Case Report

Invasive infection with *Streptococcus iniae* in Taiwan

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Received 21 January 2007
Accepted 18 April 2007

Streptococcus iniae, a common fish pathogen, rarely infects humans. In this report, a case of invasive *S. iniae* infection in a 51-year-old woman with diabetes mellitus and hepatitis C-related liver cirrhosis is described. The isolate was identified by 16S rDNA sequencing. The patient recovered after 1 week of treatment with ampicillin.

Introduction

Streptococcus iniae was first isolated in 1976 from skin lesions of Amazon freshwater dolphins (*Inia geoffrensis*) in the United States (Peir & Madin, 1976). It has been found to cause meningoencephalitis in tilapia, coho salmon, rainbow trout and yellowtail, and necrotizing myositis in red drum (Weinstein *et al.*, 1997). Many outbreaks of *S. iniae* infection with high mortality rates in aquaculture farms all over the world have been reported (Weinstein *et al.*, 1997). Asymptomatic carriage in some fish, such as barramundi and tilapia, has been observed (Shoemaker *et al.*, 2001). The bacterium was first recognized to cause human infection in 1991 in North America (CDC, 1996; Weinstein *et al.*, 1997). In this report, we describe what is believed to be the first case of invasive human *S. iniae* infection in Taiwan.

Case report

A 51-year-old woman was admitted to the Tri-Service General Hospital on July 7, 2003, because of week-long intermittent sweats, fever, dyspnoea and abdomen distension. The patient had a history of diabetes mellitus and hepatitis C-related liver cirrhosis. At admission, she had a temperature of 36.6 °C, a distended abdomen with prominent shifting dullness and a linear scar on the right thumb, but had no wound or injury on the extremities. Three days after admission, the patient had a low-grade fever up to 38 °C with abdomen distension. The blood leukocyte count was 20 000 cells ml⁻¹, and neutrophils were predominant. During a febrile chill the patient's ascitic fluid was tapped, and the leukocyte count was 1000 cells ml⁻¹.

Only blood and ascitic fluid were submitted for bacteriological culture. On the second day of incubation, the

aerobic blood culture bottle (BacT/ALERT; bioMérieux) showed Gram-positive cocci arranged in chains. The organism grew on blood agar as slightly mucoid, β -haemolytic colonies of 1 to 2 mm in diameter after 24 h of incubation at 37 °C, 5% CO₂. The isolate was positive for pyrrolidonyl arylamidase and arginine dihydrolase, and negative for catalase, bile aesculin and Voges–Proskauer tests. It was non-groupable by the Lancefield group A, B, C, D, F or G antisera. Both the Vitek (GPI) and API 20 Strep systems failed to identify the organism. The first 804 bp fragment of the 16S rRNA gene of the isolate was amplified and sequenced in our in-house laboratory. The sequence was found to be identical to that of *S. iniae* strain ATCC29178 16S rRNA gene (GenBank accession no. AF335572). The isolate was susceptible to ampicillin, penicillin, erythromycin and vancomycin, and intermediately resistant to chloramphenicol and clindamycin by antimicrobial disc diffusion tests (performed according to Clinical and Laboratory Standards Institute guidelines M100-S15 and M2-A7). Based on clinical and laboratory findings, the patient was treated with 500 mg ampicillin every 6 h for a week and recovered.

The patient described in this report was asked specifically about exposure to fresh fish or other aquatic creatures before she became ill. She did not remember having prepared fresh fish before the admission. However, her husband is a fishmonger and sells fish in the market every morning. It is possible that she acquired *S. iniae* from him, although he was not screened for *S. iniae* carriage.

Discussion

S. iniae was first recognized to cause human infections in 1995 when nine Asian patients in Toronto had invasive *S. iniae* infections (Weinstein *et al.*, 1997). Eight of these nine patients had bacterial hand cellulitis and one patient had

Table 1. Demographics of patients with reported *S. iniae* invasive infections

The patient described in this paper is indicated in bold.

Year	Country	Gender	Age (years)	Ethnicity	Underlying disease	Diagnosis	Exposure to fish	Specimen	Treatment	Reference
1991	America	Unknown	Unknown	Unknown	Unknown	Bacteraemic cellulitis	Unknown	Blood culture	Unknown	Weinstein <i>et al.</i> (1997)
1994	Canada	M	79	Unknown	Unknown	Arthritis of the knee	Unknown	Synovial fluid	Cloxacillin	Weinstein <i>et al.</i> (1997)
1995	Canada	F	74	Asian*	None	Bacteraemic cellulitis of the hand	Yes	Blood culture	Penicillin	Weinstein <i>et al.</i> (1997)
1995	Canada	F	64	Asian*	None	Bacteraemic cellulitis of the hand	Yes	Blood culture	Penicillin	Weinstein <i>et al.</i> (1997)
1995	Canada	F	40	Asian*	Chronic rheumatic heart disease	Bacteraemic cellulitis of the hand	Yes	Blood culture	Penicillin, cloxacillin	Weinstein <i>et al.</i> (1997)
1996	Canada	M	77	Asian*	Chronic rheumatic heart disease, chronic renal failure, diabetes mellitus, osteoarthritis	Endocarditis, meningitis, arthritis	Yes	Blood culture	Erythromycin, cefuroxime	Weinstein <i>et al.</i> (1997)
1996	Canada	M	80	Asian*	Diabetes mellitus, osteoarthritis	Bacteraemic cellulitis of the hand	Yes	Blood culture	Ampicillin	Weinstein <i>et al.</i> (1997)
1996	Canada	F	69	Asian*	None	Bacteraemic cellulitis of the hand	Yes	Blood culture	Cloxacillin	Weinstein <i>et al.</i> (1997)
1996	Canada	F	70	Asian*	Diabetes mellitus	Bacteraemic cellulitis of the hand	Yes	Blood culture	Ampicillin, cephalixin	Weinstein <i>et al.</i> (1997)
1996	Canada	M	71	Asian*	None	Bacteraemic cellulitis of the hand	Yes	Blood culture	Penicillin	Weinstein <i>et al.</i> (1997)
1996	Canada	F	58	Asian*	None	Bacteraemic cellulitis of the hand	Yes	Blood culture	Penicillin	Weinstein <i>et al.</i> (1997)
Un-known	Canada	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Poyart <i>et al.</i> (1998)
Un-known	Canada	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Poyart <i>et al.</i> (1998)
2000	America	M	88	Asian	Unknown	Sepsis, pneumonia	Unknown	Blood culture	Unknown	Facklam <i>et al.</i> (2005)
2001	Hong Kong	M	81	Asian Chinese	Hypertrophic obstructive cardiomyopathy, duodenal ulcer, gallstones	Bacteraemic cellulitis of the leg	Yes	Blood culture	Amoxicillin-clavulanate	Lau <i>et al.</i> (2003)
2001	Hong Kong	M	79	Asian Chinese	Alcoholism	Bacteraemic osteomyelitis	Yes	Blood culture	Penicillin, gentamicin	Lau <i>et al.</i> (2003)
2001	America	F	71	Asian	Unknown	Sepsis, cellulitis	Unknown	Blood culture	Unknown	Facklam <i>et al.</i> (2005)
2001	America	F	69	Asian	Unknown	Sepsis, cellulitis	Unknown	Blood culture	Unknown	Facklam <i>et al.</i> (2005)
2002	America	M	73	Asian	Unknown	Infected mass	Unknown	Thyroid cyst	Unknown	Facklam <i>et al.</i> (2005)
2003	America	M	63	Asian	Unknown	Toxic shock	Unknown	Blood culture	Unknown	Facklam <i>et al.</i> (2005)
2003	America	F	60	Asian	Unknown	Cellulitis	Unknown	Blood culture	Unknown	Facklam <i>et al.</i> (2005)
2003	Singapore	F	73	Asian Chinese	Unknown	Bacteraemic osteomyelitis	Yes	Tissue	Penicillin	Koh <i>et al.</i> (2004)
2003	Taiwan	F	51	Asian Chinese	Diabetes mellitus, hepatitis C-related liver cirrhosis	Bacteraemic illness	Yes	Blood culture	Ampicillin	This study

Table 1. cont.

Year	Country	Gender	Age (years)	Ethnicity	Underlying disease	Diagnosis	Exposure to fish	Specimen	Treatment	Reference
2004	Hong Kong	M	71	Asian Chinese	Chronic tophaceous gout, hypertension, hypothyroidism	Septic arthritis	Yes	Synovial fluid	Ampicillin	Lau <i>et al.</i> (2006)
2004	Hong Kong	M	74	Asian Chinese	Diabetes mellitus, alcoholic cirrhosis, hypertension	Bacteraemic cellulitis	Yes	Blood culture	Amoxicillin	Lau <i>et al.</i> (2006)
2004	America	F	82	Asian	Unknown	Cellulitis, sepsis	Unknown	Blood culture	Unknown	Facklam <i>et al.</i> (2005)

M, Male; F, female.

*Described by Weinstein *et al.* (1997); the nine Asians were eight Chinese patients and one Korean patient.

endocarditis, meningitis and arthritis (Weinstein *et al.*, 1997). Based on the data from the Centers for Disease Control and Prevention (CDC) Microbiology Laboratory, two additional cases were discovered retrospectively, including a patient from Texas, USA, with bacterial cellulitis in 1991 and a cook from Ottawa, Canada, with septic arthritis of the knee in 1994 (CDC, 1996). The first two cases of *S. iniae* infection outside North America were found in Hong Kong in 2001 (Lau *et al.*, 2003); one patient had bacterial cellulites, and the other patient had bacteraemic osteomyelitis of the spine. In 2003, another case of spinal infection caused by *S. iniae* was reported in Singapore (Koh *et al.*, 2004). In 2004, two additional cases of invasive *S. iniae* infection were reported in Hong Kong, including one patient with septic arthritis presenting as acute exacerbation of chronic gout and the other with bacterial cellulites (Lau *et al.*, 2006). As summarized in Table 1, Asian ethnic origin, old age and underlying medical conditions (such as diabetes mellitus and cirrhosis of liver) appear to be risk factors for *S. iniae* infection (Koh *et al.*, 2004; Lau *et al.*, 2003, 2006; Weinstein *et al.*, 1997). The patients median age was 71 years (mean 70.3, range 40 to 88), and the female: male ratio was 11:12. Asians (22/26, 13/26 were described as Chinese) are particularly at risk, probably because of their preference for eating fresh fish. Fresh whole fish are often purchased from the retail wet markets followed by scaling, cutting, and removal of fins and internal organs with bare hands (Lau *et al.*, 2006).

While clinicians need to be aware of the circumstances where *S. iniae* infections should be considered, microbiology laboratory staff should also pay special attention to specimens from patients with a history of fish handling. *S. iniae* grows slowly on solid medium at room temperature. Under such growth conditions, the β -haemolysis of *S. iniae* may not be evident, and it may therefore be misidentified as a viridans streptococcus and considered as a contaminant (Weinstein *et al.*, 1997). Because *S. iniae* is not included in the database of all commercial systems for bacterial identification, previously reported isolates were either unidentified or misidentified as *Streptococcus uberis*

or *Streptococcus dysgalactiae* (Facklam *et al.*, 2005; Lau *et al.*, 2003, 2006; Weinstein *et al.*, 1997). As suggested by Koh *et al.* (2004), all β -haemolytic streptococci ungroupable by the Lancefield grouping method and positive for pyrrolidonyl arylamidase should be suspected to be *S. iniae*. A recent study showed that *S. iniae* may be differentiated from other pyrrolidonyl arylamidase-positive, β -haemolytic streptococci, such as *Streptococcus pyogenes* and *Streptococcus porcinus*, by conventional tests (Facklam *et al.*, 2005). Although *S. pyogenes* can be readily identified by its group A carbohydrate antigen in Lancefield grouping, *S. porcinus* may possess groups E, P, U or V, or new antigens that are usually not included in the battery of antisera in clinical laboratories, and therefore would also be non-groupable. The Voges-Proskauer test, starch hydrolysis and acid production from sorbitol appeared to be useful for differentiation from *S. porcinus*. As PCR and sequencing techniques become more readily available in clinical laboratories, nucleic acid-based diagnostic techniques, such as 16S rDNA sequencing, should be performed to identify *S. iniae*. Several gene targets, including chaperonin 60 (Poyart *et al.*, 1998), lactate oxidase (Mata *et al.*, 2004) and 16S–23S spacer (Berridge *et al.*, 1998), have also been used successfully to identify *S. iniae*.

S. iniae isolates causing disease in fish or humans were shown to belong to a single clone by PFGE, while isolates from healthy fish were genetically diverse in Canada (Facklam *et al.*, 2005; Fuller *et al.*, 2001). *In vitro* models have shown that disease-associated *S. iniae* strains were more resistant to phagocytic clearance than non-disease-causing strains, and exhibited direct cytotoxicity to human endothelial cells unlike the non-disease-causing strains (Fuller *et al.*, 2001). Moreover, only the disease-associated strains were found to cause significant weight loss and bacteraemia in a mouse model of subcutaneous infection (Fuller *et al.*, 2001). However, the PFGE patterns of Hong Kong isolates were different from Canadian isolates (Lau *et al.*, 2003, 2006), suggesting that invasive *S. iniae* infections can be caused by strains with different genetic profiles, and perhaps a different pathogenic strain was

prevalent in Asia. Morphologically, colonies of the isolate in the present study appear larger, more β -haemolytic and mucoid than the clinical isolates from North America (Koh *et al.*, 2004; Lau *et al.*, 2003, 2006). Similar to our isolate, colonies of Hong Kong strains were also larger, more β -haemolytic and mucoid. This observation suggests that invasive *S. iniae* infections in Asia may have been missed as a result of their non-typical appearance.

A careful retrospective review of our laboratory records revealed that *S. iniae* infection in Taiwan is uncommon: in the past 3 years we have grouped 2425 β -haemolytic streptococci isolated in our laboratory and all have been typable by Lancefield group A, B, C, D, F or G antisera. However, the reported prevalence of this emerging zoonosis is likely to increase.

Acknowledgements

This study was supported by grants DOD-92-54 and TSGH-C95-40 from the Department of Defense and Tri-Service General Hospital, Taiwan, ROC.

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