Detection and typing of *Streptococcus pyogenes*

Dr K C Ng
Microbiology Division
Public Health Laboratory Services Branch
Centre for Health Protection

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Streptococcus pyogenes: Types of Infectious Disease

- **Pharyngitis, impetigo, cellulitis, necrotizing fasciitis and myositis (flesh-eating bacteria), pneumonia, bacteremia,**
- **Sacarlet fever**
- **Streptococcal toxic shock syndrome**
- **Post-suppurative complications**
  - **Rheumatic carditis (pharyngeal infections)**
  - **Acute glomerulonephritis (pharyngeal and skin infections)**

Suppurative Complications

Bacteremia

Cervical lymphadenitis

Endocarditis

Mastoiditis

Meningitis

Otitis media

Peritonsillar/retropharyngeal abscess

Pneumonia

Detection of S pyogenes

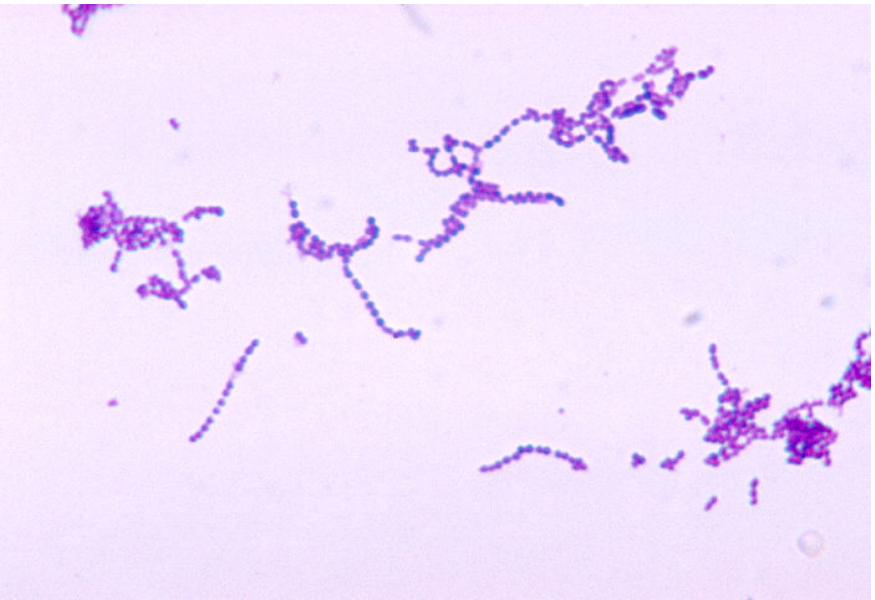
- Clinical
  - No single element of the patient's history or physical examination reliably confirms or excludes diagnosis
  - Use Centor score as clinical algorithm
- Laboratory
  - Gram stain – not useful or practical in pharyngitis but give clues in sterile site infections or collections of infectious materials
  - Throat culture – 90-95% sensitive
  - Non-culture methods
    - Allows earlier treatment
    - symptom improvement
    - reduced disease spread
  - Serology

Clinical Decision Rule for Management of Sore Throat – **Centor score**

Criteria	Points
Absence of cough	1
Tonsillar exudates or swelling	1
Swollen and tender anterior cervical nodes	1
Temperature >38°C	1

Gram stain of throat swab specimens

not useful nor practical
in pharyngitis



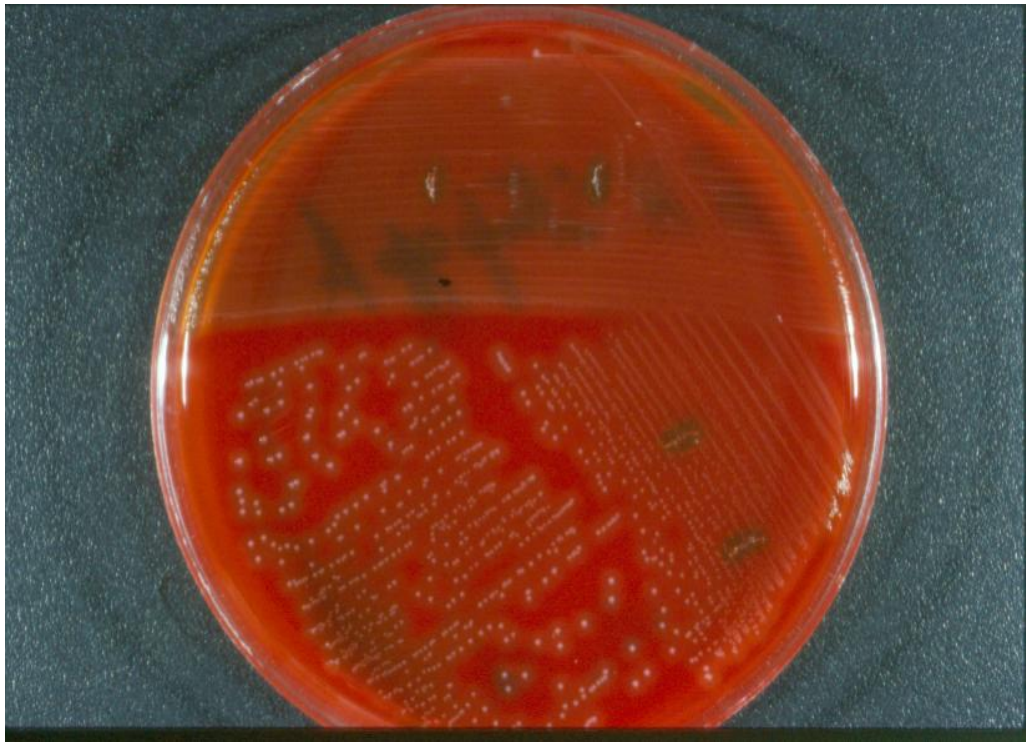
Throat culture

Deep stabs of inoculum into anaerobic portions of blood agar

Blood agar plate with trimethoprim-sulfamethoxazole – selective as growth of commensals suppressed.

Oxygen-labile streptolysin (Streptolysin O)

Oxygen-stable streptolysin (Streptolysin S)



Most GAS are bacitracin sensitive

Bacitracin-resistant group A streptococci do exist !

Haemolysis on Blood Agar

- **β-hemolysis**

  Complete clearing of blood agar due to lysis of red cells by oxygen-stable and oxygen-labile hemolysin

- **α-hemolysis**

  Greening of blood agar due to partial lysis of red cells

- **γ-hemolysis**

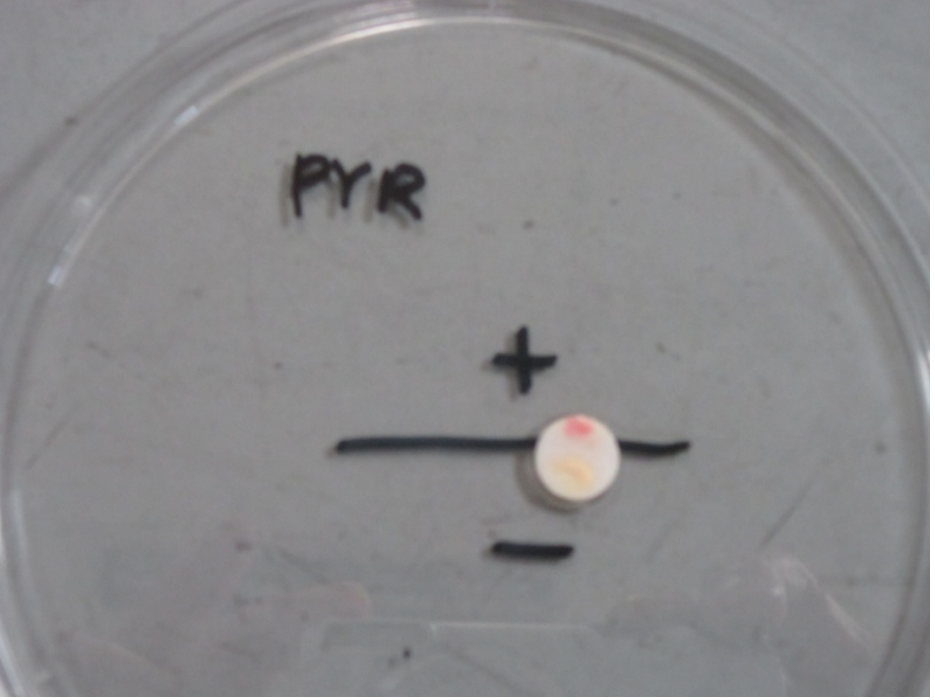
  Absence of hemolysis

Haemolysis on blood agar

- **No carbohydrate → no fermentative acids to inhibit haemolysis**
- **Blood contains NADase that inactivates NAD released by red cells, thus preventing growth of the β-hemolytic organisms *Haemophilus haemolyticus* and *H. parahaemolyticus* that require exogenous NAD**

Streptococcus:
Microbiology

- **Gram-positive cocci (round to oval shaped, occasionally elongated) -** grow in chains in liquid media
- **Catalase negative (vs Staphylococcus is catalase positive)** – 3% hydrogen peroxide
- **β-hemolytic streptococci**
  - **pyogenic large-colony (>0.5 mm in diameter) forms with Lancefield group A, C, or G antigen**
  - **small-colony (<0.5 mm in diameter) forms with Lancefield group A, C, F, or G antigen, or no Lancefield antigen**
- **Most strains sensitive to bacitracin (0.04U)**



pyrrolidonyl aminopeptidase

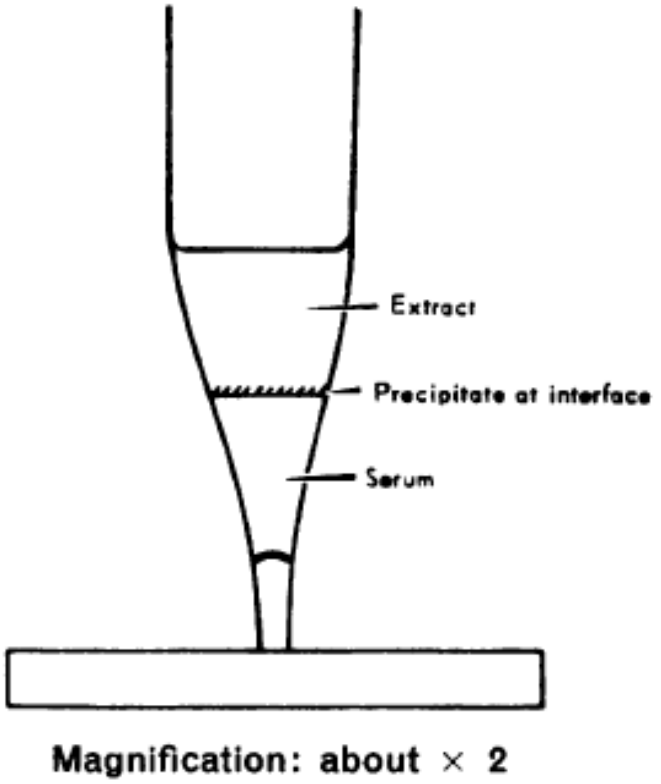
pyrrolidonyl- β-naphthylamide (PYR)

- 98% of group A streptococci and 96% of group D enterococci hydrolyze PYR.
- 98% of group B streptococci, 100% of non-group A, B and D streptococci, 100% of group D non-enterococci and 82% of viridans streptococci yield negative PYR test results

- **Hydrolysis of L-pyrrolidonyl- β-naphthylamide (PYR) with release of free β-naphthylamide**
- **Free β-naphthylamide is detected by reaction with dimethylamminocinnamaldehyde to produces a red color**

Lancefield grouping of beta-haemolytic streptococci

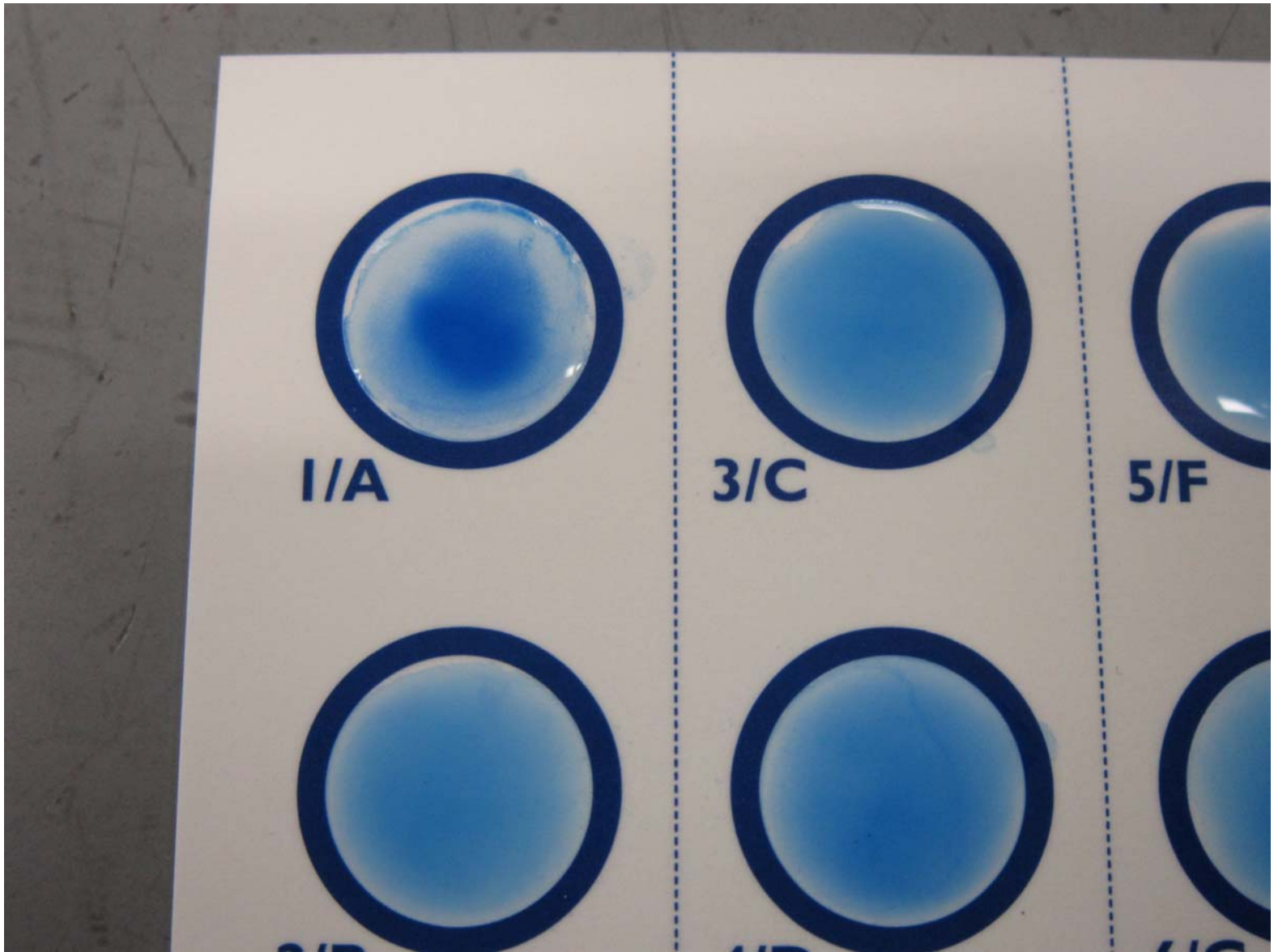
- 5-mm external diameter glass tubing drawn out to a capillary at one end and cut to a total length of about 3 cm
- The tubes are held vertically in a rack of modeling clay
- A drop of serum run in and settles at the region where the tube is constricted to the capillary.
- Extract then be layered on top
- A positive reaction appears as a clear-cut ring within 2-3 minutes



Magnification: about × 2

Bull. Org. mond. Sante' Bull. Wid Hlthi Org. 1958, 19, 153-176

Grouping of beta-haemolytic streptococci latex agglutination



Rebecca Craighill Lancefield (1895-1981)

Lancefield grouping of β-haemolytic streptococci

“Typing" streptococci with a variety of M protein-specific antibodies mixed with a streptococcal extract.

Group A streptococci were classified according to their expression of M antigens by visualizing the precipitin reactions in capillary tubes.

Non-culture method

- Commercial methods
  - Nucleic acid hybridization (Gen-Probe, San Diego, CA)
  - Rapid direct group A streptococci antigen test (RADTs)
- Moderate to heavy group A streptococci
  - comparable performance to throat culture
- Small amounts of GAS
  - Sensitivity may decrease
- Thus sensitivity and accuracy
  - is predicated on the collection of an adequate amount of material onto the collection swab
- Because of the presence of a selection bias with RADTs, selective use of these tests may also increase their sensitivity.

Rapid antigen detection tests

- All rapid tests involve an extraction step to solubilize cell wall carbohydrate and then
- Identify its presence by an immunologic reaction or molecular methods:
  - Latex agglutination
  - Enzyme immunoassay (EIA)
  - Optical immunoassay
  - Immunochromatography
  - Molecular:
    - Chemiluminescent singlestranded DNA probe *(Gen-Probe)*
    - real-time PCR method (*LightCycler Strep-A assay)*

Rapid antigen detection tests - performance

- Depends on the skill, experience, and expertise of
  - Personnel taking the throat swab and
  - Personnel performing the rapid tests
- Point of care tests – possible observer bias
- All claimed to have high sensitivities and specificities but product claim may be based on different gold standard for comparison
- Cost and facilities (for molecular tests) is also a consideration.
- Other non-group-A beta-haemolytic streptococci as a cause of “streptococcal pharyngitis” may be missed

Use of RADTs

- General ID or Paedi guidelines:
  - When a patient suspected of having GAS pharyngitis has a negative RADT, a throat culture on blood agar plate should be performed to confirm the results of the RADT
  - Physicians who use such tests without culture backup should compare their results with those of culture to validate the adequate RADT sensitivity
  - Adults have both a lower incidence of GAS pharyngitis than children and adolescents and an extremely low risk of contracting acute rheumatic fever. Negative RADT results for adults need not be confirmed with BAP cultures
- CDC –
  - All adults with pharyngitis be screened for the presence of the four “Centor criteria”.
  - Those with none or one of these criteria need not be tested or treated.

Serology
Anti-streptococcal antibodies

- Commercial kits available
- Qualitative or semi-quantitative
  - Particle-enhanced rapid screening test
- Antibody levels depend on time since onset, age, background prevalence, antibiotic treatment, other medical comorbidities
- A 2-fold or greater rise in ASO titre is considered significant in all age groups.
- Practically, not useful for diagnosing streptococcal pharyngitis but
- May be indicated to confirm previous infection in persons with suspected acute poststreptococcal glomerulonephritis or rheumatic fever
- Prompt antibiotic therapy reduce titre but not abolish antibody response

Common tests

- ASOT (Anti-streptolysin O titre)
- Haemagglutination based streptozyme test (*Streptozyme*)
  - detect antibodies against multiple extracellular streptococcal products (antihyaluronidase, antistreptokinase, antideoxyribonuclease B, anti-NADase

ASOT (Anti-streptolysin O titre)

- Peaks at 3-6 weeks
- ASO response higher in pharyngitis than skin infections
- Streptolysin O (SLO) produced by almost all GAS (and many group-C and G beta-haeomolytic streptococcus)
- All sera demonstrate a certain level of anti-streptolysin O, depending on patient age, geographical locality and the local frequency of streptococcal infections.
  - 200 IU/ml is accepted internationally as the upper limit of the “normal range” since this value was seldom exceeded in persons without clinical symptoms
  - Pre-school age children usually have values below 100 IU/ml
  - The ASO concentrations increases with age in children, reaching a peak among school children, then falling thereafter
- ↑ ASOT or equivalent → recent acute streptococcal infection, rheumatic fever, acute poststreptococcal glomerulonephritis

Typing

- No immediate clinical or therapeutic implication
- Typing of group A streptococci is useful in the investigation of both community and hospital outbreaks of group A streptococcal infection

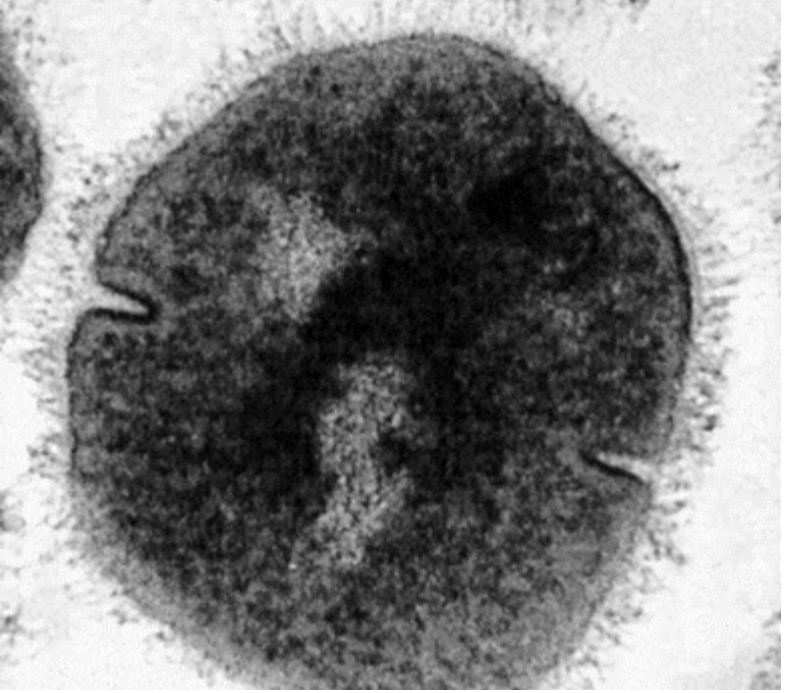
Possible Typing Methods

- Antibiogram – antibiotic susceptibility / resistance patterns
- M protein typing / *emm* typing
- T typing
- Opacity factor typing
- Molecular
  - Pulse field gel electrophoresis (PFGE)
  - MLST
  - DNA fingerprinting
  - Pyrolysis mass spctrometry

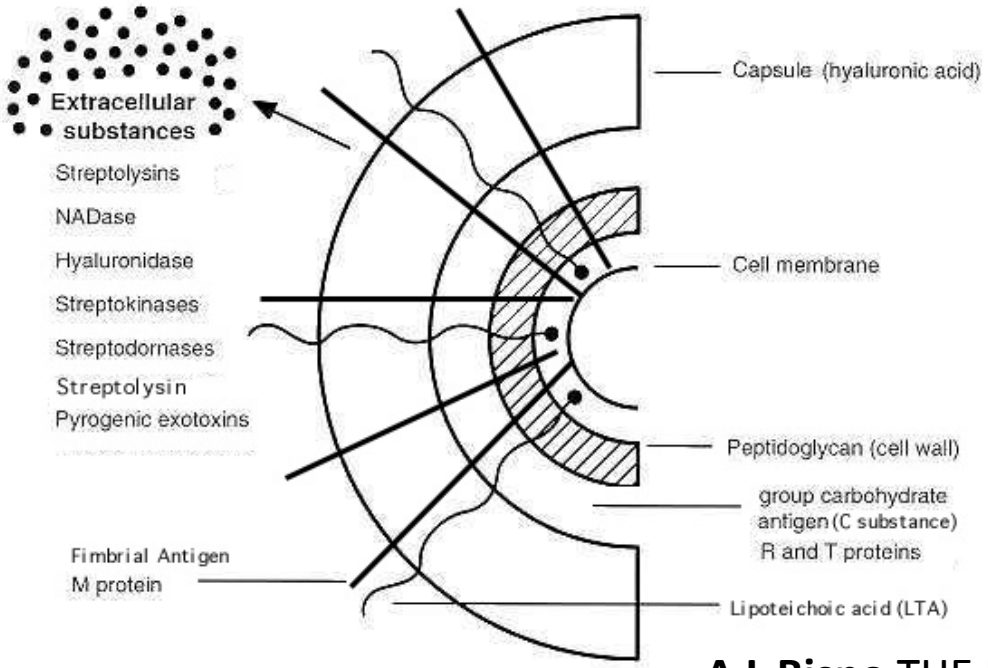
Characterization of GAS in PHLC

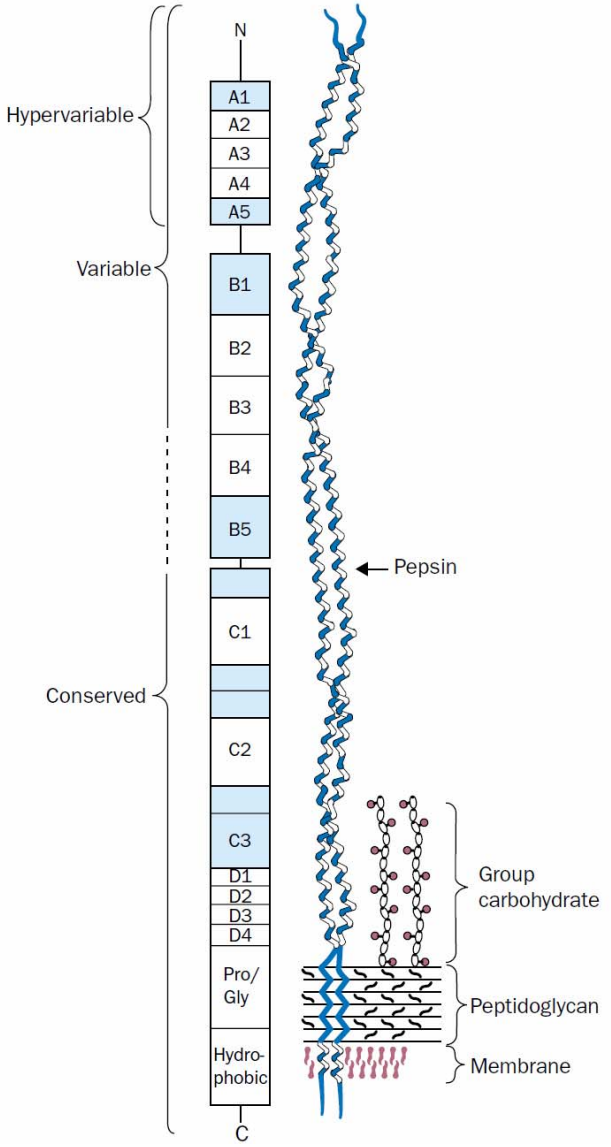
- ID of GAS: Lancefield serogrouping A, PYR positive, (bacitracin sensitive)
- M protein gene (*emm*) typing
- Antibiotic resistance
- Pulsed Field Gel Electrophoresis (PFGE)
- Virulence typing

(Detection of exotoxin, superantigenic genes)



From CDC website:
70,000X magnification electron micrograph of an ultra-thin section of *Streptococcus pyogenes*,





A L Bisno THE LANCET Infectious Diseases 2003

CDC Streptococcus Lab

- ***sof* genes from group A streptococci**
- Historically the anti-opacity factor (AOF) type, conferred by the *sof* (serum opacity factor gene) was equated with M serotype

CDC Streptococcus Lab

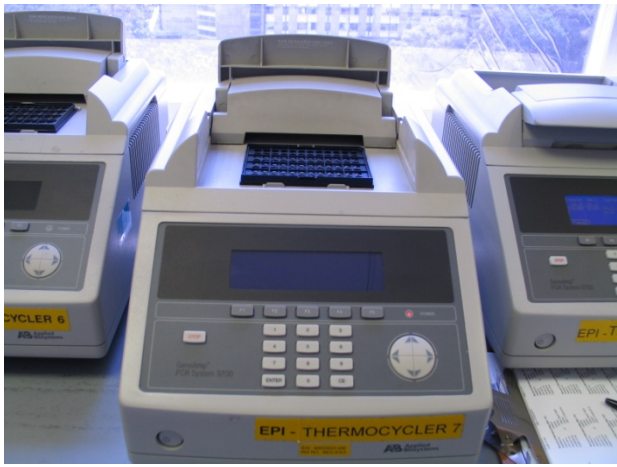
- Lancefield M protein serotyping system over the past 60 years has been very valuable
- In recent years the inherent difficulties encountered in expanding this system through conventional serologic procedures have become increasingly evident
- Using a less demanding sequence based system that is predictive of Lancefield M serotypes, the system established decades ago by Dr. Rebecca Lancefield has been extended

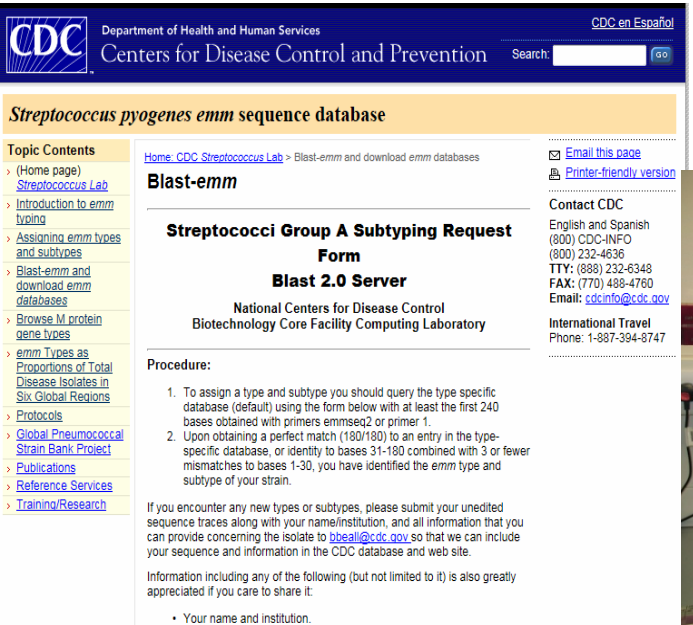
M protein gene (*emm*) typing
CDC

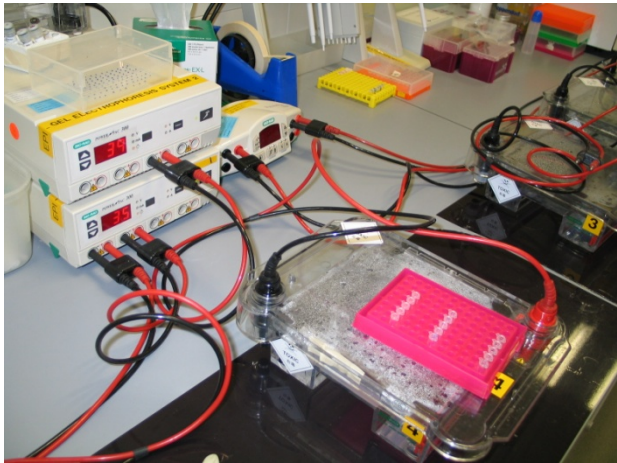
- Problems with M serotyping
  - limited availability of M typing sera
  - Newly encountered M types
  - Difficulty in interpretation
- *emm* typing, relies upon the use of the two highly conserved primers to amplify a large portion of the *emm* gene.
- The hypervariable sequence encoding M serospecificity lies adjacent to one of the amplifying primer sequences, allowing for direct sequencing.

emm typing

- The M protein types of the strains were determined by sequencing of the *emm gene* according to the CDC typing protocol
- BLAST and obtain *emm* type in CDC website

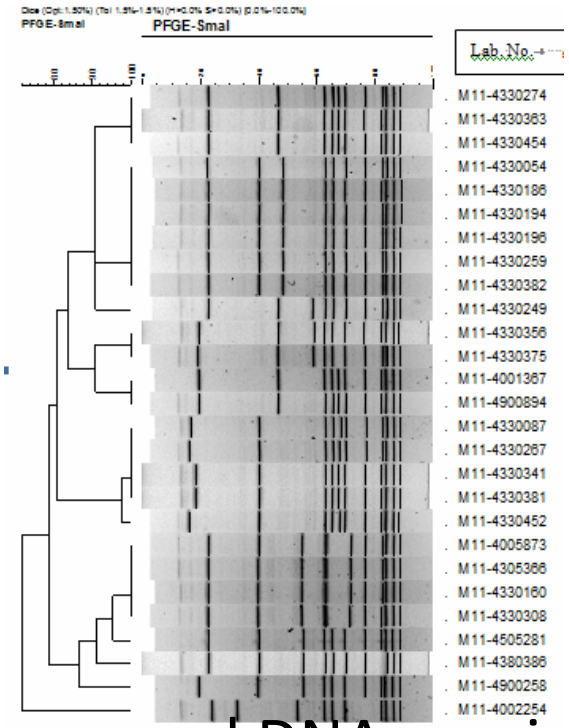






PFGE

(Pulse Field Gel Eelectrophoresis)



- Chromosomal DNA was isolated from bacterial cultures, digested with the *Sma*I restriction enzyme and electrophoresed in 1% pulsed-field certified agarose in a CHEF-DR III system
- For outbreak investigation
- Apply on isolates with same *emm* type

CHP 30 May 2011
A fatal case of scarlet fever investigated

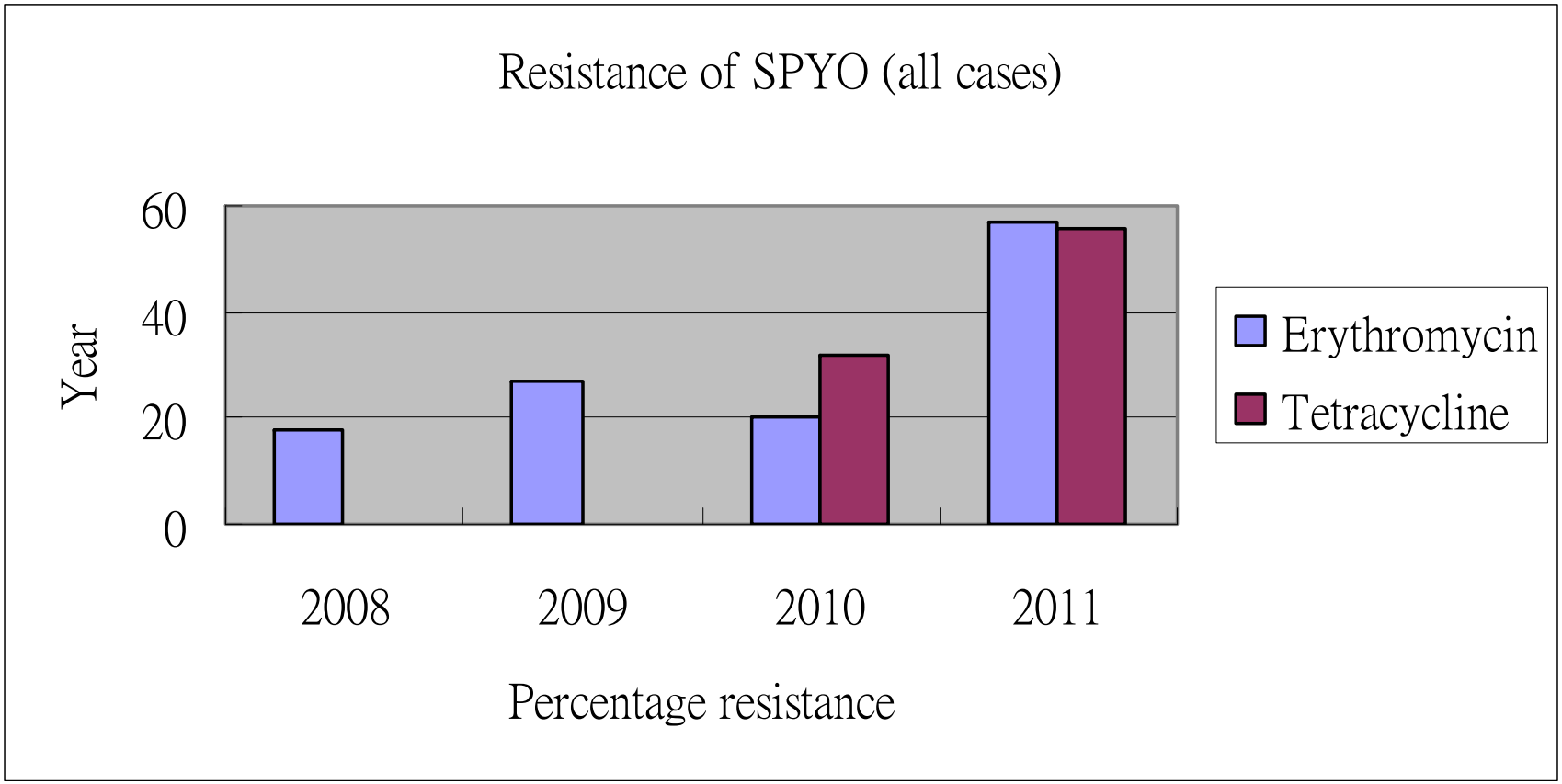
- Seven-year-old girl presented with fever, sore throat, vomiting and skin rash on May 20.
- She consulted a private doctor but her condition deteriorated.
- She was being referred and admitted to hospital on May 27.
- Her condition further deteriorated and complicated with toxic shock syndrome.
- She passed away on May 29.

Fatal case of scarlet fever
emm type 12

Streptococcus pyogenes toxin gene PCRs for the following genes

speA	speC	speH	speI	speJ	speL	spegg	sagA	smeZ	speB	speF	mf2	mf3	ssa

Antibiotic susceptibility of GAS PHLC



Note:
All susceptible to penicillin

Epidemiological analysis of Streptococcus pyogenes infections in Hong Kong

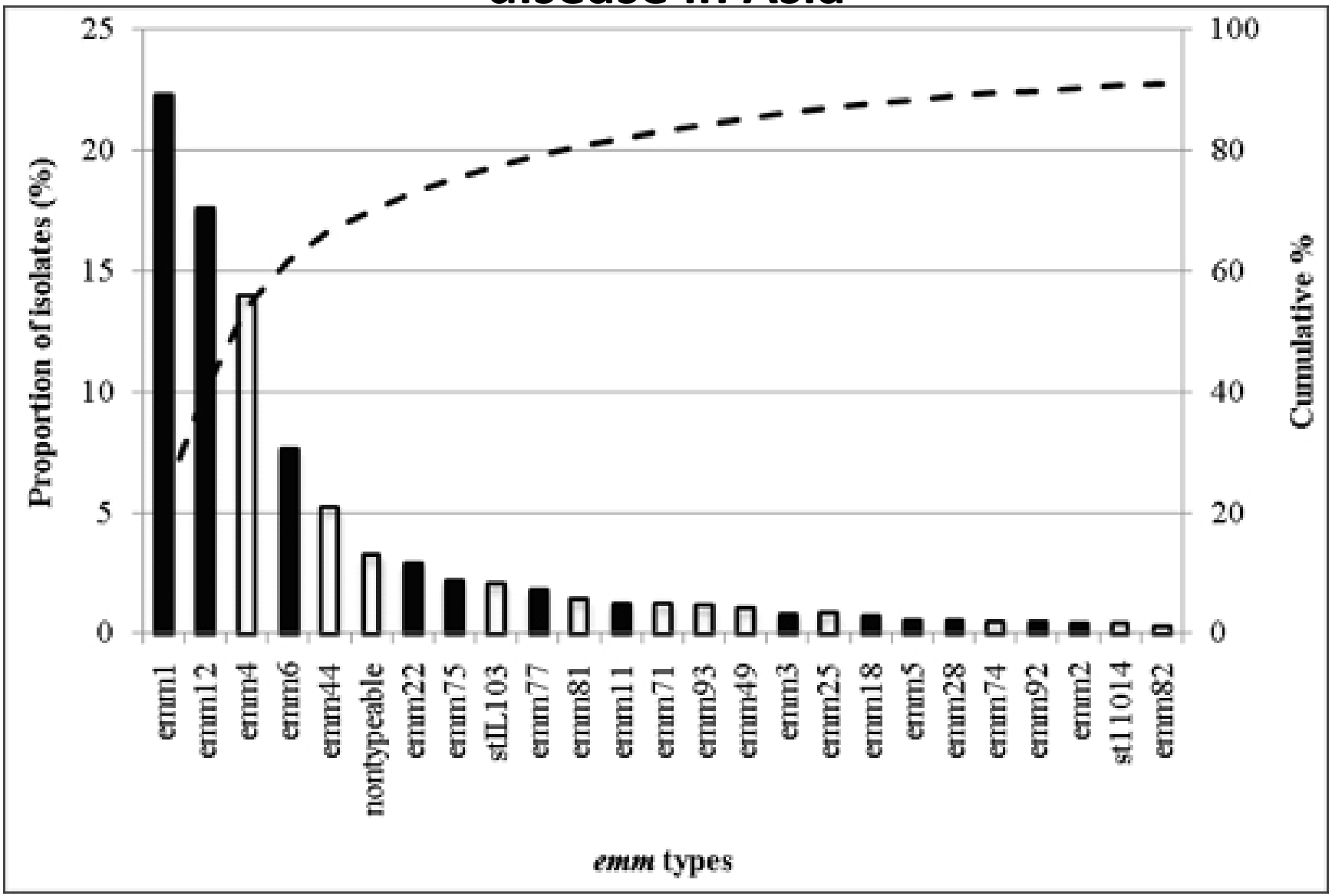
		Invasive		Non-invasive			
emm types	No. isolates	Blood	Tissue or body fluid	Pus or wound	Respiratory tract	Genital	Others
1	60	8	4	6	27	9	6
2	21	1		2	8	9	1
3	1				1		
4	40	1	1	5	14	17	2
9	2			1	1		
10	1				1		
11	4			1	1	2	
12	61	4	2	7	38	9	1
22	16	4		7	4		1
25	1			1			

Chan Janice CK. Pathology (December 2009) 41(7), pp. 681–686

M/AOF/*emm* type[a]	No. (%) of isolates		
	Invasive	Noninvasive	Total
M/*emm*1	8 (33.3)	8 (9.6)	16 (15.0)
M/AOF/*emm*4	3 (12.5)	7 (8.4)	10 (9.3)
*emm*8		1 (1.2)	1 (0.9)
*emm*11	1 (4.2)	1 (1.2)	2 (1.9)
M12	6 (25.0)	16 (19.3)	22 (20.6)
*emm*14		1 (1.2)	1 (0.9)
M18		1 (1.2)	1 (0.9)
AOF/*emm*22	1 (4.2)	2 (2.4)	3 (2.8)
*emm*25		2 (2.4)	2 (1.9)
*emm*27		1 (1.2)	1 (0.9)
AOF/*emm*28		2 (2.4)	2 (1.9)
*emm*39	1 (4.2)		1 (0.9)
*emm*42		1 (1.2)	1 (0.9)
AOF/*emm*49		5 (6.0)	5 (4.7)
M/*emm*53		5 (6.0)	5 (4.7)
AOF/*emm*58		11 (13.2)	11 (10.3)
*emm*63		1 (1.2)	1 (0.9)

Ho PL. JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2003

The 25 most common *emm* types contributing to all disease in Asia



CDC / Steer AC 2009,Lancet Infect Dis 9:611-616.

- 30 May 2011 - A fatal case of scarlet fever investigated
  - Seven-year-old girl presented with fever, sore throat, vomiting and skin rash on May 20. She consulted a private doctor but her condition deteriorated.
  - She was being referred and admitted to hospital on May 27.
  - Her condition further deteriorated and complicated with toxic shock syndrome. She passed away on May 29.
- 13 June 2011 - A severe case of scarlet fever investigated
  - Six-year-old boy presented with sore throat and generalised skin rash since June 1 and fever since June 7.
  - The boy developed septicemia and was transferred to Paediatric Intensive Care Unit on the next day.
- 21 June 2011 - Suspected fatal case of scarlet fever investigated
  - Five-year-old boy presented with fever from June 15.
  - He was admitted to hospital on June 19 for sudden deterioration in condition.
  - The boy developed toxic shock syndrome and passed away ton June 21.
  - According to his parent, the child had consulted a general practitioner for chickenpox earlier.

- Streptococcus pyogenes toxin gene PCR
  → Different for the two fatal cases
- More PFGE on more isolates
  → different patterns

Characterization of S. pyogenes amongst current scarlet fever outbreak

- Using emm typing, PFGE, 48Kb insert, virulence genes (speA, B, C, F, H, ssa)

- Data as at 2011-06-30
  - Number characterized 161
  - emm12 as the predominating type
  - Multiple PFGE patterns and
  - Multiple toxin gene profiles

Messages

- Increased notification of scarlet fever cases in 2011
- Typing of S pyogenes is useful for epidemiology purposes but usually not for clinical management
- Majority of the scarlet fever cases are *emm* type 12
- Further typing revealed multiple patterns even within the most prevalent emm type 12
- Resistance rate to erythromycin has increased as compared to previous years. Currently more than half of S. pyogenes are resistant to macrolides (e.g erythromycins) and clindamycin

My opinion

- If antibiotic is to be given empirically on clinical grounds, do not give macrolides nor clindamycin in view or high rate of resistance from current epidemiological data.
- If evolutionary change has occurred to enhance its resurgence leading to the current epidemic, this change in nature has occurred anyway. No new evidence has yet indicated the mode of transmission of GAS has changed or enhanced which warrants a new mode of prevention strategy, the infection control and prevention strategies should remain unchanged.