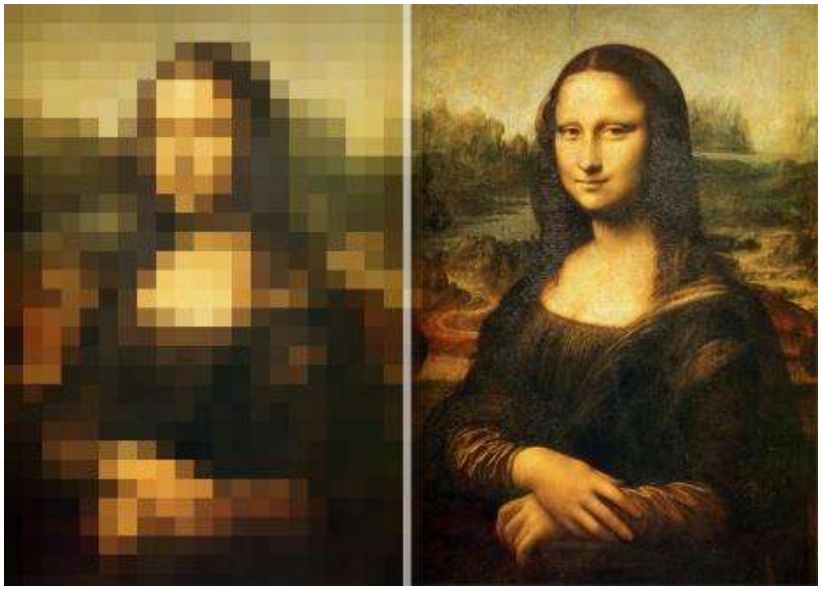

Apport du séquençage dans les épidémies hospitalières

Stephan Harbarth

62èmes journées Claude Bernard, 28 Nov 2019

Infection Control Programme, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland

Remerciements: Romain Martischang

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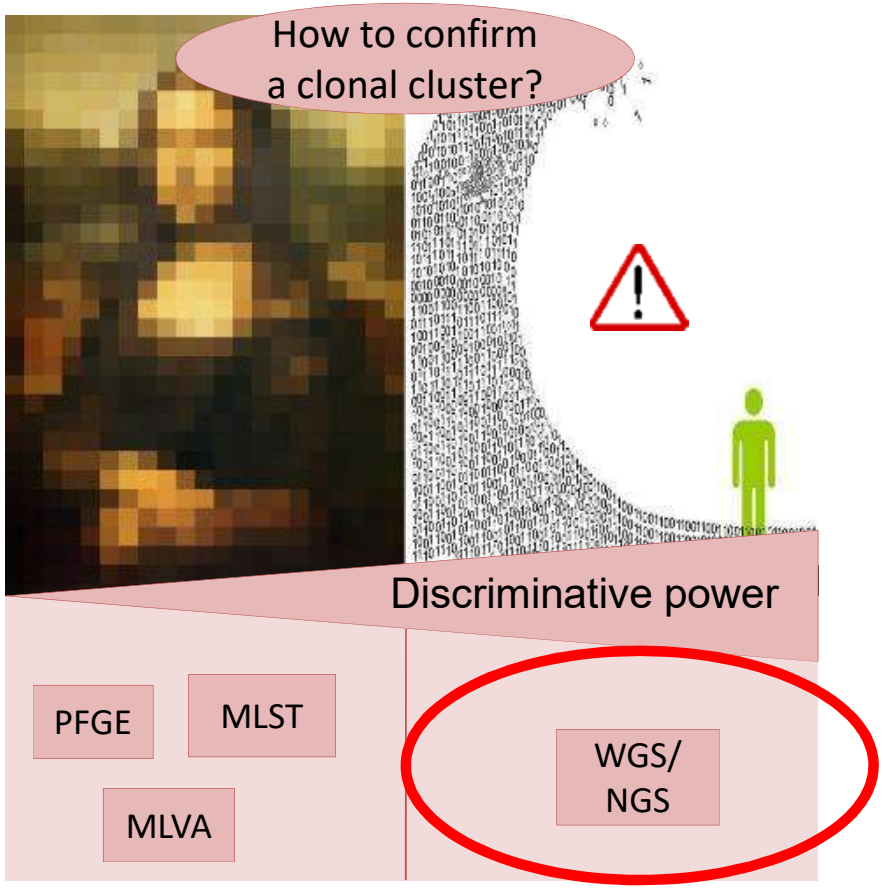
Key Words:

Hand hygiene;
World Health Organization;

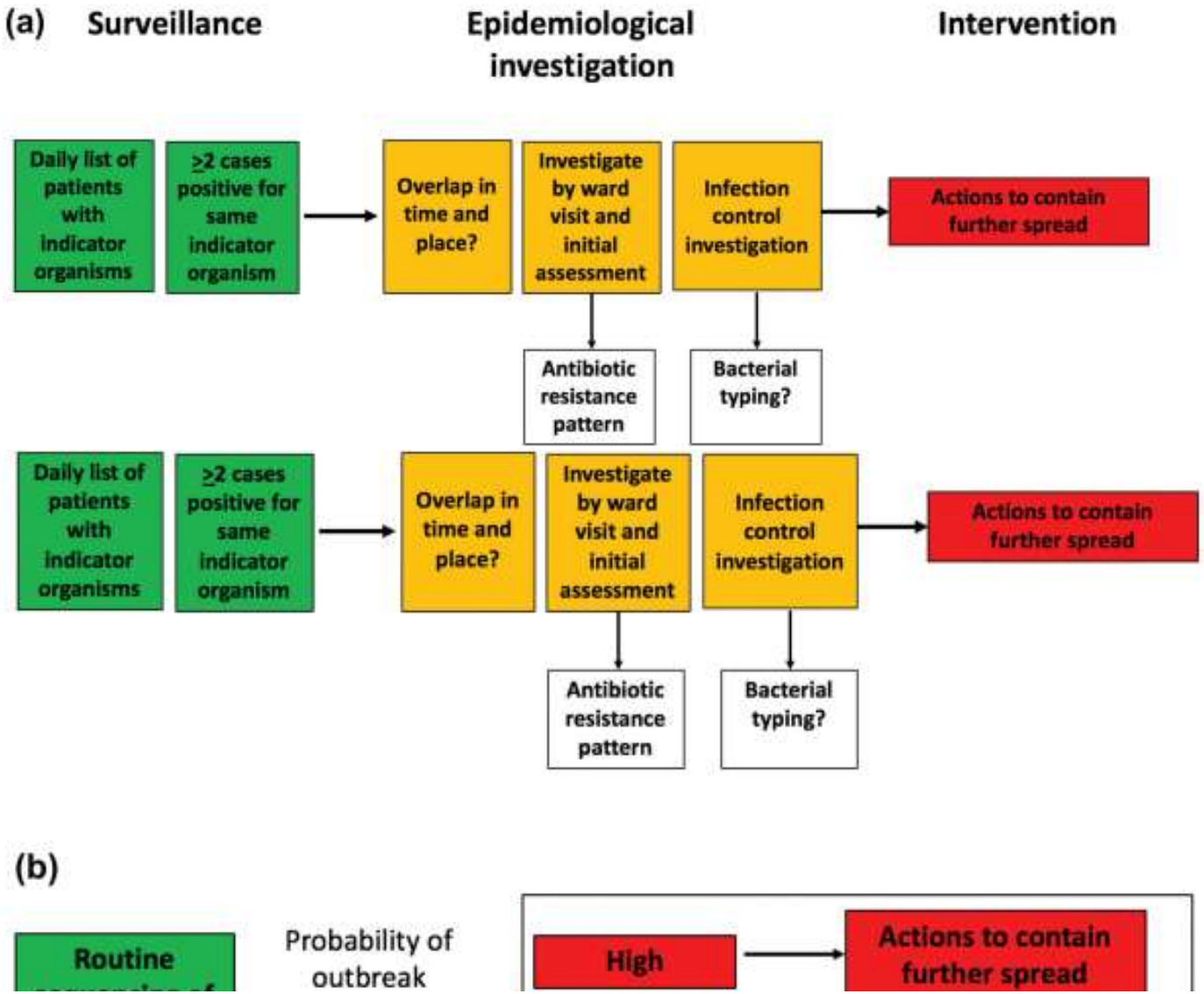
Detection and confirmation of a monoclonal nosocomial outbreak



PFGE: électrophorèse en champs pulsé
MLVA: Analyse du polymorphisme de locus répétés
MLST: Analyse de locus multiples (séquençage partiel ciblé)
WGS/NGS: séquençage de génome complet

Conventional approach to outbreak detection

NGS-based outbreak detection

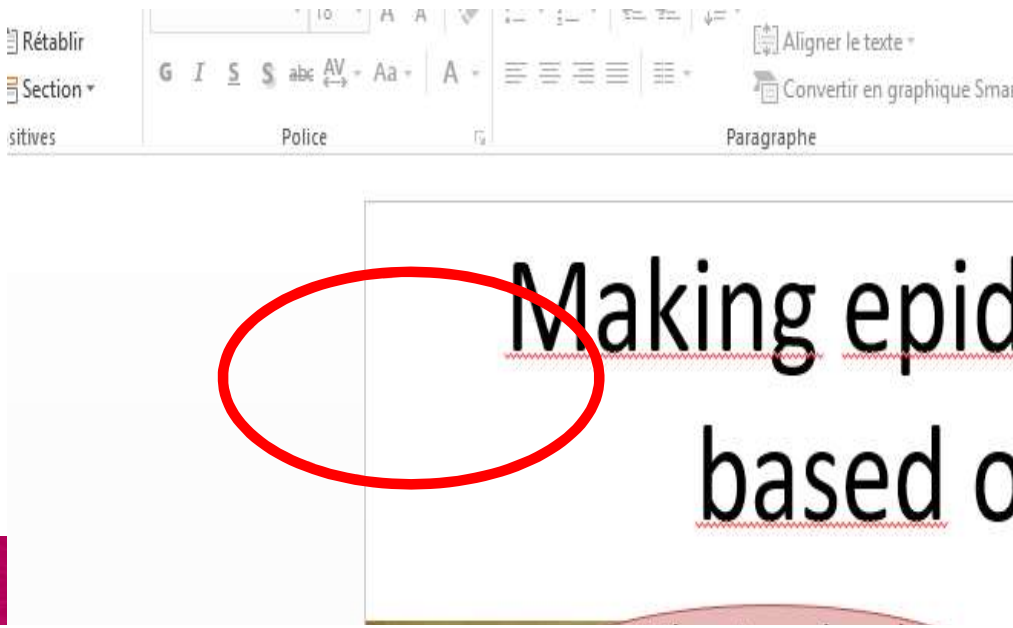


Peacock et

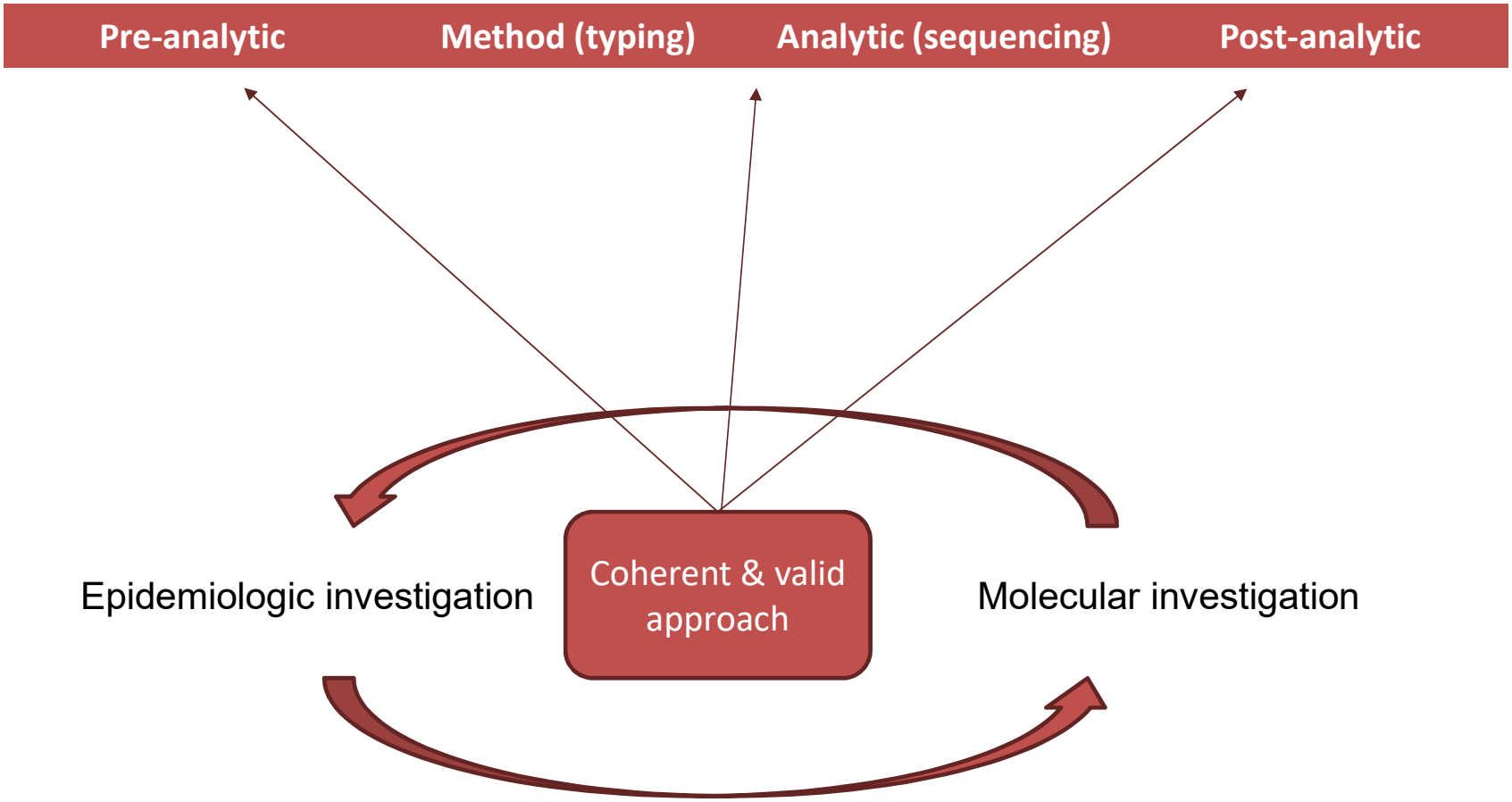
Making epidemiological inferences based on molecular data

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.



Has the time come to perform a paradigm shift towards routine NGS?


Pre-analytic
Method (typing)
Analytic (sequencing)
Post-analytic
Epidemiologic investigation
Coherent & valid approach
Molecular investigation

Pre-analytic

Indication for molecular typing?

- Do we have an epidemiologic hypothesis ?

➔ **Molecular fishing expedition?**

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 futile academic exercise ?

➔ **NGS: priority action item for changing preventive measures?**

Clinical Infectious Diseases

MAJOR ARTICLE

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,a] William G. Greendyke,[1] Benjamin A. Miko,[1] Marcus R. Pereira,[1] Susan Whittier,[5] Elizabeth C. Verna,[6] and Anne-Catrin Uhlemann[1,3]

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.

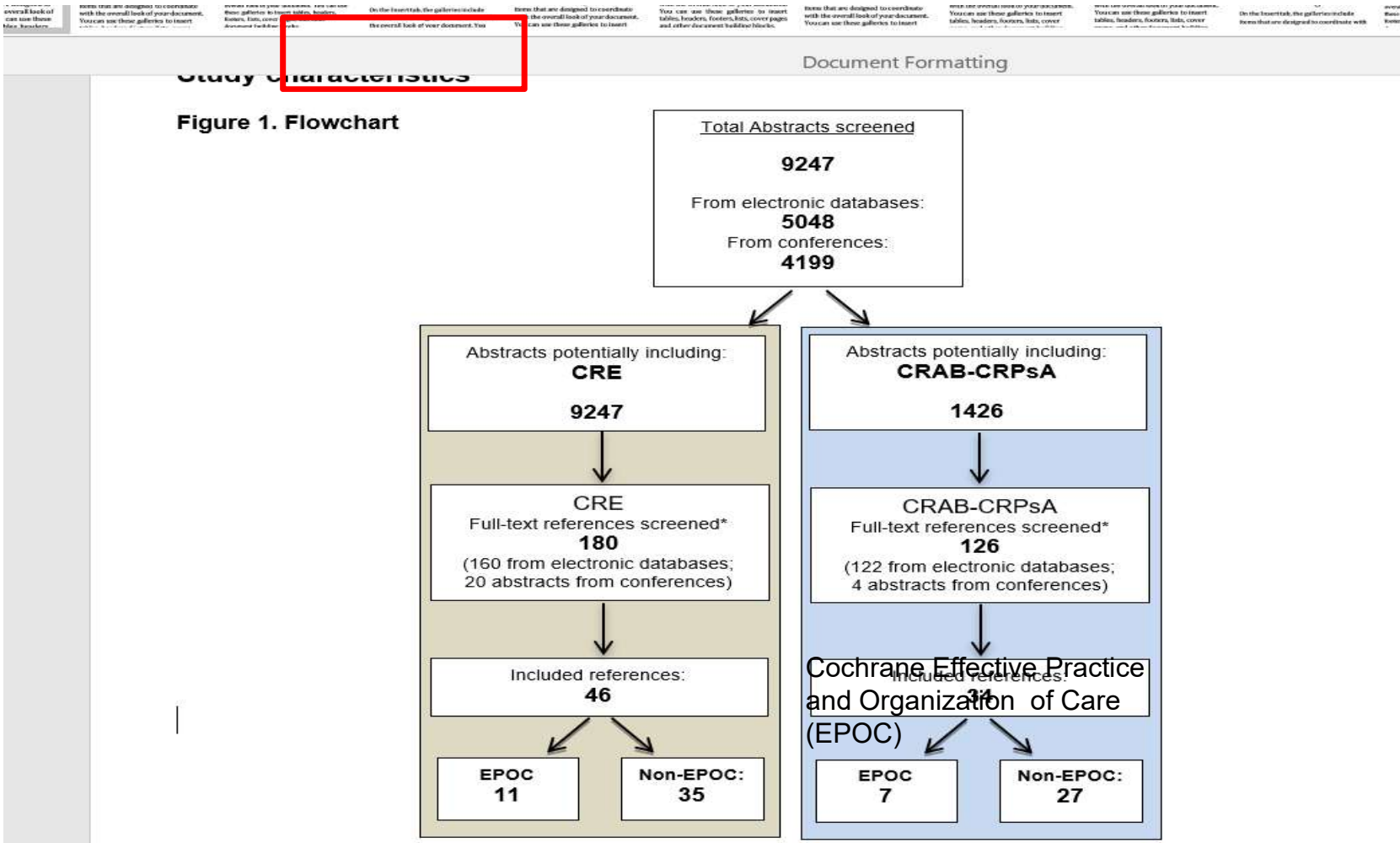
Conclusions. Active surveillance and WGS showed that MDRO colonization is a highly dynamic and complex process after LT. Understanding that complexity is crucial for informing decisions regarding MDRO infection control, use of therapeutic decolonization, and empiric treatment regimens.

WHO: CPE control

- No RCT or controlled study
- All EPOC-studies are from CRE-endemic countries: Israel, USA, Italy and Brazil
- All describe multi-faceted interventions

- EPOC: 10 interrupted time series studies
- Non-EPOC (N=36)
  - 17 Non-controlled before-after studies
  - 14 Before-after case-counts
  - 3 Modeling studies
  - 2 Longitudinal studies

CRE control review -- Flowchart



Cochrane Effective Practice and Organization of Care (EPOC)

Tomczyk S et al. Clin Infect Dis 2016

Infection control measures in high-quality CPE control studies

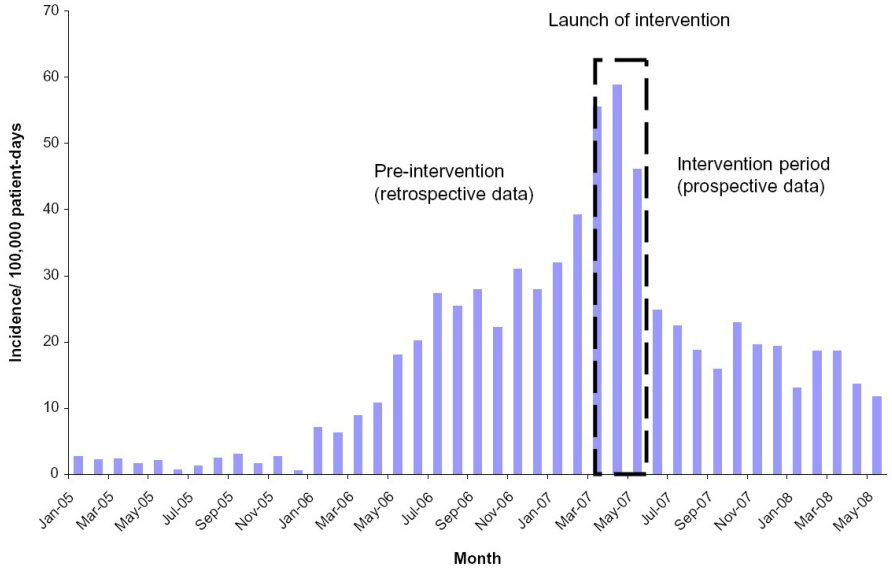
-- Systematic WHO review & meta-analysis --

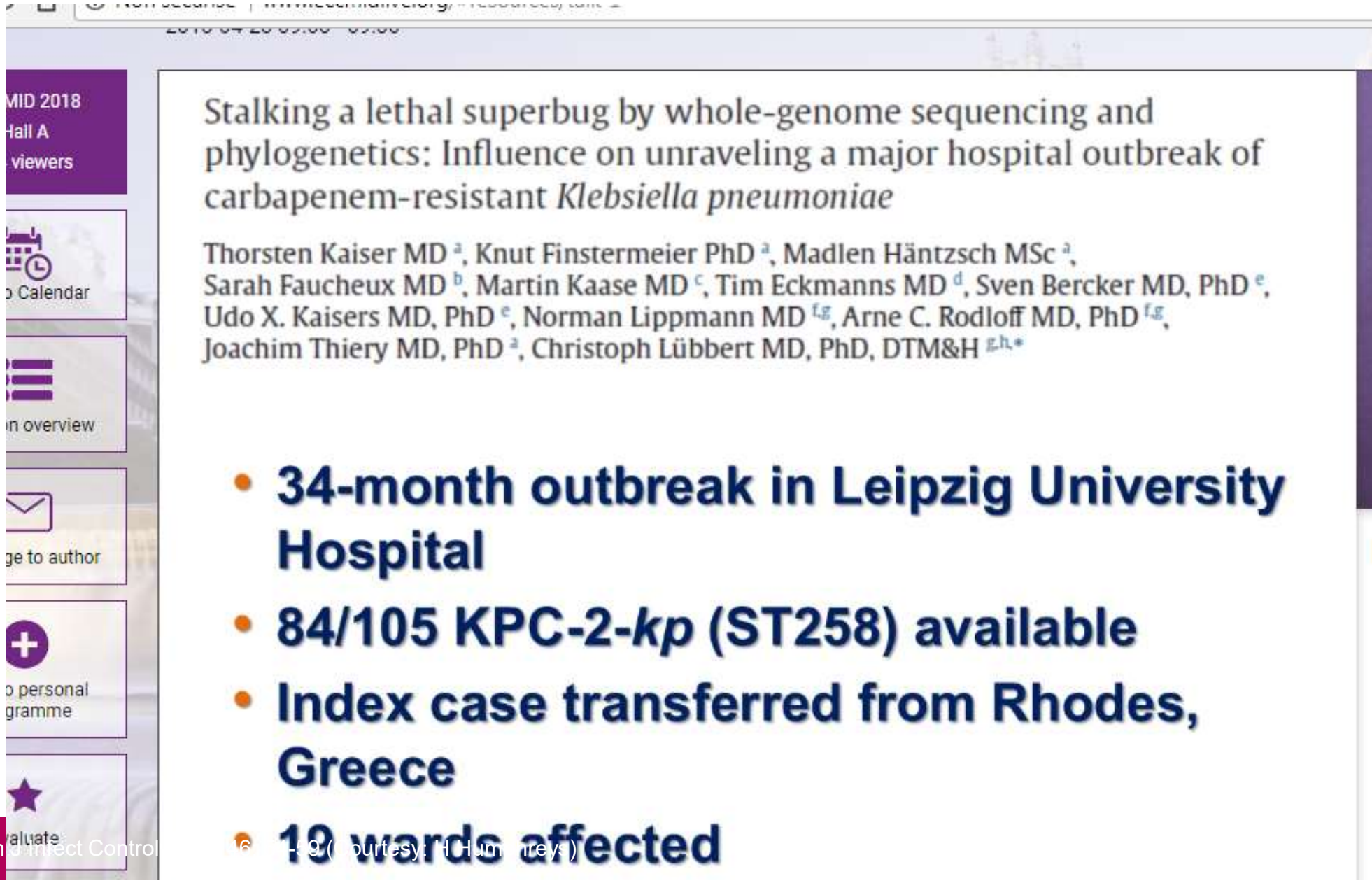
Intervention	EPOC studies
Active surveillance	10/11
Contact precautions	10/11
Cohorting	9/11
Monitoring, audit and feedback	9/11
Patient isolation	9/11
Hand hygiene education & monitoring	6/11
Education	4/11
Antibiotic stewardship	4/11
Enhanced environmental cleaning	3/11
Daily chlorhexidine gluconate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

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

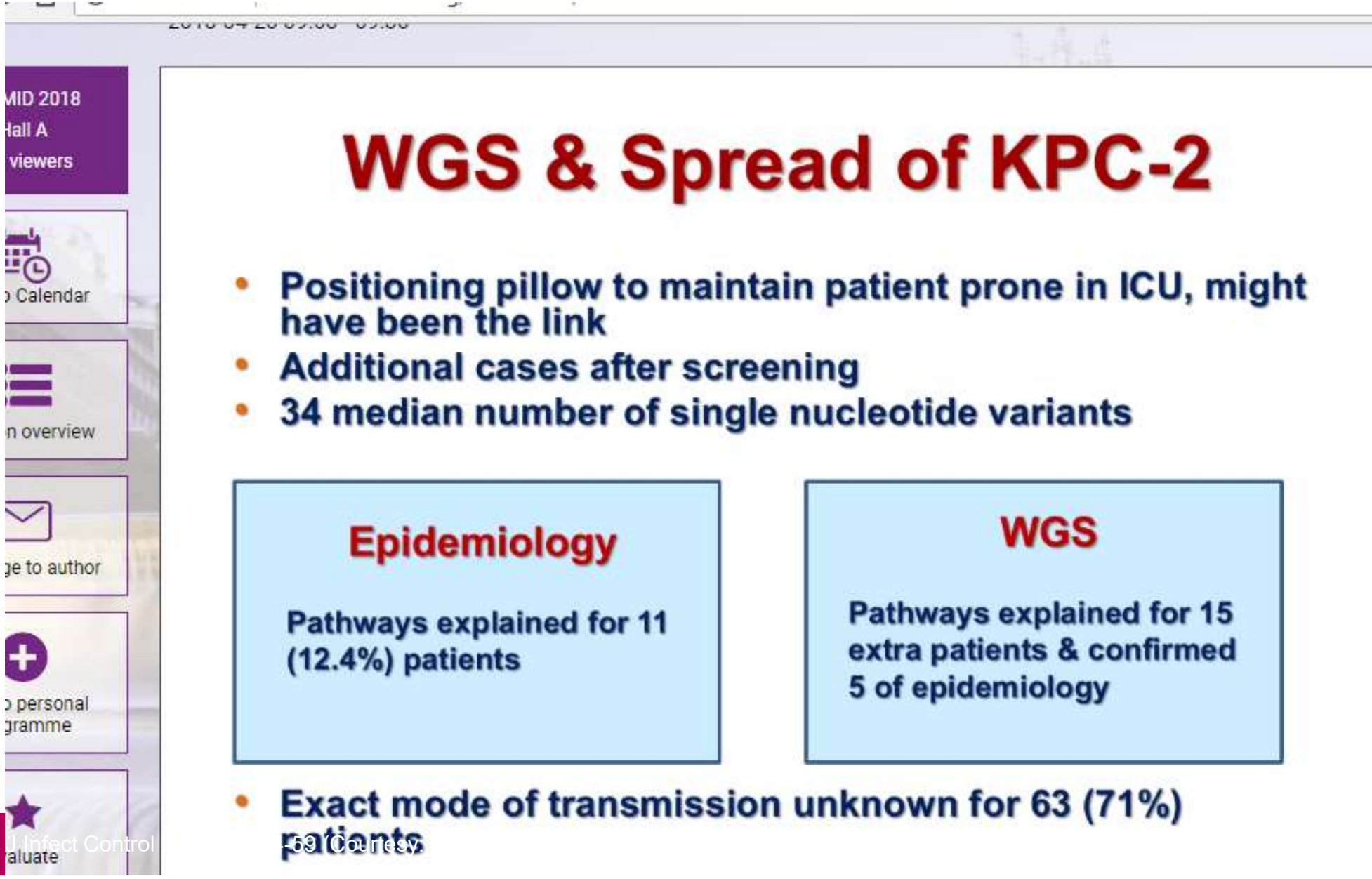
Clinical Infectious Diseases 2011;52(7):1–8

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 Group[a]





Kaiser T et al. Am J Infect Control [illegible] (courtesy: H Humphreys)



Kaiser T et al. Am J Infect Control -53 (Courtesy)

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 bias**
➔ **selection bias**
➔ **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

Michael R. Mulvey, Ph.D.
National Microbiology Laboratory
Winnipeg, MB, Canada
michael.mulvey@phac-aspc.gc.ca

Louis-Patrick Haraoui, M.D.
McGill University Health Centre
Montreal, QC, Canada

Yves Longtin, M.D.
Jewish General Hospital
Montreal, QC, Canada

- 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

Mulvey et al. NEJM 2016; 375: 2408-10

- 14 KPE isolates identified
- 9 available for sequencing:
  - 7 rectal swabs
  - 1 urine
  - 1 wound swab
- 3 species (*K. pneumoniae, K. oxytoca, and E. coli*) identified, including:
  - 3 different *K. pneumoniae* sequence types
  - 3 different incompatibility (Inc) group plasmids carrying Tn*4401*

Mulvey et al. NEJM 2016; 375: 2408-10

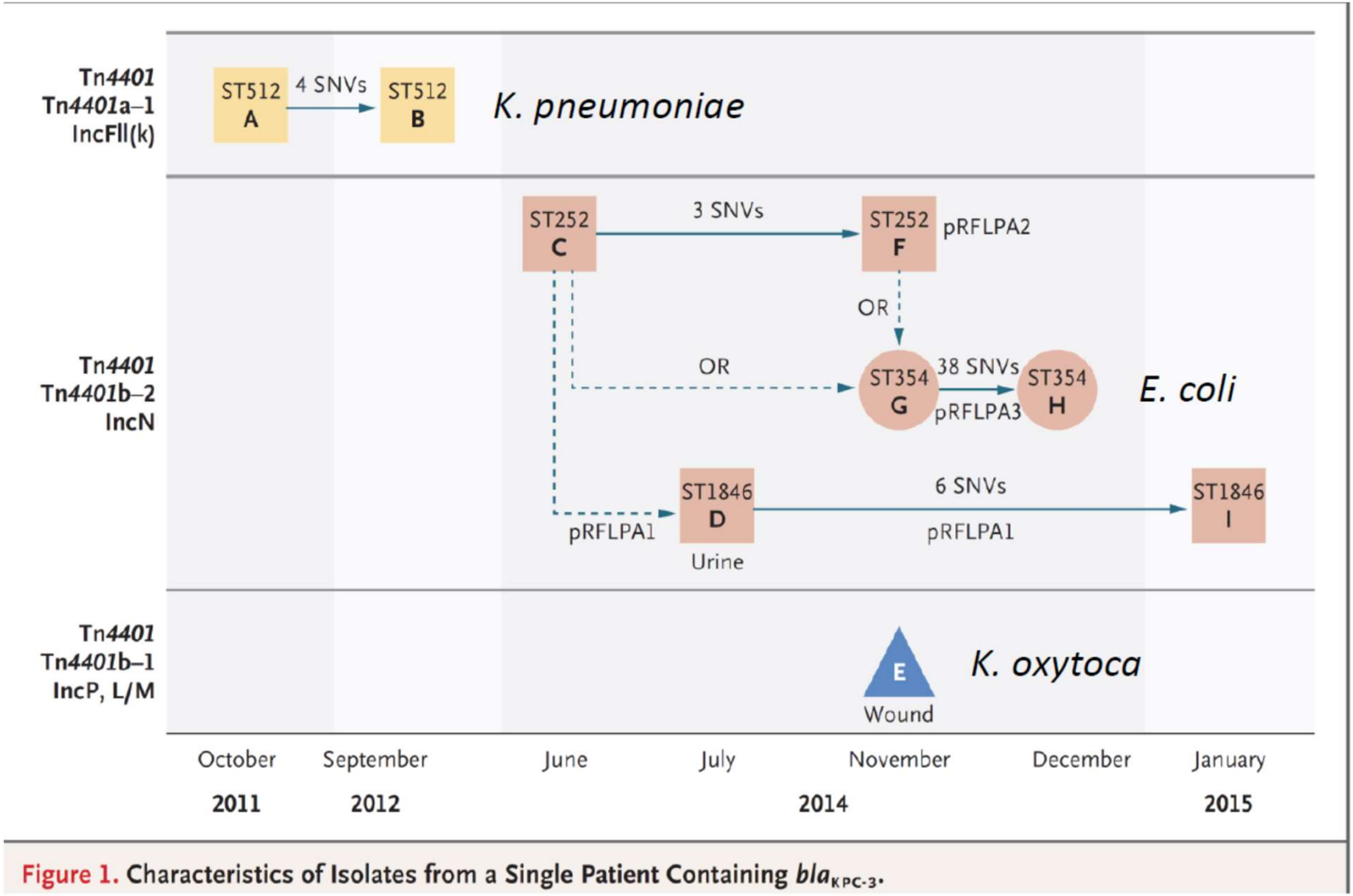


Figure 1. Characteristics of Isolates from a Single Patient Containing bla_{KPC-3}.

- Multiple isolates may have arisen from:
  - Spread of resistance element within the host and/or
  - Exposure to other KPE-colonized patients
- Limitations of outbreak investigations with traditional epidemiologic and molecular tools
- Genome sequencing necessary

Mulvey et al. NEJM 2016; 375: 2408-10

Pre-analytic | **Method (typing)**

Typing methods for outbreak investigations and epidemiologic surveillance

PFGE MLVA

Various targets

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

Monocentric outbreak

MLST

Selected loci

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

Long-term surveillance / multicenter outbreaks

PFGE: électrophorèse en champs pulsé
MLVA: Analyse du polymorphisme de locus répétés
MLST: Analyse de locus multiples (séquençage ciblé)

RAPID COMMUNICATION

Outbreak of vancomycin-resistant *Enterococcus faecium* clone ST796, Switzerland, December 2017 to April 2018

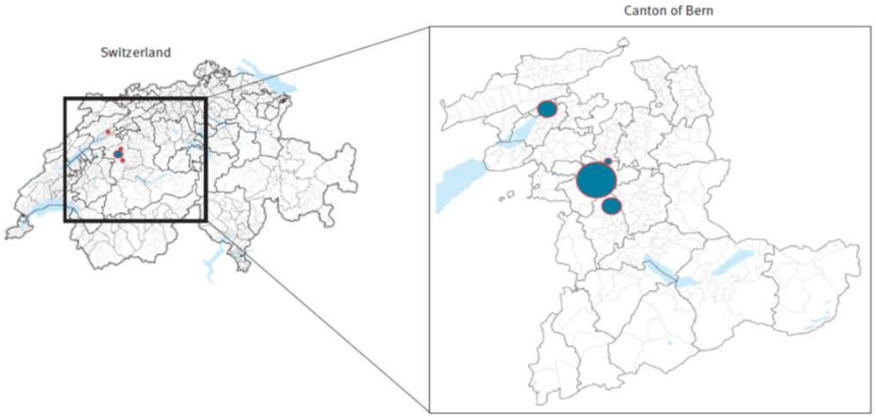
Nasstasja Wassilew[1], Helena MB Seth-Smith[2,3], Eveline Rolli[1], Yvonne Fietze[1], Carlo Casanova[4], Urs Führer[5], Adrian Egli[2,3], Jonas Marschall[1], Niccolò Buetti[1]

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 1

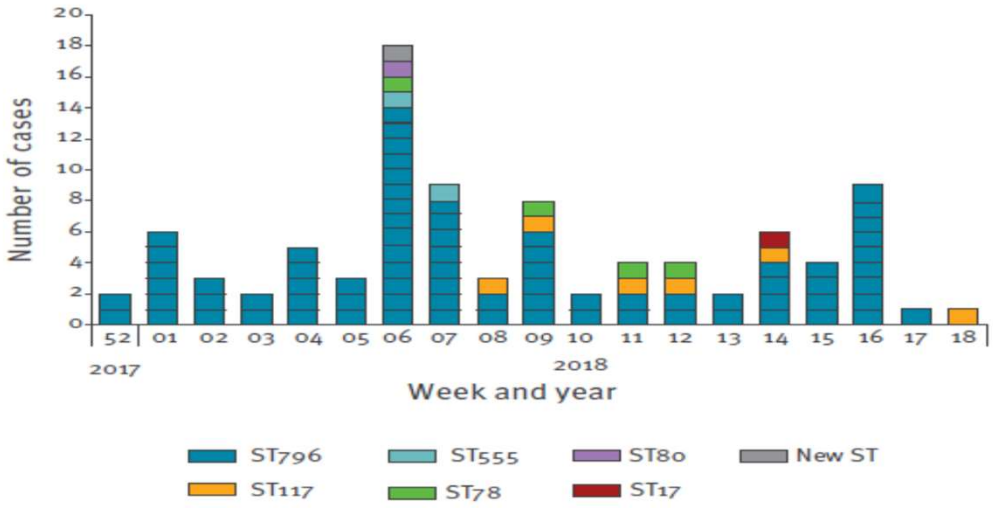
Distribution of vancomycin-resistant *Enterococcus faecium* ST796 in four different hospitals, Canton of Bern, Switzerland, 30 December 2017 to 30 April 2018 (n = 89)

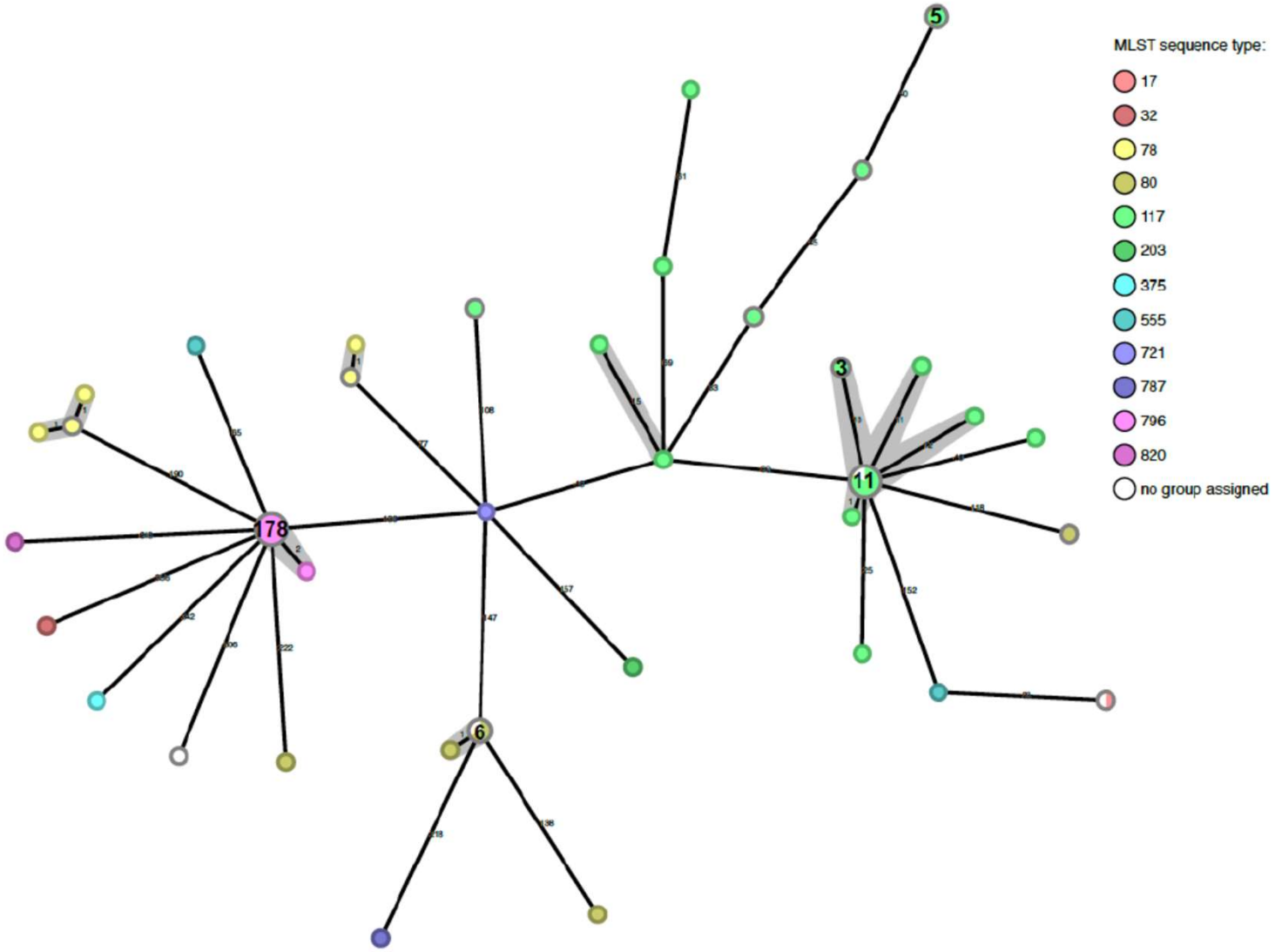


The largest outbreak at University hospital is shown by the largest circle.

FIGURE 3

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




MLST sequence type:
17
32
78
80
117
203
375
555
721
787
796
820
no group assigned
5
3
11
78
6

Pre-analytic | **Method (typing)**

Typing methods for outbreak investigations and epidemiologic surveillance

PFGE MLVA

Various targets

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

Monocentric outbreak

MLST

Selected loci

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

Long-term Surveillance / Multicenter outbreaks

WGS

Core genome

POS: High discriminatory power
CONS: Still expensive and requires special analytical skills

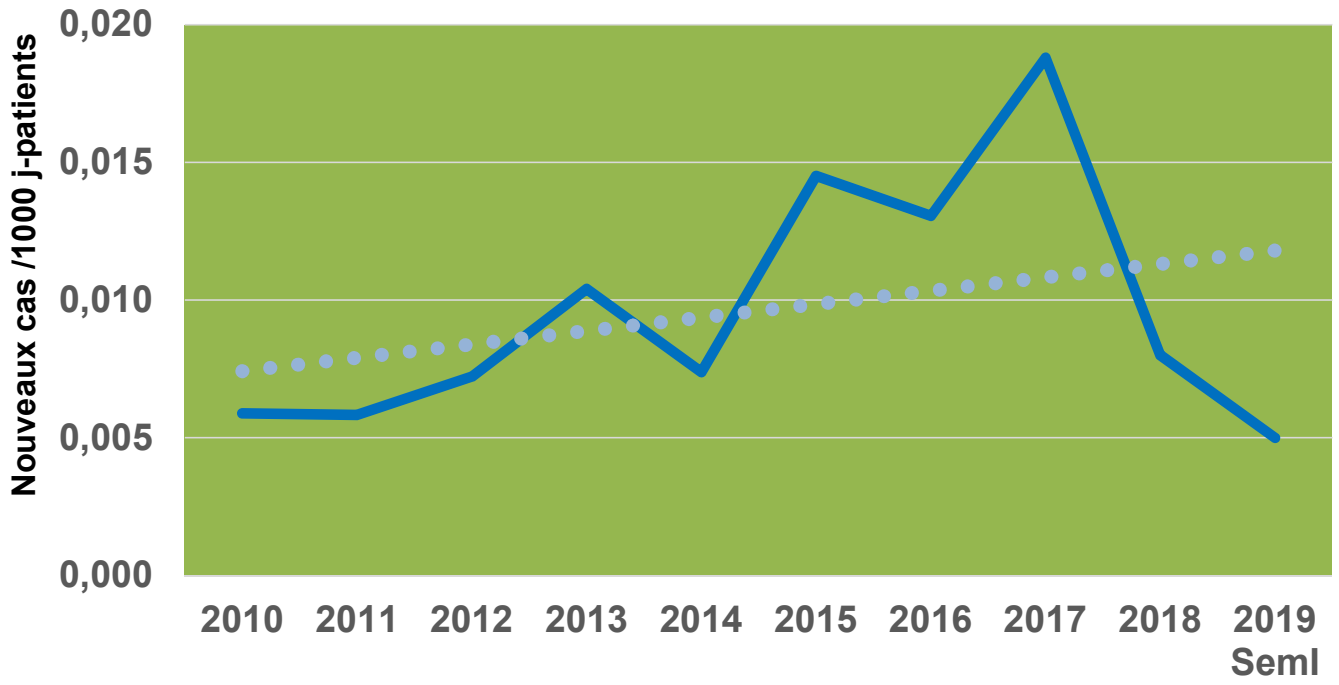
Outbreaks or epidemiologic surveillance

WGS/NGS: séquençage de génome complet

VRE outbreak

Geneva, surgical unit

Densité d'incidence de nouveaux cas VRE détectés après 48h d'admission HUG, Janvier 2010 – Juin 2019



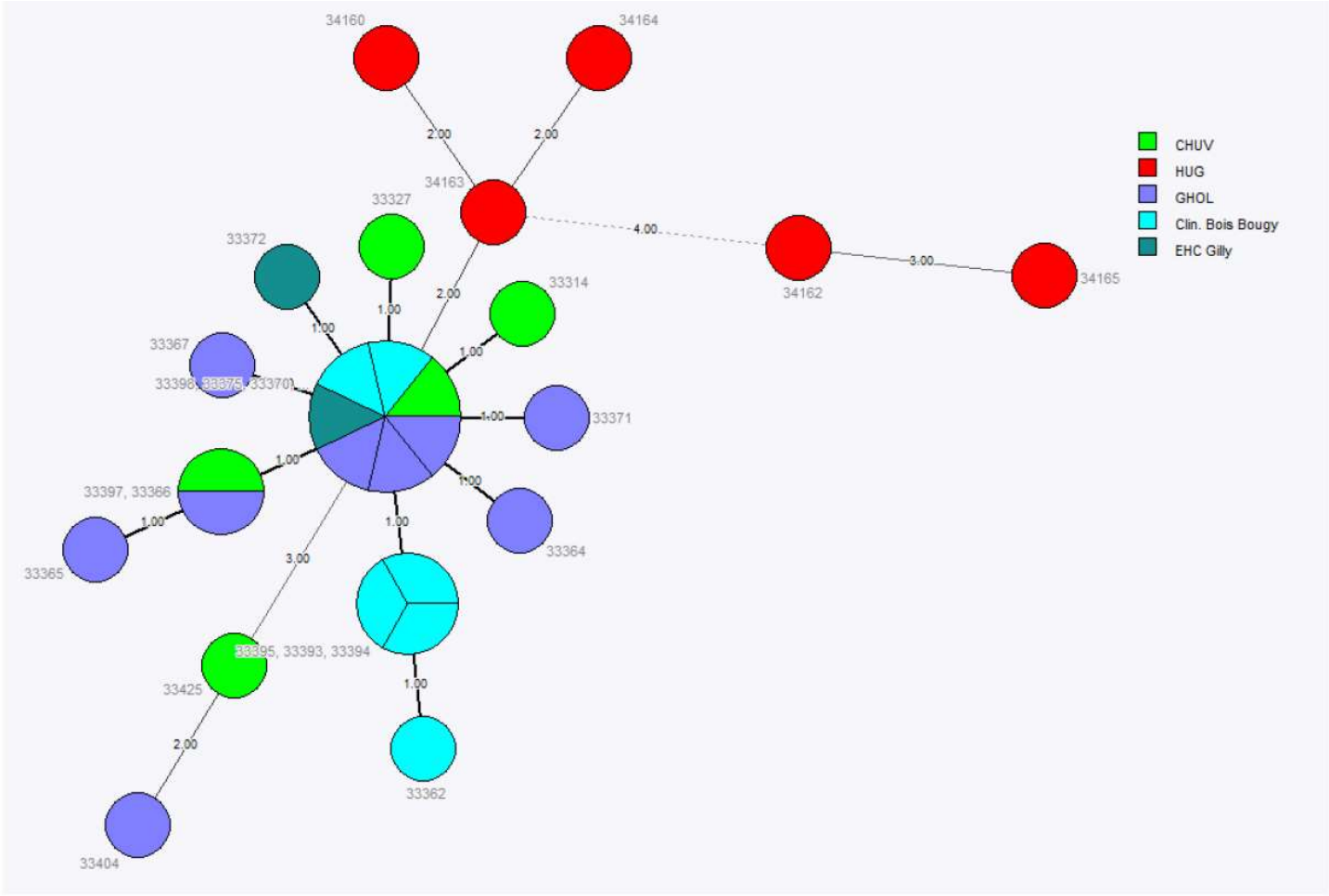


Figure 1. Arbre de maximum de parcimonie entre les souches du cluster ST117-B. La distance entre chaque souche indique le nombre de loci de différence.

Eclosions de VRE avec transmission régionale

Région du Lac Leman (2017)

nature microbiology

ARTICLES

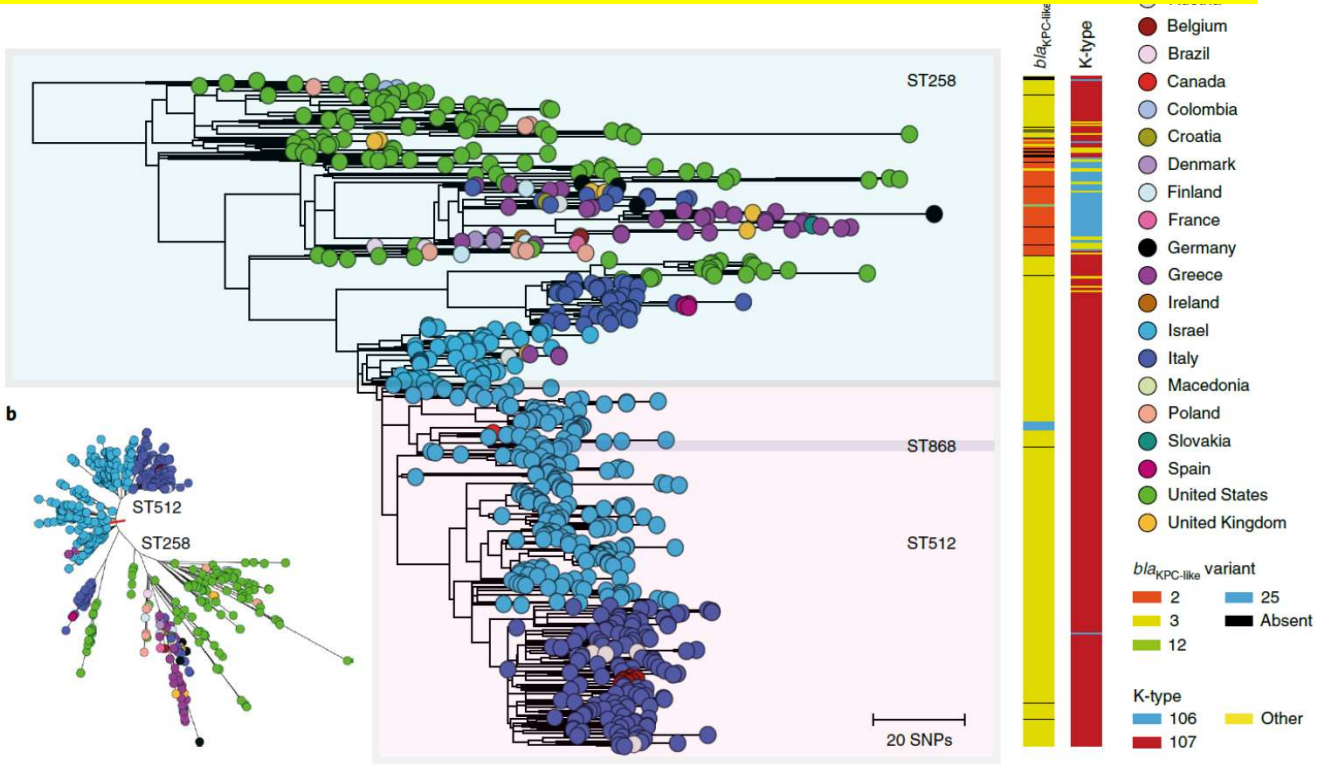
https://doi.org/10.1038/s41564-018-0230-7

Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*

Jean Y. H. Lee[1], Ian R. Monk[1], Anders Gonçalves da Silva[2,3], Torsten Seemann[3,4], Kyra Y. L. Chua[5], Angela Kearns[6], Robert Hill[6], Neil Woodford[6], Mette D. Bartels[7], Birgit Strommenger[8], Frederic Laurent[9], Magali Dodémont[10], Ariane Deplano[10], Robin Patel[11], Anders R. Larsen[12], Tony M. Korman[13], Timothy P. Stinear[1,3,15] and Benjamin P. Howden[2,3,14,15]*

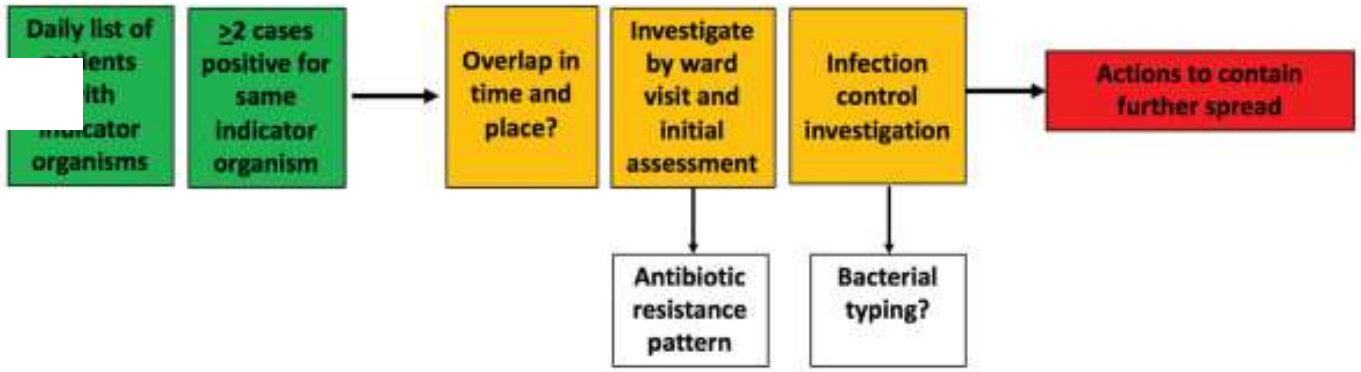
➔ Uncovered the previously unrecognized international spread of a near pandrug-resistant nosocomial pathogen, identifiable by a rifampicin-resistant phenotype.

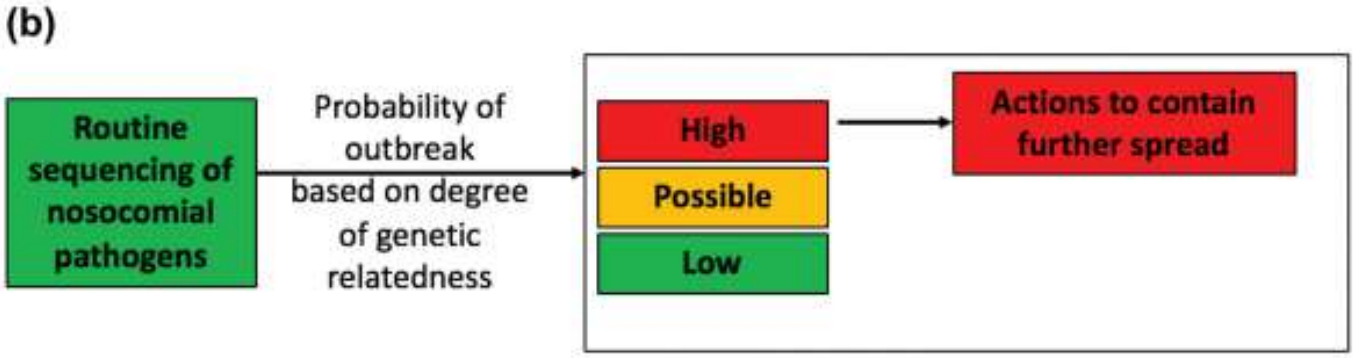
International spread of the epidemic KPC ST258/512 clone



Paradigm shift: real-life example?

NGS-based outbreak detection





Peacock et

Fig. 1. Current and proposed approach to the detection of hospital outbreaks. (a) Current practice for the detection of hospital out-

Infection Control & Hospital Epidemiology (2019), **40**, 649–655
doi:10.1017/ice.2019.75

SHEA
The Society for Healthcare Epidemiology of America

Original Article

Integration of genomic and clinical data augments surveillance of healthcare-acquired infections

Doyle V. Ward PhD[1,2], Andrew G. Hoss PhD[3], Raivo Kolde PhD[3], Helen C. van Aggelen PhD[3], Joshua Loving PhD[3], Stephen A. Smith BS[4], Deborah A. Mack RN, CIC[5], Raja Kathirvel MS[4], Jeffery A. Halperin MBA[4], Douglas J. Buell BA[6], Brian E. Wong MS[4], Judy L. Ashworth MS[4], Mary M. Fortunato-Habib BSN, MS, DNP[4], Liyi Xu PhD[3], Bruce A. Barton PhD[7], Peter Lazar BS[7], Juan J. Carmona PhD, MPH[4], Jomol Mathew PhD[7], Ivan S. Salgo MD[4], Brian D. Gross MS[4] and Richard T. Ellison III MD[2,8]

Objective: To recognize transmission clusters and identify cross-transmission events, using WGS

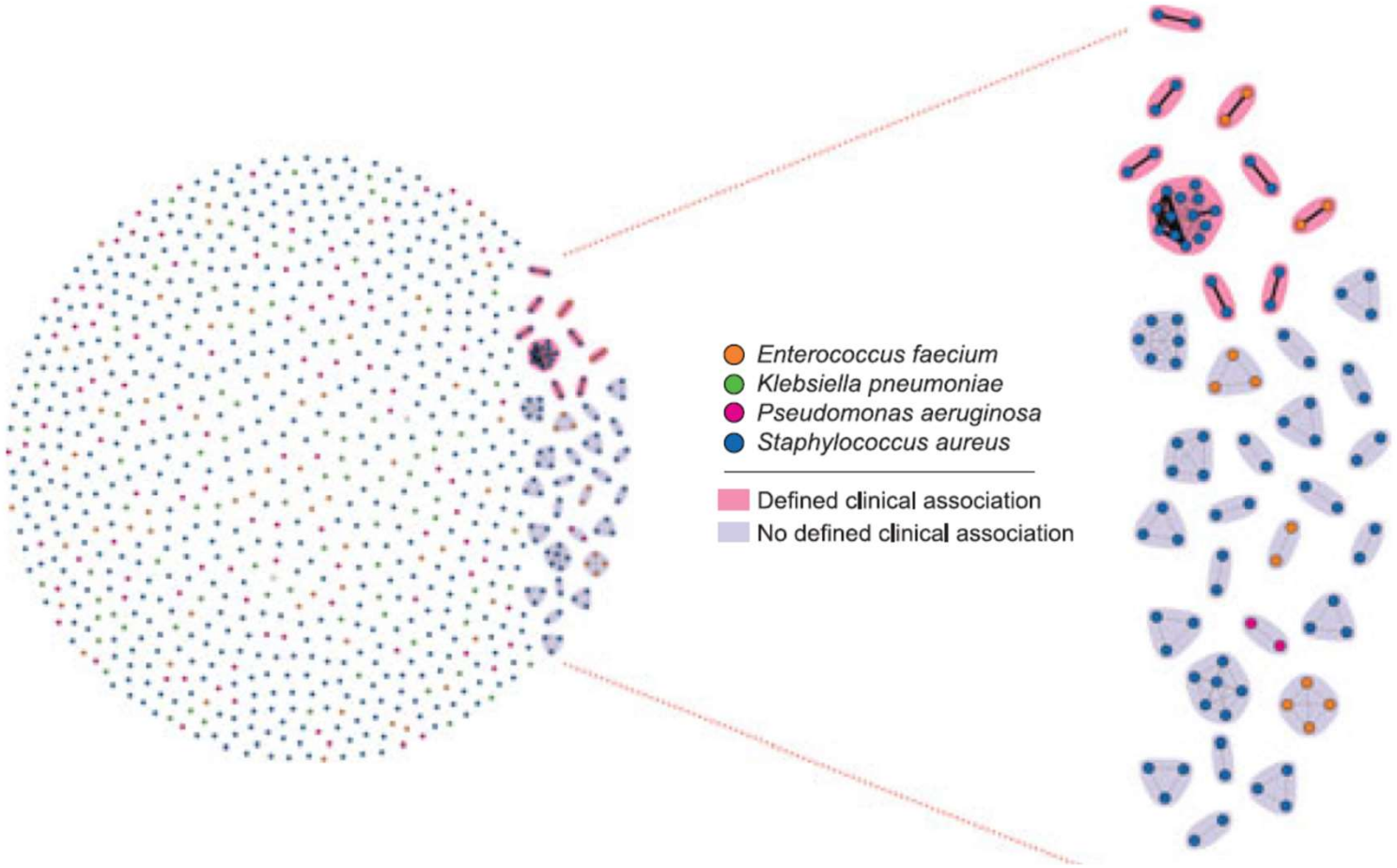
Methods (retrospective cohort study):

- Clinical isolates of *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* obtained at UMass from September 1, 2016, to September 30, 2017.
- Isolate genomes were sequenced, followed by single-nucleotide variant analysis
- Use of a cloud-computing platform for WGS analysis and cluster identification

Main results

- Most strains of the 4 studied pathogens were unrelated
  ➔Endogeneous acquisition or outside transmission
- 34 potential transmission clusters involving 96 patients:
  – 25 clusters: no clinical or epidemiological associations
  – 9 clusters: obvious clinical associations
    ➔ only 1 cluster suspected by routine manual surveillance
  – 28 *S. aureus* clusters, 5 *E. faecium* clusters, 1 cluster of *P. aeruginosa*
- Largest cluster: 21 MRSA isolates from 13 patients with community-onset MRSA infections

Identification of suspect transmission events among patients



➔ Detection bias?
➔ Missing environmental sampling?
➔ Clinical importance and impact?

Environmental reservoirs of MDRO

Infection Control & Hospital Epidemiology (2019), **40**, 47–52
doi:10.1017/ice.2018.275

Original Article

A prospective study of transmission of Multidrug-Resistant Organisms (MDROs) between environmental sites and hospitalized patients—the TransFER study

- 44% of hospital rooms contaminated with MDROs after terminal cleaning
- Transfer of bacteria between environment and patient: 18% of admissions
- Occurred early in admission

ORIGINAL ARTICLE

A *Candida auris* Outbreak and Its Control in an Intensive Care Setting

David W. Eyre, D.Phil., Anna E. Sheppard, Ph.D., Hilary Madder, F.A.N.Z.C.A., Ian Moir, Ruth Moroney, M.Sc., T. Phuong Quan, M.Sc., David Griffiths, B.Sc., Sophie George, M.Sc., Lisa Butcher, M.Sc., Marcus Morgan, M.Sc., Robert Newnham, Mary Sunderland, B.Sc., Tiphanie Clarke, B.A., Dona Foster, Ph.D., Peter Hoffman, B.Sc., Andrew M. Borman, Ph.D., Elizabeth M. Johnson, Ph.D., Ginny Moore, Ph.D., Colin S. Brown, F.R.C.Path., A. Sarah Walker, Ph.D., Tim E.A. Peto, F.R.C.P., Derrick W. Crook, F.R.C.Path., and Katie J.M. Jeffery, Ph.D.

➔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.

N Engl J Med 2018;379:1322-31.

Potential biases & challenges during the analyses

Storage → Extraction → Library preparation → Sequencing

Storage parameters:
T°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

«Library preparation methods for next generation sequencing: Tone down the bias», E.L. van Dijk, Exp Cell Research, 2014

Pre-analytic	Analytic (sequencing)	Analytic (analysis)	Post-analytic
	**cgMLST**	**wgMLST**	**hqSNP**
Discrimination	Alleles in the Core genome	Alleles in the Pan-genome	SNP
Mapping	Manually curated database (beware of new genomes)	Difficult to define alleles, constant evolution	Difficult to create a nomenclature
Inter-facility Comparability	Robust	Allele definition is sensitive to assembler and parameter choices	Sensitive to parameter variations (reference, SNP calling filters, coverage)
Reliability	Set of predefined core genes	Consider accessory genomes (variability, paralogous genes)	Depends of the reference genome

What is close enough ?

Relatedness thresholds

SNPs: nb of single-nucleotide variant differences

Table 1
Examples of relatedness criteria for wg/cgMLST and SNP typing schemes of representative clinically relevant bacteria

Organism	Relatedness threshold[a] wg/cgMLST (allele)	SNPs	References
Acinetobacter baumannii	≤8	≤3	[25,26]
Brucella spp.	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Campylobacter coli, C. jejuni	≤14	≤15	[27,28]
Cronobacter spp.	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Clostridium difficile	Epidemiologic validation in progress[b]	≤4	[29], http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst
Enterococcus faecium	≤20	≤16	[30]
Enterococcus raffinosus	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Escherichia coli	≤10	≤10	[31,32], https://enterobase.warwick.ac.uk/
Francisella tularensis	≤1	≤2	[33,34]
Klebsiella oxytoca	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Klebsiella pneumonia	≤10	≤18	[35,36]
Legionella pneumophila	≤4	≤15	[37]
Listeria monocytogenes	≤10	≤3	[38,39]
Mycobacterium abscessus		≤30	[40]
Mycobacterium tuberculosis	≤12	≤12	[41]
Neisseria gonorrhoeae	Epidemiologic validation in progress[b]	≤14	[42], http://www.applied-maths.com/applications/wgmlst
Neisseria meningitidis	Epidemiologic validation in progress[b]		http://www.cgmlst.org/ncs
Pseudomonas aeruginosa	≤14	≤37	[31,43]
Salmonella dublin	Epidemiologic validation in progress[b]	≤13	[44], https://enterobase.warwick.ac.uk/
Salmonella enterica	Epidemiologic validation in progress[b]	≤4	[45], http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst, https://enterobase.warwick.ac.uk/
Salmonella typhimurium	Epidemiologic validation in progress[b]	≤2	[46], https://enterobase.warwick.ac.uk/
Staphylococcus aureus	≤24	≤15	[47,48]
Streptococcus suis		≤21	[49]
Vibrio parahaemolyticus	≤10		[50]
Yersinia spp.	0		[51]

cg, core genome; MLST, multilocus sequence typing; SNP, single nucleotide polymorphism; wg, whole genome.
[a] 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.
[b] Proposed wg/cgMLST schemes are available online (http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst, https://enterobase.warwick.ac.uk/) but as yet have not been epidemiologically validated.

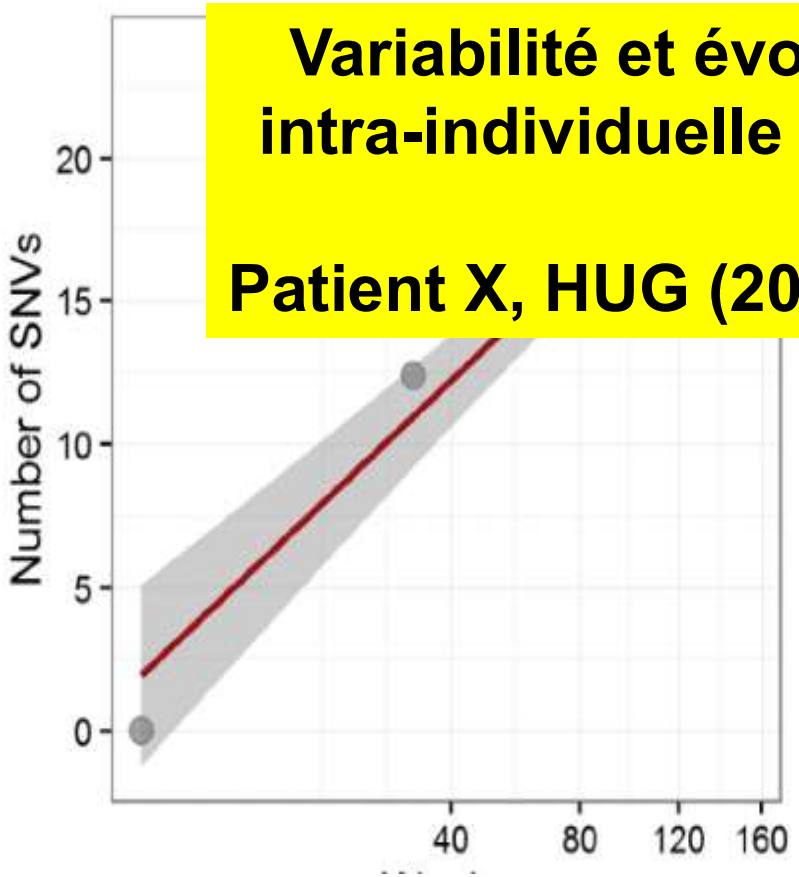
Feedback

CAVE

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

Variabilité et évolution intra-individuelle de KPC

Patient X, HUG (2012-2015)



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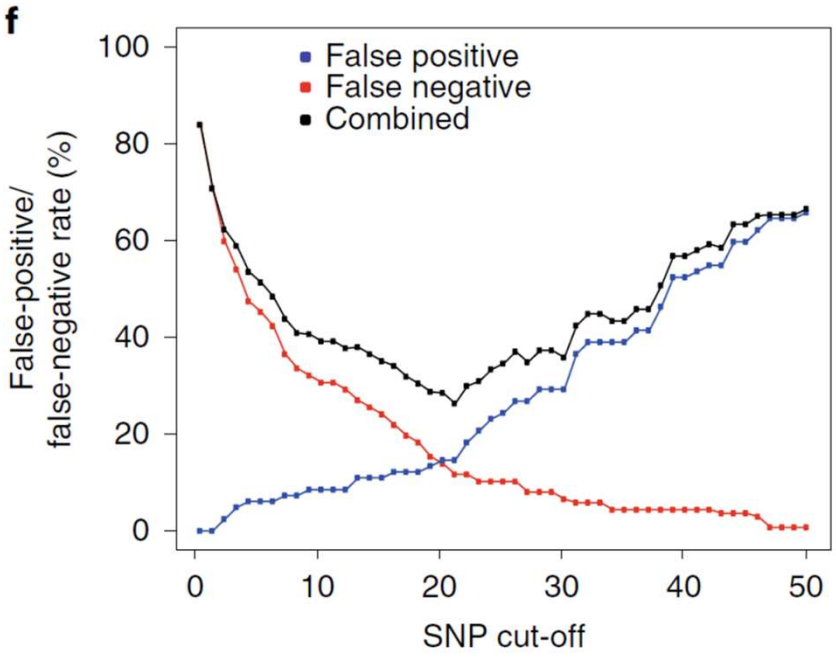
nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-019-0492-8

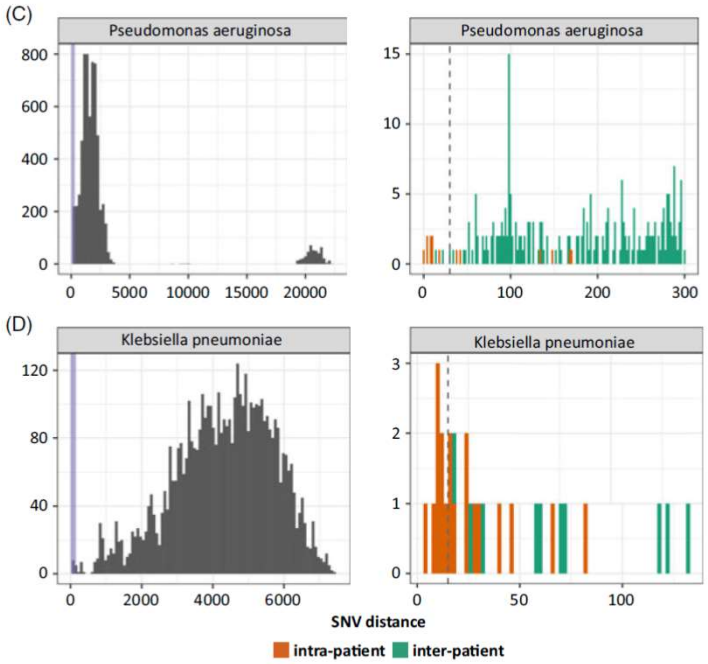
Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread

Sophia David[1], Sandra Reuter[2], Simon R. Harris[3], Corinna Glasner[4], Theresa Feltwell[3], Silvia Argimon[1], Khalil Abudahab[1], Richard Goater[1], Tommaso Giani[5], Giulia Errico[6], Marianne Aspbury[7], Sara Sjunnebo[8], the EuSCAPE Working Group[9], the ESGEM Study Group[10], Edward J. Feil[11], Gian Maria Rossolini[5,12], David M. Aanensen[1,13,14]* and Hajo Grundmann[2,4,14]*



Value of 21 SNPs for discrimination of hospital clusters

Intrapatient versus inter-patient SNP distances to inform thresholds for identification of transmission events



Threshold: 15 SNP for *Klebsiella pneumoniae*

What is close enough ?

Relatedness thresholds
SNPs: nb of single-nucleotide variant differences

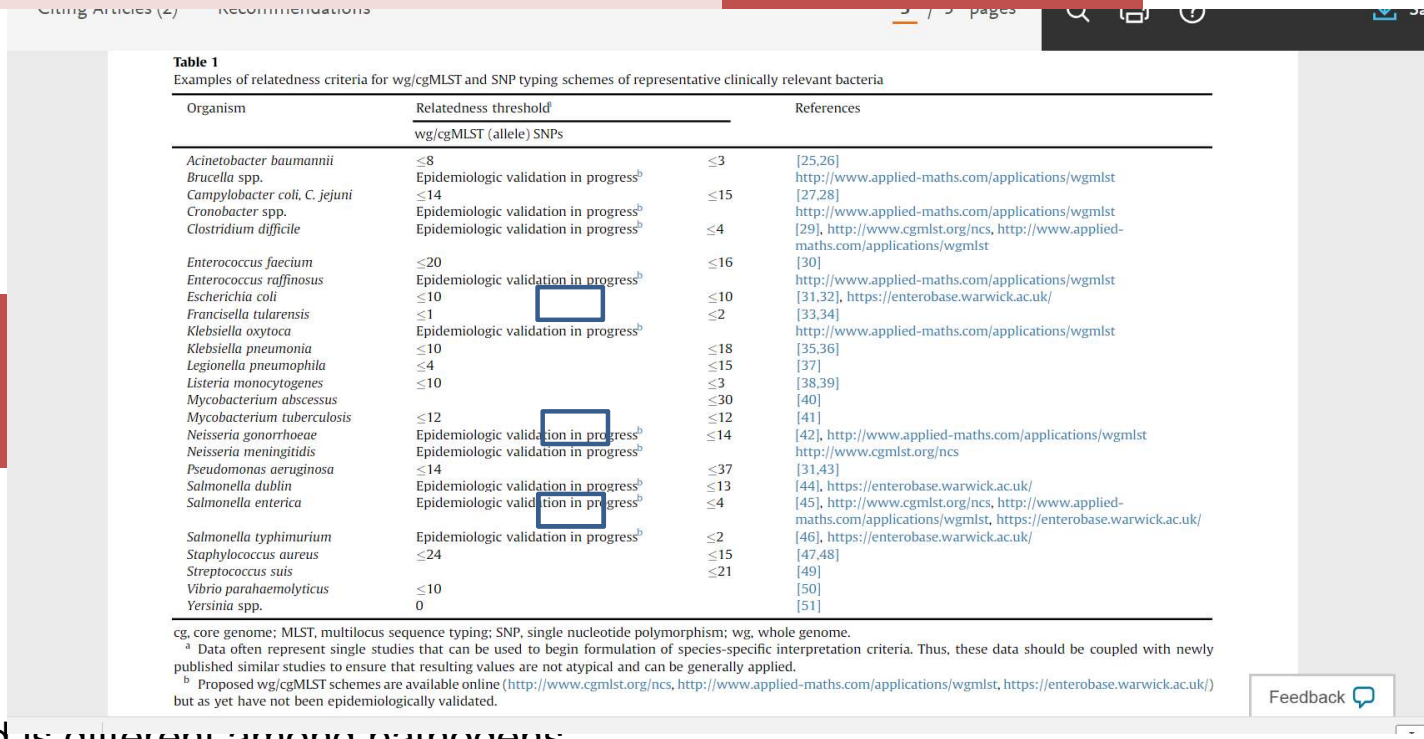


Table 1
Examples of relatedness criteria for wg/cgMLST and SNP typing schemes of representative clinically relevant bacteria

Organism	Relatedness threshold[a] wg/cgMLST (allele) SNPs		References
Acinetobacter baumannii	≤8	≤3	[25,26]
Brucella spp.	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Campylobacter coli, C. jejuni	≤14	≤15	[27,28]
Cronobacter spp.	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Clostridium difficile	Epidemiologic validation in progress[b]	≤4	[29], http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst
Enterococcus faecium	≤20	≤16	[30]
Enterococcus raffinosus	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Escherichia coli	≤10	≤10	[31,32], https://enterobase.warwick.ac.uk/
Francisella tularensis	≤1	≤2	[33,34]
Klebsiella oxytoca	Epidemiologic validation in progress[b]		http://www.applied-maths.com/applications/wgmlst
Klebsiella pneumonia	≤10	≤18	[35,36]
Legionella pneumophila	≤4	≤15	[37]
Listeria monocytogenes	≤10	≤3	[38,39]
Mycobacterium abscessus		≤30	[40]
Mycobacterium tuberculosis	≤12	≤12	[41]
Neisseria gonorrhoeae	Epidemiologic validation in progress[b]	≤14	[42], http://www.applied-maths.com/applications/wgmlst
Neisseria meningitidis	Epidemiologic validation in progress[b]		http://www.cgmlst.org/ncs
Pseudomonas aeruginosa	≤14	≤37	[31,43]
Salmonella dublin	Epidemiologic validation in progress[b]	≤13	[44], https://enterobase.warwick.ac.uk/
Salmonella enterica	Epidemiologic validation in progress[b]	≤4	[45], http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst, https://enterobase.warwick.ac.uk/
Salmonella typhimurium	Epidemiologic validation in progress[b]	≤2	[46], https://enterobase.warwick.ac.uk/
Staphylococcus aureus	≤24	≤15	[47,48]
Streptococcus suis		≤21	[49]
Vibrio parahaemolyticus	≤10		[50]
Yersinia spp.	0		[51]

cg, core genome; MLST, multilocus sequence typing; SNP, single nucleotide polymorphism; wg, whole genome.
[a] 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.
[b] Proposed wg/cgMLST schemes are available online (http://www.cgmlst.org/ncs, http://www.applied-maths.com/applications/wgmlst, https://enterobase.warwick.ac.uk/) but as yet have not been epidemiologically validated.

Feedback

CAVE

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

THUS

- <u>Consider suggested thresholds more as a guideline</u>

➔ Interprete epidemiological links on a case by case basis

➔ Interprete organism by organism (specific population genetics)

Eclosion de Klebsielles multi-R aux HUG en 2015

Cas index

Cas secondaire N° 1

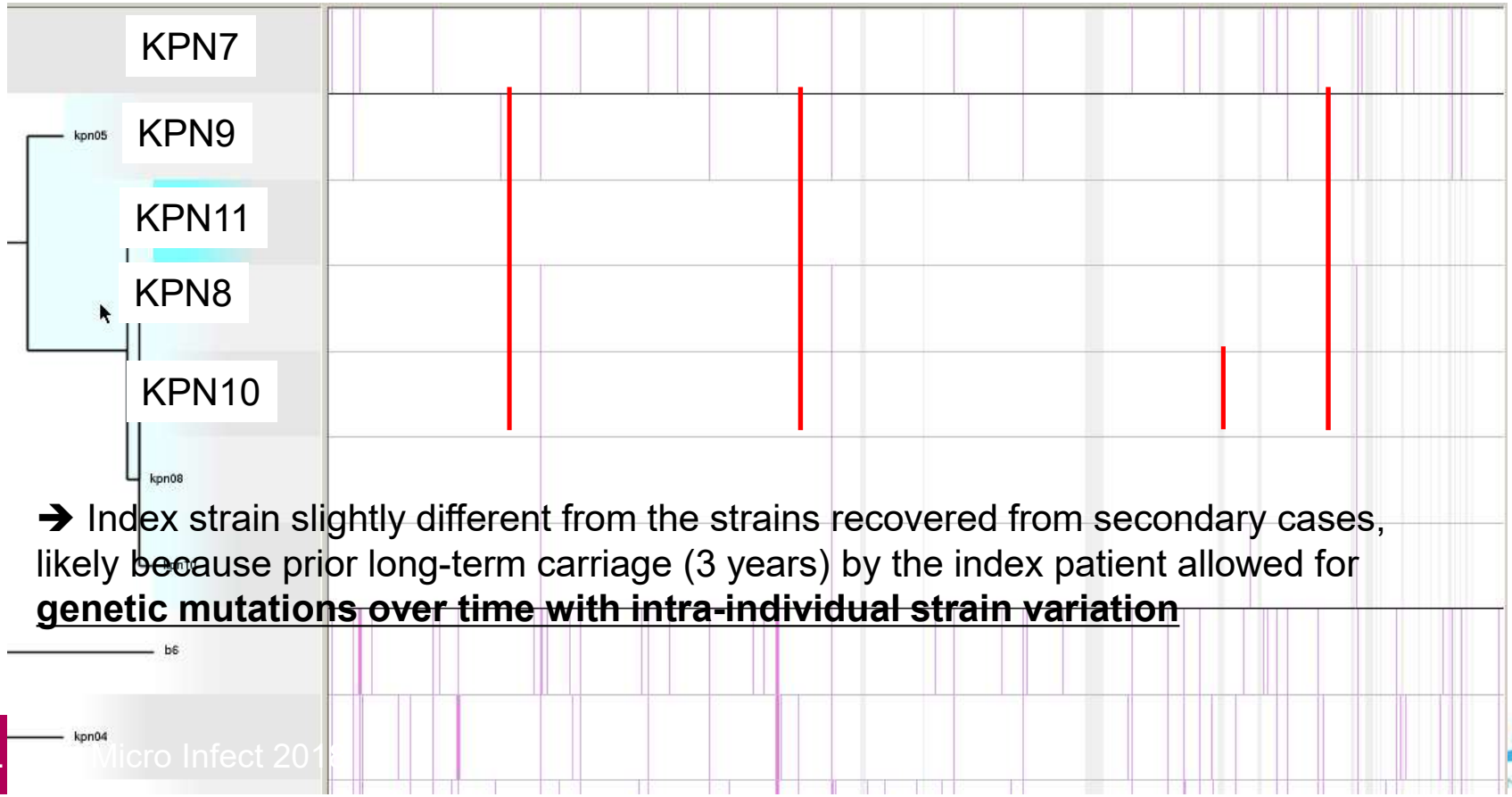
Cas secondaire N° 2

	R	Amoxicilline		
	R	Co-amoxiclav		
	R	Piperacilline		
256	R	Piperac.+tazob	256	256
	R	Cefoxitine		
	R	Cefuroxime		
	R	Ceftazidime		
	R	Ceftriaxone		
256	R	Cefepime	96	96
	R	Imipenem	>32	>32
	R	Meropeneme	>32	>32
	R	Ertapènem	32	32
	R	Aztreonam		
	R	Amikacine	2	2
	S	Gentamicine		
	R	Norfloxacine		
	R	Ciprofloxacine		
	R	Co-trimoxazole	48	48
	S	Fosfomycine	2	2
	I	Tigécycline	32	32
	R	Furanes		
8	R		0.750	0.750

SNVs analysis from whole common DNA

Whole genome analysis: KPN7 had 3 SNV differences with others, KPN10 has another SNV

Index case



➔ 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**

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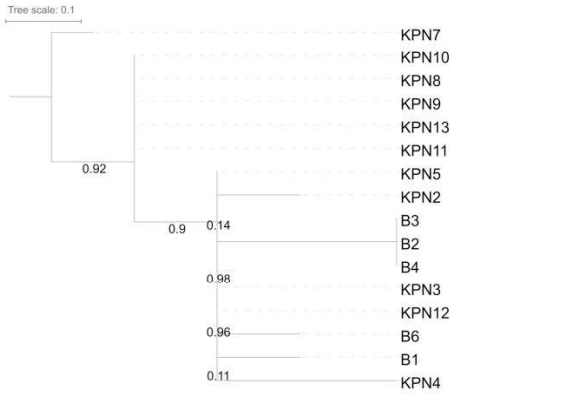
Blinded assessment of clonal relationships by wgMLST (634 genes)

Fifteen of the 18 strains belonged to ST512, while isolates KPN1, B5 and B6 belonged to ST258. From the wgMLST analysis, we first observed that KPN7 was distinct from other strains and had one SNV difference with the closest cluster that included five strains (KPN8, -9, -10, -11 and -13) (Fig. 2, Table 1). In addition, another cluster of three strains was observed (B2, B3 and B4). The other strains appeared to be unrelated at this level of analysis, with KPN1 and B5 being the most distant strains from KPN7 (Table 1); their involvement in any outbreak caused by KPN7 was thus unlikely.



Download high-res image (170KB) Download full-size image

Fig. 2. Phylogenetic tree with bootstrap values obtained by comparison of genes included in *Klebsiella pneumoniae* wgMLST. KPN7 is suspected index strain for outbreak. B5 and KPN1 do not appear on tree because of their high distance. wgMLST, whole-genome multilocus sequence typing.

SNP
and ARGs
(accessory genome)

Analysis of ARGs

All strains displayed similar antibiotic susceptibility profiles, with resistance to all β-lactams and fluoroquinolones but, for the most part, susceptibility to gentamicin, colistin and tigecycline.

shared a similar antibiotic susceptibility profile and consist harboured similar ARG content (Supplementary Table S4) strains B3 and B4 lacked the *aadA6* and *aph(3′)-Ia* genes foun plasmid KPN7_p1. Further details about resistance determin are found in the Supplementary Materials.

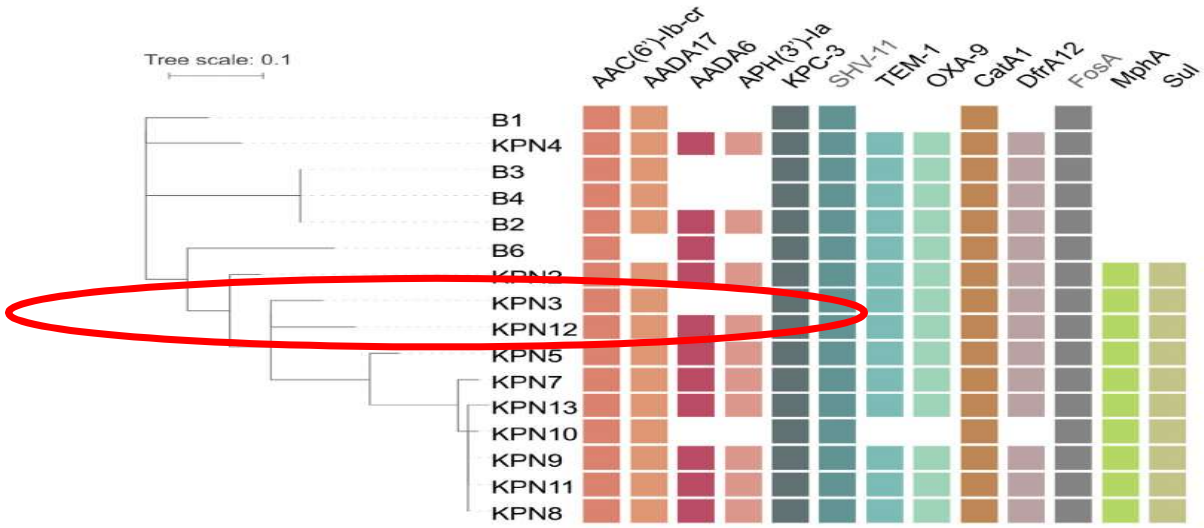


Fig. 3. Phylogenetic tree obtained by comparison of common genomes of all KPC-producing *Klebsiella pneumoniae* and associated ARGs. B5 and KPN1 do not appear on tree be of their high distance. ARGs *SHV-11* and *FosA* (in grey font) are intrinsic to *K. pneumoniae*. ARG, antibiotic resistance gene; KPC, *K. pneumoniae* carbapenemase.

2 outbreak investigations from Geneva

MRSA

CPE

Friday, June 30

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

What would you recommend for the next week – priority action items except?

A. Isolate or cohort MRSA carriers

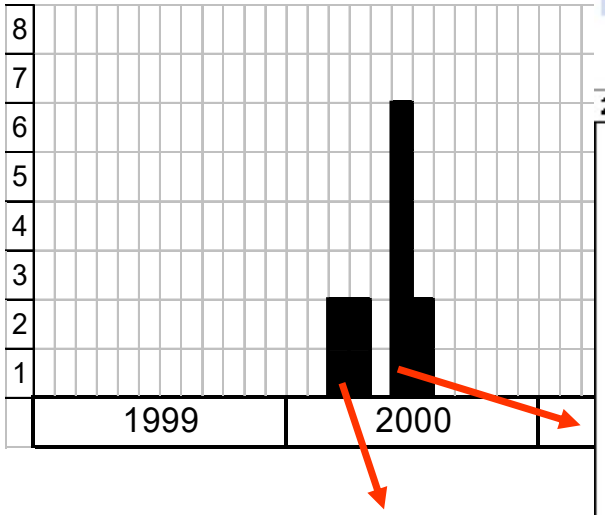
B. Reinforce hand hygiene

C. Screening of all hospitalized neonates

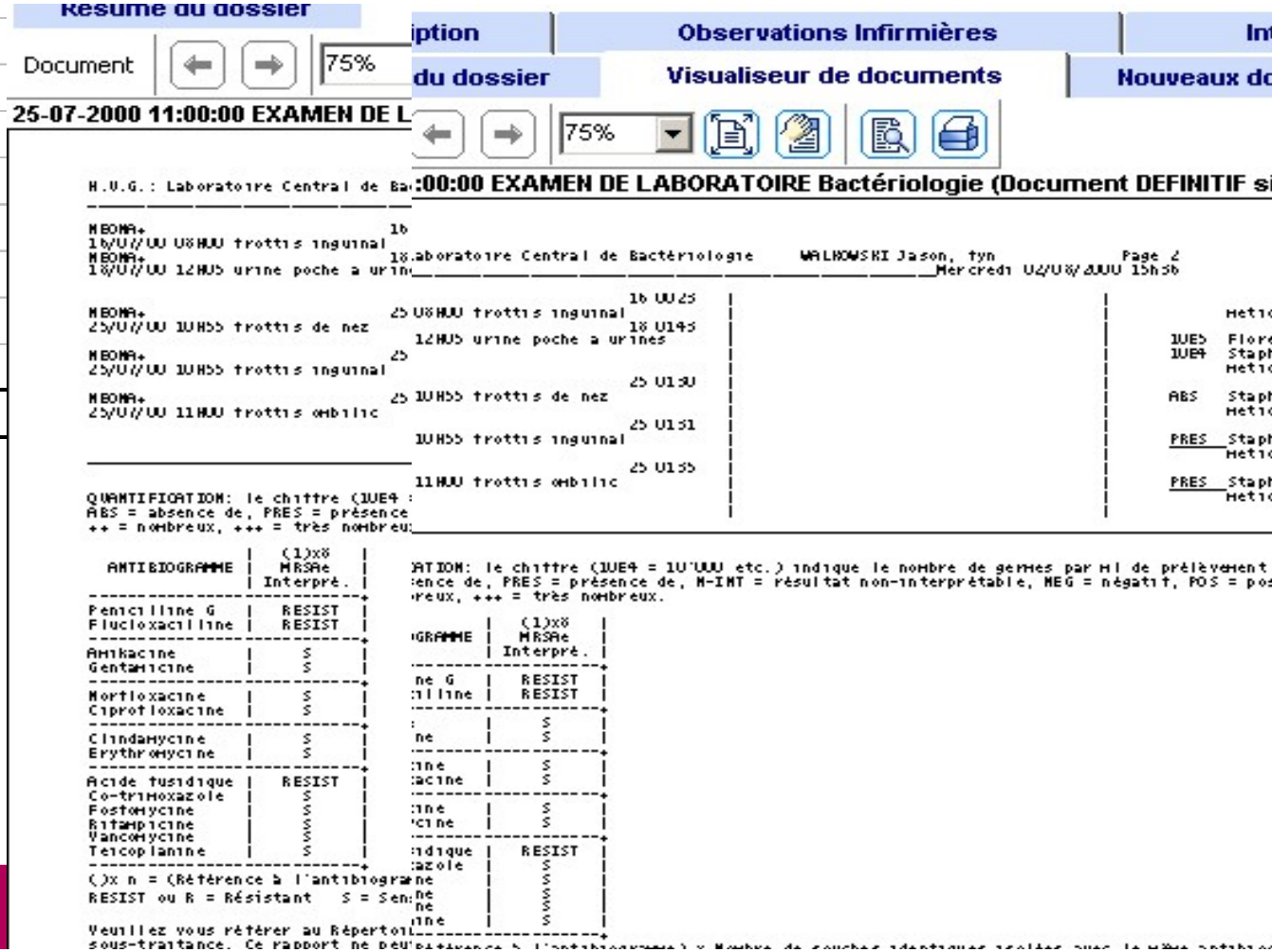
D. Implement active MRSA surveillance for all new admissions

E. **Molecular typing of MRSA isolates**

Epidemic curve
MRSA outbreak, NICU (HUG)

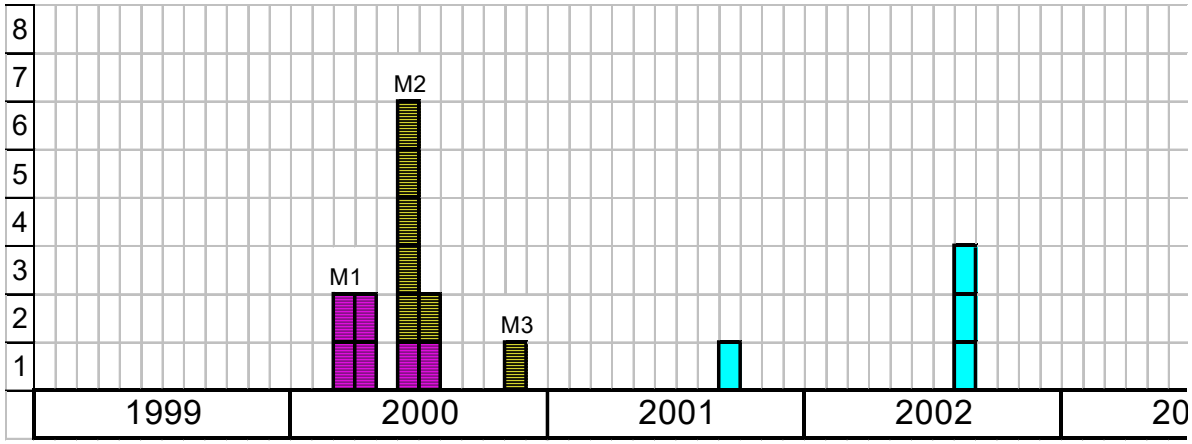


ANTIBIOGRAMMES	(1)x1 SCN. Interpré.	(2)x3 MRSAe Interpré.
Penicilline G	RESIST	RESIST
Flucloxacilline	RESIST	RESIST
Amikacine	RESIST	RESIST
Gentamicine	S	RESIST
Norfloxacine	RESIST	RESIST
Ciprofloxacine	RESIST	RESIST
Clindamycine	S	RESIST
Erythromycine	RESIST	RESIST
Acide fusidique	INTERM	S
Co-trimoxazole	RESIST	S
Fosfomycine		S
Rifampicine	S	S
Vancomycine	S	S
Teicoplanine	S	S



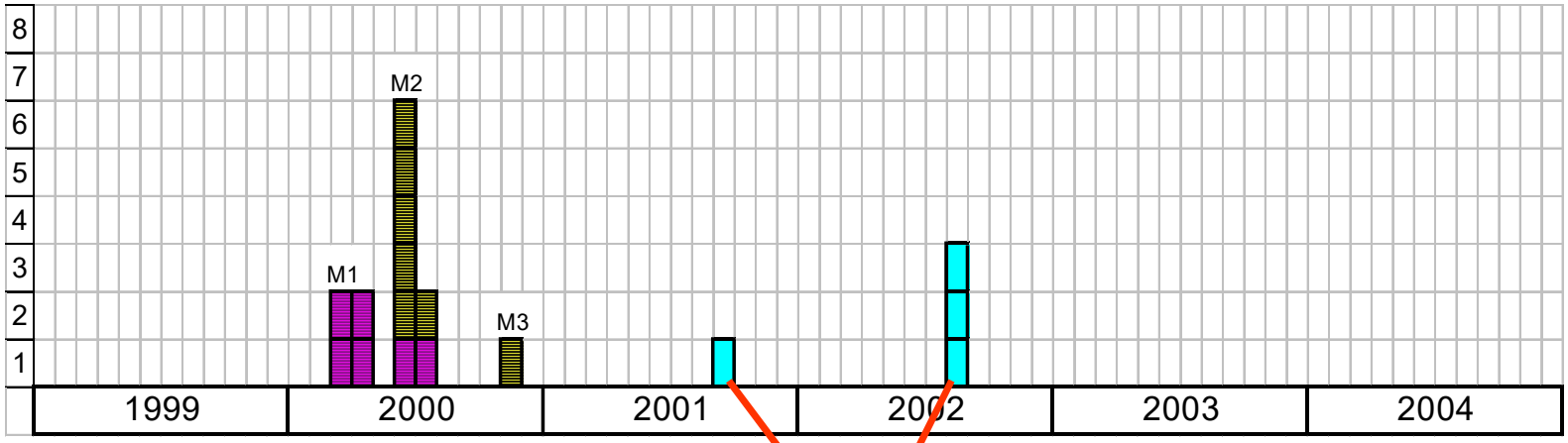
Sax et al. J Hosp Infect 2006; 63: 93-100

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)

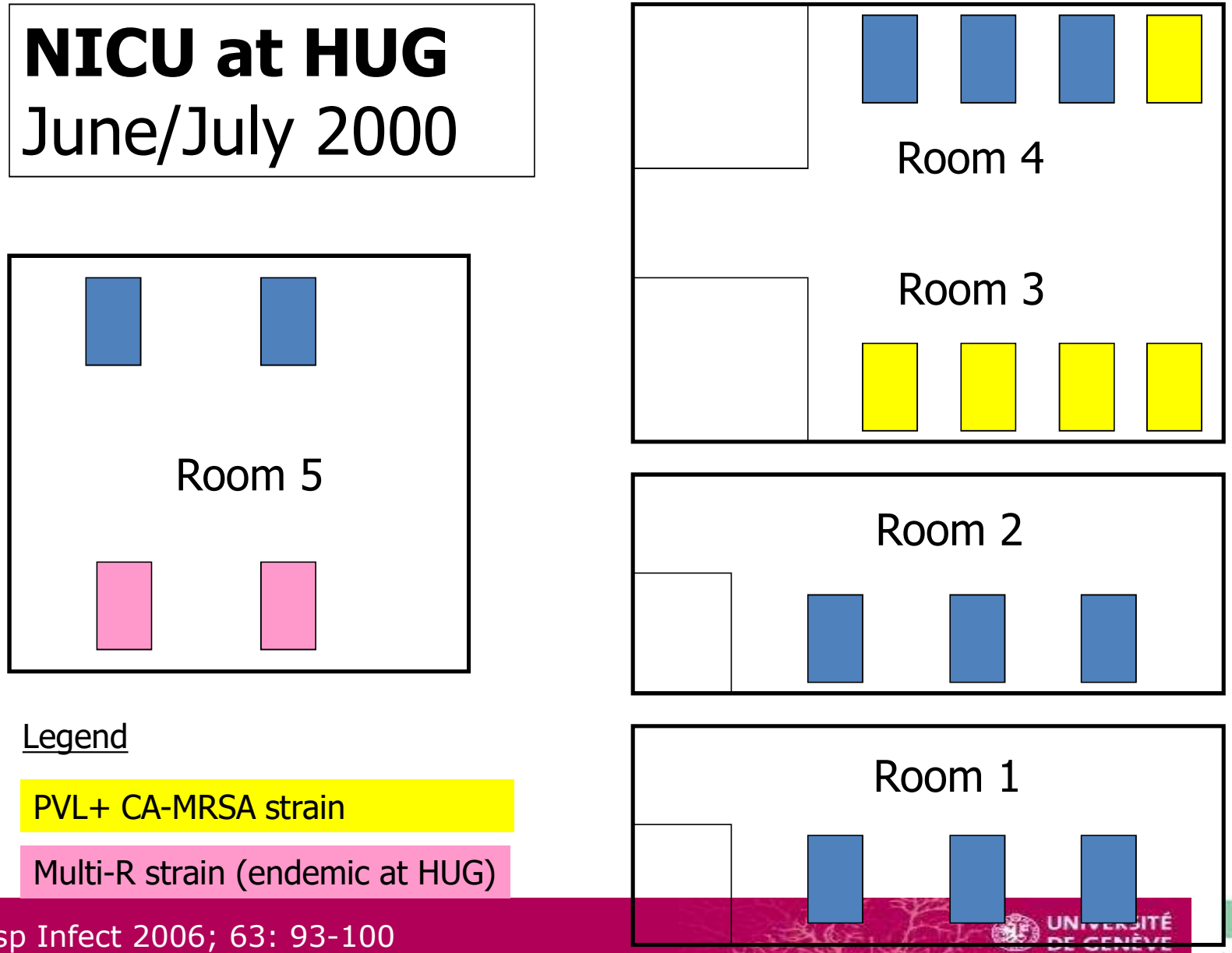
Epidemic curve
MRSA outbreak, NICU (HUG)



ANTIBIOGRAMME	(1)x1 MRSA Interpré.
Penicilline G	RESIST
Flucloxacilline	RESIST
Gentamicine	S
Norfloxacine	RESIST
Ciprofloxacine	RESIST
Clindamycine	RESIST
Erythromycine	RESIST
Acide fusidique	S
Co-trimoxazole	S
Rifampicine	S
Vancomycine	S
Teicoplanine	S

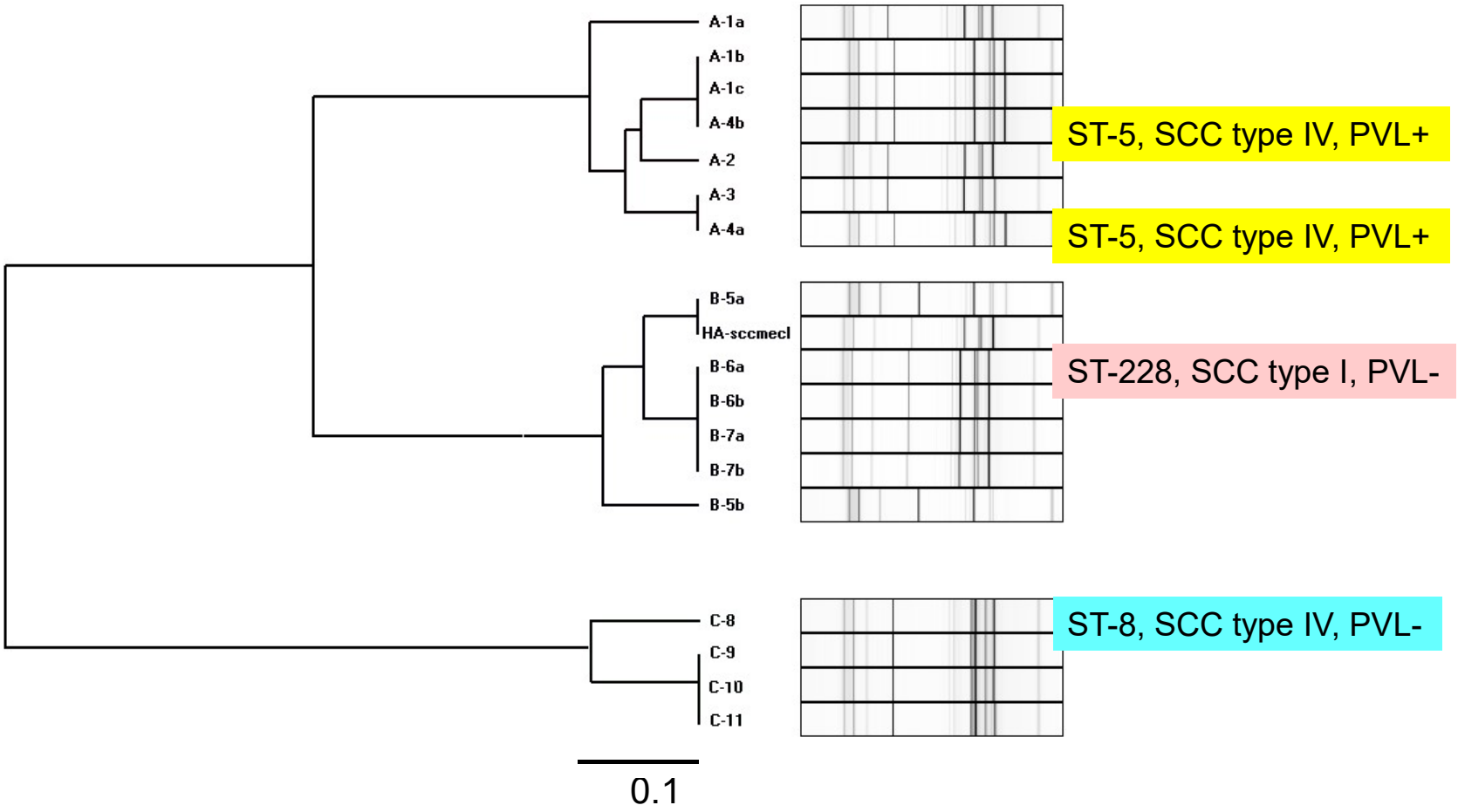
Sax et al. J Hosp Infect 2006; 63: 93-100

NICU at HUG
June/July 2000



Sax et al. J Hosp Infect 2006; 63: 93-100

Molecular Epidemiology

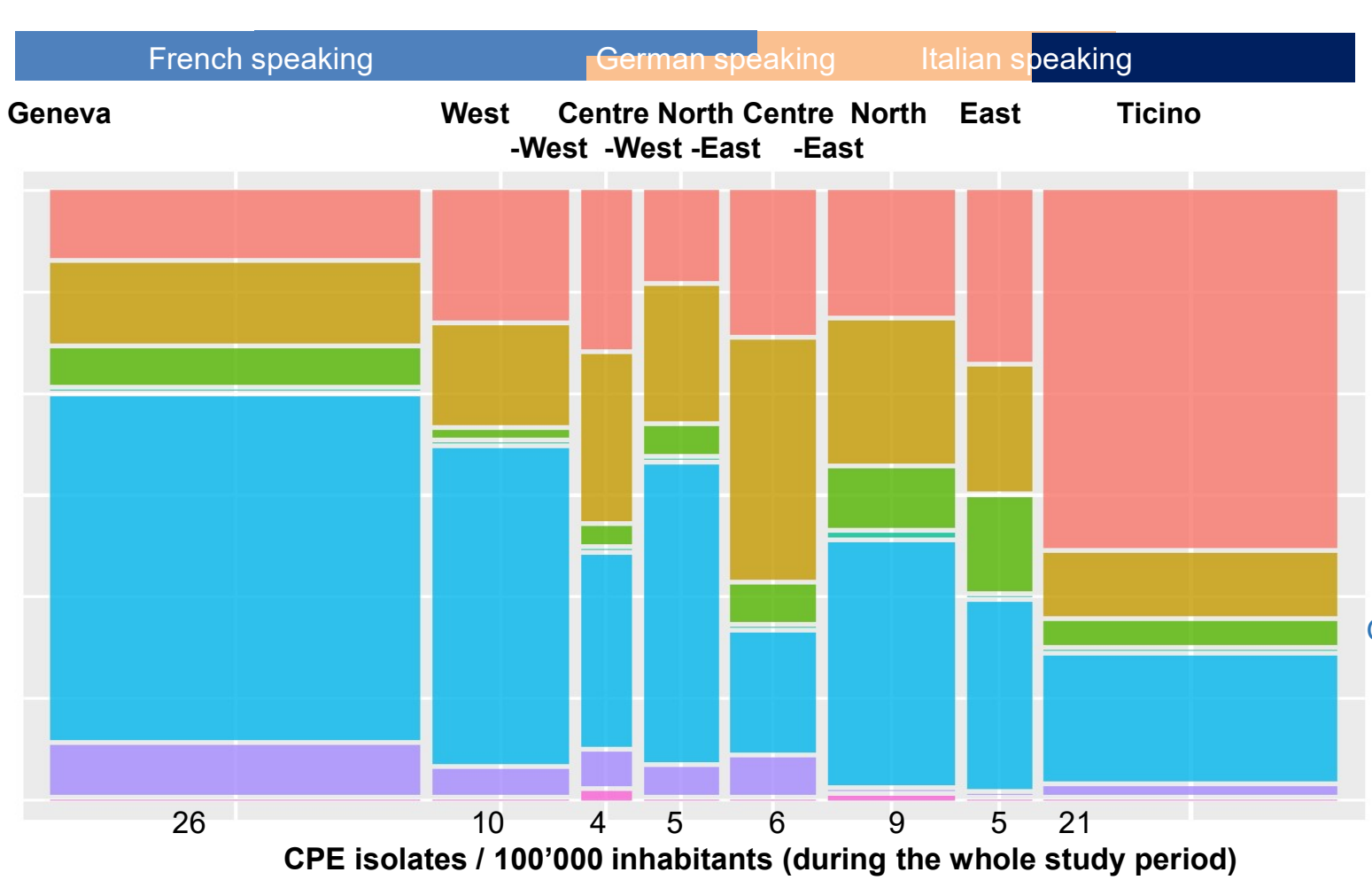


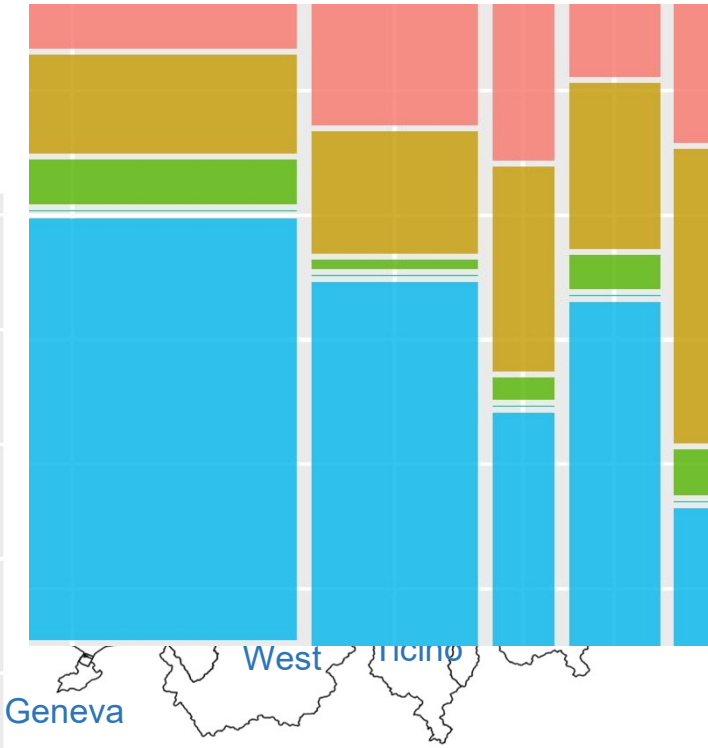
Sax et al. J Hosp Infect 2006; 63: 93-100

Orthopedic ward, Geneva

OXA-181 cluster, summer 2019

CPE isolates in CH 2013 - 2018





CH total 682 isolates
→8 isolates /100'000 inhabitants

Courtesy: A. Kronenberg (anresis.ch)

1ère investigation

04-06.19

18.06.2019 – 5AL (orthopédie septique)
Départ d'un patient porteur (M.H.) de *K.pneumoniae* KPC
Hospitalisé au 5AL du 22.05-18.06.19

➔ **Dépistage de l'unité**

Détection fortuite de 3 porteurs de carbapénémases (OXA-181)
(M.K.) *Citrobacter* sp BLSE & OXA-181 (FA) – au 5AL du 28.05-25.06.19
(M.P.) *Citrobacter* sp BLSE & OXA-181 (FA) – au 5AL du 12.04-19.06.19
(M.B.) *Citrobacter* sp BLSE & OXA-181 (FA) – au 5AL du 28.05-03.07.19

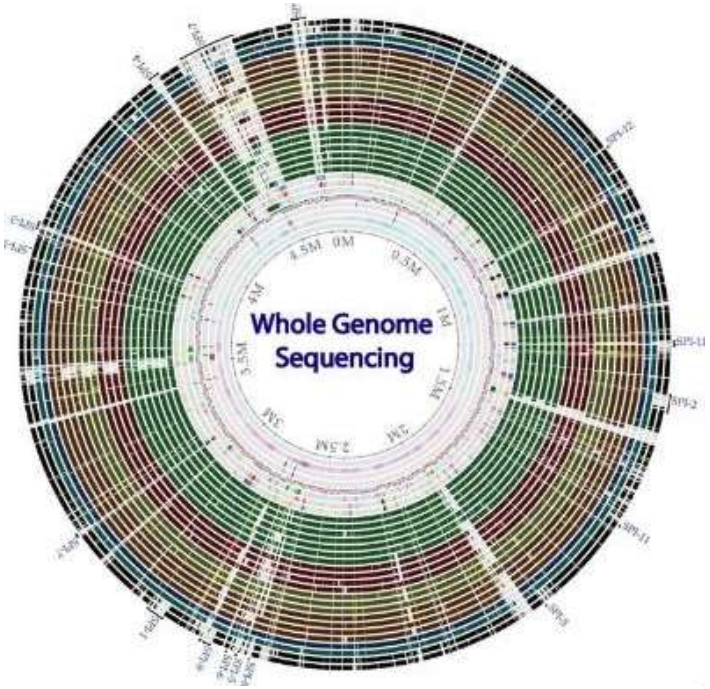
1ère investigation

03.201904-06.19

Recherche d'un cas index
(M.R.) *Klebsiella pneumoniae* OXA-181 – au 5AL du **08.03-20.03.19**

- Originaire du Sri Lanka & hospitalisation en Inde (27.02-06.03.19) MR2
- Mesures CONTACT PLUS durant son hospitalisation et son suivi
- Bonne compliance de l'équipe, mauvaise compliance du patient

Possible transfert horizontal inter-espèce du gène OXA-181 ?



Diapositive 61

MR2 MARTISCHANG Romain; 17/10/2019

1ère investigation

03.2019 04-06.19

Mais…

Citrobacter | *Klebsiella*

Profil plasmidique

	GRL-89	GRL-90	GRL-91	GRL-92
Col440I				
ColKP3				100
ColRNAI				
IncX3	100	100	100	
IncFIA				99.74
IncFIB(AP001918)				
IncFII(29)				
IncFII(pAMA1167-NDM-5)				98.08
IncY				
IncFII				
ColpVC				
IncFIB(pB171)				
IncQ1	100	100	100	
IncFIB(pQil)				100

Hypothèse épidémiologique infirmée (autre sous-type de OXA: gènes OXA-1 et OXA-232)

2ème investigation

Nouveau patient index hypothétique

(M.P.) *Citrobacter* sp BLSE & OXA-181 (FA) – au 5AL du **12.04-19.06.19**

- Circulation libre dans l'ensemble du 5AL
- Non compliance aux mesures de précaution (pt toxicomane)
- Source de contamination primaire par OXA-181 obscure

Et 1 mois plus tard…

2ème investigation

27.08.2019 – 5AL : enquête de prévalence hebdomadaire

(M.N.) *E.coli* BLSE & OXA-181 (FA) – au 5AL du **22.07-30.08.19**

- Multiples opérations (23.07, 31.07, 19.08) aux HUG
- 5 frottis anaux negatifs pour CPE (22.07, 30.07, 16.08, 13.08, 20.08)
- SANS contact direct avec les 3 autres cas OXA-181 de juin 2019
- Séquençage à tout hasard…

2ème investigation

Et…

Citrobacter sp BLSE & OXA-181
21.06.2019, 5-AL

E.coli BLSE & OXA-181
27.08.2019, 5AL

		Citrobacter			Klebsiella pneumoniae		Escherichia coli			
		GRL-89	GRL-90	GRL-91	GRL-92	GRL-93	GRL-94	GRL-95	GRL-96	Ec-101
Beta-lactam	blaSHV-33					100				
Beta-lactam	blaOXA-181	100	100	100		100	100	100	100	100
Beta-lactam	blaCTX-M-15	100	100	100	100		100		100	100
Beta-lactam	blaTEM-1B	100	100	100	100		100	100	100	
Beta-lactam	blaOXA-1				100					100
Beta-lactam	blaOXA-232				100					

	Position dans la référence				
GRL-89 Citrobacter sp. 2.6.11.28		14183-14454			20364-20989
GRL-90 Citrobacter sp. 2.6.11.27		14183-14454			20364-20988
Ec-101 Escherichia coli 2.6.16.13		14183-14454			20364-20988
GRL-95 Escherichia coli 2.3.16.21					20380-20970
GRL-91 Citrobacter sp. 2.6.11.26	13221-14457				20364-20988

2ème investigation

Et…

Citrobacter sp BLSE & OXA-181
21.06.2019, 5-AL

E.coli BLSE & OXA-181
27.08.2019, 5AL

		Citrobacter			Klebsiella pneumoniae		Escherichia coli			
		GRL-89	GRL-90	GRL-91	GRL-92	GRL-93	GRL-94	GRL-95	GRL-96	Ec-101
Beta-lactam	blaSHV-33					100				
Beta-lactam	blaOXA-181	100	100	100		100	100	100	100	100
Beta-lactam	blaCTX-M-15	100	100	100	100		100		100	100
Beta-lactam	blaTEM-1B	100	100	100	100		100	100	100	
Beta-lactam	blaOXA-1				100					100
Beta-lactam	blaOXA-232				100					

Conclusions hors du domaine accrédité

Nos résultats suggèrent la possibilité que le même plasmide a été trouvé dans les souches :

GRL-89 Citrobacter sp. 2.6.11.28
GRL-90 Citrobacter sp. 2.6.11.27
Ec-101 Escherichia coli 2.6.16.13

Les autres souches présentent un profil unique de délétions par rapport à la référence choisie.

Le séquençage à longs reads (Nanopore ou PacBio) pourrait confirmer nos hypothèses.

Hypothèse épidémiologique probablement confirmée

En attente d'une analyse plasmidique plus détaillée pour confirmer ces résultats

2ème investigation

Hypothèses :

- Réservoir plasmidique environnemental
- Non détection d'un faible inoculum de *Citrobacter* OXA-181 (transfert intra-host)

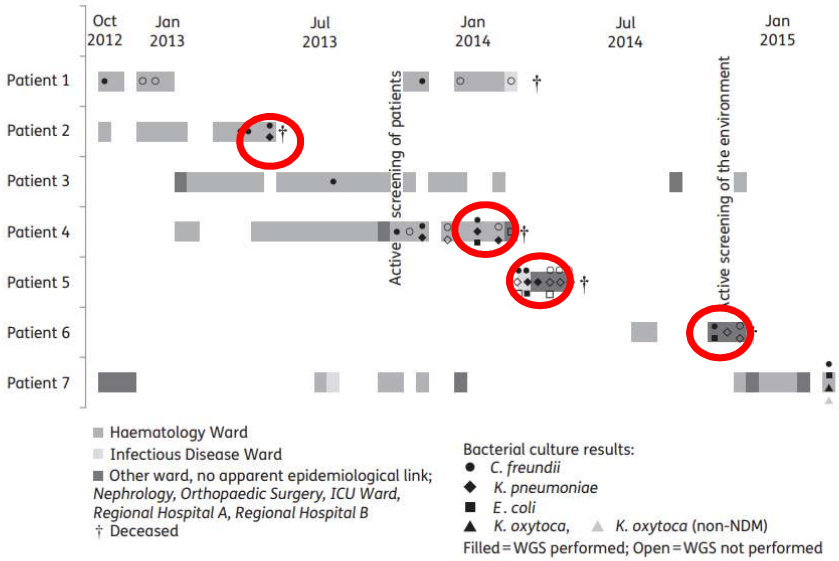


Figure 1. Timeline representing the NDM-1 *C. freundii* outbreak. Ward locations are marked in different shades of grey. Epidemiological links were detected for Patients 1, 2, 3, 4, 6 and 7 in the haematology ward and for Patients 1 and 5 in the infectious disease ward. Screening of all admitted patients and screening of the environment in the haematology ward were performed in October 2013 and November 2014, respectively, without identifying the source of the outbreak.

J Antimicrob Chemother 2016; **71**: 3117–3124
doi:10.1093/jac/dkw289 Advance Access publication 1 August 2016

Journal of Antimicrobial Chemotherapy

Use of WGS data for investigation of a long-term NDM-1-producing *Citrobacter freundii* outbreak and secondary *in vivo* spread of bla_{NDM-1} to *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*

Anette M. Hammerum[1]*, Frank Hansen[1], Hans Linde Nielsen[2], Lotte Jakobsen[1], Marc Stegger[1,3], Paal S. Andersen[1,3,4], Paw Jensen[5], Tue Kjærgaard Nielsen[6], Lars Hestbjerg Hansen[6], Henrik Hasman[1] and David Fuglsang-Damgaard[2]

[1]Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark; [2]Department of Clinical Microbiology, Aalborg University Hospital, Aalborg, Denmark; [3]Pathogen Genomics Division, Translational Genomics Research Institute (TGen), Flagstaff, AZ, USA; [4]Veterinary Disease Biology, University of Copenhagen, Copenhagen, Denmark; [5]Department of Haematology, Aalborg University Hospital, Aalborg, Denmark; [6]Department of Environmental Science, Aarhus University, Roskilde, Denmark

- Denmark: outbreak with *C.freundii* NDM-1
- Investigated by WGS
- Multiple horizontal transfers to *E.coli*, *K.pneumoniae and K. oxytoca*

Prise en charge SPCI

1ère investigation 18.06.2019 – 5AL

- Dépistage de l'unité
- Enquêtes de prévalence hebdomadaire (en plus du dépistage à l'admission en routine)
- Recherche, marquage, et dépistage à l'admission des patients (n=59) ayant croisé le premier cas index présumé
- Isolation et cohortage des patients positifs OXA-181 dans l'unité 5-AL, avec aide soignant dédié
- Nettoyage quotidien d'unité et de la salle de traitement au Tristel Fuse Surfaces (22.06.19-fin Sept.)

2ère investigation 18.06.2019 – 5AL

- Recherche, marquage, et dépistage à l'admission des patients (n=121) ayant croisé le deuxième cas index présumé

Conclusions

- **La flambée de *Citrobacter* sp producteur de carbapénèmase (OXA-181) dans l'unité du 5-AL, département de chirurgie aux HUG est à notre connaissance une première en Suisse.**

- **La documentation d'une transmission horizontale nosocomiale et inter-espèce du plasmide contenant le gène OXA-181 est aussi une première en Suisse.**

- **Bonne compliance des soignants aux mesures de précaution**
- **Mauvaise compliance des patients aux mesures de précaution**

- **Certaines limitations sont soulignées**
  - Limitations dues aux soins (unité d'orthopédie septique)
    - Transmission potentiellement augmentée (écoulements biologiques)
    - Haute densité de consommation antibiotique

  - Limitations environnementales
    - Circulation des patients et leurs familles (suivi ambulatoire en salle de traitement)
    - Absence de local dédié aux patients avec portage BMR
    - Aucun sanitaire dédié à chaque patient (ni salle de bain, ni toilettes)

Propositions SPCI

Au regard des patients à risque

- Une chambre individuelle avec sanitaire dédié pour tout patient
- Des chambres dédiées aux Mesures Spécifiques
- Une salle de traitement dédiée (avec salle d'attente à distance de l'unité)
- Un système de sécurité pour prévenir de la non compliance au respect des mesures mise en place.

Au regard du risque de futures épidémies BMR

- Maintien du dépistage BMR complet par frottis pour tout patient admis
- Maintien de l'enquête de dépistage hebdomadaire au-delà du 30 Sept 2019
- Dépister d'unité lors du départ d'un patient identifié comme porteur de MDRO même si ce patient a été hospitalisé en chambre individuelle
- Activation d'une alerte qui identifie en cas de réadmission les patients à risque d'avoir été contaminés lors d'un séjour hospitalier antérieur par un patient porteur d'une bactérie hautement multi-résistante
- De regrouper les patients en leur dédiant du personnel nécessaire en cas d'épidémie.

Surgical ward, Geneva

NDM cluster 2018-2019

BACKGROUND

From July 2018 to May 2019

3 cases **positive for NDM-producing *E.coli*** and **attributable to our hospital**

Fortuitously detected

- 1 case by routine weekly screening in the ICU
- 1 case by screening b/o additional MDRO carriage
- 1 case by urine culture in LTCF after extended hospital in a private room

No foreign travel in the past 12 months
No known risk factor for acquisition of MDRO carriage

Objective: To report an outbreak investigation guided through molecular methods

Martischang R et al. ICPIC 2019, late breaker (O45)

BACKGROUND

Cas index: Mr. G.

52 years, from United Arab Emirates

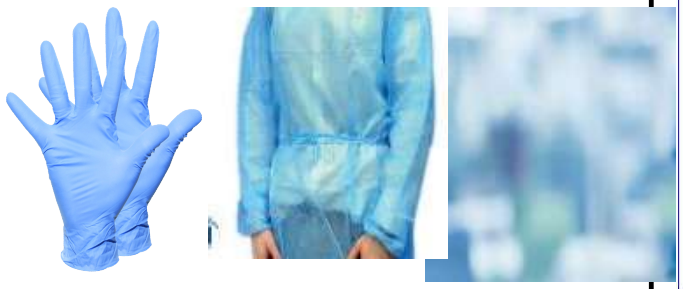
Known for inflammatory bowel disease with multiple surgical interventions

- New York (April 2017)
- Geneva (December 2017)

Transfer to Geneva University Hospitals with subsequent ICU stay (25.12.17) ➔ NDM *E.coli* pos

Rehospitalized at HUG in July 2018

Pre-emptive contact precautions



ROUGE-B M.171225.0128/1					
25/12/17 07:45 Frottis anal	ABS	Klebsiella pneumoniae carbapenemase (KPC), par PCR	ABS	Acinetobacter sp. multi-résistant	T
	PRES	[na] New Delhi metallo-beta-lactamase (NDM), par PCR	(a)	+++ Escherichia coli	(1) T
	ABS	[na] Oxacillinase 48 (OXA-48), par PCR	ABS	Enterococcus spp. vancomycine résistant	T
	ABS	[na] Oxacillinase 181 (OXA-181), par PCR			T
	ABS	[na] SPM-type carbepenemase, par PCR			T
	ABS	[na] Verona integron-encoded metallo-beta-lactamase (VIM), par PCR			T
	ABS	[na] Metallo-beta-lactamase IMP, par PCR			T
	ABS	[na] GES-type carbapenemase, par PCR			T
	ABS	[na] SME-type carbapenemase, par PCR			T

antibiogramme (CMI exprimée en mG/L)	
R	Amoxicilline AMOXIC
R	Co-amoxiclav AM.CLA
R	Piperacilline PIPERA
R	Piperac.+tazob PI+TAZ
R	Cefuroxime CEFURO
R	Ceftazidime CEFTAZ
R	Ceftolozane+tazobactam CETOZA
R	Ceftriaxone CEFTRI
R	Cefepime CEFEPI
I	Imipenem IMIPEN
I	Meropeneme MEROPE
R	Ertapènem ERTAPE
R	Aztreonam AZTREO
R	Amikacine AMIKAC
R	Gentamicine GENTAM
R	Norfloxacine NORFLO
R	Ciprofloxacine CIPROF
R	Co-trimoxazole COTRIM
S	Fosfomycine FOSFOM
S	Tigécycline TIGECY
S	Furanes FURANE
S	Colistine COLIS

Martischang R et al. ICPIC 2019, late breaker (O45)

BACKGROUND

2019					2018							
05	04	03	02	01	12	11	10	09	08	07		
											IMCUs	**Index Patient**
						ICU 1					IMCUs	**Secondary case**
					2	ICU						**Tertiary case**
Other 3												**Tertiary case**

1

2

3

81105.0124/1

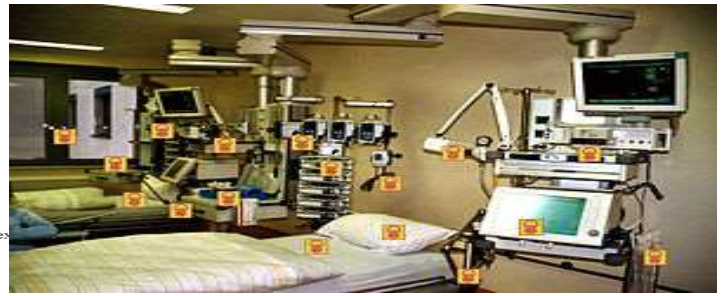
ABS	Klebsiella pneumoniae carbapenemase (KPC), par PCR	+++ Escherichia coli
PRES	(na) New Delhi metallo-beta-lactamase (NDM), par PCR	(a) +++ Klebsiella pneumoniae complexe
ABS	(na) Oxacillinase 181 (OXA-181), par PCR	
ABS	(na) Verona integron-encoded metallo-beta-lactamase (VIM), par PCR	
ABS	(na) Metallo-beta-lactamase IMP, par PCR	
ABS	(na) SPM-type carbapenemase, par PCR	
ABS	(na) GES-type carbapenemase, par PCR	
ABS	(na) SME-type carbapenemase, par PCR	
ABS	(na) GIM-type carbapenemase, par PCR	
ABS	(na) SIM-type carbapenemase, par PCR	

181121.0612/1

ABS	Klebsiella pneumoniae carbapenemase (KPC), par PCR	(c) +++ Escherichia coli
PRES	(na) New Delhi metallo-beta-lactamase (NDM), par PCR	(c) +++ Klebsiella pneumoniae complex
ABS	(na) Oxacillinase 181 (OXA-181), par PCR	
ABS	(na) Verona integron-encoded metallo-beta-lactamase (VIM), par PCR	
ABS	(na) Metallo-beta-lactamase IMP, par PCR	
ABS	(na) SPM-type carbapenemase, par PCR	
ABS	(na) GES-type carbapenemase, par PCR	
ABS	(na) SME-type carbapenemase, par PCR	
ABS	(na) GIM-type carbapenemase, par PCR	
ABS	(na) SIM-type carbapenemase, par PCR	

190503.0092/1

n de jet		
ABS	Klebsiella pneumoniae carbapenemase (KPC), par PCR	1.0E4 Escherichia coli
PRES	(na) New Delhi metallo-beta-lactamase (NDM), par PCR	1.0E2 Enterococcus fa
ABS	(na) Oxacillinase 181 (OXA-181), par PCR	
ABS	(na) Verona integron-encoded metallo-beta-lactamase (VIM), par PCR	
ABS	(na) Metallo-beta-lactamase IMP, par PCR	
ABS	(na) SPM-type carbapenemase, par PCR	
ABS	(na) GES-type carbapenemase, par PCR	
ABS	(na) SME-type carbapenemase, par PCR	
ABS	(na) GIM-type carbapenemase, par PCR	
ABS	(na) SIM-type carbapenemase, par PCR	



Probable transmission:

- 1st : **Intermediate Care Unit (IMCU)**
- 2nd and 3d : **Private room**

BACKGROUND

MET

ts' screening (July & Nov 18, May 19)
18 : Roomates screening (n=5) ➔ negative
y 19 : Entire unit screening ➔ negative

nmental screening and disinfection in the private room (May 19)
ks, bathroom, air conditioner : Negative (n=20)

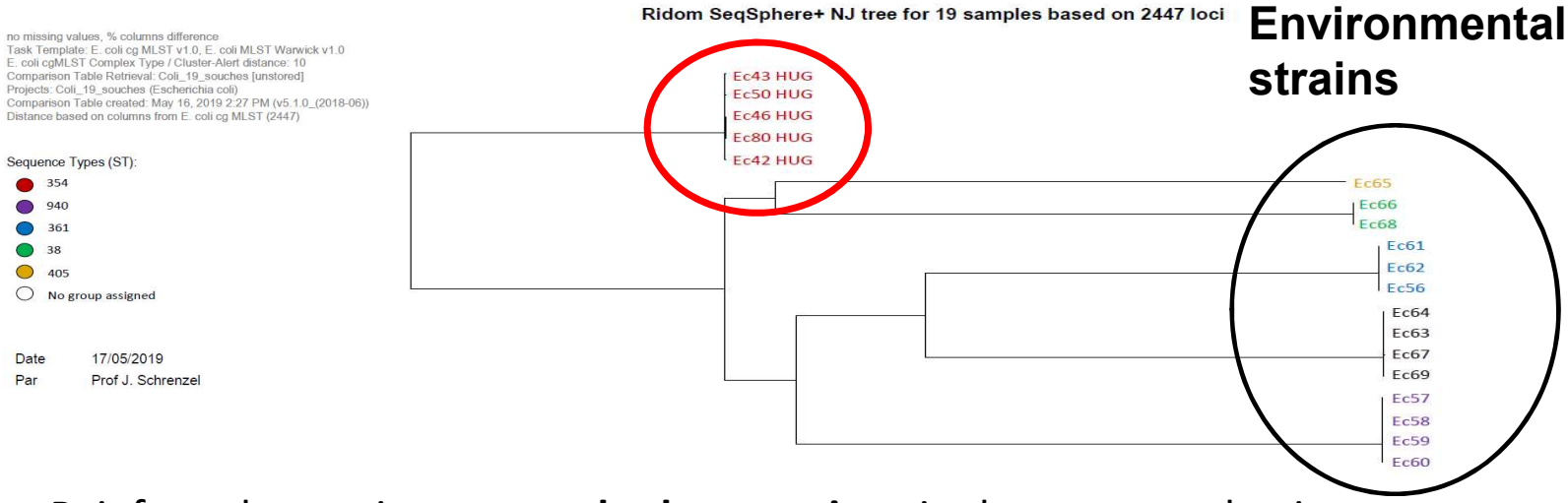
- Illumina iSeq sequencing
- cgMLST and cgSNP analyses

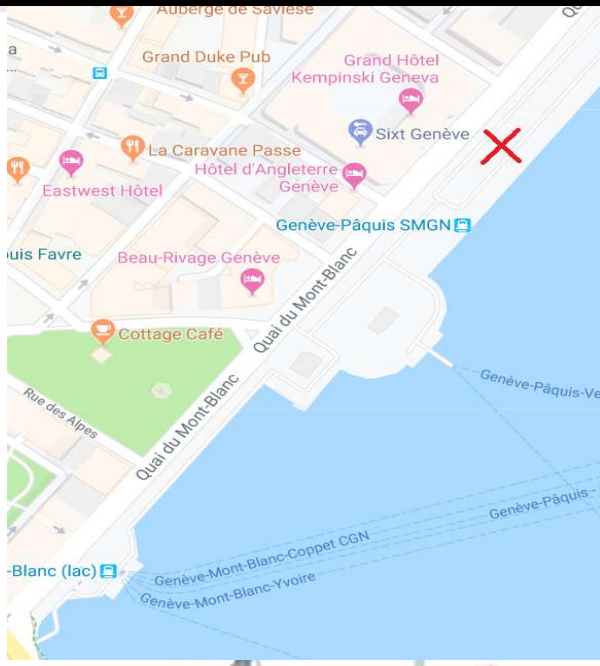
Martischang R et al. ICPIC 2019, late breaker (O45)

METHODS & RESU

E. coli ST354 NDM-1 (<10SNPs)

- Mostly from animals
- Rarely associated with carbapenemases





- Reinforced attention on **standard precautions** in the concerned units.
- Implementation of a **computerized readmission alert system** (May 19) of all patients admitted in the unit from 11.18-05.19 (n=240) with a recommendation to **screen them** at re-admission (all are negative up to now).
- One of the patients **died of surgical complications** unrelated to *E. coli* NDM-1 carriage. There was no clinical infection related to *E.coli* NDM-1.

Courtesy: J Pot

RESULTS

1st healthcare-associated **NDM-producing *E. coli*** outbreak in Switzerland

Prolonged cluster with hidden transmission detected by chance

Nosocomial transmission **despite contact precautions** for the index case

- Probable imperfect implementation of contact precautions
- Private floors are not exempt from MDROs nosocomial acquisition and may be at high risk of importation

Highlights the importance of :

- **Added value of NGS to guide investigation of an outbreak**
- **Environmental hygiene**
- **Admission screening for patients at-risk**
- **Weekly screening in ICU (shared benefits with other units/wards)**

General internal medicine ward, Geneva

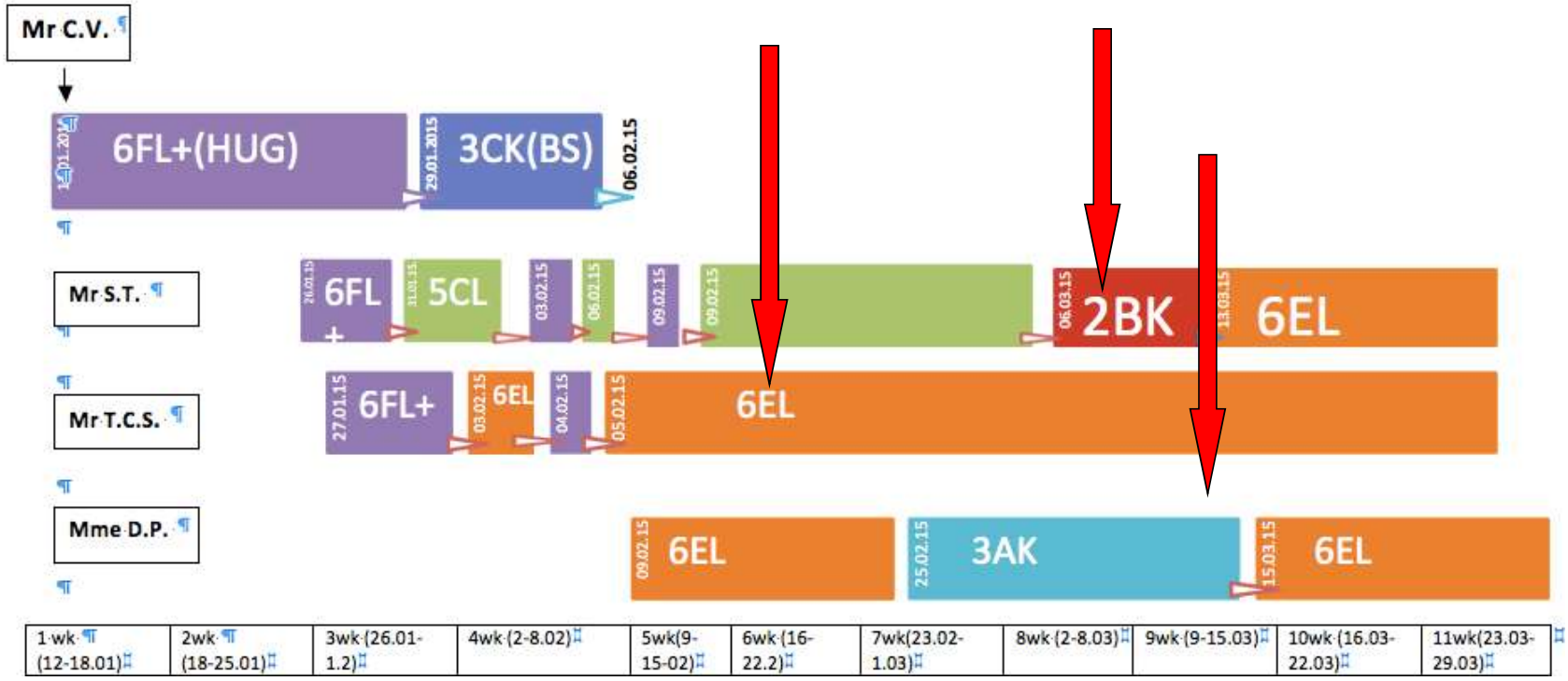
Sporadic, imported CPE
(OXA-48 cluster in 2011)

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)

Courtesy: F. Olearo, D. Pires

Spread of KPC



voir sous MS-Excel	06/03/2015 10:25:00	04/03/2015 11:45:00	04/03/2015 11:45:00	27/02/2015 19:00:00	27/02/2015 19:00:00	20/02/2015 17:00:00	19/02/2015 10:55:00	16/01/2015 18:15:00	16/01/2015 18:10:00	16/01/2015 18:10:00	16/01/2015 18:10:00	12/01/2015 12:15:00	27/12/2014 21:15:00	04/09/2014 12:00:00	21/07/2014 15:45:00	14/04/2 06:50:
	Klebsiella pneumoniae	Klebsiella pneumoniae	Proteus mirabilis	Klebsiella pneumoniae	Proteus mirabilis	Klebsiella pneumoniae	Klebsiella pneumoniae	Bacillus cereus	Pseudomonas aeruginosa	Proteus mirabilis	S. aureus méticilline-sensible (MSSA)	S. aureus méticilline-sensible (MSSA)	Escherichia coli	Escherichia coli	Pseudomonas aeruginosa	Escheri coli
	sgv ponction	frot. plaie prof.	frot. plaie prof.	frot.	frot.	liqu.	liqu.	frot. plaie prof.	frot. plaie prof.	frot. plaie prof.	frot. plaie prof.	tissu mou	urine	urine	frot. escarre	urine
Penicilline G								(32) R			R	R				
Flucloxacilline											S	S				
Amoxicilline	R	R	R	R	R	R	R			R			R	R		R
Co-amoxiclav	R	R	S	R	S	R	R			S	S	S	S	S		S
Piperacilline	R	R	S	R	I	R	R		R	S					S	
Piperac.+tazob	R	R	S	R	S	R	R		S	S			S	S	S	S
Cefalotine																
Cefoxitine	R	R	S	R	S	R	R			S			S	S		S
Cefuroxime	R	R	S	R	S	R	R			S			S	S		S
Cefuroxime axetil (oral)																
Ceftazidime	R	R	S	R	S	R	R		S	S			S	S	S	S
Ceftriaxone	R	R	S	R	S	R	R	(32) I		S			S	S		S
Cefepime	R	R	S	R	S	R	R		S	S			S	S	S	S
Imipenem	(>32) R	R	S	(32) R	(0.500) S	R	(32) R	(32) R	I	S			S	S	S	S
Meropeneme	(>32) R	R	S	(32) R	(0.032) S	R	(>32) R		I	S			S	S	S	S
Ertapènem	(>32) R	R	S	(32) R	(0.160) S	R	(>32) R			S			S	S		S
Aztreonam	R	R	S	R	S	R	R		S	S						
Amikacine	R	R	S	R	S	R	I		S	S			S	S	S	S
Gentamicine	S	S	R	S	R	S	S		S	S	S	S	S	S	S	S
Tobramycine									S						S	
Norfloxacine	R	R	S	R	S	R	R			R			R	R		R
Ciprofloxacine	R	R	S	R	S	R	R		S	R	S	S	R	R	S	R
Levofloxacine									S						S	
Clindamycine								(1) I			S	S				
Erythromycine											S	S				
Acide fusidique											S	S				
Co-trimoxazole	R	R	R	R	R	R	R			S	S	S	R	R		R
Fosfomycine	R	R	S	S	S	R	R			S			S	S		S
Rifampicine											S	S				
Tetracycline											S	S				
Tigécycline	S	S	R	S	R	S	S			R	S	S				
Vancomycine								(3) S			S	S				
Teicoplanine											S	S				
Linézolide											S	S				
Mupirocine											S	I				
Furanes	R	R	R	R	R	R	R			R			S	S		S
Colistine	(12) R	(4) R		(16) R			(0.750) S									
Carbapénèmase-OXA48							A									
Carbapénèmase-KPC							P									

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)

Brochure

- A booklet with information about KPC addressed to the patients at risk and their families

Klebsielle KPC : une bactérie multi-résistante

Ce qu'il faut savoir sur cette entérobactérie : comment la dépister et comment éviter sa transmission ?

HUG

Qu'est-ce qu'une Klebsielle KPC?

Nous sommes tous naturellement porteurs de bactéries sur notre peau, nos muqueuses et dans notre tube digestif. La plupart répondent aux antibiotiques, mais certaines deviennent résistantes. C'est le cas de la bactérie Klebsielle KPC.

Quelle est la particularité d'une Klebsielle KPC?
Cette bactérie fait partie de la flore digestive et appartient à la classe des entérobactéries. Avec l'usage des antibiotiques, elle peut devenir hautement résistante et produire des enzymes (*Klebsiella Pneumoniae* productrice de Carbapénémases = KPC) qui inhibent l'effet de la quasi-totalité des antibiotiques habituels, dits à large spectre.

Comment se transmet cette bactérie ?
La transmission se fait par voie oro-fécale lors de contacts directs ou indirects par l'intermédiaire des mains, d'aliments, d'eau, de surfaces contaminés.

Comment sait-on que l'on est porteur?
Il faut réaliser un dépistage car une personne peut être porteuse sans avoir de symptômes. Cette bactérie peut disparaître spontanément ou rester plusieurs semaines ou mois dans le tube digestif sans provoquer une infection. Parfois une infection digestive ou urinaire survient et son traitement avec des antibiotiques est alors difficile.

Comment se fait un dépistage?
Le dépistage consiste en un frottis rectal ou un prélèvement de selles qui sont analysés dans un laboratoire de bactériologie. Le résultat est disponible dans les 48 à 72 heures.

Quel est l'intérêt de faire un dépistage?
Si le dépistage est positif, votre médecin sait que vous êtes porteur de la Klebsielle KPC. Si vous contractez une infection, il prend contact avec un service spécialisé pour vous prescrire un traitement antibiotique adapté.

Que faire si vous êtes porteur d'une Klebsielle KPC?

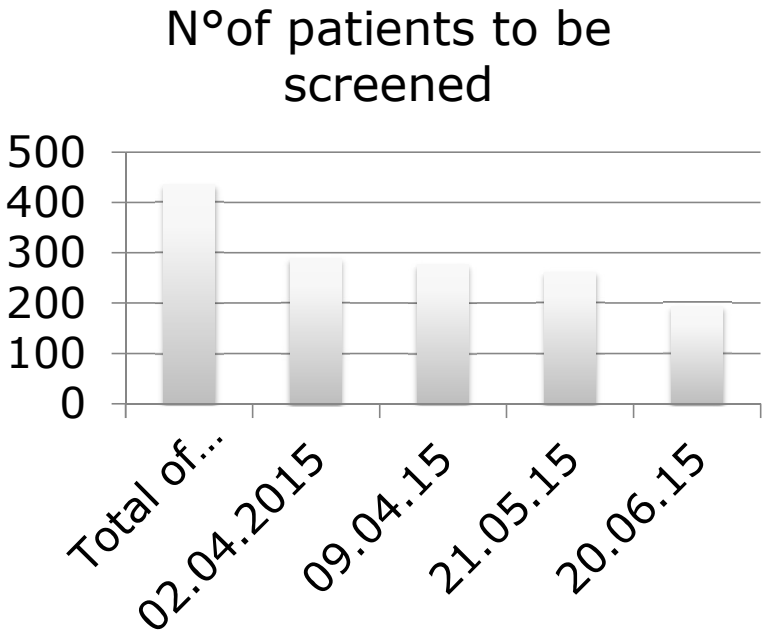
Voici les précautions à prendre chez vous pour éviter de transmettre cette bactérie à vos proches.

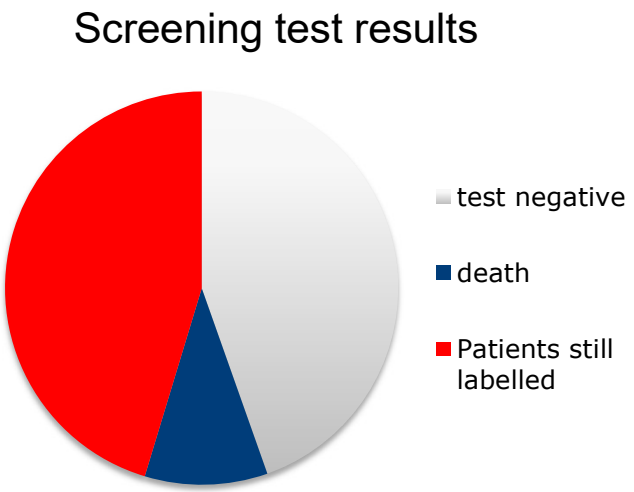
- Utilisez votre savon habituel et lavez-vous les mains chaque fois que cela est nécessaire :
  - après être allé aux toilettes
  - avant de préparer des repas et de passer à table
  - après avoir manipulé une poche à urine, du linge souillé ou un pansement
- Faites votre toilette tous les jours en utilisant votre savon habituel, mais ne partagez pas votre linge de toilette
- Lavez votre linge, à une température de 40°C minimum, en utilisant votre produit à lessive habituel
- Utilisez vos produits de nettoyage habituels pour le ménage
- Nettoyez vos sanitaires et votre salle de bains avec votre détergent habituel et rincez bien
- Entretenez normalement votre vaisselle et vos ustensiles de cuisine
- Eliminez vos pansements et vos protections souillées avec les ordures ménagères en fermant hermétiquement les sacs poubelles; n'oubliez pas de vous laver les mains après.

Y a-t-il des risques pour vos proches?
Si vous suivez les précautions ci-dessus, le risque de transmission à votre famille ou vos proches est extrêmement faible. Si vous avez des personnes fragiles dans votre entourage (nourrisson, femme enceinte, malade chronique...), demandez conseil à votre médecin.

Courtesy: F. Olearo, D. Pires

Results of extensive screening





Not one single additional KPC case detected!

Lessons learned (CPE outbreak)

- Contact precautions failed for previously identified KPC index case
- Late outbreak detection
- Screening program: logistical challenge

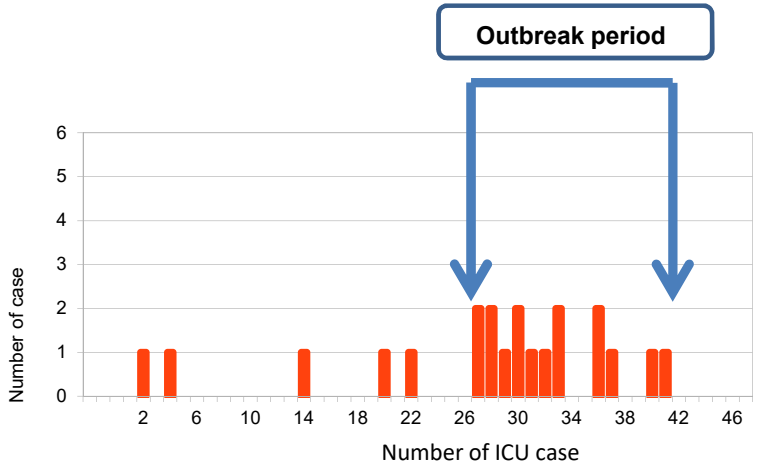
- WGS:
  - In slight contradiction to strong epidemiological evidence
  - Interesting post-hoc exercise

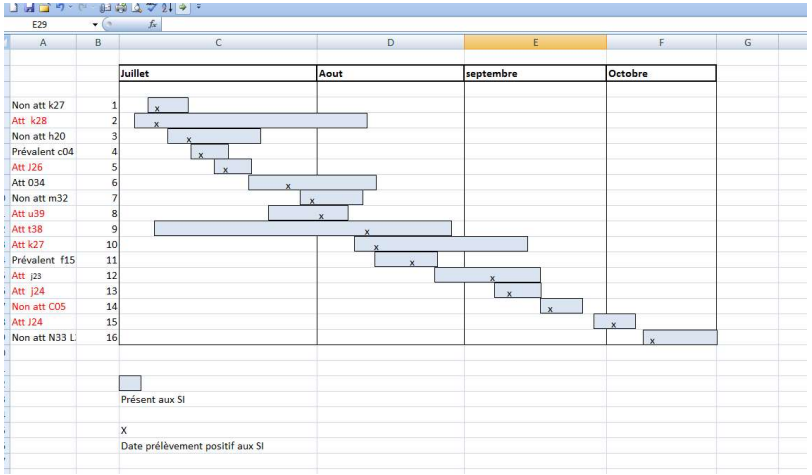
Serratia outbreak

Geneva ICU

Routine Serratia Surveillance => Epidemic curve in the ICU

Epidemiologic outbreak investigation => nosocomial transmission?

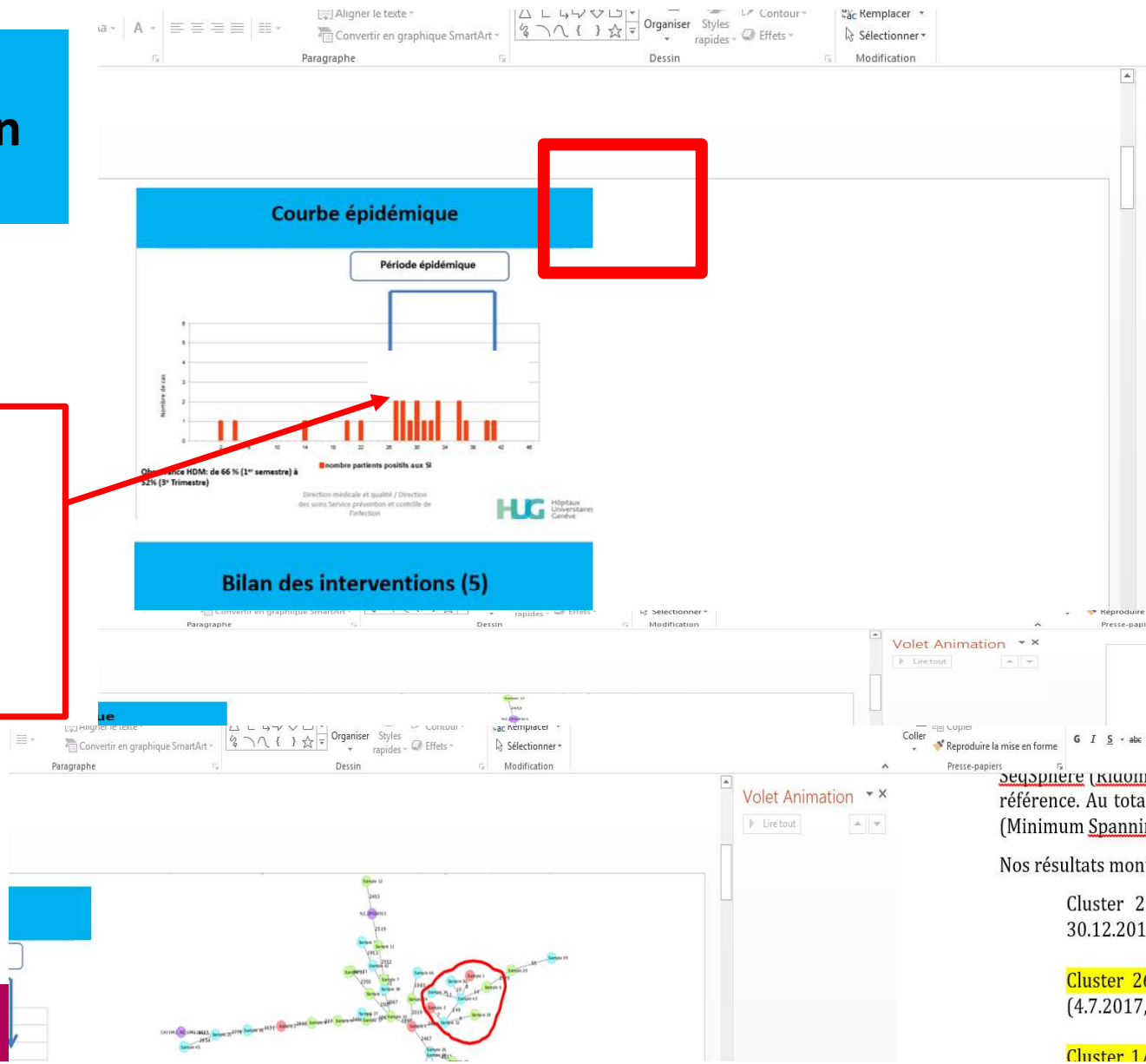




HH compliance: 66 % (1st semestre) to 52% (3d Trimestre)

Genomic investigation

-Selection and sequencing of multiple *S.marcescens* isolates (incl. outbreak strains) stored in the microbiological laboratory



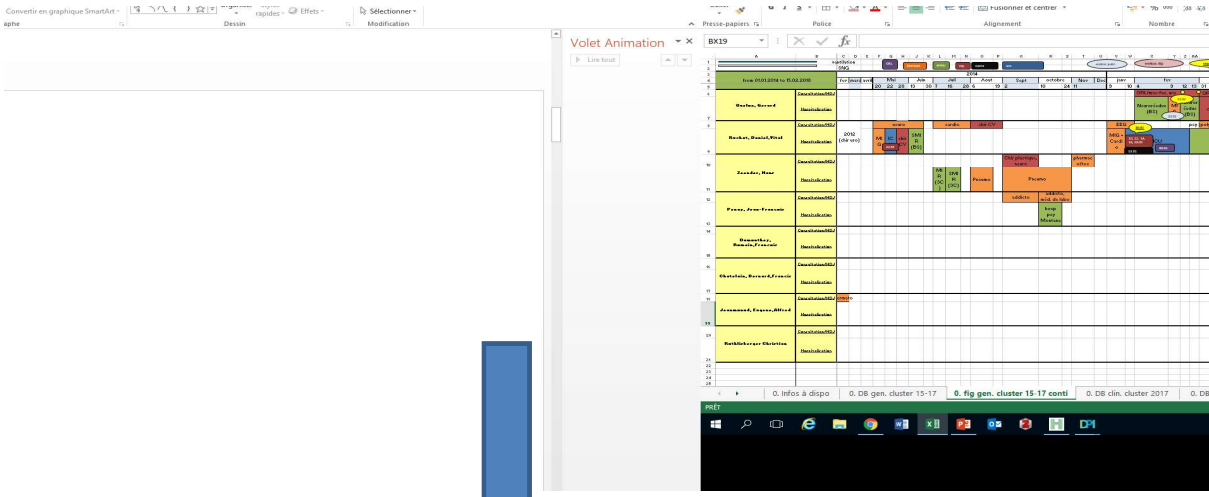
Epidemiologic investigation

-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

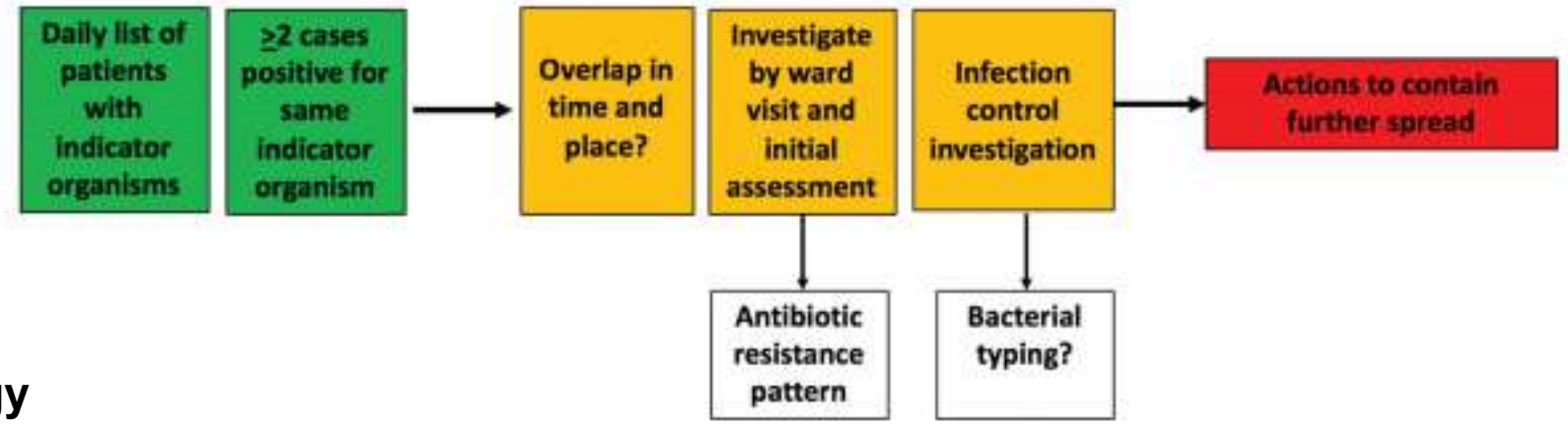
Required improvements to help hospital epidemiologists

WGS can reveal detailed spatial and temporal dynamics of nosocomial transmission events and MDRO evolution, <u>but we need</u>:

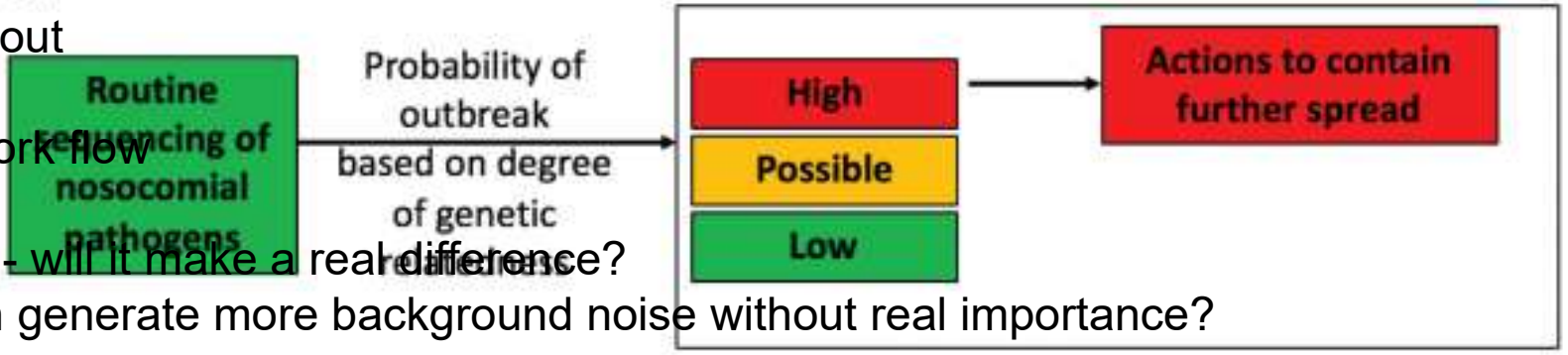
- Quality standards, proficiency testing for routine use
- Standard-operating procedures for sampling, data extraction…
- Thresholds to determine clustering and transmission events
- Shorter TAT
- Reduced costs
- Streamlined data analyses

- **<u>Most importantly</u>: demonstrate impact of WGS on preventive measures and clinical decision making** (compared to less expensive tools)

Challenges of a paradigm shift: still not prime time !



- **Surveillance strategy**
  - Clinical cultures vs active screening cultures
  - All bugs vs some bugs
- **Implementation**
  - In-house vs farm-out
  - Cost
  - Integration into work flow
- **Challenges**
  - Turn around time - will it make a real difference?
  - Will this approach generate more background noise without real importance?



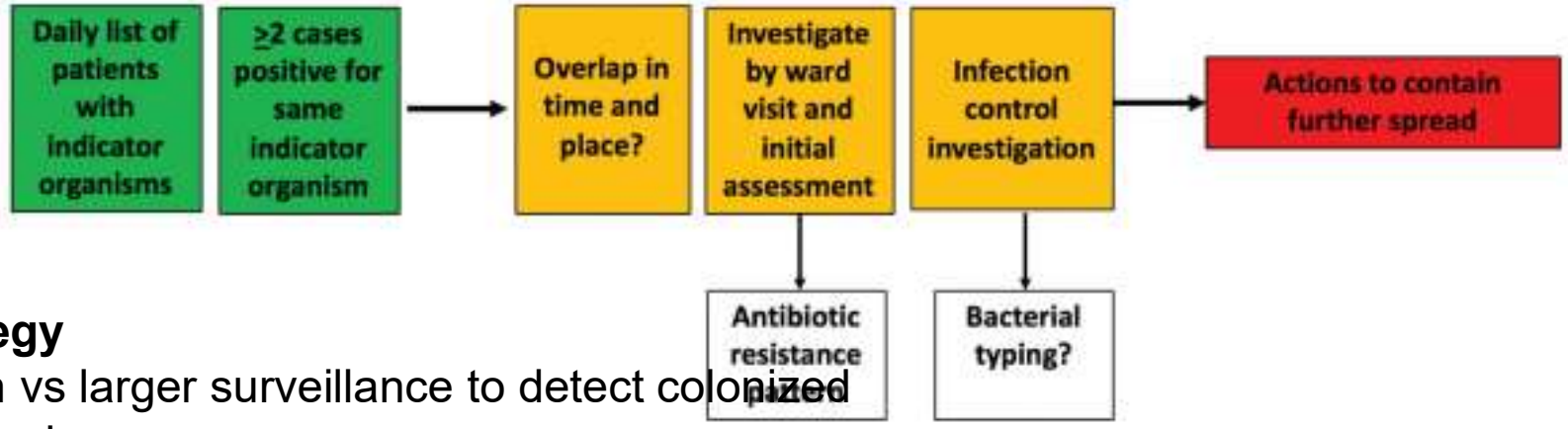
Peacock et al, Microb

Fig. 1. Current and proposed approach to the detection of hospital outbreaks. (a) Current practice for the detection of hospital outbreaks based on surveillance and epidemiology. The pattern of antibiotic resistance is commonly used as a surrogate for bacterial

Merci bcp pour votre attention!

Reserve slides

Challenges of a paradigm shift



- **Surveillance strategy**
  - Clinically-driven vs larger surveillance to detect colonized
  - All bugs vs some bugs
  - Hospital-regional landscape
- **Implementation**
  - In-house vs farm-out
  - Cost
  - Integrating into work flow
- **Challenges**
  - Turn around time - will it make a real difference?
  - Will this approach generate more work? More noise?

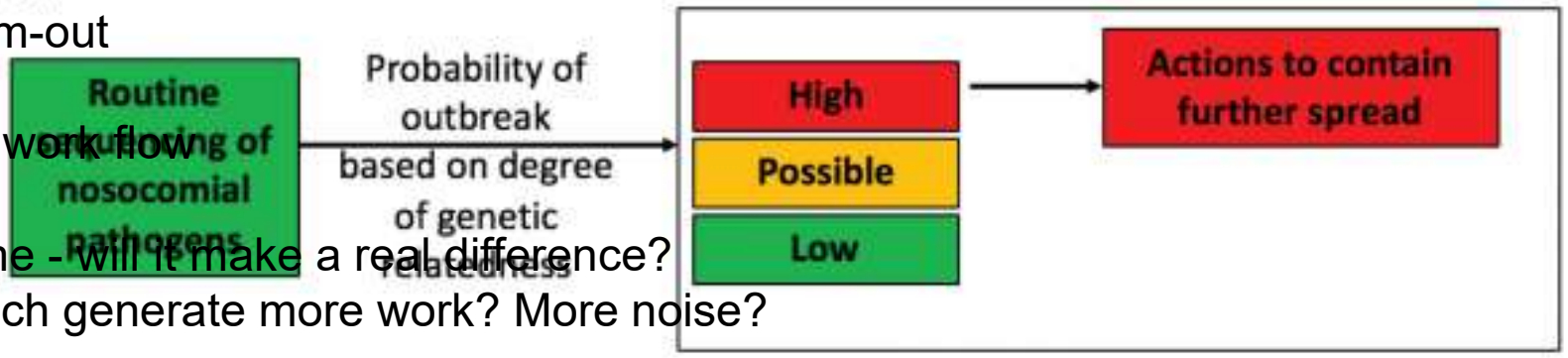


Fig. 1. Current and proposed approach to the detection of hospital outbreaks. (a) Current practice for the detection of hospital outbreaks based on surveillance and epidemiology. The pattern of antibiotic resistance is commonly used as a surrogate for bacterial

Peacock et al, Microb

Do we have an outbreak?

Epidemiological question & hypothesis
Adequate screening strategy
Correct sampling & culturing practices

Choice of typing method & platform
Be aware of multiple biases during the process
Quality control issues

Analytical approach & interpretation

- Allelic profile (ST)
- SNPs profile
- Resistance genes
- Phylogenetic trees

⇩

Threshold interpretation

⇄

Epidemiologic investigation

Future improvements to help clinicians & hospital epidemiologists

- Standardization of:
  - Quality standards, (in-silico) proficiency testing
  - Standard-operating procedures for sampling, data extraction…
  - Global consensus on a set of cluster complexes specific genomes used as to call SNPs within ST- or CC groups.
  - Nomenclature (for databases and reference genomes)

- Research agenda:
  - Thresholds to determine clusters complexes according to different pathogens
  - Comparison hqSNP, cg-MLST and wg-MLST
  - Whole genome sequencing approaches vs robust epidemiologic data
  - Automatic curation of databases (for cgMLST)

- Agreements on data sharing practices (larger outbreaks)

Pre-analytic	Analytic (sequençage)	Analytic (analyse)	Post-analytic

cgMLST	wgMLST	hqSNP

wgMLST

hqSNP

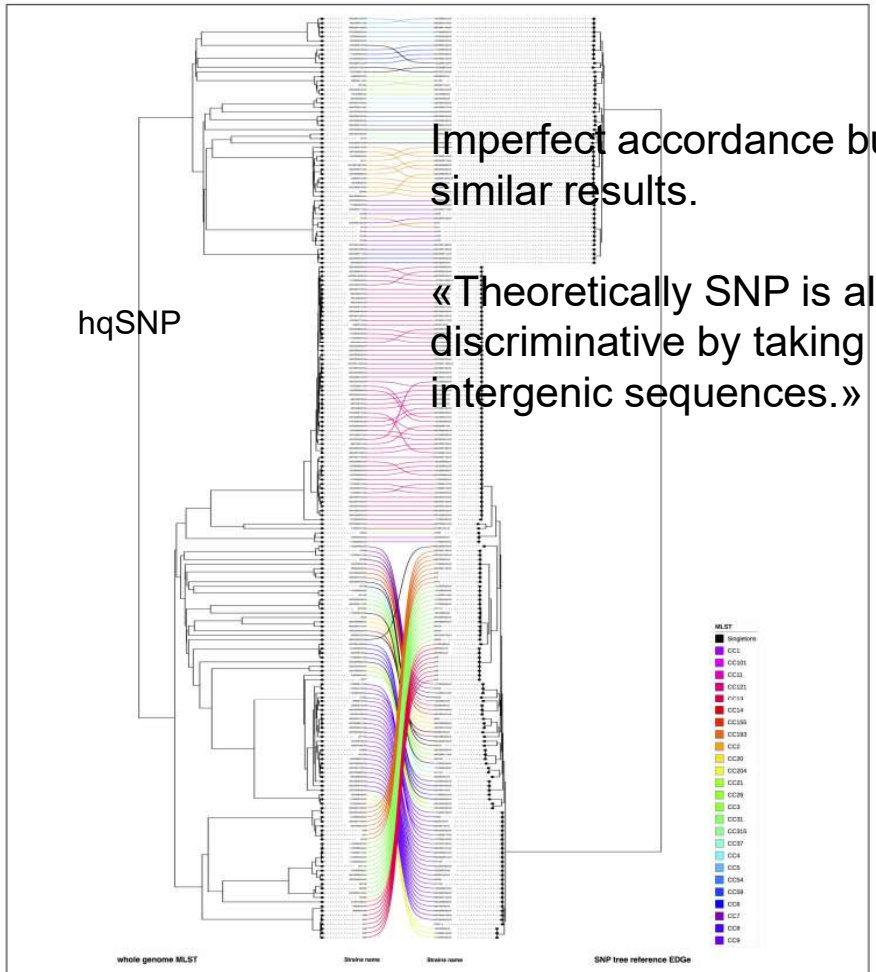


FIGURE 4 | Visual comparison of genome SNP and wgMLST. We compared genome SNP and wgMLST on the study panel using R software (on the left cgMLST and on right wgMLST). Using this face-to-face comparison, we linked corresponding strains. The connection between strains was colored according to the CC of the strains (refer to the color code). Nodes were rotated to optimize matching between corresponding strains in both trees as closely as possible. Similar clusters are connected by straight lines, while curved lines connect strains from distinct clusters.

Imperfect accordance but overall similar results.

«Theoretically SNP is also more discriminative by taking into account intergenic sequences.»

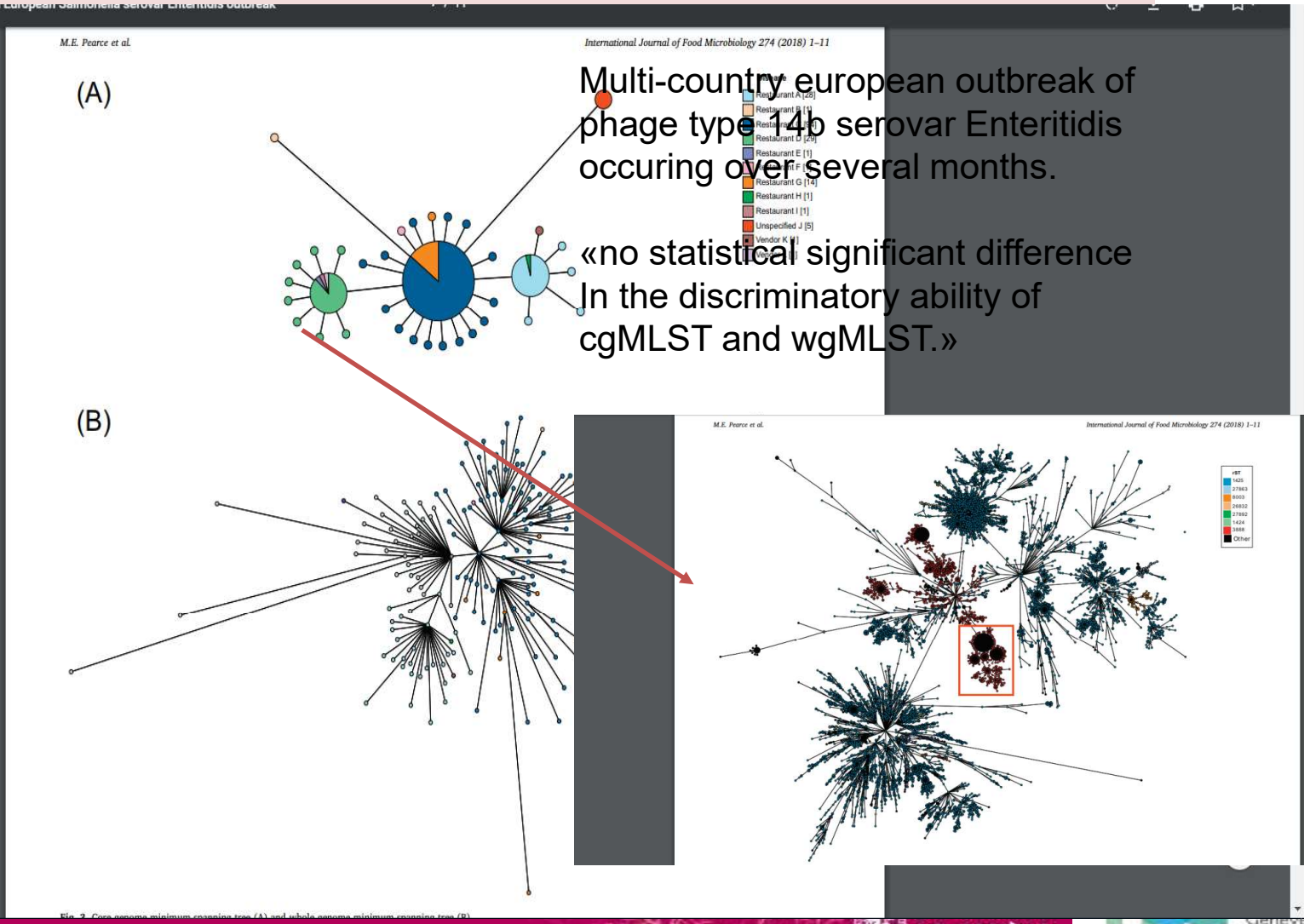
«An assesssment of different genomic approaches f

Pre-analytic	Analytic (sequençage)	**Analytic (analyse)**	Post-analytic

cgMLST	wgMLST	hqSNP

cgMLST

wgMLST



Multi-country european outbreak of phage type 14b serovar Enteritidis occuring over several months.

«no statistical significant difference In the discriminatory ability of cgMLST and wgMLST.»