BC Centre for Disease Control
An agency of the Provincial Health Services Authority

LM LABS

BCCDC Public Health Laboratory

How did it get to there from here? Understanding transmission from whole genome sequencing

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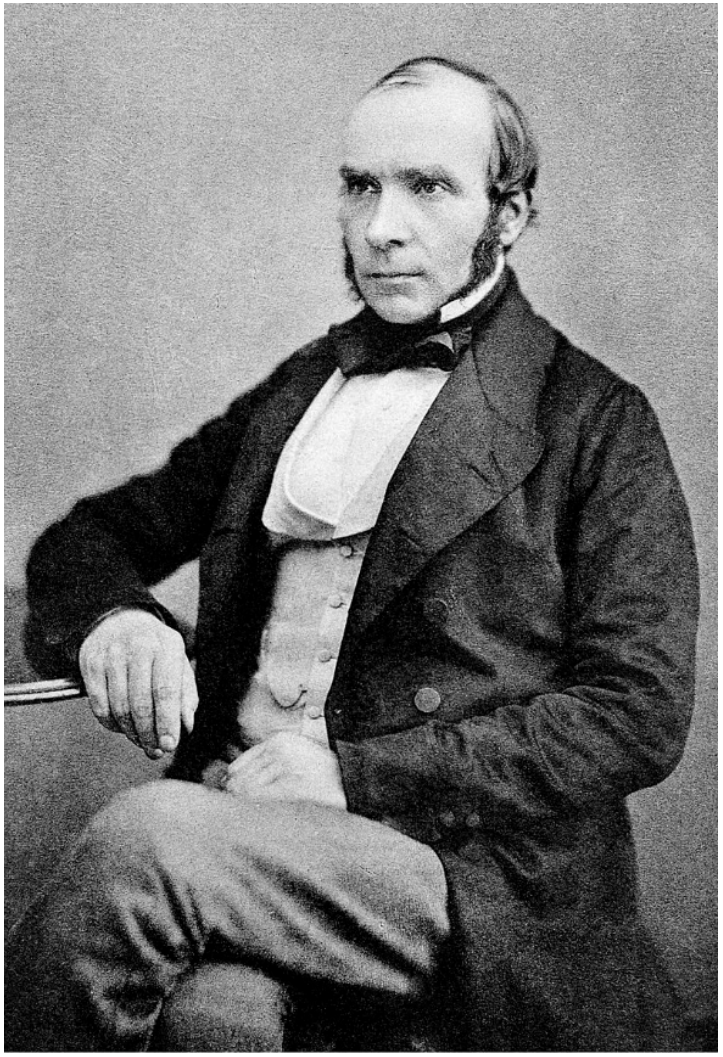
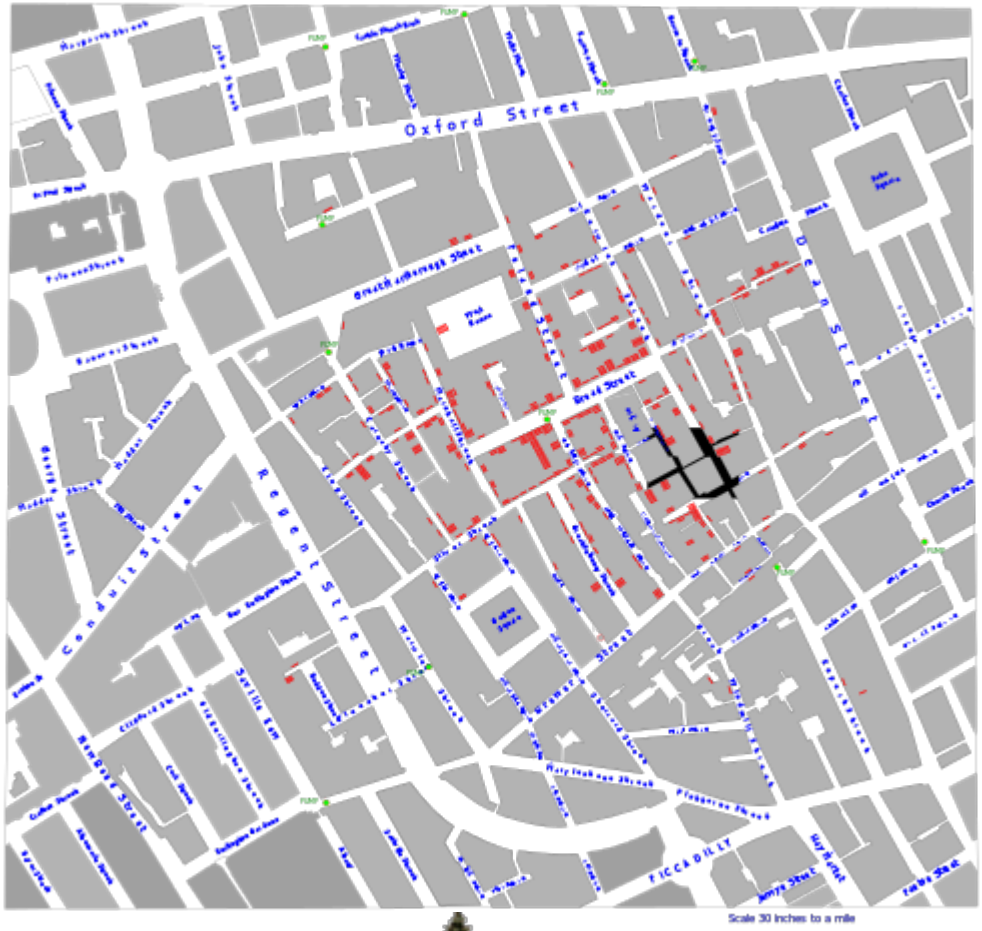
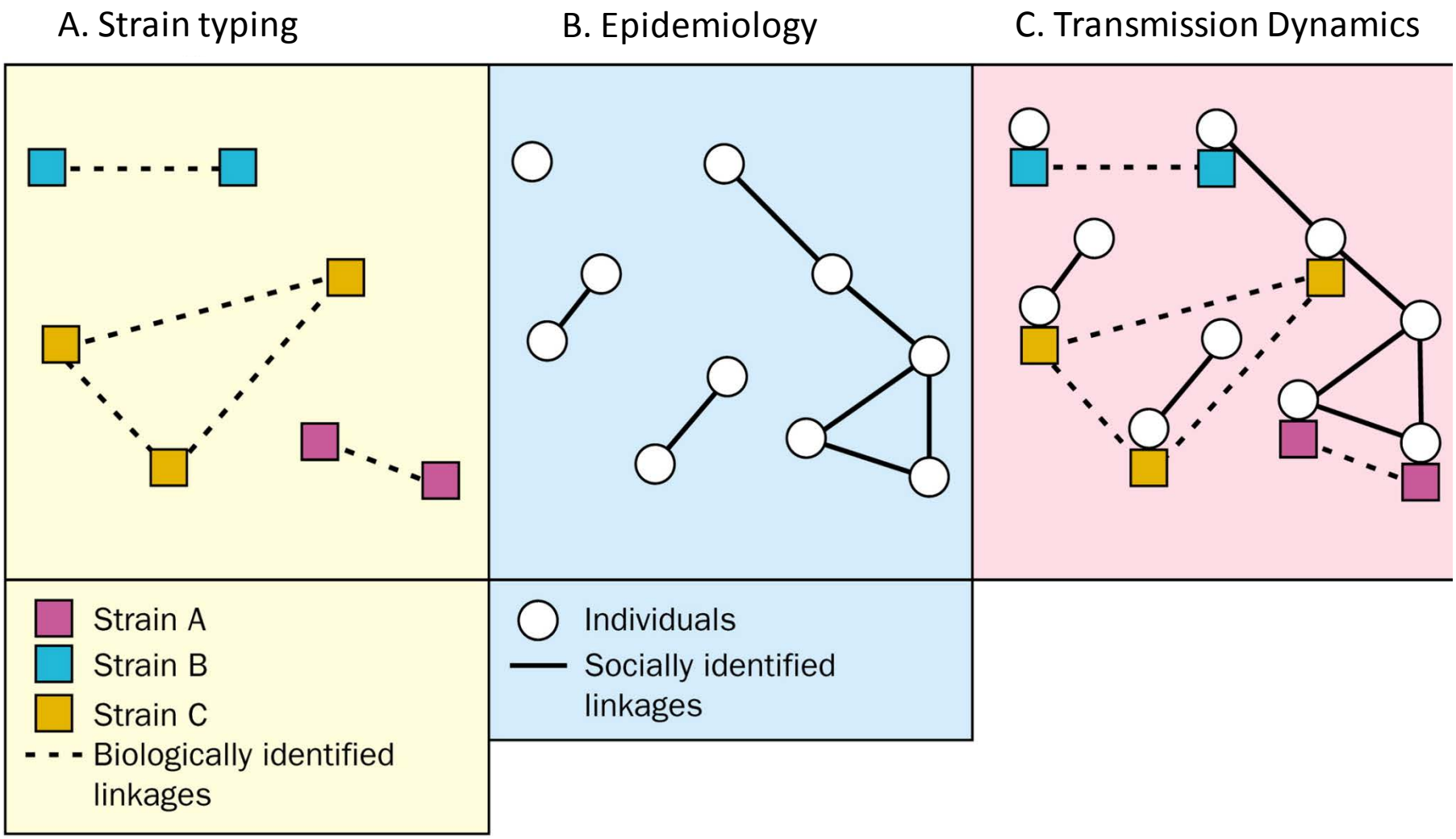
Objectives

- Molecular Epidemiology
- Evolution of Tools that Guide Outbreak Investigations
- The Role of Whole Genome Sequencing for Outbreak Investigations






A. Strain typing
B. Epidemiology
C. Transmission Dynamics
Strain A
Strain B
Strain C
Biologically identified linkages
Individuals
Socially identified linkages

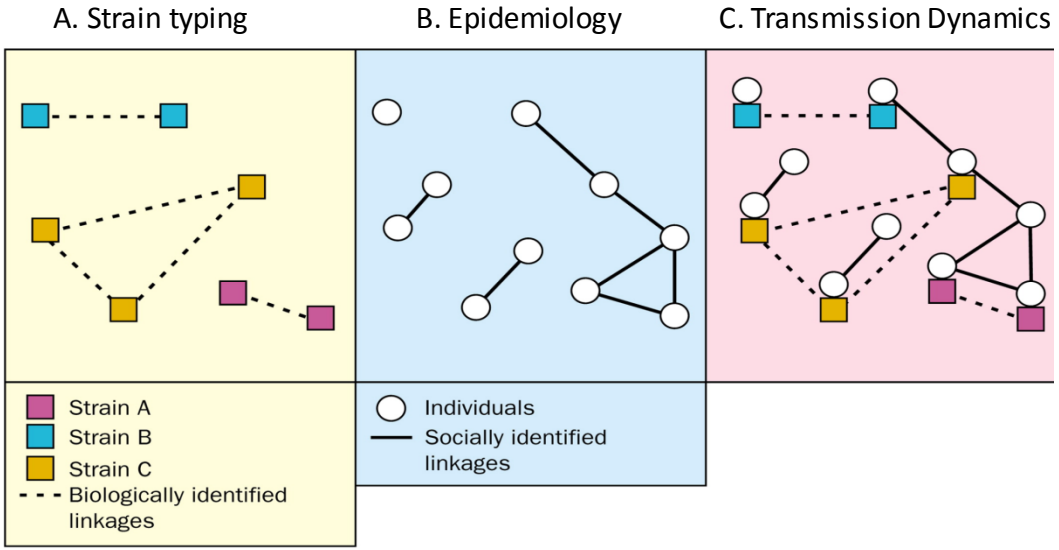
Strain Typing: By Phenotype

- Biochemicals
  - Assimilation of different biochemicals,
  - Antibiotic susceptibility profile
- Serotyping
  - Recognition by type-specific antibodies (e.g. *N. meningitidis*)
- Phage typing
  - Susceptibility to different bacteriophage

→*Lack Discriminatory Power*

Molecular Epidemiology

- Molecular tools provides info on strain relatedness
- Use of molecular methods coupled with conventional epidemiological tools, to identify potentially linked cases and aid in the investigation of outbreaks
- Choice of methods depends on pathogen, epidemiology, reference database, etc

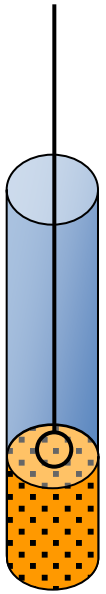


Strain Typing: By Genotype

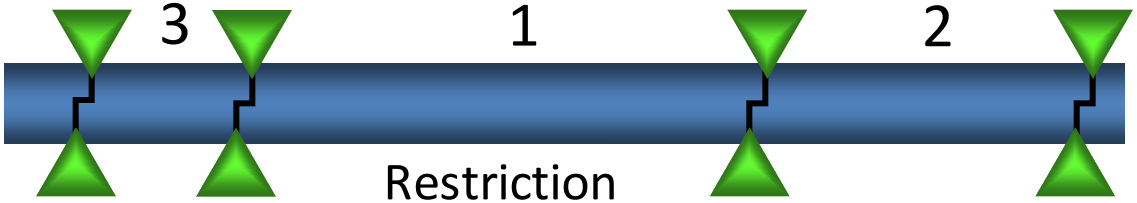
- Restriction fragment-length polymorphism (RFLP)
- Pulsed-field gel electrophoresis (PFGE)
- Random amplification of polymorphic DNA (RAPD)
- Amplified fragment length polymorphism (AFLP)
- Multilocus variable number of tandem repeats analysis (MLVA)
- Multilocus sequence typing (MLST)
- Single nucleotide polymorphism (SNP) typing
- Microarray typing

Restriction fragment-length polymorphism (RFLP)

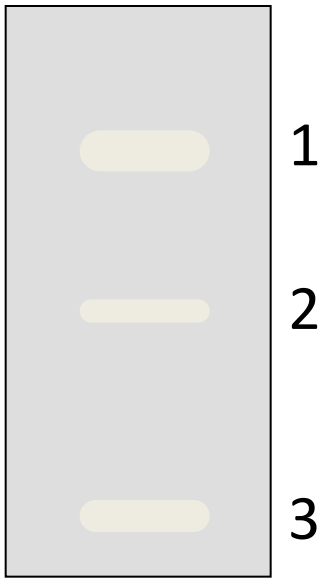
1. Grow organism



2. Fragment genome

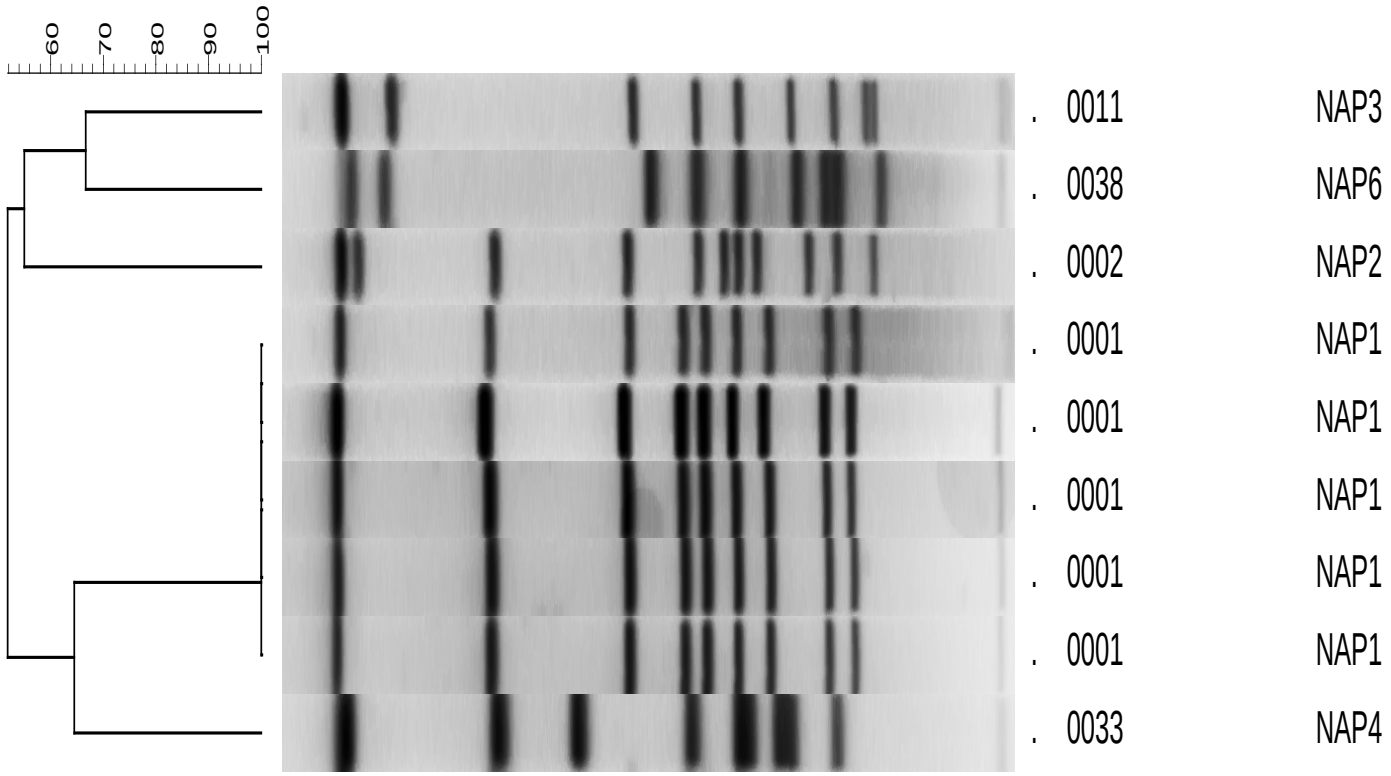


3. Run gel



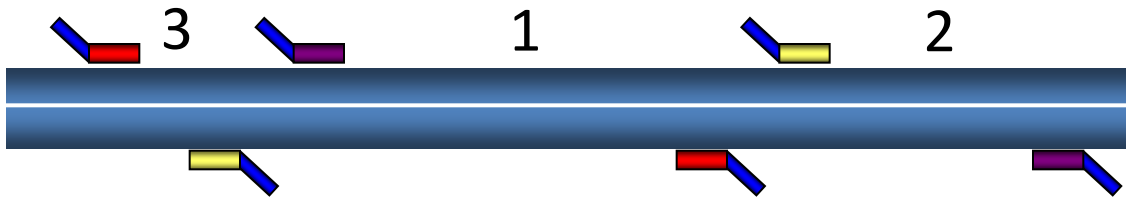
NAP1 *C. difficile* in British Columbia

At BCCDC Public Health Laboratory: DNA fingerprinting by pulsed field gel electrophoresis (PFGE)

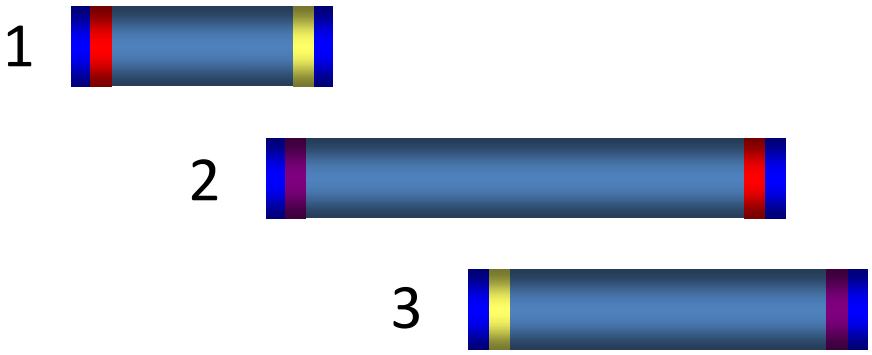


Random amplification of polymorphic DNA

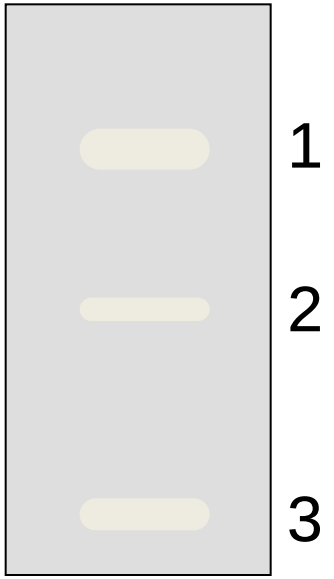
1. Amplify genome



2. Generate PCR fragments

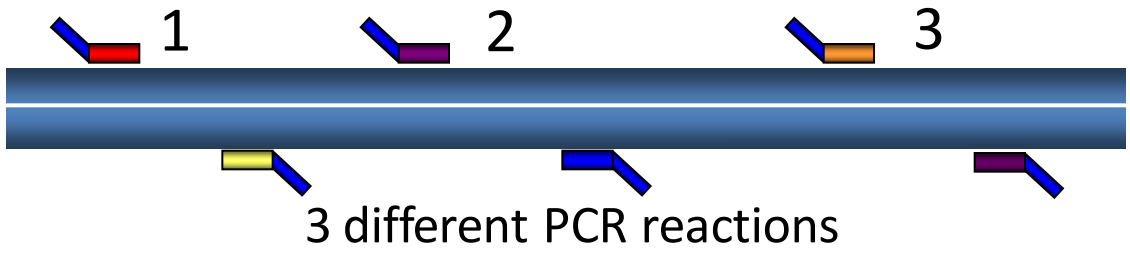


3. Run gel

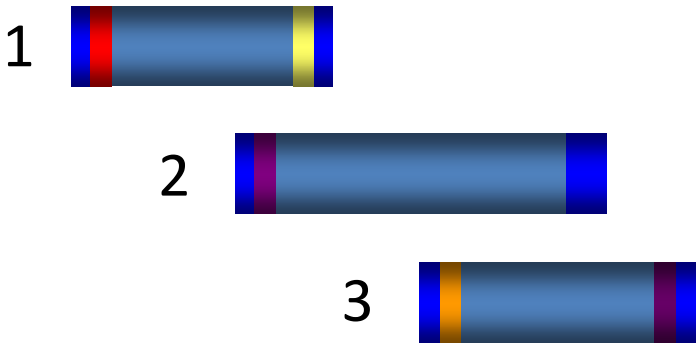


Multilocus Sequence Typing

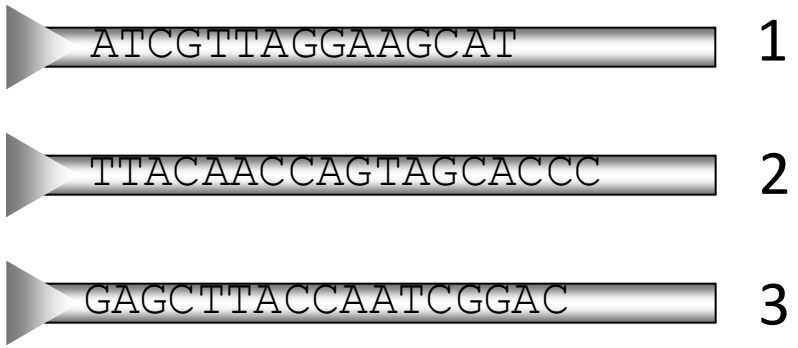
1. Amplify multiple loci



2. Generate PCR fragments



3. DNA sequencing



PFGE has limited discriminatory power

TABLE 4 Numbers of discrepancies between whole-genome sequencing and PFGE for paired-strain comparisons

	No. of strains							
	Indistinguishable		Closely related		Possibly related		Different	
Organism	Clonal by WGS	Nonclonal by WGS	Clonal by WGS	Nonclonal by WGS	Clonal by WGS	Nonclonal by WGS	Clonal by WGS	Nonclonal by WGS
VRE	55	9	0	81	0	8	0	18
MRSA	5	15	0	23	0	58	0	35
Acinetobacter baumannii	4	2	12	32	4	23	0	28
All organisms	64	26	12	136	4	89	0	81

Salipante et al. 2015. JCM vol 53 (4)

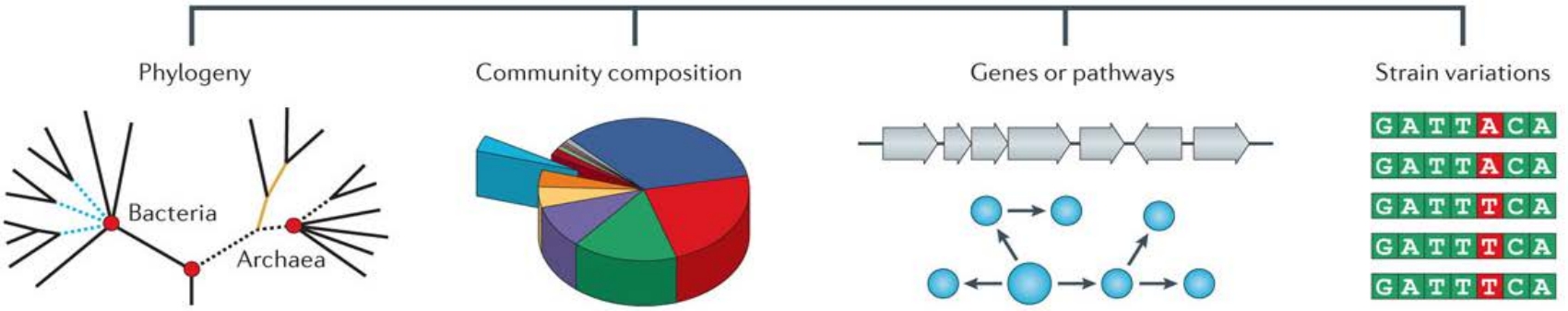
Conventional Molecular Methods and Outbreak Investigations

- Labour intensive
- Limited discriminatory power, but may be sufficient depending on scenario
- Role depends on available background database
- Laboratory's capacity/resources
- TAT needed

Highest resolution: WGS

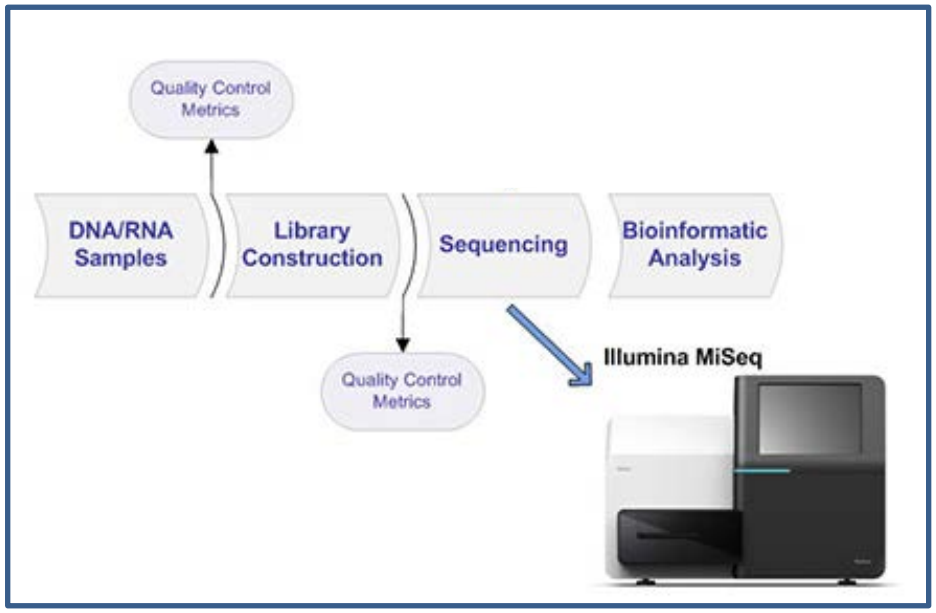
Genomics: the study of genomes (DNA)

- High throughput sequencing to obtain the nucleic acid sequences for a single organism (genome) or a mix of organisms (metagenomes)



Nature Reviews | Genetics

How do we sequence genomes?



_columbia des_citroba
_
nd_enteroba
ter_freund
cpos_inclu
british_
co
lude_citr
o
obacte
r
tish_colum
ndii_and_e
a_cpos_inc
ter_cloac
ae
terobacte
r
_freundii
olumbia_
cp

The shotgunator: http://lyorn.idyll.org/~t/assembly-exercise/index.cgi

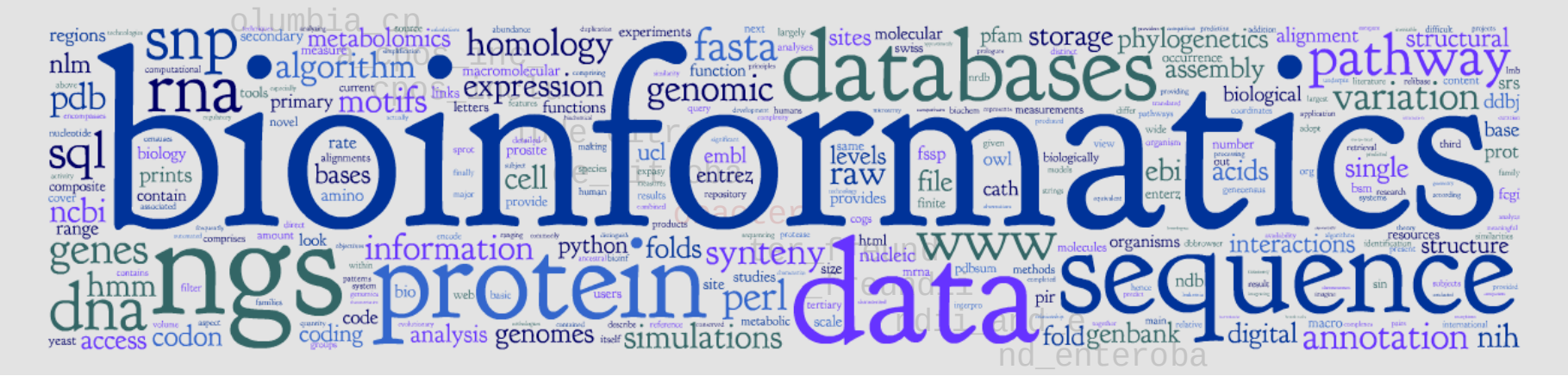
..and then we need to put it back together

```
british_co
   tish_colum
       _columbia_
         olumbia_cp
              a_cpos_inc_
                cpos_inclu
                       lude_citro
                         de_citroba
                               obacte
                               r   terobacter
                                       ter_cloacae
                                                    ter_freund
                                                       _freundii
                                                            ndii_and_e
                                                                 nd_enterob
                                                                 a
```

Consensus:
`british_columbia_cpos_include_citrobacter_cloacae + ter_freundii_and_enteroba`

..and then we need to put it back together

```
british_co
   tish_colum
      _columbia_
```



Alternative consensus:

```
british_columbia_cpos_include_citrobacter_freundii_and_enteroba
cter_cloacae
```

1. GTCACCATAGGCTAGTAGCATTGGCGACTACAA
2. GTCACCACAGGCTAGTAGCATTGGCGACTACAA
3. GTCACCATAGGCTAGTAGCATTGGCGACTACAA
4. GTCACCACAGGCTAGTAGCATTGGCGACTACAA
5. GTCACCATAGGCTAGTAGCATTGACGACTACAA

1. GTCACCATAGGCTAGTAGCATTGGCGACTACAA
2. GTCACCACAGGCTAGTAGCATTGGCGACTACAA
3. GTCACCATAGGCTAGTAGCATTGGCGACTACAA
4. GTCACCACAGGCTAGTAGCATTGGCGACTACAA
5. GTCACCATAGGCTAGTAGCATTGACGACTACAA

- Isolates 1 and 3 are identical
- Isolates 2 and 4 are identical

Genomic Clock

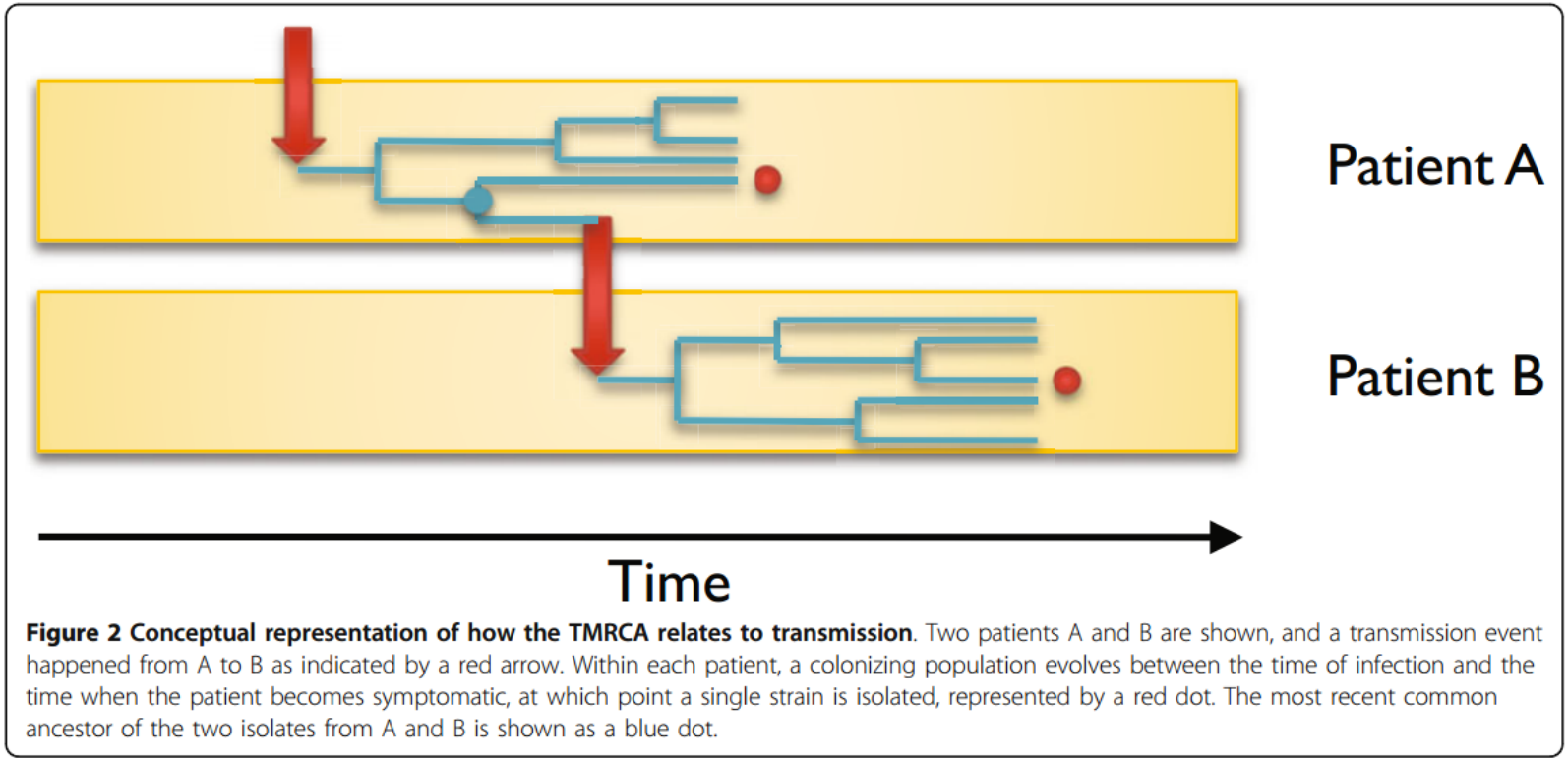
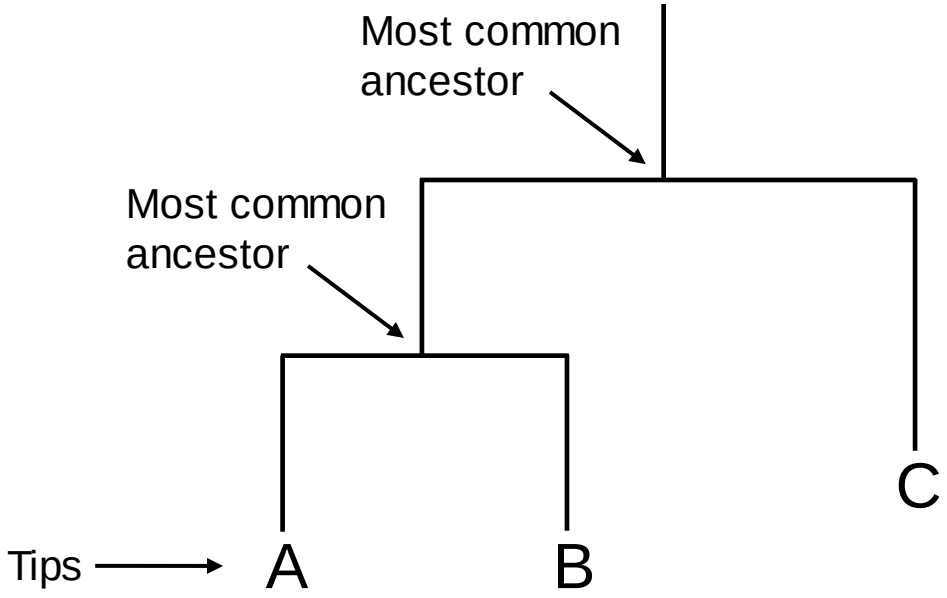
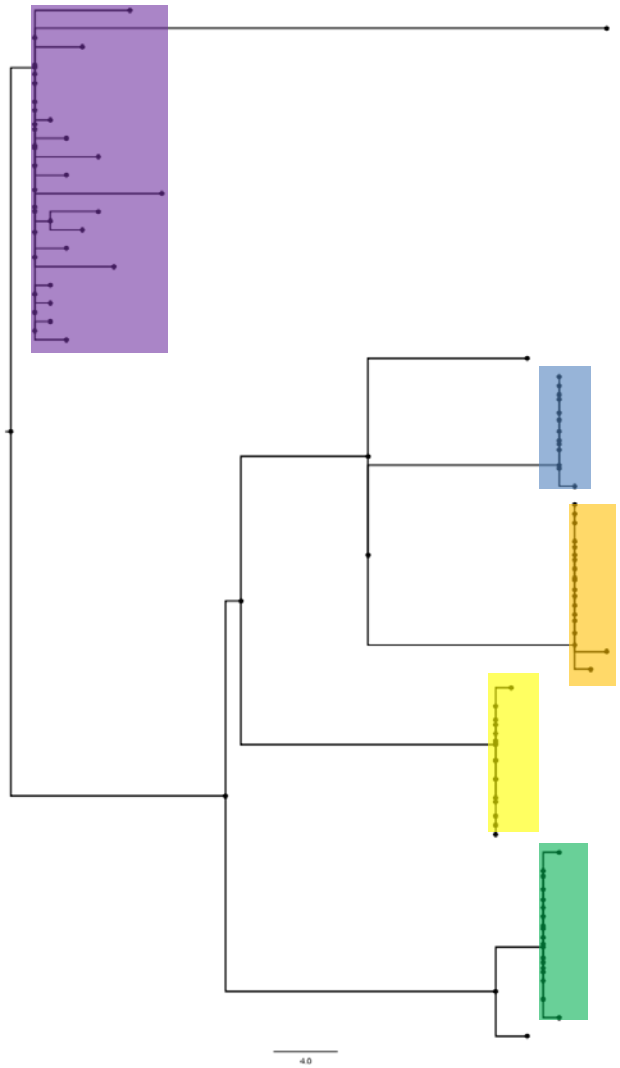


Figure 2 Conceptual representation of how the TMRCA relates to transmission. Two patients A and B are shown, and a transmission event happened from A to B as indicated by a red arrow. Within each patient, a colonizing population evolves between the time of infection and the time when the patient becomes symptomatic, at which point a single strain is isolated, represented by a red dot. The most recent common ancestor of the two isolates from A and B is shown as a blue dot.

Didelot et al. Genome Biology 2012, 13:R118

Phylogenetic trees 101



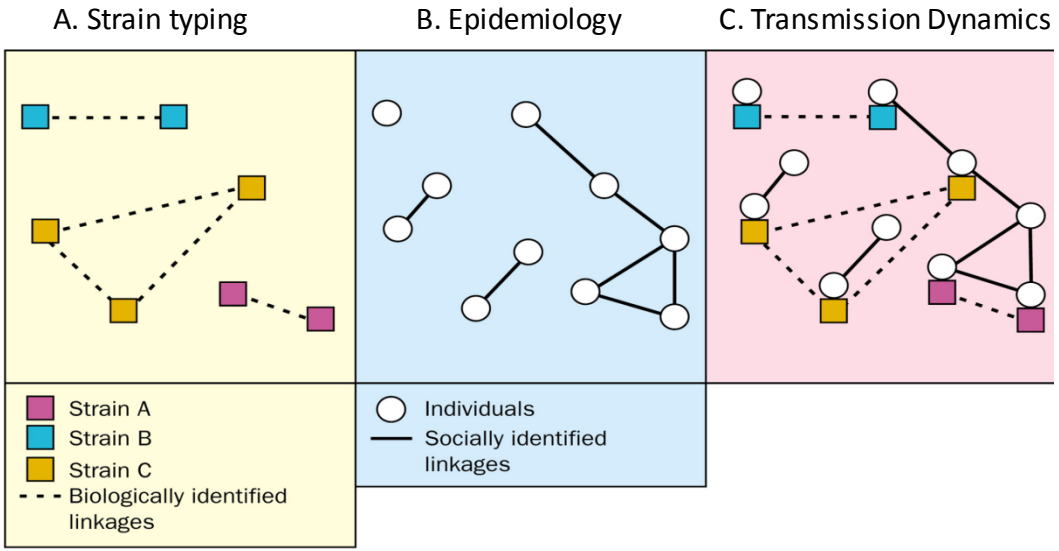


Data from: Octavia et al. J Clin Microbiol. 2015 Apr;53(4):1063-71.

Always need strong epidemiology!!!

Genomic Epidemiology

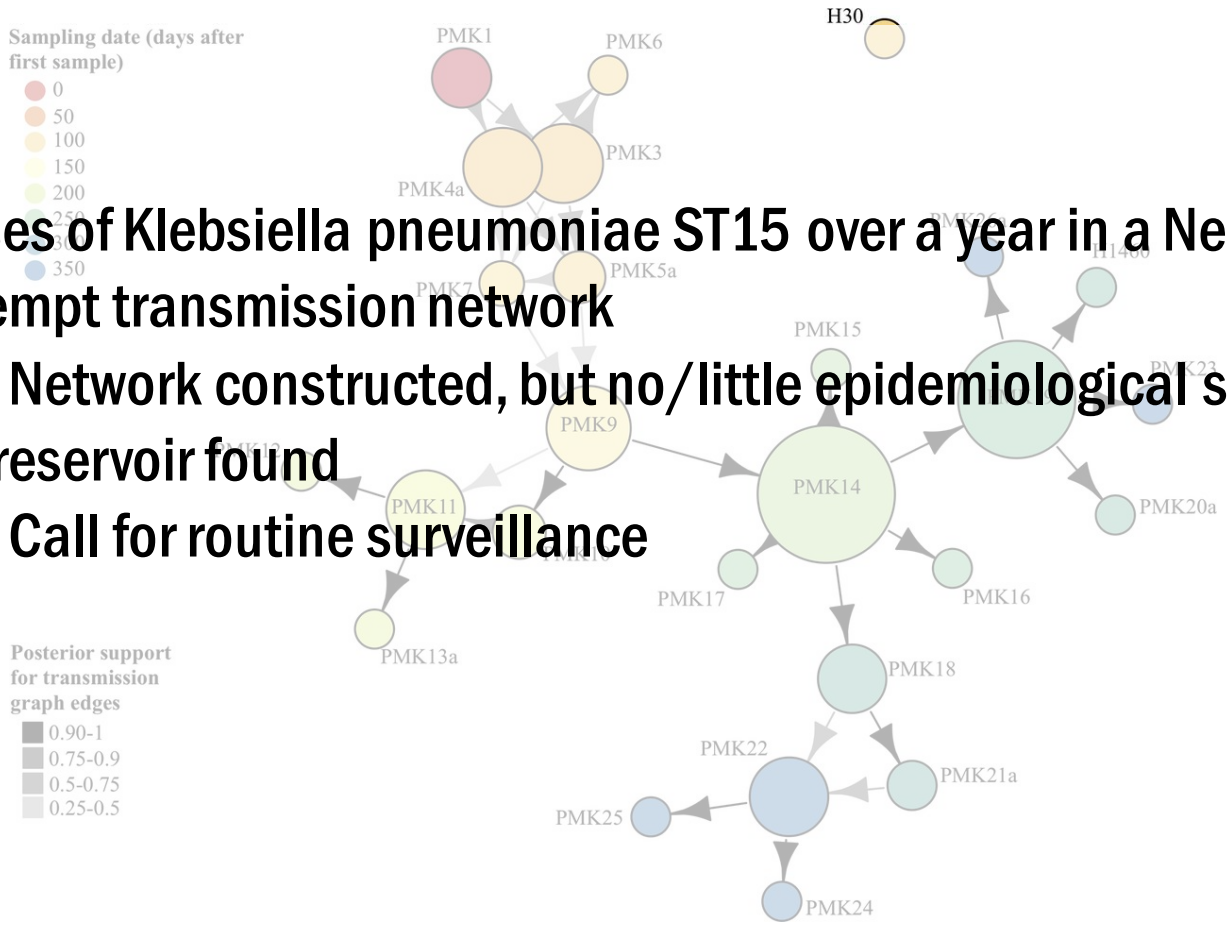
- Whole genome data provides details on strain relatedness
- Use of whole genome sequence data coupled with conventional epidemiological tools, to identify potentially linked cases and aid in the investigation of outbreaks
- Provide transmission dynamics (chain of transmission)



Stoesser et al. J Antimicrob Chemother. 2015 Apr;70(4):1008-15

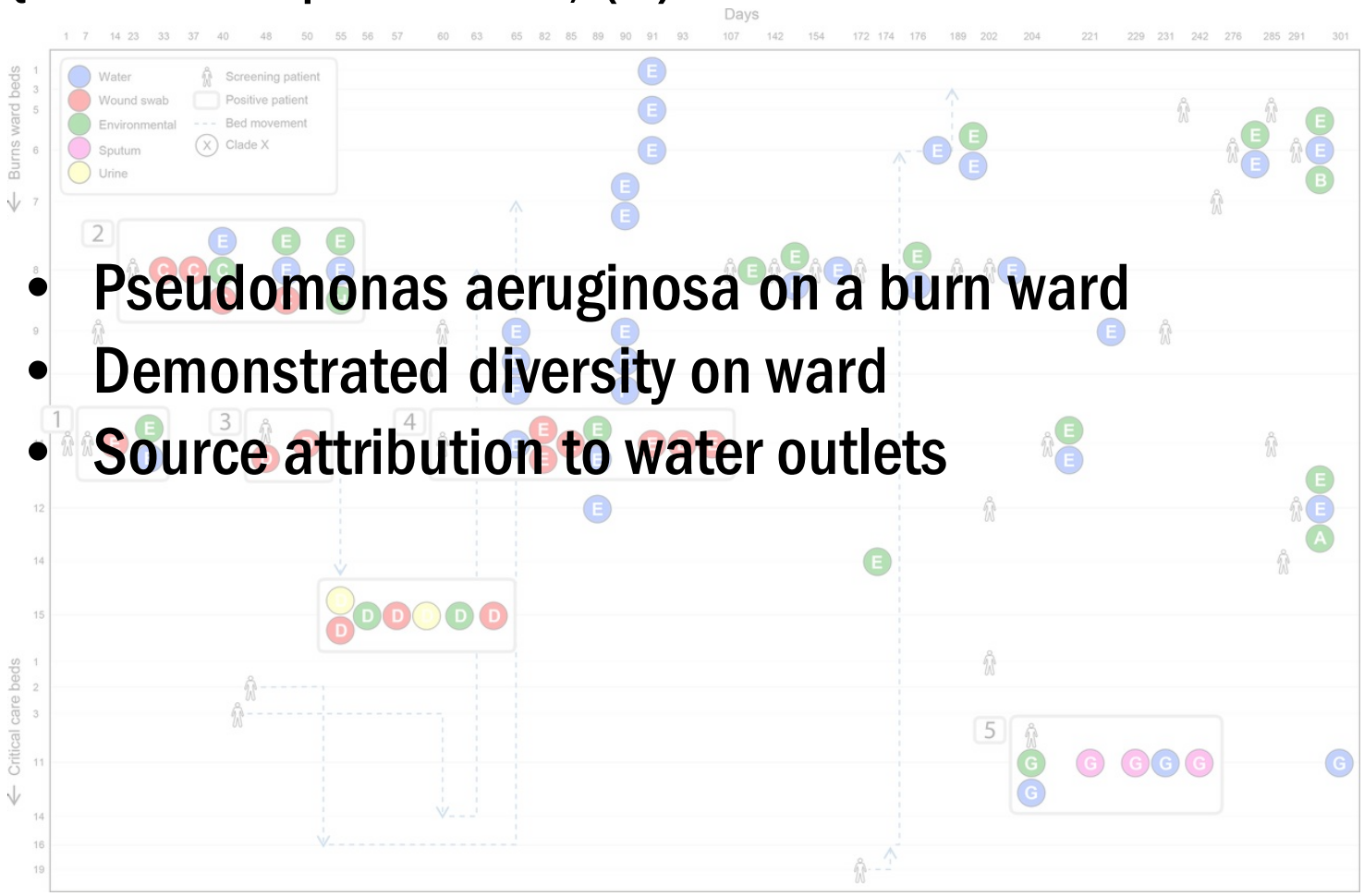
- *Enterobacter cloacae* outbreak in Nepali neonatal units
- Two clear clusters (separate) outbreaks
- One persisted even after cleaning
  - Persisting strain associated with soap dispenser

Stoesser et al. Antimicrob Agents Chemother. 2014 Dec;58(12):7347-57.



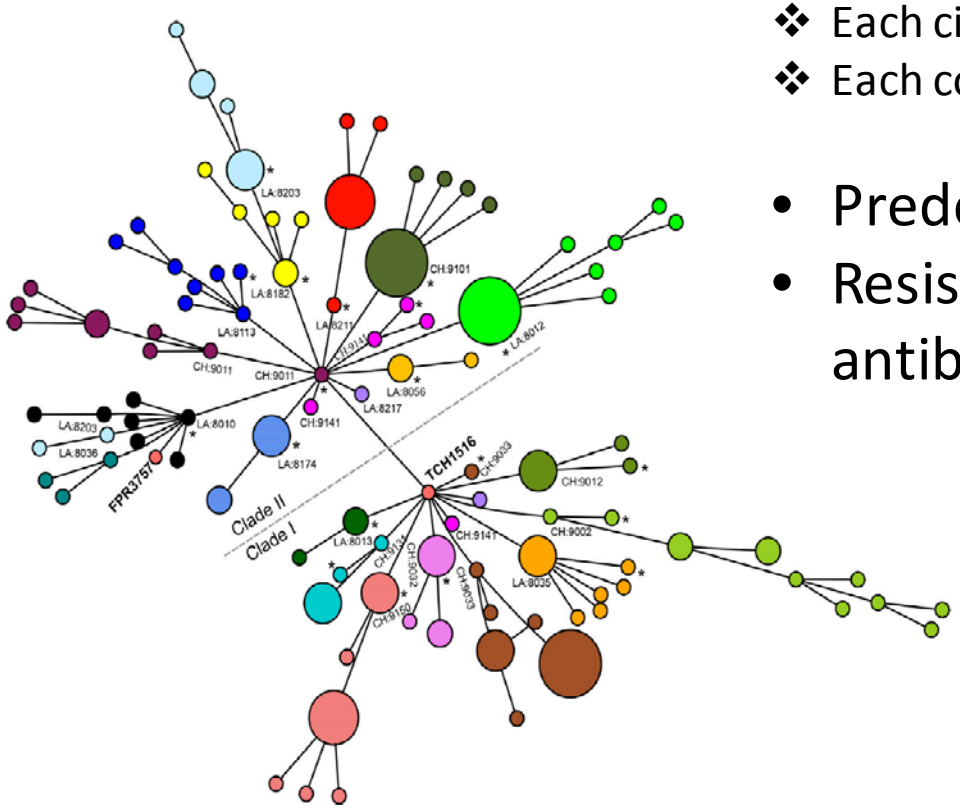
- Cases of Klebsiella pneumoniae ST15 over a year in a Nepali NICU
- Attempt transmission network
  - Network constructed, but no/little epidemiological support
- No reservoir found
  - Call for routine surveillance

Quick et al. BMJ Open. 2014 Nov 4;4(11):e006278.



- Pseudomonas aeruginosa on a burn ward
- Demonstrated diversity on ward
- Source attribution to water outlets

Household transmission of USA300 in U.S.



- Each circle = genotype
- Each colour = family

- Predominant CA-MRSA strain in U.S.
- Resistant to fluoroquinolone antibiotics

Alan et al. mBio 6(2):e00054-15

CA- vs HA- pathogens

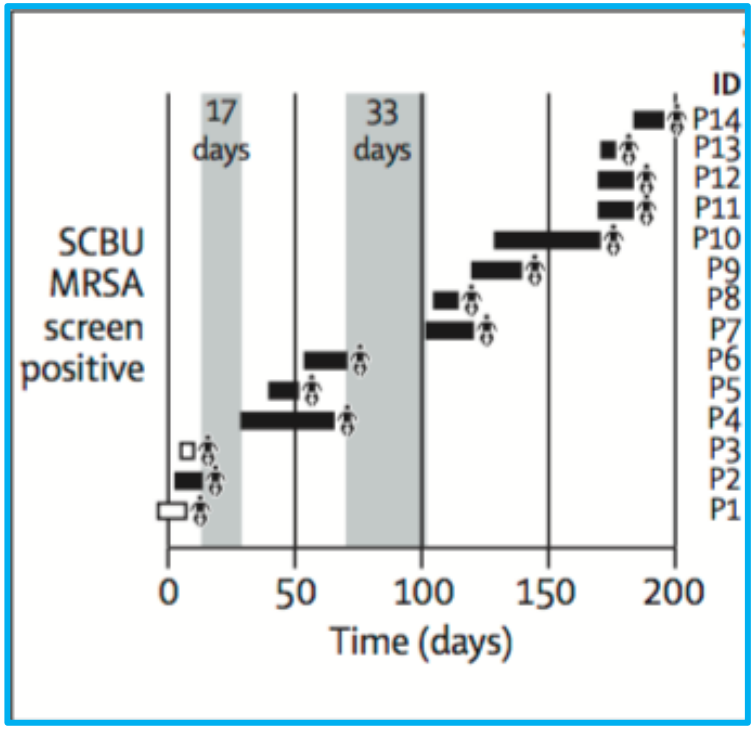
- Emergence of community associated MRSA and *C. difficile*
- CA- strains displacing previously dominant HA-strains
  - Repeat admissions and discharges?
  - Differences in transmission dynamics?
  - Epidemiology and traditional typing unable to resolved differences

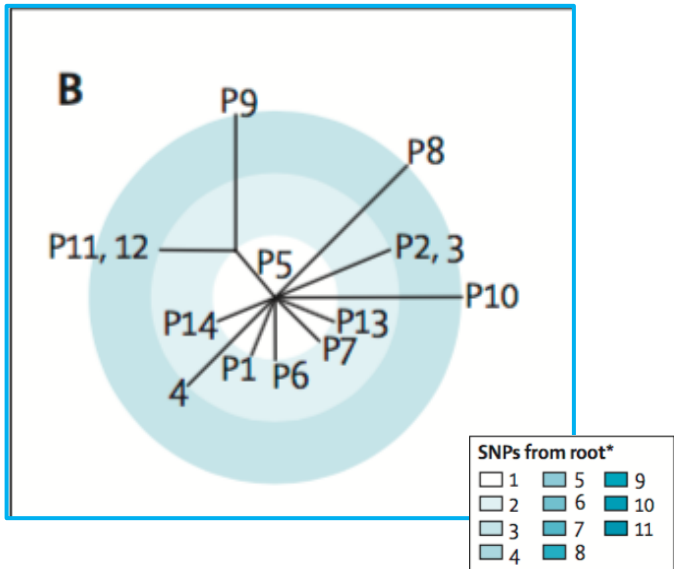
MRSA

- Goal: reduce person-to-person transmission events and control outbreaks
- Detecting transmission events critical, but current methods imperfect
  - Probability of contact with a known MRSA carrier
  - Antibiogram
  - PFGE: limited genotypes

MRSA Outbreak in Special Care Baby Unit (SCBU)

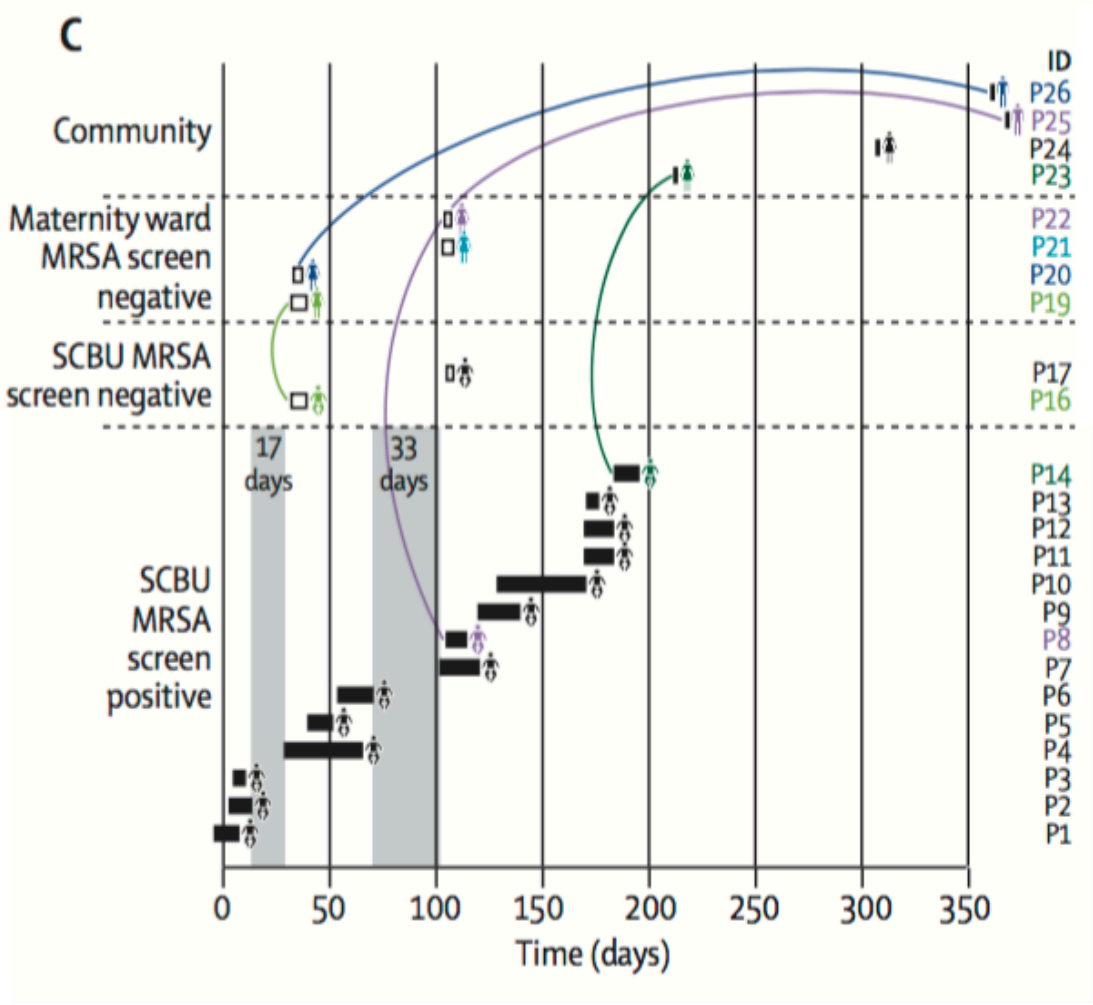
Harris et al. Lancet Infect Dis 2013; 13:130-36

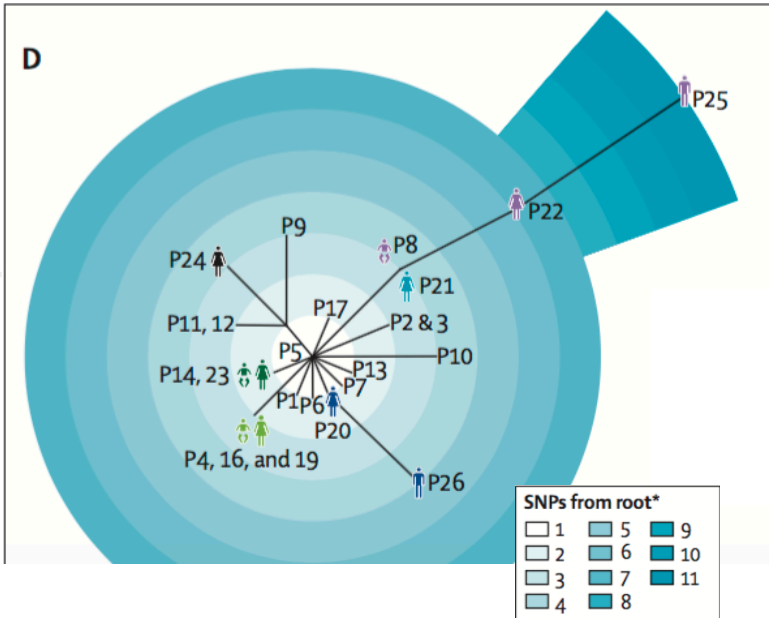




-Highly related cluster of 14 patients from SCBU
-P1 and P3 incorrectly excluded because antibiogram differed.
-Outbreak spanned the MRSA-free period (grey columns)

Social Network of MRSA Outbreak

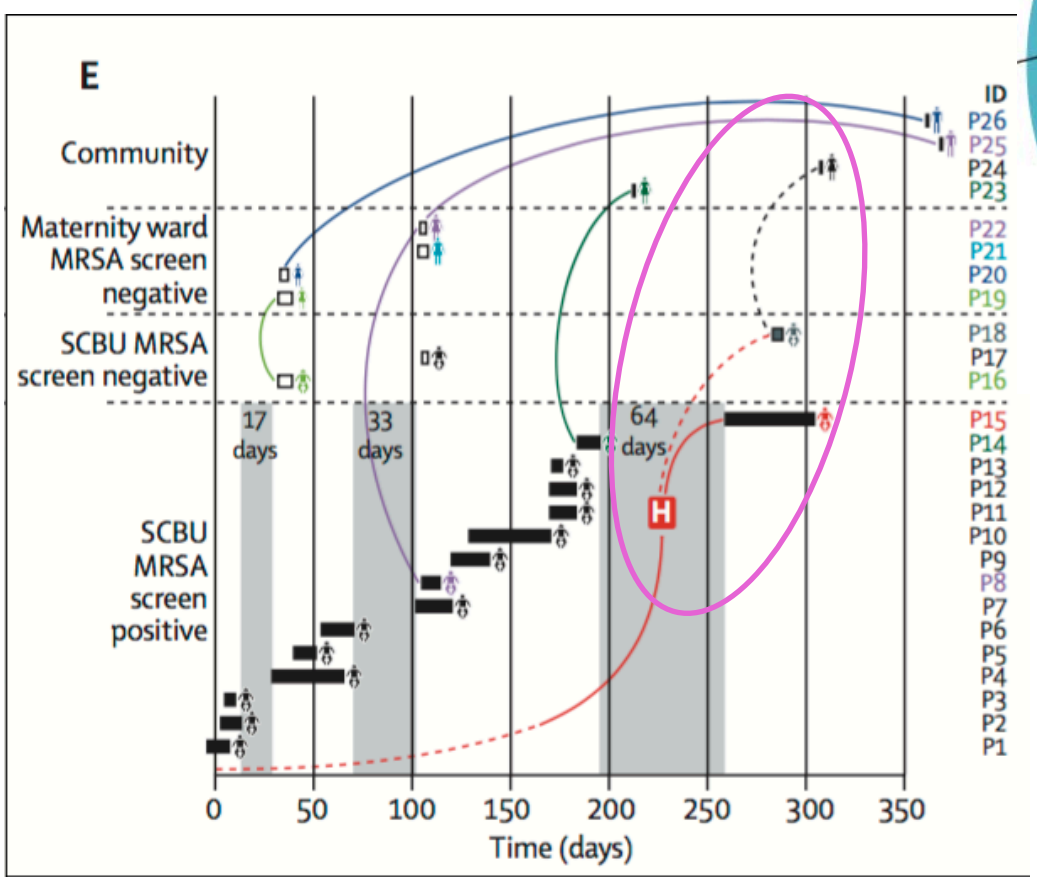


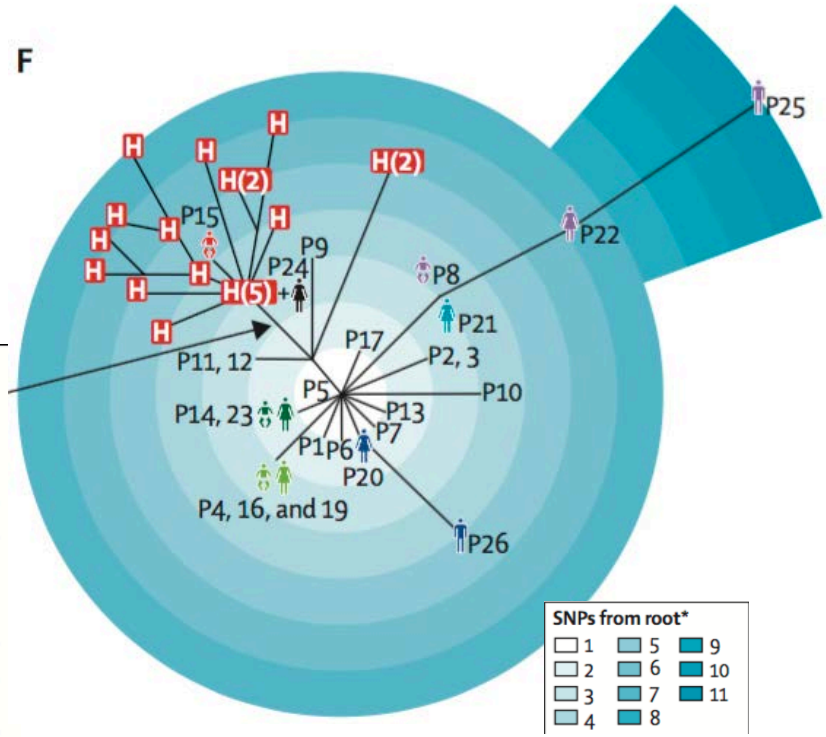


MRSA transmission
-within SCBU
-between SCBU and Maternity ward
-between SCBU, Maternity ward and community

Harris et al. Lancet Infect Dis 2013; 13:130-36

Social Network of MRSA Outbreak + Healthcare workers

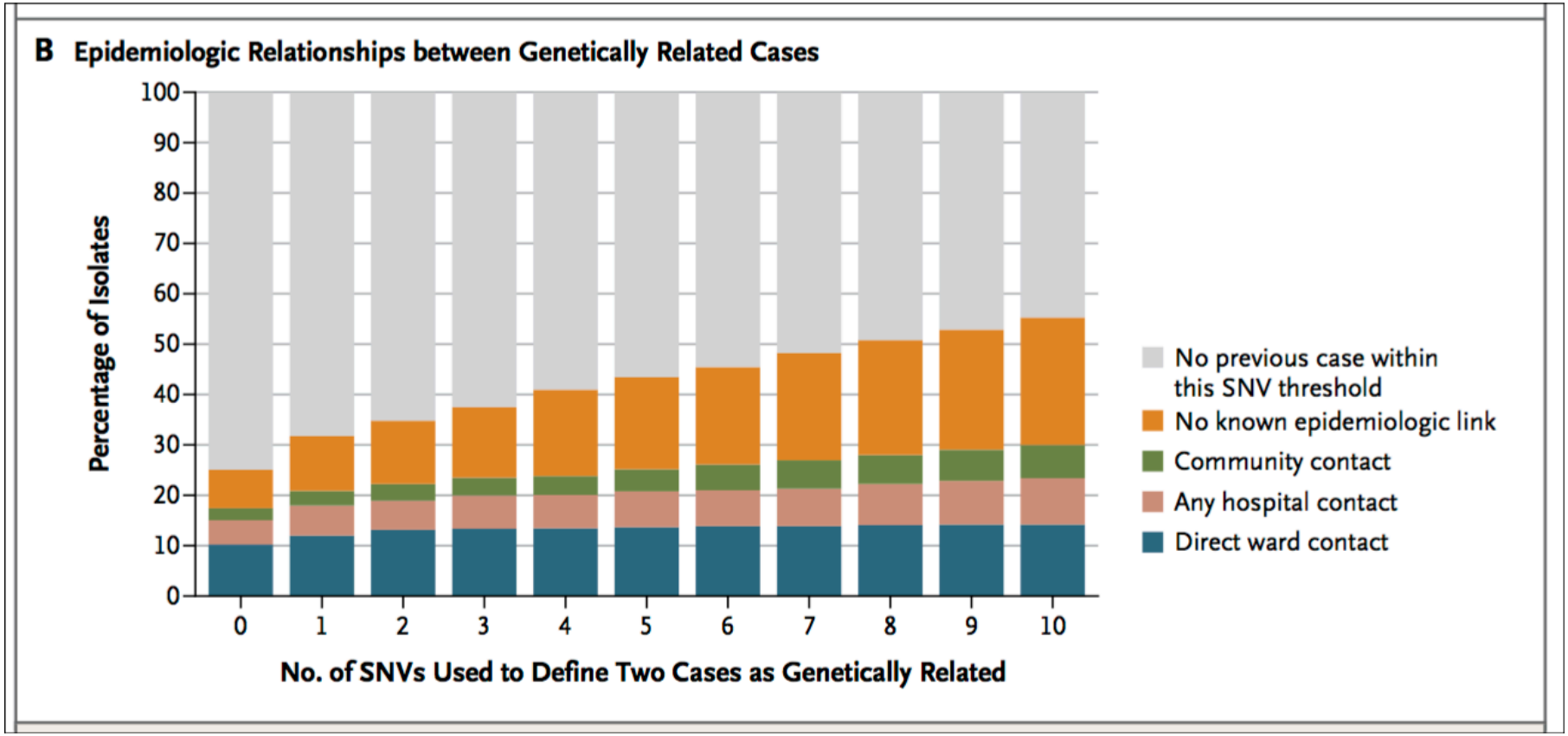




-WGS identified relatedness between P15 and P24
-transmission event after deep clean suggested staff involvement

Harris et al. Lancet Infect Dis 2013; 13:130-36

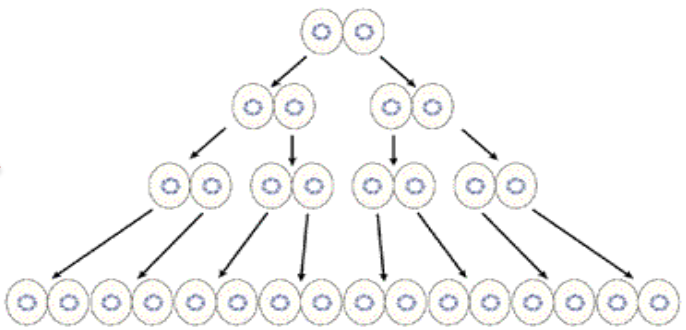
C.difficile outbreak….
not really an outbreak



Eyre et al. NEJM 2013

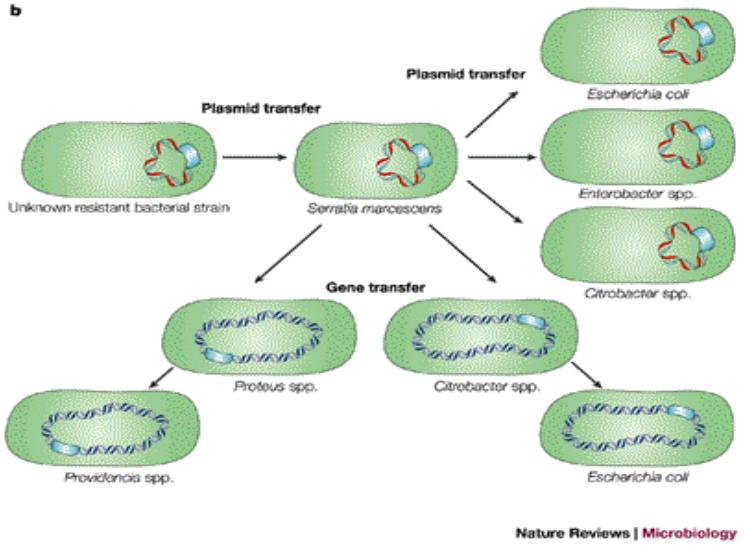
What if Bacterial Genes Transfer by Plasmids: CPO!

CLONAL SPREAD

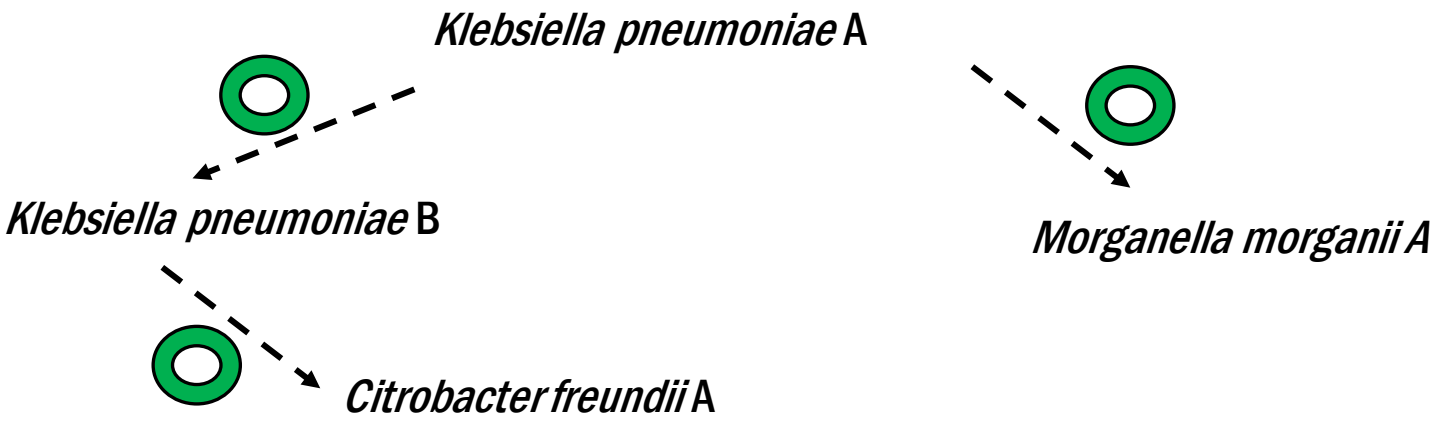


Can molecular epidemiology tools tell us how CPO is spread?

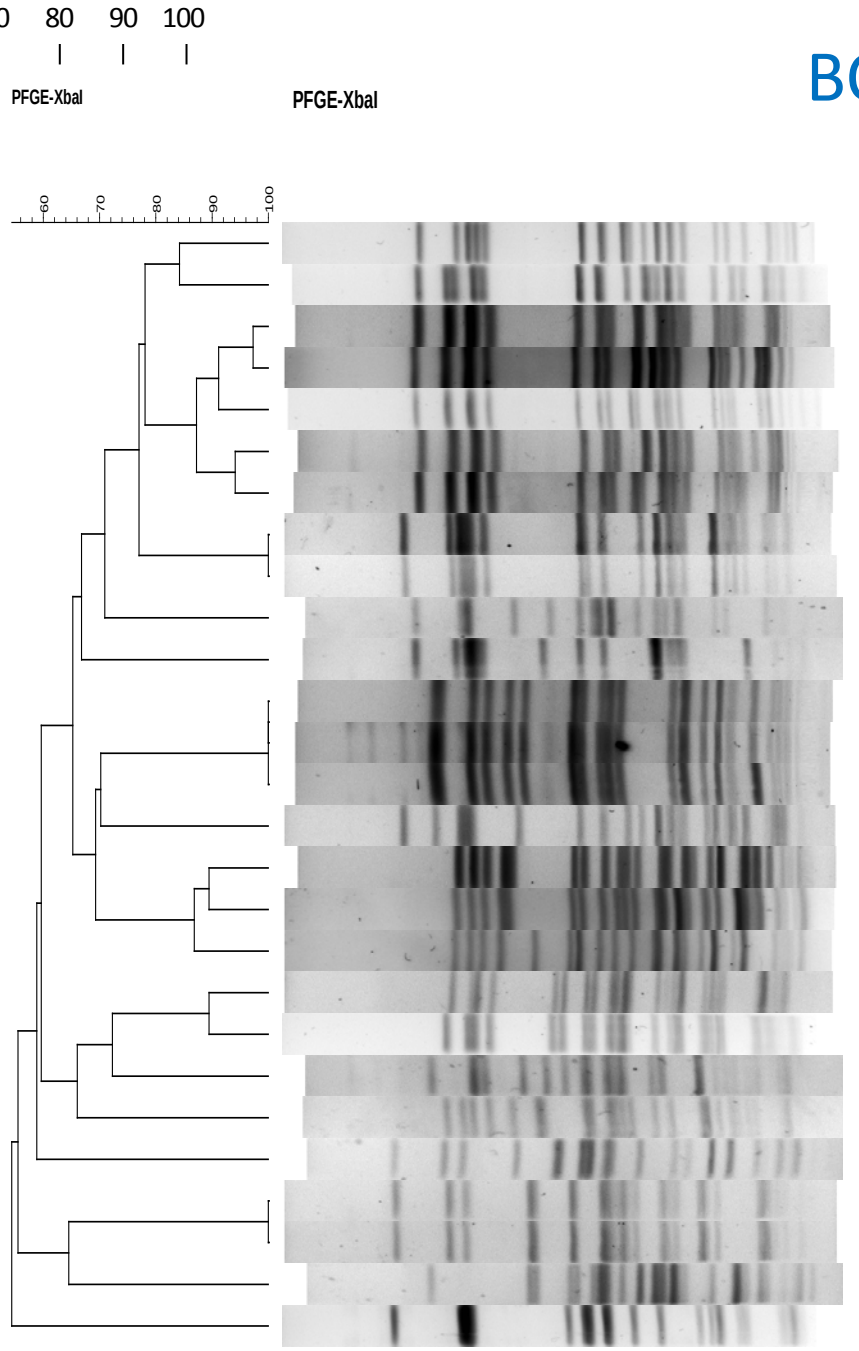
PLASMID SPREAD



MULTISPECIES OUTBREAKS

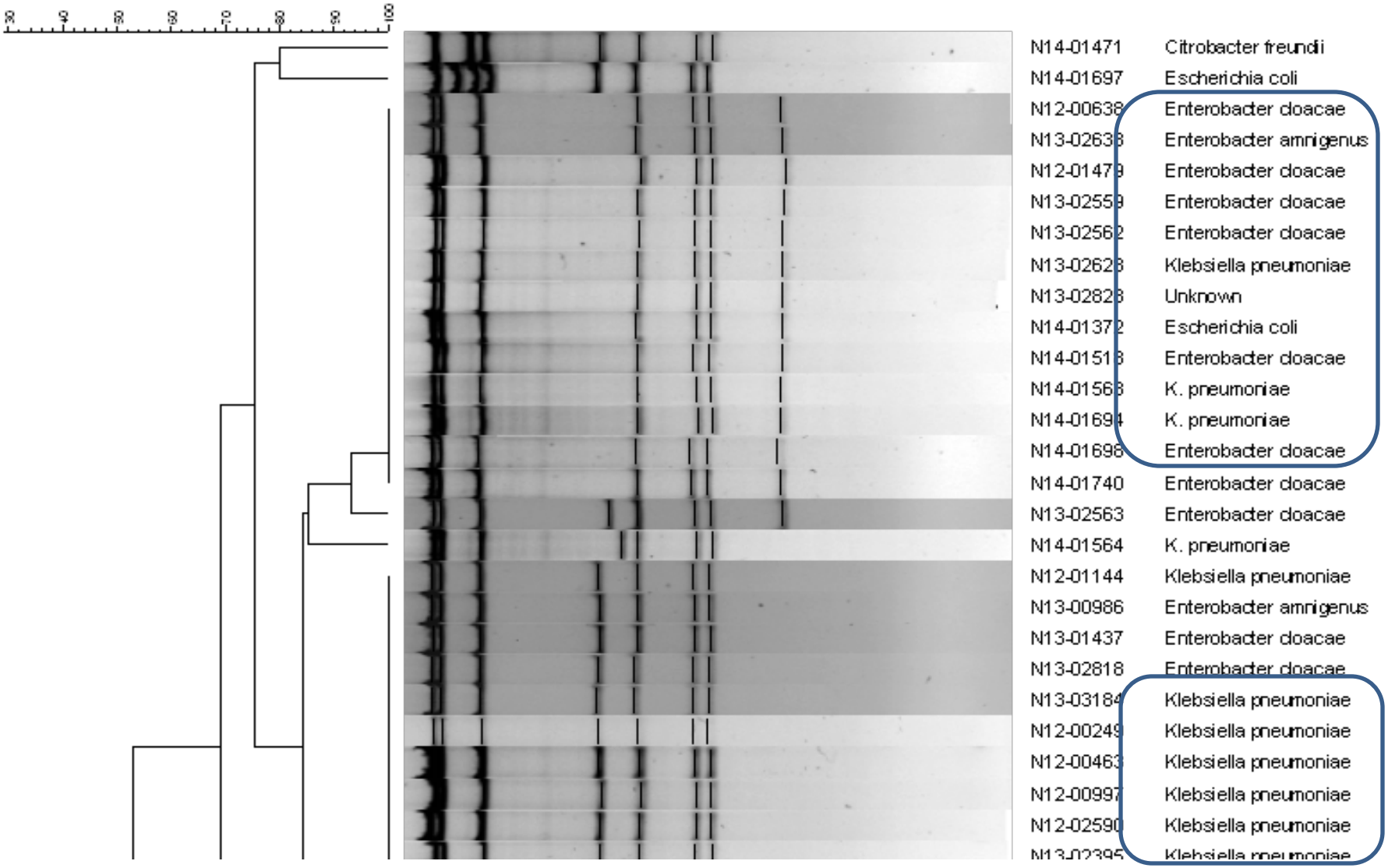


BCCDC PFGE of *K. pneumoniae*



Pt / Year	GNT	GNTP ID?	AbG ID?	Trave	Hx
Pt 8 2011	KPC	N		Y	US, multip adm
Pt 8 2011	KPC				
Pt 9 2012	NDM			Y	
Pt 38 2013	NDM			N	
Pt 9 2011	NDM	Y	Y		
Pt 38 2013	NDM				
Pt 9 2011	NDM				
Pt 6 2011	NDM	Y	N	Y	India, multip adm
Pt 6 2011	NDM				
Pt 32 2012	KPC				
Pt 35 2012	NDM				
Pt 23 2012	NDM				
Pt 20 2012	NDM	N	N		
Pt 18 2012	NDM				
Pt 3 2010	NDM				
Pt 22 2012	NDM	N			
Pt 29 2012	NDM			N	
Pt 39 2013	NDM				
Pt 2 2009	NDM	Y		Y	India
Pt 2 2008	NDM				
Pt 33 2012	NDM				
Pt 37 2013	NDM				
Pt 28 2012	NDM				
Pt 5 2010	KPC, VIN	Y		Y	Greece, hosp
Pt 5 2010	KPC, VIN				
Pt 30 2012	NDM				
Pt 7 2011	NDM				

Plasmid analysis



CPE by Region: CPHLN Data

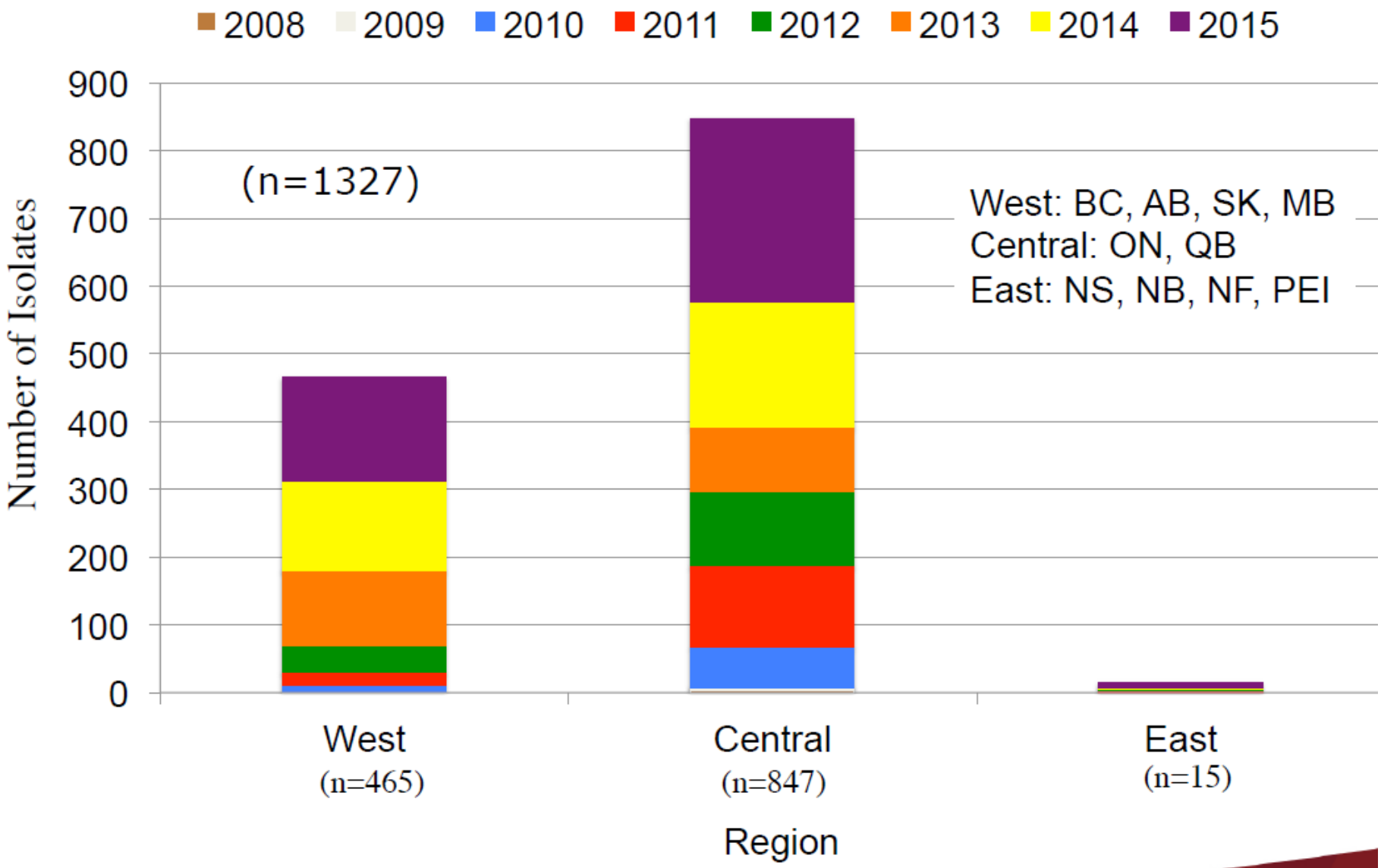
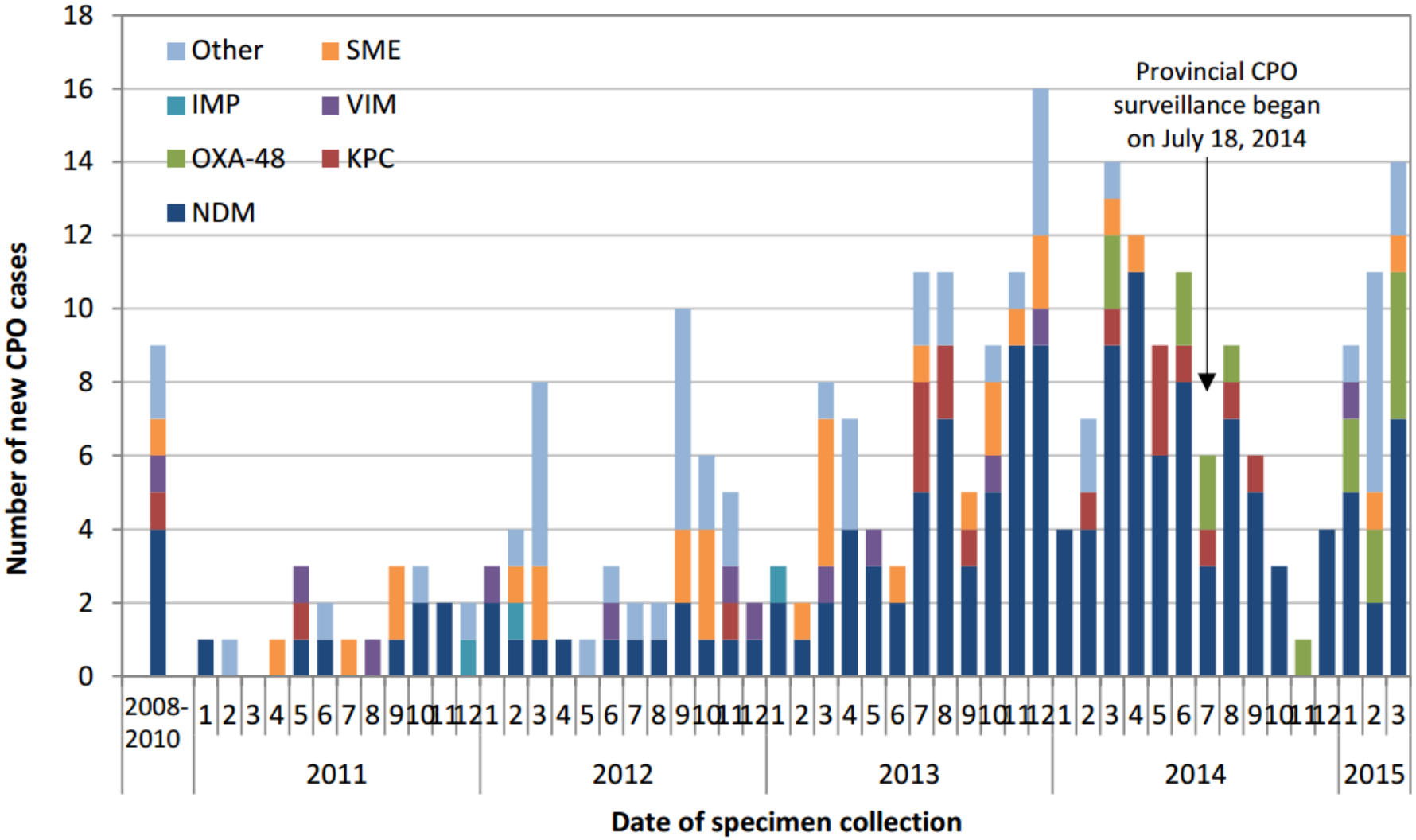


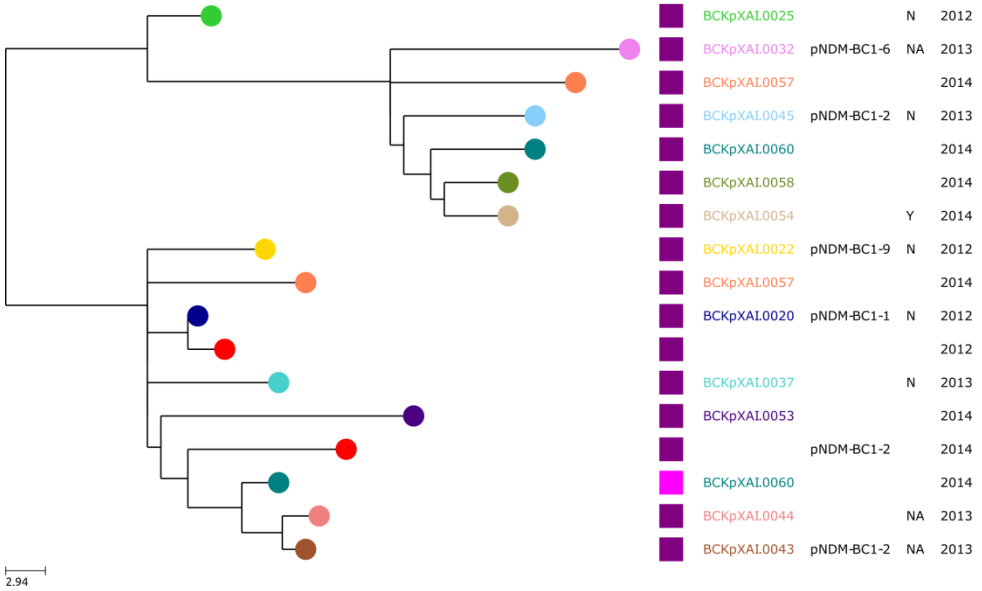
Figure 9. Number of new CPO cases by carbapenemase gene in BC, 2008 – March 2015



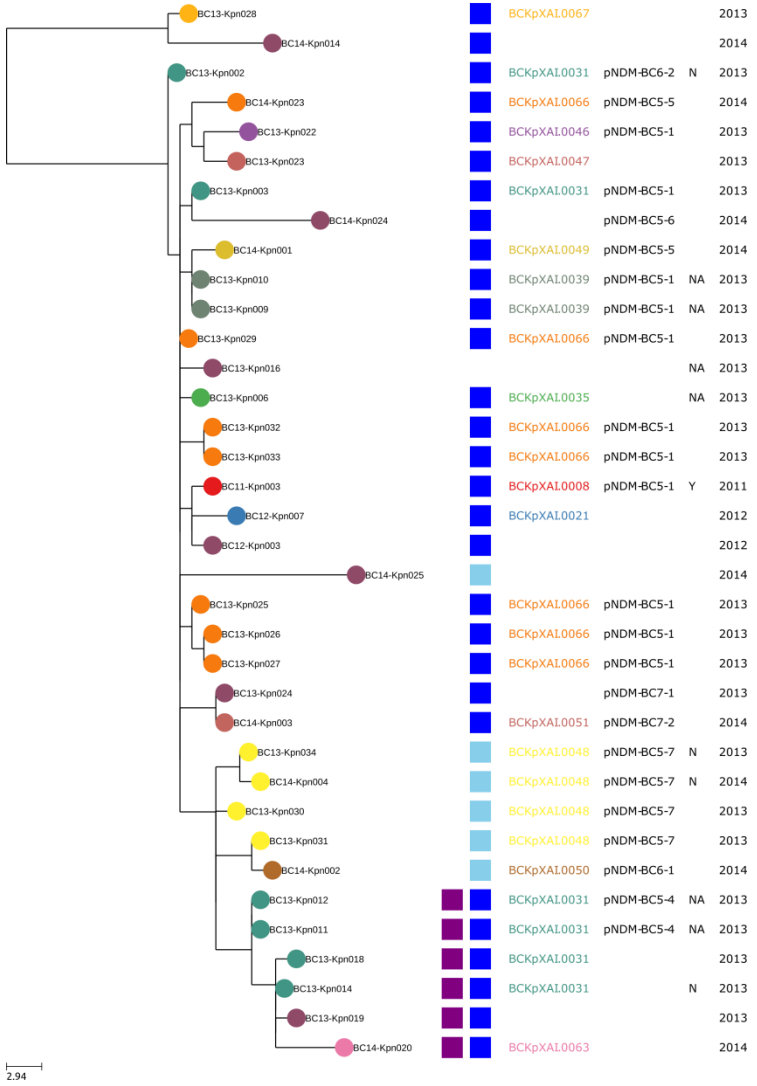
PICNet Surveillance Report 2014-15

CPO: NDM in BC

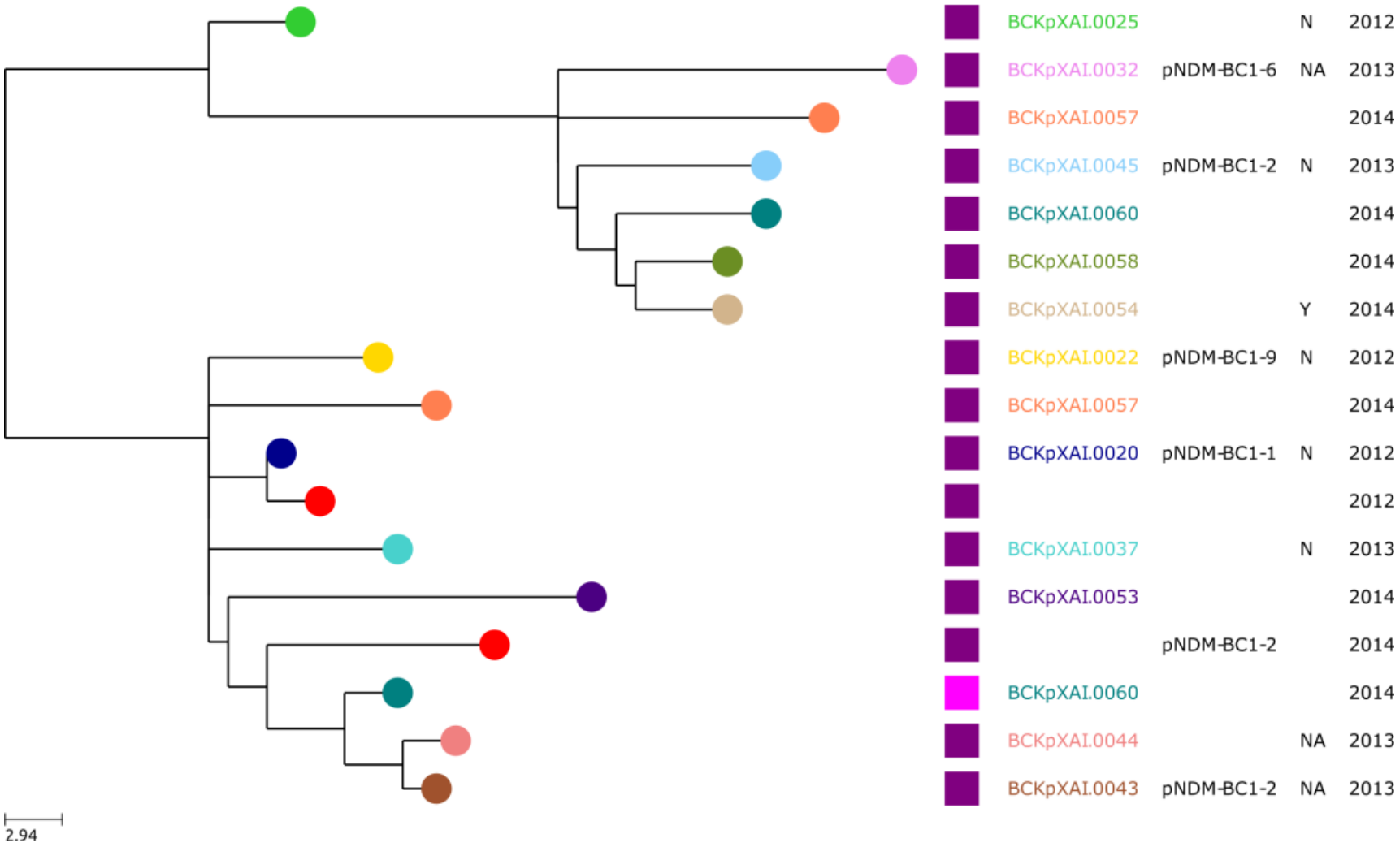
STKPN1



ST340



STKPN1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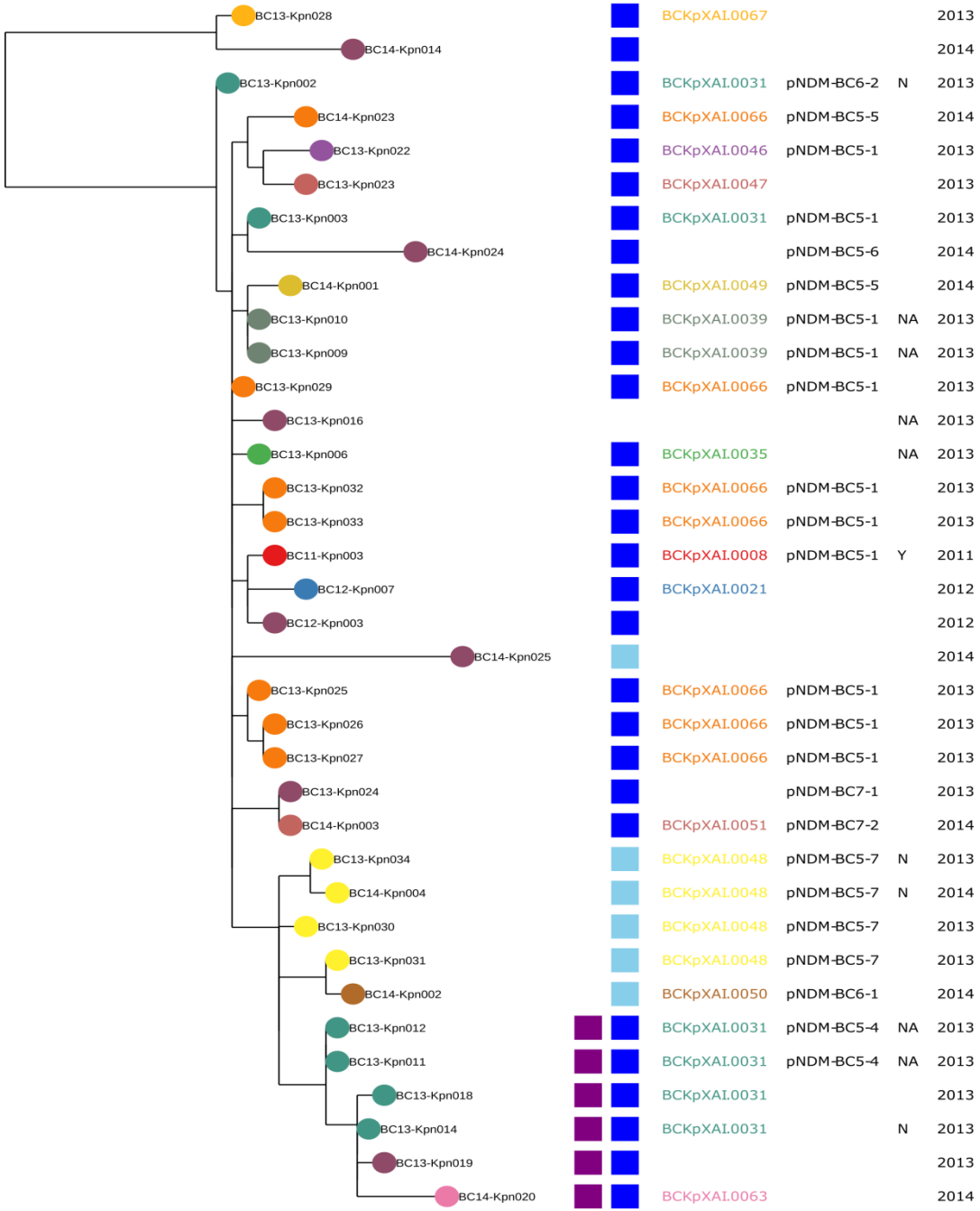

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Stay tuned for full BC NDM story....epidemiology data pending

- Goal:
  - Real-time WGS for outbreak investigation
  - Stop transmission after first few cases by finding clear connections
  - WGS provide antimicrobial susceptibility details

- Challenges:
  - Need automation
  - Costly
  - Need bioinformatics
  - More outbreaks identified, more resources needed to follow up
  - Complex biology

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THANK YOU!