Case Report

Correspondence

Zhiyong Zong
zongzhiy@scu.edu.cn or
zongzhiyong@gmail.com

Received 18 February 2011

Accepted 28 April 2011

Nosocomial peripancreatic infection associated with *Shewanella xiamenensis*

Zhiyong Zong^{1,2}

¹West China Hospital, Sichuan University, Chengdu, PR China

²State Key Laboratory of Biotherapy, Sichuan University, Chengdu, PR China

Shewanella xiamenensis, a newly established species originally from sea sediments, was repeatedly recovered from peripancreatic drainages in a patient and was the probable pathogen of hospital-acquired peripancreatic infection. A commercially available system misidentified it as *Shewanella putrefaciens*, suggesting that some previous reported cases with *S. putrefaciens* infection might have been caused by other *Shewanella* species. Precise species identification of *Shewanella* species usually requires *gyrB* sequencing.

Introduction

Shewanella xiamenensis is a newly established species of the genus *Shewanella*; the type strain of this species was found in costal sea sediments off Xiamen, Fujian province, China (Huang *et al.*, 2010). Although *Shewanella* species, especially *Shewanella algae* and *Shewanella putrefaciens*, have been recognized as human pathogens, there appear to have been no reports of the isolation of *S. xiamenensis* from any clinical samples and this species has not previously been documented as a cause of infection. However, a case of hospital-acquired infection associated with *S. xiamenensis* was recently encountered and is reported here.

Case report

A 66-year-old male patient was admitted to West China Hospital on June 2010 due to abdominal pain, distention and vomiting for 1 week. He had been diagnosed with acute pancreatitis associated with gallstones 50 days previously in this hospital, following the criteria proposed by Banks *et al.* (2006). His symptoms relieved after receiving a combination of therapy including endoscopic retrograde cholangiopancreatography (ERCP) and somatostatin. He was then discharged after hospitalization for 16 days. For this (the second) admission, he was diagnosed with recurrent pancreatitis and pancreatic pseudocyst based on clinical manifestations, serum amylase and lipase ≥ 3 times the upper limit of normal, and magnetic resonance imaging findings that revealed an 8.7×12.3 cm² cyst on the body and tail of the pancreas. The patient received a computed tomography (CT)-guided percutaneous aspiration of the pancreatic pseudocyst 6 days after this

admission and a drainage catheter was placed into the pseudocyst for continuous draining. The drainage, dark brown in colour and clear, was sent for culture but did not grow any organisms. The patient remained afebrile until 1 week after the puncture but then developed high-grade fever (>39 °C, axilla temperature) and increasing abdominal pain without additional symptoms. The white blood cell count was 12.53×10^9 l⁻¹ with 86 % neutrophils. Drainages collected by puncturing the catheter using sterile syringes were sent for culture on 8 and 9 days after the CT-guided puncture for draining. Both drainage cultures and another one collected by surgical operation later on (see below) grew the same Gram-negative bacilli and coagulase-negative staphylococci (see below for species identification). Repeated cultures of blood did not yield any organisms. Due to the onset of fever and abdominal pain and positive results of drainage cultures, this patient was diagnosed with hospital-acquired peripancreatic infection. Intravenous cefminox (2 g every 12 h) was initiated for empiric therapy until the culture results were available and was then replaced by intravenous moxifloxacin (0.4 g per d) and norvancomycin (0.4 g every 8 h). However, the patient had no response to antimicrobial treatment and received a surgical operation for debriding and draining 15 days after the CT-guided puncture. Approximately 500 ml dark brown pus was drained by surgery and the drainage was sent for culture. Following the surgical draining and meropenem (1 g every 8 h) therapy, the patient's temperature was back to normal and the abdominal pain relieved. One month after the surgery, he was in good condition and discharged.

The coagulase-negative staphylococci were identified as *Staphylococcus epidermidis* by the MicroScan Walkaway 96 SI (Siemens Healthcare Diagnostic) automated system. The *Staph. epidermidis* was resistant to oxacillin, ciprofloxacin and trimethoprim/sulphamethoxazole but susceptible to vancomycin, rifampicin, clindamycin and gentamicin. The

The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene and *gyrB* sequences of *Shewanella xiamenensis* strain WCJ25 are HQ730954 and HQ730955.

Gram-negative bacilli were identified as *Pseudomonas* spp. by the Walkaway system but as *Shewanella putrefaciens* by the Vitek II (bioMérieux) automated system. The three Gram-negative isolates recovered from three independent cultures displayed the same pattern generated by enterobacterial repetitive intergenic consensus (ERIC) PCR, which was performed as described by Versalovic *et al.* (1991). Therefore, the three isolates were actually a single strain, designated WCJ25 here. The species identification was further performed by partially sequencing the 16S rRNA gene amplified with the universal primers 27F and 1492R (Lane, 1991) and the *gyrB* gene (encoding DNA gyrase subunit B) with primers UP-1 and UP-2r (Yamamoto & Harayama, 1995). Amplicons were purified using a commercial kit (Omega) and then sequenced using an ABI 3730xl DNA Analyser (Applied Biosystems) at the Beijing Genomics Institute. Similarity searches of the sequences obtained were carried out against the GenBank, EzTaxon (Chun *et al.*, 2007) and LeBIBI (<http://pbil.univ-lyon1.fr/bibi/>) databases. The 1382 bp 16S rRNA gene sequence of strain WCJ25 was closest (99.6 % identity) to *Shewanella xiamenensis* type strain S4 and was 98.1 % identical to *Shewanella putrefaciens* type strain ATCC 8071 (Table 1). As the 16S rRNA gene sequence sometimes lacks sufficient resolution to differentiate closely related *Shewanella* species (Huang *et al.*, 2010), the more rapidly evolving *gyrB* gene was partially sequenced. The 1167 bp *gyrB* sequence of strain WCJ25 was 98.5 % identical to that of *S. xiamenensis* S4 and only 88.1 % identical to that of *S. putrefaciens* ATCC 8071 (Table 1). Based on the 16S rRNA gene and *gyrB* sequences, WCJ25 was identified as *S. xiamenensis*. Biochemical characteristics (Table 2) of WCJ25 were determined using the API 20E and 20NE systems (bioMérieux). Strain WCJ25 exhibited a biochemical pattern similar to that of *S. xiamenensis* type strain S4 (Huang *et al.*, 2010), except that WCJ25 did not utilize glucose, arabinose and caprate (Table 2). WCJ25 was susceptible to amikacin, gentamicin,

tobramycin, cefotetan, imipenem, trimethoprim/sulphamethoxazole, levofloxacin, showed intermediate resistance to piperacillin/tazobactam and ciprofloxacin, and resistant to azithromycin, ampicillin, ampicillin-sulbactam, ceftazidime, cefepime, ceftriaxone and ceftizoxime.

Due to the repeated co-isolation of *Staph. epidermidis*, *S. xiamenensis* cannot be confirmed as the definite pathogen in this case. Nonetheless, *S. xiamenensis* is very like to have been the causative pathogen of this peripancreatic infection. The genus *Shewanella* comprises many species (see <http://www.bacterio.cict.fr/s/shewanella.html>). *Shewanella* species are marine bacteria but have been reported as rare human pathogens (Holt *et al.*, 2005). The most common *Shewanella* species associated with human infections are *S. algae* and *S. putrefaciens* (Holt *et al.*, 2005), both of which have been identified as the cause of a variety of infections such as bacteraemia, pneumonia, meningitis and soft tissue infection (Brink *et al.*, 1995; Jorens *et al.*, 2004; Pagani *et al.*, 2003; Tucker *et al.*, 2010; Yilmaz *et al.*, 2007). However, the species identification of previous reports was generally performed using biochemical reactions or commercially supplied automated microbiology systems. These biochemistry-based methods may be unable to differentiate closely related *Shewanella* species, as in this case the misidentification of *S. xiamenensis* as *S. putrefaciens* by the Vitek II system. It is therefore reasonable to speculate that some previous cases reported as being due to *S. putrefaciens* may in fact have been caused by other *Shewanella* species. It seems necessary to employ sequencing of the 16S rRNA and *gyrB* genes to precisely identify *Shewanella* isolates to the species level for any incoming reports of cases with *Shewanella* infection in order to avoid misleading information.

Shewanella isolates have been found previously in various types of clinical samples, including intra-abdominal specimens (To *et al.*, 2010). However, the source of *S. xiamenensis* in this case remains undetermined. The patient had no history of contact with seawater, unlike some other

Table 1. 16S rRNA gene and *gyrB* sequence identities between strain WCJ25 and type strains of 10 *Shewanella* species

Organism*	GenBank accession no.		Identity to WCJ25 (%)	
	16S rRNA gene	<i>gyrB</i>	16S rRNA gene	<i>gyrB</i>
<i>S. xiamenensis</i> S4 ^T	FJ589031	FJ589040	99.6	98.5
<i>S. oneidensis</i> ATCC 700550 ^T	AE014299	AE014299	99.2	92.2
<i>S. hafniensis</i> NBRC 100975 ^T	AB205566	AB208056	98.3	87.8
<i>S. profunda</i> JCM 12080 ^T	AY445591	FJ589036	98.0	87.2
<i>S. putrefaciens</i> ATCC 8071 ^T	X82133	AF005669	98.1	88.1
<i>S. morhuae</i> NBRC 100978 ^T	AB205576	AB208062	97.8	86.0
<i>S. basaltis</i> DSM 14937 ^T	EU143361	FJ589041	97.8	81.2
<i>S. baltica</i> DSM 9439 ^T	AJ000214	AB231331	98.1	88.6
<i>S. glacialipiscicola</i> NBRC 102030 ^T	AB205571	AB266200	97.4	87.4
<i>S. decolorationis</i> JCM 21555 ^T	AJ609571	AJ609572	97.2	92.0

*T, type strain.

Table 2. Biochemical features of *S. xiamenensis* strains WCJ25 and S4 and *S. putrefaciens* strain ATCC 8071

+, Positive reaction; (+), weak reaction; –, negative reaction; NA, data not available. Results for *S. xiamenensis* S4 and *S. putrefaciens* ATCC 8071 were retrieved from Huang *et al.* (2010).

	<i>S.</i> <i>xiamenensis</i> WCJ25	<i>S.</i> <i>xiamenensis</i> S4	<i>S.</i> <i>putrefaciens</i> ATCC 8071
Production of			
Acetoin (Voges–Proskauer)	–	NA	NA
Arginine dihydrolase	–	NA	NA
Gelatinase	+	+	+ / –
β-Glucosidase	+	+	(+)
Hydrogen sulfide	+	NA	NA
Indole	–	NA	NA
Lysine decarboxylase	–	NA	NA
Ornithine decarboxylase	+	NA	NA
Tryptophan deaminase	–	NA	NA
Urease	–	NA	NA
Fermentation of glucose	–	+	–
Utilization of			
N-Acetyl-glucosamine	+	+	+
Adipic acid	–	NA	NA
Amygdalin	–	NA	NA
Arabinose	–	+	–
Caprate	–	+	–
Citrate	–	–	–
Galactose	–	–	+
Gluconate	–	–	–
Glucose	–	+	–
Inositol	–	NA	NA
Malate	+	+	+
Maltose	+	+	–
Mannitol	–	NA	NA
Melibiose	–	NA	NA
Phenylacetic acid	–	NA	NA
Rhamnose	–	NA	NA
Sucrose	–	NA	NA
Sorbitol	–	NA	NA
Reduction of nitrate	+	NA	NA
Oxidase	+	+	+

cases of *Shewanella* infection (Holt *et al.*, 2005). *Shewanella* was very rarely encountered locally and this case was the only one seen in 3 years, suggesting that the acquisition of *S. xiamenensis* was unlikely to have been due to patient-to-patient transmission. The co-existence of *Staph. epidermidis*, a skin commensal, indicates a possible external source of *S. xiamenensis* rather than an internal source such as migration from the gastrointestinal tract. Drainage catheters have been identified as an likely source for *Shewanella* nosocomial infections (Oh *et al.*, 2008). It is therefore

possible that *S. xiamenensis* initially colonized the catheter with *Staph. epidermidis* and later invaded ascendingly, resulting in the peripancreatic infection.

Conclusions

In summary, *Shewanella xiamenensis*, a newly recognized species from an environmental source, was repeatedly recovered from peripancreatic drainages with *Staphylococcus epidermidis* and was considered as the probable causative pathogen of peripancreatic infection. Therefore, although it was originally found in sea sediments, *S. xiamenensis* could also be a rare human pathogen. This report highlights that automatic biochemistry-based methods could misidentify closely related *Shewanella* species, and that *gyrB* sequencing is required for species identification of *Shewanella* clinical isolates. Some previous reported cases with *S. putrefaciens* infection may actually have been caused by other *Shewanella* species.

Acknowledgements

The author is grateful for Dr Chunhong Peng for collecting this isolate and thanks Ms Rujia Yu for performing the biochemical tests.

References

- Banks, P. A., Freeman, M. L. & Practice Parameters Committee of the American College of Gastroenterology (2006). Practice guidelines in acute pancreatitis. *Am J Gastroenterol* **101**, 2379–2400.
- Brink, A. J., van Straten, A. & van Rensburg, A. J. (1995). *Shewanella* (*Pseudomonas*) *putrefaciens* bacteremia. *Clin Infect Dis* **20**, 1327–1332.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Holt, H. M., Gahrn-Hansen, B. & Bruun, B. (2005). *Shewanella algae* and *Shewanella putrefaciens*: clinical and microbiological characteristics. *Clin Microbiol Infect* **11**, 347–352.
- Huang, J., Sun, B. & Zhang, X. (2010). *Shewanella xiamenensis* sp. nov., isolated from coastal sea sediment. *Int J Syst Evol Microbiol* **60**, 1585–1589.
- Jorens, P. G., Goovaerts, K. & Ieven, M. (2004). *Shewanella putrefaciens* isolated in a case of ventilator-associated pneumonia. *Respiration* **71**, 199–201.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. New York, NY: Wiley.
- Oh, H. S., Kum, K. A., Kim, E. C., Lee, H. J., Choe, K. W. & Oh, M. D. (2008). Outbreak of *Shewanella algae* and *Shewanella putrefaciens* infections caused by a shared measuring cup in a general surgery unit in Korea. *Infect Control Hosp Epidemiol* **29**, 742–748.
- Pagani, L., Lang, A., Vedovelli, C., Moling, O., Rimenti, G., Pristerà, R. & Mian, P. (2003). Soft tissue infection and bacteremia caused by *Shewanella putrefaciens*. *J Clin Microbiol* **41**, 2240–2241.
- To, K. K., Wong, S. S., Cheng, V. C., Tang, B. S., Li, I. W., Chan, J. F., Seto, W. K., Tse, H. & Yuen, K. Y. (2010). Epidemiology and

clinical features of *Shewanella* infection over an eight-year period. *Scand J Infect Dis* **42**, 757–762.

Tucker, C., Baroso, G. & Tan, P. (2010). Ventilator-associated pneumonia due to *Shewanella putrefaciens*. *Am J Health Syst Pharm* **67**, 1007–1009.

Versalovic, J., Koeuth, T. & Lupski, J. R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* **19**, 6823–6831.

Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* **61**, 1104–1109.

Yilmaz, G., Aydin, K., Bektas, D., Caylan, R., Caylan, R. & Koksai, I. (2007). Cerebellar abscess and meningitis, caused by *Shewanella putrefaciens* and *Klebsiella pneumoniae*, associated with chronic otitis media. *J Med Microbiol* **56**, 1558–1560.