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 Haliotis discus hannai (2). A third species, Shewanella xiamenensis (3), was reported as the fourth infectious 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 samples 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

References
S. haliotis
logenetic analysis of the 16S rRNA
from PCR product (JX968803). Phy
techapp.pdf) and direct sequencing
cdc.gov/EID/article/19/6/12-1607-
table Technical Appendix Table, wwwnc.
laformed by using PCR with
16S rRNA gene sequencing was per-
ated consensus primers (online
Shewanella -
talized
S. algae. Because phylogenetically re-
duced hydrogen sulfide on triple sugar
ish-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 pro-
duced hydrogen sulfide on triple sugar
iron agar. Growth at 42°C with 6.5%
NaCl suggested that this organism was
S. algae. Because phylogenetically re-
lated Shewanella species may be mis-
identified by routine biochemical tests,
the strain was confirmed by using 16S
rRNA gene sequencing.

Molecular characterization of
16S rRNA gene sequencing was per-
fomed by using PCR with Shewan-
la 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). Phy-
logenetic analysis of the 16S rRNA
gene sequence showed clustering with
S. haliotis (NR_044134T) and 99.9%
similarity and 1 base difference (on-
line Technical Appendix Figure). By
nih.gov/Blast.cgi) analysis, JX968803
showed the closest match (99.9%; 1
base difference) with Alteromonada-
ceae bacterium PH39 (AF513471).

The strain was confirmed as S.
haliotis by using additional biochemi-
test and API 20 NE System (bio-
Mérieux, Durham, NC, USA). It was
positive for ornithine decarboxylase,
gelatinase, reduction of nitrates to ni-
trites, tolerance to 6% NaCl, and as-
simulation of caprate and malate, but
negative for citrate utilization, argi-
nine dihydrolyase, lysine decarboxyl-
ase, urease, indole production, assimil-
ation of mannose, glucose, arabinose,
mannitol, maltose, adipate, and acidi-
fication of glucose. This strain was re-
sistant to polymyxin B (300 µg/disc).

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

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ceae and proposal of Pseudoalteromon-
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nov., Shewanellaceae fam. nov., Morit-
elaceae fam. nov., Ferrimonadaceae fam.
nov., Idiomarinaeae fam. nov. and Psy-
chromonadaceae fam. nov. Int J Syst Evol
dx.doi.org/10.1099/ijis.0.02997-0
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)amide, N-(4-nitro-2-phenoxyphenyl)amide, 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 JX435884) showed 99% and 100% identity, respectively, with R. typhi strain Wilmington strain (GenBank accession no. AE011797.1 (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.
**Shewanella haliotis** Associated with Severe Soft Tissue Infection, Thailand, 2012

Technical Appendix

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

<table>
<thead>
<tr>
<th>Round</th>
<th>Primer name</th>
<th>Sequence (5'→3')</th>
<th>Product (bp)</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>shew16s_99F</td>
<td>CGA GCG GCG GAC GGG TGA G</td>
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<td>CCA CTC CCA TGG TGT GAC G</td>
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<td>2</td>
<td>shew16s_349F</td>
<td>GGA GGC AGC AGT GGG GAA</td>
<td>1,077</td>
</tr>
<tr>
<td></td>
<td>shew16s_1426R</td>
<td>CCA CTC CCA TGG TGT GAC G</td>
<td></td>
</tr>
</tbody>
</table>
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) and other representative species. Numbers at branch nodes are bootstrap values. Scale bar represents number of nucleotide substitutions per site.