



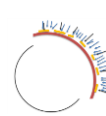
# MRE-Typisierungsmethoden

Dr. med. S. Willems

AG Krankenhaushygiene  
im Bezirksamt Tempelhof-Schöneberg  
von Berlin

08.03.2017

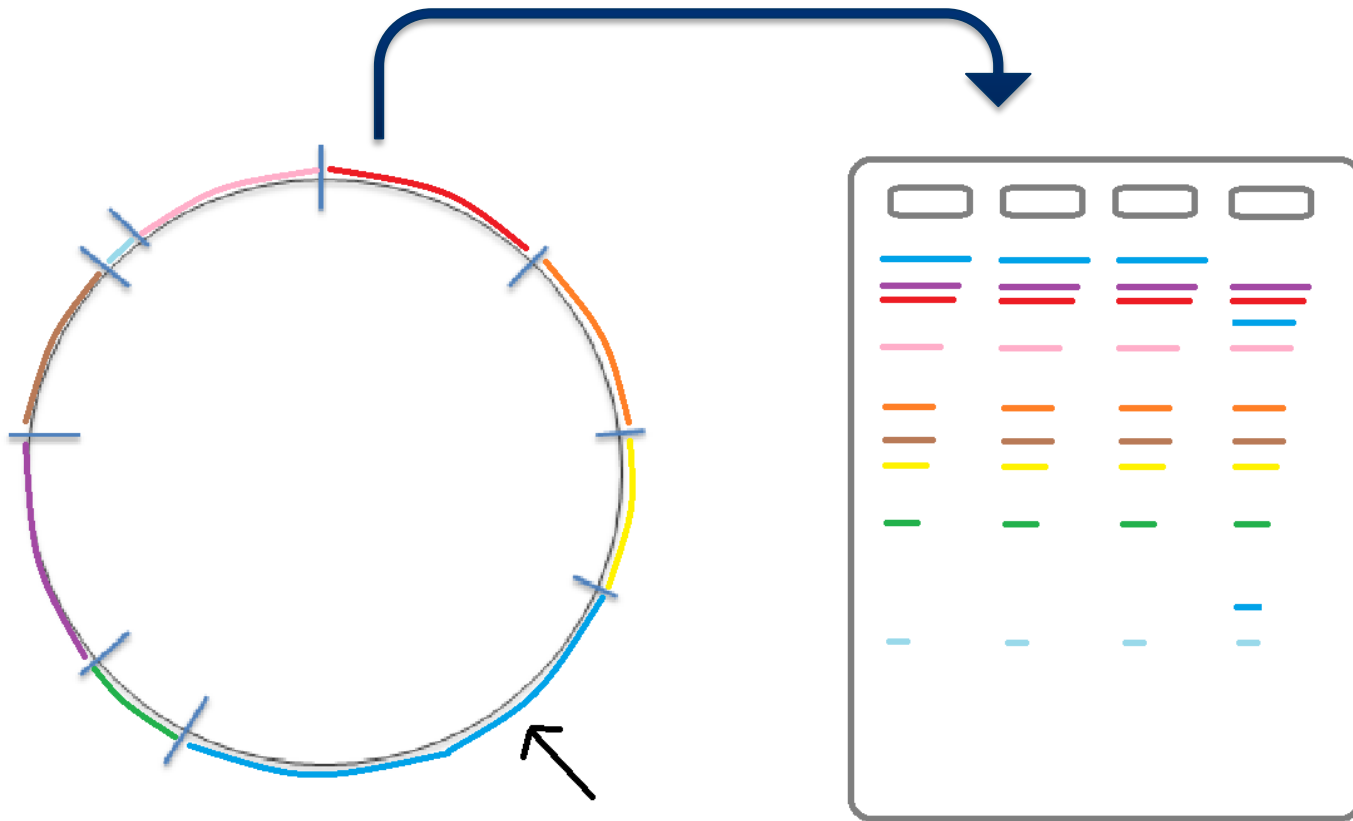


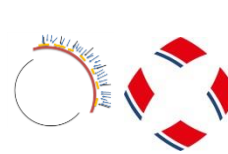


# RESISTOGRAMME DIREKTVERGLEICH

ANTIBIOTIKUM	PATIENT NUMMER										
	1	2	3	4	5	6	7	8	9	10	11
Ampicillin			r	r	r	r	r	r	r		r
Amoxicillin	r	r			r		r	r		r	
Amoxicillin/CA			r	r	r	r	r	r	r		r
Piperacillin	r	r	r	r	r	r	r	r	r	r	r
Piperacillin/Sulbactam	r	r	r	r	r	r	r	s	s	r	r
Piperacillin/Tazobactam	r	r	r	r	r	r	i	r	i		r
Cefazolin											
Cefaclor											
Cefuroxime			r	r	r	r	r		r		r
Cefuroxime - Axetil			r	r	r	r	r		r		r
Cetriaxone					r		r				
Cefotaxime	r	r	r	r	r	r	r	r	r	r	r
Cefpodoxime			r	r	r	r	r	r	r		r
Ceftazidime	r	r	r	r	r	r	r	r	r	r	r
Cefepime			r	r	r	r	r	r	r		r
Aztreonam					r	r	r				
Ertapenem			r	r	r	r	r	r	r		r
Imipenem	s	s	s	s	s	s	s	s	s	s	s
Meropenem	s	s	s	s	s	s	r	s	s	s	s
Gentamicin	r	r	r	r	r	r	r	r	s	r	r
Amikacin					r	i					
Tobramycin					r	r	r				
Ciprofloxacin	r	r	r	r	r	r	r	r	i	r	r
Levofloxacin	r	r	r	r	r	r	r	r	i	r	r
Moxifloxacin			r	r	r	r		r	s		r
Tetracycline	r	r	r	r	r	r	r	r	s	r	r
Tigecycline					i	i		r	s		i
Trimethoprim/Sulfa	r	r	r	r	r	r	r	r	s	r	r
Colistin					s	s	s				
Polymyxin B					s	s	s				
Nitrofurantoin						r					
Fosfomycin						r					
	3 MRGN	3 MRGN	3 MRGN	3 MRGN	3 MRGN	3 MRGN	4 MRGN	3 MRGN	3 MRGN	3 MRGN	3 MRGN

# PULSFELDGELELEKTROPHORESE (PFGE)

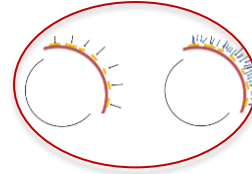




## PULSFELDDGELELEKTROPHORESE (PFGE)

**TABLE 2. Criteria for interpreting PFGE patterns**

Category	No. of genetic differences compared with outbreak strain	Typical no. of fragment differences compared with outbreak pattern	Epidemiologic interpretation
Indistinguishable	0	0	Isolate is part of the outbreak
Closely related	1	2–3	Isolate is probably part of the outbreak
Possibly related	2	4–6	Isolate is possibly part of the outbreak
Different	$\geq 3$	$\geq 7$	Isolate is not part of the outbreak



## SEQUENZBASIERTE METHODEN

### Zahlencode, Vorteil:

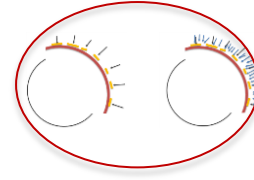
- Schnelle Technik
- Keine subjektive Interpretation, computergestützte Auswertung
- Vergleichbarkeit zwischen verschiedenen Zeitpunkten
- Vergleichbarkeit zwischen verschiedenen Laboren
- Zentrale Datenbanken möglich
- Gut abspeicherbar

**CTGGTTTTGATAAACGTTGTTGGCAAGTTAAGCC  
GAAACCGTTAAAGCTTCTCTTAAGTTAACCAGCC  
TTTTCCCGGAAAATTAGATGTAGAAGTGATCTAC  
ATGAATTCAAGATTGAATATAAAGCCAAAACCTGA  
AACCTTACCAACCACAGTTATTTCAACTTATCAG  
TTATGGCGTGCTAGATCATGTGGTACAACCTCAAT  
TAACCGATCAAAAACCTTTACCAACAGGTGAAATTGCTTCAGTTGC**

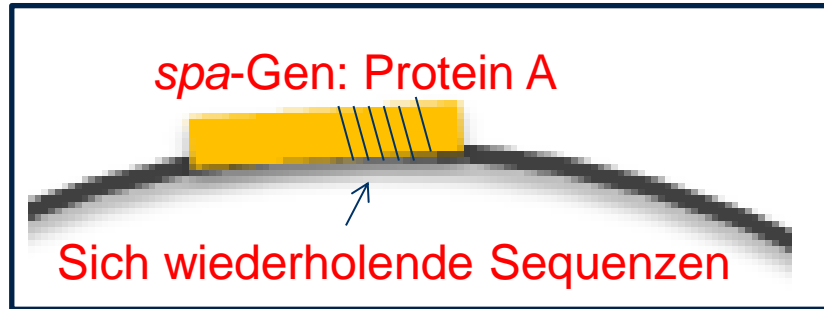


— = Adenin  
— = Thymin  
— = Cytosin  
— = Guanin  
— = Phosphat-desoxyribose Strang

DNA

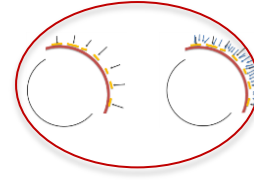


## SEQUENZBASIERTE METHODEN: SPA-TYPISIERUNG



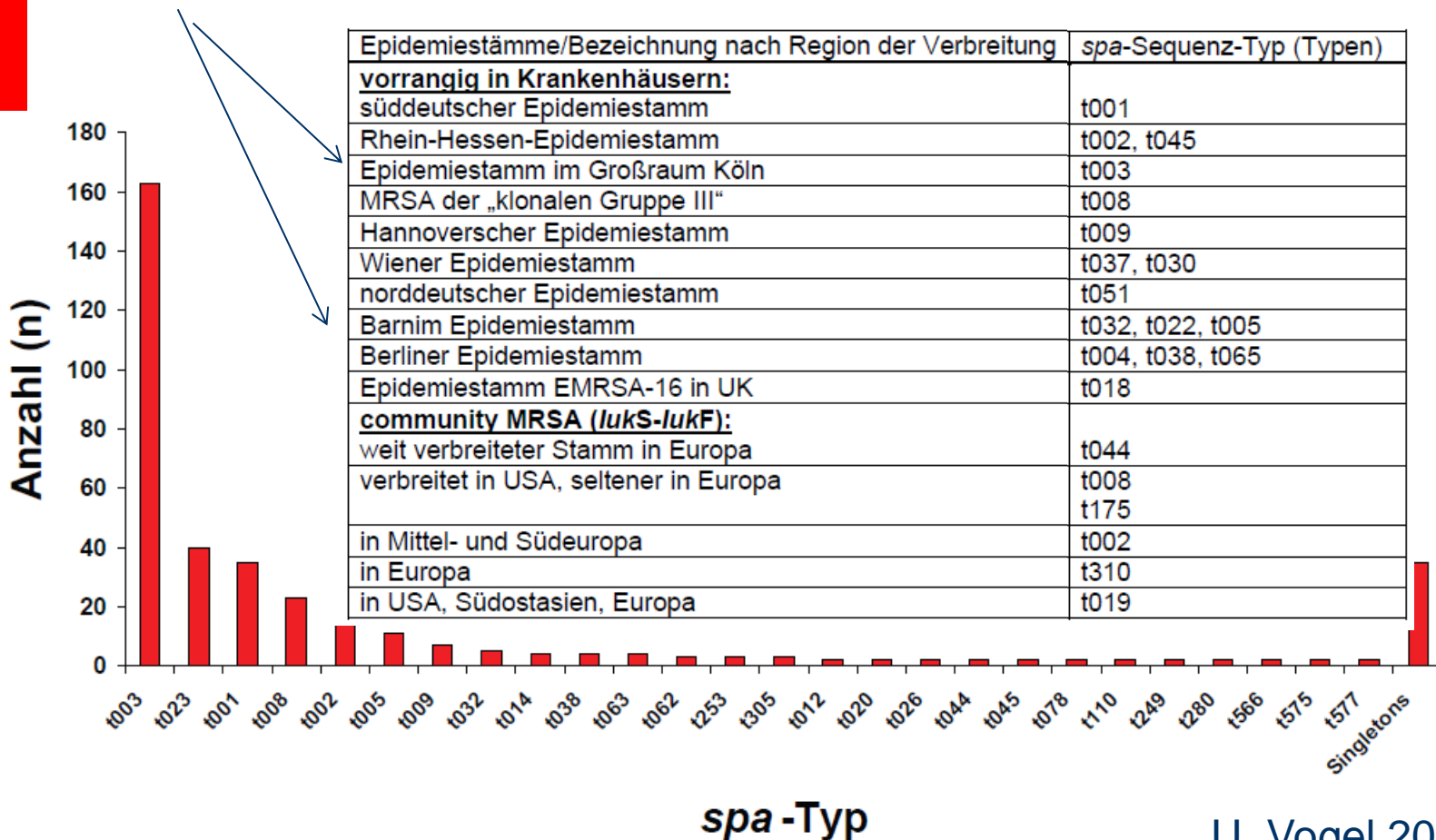
Spa-Typisierung  
MLST  
cgMLST „Ganzgenom-  
sequenzierung“

DNA



# SEQUENZBASIERTE METHODEN: SPA-TYPISIERUNG

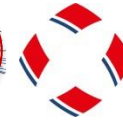
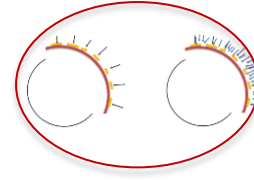
Grobe Einteilung möglich:



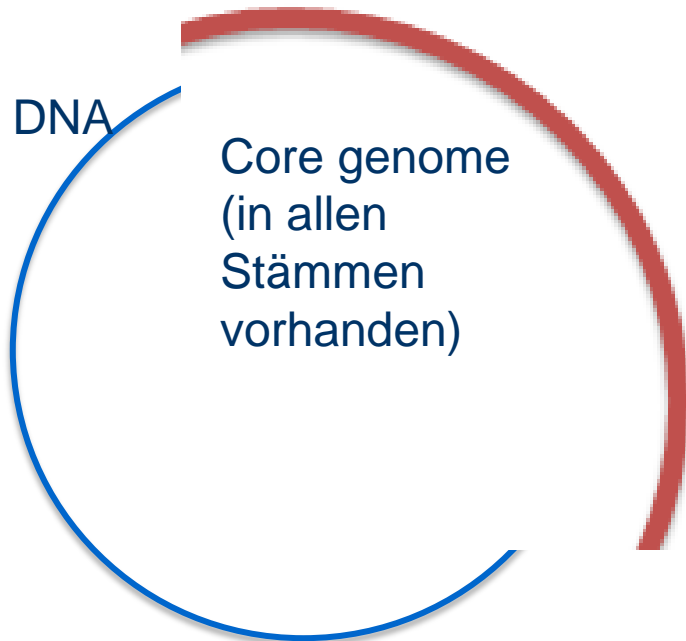
U. Vogel 2005

Abbildung 1:

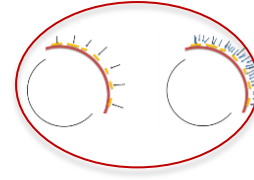
Häufigkeit von spa-Typen an der Universitätsklinik Würzburg seit April 2003.



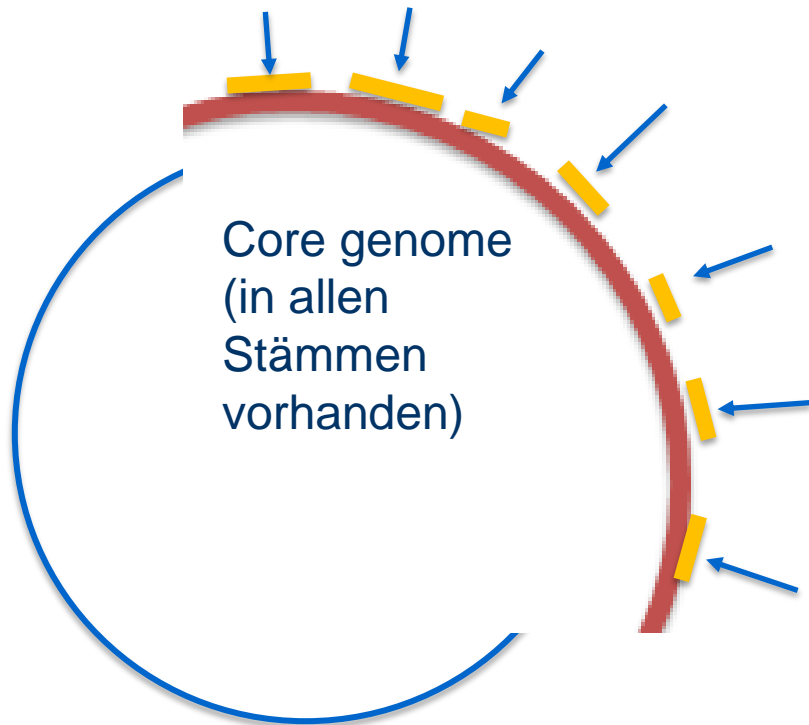
## SEQUENZBASIERTE METHODEN: MLST





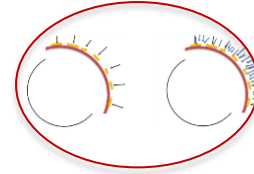


## SEQUENZBASIERTE METHODEN: MLST



„Housekeeping genes“:

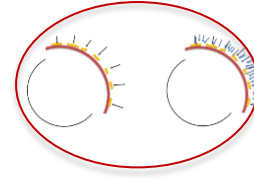
- Nicht regulierte Gene
- unabhängig vom Zelltyp und von äußeren Einflüssen exprimiert
- typischerweise Grundstoffwechsel



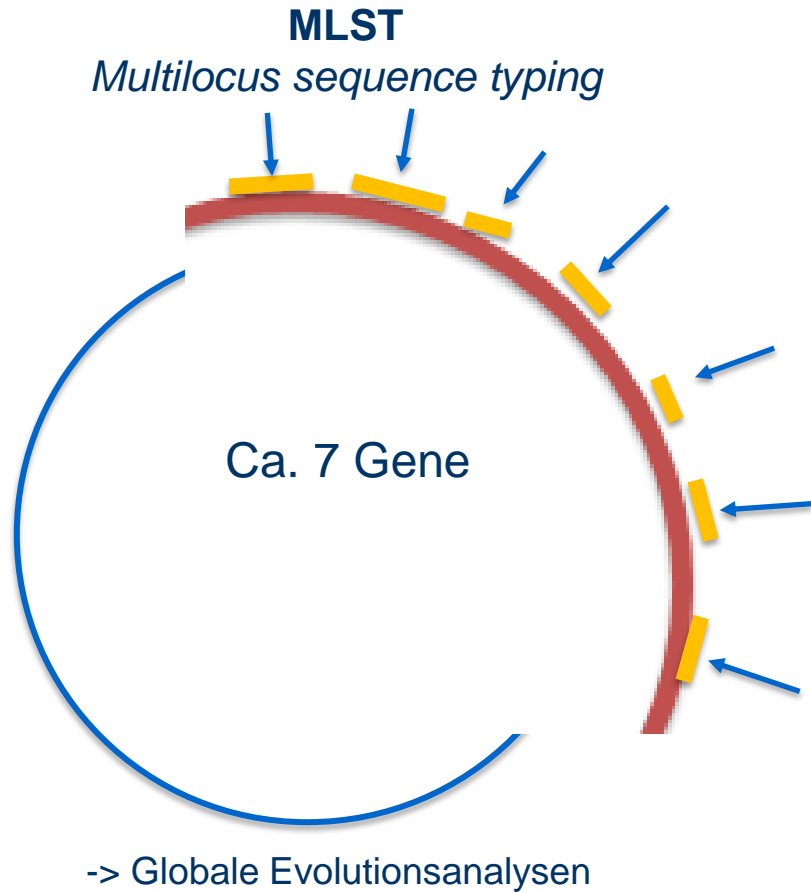
## SEQUENZBASIERTE METHODEN: MLST

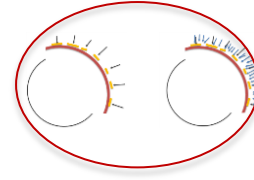
Epidemiestämme/Bezeichnung nach Region der Verbreitung	MLST	<i>spa</i> -Sequenz-Typ (Typen)
<b><u>vorrangig in Krankenhäusern:</u></b> süddeutscher Epidemiestamm	ST228	t001
Rhein-Hessen-Epidemiestamm	ST5	t002, t045
Epidemiestamm im Großraum Köln	ST225	t003
MRSA der „klonalen Gruppe III“	ST8	t008
Hannoverscher Epidemiestamm	ST254	t009
Wiener Epidemiestamm	ST239	t037, t030
norddeutscher Epidemiestamm	ST247	t051
Barnim Epidemiestamm	ST22	t032, t022, t005
Berliner Epidemiestamm	ST45	t004, t038, t065
Epidemiestamm EMRSA-16 in UK	ST36	t018
<b><u>community MRSA (lukS-lukF):</u></b> weit verbreiteter Stamm in Europa	ST80	t044
verbreitet in USA, seltener in Europa	ST8	t008
	ST1	t175
in Mittel- und Südeuropa	ST5	t002
in Europa	ST22	t310
in USA, Südostasien, Europa	ST30	t019

MLST = Multi-Locus-Sequenz-Typ



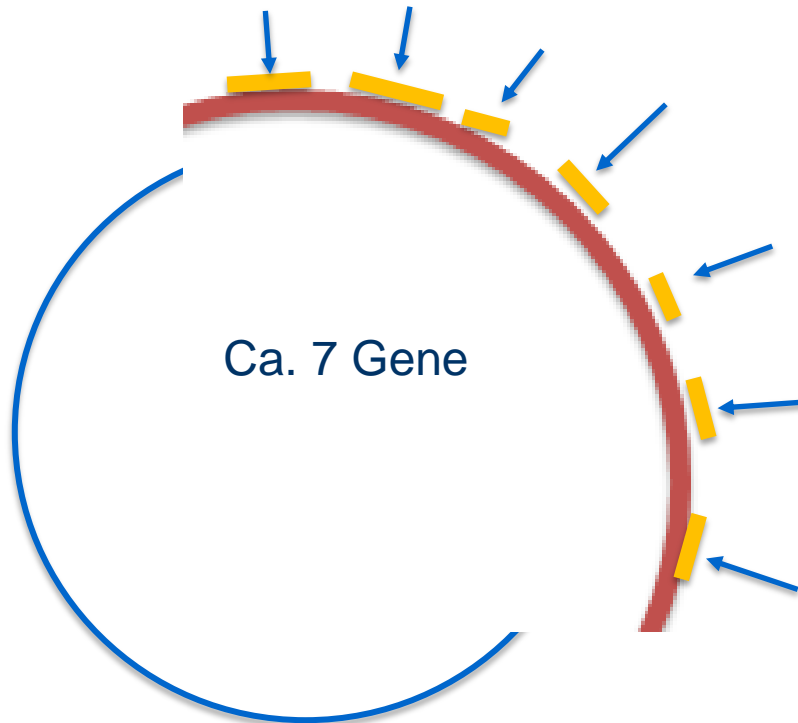
## SEQUENZBASIERTE METHODEN: MLST





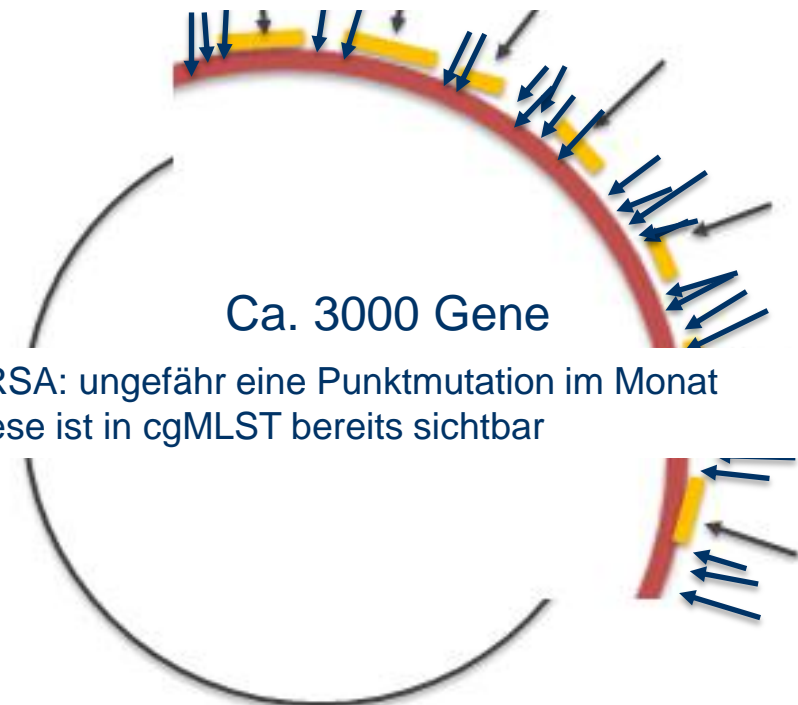
## SEQUENZBASIERTE METHODEN: MLST

**MLST**  
*Multilocus sequence typing*



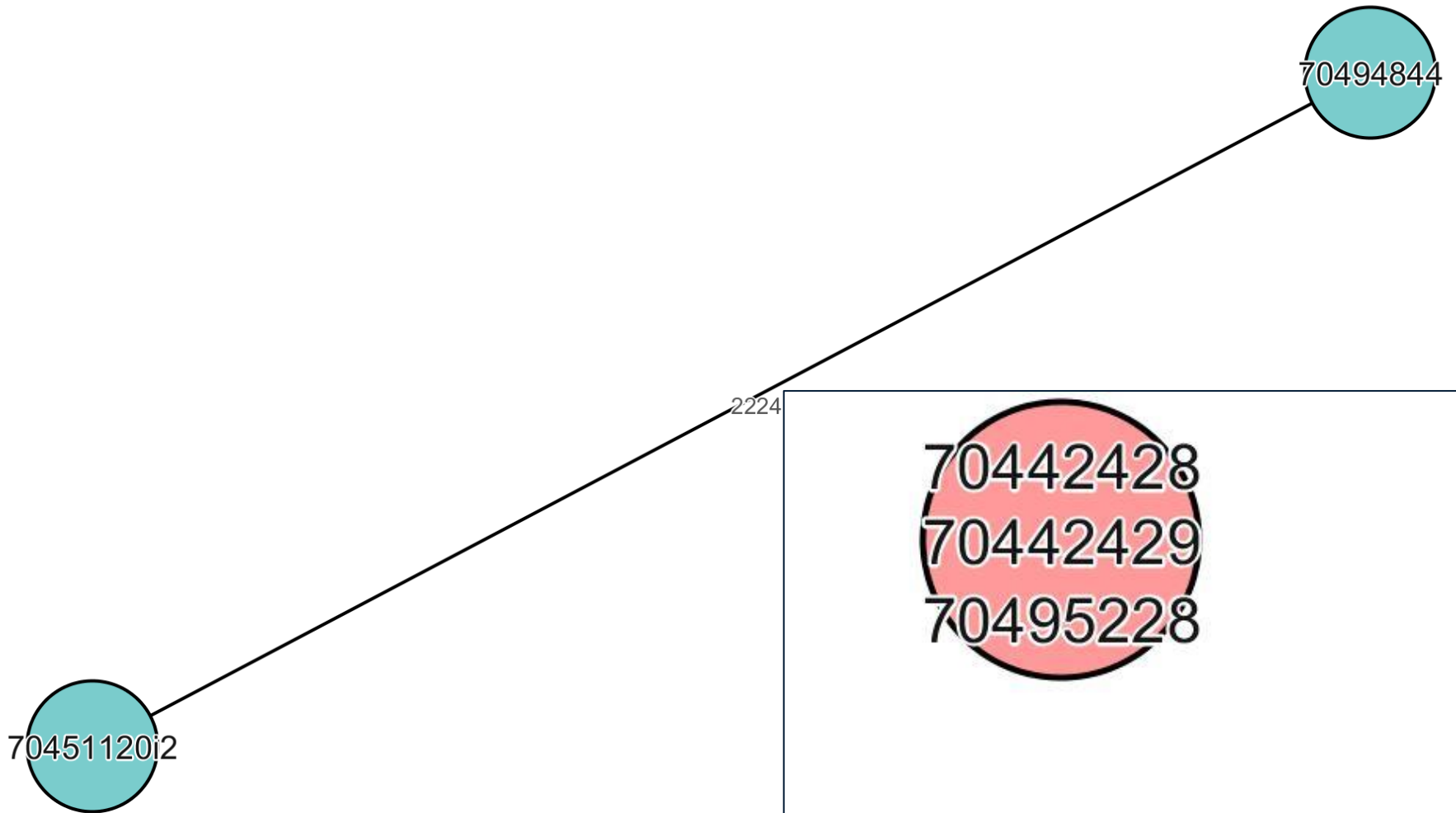
-> Globale Evolutionsanalysen

**cgMLST**  
*Core genome MLST*  
„Ganzgenomsequenzierung“

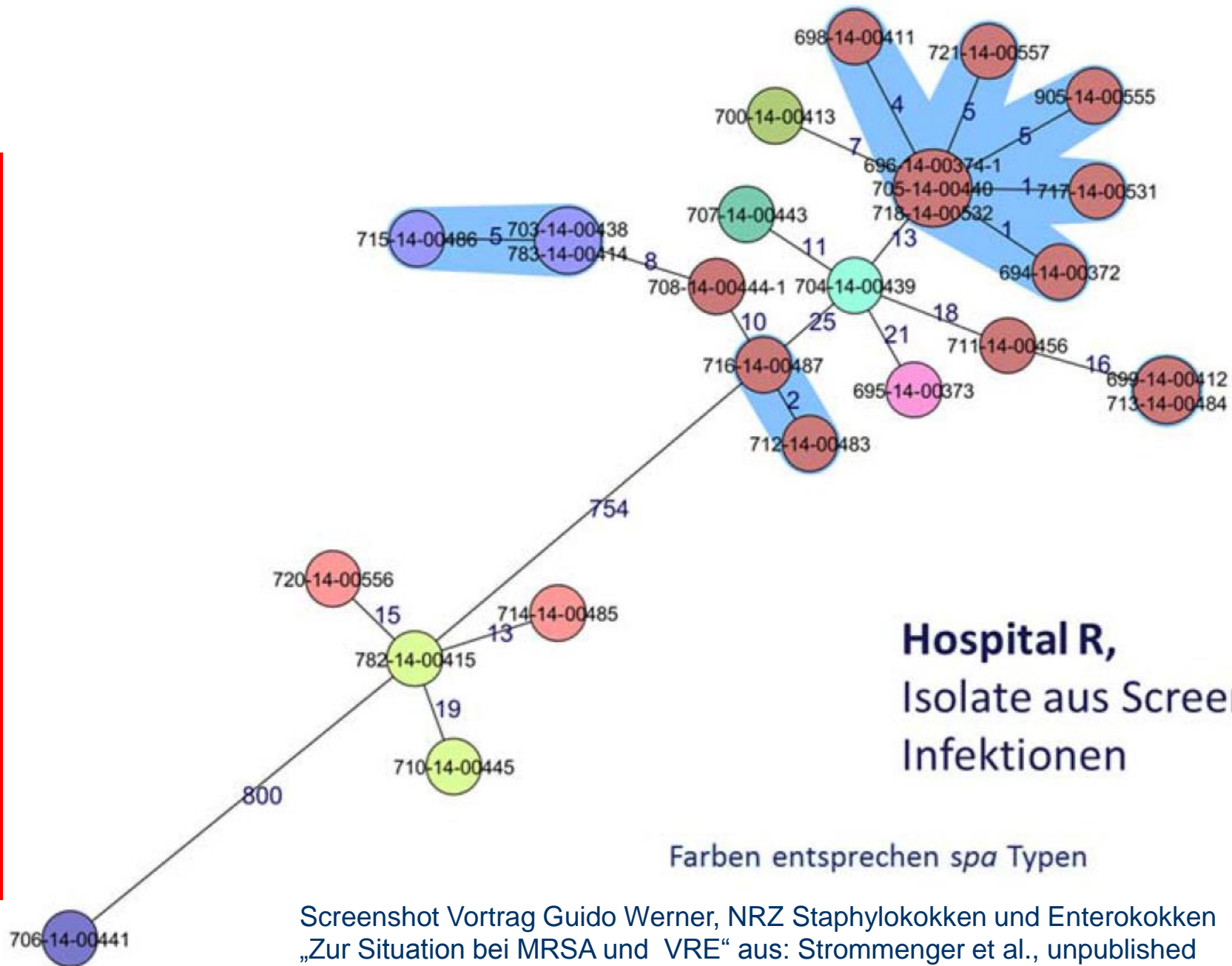


MRSA: ungefähr eine Punktmutation im Monat  
Diese ist in cgMLST bereits sichtbar

Auch variable Elemente typisiert und verglichen  
-> sehr genau



MST based on 2325 columns, pairwise ignoring missing values




# Hospital R, Isolate aus Screening und Infektionen

Farben entsprechen *spa* Typen

Screenshot Vortrag Guido Werner, NRZ Staphylokokken und Enterokokken  
„Zur Situation bei MRSA und VRE“ aus: Strommenger et al., unpublished

# Whole-Genome Sequencing Elucidates Epidemiology of Nosocomial Clusters of *Acinetobacter baumannii*

Stefanie Willems,<sup>a\*</sup> Stefanie Kampmeier,<sup>a</sup> Stefan Bletz,<sup>a</sup> Annelene Kossow,<sup>a</sup> Robin Köck,<sup>b\*</sup> Frank Kipp,<sup>a\*</sup>  Alexander Mellmann<sup>a</sup>

Institute of Hygiene, University Hospital Muenster, Muenster, Germany<sup>a</sup>; Institute of Medical Microbiology, University Hospital Muenster, Muenster, Germany<sup>b</sup>

We characterized two epidemiologically similar *Acinetobacter baumannii* clusters from two separate intensive care units (ICU) using core genome multilocus sequence typing. Clonal spread was confirmed in ICU-1 (12 of 14 isolates shared genotypes); in ICU-2, all genotypes (13 isolates) were diverse, thus excluding transmissions and enabling adequate infection control measures.

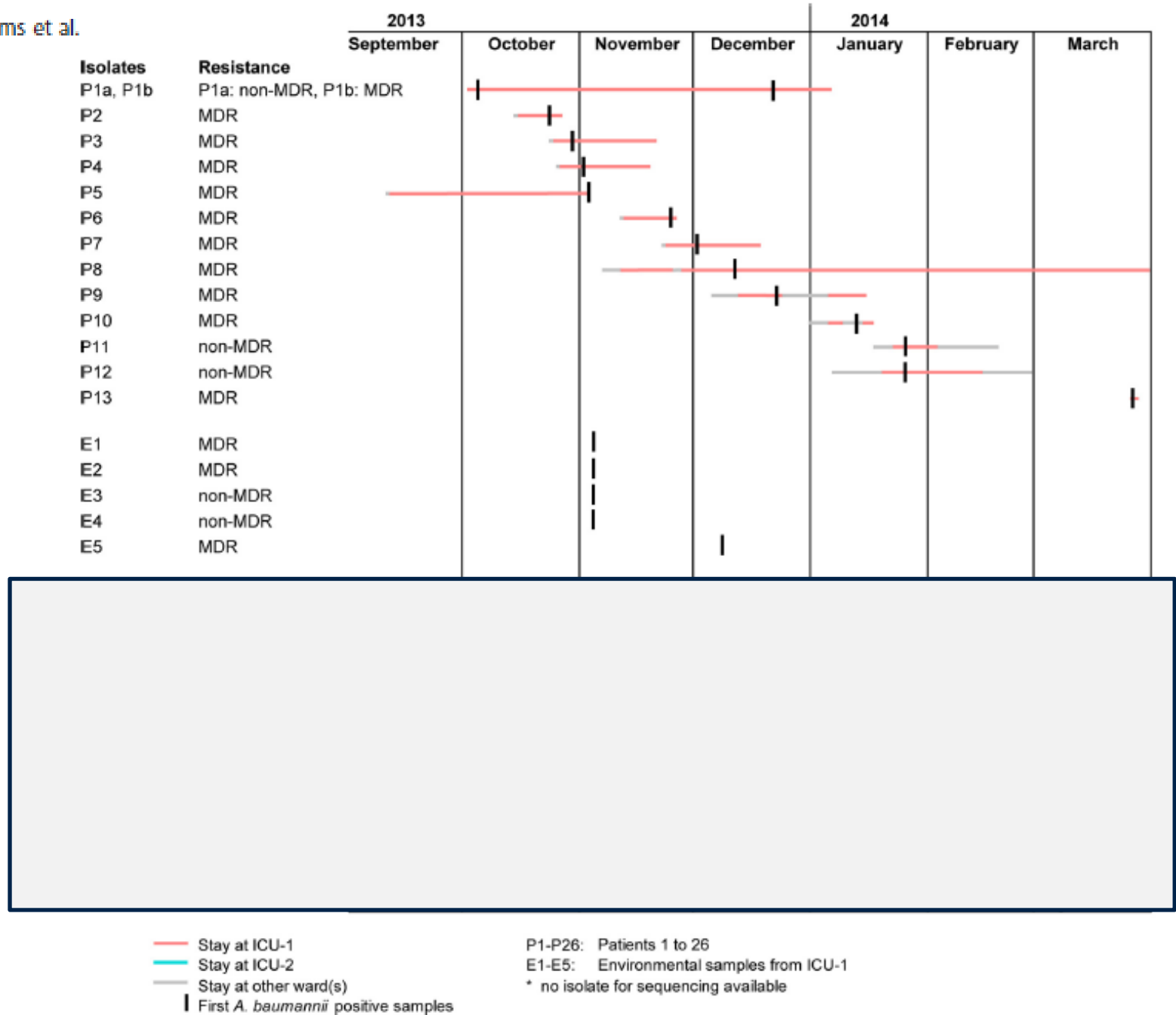
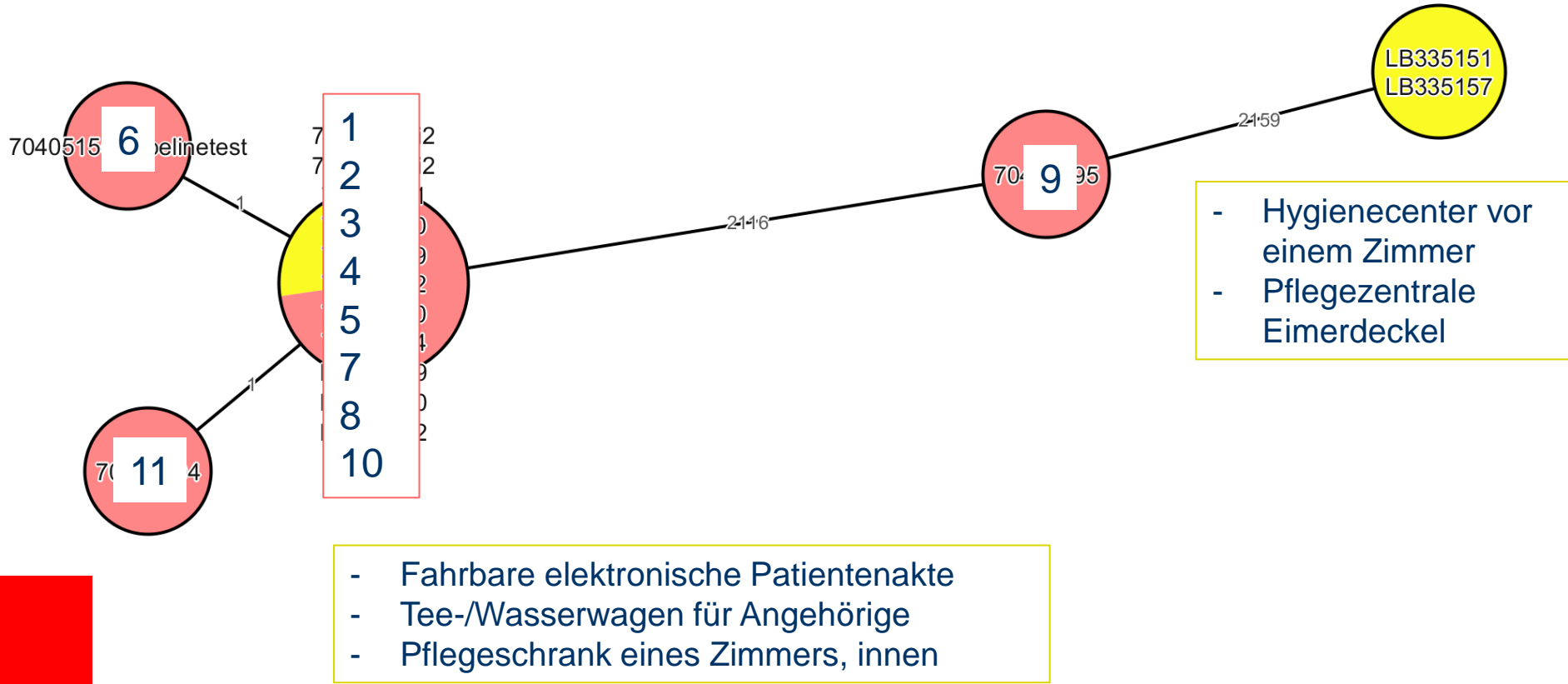




FIG 1 Linelist of all 26 patients and 5 environmental samples positive for *A. baumannii* for the two clusters in ICU-1 and ICU-2 during September 2013 and March 2014. P, patient; E, environment; MDR, multidrug-resistant phenotype (for details, see Table 1).





MST based on 3319 columns, pairwise ignoring missing values

-  Umgebungsuntersuchungen
-  3 MRGN

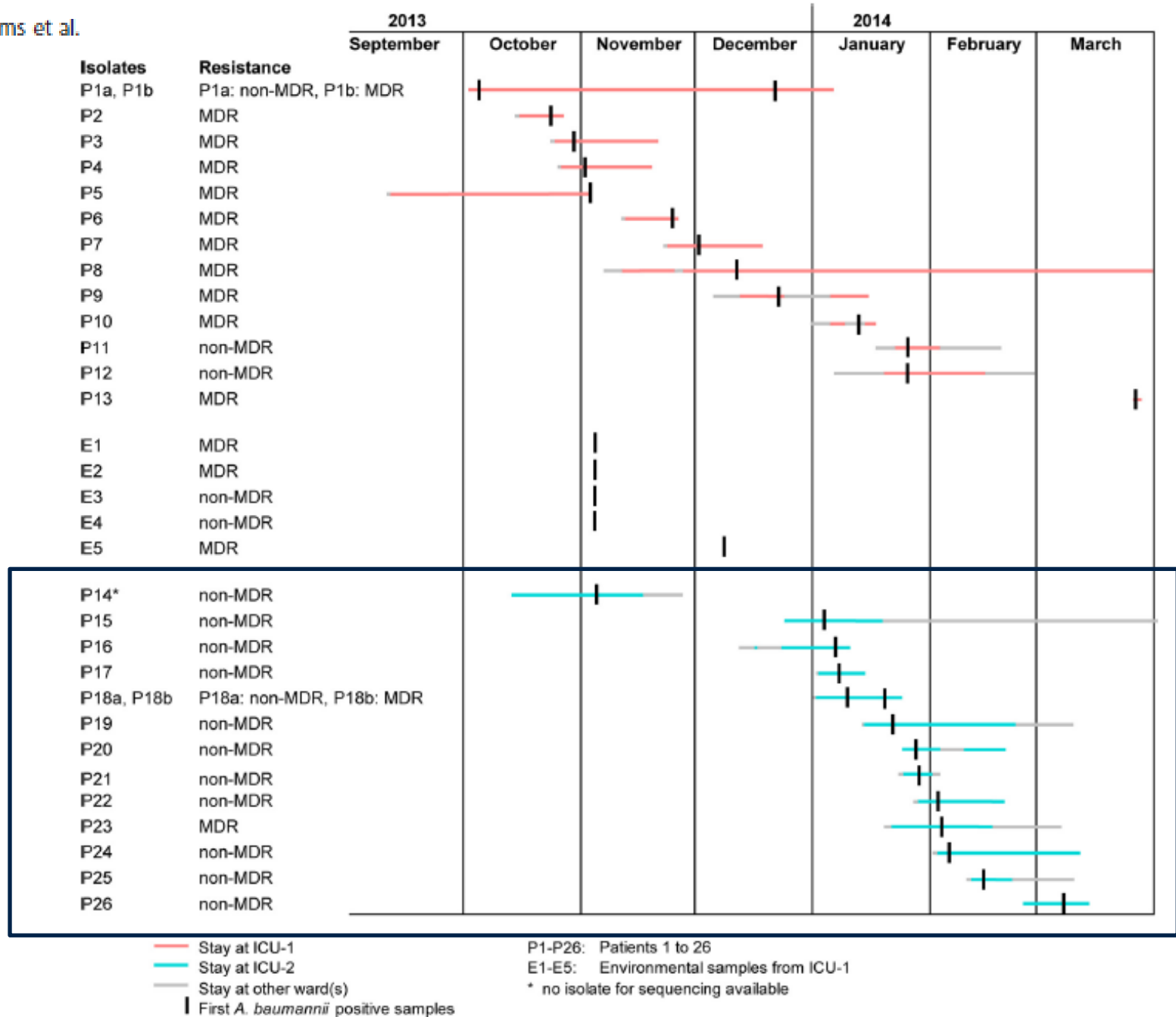


FIG 1 Linelist of all 26 patients and 5 environmental samples positive for *A. baumannii* for the two clusters in ICU-1 and ICU-2 during September 2013 and March 2014. P, patient; E, environment; MDR, multidrug-resistant phenotype (for details, see Table 1).

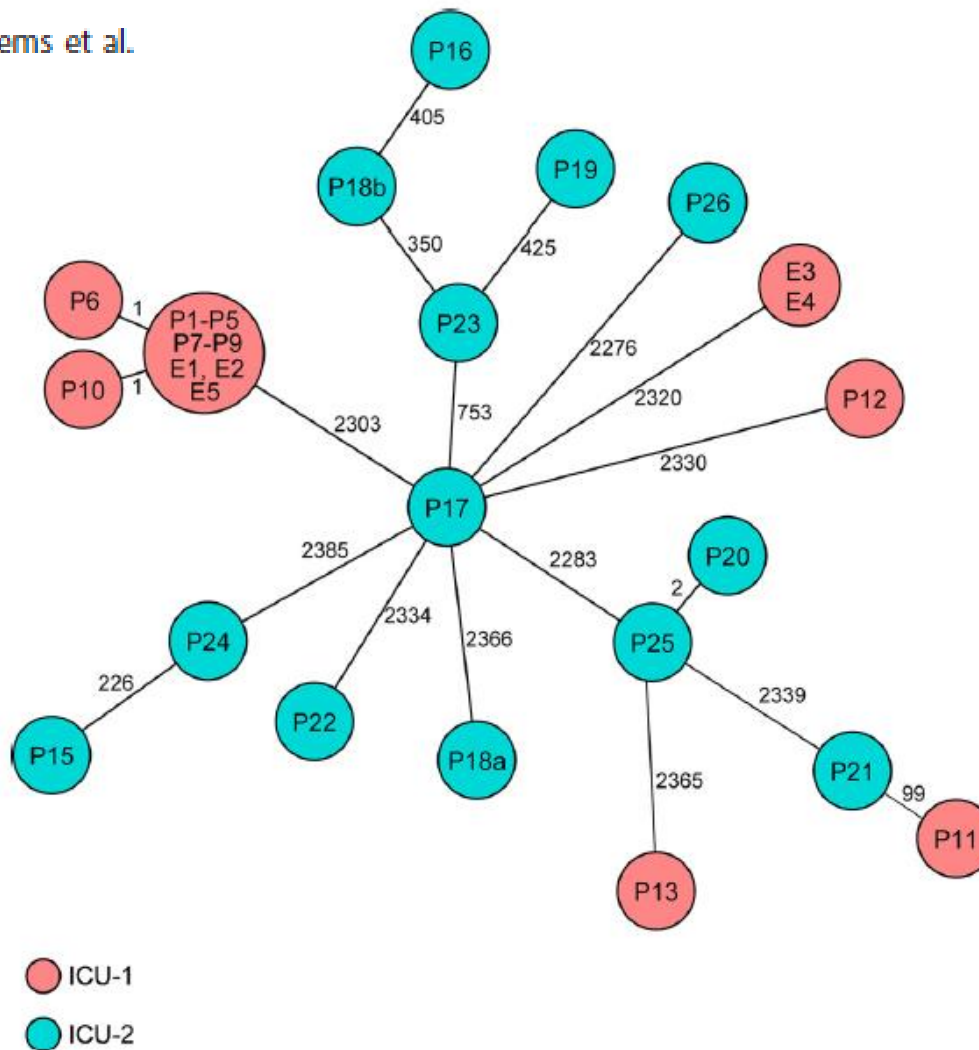


FIG 2 Minimum spanning tree of 32 *A. baumannii* isolates detected for cluster I (ICU-1) and cluster II (ICU-2) based on the allelic profiles of up to 3,319 target genes (see Tables S1 and S2 and the supplemental data set in the supplemental material) that were present in the isolates with the “pairwise ignoring missing values” option turned on in SeqSphere+ software during comparison. Each circle represents an allelic profile, i.e., the genotype, and is named by the isolate(s). The numbers on the connecting lines give the number of differing alleles. The size of the circle is proportional to the number of isolates with an identical genotype and the color of the circles represent the different wards.



# Real-Time Genome Sequencing of Resistant Bacteria Provides Precision Infection Control in an Institutional Setting

 Alexander Mellmann,<sup>a</sup> Stefan Bletz,<sup>a</sup> Thomas Böking,<sup>a</sup> Frank Kipp,<sup>a\*</sup> Karsten Becker,<sup>b</sup> Anja Schultes,<sup>c</sup> Karola Prior,<sup>c</sup> Dag Harmsen<sup>c</sup>

Institute of Hygiene, University Hospital Muenster, Muenster, Germany<sup>a</sup>; Institute of Medical Microbiology, University Hospital Muenster, Muenster, Germany<sup>b</sup>;  
Department of Periodontology and Restorative Dentistry, University Hospital Muenster, Muenster, Germany<sup>c</sup>

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- Studienintervall I: 15.10.2013 - 15.04.2014
- Studienintervall II: 15.10.2014 - 15.04.2015

Ganzgenomsequenzierung von:

- MRSA
- VRE
- 3+4 MRGN *e. coli*
- 3+4 MRGN *p. aeruginosa*



Aufhebung der Isolierung von Patienten mit 3 MRGN in Risikobereichen

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Real-Time Genome Sequencing of MDR Bacteria

TABLE 2 Comparison of transmission rates of MRSA and 3MDR-GN *E. coli* during the two study intervals

Study interval	Pathogen (no. of isolates/total no. of patient cases/no. of cases at risk wards)	No. of genotypic clusters (maximal distance for cluster recognition)	Epidemiological assessment of genotypic clusters	Total no. of cases involved in probable transmissions (%)	No. of cases in risk wards with changed infection control procedures for 3MDR-GN (%) during interval II
I	MRSA (412/397/68)	32 ( $\leq 6$ alleles)	8 clusters with probable transmissions, 16 clusters with unlikely transmissions, isolation 8 times from same patient but different colony morphology/phenotype results	23 (5.8)	15 (22.1)
	<i>E. coli</i> (102/86/51)	13 ( $\leq 10$ alleles)	1 cluster with probable transmission, 1 cluster with unlikely transmissions, isolation 11 times from same patient but different cases/colony morphology/phenotype results	2 (2.3)	2 (3.9)
II	MRSA (325/325/57)	15 ( $\leq 6$ alleles)	6 clusters with probable transmissions, 9 clusters with unlikely transmissions	14 (4.3)	6 (10.5) <sup>a</sup>
	<i>E. coli</i> (120/120/45)	8 ( $\leq 10$ alleles)	1 cluster with probable transmissions, 7 clusters with unlikely transmissions	6 (5.0)	0 (0) <sup>a</sup>

<sup>a</sup> Results of comparisons of transmission rates between interval I and II were not statistically significant for MRSA ( $P = 0.0980$ ) or MDR *E. coli* ( $P = 0.4967$ ).

# Real-Time Genome Sequencing of Resistant Bacteria Provides Precision Infection Control in an Institutional Setting

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- 202,09€ pro Patientenisolat für WGS
  - 70,4% Verbrauchsmaterialien
  - 19,9% Hardwareabschreibung und Softwarekosten
  - 9,7% Laborkosten
- Kosten pro Intervall für WGS:
  - Intervall I: 130.608,84€
  - Intervall II: 111.371,88€
- Gegenrechnung:  
Kostenberechnung bei MRE nach Herr 2003\*: 371,95€/Isobett/d

Intervall II: 56 Patienten auf Risikostationen mit 3MRGN über 17,9 Tage bei Bettenauslastung von 85,3%: Anfallende Kosten 317.180,37€

## Fazit

- WGS: schnelles, auswertbares, vergleichbares Werkzeug
- Differenzierung zufällige Häufung/realer Ausbruch
- A priori-Isolierung verhindert nicht die Übertragung
- Nicht mehr Übertragungen im Risikobereich bei Nicht-Isolierung (Ressourcen!)

Barrier precautions affect interactions between patients and caregivers. Nurses and physicians avoid direct contact with isolated patients.<sup>25,26</sup> During morning rounds, attending physicians physically examined 35% of isolated patients but 73% of other patients.<sup>26</sup> Among patients who had surgery, encounters between patients and health-care providers were about half as frequent for patients in protective isolation compared with those who were not in isolation.<sup>27</sup> The mean in-room contact time with health-care providers was 22% lower for isolated patients than non-isolated ones, although the severity of illness did not differ between groups.



## Fazit

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- Differenzierung zufällige Häufung/realer Ausbruch
- A priori-Isolierung verhindert nicht die Übertragung
- Nicht mehr Übertragungen im Risikobereich bei Nicht-Isolierung (Ressourcen!)
- Kosteneffizient