# Group B *Streptococcus* Infections Caused by Improper Sourcing and Handling of Fish for Raw Consumption, Singapore, 2015–2016

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**Learning Objectives**

Upon completion of this activity, participants will be able to:

- Evaluate clinical and epidemiological findings of a human group B *Streptococcus* outbreak in Singapore based on a microbial study of raw fish and human samples
- Determine the pathogenic potential of fish and human samples after a human group B *Streptococcus* outbreak in Singapore, based on a microbial study of these samples
- Assess the public health implications of a human group B *Streptococcus* outbreak in Singapore, based on a microbial study of raw fish and human samples.

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We assessed microbial safety and quality of raw fish sold in Singapore during 2015–2016 to complement epidemiologic findings for an outbreak of infection with group B *Streptococcus* serotype III sequence type (ST) 283 associated with raw fish consumption. Fish-associated group B *Streptococcus* ST283 strains included strains nearly identical (0–2 single-nucleotide polymorphisms) with the human outbreak strain, as well as strains in another distinct ST283 clade (57–71 single-nucleotide polymorphisms). Our investigations highlight the risk for contamination of freshwater fish (which are handled and distributed separately from saltwater fish sold as sashimi) and the need for improved hygienic handling of all fish for raw consumption. These results have led to updated policy and guidelines regarding the sale of ready-to-eat raw fish dishes in Singapore.

A major outbreak of group B *Streptococcus* (GBS) infection associated with consumption of a Chinese-style raw fish dish (*yusheng*) occurred in Singapore during 2015 and involved 238 persons during the first half of the year (*1*). The *yusheng* was typically made from sliced Asian bighead carp (*Hypophthalmichthys nobilis*) and snakehead (*Channa* spp.) and served as a side dish with porridge by food stalls within larger eating establishments. Persons with severe clinical cases had meningoencephalitis, bacteremia, and septic arthritis (*2*–*4*). GBS, or *Streptococcus agalactiae*, was identified as the causative agent (*2*,*3*).

GBS is found in ≈30% of healthy adults (*5*) and is a member of the human commensal gastrointestinal and genitourinary flora (*4*). GBS is a common cause of neonatal sepsis, is acquired by newborns from the vaginal flora of the mother, and is an increasingly common pathogen among vulnerable populations (*6*). The incidence of invasive disease in adults, particularly older adults, has been increasing (*7*,*8*). GBS is also a fish and bovine pathogen (*9*). Although GBS has been shown to colonize the gastrointestinal tract of humans linked to fish consumption (*9*), foodborne transmission leading to invasive disease has not been reported. Local epidemiologic investigations conducted separately (*2*,*3*) identified a single strain of GBS serotype III sequence type (ST) 283 as the causative agent of the outbreak in Singapore during 2015. GBS ST283 had previously been isolated from tilapia in Thailand (*10*) and in adult human cases in Hong Kong (*11*). However, GBS ST283 has not been reported to colonize the human gastrointestinal tract, although to date only 1 study of fish mongers and fish handlers has specifically looked for colonization by this strain (*12*).

We investigated microbial safety and quality of fish sold in the Singapore market during and after the outbreak during 2015 to trace the source of GBS ST283 and provide risk assessment data to support outbreak control and prevention measures. Shortly after identification of GBS ST283 as the cause of the outbreak, these data supported implementing a ban on the sale of ready-to-eat (RTE) dishes containing raw freshwater fish, as well as imposing additional requirements for sale of RTE raw fish dishes made with saltwater fish (*13*). We report the results of our analysis, which might assist the review of guidelines for handling of fish meant for raw consumption in Singapore and other countries. This report offers unique food and environmental insights into the investigation of this outbreak and complements published epidemiologic findings (*2*,*3*).

## Materials and Methods

### Collection of Fish and Fish Tank Water Samples

We collected samples of fish commonly used for raw consumption (n = 997) and fish tank water for holding live freshwater fish (n = 102) along the supply chain in Singapore during August 2015–January 2016 (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/23/12/17-0596-Techapp1.pdf). We tested samples for GBS, *Aeromonas* spp., *Listeria monocytogenes*, *Salmonella* spp., *Vibrio cholerae*, and *V. parahaemolyticus*, and determined *Escherichia coli* counts, *Staphylococcus aureus* counts, and standard plate counts (SPCs) (online Technical Appendix). We characterized selected species to determine their virulence potential (online Technical Appendix).

### Statistical Analysis

We evaluated significant differences ($p<0.05$) between bacterial counts ($\log_{10}$ CFU/g) and presence of specific foodborne bacteria by using Kruskal-Wallis, Mann-Whitney, $\chi^2$, and Fisher exact tests as appropriate. We performed analysis by using SPSS version 24.0 software (IBM, Armonk, NY, USA).

## Results

### Raw Fish Samples from Food Stalls and Restaurants/Snack Bars

Although raw freshwater and saltwater fish were served as RTE food at food stalls, only raw saltwater fish were reportedly served at restaurants/snack bars. PCR positivity rates were 43.5% (20/46) for GBS and 23.9% (11/46) for GBS serotype III in sliced fish samples from food stalls. Fish sampled from restaurants/snack bars had significantly lower rates ($p<0.05$) of 9.2% (26/282) for GBS and 0.7% (2/282) for GBS serotype III (Table). Among the 20 GBS PCR-positive samples from food stalls, 5 yielded isolates; these isolates were of serotype II ST652, serotype III ST283, serotype III ST335, and serotype V ST1 (online Technical Appendix Table 3). The GBS ST283 isolated was from a RTE sliced fish sample sold as grass carp collected from a food stall linked to a human case, as described (*12*). We did not detect GBS ST283 in samples from restaurant/snack

bars; however, we did find a range of other GBS, including serotypes Ia ST7, Ia ST103, Ia ST485, III ST651, III ST861, V ST1, V ST24, VI ST167, and VII ST1.

We found *Salmonella* serogroup B ST29 (serovar Stanley) (n = 2); *V. parahaemolyticus* (negative for *tdh*, *trh1*, and *trh2* genes) (n = 1); and non–O1 *V. cholerae* (n = 1) in freshwater fish samples from food stalls. We also isolated *V. cholerae* from saltwater fish samples, 1 from a food stall and 1 from a restaurant. We detected *L. monocytogenes* in 5 samples from restaurants/snack bars.

SPCs of most RTE raw freshwater (71.4%, 5/7) and saltwater (85.7%, 18/21) fish samples from food stalls exceeded the regulatory limit for RTE food (5 $\log_{10}$ CFU/g) in Singapore (*14*). We observed no difference in SPCs for fish slices intended for raw consumption and cooking purposes (Figure 1). We also found that 24.8% (70/282) of saltwater fish samples from restaurants/snack bars did not comply with regulatory limits for SPCs, *E. coli* counts (1.3 $\log_{10}$ CFU/g), or both (*14*). These results showed the poor quality of RTE raw freshwater and saltwater fish sold at food stalls in comparison to those sold at restaurants and snack bars.

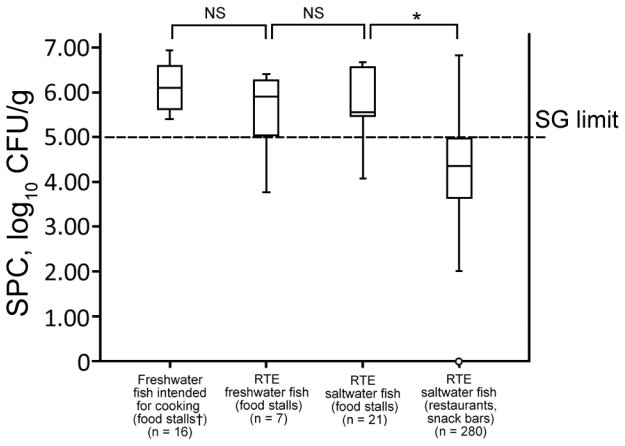


**Figure 1.** SPCs for sliced fish samples collected from various eating establishments during investigation of group B *Streptococcus* infections, Singapore, 2015–2016. Dashed horizontal line indicates regulatory limit of Singapore for SPCs for ready-to-eat foods (<5 $\log_{10}$ CFU/g) (*14*). Top and bottom of boxes in plots indicate 25th and 75th percentiles, horizontal lines indicate medians, and whiskers indicate minimum and maximum values. *p<0.05. †Food stalls housed within larger eating establishments that include hawker centers, coffee shops, and eating houses. Open circle indicates an outlier. NS, not significant (p>0.05); RTE, ready to eat; SG, Singapore government; SPCs, standard plate counts.

### Comparison of Freshwater and Saltwater Fish Samples from Fresh Produce Markets

Fish sold at food stalls were typically procured from local fresh produce markets. For the 62 samples of whole fish and fish parts we collected from these markets, we detected GBS ST283 in 28.2% (11/39) of the freshwater fish (Table), which included fish sold as tilapia, Asian bighead carp, grass carp, snakehead-haruan, snakehead-toman, and silver carp (online Technical Appendix Table 3). However, we did not detect GBS ST283 in saltwater fish. Other GBS strains detected among these fish include serotypes Ia ST7, Ia ST23, Ia ST24, and II ST28 (online Technical Appendix Table 3).

We detected *Aeromonas* spp. (48.4%, 30/62), *S. aureus* (27.4%, 17/62), non–O1 *V. cholerae* (12.9%, 8/62) and *V. parahaemolyticus* (negative for *tdh*, *trh1*, and *trh2* genes) (6.4%, 4/62) in fish samples from fresh produce markets. There was no difference in positivity rates of these organisms between freshwater and saltwater fish. We did not detect *L. monocytogenes* or *Salmonella* spp. in any fish samples collected from fresh produce markets.

Approximately 42% (15/36) of freshwater fish muscle samples had SPCs or *E. coli* counts, or both, exceeding regulatory limits for RTE food in Singapore (*14*). Positivity rates for GBS, GBS serotype III, and *E. coli*, as well as SPCs for saltwater fish, were significantly lower (p<0.05) (Figure 1; Table). *E. coli* and *S. aureus* counts for freshwater fish surfaces were significantly higher (p<0.05) than those for saltwater fish (Figure 2).

We collected 4 fish tank water samples from wet markets and supermarkets. One water sample and the live freshwater fish the tank contained were positive for GBS by PCR and non–O1 *V. cholerae* by culture; the associated fish was positive for GBS ST283 by culture. Two other fish tank water samples and the live fish the tanks contained were positive for *E. coli, S. aureus*, or both. The level of *E. coli* detected in each positive fish tank water sample was 1.3 log10 CFU/500 mL, which was greater than the l $\log_{10}$ CFU/500 mL coliform (which includes *E. coli*) limit set by the British Columbia Centre for Disease Control (*15*).

### Whole Freshwater Fish and Fish Tank Water from Ports

We tested for GBS only in whole fish and fish tank water samples collected from ports. We detected GBS ST283 in 1% (6/586) of freshwater fish samples; positive samples were from Asian bighead carps imported from and farmed in Malaysia. For 98 fish tank water samples collected from ports, 55.1% (54/98) were positive for GBS, and 6.1% (6/98) were positive for GBS ST283. Three of the GBS ST283–positive fish were kept in fish tank water that was also positive for GBS ST283.

### Comparison of Saltwater Fish from Fresh Produce Markets and Sashimi Suppliers

Our data indicate the risk for contamination of fish sold at local fresh produce markets, although saltwater fish samples from fresh produce markets had lower rates of

**Table.** Positivity rates for GBS and other foodborne bacteria in fish samples, Singapore, 2015–2016*

| | Targeted bacteria, no. positive samples/no. tested (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Characteristic | All GBS | GBS serotype III | GBS serotype III ST283 | *Aeromonas* spp.† | *E. coli* | *S. aureus* | *V. c.* | *V. p.* | *L. m.* | *Salmonella* spp. |
| Detection method | PCR | PCR | Culture, PCR | Culture | Culture | Culture | Culture | Culture | Culture | Culture |
| Ports | | | | | | | | | | |
| Freshwater fish, n = 586 | 27/586 (4.6) | 12/586 (2.0) | 6/586 (1.0) | NT | NT | NT | NT | NT | NT | NT |
| Fresh produce markets‡ | | | | | | | | | | |
| Freshwater fish, n = 39 | 30/39 (76.9)[a] | 14/39 (35.9)[b] | 11/39 (28.2) | 16/39 (41.0) | 32/39 (82.0)[c] | 11/39 (28.2) | 6/39 (15.4) | 2/39 (5.1) | 0/39 | 0/39 |
| Saltwater fish, n = 23 | 5/23 (21.7)[a] | 2/23 (8.7)[b] | 0/23 | 14/23 (60.9) | 8/23 (34.8)[c] | 6/23 (26.1) | 2/23 (8.7) | 2/23 (8.7) | 0/23 | 0/23 |
| Sashimi suppliers§ | | | | | | | | | | |
| Saltwater fish, n = 21 | 0/21 | 0/21 | 0/21 | 10/21 (47.6) | 1/21 (4.7)[c] | 0/21 | 0/21 | 0/21 | 1/21 (4.7) | 0/21 |
| Food stalls¶ | | | | | | | | | | |
| RTE freshwater fish, n = 7 | 5/7 (71.4) | 4/7 (57.1) | 1/7 (14.3) | NT | 0/7 | 0/7 (0) | 0/7 | 0/7 | 0/7 | 1/7 (14.3) |
| Freshwater fish for cooking, n = 18 | 8/18 (44.4) | 4/18 (22.2) | 0/18 | NT | 2/18 (11.1) | 0/18 | 1/18 (5.6) | 1/18 (5.6) | 0/18 | 1/18 (5.6) |
| RTE saltwater fish, n = 21 | 7/21 (33.3)[d] | 3/21 (14.3)[e] | 0/21 | NT | 0/21 | 0/21 | 1/21 (5.0) | 0/21 | 0/21 | 0/21 |
| Restaurants, snack bars | | | | | | | | | | |
| RTE saltwater fish, n = 282 | 26/282 (9.2)[d] | 2/282 (0.7)[e] | 0/282 | NT | 0/282 | 0/282 | 1/282 (0.4) | 0/282 | 5/282 (1.8) | 0/282 |

*A sample was considered positive when a specific organism was detected in ≥1 subsamples (surface, muscle, or organs) of a fish sample. Superscript letters a–e indicate a significant difference ($p<0.05$) in positivity rates of targeted bacteria between fish types. *E. coli, Escherichia coli*; GBS, group B *Streptococcus*; *L. m.*, *Listeria monocytogenes*; NT, not tested; RTE, ready to eat; *S. aureus*, *Staphylococcus aureus*; ST283, sequence type 283; *V. c.*, *Vibrio cholerae*; *V. p.*, *V. parahaemolyticus*.
†*Aeromonas caviae*, *A. hydrophila*, and *A. sobria*.
‡Fish stalls at ports and wet markets, and fresh produce sections of supermarkets, excluding sashimi and sushi counters of supermarkets.
§Companies that supplied sashimi-grade fish to restaurants and snack bars.
¶Within larger eating establishments that include hawker centers, coffee shops, and eating houses.

contamination than freshwater fish samples. The SPCs and the positivity rates for *E. coli* in saltwater fish samples from sashimi suppliers were significantly lower ($p<0.05$) than those for saltwater fish samples from fresh produce markets (Figure 3; Table), which suggested that the microbial quality of fish could be managed by improvements in handling throughout distribution channels. None of the saltwater fish muscle samples from sashimi suppliers exceeded the Singapore SPC (5 $\log_{10}$ CFU/g) and *E. coli* (1.3 $\log_{10}$ CFU/g) limits for RTE food (*14*). We did not detect GBS, *S. aureus*, *V. cholerae*, and *V. parahaemolyticus* in any fish samples collected from sashimi suppliers. However, we detected *L. monocytogenes* in 1 salmon sample.

### Characterization of GBS Isolates

We detected 6 GBS serotypes (Ia, II, III, V, VI, and VII) and 13 STs (1, 7, 23, 24, 28, 103, 167, 283, 335, 485, 651, 652, and 861) in fish (online Technical Appendix Table 3). Although most strains were within clonal complexes (1, 10, 17, 19, and 23) associated with human carriage and diseases (*16*), a total of 20 isolates from 7 sashimi samples (SGEHI2015-IV45, SGEHI2015-IV72, SGEHI2015-IV74, SGEHI2015-IV89, SGEHI2015-IV100, SGEHI2015-IV211, and SGEHI2015-IV232) did not belong to these clonal complexes. These strains had few closely related strains in the public genomic databases, and the closely related strains are mostly from animals (online Technical Appendix Figure 2).

We found GBS ST283 only among freshwater fish and water for holding freshwater fish. Genomic analyses indicated that GBS ST283 isolated from fish clustered in 2 clades (Figure 4). The first clade included 12 isolates from 6 fish from a food stall, a fresh produce market and a port, and 4 fish tank water samples from a port. Genome sequencing showed that these 12 isolates were nearly identical (0–2 SNPs and 0, 1, and 12 indels all in homopolymeric runs of >4 nt) compared with the 2.1-Mbp genome of the reference human outbreak strain, SG-M1, isolated from a meningitis patient during the GBS outbreak in Singapore during 2015 (*12,17*). Isolates that clustered into the second clade were 20 isolates from 12 fish and 2 fish tank water samples and did not include any human isolates either from this outbreak or from previous reports of human GBS infecting isolates. Sequences of these isolates showed higher intraclade diversity (57–71 SNPs and 11–33 indels) when compared with the SG-M1 genome (Figure 4).

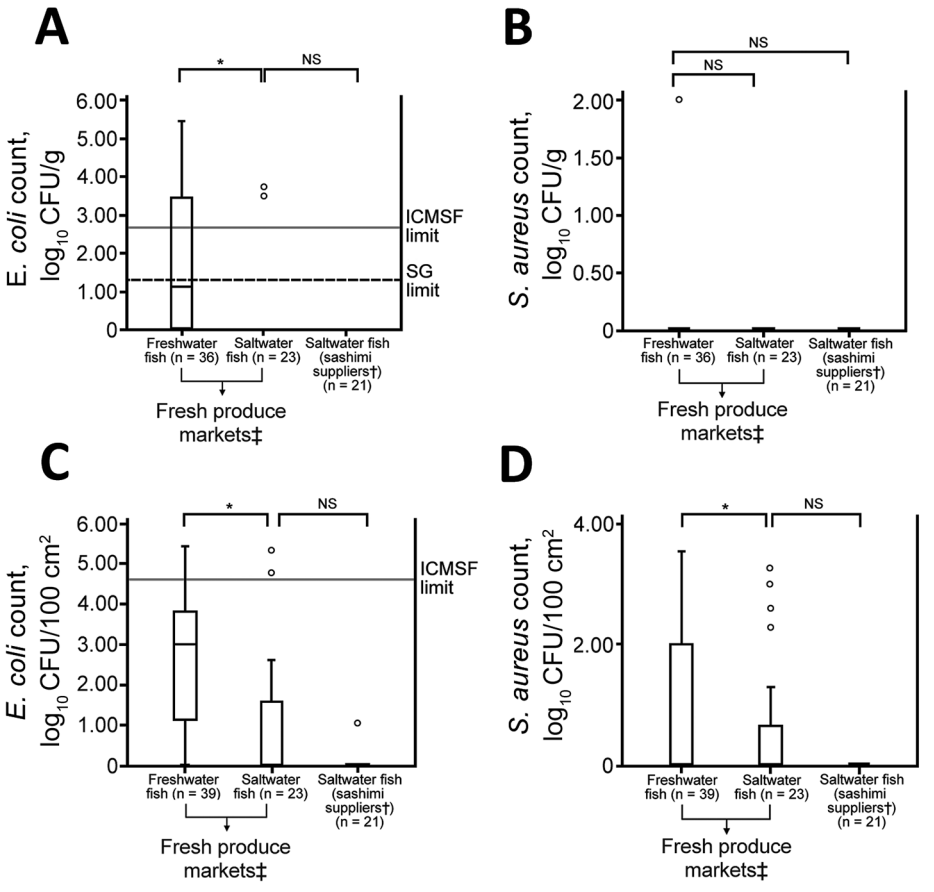


**Figure 2.** *Escherichia coli* (A and C) and *Staphylococcus aureus* (B and D) counts in fish samples (muscle and surface swabs) collected from fresh produce markets during investigation of group B *Streptococcus* infections, Singapore, 2015–2016. Solid horizontal lines indicate ICMSF limit for *E. coli* count in fresh fish intended for cooking (<2.7 $\log_{10}$ CFU/g or <4.7 log10 CFU/100 $cm^2$) (*23*). Dashed horizontal line indicates Singapore regulatory limit for *E. coli* count in ready-to-eat foods (<1.3 $\log_{10}$ CFU/g ) (*14*). Top and bottom of boxes in plots indicate 25th and 75th percentiles, horizontal lines indicate medians, and whiskers indicate minimum and maximum values. Open circles indicate outliers. *p<0.05. †Companies that supplied sashimi grade fish to restaurants and snack bars. ‡Fish stalls at ports and wet markets, as well as fresh produce sections of supermarkets, excluding sashimi and sushi counters of supermarkets. ICMSF, International Commission on Microbiological Specifications of Foods; NS, not significant (p>0.05); SG, Singapore government; SPCs, standard plate counts.

### Characterization of *S. aureus, V. cholerae*, and *V. parahaemolyticus* Isolates

We characterized 18 *S. aureus* isolates from 17 fish. All except 1 were obtained from fish surfaces. We detected >1 enterotoxin gene in two thirds of these isolates and the *sec* gene in 55.6% (10/18) of the isolates. Other enterotoxin genes (*sea, seg, seh, sei,* and *sel*) were detected at much lower rates (5.6% [1/18] to 11.1% [2/18]). We detected 4 enterotoxin genes (*sec, seg, sei,* and *sel*) in a *S. aureus* isolate obtained from the surface of a wolf herring sample collected from a port. We did not detect virulence genes (*ctxA, ctxB,* and *tcpA*) in any of the 16 non–O1 *V. cholerae* isolates from 9 fish and 1 fish tank water samples and did not detect virulence genes (*tdh, trh1,* and *trh2*) in any of the 6 *V. parahaemolyticus* isolates from 5 fish samples.

## Discussion

We found GBS ST283, the causative strain of a severe foodborne outbreak in Singapore, in the local freshwater fish supply chain that stretches from food stalls to local fresh produce markets and back to ports. Patients with GBS ST283 infections during this outbreak were more likely to show development of meningoencephalitis, septic arthritis, and spinal infection than were persons with non–GBS ST283 infections (*12*). Although this study suggested Malaysia as a source of the strain, the finding of the same ST in Hong Kong and Thailand (*10*,*11*) suggested that GBS ST283 is generally prevalent throughout the region.

Our analysis shows that there are at least 2 clades of GBS ST283 strains among fish in local markets. Fish and water strains from 1 clade were nearly identical to clinical strains from this outbreak (Figure 4). The small variability of 0–2 SNPs and 0–12 indels between fish and water strains and the reference human outbreak strain (SG-M1) is equivalent to variability observed in 131 clinical strains from the same outbreak reported elsewhere (0–5 SNPs from the SG-M1 reference) (*12*). Strains from a second clade of GBS ST283 had a difference of 57–71 SNPs and 11–33 indels when compared with the SG-M1 genome. Other GBS ST283 isolates, many collected in Hong Kong ≤17 years before this outbreak (*11*) are also different from the SG-M1 strain (≤129 SNPs) (Figure 4). We found no human-infecting isolate from Singapore or elsewhere within the second fish-associated GBS ST283 clade.

A major issue is whether all GBS ST283 strains are capable of causing invasive human disease by the foodborne route. If strains from the fish-associated clade are not pathogenic to humans, they could be used as effective controls for identifying the genetic basis of pathogenicity

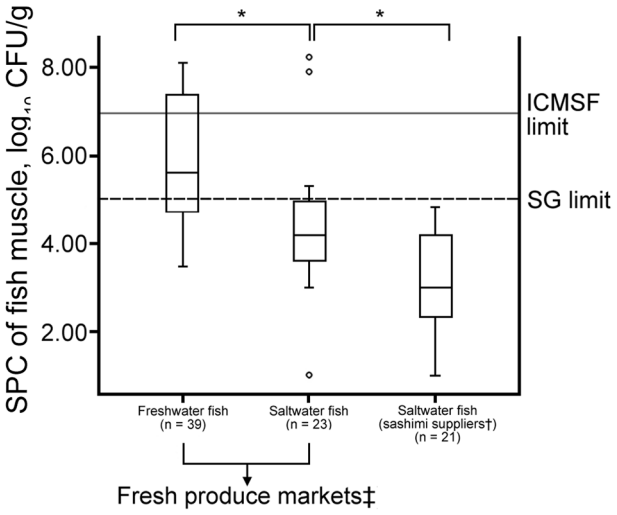


**Figure 3.** SPCs for fish samples (muscle) collected from fresh produce markets during investigation of group B *Streptococcus* infections, Singapore, 2015–2016. Solid horizontal line indicates ICMSF limit for SPCs in fresh fish intended for cooking (<7 $\log_{10}$ CFU/g) (*23*). Dashed horizontal line indicates Singapore regulatory limit for SPCs for ready-to-eat foods (<5 $\log_{10}$ CFU/g) (*14*). Top and bottom of boxes in plots indicate 25th and 75th percentiles, horizontal lines indicate medians, and whiskers indicate minimum and maximum values. Open circles indicate outliers. *p<0.05. †Companies that supplied sashimi grade fish to restaurants and snack bars. ‡Fish stalls at ports and wet markets, as well as fresh produce sections of supermarkets, excluding sashimi and sushi counters of supermarkets. ICMSF, International Commission on Microbiological Specifications of Foods; SG, Singapore government; SPCs, standard plate counts.

of the first clade and the cause of its emergence, which resulted in outbreak in Singapore in 2015. If these strains are pathogenic to humans, then broader tracking of the prevalence of GBS ST283 would be warranted.

In contrast to GBS strains that are known to cause disease outbreaks in fish (*10*,*18*), the live and whole fish from which GBS ST283 was recovered in this study did not have defects, such as corneal opacity and exophthalmia (*18*), which suggests that this ST might not be pathogenic for freshwater fish. The closest GBS fish pathogens with published genomes, GD201008–001 (*19*) and HN016 (*20*), are serotype Ia ST7 strains that are distant (>4,000 SNPs) from all ST283 strains that our group and others have identified (*12*).

Detection of 6 GBS serotypes and 13 STs showed the diversity of GBS strains in fish. Although the sample size in this study was small and our results might not represent the distribution of GBS in all fish species, our findings provide valuable data for characterizing the public health risk from consuming raw fish. No baseline information on GBS in fish was publicly available before this outbreak because fish were not a recognized source or a recognized route of transmission of GBS. Further work on GBS STs other than ST283 is underway to investigate the role of fish as a source of GBS disease in humans.

Several GBS strains from sashimi had relatively few closely related strains in the public genomic databases (online Technical Appendix Figure 2), which suggests that the GBS population associated with saltwater fish could be different from that associated with freshwater fish and humans. Another reason for this observation is that GBS from food and environmental sources are relatively undersampled in the genomic databases than those from humans.

We detected GBS serotypes Ia ST23 and Ia ST7, which are associated with human carriage (*10*), in fish samples. Although GBS ST7 has been described as a fish pathogen, the presence of GBS serotype Ia ST23 has not been reported in fish (*10*). GBS serotypes Ia ST23, and Ia ST7 and *E. coli*, which are all associated with human gut flora, suggest possible contamination of fish by effluent water.

The intentional introduction of animal feces into fish ponds as part of integrated farming (*21*,*22*) might further contribute to the complex flow of pathogens between animals and humans. Such findings point to areas for research to clarify the diversity and role of GBS strains in affecting animal and human health. For instance, GBS ST861, which was isolated from salmon in this study (online Technical Appendix Table 3), was also isolated from a clinical case in the same year in Singapore on the basis of metadata available in the PubMLST *S. agalactiae* database (http://pubmlst.org/sagalactiae/).

In addition to the finding of GBS ST283 in freshwater fish, detection of high SPCs and *E. coli* and *S. aureus* counts indicates the hazard of using such fish for preparing raw RTE dishes. Because *E. coli* is not part of the intestinal flora of cold-blooded animals (*23*), its presence suggests contamination from polluted water, unhygienic handling, or temperature abuse after harvesting. Similarly, because *S. aureus* is not part of usual fish flora, its presence on fish surfaces suggests possible transfer of human skin flora caused by unhygienic handling (*24*). We detected *V. parahaemolyticus*, an organism known to grow well in seawater but lyse rapidly in freshwater (*24*), in freshwater fish samples from fresh produce markets (5.1%, 2/39). This finding was not surprising because freshwater and saltwater fish are typically sold, handled, stored, and degutted within the same confined areas in fresh produce markets. Thus, despite lower SPCs and positive rates for *E. coli* in saltwater fish than in freshwater fish from fresh produce markets, saltwater fish procured from such environments are prone to cross-contamination.

Fish used by food stalls were generally obtained from such markets. Moreover, microbial counts for sliced fish samples from eating establishments indicated that most food stalls were not able to prepare RTE raw fish dishes of acceptable hygiene quality. Poor practices observed included

use of common chopping boards, knives, or slicers for preparing fish slices meant for raw consumption and cooking. If fish slices are contaminated, rinsing with water cannot improve their quality (online Technical Appendix).

In contrast to the quality of saltwater fish samples from fresh produce markets, all saltwater fish samples from sashimi suppliers complied with local SPCs (5 $\log_{10}$ CFU/g) and *E. coli* (1.3 $\log_{10}$ CFU/g) limits for ready-to-eat food (*14*); all samples were negative for GBS, *S. aureus*, *Salmonella* spp., and *V. parahaemolyticus*. The compliance rate among restaurants/snack bars was higher because such premises are more likely to procure fish from

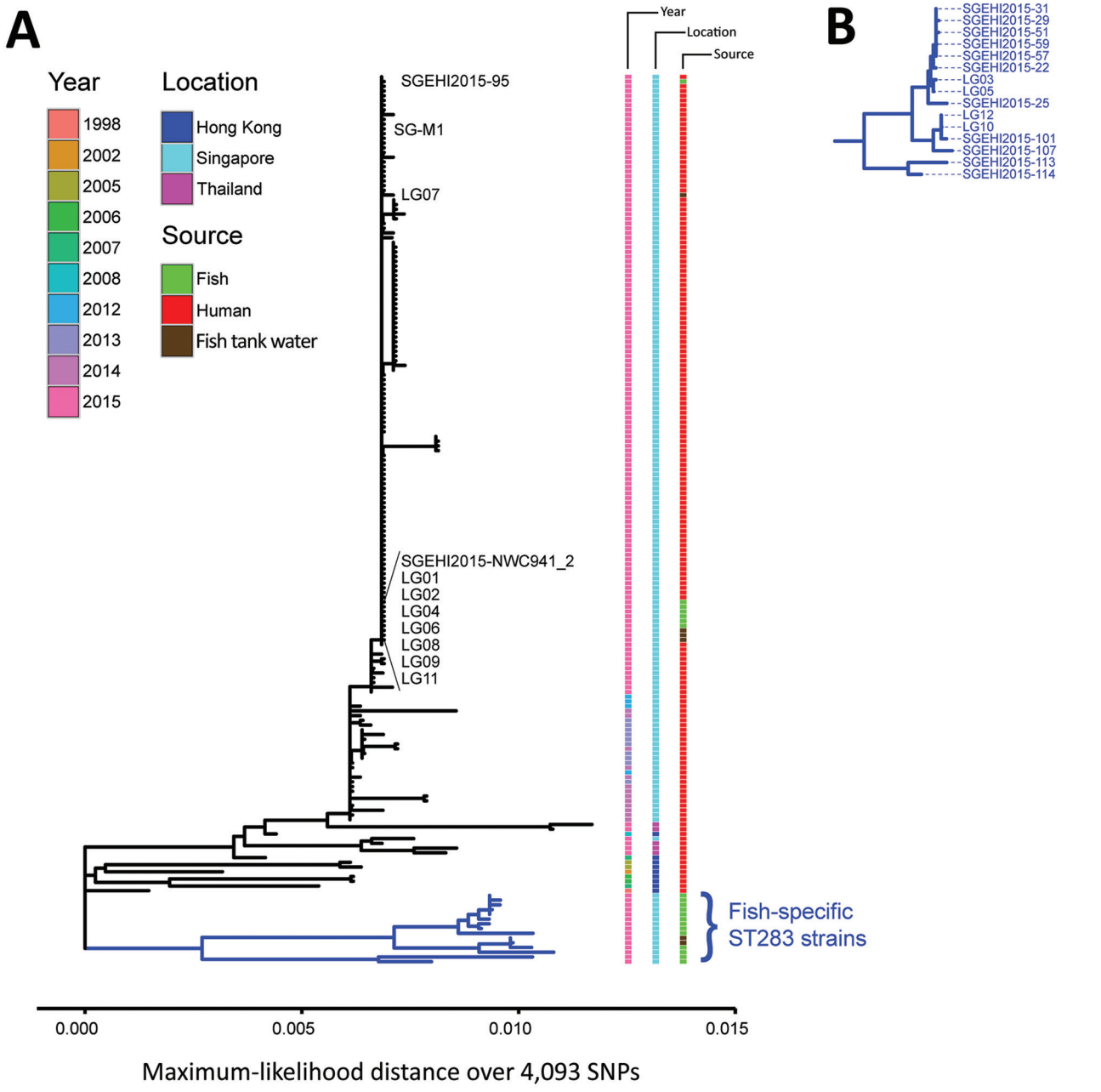


**Figure 4.** Phylogenetic analysis of group B *Streptococcus* (GBS) infections caused by improper sourcing and handling of raw fish for raw consumption, Singapore, 2015–2016. A) Maximum-likelihood single-nucleotide polymorphism (SNP)–based tree for GBS ST283 strains relative to the SG-M1 reference human outbreak strain. Year, location, and source (human or fish) for isolates are indicated. Twelve strains from 6 fish (SGEHI2015-NWC941, SGEHI2015–95, LG01, LG02, LG04, and LG06) and 4 fish tank water samples (LG07, LG08, LG09, and LG11) were nearly identical to the local reference outbreak strain SG-M1 (no SNP, 0 and 12 indels, respectively). Scale bar indicates distance over 4,093 total SNPs. B) Enlargement of blue subtree from bottom of tree in panel A showing fish GBS ST283 isolates that were different (57–71 SNPs and 11–33 indels) from the human outbreak strain. ST, sequence type.

sashimi suppliers that harvest fish from cleaner waters and adhere to stricter cold chain management practices. However, some saltwater fish samples from sashimi suppliers and restaurants were found to contain *Aeromonas* spp. (47.6%, 10/21) and *L. monocytogenes* (2.0%, 6/303), whose psychrotrophic nature has posed a challenge to the fish industry. *L. monocytogenes* is also a concern in chilled RTE food because of its ubiquity and persistence in food-processing environments (*25*).

Food and environmental findings of our study were consistent with epidemiologic findings for this outbreak (*2*,*3*). Multivariate analyses of a case–control study showed that persons who had consumed *yusheng* were more likely to acquire GBS ST283 infections than those who had not consumed *yusheng* (*2*). However, there was no strong association between GBS ST283 infections and consumption of sashimi and sushi (*2*).

Findings of this study have led to implementation of new policies in Singapore. These new policies included banning the use of freshwater fish in RTE dishes and requiring procurement of saltwater fish from suppliers for raw fish approved by the Agri-Food and Veterinary Authority of Singapore. Food stalls and food establishments providing catering services were required to stop selling RTE raw fish dishes until they complied with practices required for preparing RTE raw saltwater fish dishes.

The number of RTE fish samples collected from food stalls was limited because eating establishments were advised to stop the sale of RTE raw fish dishes containing Asian bighead carp and snakehead during July 24–December 5, 2015, while the outbreak investigation was underway (*1*). Sampling was not random because it was part of an outbreak investigation, but it was biased toward fish species and food stalls implicated in the outbreak. Thus, contamination rates might not reflect contamination rates of all fish species sold for raw consumption in the market. Similarly, testing of samples from ports and retail outlets was performed by using different protocols, which limited comparisons that could be made.

In conclusion, we detected GBS ST283, which caused a severe foodborne outbreak in Singapore in 2015, in freshwater fish, not only in food stalls and fish markets, but also in ports from which fish are imported. Comparison of human and fish isolates showed as few as 0–2 SNPs between human and fish isolates of GBS ST283 on a background of a diversity of GBS and other bacteria in freshwater fish. These data indicate the risk for contamination of raw freshwater fish and underscore the need for proper sourcing and handling of all fish for raw consumption. To control the outbreak, a ban on the sale of RTE raw freshwater fish dishes was implemented, and additional requirements were imposed for the sale of RTE raw fish dishes made with saltwater fish (*13*). Our study complements the epidemiologic findings for this outbreak (*2*,*3*) and illustrates the need for public health authorities and industries to remain vigilant regarding emerging pathogens.

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# Group B *Streptococcus* Infections Caused by Improper Sourcing and Handling of Fish for Raw Consumption, Singapore, 2015–2016

## Technical Appendix

## Materials and Methods

### Collection of Fish and Fish Tank Water Samples

#### Samples from Eating Establishments

Sliced fish samples (n = 328) from food stalls, restaurants, and snack bars were analyzed for microbiological safety and quality between freshwater and saltwater fish slices prepared by these retail eating establishments. Eating establishments linked to human cases (implicated establishments) and eating establishments that sold similar dishes but were not linked to human cases were included (control establishments). However, statistical analyses for implicated and control establishments were not performed because the number of samples from implicated establishments was small (26 food stall samples; 5 restaurant/snack bar samples). Limited samples were available because retailers were advised by health authorities to stop selling ready-to-eat raw fish dishes containing Asian bighead carp and snakehead while the outbreak investigation was underway.

#### Samples from Fresh Produce Markets and Sashimi Suppliers

Although fresh produce markets refer to fish stalls at the ports and wet markets, as well as fresh produce sections of supermarkets that food stalls are known to procure fish from, sashimi suppliers refer to companies that local restaurants and snack bars are known to procure sashimi grade fish from. Fish parts or whole fish (n = 83) were analyzed for microbiological safety and quality for freshwater and saltwater fish sampled at these locations. Subsamples consisting of surface swab specimens, muscles, or organs, were obtained from each whole fish or

fish part sample and subjected to microbial analyses (online Technical Appendix Table 2). During sampling of fish at fresh produce markets, samples of water in which live freshwater fish were kept were also collected. Microbial safety and quality of water were analyzed to assess their potential to cause cross-contamination among live fish in the market.

### Samples from Ports

A total of 586 whole freshwater fish samples and 98 holding water samples were collected at ports in Singapore. These samples were tested for group B *Streptococcus* (GBS), GBS serotype III, and GBS sequence type (ST) 283.

### Transport of Samples

Whole fish and fish part samples were purchased in their original packaging; sliced fish were collected in sterile bags, and fish tank water samples were collected in sterile bottles. All samples were transported on ice, kept refrigerated in laboratories, and analyzed on the day of collection.

### Testing of Samples

Microbiological testing of fish and fish tank water samples was performed by 3 laboratories: the Environmental Health Institute (EHI) of the National Environment Agency, the Veterinary Public Health Laboratory of the Agri-Food and Veterinary Authority of Singapore (AVA), and an accredited commercial laboratory. The Veterinary Public Health Laboratory performed testing for GBS in whole fish and fish tank water samples collected from ports, and EHI performed testing for GBS on all other samples. Standard plates counts (SPCs) for *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., *Vibrio cholerae*, and *V. parahaemolyticus* from fish slices collected from eating establishments was performed by an accredited commercial laboratory. SPCs for *E. coli*, *S. aureus*, *Aeromonas* spp., *L. monocytogenes*, *Salmonella* spp., *V. cholerae*, and *V. parahemolyticus* in whole fish and fish parts collected from ports, wet markets, supermarkets, and sashimi suppliers was performed by EHI. *S. aureus*, *Salmonella* spp., *V. cholerae*, and *V. parahaemolyticus* isolated by EHI and the accredited commercial laboratory were further characterized at EHI.

### GBS Analysis

Fish and fish tank water samples collected from ports were analyzed by Agri-Food and Veterinary Authority of Singapore by using a proprietary method that involved screening for GBS and GBS serotype III with a multiplex PCR method and isolation of viable GBS ST283 by culture method. GBS analyses on all other samples were conducted at EHI by using a modified protocol (*1*). Each fish surface swab sample was prepared by applying a premoistened sponge swab (3M Company, Maplewood. MN, USA) onto the entire surface area of each whole fish or fish part sample. The sponge swab was then suspended in 9 mL of Todd Hewitt broth (Acumedia, Lansing, MI, USA) containing colistin sulfate (7.5 μg/mL) and nalidixic acid (5 μg/mL) and homogenized by using a Stomacher (model 400; Seward Medical, Worthing, UK) with a paddle speed of 230 rpm for 30 s. A total of 25 g each of fish muscle sample, fish organ composite sample, and sliced fish sample was homogenized with 225 mL of Todd Hewitt broth containing antimicrobial drugs by using a Stomacher.

Each 500-mL fish tank water sample was concentrated by using membrane filtration with sterile 0.45-μm pore size filters made of mixed cellulose esters (Merck Millipore, Darnstadt, Germany). Membranes were then suspended in 20 mL of Todd Hewitt broth containing antimicrobial drugs. Homogenized aliquots and membranes were incubated at 37°C for 24 h. Secondary enrichment was performed by transferring 1 mL of the enriched Todd Hewitt broth to 5 mL brain heart infusion broth (Acumedia) and incubating at 37°C for 24 h. Nucleic acids were extracted from enriched brain heart infusion broth and selected preenriched sample suspensions in Todd Hewitt broth containing antimicrobial drugs by using a method described (*2*).

GBS was detected by using a singleplex PCR specific for the *dltR* gene (*3*). All GBS PCR-positive samples were cultured to obtain viable isolates. Culture involved plating of 100 μL of serially diluted (0–$10^5$ dilutions) enriched brain heart infusion broth onto Brilliance GBS Agar (Thermo Fisher Scientific, Darmstadt, Germany) and incubating the agar at 37°C for 24 h. Presumptive mauve colonies were confirmed as GBS by serologic analysis using the Streptococcal Grouping Kit (Oxoid, Basingstoke, UK) according to the manufacturer's instructions.

Although 103 of 522 enriched samples tested by EHI were positive for GBS by PCR, only 35 of these samples yielded GBS isolates when plated on chromogenic agar; GBS was not

recovered from the other 68 PCR-positive samples. A high background flora could have masked GBS in these 68 enriched samples. This masking is supported by the observation that of the 68 isolate-negative samples, 57 initially showed PCR-negative results, but then showed PCR-positive results after a 2-day enrichment. This change in results indicated low amounts of GBS in the original samples. Presampling manipulation, such as freezing, could have also contributed to the low yield.

Because recovery of viable isolates was not always successful for GBS PCR-positive samples, the enriched broth of these samples was further screened for GBS serotype III by using published primers (*4*). This screening was performed to estimate the highest possible contamination rate of GBS serotype III ST283 in fish.

A total of 84 GBS isolates were characterized by using serotyping, multilocus sequence typing (MLST), and whole-genome sequencing methods. Serotyping of GBS isolates was performed by using the IMMULEX STREP-B Antisera Set (Staten Serum Institut Diagnostica, Hillerød, Denmark) according to the manufacturer's instructions. Results were confirmed by using a published multiplex PCR protocol (*5*). MLST was performed by using published primers and Sanger sequencing (*6*). Aligned nucleotide sequences of amplified products were compared with sequences in the PubMLST *Streptococcus agalactiae* Database (http://pubmlst.org/sagalactiae/) for identification of sequence types.

Whole-genome sequencing was performed by the Genome Institute of Singapore and the Agency for Science, Technology and Research by using a NextSeq 500 sequencer (Illumina, San Diego, CA, USA) according to protocols described (*7*). A total of 1 mL of each GBS overnight culture in brain heart infusion broth was centrifuged, and the pellet of bacterial cells was lysed by using enzymatic lysis buffer at 37°C for 45 min, followed by extraction using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Genomic DNA shearing was performed by using an M220 Focused Ultrasonicator (Covaris, Woburn, MA, USA), and library preparation was performed by using the TruSeq Nano DNA LT Library Prep Kit Pooled libraries (Illumina). Samples were then sequenced by using a NextSeq 500 sequencer with 2 × 151-bp reads. All sequencing data are available in GenBank under BioProject PRJNA293392.

### Sequence Analysis

All primary sequence analysis was performed by the Genome Institute of Singapore with the Efficient Rapid Microbial Sequencing Platform. Reference-based analyses were performed by using the SG-M1 genome (*8*) as a reference. FASTQ files were mapped by using Burrows-Wheeler Aligner version 0.7.10 software (*9*). Indel realignment and single-nucleotide polymorphism (SNP) calling was performed by using Lofreq* version 2.1.2 with default parameters (*10*). Maximum-likelihood SNP trees (ignoring indels) of GBS ST283 strains were created by using SNPhylo (*11*). Neighbor-joining trees of all GBS strains were created by using APE version 3.5 (*12*). All phylogenetic trees were visualized with GGTREE version 3.2 (*13*) in R version 3.2.2 (https://www.R-project.org). Publicly available sequences of GBS were downloaded from the GenBank Short Read Archive (for Illumina datasets; http://www.ncbi.nlm.nih.gov/sra) and the Genbank FTP site (for assembled genomes; https://www.ncbi.nlm.nih.gov/genome/genomes/186?). MLST and resistance gene predictions were made by using SRST2 version 0.1.8 (*14*) for Illumina sequenced strains or manually by using BLASTN (*15*) for fully assembled reference sequences, the recommended MLST database (http://pubmlst.org/sagalactiae/) (*16*), and the ARGannot resistance gene database (*17*) included with SRST2.

### SPCs and *E. coli* and *S. aureus* Counts

The protocols used by EHI are described below. Each fish surface swab sample was prepared by applying a premoistened sponge swab onto a 10 cm × 10 cm surface area of each fish part or whole fish sample. The sponge swab was then suspended in 9 mL Butterfield buffer and homogenized by using a Stomacher. A total of 25 g of each fish muscle and sliced fish sample was homogenized with 225 mL of universal preenrichment broth (Acumedia) by using a Stomacher. Each 500-mL fish tank water sample was concentrated by membrane filtration using 0.45-μm pore size filters and suspended in 20 mL of universal preenrichment broth. SPCs and *E. coli* and *S. aureus* counts were determined performed according to described methods (*18*). The presence of the methicillin resistance gene and enterotoxin-producing genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, and *sel*) of 18 *S. aureus* isolates from 17 fish was determined by using published primers (*19–21*).

**Detection of *Aeromonas* spp., *Listeria monocytogenes, Salmonella* spp., *Vibrio cholerae*, and *V. parahaemolyticus***

Each fish surface swab sample was prepared by applying a premoistened sponge swab onto the entire surface area of each fish part or whole fish sample. The sponge swab was then suspended in 9 mL of universal preenrichment broth and homogenized by using a Stomacher. All stomached aliquots (surface swab specimens, fish muscle, fish organs, sliced fish) resuspended in universal preenrichment broth were incubated at 37°C for 24 h for detection of *Aeromonas* spp. (*A. caviae*, *A. hydrophila*, and *A. sobria*), *L. monocytogenes*, *Salmonella* spp., *V. cholerae*, and *V. parahaemolyticus*. A 10-μL loopful of an enriched culture in universal preenrichment broth was subcultured onto starch-ampicillin agar (Himedia, Mumbai, India), PALCAM agar (Acumedia), and xylose-lysine-desoxycholate (XLD) agar (Oxoid) for isolation of *Aeromonas* spp., *L. monocytogenes*, and *Salmonella* spp.; and onto thiosulfate-citrate-bile salt-sucrose agar (Acumedia) for isolation of and *V. cholerae* and *V. parahaemolyticus*. All selective agars were incubated at 37°C for 24 h, except for PALCAM agar, which was incubated at 37°C for 48 h.

After incubation, colonies on starch-ampicillin agar were flooded with diluted (1:20) iodine solution (Oxoid) to detect colonies with zones of clearance. Presumptive *Aeromonas* spp. colonies were streaked onto tryptic soy agar (Acumedia) for purification and biochemical confirmation by using API 20 E (bioMérieux, Marcy l'Étoile, France). Presumptive gray–green colonies surrounded by dark halos on PALCAM agar were picked for identification of *L. monocytogenes* by using a *Listeria* genus–specific PCR and a *L. monocytogenes* species–specific PCR that targeted the *prs* gene (*22*) and the *inlA* gene (*23*).

All other bacteria isolates, *Salmonella* spp. (n = 2), *V. cholerae* (n = 16), and *V. parahaemolyticus* (n = 6), were verified biochemically by using described methods (*18*). Sequence types of *Salmonella* spp. isolates were determined by using primers and Sanger sequencing, as well as comparison of sequences in the MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primersEnterica_html). Sixteen *V. cholerae* isolates from 9 fish and 1 fish tank water samples were screened for virulence genes (*ctxA*, *ctxB*, and *tcpA*) by using published primers (*24–26*). Six *V. parahaemolyticus* isolates from 5 fish samples were screened for virulence genes (*tdh*, *trh1*, and *trh2*) by using published primers (*27*,*28*). Nucleic acids of all bacterial isolates, including GBS, were extracted by using the DNeasy Blood and Tissue Kit (QIAGEN). Amplified product sizes were analyzed by using

the QIAxcel DNA Screening Kit (QIAGEN) or the QIAxcel DNA High Resolution Kit (QIAGEN) as appropriate.

### SPCs in Fish Pieces before and after Rinsing

We analyzed 21 sets of saltwater fish muscle samples to determine if SPC could be reduced by rinsing with water. Each set of samples consisted of 2 muscle pieces (each 5 cm × 5 cm × 1 cm). One piece served as a control (before rinsing), and the other piece was rinsed with 500 mL of sterile water. We analyzed fish muscle samples for SPCs according a described method (*18*).

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**Technical Appendix Table 1.** Characteristics of freshwater and saltwater fish tested during investigation of group B *Streptococcus* infections, Singapore, 2015–2016

| Sampling location | Type of sample | No. samples | Type of fish collected |
|---|---|---|---|
| Noneating establishment | | | |
| Port | Whole fish | 586 | Freshwater fish sold as Asian bighead carp (*Hypophthalmichthys nobilis*), grass carp (*Ctenopharyngodon idella*), Pangasius dory (*Pangasius* spp.), snakehead (*Channa* spp.), and tilapia (*Oreochromis* spp.) |
| Fresh produce markets (fish stalls at ports and wet markets and fresh produce sections of supermarkets) | Whole fish, fish parts | 39 | Freshwater fish sold as Asian bighead carp, snakehead-toman (*C. micropeltes*), snakehead-haruan (*C. striata)*, silver carp (*Hypophthalmichthys molitrix*), and tilapia |
| Fresh produce markets (fish stalls at ports and wet markets and fresh produce sections of supermarkets) | Whole fish, fish parts | 23 | Saltwater fish sold as mackerel tuna (*Euthynnus affinis*), ribbonfish (*Trichiurus* spp.), salmon (*Salmo salar*), and wolf herring (*Chirocentrus* spp.) |
| Sashimi suppliers* | Whole fish, fish parts | 21 | Saltwater fish sold as salmon and tuna (*Thunnus* spp.) |
| Eating establishment | | | |
| Food stalls in (in hawker centers, coffee shops,and eating houses) | Sliced fish | 25 | Freshwater fish sold as Asian bighead carp, grass carp, Pangasius dory, and snakehead |
| Food stalls in (in hawker centers, coffee shops,and eating houses) | Sliced fish | 21 | Saltwater fish sold as salmon and Spanish mackerel (*Scomberomorus* spp.), and wolf herring. |
| Restaurants and snack bars* | Sliced fish | 282 | Saltwater fish sold as salmon, tuna, and *tai*† |

*These locations did not sell freshwater fish meant for raw consumption.
†Generally refers to red sea bream (*Pagrus major*) in authentic Japanese cuisine, but it can be substituted with other fish by retailers in Singapore.

**Technical Appendix Table 2.** Testing of subsamples during investigation of group B *Streptococcus* infections, Singapore, 2015–2016*

| | Purpose of testing | | | |
|---|---|---|---|---|
| Characteristic | Detection of organism implicated in outbreak | Detection of food safety indicators | Detection of food hygiene indicators | Measurement of total microbial load in food |
| Testing parameter | GBS, GBS, serotype III, and GBS ST283 | *Aeromonas* spp. (*A. caviae*, *A. hydrophila*, and *A. sobria*); *Listeria monocytogenes*; *Salmonella* spp.; *Vibrio cholerae* and *V. parahaemolyticus* | *Escherichia coli* and *Staphylococcus aureus* counts | Standard plate count |
| Type of sample | | | | |
| Whole fish, fish parts | | | | |
| Organs (brain, eyes, spleen, or kidneys) | Yes | No | No | No |
| Surface swab specimen | Yes | Yes† | Yes† | No |
| Muscle | Yes | Yes† | Yes† | Yes† |
| Sliced fish | Yes | Yes‡ | Yes | Yes |
| Water | Yes | Yes‡ | Yes | Yes |

*GBS, group B *Streptococcus*; ST, sequence type.
†These parameters were tested on whole fish/fish part samples collected at only fresh produce markets and sashimi suppliers; whole fish samples collected from ports were excluded.
‡All food safety indicators were tested except for *Aeromonas* spp.

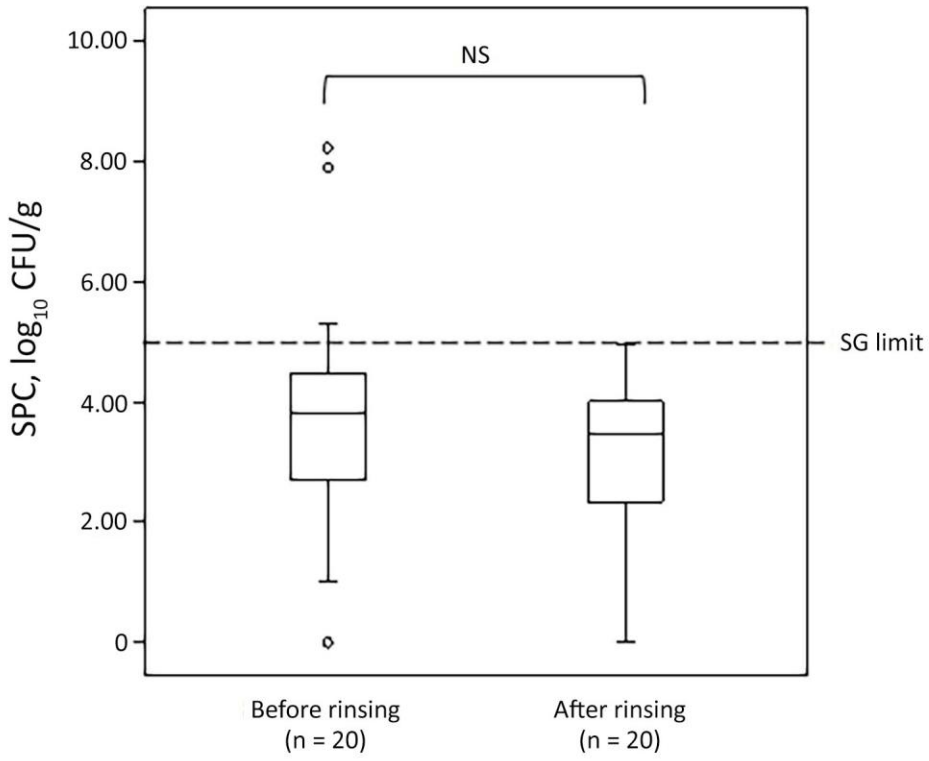
**Technical Appendix Table 3.** Characteristics of group B *Streptococcus* isolates from fish and water samples, Singapore, 2015–2016*

| Sample ID | No. isolates analyzed | Sample description | Type of sample | Sold as RTE dish | Sampling site† | Sold as freshwater fish | Serotype | ST | Antimicrobial drug resistance gene‡ |
|---|---|---|---|---|---|---|---|---|---|
| SGEHI2015–63§ | 2 | Fish parts (slab) sold as snakehead-toman | Muscle | No | Wet market stall | Yes | Ia | 7 | – |
| SGEHI2015-IV87 | 2 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | Ia | 7 | – |
| SGEHI2015-IV170 | 2 | Sliced fish sold as taï (tilapia)¶ | Muscle | Yes (sashimi) | Restaurant/snack bar | No | Ia | 7 | – |
| SGEHI2015–49§ | 1 | Fish parts (slab) sold as snakehead-toman | Surface swab | No | Wet market stall | Yes | Ia | 23 | *tetM* |
| SGEHI2015–50§ | 1 | Fish parts (slab) sold as snakehead-toman | Muscle | No | Wet market stall | Yes | Ia | 23 | *tetM* |
| SGEHI2015–243 | 10 | Whole fish sold asmackerel tuna | Surface swab | No | Fish stall (port) | No | Ia | 23 | *lsaC*, *tetM* |
| SGEHI2015-IV89 | 5 | Sliced fish sold as tuna | Muscle | Yes (sashimi) | Restaurant/snack bar | No | Ia | 103 | *tetO*# |
| SGEHI2015-IV211 | 2 | Sliced fish sold as tuna | Muscle | Yes (sashimi) | Restaurant/snack bar | No | Ia | 103 | – |
| SGEHI2015-IV232 | 2 | Sliced fish sold as salmon | Muscle | Yes (*lo-hei*) | Restaurant/snack bar | No | Ia | 103 | – |
| SGEHI2015–77§ | 1 | Fish part (slab) sold as snakehead-toman | Muscle | No | Wet market stall | Yes | II | 28 | *tetM* |
| SGEHI2015-II47 | 1 | Sliced fish sold as grass carp | Muscle | Yes (*yusheng*) | Food stall | Yes | II | 652 | *tetL*, *tetM* |
| LG01 | 1 | Whole fish sold as Asian bighead carp | Organs | No | Port | Yes | III | 283 | – |
| LG02 | 1 | Whole fish sold as Asian bighead carp | Muscle | No | Port | Yes | III | 283 | – |
| LG03 | 1 | Whole fish sold as Asian bighead carp | Surface swab | No | Port | Yes | III | 283 | – |
| LG04 | 1 | Whole fish sold as Asian bighead carp | Surface swab | No | Port | Yes | III | 283 | – |
| LG05 | 1 | Whole fish sold as Asian bighead carp | Surface swab | No | Port | Yes | III | 283 | – |
| LG06 | 1 | Whole fish sold as Asian bighead carp | Organs | No | Port | Yes | III | 283 | – |
| LG07 | 1 | Water for holding live freshwater fish | NA | NA | Port | NA | III | 283 | – |
| LG08 | 1 | Water for holding live freshwater fish | NA | NA | Port | NA | III | 283 | – |
| LG09 | 1 | Water for holding live freshwater fish | NA | NA | Port | NA | III | 283 | – |
| LG10 | 1 | Water for holding live freshwater fish | NA | NA | Port | NA | III | 283 | – |
| LG11 | 1 | Water for holding live freshwater fish | NA | NA | Port | NA | III | 283 | – |

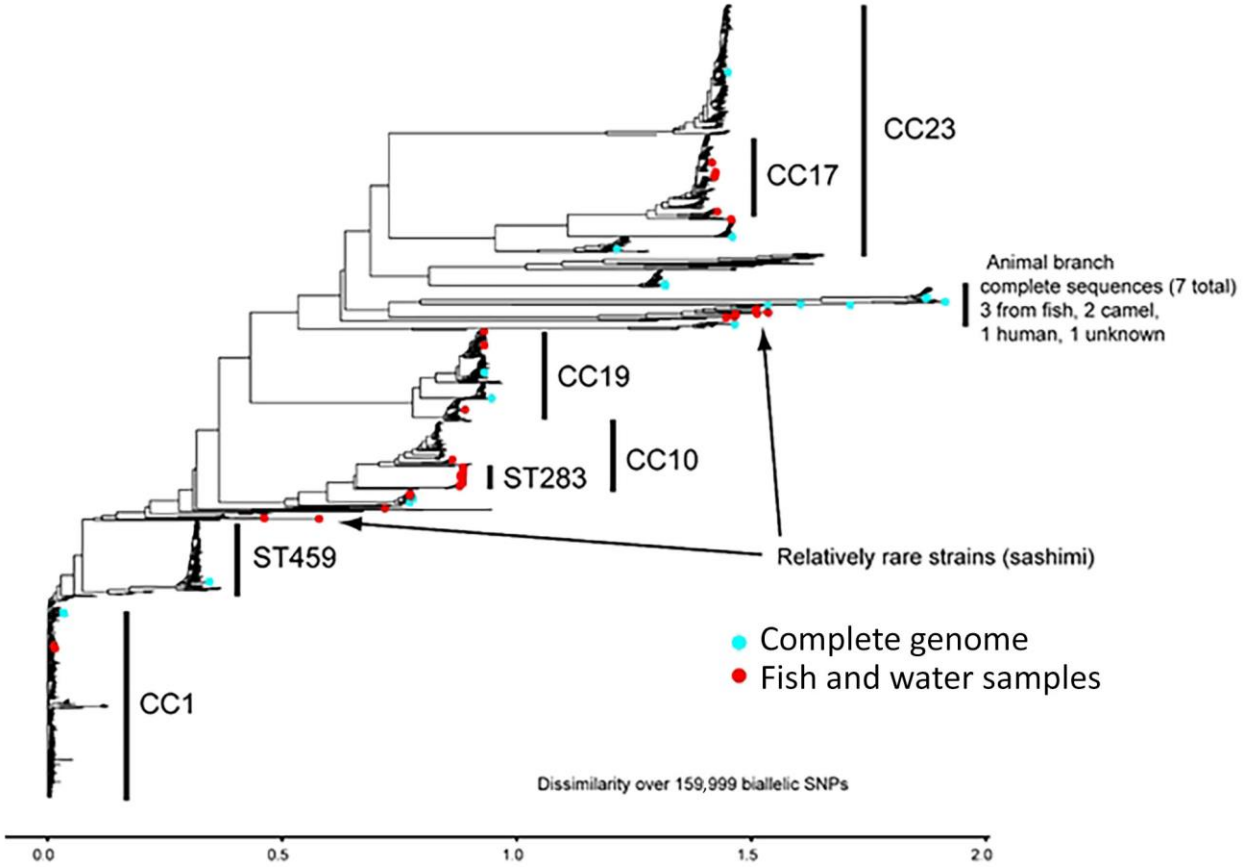
| Sample ID | No. isolates analyzed | Sample description | Type of sample | Sold as RTE dish | Sampling site† | Sold as freshwater fish | Serotype | ST | Antimicrobial drug resistance gene‡ |
|---|---|---|---|---|---|---|---|---|---|
| LG12 | 1 | Water for holding live freshwater fish | NA | NA | Port | NA | | | – |
| SGEHI2015-NWC941§ | 3 | Sliced fish sold as grass carp | Muscle | Yes (*yusheng*) | Food stall | Yes | III | 283 | – |
| SGEHI2015–107§ | 1 | Whole live fish sold as snakehead-haruan | Muscle | No | Wet market stall | Yes | III | 283 | – |
| SGEHI2015–95§ | 1 | Fish part (slab) sold as silver carp | Muscle | No | Supermarket | Yes | III | 283 | – |
| SGEHI2015–22§ | 2 | Fish part (tail) sold as Asian bighead carp | Muscle | No | Fish stall (port) | Yes | III | 283 | – |
| SGEHI2015–25§ | 1 | Fish part (head) sold as Asian bighead carp | Muscle | No | Fish stall (port) | Yes | III | 283 | – |
| SGEHI2015–51§ | 1 | Fish part (head) sold as Asian bighead carp | Surface swab | No | Supermarket | Yes | III | 283 | – |
| SGEHI2015–31§ | 4 | Fish part (slab) sold as snakehead-toman | Muscle | No | Fish stall (port) | Yes | III | 283 | – |
| SGEHI2015–57§ | 1 | Whole fish sold as black tilapia | Muscle | No | Wet market stall | Yes | III | 283 | – |
| SGEHI2015–101§ | 1 | Whole fish sold as black tilapia | Muscle | No | Wet market stall | Yes | III | 283 | – |
| SGEHI2015–113§ | 1 | Whole fish sold as black tilapia | Muscle | No | Supermarket | Yes | III | 283 | *tetM* |
| SGEHI2015–114§ | 1 | Whole fish sold as black tilapia | Organs | No | Supermarket | Yes | III | 283 | *tetM* |
| SGEHI2015–29§ | 2 | Whole fish sold as red tilapia | Organs | No | Fish stall (port) | Yes | III | 283 | – |
| SGEHI2015–59§ | 1 | Whole fish sold as black tilapia | Surface swab | No | Wet market stall | Yes | III | 283 | – |
| SGEHI2015–60§ | 1 | Whole fish sold as black tilapia | Muscle | No | Wet market stall | Yes | Ia | 24 | *tetM* |
| SGEHI2015-II33 | 1 | Sliced fish sold as snakehead | Muscle | Yes (*yusheng*) | Food stall | Yes | III | 335 | *tetM* |
| SGEHI2015-IV45 | 5 | Sliced fish sold as taï (tilapia)¶ | Muscle | Yes (sashimi) | Restaurant/snack bar | No | III | 651 | *ermB*, *tetL* |
| SGEHI2015-IV118 | 2 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | III | 861 | *lsaC*, *msrD*, *tetO* |
| SGEHI2015-IV100 | 3 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | V, Ia | 1, 485 | *tetM* |
| SGEHI2015-IV104 | 2 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | V | 1 | *tetM* |
| SGEHI2015-II55 | 2 | Sliced fish sold as wolf herring | Muscle | Yes (*yusheng*) | Food stall | No | V | 1 | *tetM* |
| SGEHI2015-II56 | 1 | Sliced fish sold as Asian bighead carp | Muscle | No | Food stall | Yes | V | 1 | *tetM* |
| SGEHI2015-IV74 | 1 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | VII | 1 | *tetM* |

| Sample ID | No. isolates analyzed | Sample description | Type of sample | Sold as RTE dish | Sampling site† | Sold as freshwater fish | Serotype | ST | Antimicrobial drug resistance gene‡ |
|---|---|---|---|---|---|---|---|---|---|
| SGEHI2015-IV72 | 2 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | VII | 1 | *tetM*, *tetO* |
| SGEHI2015-IV132 | 2 | Sliced fish sold as salmon | Muscle | Yes (sashimi) | Restaurant/snack bar | No | V | 24 | *tetM* |
| SGEHI2015-IV227 | 1 | Sliced fish sold as wolf herring | Muscle | Yes (*lo-hei*) | Restaurant/snack bar | No | VI | 167 | *tetM* |

*NA, not applicable; RTE, ready-to-eat; ST, sequence type; *lo-hei*, Lunar New Year festive dish consisting of raw fish slices served with raw vegetables and condiments; *yusheng*, Chinese style sliced raw fish dish served separately with porridge.
†Food stalls refer to stalls housed within larger eating establishments that include hawker centers, coffee shops, and eating houses. Supermarkets refer to fresh produce sections of supermarkets and exclude sashimi and sushi counters of supermarkets.
‡–, no genes encoding resistance from the ARGannot database (including those acting on aminoglycosides, β-lactams, colistin, fosfomycin, fluoroquinolones, glycopeptides, macrolides, lincosamide, streptogramines, phenicols, rifampin, sulfonamides, tetracyclines, and trimethoprim) were detected by SRST2 from the genome sequencing data.
§Isolates also described in a study (*7*) on the analysis of clinical, epidemiologic, and bacterial sequencing data obtained during investigation of group B *Streptococcus* infections.
¶Fish identified as tilapia (*Oreochromis* spp.) by using the DNA barcoding method (*29*).
#Gene encoding resistance to tetracycline (t*etO*) was detected in 3 of the 5 isolates.




**Technical Appendix Figure 1.** Group B *Streptococcus* infections caused by handling and consumption of raw fish, Singapore, 2015–2016. SPC of fish muscle before and after rinsing with water. Dashed horizontal line indicates regulatory limit of Singapore for SPCs in ready-to-eat foods (<5 $\log_{10}$ CFU/g) (*30*). Top and bottom of boxes in plots indicate 25th and 75th percentiles, horizontal lines indicate medians, and error bars indicate minimum and maximum values. Open circles indicate outliers. NS, not significant ($p>0.05$); SPC, standard plate count; SG, Singapore government.




**Technical Appendix Figure 2.** Group B *Streptococcus* infections caused by handling and consumption of raw fish, Singapore, 2015–2016. Global phylogenetic tree (neighbor-joining tree) with SNPs relative to the SG-M1 reference genome for 1,369 GBS strains. Red circles indicate strains isolated from fish and fish tank water samples sequenced during this study. Blue circles indicate complete publicly available genome sequences. All other strains are sequences from the GenBank Short Read Archive. Vertical black bars indicate CCs (*31*) and STs. Black arrows indicate several relatively rare fish strains from this study that fall outside the major CCs and have relatively few neighboring publicly available sequences. Seventeen of these strains (just below CC17) are placed among a set of strains that are more likely not to have come from humans (fish and camel strains). CC, clonal complex; GBS, group B *Streptococcus*; SNPs, single-nucleotide polymorphisms; ST, sequence type.