Diagnostics-based Infection Control

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Rapid Microbial Detection Technologies

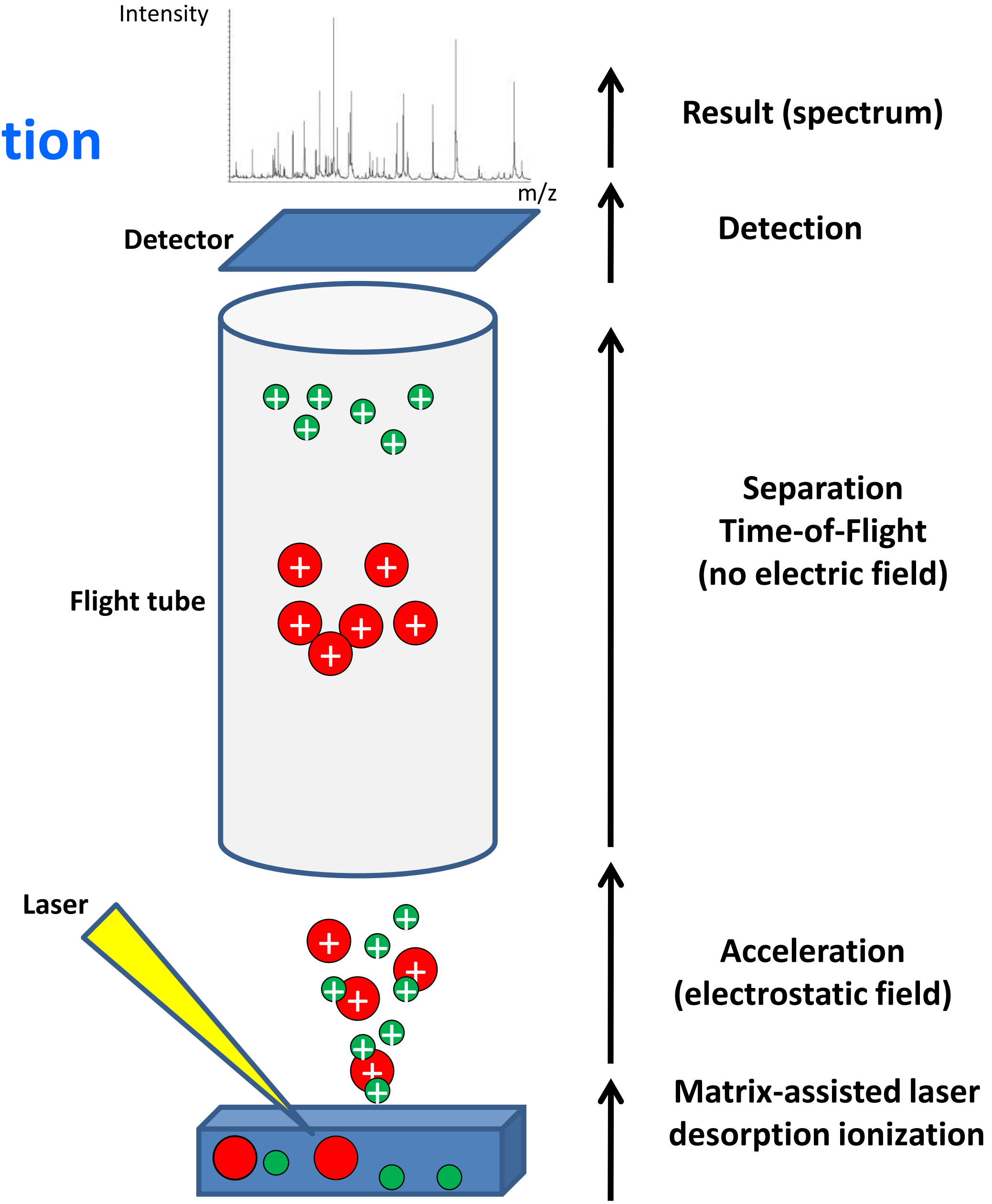
- **Antigen detection**
  - ELISA, IF, immuno-chromatography,….
- **Nucleic acid detection**
  - PCR, real-time PCR, PCR-hybridization, DNA-microarray, PCR-sequencing,…

Marc Struelens. Lecture at 6th International Conference of the Hospital Infection Society.

Agenda

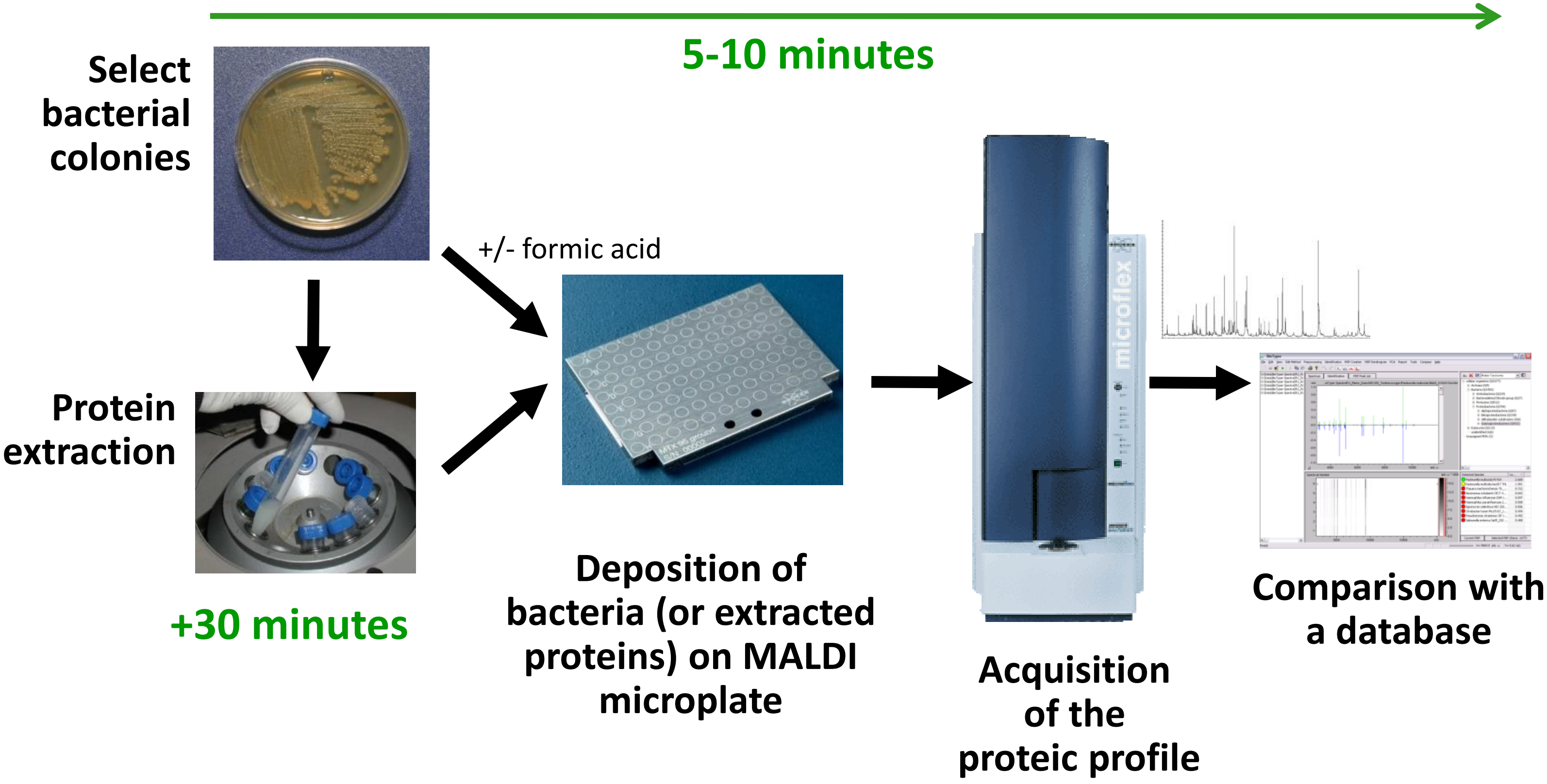
- MALDI-TOF
- Whole-Genome Sequencing
- Microbiome
- Resistome
- Future

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)



Adapted from: Croxatto, Prod'hom & Greub, FEMS Microbiol Reviews 2011

MALDI-TOF Procedure



5-10 minutes

Select bacterial colonies

Protein extraction

+30 minutes

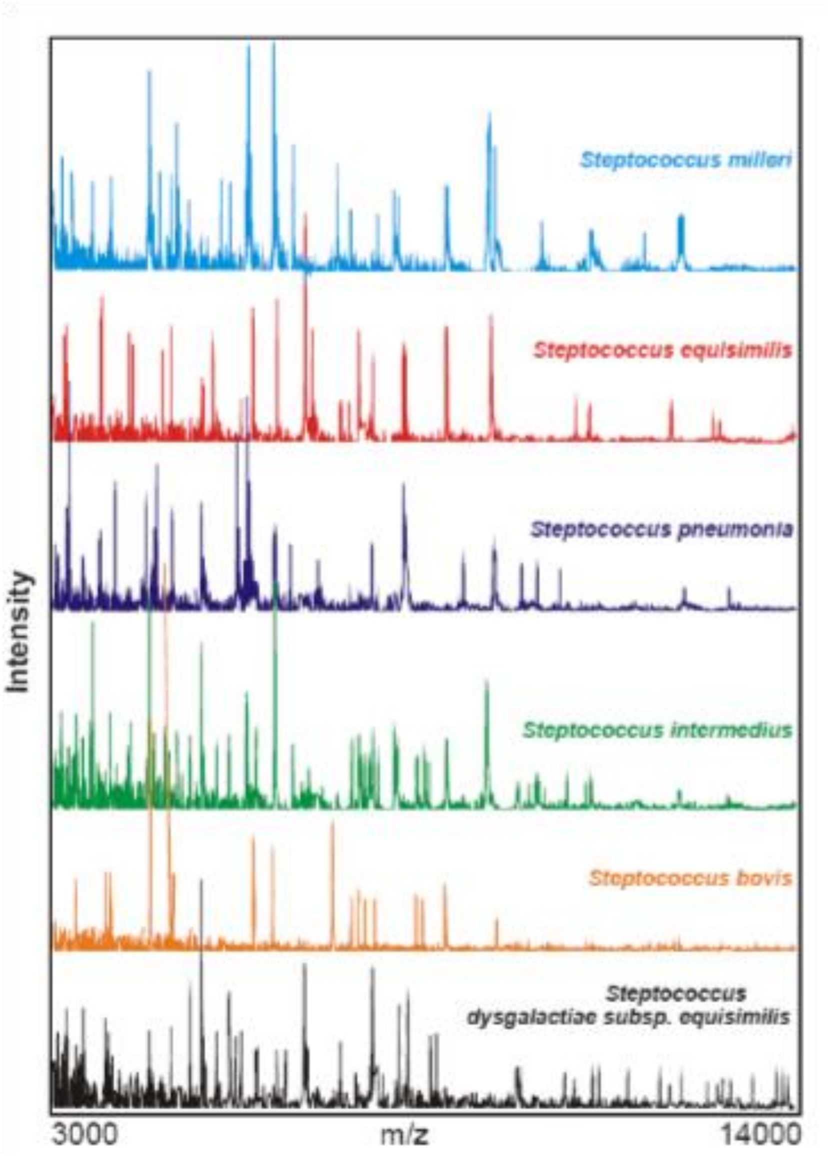
+/- formic acid

Deposition of bacteria (or extracted proteins) on MALDI microplate

Acquisition of the proteic profile

Comparison with a database

MALDI-TOF for Identification

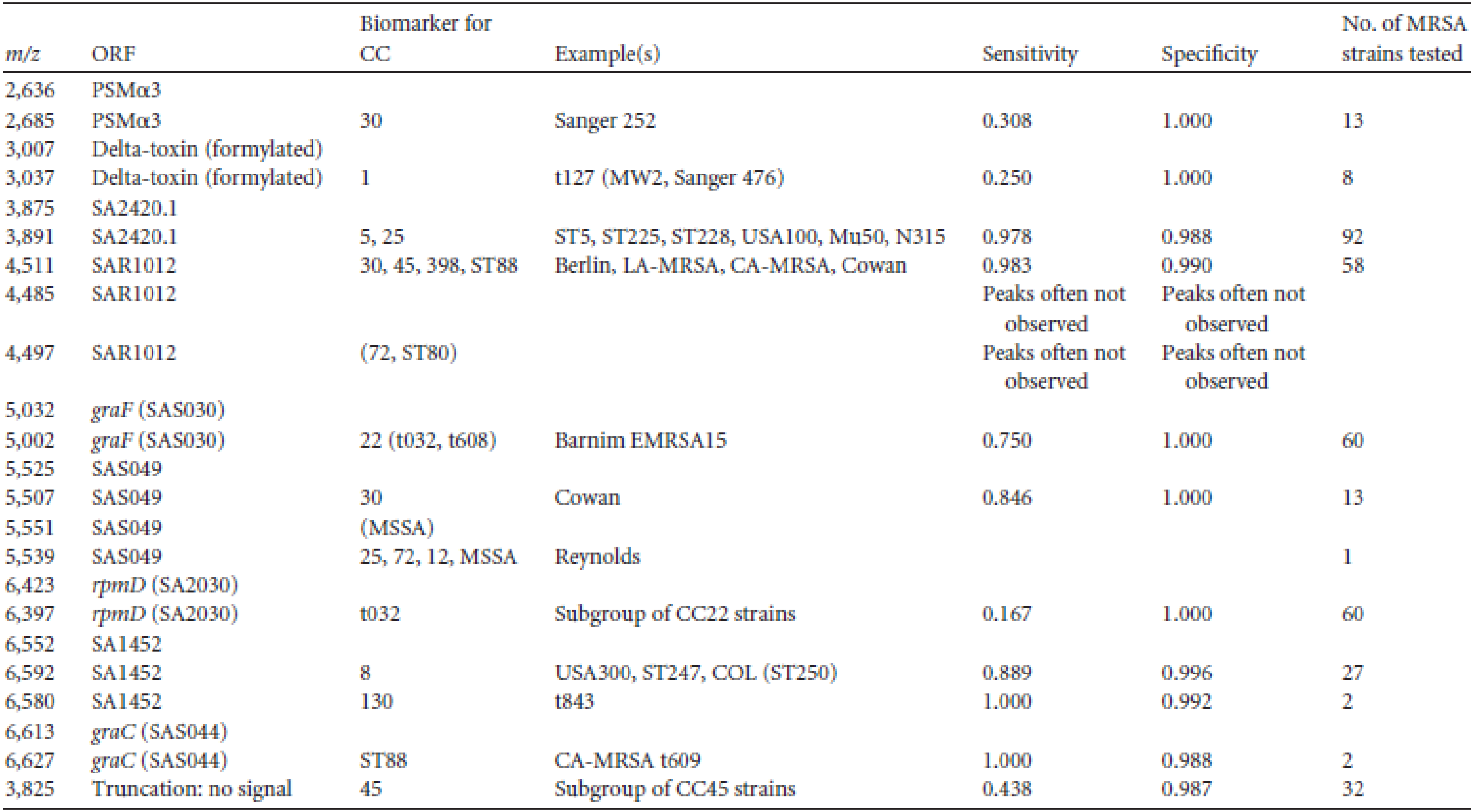


- Mass range 2,000-15, 000 Da
- 50-70% ribosomal proteins
- Obtained spectra compared with database

MALDI-TOF for Typing

- Staphylococci
- *Streptococcus pneumoniae*
- *Acinetobacter*
- *Escherichia coli*

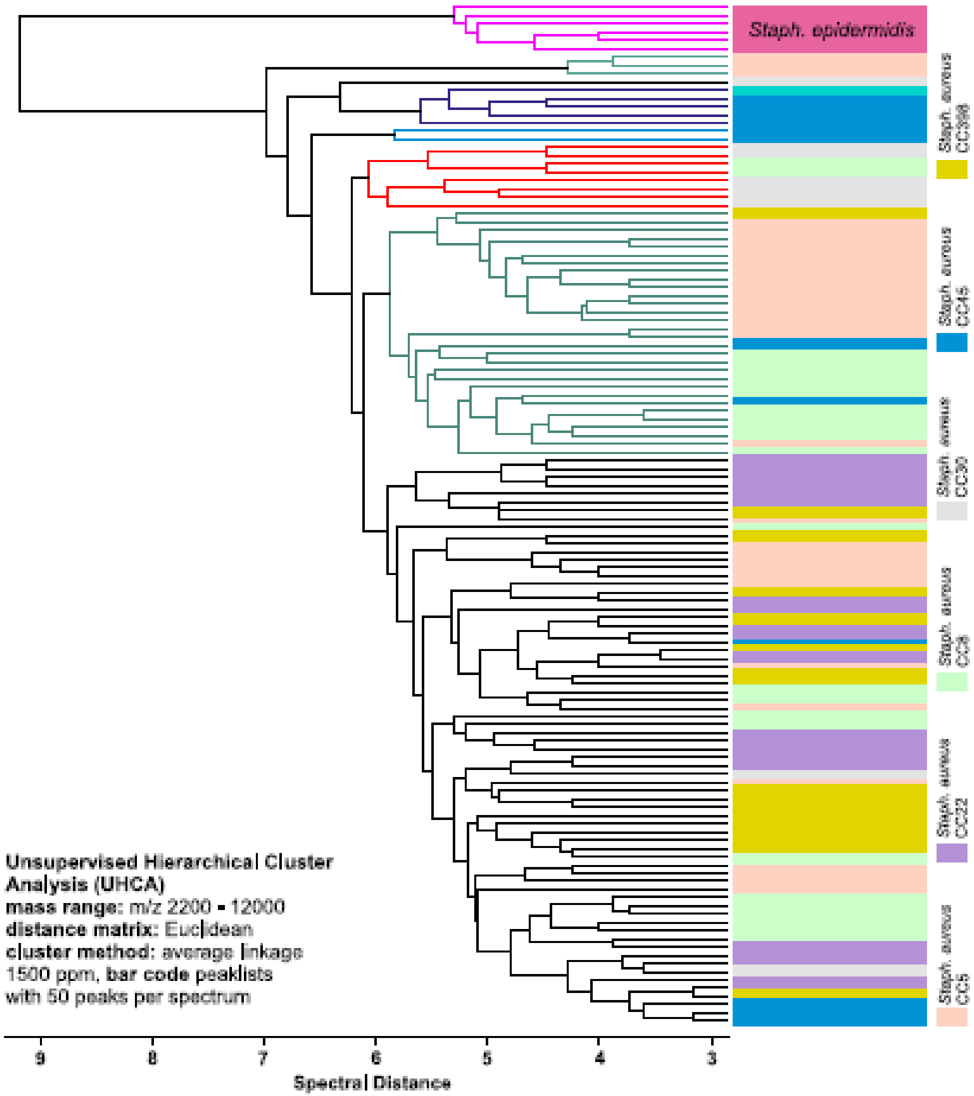
Distribution of Peak Shifts of *S. aureus* and Sensitivity and Specificity of the Markers



m/z	ORF	Biomarker for CC	Example(s)	Sensitivity	Specificity	No. of MRSA strains tested
2,636	PSMα3					
2,685	PSMα3	30	Sanger 252	0.308	1.000	13
3,007	Delta-toxin (formylated)					
3,037	Delta-toxin (formylated)	1	t127 (MW2, Sanger 476)	0.250	1.000	8
3,875	SA2420.1					
3,891	SA2420.1	5, 25	ST5, ST225, ST228, USA100, Mu50, N315	0.978	0.988	92
4,511	SAR1012	30, 45, 398, ST88	Berlin, LA-MRSA, CA-MRSA, Cowan	0.983	0.990	58
4,485	SAR1012			Peaks often not observed	Peaks often not observed	
4,497	SAR1012	(72, ST80)		Peaks often not observed	Peaks often not observed	
5,032	*graF* (SAS030)					
5,002	*graF* (SAS030)	22 (t032, t608)	Barnim EMRSA15	0.750	1.000	60
5,525	SAS049					
5,507	SAS049	30	Cowan	0.846	1.000	13
5,551	SAS049	(MSSA)				
5,539	SAS049	25, 72, 12, MSSA	Reynolds			1
6,423	*rpmD* (SA2030)					
6,397	*rpmD* (SA2030)	t032	Subgroup of CC22 strains	0.167	1.000	60
6,552	SA1452					
6,592	SA1452	8	USA300, ST247, COL (ST250)	0.889	0.996	27
6,580	SA1452	130	t843	1.000	0.992	2
6,613	*graC* (SAS044)					
6,627	*graC* (SAS044)	ST88	CA-MRSA t609	1.000	0.988	2
3,825	Truncation: no signal	45	Subgroup of CC45 strains	0.438	0.987	32

Josten et al, J Clin Microbiol 2013; 51: 1809-17

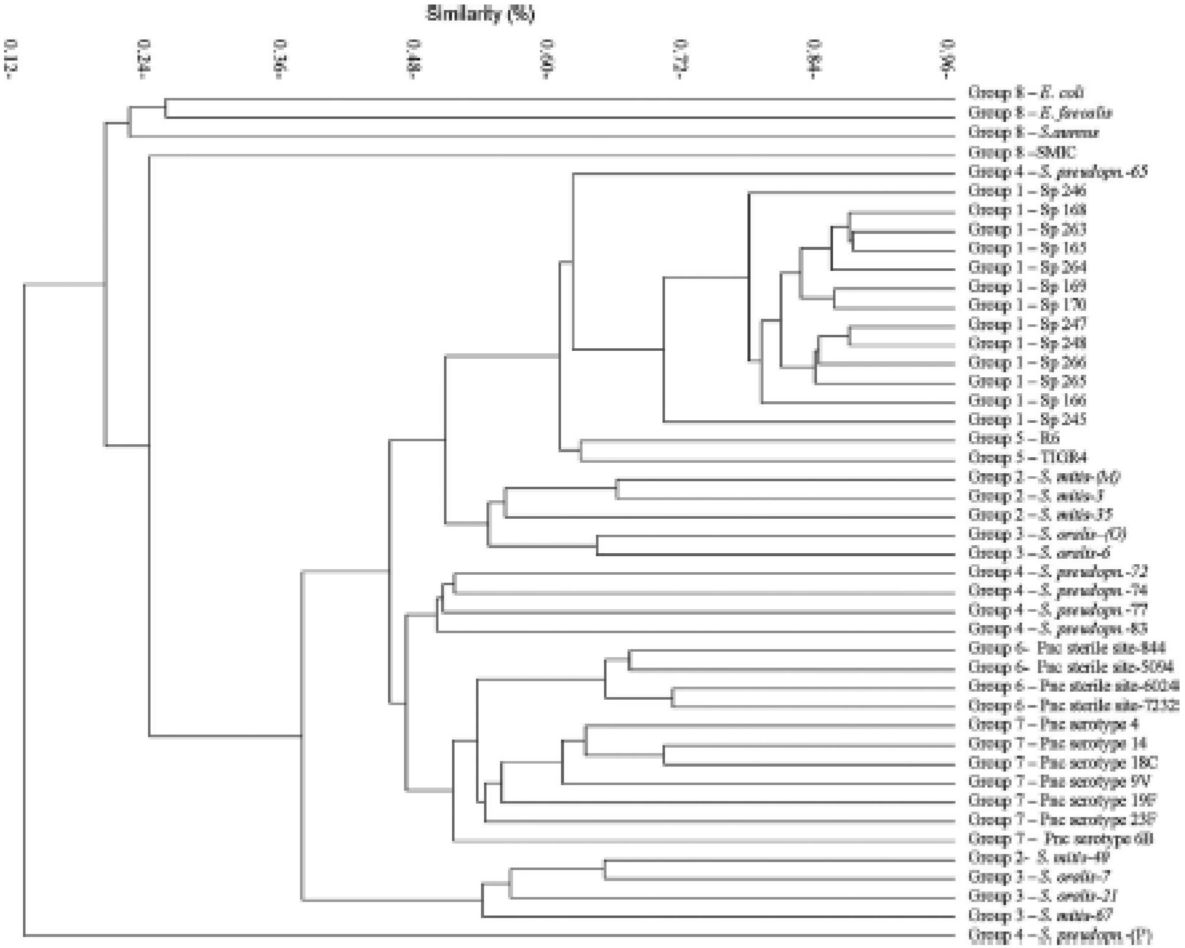
Hierarchical Clustering of *S. aureus* and *S. epidermidis* by MALDI-TOF



Unsupervised Hierarchical Cluster Analysis (UHCA)
mass range: m/z 2200 - 12000
distance matrix: Euclidean
cluster method: average linkage
1500 ppm, **bar code peaklists**
with 50 peaks per spectrum

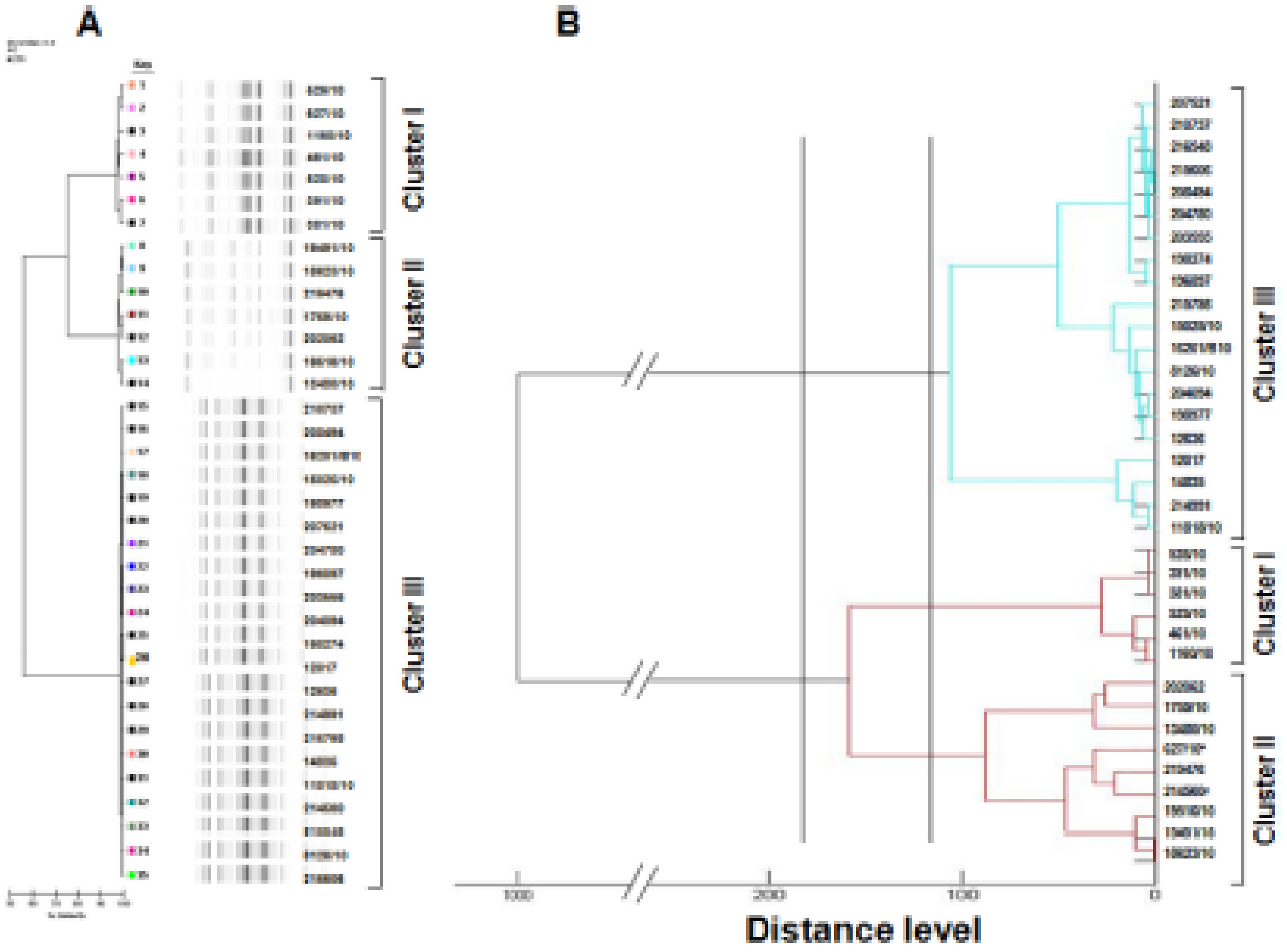
Lasch et al, J Microbiological Methods 2014; 100: 58-69

Hierarchical Clustering of *S. pneumoniae* Conjunctivitis Outbreak Isolates and Nonconjunctival Bacterial Controls by MALDI-TOF



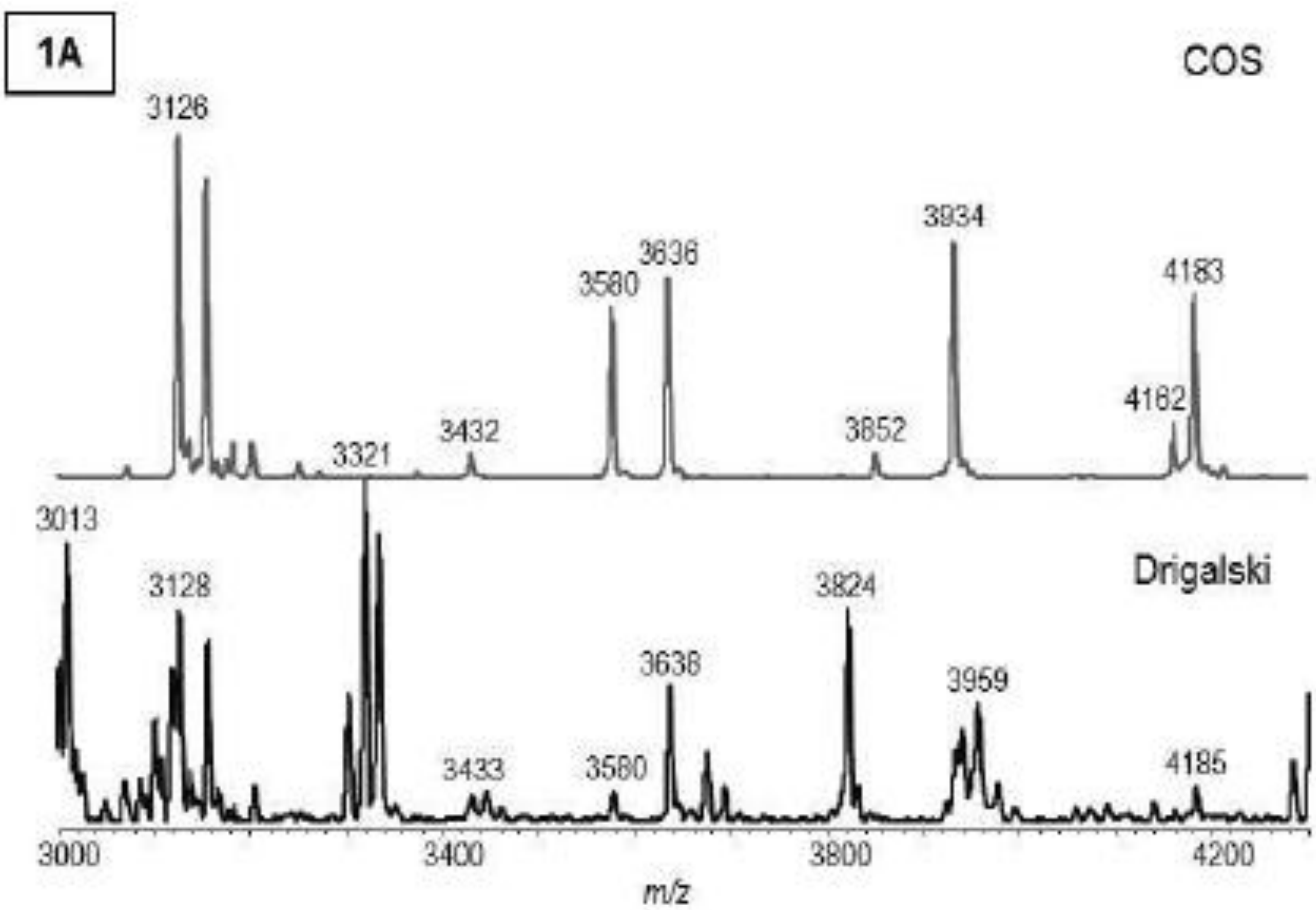
Williamson et al, Appl Environ Microbiol 2008; 74: 5891-7

Hierarchical Clustering of *Acinetobacter baumanii* by rep-PCR and MALDI-TOF



Mencacci et al, J Clin Microbiol 2013; 51:603-6

MS Spectra of *E. coli* Grown on Columbia 5% Sheep Blood Agar and Drigalski Agar



Spinali et al, J Clin Microbiol 2014

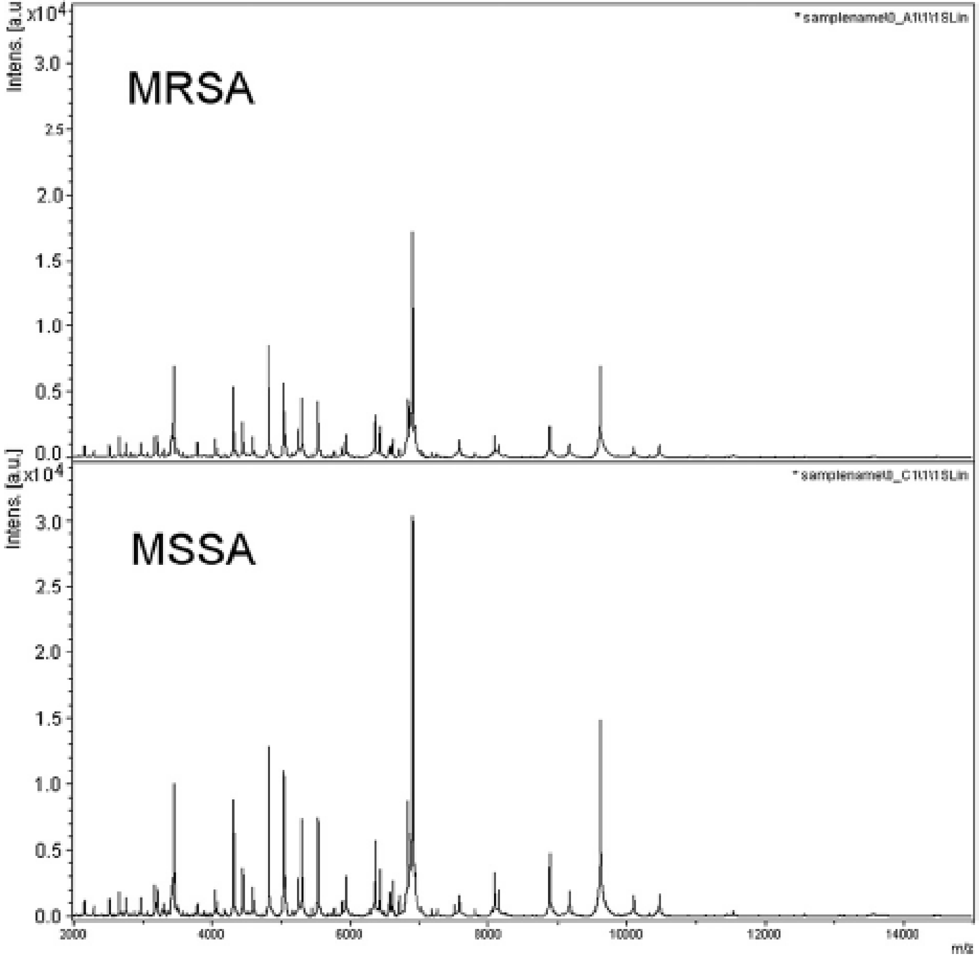
Conclusions

- **Limitations:**
  - MALDI-TOF has insufficient discriminatory power for typing of strains
  - Although discriminatory potential may depend on bacterial species
  - Lack of clear guidelines for data interpretation
  - Variation in spectra due to culture conditions, spotting techniques
  - Spectra subject to variation in protein expression and post-translational modifications and regulations
- **Expectations:**
  - No great future of using MS for strain typing

MALDI-TOF for Detection of Antibiotic Resistance

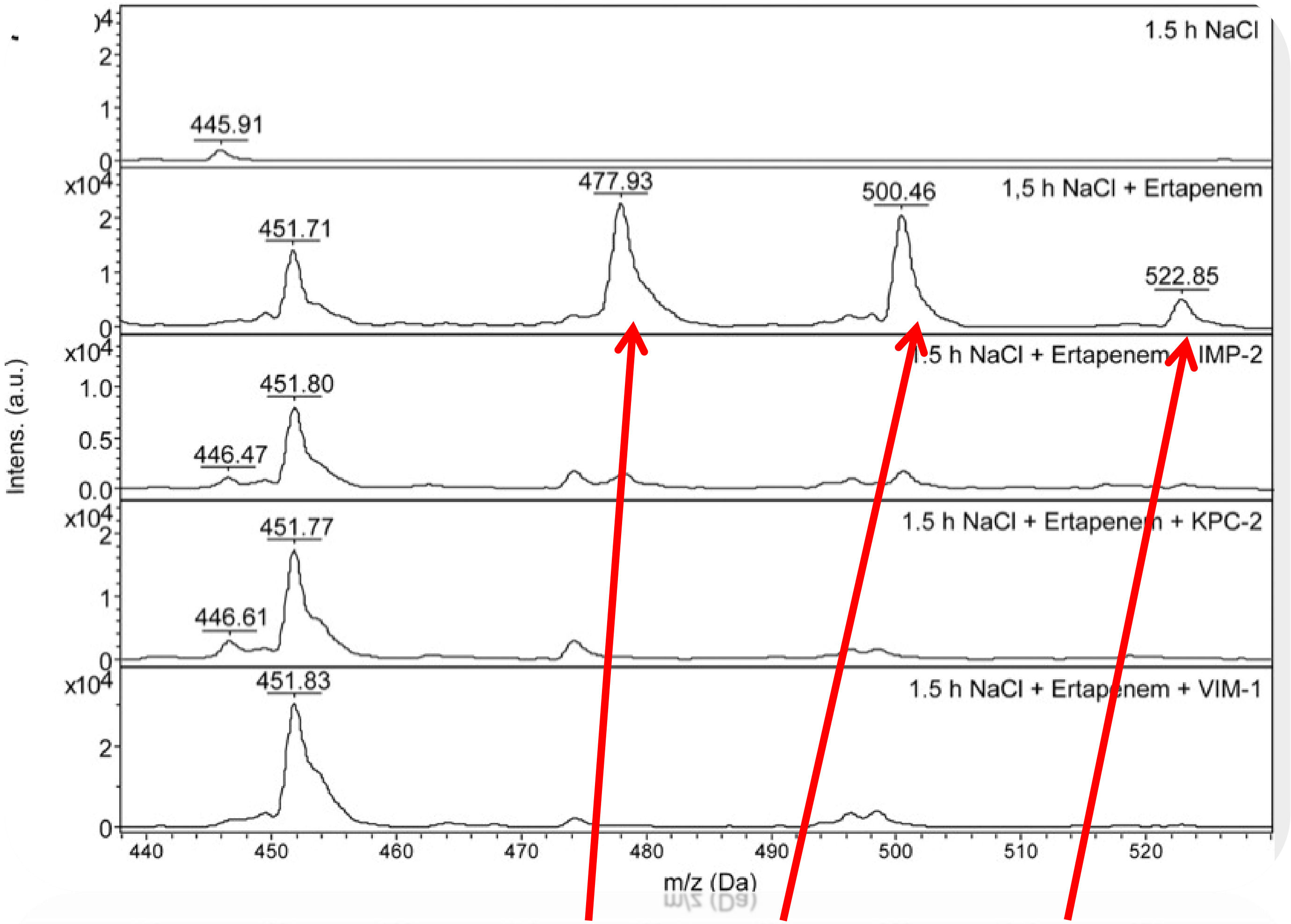
- Detection of proteins:
  - Methicillin-resistant Staphylococci
- Beta-Lactam degradation:
  - Carbapenem-resistant Gram-negative bacteria

MS Spectra of Isogenic Strains of *S. aureus* (SCC*mec*- and PBP2a harboring MRSA or lacking MSSA)



Szabados et al, J Infection 2012; 65: 400-5

Detection of Carbapenamases



NaCl

NaCl + ertapenem

IMP-2

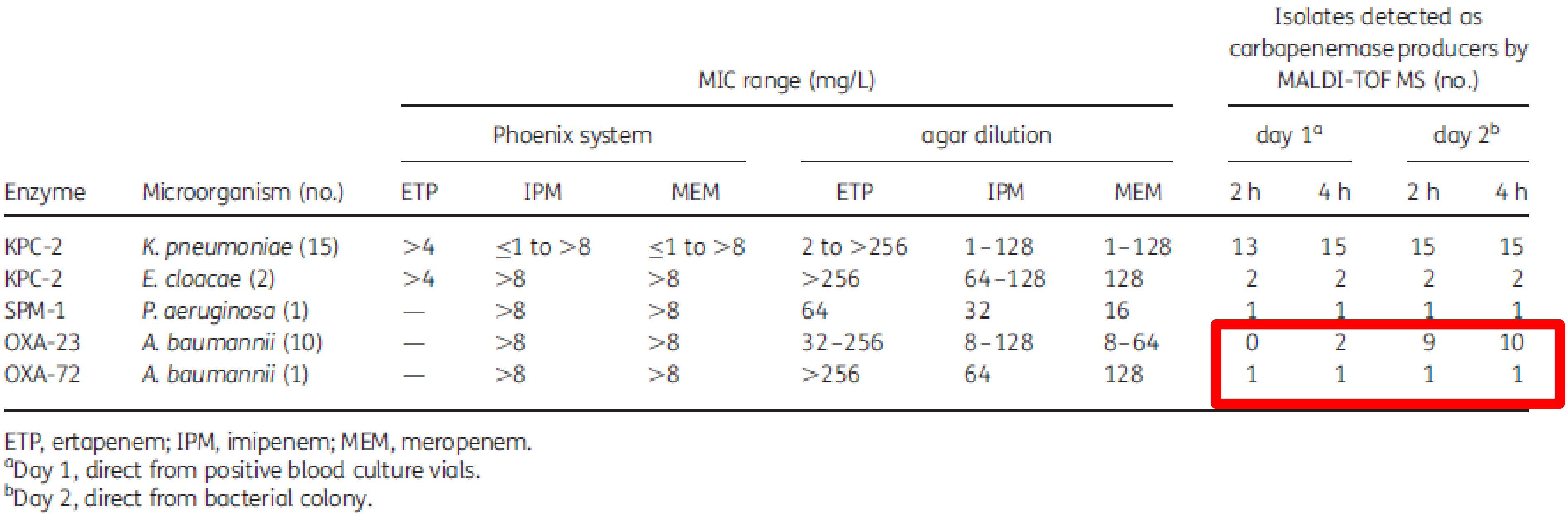
KPC-2

VIM-1

Loss of peaks at 476 Da, 498 Da and 521 Da

Burckhardt *et al.*, J Clin Microbiol 2011; 49: 3321-4

Carbapenem Susceptibility Profile and Time in Days for Detection of Carbapenemase-Producing Isolates by MALDI-TOF MS



		MIC range (mg/L)						Isolates detected as carbapenemase producers by MALDI-TOF MS (no.)			
		Phoenix system			agar dilution			day 1[a]		day 2[b]	
Enzyme	Microorganism (no.)	ETP	IPM	MEM	ETP	IPM	MEM	2 h	4 h	2 h	4 h
KPC-2	*K. pneumoniae* (15)	>4	≤1 to >8	≤1 to >8	2 to >256	1–128	1–128	13	15	15	15
KPC-2	*E. cloacae* (2)	>4	>8	>8	>256	64–128	128	2	2	2	2
SPM-1	*P. aeruginosa* (1)	—	>8	>8	64	32	16	1	1	1	1
OXA-23	*A. baumannii* (10)	—	>8	>8	32–256	8–128	8–64	0	2	9	10
OXA-72	*A. baumannii* (1)	—	>8	>8	>256	64	128	1	1	1	1

ETP, ertapenem; IPM, imipenem; MEM, meropenem.

[a] Day 1, direct from positive blood culture vials.

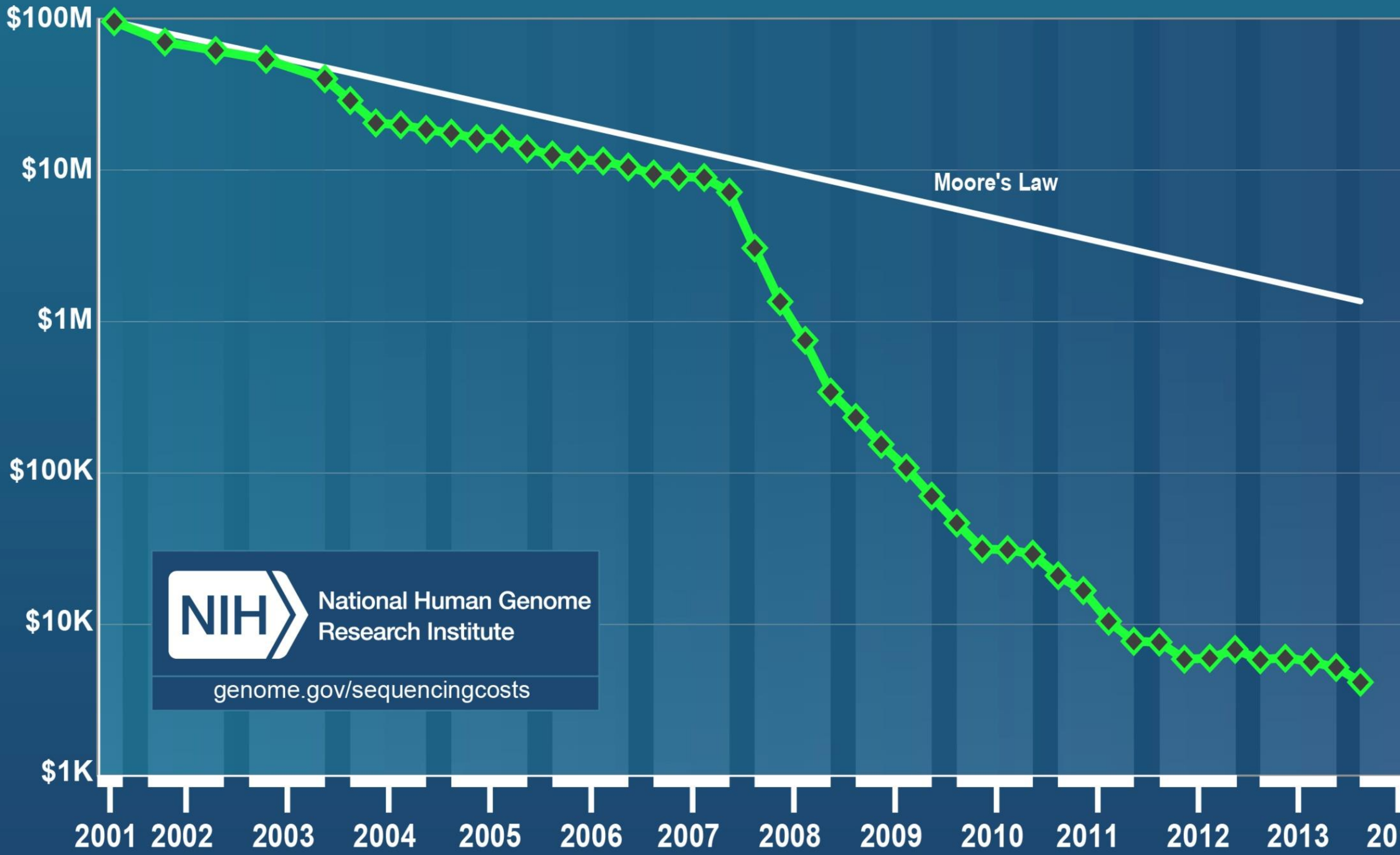
[b] Day 2, direct from bacterial colony.

Carvalhaes et al, J Antimicrob Chemother 2014; 69:2132-6

Agenda

- MALDI-TOF
- **Whole-Genome Sequencing**
- Microbiome
- Resistome
- Future

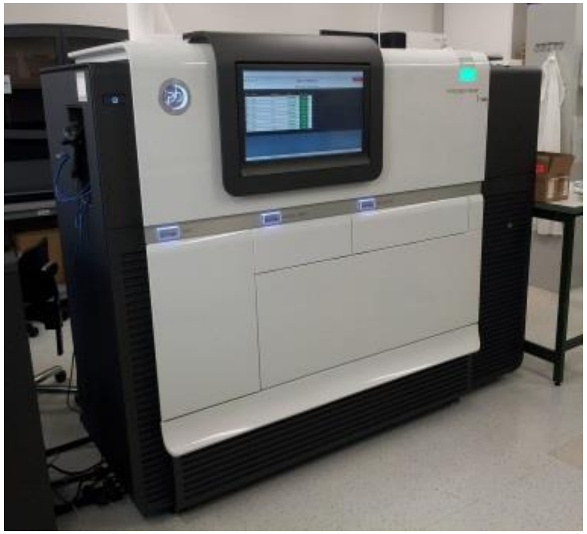
Cost per Genome (Human size)



National Human Genome Research Institute

genome.gov/sequencingcosts

NEXT GENERATION SEQUENCING



Whole-Genome Sequencing

- Detection of resistance
  - *S. aureus*
  - *P. aeruginosa*
- Typing
  - *Mycobacterium tuberculosis*
  - MRSA
  - *K. pneumoniae*
  - *A. baumannii*

WGS for *S. aureus* genetic resistance

- WGS: 992 strains collected in Brighton and Oxford
- Catalogue of known resistance genes and mutations in housekeeping genes assoc with resistance:
  - Pubmed → Genbank
  - 12 antibiotics
- Phenotypic resistance ("gold standard")
  - VITEK, disk diffusion
  - Discrepancies: E-test

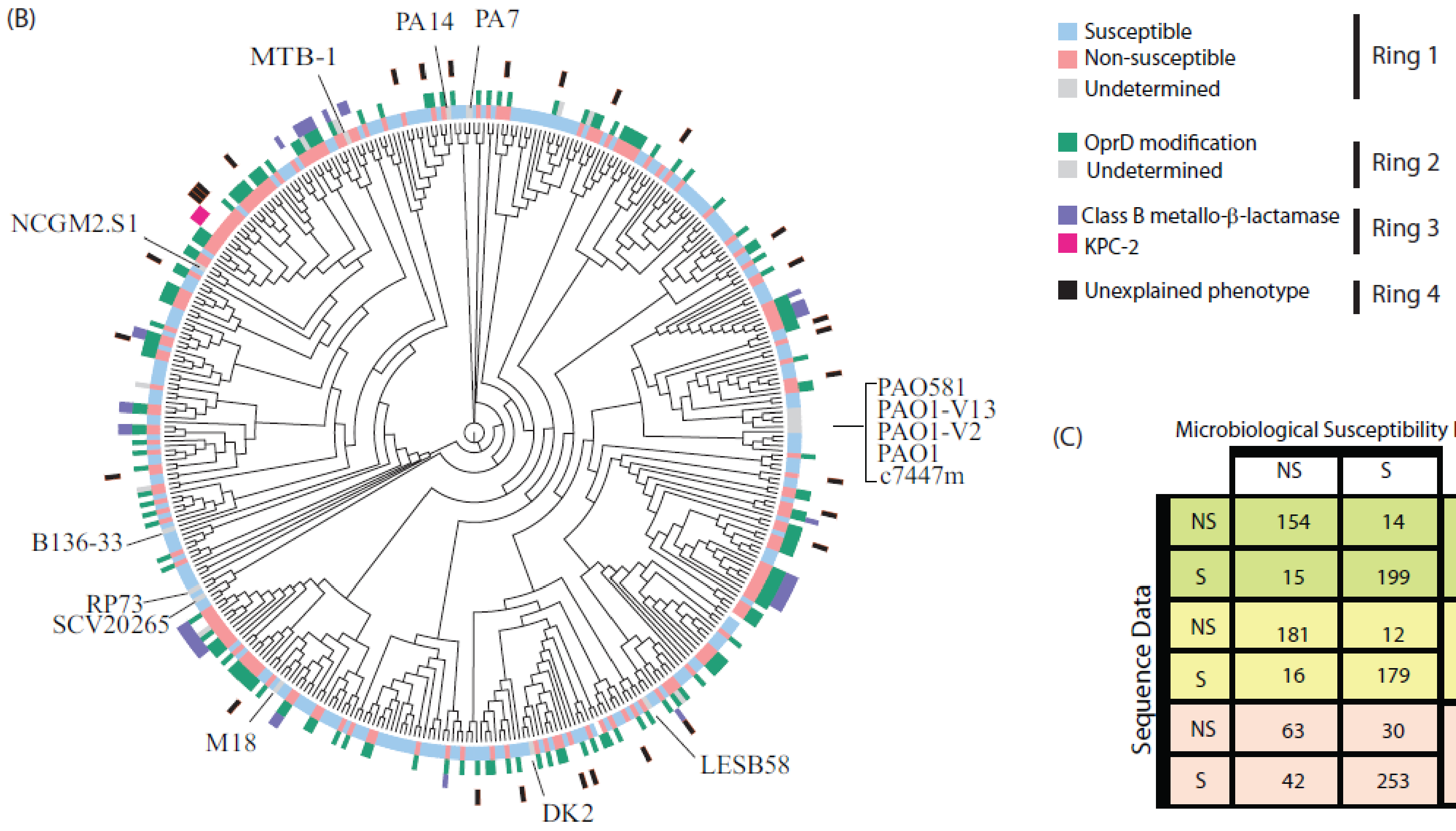
Gordon et al. J Clin Microbiol, 2014

BLINDED VALIDATION STUDY OF RESISTANCE PREDICTION FROM WGS *STAPHYLOCOCCUS AUREUS*

Derivation set of 501 and validation set of 491; here the final results of validation set after resolving discrepant results on 487 isolates

	Phenotype: resistant		**Phenotype: susceptible**		**Error Rates**	
	Genotype		**Genotype**		**ME (%)**	**VME (%)**
Antimicrobial	Susceptible	Resistant	Susceptible	Resistant		
Penicillin	2	398	84	3	3.4	0.5
Methicillin	0	55	432	0	0.0	0.0
Ciprofloxacin	2	64	421	0	0.0	3.0
Erythromycin	1	80	404	2	0.5	1.2
Clindamycin	1	76	2	0	0.0	1.3
Tetracycline	0	18	467	2	0.4	0.0
Vancomycin	0	0	491	0	0.0	n/a
Fusidic acid	1	39	445	0	0.0	2.6
Trimethoprim	0	2	200	1	0.5	0.0
Gentamicin	1	2	484	0	0.0	33.3
Mupirocin	0	2	485	0	0.0	0.0
Rifampicin	0	5	482	0	0.0	0.0
Total	8	741	4397	8	0.2	1.1

Comparison of the Phenotypic Susceptibility Profile with the Genetic Determinants that Classify *P. aeruginosa* Isolates as Susceptible or Non-Susceptible



(C)

Microbiological Susceptibility Data

Sequence Data		NS	S	
	NS	154	14	Meropenem
	S	15	199	
	NS	181	12	Levofloxacin
	S	16	179	
	NS	63	30	Amikacin
	S	42	253	

Kos et al, Antimicrob Agents Chemother 2014

Conclusions of WGS for Detection of Resistance

Limitations:

- Gene may be present, but not expressed due to unidentified alterations in regulatory region
- Alternative resistance mechanisms cannot be detected
- Complex mechanims of resistance may be difficult to detect

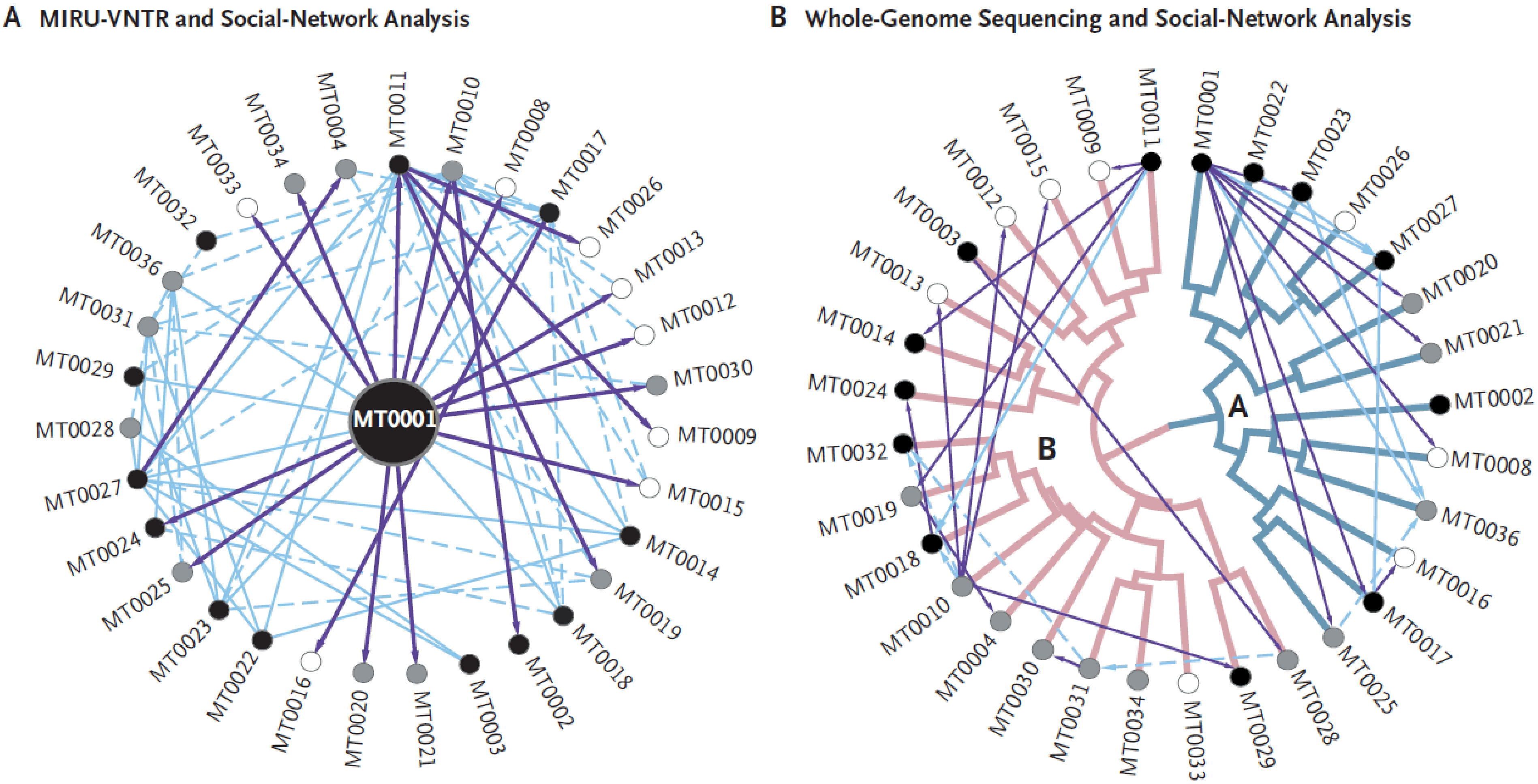
Expectations:

- Cost and turnaround time will further decrease
- WGS will replace majority of phenotypic and genotypic susceptibility tests as more genetic information becomes available

Whole-Genome Sequencing

- Detection of resistance
  - *S. aureus*
  - *P. aeruginosa*
- **Typing**
  - ***Mycobacterium tuberculosis***
  - **MRSA**
  - ***K. pneumoniae***
  - ***A. baumannii***

Putative Transmission Networks Constructed from Genotyped Data versus Whole-Genome Data of 32 Tuberculosis Patients

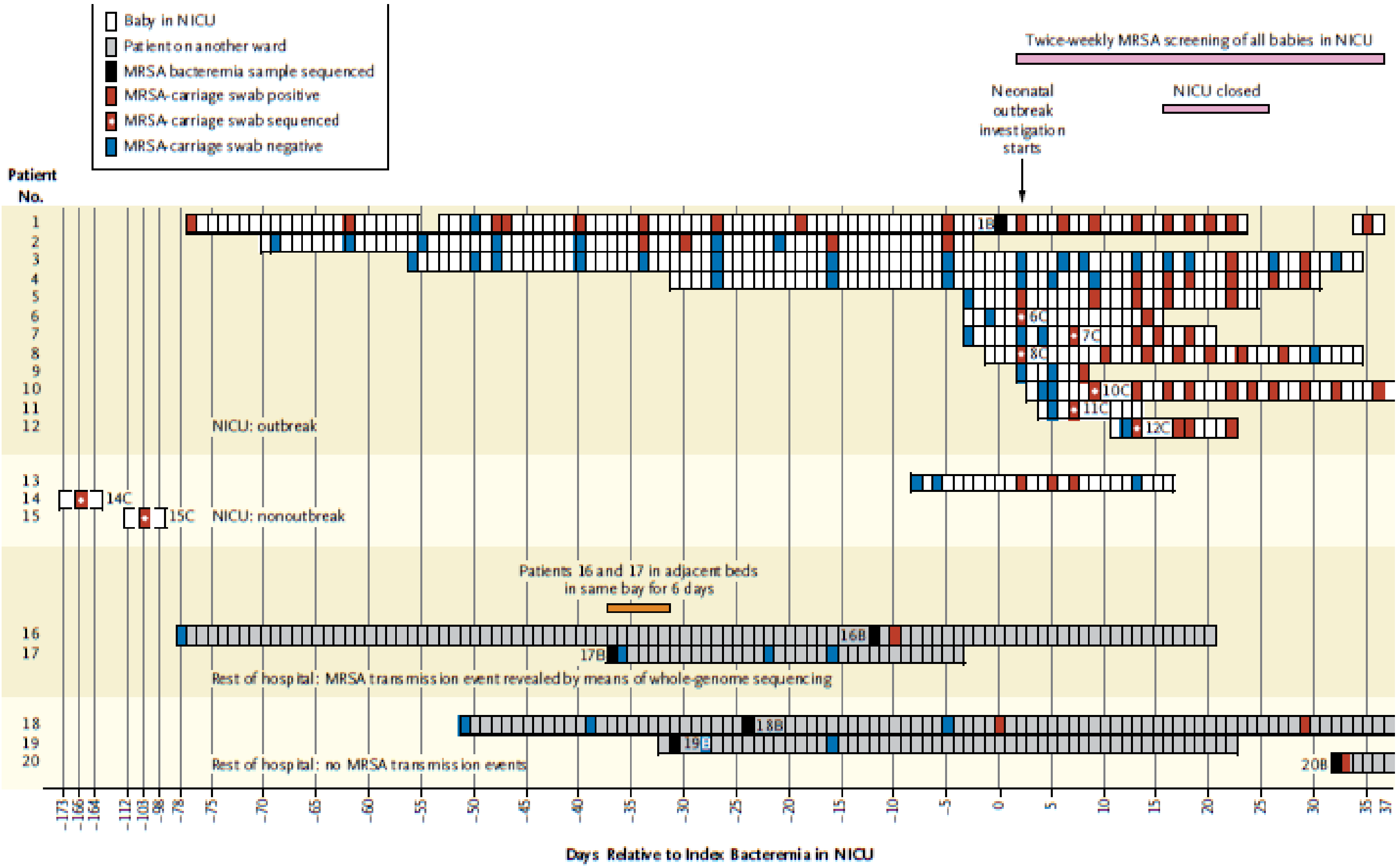


A MIRU-VNTR and Social-Network Analysis

B Whole-Genome Sequencing and Social-Network Analysis

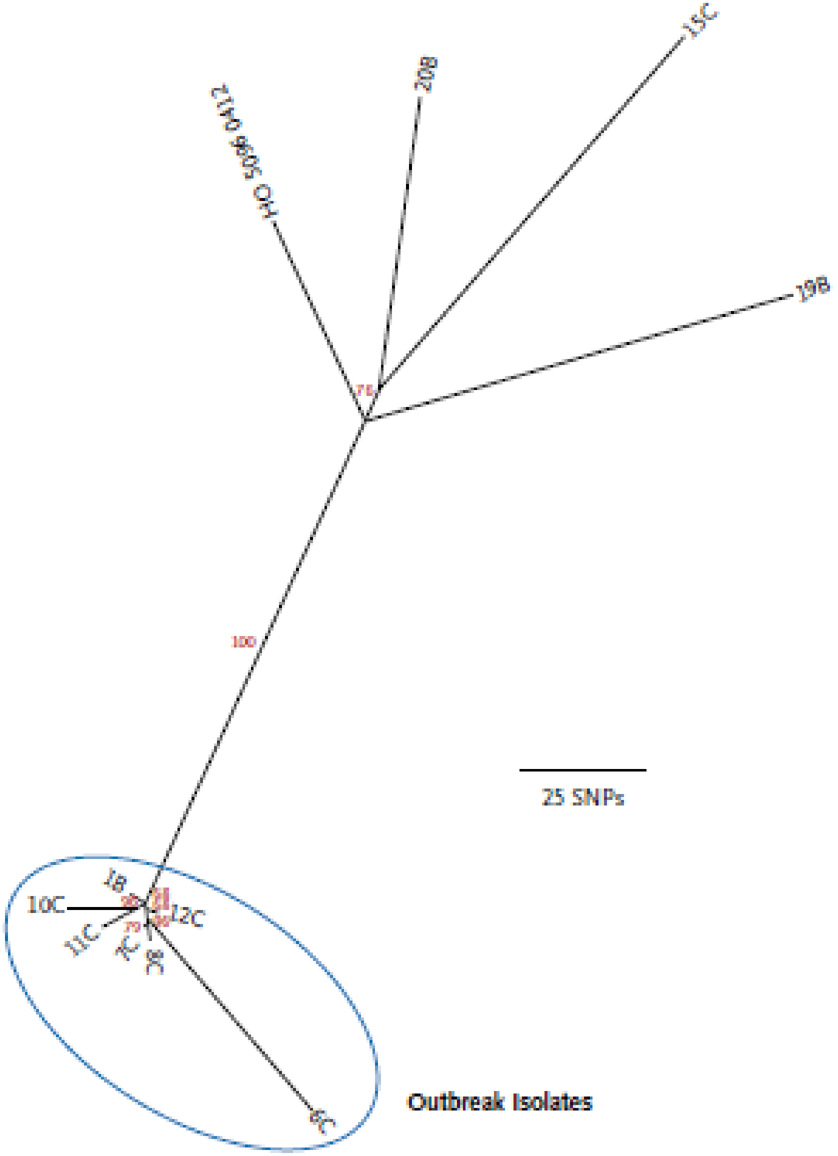
Gardy et al, NEJM 2011; 364:730-9

Timeline of the Neonatal ICU MRSA Outbreak and MRSA Bacteremia Cases on Other Wards



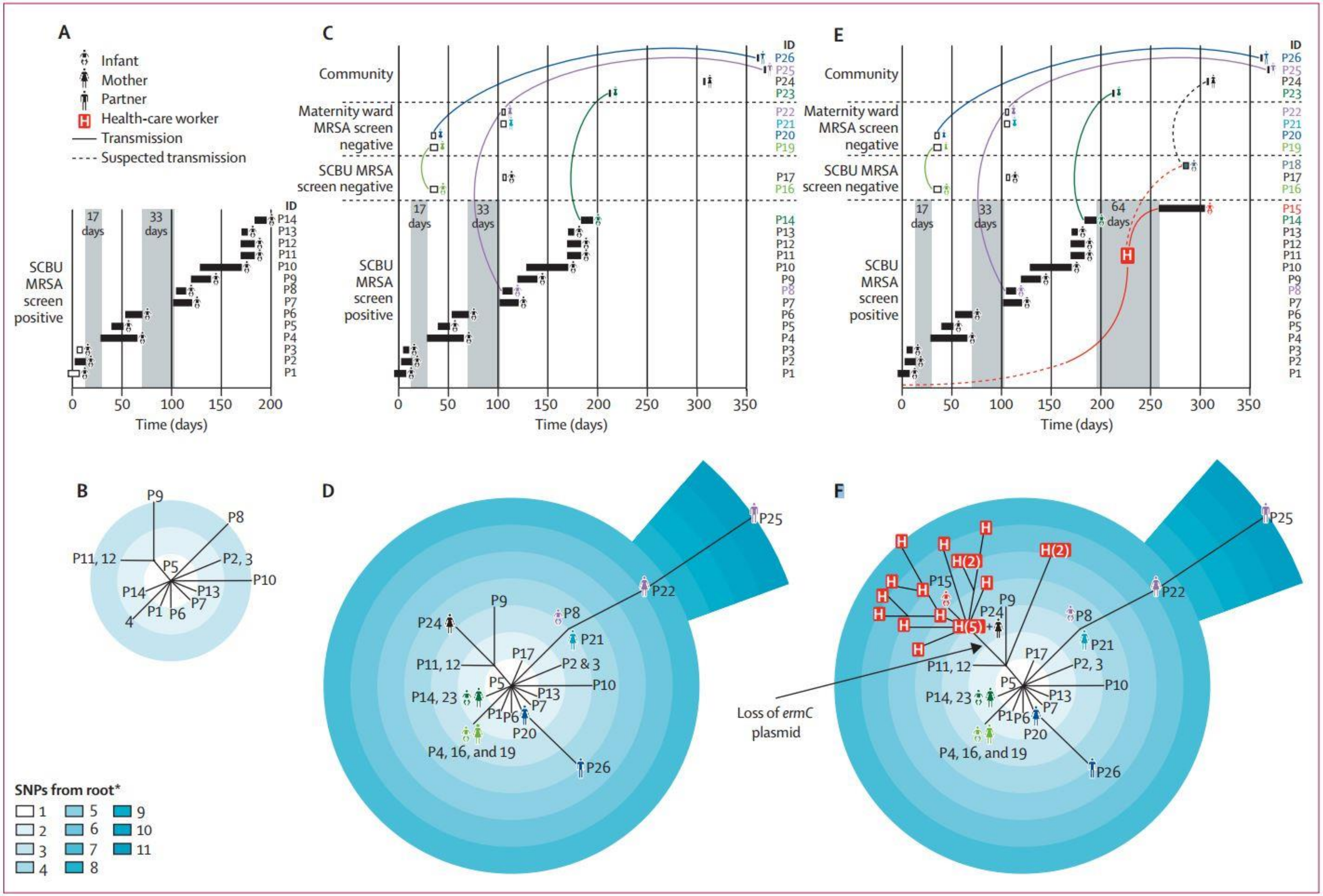
Köser et al, NEJM 2012; 366: 2267-75

Results of the Phylogenetic Analysis of the 10 MRSA Isolates of Sequence Type 22



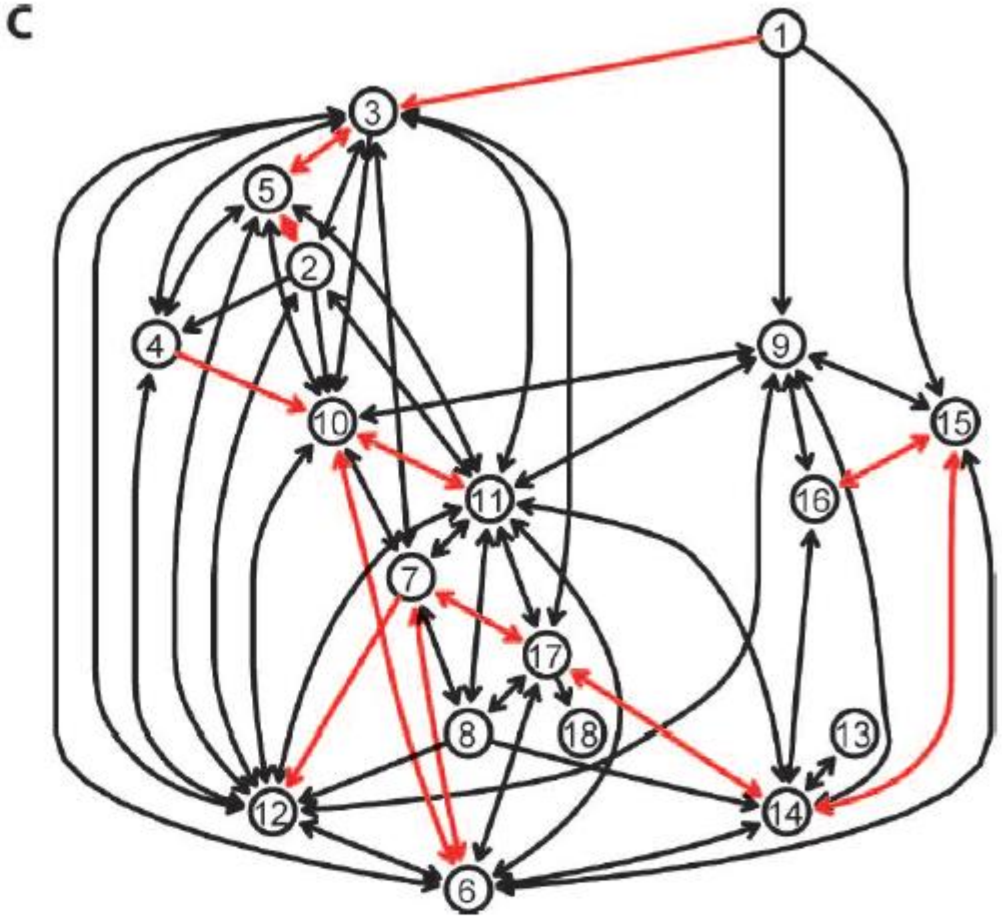
Köser et al, NEJM 2012; 366: 2267-75

Epidemiology and Phylogeny of an Outbreak of MRSA Sequence Type 2371 at a Baby Unit



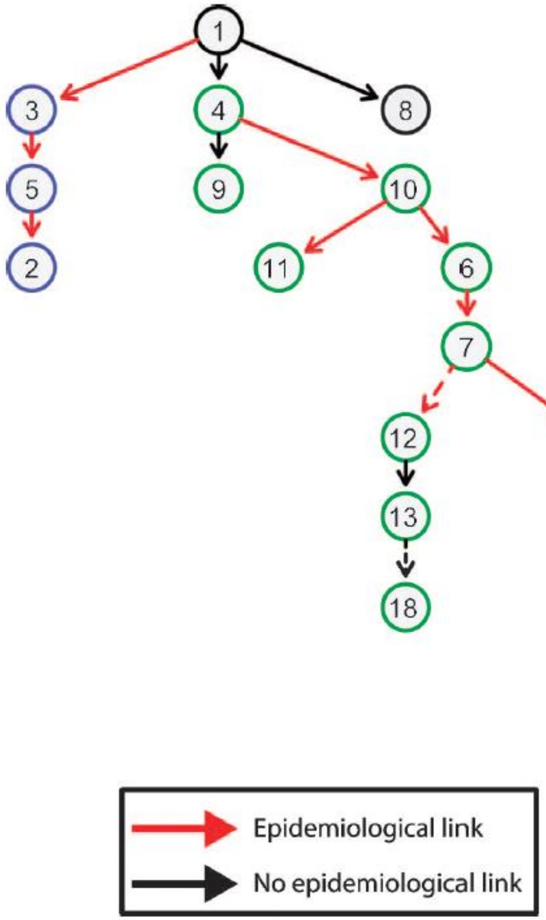
Harris LID 2013; 13: 130-36

Graph of Possible Transmission Links among Patients Infected with Carbapenem-Resistant *Klebsiella pneumoniae* during a Hospital Outbreak



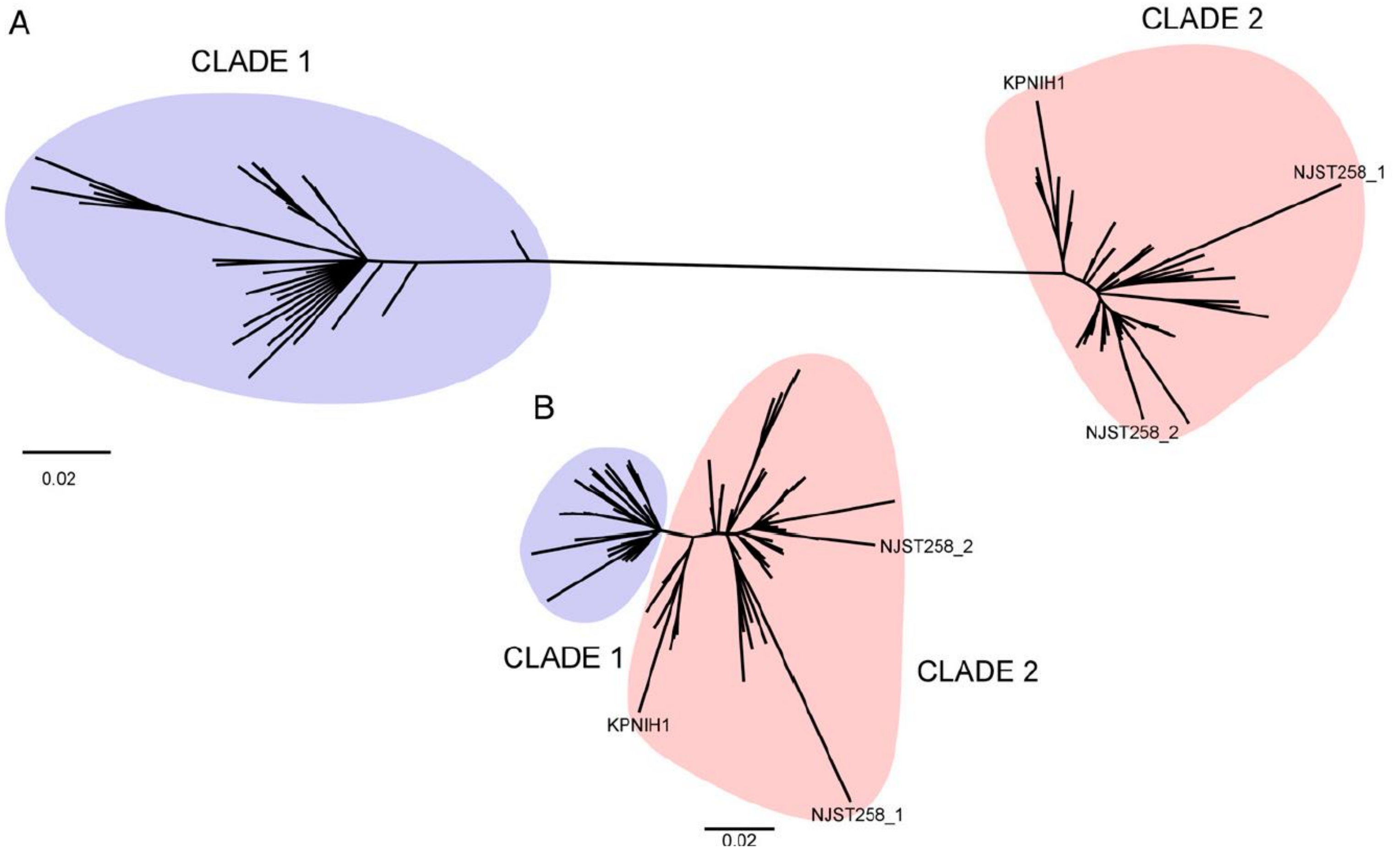
Snitkin et al, Science Transl Med 2012

Putative Map of *K. pneumoniae* Transmission Routes during an Outbreak Based on WGS and Patient Data



Snitkin et al, Science Transl Med 2012

SNP-Based Phylogenetic Analysis of Carbapenem-Resistant *K. pneumoniae* ST258 Clinical Isolates



DeLeo et al, PNAS 2014; 111:4988-4993

Microscale Evolution of *A. baumannii* within the Host

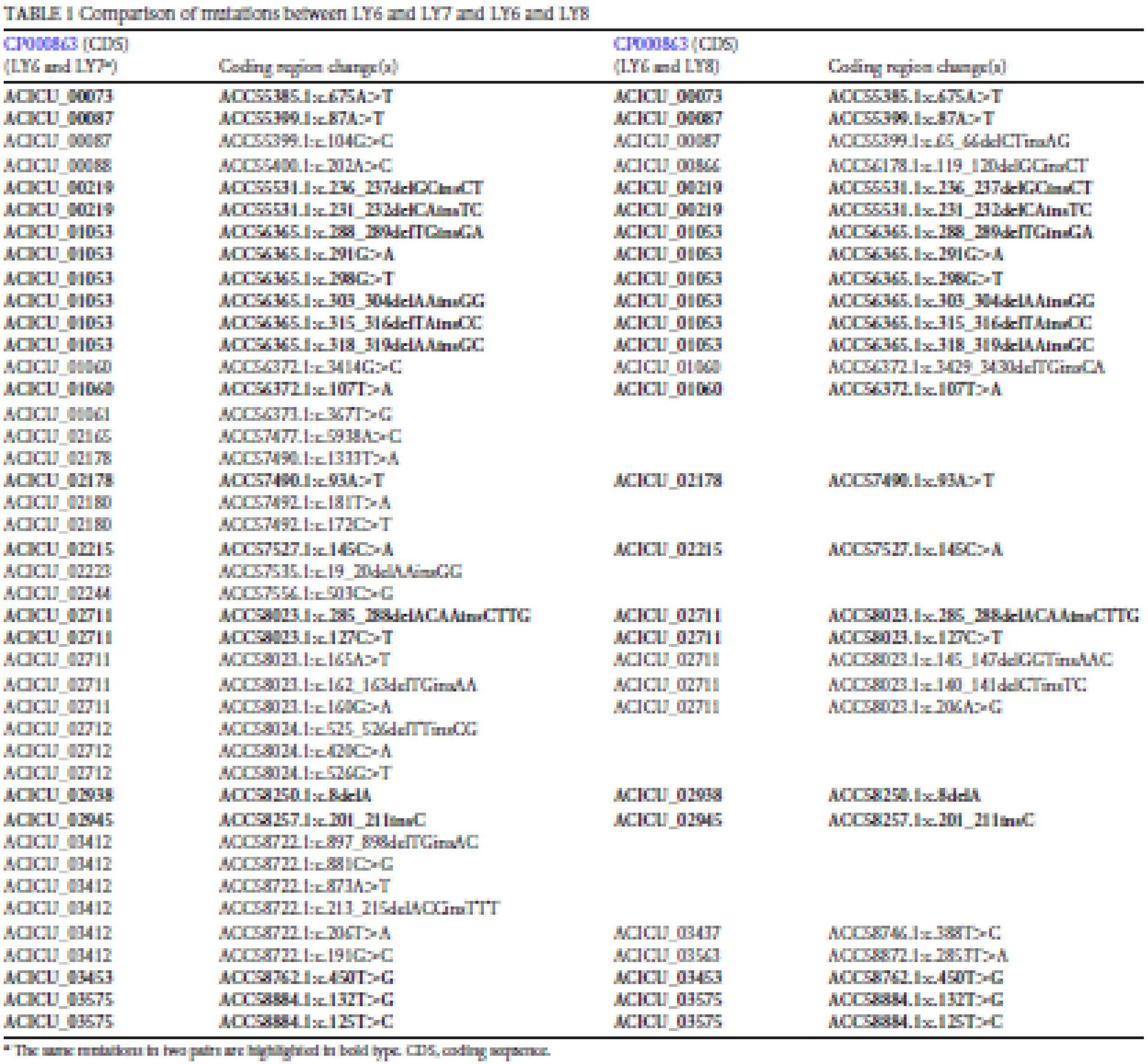


TABLE 1 Comparison of mutations between LY6 and LY7 and LY6 and LY8

CP000863 (CDS) (LY6 and LY7[a])	Coding region change(s)	CP000863 (CDS) (LY6 and LY8)	Coding region change(s)
ACICU_00073	**ACC55385.1:c.675A>T**	**ACICU_00073**	**ACC55385.1:c.675A>T**
ACICU_00087	**ACC55399.1:c.87A>T**	**ACICU_00087**	**ACC55399.1:c.87A>T**
ACICU_00087	ACC55399.1:c.104G>C	ACICU_00087	ACC55399.1:c.65_66delCTinsAG
ACICU_00088	ACC55400.1:c.202A>C	ACICU_00866	ACC56178.1:c.119_120delGCinsCT
ACICU_00219	**ACC55531.1:c.236_237delGCinsCT**	**ACICU_00219**	**ACC55531.1:c.236_237delGCinsCT**
ACICU_00219	**ACC55531.1:c.231_232delCAinsTC**	**ACICU_00219**	**ACC55531.1:c.231_232delCAinsTC**
ACICU_01053	**ACC56365.1:c.288_289delTGinsGA**	**ACICU_01053**	**ACC56365.1:c.288_289delTGinsGA**
ACICU_01053	**ACC56365.1:c.291G>A**	**ACICU_01053**	**ACC56365.1:c.291G>A**
ACICU_01053	**ACC56365.1:c.298G>T**	**ACICU_01053**	**ACC56365.1:c.298G>T**
ACICU_01053	**ACC56365.1:c.303_304delAAinsGG**	**ACICU_01053**	**ACC56365.1:c.303_304delAAinsGG**
ACICU_01053	**ACC56365.1:c.315_316delTAinsCC**	**ACICU_01053**	**ACC56365.1:c.315_316delTAinsCC**
ACICU_01053	**ACC56365.1:c.318_319delAAinsGC**	**ACICU_01053**	**ACC56365.1:c.318_319delAAinsGC**
ACICU_01060	ACC56372.1:c.3414G>C	ACICU_01060	ACC56372.1:c.3429_3430delTGinsCA
ACICU_01060	**ACC56372.1:c.107T>A**	**ACICU_01060**	**ACC56372.1:c.107T>A**
ACICU_01061	ACC56373.1:c.367T>G		
ACICU_02165	ACC57477.1:c.5938A>C		
ACICU_02178	ACC57490.1:c.1333T>A		
ACICU_02178	**ACC57490.1:c.93A>T**	**ACICU_02178**	**ACC57490.1:c.93A>T**
ACICU_02180	ACC57492.1:c.181T>A		
ACICU_02180	ACC57492.1:c.172C>T		
ACICU_02215	**ACC57527.1:c.145C>A**	**ACICU_02215**	**ACC57527.1:c.145C>A**
ACICU_02223	ACC57535.1:c.19_20delAAinsGG		
ACICU_02244	ACC57556.1:c.503C>G		
ACICU_02711	**ACC58023.1:c.285_288delACAAinsCTTG**	**ACICU_02711**	**ACC58023.1:c.285_288delACAAinsCTTG**
ACICU_02711	**ACC58023.1:c.127C>T**	**ACICU_02711**	**ACC58023.1:c.127C>T**
ACICU_02711	ACC58023.1:c.165A>T	ACICU_02711	ACC58023.1:c.145_147delGGTinsAAC
ACICU_02711	ACC58023.1:c.162_163delTGinsAA	ACICU_02711	ACC58023.1:c.140_141delCTinsTC
ACICU_02711	ACC58023.1:c.160G>A	ACICU_02711	ACC58023.1:c.206A>G
ACICU_02712	ACC58024.1:c.525_526delTTinsCG		
ACICU_02712	ACC58024.1:c.420C>A		
ACICU_02712	ACC58024.1:c.526G>T		
ACICU_02938	**ACC58250.1:c.8delA**	**ACICU_02938**	**ACC58250.1:c.8delA**
ACICU_02945	**ACC58257.1:c.201_211insC**	**ACICU_02945**	**ACC58257.1:c.201_211insC**
ACICU_03412	ACC58722.1:c.897_898delTGinsAC		
ACICU_03412	ACC58722.1:c.881C>G		
ACICU_03412	ACC58722.1:c.873A>T		
ACICU_03412	ACC58722.1:c.213_215delACGinsTTT		
ACICU_03412	ACC58722.1:c.206T>A	ACICU_03437	ACC58746.1:c.388T>C
ACICU_03412	ACC58722.1:c.191G>C	ACICU_03563	ACC58872.1:c.2853T>A
ACICU_03453	**ACC58762.1:c.450T>G**	**ACICU_03453**	**ACC58762.1:c.450T>G**
ACICU_03575	**ACC58884.1:c.132T>G**	**ACICU_03575**	**ACC58884.1:c.132T>G**
ACICU_03575	**ACC58884.1:c.125T>C**	**ACICU_03575**	**ACC58884.1:c.125T>C**

[a] The same mutations in two pairs are highlighted in bold type. CDS, coding sequence.

Wen et al, J Clin Microbiol 2014; 52:3200-8

Conclusions of WGS for Typing

- WGS can allow for the identification of (unexpected) modes of transmission
- WGS may distinguish between alternative transmission scenarios which may be critical for controlling the outbreak
- WGS can link to environmental isolates which may provide insight as to how and when contamination occurred
- WGS can provide insight into how bacterial populations respond to treatments and the evolution of resistance
- WGS can help to understand or predict host response

Expectations of WGS for Typing

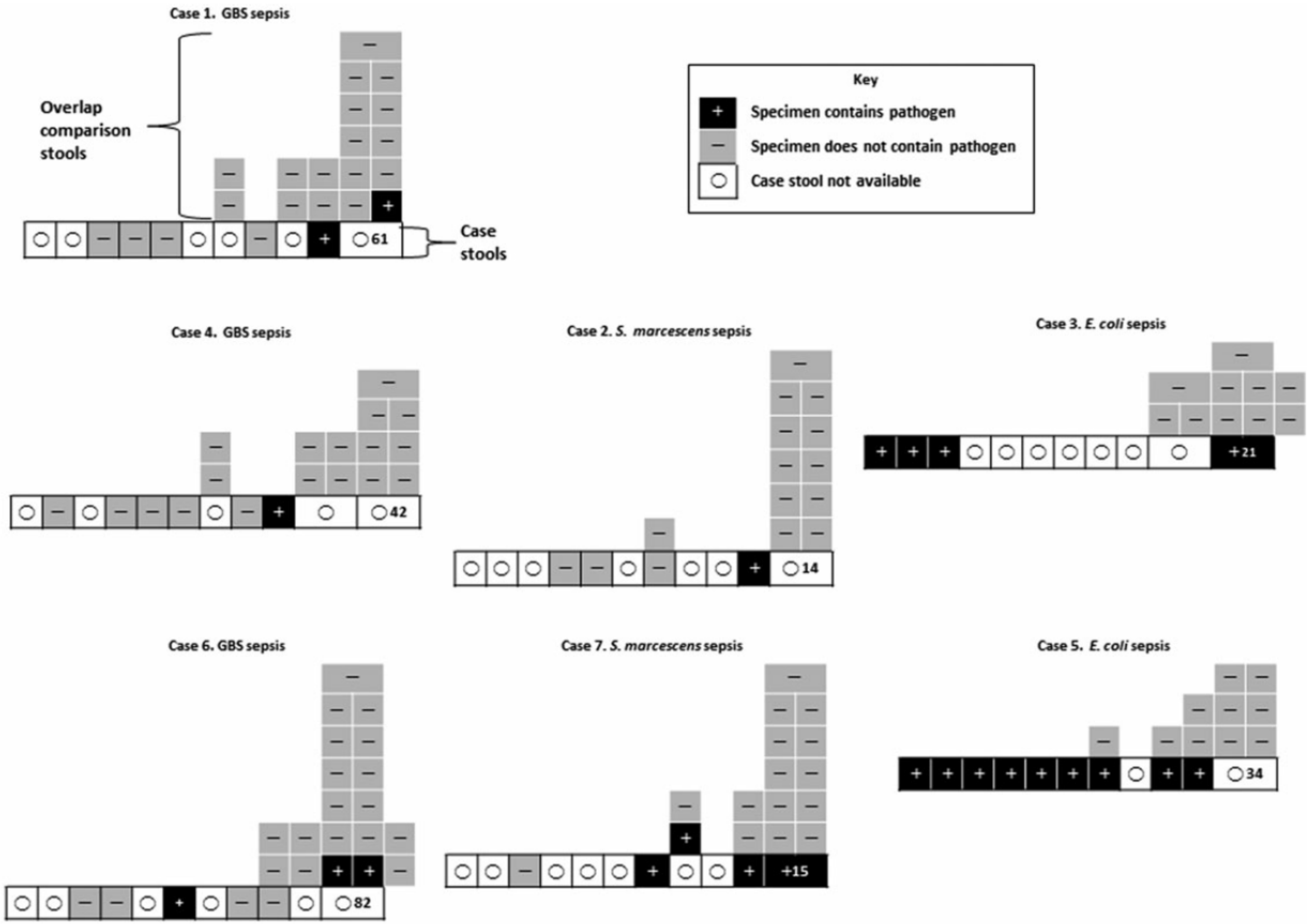
WGS will transform healthcare epidemiology and public health provided that:

- WGS data is validated versus current methods
- Data are provided real-time
- At affordable cost
- Solutions for processing and storage of data
- Clinically relevant information is provided
- Data Interpretation is fully automated and can be done without specialist knowledge

Agenda

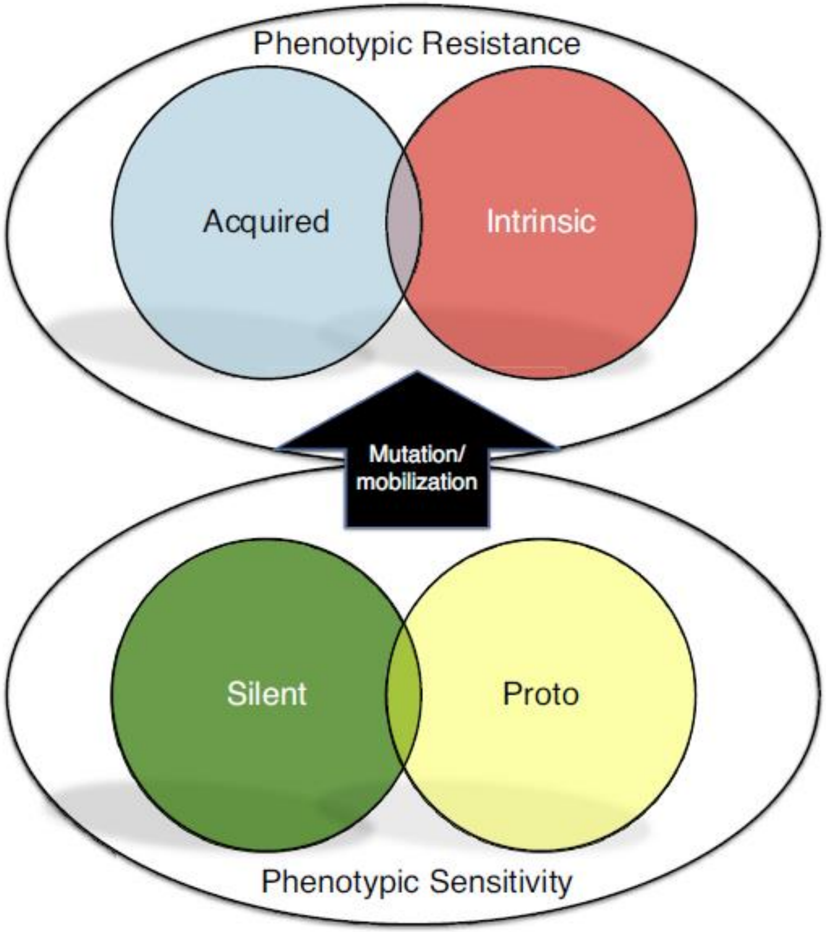
- MALDI-TOF
- Whole-Genome Sequencing
- **Microbiome**
- **Resistome**
- Future

Gut Microbiome Predicts Bacteremia and Helps Preventing Spread of Organisms



Carl et al, CID 2014; 58:1211-8

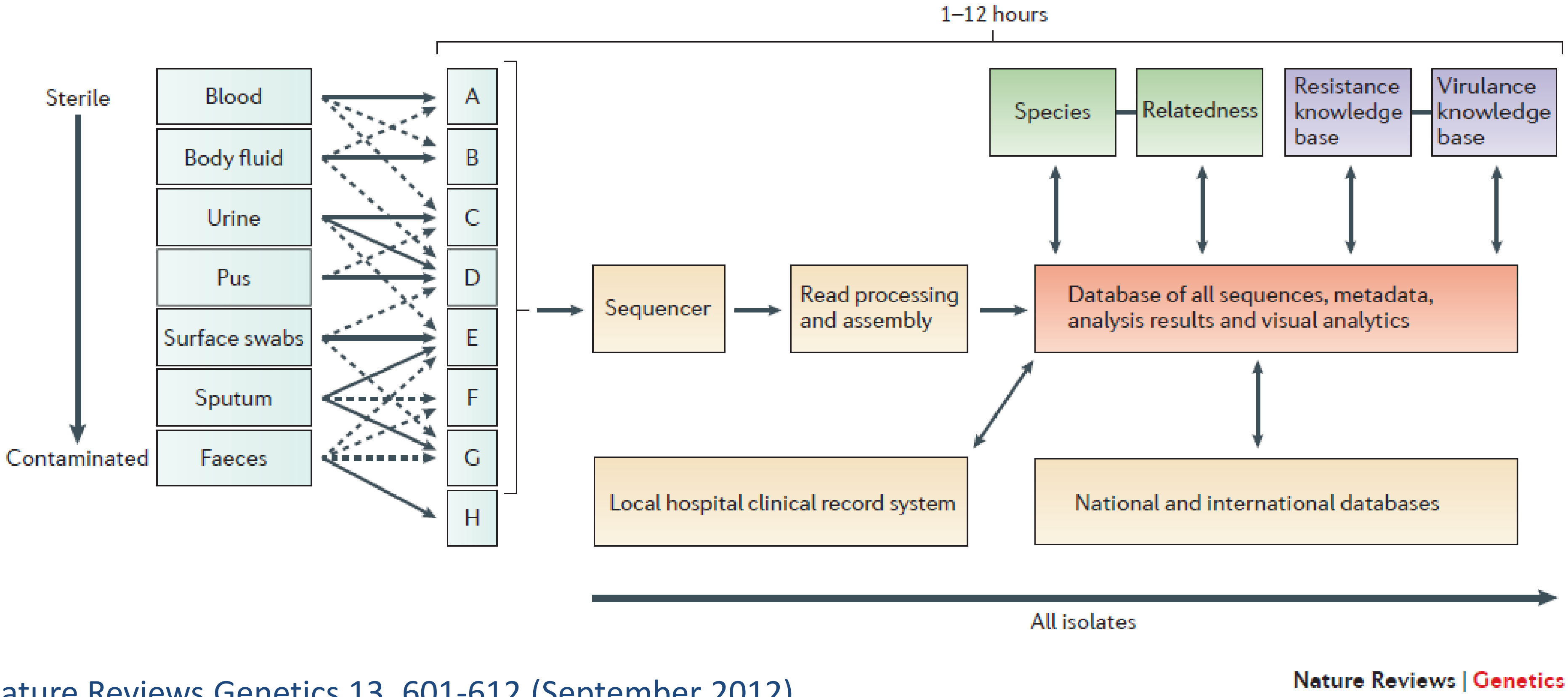
The Antibiotic Resistome



Perry et al, Curr Opinion Microbiol 2014; 21: 45-50

Future Practice

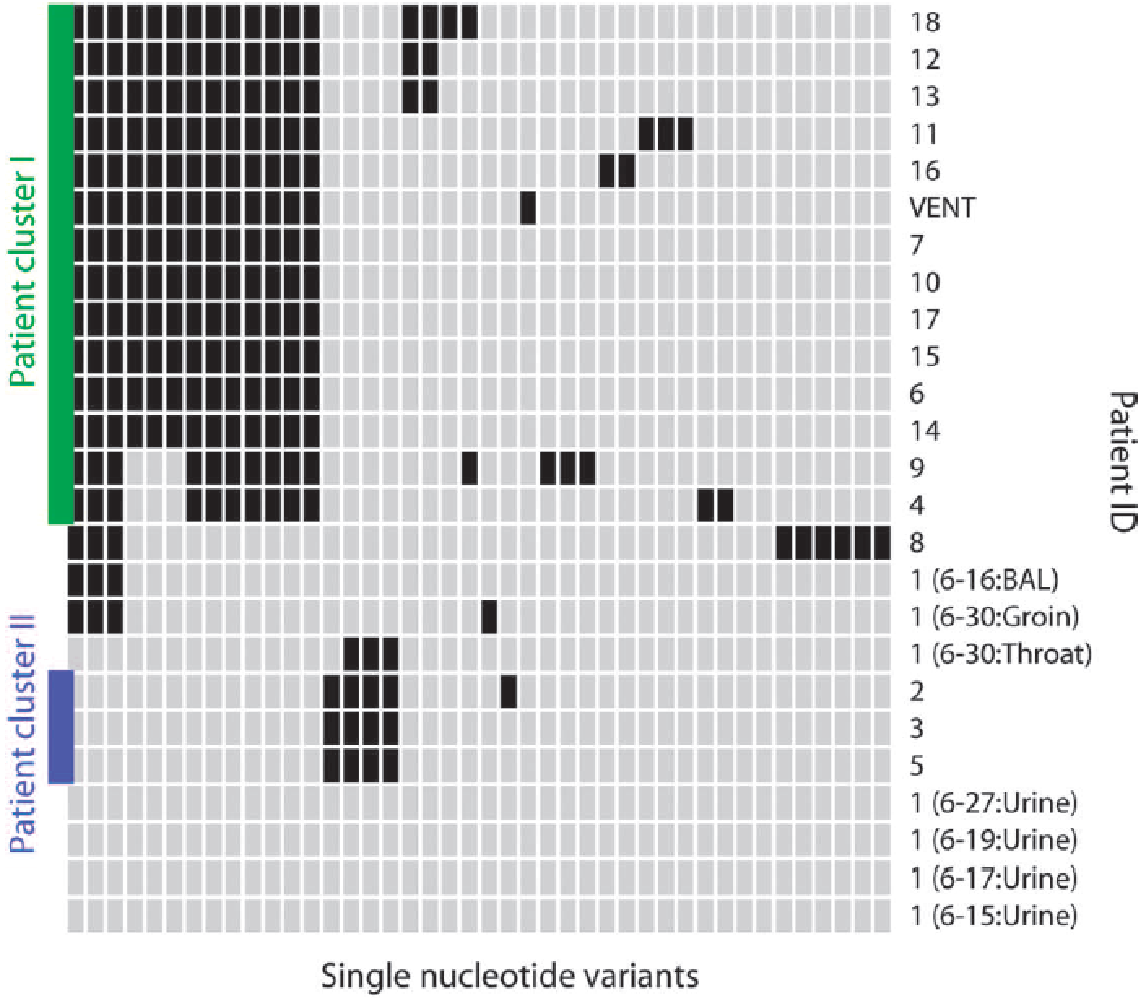
IN ONE STEP GENERATE THE COMPLETE DIAGNOSTIC, TYPING AND SURVEILLANCE INFORMATION



Nature Reviews Genetics 13, 601-612 (September 2012)

Thank You

Single Nucleotide Variants Identified in *K. pneumoniae* Genomes from Different Patients and Body Sites



Snitkin et al, Science Transl Med 2012