How can WGS help in nosocomial outbreaks? The point of view of the hospital epidemiologist

S. Harbarth & R. Martischang
Geneva, 19 October 2018

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Making epidemiological inferences based on molecular data

We expect epidemiologically linked isolates to be genetically identical or similar, therefore:

We expect the bacterial population to have a clonal structure
=> Detection of monoclonal clusters of isolates.
Making epidemiological inferences based on molecular data

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We expect the bacterial population to have a clonal structure
=> Detection of monoclonal clusters of isolates.

Discriminative power

- hqSNP
- wgMLST
- MLST
- MLVA
- PFGE
- WGS
Pre-analytic Method (typing) Analytic (sequencing) Post-analytic

Epidemiologic investigation Coherence & right approach Molecular investigation
Indication for molecular typing?

- Do I have an epidemiologic hypothesis?

→ Molecular fishing expedition?
Indication for molecular typing?

- Do I have an epidemiologic hypothesis?

→ Molecular fishing expedition?

- Do I expect any impact on infection control interventions? Or futile academic exercise?

→ Priority action item for changing preventive measures?
Genomic Surveillance Reveals Diversity of Multidrug-Resistant Organism Colonization and Infection: A Prospective Cohort Study in Liver Transplant Recipients

Nenad Macesic,1,2 Angela Gomez-Simmonds,1 Sean B. Sullivan,1,3 Marla J. Giddins,1,3 Samantha A. Ferguson,1 Gautam Korakavi,1 David Leeds,1 Sarah Park,1 Kevin Shim,1 Madeleine G. Sowash,1 Melanie Hofbauer,1 Ryan Finkel,1 Yue Hu,1 Jared West,1 Nora C. Toussaint,4,5 William G. Greendyke,1 Benjamin A. Meko,1 Marcus R. Pereira,1 Susan Whittier,5 Elizabeth C. Verna,6 and Anne-Catrin Uhlemann1,3

**Results.** We collected 998 stool samples and 119 rectal swabs from 128 patients. MDRO colonization was detected in 86 (67%) patients at least once and was significantly associated with subsequent MDRO infection (0 vs 19.8%, $P = .002$). Child-Turcotte-Pugh score at LT and duration of post-LT hospitalization were independent predictors of both MDRO colonization and infection. Temporal dynamics differed between MDROs with respect to onset of colonization, clearance, and infections. We detected an unexpected diversity of CRE colonizing isolates and previously unrecognized transmission that spanned Ceph-RE and CRE phenotypes, as well as a cluster of mcr-1–producing isolates.

**Conclusions.** Active surveillance and WGS showed that MDRO colonization is a highly dynamic and complex process after LT. Understanding that complexity is crucial for informing decisions regarding MDRO infection control, use of therapeutic decolonization, and empiric treatment regimens.
Infection control measures in high-quality CPE control studies -- Systematic WHO review & meta-analysis --

<table>
<thead>
<tr>
<th>Intervention</th>
<th>EPOC studies</th>
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<tbody>
<tr>
<td>Active surveillance</td>
<td>10/11</td>
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<tr>
<td>Contact precautions</td>
<td>10/11</td>
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<tr>
<td>Cohorting</td>
<td>9/11</td>
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<tr>
<td>Monitoring, audit and feedback</td>
<td>9/11</td>
</tr>
<tr>
<td>Patient isolation</td>
<td>9/11</td>
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<tr>
<td>Hand hygiene education &amp; monitoring</td>
<td>6/11</td>
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<tr>
<td>Education</td>
<td>4/11</td>
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<tr>
<td>Antibiotic stewardship</td>
<td>4/11</td>
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<tr>
<td>Enhanced environmental cleaning</td>
<td>3/11</td>
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<tr>
<td>Daily chlorhexidine gluconate baths</td>
<td>3/11</td>
</tr>
<tr>
<td>Flagging positive patients in medical record (alerts)</td>
<td>3/11</td>
</tr>
<tr>
<td>Environmental surveillance</td>
<td>1/11</td>
</tr>
<tr>
<td>Temporary ward closure</td>
<td>1/11</td>
</tr>
</tbody>
</table>

Containment of a Country-wide Outbreak of Carbapenem-Resistant *Klebsiella pneumoniae* in Israeli Hospitals via a Nationally Implemented Intervention

Clinical Infectious Diseases 2011;52(7):1–8

Mitchell J. Schwaber,1 Boaz Lev,2 Avi Israeli,2 Ester Solter,1 Gill Smollan,1 Bina Rubinovitch,1 Itamar Shalit,1 Yehuda Carmeli,1 and the Israel Carbapenem-Resistant Enterobacteriaceae Working Groupa
Stalking a lethal superbug by whole-genome sequencing and phylogenetics: Influence on unraveling a major hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae*

Thorsten Kaiser MD\textsuperscript{a}, Knut Finstermeier PhD\textsuperscript{a}, Madlen Häntzsch MSc\textsuperscript{a}, Sarah Faucheux MD\textsuperscript{b}, Martin Kaase MD\textsuperscript{c}, Tim Eckmanns MD\textsuperscript{d}, Sven Bercker MD, PhD\textsuperscript{e}, Udo X. Kaisers MD, PhD\textsuperscript{e}, Norman Lippmann MD\textsuperscript{f,g}, Arne C. Rodloff MD, PhD\textsuperscript{f,g}, Joachim Thiery MD, PhD\textsuperscript{a}, Christoph Lübbert MD, PhD, DTM&H\textsuperscript{g,h,*}

- 34-month outbreak in Leipzig University Hospital
- 84/105 KPC-2-kp (ST258) available
- Index case transferred from Rhodes, Greece
- 19 wards affected
WGS & Spread of KPC-2

- Positioning pillow to maintain patient prone in ICU, might have been the link
- Additional cases after screening
- 34 median number of single nucleotide variants

**Epidemiology**

Pathways explained for 11 (12.4%) patients

**WGS**

Pathways explained for 15 extra patients & confirmed 5 of epidemiology

- Exact mode of transmission unknown for 63 (71%) patients
Pre-analytic

**Indication for molecular typing?**

- Do I have an epidemiologic hypothesis?

  ➔ Molecular fishing expedition?

- Do I expect any impact on infection control interventions? Or simply academic exercise?

  ➔ Priority action item for modified preventive measures?

**Sampling?**

- Do I have a strong, robust sampling strategy? (Who, How, When)

  ➔ Avoid detection & selection & misclassification bias

- Adequate screening for asymptomatic carriers

- If possible, select the right colonies by selective cultures (multiresistant organisms).

- How many morphologically similar isolates to sample from the same clinical culture?
Multiple Variants of *Klebsiella pneumoniae* Producing Carbapenemase in One Patient

- 82 yr man
- Admitted to the same hospital 21 times (2011-2015)
- Often grouped with patients colonized with KPC-producing Enterobacteriaceae (KPE)
- Numerous antibiotic courses
Typing methods for outbreak investigations and epidemiologic surveillance

**PFGE**
- **POS:** Established method
- **CONS:** Little discriminatory power, cannot establish exact transmission routes

**MLVA**
- **POS:** Robust, reproducible method; allows to observe longterm trends
- **CONS:** Little discriminatory power

**MLST**
- Various targets

Monocentric outbreak

Long-term Surveillance / Multicenter outbreaks
Outbreak of vancomycin-resistant *Enterococcus faecium* clone ST796, Switzerland, December 2017 to April 2018

Nasstasja Wassilew, Helena MB Seth-Smith, Eveline Rolli, Yvonney Fietze, Carlo Casanova, Urs Führer, Adrian Egli, Jonas Marschall, Niccolò Buetti

1. Department of Infectious Diseases, University Hospital Bern, Bern, Switzerland
2. Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland
3. Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland
4. Institute for Infectious Diseases, University of Bern, Bern, Switzerland
5. Infectious Diseases Department, Biel Hospital, Biel, Switzerland

Correspondence: Niccolò Buetti (niccolo.buetti@gmail.com)

**Figure 3**

Epidemic curve of vancomycin-resistant enterococci (VRE) cases by sequence type, Canton of Bern outbreak, Switzerland, December 2017–April 2018 (n = 89)

The largest outbreak at University hospital is shown by the largest circle.
Typing methods for outbreak investigations and epidemiologic surveillance

**PFGE**
- **POS:** Established method
- **CONS:** Little discriminatory power, cannot establish exact transmission routes

**MLVA**
- **POS:** Various targets

**MLST**
- **POS:** Robust, reproducible method; allows to observe long-term trends
- **CONS:** Little discriminatory power

**WGS**
- **POS:** Core genome
- **CONS:** High discriminatory power
- **CONS:** Still expensive and requires special analytical skills

- **Monocentric outbreak**
- **Long-term Surveillance / Multicenter outbreaks**
- **Outbreaks or epidemiologic surveillance**
Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*

Jean Y. H. Lee¹, Ian R. Monk¹, Anders Gonçalves da Silva²,³, Torsten Seemann⁴, Kyra Y. L. Chua⁵, Angela Kearns⁶, Robert Hill⁶, Neil Woodford⁶, Mette D. Bartels⁷, Birgit Strommenger⁸, Frederic Laurent⁹, Magali Dodémont¹⁰, Ariane Deplano¹⁰, Robin Patel¹¹, Anders R. Larsen¹², Tony M. Korman¹³, Timothy P. Stinear¹,³,¹⁵ and Benjamin P. Howden²,³,¹⁴,¹⁵*

» Uncovered the previously unrecognized international spread of a near pandrug-resistant nosocomial pathogen, identifiable by a rifampicin-resistant phenotype.
The transmission of *C. auris* was found to be linked to reusable axillary temperature probes, indicating that this emerging pathogen can persist in the environment and be transmitted in health care settings.
Bias during the process

Storage → Extraction → Library preparation → Sequencing

Storage parameters:
- $T^\circ C$, time, media, UV, container

Extraction parameters:
- enzymatic, mechanical (fav. GPB)

Size selection:
- gels (melting~dec. AT-rich sequences)

Library preparation:
- PCR approaches (heterogeneous affinities)

The right platform

Illumina
- Accurate
- Takes time (days)

454, Ion torrent
- ! False positive variant calling

PacBio, MinION
- ! False positive variant calling

Next-generation sequencing technologies and their application to the study and control of bacterial infections», J. Besser, CMI, 2018

Prof. SCHRENZEL Jacques

What is close enough?

**Relatedness thresholds**

- **SNPs:** nb of SNPs differences
- **MLST:** nb of alleles variations

**Pre-analytic**

**Method (typing)**

**Analytic (WGS)**

**Post-analytic**

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**BUT**

- Intra-individual variation
- Genetic recombination events
- Clock speed is different among pathogens

**THUS**

- Consider suggested thresholds more as a guideline
  - Interprete epidemiological links on a case by case basis
  - Interprete organism by organism (specific population genetics)

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«Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches», A.C.Schürch, CMI, 2018

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Table 1

<table>
<thead>
<tr>
<th>Organism</th>
<th>Relatedness threshold</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>(&lt;8)</td>
<td>[25,26] <a href="http://www.applied-maths.com/applications/wgmst">http://www.applied-maths.com/applications/wgmst</a></td>
</tr>
<tr>
<td><em>Campylobacter coli, C. jejuni</em></td>
<td>(&lt;14)</td>
<td>[27,28] <a href="http://www.applied-maths.com/applications/wgmst">http://www.applied-maths.com/applications/wgmst</a></td>
</tr>
<tr>
<td><em>Enterococcus raffinosus</em></td>
<td>(&lt;10)</td>
<td>[31,32] <a href="https://enterobase.warwick.ac.uk/">https://enterobase.warwick.ac.uk/</a></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>(&lt;10)</td>
<td>[33,34] <a href="http://www.applied-maths.com/applications/wgmst">http://www.applied-maths.com/applications/wgmst</a></td>
</tr>
<tr>
<td><em>Francisella tularensis</em></td>
<td>(&lt;1)</td>
<td>[35] <a href="http://www.cgmist.org/ncs">http://www.cgmist.org/ncs</a></td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>(&lt;1)</td>
<td>[36]</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>(&lt;10)</td>
<td>[37] <a href="http://www.applied-maths.com/applications/wgmst">http://www.applied-maths.com/applications/wgmst</a></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>(&lt;3)</td>
<td>[40]</td>
</tr>
<tr>
<td><em>Mycobacterium abcessus</em></td>
<td>(&lt;30)</td>
<td>[41]</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>(&lt;12)</td>
<td>[42] <a href="http://www.applied-maths.com/applications/wgmst">http://www.applied-maths.com/applications/wgmst</a></td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>(&lt;12)</td>
<td>[43] <a href="http://www.cgmist.org/ncs">http://www.cgmist.org/ncs</a></td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>(&lt;14)</td>
<td>[44] <a href="https://enterobase.warwick.ac.uk/">https://enterobase.warwick.ac.uk/</a></td>
</tr>
<tr>
<td><em>Salmonella dublin</em></td>
<td>(&lt;13)</td>
<td>[46] <a href="https://enterobase.warwick.ac.uk/">https://enterobase.warwick.ac.uk/</a></td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>(&lt;4)</td>
<td>[47]</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>(&lt;24)</td>
<td>[48]</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>(&lt;15)</td>
<td>[49]</td>
</tr>
<tr>
<td><em>Streptococcus suis</em></td>
<td>(&lt;10)</td>
<td>[50]</td>
</tr>
<tr>
<td><em>Vibrio parahaemolyticus</em></td>
<td>(&lt;21)</td>
<td>[51]</td>
</tr>
<tr>
<td><em>Yersinia spp.</em></td>
<td>(0)</td>
<td>[52]</td>
</tr>
</tbody>
</table>

\(c\_g\): core genome; MLST: multi locus sequence typing; SNP: single nucleotide polymorphism; wg: whole genome.

* Data often represent single studies that can be used to begin formulation of species-specific interpretation criteria. Thus, these data should be coupled with newly published similar studies to ensure that resulting values are not atypical and can be generally applied.

* Proposed wg/cgMLST schemes are available online (http://www.cgmist.org/ncs, http://www.applied-maths.com/applications/wgmst, https://enterobase.warwick.ac.uk/) but as yet have not been epidemiologically validated.
Outbreak investigations from Geneva

MRSA
CPE
VRE
Serratia
Phone rings:

„Hello…! We have a problem down here at the NICU – can you help us?“
“Do you want to speak to the man in charge or the nurse who knows what’s going on?”
Veni vidi ... (vici ?)
Julius Caesar, De Bello Gallico

You go in there and have a look...

- **What?**  
  - MRSA outbreak

- **Who?**  
  - 11 neonates, 2 mothers

- **Where?**  
  - NICU & nursery

- **When?**  
  - Over the last 3 months

- **Severe?**  
  - No – mostly carriage
What would you recommend for the next week – priority action items except?

A. Isolate or cohort MRSA carriers
B. Reinforce hand hygiene
C. Screening of all hospitalized neonates
D. Implement active MRSA surveillance for all new admissions
E. Molecular typing of MRSA isolates
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C. Screening of all hospitalized neonates
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E. Molecular typing of MRSA isolates
Epidemic curve
MRSA outbreak, NICU (HUG)

Sax et al. J Hosp Infect 2006; 63: 93-100
Control measures
MRSA outbreak, NICU (HUG)

A. Isolate or cohort MRSA carriers
B. Reinforce hand hygiene
C. Screening of all neonates & mothers
D. Implement active MRSA surveillance (admission & discharge)

Sax et al. J Hosp Infect 2006; 63: 93-100
Epidemic curve
MRSA outbreak, NICU (HUG)

Sax et al. J Hosp Infect 2006; 63: 93-100
NICU at HUG
June/July 2000

Legend
- PVL+ CA-MRSA strain
- Multi-R strain (endemic at HUG)

Molecular Epidemiology

ST-5, SCC type IV, PVL+

ST-228, SCC type I, PVL-

ST-8, SCC type IV, PVL-

Sax et al. J Hosp Infect 2006; 63: 93-100
General internal medicine ward, Geneva

Sporadic, imported CPE (OXA-48 cluster in 2011)
KPC outbreak in Geneva, 2015

• Mr CV, Italian origin, known KPC carrier since 2012
• Admitted in January 2015 for severe KPC urosepsis
• Control measures were applied (private room)
Case TC: KPC cross-transmission despite single room isolation of index patient

Sepsis due to Colistin-R KPC, HUG, March 2015
Total: 3 cases of KPC cross-transmission
(2 clinical infections + 1 asymptomatic colonization)

Need of a bundled intervention

- Contact tracing with widespread screening
- Cohorting / strict contact precautions
- Electronic re-admission alert system
- Information (HCWs, patients, families)

Courtesy: F. Olearo, D. Pires
Results of extensive screening

N° of patients to be screened

Not one single additional KPC case detected!

Screening test results

- test negative
- death
- Patients still labelled

Not one single additional KPC case detected!
Whole genome analysis: KPN7 had 3 SNV differences with others, KPN10 has another SNV

- Index strain slightly different from the strains recovered from secondary cases, likely because prior long-term carriage (3 years) by the index patient allowed for genetic mutations over time with intra-individual strain variation

*ParSNP, all strains against strain KPN7 (index case)  E. Ruppé et al. Clin Micro Infect 2018
VRE outbreak

- Geneva, surgical unit
Incidence density of hospital-acquired VRE HUG, January 2010 – June 2018

Nouveaux cas VRE/1000 j-patients

Service PCI - HUG
Figure 1. Arbre de maximum de parcimonie entre les souches du cluster ST117-B. La distance entre chaque souche indique le nombre de loci de différence.

Courtesy: D. Blanc - CHUV
Serratia outbreak

Geneva ICU
Routine Serratia Surveillance => Epidemic curve in the ICU

Epidemiologic outbreak investigation => nosocomial transmission?

Outbreak period

HH compliance: 66% (1st semestre) to 52% (3rd Trimestre)
Selection and sequencing of multiple *S. marcescens* isolates (incl. outbreak strains) stored in the microbiological laboratory


**Cluster 1/3/6/18/30/32/34/43**: 8 isolats, tous provenant d’échantillons respiratoires mais de différents patients, prélevés dans un intervalle de deux ans (29.08.2015, 30.01.2015, 30.05.2016, 26.10.2016, 10.08.2017, 29.08.2017, 21.03.2017, 17.08.2017). Dans ce cluster, les cgSNPs ont été trouvés en 20 positions (sur un total de 1’485’517 positions), avec 0-8 cgSNPs dans les comparaisons par paires de souches (Table1). Le Neighbour-joining tree (cgSNP) est représenté dans la Figure 2. Les profils cgMLST sont identiques pour les isolats 34 et 43.
Data collection to retrieve epidemiological links based on genomic data (small monoclonal cluster from 2015 to 2017)

-Epidemiological investigation based on geospatial, microbiological and medical information (respiratory procedures, respiratory therapy, surgery etc…)

BUT

- Selection & detection bias (missing cases)
- Misclassification bias
- Information bias (retrospective study)
Conclusions
WGS can reveal detailed spatial and temporal dynamics of nosocomial transmission events and MDRO evolution, but we need:

- Quality standards, proficiency testing for routine use
- Standard-operating procedures for sampling, data extraction…
- Agreements on data sharing practices (larger outbreaks)
- Shorter TAT
- Reduced costs
- Streamlined data analyses
- Thresholds to determine clustering and transmission events

Most importantly: demonstrate impact of WGS on preventive measures and clinical decision making (compared to less expensive tools)
Thanks for your attention!