Bacterial infections:
MRSA in NICU

Alex Outhred
HK Paediatric Infection Control Workshop 2016

How to allocate infection control resources?

- Greatest prevention of harm
  - focus on problems that cause significant harm
  - focus on problems that have effective interventions
- Targets
  - bugs
  - hand hygiene
  - patient environment - cleaning etc.
  - devices - venous and urinary catheters
- Be aware of biases
  - human nature to focus on most interesting or easiest aspects of problem
  - cannot eliminate but can try to compensate
  - importance of multidisciplinary teams

MRSA in neonatal intensive care

- Hospital in Australia
- Small neonatology unit, ~20 patients
  - no deliveries at hospital
  - most patients transferred from other NICUs for tertiary investigations (often short admissions) or surgery (sometimes long admissions)
  - some admissions via emergency department
  - snapshot median length of stay = 9 days
  - mixture of HDU and ICU beds
- 23 patients identified with MRSA over ~1 year, including some temporal clusters

Epidemic curve
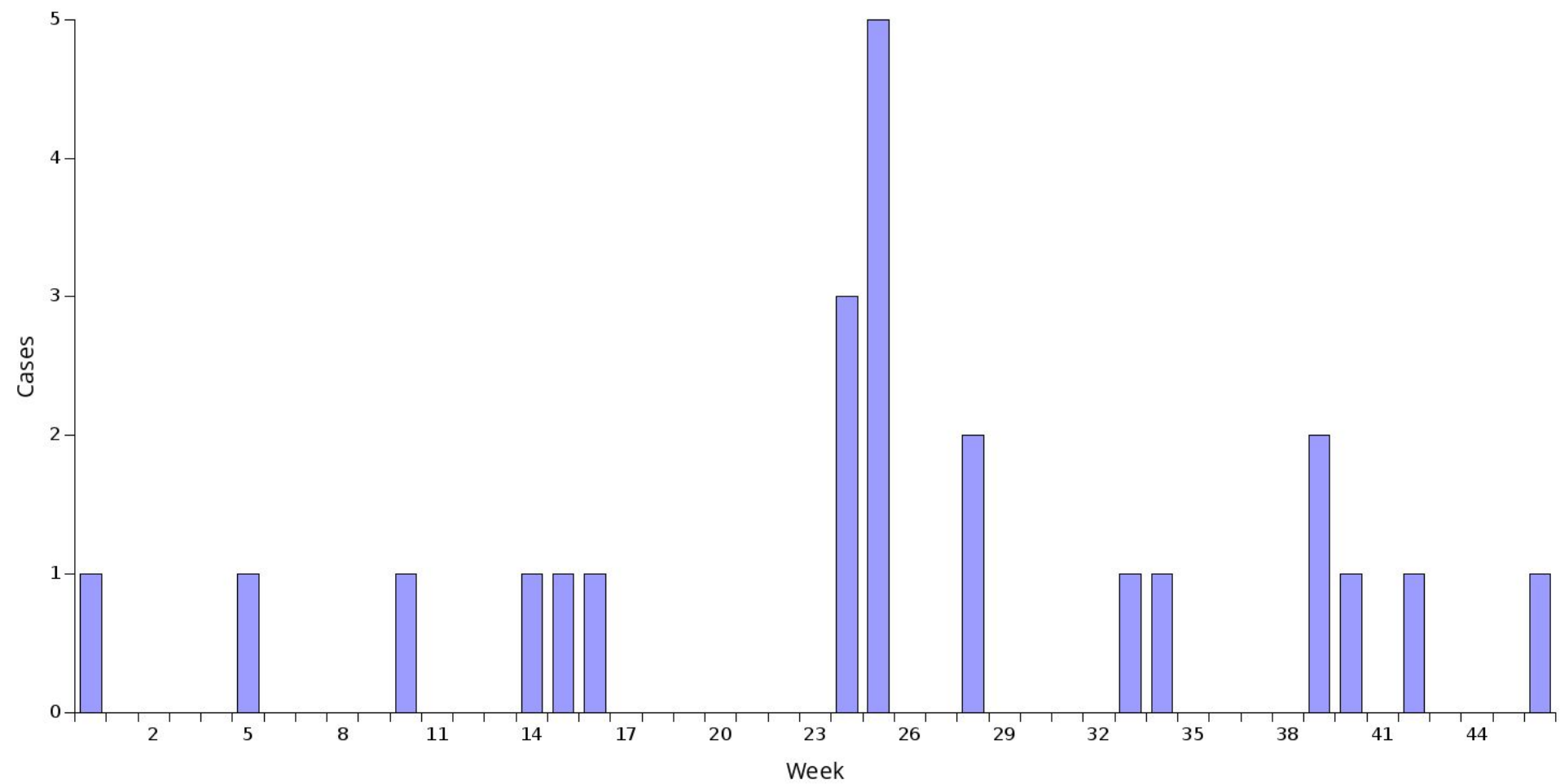
Epidemic curve: sufficient information?

- Basic epidemic curve
  - suggests there may be a problem, esp. weeks 24-25
  - not clear if situation has returned to baseline or still excess cases
- Staff know that some cases were transferred into unit
- Temporal patterns might not be true: diversity in MRSA
- Can the laboratory help?
  - most laboratories can provide susceptibility data for MRSA isolates
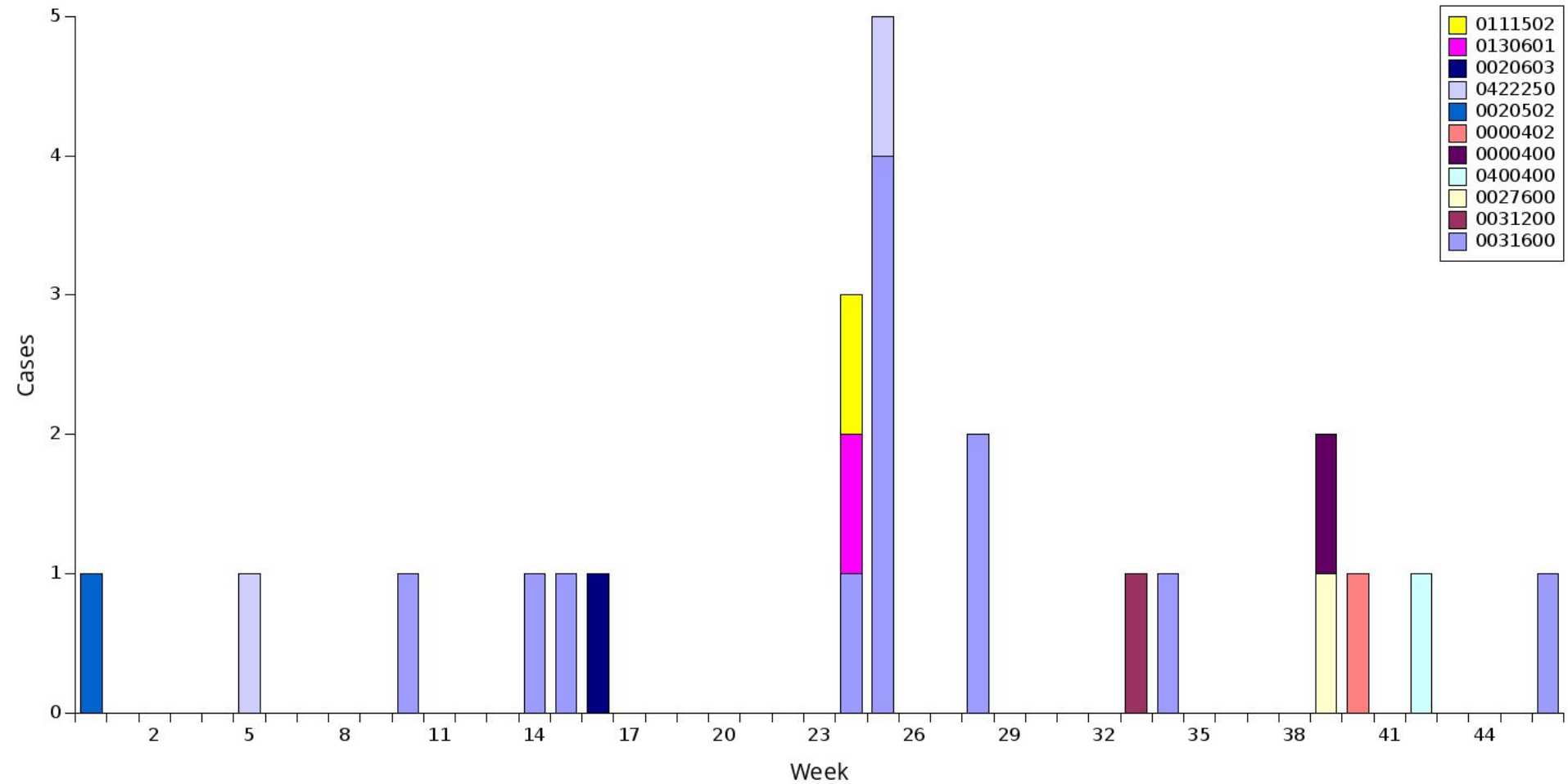 - would this help discriminate?

Epidemic curve: resistance pattern
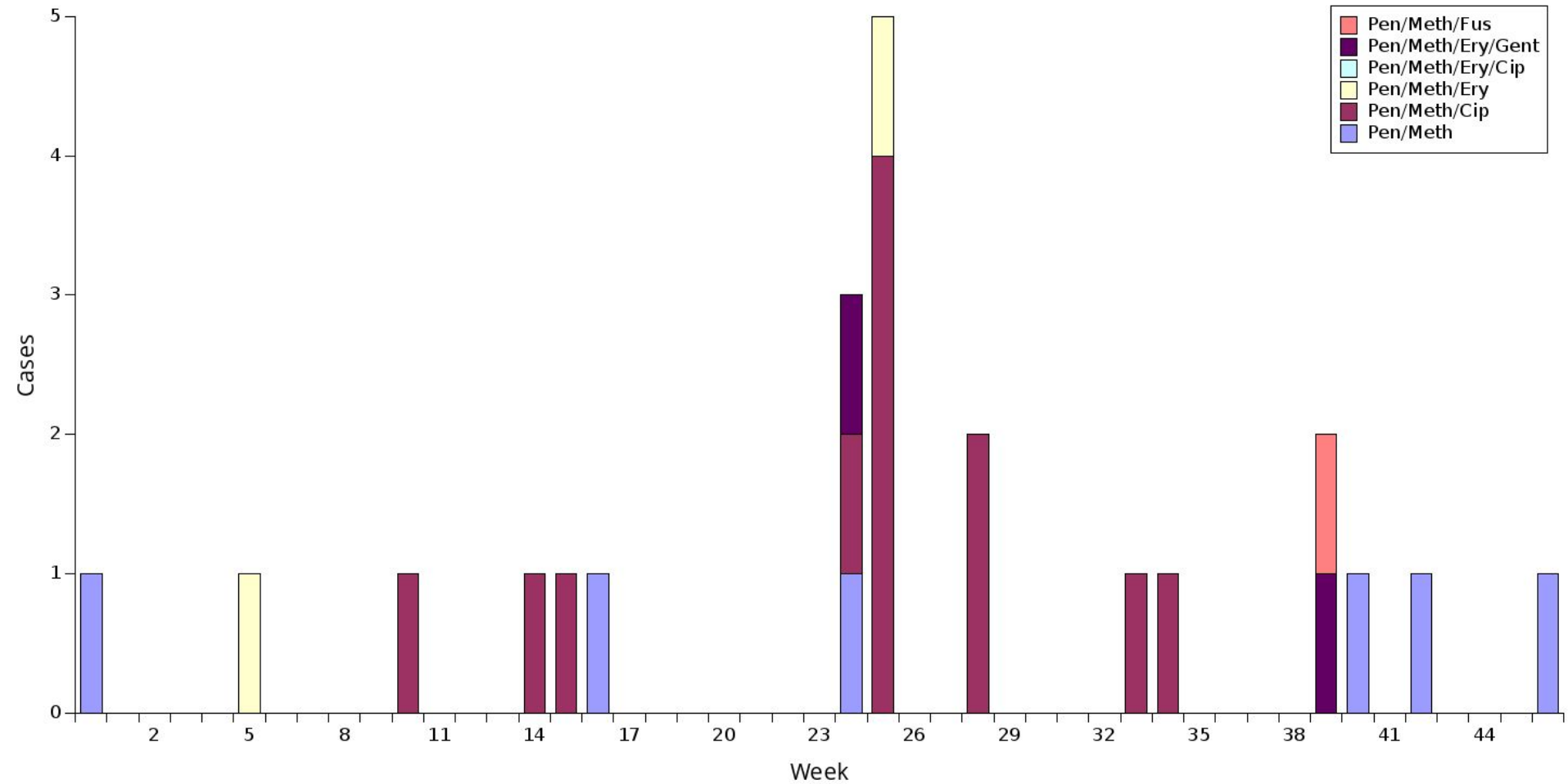
Epidemic curve with susceptibilities: enough?

- Six different susceptibility patterns
  - but all oligo-resistant
  - pen/meth/cipro-resistant are most concerning - 12 of 23
    - ST22 vs ST36 vs ST93 (occasionally R/cipro)?
  - pen/meth-resistant next most concerning - 6 of 23
    - probably mostly community strains, but...
- Numerous publications showing poor stability of resistance patterns
  - eg. loss of a single resistance mechanism from a strain that typically possesses it
  - could any of the pen/meth-resistant isolates be related to the pen/meth/cipro-resistant isolates?
- Consider performing typing
  - in this case, there was access to "binary typing"
    - reverse line blot method of detecting certain genetic elements in *S. aureus*.

nuc	mecA	pvl	Binary Typing Result	% of all isolates	(Predicted)* MLST		SCC mec	spa
🟩	🟩		13184	11.90%	22	🟢	IV	t032
🟩	🟩		20737					
🟩	🟩		263554					
🟩	🟩	🟦	37634	9.75%	93	🟢	IV	t202
🟩	🟩		5120	0.34%	953	🔴	IV	t267

Binary typing individual probe results^

Probe labels: seaSp, seaAp, secSp, secAp, sedSp, sedAp, pvlSp, pvlAp, N046Sp, N046Ap, SAV0881Sp, SAV0881Ap, PV83ORF2Sp, PV83ORF2Ap, SAV0858Sp, SAV0858Ap, phi11-458Sp, phi11-458Ap, sIORD257Sp, sIORF257Ap, SA1801Sp, SA1801Ap, SAV1974Sp, SAV1974Ap, sIORF182Sp, sIORF182Ap, ccrAB1Sp, ccrAB1Ap, ccrCSp, ccrCAp, mecRISp, mecRIAp, E007Sp, E007Ap, CQ002Sp, CQ002Ap, adbSp

Epidemic curve: binary typing

Epidemic curve with binary typing: enough?

- Appears to be more discriminatory
  - 5 different susceptibility patterns amongst 23 isolates
  - 11 different binary types amongst 23 isolates
- The largest group still has 12 of 23 isolates; matches ST22/EMRSA-15
  - but the members of the group are not identical to those using susceptibility profile
  - one isolate has moved each way
    - 1 of 12 by suscept is not included by binary typing, and
      1 of 12 by binary typing is not included by suscept
- However, binary typing can also show similarity of nonidentical types
  - the isolate in week 33 that was excluded from the susceptibility group using binary typing is very similar to the 12 members of the large group
  - in other words, if we relax the discriminatory power of binary typing, the large group expands to 13 isolates
- Still uncertainty
  - a better typing method (eg. WGS) would give us more discrimination
  - likely transmission of ST22 within the unit from week 24 to week 28, perhaps more

Typing methods for MRSA

- susceptibility profile
- MLVA
- *spa* typing
- MLST
- binary typing
- RAPD
- whole-genome sequencing

Why does typing matter?

- Where is the MRSA coming from? Which sources are most important?
    - transmission between patients within unit
    - transmission from healthcare workers within unit
    - transmission from family members within unit
    - introductions by transfer from other units
    - acquisition near time of delivery
- Combination of enhanced sampling and typing
- Helps decide resource allocation to reduce MRSA burden

MRSA transmission in hospitals: insights from WGS

- Other typing methods have poor resolution compared to WGS
  - including *spa* typing, MLVA, MLST, PFGE, binary typing
  - Clusters, transmission found by other typing methods are often rejected using WGS
- *S. aureus* accumulates WGS variants (SNPs) quite slowly
  - 3 to 12 SNPs per core genome per year, or 1 SNP every 4-19 weeks
  - Therefore, even with WGS it won't be possible to characterise all transmission events
- Some individuals can be colonised with a "cloud" of related clones
  - Eg. when persistently colonised at multiple anatomical sites
  - If you don't sample multiple colonies, sites, & times this will be missed
   - and if you miss this, you may misinterpret transmission patterns

The Stealthy Superbug: the Role of Asymptomatic Enteric Carriage in Maintaining a Long-Term Hospital Outbreak of ST228 Methicillin-Resistant *Staphylococcus aureus*

Laurence Senn,[a] Olivier Clerc,[a] Giorgio Zanetti,[a] Patrick Basset,[a] Guy Prod'hom,[b] Nicola C. Gordon,[c] Anna E. Sheppard,[c] Derrick W. Crook,[c] Richard James,[d] Harry A. Thorpe,[e] Edward J. Feil,[e] Dominique S. Blanc[a]

Hospital Preventive Medicine Service, University Hospital of Lausanne, Lausanne, Switzerland[a]; Institute of Microbiology, University Hospital of Lausanne, Lausanne, Switzerland[b]; NIHR Oxford Biomedical Research, John Radcliffe Hospital, Oxford, United Kingdom[c]; Department of Physics and Centre for Networks and Collective Behaviour, University of Bath, Bath, United Kingdom[d]; Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom[e]

ABSTRACT Whole-genome sequencing (WGS) of 228 isolates was used to elucidate the origin and dynamics of a long-term outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 228 (ST228) SCC*mec* I that involved 1,600 patients in a tertiary care hospital between 2008 and 2012. Combining of the sequence data with detailed metadata on patient admission and movement confirmed that the outbreak was due to the transmission of a single clonal variant of ST228, rather than repeated introductions of this clone into the hospital. We note that this clone is significantly more frequently recovered from groin and rectal swabs than other clones ($P < 0.0001$) and is also significantly more transmissible between roommates ($P < 0.01$). Unrecognized MRSA carriers, together with movements of patients within the hospital, also seem to have played a major role. These atypical colonization and transmission dynamics can help explain how the outbreak was maintained over the long term. This "stealthy" asymptomatic colonization of the gut, combined with heightened transmissibility (potentially reflecting a role for environmental reservoirs), means the dynamics of this outbreak share some properties with enteric pathogens such as vancomycin-resistant enterococci or *Clostridium difficile*.

IMPORTANCE Using whole-genome sequencing, we showed that a large and prolonged outbreak of methicillin-resistant *Staphylococcus aureus* was due to the clonal spread of a specific strain with genetic elements adapted to the hospital environment. Unrecognized MRSA carriers, the movement of patients within the hospital, and the low detection with clinical specimens were also factors that played a role in this occurrence. The atypical colonization of the gut means the dynamics of this outbreak may share some properties with enteric pathogens.
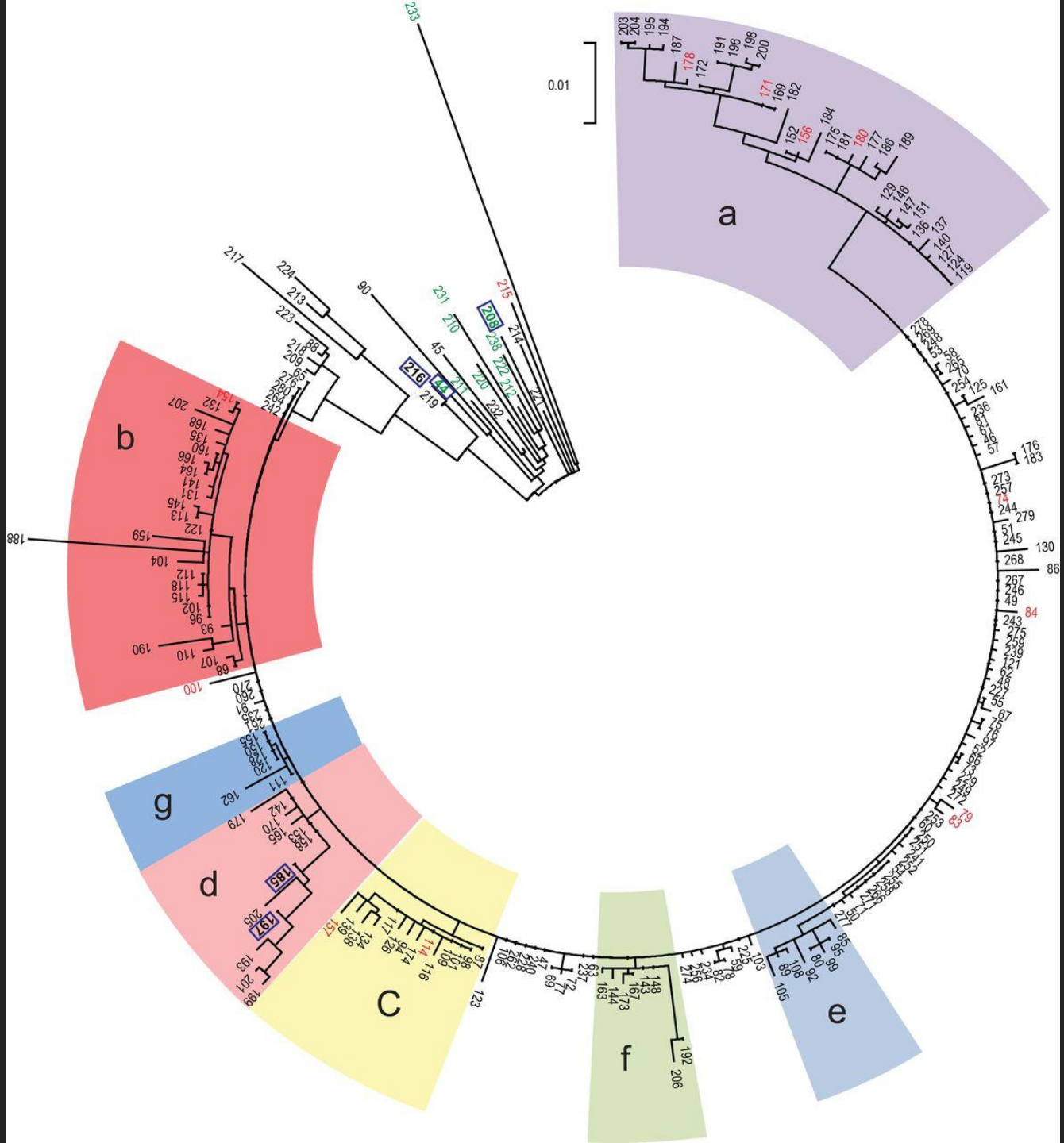
Senn L, Clerc O, Zanetti G, Basset P, Prod'hom G, Gordon NC, et al. doi:10.1128/mBio.02039-15
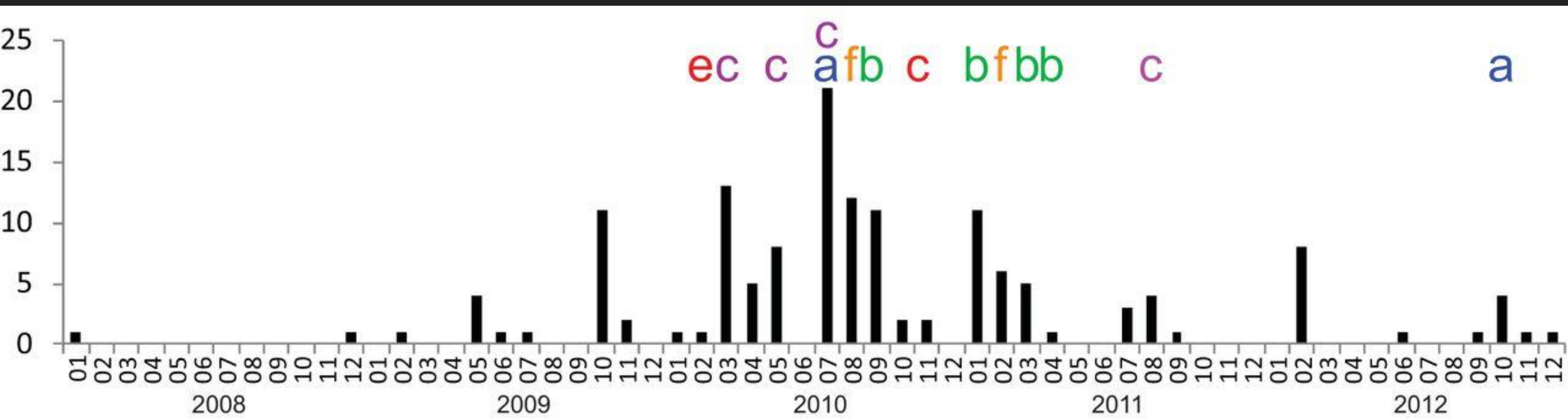
What was revealed by typing: Senn et al.

- Huge outbreak of an unusual strain (ST228) spanning 5 years
- Extensive transmission with relatively little clinical disease
- Evolved into seven different sublineages
  - sometimes several lineages within a single ward in a short space of time
- (Enteric colonisation may have been important)
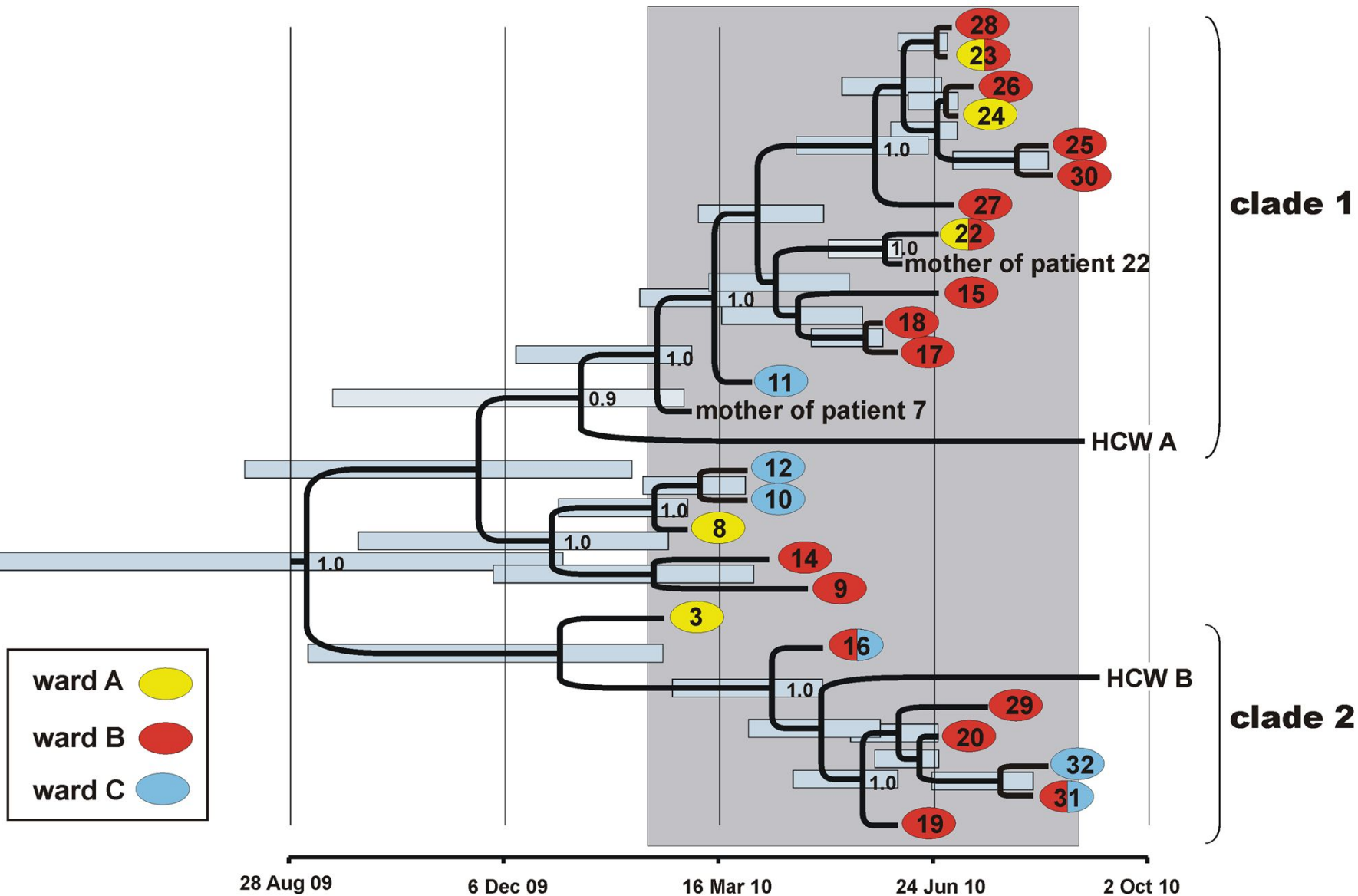
What was revealed by typing: Nübel et al.

- Healthcare workers acquired MRSA from patients
- Did not appear to contribute to transmission in this case
- Mothers were often colonised with the same strains as their infant - but who became colonised first?

Whole-Genome Sequencing for Outbreak Investigations of Methicillin-Resistant *Staphylococcus aureus* in the Neonatal Intensive Care Unit: Time for Routine Practice?

Taj Azarian, MPH, PhD;[1,2] Robert L. Cook, MD;[1,2] Judith A. Johnson, PhD;[3,2] Nilmarie Guzman, MD;[4] Yvette S. McCarter, PhD;[5] Noel Gomez;[6] Mobeen H. Rathore, MD;[7,8] J. Glenn Morris Jr., MD;[2,4] Marco Salemi, PhD[2,3]

BACKGROUND. Infants in the neonatal intensive care unit (NICU) are at increased risk for methicillin-resistant *Staphylococcus aureus* (MRSA) acquisition. Outbreaks may be difficult to identify due in part to limitations in current molecular genotyping available in clinical practice. Comparison of genome-wide single nucleotide polymorphisms (SNPs) may identify epidemiologically distinct isolates among a population sample that appears homogenous when evaluated using conventional typing methods.

OBJECTIVE. To investigate a putative MRSA outbreak in a NICU utilizing whole-genome sequencing and phylogenetic analysis to identify recent transmission events.

DESIGN. Clinical and surveillance specimens collected during clinical care and outbreak investigation.

PATIENTS. A total of 17 neonates hospitalized in a 43-bed level III NICU in northeastern Florida from December 2010 to October 2011 were included in this study.

METHODS. We assessed epidemiological data in conjunction with 4 typing methods: antibiograms, PFGE, *spa* types, and phylogenetic analysis of genome-wide SNPs.

RESULTS. Among the 17 type USA300 isolates, 4 different *spa* types were identified using pulsed-field gel electrophoresis. Phylogenetic analysis identified 5 infants as belonging to 2 clusters of epidemiologically linked cases and excluded 10 unlinked cases from putative transmission events. The availability of these results during the initial investigation would have improved infection control interventions.

CONCLUSION. Whole-genome sequencing and phylogenetic analysis are invaluable tools for epidemic investigation; they identify transmission events and exclude cases mistakenly implicated by traditional typing methods. When routinely applied to surveillance and investigation in the clinical setting, this approach may provide actionable intelligence for measured, appropriate, and effective interventions.
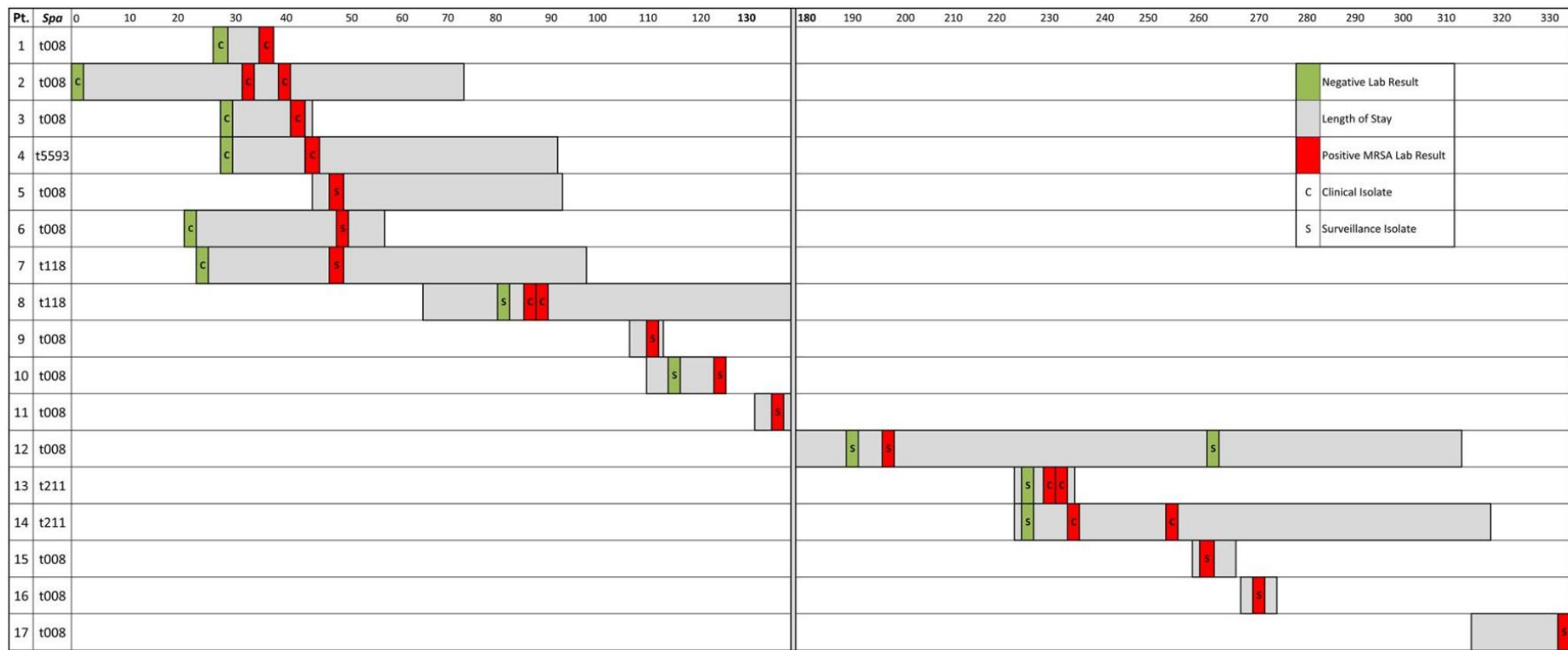
FIGURE 1. Detailed timeline for putative neonatal intensive care unit (NICU) outbreak. Lengths of stay are indicated in grey for the 17 pulse-field gel electrophoresis type USA300 isolates. *Spa*-types are indicated next to the patient number. Positive (red) and previously negative (green) surveillance (S) and clinical (C) isolates are illustrated. A 37-day gap between the 2 discrete outbreak periods is designated with a double vertical line shaded in gray.
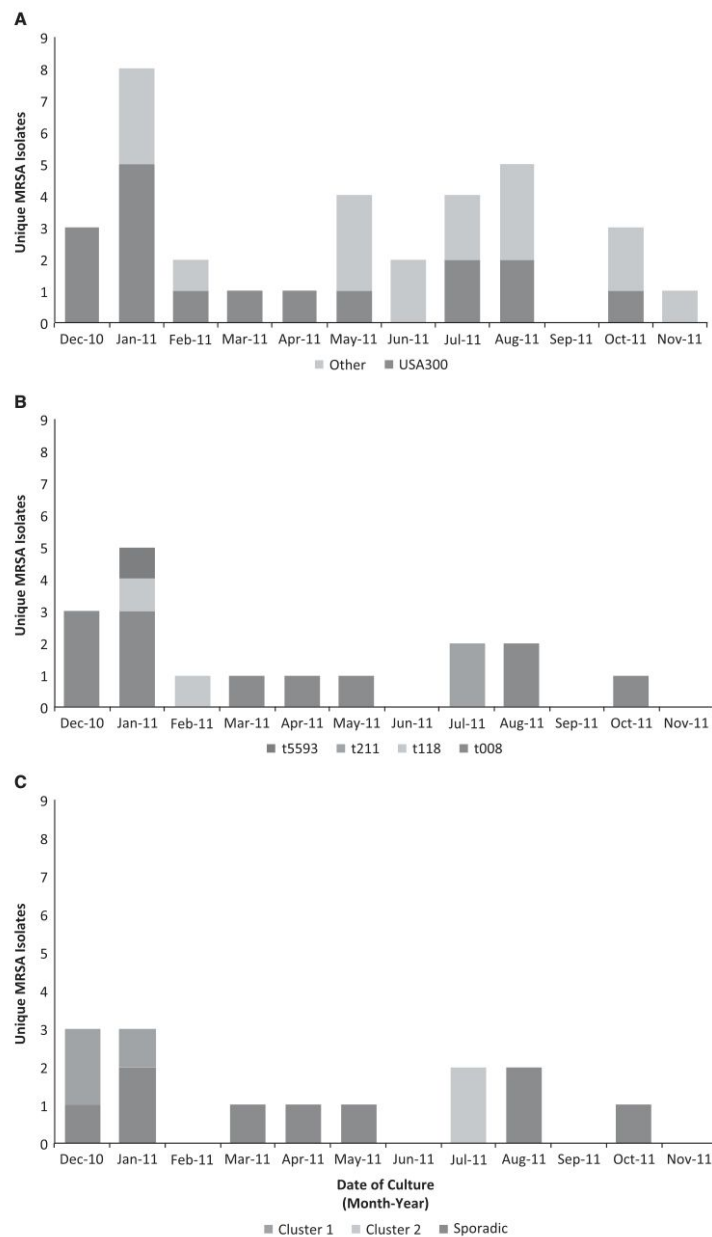
FIGURE 3. Epidemic curve of putative (neonatal intensive care unit) NICU outbreak incorporating increasing levels of resolution from molecular and whole-genome sequencing (WGS) analysis. (A) Epidemic curve of 34 cases using dates of incident clinical or surveillance MRSA-positive laboratory results. Cases identified as USA300 using pulsed-field gel electrophoresis (PFGE) (n = 17) during the primary outbreak investigation are indicated in blue. (B) Epidemic curve of 17 PFGE-typed USA300 cases stratified by *spa*-type conducted retrospectively to identify 5 non-*t008 spa*-types among the 17 PFGE-typed USA300 isolates. (C) Epidemic curve of 10 remaining PFGE-typed USA300 and *spa*-type *t008* isolates further stratified by results from phylogenetic analyses. Cluster 1 (patients 1, 2, and 6) represent epidemiological linkages based on phylogenetic data (eg, SNP distances) and epidemiological assessment (eg, overlapping lengths of stay).

What was revealed by typing: Azarian et al.

- Initially investigated 17 isolates that appeared to form a cluster (all USA300)
- However, the more closely they looked, the more separate introductions into their unit they found.
- Ultimately there was one cluster of 3 patients and one cluster of 2 patients (twins).
- Without performing the typing (WGS), infection control response may have targeted HCW hand hygiene as first priority.
    - This is a good priority to have, but as they found <4 transmissions, their performance may have been close to optimal already
    - If the problem is multiple reintroductions of community strains, a different response is required
    - How to address acquisition from family members?

Azarian T, Cook RL, Johnson JA, Guzman N, McCarter YS, Gomez N, et al. *Whole-Genome Sequencing for Outbreak Investigations of Methicillin-Resistant Staphylococcus aureus in the Neonatal Intensive Care Unit: Time for Routine Practice?* Infection Control & Hospital Epidemiology. 2015 Jul;36(07):777–85.
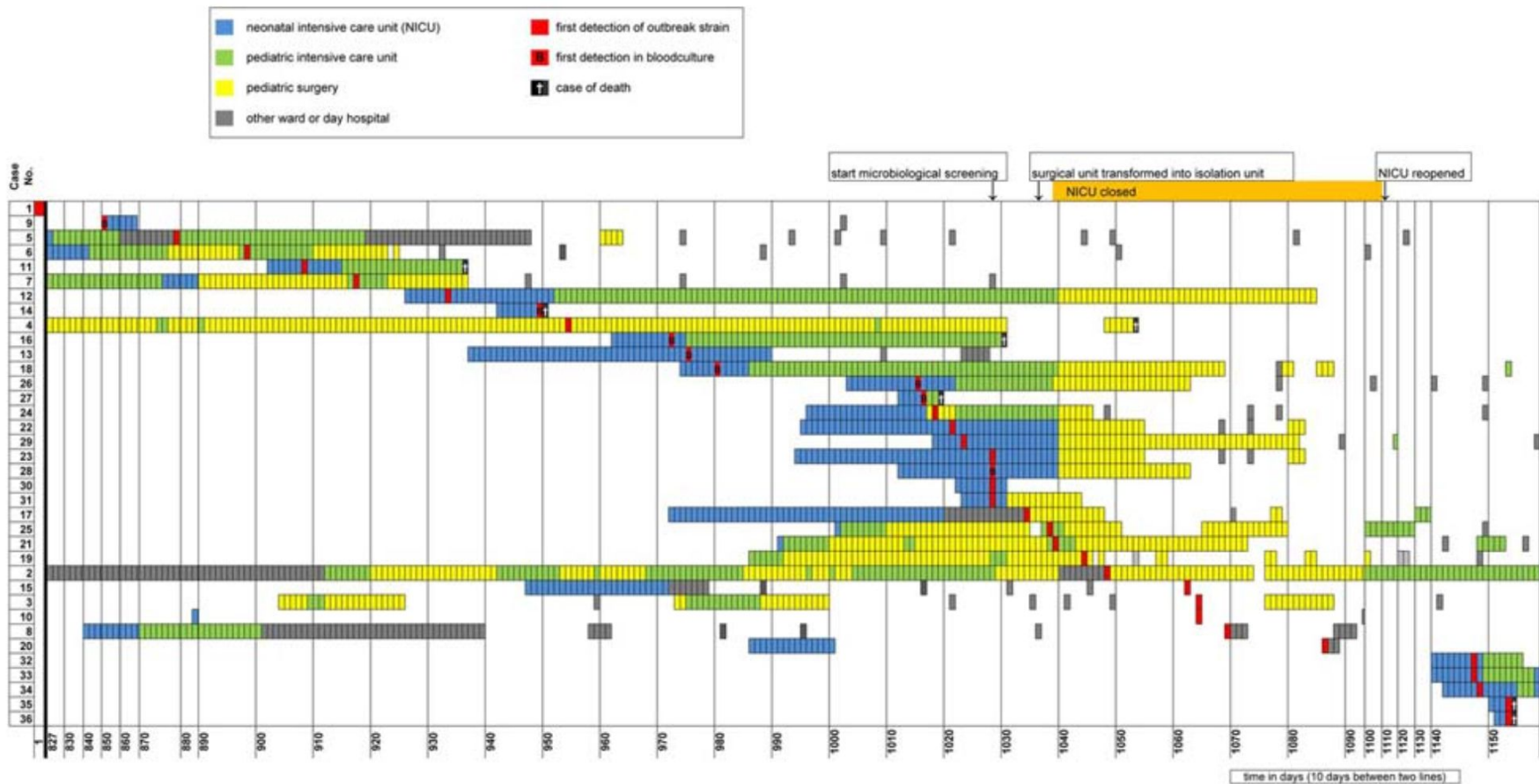
Figure 1 Timeline of the outbreak. The index patient (case 1), isolate from ≈01.01.2009 (day 1 of the outbreak) was retrospectively found to belong to the outbreak. Eight hundred and fifty days later the second case was tested positive. Black line between day 1 and day 827 represents time span with no additional information on cases. Time intervals of 10 days between vertical lines compressed whenever additional information was not lost. Data on case 37 are not presented in this figure. (Note: After discharge many infants came back to hospital for day hospital visits during which they were treated by members of the same team treating infants on neonatal intensive care unit (NICU) and paediatric intensive care unit. Sixty-five per cent of cases were on the same ward as another case within 7 days prior to first detection. Thirty-three cases were exposed to the NICU.)
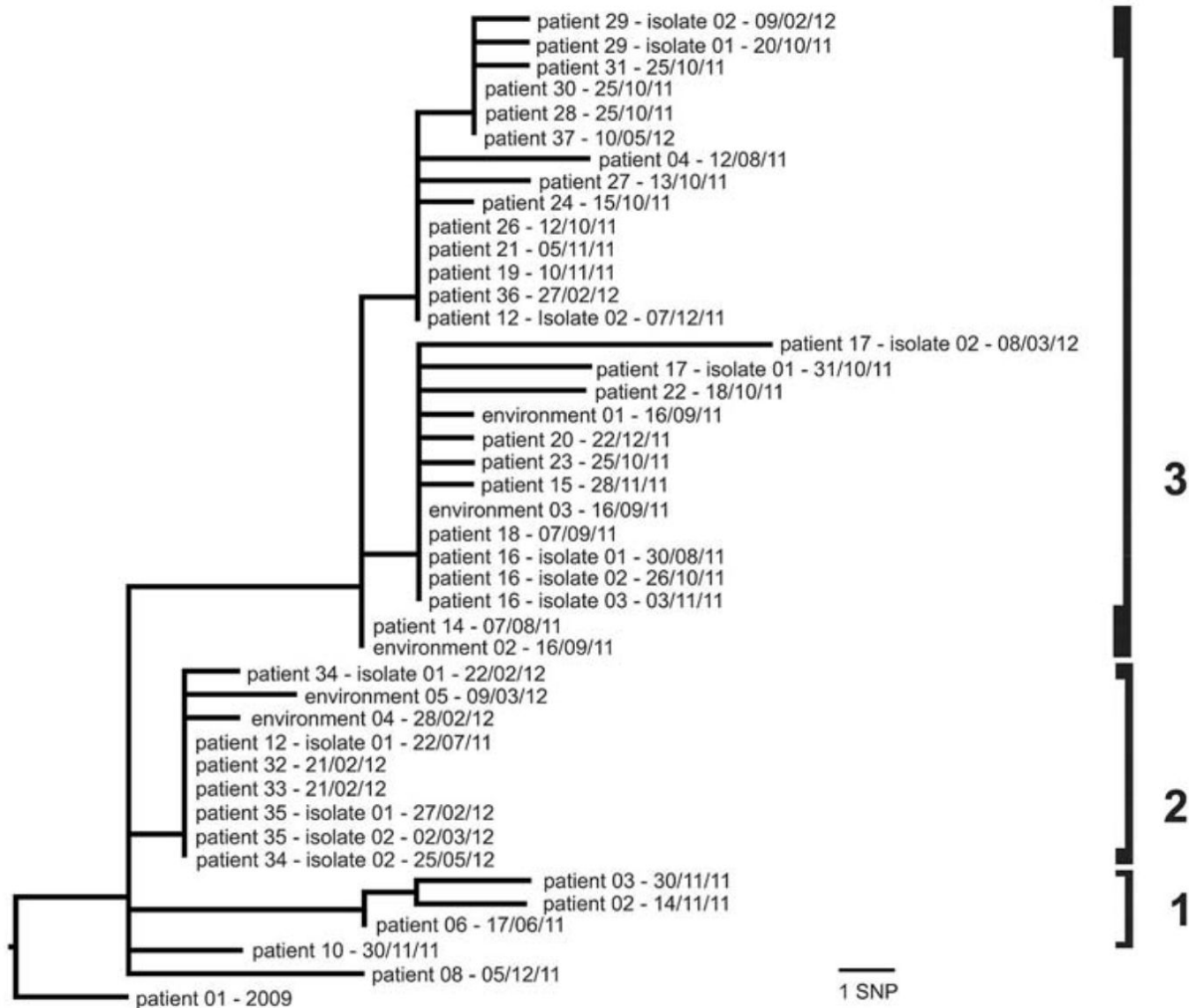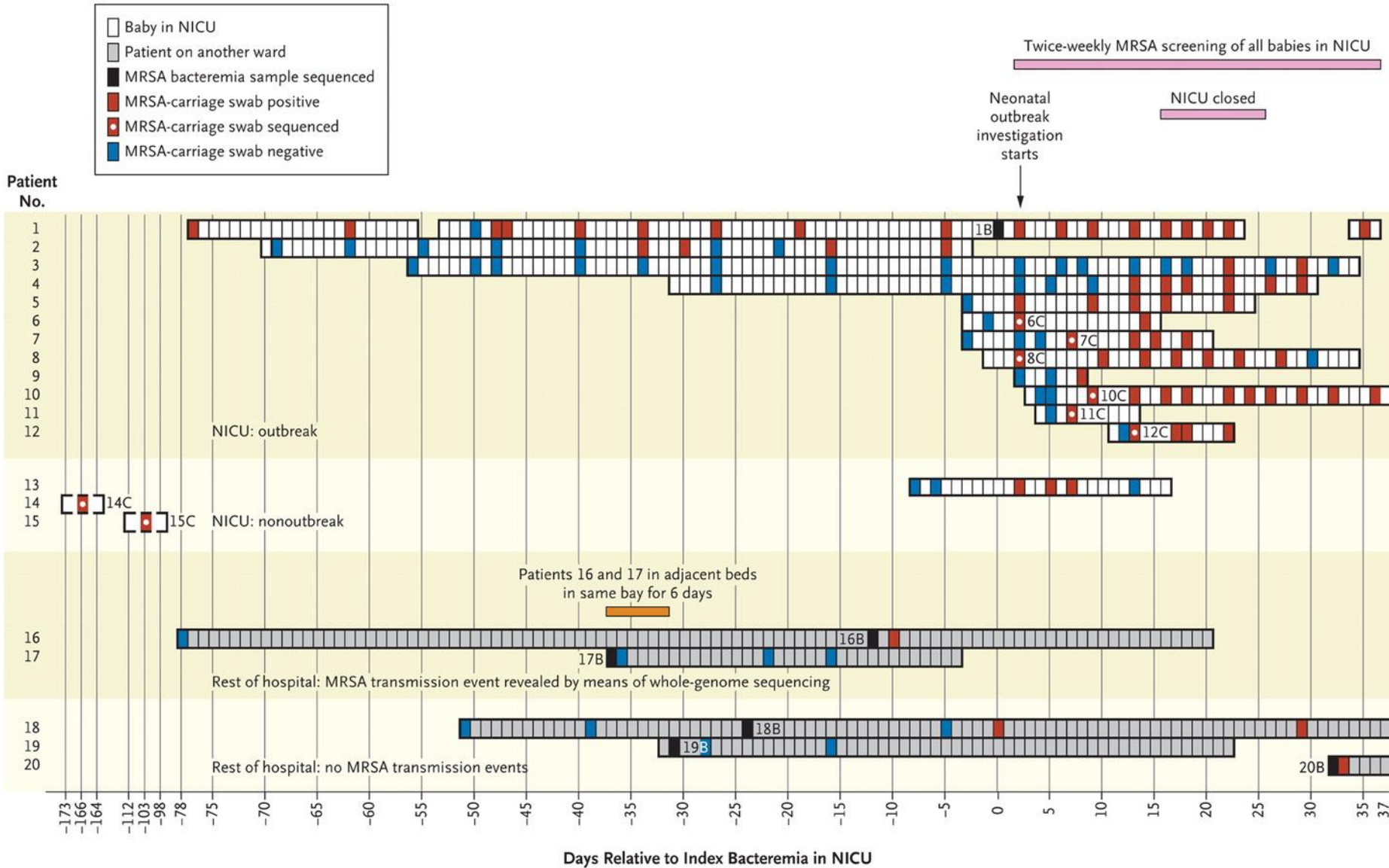
Figure 2 Maximum-likelihood phylogenetic tree based on sequence variation in the core genome (5.2 Mio base pairs) from ESBL-producing *Klebsiella pneumoniae* isolates representing the outbreak investigated. The tree was rooted by using isolate 316/12 (ST514, collected in Poland in 1996). Patient numbers, environmental sample numbers and isolation dates are indicated. Where multiple isolates from individual patients were available, these are numbered consecutively. Phylogenetic clades 1, 2 and 3 are indicated. SNP, single nucleotide polymorphism; ST, sequence type.

What was revealed by typing: Haller et al.

- Outbreak of ESBL *Klebsiella pneumoniae* associated with bacteraemia and deaths.
- Unlike Azarian experience, all isolates were closely related over 3-year period
- Ongoing transmission occurring within the unit - not point source or multiple introductions.
- Transmission continued despite closure, renovation and reopening of unit.
- Devastating findings for the unit.

Haller S, Eller C, Hermes J, Kaase M, Steglich M, Radonić A, et al. *What caused the outbreak of ESBL-producing Klebsiella pneumoniae in a neonatal intensive care unit, Germany 2009 to 2012? Reconstructing transmission with epidemiological analysis and whole-genome sequencing.* BMJ Open. 2015 May 1;5(5):e007397.

Köser CU, Holden MTG, Ellington MJ, Cartwright EJP, Brown NM, Ogilvy-Stuart AL, et al.
doi: 10.1056/NEJMoa1109910

Insights gained from WGS studies

- Diversity in clusters and outbreaks
- Dominant method of transmission can vary with patient, setting, strain
- Results often obtained too late to influence infection control response
  - but in some cases, the results could have led to altered response

Lab aspects: overview

- Use appropriate screening method
  - Culture with selective agar generally cheaper with good sensitivity, specificity
  - PCR screening can have advantages if turnaround time is short, result is acted upon promptly and effectively
    - but culture still necessary if typing might be needed
- All screening methods dependent upon specimen quality
  - Specimen collectors must be trained
  - Multiple sites should be sampled
    - different patients and strains ⇒ diversity in sites of colonisation

Role of microbiome

- Likely to be important for colonisation resistance
  - neonates, esp. premature, serve as blank slate for colonisation by MROs - founder effects?
  - "normal" flora (ie. diverse, low-virulence microbiome) may be protective
  - direct effects - different microbes compete for nutrients and secrete inhibitory compounds
  - indirect effects - microbiome has major influence on immune system
- Many influences on microbiome:
  - caesarean vs vaginal delivery
  - premature vs term
  - breastmilk vs formula
  - skin to skin contact
  - antimicrobial exposure in mother and neonate
- Microbiome complexity leads to challenges
  - probiotics generally simple mixtures, not representative
  - microbiota transfer - hard to define, hard to conduct trials
  - parental source may be acceptable

Recent literature on microbiome

- Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. *Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life*. doi:10.1016/j.chom.2015.04.004
  - Mode of delivery and breastfeeding have long-term influence on microbiome
- Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, et al. *Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer*. doi:10.1038/nm.4039
  - Demonstration that parental microbiome can be transferred to neonate
- Heida FH, Zoonen AGJF van, Hulscher JBF, Kiefte BJC te, Wessels R, Kooi EMW, et al. *A Necrotizing Enterocolitis-Associated Gut Microbiota Is Present in the Meconium: Results of a Prospective Study*. doi:10.1093/cid/ciw016
  - Enteric Staphylococci may be protective against NEC

My approach to MRSA in NICUs

- Think you might have a problem?
  - collect more data!
  - adopt regular screening (eg. weekly) in addition to admission and discharge screening
  - ensure specimen collection follows best practice
    - sample multiple sites
    - use pre-moistened swab for dry sites
  - record each case as "external acquisition", "local acquisition" and specimen date
  - individual patient rooms best
    - if impossible, cohort like with like eg. same antibiogram
    - if impossible, cohort all MRSA together (this is not good, only if no alternative)
  - reinforce standard precautions including adequate staffing, excellent hand hygiene, prompt and thorough cleaning, optimal central line practices
  - audit practices and shared facilities, walk around in small teams and try to think of everything -
    - toilets and changing areas (what happens to a soiled nappy?)
    - feed preparation areas
    - parent rest areas
    - laundry (eg. what happens to soiled woollen booties and beanies?)

My perspective cont'd

- Still problems after easy stuff has been done, or problems getting worse quickly?
  - screen family members
  - try to screen HCW too
  - arrange typing, I suggest straight to WGS
    - ~$100USD/isolate not cheap,
      - but compared to overall NICU costs?
      - cost of closing unit?
    - ideally get results within a week, within a month should be straightforward
- If low rates of MRSA disease, don't panic.
- If serious MRSA infections eg. bacteraemia in >2 probably linked cases?
  - start high-level discussions about closing unit to new admissions
  - needs to be a shared or executive-level decision