How should we best use molecular tests in infection control?

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for

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Outline

- Molecular tools available for hospital infection control
- How should we apply them
  - Rapid diagnostics including drug susceptibility
  - Surveillance
  - Molecular epidemiological studies
- The future
  - Next Gen Sequencing
  - Markers of infectiousness
  - Markers of virulence

Molecular tools

The ‘omics’ (genomics, transcriptomics, proteomics, and metabolomics)—laboratory techniques are very rapidly developing fields used to study the structure, function, and dynamic interactions of microorganisms and their genomes

- **Genomics** is the study of an organism’s genome, be that deoxyribonucleic acid (DNA), ribonucleic acid (RNA), whole-genome sequencing (**WGS**) using next-generation sequencing (**NGS**) techniques.
- **Transcriptomics** studies the expression of an organism’s genes, examining the patterns of individual gene expression and the relative abundance of RNA transcripts, including messenger, ribosomal, and transfer RNAs.
- **Proteomics** involves analysis of the proteins expressed by an organism, which reflect gene transcription, translation, and post-translational modification.
- **Metabolomics** is concerned with a cell’s metabolic (that is, chemical) fingerprint.

Molecular tools

Typing techniques:

•Direct comparison of DNA/RNA sequences using Whole Genome Sequencing (WGS) to identify

Single Nucleotide Polymorphisms (SNPs)
16S ribosomal RNA (rRNA) sequencing

•**Multilocus sequence typing (MLST**) can distinguish between Shiga toxin-producing strains of *Shigella* and *Escherichia coli*

•Measurement of the accumulation of pathogen-specific genetic targets amplified by polymerase chain reaction (PCR) using labelled nucleotides

• Gel electrophoresis in (e.g. (**MIRU-VNTR**), for *Mycobacterium tuberculosis*), and to identify restriction fragment length polymorphisms (**RFLP**) (partially discriminatory for *Shigella* species)

• Probe Matrix Hybridization: measures binding to known single-strand nucleic acid sequences by single-strand fragments of “test” microbial’s genome (e.g. DNA microarray or ‘gene chip’)

Potential Applications

1. Rapid diagnosis
2. Surveillance
   - Trends and monitoring
   - Hypothesis driven epidemiological research e.g. PVL *S aureus*
   - Monitoring new virulent trends, drug resistance, vaccine escape, diagnostic escape
3. Investigating transmission:
   - Prospective public health led cluster investigation and identification of super spreaders / undetected events
   - Outbreak investigation
   - Environmental spread
   - Direction of transmission or Excluding transmission
4. Understanding pathogenesis (virulence, drug resistance etc.)

Transmission investigation

We use these tests to inform epidemiological hypotheses including the effectiveness of infection control/antimicrobial stewardship interventions

PLEASE AVOID THE TERM

“epidemiological typing”

1. Rapid diagnosis

- Rapid detection of infections to inform isolation, decolonisation or focused therapy e.g. multiply drug-resistant organisms such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococcus (VRE).

1. Rapid diagnosis

- Remember other measures are more important than the type of test used e.g. environmental disinfection, hand hygiene, contact precautions, bundles of measures to prevent bloodstream infections and ventilator-associated pneumonia, and decolonization
- They key added value is in time to diagnosis….
  advertised analytic turn around vs actual turn around time

1. Rapid diagnosis

Multiply drug-resistant gram-negative rods are increasingly important because of the complexity and variability of the resistance determinants – no good commercial tests yet.

Clostridium difficile is a good candidate for molecular testing as existing enzyme immunoassays (EIAs) are just not sensitive enough. Remember false positives – only test diarrheal samples etc.

2. Surveillance

Information for Action

- Capacity for bioinformatics and analysis preferably software that generates timely and useful information from genetic data.
- Such analysis should take into account that all information is either:
  - of public health relevance and actionable
  - valid but not actionable
  - of unknown significance
- Effective ways to convey meaningful information need to be developed and training for appropriate specialists

"Effective & timely communication and Competency"

2. Surveillance

- Epidemiological information (e.g. time, place, person, instrumentation, IPC, AS)
- Carriers versus disease (70% carriers and cross-infectors)
- Ease of isolation …. although…
- Where analytical epidemiological studies are undertaken:
  - BIAS: selection bias (convenience samples)
  - CHANCE: sample size, background variability
    e.g. "invaders" from community with same EMRSA?
  - CONFOUNDING factors
    e.g. other interventions confusing (e.g. isolation introduced as well as bundles)
- Use of prevalent cases (a convenience sample)
- VALIDITY: repeatability (intra and inter); standardised methods
- Reporting statement: STROME-ID


Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID): an extension of the STROBE statement

Nigel Field, Ted Cohen, Marc J Struelens, Daniel Palm, Barry Cookson, Judith R Glynn, Valentina Gallo, Mary Ramsay, Pam Sonnenberg, Duncan MacCannell, Andre Charlett, Matthias Egger, Jonathan Green, Paolo Vineis, Ibrahim Abubakar

THE LANCET Infectious Diseases

2. Surveillance: monitoring

- Emergence of resistant strains
  - Plotting the SNP distance from the phylogenetic root of the tree against time, Harris *et al.* estimated the emergence of the ST239 clone of MRSA to the mid-1960s, consistent with the increased use of antibiotics and the first detection of MRSA***
- Emergence of vaccine escape variants
  - whole-genome sequences of a clone *Streptococcus pneumoniae and showed* that the generation of vaccine-escape variants had occurred within the population prior to the introduction of the antipneumococcal vaccine**
  - Baseline analysis possible (e.g. Malawian strains of pneumococci sequenced prior to vaccine introduction*)
- Emergence of 'diagnostic escape' e.g.*Chlamydia trachomatis* and *Neisseria gonorrhoeae* *due to genes varying.*

(MRSA SCCmecs also relevant e.g. bovine MRSA SCCmecC)

*PLoS One. 2012;7(9):e44250

***Science 331(6016),* 430–434 (2011).

****Science 327(5964), 469–474 (2010).*

3 Transmission

- Reasonable to assume that it can establish when infections are *not* caused by the same strain
- Establishing transmission of strains (can be multiple)
- Consider:
  - availability of other information to determine direction of spread (incubation, infectiousness, other sources, variability)
  - Chance - how much background variation?
  - Molecular clock – how much difference is expected?

but WGS holds promise beyond previous molecular epidemiological tools to address transmission

3 Transmission: Basic assumptions in molecular epidemiology

- Strains with a common origin are more similar than strains with different origins
- Degree of similarity tells us about common origins and time since divergence
- Depending on the question, we need to decide on using slower or faster evolving markers

3. Transmission: Role of molecular tests depends on the source of the hospital acquired infection

- auto-infection—acquired from an endogenous source
- cross-infection—acquired from an exogenous source
- environmental—acquired from the environment.

3. Transmission: Investigating Clusters

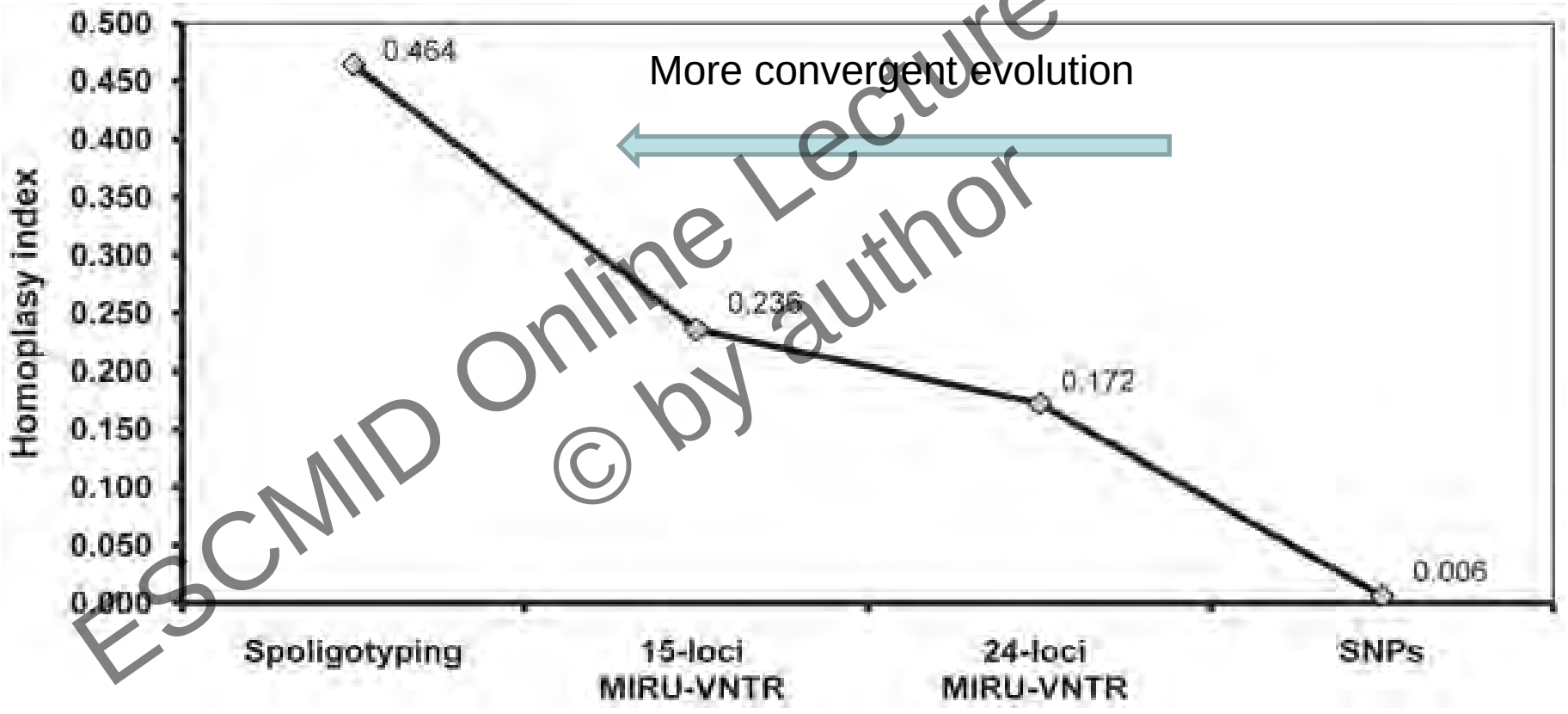
- Universal prospective typing (stored strains can be very valuable e.g. BSI isolates)
- How quickly can we get the results (sequencing from patient samples, processing and analysis and delivery of results to clinical/public health teams to act)
- Cost effectiveness

3. Transmission: Investigating Clusters

- How complete is the population?
  - –incomplete ascertainment
  - – contact tracing
  - – case definition
  - – immigration and emigration (short lengths of stay!)
  - – time window
- Resolution (sensitivity versus specificity)

3. Transmission: Investigating Clusters

Comparison of the homoplasty index (HI) across the different TB genotyping methods.



Comas I, Homolka S, Niemann S, Gagneux S (2009) Genotyping of Genetically Monomorphic Bacteria: DNA Sequencing in Mycobacterium tuberculosis Highlights the Limitations of Current Methodologies. PLoS ONE 4(11): e7815. doi:10.1371/journal.pone.0007815
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0007815

Convergent evolution in DR locus – limitation of spoligotyping. Fenner et al

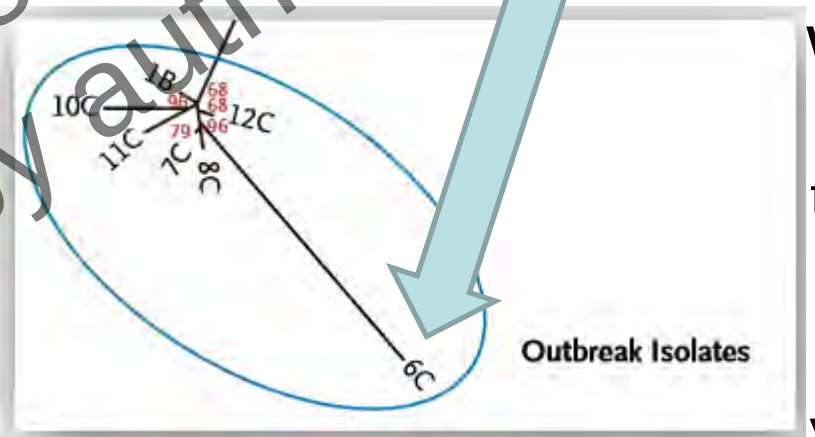
3. Transmission: Investigating Outbreak

- Provide timely information on:
  - evolutionary origin
  - transmission route
  - pathogenic potential
  - resistance information

3. Transmission: Investigating Outbreak

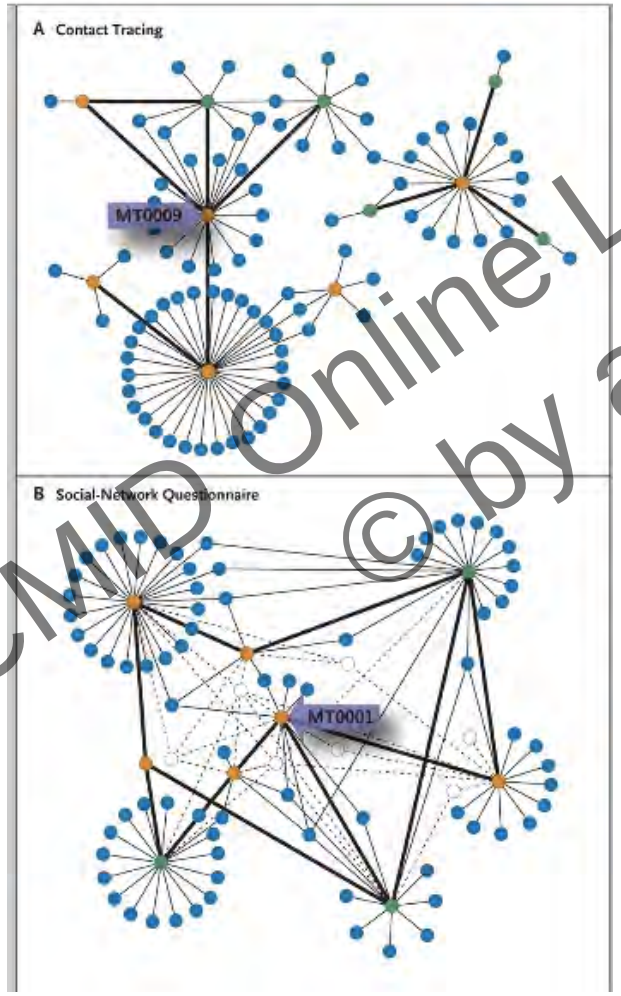
MRSA

- Neonatal outbreak**
  - Revealed a cluster of outbreak isolates/missed transmission: 1.5d to produce results: hyper-mutator found: *spa* not reported. $150/isolate
- Outbreak involving r W***
  - Link to mother in pos
    member of staff (*BC*: t?)
- But must compleme
  - High carriage rate of
    e.g. genomic analys y
    associated epidemic USA300 : very few genetic variations (11–48 SNPs). Arginine catabolic mobile element (ACME) growth advantages, from CNS perhaps? *



**Koser et al NEJM. 2012 Jun 14;366(24):2267-75
***Harris et al Lancet ID 2013 Feb;13(2):130-6
*Diep et al Lancet. 2006;367(9512):731-9

3. Transmission:
Investigating Tuberculosis Outbreak



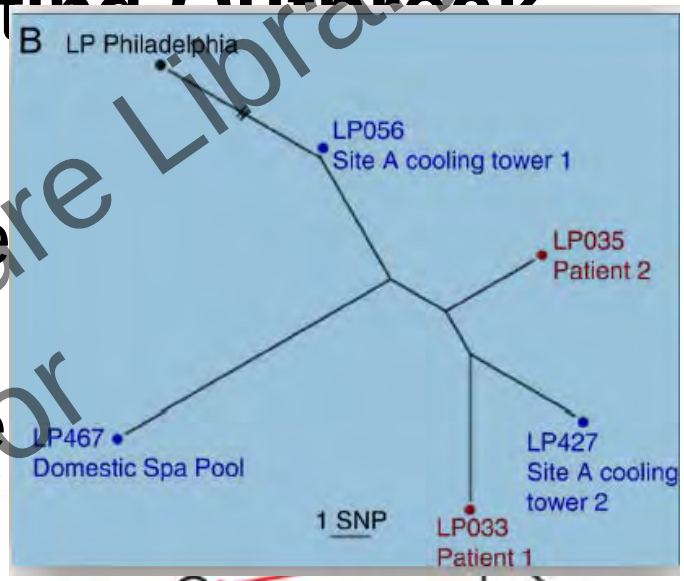
Social network analysis and WGS – led to identification of mode of spread and potential super spreaders

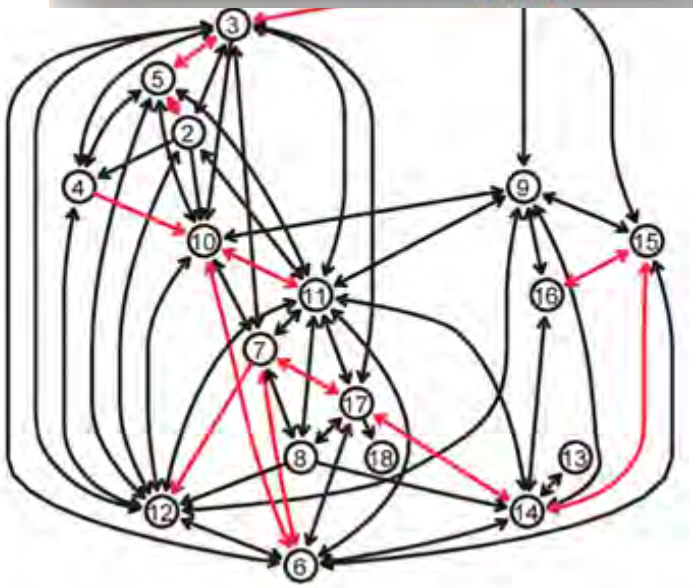
Gardy et al NEJM. 2011;364(8):730-9

3. Transmission: Investigating Outbreak

- Hospital Outbreak of Carbape
  *Klebsiella pneumoniae**
  - Single source from a patient, ve
- Legionella**
  - Retrospective WGS able to ra
    outbreak from non-outbreak is
    environmental source

  COMMENT
  - No mention of the TIMELINES





*Sci Transl Med. 2012 Aug 22;4(148):148
** BMJ Open 2013;3:e002175

4. Pathogenesis

a. Top down virulence investigation:

- Population impact of strains with
  - Higher morbidity (e.g. toxin production)
  - Higher mortality
  - Increased transmission
  - Drug resistance
  - Higher rates of mutation
- Models for investigating virulence based on WGS information developing
  - e.g. *S aureus* work CC398 strains by Priest et al (developed model, tested it and used it for prediction: 5-10 years work still to do)

Nat Rev Microbiol. 2012 Nov;10(11):791-7. doi: 10.1038/nrmicro2880.

4. Pathogenesis

b. Recurrence:

- Compare strains from initial and recurrent episode of disease in an individual
- Same strain: relapse or reinfection (depends on background variability)
- Different strain: reinfection with new strain, mutation - how different is different?
  - >40SNPs likely to be a different *S. aureus* (Moore et al, J Hosp Infect (in press))
  - Can be very complex e.g. NEC [Raveh-Sadka DOI: 10.7554/eLife.0547],
- Mixed infection

4. Pathogenesis

c. Drug resistance:

- Russian WGS of TB – fluoroquinolone resistance through acquisition and not transmission**
  - Drug therapy is driving evolution
  - Selection of evolution of same virulence determinants and quinolone resistance genes

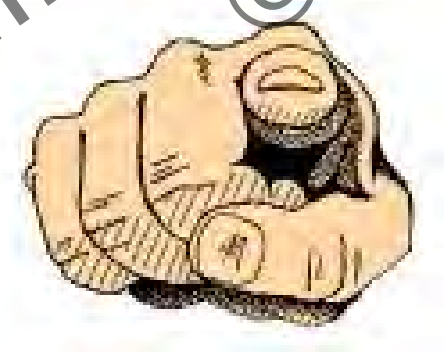
** Casali N et al, Genome Research Jan 31, 2012,
doi: 10.1101/gr.128678.111
http://www.ncbi.nlm.nih.gov/pubmed/22294518

Other issues

- Epigenetics- alteration of gene expression not due to sequence variation e.g. methylation of DNA
- Influence of host genetics
- Influence of environment on phenotypes – microbiome, metagenomics and systems biology
- Drug resistance and fitness costs

Ethical and legal issues

- True “Fingerprinting” rarely possible: e.g. helicobacter
- Careful we don’t mistakenly fingerpoint



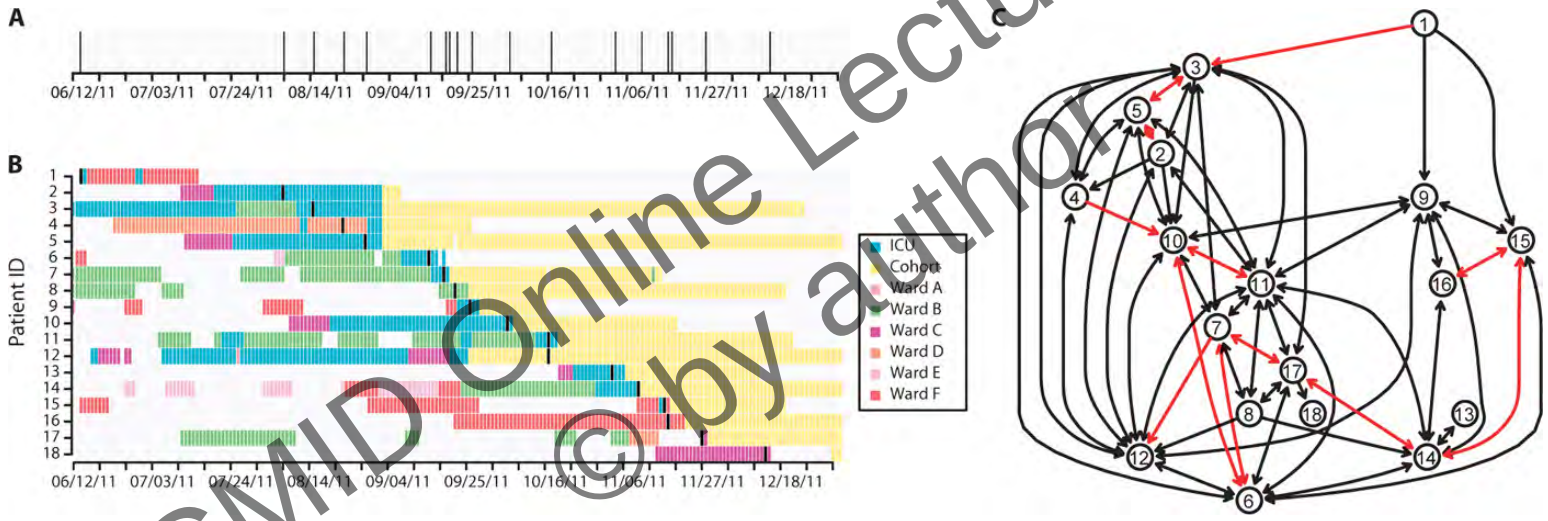
Conclusions

- Lots of promise and progress
- But
  - Automated processing and int… n (including application of system biol… nd virulence/resistance)
  - Nomenclature
  - Sequenci…
  - Co… tive of univ…
  - Data … of WGS in analytical epidem…
  - Have cle… es to deal with ethics
  - Does not re…ace the rest of infection control!

Typing to explore epidemiological hypotheses
NOT
"Epidemiological typing!"

Thanks for your attention

Fig. 1 Patient location and overlap during the outbreak.



Evan S. Snitkin et al., Sci Transl Med 2012;4:148ra116

Science Translational Medicine AAAS

Published by AAAS

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

Harris SR[1], Cartwright EJ, Török ME, Holden MT, Brown NM, Ogilvy-Stuart AL, Ellington MJ, Quail MA, Bentley SD, Parkhill J, Peacock SJ.

Author information

Abstract

BACKGROUND:

The emergence of meticillin-resistant Staphylococcus aureus (MRSA) that can

1.5d to produce results

- After extracting DNA from
- an overnight culture, it took us approximately
- 1.5 days to prepare the DNA libraries and sequence
- the isolates, although for fewer samples and
- shorter sequence-read lengths, faster protocols
- could be used that would reduce the time period to
- under a day. The approximate cost of all the ma

Diep USA 300

- USA300, a clone of meticillin-resistant *Staphylococcus aureus*, is a major source of community-acquired infections in the USA, Canada, and Europe. Our aim was to sequence its genome and compare it with those of other strains of *S aureus* to try to identify genes responsible for its distinctive epidemiological and virulence properties.
- **Methods**