

# PCR IN PNEUMOCOCCAL DISEASE DIAGNOSIS (& SURVEILLANCE)

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# Current diagnostic approach

- IPD case definition:
  - ▣ Isolation of *Streptococcus pneumoniae* or detection of nucleic acid from a normally sterile site (blood, CSF, aspirated body fluid).
- Advantage of culture:
  - ▣ Isolate for serotyping & susceptibility; inexpensive
- Disadvantage of culture:
  - ▣ Insensitive and takes longer
- What about nonsterile sites (pneumonia etc.)?
- Is detection of *S. pneumoniae* enough?

# Pneumococcal PCR

- Sensitive, specific, rapid
- Targets
  - ▣ *pyl* – pneumolysin (in *S. pseudopneumoniae*)
  - ▣ *lytA* – autolysin
  - ▣ *pspA* – pneumococcal surface protein
- Sensitivity: <1-~10 cfu/mL; specificity *lytA*>*pspA*>*pyl*
- Multiplex:
  - ▣ other pathogens (e.g. meningitis)
  - ▣ antibiotic R/S genes (e.g *pbp2b*; *ermB*; *mef*)

# Evaluation

- Sterile sites (e.g. CSF, pleural fluid)
  - PCR is now “gold standard”
    - more sensitive (100%) than Gram stain (60%)
    - faster and more sensitive than culture
      - depends on previous antibiotics
    - highly specific
- Nonsterile sites (e.g. sputum, NPA/S)
  - How to distinguish colonisation from infection?
  - ?Alternatives e.g. pneumococcal antigen tests?

# Bacteraemia/pneumonia diagnosis

- Comparison of Binax pneumococcal urinary antigen with *pyl/lytA* PCR on EDTA blood

Condition (N)	Binax +ve (urine)	PCR +ve (blood)
Pneumococcal bacteraemia (58)	51 (sens 88%)	31 (sens 54%)
Non-pneumococcal bacteraemia (51)	2 (spec 96%)	2 (spec 96%)
Community-acquired pneumonia (77)	21 (27%)	6 (8%)

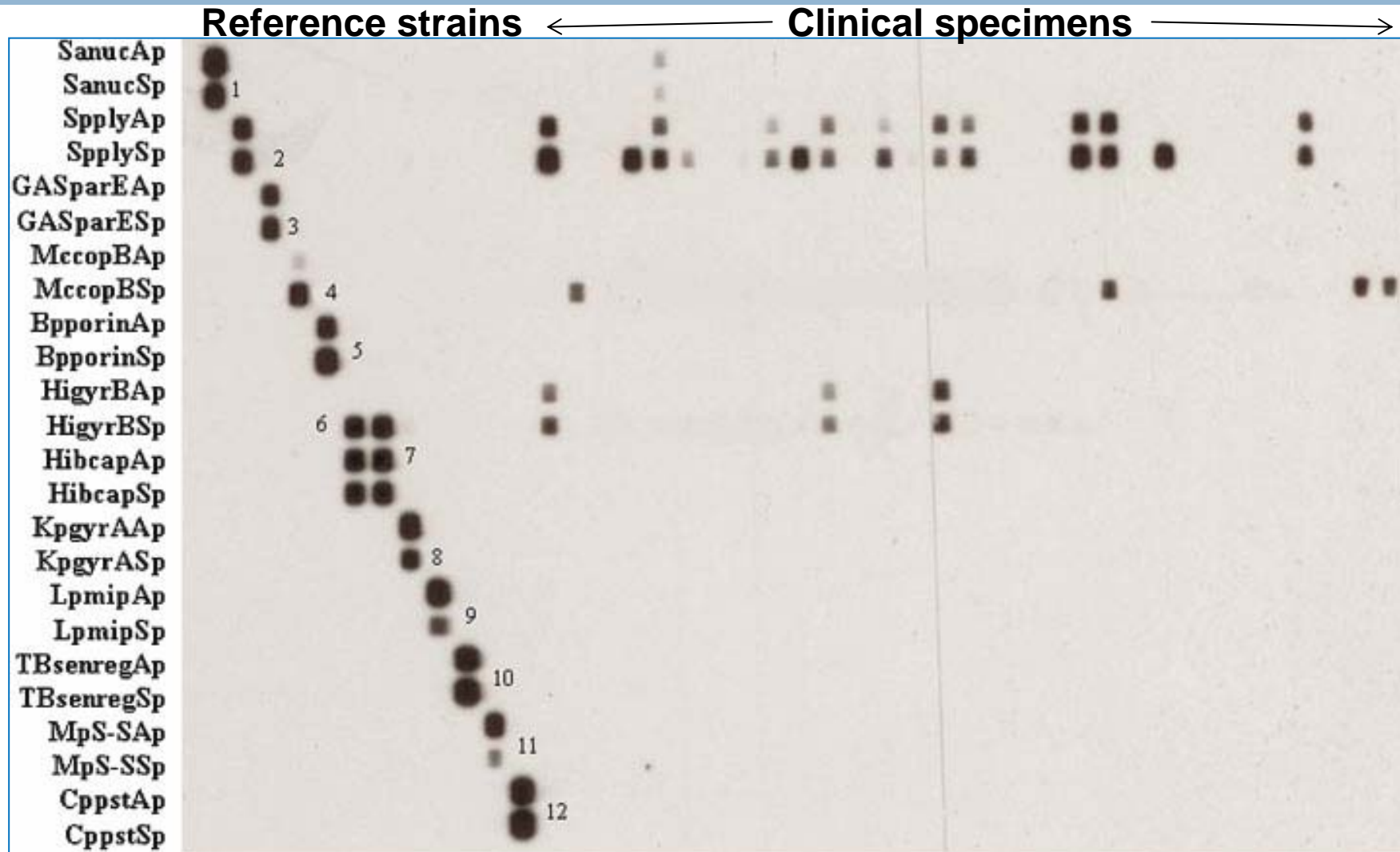
# Nonsterile (respiratory) specimens

Wang et al & Gilbert *Pediatr Pulmonol* 2008;43:150

- 100 NPAs Chinese children <5 yrs; pneumonia
- Most already on antibiotics
- Investigation: culture; mPCR/RLB
  - 12 primer pairs: *S. pneumoniae*; *H. influenzae* (+ Hib);  
*M. catarrhalis*; *S. pyogenes*; *S. aureus*; *B. pertussis*;  
*M. tuberculosis*; *M. pneumoniae*; *C. pneumoniae*;  
*L. pneumophila*; *P. aeruginosa*; *K. pneumoniae*
  - Amplicons - reverse line blot: membrane macroarray

# Results - mPCR/RLB

Wang et al 2008



# Results

Wang et al 2008

- One or more pathogens identified: 65%
  - ▣ Culture – 23% (St pn 13, Hi 5)
  - ▣ mPCR/RLB +ve 63%
    - St pn 52 [alone 23]; Hi 28 [alone 11]
  - ▣ 8 other species (not *L. pneumophila*) identified
  - ▣  $\geq 2$  species 13 specimens
- Advantages: mixed pathogens; sensitive
- Disadvantages: ?colonising vs infecting?
- Suitable for surveys > individual diagnosis

# Real-time quantitative (R-Q) PCR

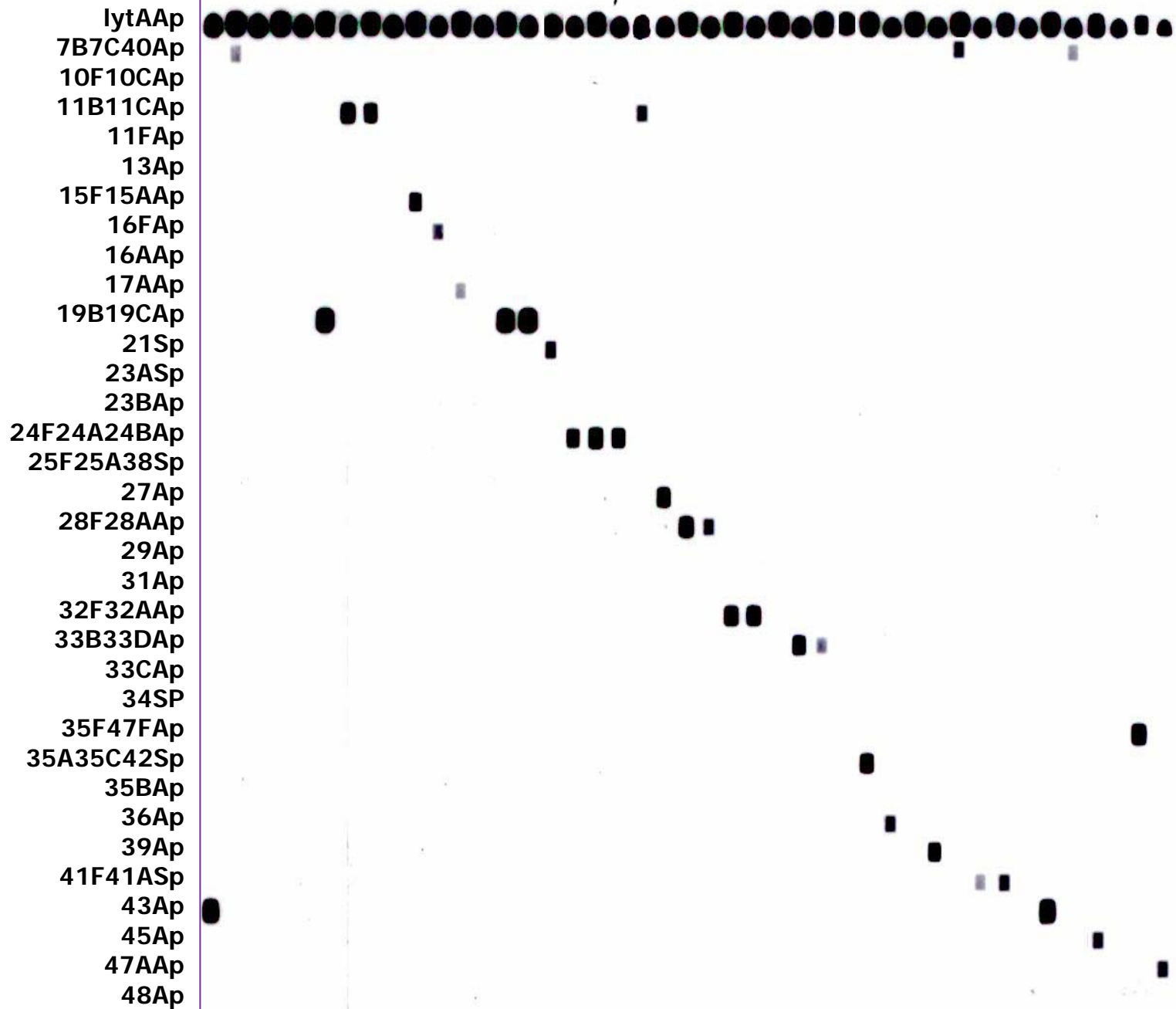
Johansson et al *Diag Microbiol Infect Dis* 2008;60:255-61

- 184 patients - community-acquired pneumonia
- Pneumococcal pneumonia 70 (38%)
  - ▣ **Blood culture 15% (27/179)**
  - ▣ **NPS culture 27% (42/158)**
  - ▣ **Pneumococcal urinary antigen 20% (33/169)**
  - ▣ **Sputum culture  $\geq 10^5$  cfu/mL 15% (19/128)**
  - ▣ **Sputum RQ-PCR  $\geq 10^5$  cfu/mL 27% (34/127)**
    - Of these 24/34 +ve by another method

# Australian Research Network on Empyema (ARNiE)\*

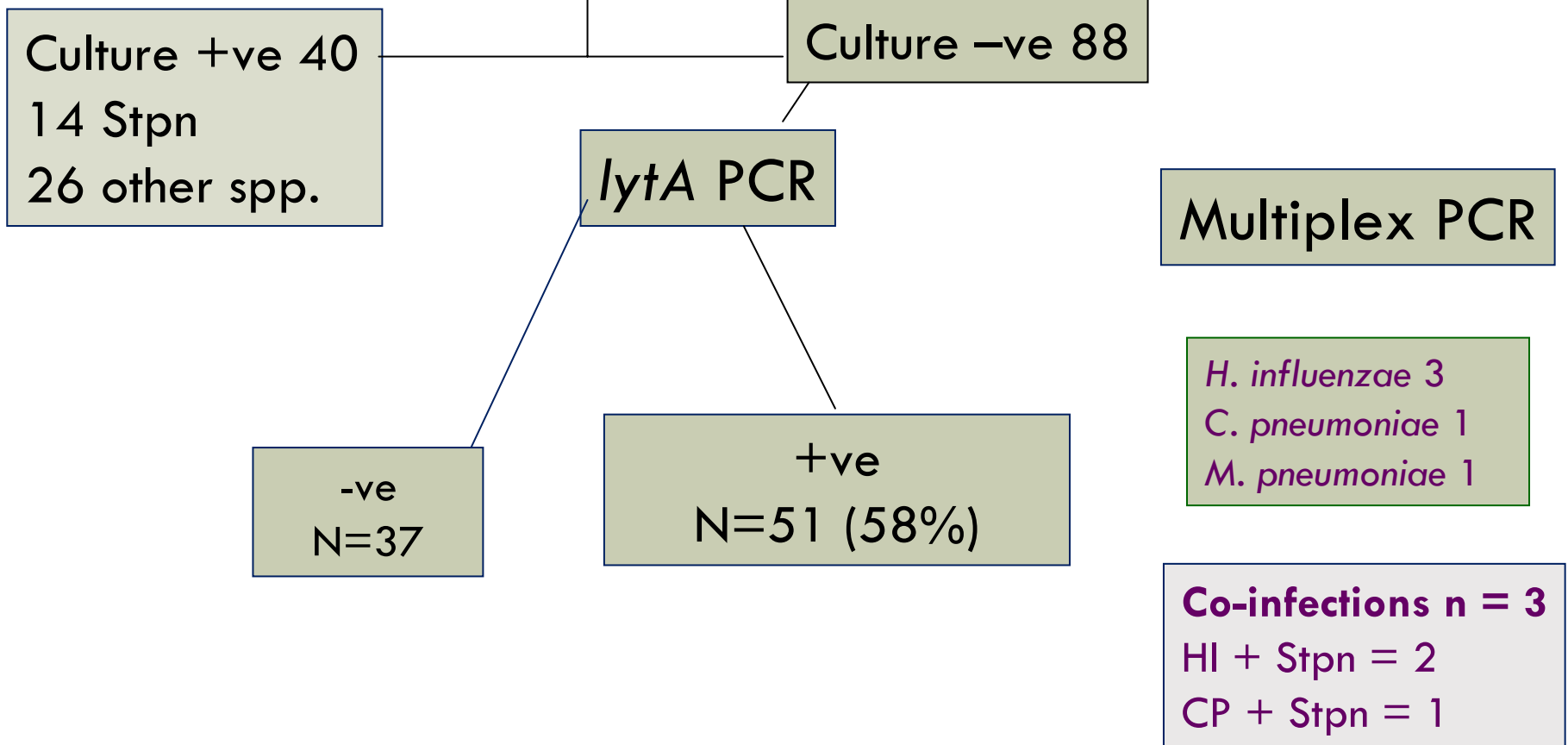
- Aim – causes (incl. pneumococcal serotypes)
- 13 Australian Children's Hospitals study
- Pleural fluid – culture & molecular testing
  - ▣ *lytA* PCR + multiplex PCR for Hi, Mp, Cp
  - ▣ *lytA* +ve – serotype identified
    - mPCR/RLB: 2 sets of primers for all 90 serotypes (+*pyl*)
      - 23 for common serotypes; 34 for less common
      - (32 crossreacting)
      - Amplicons detected by RLB

\*supported by GlaxoSmithKline



# ARNiE – preliminary results

Pleural fluid specimens: N = 128



# ARNiE – preliminary results (summary)

## Positive Stpn result

Culture = 14 (11%)

PCR = 65 (51%)

## Serotype identification

**n = 40+/65 (19A, 3, 7)**

- 33 by mPCR/RLB
- +  $\geq 7$  by *cpsAB/wzg* sequence

# Conclusions

- Pneumococcal PCR sensitive, specific, rapid
- Gold standard for sterile sites
  - ▣ Allows serotype identification
- ? Less sensitive/specific than pneumococcal UA for diagnosis of pneumococcal pneumonia in adults
- mPCR/RLB can identify multiple respiratory pathogens - studies of pneumonia aetiology

# Acknowledgements

## **CIDM**

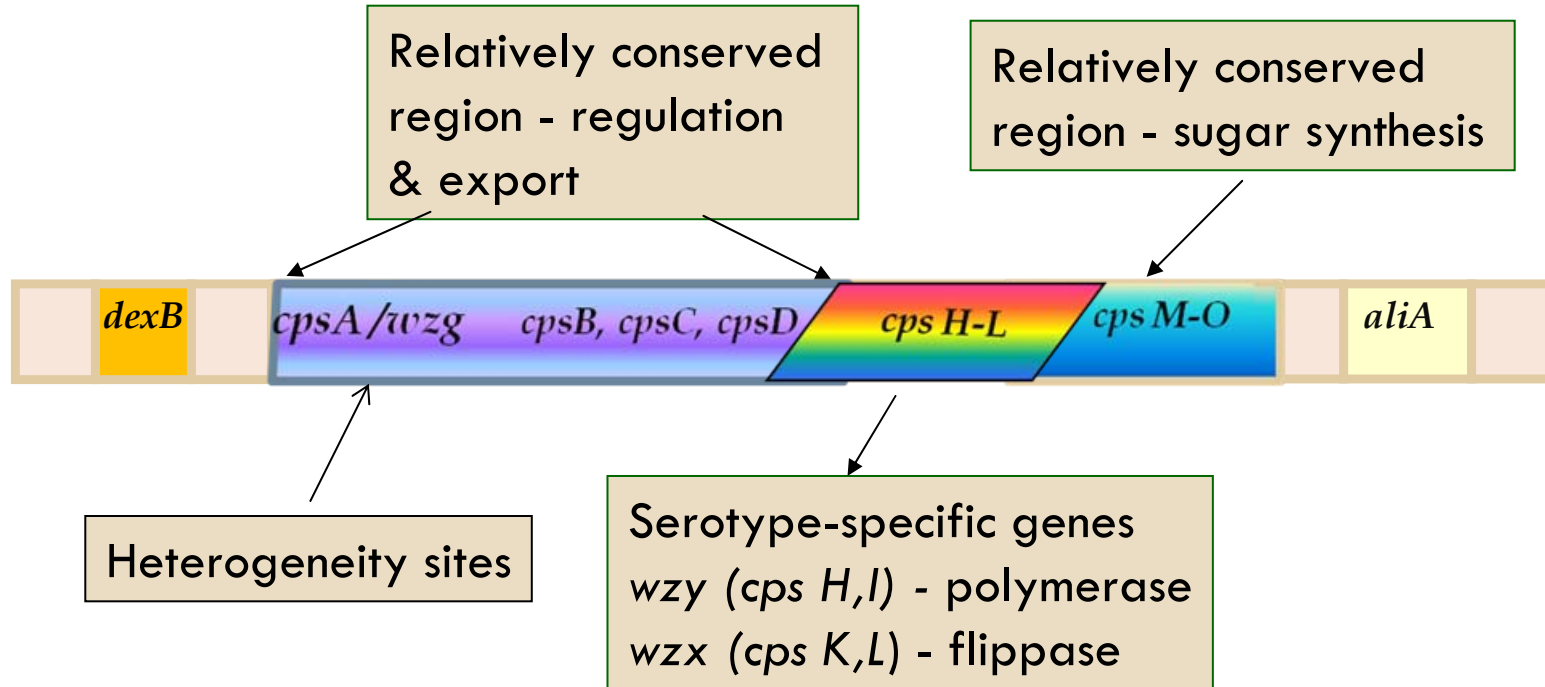
- mPCR/RLB: Fanrong Kong
  - Serotype identification: Fei Zhou
  - Respiratory pathogens: Yajuan Wang (Beijing CH)
- Empyema PCRs – Julia Warning, Kiran Thapa
- Serotyping – Shahin Oftadeh

## **Collaborators**

- ARNiE study – Adam Jaffe, Roxanne Strachan

# Pneumococcal *cps* cluster

## – serotype identification



- Cassette structure - flanked by *dexB*, *aliA*
- Relatively conserved genes at each end but variable in the middle
  - *cps A-D* all serotypes; *cps M-O* most serotypes
  - *cps H-L* serotype specific (exc serotype 3)