







CLINICAL PRACTICE ARTICLE

**REVISED** **Invasive *Streptococcus agalactiae* ST283 infection after fish consumption in two sisters, Lao PDR [version 2; peer review: 2 approved]**

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**Abstract**

**Background:** *Streptococcus agalactiae* is a normal commensal of the human gastro-intestinal and female genital tracts. It causes serious disease in neonates and pregnant women, as well as non-pregnant adults. Food-borne outbreaks have also been described. A link between invasive Group B streptococcus (GBS) infection in humans caused by *S. agalactiae* serotype III-4, sequence type 283 (ST283) and the consumption of raw fresh-water fish was first described in Singapore in 2015.

**Case presentation:** We report the simultaneous occurrence of acute fever and myalgia in two sisters who were visiting Laos. Both were found to have invasive GBS ST283 infection, confirmed by blood culture. Infection was temporally linked to fish consumption. They responded well to intravenous antibiotics within 48 hours.

**Conclusions:** Food-borne transmission of *Streptococcus agalactiae* is an important and under-recognised source of serious human disease throughout Southeast Asia and possibly beyond.

**Keywords**

*Streptococcus agalactiae*, food-borne infection, Laos

**Open Peer Review****Approval Status**  

1

2

**version 2**

(revision)

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**version 1**

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view



view

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Any reports and responses or comments on the article can be found at the end of the article.

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**Competing interests:** No competing interests were disclosed.

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**REVISED Amendments from Version 1**

This version (V2) includes some additional clinical details and information about the outcome of the two patients after discharge. We also added an explanation in the discussion about why septic arthritis related to her steroid injections was considered unlikely in the sister with a previous history of joint disease.

**Any further responses from the reviewers can be found at the end of the article**

**Background**

*Streptococcus agalactiae*, or Group B streptococcus (GBS), is a normal commensal of the human gastro-intestinal and female genital tracts. It is also widely distributed in a variety of other animal species<sup>1</sup>. In humans, GBS can cause serious, invasive infection in neonates and pregnant women. In addition, GBS causes systemic infection, including meningitis, septic arthritis, soft tissue infection and endocarditis, in patients with underlying diseases, e.g. type II diabetes or malignancy<sup>2-4</sup>.

A link between GBS invasive infection in humans and food consumption was first described in Singapore in 2015<sup>5</sup>. An outbreak caused by a single virulent GBS strain (serotype III-4, sequence type 283 (ST283)) was strongly associated with raw fish consumption. The outbreak included cases of sepsis and meningitis in previously healthy adults<sup>5-7</sup>. GBS ST283 has recently been described as a cause of invasive human infection throughout SE Asia, including the Lao People's Democratic Republic (Laos), where there is a long-standing tradition of raw fish consumption<sup>8</sup>. We describe two sisters who became infected with GBS ST283 after consuming fish dishes while visiting Laos from the United States.

**Case report**

In early November 2018, the Microbiology laboratory at Mahosot Hospital, Vientiane, Laos received blood culture specimens from two adult inpatients. The patients were Asian sisters, housewives, aged 58 and 55 years, both former Lao refugees and now resident in the USA, who were visiting friends and family in Laos. GBS was isolated from both sets of blood cultures and these were reported promptly to the clinical team on the Infectious Diseases ward.

On further enquiry, the sisters had stayed for two days in Vientiane before travelling to the popular resort town of Vangvieng, north of the capital city, with their relatives. The family (approximately 10 people) had a meal on their first day in Vangvieng called "Larb Pa Nam Khong" (Mekong fish salad). Larb is a signature dish of Laos and North-East Thailand. Larb can be prepared with any kind of fish or meat, which may be raw, semi-cooked or cooked, which is then mixed with other ingredients such as roasted rice, chilli powder, fermented fish, chopped yard long beans, mint leaves and lime juice<sup>9</sup>. Both sisters ordered cooked Larb, but did not know exactly what kind of fish was included in the dish, and then, that evening, the family had dinner in a hotel restaurant which included raw salmon.

The following day, the 58-year-old-sister developed generalized muscle pains, particularly in the large muscles of her arms

and legs. She was unable to walk or raise her arms. This was associated with nausea and vomiting, abdominal cramps and three episodes of watery diarrhoea, without blood or mucus, but no fever. The patient had no significant past medical history, no underlying diseases, was not taking any medications and had no history of allergy.

On the same day, her younger sister, aged 55 years, developed a fever, myalgia and back pain which radiated down both legs, but she had no other symptoms. She had been diagnosed in the USA seven years previously with sciatic nerve root compression and osteo-arthritis by magnetic resonance imaging. She regularly took low dose aspirin and simvastatin (20 mg) for cardiovascular disease prophylaxis. She reported receiving steroid injections into her right knee and ankle for osteo-arthritis shortly before leaving the USA. No other family members were affected.

Both sisters were admitted initially to the provincial hospital in Vangvieng, but were rapidly referred to the Infectious Diseases ward at Mahosot Hospital in Vientiane. On physical examination, no fever (body temperature of 37°C) and no abnormalities were found in the 58-year-old sister. The 55-year-old sister had fever with body temperature of 38°C and inflammation, swelling, redness and pain of the right knee and ankle, although the clinical signs and symptoms were not considered consistent with established septic arthritis and there were no joint effusions so joint aspiration was not performed. Investigations included full blood count, liver function tests, urea, electrolytes, and abdominal ultrasound. All were reported as normal in both patients. In addition, blood was cultured immediately on admission and both patients were started on ceftriaxone 2 g and gentamicin 160 mg (both IV every 24 hours). Oral albendazole 400mg for three days was also commenced to cover for the possibility of intestinal parasitosis.

Both sets of blood cultures flagged as positive within 24 hours, using the BD BACTEC Automated Blood Culture System, and long chains of Gram-positive cocci were seen on Gram staining. Group B  $\beta$ -haemolytic streptococci were isolated subsequently from both patients and confirmed by API 20 STREP (bioMérieux, Basingstoke, UK) (profile 3063014, 99.9% ID). Results were reported promptly to the clinicians on the Infectious Diseases ward. Both isolates were susceptible to penicillin, chloramphenicol, erythromycin and, unusually for GBS, tetracycline, by disc testing, according to the methods of the European Committee on Antimicrobial Susceptibility Testing version 8.0 (2018). Both patients improved within two days of starting empirical treatment with ceftriaxone and gentamicin. They remained in hospital for four days without complications and were discharged well, on oral cefixime 500mg daily for a further 10 days and returned to the USA shortly after discharge.

A food source was considered most likely for these concurrent GBS infections. Unfortunately, no food samples were available for culture. The DNA of the two GBS isolates were extracted and identified as ST283 using a specific polymerase chain reaction (PCR) test at the Tan Tock Seng Hospital (TTSH), Singapore. The two DNA isolates were sent to the Genome Institute of Singapore (GIS), Singapore, for whole genome sequencing (WGS) and confirmed to be ST283 and to cluster with previous GBS ST283 from Lao PDR.

## Discussion

Our report highlights two simultaneous cases of invasive infection by GBS ST283, the same sequence type associated with food-borne transmission in Singapore in 2015. Our patients were middle aged, with no known medical co-morbidities and presented with predominant symptoms of acute musculoskeletal pain, consistent with previous descriptions<sup>6,7</sup>. Of note, a retrospective study in Laos showed 32% of adult patients confirmed with GBS ST283 had meningitis<sup>10</sup>. Our two cases were investigated early and received prompt appropriate treatment before developing more severe disease. Other family members who shared the meal were asymptomatic; potential explanations may include chance or a degree of acquired resistance or immunity in local residents due to repeated exposure. The possibility that the younger sister had been infected during the joint injections in the USA was considered unlikely as she did not report an exacerbation of her joint symptoms following the injection and did not present with signs and symptoms consistent with acute septic arthritis, including the absence of joint effusions meaning that aspiration was not attempted.

Although GBS is a well-known cause of bacteraemia in older adults and those with co-morbidities, both food-borne infection and community-acquired infection in otherwise healthy patients are under-recognised<sup>11,12</sup>. The outbreak of invasive GBS infection in Singapore in 2015 provided the initial observation<sup>5-7,13</sup>. The investigation of that outbreak revealed that a virulent strain of GBS, ST283, caused serious infections in previously healthy and younger adults, including meningo-encephalitis, endocarditis and septic arthritis. It was demonstrated later that infection in these patients was significantly associated with raw freshwater fish consumption<sup>5-7</sup>.

A whole genome sequencing study on GBS isolates, cultured from human and animal samples throughout Southeast Asia,

revealed that 76% of human isolates from Lao PDR from 2000–2017 were ST283<sup>8,10</sup>. The study demonstrated the widespread presence of GBS ST283 in human bacteraemic infections and also in fish, raising the possibility that the majority of adult GBS sepsis cases in the region are foodborne. In SE Asian countries GBS ST283 has been isolated from farmed freshwater fish, particularly Tilapia<sup>8,13</sup>, which is a common ingredient of many fish dishes in Laos.

The concurrent occurrence of the two cases presented here, following consumption of fish dishes, is intriguing and highly suggestive that fish were the source, although unfortunately we could not test the fish they consumed to confirm this. Nonetheless, a clear link between fish consumption and invasive human GBS infection has not been described previously in Laos, and transmission routes have not been studied outside Singapore. Further study is required to confirm the link definitively.

## Consent

Written informed consent for publication of their clinical details was obtained from the patients.

## Data availability

All data underlying the results are available as part of the article and no additional source data are required.

## Acknowledgements

We would like to thank the patients for agreeing for their stories to be published. Also, thanks to Dr Siho Sisouphone, Dr Ko Chang, and Dr Chirapha Darasavath, who provided history and clinical details of the patients and Professor Elizabeth Ashley, who reviewed and commented on this report.

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# Open Peer Review

Current Peer Review Status:  

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## Version 1

Reviewer Report 20 June 2022

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**Kirsty Le Doare** 

Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St George's, University of London, London, UK

This is a very well written case report of two sisters with invasive GBS disease with temporal association to raw fish consumption. Although the authors were not able to identify the GBS in the fish dishes, they have made a good attempt to describe the associations with this phenomenon.

For the 55 year old sister, she had steroid injections prior to arrival in Laos. How long before and was she also investigated for septic arthritis as she had fever and pain in these joints? Could the infection be due to seeding following the steroid injections?

I have no other comments and look forward to seeing the full genetics when available as this will add to the debate about zoonotic infection with GBS in SE Asia.

**Is the background of the cases' history and progression described in sufficient detail?**

Yes

**Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?**

Yes

**Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?**

Yes

**Is the conclusion balanced and justified on the basis of the findings?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Group B Streptococcal infection and vaccines, neonatal and maternal infections

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 09 Nov 2022

**Manophab Luangraj,**

Thanks for your great comments and suggestions. Regarding to your comments and queries, please see our responses below.

**For the 55 year old sister, she had steroid injections prior to arrival in Laos. How long before and was she also investigated for septic arthritis as she had fever and pain in these joints? Could the infection be due to seeding following the steroid injections?**

*We do not have details as to precisely how long before coming to Laos she had the injections, although it was shortly before travel. As noted in our response to Reviewer one, she did not have clinical signs and symptoms consistent with established acute septic arthritis and there was no evidence of joint effusions. In the circumstances, aspiration of the joint was not performed. She also did not report any exacerbation of her joint symptoms following the injections. We therefore feel that it is unlikely that her infection was the result of seeding her joints during the injections and have added a comment to this effect in the case report. ("Discussion" section, line 9-13 of 1<sup>st</sup> paragraph)*

**Competing Interests:** No competing interests were disclosed.

Reviewer Report 11 May 2022

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**Ho-Man Yeung**

Department of Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA

Overall, this is a very well written case of two women traveling to Laos who were subsequently diagnosed with GBS bacteremia. The history including the traveling and food consumption history were clearly written. Both patients had gastrointestinal symptoms the day after consumption of contaminated fish, found to have bacteremia, that was treated with 14 days of antibiotics.

It is possible that the patients had viral gastroenteritis, followed by secondary GBS translocation seeding to the bloodstream. However, the authors did a great job establishing a temporal connection of infection to food consumption with circumstantial evidence, even without specimen

of the contaminated food. The fact that both sisters had same presentation further support a primary GBS infection.

I have several suggestions/clarifications.

1. What was the clinical outcome after treatment? I presume they were discharged and returned to the USA.
2. Did the younger patient also have septic arthritis? Her exam finding in the setting of bacteremia would be suspicious.
3. It would be useful to include information about the virulence factors of GBS leading to gastrointestinal infection.

**Is the background of the cases' history and progression described in sufficient detail?**

Yes

**Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?**

Yes

**Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?**

Yes

**Is the conclusion balanced and justified on the basis of the findings?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medicine

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 09 Nov 2022

**Manophab Luangraj,**

Thanks for your great comments and suggestions through our case report. Regarding to your comments and queries, please see our answers below the questions.

**Q1: What was the clinical outcome after treatment? I presume they were discharged and returned to the USA.**

*The two patients improved clinically after IV treatment with 4 doses of ceftriaxone. They were discharged on oral cefixime to complete a 2-week course as outpatients. We heard later from their Lao cousin that they returned to the USA a few days after discharge. We have added this information into the case report ("case report" section, line 11 of 6<sup>th</sup> paragraph)*

**Q2: Did the younger patient also have septic arthritis? Her exam finding in the setting of bacteremia would be suspicious.**

*Even though the younger patient had underlying osteo-arthritis, on admission to hospital she did not have clinical signs and symptoms consistent with established acute septic arthritis and there was no evidence of joint effusions. In the circumstances, aspiration of the joint was not performed. We cannot therefore exclude very early stage septic arthritis and have added a comment to this effect in the case report ("case report" section, line 5-7 of 5<sup>th</sup> paragraph).*

**Q3: It would be useful to include information about the virulence factors of GBS leading to gastrointestinal infection.**

*Substantial sequencing of ST283 has been done on opportunistic collections from both human and non-human sources throughout Southeast Asia. Initial large-scale genomic comparisons have shown several thousand genetic changes (including both SNPs and larger changes such as gene gain/loss and prophage gain/loss) that are conserved among all human disease-causing ST283; from a simple sequence analysis point of view, any individual or combination of these changes could be related to virulence. The addition of these two cases does not offer substantial additional data to answer this question of ST283 virulence, which will likely require experimental models of infection. Therefore, we prefer not to use these two genome sequences to speculate on virulence. The value of this case report instead is in terms of validating the hypothesis of foodborne transmission in countries outside Singapore, which is currently the only place where it has been confirmed.*

**Competing Interests:** No competing interests were disclosed.