Title

2015 epidemic of severe *Streptococcus agalactiae* ST283 infections in Singapore associated with the consumption of raw freshwater fish – A detailed analysis of clinical, epidemiological and bacterial sequencing data

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Running title

Outbreak of S. *agalactiae* ST283 infection

Summary

ST283 is a zoonotic *Streptococcus agalactiae* clone capable of colonizing and infecting farmed freshwater fish, causing unusually severe invasive disease in humans. It caused a large foodborne outbreak in Singapore and poses both a regional and potentially international threat.

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Abstract

Background: *Streptococcus agalactiae* (GBS) has not been described as a foodborne pathogen. However, in 2015, a large outbreak of severe invasive sequence type (ST) 283 GBS infections in adults epidemiologically linked to the consumption of raw freshwater fish occurred in Singapore. We attempted to determine the scale of the outbreak, define the clinical spectrum of disease, and link the outbreak to contaminated fish.

Methods: Time-series analysis was performed on microbiology laboratory data. Food-handlers and fishmongers were screened for enteric carriage of GBS. A retrospective cohort study was conducted to assess differences in demographic and clinical characteristics of patients with invasive ST283 and non-ST283 infections. Whole genome sequencing was performed on human and fish ST283 isolates from Singapore, Thailand and Hong Kong.

Results: The outbreak was estimated to have started in late January 2015. Within the study cohort of 408 patients, ST283 accounted for 35·8% of cases. Patients with ST283 infection were younger and had fewer co-morbidities but were more likely to develop meningoencephalitis, septic arthritis and spinal infection. Of 82 food-handlers and fishmongers screened, none carried ST283. Culture of 43 fish samples yielded 13 ST283-positive samples. Phylogenomic analysis of 161 ST283 isolates from humans and fish revealed they formed a tight clade distinguished by 93 SNPs.

Conclusions: ST283 is a zoonotic GBS clone associated with farmed freshwater fish, capable of causing severe disease in humans. It caused a large foodborne outbreak in Singapore and poses both a regional and potentially more widespread threat.

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Introduction

Streptococcus agalactiae, also known as Group B Streptococcus (GBS), is a β-hemolytic streptococcus that colonizes the digestive and urinary tracts of approximately one-third of the human population (1). It is also a principal cause of bovine mastitis and invasive disease in fish (2, 3). GBS is a well-known cause of severe infections in neonates, the elderly and the immunocompromised (1, 4, 5). The prevalence of infections has increased over the past two decades in adults with co-morbidities (1, 5, 6), commonly manifesting as occult bacteremia, skin and soft tissue infections (SSTIs) and urinary tract infections (UTIs), whereas severe clinical syndromes such as meningoencephalitis are rare (1, 5-12).

Most human strains belong to a few tetracycline-resistant clones that have disseminated worldwide and evolved separately from bovine clones (2). Capsular serotype III GBS has been associated with severe disease in non-pregnant adults in Hong Kong and Singapore (13-15). In Hong Kong, multilocus sequence type (ST) 283 accounted for 27·4% of 73 serotype III GBS causing invasive infections in adults between 1993 and 2003 (15). Five adult meningitis cases from Singapore in 1998 were caused by ST11, a single-locus variant of ST283 (13, 16). The only other two human cases of ST283 GBS infections reported in the literature were from a study conducted in France between 2002 and 2007 (17). The source of human ST283 GBS has never been ascertained. It is not known to colonize the gastrointestinal or urinary tract in humans, but has previously been recovered from farmed freshwater fish in Southeast Asia (2).

In 2015, a large outbreak of severe invasive ST283 GBS infections in non-pregnant adults occurred in Singapore (18). In a retrospective study involving 40 case-patients and 58 controls conducted by public health authorities as part of the nationwide outbreak investigation, consumption of Asian bighead carp (*Hypophthalmichthys nobilis*) and Snakehead fish (*Channa*

spp.) in the Chinese-style raw fish dish "yusheng" was strongly associated with ST283 GBS bacteremia (adjusted OR: 25·92) (18). The outbreak rapidly abated after local health authorities advised retailers to stop the sale of raw fish dishes using the two implicated species.

In this study, we attempted to determine the scale of the GBS outbreak in Singapore, define the clinical spectrum of disease associated with ST283 GBS, and further explore the link between human cases and fish consumption using whole genome sequencing (WGS). We also obtained historical and recent ST283 GBS isolates from Hong Kong where human infections continue to occur sporadically (15), and ST283 GBS isolates from Thailand where clinicians had reported severe infections occurring in adults (19-21). These were sequenced with local human and fish isolates in order to understand the regional molecular epidemiology and ascertain the public health risk posed by this particular GBS clone.

Methods

The various investigations are briefly described below, with more detailed explanations provided in the Materials and Methods section in the Supplementary Appendix.

Time-series analysis

We carried out change point analyses on weekly time-series microbiology laboratory non-duplicate GBS data recorded between 1st January 2011 and 29st August 2015 to estimate the onset and scale of the outbreak. Data were contributed by five public sector hospitals, collectively accounting for approximately 70% of acute care hospital beds in Singapore. The cases were split into "bacteremia" (i.e. from blood cultures) and "non-bacteremia" categories. (Supplementary Appendix).

Fishmonger and food handler screening

Food handlers and fishmongers from retail establishments epidemiologically linked to the outbreak underwent stool screening in order to assess if colonised food handlers/fishmongers could be the source of infection. GBS isolation from stool was performed using CHROMagar StrepB (Chromagar, Becton, Dickinson, Sparks, USA). All isolated GBS were sent to the National Public Health Laboratory (NPHL) for capsular serotyping (22-24). Isolates belonging to serotype III underwent multilocus sequence typing (MLST) (16).

Raw fish sampling

Raw freshwater fish samples were collected from fish ports, wet markets, supermarkets and eating establishments in Singapore between August and December 2015, and cultured for GBS, following a modified published protocol (25) (Supplementary Appendix). Capsular serotyping and MLST were performed on recovered GBS isolates to determine if ST283 GBS was present in fish. Four isolates from "yusheng" (raw-fish dish) samples obtained from a foodstall epidemiologically linked to the outbreak were subsequently sequenced.

Retrospective cohort study

In order to define the clinical and epidemiological spectrum of ST283 GBS infections, we performed a retrospective cohort study of adult patients with invasive GBS infection from 1st January 2011 to 31st August 2015 at Singapore General Hospital (SGH), Changi General Hospital (CGH) and Tan Tock Seng Hospital (TTSH). Invasive disease was defined by isolation of GBS from blood and/or other sterile sites. Patients whose GBS isolates were not archived

were excluded. We reviewed the patients' clinical charts, collecting clinical, demographic and epidemiological data via a standardized template (Supplementary Appendix).

Isolates were confirmed as GBS using MALDI-TOF mass spectrometry (Bruker), with capsular serotype determined by PCR (22-24). ST283 status was initially defined by either MLST or a proprietary ST283-specific PCR test based on unique sequences developed after whole-genome sequencing (WGS) of an earlier outbreak isolate (Alexander Lezhava, personal communication) (26).

To assess differences in demographic and clinical characteristics between patients infected with ST283 as opposed to other GBS clones, and between patients who died or survived, the permutation test was used for continuous variables, while Fisher's exact test or Chi-square test was used for categorical variables where appropriate. A multivariate logistic regression model was also fitted to identify potential predictors of mortality. Variable selection for the final multivariate logistic regression model was carried out as previously described (27). All analyses were performed in the R statistical environment, version 3.2.0.

GBS isolates from Thailand and Hong Kong

Four and nine ST283 GBS isolates from Thailand and Hong Kong respectively were investigated. The Thai isolates were obtained from a subset of patients diagnosed with severe invasive GBS infections in 2015 whereas the Hong Kong isolates were collected from patients with invasive GBS infection between 1998 and 2008.

Whole Genome Sequencing

ST283 isolates were sequenced using PacBio SMRT or Illumina sequencers (Supplementary Appendix) to determine the genomic epidemiology of fish and human isolates. Publicly available genome sequences (28-32) were downloaded from GenBank RefSeq or the Genbank Short Read Archive (SRA) for comparison. Both reference-based and assembly-based analyses were done (Supplementary Appendix).

Ethics

The Singhealth Centralised Institute Review Board approved the study (approval ID: 2015/2642).

Results

Onset of Singapore outbreak

We estimated that the outbreak began in the week of 25 January 2015 (95% CI, week of 4 January – week of 22 February 2015). Prior to the outbreak, there were on average 2·8 (95% CI, 2·6-3·1) bacteremias per week, which increased to 36·3 (95% CI, 29·6-44·1) per week at the peak of the outbreak during the week of 5 July 2015 (Figure 1A). Non-bacteremic GBS infections did not increase throughout this period (Figure 1B). The full distribution of weekly cases between January 2011 and August 2015 is shown in Figure S1, Supplementary Appendix.

Epidemiologic investigations

Four food handlers (n = 68) and four fishmongers (n =14) were colonized within the gastrointestinal tract by non-ST283 GBS.

Of 43 freshwater fish sampled, ST283 GBS isolates were recovered from two samples of "yusheng" – prepared using Asian bighead carp and fish sold as grass carp – collected from an implicated retail stall, one Snakehead fish from a wet market stall which supplied fish to hawker stalls and 10 fish samples from other food establishments not directly related to the outbreak.

Clinical characteristics of ST283 GBS infection

There were 146 cases (35·8%) of invasive ST283 GBS infections among 408 cases in the retrospective cohort. No patients from SGH were included prior to 2015 as GBS isolates from this hospital were only archived from 2015 onwards, but there were 61 ST283-infected patients in SGH in the first eight months of 2015. In the other two hospitals, the number of ST283-infected patients increased dramatically from three in 2012 to 59 in the first eight months of 2015, with 9 and 14 cases in the intervening years. The proportion of ST283-infected patients among all patients with invasive GBS infection also increased over time – 7·9% in 2012, 15·5% in 2013, 18·9% in 2014 and 55·1% in 2015.

The demographic and clinical characteristics of the cohort are shown in Table 1. Compared to patients with non-ST283 GBS, ST283-infected patients were more likely to be younger and of Chinese ethnicity. They also had fewer overall co-morbidities resulting in lower Charlson co-morbidity scores (33).

ST283 GBS infections were more commonly associated with meningoencephalitis, native joint septic arthritis and spinal infection, whereas patients with invasive non-ST283 infections were more likely to have SSTIs and/or UTIs. Within the clinical cohort there were five fatalities (3·4%) due to ST283 infection – three patients had a primary bacteremia with no clear source and the remaining two had meningoencephalitis. Risk factors associated with inpatient mortality

included older age, higher Charlson co-morbidity scores, intensive care unit (ICU) admission, inotropic support, mechanical ventilation, elevated serum urea and creatinine, and high Pitt bacteremia score (34) (Table S2, Supplementary Appendix). On multivariate analysis, higher Charlson co-morbidity scores, inotropic support, mechanical ventilation, elevated serum urea, and high Pitt bacteremia scores were associated with increased mortality (Table 2). When infection with ST283 GBS was forced into the multivariate model as a variable, it was neither protective nor harmful (adjusted OR: 1·10, 95% CI: 0·26-4·23).

Genomic analysis of ST283 GBS

There were 161 ST283 isolates sequenced, including 144 isolates from the clinical cohort, four isolates from Thailand, nine isolates from Hong Kong, and four "yusheng" isolates (Table S3, Supplementary Appendix). With the inclusion of sequencing data from publicly available GBS strains (28-32), phylogenetic analysis demonstrated that ST283 formed a distinct clade within the CC10 clonal complex (Figure S2, Supplementary Appendix).

Genomic analysis of the ST283 isolates showed strains differed by a maximum of 93 single nucleotide polymorphisms (SNPs) and 6 small insertions/deletions (indels). Two separate analyses of ST283 strains showed little evidence of recombination (0·8-1·9% of the entire genome), as similarly noted for several other pathogenic clones of GBS (28, 35). The ST283 genomes were separated from non-ST283 genomes by at least 2,000 SNPs (Figure S2, Supplementary Appendix).

All Singaporean human isolates from 2015 differed by a maximum of 14 SNPs (Figure 2), except for one isolate (SG-M261) which clustered with isolates from Thailand - the patient did not have a history of recent travel to Thailand. The Singaporean isolates from 2012-2014 were

closely related to the 2015 Singaporean outbreak isolates, and in the phylogenetic reconstruction they formed a basal cluster to the later isolates. Notably the four fish isolates (SGM-487, SGEHI2015-NWC941-2, SGEHI2015-NWC941-3 and SGEHI2015-NWC941-4) recovered from two "yusheng" samples were found within the main cluster of isolates from 2015. The Hong Kong and Thai isolates were more diverse and represent a broader sampling of the diversity of ST283, being from a different time period. Bayesian phylogenetic analysis of the ST283 strains with a subset of CC10 strains predicted a clock rate of 2·7 SNPs/Mbp/year (95% range 1·8-3·5).

Discussion

GBS has previously been implicated only in small nosocomial outbreaks, particularly among neonates (36-38). This GBS outbreak was unique on several fronts. The outbreak occurred in the community and is by far the largest ever reported. Foodborne transmission of GBS from fish to humans had previously been hypothesized, but never conclusively proven (3). While a previously published case-control study demonstrated a strong epidemiologic association between the invasive GBS outbreak and the consumption of "yusheng" in Singapore (18), genomic analysis of fish and human isolates in this study provides direct evidence of the link. These two studies, combined with the rapid outbreak resolution and return to baseline rates after local retailers had stopped the sale of "yusheng", make a convincing and virtually causal link between the consumption of raw freshwater fish and ST283 GBS infection. The phylogenetic relationship revealed by the genomic analysis of the Singapore outbreak with the Thai and Hong Kong isolates, coupled with reports of severe human infections in Thailand (21) and the presence of ST283 GBS in farmed freshwater fish in Southeast Asia (2), point to a regional infectious disease threat with the potential for further international spread given the global importance of

freshwater aquaculture and complexity of the global food trade network (39). In the case of the 2015 outbreak, the genomic evidence suggests a point source, however the analysis of isolates from Singapore in preceding years, indicate that closely related isolates were causing disease, albeit at far lower incidence. This suggests that there was an established reservoir of ST283, likely associated with the production and processing of fish, which prior to the outbreak, had been the source of the sporadic cases for a number of years.

Although the outbreak started in early 2015, ST283 GBS has caused human disease in Singapore since at least 2012. The acute trigger that resulted in the large outbreak in 2015 is unknown. The local tradition of eating "Yusheng" is believed to have been brought to Singapore by migrants from China, and has been practiced in Singapore for decades. Saltwater wolf herring was originally used but this was gradually switched to the Asian bighead carp and Snakehead fish. Hong Kong banned the consumption of raw freshwater fish almost forty years ago due to risk of infection by the liver fluke *Chlonorchis sinesis* (40), possibly explaining the sporadicity of ST283 infection observed there (15).

The source of the GBS in the freshwater fish remains unknown. ST283 GBS and its single locus-variant ST491 were first described as fish pathogens in farmed tilapia in Thailand and Vietnam respectively (2), and therefore it is possible that this strain of GBS may be introduced to freshwater fish during their farming.. An alternative possibility is that the fish were contaminated with ST283 GBS at some point during the logistic chain of import and processing. The results from food-handlers and fishmongers suggest that possible contamination of fish may lie further up the supply chain. However, to our knowledge, upstream investigations conducted by public health authorities at overseas fish farms and processing plants could not conclusively identify the point of contamination.

Our study further contributes evidence towards the understanding of ST283 as a uniquely virulent GBS clone (15). The lack of increase in non-blood clinical isolates during the period of the outbreak (Figure 1B) suggests that ST283 GBS primarily causes severe invasive disease in humans. The spectrum of severe clinical disease is also noteworthy, with significantly higher rates of meningoencephalitis, septic arthritis and spinal infection notwithstanding that those infected were younger and healthier than patients with non-ST283 GBS infections. Among the patients with ST283 infection in the clinical cohort, there were five fatalities that occurred during the outbreak period. The overall inpatient mortality was higher in patients with non-ST283 GBS (9·5 % vs. 3·4%, p=0.038), likely explained by a differing patient profile between the groups and supported by results of the multivariate analysis of predictors of mortality (Table 2). The differing epidemiological profile, where >95% of those infected with ST283 GBS were of Chinese ethnicity, may well be due to an ethnic difference in consumption of "yusheng".

Genomic analysis suggests that ST283 is highly clonal, with low levels of recombination and indels, similar to many other pathogenic clones of GBS (28, 35). The clonality of the outbreak ST283 strain may indicate a point source or very recent emergence and expansion within the context of existing ST283 strains in Southeast Asia. Like other pathogenic clones of GBS, ST283 appears to be evolving mostly via SNP mutations and at a similar rate with respect to time (28). Comparison of the SG-M1 genome (26) with other closely related strains showed multiple regions of difference that could be responsible for higher virulence of the ST283 strains, including several integrated prophages, surface proteins, and a previously identified region that may have been horizontally transferred from *Streptococcus anginosus* (2).

Our study had several limitations. We were not able to collect detailed information of food exposures from the clinical cohort as this was a retrospective study. We were also not able to

ascertain the exact source of the ST283 GBS causing the Singapore outbreak. Although genomic differences between ST283 and non-ST283 GBS were identified, these will need to be further explored in pathogenicity studies to determine the exact genes responsible for the differences in clinical presentations between different GBS clones.

In summary, ST283 is a zoonotic Asian GBS clone capable of colonizing and infecting various farmed freshwater fish, causing unusually severe invasive disease in humans. It caused a large foodborne outbreak in Singapore in 2015 and poses a regional as well as potential international threat given the complexity of the global food trade network. It can be added to the list of infectious disease reasons for avoiding the consumption of raw freshwater fish.

Contributors

SK, TB, SLC, THK and LYH were involved in the conception and design of the study. TB, WYT, MLC and LCN performed the serotyping of isolates. SLC, KSM and CWW performed the whole genome sequencing. SK, MK, THK, TYT, MLC, LCN, HB, AA, NS, MI, JT and TB were involved in acquisition of the data and SK, SLC, MH, LYH, and TB were involved in the analysis and interpretation of the data. CTL performed the statistical analysis.

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Conflict of Interests

LYH is the director of the Singapore Infectious Diseases Initiative but had no role in reviewing or awarding the project application. SLC and TB have minority interests in a technology disclosure for methods to detect ST283 GBS. Other authors have no conflicts of interest to declare.

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Tables

Table 1. Demographic, clinical and microbiological characteristics of study population

Variable	ST283 (n=146)	Non-ST283 GBS (n=262)	p-value
Demographics			
Gender			0·84
Male	83 (56·8)	153 (58·4)	
Ethnicity			<0·001
Chinese	139 (95·2)	177 (67·6)	
Non-Chinese	7 (4·8)	85 (32·4)	
Median age, years (IQR)	61·0 (50·0, 68·0)	69.5 (57·3, 78·0)	<0·001
Consumption of raw fish [a]			<0·001
Yes	26 (17·8)	0 (0·0)	
No	6 (4·1)	29 (11·1)	
Unknown	114 (78·1)	233 (88·9)	
Travel history [a]			<0·001
Yes	15 (10·3)	1 (0·4)	
No	71 (48·6)	153 (58·4)	
Unknown	60 (41·1)	108 (41·2)	
Comorbidities			
Diabetes Mellitus	29 (19·9)	156 (59·5)	<0·001
Hypertension	61 (41·8)	172 (65·6)	<0·001
Hyperlipidemia	59 (40·4)	143 (54·6)	0·008
Ischemic heart disease	13 (8·9)	93 (35·5)	<0·001
Valvular heart disease	6 (4·1)	18 (6·9)	0·359

Chronic lung disease [b]	2 (1·4)	19 (7·3)	0·019
Chronic kidney disease [c]	11 (7·5)	65 (24·8)	<0·001
Chronic liver disease [d]	1 (0·7)	19 (7·3)	0·003
HIV	0 (0·0)	1 (0·4)	1·000
Solid-organ tumor	9 (6·2)	49 (18·7)	<0·001
Leukemia/Lymphoma	0 (0·0)	5 (1·9)	0·165
Transplant recipient [e]	1 (0·7)	0 (0·0)	0·358
Charlson Co-morbidity Score			<0·001
0	32 (21·9)	5 (1·9)	
1	28 (19·2)	13 (5·0)	
2	26 (17·8)	21 (8·0)	
≥3	60 (41·1)	223 (85·1)	
Receipt of chemotherapy [f]	0 (0·0)	2 (0·8)	0·593
Systemic steroid use [f]	1 (0·7)	4 (1·5)	0·659
Surgery [f]	3 (21)	1 (0·4)	0·133
OGD [f]	1 (0·7)	1(0·4)	1·000
Colonoscopy [f]	0 (0·0)	1 (0·4)	1·000
Cystoscopy [f]	0 (0·0)	1 (0·4)	1·000
Admission characteristics			
Symptom duration of less than one week prior to admission	126 (86·3)	195 (74·4)	0·007
Required ICU admission	20 (13·7)	31 (11·8)	0·696
Inotropic support	17 (11·6)	39 (14·9)	0·446
Ventilatory support	10 (6·8)	24 (9·2)	0·533
Urgent renal replacement therapy	4 (2.7)	3 (1·1)	0·255
Median Pitt Bacteremia Score (IQR)	1 (0, 2)	1 (0, 2)	0·947

Laboratory parameters at presentation			
Median WBC count (x10^9/L) (IQR)	13·40 (9·84, 17·98)	14·25 (9·83, 18·58)	0·198
Median platelet count (x10^9/L) (IQR)	177.0 (134·8, 241·0)	224.0 (153·0, 336·2)	<0·001
Median creatinine (µmol/L) (IQR)	92·0 (64·0, 122·8)	117·0 (85·0, 204·0)	<0·001
Median urea (mmol/L) (IQR)	5·30 (3·53, 8·30)	8·45 (5·00, 14·85)	<0·001
Median CRP (mg/L) (IQR)	211·0 (113·0, 318·8)	124·0 (22·05, 213·60)	<0·001
Median procalcitonin (µg/L) (IQR)	6·00 (2·00, 27·34)	3·70 (0·93, 14·27)	0·096
GBS serotype			N.A
III	146 (100)	44 (16·9)	
Ia	-	38 (14·6)	
Ib	-	17 (6·5)	
II	-	35 (13·5)	
IV	-	1 (0·4)	
V	-	55 (21·2)	
VI	-	58 (22·3)	
VII	-	12 (4·6)	
Clinical Syndrome			
Bacteremia without specific focus	33 (22·3)	61 (23·5)	0·81
Meningitis/ Meningoencephalitis	29 (19·9)	0 (0·0)	<0·001

Endocarditis (native valve)	15 (10·3)	13 (5·0)	0·064
Endovascular infection [g]	1 (0·7)	2 (0·8)	1·000
Endophthalmitis	6 (4·1)	3 (1·1)	0·075
Pneumonia	1 (0·7)	3 (1·1)	1.000
Skin and soft tissue infection	27 (18·5)	110 (42·0)	<0·001
Native joint septic arthritis	44 (30·1)	13 (5·0)	<0.001
Prosthetic joint septic arthritis	1 (0·7)	1 (0·4)	1·000
Spinal infection [h]	12 (8·2)	5 (1·9)	0·005
Lower urinary tract infection	5 (3·4)	31 (11·8)	0·007
Upper urinary tract infection [i]	8 (5·5)	12 (4·6)	0·870
Hepatobiliary infection	1 (0·7)	2 (0·8)	1·000
Inpatient mortality	5 (3·4)	25 (9·5)	0·038

NOTE. Data are number (%) of cases, unless otherwise stated.

Abbreviations: IQR -interquartile range; HIV - human immunodeficiency virus infection; OGD - oesophagogastroduodenoscopy; ICU - intensive care unit; WBC - white blood cell; CRP - C-reactive protein; GBS-Group B Streptococcus; N.A – not applicable

[a] *p*-value calculated following exclusion of unknown cases

[b] Includes, but is not limited to chronic obstructive pulmonary disease, interstitial lung disease and bronchiectasis

[c] Includes, but is not limited to dialysis and chronic renal insufficiency

[d] Includes, but is not limited to cirrhosis and chronic hepatitis

[e] Includes, but is not limited to renal transplant, hematopoietic stem cell transplant and liver transplant

[f] Within one month prior to hospital presentation

[g] Includes aortitis and aortic mycotic aneurysm

[h] Includes spondylodiscitis, vertebral osteomyelitis, epidural and para-spinal abscess

[i] Includes pyelonephritis and renal abscess

Table 2. Predictors of mortality by multivariate logistic regression analysis

Characteristic	Crude OR (95% CI)	Adjusted OR (95%CI)
Charlson Comorbidity Score	1·29 (1·13, 1·46)	1·35 (1·16, 1·59)
Inotropic support	13·42 (6·08, 30·70)	3·60 (1·09, 11·51)
Ventilatory support	13·00 (5·54, 30·44)	4·54 (1·25, 15·99)
Elevated serum creatinine	1·002 (1·000, 1·004)	0·996 (0·991, 1·000)
Elevated serum urea	1·08 (1·04, 1·12)	1·07 (1·03, 1·13)
Pitt Bacteremia Score	1·49 (1·29, 1·76)	1·24 (1·01, 1·54)

Figure Legends

Figure 1. Weekly counts of non-duplicate *Streptococcus agalactiae* infections based on laboratory records of five public sector hospitals in Singapore, January 2014 to August 2015. (A) Blood culture results. (B) All non-blood culture results.

Figure 2. Phylogenetic tree based on core genome SNPs of all *Streptococcus agalactiae* ST283 human isolates from the Singapore clinical cohort, Thailand, and Hong Kong and *Streptococcus agalactiae* ST283 fish isolates from fish samples from a retail stall epidemiologically linked to the outbreak. All ST283 strains listed in Supplementary Table S3 were used to create a maximum likelihood tree, using A909 as an outgroup (not shown for clarity). Coloured boxes to the right of each strain name indicate the year, country, and host species for the isolate. The four fish isolates are SG-M487, SGEHI2015-NWC941-2, SGEHI2015-NWC941-3 and SGEHI2015-NWC941-4, which were isolated from Asian bighead carp and fish sold as grass carp obtained from an eating establishment epidemiologically linked to the outbreak. The scale bar at the bottom indicates the fraction of SNP differences among all core genome polymorphic positions (4020 positions in total).

Figure 1 (.tif and .eps files only)

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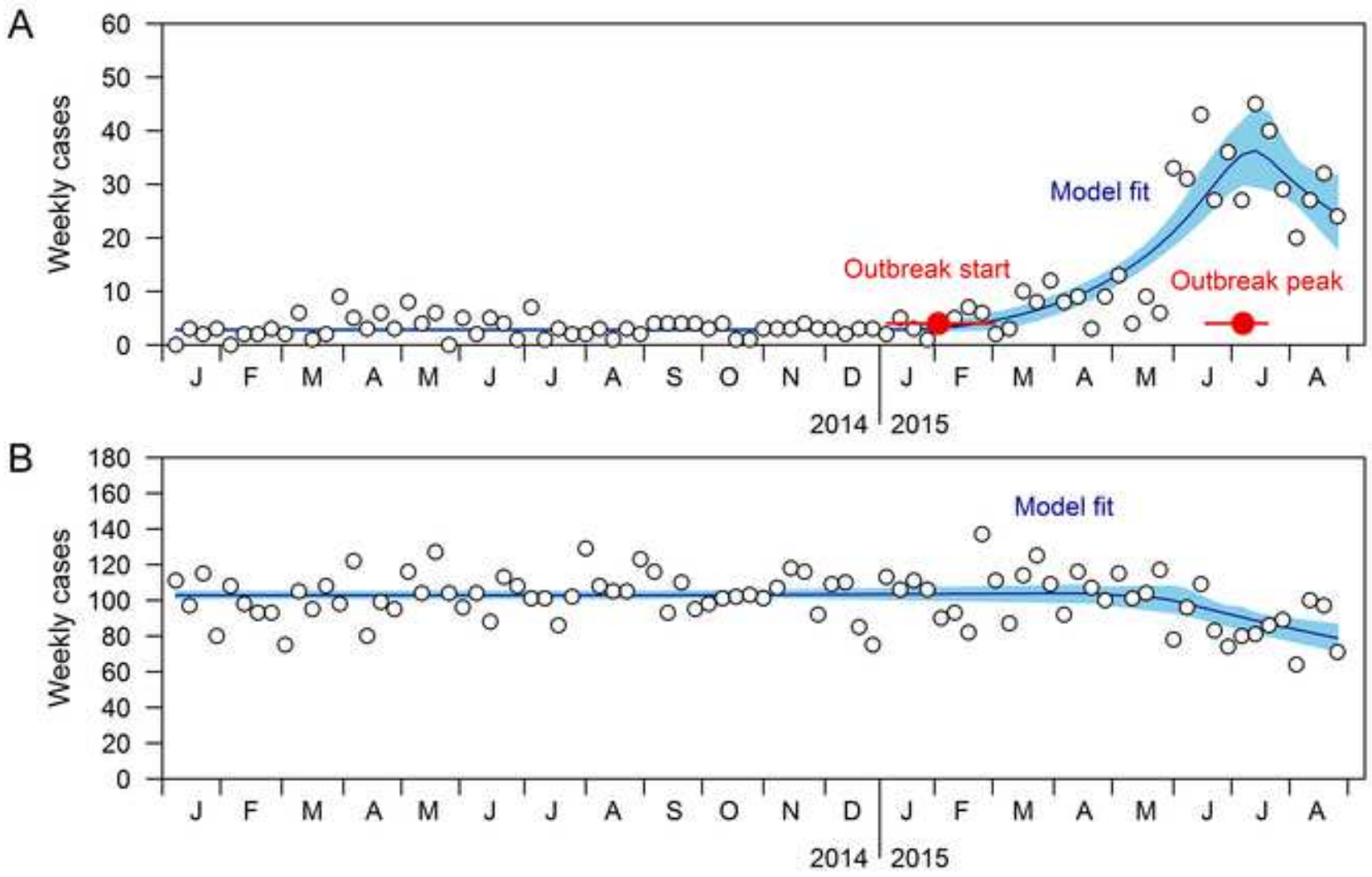

Figure 2 (.tif and .eps files only)

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