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Polymicrobial Bloodstream Infection with *Eggerthella lenta* and *Desulfovibrio desulfuricans*[▽]

Karin Liderot,[1] Martin Larsson,[2] Stina Boräng,[1] and Volkan Özenci[1]*

Division of Clinical Microbiology F 72, Karolinska Institutet, Karolinska University Hospital, Huddinge, SE 141 86 Stockholm, Sweden,[1] and Department of Internal Medicine, Stockholm South General Hospital, Stockholm, Sweden[2]

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The advancement in culture identification methods has made possible the culture and identification of slow-growing anaerobic bacteria in clinical samples. Here, we describe a case of polymicrobial bloodstream infection (BSI) caused by *Eggerthella lenta* and *Desulfovibrio desulfuricans*, identified by API 20A and Vitek 2 systems and by 16S rRNA sequencing.

CASE REPORT

The patient was an 86-year-old woman, living alone though provided with community home care assistance. In spring of 2009, the patient was admitted to hospital twice. The first time, she presented with dyspnea, an obstructive breathing pattern, confusion, and a high fever. She was admitted to a geriatric hospital where she stayed for 2 weeks and was initially treated with trimethoprim-sulfamethoxazole. Treatment was switched to cefuroxime after 3 days due to treatment failure, and the course was finished with amoxicillin. One week after getting discharged from the geriatric hospital, she was admitted to Stockholm South General Hospital. She presented with a fever, dyspnea, and this time with diarrhea as well. Upon examination, a decubital wound, not described earlier, which was 4 by 3 cm wide and 1 cm deep, was found in the sacrum area. She had a temperature of 39.3°C, a heart rate of 104 beats/minute, a blood pressure of 106/54 mm Hg, and a breathing frequency of 35 breaths/minute. Her white cell count at admission was 20×10^9/liter, and her C-reactive protein level was 104 mg/liter. Initially, the clinical picture together with the laboratory results was interpreted as a bloodstream infection (BSI) derived from an airway infection. However, the wound found in the sacrum, which appeared infected, was considered an alternative source for the infection. Cultures were taken from the blood, the nasopharynx, and the decubital wound. Growth was reported to occur in one blood culture vial on day 3. The nasopharynx culture showed no pathogenic bacteria, and the wound culture showed mixed bacterial flora containing several different types of *Enterobacteriaceae*. The antibiotic treatment was started with cefuroxime and then switched to amoxicillin after 2 days. The patient was discharged when both the clinical and the laboratory parameters, including C-reactive protein level and white cell count, were normalized after antibiotic treatment.

Blood samples were cultured in a BacT/Alert 3D (bioMérieux, Inc., Durham, NC) automated blood culture system. After 52 h of incubation, one of two anaerobic blood culture vials signaled positive. Gram staining revealed Gram-positive bacilli. Broth from the anaerobic bottle was subcultured onto blood agar plates incubated in air, chocolate agar plates were incubated in 5% CO_2, and blood agar plates were incubated in an anaerobic jar. After 48 h of incubation at 37°C, no growth was seen on the plates incubated in air and in those incubated in CO_2. A tiny growth was seen in the plates incubated in the anaerobic jar, which was inhibited by a metronidazole disc (Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom). The plate was reincubated anaerobically for three more days. At closer inspection after a total of 5 days of incubation, two different colony morphologies were observed. Gram staining showed that one consisted of short Gram-positive bacilli without spores and the other of short, slightly curved Gram-negative bacilli. The two bacterial isolates were then subcultured separately and tested for identification and antibiotic susceptibility. The Gram-positive bacilli formed gray smooth colonies, whereas the Gram-negative bacilli formed gray-green smooth colonies with a strong sulfurous odor. The aerobic blood culture vials and the second anaerobic vial remained negative until the end of the culture period.

Both API 20A (bioMérieux) and the Vitek 2 system (bioMérieux) could identify the Gram-positive bacilli as *Eggerthella lenta* with 94% and 99% probability, respectively. However, the Gram-negative bacilli could not be identified by the Vitek 2 system.

16S rRNA PCR and sequencing were performed with both of the isolates. Bacterial DNA was extracted using the automated MagNA Pure LC system, a MagNA Pure LC total nucleic acid isolation kit (Roche Diagnostics AB, Mannheim, Germany), according to the manufacturer's instructions. PCR amplification was performed by adding 3 μl of the extract to a master mix containing 10 μM each primer (5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-CCGTCAATTCMTTTRAGTTT-3′) together with 25 μl HotStarTaq master mix (Qiagen, Hilden, Germany) to give a final volume of 50 μl. After initial denaturation, the thermocycling parameters were 32 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were purified using MicroCon YM-100 columns (Millipore, Billerica, MA) according to the manufacturer's instructions. Se-

* Corresponding author. Mailing address: Division of Clinical Microbiology F 72, Karolinska Institutet, Karolinska University Hospital, Huddinge, SE 141 86 Stockholm, Sweden. Phone: 46858581147. Fax: 46858581125. E-mail: volkan.ozenci@karolinska.se.

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quencing of both strands was carried out using an ABI Prism BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) with a GeneAmp 9700 thermocycler (Applied Biosystems). Sequencing primers used in the two reactions were 1 μM (each) 5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-GWATTACCGCGGCKGCTG-3′, respectively. The sequence cycling products were analyzed by capillary electrophoresis and fluorescence detection with an Applied Biosystems ABI 3130xl genetic analyzer. The fluorescence data were analyzed with the Sequencer Software program (version 4.5; Gene Codes Corporation, Ann Arbor, MI). A BLAST search (1) showed 99% nucleotide identity to previously registered sequences of the 16S rRNA gene of *Desulfovibrio desulfuricans* (577 of 580 bases) for the Gram-negative bacilli and 99% nucleotide identity to *E. lenta* (381 of 382 bases) for the Gram-positive bacilli.

Phenotypical testing revealed that both bacterial isolates were catalase (15%) negative, bile sensitive (Diatabs; Rosco Diagnostica A/S, Taastrup, Denmark), spot indol negative, and reduced nitrate (Diatabs). *E. lenta* was nonmotile and *D. desulfuricans* was motile when tested with a wet mount. *D. desulfuricans* was desulfoviridin positive, as indicated by red fluorescence under UV light (365 nm) after addition of a drop of 2 M NaOH. When tested with disc diffusion method, zones of inhibition against kanamycin (Neo-Sensitabs; Rosco Diagnostica A/S, Taastrup, Denmark) and metronidazole were observed with both isolates. *E. lenta* showed a zone of inhibition against vancomycin (Oxoid) but not against colistin (Oxoid). *D. desulfuricans* was totally resistant to both vancomycin and colistin. The disk diffusion tests described above were performed only for identification purposes. None of the bacteria produced beta-lactamase when tested with a nitrocefin disc (bioMérieux). Antibiotic susceptibilities were determined by Etest (bioMérieux) with IsoSensitest agar (Oxoid) supplemented with 5% defibrinated horse blood and 20 mg/liter NAD (b-NAD). The Etest results are shown in Table 1.

TABLE 1. Etest MICs for the bloodstream isolates

Antibiotic	MIC (mg/liter)[a] for:	
	Eggerthella lenta	*Desulfovibrio desulfuricans*
Amoxicillin	1.0 (4, 8)	0.50 (0.5, 2)
Amoxicillin-clavulanic acid	1.0 (4, 8)	0.125 (4, 8)
Cefotaxime	>256 (ND)	1.0 (ND)
Chloramphenicol	16 (8, 8)	16 (8, 8)
Clindamycin	0.25 (4, 4)	0.25 (4, 4)
Imipenem	1.0 (2, 8)	0.50 (2, 8)
Metronidazole	1.0 (4, 4)	0.016 (4, 4)
Moxifloxacin	1.0 (ND)	>32 (ND)
Penicillin G	1.0 (0.25, 0.5)	NT
Piperacillin-tazobactam	32 (8, 16)	64 (8, 16)

[a] The first and second values in parentheses are the MIC breakpoints defined by the Swedish Reference Group for Antibiotics (SRGA) for categorization as susceptible (≤MIC breakpoint) and resistant (>MIC breakpoint), respectively. The SRGA MIC breakpoints for anaerobic Gram-positive and Gram-negative bacteria were used for *E. lenta* and *D. desulfuricans*, respectively. ND, not defined; NT, not tested.

E. lenta, previously named *Eubacterium lentum*, is a Gram-positive bacillus that occurs singly, in pairs, and in short chains (4, 9). It is an obligate anaerobe without spores or flagella. *D. desulfuricans* is a slightly curved Gram-negative bacillus. It is a motile, strictly anaerobic sulfate-reducing bacterium. Both *Eggerthella* spp. and *Desulfovibrio* spp. can be part of the normal intestinal flora. *Desulfovibrio* spp. are also environmental bacteria present in soil and water.

The traditional phenotypic methods have limited value in trying to identify slow-growing anaerobic bacteria. The modern identification methods in combination with molecular techniques have been shown to be helpful in this regard (3, 5, 6). Here, we describe a case of polymicrobial BSI with *E. lenta* and *D. desulfuricans*. To our knowledge, this is the first published case of a polymicrobial BSI caused by these bacteria. *E. lenta* could be identified by API 20A, Vitek 2, and 16S rRNA sequencing. *D. desulfovibrio* is not included in the Vitek 2 database and could be identified only by 16S rRNA sequencing.

The biochemical properties and antibiotic susceptibilities of the isolates concurred well with previous publications (3, 5, 6, 7, 10). *E. lenta* and *D. desulfuricans* are mostly mentioned in relation to diseases in the gastrointestinal tract and the hepatobiliary tract. They are probably underreported as a part of a multibacterial flora in abdominal abscesses, probably overgrown by less fastidious bacteria on culture plates (3, 5, 6, 10). Both *E. lenta* and *D. desulfuricans* have previously been described to cause bacteremia. There are two articles (including one case report) describing *E. lenta* bacteremia that have been published since the name changed from *Eubacterium lentum* in 1999 (2, 5). Most of the patients with *E. lenta* bacteremia seem to have intra-abdominal sources of bacteria. Two cases of human bacteremia with *D. desulfuricans* have been described (3, 8). The second patient was diagnosed by 16S rRNA sequencing. The first patient had an intra-abdominal source of infection. In the second patient, an intra-abdominal source was suspected but not confirmed. Another possible way for slow-growing anaerobic bacteria to enter the bloodstream is through infected decubital wounds near the anal canal, previously described for *E. lenta* (5). This could have been the case with our patient since she had a decubital wound in the sacrum that appeared infected. The wound sample, described here, was cultured anaerobically only for 48 h as in the routine protocol. Therefore, the presence of these two slow-growing organisms could not be determined. Moreover, growth of mixed bacterial flora composed of several different types of *Enterobacteriaceae* was observed.

This case underlines the significance of new diagnostic methods in the identification of rare, slow-growing anaerobic bacteria from patients with BSI. Identification of these bacteria might be clinically relevant. The new identification methods may also improve our knowledge of the epidemiology and pathogenicity of slow-growing anaerobic bacteria.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Chan, R. C., and J. Mercer.** 2008. First Australian description of *Eggerthella lenta* bacteremia identified by 16S rRNA gene sequencing. Pathology **40:** 409–410.
3. **Goldstein, E. J., D. M. Citron, V. A. Peraino, and S. A. Cross.** 2003. *Desulfovibrio desulfuricans* bacteremia and review of human Desulfovibrio infections. J. Clin. Microbiol. **41:**2752–2754.
4. **Kageyama, A., Y. Benno, and T. Nakase.** 1999. Phylogenetic evidence for the

transfer of *Eubacterium lentum* to the genus *Eggerthella* as *Eggerthella lenta* gen. nov., comb. nov. Int. J. Syst. Bacteriol. **49:**1725–1732.

5. **Lau, S. K., P. C. Woo, A. M. Fung, K. M. Chan, G. K. Woo, and K. Y. Yuen.** 2004. Anaerobic, non-sporulating, bacteremia characterized by 16S rRNA gene sequencing. J. Med. Microbiol. **53:**1247–1253.
6. **Lau, S. K., P. C. Woo, G. K. Woo, A. M. Fung, M. K. Wong, K. M. Chan, D. M. Tam, and K. Y. Yuen.** 2004. *Eggerhella 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.
7. **Liebetrau, A., A. C. Rodloff, J. Behra-Miellet, and L. Dubreuil.** 2003. In vitro activities of a new des-fluoro(6) quinolone, garenoxacin, against clinical anaerobic bacteria. Antimicrob. Agents Chemother. **47:**3667–3671.
8. **Porschen, R. K., and P. Chan.** 1977. Anaerobic vibrio-like organisms cultured from blood: Desulfovibrio desulfuricans and Succinivibrio species. J. Clin. Microbiol. **5:**444–447.
9. **Wade, W. G., J. Downes, D. Dymock, S. J. Hiom, A. J. Weightman, F. E. Dewhirst, B. J. Paster, N. Tzellas, and B. Coleman.** 1999. The family *Coriobacteriaceae*: reclassification of *Eubacterium exiguum* (Poco *et al*. 1996) and *Peptostreptococcus heliotrinreducens* (Lanigan 1976) as *Slackia exigua* gen. nov., comb. nov. and *Slackia heliotrinireducens* gen. nov., comb. nov., and *Eubacterium lentum* (Prevot 1938) as *Eggerthella lenta* gen. nov., comb. nov. Int. J. Syst. Bacteriol. **49:**595–600.
10. **Warren, Y. A., D. M. Citron, C. V. Merriam, and E. J. Goldstein.** 2005. Biochemical differentiation and comparison of Desulfovibrio species and other phenotypically similar genera. J. Clin. Microbiol. **43:**4041–4045.