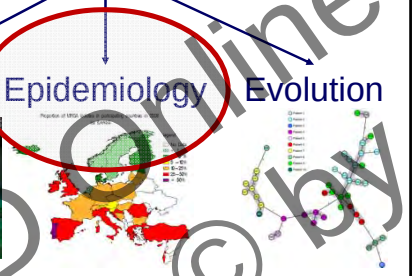
Typing in Microbiology

Alexander Mellmann
Institute for Hygiene, University Hospital Münster, Germany

Applications for typing - the “genetic fingerprint”

Forensics Epidemiology Evolution



Typing questions & suitable methods

Questions	Suitable methods	Required discriminatory power	Time span
Outbreak investigations Short-term/local surveillance Control of hygiene measures	PFGE, RFLP, AFLP, RA-PCR, VNTR, SLST, micro-array, WGS	high	weeks - month

Guidelines for the validation and application of typing methods for use in bacterial epidemiology

A. van Belkum[1], P. T. Tassios[2], L. Dijkshoorn[3], S. Haeggman[4], B. Cookson[5], N. K. Fry[6], V. Fussing[7], J. Green[8], E. Feil[9], P. Gerner-Smidt[10], S. Brisse[11] and M. Struelens[12] for the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group on Epidemiological Markers (ESGEM)

[1]Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands, [2]National and Kapodistrian University of Athens, Department of Microbiology, Athens, Greece, [3]Leiden University Medical Center, Department of Infectious Diseases, Leiden, The Netherlands, [4]Swedish Institute for Infectious Disease Control, Department of Bacteriology, Solna, Sweden, [5]Laboratory of Health Care Associated Infections, [6]Respiratory and Systemic Infection Laboratory, Health Protection Agency, Centre for Infections, London, UK, [7]Novo Nordisk, QC Microbiology SDK, Novo Alle, Bagsvaerd, Denmark, [8]Statistics, Modelling and Bioinformatics Department, Health Protection Agency, Centre for Infections,London, [9]University of Bath, Department of Biology, Bath, UK, [10]Centers for Disease Control and Prevention, Foodborne and Diarrheal Diseases Branch, Divison of Bacterial and Mycotic Diseases, Atlanta, GA, USA, [11]Institut Pasteur, Unit BBPE28, Paris, France and [12]Université Libre de Bruxelles, Hôpital Erasme, Bacteriologie,Brussels, Belgium

"…It must be emphasised that typing results can never stand alone and need to be interpreted in the context of all available epidemiological, clinical and demographical data relating to the infectious disease under investigation…."

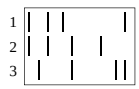
van Belkum et al., Clin Microbiol Infect 2007

Methods

Phenotyping
- Morphology
- Resistance patterns
- Serotyping
- Phage typing
- Lipopolysaccharide and fatty acid profiles
- Protein profiles
- MLEE

Genotyping
- Hybridization-based
  - Single probe → array
- Fragment-based
  - PFGE
  - RA-PCR
  - VNTR/MLVA
- Sequence-based
  - SLST, MLST
  - WGS

Clustering of band-based typing data



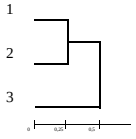
Normalized gel picture

Band presence in pattern 2	Band presence in pattern 1: +	Band presence in pattern 1: -
+	a	b
-	c	d

$s_J = \frac{a}{a+b+c}$ Jaccard coefficient

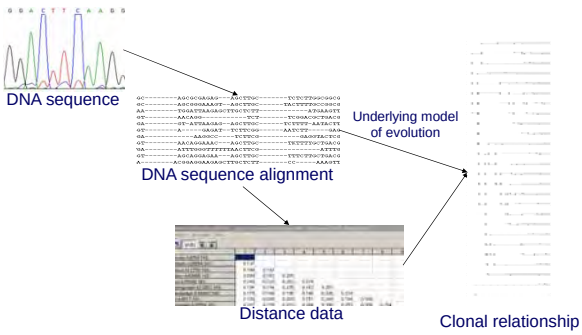
$s_D = \frac{2a}{2a+b+c}$ Dice coefficient

Calculation of similarity



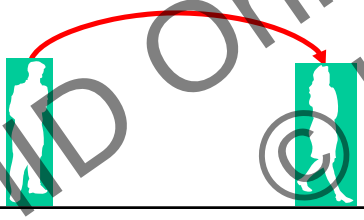
Clonal relationship

Clustering of sequence-based typing data

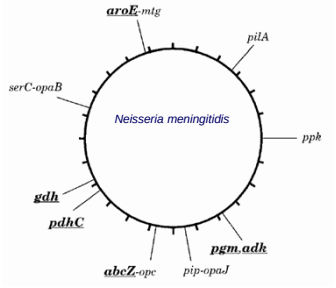


Issues in interpretation

- What constitutes a “significant” difference?
- Could a “single genetic event” occur during transfer of an organism from patient-to-patient?



Multilocus sequence typing (MLST)



Maiden et al., PNAS 1998

Today: > 80 MLST schemes for different organisms published and hosted at pubmlst.org, www.mlst.net, http://bigsdb.web.pasteur.fr and other databases

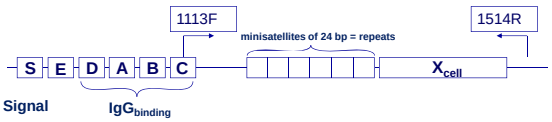
Multilocus sequence typing (MLST)

- long term epidemiology, replaced MLEE
- sequences of 5-7 hk-genes distributed over the whole genome (selectively neutral, slowly evolving, recombination independent loci)
- identical gene sequences = same allele number
- combination of alleles at each locus = sequence type (ST)
- related STs are grouped into clonal complexes
- single expanding central web-based MLST database; can be interrogated electronically but is curated manually

Suitable questions for MLST

- Where does my outbreak clone "fit" into the whole population?
  - availability of the MLST datasets on the internet
  - ease of comparability of sequence data
- What kind of population structure has a certain species?
  - clonal vs. panmictic population structure
  - evolution of a certain species

S. aureus protein A gene (*spa*)

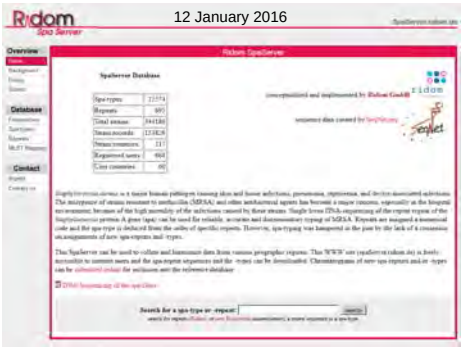


Frénay et al., Eur J Clin Microbiol Infect Dis 1996
Shopsin et al., J Clin Microbiol 1999

Repeat succession = *spa* type
VNTR = variable number of tandem repeats (multiple VNTR = MLVA)

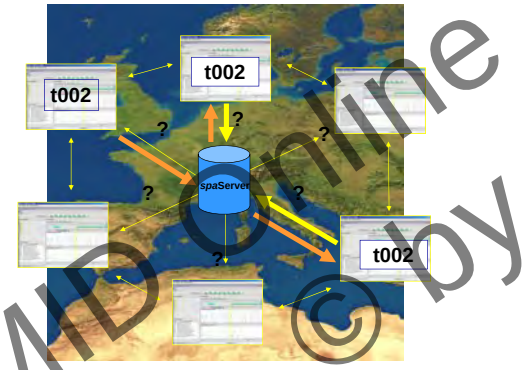
- singlelocus sequence typing (SLST)
- fast (< 2 d) and cheap in comparison to MLST or PFGE
- *spa* is stable and highly discriminatory (outbreak detection)

*spa*Server ensures a uniform and unambiguous typing nomenclature

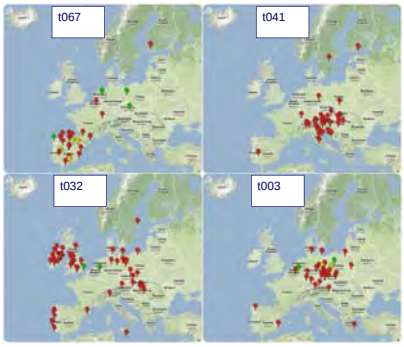


http://spaServer.ridom.de

spaServer: nomenclature and typing database

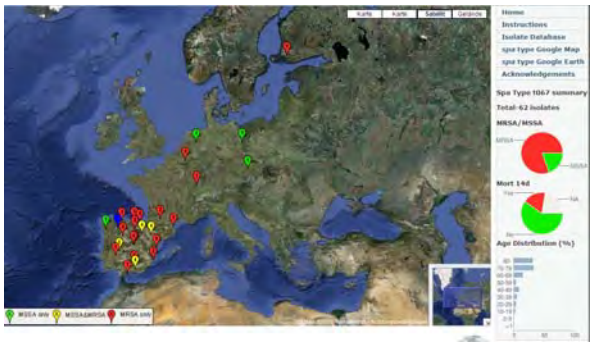


Spread of different *spa* types in Europe



Grundmann et al., PLoS Med 2010

Spread of *spa* t067

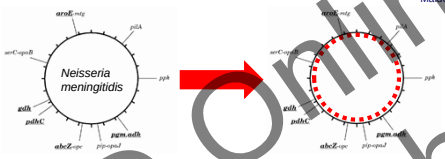


Grundmann et al., PLoS Med 2010

WGS enables expansion of allelic typing: MLST → core genome MLST (cgMLST)

- MLST: 5-7 housekeeping genes → mainly for population genetics and evolutionary studies

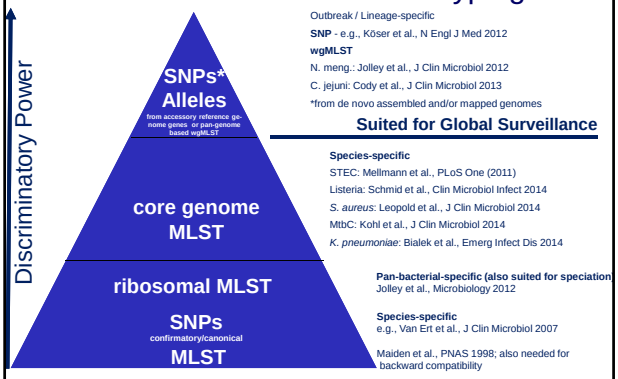
Maiden et al., PNAS 1998



- cgMLST: genome-wide gene-by-gene typing → high discrimination for cluster investigations and in-depth evolutionary studies

Maiden et al., Nature Rev Microbiol 2013
Mellmann et al., Plos ONE 2011

Standardized hierarchical typing



Outbreak / Lineage-specific
SNP - e.g., Köser et al., N Engl J Med 2012
wgMLST
N. meng.: Jolley et al., J Clin Microbiol 2012
C. jejuni: Cody et al., J Clin Microbiol 2013
*from de novo assembled and/or mapped genomes

Suited for Global Surveillance

Species-specific
STEC: Mellmann et al., PLoS One (2011)
Listeria: Schmid et al., Clin Microbiol Infect 2014
S. aureus: Leopold et al., J Clin Microbiol 2014
MtbC: Kohl et al., J Clin Microbiol 2014
K. pneumoniae: Bialek et al., Emerg Infect Dis 2014

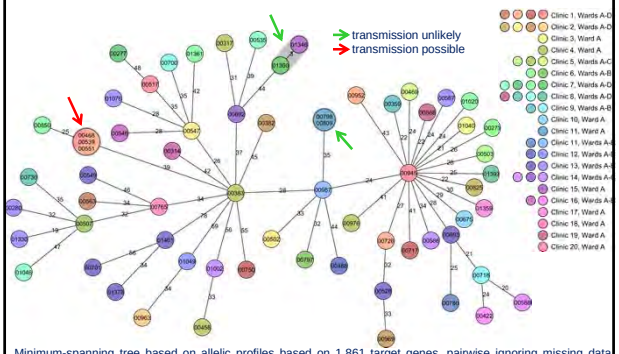
Pan-bacterial-specific (also suited for speciation)
Jolley et al., Microbiology 2012

Species-specific
e.g., Van Ert et al., J Clin Microbiol 2007

Maiden et al., PNAS 1998; also needed for backward compatibility

For hierarchical microbial typing see also: Maiden et al. Nat Rev Microbiol 2013

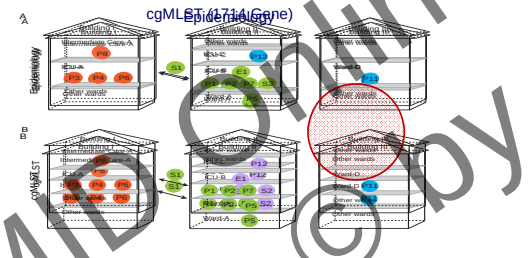
Diversity of LA-MRSA of spa Type t011 (n=66, isolated Oct 13 – Apr 14, Muenster)



Minimum-spanning tree based on allelic profiles based on 1,861 target genes, pairwise ignoring missing data (mean missing targets: 35); cluster threshold ≤ 6 differing alleles

NGS provides novel insights into infection control and prevention

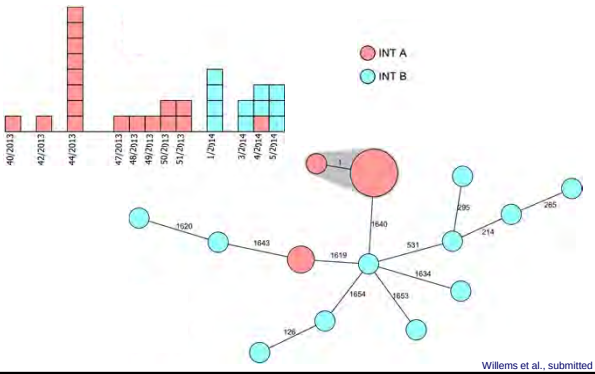
- Cluster of MRSA (all spa t001, Aug-Sept 2003)



P = Patient
S = Staff
E = Environment

Leopold et al., J Clin Microbiol 2014

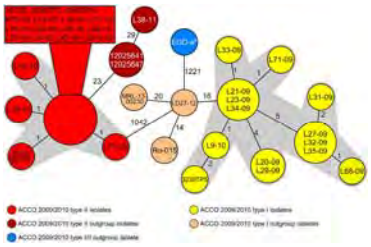
Prospective NGS enables focused infection control - example "*A. baumannii*"



Willems et al., submitted

NGS-based typing for foodborne infections, standardized nomenclature

- cgMLST nomenclature for *L. monocytogenes*



Minimum-spanning tree illustrating the phylogenetic relationship based on the cgMLST allelic profiles of 33 *L. monocytogenes* isolates from a two-clone outbreak associated with acid curd cheese (ACCO). Outgroup isolates per outbreak (with identical PFGE profiles and serotypes) are shown in comparison to the reference strain EGD-e. Closely related genotypes (≤10 allele difference) are shaded in gray.

Ruppitsch et al., J Clin Microbiol 2015

Conclusions

- Depending on the question, typing enhances epidemiological investigations to unravel the spread of pathogens
- Sequence-based methods are today state-of-the-art
- Challenge to define a significant difference between closely related strains
- Standardization is still a great issue - cgMLST approach enables standardized nomenclature to facilitate inter-laboratory exchange of data

Acknowledgements

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  - S. Leopold (now Jackson Laboratory, USA), T. Böking, S. Willems, S. Bletz and the “Sequencing” Lab
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