), aminoglycosides (gentamicin, amikacin and netilmicin), ciprofloxacin, piperacillin-tazobactam, trimethoprim-sulphamethoxazole and carbapenems (Imipenem, Meropenem, Ertapenem). Meanwhile, a repeat blood culture after 24 h also yielded pure growth of *E. vulneris*. Based on the susceptibility report, the initial therapy was continued. After 7 days of the antibiotic treatment, the infant improved clinically with lowering of fever, normal skin turgor and a sucking reflex. The antibiotics were discontinued on day 10 and the infant was discharged on oral feeds. Her follow-up blood culture at the time of discharge was sterile.

Molecular characterization

The *E. vulneris* isolate was screened for virulence markers encoding heat-labile (LT) and heat-stable (ST) enterotoxins [1] and haemolysin (*hlyA*) [2] associated with diarrhoea and sepsis. The DNA extracted from the culture grown overnight in tryptic soy broth at 37°C in a CO_2 incubator is used as template. The primer sequences were: LT-F 5′-GGCGACAGATTATACCGTGC-3′; LT-R 5′-CCGAATTCTGTTATATATGTC-3′; ST-F 5′-TTAATAGCACCCGGTACAAGCAGG-3′; ST-R 5′-CTTGACTCTTCAAAAGAGAAAATTAC-3′; hlyA F 5′-AACAAGGATAAGCACTGTTCTGGCT-3′; hlyA R 5′-ACCATATAAGCGGTCATTCCCGTCA-3′.

The PCR for LT and ST toxin genes was performed using a predefined program with an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 45 min and final extension at 72° for 5 min with following primer pairs. For haemolysin, the reaction conditions were initial denaturation at 95° for 5 min, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55° for 30 seconds, extension at 72°C for 1 min and final extension at 72° for 5 min. The isolate was found to be positive for LT toxin and haemolysin (*hlyA*) but it was negative for ST toxin (Fig. 1).

Discussion

Escherichia vulneris is an opportunistic human pathogen and there are limited clinical reports of human infections worldwide [3–5]. A review of *E. vulneris* infections reported in humans worldwide is presented in Table 1. In humans, *E. vulneris* was initially isolated from infected wounds, in association with other bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, streptococci, enterococci and *Enterobacter* spp., *Acinetobacter lwoffii and Cedecea neteri* [4,6]. Later, *E. vulneris* was also isolated from other clinical specimens, such as stool, sputum, urine, vaginal swabs and throat swabs, where it was thought to be a colonizer [3]. As *E. vulneris* failed to induce soft-tissue infections or lethality in mice on pathogenicity testing, the clinical significance of this species was doubted [4]. However subsequent studies showed *E. vulneris* as the sole pathogen in clinical cases of urosepsis, osteomyelitis, intravenous catheter-related bacteraemia,

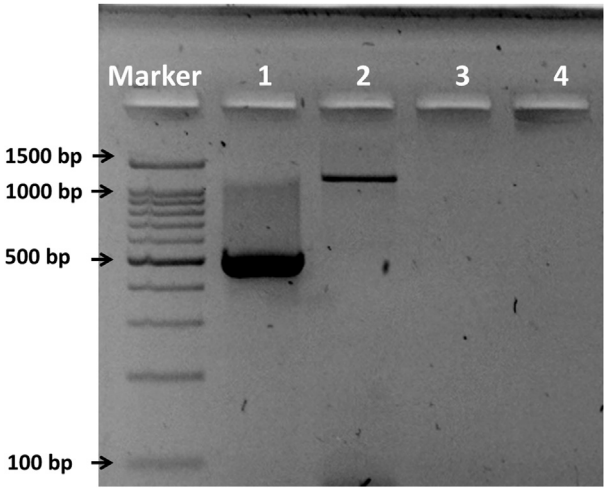

FIG. 1. Gel picture showing *Escherichia vulneris* positive for heat-labile (LT) toxin (508 bp, Lane 1); hlyA toxin (1177 bp, Lane 2); and negative for heat-stable (ST) toxin (Lane 3); PCR mixture without the template was taken as a negative control (Lane 4); 100-bp ladder was used as a DNA marker. PCR products were run on 1.8% agarose gel and visualized under UV Transilluminator and pictured using a gel documentation system.