1 **TITLE**

2 A FISHY TALE – A MAN WITH STREPTOCOCCUS HALICOERI EMPYEMA

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In 2004, veterinary laboratories in the United Kingdom reported a novel Lancefield group B streptococcus, *Streptococcus halichoeri* in seals. We report a case of *Streptococcus halichoeri* causing post-operative empyema in a patient. A search of the literature revealed that this is the first case of *S. halichoeri* ever reported in humans.

CASE REPORT

Our patient was a 45 year old Chinese male who presented with sharp left sided chest pain in October 2012. He was referred to a tertiary hospital centre by a family physician for a left third rib lesion seen on his chest radiograph. He was otherwise asymptomatic, with no significant cough or loss of weight. He had a past medical history of diabetes mellitus, hypertension and hyperlipidemia. He worked as a supervisor in a chemical factory, and kept a pet fish at home. Physical examination was unremarkable. Computed tomography (CT) of the thorax reported a diffusely expanded lesion in the body of the left third rib with endosteal scalloping of the cortex and soft tissue attenuation internally, and septations within the lesion. This appeared long standing and was suggestive of a benign process. There was also 8mm lucency with sclerotic margins seen in the left fifth rib. A full body PET computed tomography revealed an expanded, lytic lesion in the left
3rd rib with cortical disruption. Pre operative laboratory tests showed a white blood cell count (WBC) of 11.9 x10^9 /L, with neutrophils of 8.6 x10^9/L.

He underwent an elective left thoracotomy and excision biopsy of the left third rib lesion in March 2013, and had a left chest tube inserted intra operatively. Histology reported a benign osteofibrous lesion compatible with fibrous dysplasia. Three days after his surgery, he developed a fever with cough. He had persistent haemoserous drainage from the chest tube. Inflammatory markers were elevated, WBC 17 x10^9/L, C reactive protein (CRP) 271 mg/L.

Pleural fluid sent for Gram stain and bacterial culture revealed numerous neutrophils and gram positive cocci in chains. After 24 hours incubation on sheep blood agar, tiny white non-haemolytic colonies were isolated. The organism was unidentifiable by the VITEK 2 (bioMerieux) or by the API Streptocccal commercial kit (API 20 STREP bioMerieux SA, Marcy l’Etoile, France). Sequencing of the 16s rRNA gene also failed to yield a result. Lancefield grouping saw this organism as belonging to Lancefield group B. Acid was produced from mannitol and ribose but not from L-arabinose, glycogen, inulin, raffinose, sorbitol or trehalose. The organism produced arginine dihydrolase and acetoin but no activity was detected for β–galactosidase, β-galactosidase or β-glucuronidase. It also did not hydrolyse hippurate. We subjected it to matrix-assisted laser desorption ionization, using the Bruker Biotype system with version 3.1 software and database and this gave a score of 2.227 for Streptococcus halichoeri. It was sensitive to penicillin (minimum inhibitory concentration (MIC) 0.016mg/L) and levofloxacin (MIC 0.5mg/L).
The patient recalled handling of large freshwater fish (flowerhorn cichlids, also known as *luo han*), washing of large styrofoam boxes containing the fish from a local fish farm a week prior to his admission. During his hospital stay, his fish, boxes and fish tank had been discarded.

He was started on oral levofloxacin in view of a possible previous drug allergy to ampicillin. After a week of levofloxacin, his WBC remained elevated, and a repeat CT thorax showed a left loculated pleural effusion with compressive atelectasis of the adjacent left lower lobe. A second chest drain was inserted under radiological guidance on post operative day 11. Repeated pleural fluid cultures persistently grew *Streptococcus halichoeri*. Intravenous ceftriaxone 2g daily was commenced after 10 days of levofloxacin. Chest tube pleural fluid drainage improved significantly, and his inflammatory markers trended downwards with ceftriaxone. He received a total of 4 weeks of intravenous ceftriaxone with no recurrence of pleural effusion on outpatient follow up.

Zoonotic infections caused by *Streptococcus* species is a gradual growing concern of emerging infections with few outbreaks in the recent years. The major species include *Streptococcus canis*, *Streptococcus equi sub. zooepidemicus*, *Streptococcus iniae* and *Streptococcus suis*.
Streptococcus suis is a major porcine pathogen, and is the most commonly isolated bacteria in tonsils of swine. It has been increasingly recognized as an emerging zoonotic pathogen especially in Asia. S. suis can be transmitted to human beings via direct contact, usually in people who handle or eat pork. S. suis has been reported to cause bacterial meningitis who worked as a butcher and another after a swine bite, endocarditis in three patients who had a history of undercooked pork consumption, streptococcal toxic shock syndrome, and pneumonia and empyema.

Streptococcus iniae is a serious aquatic pathogen that rarely infects humans, causing outbreaks and mortality in wild reef fish and farmed marine aquaculture. It was first reported to cause invasive human infections in 1995, affecting nine patients, of which eight had hand cellulitis, one with endocarditis, meningitis and arthritis. Since then, there have been reports of Streptococcus iniae causing soft tissue infection in human resulting from fresh seafood contact in Taiwan, two reports of osteomyelitis and discitis.

While common pathogens causing human infection acquired from tropical fishes through open wounds include Mycobacterium marinum, Streptococcus iniae, Vibrio vulnificus and Vibrio daceala, Aeromonas hydrophila, Edwardsiella tarda, Erysipelothrix rhusiopathiae, Streptococcus halichoeri has not been known to be a problem in humans.

Streptococcus halichoeri was first reported in seals in Inverness and Cornwall veterinary laboratories in 2004 having been isolated from wounds on grey seals that had been...
inflicted by other seals. Hence it was hypothesized that the organism could be present on the teeth and skin of grey seals. Although there was no evidence of human infections with the organism, an alert was published in the United Kingdom’s Communicable disease Report informing healthcare workers to consider *S. halichoeri* in cases of seal bites.

It remains an open question if the patient’s recent fish contact is a possible source of his *Streptococcus halichoeri* infection.

Routine methods used in the microbiology laboratory may not identify *S. halichoeri*. The commonly used identification systems such as the API 20 Strep (bioMerieux) or Vitek 2 (bioMerieux) are unable to identify this organism as it is not included in their database.

We found that 16s rRNA gene sequencing was not helpful in this case but were pleasantly surprised that MALDI-TOF MS was able to identify this organism with such a good score. Since this was the first time our laboratory had isolated such an organism, we proceeded to perform a variety of biochemical tests to confirm the identity of the organism. *S. halichoeri* is a non-beta-haemolytic Lancefield group B streptococcus and produces acid from cyclodextrin, mannitol, maltose, ribose and pullulan. They are also Voges-Proskauer positive and show activity for arginine dihydrolase, alkaline phosphatase and pyrrolidonyl arylamidase.
Since not all laboratories may have the capability of MALDI-TOF mass spectrometry, they will need to rely on conventional tests to pick up cases of *S. halichoeri*. One indication that the laboratory might have isolated a strain of *S. halichoeri* is that like *S. agalactiae*, it is a Lancefield group B Streptococcus. However unlike *S. agalactiae*, it is non-beta haemolytic and does not hydrolyse hippurate. Such a finding can then trigger further testing with the above biochemical tests to confirm if one is dealing with *S. halichoeri*.

This is the first case of *Streptococcus halichoeri* found in humans. However it could be under diagnosed depending on individual laboratories identification methods. A detailed history of contact with fish and marine animals should be taken in all cases of infection caused by this organism to determine if *Streptococcus halichoeri* could potentially be an emerging zoonotic infection.

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