Workshop
Typing, resistance and virulence

Alexander Mellmann
Institute for Hygiene, University Hospital Münster, Germany

What is typing in microbiology and infectious diseases?

- Ability to discriminate among related bacterial isolates for epidemiological surveillance
- Various technologies used during the last decades to answer different questions – short/long-term settings etc.
- Until the use of WGS data, no method fits to all questions
- Typing should be only in context with epidemiological data

Criteria for evaluation of typing systems

- Typeability
- Reproducibility
- Stability
- Discriminatory power
- Epidemiologic concordance and typing system concordance

Struelens et al., Clin Microbiol Infect 1996
van Belkum et al., Clin Microbiol Infect 2007

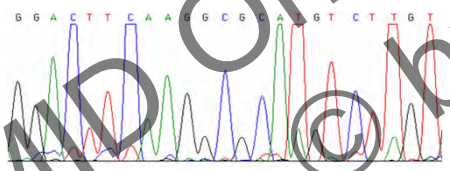
Discriminatory power

- Resolving power of a single method
- Calculation of the discriminatory index (DI)
- DI is the average probability that the typing system will assign different types to two unrelated strains randomly sampled in the population
- DI is dependent on the number of types and on the homogeneity of frequency distribution of strains into types

Hunter et al., J Clin Microbiol 1988
Struelens et al., Clin Microbiol Infect 1996
van Belkum et al., Clin Microbiol Infect 2007

Example

- 36 *Campylobacter* isolates of three documented outbreaks and 30 consecutive strains from 12 sporadic cases of campylobacteriosis were analyzed by PFGE, MLST, *flaA*, and *flaB* SVR sequencing.



Example DI

- Resolving power of PFGE, MLST, *flaA*, and *flaB* typing schemes (DI)

Method	No. of types	No. of most frequent type	DI
PFGE	18	8	0.944
MLST	14	12	0.886
flaA	13	6	0.920
flaB	12	8	0.902

(*C. jejuni*, n = 42)

Mellmann et al., J Clin Microbiol 2004

Concordance analysis

- Quantification of the epidemiologic concordance: Probability that epidemiologically related strains derived from presumable single-clone outbreaks are determined to be similar enough to be classified into the same clones
- Quantification of the typing system concordance: Percentage of cross-classification concordance between the “gold standard” method and the evaluated method

Robinson et al., J Mol Evol 1998
Struelens et al., Clin Microbiol Infect 1996

Cross-classification concordance - PFGE vs. MLST / *flaA* / *flaB* types

Method	No. of types	No. of most frequent type	DI	Concordance with PFGE (%)
PFGE	18	8	0.944	-----
MLST	14	12	0.886	93.96
flaA	13	6	0.920	94.77
flaB	12	8	0.902	**95.82**

(*C. jejuni*, n = 42; all possible pairs, n = 861)

Mellmann et al., J Clin Microbiol 2004

Calculation of Concordance by pair-wise comparisons

Strain	PFGE pattern	MLST ST
1	A	1
2	A	1
3	B	1
4	B	2
5	C	3

Calculation of Concordance by pair-wise comparisons

Isolat	PFGE pattern	MLST ST
1	A	1
2	A	1
3	B	1
4	B	2
5	A	3

PFGE versus MLST		MLST	
		match	mismatch
PFGE	same	a	b
	different	c	d

Isolat	1	2	3	4	5
1	--				
2	a	--			
3	c	c	--		
4	d	d	b	--	
5	b	b	d	d	--

Calculation of Concordance by pair-wise comparisons

Isolat	1	2	3	4	5
1	--				
2	a	--			
3	c	c	--		
4	d	d	b	--	
5	b	b	d	d	--

PFGE versus MLST		MLST	
		match	mismatch
PFGE	same	a	b
	different	c	d

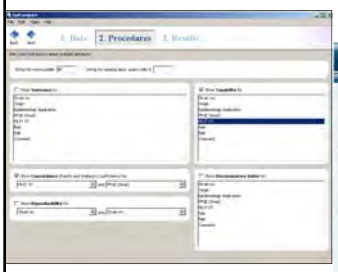
PFGE versus MLST		MLST	
		match	mismatch
PFGE	same	1	3
	different	2	4

Concordance (%) = (a+d) / (a+b+c+d) * 100

= 50%

Robinson et al., J Mol Evol 1998
Adjusted Rand: Carrico et al., J Clin Microbiol 2006

Free tools for calculations



http://www.ridom.de/

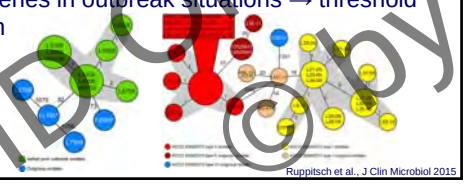
http://darwin.phyloviz.net/ComparingPartitions/index.php?link=Home

How to define thresholds for similarity? Is 99.XX % similarity still “the same”?

- Typing scheme should cover the diversity within a species → set of genes useful for typing
- Necessity of well-characterized strains
  - outbreaks (high-quality epi-data) + outgroups
  - sporadic cases
  - → threshold determination

core genome MLST definition and evaluation (*L. monocytogenes*)

- Selection of genes present in all reference strains, serotypes, MLST groups etc.
- Test of these genes using a second diverse set of strains to evaluate representativeness of genes (≥ 95 % genes present in all isolates)
- Use of genes in outbreak situations → threshold definition



Ruppitsch et al., J Clin Microbiol 2015

Questions from the participants

- I have chosen these workshops because i think it fits best with my interest and with future research-projects at my department. Hoping to get more knowledge about NGS and how to use and interpret results with focus on the detection of antimicrobial resistance.
- e.g.
  - Mykrobe (www.mykrobe.com)
  - Center for Genomics Epidemiology (https://cge.cbs.dtu.dk/services/)