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Shewanella haliotis Associated with Severe Soft Tissue Infection, Thailand, 2012

To the Editor: Marine bacteria of the family Shewanellaceae, genus *Shewanella*, are gram-negative, motile bacilli that grow aerobically or anaerobically and produce hydrogen sulfide (*1*). Organisms belonging to a *Shewanella* species were first isolated in 1931 by Derby and Hammer from dairy products and classified as *Achromobacter putrefaciens* (*2*). Members of *Shewanella* species usually are found in marine environments in warm climates or during summer in temperate climates (*3*). In humans, most *Shewanella* species infections occur in skin and soft tissues (*4*). One species (*S. algae*) and possibly a second (*S. putrefaciens*) have been isolated from human samples on multiple occasions (*5*). A third species, *S. haliotis*, was implicated in human infections during 2010 (*6*) and *S. xiamenensis* was reported as the fourth infectious species among humans during 2011 (*7*). *S. haliotis* is a novel bacterial species that was isolated from the gut microflora of abalones (*Haliotis discus hannai*) in 2007 (*8*). We report the second description, to our knowledge, of S. haliotis involved in human disease.

In September 2012, a 52-year-old woman, living in Bangkok, Thailand, was hospitalized after experiencing drowsiness for 2 hours. She had a low-grade fever, chills, and swelling, erythema, and tenderness in her left leg. During the previous week, she had handled fresh seafood in a market and had eaten cooked mackerel. She denied having eaten uncooked food or wading into flooded areas or the sea. She had undergone orthotopic liver transplantation 6 months previously to excise hepatocellular carcinoma related to Child-Pugh class C hepatitis C cirrhosis; since that procedure, she had been under treatment with immunosuppressive drugs. She also had diabetes, hypertension, and nephrotic syndrome. Physical examination revealed that in addition to above-named symptoms, multiple blisters were noted (Figure, panel A). Her oral temperature was 37.8°C, blood pressure 80/40 mm Hg, pulse was 110 bpm, and respiratory rate was 24 breaths/minute. A complete blood count showed a leukocyte count of 2,250 cells/μL (91.2% neutrophils). Despite adequate rehydration, monitored by central venous pressure, the patient required norepinephrine to stabilize her vital signs. The clinical diagnosis of her condition was septic shock with suspected necrotizing fasciitis.

After tissue and blood samples were collected and submitted for microbiological analysis, shock resuscitation and an emergency fasciotomy (Figure, panel B) were performed, and antimicrobial drug treatment with meropenem and vancomycin was started. Surgeons did not confirm the suspected necrotizing fasciitis. Two sets of blood cultures and fluid culture sampled from the left leg identified *S. algae* by conventional biochemical methods. The MICs of antimicrobial drugs were determined by Etest (bioMérieux, Solna, Sweden). This strain was susceptible to ciprofloxacin (0.25

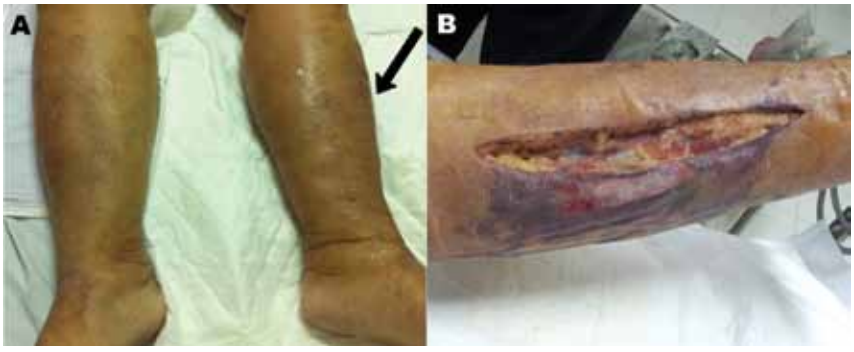

Figure. *Shewanella haliotis* severe soft tissue infection of woman in Thailand, 2012. The patient sought treatment for painful erythematous swelling of the left leg. A) Arrow indicates affected area. B) Postsurgical fasciotomy wound with necrotic tissue.

mg/L), piperacillin-tazobactam (1.0 mg/L), ceftriaxone (1.0 mg/L), and meropenem (0.38 mg/L). The patient had fever for the first 2 days of hospitalization. After 2 weeks of treatment, the antimicrobial drug was switched to oral ciprofloxacin; treatment was continued after dressing and debridement of the fasciotomy wound.

The organism produced yellowish-brown mucoid colonies on sheep blood agar and chocolate agar after 18 hours of incubation at 35°C under CO2 atmosphere. MacConkey agar showed non–lactose-fermenting colonies that were oxidase-positive, motile, and produced hydrogen sulfide on triple sugar iron agar. Growth at 42°C with 6.5% NaCl suggested that this organism was S. algae. Because phylogenetically related *Shewanella* species may be misidentified by routine biochemical tests, the strain was confirmed by using 16S rRNA gene sequencing.

Molecular characterization of 16S rRNA gene sequencing was performed by using PCR with *Shewanella* species consensus primers (online Technical Appendix Table, wwwnc.cdc.gov/EID/article/19/6/12-1607-Techapp.pdf) and direct sequencing from PCR product (JX968803). Phylogenetic analysis of the 16S rRNA gene sequence showed clustering with *S. haliotis* (NR_044134T) and 99.9% similarity and 1 base difference (online Technical Appendix Figure). By using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis, JX968803 showed the closest match (99.9%; 1 base difference) with Alteromonadaceae bacterium PH39 (AF513471).

The strain was confirmed as *S. haliotis* by using additional biochemical tests and API 20 NE System (bioMérieux, Durham, NC, USA). It was positive for ornithine decarboxylase, gelatinase, reduction of nitrates to nitrites, tolerance to 6% NaCl, and assimilation of caprate and malate, but negative for citrate utilization, arginine dihydrolase, lysine decarboxylase, urease, indole production, assimilation of mannose, glucose, arabinose, mannitol, maltose, adipate, and acidification of glucose. This strain was resistant to polymyxin B (300 μg/disc).

More than 50 species of Shewanella have been reported. The route of *Shewanella* infection is associated with direct contact with the organism through seawater or ingestion of raw seafood (*9*). Japan reported 1 case of *S. haliotis* infection in an elderly patient in whom *Vibrio vulnificus* infection was initially suspected (*6*), and various clinical manifestations of *S. algae* infection have been reported (*5*). Community- and hospital-acquired infection with *Shewanella* species from contaminated medical devices have also been reported (*10*). *S. haliotis* and *S. algae* are closely related organisms; discriminating between them on the basis of biochemical tests is difficult. Molecular characterization of 16S rRNA gene sequencing can be used to differentiate the 2 species. In summary, this case suggests that immune-compromised persons in tropical climates could be susceptible to *S. haliotis* soft tissue infection in the absence of typical exposures.

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Murine Typhus in Humans, Yucatan, Mexico

To the Editor: *Rickettsia typhi* is the causal agent of murine typhus, a febrile illness affecting humans worldwide (*1*). In Mexico, recent studies demonstrated a 14% prevalence of antibodies against typhus group rickettsiae in healthy adult blood donors in Mexico City, and a recent nonfatal case of endemic typhus was reported in Yucatan (*2*,*3*).

In May 2011, a 42-year-old woman and her 12-year-old son sought care at the clinical service of the Autonomous University of Yucatan. They had malaise, headache, fever (39°C), fibromyalgia, sore throat, and fatigue and an erythematous rash on the chest that after 6 days spread to the abdomen and extremities.

Dengue fever was diagnosed, and the patients were treated empirically with acyclovir, methanesulfonamide, N-(4-nitro-2-phenoxyphenyl) and clarithromycin. Dengue could not be confirmed by laboratory testing.

Murine typhus was diagnosed on the basis of PCR amplification and immunofluorescent assay for antibodies to *R. typhi*. *Rickettsia* species was determined by sequencing of rickettsial genes. Three serum samples were collected from the woman (8, 12, and 16 days after illness onset) and 1 from the boy (8 days) in 3.8% sodium citrate as anticoagulant, and DNA was extracted immediately by QIAamp DNA Blood Mini Kit (QIAGEN Valencia, CA, USA) in accordance with the manufacturer's instructions. Single-step PCR amplification was performed by using genus-specific primers for the rickettsial 17-kDa protein and citrate synthase (*gltA*) genes as reported (*4*)

Sequences of the citrate synthase and 17-kDa PCR products were compared at the National Center for Biotechnology Information BLAST software (*5*). Three PCR amplicons of both genes were fully sequenced and compared with sequences in GenBank. The 17-kDa and citrate synthase fragment sequences (GenBank accession nos. JX198507 and JX458814) showed 99% and 100% identity, respectively, with *R. typhi* strain Wilmington strain (GenBank accession no. AE017197.1) (Table).

Immunofluorescent assay was performed by using *R. rickettsii* and *R. typhi* antigen fixed on slides. We examined the serum samples for IgG and IgM, assessing reactivity of γ chain–specific and m heavy chain–specific secondary conjugates, respectively, with rickettsial antigens. All 3 samples from the woman and the sample from the boy contained antibodies to *R. typhi* (Table). Both patients were treated with 100 mg of oral doxycycline 2×/day for 7 days (boy), and 10 days (woman); symptoms improved in 72 hours for the child. The woman's symptoms resolved completely in 5 days.

Typhus has been endemic in Mexico since before the conquest period (*6*). Socioeconomic aspects play a major role in zoonotic diseases, such as rickettsioses, especially in their distribution in urban and suburban areas because of factors such as marginalized communities, animal breeding, education levels, poverty, and social exclusion from health systems.

Overcrowding resulting from migration from rural areas to large urban centers contributes to increased zoonoses in urban areas. Also contributing is the ecologic imbalance of flora and fauna associated with deteriorating sanitary conditions in areas where mammals involved in the cycle of *R. typhi*, such as rodents and opossum, may live in the same habitat as humans and colonize backyards, waste deposit area, and areas around the neighborhoods where they can find food. The concurrence and presence of mammals, vectors, and humans may contribute to maintaining transmission of endemic typhus in a reduced area, with the possibility to cause outbreaks

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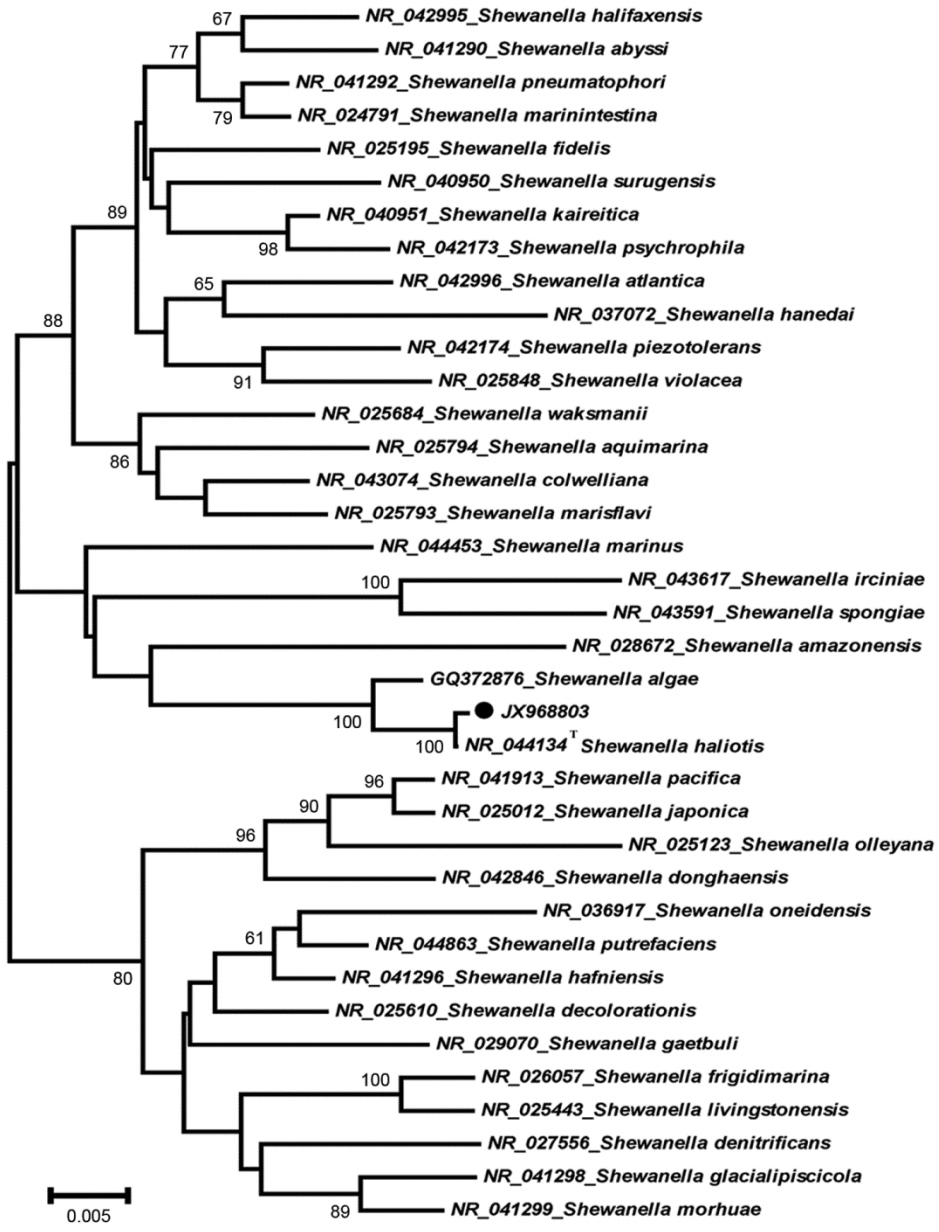
Technical Appendix

Specific *Shewanella* Consensus Primer Sets for 16S rRNA Gene Sequencing and Phylogenetic Analysis

Technical Appendix Table. Specific *Shewanella* consensus primer sets for 16S rRNA gene sequencing performed during investigation of soft tissue infection, Thailand, YEAR**.**

Round	Primer name	Sequence (5'→3')	Product (bp)
1	shew16s_99F	CGA GCG GCG GAC GGG TGA G	1,327
	shew16s_1426R	CCA CTC CCA TGG TGT GAC G	
2	shew16s_349F	GGA GGC AGC AGT GGG GAA	1,077
	shew16s_1426R	CCA CTC CCA TGG TGT GAC G	



Technical Appendix Figure. Phylogenetic analysis of *Shewanella* spp. 16S rRNA gene sequencing (1,077 base pairs). Closed circle indicates the strain in this study compared with the *S. haliotis* prototype species (NR_044134^T) and other representative species. Numbers at branch nodes are bootstrap values. Scale bar represents number of nucleotide substitutions per site.