Group B *Streptococci* in Sushi and Sashimi

Dear Editor,

A large outbreak of invasive Group B *streptococcus* (GBS) infection occurred in Singapore in 2015 which was associated with the consumption of Chinese-style raw fish (yusheng) porridge.[1] The GBS clone responsible belonged to *serotype III, ST283*.[2] We sought to determine if GBS could also be found in Japanese-style sushi and sashimi.

We collected 17 fish sold ready-to-eat (12 salmon, 4 'tai', 1 tilapia) from various Japanese food outlets (n = 8), and supermarkets (n = 4). Fish purchased were kept in their original containers and immediately transported by ice-box back to the laboratory. The fish species were chosen because GBS tends to cause a problem with freshwater fish. 'Tai' is normally understood to be a saltwater fish, the red sea bream (*Pagrus major*). However, we wanted to know if cheaper freshwater fish was being used as a substitute for genuine 'tai' as this is a common practice in many countries.[3]

The fish meat was processed using a modification of the protocol described by van der Mee-Marquet et al;[4] 25 g of fish was macerated in 225 mL of Todd-Hewitt broth containing 8 µg/mL polymixin B and 32 µg/mL nalidixic acid using a stomacher before incubating 24 hours at 35˚C to 37˚C. The next day, 1 mL of the broth was removed and transferred to 5 mL of brain heart infusion containing 8 µg/mL polymixin B and 32 µg/mL nalidixic acid, and incubated at 35˚C to 37˚C for a further 24 hours. The following day, a loopful of the broth was plated onto chromID® Strepto B (bioMérieux) agar plates which were incubated at 35˚C to 37˚C for up to 48 hours. Pink, red, and violet colonies were selected for further workup. Polymerase chain reaction (PCR) serotyping was performed directly on deoxyribonucleic acid (DNA) extracted from the incubated broth if no GBS was isolated.[5]

Table 1. Characteristics of Group B *Streptococci* Isolated from Fish Meat

Fish Species	Salmon	Tilapia
PCR serotype	*II*	*Ia*
MLST	*ST1*	*ST7*
Antimicrobial susceptibility		
Penicillin	S*	S
Erythromycin	S	S
Clindamycin	S	S
Tetracycline	R†	S

MLST: Multi-locus sequence typing; PCR: Polymerase chain reaction
*Susceptible.
†Resistant.

We were able to isolate GBS from 2 samples (salmon and tilapia). The isolates were identified using matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) and characterised by antimicrobial susceptibility testing,[6] PCR serotyping, and multi-locus sequence typing (MLST).[7] The results are summarised in Table 1. In addition, we were able to obtain PCR sequences consistent with GBS belonging to *serotype Ia* and *MLST ST7* from broth culture of 'tai' meat even though culture was unsuccessful.

PCR amplification and sequencing for the cytochrome *c* oxidase subunit *I gene* was performed on DNA extracts of the 4 'tai' samples.[8] The sequences were compared with those in the GenBank and the Fish-Bol database (http://www.fishbol.org/). Two samples were red sea bream *(Pagrus major)*. One was crimson snapper *(Lutjanus erythropterus)*, this was the broth sample that had sequences for *serotype Ia, ST7* GBS. The other sample was a Nile tilapia *(Oreochromis niloticus)*.

Foxman et al showed that fish consumption was an independent risk factor leading to the colonisation of the human gut with *serotype Ia* and *Ib* GBS.[9] *Serotype Ia, ST7* GBS is a well known fish pathogen and may be just emerging as a human pathogen.[10,11] The clinical significance of *serotype II, ST1* GBS is uncertain. It is possible that this is not a fish-specific clone and may have resulted from human contamination.

In this study, we did not find any virulent *serotype III, ST283* GBS. However, this could also be because the sample size was small, and it is improper to draw a firm conclusion on the absence of the virulent *serotype III, ST283* GBS, from fish meat. It is an important principle in science that the absence of evidence of a marker (or phenomenon) does not constitute evidence of the absence of that marker (or phenomenon). We were able to show the presence of other GBS and evidence of fish substitution, so there remains a potential for zoonotic GBS infection from the consumption of sushi/sashimi.

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