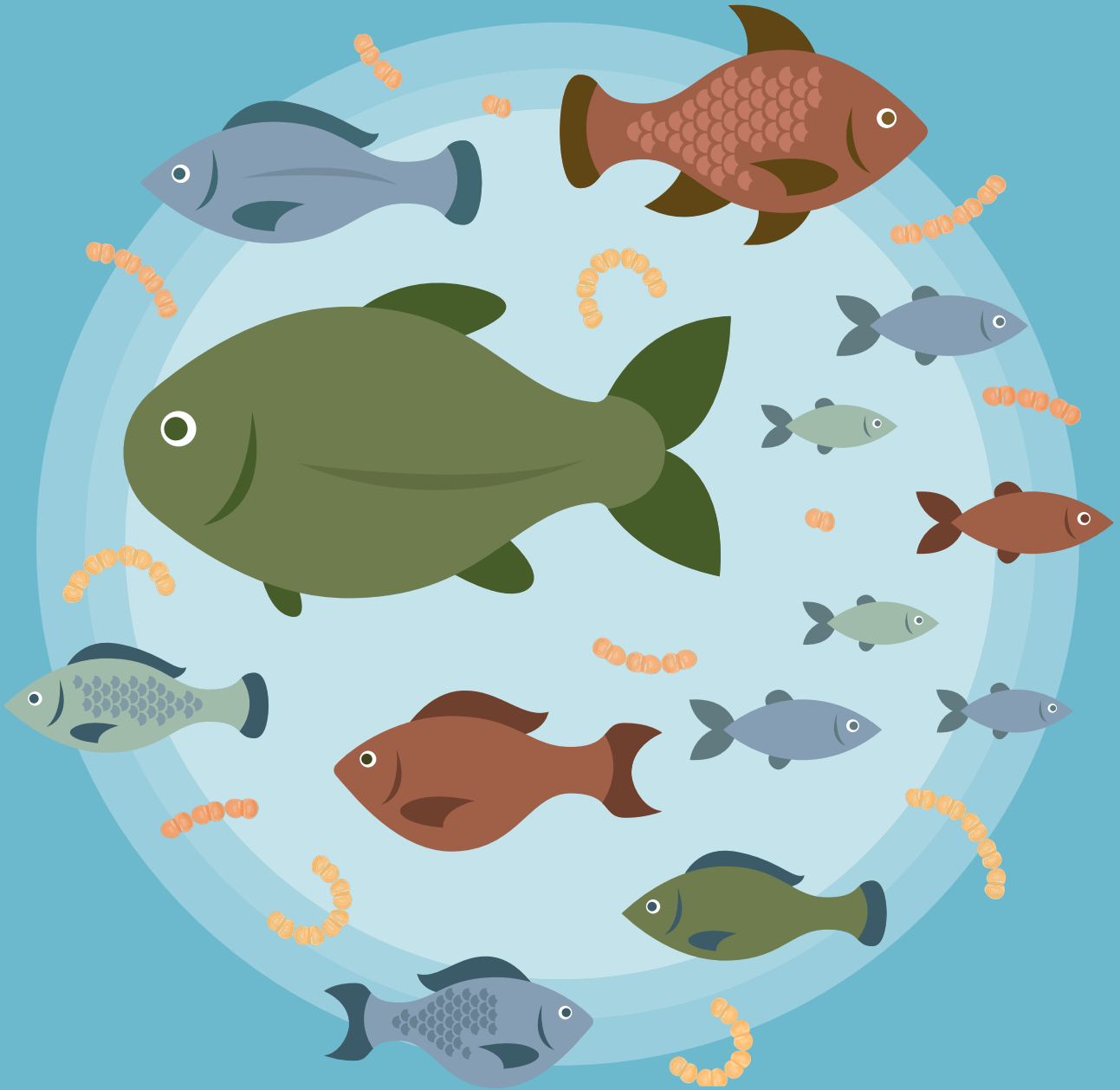Food and Agriculture
Organization of the
United Nations

RISK PROFILE

Group B *Streptococcus* (GBS)
Streptococcus agalactiae
sequence type (ST) 283
in freshwater fish

RISK PROFILE

Group B *Streptococcus* (GBS) *Streptococcus agalactiae* sequence type (ST) 283 in freshwater fish

ABSTRACT

In Singapore during 2015, Group B *Streptococcus* (GBS) sequence type 283 (ST283) caused the only reported foodborne outbreak of invasive GBS disease. Over 20 percent of cases were healthy adults without comorbidities, which is unusual for GBS. The outbreak was linked to the consumption of raw freshwater fish. Subsequent investigations found that ST283 GBS has been common among GBS causing disease in humans and in tilapia across Southeast Asia for at least 20 years whereas it was almost non-existent outside this region. Given the novelty of the outbreak, this risk profile consolidates the current knowledge to identify data gaps about GBS ST283 along the freshwater fish supply chain in Southeast Asia.

Although GBS fish infection can present with few clinical signs of disease, outbreaks of GBS in high intensity tilapia aquaculture can result in severe infection with mortalities of up to 80 percent. These outbreaks are largely undocumented but likely have a wide effect on aquaculture, given its economic and social importance across Southeast Asia.

There is also a lack of data on patterns of fish consumption, including frequency, amount, preparation and consumer demographics. Nevertheless, consumption of non-heat-treated freshwater fish is common in Southeast Asia. Given the multitude of data gaps, the risk posed by GBS ST283 from consumption of freshwater fish remains highly uncertain. Potential risk management options start with the application of good aquaculture practices and good food safety measures throughout the supply chain.

Keywords: food safety, foodborne diseases, *Streptococcus agalactiae,* Group B *Streptococcus* (GBS), ST283, freshwater fish, aquaculture, raw fish, Southeast Asia, risk profile.

CONTENTS

ACKNOWLEDGEMENTS

ABBREVIATIONS AND ACRONYMS

AMR	antimicrobial resistance
CC	clonal complexes
CFU	colony forming unit
CRL	central reference laboratory
DALY	disability-adjusted life years
DLV	double locus variants
GBS	Group B *Streptococcus*
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practices
HACCP	hazard analysis and critical control point
ICMSF	The International Commission on Microbiological Specifications for Foods (ICMSF)
ID	infectious or illness dose
LD50	median lethal dose
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
MLST	multilocus sequence typing
MPa	megapascal pressure unit
qPCR	quantitative polymerase chain reaction
SNP	single-nucleotide polymorphisms
SLV	single locus variants
ST	sequence type
ST283	sequence type 283

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DECLARATION OF INTERESTS

All experts completed a Declaration of Interests form in advance of the work. Based on the information provided, three of the 35 experts declared a potential interest relevant to the topic of the meeting; and nine experts declared that they are involved in relevant research with public entities. However, these were not considered by FAO to present any conflict in light of the objectives of the work. Nevertheless, these declared interests and the full copies of the Declaration of Interest forms were brought to the attention of all the experts and collaborators involved in the initiative. All the experts confirmed that they participated in the initiative in their individual capacities and not as representatives of their country, government or organization.

EXECUTIVE SUMMARY

In 2015, *Streptococcus agalactiae*, also referred as Group B *Streptococcus* (GBS), caused an outbreak involving at least 146 people in Singapore. The strain responsible was identified as sequence type 283 (ST283). This outbreak was remarkable, both because it was the first documented foodborne outbreak of invasive disease caused by GBS, and because it caused invasive disease in otherwise healthy adults. Disease included serious infections such as septic arthritis and meningitis. Epidemiological investigations pointed to a strong link with the consumption of raw freshwater fish. An advisory was issued in July 2015 warning the public not to eat raw freshwater fish and the outbreak quickly abated. A resurgence of cases led to new legislation in December 2015 banning the sale of raw freshwater fish as a ready-to-eat food. However, further small numbers of cases of ST283 continued to be identified in Singapore, with a surge of 18 cases seen in July 2020, indicating ongoing risk. The source of these more recent infections has not been ascertained, but the cases denied eating raw freshwater fish when interviewed by officials.

Investigations since 2015 have shown that invasive GBS ST283 disease is common in other countries and areas in and around Southeast Asia including China, Hong Kong SAR, Lao People's Democratic Republic, Thailand, and Viet Nam. In contrast, very few cases have been reported beyond the region, despite numerous appropriate typing studies having been undertaken in Africa, mainland China, Europe, and North and South America.

FAO commissioned this risk profile to document the current state of knowledge on the presence and transmission of GBS ST283 along the freshwater fish supply chain (covering aquaculture and wild capture, transport, processing, retail, preparation, and consumption) and to identify relevant data gaps.

GBS (not specifically ST283 GBS) is commonly carried by healthy men and women. Since the 1960s, GBS has been recognized as a major cause of neonatal sepsis and infection of pregnant and parturient women around the world. In addition, GBS has emerged since the 1990s as a common cause of sepsis amongst adults with comorbidities, but it is very uncommon in healthy adults. In contrast, GBS ST283 causes invasive disease in previously healthy adults.

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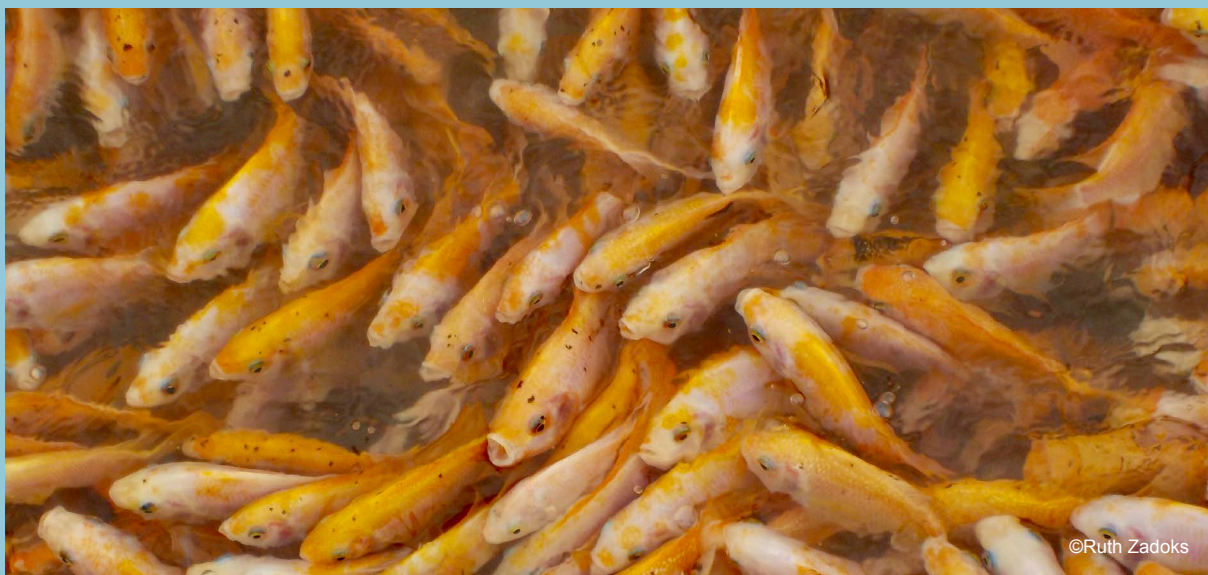
Very few studies have been undertaken in relation to the growth, survival, and inactivation of GBS in general. However, published information indicates that streptococci are not heat resistant and do not survive pasteurization; in particular, GBS ST283 is more heat sensitive than *Escherichia coli* O157:H7 and *Listeria monocytogenes*. In addition, GBS can grow at salinity concentrations up to 5.5 percent NaCl and over a wide pH range, though they are less acid resistant than *E. coli* O157:H7 and *L. monocytogenes*.

Tilapias are freshwater fish. They are farmed in aquaculture in farms across Southeast Asia. GBS ST283 has been identified in tilapia farmed in Malaysia, Thailand, and Viet Nam, and may also be present in other aquaculture industries, having been detected in a range of freshwater species. Infection of fish with *Streptococcus* spp., including GBS, can be varied, and clinical signs are wide ranging. Infection may occur at any fish age, and all life cycle stages may contribute to transmission, which can be via the aquatic environment and human effluent. Aquaculture outbreaks of GBS can result in mortalities of up to 80 percent. This is of particular importance given that Southeast Asia is one of the world's major freshwater fish-producing regions and that Nile tilapia is an important economic species across the region. In Southeast Asia freshwater fish, including tilapia, are mainly produced for domestic consumption.

Given the economic importance of freshwater fish, some data exist in relation to the control of GBS in the aquatic environment. In particular, the risk of GBS disease in fish can be reduced through good water quality management and good fish husbandry. In relation to water quality, the risk of GBS disease in fish increases with high water temperature, high water acidity/alkalinity, and water pollution. Good husbandry practices include treatment of pond water and fry prior to introduction in the aquaculture environment; cleaning of production units and equipment between batches; low stress fish handling and appropriate stocking densities; use of appropriate feed and feeding amounts; removal of dead and moribund fish; and implementation of good biosecurity practices. However, it is noted that implementation of good husbandry practices alone may not suffice to prevent GBS disease in fish or consumers.

Investigation of the occurrence of GBS and GBS ST283 in the production and supply chain of freshwater fish reveals a lack of robust data, including the exact mechanism by which GBS causes disease in fish; prevalence in freshwater fish (including fish without clinical signs and sick fish) within wild and farm environments, regions, and countries; the effects of processing factors; and prevalence and concentration of GBS in or on fish through the supply chain, including retail. In addition, there

is a lack of data in relation to consumption patterns including frequency, amount, and preparation (raw, preserved, heat treated, etc.) of fish before consumption. In particular, the effects of various preparation (such as addition of lime juice) and preservation (such as fermentation) practices on GBS concentration. Similarly, the demographics of the consuming population are not well described, although it is noted that consumption of raw (not heat treated) freshwater fish is common in Southeast Asia and may be regarded as normal in some cultures. As an example, the outbreak in Singapore was largely concentrated amongst older Chinese people who are more likely to consume raw freshwater fish with porridge.

The mechanism by which GBS (and specifically GBS ST283) cause infection and invasive disease, especially foodborne infection and disease, is not understood. No data are available for the dose–response relationship of GBS ST283 in humans after oral ingestion and only limited data exist for animal models. Robust data on GBS ST283 epidemiology are not available because of limited healthcare, diagnostic, bacterial typing, and reporting infrastructure. As a result, invasive GBS ST283 in Southeast Asia may be a neglected tropical disease, whereby lack of data perpetuates lack of investment in reporting, and vice versa. More fundamentally, it is not understood whether the severity of disease associated with the GBS ST283 outbreak in Singapore relates to this specific strain or to other factors such as the mode of transmission or level of contamination.

Due to the overall lack of quantitative data, a qualitative risk assessment was attempted for GBS ST283 disease resulting from consumption of raw freshwater fish. The disease can be categorized as severe, but the likelihood of exposure to levels high enough to cause disease is highly uncertain, resulting in a subsequent risk rating that could range from low to high. In addition, consumption of fermented fish or fish that have been prepared with spices and condiments is rated to have equal risk as consumption of raw freshwater fish – this conservative estimate was based on the wide-ranging pH tolerance of GBS ST283, though this effect was also very uncertain. In contrast, consumption of partially or fully heat-treated fish is rated to be lower in risk – this was based on the heat susceptibility of GBS ST283, though the effect depends on the extent of heat treatment.

The lack of reliable quantitative data also limits the risk management options that can be recommended. Risk mitigation options likely to have some effects are based on the application of Good Aquaculture Practices during production, Good Hygienic Practices, Good Manufacturing Practices, and a Hazard Analysis and Critical Control Point system during processing, transport, and retail. Whereas consumption of raw fish was clearly related to the GBS ST283 disease outbreak in Singapore, consumption of raw fish is widely practiced in Southeast Asia because of its cultural importance, despite long-standing efforts to use public health campaigns to reduce the practice. It has been speculated that participatory approaches involving strategic risk communications and community engagement may prove more effective in changing food practices associated with raw fish consumption, though these remain to be tested. Risk management options with potential at the farm level include vaccination, dietary supplements (probiotics), glycoinhibitors, and GBS surveillance, although their cost–benefit needs to be evaluated.

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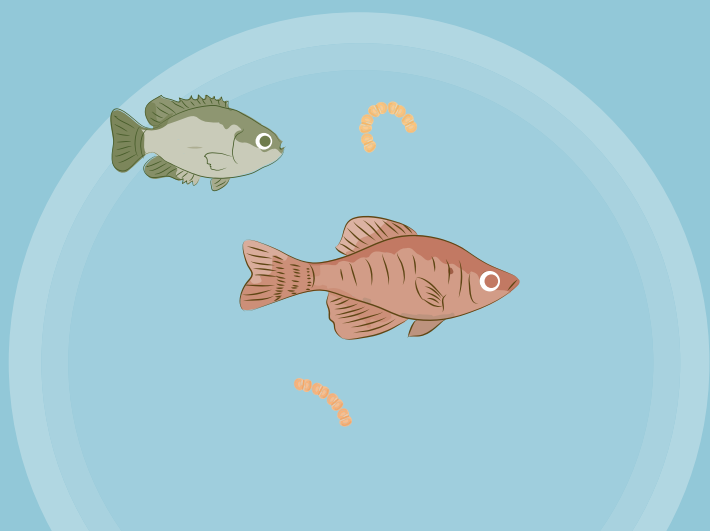
1 INTRODUCTION

1.1. Background of the food safety issue

The only reported foodborne outbreak of invasive disease caused by *Streptococcus agalactiae*, also called Group B *Streptococcus* (GBS), occurred in Singapore in early 2015. It resulted from a highly unusual strain of GBS, denoted as sequence type 283 (throughout this document, this will be abbreviated as ST283). The outbreak was remarkable, both because it was foodborne and because it involved invasive disease in many otherwise healthy adults – over 20 percent of invasive ST283 cases were healthy adults, but only 2 percent of invasive non-ST283 GBS disease was seen in healthy adults. This suggests that ST283 is more virulent than other strains of GBS. Investigations since then have shown that GBS ST283 is prevalent in people in other countries/areas in and around Southeast Asia including China, Hong Kong SAR, Lao People's Democratic Republic, Thailand, and Viet Nam (Barkham *et al.*, 2019, Kalimuddin *et al.*, 2017).

Epidemiological investigations of the Singapore outbreak pointed to a strong link with consumption of raw freshwater fish, which is a popular dish amongst older Chinese Singaporeans as a complement to porridge. An advisory in July 2015 warning the public to avoid eating raw freshwater fish curtailed the outbreak. A resurgence of cases led to new legislation in December 2015, banning the sale of raw freshwater fish as a ready-to-eat food. (Rajendram *et al.*, 2016, Tan *et al.*, 2016). ST283 has been identified in tilapia farms in Malaysia, Thailand, and Viet Nam and may also be present in other aquaculture industries (Barkham *et al.*, 2019). Although ST283 transmission routes have not been studied outside of Singapore, the finding of ST283 in humans and fish in the Southeast Asia region, where consumption of raw fish is common, and the very rare occurrence of ST283 outside the region, suggests a relationship between freshwater fish consumption and ST283 infection in humans.

Consequently, this emerging foodborne pathogen has the potential for a significant public health effect in the Southeast Asia region. In addition, given the growth in aquaculture in the region and increasing consumption of fish, it has considerable potential to affect food security and livelihoods in Southeast Asia. Similarly, exports outside the region, of live tilapia to farms and of tilapia for human consumption, may pose a global hazard. However, as will be shown in this risk profile, significant data gaps with respect of our understanding of foodborne GBS ST283 hinder a full assessment of risk to support risk management decision-making. GBS ST283 potentially affects six items of the United Nations (UN) Sustainable Development Goals (SDGs): No Poverty, Zero Hunger, Good Health and Well-Being, Decent Work and Economic Growth, Responsible Consumption and Production, and Life Below Water.

1.2. Scope and purpose of this risk profile

To provide scientific information for risk managers, pertinent information related to the public health risk posed by fish-derived GBS ST283 in various countries is collated in this document. The scope of this risk profile is restricted to freshwater fish, which can be farmed or commercially or non-commercially caught in the wild. In particular, tilapia is the freshwater fish species of primary focus because of its high production, common consumption, and relatively high prevalence of GBS ST283. GBS ST283 has also been found in other aquatic host species, including frogs and marine fish, but these have not yet been linked to foodborne disease and hence these aquatic species are not included. There is no evidence of GBS in other aquatic species, for example crustaceans, shellfish or other invertebrates, or macroalgae.

The purpose of this risk profile is to provide comprehensive information, through a critical review of currently available evidence, to address the risk management question of:

What is the risk to human health posed by GBS ST283 through consumption of freshwater fish in and around Southeast Asia?

In this document, the current state of knowledge about the presence and transmission of GBS ST283 along the freshwater fish supply chain is described, covering aquaculture and wild capture, transport, processing, retail, preparation, and consumption. Different preparation and consumption methods are considered as far as possible. A preliminary assessment of the current public health risk is presented by integrating available information, and data gaps are summarized. It should be noted that this risk profile does not represent a full risk assessment but could be used to assist risk managers in ascertaining the potential need for a full risk assessment. Possible control measures are described to support risk management.

Where possible, information is specifically based on GBS ST283. Where such information is not available, data were considered from GBS, other streptococcal species affecting fish, other streptococcal species not affecting fish, and other bacterial or non-bacterial foodborne pathogens from fish, with this order presumed to reflect a hierarchy of strength of evidence.
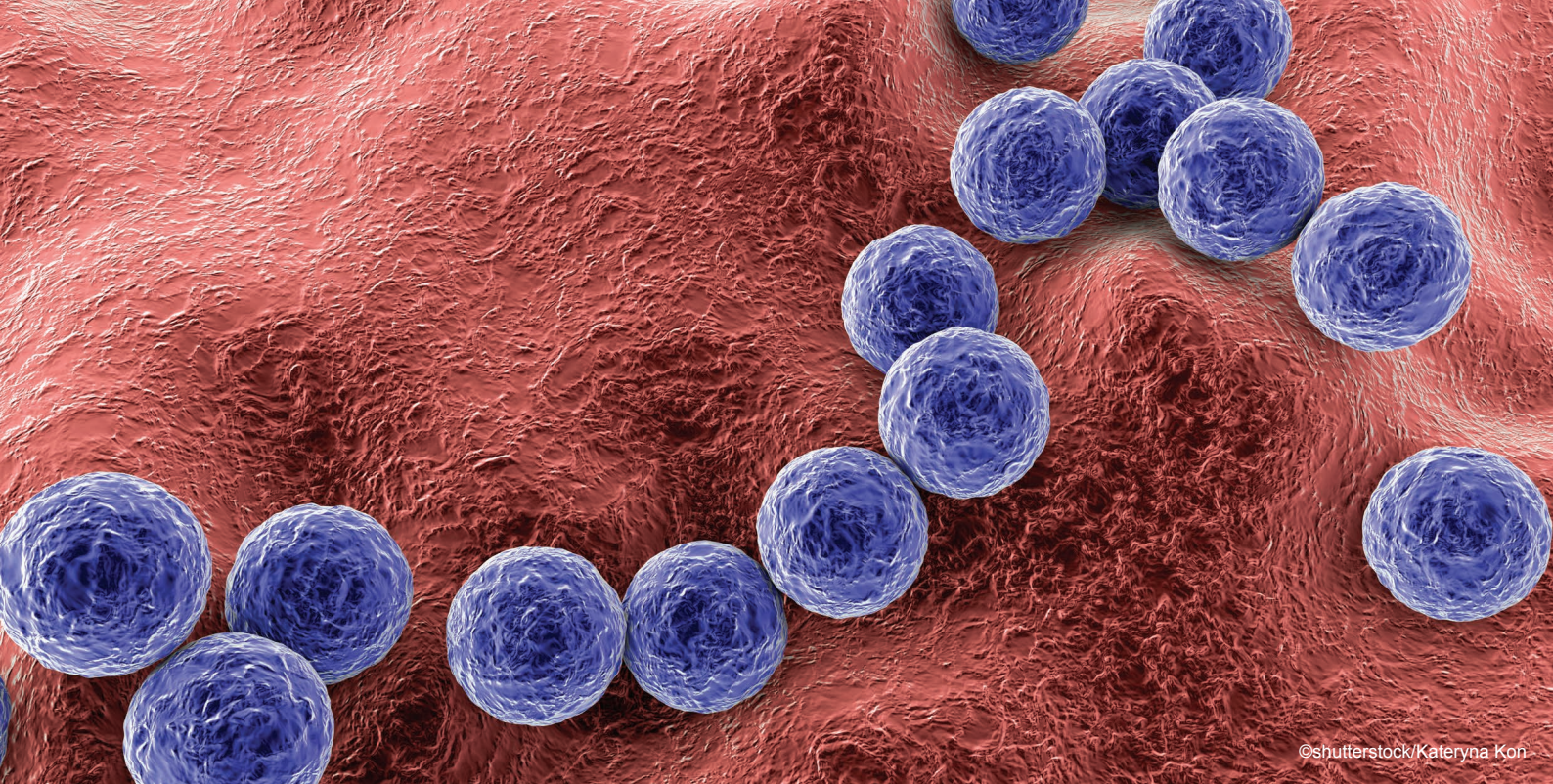
2 HAZARD-FOOD COMMODITY COMBINATION OF CONCERN

2.1. The hazard of concern: Group B *Streptococcus* sequence type 283

KEY POINTS:

- GBS (not specifically ST283) is commonly carried by healthy men and women.
- GBS (not specifically ST283) is a major cause of neonatal invasive disease around the world.
- GBS (not specifically ST283) has emerged as a common cause of sepsis among adults with comorbidities, although it is uncommon in healthy adults.
- GBS has been reported in mammals, reptiles, amphibians, bony fish and cartilaginous fish (rays), but not in birds. GBS ST283 and closely related types have only been reported in humans, fishes, and frogs.
- Streptococci in general are not heat resistant and do not survive pasteurization; in particular, GBS ST283 is more heat sensitive than *Escherichia coli* O157:H7 and *Listeria monocytogenes*.
- GBS can grow over a wide pH range but is less acid resistant than *E. coli* O157:H7 and *L. monocytogenes*.
- GBS can grow at salinity concentrations up to 5.5 percent NaCl (sea water is on average around 3.5 percent).
- GBS can survive well at temperatures as cold as −70 °C.

2.1.1. The microorganism

2.1.1.1. Characteristics of the organism

GBS is an encapsulated, gram-positive, non-motile coccus, arranged in pairs and chains. It is a facultative anaerobe that is catalase and oxidase negative. Most human GBS isolates derived from clinical disease, including ST283, are beta-haemolytic on sheep blood agar, and this is commonly used as a diagnostic characteristic in medical microbiology. A minority of human carriage isolates, some bovine isolates, and many isolates derived from aquatic host species are non-haemolytic.

GBS is part of the normal microbiota of the healthy gastrointestinal tract and colonizes the urogenital tract of men and women (Bliss *et al.*, 2002, Lyhs *et al.*, 2016). However, it is opportunistic and may spread to other body sites, including the bloodstream, brain, and joints, where it can cause serious infections, as well as skin ulcers and wounds. GBS was identified as an important neonatal pathogen in the 1960s and is a leading cause of neonatal meningitis and sepsis. Invasive GBS infections also affect pregnant women, especially peripartum. Over the last 30 years, GBS has emerged as a common cause of invasive sepsis amongst adults with comorbidities, although it is uncommon in healthy non-pregnant adults.

The World Health Organization classifiers for GBS disease in ICD-10 Version:2016 include: A40.1 ("Sepsis due to *Streptococcus* group B"), B95.1 ("*Streptococcus*, group B, as the cause of diseases classified to [sic] other chapters"), and specific neonatal classifications, such as P23.3 ("Congenital pneumonia due to *Streptococcus*, group B") and P36.0 ("Sepsis of newborn due to *Streptococcus*, group B"). No separate classification of foodborne GBS or GBS ST283 is included (WHO, 2016).

2.1.1.2. Subtyping of Group B *Streptococcus*

GBS can be classified at the subspecies level using a variety of typing methods. Different methods classify GBS types in different ways and correlation between the bacterial characteristics targeted by different methods is limited. Capsular typing (serotyping) and multilocus sequence typing (MLST) are the two most common systems in use. Whereas serotyping will remain useful as a result of the possible advent of vaccines targeting the capsular polysaccharide, MLST is much more discriminatory. It has been used widely over the past two decades to define GBS sequence types (STs) (Barkham *et al.*, 2019, Da Cunha *et al.*, 2014, Delannoy *et al.*, 2013). Other typing methods include antimicrobial resistance (AMR) profiling, virulence gene and surface protein gene typing, and insertion sequence typing, potentially further discriminating among strains sharing the same ST, although many of the virulence factors that are used in typing of human isolates are absent from animal isolates. More recently, whole genome sequencing (WGS) has been used to infer capsule type, ST, AMR, and virulence and surface protein genes (Ashton *et al.*, 2015, Barkham *et al.*, 2019, Furfaro *et al.*, 2019, Sigaúque *et al.*, 2018). WGS offers a much-improved ability to distinguish closely- related bacteria using single-nucleotide polymorphisms (SNPs), or whole or core genome MLST approaches.

GBS serotyping has identified ten capsular types: Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX. All isolates of ST283 and closely-related STs (see Section 2.1.1.3) belong to a subtype of serotype III, designated serotype III-4; this subtype has not been described in any other GBS lineage, so it corresponds to ST283 and closely-related STs. Based on an analysis of WGS data, ST283 appears to be a recently-emerged clone, with emergence dating back to the 1980s, coinciding with the intensification of aquaculture (Barkham *et al.*, 2019). ST283 is one of only three clades of GBS that cause disease in fish, the others being ST7 and its close relatives (which are serotype Ia), and CC552 (serotype Ib) (Delannoy *et al.*, 2013).

2.1.1.3. Nomenclature of sequence type 283

Multilocus sequence typing (MLST) was developed before whole genome sequencing became readily available. MLST is based on sequencing multiple parts (loci) of a genome, as it used to be easier than sequencing a whole genome, and targets a defined set of genes that are generally, but not always, conserved across the entire species. For GBS, MLST involves looking at the sequences of seven loci, in seven genes; for each gene, or locus, the sequence can vary in different GBS. A variant is called an allele, and each allele is given a numerical name, such as 1, 2, 3, 4 and so on. The alleles are named in the order that they are registered in a central register, so the numerical proximity of alleles does not imply that they are more similar to each other than alleles with more distant numerical names. Each of the seven loci can have multiple alleles in different GBS. MLST will thus provide seven numbers, one for each of the seven loci.

The set of seven allele numbers for these genes constitutes an allelic profile that defines a specific ST. For example, ST283 refers to the allelic profile 9-5-7-1-3-3-2 (which corresponds to allele numbers at the seven loci: *adhP, pheS, atr, glnA, sdhA, glcK,* and *tkt*, respectively). Single locus variants (SLV) match exactly at six of seven of these alleles, and double locus variants (DLV) at five of seven. Historically, similarities between allelic profiles have been used to define clonal complexes (CC) (Feil *et al.*, 2004). For example, fish can be affected by CC7, which includes and is named for ST7; other STs in CC7 include ST6, ST500, and ST735, all of which are SLVs of ST7. Similarly, CC552 includes and is named for ST552, but also includes ST246, ST258, ST259, ST260, ST261, ST553, and a growing number of other STs. ST260 and ST553 are SLVs of ST552, whereas ST246, ST259, and ST261 are DLVs of ST552, and ST258 is a triple locus variant of ST552.

However, MLST only samples seven loci, which constitute a small proportion of the whole genome, and any two bacteria with similar MLST profiles may have differences in other parts of the genome. As WGS has become more common, it has become clear that some STs that would normally be considered part of a CC, based on similar MLST profiles, are not actually closely related. Specifically, for ST283, analysis of whole genome sequences has identified the following STs reported in the public MLST database as closely related.

- ST491 (an SLV of ST283), and ST1311 (an SLV of ST491, and a DLV of ST283). Only one example of ST491 and ST1311 have been reported; they were isolated from tilapia in Viet Nam in 2006 and 2016, respectively, (Barkham *et al.*, 2019, Delannoy *et al.*, 2013).
- ST739 (an SLV of ST283). Only one example of ST739 has been reported; it was isolated in 2014 from a diseased tiger frog farmed for human consumption in Guangdong, China (Barkham *et al.*, 2019).

In contrast, the following SLVs of ST283 in the public MLST databases are not closely related by analysis of WGS data, or have no other data to evaluate:

- ST160 is of unknown origin and no genomic data are available;
- ST690 is represented by a human isolate but no genomic data are available;
- ST751 is represented by human isolates from Europe, which are serotype II; and
- WGS data indicate that ST751 is not closely related to ST283 (Barkham *et al.*, 2019, Delannoy *et al.*, 2013, Jones *et al.*, 2003).

For simplicity, ST283 (as opposed to CC283) will be used in the remainder of the document, but the closely related STs including ST491, ST739, and ST1311 will hereafter be understood to be included when ST283 is mentioned. Note that, because of prevailing usage in the literature, we will still refer to CC7 and CC552, which accordingly both include more than one ST.

2.1.1.4. Antibiotic susceptibility of Group B *Streptococcus* in humans and fish

The prevalence of AMR in GBS varies according to host, age and geographical location. In human and bovine medicine, beta-lactams are the choice of treatment of GBS (except in patients with suspected or confirmed penicillin allergy), although there are a small but growing number of reports of human, bovine, and piscine GBS with reduced penicillin susceptibility because of changes in penicillin binding proteins (Li *et al*., 2020).

For GBS ST283, a search of the relevant literature revealed that there are no reports to date of resistance to erythromycin, clindamycin, or penicillin (T. Barkham, personal communication, 2020). Data on tetracycline resistance is limited. Notably, for GBS in general (not specifically ST283), tetracycline resistance has been described as a marker of human host adaptation (Da Cunha *et al*., 2014). The vast majority of ST283 isolated before 2006 was also resistant to tetracycline. However, amongst recent isolates, only isolates from Viet Nam and Malaysia remain resistant, whereas isolates from Lao People's Democratic Republic and Thailand are susceptible. Isolates from Singapore show variable tetracycline resistance, presumably because of importation from different countries (Barkham *et al*., 2019). The presence and/or persistence of tetracycline resistance is suspected to be related to the use of tetracyclines in aquaculture (Thi Kim Chi *et al*., 2017). However, similar antimicrobial use is reported in aquaculture in Thailand and Viet Nam, but resistance to tetracycline amongst human ST283 isolates from Thailand disappeared after 2012 (Rico *et al*., 2014). This loss of tetracycline resistance after 2012 was also seen in human ST283 isolates from Lao People's Democratic Republic and Singapore (Barkham *et al*., 2019).

2.1.1.5. Virulence of Group B *Streptococcus* sequence type 283

Based on published and unpublished genomic analyses conducted to date, ST283 shares many virulence factors with other GBS strains, including the presence of a capsule, pili, a hemolysin operon, and the virulence genes *hylB* (hyaluronidase), *scpB* (C5a peptidase), *lmb* (laminin binding protein), and *bca* (C protein alpha-antigen) (Edwards and Baker, 2020, Jensen, 1982, Mehershahi *et al*., 2015). ST283 GBS and other non-ST17 GBS possess the *bibA* gene (*bibA* adhesin), but not the *hvgA* gene (hypervirulent GBS adhesin) that is found in ST17, the major neonatal clade of GBS. Unique to ST283 is the consistent integration of a prophage at the 5'-end (five prime end) of the hyaluronidase gene *hylB*. It has been hypothesized that this prophage may play a role in regulation of the transcription and expression of *hylB* (Crestani, Forde and Zadoks, 2020).

Virulence of ST283 in fish almost certainly has a different molecular basis than virulence in humans. A mobile genetic element named Locus 3 is associated with fish-pathogenic GBS strains of all clades, i.e. CC7, ST283, and CC552 (Delannoy *et al*., 2016). This locus contains the Leloir operon, which is associated with galactose metabolism, a feature that is enriched in fish isolates compared to those from other host species (Richards *et al*., 2019). Locus 3 is also present in ST283 from humans (Crestani, personal communication) and has occasionally been reported in other isolates, for example GBS ST1 from cattle, ST6 and ST7 from humans, and ST399 from a dolphin (Delannoy *et al*., 2016).
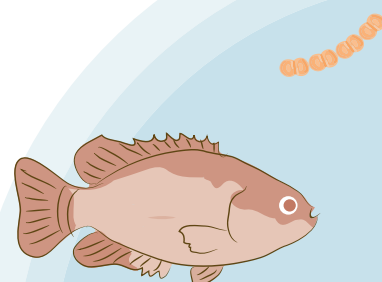
2.1.1.6. Natural habitats and hosts of Group B *Streptococcus*

GBS was named "*agalactiae*" (meaning "no milk" in Greek) because it causes udder infections in dairy cows, causing significant reductions in milk yields. GBS has also been reported in other mammals (camels, horses, dogs, cats, monkeys, seals, whales, dolphins, rabbits, guinea pigs, rats, and mice), reptiles (crocodiles, monitor lizards), amphibians (frogs), bony fishes, and cartilaginous fishes (rays), but not in birds.

GBS ST283 strains have only been reported for humans, fish, and frogs (Barkham *et al.*, 2019). GBS ST283 has mostly been detected in freshwater species, but some isolates are from marine species (Zadoks *et al.*, 2020). In the environment, GBS survival is thought to be limited, and its presence in water or other environmental sources is largely attributed to contamination with human sewage or animal waste (Jafar *et al.*, 2008, Jensen and Berg, 1982, Jorgensen *et al.*, 2016). GBS (not specifically ST283) may occur in soil, water, sediment, and other environmental sources (Lyhs *et al.*, 2016).

2.1.2. Growth and survival characteristics

For *Streptococcus* spp. in general, and GBS and GBS ST283 specifically, there are few published studies reporting how environmental conditions affect the growth and survival of the organism. The major predictive microbial modelling databases namely ComBase (ComBase Team, 2019), pathogen modeling program (USDA) and Sym'Previus (Sym'Previus Team) do not include records for *Streptococcus* spp. or GBS in food or broth media. The main experimental studies are summarized in Table 1 and Table 2 and in proceeding sections.

Table 1. Limits for growth of GBS (not specifically ST283) when other conditions are near optimum

	MINIMUM	OPTIMUM	MAXIMUM
Temperature (°C)	5	30	45
pH	3	7	11
Water activity[1]	>0.91	Uncertain	>0.99
NaCl (percent)	0	0.5	5.5

[1] For *Streptococcus* spp. generally.
Source: Laith *et al.*, 2017.
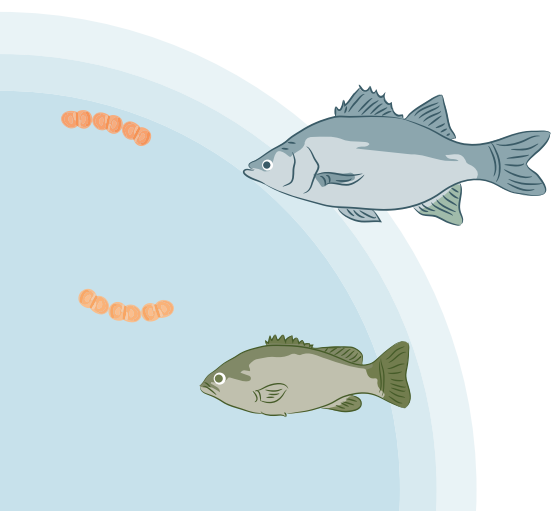
2.1.2.1. Temperature

Bacteria of the genus *Streptococcus* in general, and GBS specifically, do not survive pasteurization. Experimental studies on human milk have shown how both low-temperature (62 °C) long-time (30 minutes) pasteurization (LTLT) and high-temperature (72 °C) short-time (16 seconds) pasteurization (HTST) are capable of effectively reducing high GBS loads below the detectable limit (>6 log reduction after 4 seconds at 72 °C; >5 log reduction after 5 minutes at 62.5 °C) (Terpstra *et al.*, 2007, Wills *et al.*, 1982).

The effects of dipping raw fish slices in warm porridge, which was the practice associated with the outbreak in Singapore in 2015, were simulated at 56.4 °C by Zwe *et al.* (2019). The results from tests to identify the heat resistance of different GBS strains were expressed as the decimal reduction time (D-value). This is the time required to achieve a log reduction, which is equivalent to killing 90 percent of the GBS, so an organism with a smaller D-value is killed faster and is less heat resistant. The results indicated that all GBS strains tested were less heat resistant than *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Although GBS ST283 had the lowest measured D-values for heat resistance, these were not significantly different from the values for the GBS ST651 strain (Table 2).

Table 2. Decimal reduction times (in minutes) for various pathogens under different conditions

ORGANISM	D-VALUE AT pH 7.0 AT 56.4 °C	D-VALUE AT pH 2.35 AT 37 °C
GBS ST283(a)	0.72 ± 0.06	0.32 ± 0.13
GBS ST283(b)	0.72 ± 0.06	0.68 ± 0.28
GBS ST335	0.88 ± 0.12	0.60 ± 0.01
GBS ST651	0.74 ± 0.12	0.44 ± 0.08
E. coli O157:H7	11.44 ± 1.19	N.M.
L. monocytogenes	9.78 ± 0.30	23.69 ± 1.72

Source: Zwe *et al.*, 2019.

Vegetative cells of streptococci are generally resistant to freezing and frozen storage (Speck and Ray, 1977). Evans, Klesius and Shoemaker (2004) confirmed 100 percent survival of GBS from organs of naturally infected mullet after nine months storage at –20 °C to –70 °C, and culture-positive samples were recovered from experimentally infected tilapia up to 180-day post-freezing at –70 °C.

GBS isolated from fish (naturally infected hybrid tilapia) grew at temperatures ranging from 5 °C to 45 °C, with the optimal growth being observed at 30 °C (Laith *et al.*, 2017). GBS CC552 grows at 28 °C but not at 37 °C, whereas CC7 and ST283 can grow at either temperature.

2.1.2.2. pH

Based on GBS pathophysiology in humans, GBS have successfully adapted to varying pH levels ranging from the acidic environment of the vaginal mucosa (pH 4 ± 0.5) to the almost neutral environments of the respiratory tract and human blood (Shabayek and Spellerberg, 2017). One report, using a gastric-equivalent pH of 2.35, indicated that all GBS strains tested were less acid resistant than *E. coli* O157:H7 and *L. monocytogenes*, when tested at 37 °C. These results are surprising, as *L. monocytogenes* is generally not reported to tolerate such a low pH, but interpretation of experimental work on acid resistance can be complicated as bacteria can adjust to a lower pH, if given time. The two GBS ST283 strains tested in this work differed significantly in their acid tolerance; the lowest measured D-value for acid tolerance was one of the GBS ST283 strains, but this again was not significantly different from GBS ST651 (Table 2) (Zwe *et al.*, 2019). GBS from naturally infected hybrid tilapia showed growth at a pH ranging from 3 to 11, but with an optimum of 7 (Table 1) (Laith *et al.*, 2017). The relevance of these experiments to acid tolerance in the natural environment, such as in food dishes, is unknown. Also, an acidic pH (pH 5) was shown to be required for adhesion of an encapsulated GBS strain (SaTiBe08-18, ST261, serotype Ib) to epithelial cells on tilapia intestinal explants (Barato *et al.*, 2016).

2.1.2.3. Water activity

Water activity (aw) is a critical parameter affecting microbial activity. Precise minimum aw requirements for growth and survival of GBS ST283 and GBS in general are unknown, however, aw values > 0.91 are typically needed to support growth and survival of most gram-positive bacteria (Roos, 2003).

2.1.2.4. Salinity

GBS can grow at NaCl concentrations from 0 to 5.5 percent, as shown in Table 1 (Laith *et al.*, 2017). In agreement with those results, GBS from freshwater red hybrid tilapias can survive at 0 to 1 percent NaCl but not at 6 percent to 8 percent NaCl (Amal, 2007), whereas GBS isolated from marine water silver pomfret (*Pampus argentusa*) could grow in culture media at 3 percent to 4 percent NaCl (Azad *et al.*, 2012, Duremdez *et al.*, 2004). These laboratory results support the possibility of GBS occurring in fish from brackish or marine water, as demonstrated for CC552 in many fish species (Bowater *et al.*, 2012).

2.1.2.5. High pressure

Exposure of bacteria to high pressure causes damage to the cell membrane with consequent denaturation of proteins and cell death. For this reason, because of the presence of a hard cell wall, gram-positive bacteria are generally more resistant to high pressure than gram-negative ones. Experimental data on *S. agalactiae* ATCC 12927 (not an ST283 strain) in human milk treated by means of high-pressure processing (HPP) showed an 8-log reduction after four minutes at 400 megapascal pressure units (MPa) (Viazis, Farkas and Jaykus, 2008).

2.2. The food: freshwater fish

> **KEY POINTS:**
>
> - Southeast Asia is one of the world's major freshwater fish producing regions.
> - Nile tilapia can be considered a regional economically-important species because several Southeast Asian countries are major producers.
> - Southeast Asian freshwater fish, including tilapia, are mainly produced for domestic consumption.
> - GBS ST283 has been found in tilapia, including those that appear healthy, and other aquaculture species in Southeast Asia.

2.2.1. Supply chain of freshwater fish

Southeast Asia has abundant aquatic resources, including five major river systems. Their drainage basins and large tributaries, as well as the climate, are highly supportive of capture fishery and aquaculture of freshwater fish. Tilapia is of the greatest importance for this risk profile because it is a major freshwater aquaculture species, with the third highest tonnage of 4 525.4 thousand tonnes globally in 2018 (FAO, 2020b) and because GBS, including ST283, is a major pathogen of tilapia.

Tilapia production is highly concentrated in a few countries and the major tilapia-producing countries accounted for approximately 98 percent of the global farmed tilapia production in 2016, of which approximately 95 percent was sold domestically (El-Sayed, 2019). Although exact data are scarce, small-scale farms, which are thought to contribute between 30 percent and 80 percent of the total aquaculture production volume (HLPE, 2014, Phillips *et al.*, 2016), apply more traditional farming practices with weaker links to markets than large-scale commercial operations (Ali *et al.*, 2018). Sampling has been insufficient to allow comment on whether GSB ST283 is more common in small-scale or large-scale farms.

The supply chain for freshwater fish, despite some differences between species, harvesting source (capture versus aquaculture), and countries, can generally be divided into the following stages (Tint *et al.*, 2020):

(i)	Fish production, either through capture fisheries or aquaculture
(ii)	Harvesting
(iii)	Transportation
(iv)	Processing
(v)	Storage, transportation, and retail

These stages are described in more detail in the following sections. A general overview is presented in Figure 1.

Figure 1. General representation of the freshwater fish supply chain
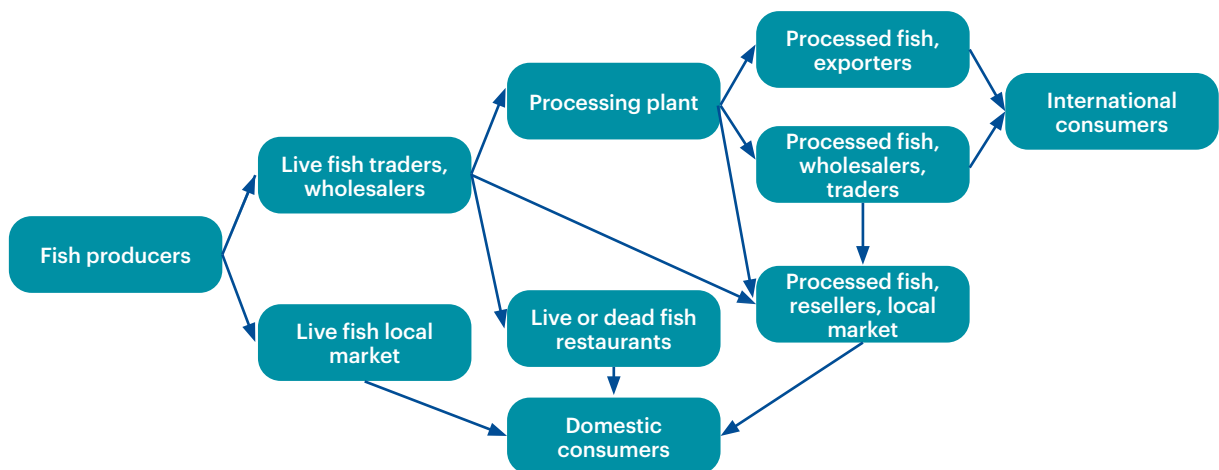
2.2.1.1. Production

Production systems for freshwater fish are diverse and range from traditional small-scale culture to intensive high-density stocking systems. Fish such as tilapia can be cultured in cages in rivers or lakes, earthen ponds or geomembrane ponds with open systems, recirculation aquaculture systems, in-pond raceway systems, biofloc technology systems, aquaponic systems, or rice paddies (Abakari *et al.*, 2020, He *et al.*, 2020, Liang and Chien, 2013, Wambua *et al.*, 2020). This can be done in monoculture, or polyculture with other herbivorous or omnivorous fish and shrimp, as well as other animal species, such as frogs, or rice (Stickney, 2013). However, unless well managed, polyculture can result in poorer water quality compared with monoculture (Chang *et al.*, 2020). Details of fish health management and GBS control are given in Section 2.3.

2.2.1.2. Harvesting

Harvesting and capturing techniques applied for freshwater fish can differ depending on species. In contrast, the handling conditions along the supply chain are relatively similar between species despite the ethnic diversity of consumers in Southeast Asia.

In capture fisheries, harvest methods are diverse, including nets, hooks and traps, and depend on local practices and commercial conditions (Borderías and Sánchez Alonso, 2011, Hortle, 2009). For farmed fish, harvest methods vary depending on stocking density and the farm type, for example pond, cage, or raceway. In intensive production systems, harvesting is normally a single event performed at the end of the production cycle. The methods employed include netting or pumping the live fish into another holding unit or transportation vehicle followed by shipping to a secondary processing plant. Intensive aquaculture farms usually practice monoculture and are more commonly export-market orientated or focused on premium products on the domestic market, sold via retailers. Premium products have more requirements, as they are marketed and sold at a higher price point (FAO, 2018). Small-scale production units often practice a partial harvest, selecting fish when they reach the desired market size and selling at local markets or to traders. These are destined for the domestic market.

2.2.1.3. Transportation

Fish destined for international markets are typically starved for 24 hours to 48 hours prior to transportation to a processing plant. The starvation period depends on the water temperature and species. Starvation reduces the microbial load in the fish and reduces post-mortem autolysis from digestive enzymes, which can result in off-flavours (Borderías and Sánchez-Alonso, 2011). Starvation prior to transportation is not commonly practiced in fish harvested from small-scale fish farms. The fish are transported as live animals, usually at night, in a wide range of containers with or without supplemental oxygen, in a small volume of farm water. In some cases, the transportation water will be cooled. The transportation distances are usually short (within the same district). Stressful conditions during harvest and transport can exacerbate stress in the animals and compromise the quality of the product (Borderías and Sánchez-Alonso, 2011). In some cases, freshwater fish are killed directly after harvest and distributed to local fish markets, restaurants, or directly to end consumers. The fish will usually be placed on ice flakes and transported in insulated iceboxes.

2.2.1.4. Processing

Processing consists of three stages (pre-processing, primary processing, and secondary processing), and all processing methods involve a degree of handling of the raw product. Pre-processing involves inspection of the product, washing, and grading and can be performed on the ship or at the wet market, restaurant, or processing plant.

Primary processing involves removal of viscera, head, tail, fins, scales, skin, and bones. In large-scale processing plants, some of these steps may be automated, whereas in small-scale operations and at retail outlets and restaurants these steps are performed manually. Retailers at wet markets and in some supermarkets will kill and gut fish and sell them as fresh whole fish or as a cooked and ready-to-eat (RTE) product (Abdullah, Idrus and Mardi, 1978). At processing facilities, fish are held in holding tanks until killing, gilling, and gutting; head removal is optional depending on the final product form. Whole fish are then chilled or processed further.

Secondary processing results in value-added products, such as fresh, frozen, or heat-treated fillets (including plain, crumbed, or battered), and salted, dried, smoked, or canned products. The degree of processing depends on the species and final market destination.

2.2.1.5. Shelf-life

Information on shelf-life and transportation of freshwater fish is limited (Jimenez-Ruiz *et al*., 2020). Gerges, Selim and Osman (2016) found the shelf-life of tilapia fillets to be seven days when stored at 2 °C, whereas Cyprian *et al*. (2013) observed the shelf-life of air-packed fresh fillet to be 20 days when stored at –1 °C and 13 days to 15 days when stored at +1 °C.

2.2.2. Production statistics

Over the past 20 years, global freshwater fisheries production increased from 6.4 million tonnes (average between 1986 and 1995) to 12.0 million tons (2018), whereas freshwater aquaculture increased from 8.6 million tonnes to 51.3 million tonnes. Much of this growth occurred in East Asia and Southeast Asia, where production of cultured freshwater fish increased from 5.1 million tonnes to 43.4 million tonnes (FAO, 2020b). Indonesia, the Philippines, Viet Nam, and Thailand are the biggest producers of farmed and captured tilapia species in the region (World Bank, 2013).

The value of aquaculture for freshwater fish exceeded USD 13.6 billion in 2018, and accounted for 0.46 percent of regional GDP (FAO, 2020a, World Bank, 2020). In individual mainland Southeast Asian countries, this contribution can be greater (1.76 percent–2.92 percent for Viet Nam, Myanmar,

and Cambodia). Many Southeast Asian governments are expected to promote culture-based fishery production as an alternative to captured fishery, which has plateaued in production for many years (World Bank and Ministry of Planning and Investment of Vietnam, 2016). Thus, freshwater fish are of great importance to regional economies as well as to food security, and foodborne GBS may pose a threat to food production, food safety, and economic growth.

2.2.3. International trade

International trade in fish and fish products is essential for the economies of many countries and regions. Many developing countries, particularly in Southeast Asia, are seeing a steady increase in international trade, with faster rates of growth compared with developed countries. China, the rest of East Asia, Southeast Asia, and South America increased their combined share of global exports of fish and fish products from 38 percent to 54 percent between 1976 and 2018. Rapid growth of aquaculture is projected over the next decade in Southeast Asia as well as South Asia and Latin America (FAO, 2020b, World Bank, 2013).

However, only a small fraction of freshwater fish production is traded internationally. According to FAO, freshwater fish commodities of about 894 535 tons were exported from Southeast Asian countries (Figure 2), which accounted for only 10.22 percent of the total freshwater fish production of the region in 2018 (FAO, 2020a). Moreover, 674 580 tons (75.5 percent) of these export commodities were catfish, mainly as frozen fillet (FAO, 2020a). Therefore, despite the relatively high production, export of tilapia and carps was limited. These data emphasize that Southeast Asian freshwater fish are mainly destined for domestic consumption.

Importation of freshwater fish products by Southeast Asian countries is lower than exports (Figure 2). Amongst Southeast Asian countries, Thailand is the biggest importer (37.8 percent) in the region, followed by the Philippines (20.0 percent) and Singapore (19.0 percent). The major imported commodity by Southeast Asian countries was also catfish (particularly frozen fillets), most of which was imported from Viet Nam (FAO, 2020a).

Figure 2. Import and export quantities of freshwater fish products by Southeast Asian countries between 2010 and 2018
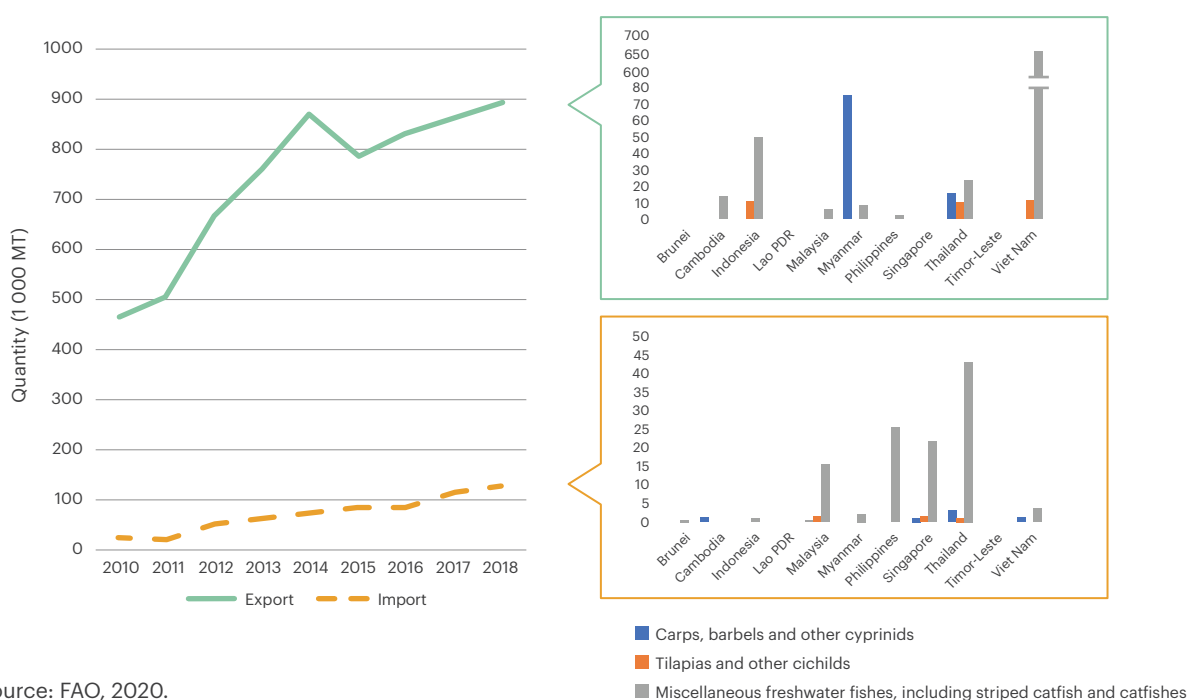
Source: FAO, 2020.

There is a rising trend for import and export of prepared or preserved tilapia products, including frozen whole fish and fillets (Figure 3). Currently, consumer preferences in many Southeast Asian countries are changing from traditional, small-sized, unprocessed fresh fish to larger-sized fish sold as fresh, chilled, or frozen products (El-Sayed, 2019).

Figure 3. Global tilapia exports and imports
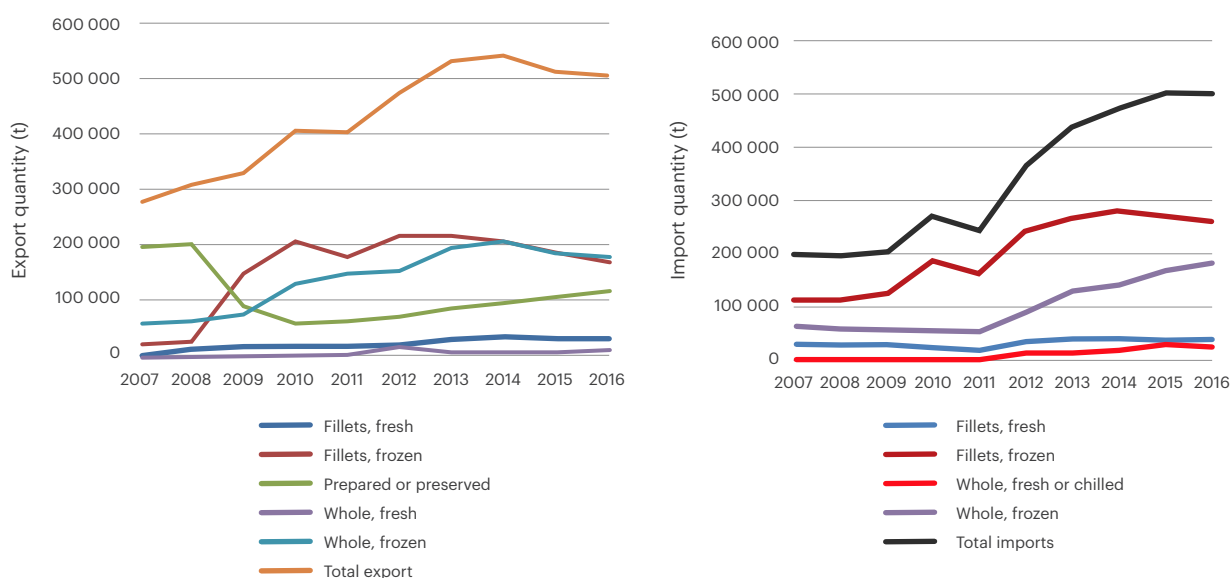
Source: FAO, 2018.

Importantly, statistics for international trade of live fry and broodstock for hatchery and grow-out farming are not available. In the case of tilapia, fish stocks have been traded across the continent to expand commercialization of tilapia in African countries where hatcheries are not readily available (Mapfumo, 2018). Of note, importation of tilapia broodstock from Asia was speculated to be the source of fish-related outbreaks of GBS ST283 in Brazil in 2016 and 2017 (Leal *et al.*, 2019).

2.2.4. Consumption

2.2.4.1. Volume

In 2017, finfish consumption was over 20 kg per capita per year in Oceania, Asia, North America, and Europe versus approximately 10 kg per capita in Africa, Latin America, and the Caribbean (FAO, 2020b). Annual consumption of fish in inland countries is less than 1 kg of fish per capita despite advances in logistics and supply chains (FAO, 2020b). Freshwater fish remain very important to food security in many low-income and middle-income countries (FAO, 2020b, Hasselberg *et al.*, 2020).

2.2.4.2. Food preparation

Broadly speaking, fish dishes can be divided into several categories based on the preparation, including raw, preserved (for example salted and/or fermented), smoked (cold or hot), and heat treated. As observed during the GBS ST283 outbreak in Singapore, the term "cooked" can be understood in different ways in different languages and cultures, for example as "heat treated" or "prepared, but not necessarily with heat treatment". Therefore, use of the term "cooked" may lead to misunderstanding in the context of food safety. Heat-treated fish is not known to pose a food safety risk if sufficiently heated and protected from re-contamination and is not described in detail here.

Raw fish dishes

The consumption of raw fish is particularly associated with the food practices of the Mekong basin (Grundy-Warr *et al.*, 2012). One example is *yu sheng*, which was associated with the 2015 outbreak of GBS ST283 disease in Singapore (Kalimuddin *et al.*, 2017). There are a range of preparation techniques, but this dish is typically similar to a tossed salad containing raw fish. Another traditional raw fish dish, called *koi pla*, is commonly eaten in northeast Thailand and Lao People's Democratic Republic. This dish is made from chopped raw fish meat mixed with chili, lemon-juice, spices, and herbs. It is relatively easy to prepare and popular in fishing and farming villages, where local freshwater fish is easily and cheaply available.

Preserved fish dishes

Apart from the raw *koi pla*, Kaewpitoon *et al.* (2008) identify the following dishes which do not involve heat treatment and are prepared from cyprinid fish (i.e. carp-like fishes and minnow types):
- *pla som*, or sour fish, is moderately fermented fish that is stored for a few weeks; and
- *pla ra* and *jaewbhong* are fish sauces or seasonings that use salted fish with other ingredients and are stored and fermented for over three months.

Anecdotal evidence indicates that *pla ra* and *jaewbhong* are consumed daily by a high number of people in the Northeast of Thailand and lowland Lao People's Democratic Republic. Although Nile tilapia is a cyprinid fish, only a fraction of its production is used in such dishes (0.32 percent), and they are more commonly prepared with striped snakehead, silver barb, climbing perch, and a variety of local freshwater fish (1.58 percent to 5.96 percent of production volume).

Various forms of freshwater preserved fish are also consumed in Sarawak, Malaysia. Examples include *kasom ikien*, eaten by the Bidayuh, and *kasam ikan*, which is eaten by the Iban. These dishes would commonly be eaten as an accompaniment to a rice meal, and for poorer families, might at times represent the sole source of protein in a typical meal.

2.2.5. Freshwater fish associated with Group B *Streptococcus* sequence type 283 occurrence

Identification of GBS ST283 in freshwater fish has been reported from a number of species listed here, though it should be noted that detection at retail could be a result of cross-contamination from other sources. In addition, identification of fish species may not be accurate as substitution of high-value species with less expensive fish is common (Barkham *et al.*, 2019). The list is sorted in order of increasing strength of evidence for infection of fish, i.e. from potential contamination of tissues post-harvest to evidence of organ invasion during clinical disease of live fish:

- muscle sold as grass carp (*Ctenopharyngodon idella*) – three different samples from a food stall in Singapore (Chau *et al.*, 2017);
- muscle sold as silver carp (*Hypophthalmichthys molitrix*) – one sample from a supermarket in Singapore (Chau *et al.*, 2017);
- surface swabs, muscle, and organs sold as Asian bighead carp (*Hypophthalmichthys nobilis*) – six different samples from the port in Singapore (Chau *et al.*, 2017);
- surface swabs, muscle, and organs of black tilapia (*Oreochromis niloticus*) in supermarkets and wet markets in Singapore (Barkham *et al.*, 2019, Chau *et al.*, 2017);
- organs in red tilapia (*Oreochromis* sp.) in supermarkets and wet markets in Singapore (Barkham *et al.*, 2019, Chau *et al.*, 2017);
- all life stages of red tilapia (*Oreochromis* spp.), regardless of obvious clinical manifestation (Pradeep *et al.*, 2016);
- organs, for example brain and kidney, of clinically diseased black tilapia and red tilapia from fish farms in Malaysia, Viet Nam, and Brazil (Barkham *et al.*, 2019, Leal *et al.*, 2019); and

• organs of Mekong giant catfish (*Pangasianodon gigas*) with clinical disease (Zadoks *et al.*, 2020).

In addition to the above freshwater fish, freshwater frogs (*Hoplobatrachus rugulosus, H. chinensis*) and marine species (*Lates calcarifer*; known as barramundi, Asian seabass, and giant sea perch) have been reported to have been affected by GBS ST283 (Barkham *et al.*, 2019, Zadoks *et al.*, 2020). A wide variety of marine fish is affected by GBS CC7 and CC552 (Bowater *et al.*, 2012, Delannoy *et al.*, 2013, Plumb *et al.*, 1974), but their identity is beyond the scope of this risk profile.

2.3. Risk factors for Group B *Streptococcus* sequence type 283 pre-harvest

KEY POINTS:

• The risk of GBS disease in fish can be reduced through good water quality and good fish husbandry management.
• The risk of GBS disease in fish increases with high water temperature, high water acidity/alkalinity, and water pollution.
• Husbandry-based risk reduction measures may not suffice to completely prevent GBS disease in fish or people.
• Tilapia at every life stage, including fry, may harbour GBS regardless of obvious clinical manifestations.

The occurrence of pathogens in the fish environment alone is not sufficient to cause a disease outbreak. Various stressors usually play a significant role in outbreaks of infectious diseases in fish populations (Kumar *et al.*, 2015, Yanong and Francis-Floyd, 2020). Some common stressors that have been linked with streptococcal outbreaks include:

• Poor water quality, such as high-water temperatures (Alsaid *et al.*, 2013, Amal *et al.*, 2015), low dissolved oxygen concentration (Amal *et al.*, 2015), high ammonia concentrations (Amal *et al.*, 2015), and high acidity/alkalinity (pH <6 or >8) (Alsaid *et al.*, 2013, Amal *et al.*, 2015)
• Poor husbandry, including high stocking densities and rough handling and harvesting of fish (Shoemaker, Evans and Klesius, 2000).

Whereas *Streptococcus* spp. have long been recognized and studied as fish pathogens, including to some extent GBS CC7 and CC552, ST283 is more recent. Thus, relatively little information about the effects of water quality and husbandry practices on GBS ST283 is available. Consequently, the effects of water quality and husbandry practices are discussed in the following sections in a general way as they relate to fish health, and in relation to GBS and ST283 specifically where the information exists.

2.3.1. Water quality

Water quality is an important factor in all aquaculture systems (Boyd and Tucker, 2012, El-Sayed, 2019). Poor water quality is a stressor for the immune system, increasing the susceptibility of cultured animals to infection (Amal *et al.,* 2015). In addition, poor water quality, such as the presence of uneaten fish food and reduced oxygen levels, can promote the growth of various pathogenic bacteria in fish and the surrounding aquatic environment (Glibert *et al.,* 2002, Ismail *et al.,* 2016b).

2.3.1.1. Temperature

High water temperature is associated with increased mortality of cultured or wild fish because of GBS in tropical countries. GBS disease in fish is considered a "warm water" streptococcosis. This group of diseases causes mortality at temperatures higher than 15 °C. Other pathogens that cause warm water streptococcosis include *Lactococcus garvieae* (synonym *Enterococcus seriolicida*), *Streptococcus iniae* (obsolete synonym *S. shiloi*), and *S. parauberis*. By contrast, "cold water" streptococcosis occurs at temperatures below 15 °C and is caused by *Vagococcus salmoninarum* or *Lactococcus piscium* (Romalde *et al.,* 2008).

High water temperatures stress fish and reduce their immunity against infection by GBS. In addition, high water temperatures also promote the growth of GBS. Observational data from Brazil and Malaysia indicate that GBS infections in cultured tilapia were associated with water temperatures above 27 °C (Amal *et al.,* 2015, Mian *et al.,* 2009).

2.3.1.2. Dissolved oxygen

The water temperature in freshwater fish aquaculture generally ranges from 24 °C to 32 °C, with a pH between 7 and 8. Dissolved oxygen levels should be between 4 mg/L and 8 mg/L (Santos *et al.,* 2019, Tavares *et al.,* 2018). Low dissolved oxygen can be a substantial stressor in aquaculture systems, which is why some farms use artificial aeration.

Under experimental challenge conditions, Nile tilapia exposed to sub-lethal dissolved oxygen levels (≤1 mg/L) had significantly higher mortality and blood glucose levels as a result of infection by GBS than fish exposed to acceptable levels (4 mg/L to 6 mg/L) (Evans, Shoemaker and Klesius, 2003). Likewise, elevated levels of dissolved oxygen were associated with lower isolation of GBS in a field study of cultured red hybrid tilapias (Amal *et al.,* 2015).

2.3.1.3. Ammonia

Ammonia is a waste product of fish and can also result from the breakdown of unutilized feed. High ammonia concentration in the water is usually related to poor environmental conditions of the fish growing area because of nearby residential or agricultural activity and disposal of household waste. The massive mortality of wild mullet (*Liza klunzingeri*) caused by GBS in Kuwait Bay was associated with elevated ammonium concentrations (0.11 mg/L to 0.33 mg/L) (Glibert *et al.,* 2002). A higher ammonia concentration (0.24 mg/L) was also significantly correlated ($r = 0.5085$; $p < 0.01$) with increased isolation of GBS in cultured red hybrid tilapia in Malaysia (Amal *et al.,* 2015).

2.3.1.4. pH

A field study of cultured red hybrid tilapia in selected lakes in Malaysia, where ST283 is the predominant GBS type in fish, found increased GBS isolation with higher water pH during the fish culture period (Amal *et al.,* 2015). Experimental work supports this observation, in that mortality of tilapia after experimental challenge with ST283 was lowest at pH 7.5 (40 percent), but increased to 60 percent at pH 8.5. Mortality was similarly high at an acidic pH of 6.5 (70 percent), and highest at a pH of 5.5 (95 percent) (Phuoc *et al.,* 2020).

2.3.1.5. Salinity

Tilapia are freshwater fish by nature. Exposure to high salinity poses a metabolic burden and may predispose tilapia to infection and mortality (El-Leithy *et al.*, 2019). To expand the area where tilapia can be farmed, salt-tolerant varieties of some tilapia species have been produced. Salt-tolerant tilapia can cope with salinity levels of up to 12 g/L, but this comes at a metabolic cost such as negative effect on growth performance (Rahmah *et al.*, 2020). The impact of salinity and salt tolerance of tilapia on the risk of GBS (and GBS ST283) infection requires further investigation.

2.3.2. Husbandry

Good fish farm management will control and prevent, or at least reduce, the introduction, multiplication, and transmission of GBS in the fish growing environment. Common husbandry practices that have been associated with streptococcosis (including disease caused by GBS) or its prevention are discussed in Section 2.3.2.1 to Section 2.3.2.9. However, husbandry-based risk reduction measures alone may not suffice to prevent GBS disease in fish or people and should be combined with other measures for comprehensive management.

2.3.2.1. Treatment of water

The water supply should be treated before fish fry or brood stocks are introduced into the hatchery or farm as the water, pond sediment, fish faeces, and fish fry may introduce *Streptococcus* spp. into the fish farm (Amal *et al.*, 2013, Nguyen and Kanai, 1999, Nguyen, Kanai and Yoshikoshi, 2002). Tilapia at every life stage, including fry, may harbour GBS regardless of any obvious clinical manifestations (Pradeep *et al.*, 2016), and transmission of GBS is possible between the infected fish fry and the adult fish (Musa *et al.*, 2009, Nguyen, Kanai and Yoshikoshi, 2002).

2.3.2.2. Cleaning of production units and equipment

Periodic cleaning and disinfection of production units and equipment decreases the chance of GBS introduction (Zamri-Saad *et al.*, 2014). Before the introduction of new fry, cleaning of the cage nets, tanks, and ponds by physical cleaning and drying is recommended; additional liming, with calcite or dolomite, is also recommended for ponds. All equipment such as scoop nets, buckets, small tanks, containers, etc., should be regularly cleaned using common commercial bleach.

In Southeast Asia, aquaculture farmers, who are mostly small scale, typically treat ponds once a year, at most. The treatment includes dredging up pond sediment and fertilizing and liming the pond. Water is drawn from public waterways, such as irrigation canals or rivers (Hishamunda *et al.*, 2009), and the pond is rested for a couple of weeks before starting the new crop. In the case of river cage culture, which is the predominant system in Viet Nam's Mekong region and along the Perfume River (Huong River) in Thua Thien Hue province, routine management consists only of net cleaning and drying.

2.3.2.3. Fish handling

Fish mucus acts as an antibacterial agent and a physical barrier between pathogenic organisms in the water and the fish (Francis-Floyd, 2002). Rough handling can injure fish skin by removing the skin mucus and scale, thus creating a direct route for GBS infection (Xu, Shoemaker and Klesius, 2007).

2.3.2.4. Stocking

High productivity in fish farming is achieved by balancing stocking density with survival rate. The optimal fish stocking density depends on cage size, fish size, and the aquaculture system. Generally, when mortality is high, lowering stocking density helps to reduce both the level of stress in fish and the pathogen load in the population (Shoemaker, Evans and Klesius, 2000). High fish stocking density (≥ 11.2 g/L) significantly increases the rate of mortality in tilapia exposed to *S. iniae* (Shoemaker, Evans and Klesius, 2000). Specific thresholds for GBS have not been reported and may differ between fish species, production systems, and growth stages.

2.3.2.5. Feeding

Partial reduction or discontinuation of feeding can also reduce mortality during streptococcosis outbreaks. Infected fish tend to have a lower appetite. Uneaten (or excess) feed can lead to deterioration of water quality, creating stress (for example high ammonia levels) for the fish as well as a favourable environment for the proliferation of bacteria. Contaminated feed is another source of infection, as seen with the use of contaminated "trash" fish as feed, which has been implicated in outbreaks of *Streptococcus* in the Republic of Korea (Kim *et al.*, 2007).

2.3.2.6. Removal of sick and dead fish

Good fish farm management involves removal of morbid and dead fish as frequently as possible, which can be a challenge for extensive farms with many cages/ponds and high densities of cultured fish. Mortalities need to be disposed of appropriately, such as by incineration or burying in quick lime. Equipment, for example nets, used to remove the dead fish need to be properly disinfected before further use in other fish-holding facilities. If not, infection is likely to be spread across the aquaculture facility. The presence of dead infected fish can result in the infection of healthy fish. Horizontal transmission of the pathogens between fish is also thought to occur (Xu, Shoemaker and Klesius, 2007), potentially through cannibalism of infected dead fish.

2.3.2.7. Treatment

Antibiotics are only effective in treating outbreaks of streptococcosis if applied early. Because infected fish have reduced appetite, oral antibiotic treatments are less effective. Therefore, antibiotics only partially control mortality during the period of application, with mortality usually increasing after treatment cessation. As a result, farmers are often tempted to extend the duration of antibiotic application or use higher doses (Zamri-Saad *et al.*, 2014).

2.3.2.8. Vaccination

Vaccination has been demonstrated to control GBS in cultured fish around the world, both in laboratory and field trials (Evans, Klesius and Shoemaker, 2004, Ismail *et al.*, 2016a, Ismail *et al.*, 2017, Liu *et al.*, 2016, Wang *et al.*, 2020). However, vaccination is not commonly used by small-scale and medium-scale fish farmers because of the cost, limited knowledge, and limited availability of the vaccine in their area or country. For example, in Brazil, estimated production costs would increase by between 2.82 percent and 3.57 percent if vaccination was to be implemented (C.A.G. Leal, personal communication, 2020). Moreover, according to the manufacturers, commercially available vaccines are not effective against both haemolytic (CC7, ST283) and non-haemolytic (CC552) GBS, so strain typing would be needed to select appropriate vaccines, further increasing cost. In addition, laboratory infrastructure for diagnosis and strain typing is lacking in the aquaculture industry in Southeast Asia, both for GBS and other pathogens.

2.3.2.9. Biosecurity

Biosecurity involves practices, procedures, and policies used to prevent the introduction of infectious diseases (Dvorak, 2009). This applies at both the farm level and the national level. The global distribution of GBS CC552 is attributed to the international distribution of tilapia brood stock to support aquaculture development (Kawasaki *et al.*, 2018). Likewise, importation of tilapia from Asia is considered a likely source of fish-related outbreaks of GBS ST283 in Brazil from introduced carrier fish from Asia (Leal *et al.*, 2019).

Effective biosecurity measures can reduce the risk of introduction and the economic effects of diseases. Fish movement, water sources, fish health, equipment/vehicles, fish feed, and vectors (human and animal) are the main risk factors for disease introduction and spread in aquaculture facilities. The use of good biosecurity practices in hatcheries and on farms can thus protect fish and associated investments (Bondad-Reantaso, Arthur and Subasinghe, 2012).

2.4. Presentation and transmission of Group B *Streptococcus* sequence type 283 in fish pre-harvest

KEY POINTS:

- Fish infected with GBS may or may not show clinical signs and such clinical signs are wide ranging.
- Only apparently healthy fish will usually be sold.
- GBS may occur at all stages of the fish life cycle and all life cycle stages may contribute to transmission.
- GBS may be transmitted to humans via the aquatic environment and human effluent.

Given the relatively recent apparent emergence of GBS ST283, there is a lack of data on the prevalence and concentration of the organism in the fish supply and value chain. In most studies, *S. agalactiae* are isolated from clinically affected fish by culture methods and bacterial isolates are then subjected to MLST or WGS, which are not always affordable or feasible typing methods for all. Methodologies that can be used specifically to enumerate ST283 from fish or food have not been described so far. This may become possible in future, when genetic/virulence markers specific to this clonal complex are identified and technologies such as colony hybridization, quantitative polymerase chain reaction (qPCR), digital droplet PCR, or Most Probable Number (MPN) combined with PCR can be applied for quantitative determination of ST283 in association with fish. It is not known whether ST283 can co-occur with other GBS clonal complexes (CCs) in individual fish or at the farm level. Within countries, multiple types co-exist (Syuhada *et al.*, 2020), although the prevalence of types may differ between regions or river systems (Phuoc *et al.*, 2020).

2.4.1. Presentation of Group B *Streptococcus* infection and disease in fish

2.4.1.1. Clinical manifestations

The clinical signs of GBS ST283 infection in fish are similar to those for other GBS infections. Clinical signs of disease are a combination of behavioural abnormalities with changes in the external and internal organs of the infected fish. For GBS generally, the clinical signs and gross and histopathological lesions are variable and depend on the host species, fish age, and stage of the infection. In GBS ST283 outbreaks, diseased fish show signs of lethargy, melanosis, anorexia, ataxia (erratic swimming), unilateral or bilateral exophthalmia, corneal opacity, and peri-ocular or skin haemorrhages (Figure 4). Some fish have abdominal distension as a result of ascites (Chideroli *et al.*, 2017, Delannoy *et al.*, 2013, Leal *et al.*, 2019). Current reports of ST283 in fish are primarily based on organ examination of clinically sick fish (Barkham *et al.*, 2019, Delannoy *et al.*, 2013, Syuhada *et al.*, 2020). In fish farming, only apparently healthy-looking fish will usually be sold, limiting consumer exposure to GBS from clinically affected fish.

Figure 4. Clinical manifestations of GBS disease in tilapia caused by GBS ST283

Notes: A) High mortality in floating cage tilapia farms in Brazill; B) Erratic swimming of moribund red tilapia in Malaysia; C) Ascites in tilapia farms in Brazil; and D) Exophthalmia in moribund red tilapia in Malaysia.

2.4.1.2. Mortality

In general, GBS causes up to 80 percent mortality in affected fish populations (Amal and Zamri-Saad, 2011, Amal *et al.*, 2015, Zamri-Saad, Amal and Siti-Zahrah, 2010). Cumulative mortality of 30 percent to 80 percent in China (Li *et al.,* 2014) and 60 percent to 70 percent in tilapia rearing cages in Malaysia (Siti-Zahrah *et al.*, 2005) have been reported.

With respect to GBS ST283, mortality rates ranging from 25 percent to 35 percent during the grow-out stage are commonly reported by tilapia producers in Brazil (Leal *et al.*, 2019); during outbreaks this ranges between 10 percent and 80 percent (Chideroli *et al.*, 2017). In northern Thailand, daily mortality was 0.5 percent to 1.5 percent during a streptococcosis outbreak (predominantly caused by ST283) in tilapia river-based cage farms (Niu *et al.*, 2020).

Reporting of GBS is largely opportunistic, driven by the interests and availability of researchers and diagnostic facilities. There is currently no obligation or opportunity for routine surveillance.

DATA GAPS:

- Incidence and geographic distribution of GBS disease on fish farms is unknown.
- Data on the extent of morbidity as a result of GBS during outbreaks across species and production systems do not exist.
- There are no systematic data being collected on mortality as a result of GBS during outbreaks across species, production systems and countries.

2.4.1.3. Non-clinical presentation in fish

There are several reports of detection of ST283 or other strains of GBS in fish or seafood at retail. GBS ST283 has been isolated from apparently healthy retail fish in Southeast Asia (Barkham *et al.*, 2019, Chau *et al.*, 2017). Possible explanations include that those fish were in the early stages of infection, that GBS ST283 could have induced an apparently healthy carrier state in part of the fish population, or that post-harvest contamination occurred, for example from other fish or human carriers. In France, GBS was found in 2 of 17 prepared seafood dishes but not in any of 41 fresh or RTE fish samples (van der Mee-Marquet *et al.*, 2009); this limited data was interpreted by the authors of that study as suggesting that handlers or non-seafood ingredients were the most likely source of contamination. In addition, evidence of low level or sub-clinical infection in fish is also available. For example, GBS ST283 was identified in 10 of 28 (35.7 percent) red hybrid tilapia in Malaysia (on-farm) even though the fish did not show any external or internal signs of disease (Barkham *et al.*, 2019). Furthermore, GBS ST283 was found in 3 of 20 apparently healthy fish that were sampled during an outbreak caused by coinfection of tilapia lake virus, *Aeromonas hydrophila*, and *Streptococcus agalactiae* in cultured red hydrid tilapia in Selangor, Malaysia (Barkham *et al.*, 2019, Basri *et al.*, 2020).

Detection of GBS in the muscle tissue of outwardly healthy fish has also been reported for CC7, including muscle abscesses in chronically infected fish or yellow and dark red nodules in the muscle near the vertebra (Junior *et al.*, 2020, Li *et al.*, 2014). GBS serotype Ia, presumed to be CC7, was detected in 146 of 229 (64 percent) of healthy adult/commercial tilapia in China (Sun *et al.*, 2016). Fish originated from ten farms in five districts and weighed 2 kg to 3 kg, which is well above the typical market weight. It has been postulated that the gastrointestinal epithelium is the main route of entry for GBS into fish (Iregui *et al.*, 2016). There are significant knowledge gaps with regards to the distribution of GBS in live fish (site, prevalence, and impact of host and environmental factors) and in fish post-harvest (introduction, multiplication, and removal and inactivation of GBS).

DATA GAPS:

- Prevalence and distribution (body sites) of GBS ST283 in apparently healthy fish is unknown.
- Host and environmental factors affecting prevalence and distribution of GBS ST283 in apparently healthy fish have not been studied.
- Sources and mechanisms of introduction and amplification of GBS ST283 in retail fish are unknown.

2.4.2. Transmission of Group B *Streptococcus* sequence type 283 in fish

2.4.2.1. Transmission via fish

In Southeast Asia (Philippines, Malaysia) and elsewhere (Brazil), GBS can be found in fish at the hatchery and grow-out stages (Amal *et al.*, 2013, Legario *et al.*, 2020, Mian *et al.*, 2009). GBS ST283 has been detected in tilapia with live weight ranging from 7 g to 1 206 g, including large fingerlings and broodstock (C.A.G. Leal, personal communication, 2020). In Malaysia, where ST283 is the most common type of GBS in fish, transmission from a hatchery to a grow-out farm has been documented (Amal *et al.*, 2013).

It is not clear how transmission between fish in grow-out farms occurs. Based on studies using GBS CC552 in tilapia, it has been suggested that the fish gut is the main source of GBS (Iregui *et al.*, 2016), but transmission in the farm to other fish through the oral route is not clear. Oral challenge of Queensland grouper with GBS CC552 did not induce disease (Delamare-Deboutteville *et al.*, 2015). Potential explanations include the importance of stressors to induce disease after ingestion or gut colonization, differences between fish species, and alternative routes of transmission, for example through cannibalism or via water.

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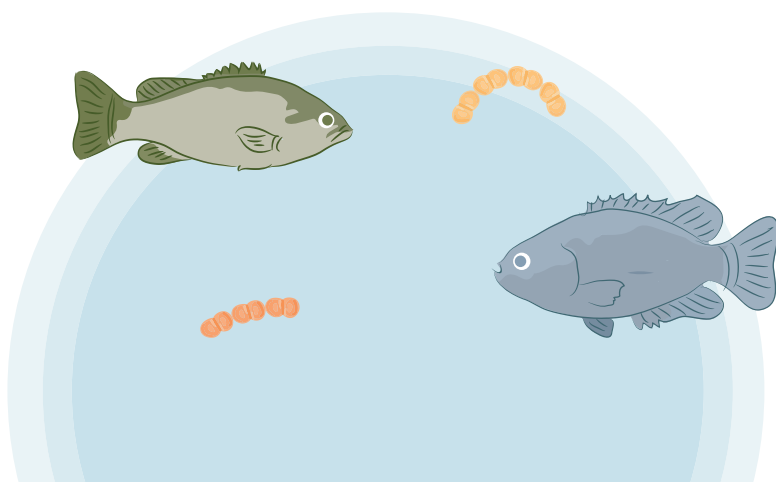
2.4.2.2. Transmission via the environment

Transmission via the growing water, for example river, has not been documented but is thought to be very likely and could explain why ST283 is common in several provinces along the Mekong river in Viet Nam or the Mae Klong and Chao Phraya rivers in central Thailand, where the use of floating cages for tilapia cultivation is widespread. The possibility of environmental transmission is supported by an outbreak of ST7 in wild mullet (*Liza klunzingeri*) and farmed sea bream (*Sparus auratus*), which was attributed to contaminated sea water (Evans *et al.*, 2002, Jafar *et al.*, 2008). In experimental challenge studies, including published and unpublished studies, all types of GBS affecting fish, including ST283, can be transmitted by cohabitation of infected and non-infected fish (Mian *et al.*, 2009) or through immersion of fish in water with GBS (Delamare-Deboutteville *et al.*, 2015). Human effluent may contribute to exposure of fish to GBS unintentionally, for example through outhouses or sewage spills, or intentionally through fish-pond toilets, which are used in some areas to recycle nutrients (Delannoy *et al.*, 2013, Jafar *et al.*, 2008).

DATA GAPS:

- The exact mechanism or combination of mechanisms, for example direct contact, waterborne, or through ingestion, of GBS infection in fish is unknown.
- No data exist in relation to the likelihood and effect of wastewater and surface water contamination on GBS contamination of growing waters.

2.5. Risk factors for Group B *Streptococcus* sequence type 283 in fish post-harvest

KEY POINTS:

- Apart from heat-treatment, there are essentially no data on the effect of any processing steps on GBS presence or levels.
- Baseline data on the prevalence and concentrations at retail do not exist.
- High levels of fish-borne fluke infection in fish and people in regions of Southeast Asia suggest that consumption of raw or preserved fish, without proper heat treatment, is common in many parts of Southeast Asia.
- Human disease was linked to the consumption of raw fish contaminated with GBS ST283 as part of a large outbreak in Singapore.

Processing steps have been described in Section 2.2.1.4. Heat treatment kills GBS, whereas freezing does not. Apart from freezing or heat treatment, there are no studies on the effects of processing and preservation methods on the presence and concentration of GBS, including GBS ST283, on and in freshwater fish.

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2.5.1. Retail and food service

A Nigerian study of tilapia samples (200 g to 500 g) obtained from fishermen showed fish stored for up to 15 days on ice or 12 hours at ambient temperature remained in acceptable condition, with total microbial loads not greater than 10^6 CFU/g (Adoga, Joseph and Samuel, 2010). However, monitoring total bacterial loads is not a reliable way to assess the safety of the product and to assess whether any temperature abuse has occurred that could lead to potential growth of pathogens.

In Singapore, the occurrence of GBS in raw fish sold in different retail outlets was investigated in two studies. Kalimuddin *et al*. (2017) analysed 43 fish samples from fish ports, wet markets, supermarkets, and eating establishments between August and December 2015. Thirteen samples (30 percent) were positive for GBS ST283. Subsequently, the microbiological quality of raw fish sold in ports, markets, and restaurants was investigated (Chau *et al*., 2017). GBS ST283 was detected in 18 of 997 (1.8 percent) samples (all freshwater fish), one from a ready-to-eat dish sold at a food stall, six from fresh fish sold in ports, and eleven from fresh fish products sold in markets (Chau *et al*., 2017). All fish were apparently healthy and post-harvest contamination could not be excluded. Environmental and handling conditions that may promote the presence and load of GBS ST283 in freshwater fish are yet to be studied. However, fish purchased from produce markets and food stalls, with relatively poor handling and hygiene practices (as inferred from high standard plate counts and high *E. coli, S. aureus*, and *Salmonella* spp. occurrence), had higher rates of GBS contamination (12/108) than those from ready-to-eat food prepared in restaurants and snack bars (0/282) (Chau *et al*., 2017). Similarly, a higher prevalence of *E. coli* (72 percent versus 44 percent) and *Salmonella* spp. (59 percent versus 23 percent) was found in retail food purchased from traditional open (wet) markets compared with modern supermarkets, respectively, in Thailand (Ananchaipattana *et al*., 2012).

Among 102 fish tank water samples analysed in Singapore, 55.1 percent (54/98) from the port were positive for GBS and 6.1 percent (6/98) were positive for GBS ST283. Three of four fish tank water samples from markets and supermarkets were positive for GBS ST283, and fish from the corresponding tanks were also positive for GBS ST283 (Chau *et al*., 2017).

Nanayakkara *et al* (2018) studied the occurrence of GBS in fish at wet markets in China, Hong Kong SAR. GBS was detected in 20 out of 53 tilapia (*Oreochromis mossambicus*) and 9 out of 52 big head carp (*Hypophthalmichthys nobilis*), respectively. Among 64 isolates, most belonged to serotype Ia (52 percent) or undetermined serotypes (39 percent); only one may have belonged to serotype III, but there was no further information at the serosubtype or ST level reported.

- Baseline data on the prevalence and concentration of GBS and GBS ST283 in raw fish at retail do not exist.
- There exists only limited data on the association of GBS contamination and retail outlet and environmental hygiene conditions.
- No information exists about the prevalence and concentration of GBS contamination of different fish parts (fillet, viscera, gills, etc.).
- Post-process handling and storage conditions and durations and their impact on GBS load have not been systematically studied.

2.5.2. Food preparation and consumption

In relation to consumption of freshwater fish, the demographics of the consuming population, serving sizes, frequencies, and formats in which these are consumed are largely unknown.

2.5.2.1. Food preparation

Many local cultures in Southeast Asia commonly consume uncooked or undercooked (i.e. not heat-treated) fish. This may be particularly common in rural communities that are proximal to rivers, lakes, or other water sources where fish can be grown and caught. In addition, there is a paucity of published information on the effect of various food preparation practices on GBS viability or concentrations.

Consumption of Asian bighead carp (*Hypophthalmichthys nobilis*) and snakehead fish (*Channa* sp.) in the Chinese-style raw fish dish *yu sheng* was strongly associated with ST283 GBS bacteraemia (adjusted odds ratio of 25.92) during the 2015 outbreak in Singapore (Kalimuddin *et al.*, 2017). Discontinuation of the sale of RTE raw river fish dishes abated the outbreak. However, it remains possible that these two particular fish species may have been contaminated during handling. Regardless, the ultimate origin of the GBS ST283 in that outbreak remains unknown. In addition, the identification of the fish species was uncertain, and the practice of selling fillets from a cheaper fish in place of a more expensive fish is considered prevalent in Southeast Asia.

It is unknown how preparation methods that do not involve heat treatment, such as fermentation or the use of spices, affect the prevalence and concentration of GBS ST283 and the subsequent likelihood of foodborne infection. However, epidemiological research identified a descending risk of infection with the liver fluke *Opisthorchis viverrini*, with uncooked *koi pla* posing the highest risk, followed by *pla som* (moderately fermented, stored for a few days to weeks), *pla ra* (extensively fermented, highly salted fish, stored for at least two to three months), and *jaewbhong* (fully preserved) (Sithithaworn and Haswell-Elkins, 2003). Although GBS survival may differ from parasite survival under these conditions, in the absence of specific data, the rates of parasitic disease may serve as a rough proxy for the risk of foodborne GBS ST283 disease.

2.5.2.2. Serving size and consumption patterns

There is scant evidence regarding the consumption frequency of various types of fish dishes. Although *yu sheng* has been associated with Lunar New Year (typically using salmon during this holiday; GBS has never been identified in salmon, which is a cold-water marine fish), it is enjoyed across Southeast Asia throughout the year when freshwater fish are more likely to be used. In North and Northeast Thailand, *koi pla* is reserved for special occasions; *pla som* is eaten several times a week; and *pla ra* and *jaewbhong* are consumed daily by a large number of people (Kaewpitoon *et al.*, 2008).

The widespread prevalence of *Opisthorchis viverrini*, a liver fluke causing cholangiocarcinoma (bile duct cancer), is indicative of the ongoing cultural practice of eating raw river fish in northern parts of Thailand, southern and central Lao People's Democratic Republic, and other parts of the Mekong basin, including Cambodia and both northern and southern Viet Nam (Sithithaworn *et al.*, 2012a, Suwannahitatorn *et al.*, 2019, Wang, Feng and Sithithaworn, 2013). In endemic areas, the prevalence of *O. viverrini* can be as high as 70 percent in cyprinid fish (which includes tilapia) and 85 percent in people (Saenna *et al.*, 2017, Sithithaworn *et al.*, 2012b).

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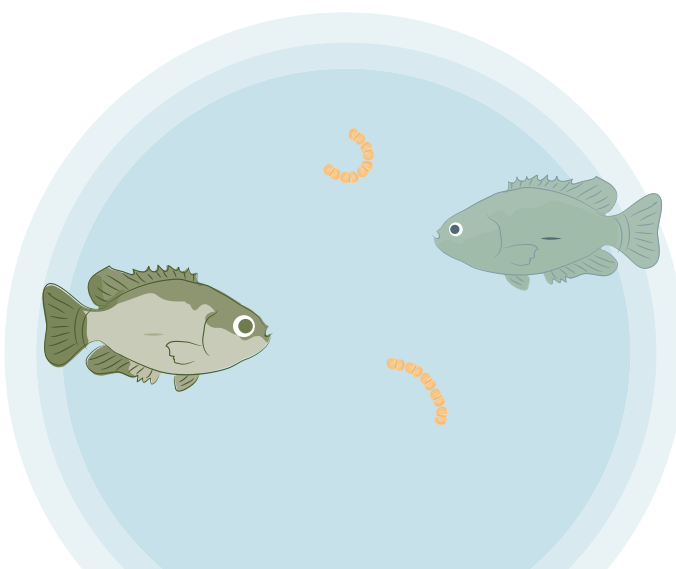
3 DESCRIPTION OF ADVERSE HEALTH EFFECTS IN HUMANS AS A RESULT OF GROUP B *STREPTOCOCCUS* SEQUENCE TYPE 283 INFECTION

3.1. Characteristics of the disease

KEY POINTS:

- GBS ST283 causes serious invasive disease, including sepsis, septic arthritis, and meningitis.
- GBS ST283 causes disease in adults with relatively few or no underlying comorbidities, i.e. the general population appears susceptible to infection with GBS ST283.
- The biological mechanism for how ingestion of food contaminated with GBS can lead to sepsis is not understood.

3.1.1. Outcome of exposure

GBS ST283 infection linked to fish consumption presents with bacteraemia and sepsis in adults. When 146 adult ST283 cases involved in a foodborne outbreak of invasive GBS disease were compared with 262 non-ST283 GBS cases in a retrospective cohort study in Singapore, it was found that ST283 cases were more commonly associated with meningoencephalitis (19.9 percent versus 0.0 percent), endocarditis (10.3 percent versus 5.0 percent), septic arthritis (30.1 percent versus 5.0 percent), and spinal infections (8.2 percent versus 1.9 percent) (Kalimuddin *et al.*, 2017). ST283-infected patients were more likely to be younger (median age 61 years versus 69.5 years), of Chinese ethnicity (95 percent versus 67 percent), and to have fewer comorbidities than the non-ST283 GBS cohort: 21.9 percent of ST283 cases, but only 1.9 percent of non-ST283 cases were in people with a comorbidity score of "zero" (meaning they were healthy, without comorbidities). Mortality was lower in ST283 cases compared with non-ST283 GBS cases (3.4 percent versus 9.5 percent), which probably reflects the healthier status of people affected by ST283, but some survivors suffered from complications of sepsis, such as loss of limbs. (Kalimuddin *et al.*, 2017).

A retrospective study of invasive GBS isolates from six Southeast Asian countries showed similar clinical data: 345/357 (97 percent) patients with ST283 were adults, and 20 percent to 64 percent had comorbidities, but comparative rates for non-ST283 disease were not reported. Septic arthritis was most prevalent (23 percent to 39 percent), followed by meningitis (10 percent to 35 percent) and endocarditis (4.5 percent to 10 percent) (Barkham *et al.*, 2019). Previous reports indicated that GBS septic arthritis is unusual in otherwise healthy adults (Clerc *et al.*, 2011).

In a smaller study of 44 adults with ST283 infection from China, Hong Kong SAR (Ip *et al.*, 2016), sepsis was the most common serious complication (38.6 percent), followed by septic arthritis (22.7 percent) and meningitis (15.9 percent). The mean age was 63 years (range 23 years to 96 years). A relatively high proportion of patients (36.4 percent) did not have underlying diseases reported, but the overall mortality was much higher than in Singapore (27.3 percent versus 3.4 percent).

These differences have not been explained, but could be related to the mode of acquisition. The outbreak in Singapore was associated with the consumption of contaminated fish, but transmission was not reported from China, Hong Kong SAR. It is unclear whether the severity of disease relates to specific virulence properties of the organism itself, or whether the mode of transmission and/or level of contamination might play a role.

Non-invasive disease caused by ST283 appears rare to date; the few isolates from pus swabs were associated with confirmed diagnoses of cellulitis, osteomyelitis, spondylitis, and mycotic aneurysm (Ip *et al.*, 2006). However, typing of non-invasive GBS is unusual, so the absence of reports may reflect an absence of data rather than absence of GBS from non-invasive disease.
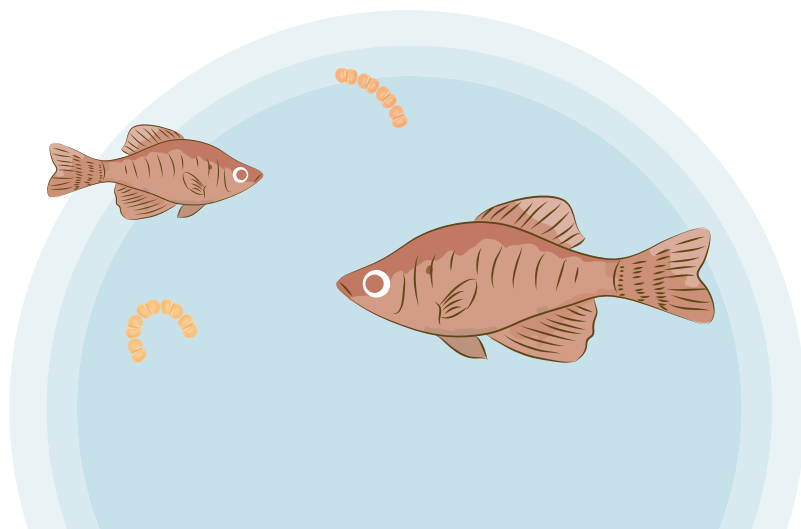
- Incidence of GBS, including GBS ST283, as a cause of sepsis and other forms of invasive disease in humans in Southeast Asia has not been quantified.
- The occurrence of GBS ST283 in non-invasive manifestations of GBS disease is unknown.
- Factors contributing to the severity of GBS ST283 disease in humans are poorly understood; this includes human factors, GBS virulence properties, mode of transmission, and dose.

3.1.2. Susceptible population

In adults with non-ST283 GBS, a high proportion of invasive GBS disease is associated with acute or chronic risk factors, including pregnancy, with less than 10 percent of cases occurring in non-pregnant adults without comorbidities (Collin, Shetty and Lamagni, 2020, Jump *et al.*, 2019). Commonly reported predisposing conditions include diabetes, cardiovascular disease, malignancy, lung and kidney disease, and being under- or overweight. Between 10-24 percent of non-pregnant adult disease is potentially linked to healthcare interventions, particularly surgery, with residents of long-term care facilities also noted as being at higher risk of infection (Collin, Shetty and Lamagni, 2020, Dahl, Tessin and Trollfors, 2003, Henning *et al.*, 2001, Skoff *et al.*, 2009, Tyrrell *et al.*, 2000).

In contrast, GBS ST283 disease associated with fish consumption affects healthy people, without comorbidities. When ST283 and non-ST283 GBS bacteraemic cases were compared, it was found that 21.9 percent of ST283 cases were adults without any comorbidities, whereas only 1.9 percent of non-ST283 GBS bacteraemic cases were people without comorbidities (Kalimuddin *et al.*, 2017).

As a fish-borne pathogen, populations at risk are likely to be those consuming raw freshwater fish. The age profile of cases in the Singapore outbreak was older than the general population (median age 61 years in the outbreak compared to 37 years in the general population) (Kalimuddin *et al.*, 2017, Singapore Department of Statistics, 2015), which likely reflects a difference in exposure – the raw fish dish *yu sheng* is a traditional dish that is more popular among older Singaporeans. Differences in exposure similarly likely explain the predominance of ethnic Chinese cases in the Singapore outbreak (95 percent versus 74 percent in the general population). Based on the limited foregoing information it is concluded that the general population is susceptible to foodborne GBS ST283 infection.

3.1.3. Mechanisms of infection and disease

The biological mechanism for how ingestion of food contaminated with GBS can lead to sepsis is not understood. For early onset neonatal disease, gut colonization, followed by vaginal colonization and vertical (mother-to-child) transmission is considered the source and route of transmission, so potential faecal-vaginal transmission of ST283 warrants close monitoring. A recent review found rectovaginal carriage rates of GBS in Southeast Asia to be 14 percent, based on 4 591 women screened (Russell *et al.*, 2017). However, MLST data were not reported.

Similarly, a review of data from China showed maternal GBS colonization rates ranged from 4 percent to 15 percent, but ST283 was not found (Huang *et al.*, 2019). However, cases of neonatal disease resulting from ST283 have been recorded from China, Hong Kong SAR and Lao People's Democratic Republic, suggesting that vertical transmission may be possible (Barkham *et al.*, 2019, Ip *et al.*, 2016). Data are lacking on person-to-person transmission of ST283 in adults, and there are limited data regarding gastrointestinal carriage in humans; the sole published report found that none of 83 stool

samples collected from food handlers and fishmongers during the Singapore outbreak contained GBS ST283 (Kalimuddin *et al.*, 2017). However, unpublished data found ST283 in five of 184 (2.7 percent) stool samples collected in Northern Thailand (Timothy Barkham – personal communication).

Although not commonly considered a hospital pathogen, GBS is increasingly being recognized as a source of hospital outbreaks. GBS clusters among adult patients have been reported in healthcare settings, indicating possible nosocomial transmission (Baraboutis *et al.*, 2010, Denton *et al.*, 1993, Nagano *et al.*, 2012). Although transmission of GBS between asymptomatic human carriers is known to be associated with sexual activity, sources and transmission routes of GBS infection in adults or in hospitals are poorly understood (Collin *et al.*, 2019).

DATA GAPS:

- Sources and routes of transmission in people who deny eating raw river fish have not been studied adequately.
- There are few data on gastrointestinal carriage of GBS ST283 in apparently healthy individuals.
- The mechanism of how consumption of GBS leads to disease, and serious complications, is not understood.

3.1.4. Nature and availability of treatment

Treatment of GBS ST283 is similar to that for GBS disease caused by other types. Primarily, treatment is with antibiotics to which the species remains largely susceptible, including penicillins or cephalosporins, with or without gentamicin (where indicated). Depending on the susceptibility profile of the isolate, macrolides and lincosamides may also be used. Treatment is often prolonged to at least three weeks in cases with a deep-seated focus, such as bone and joint infections. Organ supportive management and source control by debridement/drainage are often required. ST283 resistance to tetracyclines varies (see Section 2.1.1.4), but this is not relevant to case management as tetracyclines are not used for this purpose. GBS ST283 is generally susceptible to macrolides, although resistance is common among other GBS serotypes, particularly in Southeast Asia.

Although foodborne GBS infection can be treatable, access to healthcare is a key determinant of infection outcome for individuals and populations in Southeast Asia. Furthermore, access to healthcare is crucial for gathering epidemiological data to identify and quantify occurrence. The members of the Association of South East Asian Nations (ASEAN) differ markedly in availability and accessibility of healthcare: Malaysia, Singapore, and Viet Nam have more than ten physicians per 10 000 population, whereas Cambodia, Lao People's Democratic Republic, and Thailand have fewer than five per 10 000 (WHO, 2015). Progress towards universal health coverage is also uneven, with out-of-pocket expenditure on healthcare in 2012 remaining above 50 percent in Cambodia, Lao People's Democratic Republic, Myanmar, and Singapore (WHO, 2015). Availability of laboratory and diagnostic facilities also varies, and even Singapore, with its strong biomedical infrastructure, took several months to recognize the 2015 outbreak (Kalimuddin *et al.*, 2017).

3.2. Epidemiology of Group B *Streptococcus* sequence type 283 infection

> **KEY POINTS:**
>
> - Robust epidemiological data for GBS (both generally and ST283) infections do not exist in Southeast Asia because of limited healthcare, diagnostic, bacterial typing, and reporting infrastructure.
> - The lack of routine application of MLST to GBS isolates leads to substantial uncertainty with respect to the role of ST283 in human disease and its infection routes, including the extent of foodborne infection.
> - Based on retrospective analysis of opportunistic strain collections, GBS ST283 detection in collections of invasive human GBS isolates appears to be largely limited to Southeast Asia.

3.2.1. Surveillance for Group B *Streptococcus*/Group B *Streptococcus* sequence type 283 infection in the Southeast Asian region

GBS disease is not notifiable in Southeast Asia and identification relies on voluntary surveillance systems and submission of isolates to a central reference laboratory (CRL). There are a multitude of reasons for the lack of robust data on the extent of GBS ST283 infections, including foodborne infections, in Southeast Asia. These include the following:

- limited laboratory support, especially in rural settings and small hospitals;
- suitable specimens prior to treatment are often not collected, especially if diagnostic testing incurs additional costs, i.e. a patient's out-of-pocket costs;
- the use of prior "over-the-counter" antibiotics may inhibit the recovery of GBS in culture;
- laboratories may not have the resources to correctly identify GBS and may report them simply as "*Streptococcus* species" or misidentify them as other bacterial species;
- laboratories may not proceed to Lancefield serologic typing, or do not apply the whole panel, which can result in misidentification of group B versus other groups;
- isolates are not routinely subjected to MLST, or other modes of typing, necessary for the identification of ST283 (Barkham *et al.*, 2019);
- advanced identification tools such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and WGS are limited to CRLs and larger hospital laboratories, and MALDI-TOF-MS does not routinely provide subtyping data; and
- lack of adequate resources, such as -80 °C freezers, and efficient logistics limit rapid transport of isolates to a CRL.
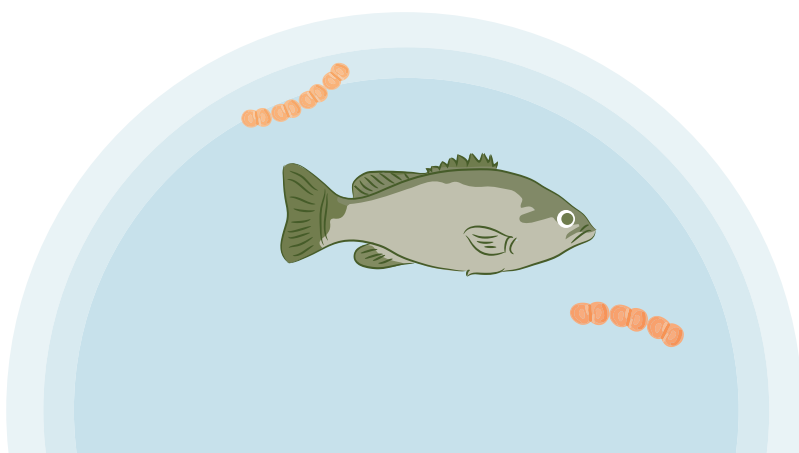
3.2.2. Frequency and sources of Group B *Streptococcus* sequence type 283 infection

In 2002, the first description of GBS capsular type III, subtype 4, from three isolates with undefined epidemiology was reported by the *Streptococcus* Laboratory in New South Wales, Australia (Kong *et al*., 2002); the isolates were subsequently confirmed to be ST283. Since then, GBS ST283 has been widely detected across Southeast Asia from humans, but less frequently elsewhere (Barkham *et al*., 2019, Kalimuddin *et al*., 2017). A summary of ST283 detection in opportunistic GBS collections from invasive human GBS isolates from various countries is shown in Table 3. These studies show a relatively high percentage of GBS ST283 detected from invasive human GBS isolates from Southeast Asian countries, but a very low detection of GBS ST283, despite numerous appropriate typing studies, amongst 4 198 GBS in Africa, China, Europe, and North and South America. Only five invasive GBS ST283 have been reported in humans outside Southeast Asia/China, Hong Kong SAR, namely in France with two cases (Salloum *et al*., (2010), the UK with 1 case (Genbank ERR1742070), the USA with one case (McGee *et al*. (2021)), and the Netherlands with one case (Genbank ERR1659855). None of these cases had associated epidemiological data, so prior travel to Southeast Asia cannot be ruled out.

Table 3. ST283 detection in GBS collections from invasive human GBS isolates

COUNTRY/REGION	SAMPLE SIZE (GBS ISOLATES)	NUMBER (PERCENT) OF ISOLATES THAT ARE ST283	SOURCE
China, Hong Kong SAR	437	50 (11 percent)	Ip *et al*. (2016)
Lao People's Democratic Republic	38	29 (76 percent)	Barkham *et al*. (2019)
Singapore 2001–2010	331	21 (6 percent)	Barkham *et al*. (2019)
Singapore 2011–2015	408	146 (36 percent)	Kalimuddin *et al*. (2017); McGee *et al*. (2021)
Thailand	139	102 (73 percent)	Barkham *et al*. (2019)
Viet Nam	13	4 (31 percent)	Barkham *et al*. (2019)
Malaysia	18	0 (0 percent)	Eskandarian *et al*. (2013)
Africa, mainland China, Europe, and North and South America	4 198	5 (0.1 percent)	Barkham *et al*. (2019)

Source: Zwe *et al*., 2019.

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Sporadic cases continue to be identified in Southeast Asia, with one case of ST283 meningitis in Myanmar (T. Barkham, personal communication, 2020), and a report of two adult sisters who developed ST283 meningitis within weeks of returning home to Lao People's Democratic Republic after an extended period abroad (T. Barkham, personal communication, 2020). Sporadic ST283 bacteraemia/meningitis cases have continued to be noted in recent years from one hospital in China, Hong Kong SAR (M. Ip, personal communication, 2020). No epidemiological and/or food exposure data are available for these sporadic cases.

Unpublished data from one hospital in Singapore demonstrate the chronicity of ST283: it showed an ongoing occurrence of 2 to 15 GBS ST283 cases per year from 1998 to 2019 (excluding the outbreak year in 2015). More recent data from Singapore showed a sudden increase, with 18 cases reported from the whole country in one month, in July 2020 (Singapore Ministry of Health and Singapore Food Agency, 2020); notably, a ban on the sale of freshwater fish for raw consumption (introduced in 2015) was still in effect. Furthermore, Singapore had also implemented escalating travel restrictions beginning in January 2020 because of COVID-19; by April 2020, no leisure travel out of Singapore was allowed, and this extended beyond July 2020, limiting the likelihood that these cases of GBS ST283 were acquired overseas. These events suggest that a local reservoir, or imported source of GBS ST283, was present in Singapore at that time. Investigation of these cases by the Singapore Ministry of Health did not reveal any epidemiological links, although patients may have been less likely to admit to eating raw freshwater fish.

DATA GAPS:

- Consolidated data do not exist on food consumption, travel history, or other risk factors for sporadic cases of GBS ST283 infection.

3.2.2.1. Group B *Streptococcus* sequence type 283 outbreaks in humans in Southeast Asian countries and sources

The first reported outbreak of GBS ST283 was in Singapore in 2015 (Rajendram *et al.*, 2016, Tan *et al.*, 2016). However, Wilder-Smith *et al.* (2000) reported an increase in adult GBS meningitis in 1998 in Singapore and China, Hong Kong SAR; these were initially described as ST11 (Jones *et al.*, 2003), but were subsequently shown to be ST283 (Barkham *et al.*, 2018). In addition, Louthrenoo *et al.* (2014) described GBS as an "emerging cause of septic arthritis" in Thailand as they found four GBS cases between 1990 and 2008, and an additional 34 cases between 2008 and 2010. It was notable that 14 of these cases did not have any comorbidities that might predispose them to joint infection.

Reports that studied transmission are limited to the Singapore outbreak, where case-control studies, the response to an intervention (Rajendram *et al.*, 2016, Tan *et al.*, 2016), and genomic analyses (Kalimuddin *et al.*, 2017) all supported GBS ST283 acquisition from consumption of raw freshwater fish. Subsequent genomic analysis of all reported ST283 with whole genome sequencing data, including GBS from humans and tilapia, shows that ST283 forms a monophyletic clade (Barkham *et al.*, 2019), but transmission itself was not studied. Previous work on GBS in general has pointed at fish as a potential source of GBS that colonize humans (Foxman *et al.*, 2007). However, molecular typing studies have found little evidence of shared clones between fish and humans except for ST283 (potentially a zoonosis) and ST7, whose populations in fish and humans were shown to be closely related at the genome level, but for which there is no epidemiological indication of zoonotic transmission. Our understanding of GBS transmission in the food chain suffers from a lack of data, probably as it has not previously been regarded as a foodborne threat.
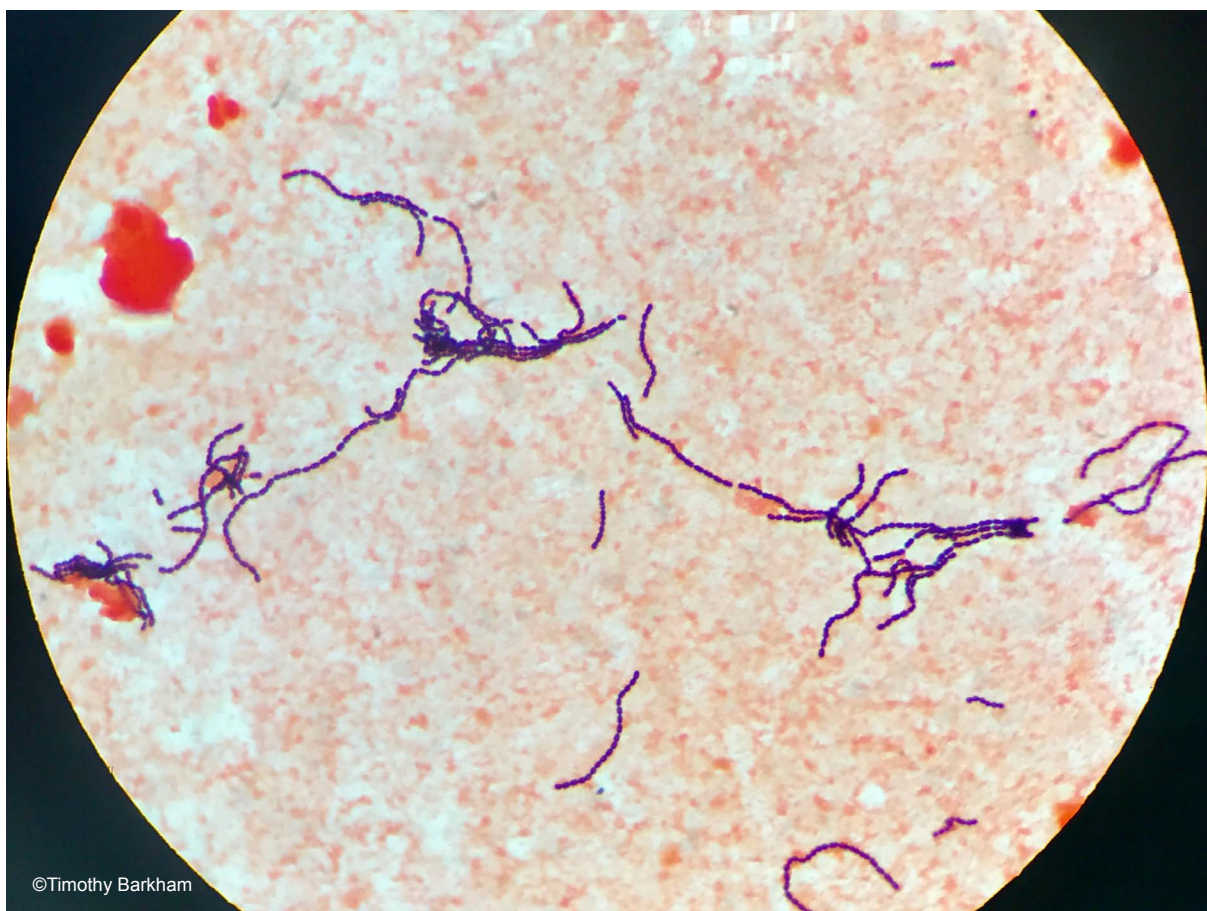
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3.2.3. Disease burden and economic impact of Group B *Streptococcus* sequence type 283 infection in humans

Population based GBS bacteraemia rates are only available for Nakhon Phanom Province, Thailand (O. Sangwichian, personal communication, 2020) and the United Kingdom of Great Britain and Northern Ireland (Public Health England, 2015, Public Health England, 2016, Public Health England, 2017), and these are shown in Table 4. A total of 73 percent of the invasive GBS isolates from Thailand in 2014 were ST283; thus, ST283 infections alone account for the two- to three-fold higher rate of GBS bacteraemia in Thailand compared with the United Kingdom of Great Britain and Northern Ireland. However, the proportion contributed by foodborne exposure is not known.

Table 4. GBS bacteraemia rates per 100 000 in Nakhon Phanom Province, Thailand, and the United Kingdom based on expert opinion

YEAR	GBS BACTERAEMIA RATE (PER 100 000)	
	NAKHON PHANOM PROVINCE, THAILAND	UNITED KINGDOM
2014	7.8	2.8
2015	6.7	3.1
2016	7.4	3.7

Source: O. Sangwichian, personal communication, 2020; Public Health England, 2015; Public Health England, 2016; and Public Health England, 2017.

No information exists to estimate the health and economic effects of GBS ST283 infection in humans globally. Information is needed to calculate disability-adjusted life years (DALY) related to foodborne GBS ST283 infection (Devleesschauwer *et al*., 2014a, Devleesschauwer *et al*., 2014b), including demographic information (such as age and gender); domain-specific annual incidence; hospitalization, complications, and mortality rate; associated disability weights; and durations of the disability. Furthermore, there are no data on the preventive and treatment costs and loss in annual income because of GBS ST283, which are all needed to estimate the health and economic effects of GBS ST283.

DATA GAPS:

- There are no published data on the public health burden of foodborne GBS ST283 infections.
- Information to calculate DALY does not exist.
- No economic information, including treatment cost and loss of income, exists in relation to foodborne GBS ST283 infections.

3.3. Dose–response

KEY POINTS:

- No data are available for the dose–response relationship of GBS, including GBS ST283, in humans after oral ingestion.
- Limited data exist for GBS ST283 and other *Streptococcus* spp. infection in animal models, but these may not be representative of the situation in humans.

There are no challenge studies of GBS ST283 in humans or in laboratory models that can be used to estimate an oral dose–response model for GBS ST283 infection/disease.

The following publications report on *in vivo* infection models or median lethal dose (LD50), though these are of limited use for extrapolation to human GBS ST283 infection via the oral route.

- A wax moth (*Galleria mellonella*) larvae model showed that both ST17 and ST283 strains had lower LD50 than other sequence types of GBS. Notably, ST283 strains had a lower LD50 (1×10^5 colony forming units (CFU)) than ST17 strains (1×10^6 CFU) (Six *et al.*, 2019).
- An intraperitoneal infection model in red hybrid tilapia resulted in an LD50 of 315 CFU for ST283 GBS, compared to 435 CFU for ST7 (Syuhada *et al.*, 2020).
- In a second intraperitoneal challenge model in red hybrid tilapia, ST283 had an LD60 of 1.5×10^3 to 2×10^3, compared to 3×10^5 CFU for a strain from CC552. There was no virulence in fish when a human isolate was tested in this model (the human isolate was ST651, which is not from a CC that is associated with fish) (Phuoc *et al.*, 2020).
- An intraperitoneal infection model of CD1 mice resulted in a LD50 of 3×10^6 CFU (Yang *et al.*, 2019).
- An inoculum of 1×10^7 CFU in ICR six weeks old mice resulted in 90 percent mortality for ST283 strains ten days post inoculation, whereas CC1 and CC103 caused no mortality (Yang *et al.*, 2020).

Experimental challenge studies with ST283 in humans are not possible for ethical reasons, but extrapolation of results from animal studies to humans may have limited validity because of differences in physiology, for example stomach pH and immune systems. In addition, many challenge studies did not use oral exposure and alternative routes of transmission may not reflect the impact of exposure to gastric acid, or the role of adherence and invasion of the gut epithelium.

DATA GAPS:

- No dose–response model exists for GBS ST283 infection in humans via the oral route.

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4 ASSESSMENT OF RISK

KEY POINTS:

- Despite the large number of data gaps that exist in relation to GBS ST283 throughout the food chain, a qualitative risk assessment using a risk matrix was attempted.
- The likelihood of a portion of food containing enough GBS ST283 organisms to cause infection/disease was highly uncertain.
- The severity of the disease caused by GBS ST283 can be considered to be "severe" according to the life-threatening nature and substantial complications.
- Using a four-category scale risk matrix, the risk was estimated to be at least "low" for consumption of raw fish, although the risk could be higher depending on the likelihood of infection, the prevalence and concentration of GBS ST283 contamination in fish, and the health condition of the consumers.
- Consumption of fermented fish or fish that has been traditionally prepared with other spices/condiments was rated as equally high risk as raw freshwater fish. This conservative estimate was based on the wide-ranging pH tolerance of GBS ST283, although this was also very uncertain.
- Consumption of partially or fully heat-treated fish was rated lower in risk than consumption of raw freshwater fish. This estimate was based on the heat susceptibility of GBS ST283, though this was also uncertain, and the effect depends on how much heat treatment the fish receives.

Historically, GBS disease in humans mainly affects infants in the materno-foetal unit and people with comorbidities. GBS ST283 appears different from other strains of GBS in a number of ways:

- it causes invasive disease in non-pregnant adults without comorbidities;
- it caused the reported foodborne outbreak of invasive GBS disease;
- in Singapore, it was shown to be transmitted from raw freshwater fish to humans; and
- GBS ST283 is one of only three major groups of GBS that cause fish mortality.

GBS ST283 in humans is largely restricted to Southeast Asia, where it is widespread and accounts for 11 percent to 76 percent of all invasive GBS disease since records began. The transmission of GBS ST283 has only been studied in Singapore. If the GBS ST283 infections outside of Singapore are also acquired via the foodborne route, then GBS is predominantly a foodborne disease in at least these parts of Southeast Asia.

As noted throughout this document, available data for ST283 are largely based on opportunistic, pre-existing collections of GBS. The human collections were largely found in laboratories funded from overseas and may not reflect epidemiology in other parts of the countries studied. GBS were recovered from both humans and fish in Thailand and Viet Nam, but data are otherwise disjointed in time and place, and apart from some of the Thai data, are not population-based. Data on ST283 related outbreaks in aquaculture are largely anecdotal, apart from data which shows ST283 is widespread in tilapia farms across numerous regions in Thailand (Dangwetngam *et al*., 2016, Kannika *et al*., 2017, Kayansamruaj *et al*., 2019, Suanyuk *et al*., 2008). The data are not sufficient to estimate the burden of disease across Southeast Asia, either in humans or aquaculture. Data on ST283 were not found from Cambodia, Indonesia, or the Philippines in the peer-reviewed literature, although there is evidence for GBS infections in fish in some of those countries. Some literature suggests occurrence of ST283 in humans but without molecular confirmation; for example, a report from Thailand noted unusual cases of septic arthritis caused by GBS but without typing data (Louthrenoo *et al*., 2014) and isolates are no longer available. Transmission routes were only studied in Singapore.

4.1. Risk from Group B *Streptococcus* sequence type 283 in freshwater fish

The "simplest" qualitative risk assessment combines:

- the **likelihood of infection**, that is the likelihood of the hazard being present at the time of consumption in sufficient numbers to cause disease– in other words prevalence and concentration of the hazard in relation to the ID50; and
- **severity** of the resulting disease following ingestion of the hazard.

4.1.1. Likelihood of infection

The likelihood of infection is a result of the contamination (prevalence and concentration) being propagated – including any increases and decreases – through the food chain from the aquatic environment (either aquaculture or wild capture), in conjunction with the dose required to generate infection in humans. In a quantitative risk assessment this can be done through mathematical models, whereas in qualitative risk assessment each step in the food chain is described by non-numerical descriptors of the likelihood such as "high", "medium", and "low". Depending on the situation, a different likelihood scale including more descriptors (for example "very low") can be used to increase the level of resolution of the outcome.

Sumner, Ross and Ababouch (2004) defined qualitative risk assessment as one that "is based on data which, although forming an inadequate basis for numerical risk estimations, nonetheless, when conditioned by prior expert knowledge and identification of attendant uncertainties, permits risk ranking or separation into descriptive categories of risk." Hence, qualitative risk assessments mainly rely on logical and reasoned discussion of the available evidence. Despite the limitations introduced by the subjective choices that need to be made, they represent a valuable approach for a systematic assessment of the risk in data-scarce settings or when a rapid response is needed to tackle new or urgent threats.

It should also be noted that the likelihood aspect needs to take into account, as best as possible, information about the dose–response model, or ID50. That is because a hazard that has a high ID50 (for example *Listeria monocytogenes*), requires higher contamination levels to be present on/in the food at the time of consumption than a hazard that has a low ID50 (for example, norovirus).

Based on the information provided in Section 2 of this document, it is currently not possible to ascertain a quantitative or qualitative description of the likelihood of GBS ST283 infection in humans in relation to the consumption of freshwater fish. However, given that GBS ST283 is not heat-resistant (Section 2.3) and even exposure to mildly elevated temperatures for a short time appears to be effective in reducing the microbial load in contaminated products, it can be concluded that consumption of raw freshwater fish will have a higher likelihood to result in infection than freshwater fish that has received some type of heat treatment, for example dipping in a hot liquid/porridge or fully cooked (note that GBS grows at 37 °C, so certain temperatures may promote multiplication, rather than reducing bacterial load). In contrast, GBS are adapted to a large range of pH values and the effect of pH-modifying or other preparation and preservation practices, for example addition of lime juice/garlic/chili or fermentation, is uncertain. Assuming that these practices have no effect on GBS concentration, compared with raw fish, results in a conservative approach.

4.1.2. Severity of disease

The International Commission on Microbiological Specifications for Foods (ICMSF) (2018) proposed a classification for foodborne hazards as:

- **Severe** (either for general population or a restricted sub-population): life threatening or substantial chronic sequelae or long duration (for example Shiga-toxigenic *E. coli*, *Vibrio cholera* O1 for the general population or Entero-pathogenic *E. coli* in infants).
- **Serious**: incapacitating but not life threatening; sequelae infrequent; moderate duration (for example *Salmonella, Shigella, L. monocytogenes*).
- **Moderate**: not usually life threatening; no sequelae; normally short duration; symptoms are self-limiting; can result in severe discomfort (for example *Staphylococcus aureus* toxin, *Vibrio parahaemolyticus, Bacillus cereus* toxin, enteropathogenic *E. coli*).

Given the information provided in Section 3, it is concluded that GBS ST283 can be considered a Severe hazard for the general population.
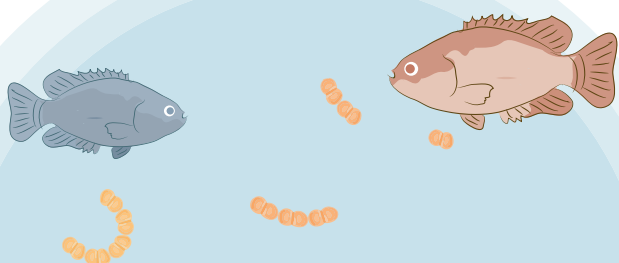
4.1.3. Risk estimation

The likelihood and severity are commonly combined using a risk matrix, such as that shown in Table 5. There exists no standardized risk matrix and hence the number of descriptions of the likelihood and severity categories can differ between different risk matrices in use. However, irrespective of the risk matrix that is used, a common feature of all risk matrices is that the level of risk increases as both likelihood and severity increase (i.e. from bottom left to top right in Table 5). Although this type of assessment utilizes descriptive terms for likelihood and severity, these can also be mapped to numerical information, especially in relation to likelihood, for example likelihood < 1 percent ≡ "Very unlikely", 1–10 percent ≡ "Unlikely", etc. (FAO/WHO, 2021). Alternative risk assessment methods, for example Risk Ranger (Ross and Sumner, 2002), require more detailed data and information than a qualitative assessment using a risk matrix.

Table 5. Risk matrix for qualitative estimation of the risk as a function of the likelihood of infection per serving and severity of consequences

LIKELIHOOD OF INFECTION	SEVERITY		
	MODERATE	SERIOUS	SEVERE
Very likely	Medium	High	High
Likely	Medium	Medium	High
Possible	Low	Medium	Medium
Unlikely	Low	Low	Medium
Very unlikely	Very low	Low	Low

As explained above, because of the recent emergence of GBS ST283 as a foodborne pathogen, the substantial lack of systematic data prevents an accurate estimation of the likelihood of foodborne GBS ST283 infection in humans. In particular, from Sections 2 and 3 it is apparent that there is a lack of data in relation to:
- prevalence and concentration of GBS ST283 on raw freshwater fish at retail or at the point of consumption;
- the effects of different fish presentation and preparation practices on GBS ST283 concentration; and
- the dose–response model or ID50 of GBS ST283 in the general population associated with ingestion.

Nevertheless, the disease caused by GBS ST283 in the general population is severe. Using the risk matrix in Table 5 for illustrative purposes, the potential risk from consumption of raw freshwater fish could range from low to high, depending on the actual likelihood of infection. Though the actual risk outcome is highly uncertain given the current lack of data, it should be noted that a higher likelihood of infection will generally result in a higher risk. Hence:
- consumption of raw fish will have a risk that is at least as high as the risk of consumption of fish that has either been fermented or prepared using traditional practices; and
- consumption of partially or fully heat-treated freshwater fish will have a lower risk than consumption of raw fish, and the effect will depend on the time and temperature of the heat treatment.

As a reality check, it is noted that these observations align with the fact that GBS ST283 has resulted in an outbreak of foodborne GBS ST283 diseases in Singapore in 2015, affecting 146 people, linked to consumption of raw freshwater fish. Hence, it can be concluded that although the actual likelihood of infection remains highly uncertain, the risk posed by GBS ST283 to the general population of Southeast Asia is at least "low" and efforts should be made to reduce the uncertainty in those data gaps that prevent a more accurate evaluation of the risk (see Section 4.2).

4.2. Data gaps

The following list provides information about important data gaps (identified in previous Sections) to better estimate the risk. These data gaps are listed in "reverse order", meaning starting closest to the consumer and then moving back in the food chain to production. This was done to focus future data collection efforts on those gaps that are closest to the consumer and thus allow more immediate benefit in terms of estimating risk. Nevertheless, data further back in the food chain will be valuable in relation to better understanding both the mechanisms of food contamination and the potential benefit of risk mitigation measures throughout the food chain.

- Dose–response model for GBS ST283 in humans: currently no information exists about a dose–response model for ingestion of GBS ST283 nor for ID50 (median Infectious or illness dose).
- Susceptibility and risk factors: the reason that the 2015 outbreak in Singapore was notable was because of the infection and disease of healthy adults, which suggests that the general population is susceptible to ST283. However, no information exists that indicates whether particular sub-populations, such as the elderly, very young, or immune-compromised people, are at a higher risk than the general population. In addition, there exist little data on food consumption, travel history, or other risk factors for GBS ST283 infection.
- Disease burden: with the exception of limited data from Thailand, the disease burden related to GBS ST283 in Southeast Asian countries is not well quantified, especially related to foodborne infection. Furthermore, information to calculate the DALY for GBS ST283 does not exist, nor does economic information, including treatment cost and loss of income.
- Incidence of disease: incidence of GBS, including GBS ST283, as a cause of sepsis and other forms of invasive disease in humans in Southeast Asia has not been quantified. The occurrence of GBS ST283 in non-invasive manifestations of GBS disease is unknown and factors contributing to the severity of GBS ST283 disease in humans, for example host factors, virulence properties, mode of transmission, or dose, are poorly understood. In addition, the reason why GBS ST283 related disease has mainly been reported in Southeast Asia and not other geographical regions is not understood.
- Human infection and shedding: there currently are no data related to the prevalence of GBS ST283 carriage in humans or the concentration of GBS ST283 in faeces, including the duration of shedding, especially following invasive infection and during asymptomatic infection. Robust data on GBS ST283 epidemiology are not available because of limited healthcare, diagnostic, bacterial typing, and reporting infrastructure.
- Mechanism of infection: the mechanism(s) of how consumption of fish contaminated with GBS ST283 infects and causes disease in humans is not understood. The potential for other GBS strains to cause similar disease, associated with consumption of contaminated fish, is not known.
- Size and demographics of consuming population: no systematic data exist documenting the size of the consuming population, nor their demographic characteristics, cultural identities, and geographic variables, such as coastal or inland location. Ideally this information would be country specific.

- Food preparation and consumption practices: although the Singapore outbreak was related to consumption of raw freshwater fish, the exact food preparation and consumption practices are unknown. In addition, the extent to which different practices mitigate the risk, for example dipping into hot liquids, is also not known. Ideally this information would be country and/or culture specific.
- Frequency of consumption and serving size: no information exists about how frequently consumers of freshwater fish consume the product in various preparation formats, and how big the serving size is (including mean and variability in serving size). Ideally this information would be country specific.
- Prevalence and concentration of GBS ST283 at retail: sampling at the port and in markets after the 2015 Singapore outbreak indicated higher prevalence of GBS ST283 detection at markets than at the port. However, this work was limited in scope and no concentration data was obtained. To undertake a risk assessment, systematic data on the prevalence and concentration of GBS ST283 on freshwater fish, including specific fish parts, are required. Ideally this information would be country specific.
- Trade volumes: there are only limited data on the production and trade volumes of freshwater fish, by species and product format. This information is needed to better understand risk pathways and likely exposures.
- Post-process handling (including storage and transport): no data are currently available to indicate the actual conditions of post-process handling, storage, and transport (in particular, temperature and duration of storage and transport). Ideally this information would be country specific.
- Effect of processing: the effects of freshwater fish processing, for domestic and international markets, on factors affecting contamination of fish meat with GBS ST283 are not supported by data, and thus are poorly understood.
- Where in the fish is GBS ST283 found: given the opportunity for cross-contamination of samples during testing, there currently exists no good data on the distribution (body sites) of GBS ST283 in sick and apparently healthy fish.
- Probability of contamination of live fish: there are currently no systematic country-specific and species-specific data on the prevalence and concentration of GBS ST283 within and between fish farms and ponds/cages, and wild-caught fish. In particular, such work would investigate colonization or infection of fish with and without clinical signs which are more likely to be sold for human consumption. Nevertheless, investigations of GBS ST283-related outbreaks, including the extent of morbidity and mortality, in aquaculture will also be important to better understand the disease in fish, which may assist in on-farm prevention and mitigation measures.
- Risk-factors for fish infections: a better understanding is required of which factors contribute to the contamination of aquaculture environments and subsequent infection of fish, including apparently healthy fish. This should include the effect of co-infections with other organisms, and changes in the microbiome of the fish and their environment, on GBS.
- Mechanism of fish infection: the mechanism(s), for example direct contact, waterborne, or through ingestion, of GBS infection in fish is unknown, and no data exist in relation to the likelihood and effect of wastewater and surface water contamination on GBS contamination of growing waters.
- Occurrence of ST283 in humans in countries other than Southeast Asia, especially countries where ST283 has already been detected in cultured fish (Brazil, South America).
- The risks associated with exports from Southeast Asia.
- A lack of understanding of the knowledge of the fish farmers in different settings such as best practices, signs of a diseased population or individual fish, implications of poor husbandry.
- The barriers to good farming processes.

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5 IMPLICATIONS FOR RISK MANAGEMENT

5.1. Existing control measures

Given the lack of data related to GBS and GBS ST283 contamination of fish, fish products, and the supply chain, risk management options for this organism are fairly general. Hence, risk management options are based on the application of:

- Good Aquaculture Practices (GAqP) during production (Joint Institute For Food Safety And Applied Nutrition (JIFSAN), 2016); and
- Good Hygienic Practices (GHP), Good Manufacturing Practices (GMP), and a Hazard Analysis and Critical Control Point (HACCP) system during production, transport, and retail, especially as they relate to fish and fishery products (FAO/WHO, 2020).

It is recognized that these systems are more likely to be applied in large-scale production systems and the export supply chain than small-scale production for domestic consumption. In addition, hygienic practices in the fish value chain may be monitored using total bacterial counts or indicator bacteria such as *Escherichia coli*. Currently, there are no data to indicate if and to what extent GBS ST283 can originate from faecal contamination, for example related to contamination from food handlers or contamination of the growing environment. In addition, no data exist to indicate the relation, if any, between total bacterial counts, *E. coli* counts, and prevalence or concentration of GBS in fish. Nevertheless, there are several potential risk management options specific to GBS in fish production that are discussed in Section 5.1.4. However, their efficacy needs to be verified and validated.

5.1.1. Production, including wild capture

Prevention and control of a disease is a multifactorial process and requires an integrated health management approach (Wendover, 2009).

Practices that contribute to good aquaculture practices and thus have the potential to reduce food safety risks at the production stage are listed below, although it is recognized that their applicability may differ between countries and production systems, for example pond versus river cage based systems:

- Maintain good on-farm biosecurity for aquaculture production.
- Practice all-in-all-out harvest, including cleaning of ponds and equipment and fallow periods between production cycles.
- Use appropriate stocking density.
- Provide adequate nutrition to meet fish growth requirements and limit feed wastage; do not overfeed the fish.
- Use commercial sources of feed and store feed properly and use before expiry date.
- Maintain good water quality.
- Use health-certified animal stocks (with or without laboratory testing).
- Ensure compliance with good hygiene and sanitation practices on the farms.
- Include acclimation periods where animals purchased from hatcheries are stocked and monitored (one to five days) for any behavioural or clinical signs prior to stocking into the farm.
- Use a single stocking time with no inclusion of wild fish stocks.
- Train staff in all relevant aspects of fish production, fish health, and food safety as it relates to fish farming.
- Provide appropriate facilities for the operation, including toilet facilities and facilities for storage of feed and chemicals.
- Control invasive species of the farm/pond and remove wild fish from the site and around the farm in river-based cages.
- Remove moribund and dead fish frequently, and dispose of the corpses appropriately.
- Strictly control chemical and veterinary treatments (water and animal) and observe withholding periods.
- Avoid transmission of antimicrobials, residues, or resistance genes between humans, fish and other animal species through waste run-off or husbandry.
- Reduce stress during harvest and harvest correctly. Chill fish quickly and handle appropriately to reduce likelihood of contamination with human foodborne pathogens.

5.1.2. Transport, processing and retail

General measures relevant to bacterial contamination of seafood intended for raw consumption are described in some detail in "Risk Assessment of *Vibrio parahaemolyticus* in seafood" (FAO/WHO, 2011). Note that the term "seafood" includes freshwater fish. The occurrence of pathogens in the final product at the point of consumption is mainly affected by, first, the temperature during processing/transport and, second, the potential for cross-contamination during transport and preparation. Cold chain systems and the introduction of preventive hygienic measures to avoid cross-contamination are well known for most food processing and transport systems and these will reduce the risk of foodborne disease by preventing bacterial growth.

Various government efforts in Southeast Asian countries have been made to initiate the implementation of HACCP in the fish processing industries. The application of the HACCP concept is unique to each process and factory. A detailed study of process flow is necessary to identify the hazards and the points where control can be exerted. However, as noted above, HACCP principles are general and not specific to GBS ST283.

5.1.3. Consumption

The outbreak of foodborne GBS ST283 infection in humans was linked to the consumption of raw fish (Kalimuddin *et al.*, 2017). The policies subsequently implemented in Singapore included banning the use of freshwater fish in ready-to-eat (RTE) dishes and requiring procurement of saltwater fish from suppliers for raw fish approved by Singapore authorities. In addition, catering services were required to comply with practices required for preparing RTE raw saltwater fish dishes (Chau *et al.*, 2017). These regulatory actions were in direct response to the foodborne outbreak and brought it to an end. However, it should be noted that despite these measures, there is unpublished evidence from one hospital in Singapore that GBS infections are still higher than prior to the 2015 outbreak. This may imply the existence of another source of infection or that the existing control measures are not completely effective. As noted in Section 3.2.2, investigation of these cases by the Singapore MOH did not reveal any epidemiological links, although patients may have been less likely to admit to eating raw freshwater fish.

As the food safety risk associated with GBS ST283 is a recently detected phenomenon, no evaluations of related public health interventions have been conducted. However, as noted in Section 2.5.2, consumption of raw and not fully heat-treated fish dishes in Southeast Asia is widespread, which is quantified by the proxy of parasitic infections, such as liver fluke and anisakis (Jongsuksuntigul and Imsomboon, 2003). Control measures for these infections, which may be applicable to GBS ST283, include public health campaigns to promote avoidance of eating raw fish and promoting hygienic defecation measures to prevent transmission into the natural environment that serves to exacerbate the cycle of infection (Jongsuksuntigul and Imsomboon, 2003). However, despite long-standing efforts, this remains a significant problem in the region.

Public health messages appear to be less effective, largely because of campaigns being developed in metropolitan centres, with little understanding of the socio-cultural drivers for the food practices and associated behaviours. For example, additional meanings may be attached to the practice of raw fish consumption associated with traditional identities, food security, and community resilience. Consequently, there is a need to pursue a whole systems approach to any public health campaigns, including medical, natural and social sciences, and humanities perspectives (Asavarut *et al.*, 2016, Sripa and Echaubard, 2017). Participatory approaches might be better suited to developing public health campaigns in local idioms, rather than imposing these from remote, more affluent centres (Asavarut *et al.*, 2016). Consequently, participatory approaches may prove more effective in attempting to change food practices associated with raw fish consumption.

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Further potential control measures may include modifications to food preparation informed by the small body of literature on the effects of common food ingredients, such as garlic (Ankri and Mirelman, 1999), ginger (Islam *et al.*, 2014), and lime juice (Oikeh *et al.*, 2016), on GBS ST283 loads and viability in food items. It is possible that recipe adaptation may achieve a route to infection control that is more acceptable to communities than messages of avoidance. However, all of these approaches require further investigation.

In Southeast Asia, various traditional preservation and preparation methods of fish and fish-based products are practiced (Abdullah, Idrus and Mardi, 1978). However, as stated above, it is not known how effective the following traditional approaches are in controlling GBS ST283.

- **Salting and drying**: in the salting and dying process, both fresh and marine water fish are used. The process includes removal of fish gills and guts, and the fish are split and opened in a butterfly fashion. Then, layers of coarse salt are applied to both sides of the fish, which is then left to dry in direct sunlight for up to seven days depending on the size of the fish and intensity of the sunlight. The fish are then washed to remove the remaining salt and spread out to dry again. The fish usually will be fried or cooked at a high temperature before being consumed.
- **Drying**: in this process, the fish are dried at ambient conditions without the addition of salt. The fish used in this process come from fresh and marine waters. The fish usually are fried or cooked at a high temperature before being consumed.
- **Smoking**: this method of curing is very country specific and not commonly used in Southeast Asia. Fish can be soft or hard smoked, depending on the smoking time and temperature. Soft smoked fish is usually later cooked at high temperature, for example in a curry, or by direct heating, before being consumed. Hard, or dry, smoked fish is more likely to be consumed without further heat treatment.
- **Fermentation**: fish fermentation or fish sauce is usually prepared by fermenting with salt.
- **Pickling**: pickling or conserving in vinegar and salt is again limited in this region. Most of these products seen in the region are imported.
- **Seasoning raw fish**: raw fish dishes are usually prepared by mixing with various seasonings such as garlic, salt, chili, and lime juice.

Drawing on the approaches to liver fluke control, there may be an opportunity to devise a range of interventions including pharmacological and public health approaches to managing GBS ST283 infection. From a medical perspective, human vaccines may present a route to preventing disease, although promoting messages about symptom recognition, early detection, and early treatment may be more realistic and cost–beneficial for controlling infection and limiting the potential for complications.

5.1.4. Potential risk management options

5.1.4.1. Vaccination of fish

Numerous experimental GBS vaccines have been described in the literature, based on a variety of vaccine technologies. Commercially available vaccines target GBS serotype Ib (CC552) or GBS serotype Ia (CC7). There is limited cross-protection between non-haemolytic (serotype Ib, CC52) and beta-haemolytic strains (serotype Ia, CC7) but vaccine producers claim cross-protection between different beta-haemolytic types of GBS, i.e. CC7 and ST283.

Vaccination programmes with commercial and autogenous vaccines have been carried out to control GBS ST283 outbreaks in Brazil. The success of those programmes depends on the water temperature during outbreaks. Vaccinated fish raised in endemic floating cage farms under water temperatures below 30 °C have shown survival rates from 85 percent to 92 percent in the grow-out phase. In contrast, in seasons (mainly summer) when water temperatures rise above 31 °C, survival rates of 70 percent to 75 percent are obtained, which is close to the survival in unvaccinated fish (C.A.G. Leal, personal communication, 2020).

The additional production cost of vaccination of about 3 percent, and experiences of vaccine failure because of a mismatch between the vaccine strain and the strain causing disease on-farm, has limited the widespread uptake of vaccination, especially by small-scale producers.

5.1.4.2. Dietary supplements

Dietary supplements such as immunostimulants have been incorporated into fish feed to enhance the immune response of the host, thus reducing disease susceptibility. However, to date there are no data on immunostimulants against GBS ST283.

Some microbial cultures have been shown to better combat a wide range of fish bacterial pathogens, including GBS. For example, *Bacillus* spp. are commonly used for microbial control in animals and plants, and it has been shown experimentally to help combat GBS in fish (Kuebutornye *et al.*, 2020). *Bacillus* spp. exhibit an anti-GBS effect via mechanisms such as enhancement of host immune status (Kim, Subramanian and Heo, 2017) and excretion of metabolites inhibiting the growth of GBS (Yi *et al.*, 2018). The promising effect of microbial cultures has been observed in both *in vitro* and *in vivo* studies for tilapia and other freshwater fish (Kuebutornye *et al.*, 2020, Thy *et al.*, 2017, Xia *et al.*, 2020), although it may be constrained by fluctuations in water quality (Srisapoome and Areechon, 2017).

5.1.4.3. Glycoinhibitors

Glycoinhibitors of adherence of GBS in fish have been proposed as an effective measure to avoid GBS infection in tilapia (Barato *et al.*, 2016, Iregui and Barato, 2017, Vásquez-Machado, Barato-Gómez and Iregui-Castro, 2019). According to this patent, specific sugars and one lectin, which block the adherence of GBS to intestinal epithelium of farmed tilapia, can be used to prevent bacterial adhesion and invasion in the fish. The concept was developed in Colombia, where CC552, but not ST283, has been reported in fish (Barato *et al.*, 2015). The approach is based on the notion that the main route of infection is oral and preceded by intestinal colonization (Barato *et al.*, 2016, Vásquez-Machado, Barato-Gómez and Iregui-Castro, 2019). Some challenge studies provide strong support for this route of infection (Iregui *et al.*, 2016), whereas other studies are equivocal (Soto *et al.*, 2016) or suggest that immersion of fish in GBS-contaminated water (as opposed to oral exposure) is associated with mortality (Delamare-Deboutteville *et al.*, 2015). Similar studies are yet to be undertaken for ST283.

5.1.4.4. Selective breeding

Selective breeding of tilapia to improve resistance against *Streptococcus* is in progress (Suebsong *et al.*, 2019) with promising trends in resistance improvement from first to third generations of hybrid offspring (P. Kayansamruaj, personal communication, 2020).

5.1.4.5. Group B *Streptococcus* surveillance

A culture-based surveillance and control programme for GBS in a Danish dairy herd resulted in a reduction in the herd-level frequency of GBS infections (mastitis) from 4 percent in 1966 to 0.5 percent in 2000. The programme was initially voluntary but later mandatory and illustrates that mandatory nation-wide surveillance and control may help to reduce GBS prevalence, at least in some animal production systems (Mweu, 2014). A cost–benefit analysis of surveillance and control measures in fish has not been reported, and is unlikely to be a cost-effective option in Southeast Asia, especially as between-farm transmission in river systems would be very difficult to control.

In contrast, establishing a comprehensive programme for public health surveillance of human GBS disease is of fundamental importance as a means of providing early warning of potential outbreaks and for assessing epidemiological patterns of disease, including exposure through consumption of fish. Developing infrastructure to facilitate surveillance of GBS strain types is of fundamental importance in addressing key data gaps and providing rapid response to emerging threats.

REFERENCES

Abakari, G., Luo, G., Meng, H., Yang, Z., Owusu-Afriyie, G., Kombat, E. O. & Alhassan, E. H. 2020. The use of biochar in the production of tilapia (*Oreochromis niloticus*) in a biofloc technology system-BFT. *Aquacultural Engineering, 91*: 102123. 10.1016/j.aquaeng.2020.102123.

Abdullah, M. I., Idrus, A. Z. & Mardi, S. The fish processing industry in Peninsular Malaysia. Indo-Pacific Fishery Commission Symposium on Fish Utilization Technology and Marketing in the IPFC Region, 8-11 March 1978 Manila. [Accessed 2020].

Adoga, I. J., Joseph, E. & Samuel, O. F. 2010. Storage life of tilapia (*Oreochromis niloticus*) in ice and ambient temperature. *Researcher, 2(5): 39-44.*

Ali, H., Upraity, V., Gurung, S., Dhar, G. C. & Belton, B. 2018. Making sense of the market: Assessing the participatory market chain approach to aquaculture value chain development in Nepal and Bangladesh. *Aquaculture, 493: 395-405.*

Alsaid, M., Daud, H., Mohamed, N., Bejo, S. K., Mohamed, Y. & Abuseliana, A. 2013. Environmental factors influencing the susceptibility of red hybrid tilapia (*Orechromis sp.*) to Streptococcus agalactiae infection. *Advanced Science Letters, 19(12): 3600-3604.*

Amal, M. N. A. 2007. I*solation and identification of Streptococcus spp. isolated from red tilapia (Oreochromis sp.). Bachelor of Science in Agrotechnology (Aquaculture) Undergraduate thesis, Universiti Malaysia Terengganu.* [Accessed 2020].

Amal, M. N. A. & Zamri-Saad, M. 2011. Streptococcosis in tilapia (*Oreochromis niloticus): A review. Pertanika Journal of Tropical Agricultural Science, 34(2): 195 - 206.*

Amal, M. N. A., Zamri-Saad, M., Zahrah, A. S. & Zulkafli, A. R. 2015. Water quality influences the presence of *Streptococcus agalactiae in cage cultured red hybrid tilapia, Oreochromis niloticus × Oreochromis mossambicus. Aquaculture Research, 46(2): 313-323.* 10.1111/are.12180.

Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A. & Zulkafli, A. R. 2013. Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus (L.)× Oreochromis mossambicus (Peters),* farm. *Journal of Fish Diseases, 36(8): 735-739.*

Ananchaipattana, C., Hosotani, Y., Kawasaki, S., Pongsawat, S., Md. Latiful, B., Isobe, S. & Inatsu, Y. 2012. Prevalence of foodborne pathogens in retailed foods in Thailand. *Foodborne pathogens and disease, 9(9): 835-840.*

Ankri, S. & Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. *Microbes and infection, 1(2): 125-129.*

Asavarut, P., Norsworthy, P. J., Cook, J., Taylor-Robinson, S. D. & Harrison, R. V. 2016. Diet and disease: Tansgressing boundaries between science and society - understanding neglected diseases through the lens of cultural studies and anthropology. *Medical Humanities, 42: 181-183.*

Ashton, P. M., Peters, T., Ameh, L., McAleer, R., Petrie, S., Nair, S., Muscat, I., de Pinna, E. & Dallman, T. 2015. Whole genome sequencing for the retrospective investigation of an outbreak of Salmonella Typhimurium DT 8. *PLoS currents, 7.*

Azad, I. S., Al-Marzouk, A., James, C. M., Almatar, S., Al-Gharabally, H. & Qasem, J. A. 2012. Outbreak of natural streptococcosis in hatchery produced silver pomfret (*Pampus argenteus Euphrasen) larvae in Kuwait. Aquaculture, 330: 15-20.*

Baraboutis, I. G., Doris, K., Papanikolaou, K., Tsagalou, E. P., Chatsiou, K., Papathanasiou, E., Platsouka, E., Papastamopoulos, V., Belesiotou, H. & Apostolou, T. 2010. An outbreak of hemodialysis catheter-related bacteremia with sepsis caused by *Streptococcus agalactiae in a hemodialysis unit. International Journal of Infectious Diseases, 14(5): e418-e422.*

Barato, P., Martins, E. R., Melo-Cristino, J., Iregui, C. A. & Ramirez, M. 2015. Persistence of a single clone of *Streptococcus agalactiae causing disease in tilapia (Oreochromis sp.) cultured in Colombia over 8 years. Journal of Fish Diseases, 38(12): 1083-7.* 10.1111/jfd.12337.

Barato, P., Martins, E. R., Vasquez, G. M., Ramirez, M., Melo-Cristino, J., Martínez, N. & Iregui, C. 2016. Capsule impairs efficient adherence of *Streptococcus agalactiae to intestinal epithelium in tilapias Oreochromis sp. Microbial Pathogenesis, 100: 30-36. 10.1016/j.micpath.2016.08.040.*

Barkham, T., Sheppard, A., Jones, N. & Chen, S. 2018. *Streptococcus agalactiae that caused meningitis in healthy adults in 1998 are ST283, the same type that caused a foodborne outbreak of invasive sepsis in 2015: An observational molecular epidemiology study. Clinical Microbiology and Infection. 10.1016/j.cmi.2018.04.006.*

Barkham, T., Zadoks, R. N., Azmai, M. N. A., Baker, S., Bich, V. T. N., Chalker, V., Chau, M. L., Dance, D., Deepak, R. N., van Doorn, H. R., Gutierrez, R. A., Holmes, M. A., Huong, L. N. P., Koh, T. H., Martins, E., Mehershahi, K., Newton, P., Ng, L. C., Phuoc, N. N., Sangwichian, O., Sawatwong, P., Surin, U., Tan, T. Y., Tang, W. Y., Thuy, N. V., Turner, P., Vongsouvath, M., Zhang, D., Whistler, T. & Chen, S. L. 2019. One hypervirulent clone, sequence type 283, accounts for a large proportion of invasive *Streptococcus agalactiae isolated from humans and diseased tilapia in Southeast Asia. PLOS Neglected Tropical Diseases, 13(6): e0007421. 10.1371/journal.pntd.0007421.*

Basri, L., Nor, R. M., Salleh, A., Saad, M. Z., Barkham, T. & Amal, M. N. A. 2020. Co-infections of tilapia lake virus, *Aeromonas hydrophila and Streptococcus agalactiae in farmed feed hybrid tilapia. Animals, 10(11): 2141.*

Bliss, S. J., Manning, S. D., Tallman, P., Baker, C. J., Pearlman, M. D., Marrs, C. F. & Foxman, B. 2002. Group B *Streptococcus colonization in male and nonpregnant female university students: a cross-sectional prevalence study. Clinical Infectious Diseases, 34(2): 184-90. 10.1086/338258. Epub 2001 Dec 5.*

Bondad-Reantaso, M. G., Arthur, J. R. & Subasinghe, R. P. 2012. Improving biosecurity through prudent and responsible use of veterinary medicines in aquatic food production. *FAO Fisheries and Aquaculture Technical Paper, (547): I,III,IV,VII,VIII,IX,X,XI,XII,XV,1-9,11-67,69-89,91-117,119-153,155-165,167-203,205-207.*

Borderías, A. J. & Sánchez-Alonso, I. 2011. First processing steps and the quality of wild and farmed fish. *Journal of food science, 76(1): R1-R5.*

Bowater, R. O., Forbes-Faulkner, J., Anderson, I. G., Condon, K., Robinson, B., Kong, F., Gilbert, G. L., Reynolds, A., Hyland, S. & McPherson, G. 2012. Natural outbreak of *Streptococcus agalactiae (GBS) infection in wild giant Queensland grouper, Epinephelus lanceolatus (Bloch), and other wild fish in northern Queensland, Australia. Journal of fish diseases, 35(3): 173-186.*

Boyd, C. E. & Tucker, C. S. 2012. *Pond aquaculture water quality management. Springer Science & Business Media2012].*

Chang, Y., Wang, W., Liu, X., Du, F. & Yao, D. 2020. Effects of different pond aquaculture systems on water environments, and suggestions for structural adjustments. *Polish Journal of Environmental Studies, 29(1): 571-577.*

Chau, M. L., Chen, S. L., Yap, M., Hartantyo, S. H. P., Chiew, P. K. T., Fernandez, C. J., Wong, W. K., Fong, R. K., Tan, W. L., Tan, B. Z. Y., Ng, Y., Aung, K. T., Mehershahi, K. S., Goh, C., Kang, J. S. L., Barkham, T., Leong, A. O. K., Gutierrez, R. A. & Ng, L. C. 2017. Group B *Streptococcus infections caused by improper sourcing and handling of fish for raw consumption, Singapore, 2015-2016. Emerging Infectious Diseases, 23(12). 10.3201/eid2312.170596.*

Chideroli, R. T., Amoroso, N., Mainardi, R. M., Suphoronski, S. A., de Padua, S. B., Alfieri, A. F., Alfieri, A. A., Mosela, M., Moralez, A. T. P., de Oliveira, A. G., Zanolo, R., Di Santis, G. W. & Pereira, U. P. 2017. Emergence of a new multidrug-resistant and highly virulent serotype of *Streptococcus agalactiae in fish farms from Brazil. Aquaculture, 479: 45-51. 10.1016/j.aquaculture.2017.05.013.*

Clerc, O., Prod'hom, G., Greub, G., Zanetti, G. & Senn, L. 2011. Adult native septic arthritis: A review of 10 years of experience and lessons for empirical antibiotic therapy. *Journal of Antimicrobial Chemotherapy, 66(5): 1168-73. 10.1093/jac/dkr047.*

Collin, S. M., Lamb, P., Jauneikaite, E., Le Doare, K., Creti, R., Berardi, A., Heath, P. T., Sriskandan, S. & Lamagni, T. 2019. Hospital clusters of invasive Group B streptococcal disease: A systematic review. *Journal of Infection, 79(6): 521-527.*

Collin, S. M., Shetty, N. & Lamagni, T. 2020. Invasive group B *Streptococcus infections in adults, England, 2015-2016. Emerging Infectious Diseases, 26(6): 1174-1181. 10.3201/eid2606.191141.*

ComBase Team 2019. ComBase: A web resource for quantitative and predictive food microbiology. 2020 https://data.nal.usda.gov/dataset/combase-web-resource-quantitative-and-predictive-food-microbiology [Accessed 6 March 2021].

Crestani, C., Forde, T. L. & Zadoks, R. N. 2020. Development and application of a prophage integrase typing scheme for Group B *Streptococcus*. *Frontiers in microbiology, 11: 1993*.

Cyprian, O., Lauzon, H. L., Jóhannsson, R., Sveinsdóttir, K., Arason, S. & Martinsdóttir, E. 2013. Shelf life of air and modified atmosphere-packaged fresh tilapia (*Oreochromis niloticus*) fillets stored under chilled and superchilled conditions. *Food Science & Nutrition,* 1(2): 130-140.

Da Cunha, V., Davies, M. R., Douarre, P. E., Rosinski-Chupin, I., Margarit, I., Spinali, S., Perkins, T., Lechat, P., Dmytruk, N., Sauvage, E., Ma, L., Romi, B., Tichit, M., Lopez-Sanchez, M. J., Descorps-Declere, S., Souche, E., Buchrieser, C., Trieu-Cuot, P., Moszer, I., Clermont, D., Maione, D., Bouchier, C., McMillan, D. J., Parkhill, J., Telford, J. L., Dougan, G., Walker, M. J., Consortium, D., Holden, M. T. G., Poyart, C. & Glaser, P. 2014. *Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline. Nature Communications, 5: 4544. 10.1038/ncomms5544.*

Dahl, M. S., Tessin, I. & Trollfors, B. 2003. Invasive group B streptococcal infections in Sweden: Incidence, predisposing factors and prognosis. *International Journal of Infectious Diseases, 7(2): 113-119.*

Dangwetngam, M., Suanyuk, N., Kong, F. & Phromkunthong, W. 2016. Serotype distribution and antimicrobial susceptibilities of *Streptococcus agalactiae isolated from infected cultured tilapia (Oreochromis niloticus) in Thailand: Nine-year perspective. Journal of Medical Microbiology, 65(3): 247-54. 10.1099/jmm.0.000213.*

Delamare-Deboutteville, J., Bowater, R., Condon, K., Reynolds, A., Fisk, A., Aviles, F. & Barnes, A. C. 2015. Infection and pathology in Queensland grouper, *Epinephelus lanceolatus,(Bloch), caused by exposure to Streptococcus agalactiae via different routes. Journal of fish diseases, 38(12): 1021-1035.*

Delannoy, C. M., Crumlish, M., Fontaine, M. C., Pollock, J., Foster, G., Dagleish, M. P., Turnbull, J. F. & Zadoks, R. N. 2013. Human *Streptococcus agalactiae strains in aquatic mammals and fish. BMC Microbiology, 13: 41. 10.1186/1471-2180-13-41.*

Delannoy, C. M., Zadoks, R. N., Crumlish, M., Rodgers, D., Lainson, F. A., Ferguson, H. W., Turnbull, J. & Fontaine, M. C. 2016. Genomic comparison of virulent and non-virulent *Streptococcus agalactiae in fish. Journal Fish of Diseases, 39(1): 13-29. 10.1111/jfd.12319.*

Denton, M., Hawkey, P. M., Hoy, C. M. & Porter, C. 1993. Co-existent cross-infection with *Streptococcus pneumoniae and group B streptococci on an adult oncology unit. Journal of Hospital Infection, 23(4): 271-278.*

Devleesschauwer, B., Havelaar, A. H., De Noordhout, C. M., Haagsma, J. A., Praet, N., Dorny, P., Duchateau, L., Torgerson, P. R., Van Oyen, H. & Speybroeck, N. 2014a. Calculating disability-adjusted life years to quantify burden of disease. *International journal of public health, 59(3): 565-569.*

Devleesschauwer, B., Havelaar, A. H., Maertens de Noordhout, C., Haagsma, J. A., Praet, N., Dorny, P., Duchateau, L., Torgerson, P. R., Van Oyen, H. & Speybroeck, N. 2014b. DALY calculation in practice: A stepwise approach. *International Journal of Public Health, 59(3): 571-4. 10.1007/s00038-014-0553-y.*

Duremdez, R., Al-Marzouk, A., Qasem, J. A., Al-Harbi, A. & Gharabally, H. 2004. Isolation of *Streptococcus agalactiae from cultured silver pomfret, Pampus argenteus (Euphrasen), in Kuwait. Journal of Fish Diseases, 27(5): 307-310.*

Dvorak, G. 2009. Biosecurity for aquaculture facilities in the North Central region. *North Central Regional Aquaculture Center Extension Fact Sheets Series #115.* https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1007&context=ncrac_factsheets [Accessed 6 March 2021].

Edwards, M. S. & Baker, C. J. 2020. *Streptococcus agalactiae (group B Streptococci) Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases, 9th Edition, pp. 2505-2512. Philadelphia, Elsevier. [Accessed 2020].*

El-Leithy, A. A., Hemeda, S. A., Abd El Naby, W. S., El Nahas, A. F., Hassan, S. A., Awad, S. T., El-Deeb, S. I. & Helmy, Z. A. 2019. Optimum salinity for Nile tilapia (*Oreochromis niloticus) growth and mRNA transcripts of ion-regulation, inflammatory, stress-and immune-related genes. Fish Physiology and Biochemistry, 45(4): 1217-1232.*

El-Sayed, A.-F. M. 2019. Tilapia culture. Academic Press [Accessed 2020].

Eskandarian, N., Neela, V., Ismail, Z., Puzi, S. M., Hamat, R. A., Desa, M. N. & Nordin, S. A. 2013. Group B streptococcal bacteremia in a major teaching hospital in Malaysia: A case series of eighteen patients. *International Journal of Infectious Diseases, 17(9): e777-80. 10.1016/j.ijid.2013.01.011.*

Evans, J., Klesius, P., Gilbert, P., Shoemaker, C., Al Sarawi, M., Landsberg, J., Duremdez, R., Al Marzouk, A. & Al Zenki, S. 2002. Characterization of β-haemolytic group B *Streptococcus agalactiae in cultured seabream, Sparus auratus L., and wild mullet, Liza klunzingeri (Day), in Kuwait. Journal of Fish Diseases, 25(9): 505-513.*

Evans, J. J., Klesius, P. H. & Shoemaker, C. A. 2004. Efficacy of *Streptococcus agalactiae (group B) vaccine in tilapia (Oreochromis niloticus) by intraperitoneal and bath immersion administration. Vaccine, 22(27-28): 3769-3773.*

Evans, J. J., Shoemaker, C. A. & Klesius, P. H. 2003. Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae in Nile tilapia Oreochromis niloticus. Journal of Aquatic Animal Health, 15(3): 202-208.*

FAO 2018. Globefish highlights-A quarterly update on world seafood markets (3rd issue 2018).1-72.http://www.fao.org/3/CA1531EN/ca1531en.pdf [Accessed 6 March 2021].

FAO 2020a. Global production by production source 1950–2018 (FishstatJ). Rome: http://www.fao.org/fishery/statistics/software/fishstatj/en [Accessed 6 March 2021].

FAO 2020b. The state of world fisheries and aquaculture 2020: Sustainability in action. Rome: https://doi.org/10.4060/ca9229en [Accessed 3 March 2021].

FAO/WHO 2011. Risk assessment of *Vibrio parahaemolyticus in seafood-interpretative summary and technical report. Microbological Risk Assessment series; No. 16. Rome: [Accessed 6 March 2021].*

FAO/WHO 2020. Code of practice for fish and fishery products. Rome.1-372. [Accessed 2020].

FAO/WHO 2021. Microbiological risk assessment guidance for food: Joint FAO/WHO expert meetings on microbiological risk assessment (JEMRA) on methodologies of microbiological risk assessment [In preparation]. [Accessed 2021].

Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P. & Spratt, B. G. 2004. eBURST: Inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of Bacteriology, 186(5): 1518-1530.*

Foxman, B., Gillespie, B. W., Manning, S. D. & Marrs, C. F. 2007. Risk factors for group B streptococcal colonization: potential for different transmission systems by capsular type. *Annals of Epidemiology, 17(11): 854-62. 10.1016/j.annepidem.2007.05.014.*

Francis-Floyd, R. 2002. Stress – Its role in fish disease. *University of Florida IFAS Extension Circular 919.* https://edis.ifas.ufl.edu/fa005 [Accessed 6 March 2021].

Furfaro, L. L., Chang, B. J., Kahler, C. M. & Payne, M. S. 2019. Genomic characterisation of perinatal Western Australian *Streptococcus agalactiae isolates. PLoS one, 14(10): e0223256.*

Gerges, T. M., Selim, A. & Osman, M. 2016. Improvement the shelf life of tilapia fillets stored at chilling condition. *Benha Veterinary Medical Journal, 31(2): 45-55.*

Glibert, P. M., Landsberg, J. H., Evans, J. J., Al-Sarawi, M. A., Faraj, M., Al-Jarallah, M. A., Haywood, A., Ibrahem, S., Klesius, P. & Powell, C. 2002. A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: The roles of bacterial disease, harmful algae, and eutrophication. *Harmful Algae, 1(2): 215-231.*

Grundy-Warr, C., Andrews, R. H., Sithithaworn, P., Petney, T. N., Sripa, B., Laithavewat, L. & Ziegler, A. D. 2012. Raw attitudes, wetland cultures, life-cycles: Socio-cultural dynamics relating to *Opisthorchis viverrini in the Mekong Basin. Parasitology International, 61(1): 65-70.*

Hasselberg, A. E., Aakre, I., Scholtens, J., Overå, R., Kolding, J., Bank, M. S., Atter, A. & Kjellevold, M. 2020. Fish for food and nutrition security in Ghana: Challenges and opportunities. *Global Food Security, 26: 100380.*

He, R. Z., Xu, J., Wang, J. & Li, A. X. 2020. Quantitative detection of streptococcosis infection in dead samples of Nile tilapia (*Oreochromis niloticus). Journal of Applied Microbiology, 129(5): 1157-1162.*

Henning, K. J., Hall, E. L., Dwyer, D. M., Billmann, L., Schuchat, A., Johnson, J. A. & Harrison, L. H. 2001. Invasive group B streptococcal disease in Maryland nursing home residents. *Journal of Infectious Diseases, 183(7): 1138-1142.*

Hishamunda, N., Bueno, P. B., Ridler, N. & Yap, W. G. 2009. *Analysis of aquaculture development in Southeast Asia. Food and Agriculture Organization of the United Nations (FAO)2009].*

HLPE 2014. Aquaculture for food security and nutrition. *A report by the High Level Panel of Experts on Food Security and Nutrition. Rome: Food and Agriculture Oganization [Accessed 2021].*

Hortle, K. G. 2009. Chapter 9 - Fisheries of the Mekong River Basin. In Campbell, I. C., ed. *The Mekong: Biophysical environment of an international river basin, pp. 197-249. San Diego, Academic Press. Available:* https://www.sciencedirect.com/science/article/pii/B9780123740267000097 [Accessed 2021].

Huang, J., Lin, X. Z., Zhu, Y. & Chen, C. 2019. Epidemiology of group B streptococcal infection in pregnant women and diseased infants in mainland China. *Pediatrics and Neonatology, 60(5): 487-495. 10.1016/j.pedneo.2019.07.001.*

International Commission on Microbiological Specifications for Foods (ICMSF) 2018. Microorganisms in foods 7: Microbiological testing in food safety management.

Ip, M., Ang, I., Fung, K., Liyanapathirana, V., Luo, M. J. & Lai, R. 2016. Hypervirulent clone of group B *Streptococcus serotype III sequence type 283, Hong Kong, 1993-2012. Emerging Infectious Diseases, 22(10): 1800-3. 10.3201/eid2210.151436.*

Ip, M., Cheuk, E. S., Tsui, M. H., Kong, F., Leung, T. N. & Gilbert, G. L. 2006. Identification of a *Streptococcus agalactiae serotype III subtype 4 clone in association with adult invasive disease in Hong Kong. Journal of Clinical Microbiology, 44(11): 4252-4. 10.1128/JCM.01533-06.*

Iregui, C. A., Comas, J., Vásquez, G. M. & Verjan, N. 2016. Experimental early pathogenesis of *Streptococcus agalactiae infection in red tilapia Oreochromis spp. Journal of Fish Diseases, 39(2): 205-215.*

Iregui, C. C. A. & Barato, G. P. A. 2017. Glycoinhibitors of adherence of *Streptococcus in fish. Google Patents.* WO/2017/109587 [Accessed 2020].

Islam, K., Rowsni, A. A., Khan, M. M. & Kabir, M. S. 2014. Antimicrobial activity of ginger (*Zingiber officinale) extracts against food-borne pathogenic bacteria. International Journal of Science, Environment and Technology, 3(3): 867-871.*

Ismail, M. S., Siti-Zahrah, A., Syafiq, M. R. M., Amal, M. N. A., Firdaus-Nawi, M. & Zamri-Saad, M. 2016a. Feed-based vaccination regime against streptococcosis in red tilapia, *Oreochromis niloticus x Oreochromis mossambicus. BMC Veterinary Research, 12(1): 194.*

Ismail, M. S., Syafiq, M. R., Siti-Zahrah, A., Fahmi, S., Shahidan, H., Hanan, Y., Amal, M. N. A. & Saad, M. Z. 2017. The effect of feed-based vaccination on tilapia farm endemic for streptococcosis. *Fish & Shellfish Immunology, 60: 21-24.*

Ismail, N. I. A., Amal, M. N. A., Shohaimi, S., Saad, M. Z. & Abdullah, S. Z. 2016b. Associations of water quality and bacteria presence **in cage cultured red hybrid tilapia,** *Oreochromis niloticus × O. mossambicus. Aquaculture Reports, 4: 57-65.*

Jafar, Q. A., Sameer, A. Z., Salwa, A. M., Samee, A. A., Ahmed, A. M. & Al-Sharifi, F. 2008. Molecular investigation of *Streptococcus agalactiae isolates from environmental samples and fish specimens during a massive fish kill in Kuwait Bay. Pakistan Journal of Biological Sciences, 11(21): 2500-4.*

Jensen, N. E. 1982. Experimental bovine group B streptococcal mastitis induced by strains of human and bovine origin. *Nordisk Veterinaermedicin, 34(12): 441-450.*

Jensen, N. E. & Berg, B. 1982. Sewage and aquatic biotopes as potential reservoirs of group B *Streptococci [zoonosis, cattle]. Dansk Veterinaertidsskrift, 65(5): 197-200.*

Jimenez-Ruiz, E. I., Maeda-Martínez, A. N., Ocaño-Higuera, V. M., Sumaya-Martinez, M. T., Sanchez-Herrera, L. M., Fregoso-Aguirre, O. A., Rincones-López, J. E. & Palomino-Hermosillo, Y. A. 2020. Shelf life of fresh fillets from eviscerated farmed tilapia (*Oreochromis niloticus) handled at different pre-filleting times. Journal of Food Processing and Preservation: e14529.*

Joint Institute For Food Safety And Applied Nutrition (JIFSAN) 2016. Good Aquacultural Practices (GAqP). https://jifsan.umd.edu/training/international/courses/gaqp/manuals [Accessed 15 February 2021].

Jones, N., Bohnsack, J. F., Takahashi, S., Oliver, K. A., Chan, M. S., Kunst, F., Glaser, P., Rusniok, C., Crook, D. W., Harding, R. M., Bisharat, N. & Spratt, B. G. 2003. Multilocus sequence typing system for group B *Streptococcus. Journal of Clinical Microbiology, 41(6): 2530-6. 10.1128/jcm.41.6.2530-2536.2003.*

Jongsuksuntigul, P. & Imsomboon, T. 2003. Opisthorchiasis control in Thailand. *Acta tropica, 88(3): 229-232.*

Jorgensen, H. J., Nordstoga, A. B., Sviland, S., Zadoks, R. N., Solverod, L., Kvitle, B. & Mork, T. 2016. *Streptococcus agalactiae in the environment of bovine dairy herds – Rewriting the textbooks? Veterinary Microbiology, 184: 64-72. 10.1016/j.vetmic.2015.12.014.*

Jump, R. L. P., Wilson, B. M., Baechle, D., Briggs, J. M., Banks, R. E., Song, S., Zappernick, T. & Perez, F. 2019. Risk factors and mortality rates associated with invasive group B *Streptococcus infections among patients in the US Veterans Health Administration. JAMA Network Open, 2(12): e1918324. 10.1001/jamanetworkopen.2019.18324.*

Junior, J. A. F., Leal, C. A. G., de Oliveira, T. F., Nascimento, K. A., de Macêdo, J. T. S. A. & Pedroso, P. M. O. 2020. Anatomopathological characterization and etiology of lesions on Nile tilapia fillets (*Oreochromis niloticus*) caused by *bacterial pathogens. Aquaculture: 735387.*

Kaewpitoon, N., Kaewpitoon, S. J., Pengsaa, P. & Sripa, B. 2008. *Opisthorchis viverrini: The carcinogenic human liver fluke. World Journal of Gastroenterology, 14(5): 666.*

Kalimuddin, S., Chen, S. L., Lim, C. T. K., Koh, T. H., Tan, T. Y., Kam, M., Wong, C. W., Mehershahi, K. S., Chau, M. L., Ng, L. C., Tang, W. Y., Badaruddin, H., Teo, J., Apisarnthanarak, A., Suwantarat, N., Ip, M., Holden, M. T. G., Hsu, L. Y., Barkham, T. & Singapore Group, B. S. C. 2017. 2015 epidemic of severe *Streptococcus agalactiae sequence type 283 infections in Singapore associated with the consumption of raw freshwater fish: A detailed analysis of clinical, epidemiological, and bacterial sequencing data. Clinical Infectious Diseases, 64(suppl_2): S145-S152. 10.1093/cid/cix021.*

Kannika, K., Pisuttharachai, D., Srisapoome, P., Wongtavatchai, J., Kondo, H., Hirono, I., Unajak, S. & Areechon, N. 2017. Molecular serotyping, virulence gene profiling and pathogenicity of *Streptococcus agalactiae isolated from tilapia farms in Thailand by multiplex PCR. Journal of Applied Microbiology, 122(6): 1497-1507. 10.1111/jam.13447.*

Kawasaki, M., Delamare-Deboutteville, J., Bowater, R. O., Walker, M. J., Beatson, S., Ben Zakour, N. L. & Barnes, A. C. 2018. Microevolution of *Streptococcus agalactiae ST-261 from Australia indicates dissemination via imported tilapia and ongoing adaptation to marine hosts or environment. Applied and Environmental Microbiology, 84(16). 10.1128/AEM.00859-18.*

Kayansamruaj, P., Soontara, C., Unajak, S., Dong, H. T., Rodkhum, C., Kondo, H., Hirono, I. & Areechon, N. 2019. Comparative genomics inferred two distinct populations of piscine pathogenic *Streptococcus agalactiae, serotype Ia ST7 and serotype III ST283, in Thailand and Vietnam. Genomics, 111(6): 1657-1667. 10.1016/j.ygeno.2018.11.016.*

Kim, D.-H., Subramanian, D. & Heo, M.-S. 2017. Dietary effect of probiotic bacteria, *Bacillus amyloliquefaciens-JFP2 on growth and innate immune response in rock bream Oplegnathus fasciatus, challenged with Streptococcus iniae. The Israeli Journal of Aquaculture, 69: 11.*

Kim, J. H., Gomez, D. K., Choresca Jr, C. H. & Park, S. C. 2007. Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. *Aquaculture, 272(1-4): 105-110.*

Kong, F., Gowan, S., Martin, D., James, G. & Gilbert, G. L. 2002. Serotype identification of group B *Streptococci by PCR and sequencing. Journal of Clinical Microbiology, 40(1): 216-226. 10.1128/jcm.40.1.216-226.2002.*

Kuebutornye, F. K. A., Abarike, E. D., Lu, Y., Hlordzi, V., Sakyi, M. E., Afriyie, G., Wang, Z., Li, Y. & Xie, C. X. 2020. Mechanisms and the role of probiotic *Bacillus in mitigating fish pathogens in aquaculture. Fish Physiology and Biochemistry, 46: 819-841.*

Kumar, P., Thirunavukkarasu, A. R., Subburaj, R. & Thiagarajan, G. 2015. Concept of stress and its mitigation in aquaculture. *Advances in marine and brackishwater aquaculture. Springer.95-100. [Accessed 2020].*

Laith, A. A., Ambak, M. A., Hassan, M., Sheriff, S. M., Nadirah, M., Draman, A. S., Wahab, W., Ibrahim, W. N., Aznan, A. S., Jabar, A. & Najiah, M. 2017. Molecular identification and histopathological study of natural *Streptococcus agalactiae infection in hybrid tilapia (Oreochromis niloticus). Veterinary World, 10(1): 101-111. 10.14202/vetworld.2017.101-111.*

Leal, C. A. G., Queiroz, G. A., Pereira, F. L., Tavares, G. C. & Figueiredo, H. C. P. 2019. *Streptococcus agalactiae sequence type 283 in farmed fish, Brazil. Emerging Infectious Diseases, 25(4): 776-779. 10.3201/eid2504.180543.*

Legario, F. S., Choresca Jr, C. H., Turnbull, J. F. & Crumlish, M. 2020. Isolation and molecular characterization of streptococcal species recovered from clinical infections in farmed Nile tilapia (*Oreochromis niloticus*) in the Philippines. *Journal of Fish Diseases, 43(11): 1431-1442.*

Li, C., Sapugahawatte, D. N., Yang, Y., Wong, K. T., Lo, N. W. S. & Ip, M. 2020. Multidrug-resistant *Streptococcus agalactiae strains found in human and fish with high penicillin and cefotaxime non-susceptibilities. Microorganisms, 8(7): 1055.*

Li, Y. W., Liu, L., Huang, P. R., Fang, W., Luo, Z. P., Peng, H. L., Wang, Y. X. & Li, A. X. 2014. Chronic streptococcosis in Nile tilapia, *Oreochromis niloticus (L.), caused by Streptococcus agalactiae. Journal of Fish Diseases, 37(8): 757-63. 10.1111/jfd.12146.*

Liang, J.-Y. & Chien, Y.-H. 2013. Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia–water spinach raft aquaponics system. *International Biodeterioration & Biodegradation, 85: 693-700*.

Liu, H., Zhang, S., Shen, Z., Ren, G., Liu, L., Ma, Y., Zhang, Y. & Wang, W. 2016. Development of a vaccine against *Streptococcus agalactiae in fish based on truncated cell wall surface anchor proteins. Veterinary Record, 179(14): 359-359*.

Louthrenoo, W., Kasitanon, N., Wangkaew, S., Hongsongkiat, S., Sukitawut, W. & Wichainun, R. 2014. *Streptococcus agalactiae: An emerging cause of septic arthritis. Journal of Clinical Rheumatology, 20(2): 74-8. 10.1097/RHU.0000000000000071*.

Lyhs, U., Kulkas, L., Katholm, J., Waller, K. P., Saha, K., Tomusk, R. J. & Zadoks, R. N. 2016. *Streptococcus agalactiae serotype IV in humans and cattle, Northern Europe. Emerging Infectious Diseases, 22(12): 2097-2103. 10.3201/eid2212.151447*.

Mapfumo, B. 2018. Tilapia trade: Global and regional trends. FAO/ASTF GCP/RAF/510/MUL: Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture: http://www.fao.org/fi/static-media/MeetingDocuments/TiLV/dec2018/Default.html [Accessed 30 November 2020].

McGee, L., Chochua, S., Li, Z., Mathis, S., Rivers, J., Metcalf, B., Ryan, A., Alden, N., Farley, M. M. & Harrison, L. H. 2021. Multistate, population-based distributions of candidate vaccine targets, clonal complexes, and resistance features of invasive group B *streptococci within the United States, 2015–2017. Clinical Infectious Diseases, 72(6): 1004-1013*.

Mehershahi, K. S., Hsu, L. Y., Koh, T. H. & Chen, S. L. 2015. Complete genome sequence of *Streptococcus agalactiae serotype III, multilocus sequence type 283 strain SG-M1 Genome Announcement, 3(5). 10.1128/genomeA.01188-15*.

Mian, G. F., Godoy, D. T., Leal, C. A., Yuhara, T. Y., Costa, G. M. & Figueiredo, H. C. 2009. Aspects of the natural history and virulence of *S. agalactiae infection in Nile tilapia. Veterinary Microbiology, 136(1-2): 180-3. 10.1016/j.vetmic.2008.10.016*.

Musa, N., Wei, L. S., Musa, N., Hamdan, R. H., Leong, L. K., Wee, W., Amal, M. N., Kutty, B. M. & Abdullah, S. Z. 2009. Streptococcosis in red hybrid tilapia (*Oreochromis niloticus) commercial farms in Malaysia. Aquaculture Research, 40(5): 630-632*.

Mweu, M. 2014. *Streptococcus agalactiae infection in the population of Danish dairy cattle herds: An epidemiological inquiry. PhD, University of Copenhagen. [Accessed 6 March 2021]*.

Nagano, N., Nagano, Y., Toyama, M., Kimura, K., Tamura, T., Shibayama, K. & Arakawa, Y. 2012. Nosocomial spread of multidrug-resistant group B *streptococci with reduced penicillin susceptibility belonging to clonal complex 1. Journal of Antimicrobial Chemotherapy, 67(4): 849-856*.

Nanayakkara, D., Li, C., Luo, M., Yang, Y. & Ip, M. 2018. Isolation and characterization of *Streptococcus agalactiae from freshwater fish procured from wet markets in Hong Kong*. https://www.researchgate.net/profile/Dulmini-Sapugahawatte/publication/331669113_Isolation_and_characterization_of_Streptococcus_agalactiae_from_freshwater_fish_procured_from_wet_markets_in_Hong_Kong/links/5d08937492851cfcc61f77ce/Isolation-and-characterization-of-Streptococcus-agalactiae-from-freshwater-fish-procured-from-wet-markets-in-Hong-Kong.pdf [Accessed 6 March 2021].

Nguyen, H. T. & Kanai, K. 1999. Selective agars for the isolation of *Streptococcus iniae from Japanese flounder, Paralichthys olivaceus, and its cultural environment. Journal of Applied Microbiology, 86(5): 769-776*.

Nguyen, H. T., Kanai, K. & Yoshikoshi, K. 2002. Ecological investigation of *Streptococcus iniae in cultured Japanese flounder (Paralichthys olivaceus) using selective isolation procedures. Aquaculture, 205(1-2): 7-17*.

Niu, G., Khattiya, R., Zhang, T., Boonyayatra, S. & Wongsathein, D. 2020. Phenotypic and genotypic characterization of *Streptococcus* spp. isolated from tilapia (*Oreochromis* spp.) cultured in river-based cage and earthen ponds in Northern Thailand. *Journal of Fish Diseases, 43(3): 391-398*.

Oikeh, E. I., Omoregie, E. S., Oviasogie, F. E. & Oriakhi, K. 2016. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. *Food science & nutrition, 4(1): 103-109*.

Phillips, M., Subasinghe, R., Tran, N., Kassam, L. & Chan, C. 2016. Aquaculture big numbers. FAO Fisheries and Aquaculture Technical Paper; 601. http://www.fao.org/3/i6317e/i6317e.pdf [Accessed 6 March 2021].

Phuoc, N. N., Linh, N. T. H., Crestani, C. & Zadoks, R. N. 2020. Effect of strain and enviromental conditions on the virulence of *Streptococcus agalactiae (group B Streptococcus; GBS) in red tilapia (Oreochromis sp.). Aquaculture, 534: 736256.*

Plumb, J. A., Schachte, J. H., Gaines, J. L., Peltier, W. & Carroll, B. 1974. *Streptococcus sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. Transactions of the American Fisheries Society, 103(2): 358-361.*

Pradeep, P. J., Suebsing, R., Sirthammajak, S., Kampeera, J., Jitrakorn, S., Saksmerprome, V., Turner, W., Palang, I., Vanichviriyakit, R. & Senapin, S. 2016. Evidence of vertical transmission and tissue tropism of streptococcosis from naturally infected red tilapia (*Oreochromis spp.). Aquaculture Reports, 3: 58-66.*

Public Health England 2015. Voluntary surveillance of pyogenic and non-pyogenic streptococcal bacteraemia in England, Wales and Northern Ireland: 2014. 9 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/478808/hpr4115_strptcccs.pdf [Accessed 6 March 2021].

Public Health England 2016. Surveillance of pyogenic and non-pyogenic streptococcal bacteraemia in England, Wales and Northern Ireland: 2015. 10 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/572847/hpr4116_strptccc-crrctd.pdf [Accessed 6 March 2021].

Public Health England 2017. Laboratory surveillance of pyogenic and non-pyogenic streptococcal bacteraemia in England, Wales and Northern Ireland: 2016. 11 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/660589/hpr4117_pnp-strptccc.pdf [Accessed 6 March 2021].

Rahmah, S., Liew, H. J., Napi, N. & Rahmat, S. A. 2020. Metabolic cost of acute and chronic salinity response of hybrid red tilapia *Oreochromis sp. larvae. Aquaculture Reports, 16: 100233.*

Rajendram, P., Mar Kyaw, W., Leo, Y. S., Ho, H., Chen, W. K., Lin, R., Pratim, P., Badaruddin, H., Ang, B., Barkham, T. & Chow, A. 2016. Group B *Streptococcus sequence type 283 disease linked to consumption of raw fish, Singapore. Emerging Infectious Diseases, 22(11): 1974-1977. 10.3201/eid2211.160252.*

Richards, V. P., Velsko, I. M., Alam, M. T., Zadoks, R. N., Manning, S. D., Pavinski Bitar, P. D., Hassler, H. B., Crestani, C., Springer, G. H., Probert, B. M., Town, C. D. & Stanhope, M. J. 2019. Population gene introgression and high genome plasticity for the zoonotic pathogen *Streptococcus agalactiae. Molecular Biology and Evolution, 36(11): 2572-2590. 10.1093/molbev/msz169.*

Rico, A., Oliveira, R., McDonough, S., Matser, A., Khatikarn, J., Satapornvanit, K., Nogueira, A. J., Soares, A. M., Domingues, I. & Van den Brink, P. J. 2014. Use, fate and ecological risks of antibiotics applied in tilapia cage farming in Thailand. *Environmental Pollution, 191: 8-16. 10.1016/j.envpol.2014.04.002.*

Romalde, J. L., Ravelo, C., Valdés, I., Magariños, B., de la Fuente, E., San Martín, C., Avendaño-Herrera, R. & Toranzo, A. E. 2008. *Streptococcus phocae, an emerging pathogen for salmonid culture. Veterinary Microbiology, 130(1-2): 198-207.*

Roos, Y. H. 2003. Water activity | Effect on food stability *Encyclopedia of Food Sciences and Nutrition (Second Edition). Academic Press. [Accessed 2020].*

Ross, T. & Sumner, J. 2002. A simple, spreadsheet-based, food safety risk assessment tool. *International Journal of Food Microbiology, 77(1-2): 39-53.*

Russell, N. J., Seale, A. C., O'Driscoll, M., O'Sullivan, C., Bianchi-Jassir, F., Gonzalez-Guarin, J., Lawn, J. E., Baker, C. J., Bartlett, L. & Cutland, C. 2017. Maternal colonization with group B *Streptococcus and serotype distribution worldwide: Systematic review and meta-analyses. Clinical Infectious Diseases, 65(suppl_2): S100-S111.*

Saenna, P., Hurst, C., Echaubard, P., Wilcox, B. A. & Sripa, B. 2017. Fish sharing as a risk factor for *Opisthorchis viverrini infection: Evidence from two villages in north-eastern Thailand. Infectious Diseases of Poverty, 6(1): 1-9.*

Salloum, M., van der Mee-Marquet, N., Domelier, A. S., Arnault, L. & Quentin, R. 2010. Molecular characterization and prophage DNA contents of *Streptococcus agalactiae strains isolated from adult skin and osteoarticular infections. Journal of Clinical Microbiology, 48(4): 1261-9. 10.1128/JCM.01820-09.*

Santos, J. F., Assis, C. R. D., Soares, K. L. S., Rafael, R. E. Q., Oliveira, V. M., de Vasconcelos Filho, J. E., França, R. C. P., Lemos, D. & Bezerra, R. S. 2019. A comparative study on Nile tilapia under different culture systems: Effect on the growth parameters and proposition of new growth models. *Aquaculture, 503: 128-138.*

Shabayek, S. & Spellerberg, B. 2017. Acid stress response mechanisms of group B *Streptococci. Frontiers in Cellular and Infection Microbiology, 7: 395.*

Shoemaker, C. A., Evans, J. J. & Klesius, P. H. 2000. Density and dose: Factors affecting mortality of *Streptococcus iniae infected tilapia (Oreochromis niloticus). Aquaculture, 188(3-4): 229-235.*

Sigaúque, B., Kobayashi, M., Vubil, D., Nhacolo, A., Chaúque, A., Moaine, B., Massora, S., Mandomando, I., Nhampossa, T. & Bassat, Q. 2018. Invasive bacterial disease trends and characterization of group B streptococcal isolates among young infants in southern Mozambique, 2001–2015. *PloS one, 13(1): e0191193.*

Singapore Department of Statistics 2015. General household survey. https://www.singstat.gov.sg/publications/ghs/ghs2015 [Accessed 6 March 2021].

Singapore Ministry of Health & Singapore Food Agency 2020. Advisory on the increase in the number of invasive group B *Strepcoccus cases. MOH news highlights:* https://www.moh.gov.sg/news-highlights/details/advisory-on-the-increase-in-the-number-of-invasive-group-b-*Streptococcus*-cases [Accessed 6 March 2021].

Sithithaworn, P., Andrews, R. H., Van De, N., Wongsaroj, T., Sinuon, M., Odermatt, P., Nawa, Y., Liang, S., Brindley, P. J. & Sripa, B. 2012a. The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitology International, 61(1): 10-16.*

Sithithaworn, P. & Haswell-Elkins, M. 2003. Epidemiology of *Opisthorchis viverrini. Acta Tropica, 88(3): 187-194.*

Sithithaworn, P., Ziegler, A. D., Grundy-Warr, C., Andrews, R. H. & Petney, T. N. 2012b. Changes to the life cycle of liver flukes: Dams, roads, and ponds. *The Lancet Infectious Diseases, 12(8): 588.*

Siti-Zahrah, A., Padilah, B., Azila, A., Rimatulhana, R. & Shahidan, H. Multiple streptococcal species infection in cage-cultured red tilapia, but showing similar clinical sign. M. G. Bondad- Reantaso, C. V. Mohan, M. Crumlish, & R.P. Subasinghe (Eds.), Disease in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines, 2005 Colombo, Sri Lanka. 332-339. [Accessed 2020].

Six, A., Krajangwong, S., Crumlish, M., Zadoks, R. N. & Walker, D. 2019. Galleria mellonella as an infection model for the multi-host pathogen *Streptococcus agalactiae reflects hypervirulence of strains associated with human invasive disease. Virulence, 10(1): 600-609.* 10.1080/21505594.2019.1631660.

Skoff, T. H., Farley, M. M., Petit, S., Craig, A. S., Schaffner, W., Gershman, K., Harrison, L. H., Lynfield, R., Mohle-Boetani, J., Zansky, S., Albanese, B. A., Stefonek, K., Zell, E. R., Jackson, D., Thompson, T. & Schrag, S. J. 2009. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. *Clinical Infectious Diseases, 49(1): 85-92.* 10.1086/599369.

Soto, E., Zayas, M., Tobar, J., Illanes, O., Yount, S., Francis, S. & Dennis, M. M. 2016. Laboratory-controlled challenges of Nile tilapia (*Oreochromis niloticus) with Streptococcus agalactiae: Comparisons between immersion, oral, intracoelomic and intramuscular routes of infection. Journal of Comparative Pathology, 155(4): 339-345.* https://doi.org/10.1016/j.jcpa.2016.09.003.

Speck, M. L. & Ray, B. 1977. Effects of freezing and storage on microorganisms in frozen foods: A review. *Journal of Food Protection, 40(5): 333-336.*

Sripa, B. & Echaubard, P. 2017. Prospects and challenges towards sustainable liver fluke control. *Trends in Parasitology, 33(10): 799-812.*

Srisapoome, P. & Areechon, N. 2017. Efficacy of viable *Bacillus pumilus isolated from farmed fish on immune responses and increased disease resistance in Nile tilapia (Oreochromis niloticus): Laboratory and on-farm trials. Fish & Shellfish Immunology, 67: 199-210.*

Stickney, R. R. 2013. Polyculture in aquaculture. In Christou, P., Savin, R., Costa-Pierce, B. A., Misztal, I. & Whitelaw, C. B. A., eds. *Sustainable Food Production, pp. 1366-1368. New York, NY, Springer New York. Available:* https://doi.org/10.1007/978-1-4614-5797-8_176 [Accessed 6 March 2021].

Suanyuk, N., Kong, F., Ko, D., Gilbert, G. L. & Supamattaya, K. 2008. Occurrence of rare genotypes of *Streptococcus agalactiae in cultured red tilapia Oreochromis sp. and Nile tilapia O. niloticus in Thailand—Relationship to human isolates? Aquaculture, 284(1): 35-40.* https://doi.org/10.1016/j.aquaculture.2008.07.034.

Suebsong, W., Poompuang, S., Srisapoome, P., Koonawootrittriron, S., Luengnaruemitchai, A., Johansen, H. & Rye, M. 2019. Selection response for *Streptococcus agalactiae resistance in Nile tilapia Oreochromis niloticus. Journal of Fish Diseases, 42(11): 1553-1562.*

Sumner, J. L., Ross, T. & Ababouch, L. 2004. Application of risk assessment in the fish industry. FAO Fisheries Technical Paper 442: http://www.fao.org/3/y4722e/y4722e00.htm [Accessed 6 March 2021].

Sun, J., Fang, W., Ke, B., He, D., Liang, Y., Ning, D., Tan, H., Peng, H., Wang, Y. & Ma, Y. 2016. Inapparent *Streptococcus agalactiae infection in adult/commercial tilapia. Scientific Reports, 6: 26319.*

Suwannahitatorn, P., Webster, J., Riley, S., Mungthin, M. & Donnelly, C. A. 2019. Uncooked fish consumption among those at risk of *Opisthorchis viverrini infection in central Thailand. PLoS ONE, 14(1): e0211540.*

Sym'Previus Team Sym'Previus: Predictive models for food microbiology. https://symprevius.eu/en/ [Accessed 6 March 2021].

Syuhada, R., Zamri-Saad, M., Ina-Salwany, M. Y., Mustafa, M., Nasruddin, N. N., Desa, M. N. M., Nordin, S. A., Barkham, T. & Amal, M. N. A. 2020. Molecular characterization and pathogenicity of *Streptococcus agalactiae serotypes Ia ST7 and III ST283 isolated from cultured red hybrid tilapia in Malaysia. Aquaculture, 515: 734543. 10.1016/j. aquaculture.2019.734543.*

Tan, S., Lin, Y., Foo, K., Koh, H. F., Tow, C., Zhang, Y., Ang, L. W., Cui, L., Badaruddin, H., Ooi, P. L., Lin, R. T. & Cutter, J. 2016. Group B *Streptococcus serotype III sequence type 283 bacteremia associated with consumption of raw fish, Singapore. Emerging Infectious Diseases, 22(11): 1970-1973. 10.3201/eid2211.160210.*

Tavares, G. C., Carvalho, A. F., Pereira, F. L., Rezende, C. P., Azevedo, V. A. C., Leal, C. A. G. & Figueiredo, H. C. P. 2018. Transcriptome and proteome of fish-pathogenic *Streptococcus agalactiae are modulated by temperature. Frontiers in Microbiology, 9: 2639.*

Terpstra, F. G., Rechtman, D. J., Lee, M. L., Hoeij, K. V., Berg, H., Engelenberg, F. A. V. & Wout, A. B. V. T. 2007. Antimicrobial and antiviral effect of high-temperature short-time (HTST) pasteurization applied to human milk. *Breastfeeding Medicine, 2(1): 27-33. 10.1089/bfm.2006.0015.*

Thi Kim Chi, T., Clausen, J. H., Van, P. T., Tersbøl, B. & Dalsgaard, A. 2017. Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. *Aquaculture Reports, 7: 40-47.* https://doi.org/10.1016/j. aqrep.2017.05.003.

Thy, H. T. T., Tri, N. N., Quy, O. M., Fotedar, R., Kannika, K., Unajak, S. & Areechon, N. 2017. Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens 54A, and Bacillus pumilus 47B on growth, innate immunity and stress responses of striped catfish (Pangasianodon hypophthalmus). Fish & Shellfish Immunology, 60: 391-399.*

Tint, K. K., Ngin, K., Sapari, A., Souliphone, K., Suwannapoom, S., Viron, J. G., Thanh, V. T. P. & Chumchuen, S. V. 2020. Fish trade practices: Southeast Asian perspective. *Fish for the People, 18(2): 9-20.*

Tyrrell, G. J., Senzilet, L. D., Spika, J. S., Kertesz, D. A., Alagaratnam, M., Lovgren, M. & Talbot, J. A. 2000. Invasive disease due to group B streptococcal infection in adults: Results from a Canadian, population-based, active laboratory surveillance study -1996. Sentinel Health Unit Surveillance System Site Coordinators. *The Journal of Infectious Diseases, 182(1): 168-173.*

USDA Pathogen modeling program (PMP). Pennsylvania, USA: https://pmp.errc.ars.usda.gov/PMPOnline.aspx#nogo [Accessed 6 March 2021].

van der Mee-Marquet, N., Domelier, A. S., Salloum, M., Violette, J., Arnault, L., Gaillard, N., Bind, J. L., Lartigue, M. F., Quentin, R. & Bloodstream Infection Study Group of the Reseau des Hygienistes de la Region, C. 2009. Molecular characterization of temporally and geographically matched *Streptococcus agalactiae strains isolated from food products and bloodstream infections. Foodborne Pathogens and Disease, 6(10): 1177-83. 10.1089/fpd.2009.0287.*

Vásquez-Machado, G., Barato-Gómez, P. & Iregui-Castro, C.** 2019. Morphological characterization of the adherence and invasion of *Streptococcus agalactiae to the intestinal mucosa of tilapia Oreochromis sp.: An in vitro model. Journal of Fish Diseases, 42(9): 1223-1231.*

Viazis, S., Farkas, B. E. & Jaykus, L. A. 2008. Inactivation of bacterial pathogens in human milk by high-pressure processing. *Journal of Food Protection, 71(1): 109-118.*

Wambua, D. M., Home, P. G., Raude, J. M. & Ondimu, S. 2020. Environmental and energy requirements for different production biomass of Nile tilapia (*Oreochromis niloticus) in recirculating aquaculture systems (RAS) in Kenya. Aquaculture and Fisheries, In Press.*

Wang, Q., Fu, T., Li, X., Luo, Q., Huang, J., Sun, Y. & Wang, X. 2020. Cross-immunity in Nile tilapia vaccinated with *Streptococcus agalactiae and Streptococcus iniae vaccines. Fish & Shellfish Immunology, 97: 382-389. 10.1016/j. fsi.2019.12.021.*

Wang, Y.-C., Feng, C.-C. & Sithithaworn, P. 2013. Environmental determinants of *Opisthorchis viverrini prevalence in northeast Thailand*. Geospatial Health, 8(1): 111-123.

Wendover, N. 2009. Managing tilapia health in commercial systems. *The Fish Site, 19 November 2009. Available:* https://thefishsite.com/articles/managing-tilapia-health-in-commercial-systems [Accessed 16 February 2021].

WHO 2015. World health statistics 2015. https://www.who.int/docs/default-source/gho-documents/world-health-statistic-reports/world-health-statistics-2015.pdf [Accessed 1 March 2021].

WHO. 2016. *International statistical classification of diseases and related health problems 10th revision (ICD-10) [Online]. Geneva, Switzerland Available:* https://icd.who.int/browse10/2016/en#/ [Accessed 15 February 2021].

Wilder-Smith, E., Chow, K. M., Kay, R., Ip, M. & Tee, N. 2000. Group B streptococcal meningitis in adults: Recent increase in Southeast Asia. *Australian and New Zealand Journal of Medicine, 30(4): 462-5.*

Wills, M. E., Han, V. E. M., Harris, D. A. & Baum, J. D. 1982. Short-time low-temperature pasteurisation of human milk. *Early Human Development, 7(1): 71-80.* https://doi.org/10.1016/0378-3782(82)90009-3.

World Bank 2013. Fish to 2030: Prospects for fisheries and aquaculture. *Agriculture and Environmental Services Discussion Paper 03.* http://www.fao.org/3/i3640e/i3640e.pdf [Accessed 6 March 2021].

World Bank 2020. GDP: World Bank national accounts data, and OECD National Accounts data files. https://data.worldbank.org/indicator/NY.GDP.MKTP.CD [Accessed 30 November 2020].

World Bank & Ministry of Planning and Investment of Vietnam 2016. Vietnam 2035: Toward prosperity, creativity, equity, and democracy. Washington, DC: https://openknowledge.worldbank.org/handle/10986/23724 [Accessed 6 March 2021].

Xia, Y., Wang, M., Gao, F., Lu, M. & Chen, G. 2020. Effects of dietary probiotic supplementation on the growth, gut health and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus). Animal Nutrition, 6(1): 69-79.*

Xu, D. H., Shoemaker, C. A. & Klesius, P. H. 2007. Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus (L.). Journal of Fish Diseases, 30(4): 233-238.*

Yang, Y., Luo, M., Zhou, H., Li, C., Luk, A., Zhao, G., Fung, K. & Ip, M. 2019. Role of two-component system response regulator bceR in the antimicrobial resistance, virulence, biofilm formation, and stress response of group B *Streptococcus. Frontiers in Microbiology, 10: 10. 10.3389/fmicb.2019.00010.*

Yang, Y., Yeoh, Y. K., Li, C., Sapugahawatte, D., Rothen, J., Morach, M., Stephan, R., Schmitt, S., Ewers, C., Reyes Vélez, J., Urs, G., Crespo, M., Crumlish, M., Revathi, G., Regli, W., Luo, M., Lin, Z., Zhou, H., Fung, K. & Ip, M. 2020. Comparative genomics and virulence of human and animal group B *Streptococcus (Streptococcus agalactiae).* https://www.researchgate.net/publication/341726516_Comparative_genomics_and_virulence_of_human_and_animal_Group_B_*Streptococcus_Streptococcus*_agalactiae [Accessed 6 March 2021].

Yanong, R. P. E. & Francis-Floyd, R. 2020. Streptococcal infections of fish. *Series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.* https://edis.ifas.ufl.edu/pdffiles/FA/FA05700.pdf [Accessed 6 March 2021].

Yi, Y., Zhang, Z., Zhao, F., Liu, H., Yu, L., Zha, J. & Wang, G. 2018. Probiotic potential of *Bacillus velezensis JW: Antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on Carassius auratus. Fish & Shellfish Immunology, 78: 322-330.*

Zadoks, R. N., Barkham, T., Crestani, C., Nguyen, N. P., Sirmanapong, W. & Chen, S. L. Population growth, climate change and intensification of the aquaculture industry as drivers of invasive disease emergence in humans in Southeast Asia. The 6th World One Health Congress, 30 October - 3 November 2020 Virtual meeting. [Accessed 2020].

Zamri-Saad, M., Amal, M. N. & Siti-Zahrah, A. 2010. Pathological changes in red tilapias (*Oreochromis spp.) naturally infected by Streptococcus agalactiae. Journal of Comparative Pathology, 143(2-3): 227-9. 10.1016/j.jcpa.2010.01.020.*

Zamri-Saad, M., Amal, M. N. A., Siti-Zahrah, A. & Zulkafli, A. R. 2014. Control and prevention of streptococcosis in cultured tilapia in Malaysia: A review. *Pertanika Journal of Tropical Agricultural Science, 37(4).*

Zwe, Y. H., Goh, Z. H. E., Chau, M. L., Aung, K. T. & Yuk, H.-G. 2019. Survival of an emerging foodborne pathogen: Group B *Streptococcus (GBS) serotype III sequence type (ST) 283—under simulated partial cooking and gastric fluid conditions. Food Science and Biotechnology, 28(3): 939-944.*