Whole genome sequencing & new strain typing methods in IPC
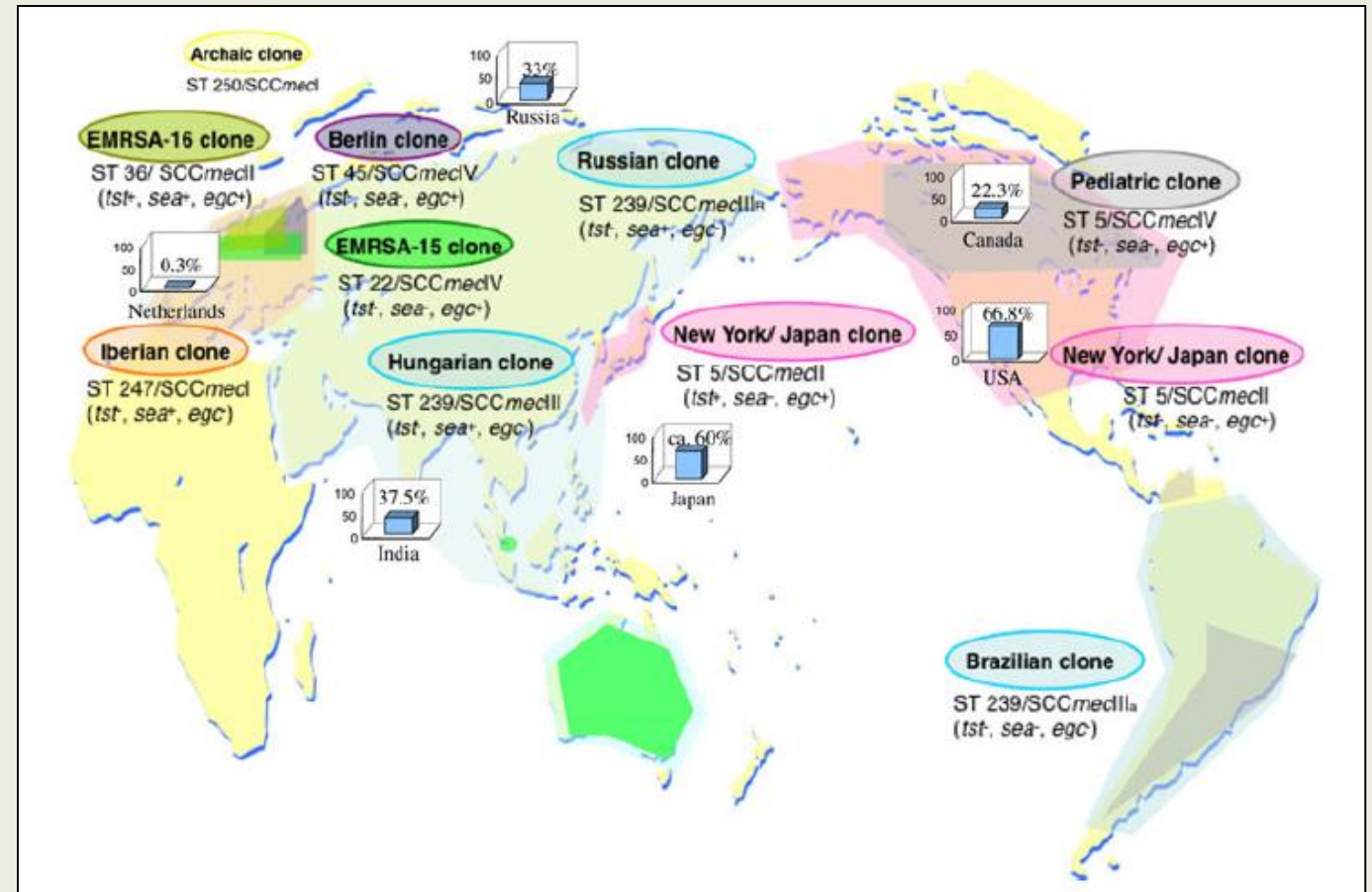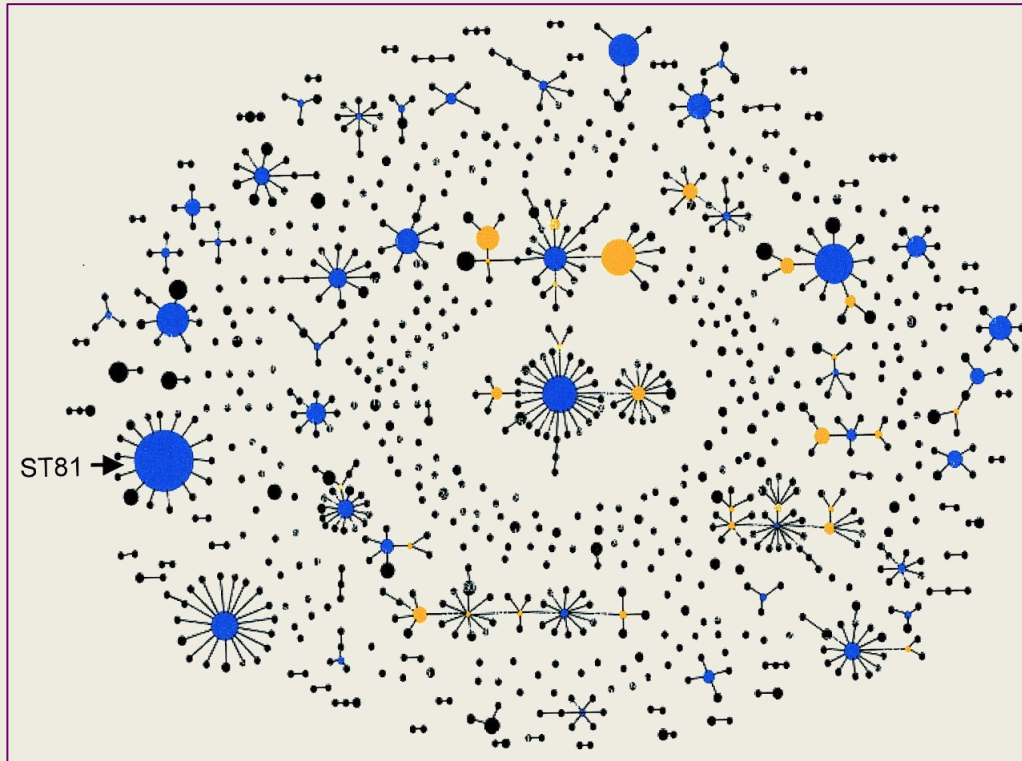
Lyn Gilbert

ACIPC conference Hobart,

November 2015
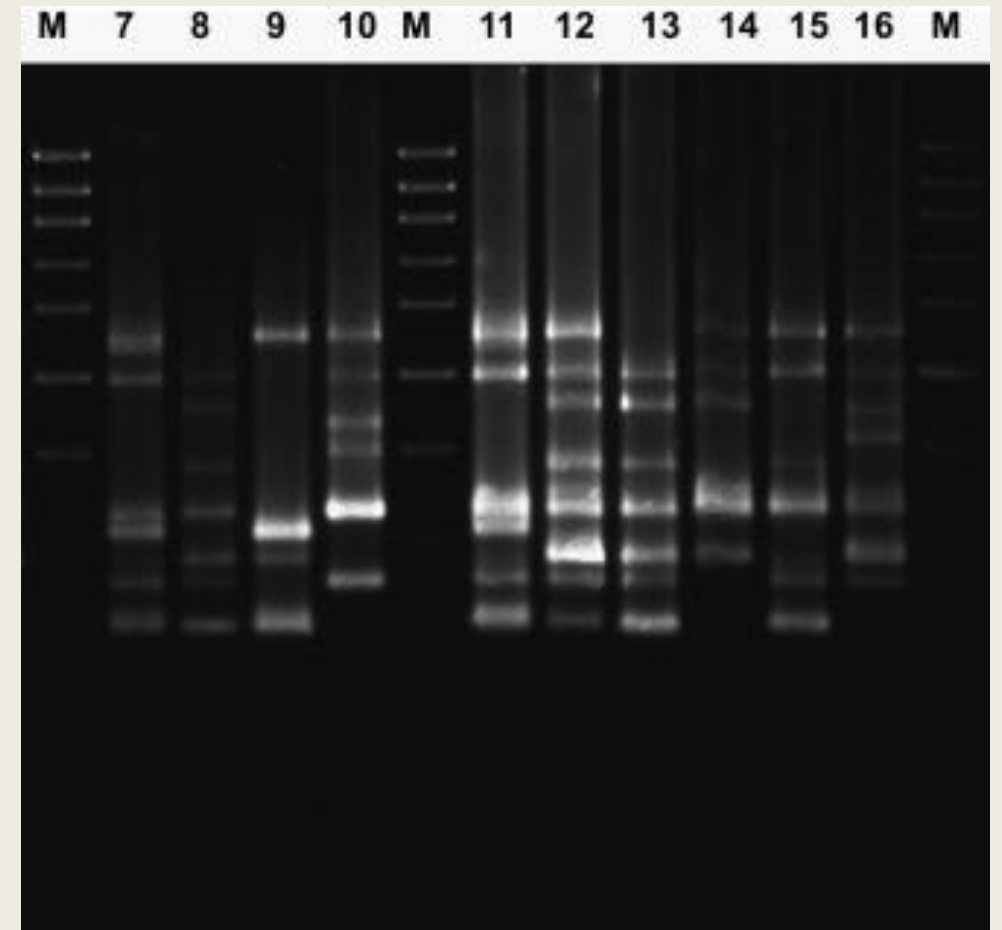
Why do strain typing?

- Evolution, population genetics, geographic distribution

Why strain typing?

- Track prevalence, distribution of "epidemic" strains
- e.g: *C. difficile* ribotype 027

Why strain typing?

- Detect toxin gene e.g: PVL in *S. aureus*
  - management e.g. add clindamycin (antitoxin activity)

Review

Pragmatic management of Panton–Valentine leukocidin-associated staphylococcal diseases

Y. Gillet[a], O. Dumitrescu[b,c,d], A. Tristan[b,c,d], O. Dauwalder[b,c,d], E. Javouhey[a], D. Floret[a], F. Vandenesch[b,c,d], J. Etienne[b,c,d], G. Lina[b,c,d,*]

[a] Division of Pediatric Intensive Care, Hôpital Femme Mère Enfant, Bron, France
[b] Université de Lyon, Centre National de Référence des Staphylocoques, Lyon, France
[c] INSERM U851, IFR128, Lyon, France   International Journal of Antimicrobial Agents 38 (2011) 457–464
[d] Hospices Civils de Lyon, Lyon, France

Strain typing in infection control

Patient develops MRSA bacteraemia, 2 weeks post admission

- No admission screening.
- Prevalence of MRSA colonisation in ward is 20%.
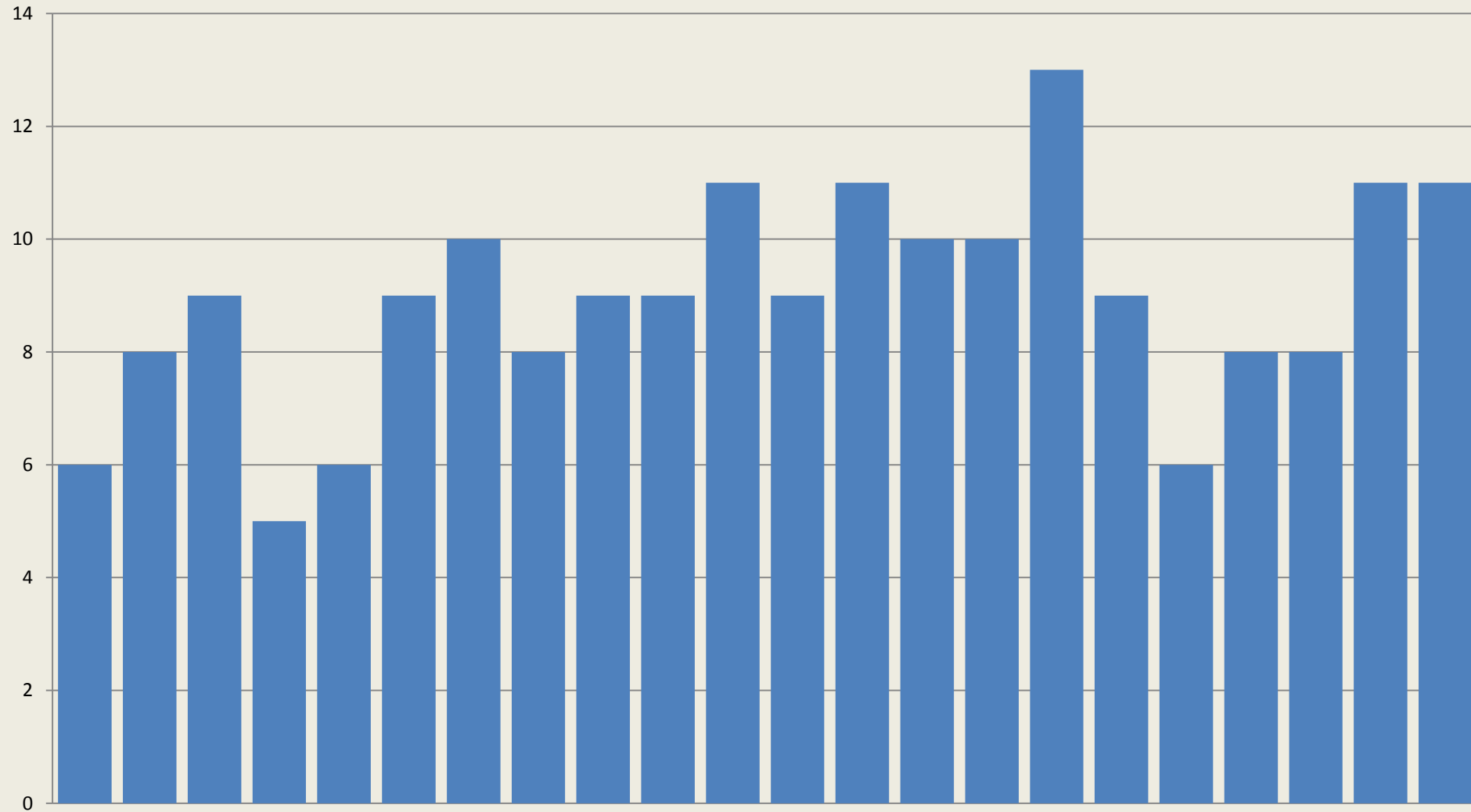- ?Hospital or community-acquired ?source patient
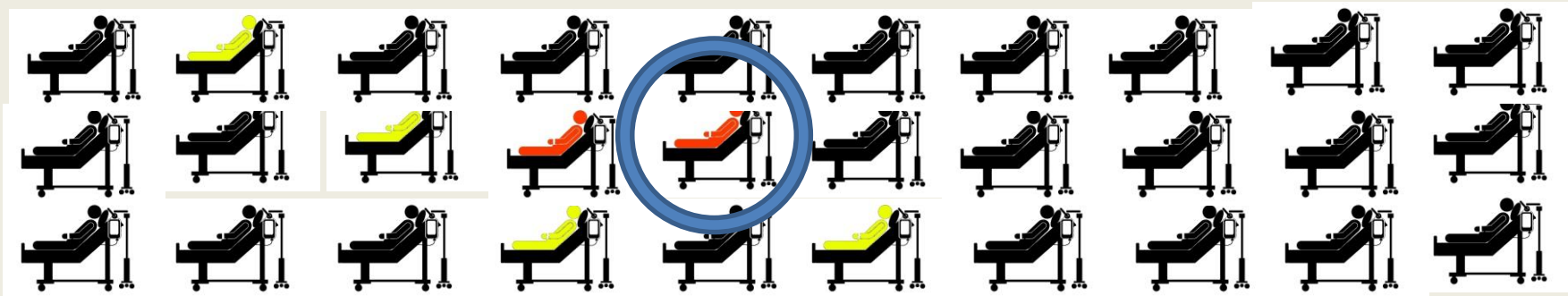
Identify outbreaks and transmission events:

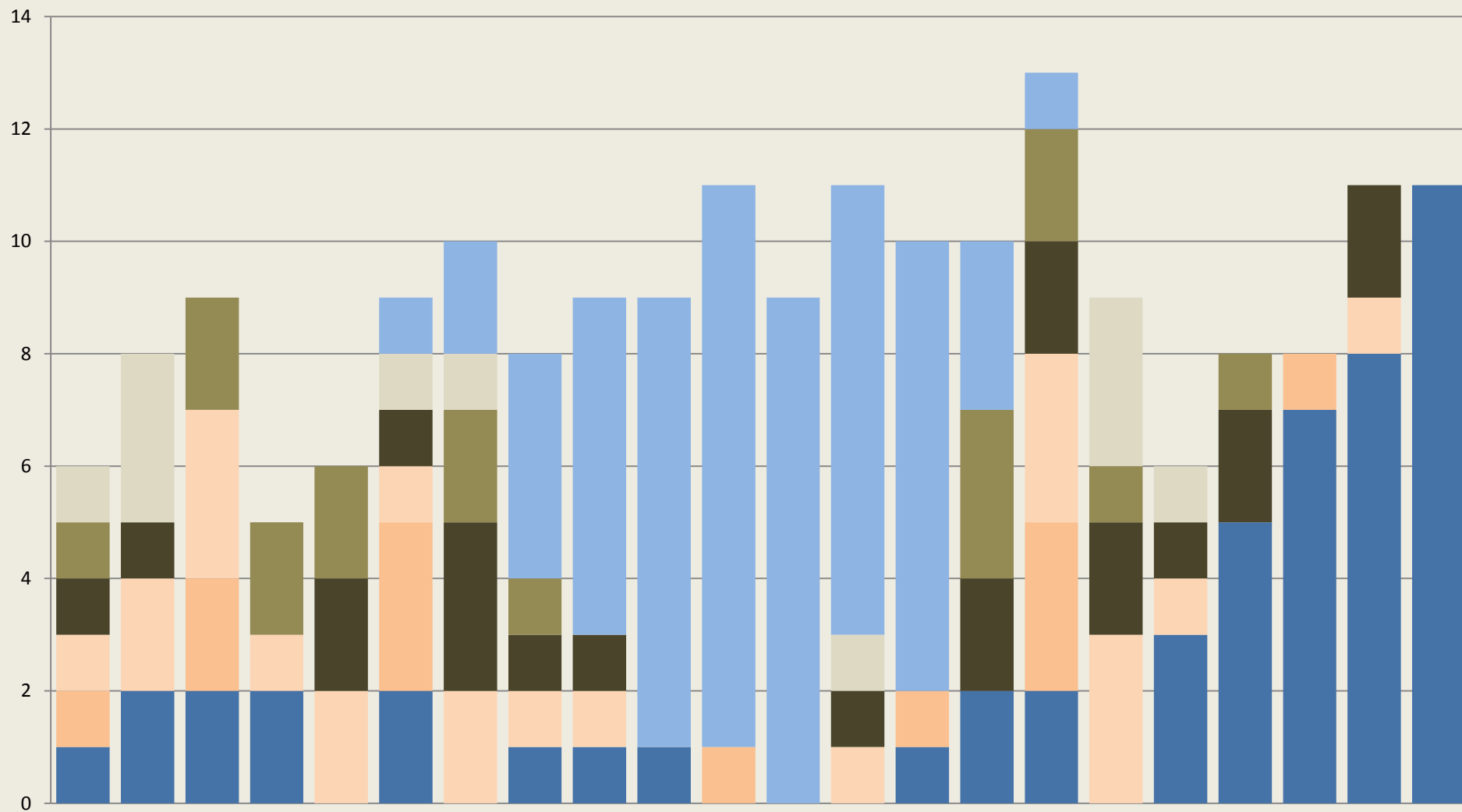– Rapid feedback to clinicians; increase engagement

Prospective Strain Typing

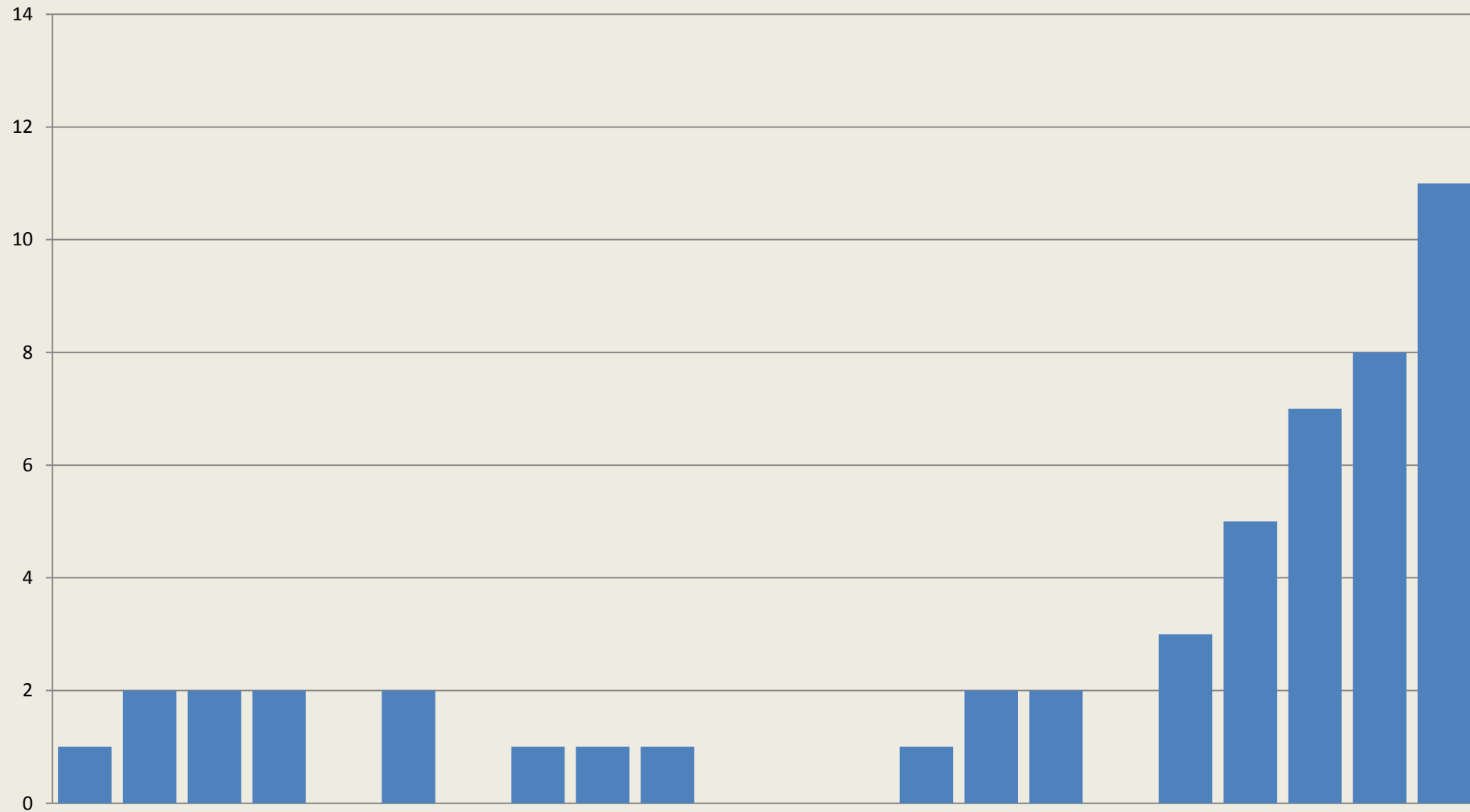**New MRSA isolates per week for hospital inpatients**

Prospective Strain Typing

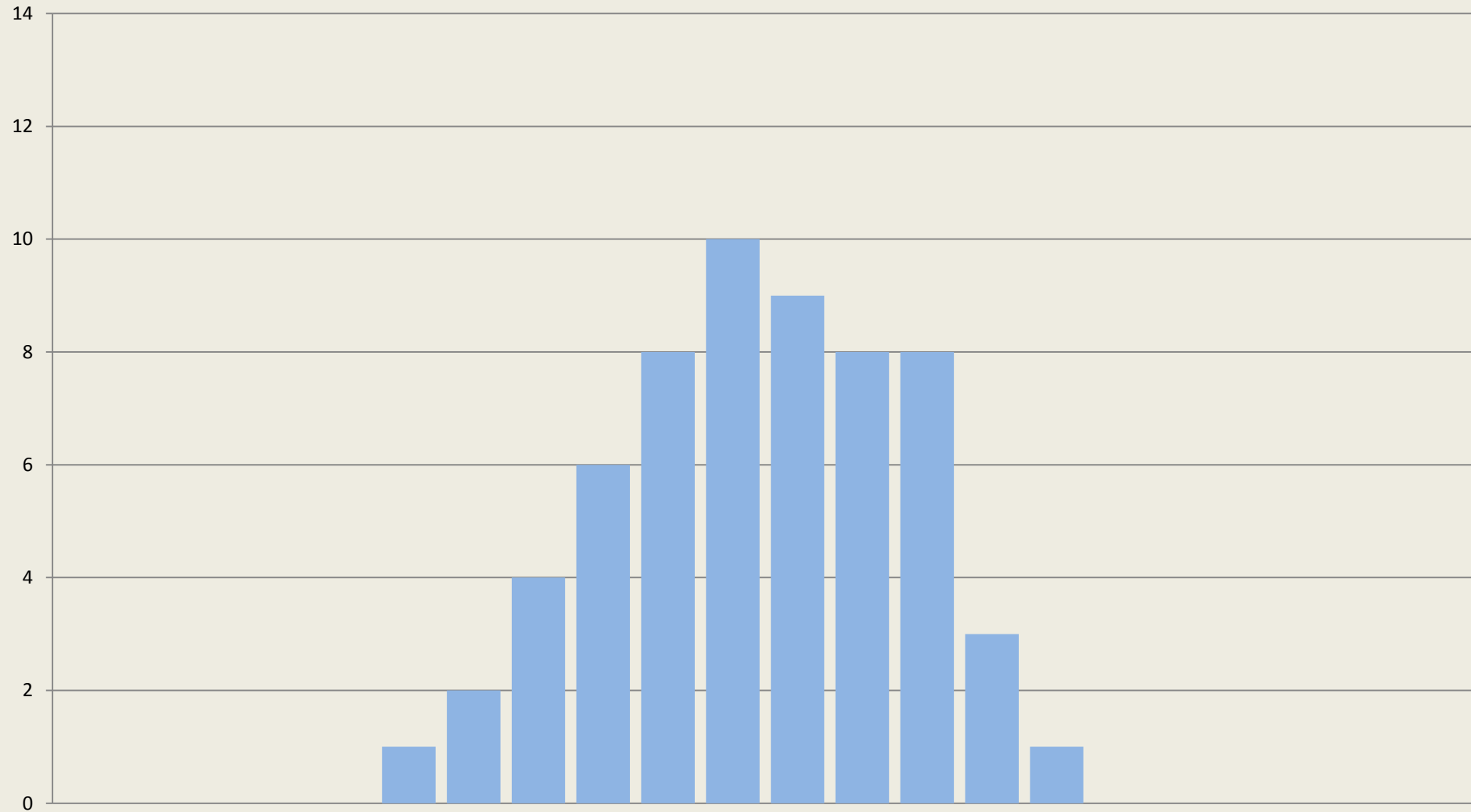**New MRSA isolates per week for hospital inpatients**

Prospective Strain Typing

New Strain A MRSA isolates per week for hospital inpatients

Prospective Strain Typing

New Strain G MRSA isolates per week for hospital inpatients

Pulsed field gel electrophoresis (PFGE)

- Extract DNA; digest with restriction enzyme
- Different sized fragments; separate on a gel

Image from www.xyz.co.uk
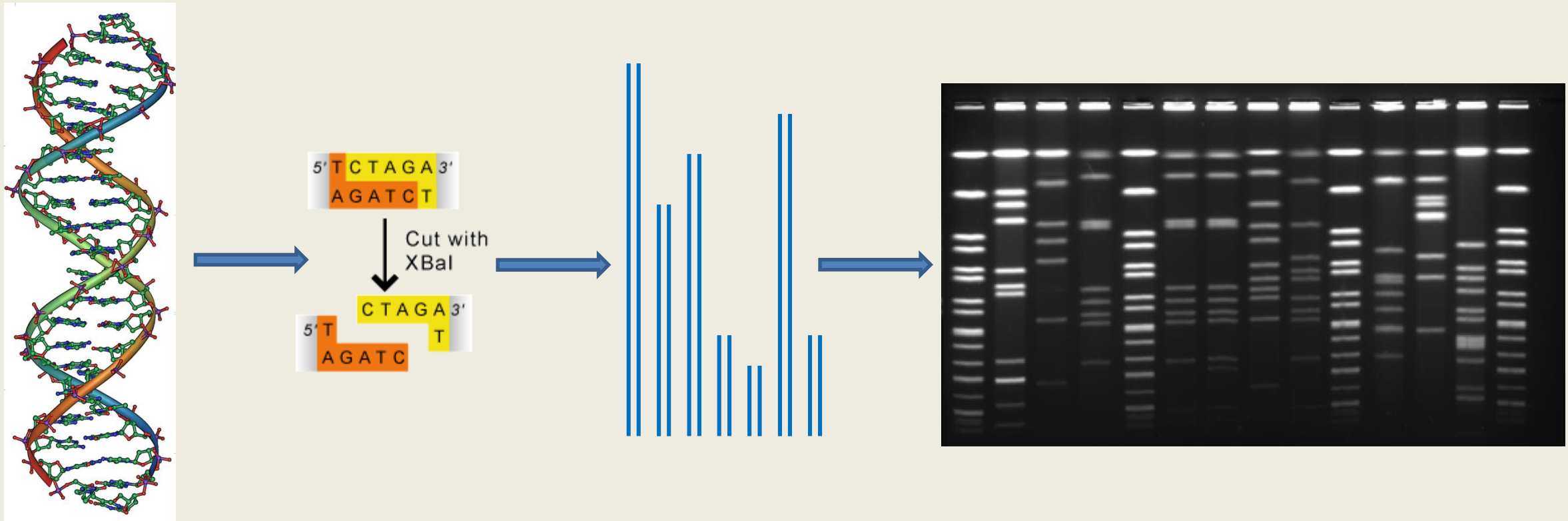
Pulse field gel electrophoresis (PFGE)

- Advantage: highly discriminatory
- Disadvantages
  - Labour intensive
  - Expensive (>$100 per isolate)
  - Low-throughput (10-12 isolates)
  - Turnaround time ~4-5 days
  - Interpretation subjective
  - Reproducibility poor

Binary typing using mPCR/RLB

- Multiplex PCR- reverse line blot assay
  - (mPCR/RLB)
- Up to 43 targets in single mPCR
  - products identified by attachment to probes on a membrane
- 43 isolates per membrane
- Inexpensive (consumables ~$2)
- Rapid TAT ~10 hours
- Reproducible; easy to interpret
- Results can be shared

Kong & Gilbert 2006. Nature Protocols, 1:2668
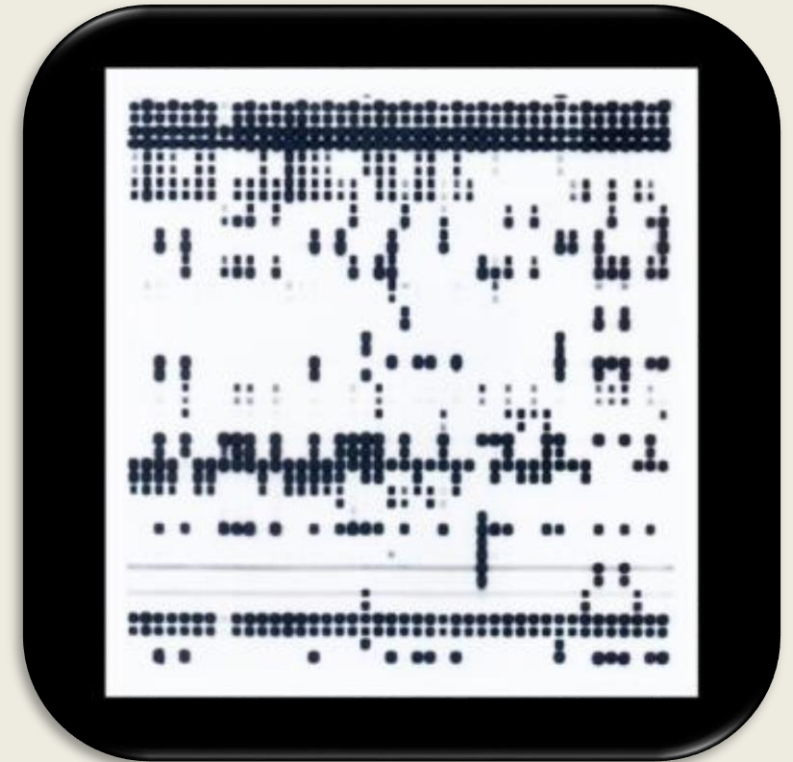
MRSA binary typing system

- High discriminatory power
  - comparable with PFGE
- 19 targets
  - 4 toxin genes incl. **pvl**
  - 9 phage-derived open reading frames
  - 6 SCC*mec* elements
  - *nuc* and *mecA* as controls
- Strain type infers MLST
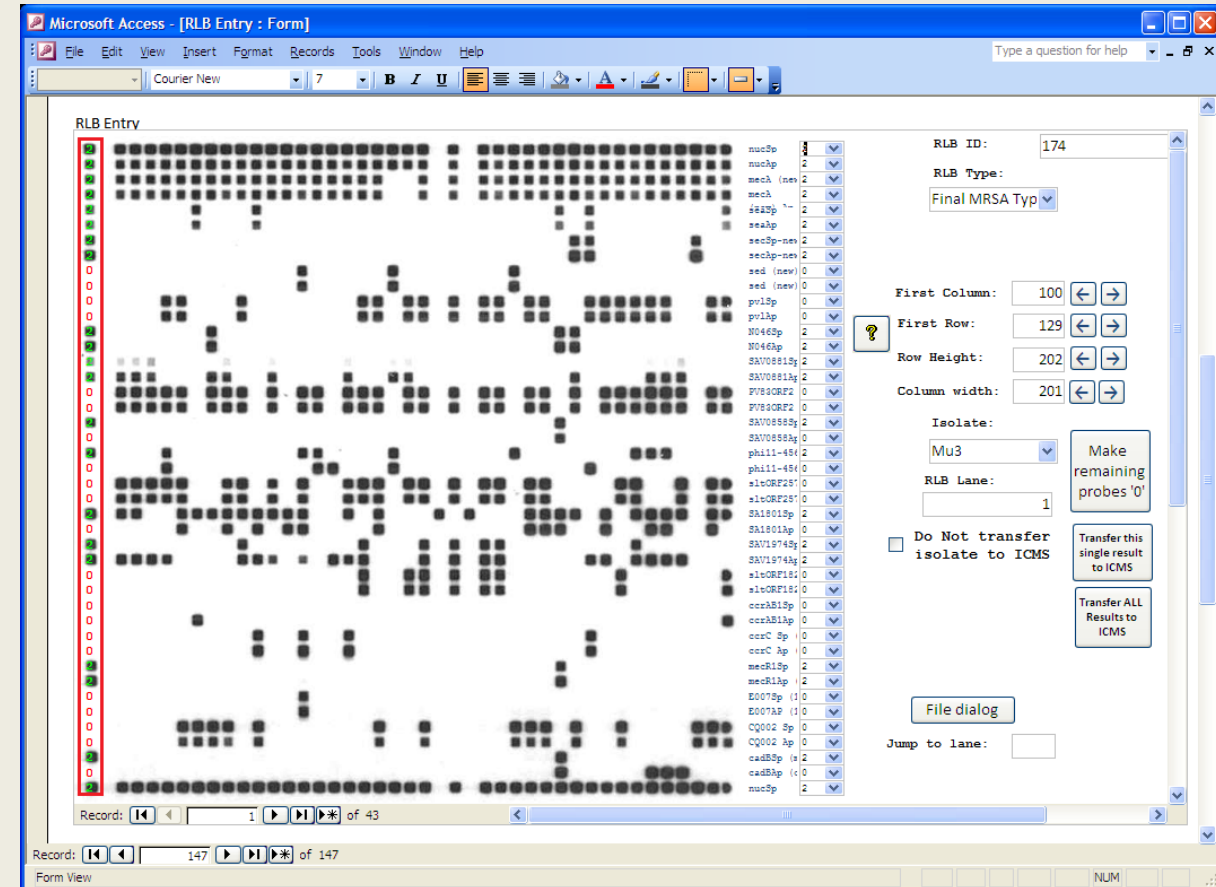
Strain typing to understand MRSA transmission

- 3 surgical wards: high colonisation/infection rate
- MRSA point prevalence survey
  - patients screened
  - environmental swabs
    - after "terminal" cleaning of isolation rooms

■ Patient Isolates

● Environmental Isolates

✤ Staff Isolate

15
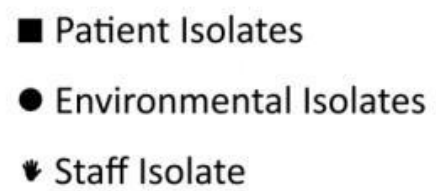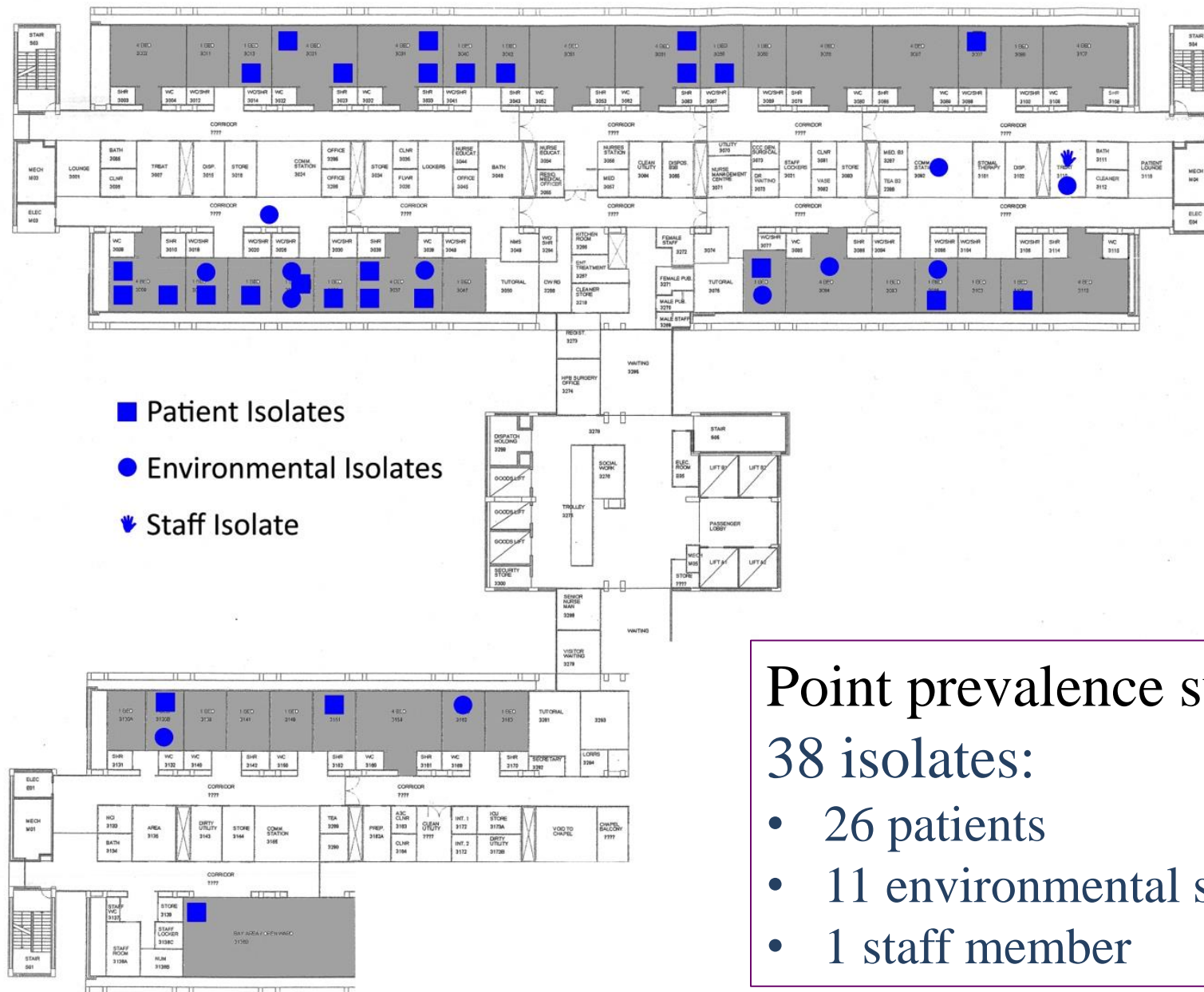
Point prevalence survey

38 isolates:

- 26 patients
- 11 environmental swabs
- 1 staff member

■ Patient Isolates

● Environmental Isolates

♥ Staff Isolate
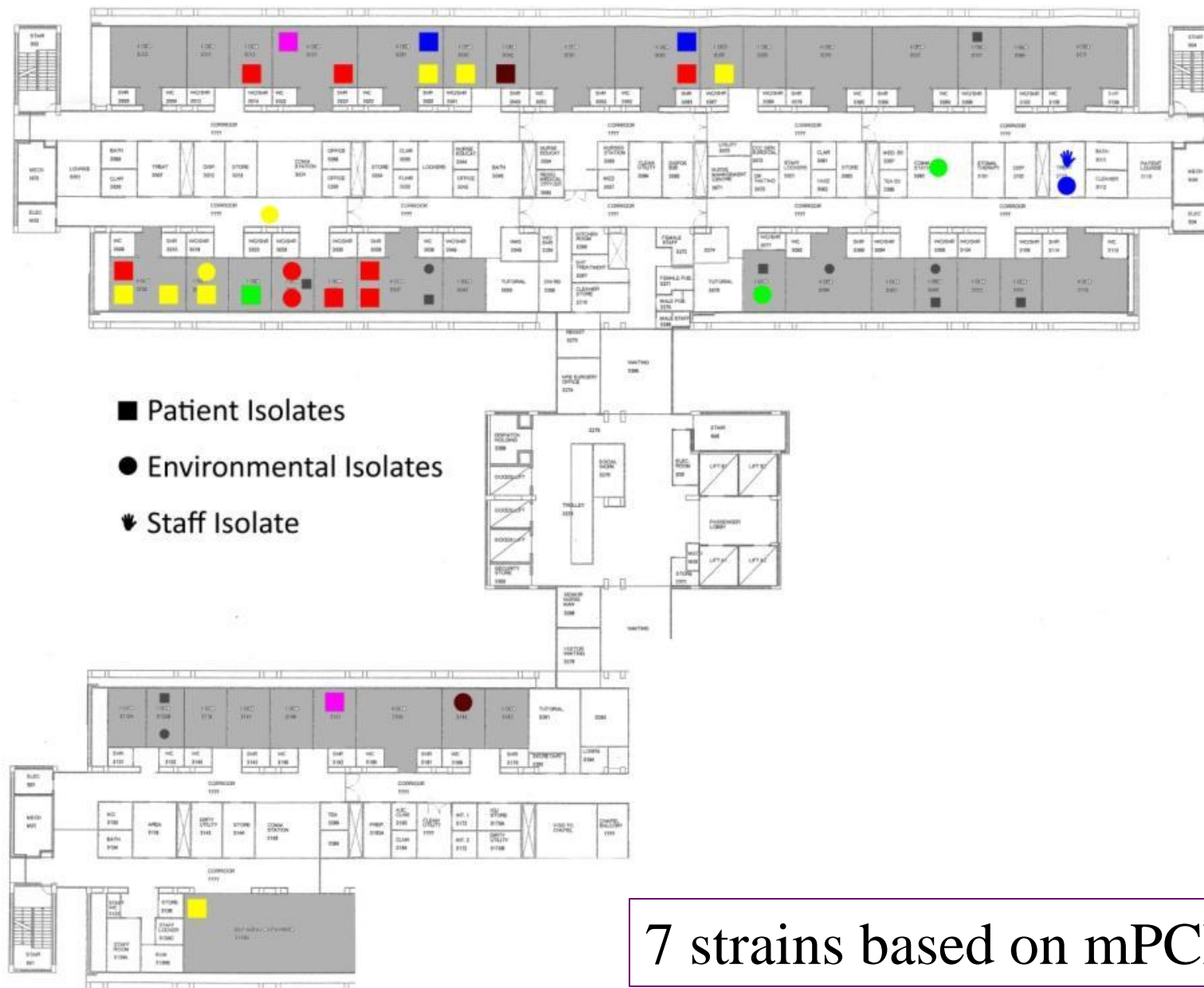
7 strains based on mPCR/RLB

17

■ Patient Isolates

● Environmental Isolates

♛ Staff Isolate

■ Patient Isolates

● Environmental Isolates

✋ Staff Isolate
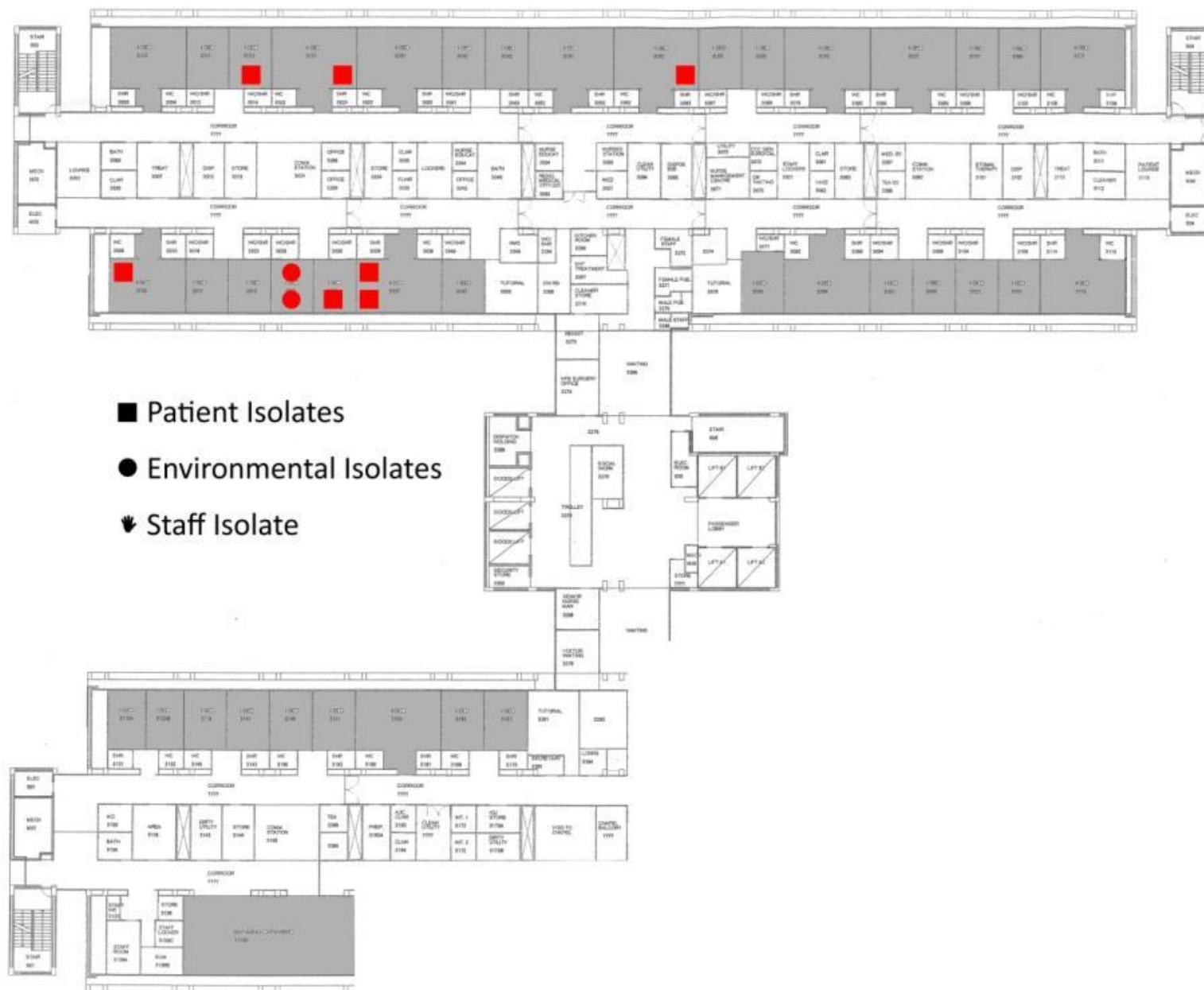
Interventions to reduce MRSA prevalence

- Strain carried by patients = environmental strains
  - e.g. hand sets
- Infection control interventions:
  - hand hygiene
  - enhanced environmental cleaning (vaporised H_2O_2)
  - video-reflexive ethnography
- Subsequent decrease in MRSA colonisation rates
- Binary typing - macro- & microepidemiology

Serial MRSA point prevalence surveys

Point prevalence surveys in 3 surgical wards by multilocus sequence type (MLST)

No. of patients

Vapourised H_2O_2

Videoreflexive ethnography

- 38% (11/3/13)
- 13% (15/7/13)
- 17% (4/11/13)
- 7% (19/3/14)
- 17% (10/9/14)
- 10% (14/4/15)

Legend:
- 239
- 22
- 45
- na
- 73
- 93
- 20
- 5
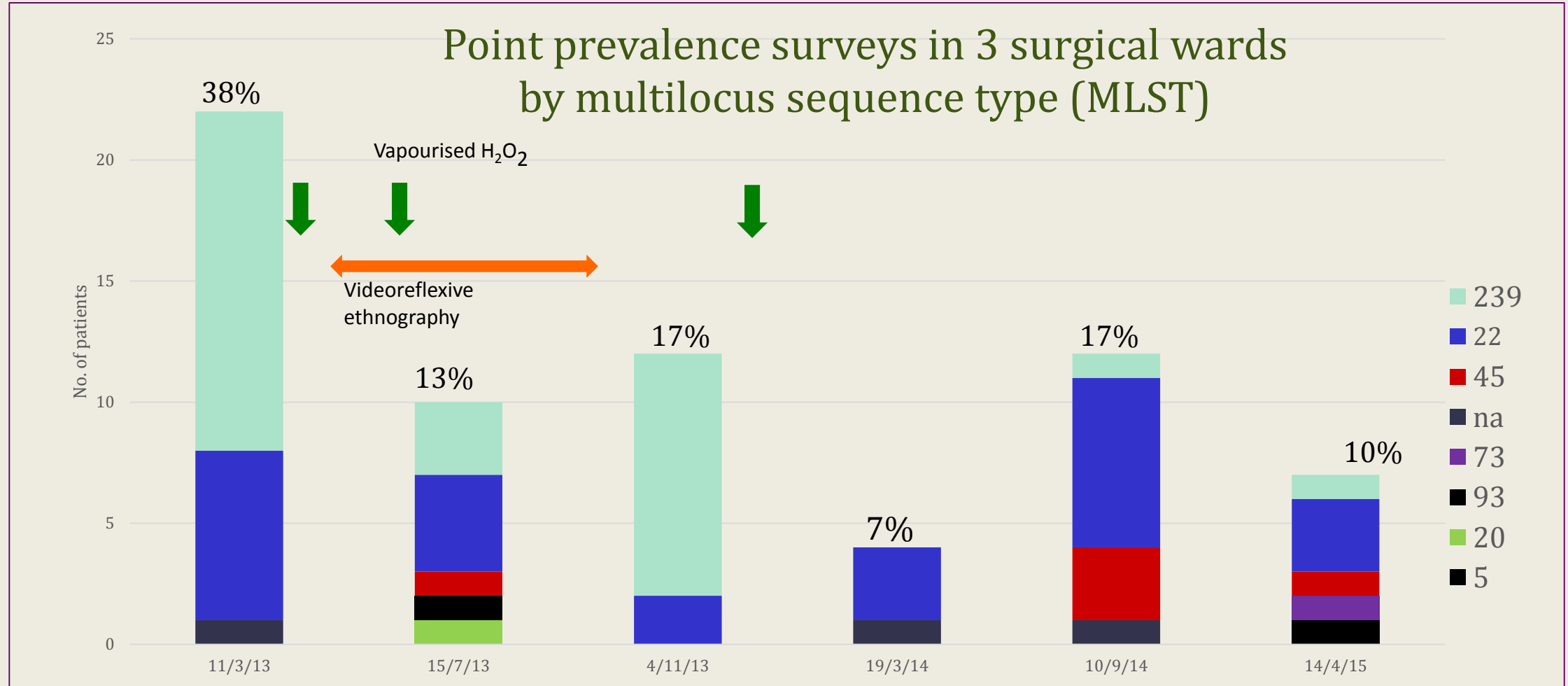
Sequence based typing

- Sequencing of one/several genes or whole genome
- Highly reproducible
  - amenable to sharing via databases
- Becoming less expensive and labour intensive
- Discriminatory power depends on....
  - which genes; how many

Multilocus sequence typing

- 7 "housekeeping" genes
  - macroepidemiology
  - not discriminatory enough for outbreak investigation
- Each product - allele number
  - comparison with known sequences in the MLST database
- Combination of 7 allele numbers = MLST

Whole genome sequencing

- 1st bacterial genome sequenced 1995 *Haemophilus influenzae*
    - = 1.8 megabase pairs (Mbp)
    - Cost >$1,000,000
    - Time taken >1 year

- *S. aureus. E. faecium* ~3 Mbp
- *E. coli; K. pneumoniae* 4.5-6 Mbp

- WGS: <$100 per isolate
    - ~many isolates; hours-days
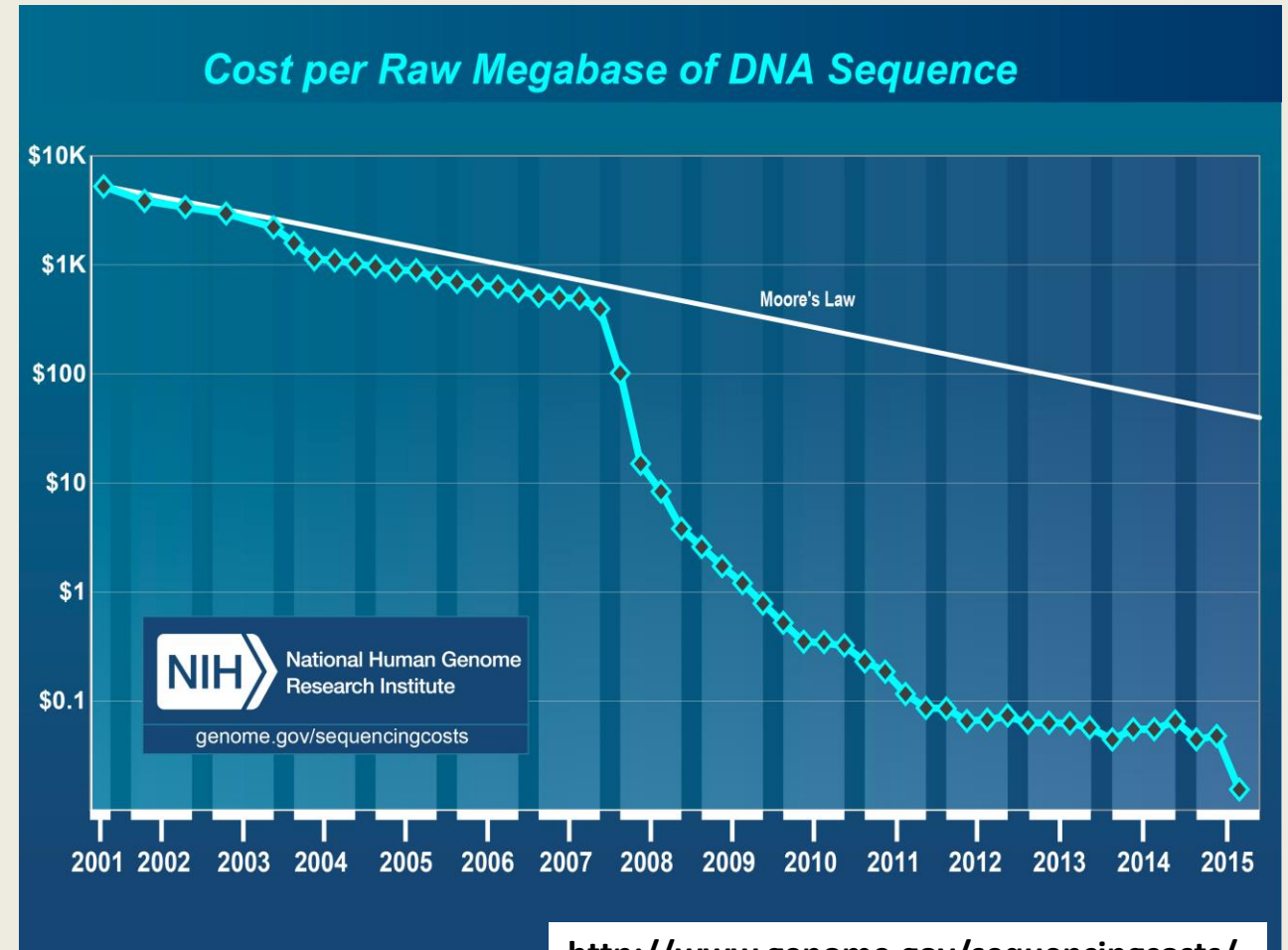
http://www.genome.gov/sequencingcosts/

WGS for outbreak investigation?

1. Discriminatory power
    = ability to distinguish unrelated isolates
    • more DNA examined more easily isolates distinguished

WGS for outbreak investigation?

1. Discriminatory power
 = ability to distinguish unrelated isolates

PFGE:
0.01% of
genome
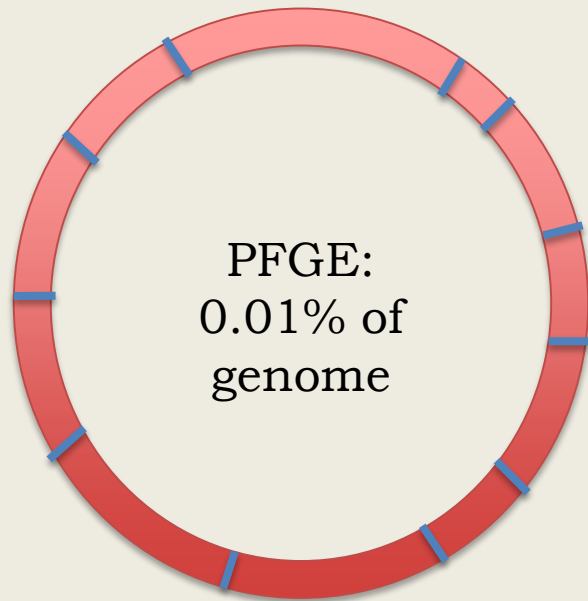
No useful genetic data

WGS for outbreak investigation?

1. Discriminatory power
= ability to distinguish unrelated isolates

PFGE:
0.01% of
genome

Binary
typing:
0.1% of
genome
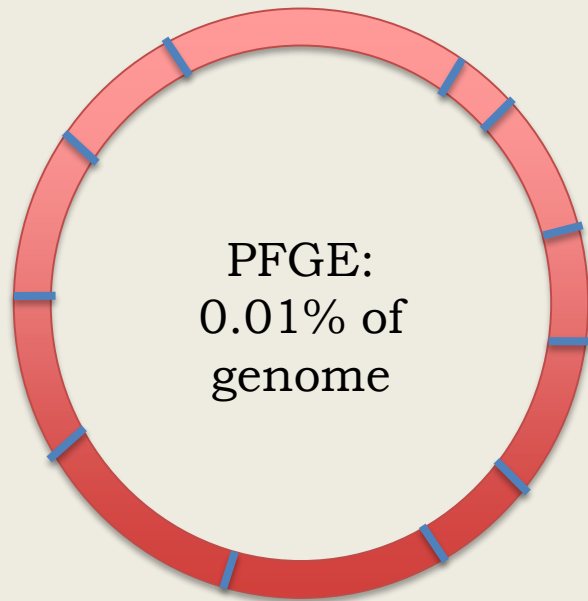
No useful genetic data
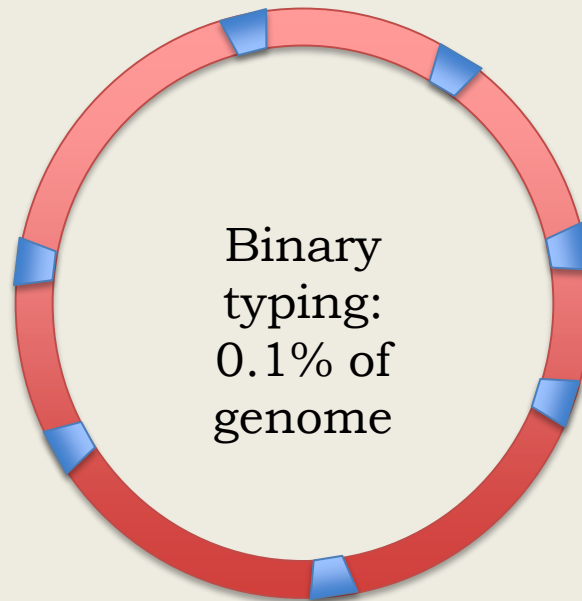
Can identify specific genes
- infer phenotypes

WGS for outbreak investigation?

1. Discriminatory power
= ability to distinguish unrelated isolates

PFGE:
0.01% of
genome

Binary
typing:
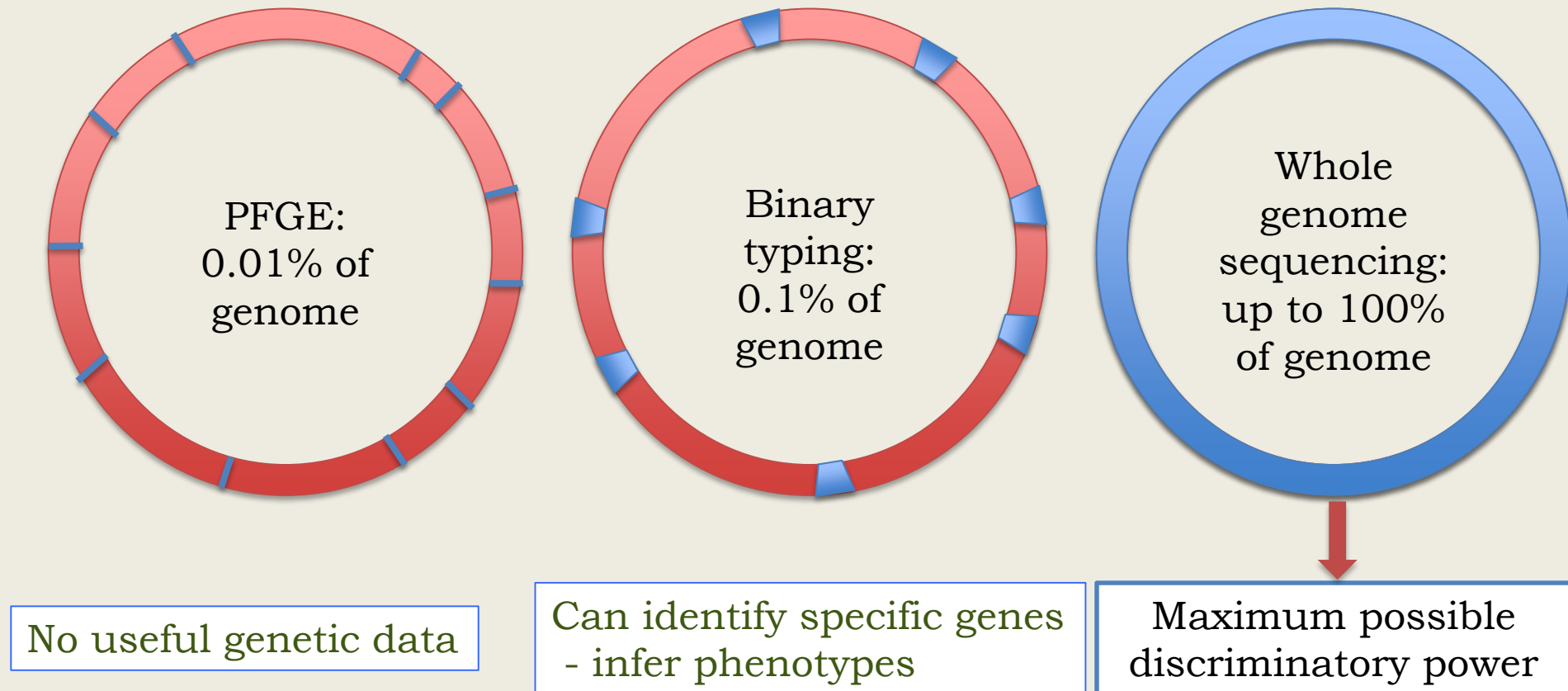0.1% of
genome

Whole
genome
sequencing:
up to 100%
of genome

No useful genetic data

Can identify specific genes
- infer phenotypes

Maximum possible
discriminatory power

Why WGS for outbreak investigation?

2. Information about direction of transmission

Position	290596	414323	535960	665169	1442748	1474315	1490359	1491399	1628185	1859577	1894645	2505244	2564234
Isolate Y	A	A	G	C	G	A	G	G	T	A	G	T	C
Isolate E	A	A	T	C	G	A	G	G	T	A	G	C	C
Isolate K	A	A	G	C	G	A	G	G	T	T	G	T	C
Isolate W	A	G	G	C	G	A	G	G	T	T	G	T	C
Isolate Q	A	G	G	C	G	G	G	G	T	T	G	T	C

Whole genome sequencing

PFGE   Binary typing

Why WGS for outbreak investigation?

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Isolate W	A	G	G	C	G	A	G	G	T	T	G	T	C
Isolate Q	A	G	G	C	G	G	G	G	T	T	G	T	C

Whole genome sequencing

PFGE   Binary typing

Why WGS for outbreak investigation?

3. Provides copious additional information

- Bacterial identification; antibiotic susceptibility
- Virulence factors; phylogenetic data

4. Comparable with alternative methods

- Turnaround time; cost; throughput
- Reproducibility; digitisation/databases
- (Ease of analysis & interpretation)
  - currently main limitation

Outbreak investigation - SCU

Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study

Simon R Harris*, Edward J P Cartwright*, M Estée Török, Matthew T G Holden, Nicholas M Brown, Amanda L Ogilvy-Stuart, Matthew J Ellington, Michael A Quail, Stephen D Bentley, Julian Parkhill†, Sharon J Peacock†

Lancet Infect Dis 2013; 13: 130–36

- 12 infants with MRSA
- 3 time periods over 6 m – with gaps
- Links suspected (antibiogram); gaps unexplained
- Additional cases after "deep" cleaning etc.
- Total cases identified by IPC team = 17

Outbreak investigation - SCU

Whole-genome sequencing for analysis of an outbreak of meticillin-resistant *Staphylococcus aureus*: a descriptive study

Simon R Harris*, Edward J P Cartwright*, M Estée Török, Matthew T G Holden, Nicholas M Brown, Amanda L Ogilvy-Stuart, Matthew J Ellington, Michael A Quail, Stephen D Bentley, Julian Parkhill†, Sharon J Peacock†

Lancet Infect Dis 2013; 13: 130–36

- WGS: 14 = new MLST; 1 variant c.f. ST22 + PVL
  - closely related cluster (20 SNPS)
  - 3 excluded
- Definition of wider outbreak
  - WGS identified 26 additional cases
  - transmission within SCU, mothers, community
  - staff member colonised with outbreak strain
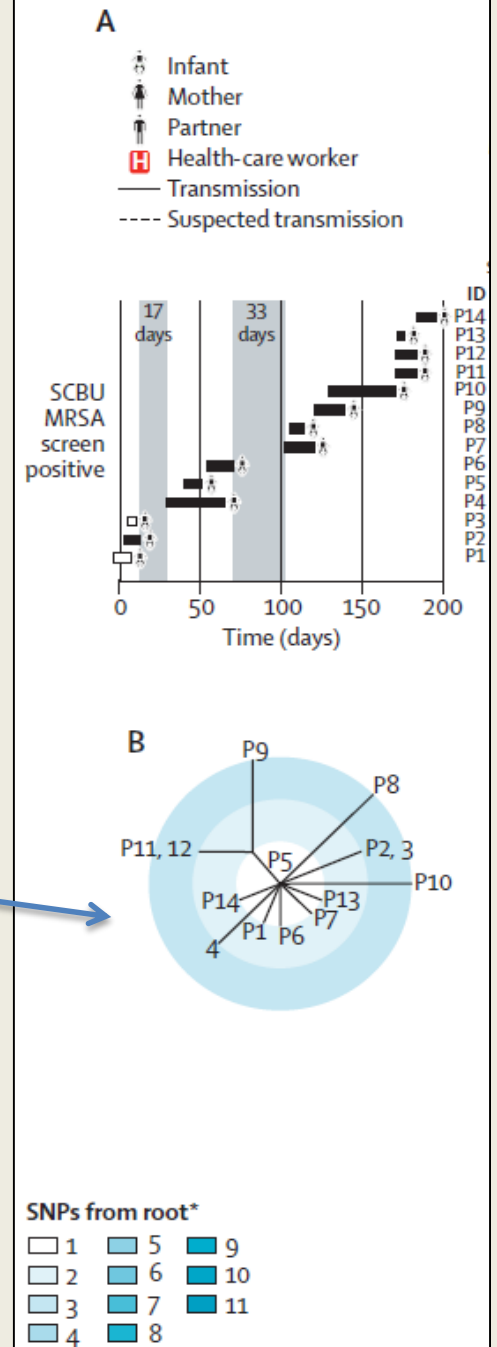
Conclusions

- Typing discriminates strains within species
- New methods, incl. WGS (soon):
  - rapid, inexpensive – prospective surveillance
- Some methods can identify (from isolates or specimens):
  - species, antibiotic susceptibility/resistance
  - nosocomial transmission events & outbreaks
  - hypervirulent, resistant or outbreak strains
- WGS: will soon replace other methods
  - New insight into infectious disease epidemiology
  - ?personalised medicine for infectious disease