

Streptococcus agalactiae ST283
causing invasive sepsis in healthy
adults is widespread in SE Asia

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Streptococcus agalactiae

- A bacterium!
 - Mastitis in cows
 - Group B streptococcus, GBS
- Is well known
 - **Neonatal** sepsis
 - 1960s
 - Sepsis in **older adults** with **co-morbidities**
 - 1990s

Different concept

- Invasive
- Healthy
- Younger adults
- Community

Singapore outbreak, 2015

Associated – raw fish

GBS, ST283

- Today

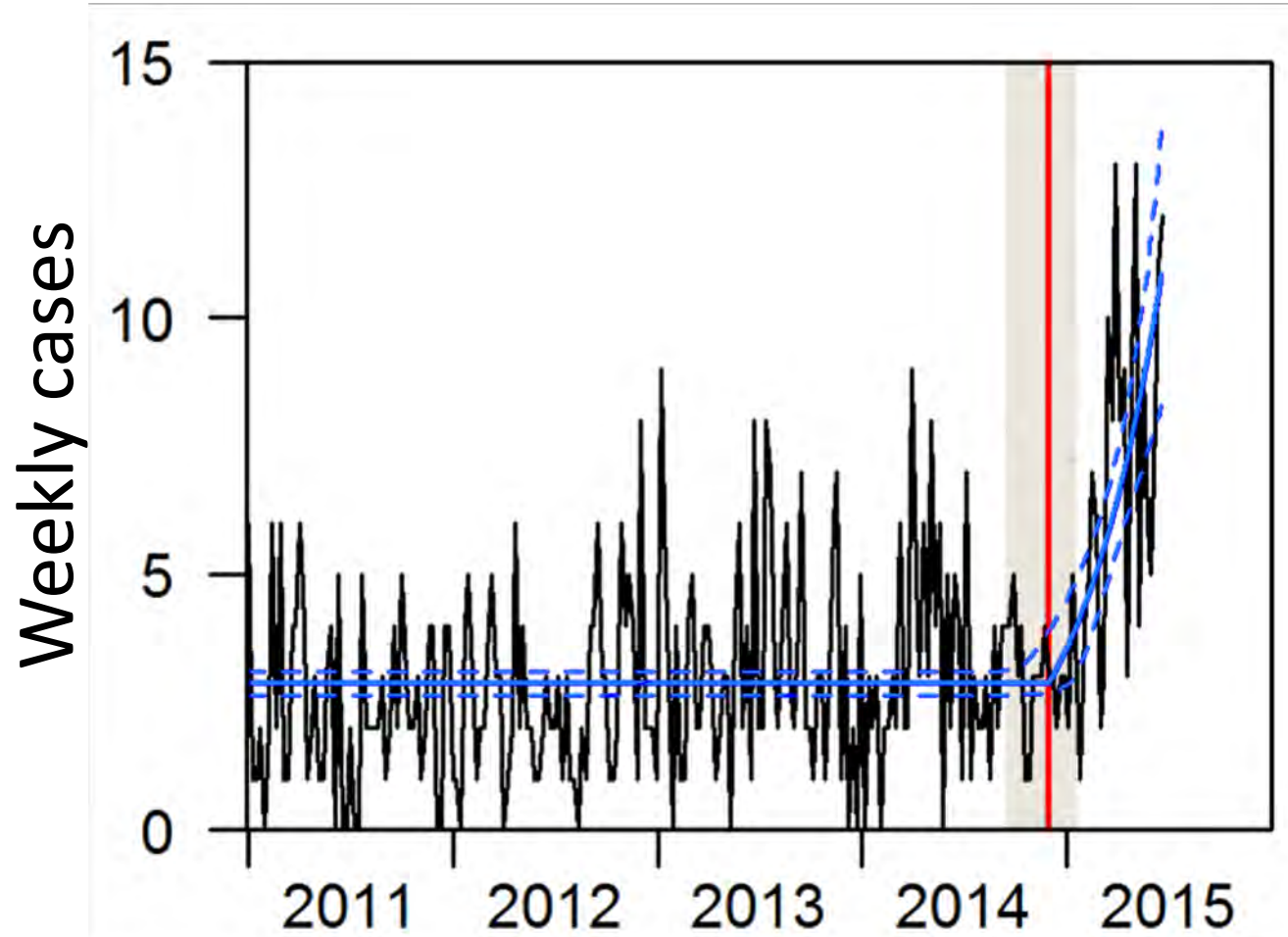
Widespread in SE Asia, for decades

The Singapore GBS outbreak

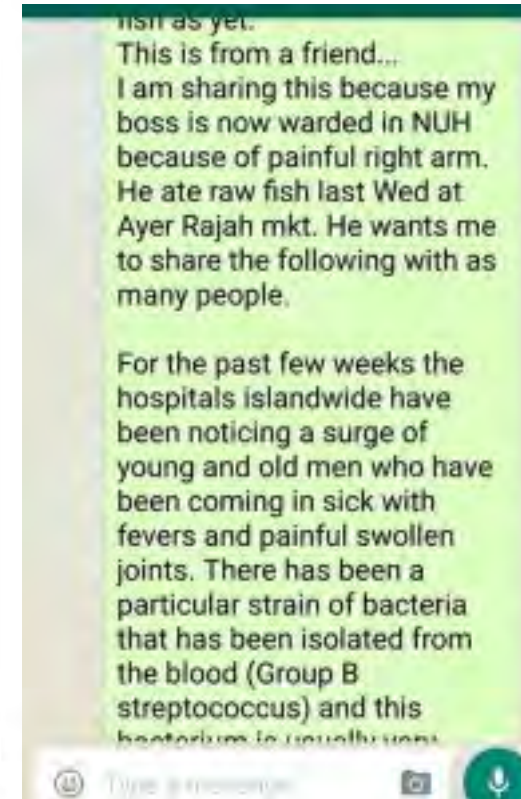
- 2015
- Surge of invasive GBS cases
 - ST283
- Had to be foodborne

GBS blood isolates

Clinical Infectious Diseases 2017;64(S2):S145–52



Social media



Case control studies

Bacteraemia	ST283	Non-ST283	p
Yusheng consumption	(40)	(58)	<0.001 aOR 11.38 (2.76–46.98)
Yes	18	8	
No	22	50	

EID 2016, 22; 11: 1970



- Official advisory
– Avoid Yusheng

Singapore GBS outbreak curve

Tan S et al. EID 2016, 22; 11: 1970

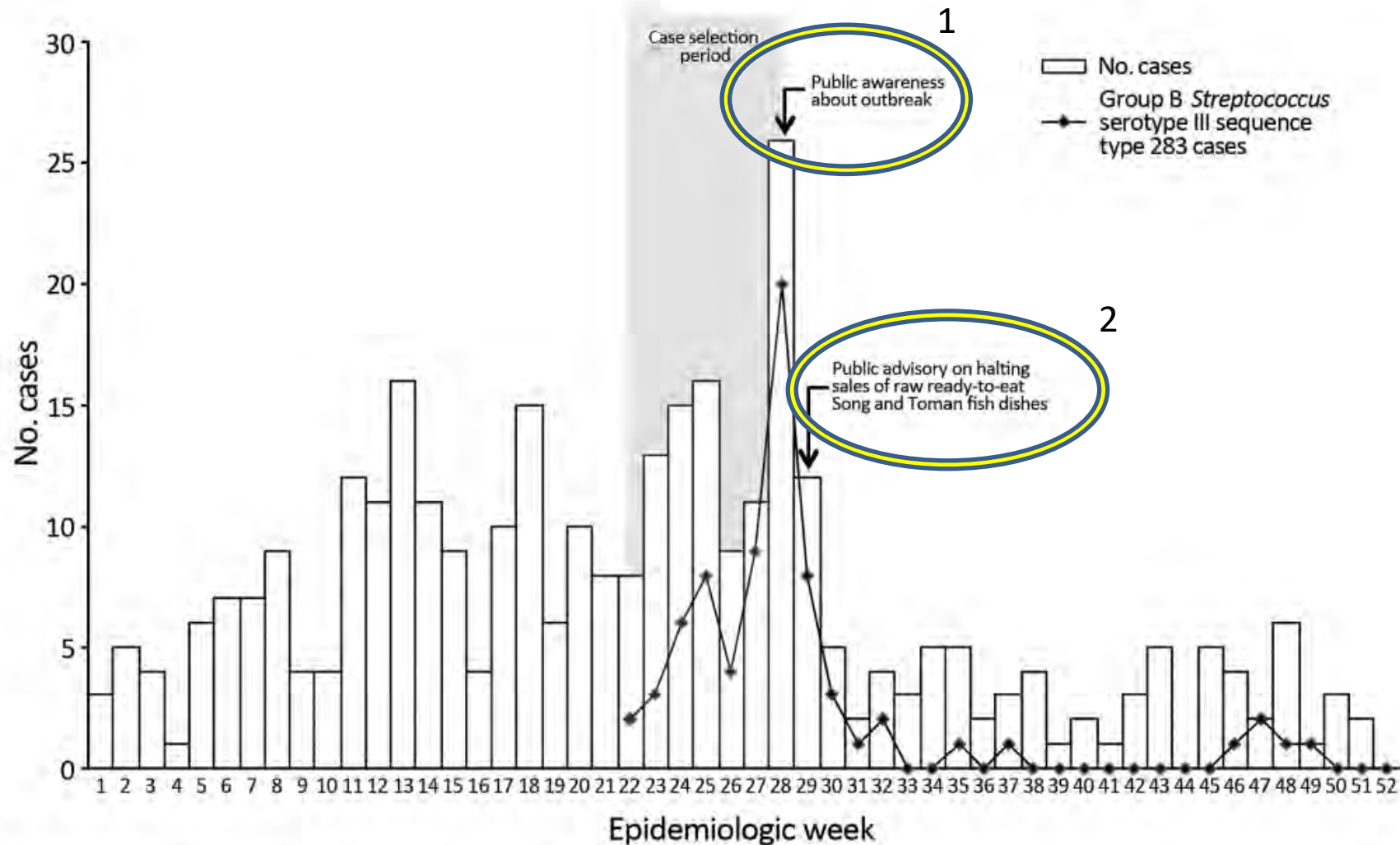


Figure. Timeline of group B *Streptococcus* bacteremia cases reported in 6 public hospitals, Singapore, epidemiologic weeks 1–52, 2015

2015 Review of ST283; cited x 3

- Humans
 - Hong Kong
 - 20 cases of invasive disease
 - non-pregnant adults
 - France
 - Two cases, osteoarticular infection
- Fish
 - One from Thailand
 - One from Vietnam

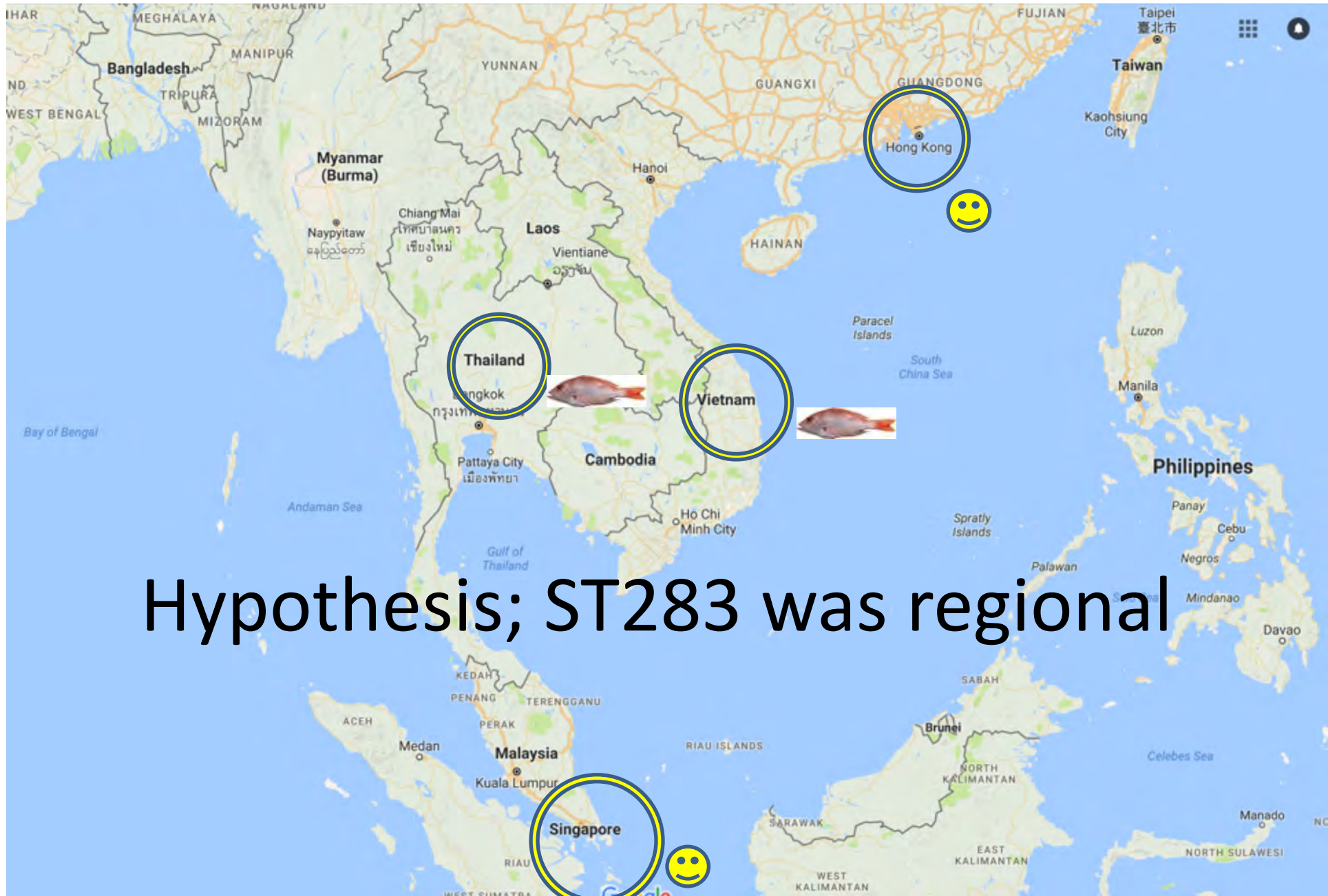
Literature on 'absence'

- MLST data
 - Africa, China, Europe, N. America, S. America
 - 4,104 human GBS
 - 1,254 animal GBS
- SE Asia under-represented
 - Only 10 GBS isolates

So the SEA data is unreliable!

GBS meningitis

- 11 adults
 - 1998
 - Singapore & Hong Kong
 - *No co-morbidities*
- They were ST283
 - Aust NZ J Med 2000
 - JCM 2003
 - CMI 2018

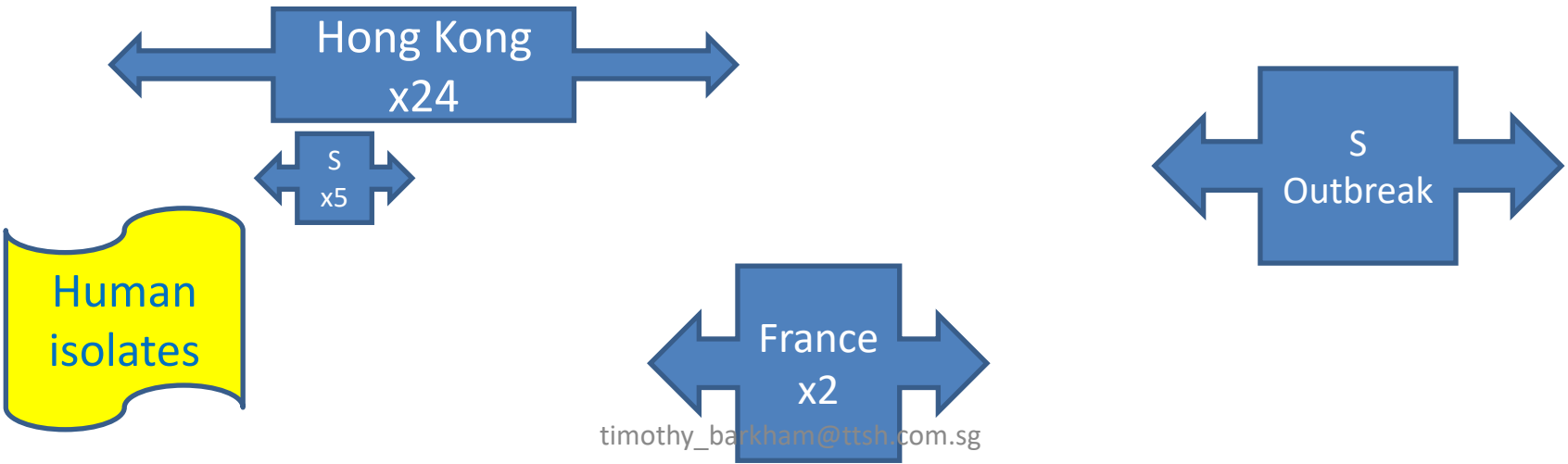
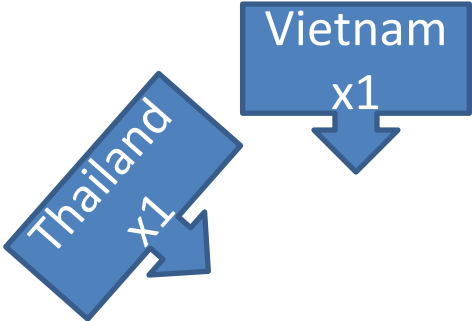


Hypothesis; ST283 was regional

ST283 timeline

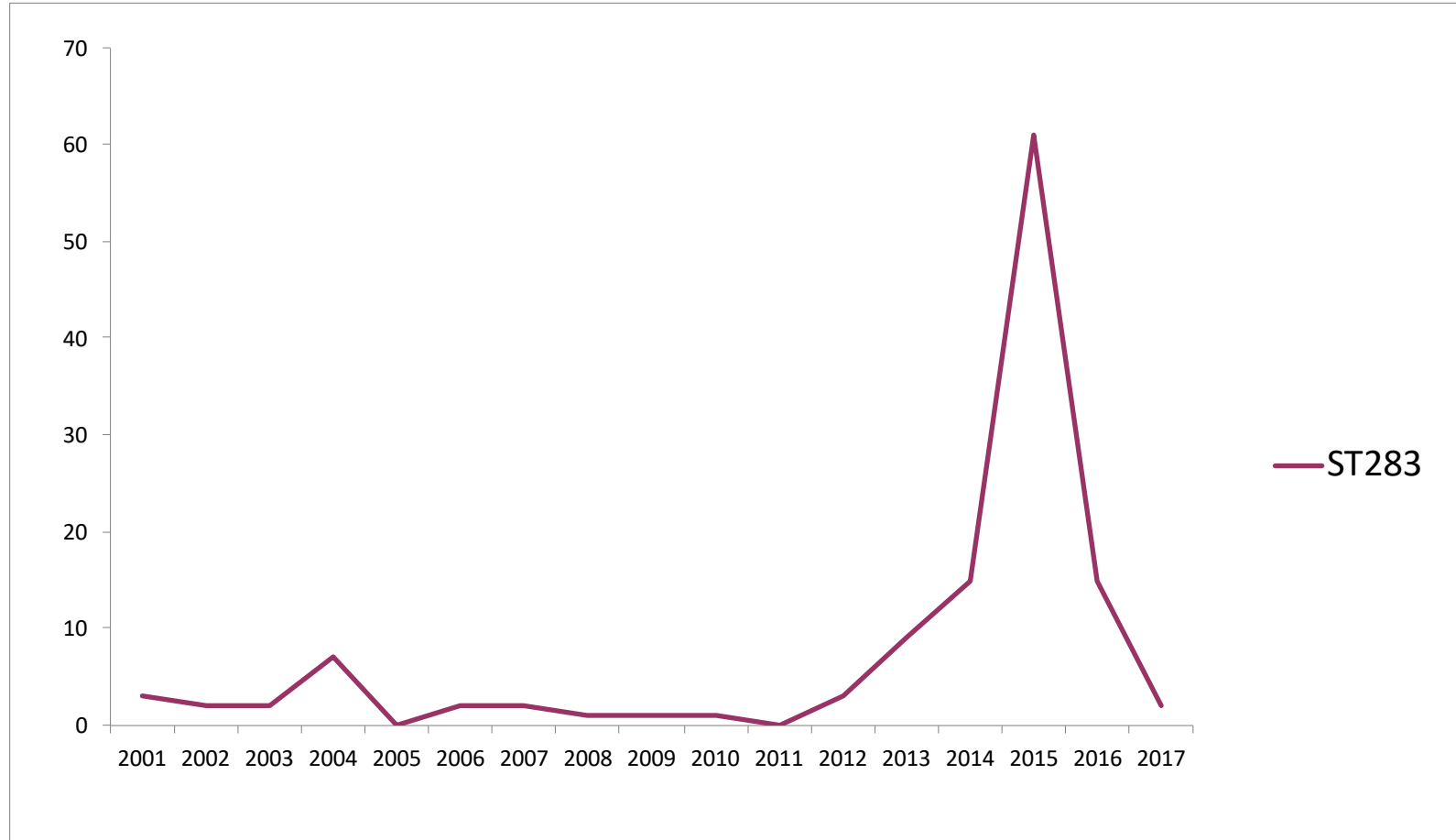
Fish isolates

Singapore fish 2015;
 ST283 = 11/39 freshwater fish in Markets - 28%
 ST283 = 6/586 freshwater fish in Ports - 1%
 ST283 - 1/7 RTE freshwater fish in Hawker stalls - 14%

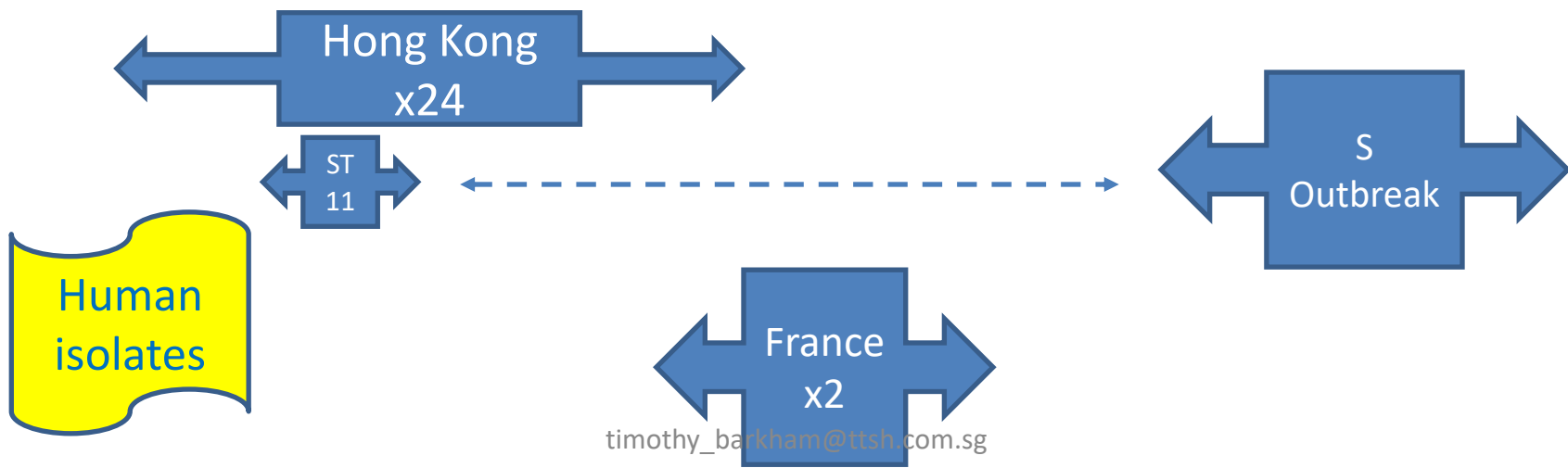
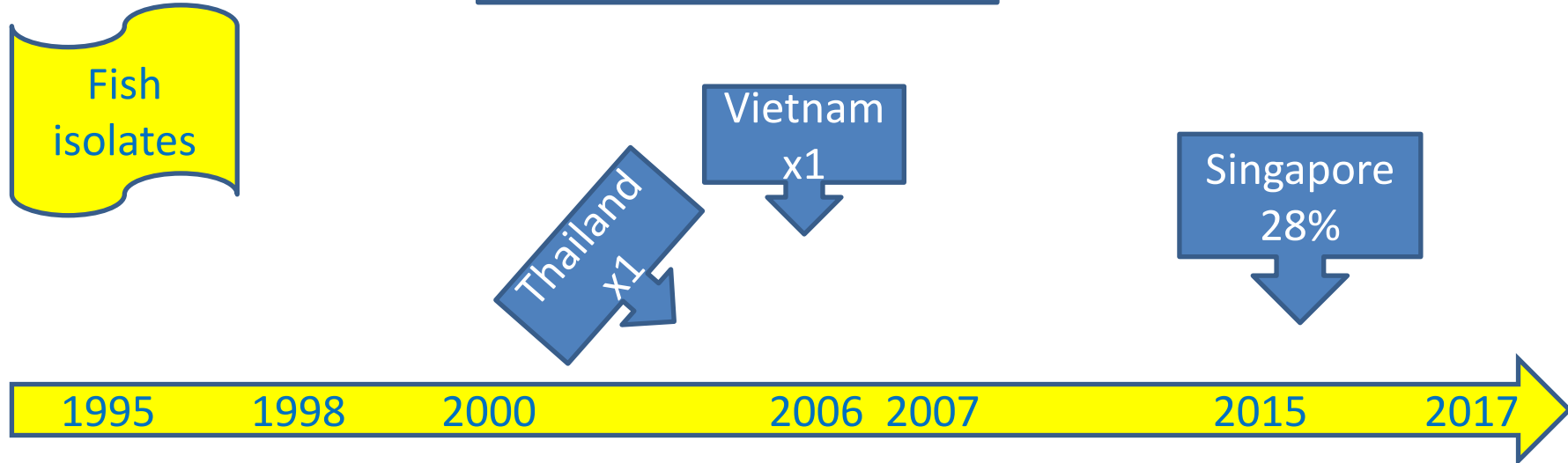


Human isolates

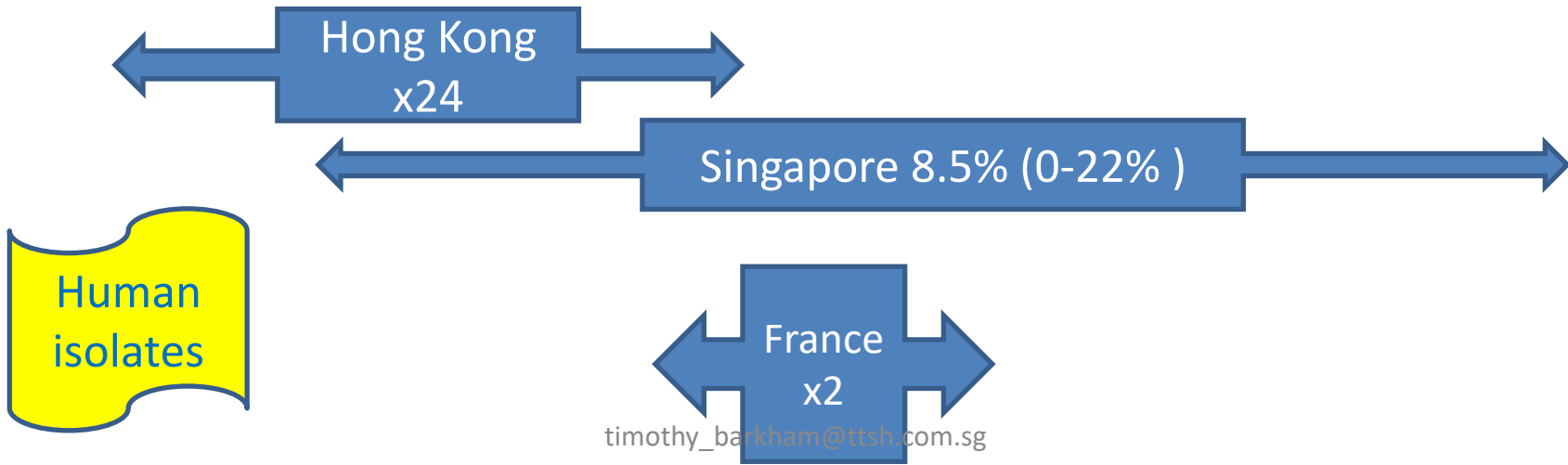
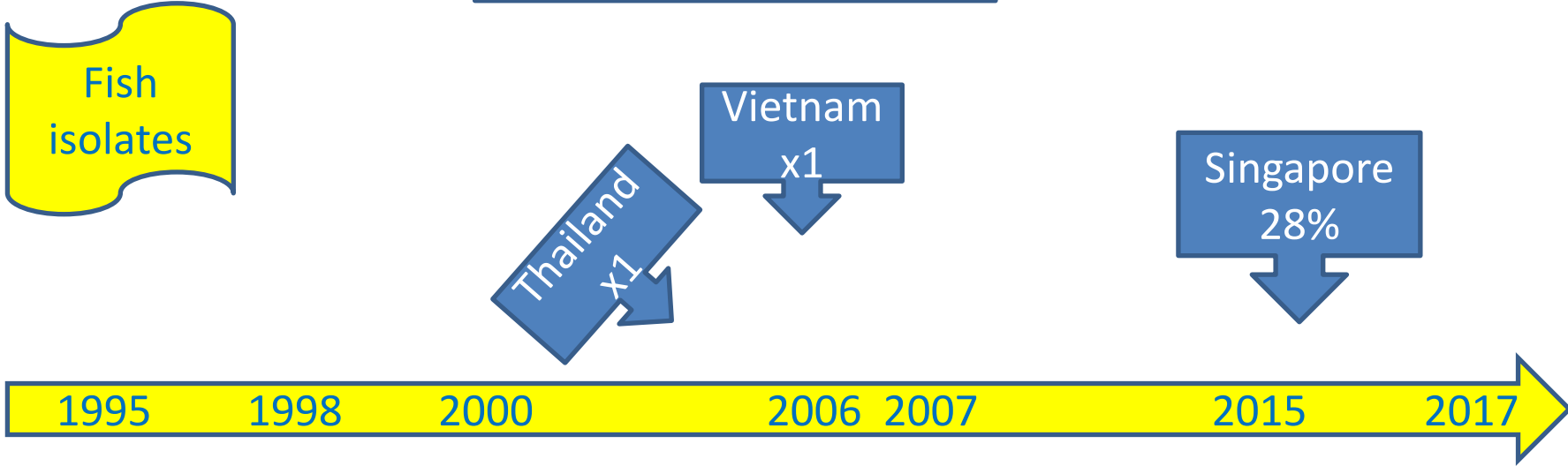
Historical invasive Singapore



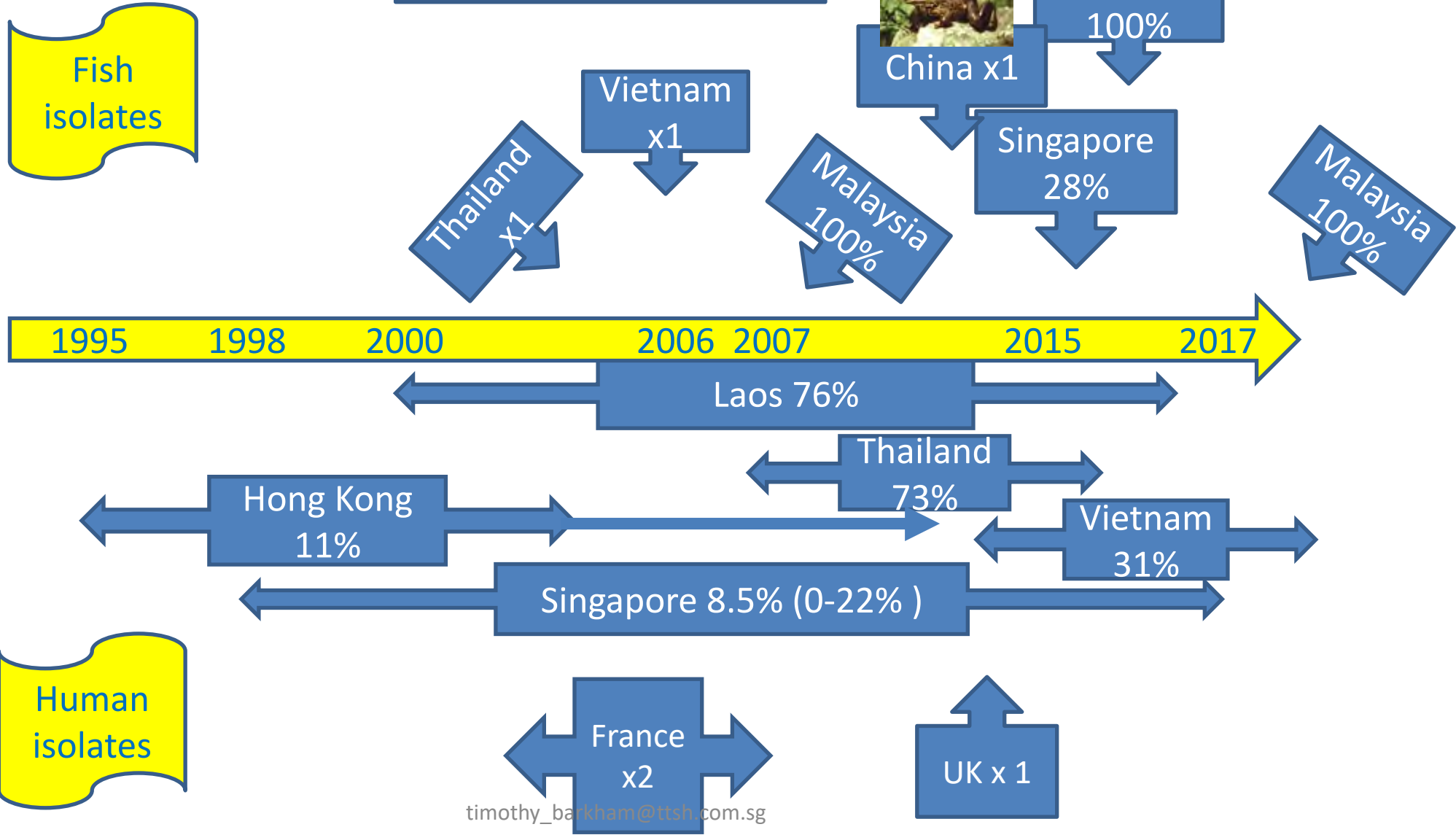
ST283 timeline



ST283 timeline

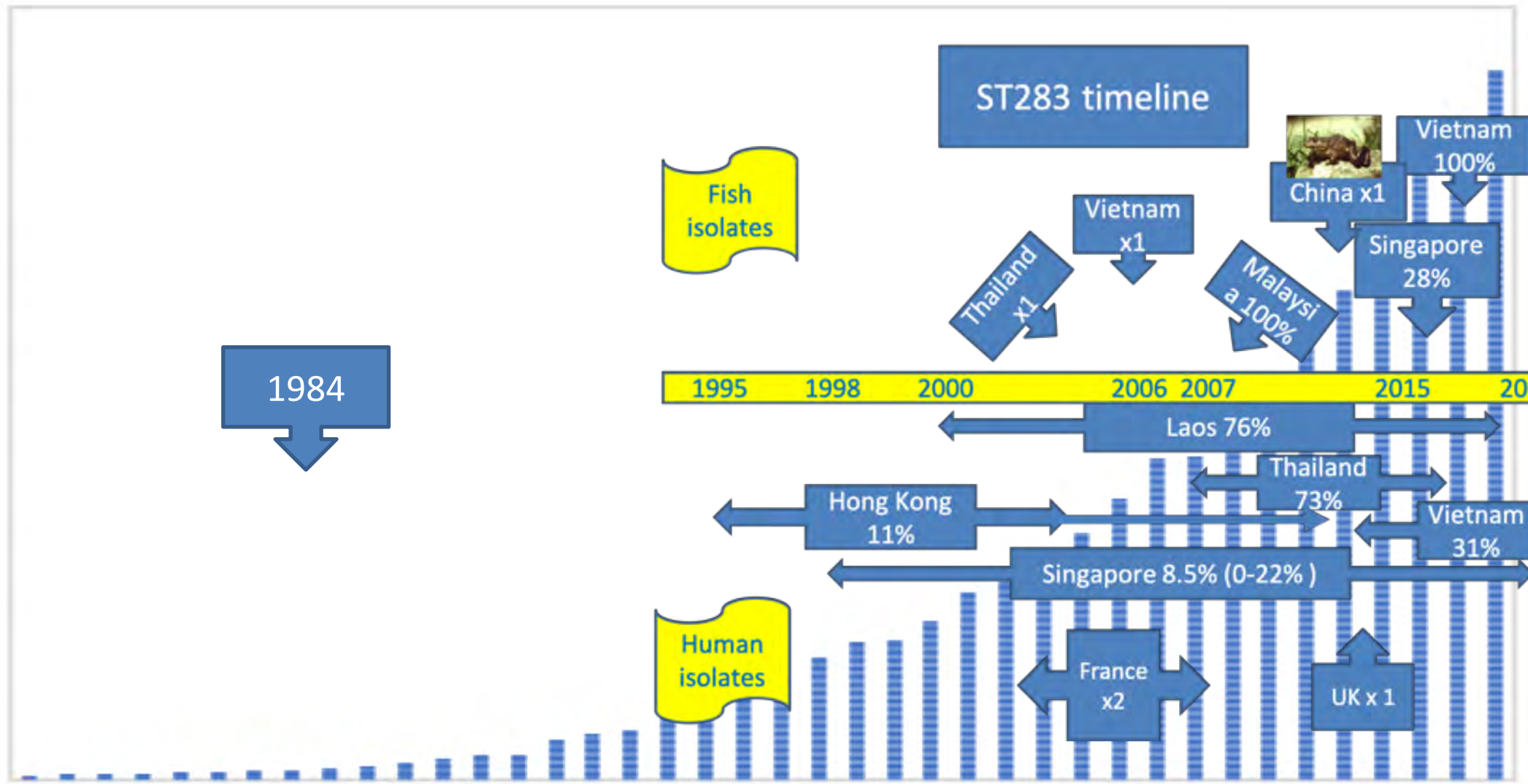


ST283 timeline





Tonnes (x100,000)



Older data from Thailand

- Serotype III
 - Emerging serotype in 2003-2006
 - Caused over 56% of fish disease in multiple sites
 - No ST data
 - The only serotype III to cause outbreaks in fish
 - Is ST283
- Reports of adult onset GBS septic arthritis
 - Without typing data
 - 1990 to 2010
 - 14 of 38 cases had no comorbidities that might predispose to joint infections

Kannika K, et al. *J Appl Microbiol* 2017; **122**(6): 1497-507.

Louthrenoo W, et al.. *J Clin Rheumatol* 2014; **20**(2): 74-8.



GBS bacteraemia rates relative to *E. coli*; Thailand, Singapore and Britain.

	<u>2014</u>	<u>2015</u>	<u>2016</u>
Nakhon Phanom, Thailand	No. (%)	No. %	No. %
GBS/ <i>E. coli</i>	60/426 (14)	61/455 (13)	75/547 (14)
Singapore			
GBS/ <i>E. coli</i>	55/1065 (5)	104/1141 (9)	56/1226 (5)
Britain	Rate (%)	Rate (%)	Rate (%)
GBS/ <i>E. coli</i>	2.8/54.8 (5)	3.1/59 (5)	3.7/64.9 (6)

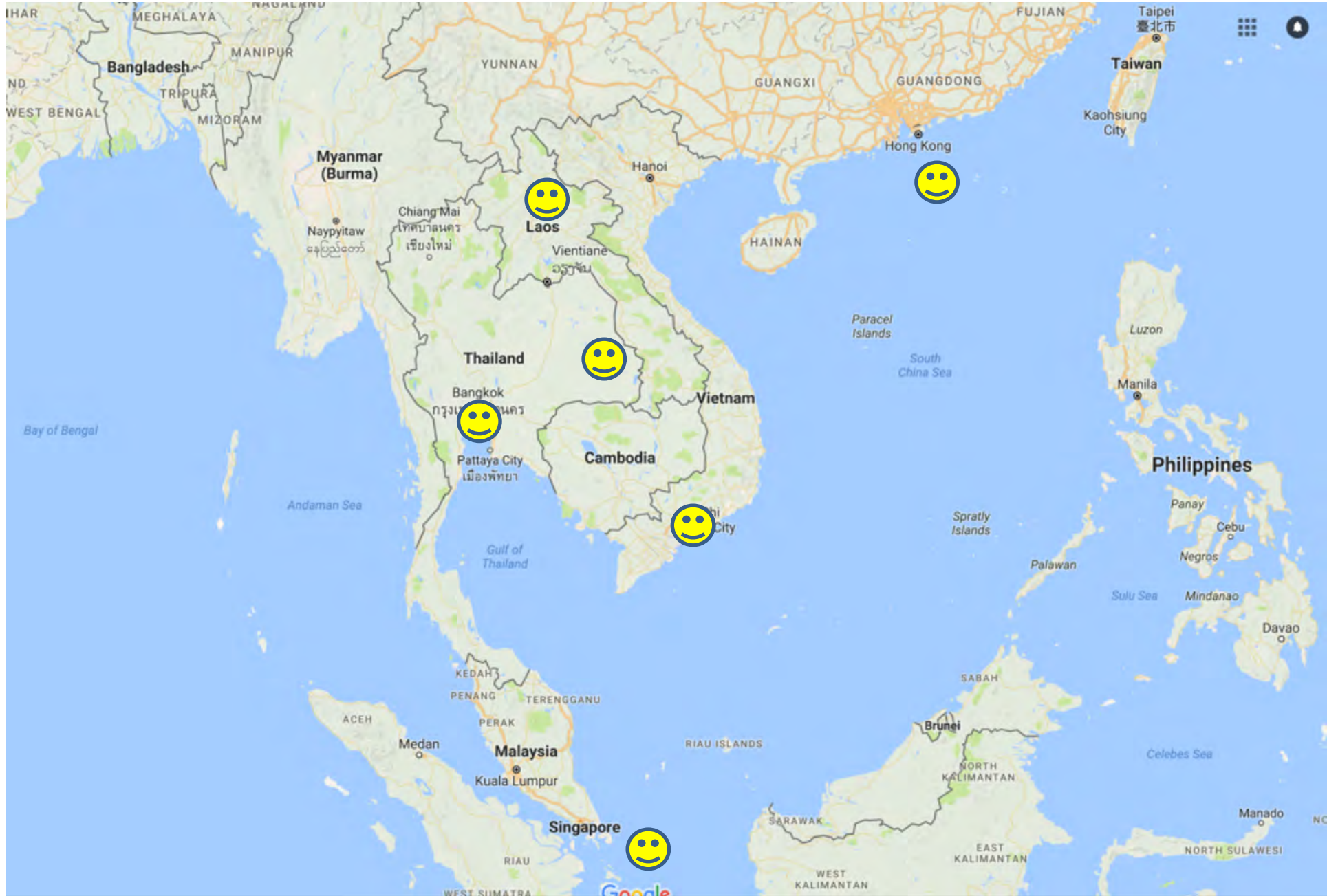
Note; Note similar ‘%’ for Singapore and UK, but Thai data is ~x3 fold higher.



GBS bacteraemia rates relative to *E. coli*; Thailand, Singapore and Britain.

	<u>2014</u>	<u>2015</u>	<u>2016</u>
Nakhon Phanom, Thailand	No. (%)	No. %	No. %
GBS/ <i>E. coli</i>	60/426 (14)	61/455 (13)	75/547 (14)
GBS (excluding ST283)/ <i>E. coli</i>	16/426 (4)	ND	ND
 Singapore			
GBS/ <i>E. coli</i>	55/1065 (5)	104/1141 (9)	56/1226 (5)
 Britain	Rate (%)	Rate (%)	Rate (%)
GBS/ <i>E. coli</i>	2.8/54.8 (5)	3.1/59 (5)	3.7/64.9 (6)

Note: after excluding ST283, GBS ‘%’ for Thailand is now similar to Singapore and UK
 So, ST283 results in a three fold higher rate in Thailand than in Singapore or UK



Yes; human ST283 is regional

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Yes; aquaculture ST283 is regional

Summary

- ST283
 - Widespread in SE Asia
 - Absent elsewhere ...
 - Decades
- Large population at risk
- N.b. did not study transmission



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Interpretation

GBS is primarily a foodborne infection, associated with aquaculture, in parts of SE Asia

Hurting people, fish
Economic losses in aquaculture

Factors: Pathogen, Infected food, consumption of raw food

This shows they do eat a lot of raw fish!

Thai doctors battle raw fish dish that kills 20,000 a year

Villagers being screened for cancer-causing parasite, kids taught risks of eating raw fish

KALASIN (Thailand) • It was not until he got to medical school that Dr Narong Khuntikeo finally discovered what caused the liver cancer that took both of his parents' lives: their lunch.

Like millions of Thais across the rural north-east, his family regularly ate koi pla – a dish made of raw fish ground with spices and lime.

The pungent meal is quick, cheap and tasty, but the fish is also a favourite feast for parasites that can cause a lethal liver cancer,

killing up to 20,000 Thais annually.

Most hail from the north-east, a large, poor region known as Isaan where people have dined on koi pla for generations and now has the highest reported incidence of cholangiocarcinoma (CCA) – bile duct cancer – in the world.

One of the major causes of CCA is a parasitic flatworm – or fluke – which is native to the Mekong region and found in many freshwater fish.

Once eaten, the worms can em-

bed undetected in the bile ducts for years causing inflammation that can, over time, trigger the aggressive cancer, according to the World Health Organisation.

"It's a very big health burden around here... it affects families, education and socio-economic development," said Dr Narong, who went on to become a liver surgeon to battle the scourge.

"But nobody knows about this because they die quietly, like leaves falling from a tree."

After seeing hundreds of hopeless late-stage cases on the operating table, Dr Narong is now marshalling scientists, doctors and anthropologists to attack the "silent



Millions of Thais across the rural north-east regularly eat koi pla, a local dish made of raw fish ground with spices and lime. The pungent meal is quick, cheap and tasty, but it is also a favourite feast for parasites. PHOTO: AGENCE FRANCE-PRESSE

killer" at the source.

They are fanning out across Isaan provinces to screen villagers for the liver fluke, and warn them of the perils of koi pla and other risky fermented fish dishes.

But changing eating habits is no easy task in a region where the love for Isaan's famously chilli-laden cuisine runs deep.

Health officials are pinning their hopes on the next generation, targeting children with a new school curriculum that uses cartoons to teach them the risks of eating raw fish.

For the elderly, the target is to catch infections through screening before it is too late.

Dr Narong and his team have de-

veloped urine tests to detect the presence of the parasite, which has infected up to 80 per cent of some Isaan communities.

They have also spent the past four years trucking ultrasound machines around the region to examine the livers of villagers who live far from public hospitals.

AGENCE FRANCE-PRESSE

Singapore since 2015

- Tan Tock Seng Hospital
- 2015 $44/69 = 64\%$ (Jan to August)
- 2016 $17/58 = 29\%$
- 2017 $2/58 = 3\%$
- 2018 $7/56 = 13\%$

N.b. Illegal to sell RTE fish for raw consumption, since late 2015

N.b. Malaysia ST283 from 6/6 diseased fish in 2018, from a different state than earlier data

... is it spreading?

- Outbreak 2016 in six fish farms in Brazil
 - High mortality
 - ST data not reported
 - Limited sequences clustered most closely with JP17
 - JP17 is an ST283 from a tilapia from Thailand

Questions

- Can we assess transmission?
 - From fish to humans or vice versa
- Is there another reservoir of ST283?
 - A common source contaminating food
 - e.g. chicken meat, chicken faeces
 - food or dumped into waterways
- Are other human GBS acquired from food?
 - In places with suitable conditions
 - Temasek grant

Plans

- SEA consortium – ‘SEA-BeaSt’
 - Meeting held in December 2018 (in Singapore)
 - Funded by Global Challenges Research Fund
 - through the University of Scotland
 - Reps from Malaysia, Lao PDR, Vietnam, Thailand, Cambodia & Singapore.
- Human and animal aspects
 - Reservoirs, Transmission
 - Carriage in the gut
 - Vaccines, Pathogenicity

Specific questions

- Public health significance?
- Activity in other countries?
- Existing surveillance?
- Aquaculture burden?
- What should be done?
- Still cases in Singapore. Lack of data/rates.
- Almost 'Nil'; note SEA-BeaSt initiative
- Poor. Note Thai data overlooked; see GBS data buried in a poster aimed at a different bacterium!
- Anecdotal economic impact on fish farmers.
- Surveillance, to define the size of the problem

Bacteremia due to *Burkholderia pseudomallei* in Nakhon Phanom Thailand 2014-2016

Ornuma Sangwichian¹, Payaowadee Abthaisong², Nanthanat Techawetcharoen², Chayada Viyasing², Warunee Poramart², Baramheht Piralam², Anchalee Jatapai³, Pongpun Sawatwong² and Toni Whistler¹

¹Strengthening Laboratory Capacity Program, Thailand MOPH - U.S. CDC collaboration

²Project staff of Provincial Health Office, Nakhon Phanom, Thailand

³International Emerging Infections Program, Thailand MOPH - U.S. CDC collaboration

INTRODUCTION

The Strengthening Laboratory Capacity Program (SLCP) of the Thailand MOPH - U.S. CDC Collaboration in partnership with the Nakhon Phanom (NP) Provincial Health Office has conducted blood stream infections (BSI) surveillance to enhance the detection of blood borne pathogens since 2005.

Burkholderia pseudomallei is the causative agent of melioidosis, a serious disease of humans and animals that occurs primarily in Southeast Asia, northern Australia and other tropical areas. *B. pseudomallei* is a Gram negative bacillus showing bipolar staining, present in the environment in endemic areas. Disease occurs after bacterial contamination through breaks in the skin, or by inhalation or ingestion (1). The highest documented infection rate is in Northeastern Thailand and incidence of human cases is increasing every year (2).

The objective of this study was to investigate the prevalence of *B. pseudomallei* bacteremia in hospitalized patients in Nakhon Phanom province from 2014-2016.

METHODS

Blood specimens were collected at 12 hospitals in NP, inoculated into the appropriate bottle at volumes recommended by the manufacturer, and transported to the SLCP lab within 12 hours at 25-30°C

Transport to SLCP NP lab within 12 hours at 25 - 30°C

2014:
BacT/ALERT 3D
(Biomérieux, France)



2015-2016:
BACTEC FX
(Becton Dickinson, USA)



All positive of blood cultures were sub-cultured onto sheep blood, chocolate and MacConkey agar plates and incubate overnight at 35°C. Colonies suspected to be *B. pseudomallei* were confirmed as such using manual biochemical assays and the Analytical Profile Index (API®) system (Biomérieux, France).

Manual biochemical assays



Table 2. The most common isolates 2014-2016

Pathogen	2014		2015		2016	
	No. Isolates	%	No. Isolates	%	No. Isolates	%
<i>Escherichia coli</i>	426	24.1	455	21.6	547	30.3
<i>Burkholderia pseudomallei</i>	134	7.6	194	9.2	219	12.1
<i>Klebsiella pneumoniae</i>	111	6.3	142	6.7	165	9.1
<i>Staphylococcus aureus</i>	73	4.1	127	6.0	109	6.0
<i>Streptococcus agalactiae</i>	60	3.4	61	2.9	75	4.2
<i>Streptococcus pneumoniae</i>	37	2.1	50	2.4	40	2.2
<i>Acinetobacter baumannii</i>	25	1.0	35	2.3	47	2.0
<i>Streptococcus pyogenes</i>	18	1.0	49	2.3	36	2.0
<i>Enterococcus</i> species	27	1.5	19	0.9	52	2.9
<i>Salmonella</i> species	31	1.8	25	1.2	36	2.0
Other pathogens*	822	46.6	953	45.2	480	26.6

* This includes mold, fungi, *Enterobacter* spp., *Vibrio*, *Haemophilus*, *Pseudomonas* spp. and *Proteus* spp.

Table 3. Prevalence of *B. pseudomallei* bacteremia in NP 2014-2016

Year	<i>B. pseudomallei</i> cases	Population*	Prevalence per 100,000 people	p-value**
2014	104	705,741	14.7	0.062
2015	125	717,164	17.4	
2016	157	718,909	21.8	
Total 3 years	386	2,141,814	18.0	
Age (Years)				
< 15	4	432,409	0.9	
15-24	5	317,706	1.6	
25-34	26	326,105	8	
35-44	64	378,413	16.9	
45-54	96	310,835	30.9	
55-64	111	201,113	55.2	
65-74	62	113,231	54.8	
> 75	18	62,002	29	

* GIS Health Data Nakhon Phanom <http://203.157.176.8/giscenter/pop.php>

** Linear regression for trend analysis

Table 4. Percent susceptibility of *B. pseudomallei*

Year	Percent Susceptible (No. tested)	
	Trimethoprim/Sulfamethoxazole	Ceftazidime
2014	99.0 (104)	100 (114)

Wow!

S. aureus = 73

GBS = 60

Astonishing that GBS is almost as common as *S. aureus*.

This is not normal, totally off limits!

... but maybe it is normal for this area, due to ST283!

We learn!

Genomic insights into a foodborne outbreak of Group B Streptococcus infection in Singapore – and beyond

Swaine Chen
National University of Singapore
Genome Institute of Singapore
190227

Key questions

- Is this really foodborne?
- Extent of ST283?
- Why is ST283 causing disease?

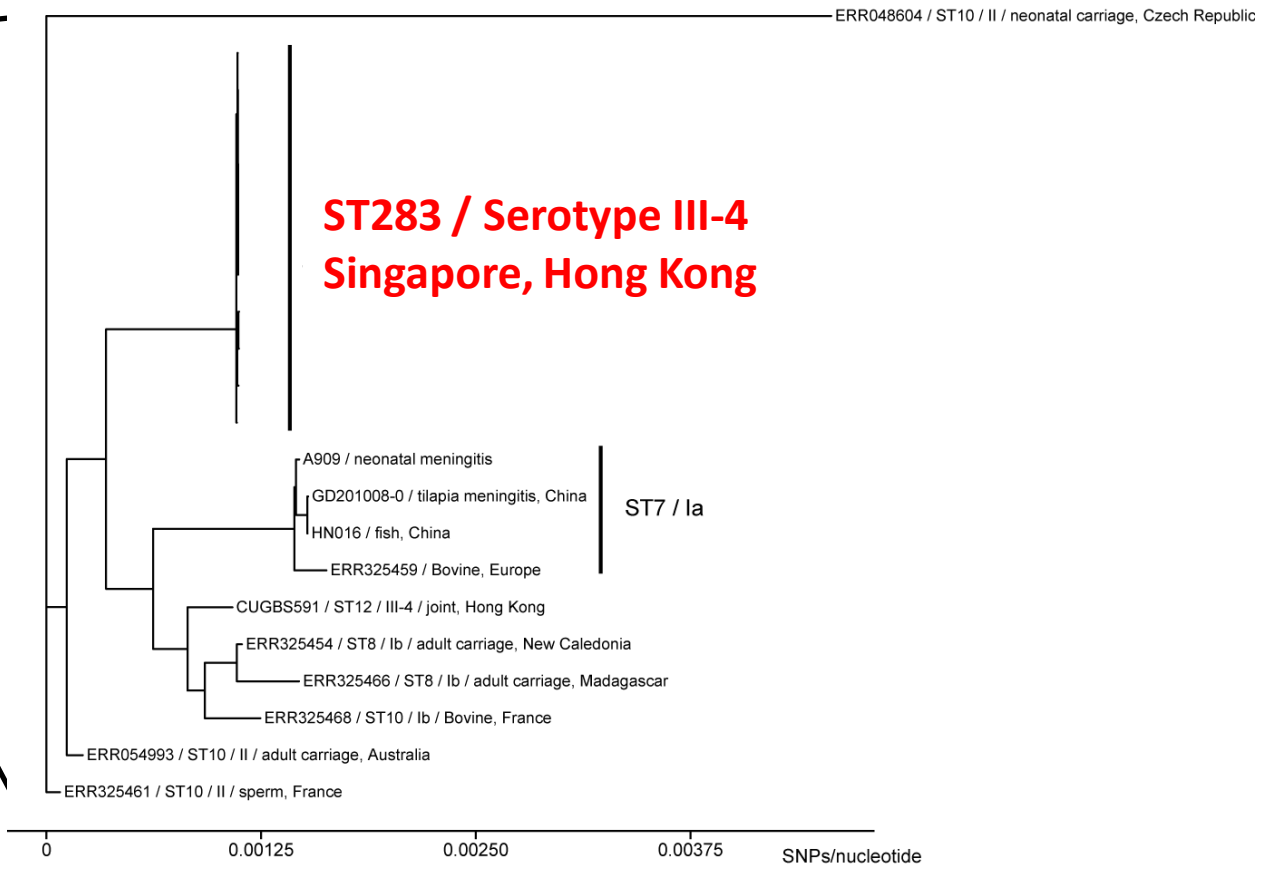
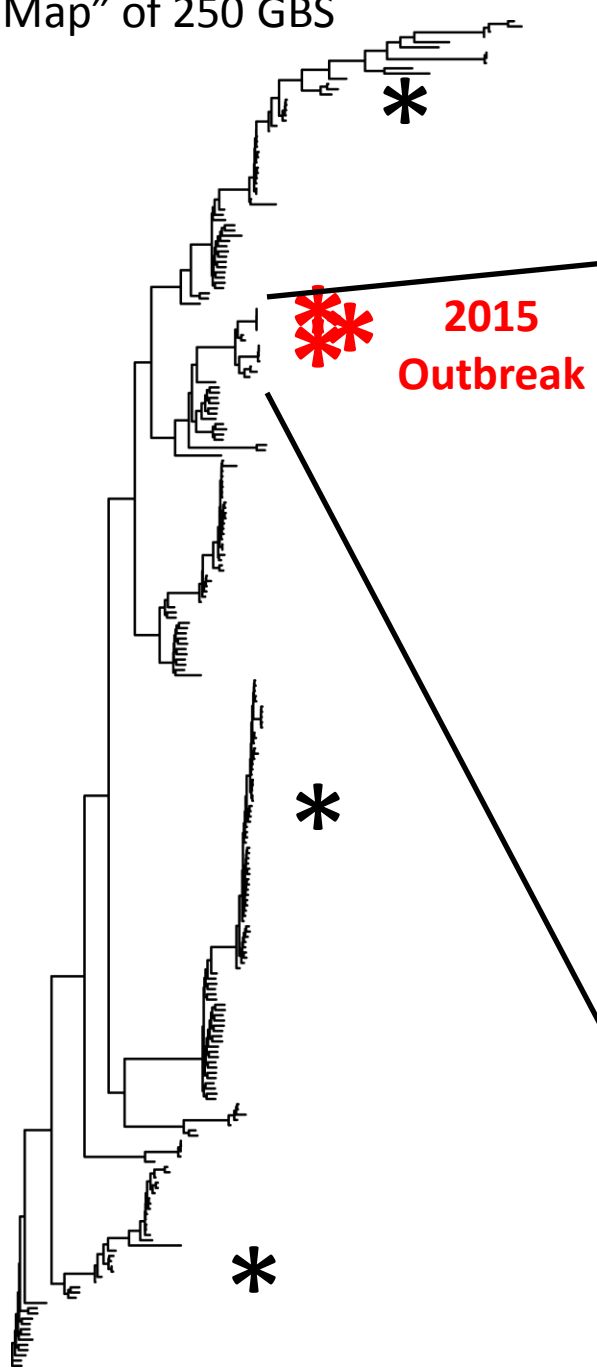


WGS data

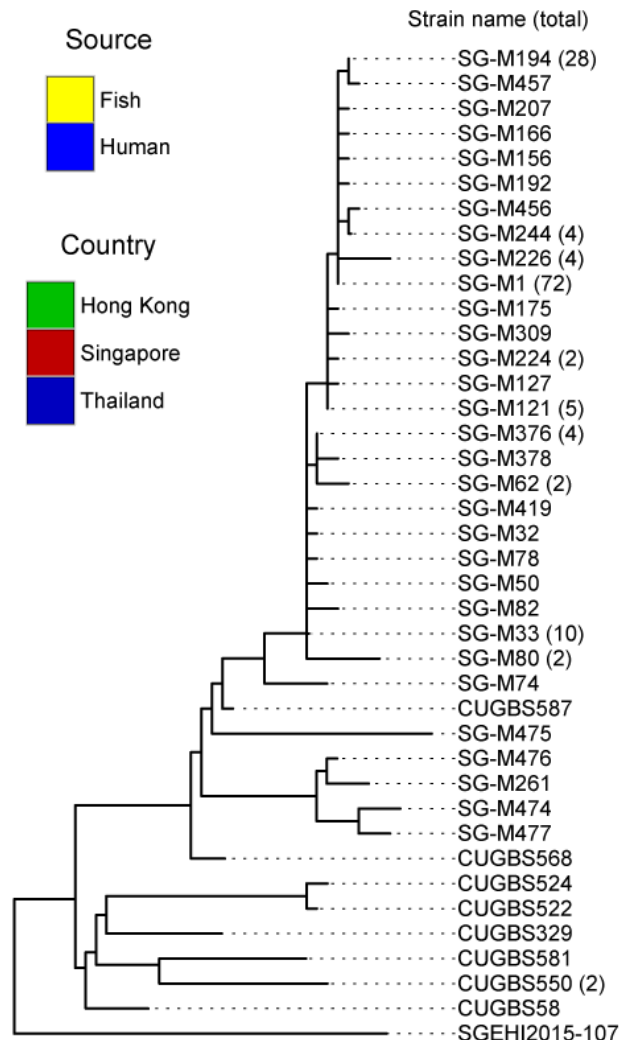
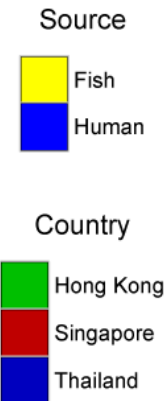
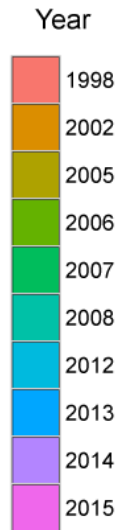
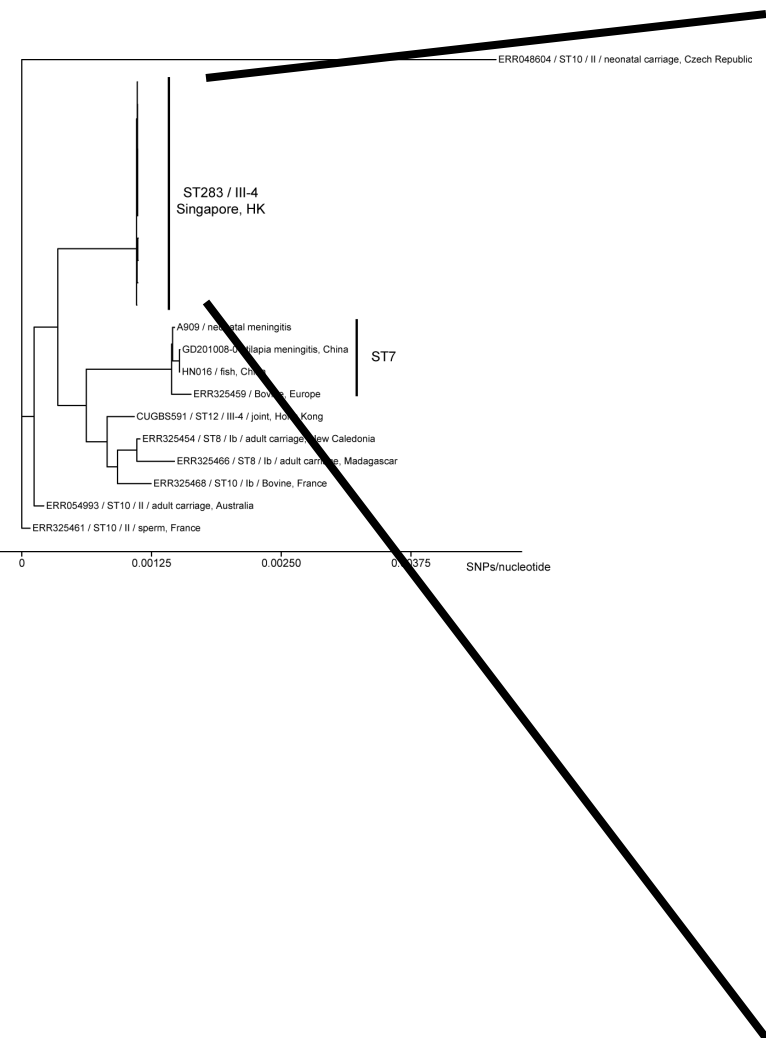
- 157 Human isolates
 - 144 from Singapore (2012-2015)
 - 9 from Hong Kong (1998-2007)
 - 4 from Thailand (2015)
- 88 Fish isolates from Singapore
 - 12 from point of import (2015)
 - 76 from wholesale or retail (2015)

"Map" of 250 GBS

One outbreak strain found in fish and humans



One outbreak strain found in fish and humans



Singapore 2015

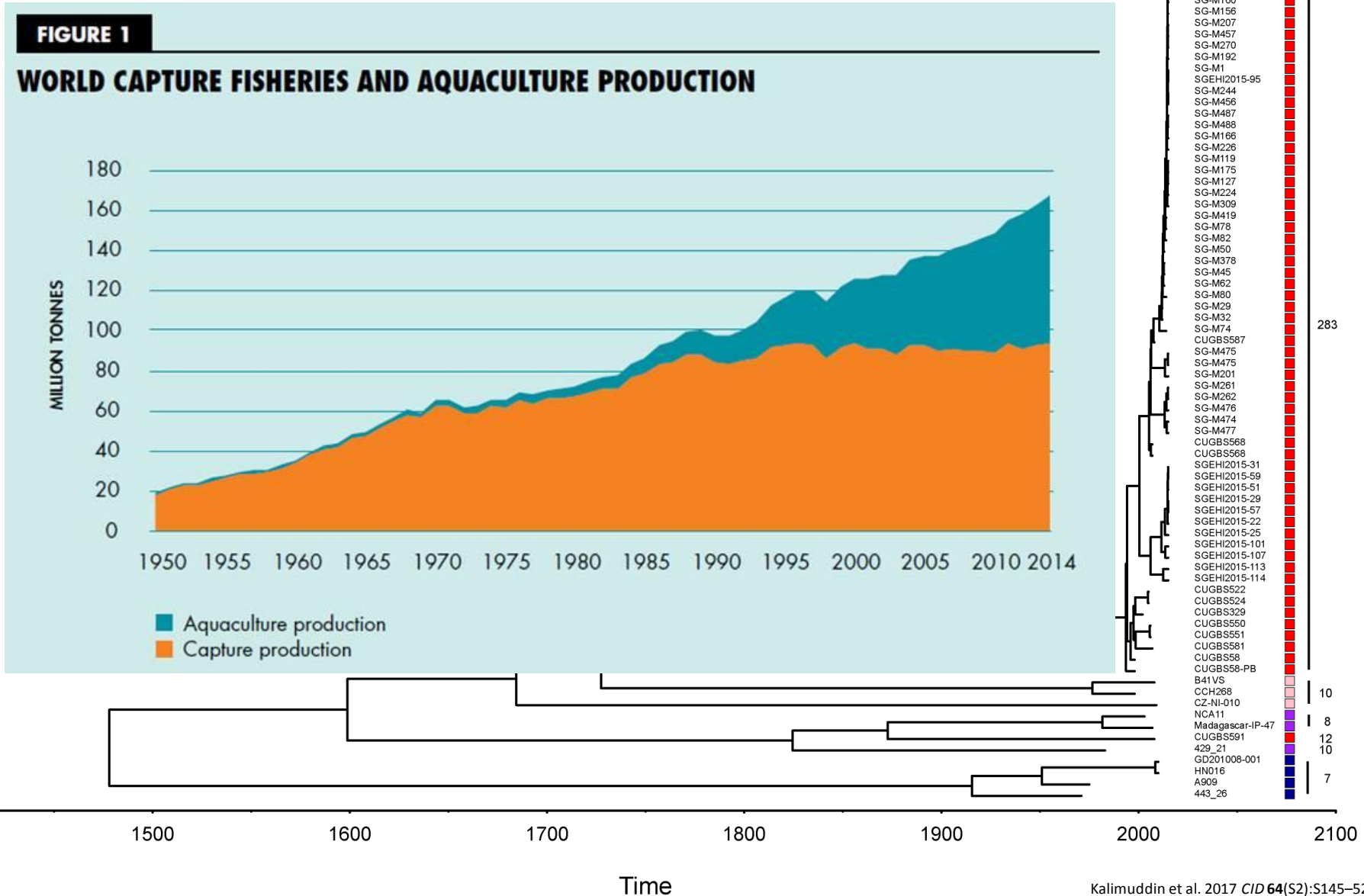
SG, 2012-2014

Thailand 2015

HK, 1998-2006

Historical isolates

ST283 emerged in 1984





Tan Tock Seng
HOSPITAL

ST283 timeline

Fish
isolates



Thailand
x1

Vietnam
SLV283

Malaysia
100%

China x1

Singapore
30%

Vietnam
100%

Laos 76%

Thailand
73%

Hong Kong
11%

Vietnam
31%

Singapore 8.5% (0-22%)

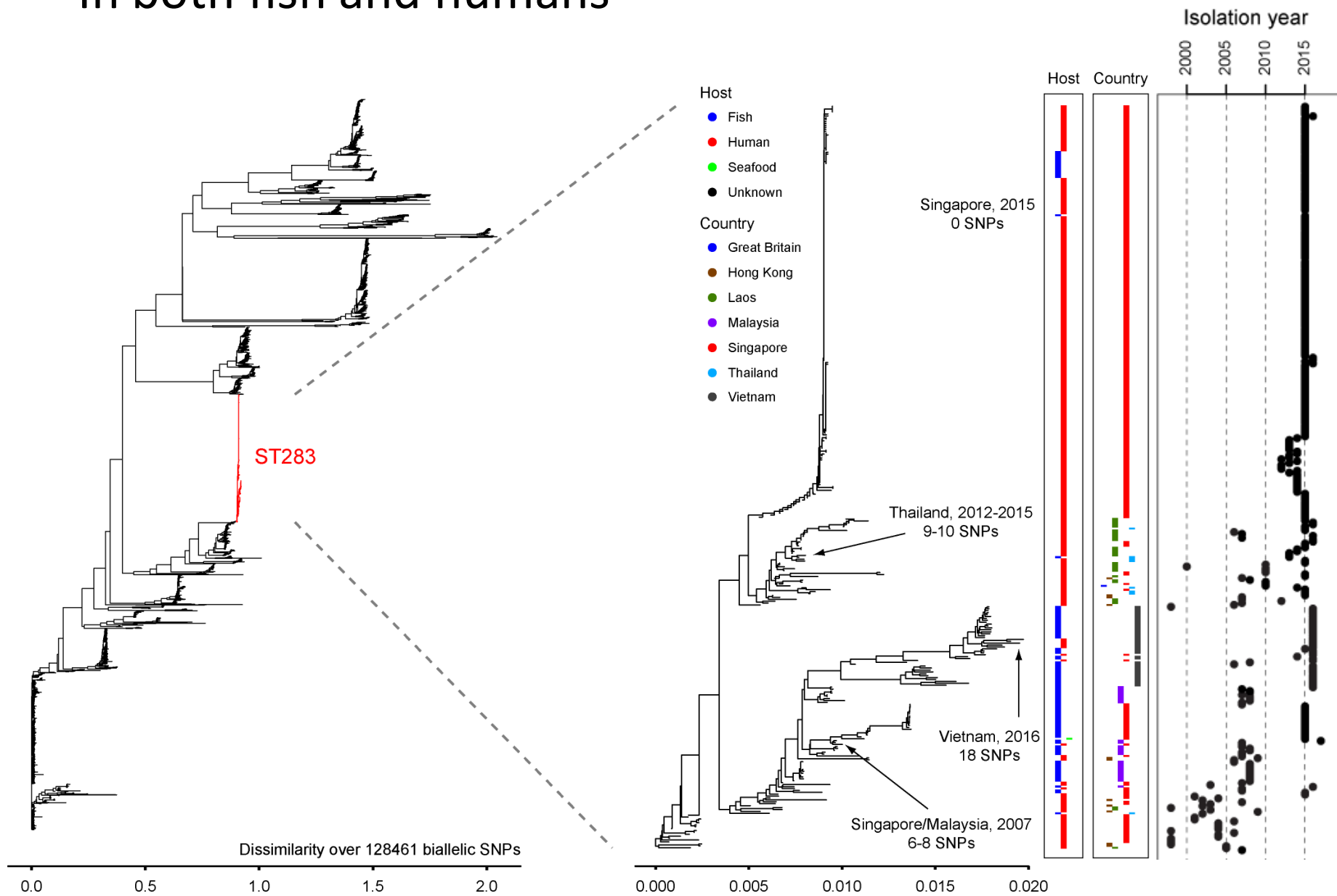
Human
isolates

France
x2

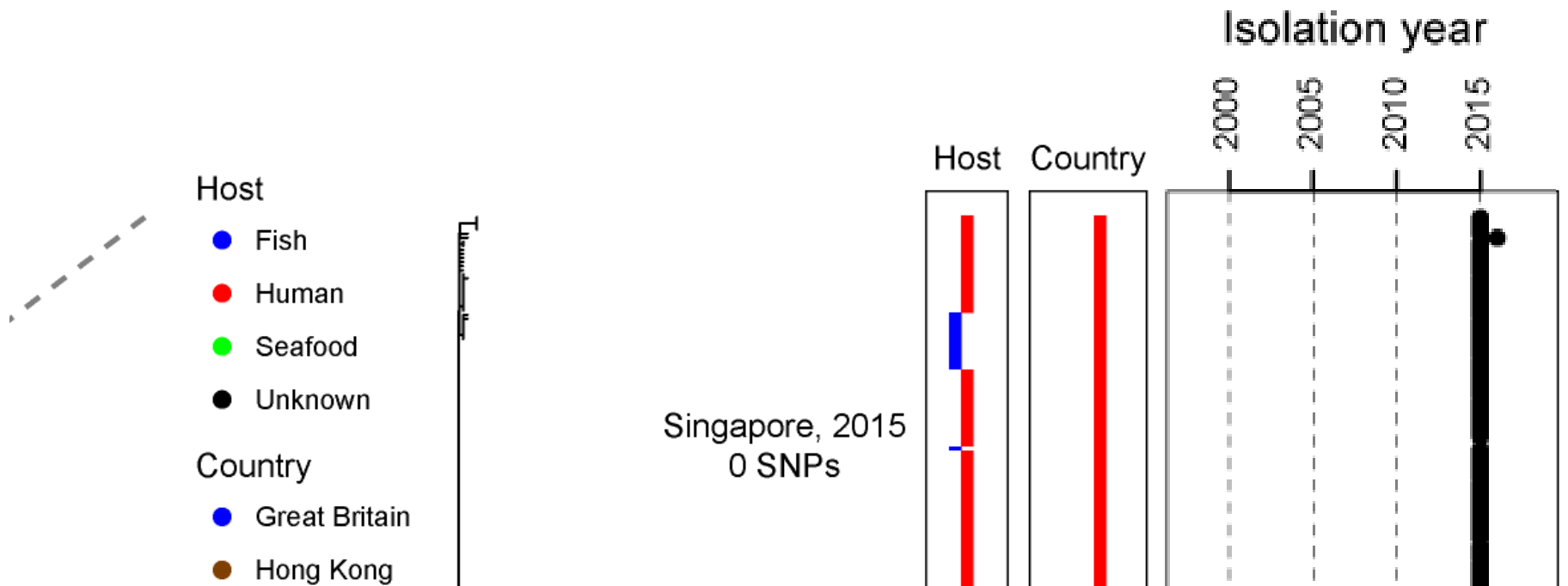
UK x1

ST283 is only in SEA In both fish and humans

389 ST283 Genomes

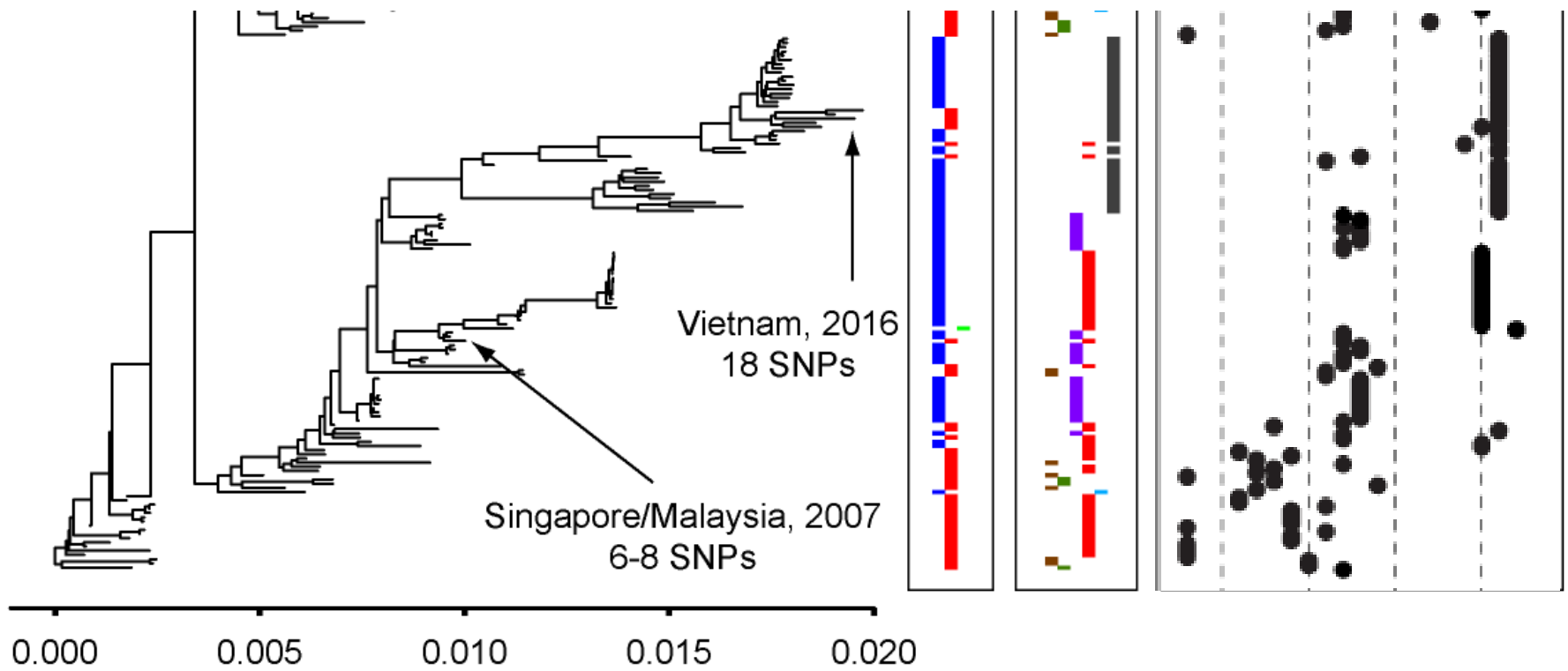


ST283 is only in SEA In both fish and humans



ST283 is only in SEA

In both fish and humans



2015 GBS outbreak

- Is this really foodborne?
 - Identical strain
 - Epidemiology
- Extent of ST283?
 - Throughout SE Asia
 - 1984, rapidly expanding
- Why is ST283 causing disease?
 - Ongoing!



GBS ST283 in SEA: An unrecognized regional problem

- GBS can be foodborne
- GBS infects healthy adults
- Adult is 90% of GBS
- ST283 is >50% of adult
- SEAsia at high risk
 - Culture, Food
 - Disproportionately LMIC
 - Food security
 - Fish exports
 - Economic development



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 - Roland Jureen - NUHS

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 - Chau Man Ling – NEA
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- Vicki Chalker – England
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