



Bridging the gaps in Bioinformatics

Genetic characterization of *Staphylococcus aureus*

<May 2 2023>

Objectives

Specific objectives of this session:

- Learn about *S. aureus* as a human commensal and pathogen
- Learn about MRSA virulence and pathogenesis
- Learn about MRSA typing and evolution
- Learn how to apply typing of MRSA for public health purposes

Related to the course objectives

Learn about bioinformatics tools applied in public health

Outline

This session consists of the following elements

- *S. aureus* as a human commensal and pathogen
 - Resistance to antimicrobials- beta-lactams
 - Typing of MRSA for public health purposes
 - *SCCmec*
 - Pathogenesis and virulence
 - Evolution of MRSA
1. Group exercise integrating bioinformatic tools to annotate *S. aureus* genomes and interpretation of results in a public health context

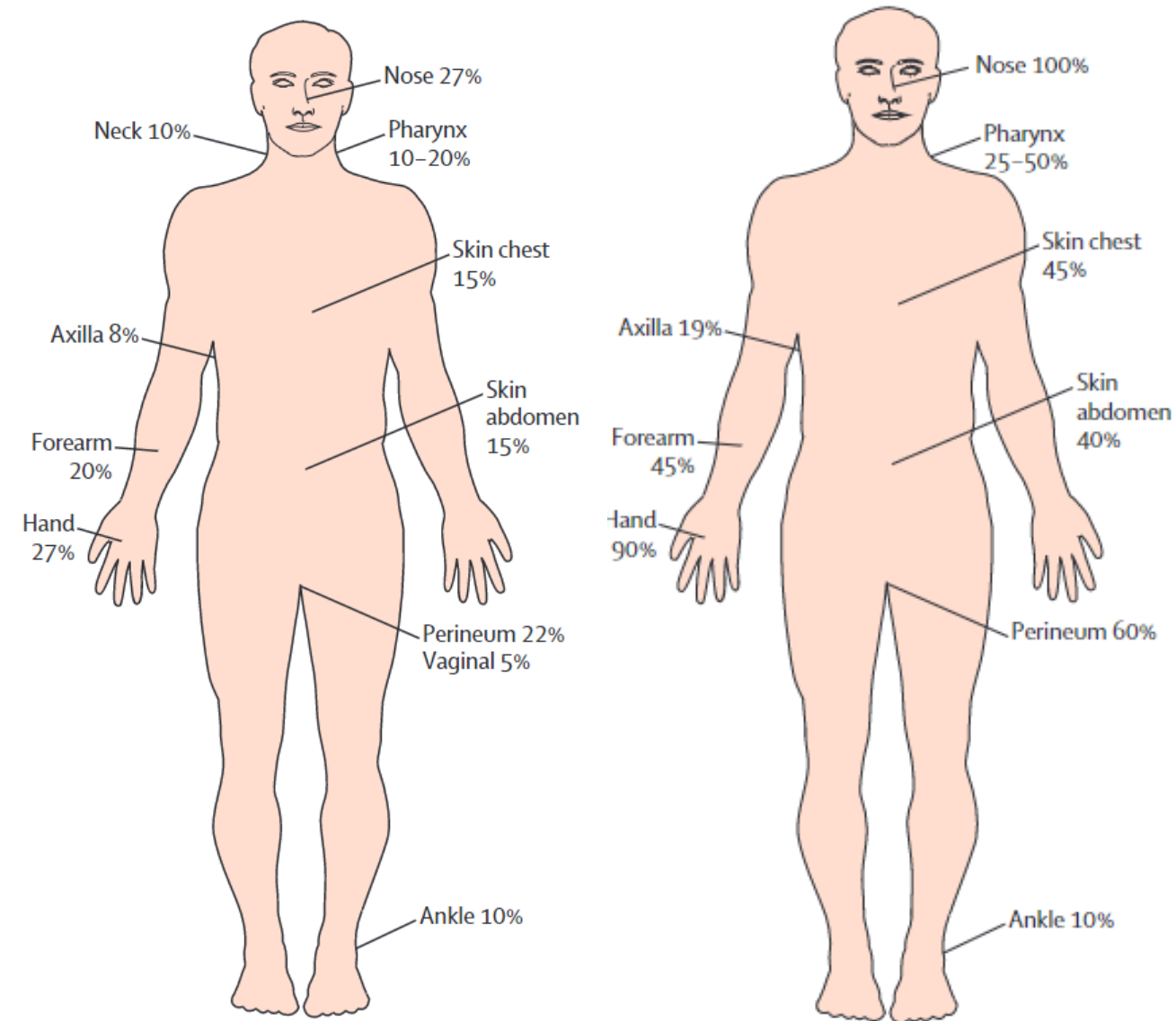


Outline

- *S. aureus* as a human commensal and pathogen
- Resistance to antimicrobials- beta-lactams
- Typing of MRSA for public health purposes
- *SCCmec*
- Pathogenesis and virulence
- Evolution of MRSA

ILOs: after the talk you will have a overview of MRSA typing, genetics and evolution

Colonization



Frequency of *S. aureus* carriage

- 20 % permanent (colonization)
 - 30 % intermittent (contamination)
 - 50 % never
-
- Human Genetics,
 - Microbial genetics
 - microbial community state types?
 - Colonizing strain cause infection

S. aureus infections

Skin and soft tissue infections

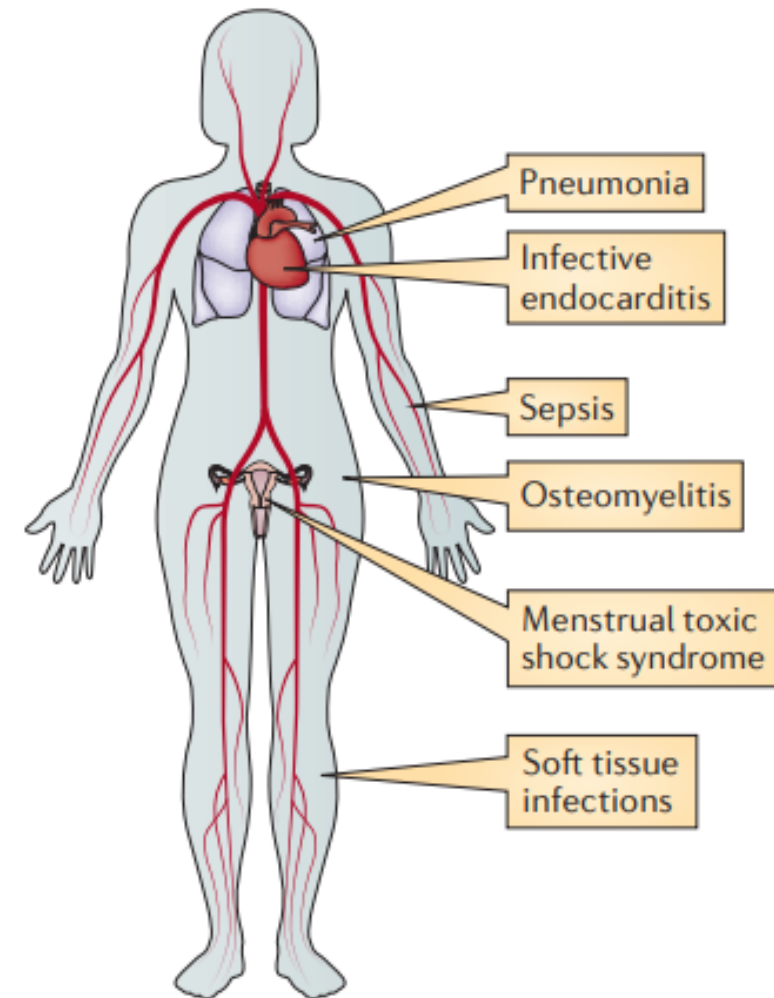
- Impetigo, furunculosis, abscesses
- **Wound infections, SSSS**

Severe infections

- BSI (30 day all course fatality: ca. 25%)
- Pneumonia, endocarditis, osteomyelitis

Toxic diseases

- Toxic shock syndrome
- Food poisoning



Virulence factors

- Adhesins- MSCRAMMs

(microbial surface components recognizing adhesion matrix molecules)

fibrinogen-, collagen- binding proteins

- Extracellular enzymes

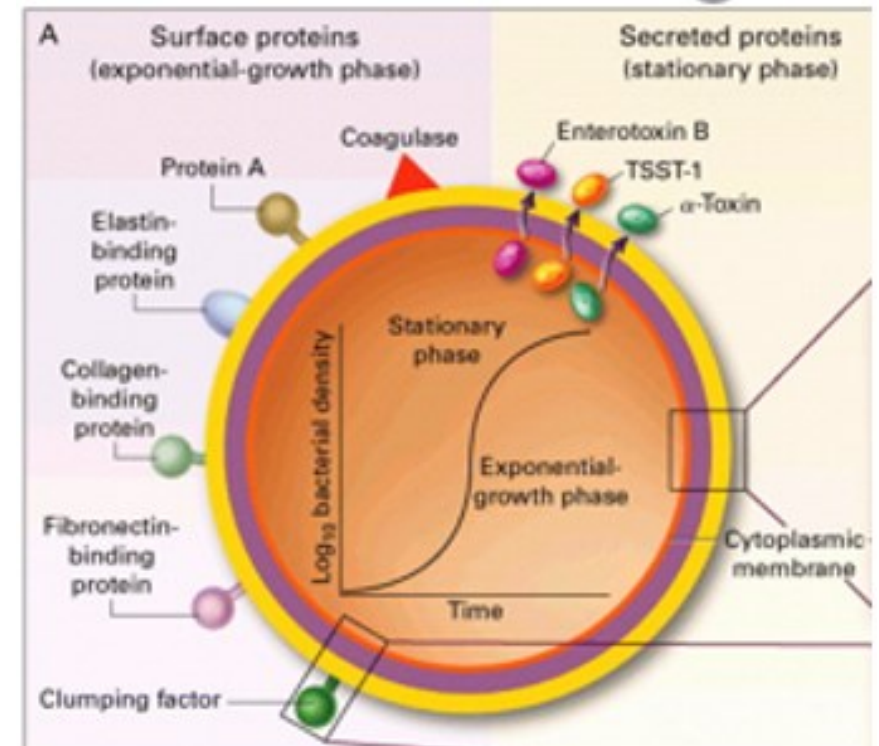
– coagulases, proteases, hemolysins

- Toxins

TSST, Eta/b, enterotoxins, PVL

- Immune evasion

Protein A binds AB fc-region, IEC: sak,chp,scn



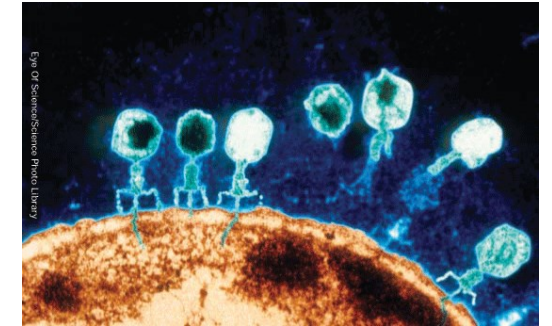
Virulence factors

Mobile Genetic elements (MGEs) and horizontal gene transfer (HGT)

- conjugation of conjugative plasmids
- transduction by bacteriophage

Restriction- modification (RM) barriers prevent HGT

RM systems are “lineage specific”



Consequently many virulence factors are lineage specific

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Username
Password

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VirulenceFinder 1.5

View the [version history](#) of this server.

The database is curated by:
Flemming Scheutz, SSI
([click to contact](#))

Select species

Listeria
S. aureus
Escherichia coli
Enterococcus

Select threshold for %ID


90 %

Select minimum length

60 %

Select type of your reads

Assembled Genome/Contigs*

 Isolate File

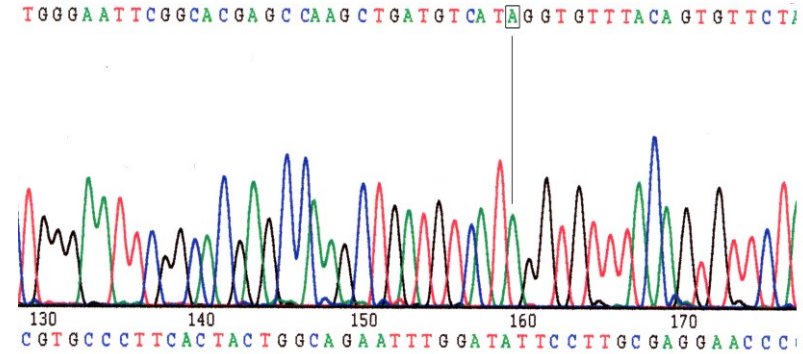
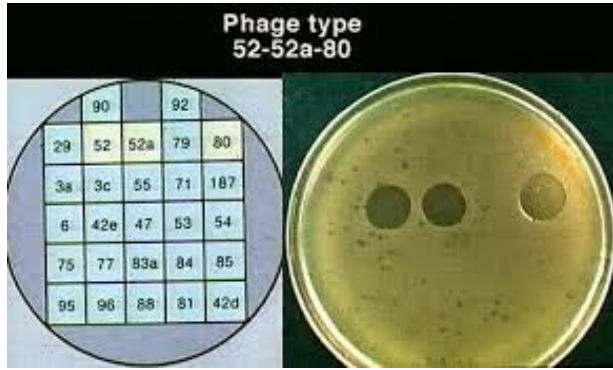
| Name | Size | Progress | Status |
|------|------|----------|--------|
| | | | |

 Upload

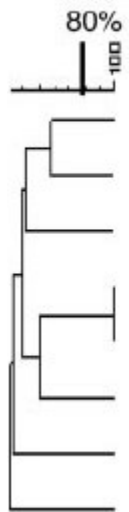
 Remove

Typing of *Staphylococcus aureus*

From Phages to WGS



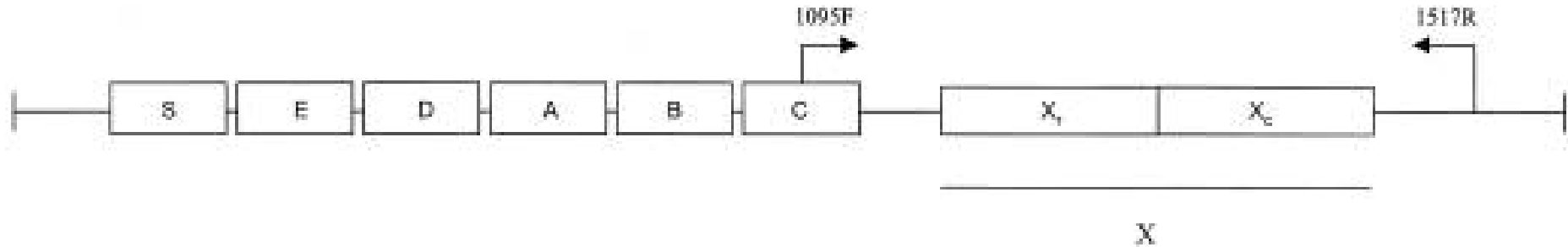
Typing Method



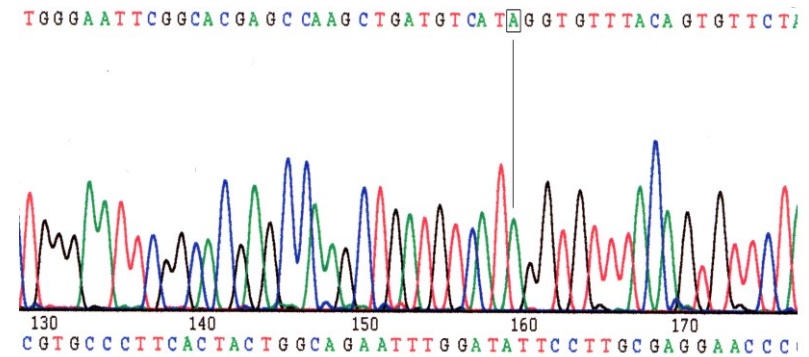
| MLVA | PFGE | Type |
|-------------------|-------------------|---------------|
| [banding pattern] | [banding pattern] | USA700 |
| [banding pattern] | [banding pattern] | USA200 |
| [banding pattern] | [banding pattern] | USA600 |
| [banding pattern] | [banding pattern] | USA300 |
| [banding pattern] | [banding pattern] | USA500 |
| [banding pattern] | [banding pattern] | USA1100 |
| [banding pattern] | [banding pattern] | USA100 |
| [banding pattern] | [banding pattern] | USA400 |



Spa typing



Staphylocococcus protein A- Surface protein
–Variable X region
1 to 25 repeats
No, sequence and succession determines the
spa type



Overview

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Background

Server Policy

Database

Frequencies

Spa-types

Repeats

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Privacy Policy

Imprint

Ridom SpaServer

SpaServer Database

| | |
|-------------------|--------|
| Spa-types: | 21092 |
| Repeats: | 836 |
| Total strains: | 458782 |
| Strain records: | 190478 |
| Strain countries: | 162 |
| Registered users: | 897 |
| User countries: | 71 |

conceptualized and implemented by **Ridom GmbH**



sequence data curated by **SeqNet.org**



Result: Spa-type: t044

Spa-Type Repeats Succession: 07-23-12-34-34-33-34

(Kreiswirth IDs: UJGBBPB)

SpaTyper- CGE tool



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spaTyper 1.0

Software version: (.)

Database version: ([2023-05-01](#))

Sequencing Platform Select the sequencing platform used to generate the uploaded reads. (Note preassembled reads)

Due to CPU requirements for assembly this tool will only allow preassembled reads as input

Assembled Genome/Contigs*

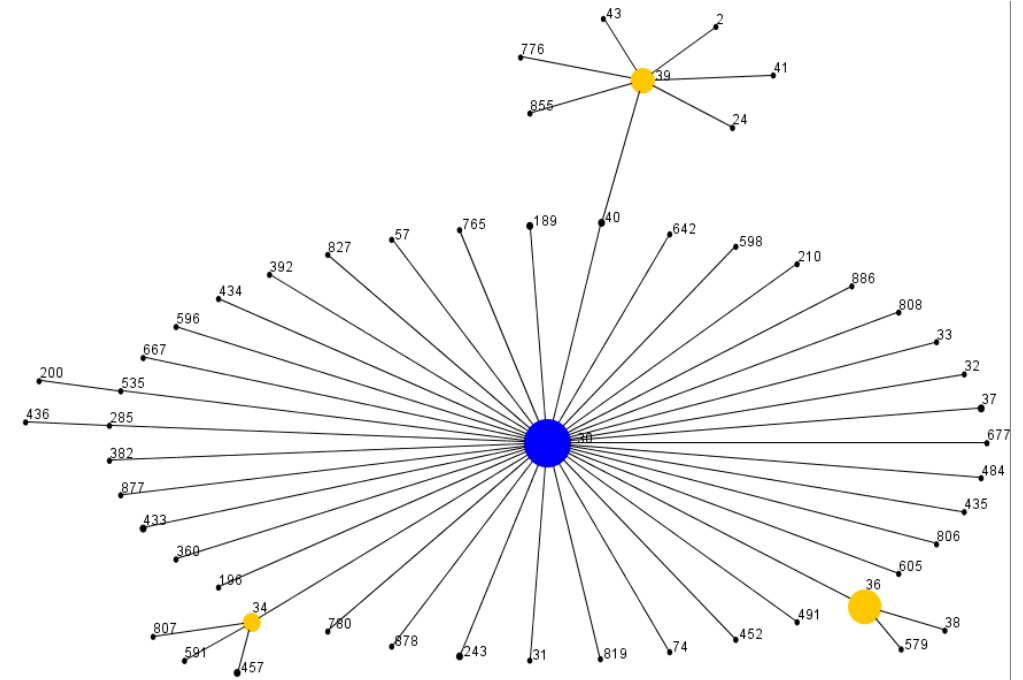
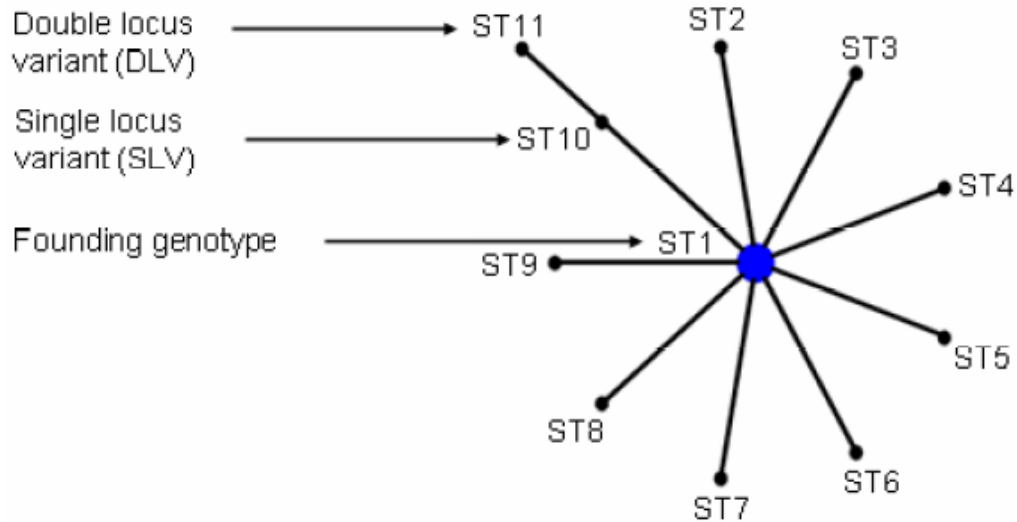
| Name | Size | Progress |
|------|------|----------|
|------|------|----------|

Genetic lineages

Sequence type

- MLST(ST), n > 2200
- Clonal Complexes

| Chromosomal Genes and Allelic Designation | | | | | | | ST | CC |
|---|-------------|-------------|------------|------------|-------------|-------------|-----|----|
| <i>arcC</i> | <i>aroE</i> | <i>glpF</i> | <i>gmk</i> | <i>pta</i> | <i>tpiA</i> | <i>yqiL</i> | | |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 4 | 1 | 4 | 12 | 1 | 10 | 5 | 5 |
| 3 | 3 | 1 | 1 | 4 | 4 | 3 | 8 | 8 |
| 3 | 3 | 1 | 1 | 4 | 4 | 16 | 250 | 8 |
| 3 | 3 | 1 | 12 | 4 | 4 | 16 | 247 | 8 |



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MLST 2.0

Service

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[Article abstract](#)

[Citations](#)

Software version: 2.0.9 ([2022-05-11](#))

Database version: ([2023-05-01](#))

MLST allele sequence and profile data is obtained from [PubMLST.org](#).

Momentanously, the species *Lactococcus Lactis* is unavailable.

Select MLST configuration

Staphylococcus aureus ▼

Methicillin Resistant Staphylococcus aureus- MRSA

Staphylococcus aureus and β -lactams

β -lactams including penicillins and cephalosporins used since the early 1940s and late 1950s, respectively.

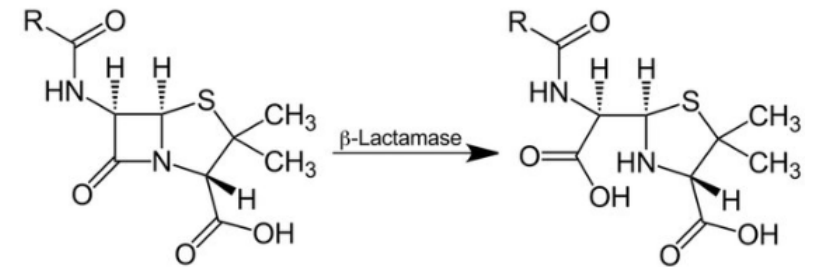
Penicillins binds to penicillin binding proteins (PBPs) causing discontinuation of cell wall synthesis and killing of the bacteria

Penicillin resistance

blaZ - usually plasmid-borne

Penicillinase

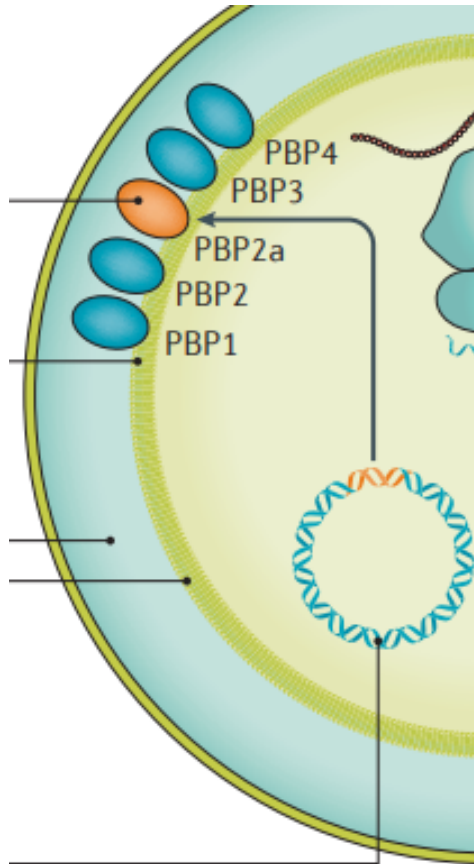
Enzymatic cleavage of the β -lactam ring



Susann Skagseth

β -lactam resistance in *S. aureus*

Methicillin resistance- MRSA

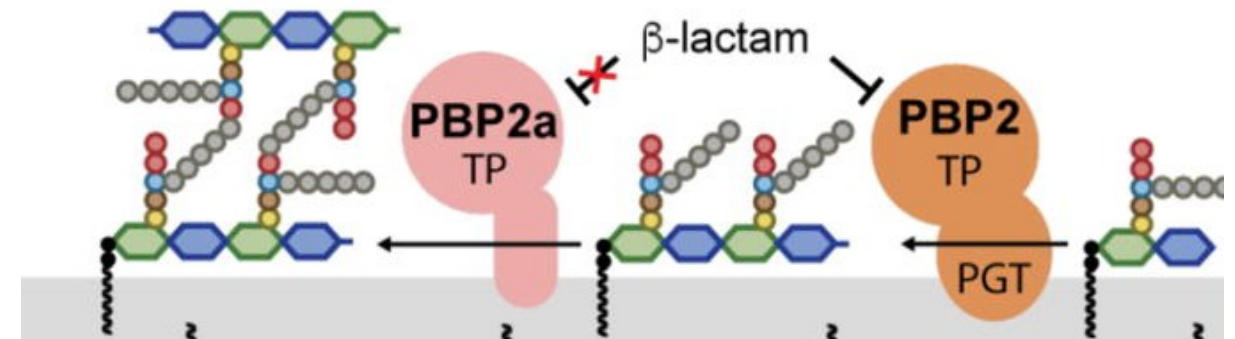


mecA or *mecC*

Present on *SCCmec*

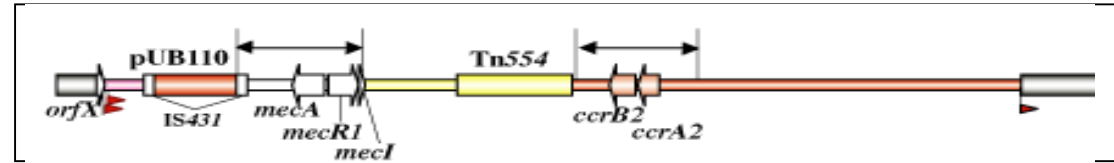
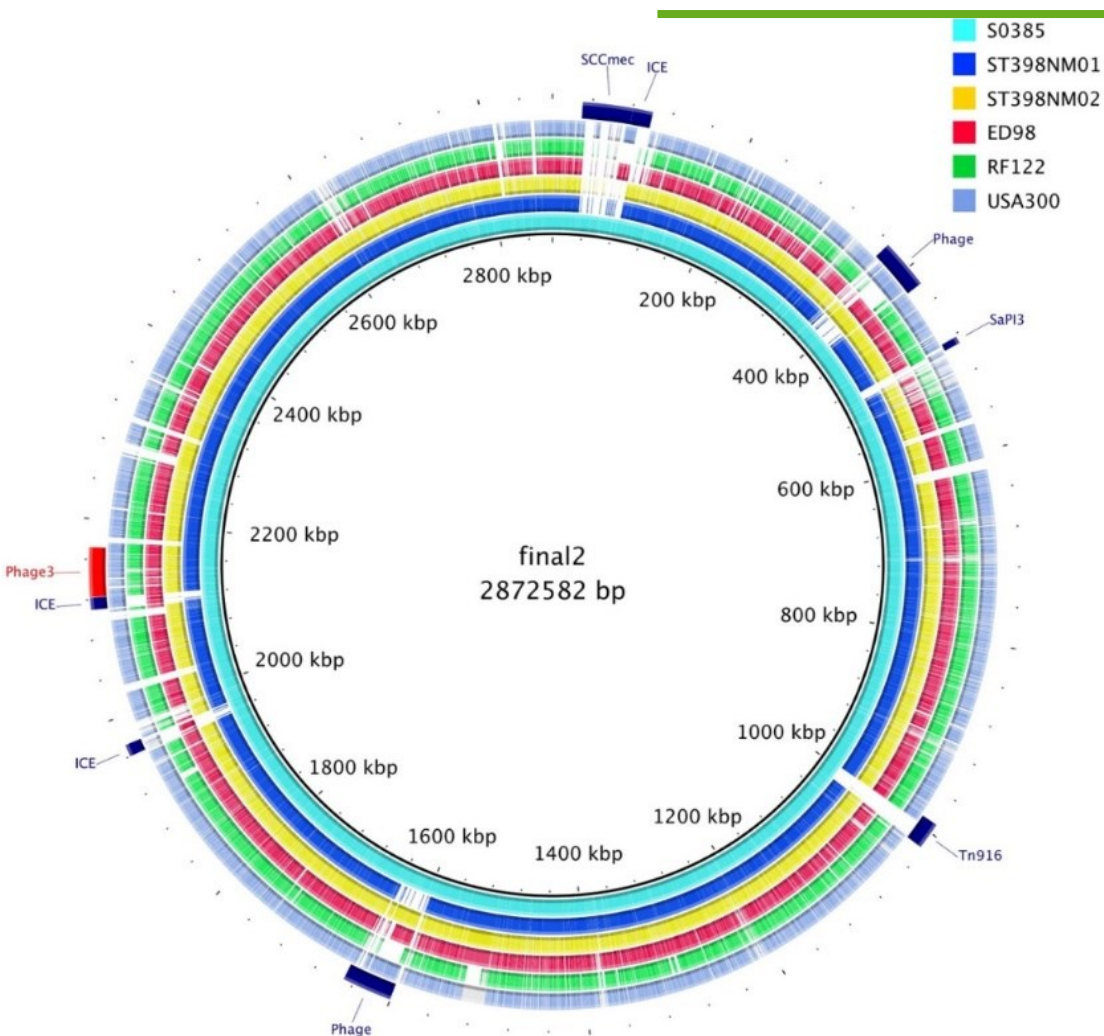
PBP2a or PBP2c

Low affinity for penicillins and cephalosporins



MRSA nomenclature

Staphylococcal Cassette Chromosome (*SCC_{mec}*)



Nomenclature- ST and *SCC_{mec}* type
i.e ST8-IV

1961 The first MRSA report



DRUGS

BRITISH
MEDICAL JOURNAL

JAN. 14, 1961

Correspondence

Because of heavy pressure on our space, correspondents are asked to keep their letters short.

“Celbenin”-resistant Staphylococci

SIR,—The Staphylococcus Reference Laboratory receives for phage-typing large numbers of strains of staphylococci, and it seemed that this material might usefully be examined to see whether any strains resistant to the new penicillinase-resistant penicillin (BRL 1241, “celbenin”) were in circulation at about the time of introduction of the new antibiotic.

vaning.—I am, etc.,

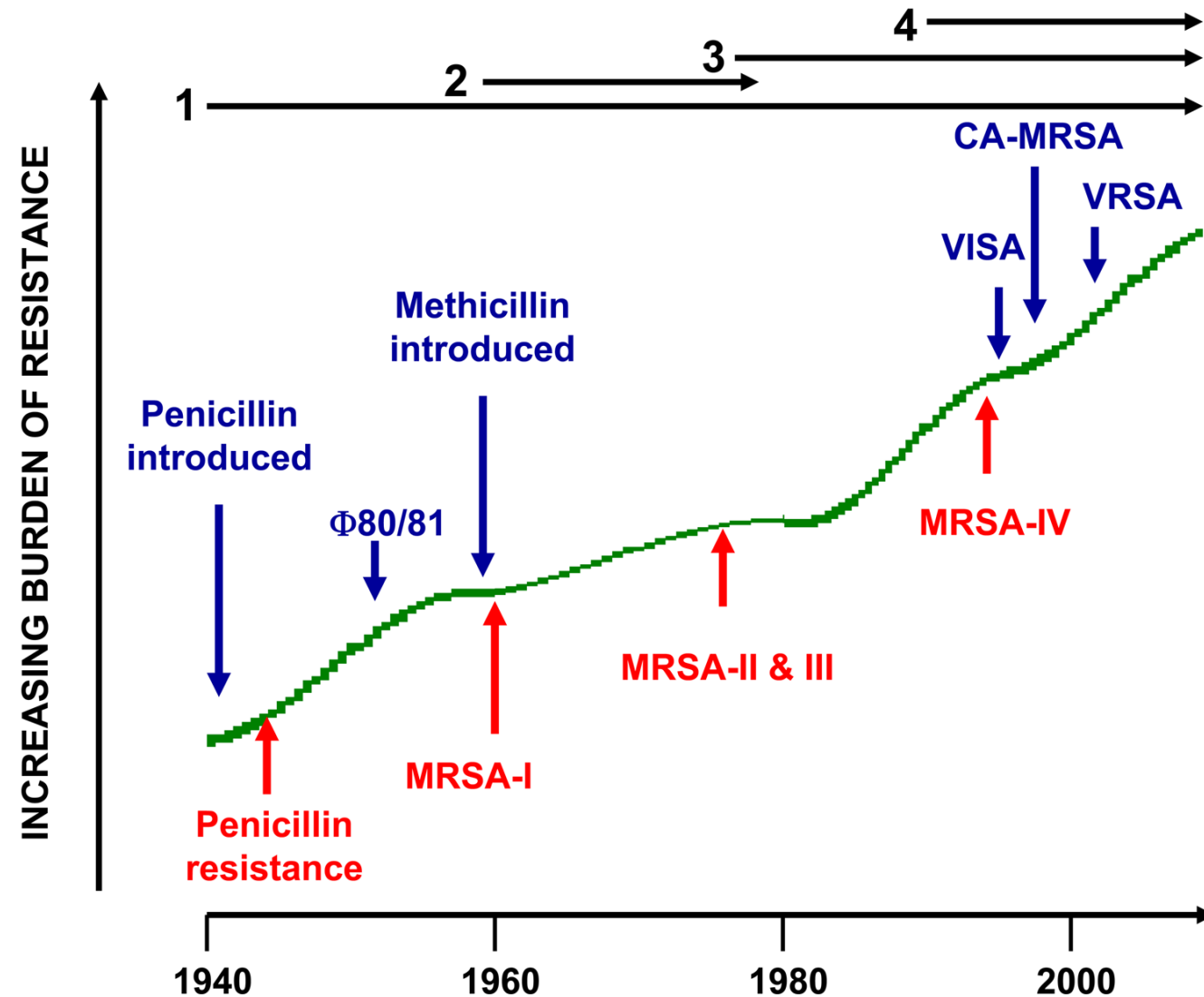
M. PATRICIA JEVONS.

Staphylococcus Reference Laboratory,
Colindale, London N.W.9.

Sensitivity to Celbenin in Tube Tests with Doubling Dilutions

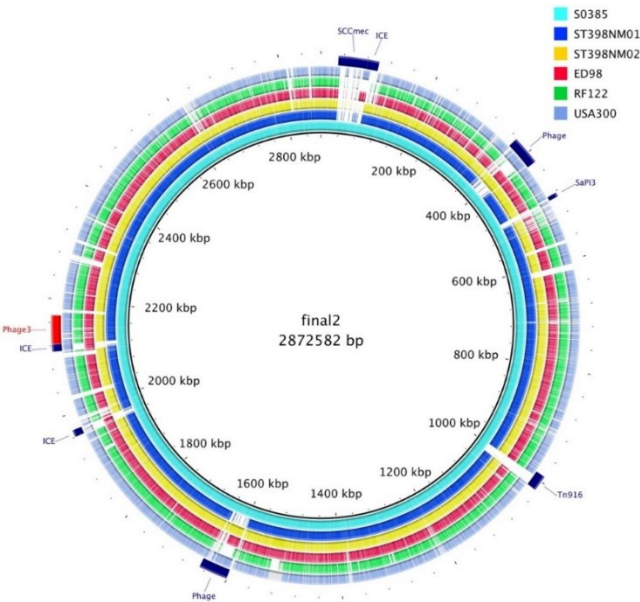
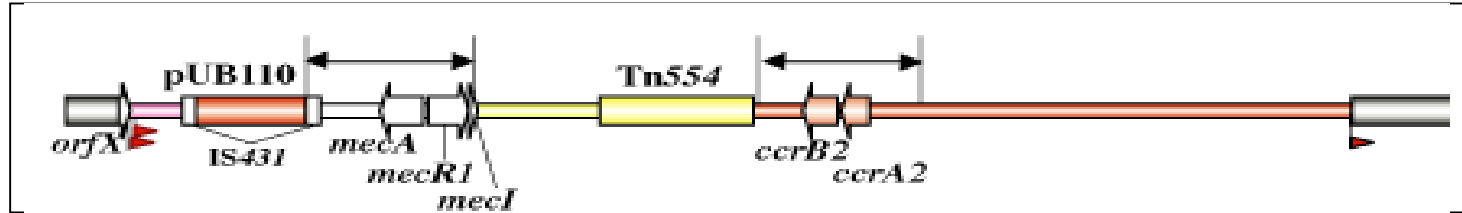
| Strain | Date of Isolation | Source | Site | Minimal Inhibitory Concentration of Celbenin ($\mu\text{g. per ml.}$) | |
|--------|-------------------|-----------|---------------------|---|----------|
| | | | | Colony a | Colony b |
| 13136 | 2/10/60 | Patient A | Nephrectomy wound | 12.5 | 6.5 |
| 13137 | 2/10/60 | Nurse B | Finger infection .. | 12.5 | 25.0 |
| 10395 | 21/7/60 | Patient C | Nose | 6.25 | 6.25 |
| 10396 | 5/7/60 | .. C | Eczematous skin .. | 3.125 | 3.125 |
| 14083 | 28/10/60 | .. C | Nose | 1.60 | 1.60 |
| 14668 | 8/11/60 | .. C | | 25.0 | 25.0 |

MRSA evolution



SCC*mec* and epidemiology

Mobile genetic element, containing *mecA/C*, 15 major types I-XV



| SCC <i>mec</i> type | <i>ccr</i> type | <i>mec</i> class | Size (kb) | Additional resistance determinants |
|---------------------|------------------|------------------|-----------|---|
| I | <i>ccrAB1</i> | A | 36 | None |
| II | <i>ccrAB2</i> | B | 39 | Erythromycin, aminoglycosides |
| III | <i>ccrAB3</i> | B | 67 | Macrolide, tetracycline, cadmium, mercury |
| IV | <i>ccrAB2</i> | A | 21-24 | None (IVc: gentamicin) |
| V | <i>ccrC</i> | C | 27 | None |
| Vc | <i>ccrC2</i> & 5 | C | 47 | Tetracycline, heavy metals |

Structure of SCC_{mec} and J regions

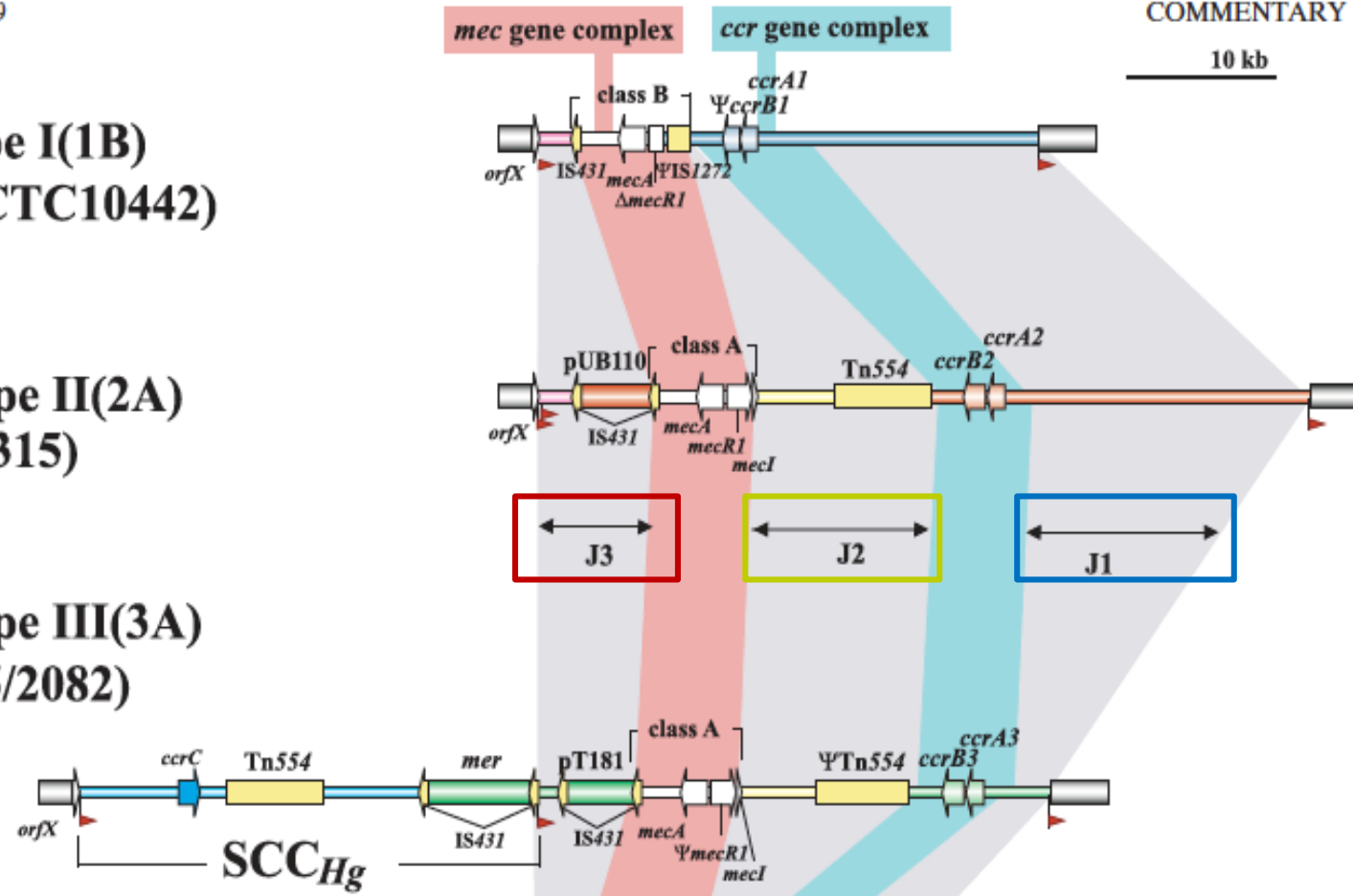
VOL. 53, 2009

COMMENTARY 4963

Type I(1B)
(NCTC10442)

Type II(2A)
(N315)

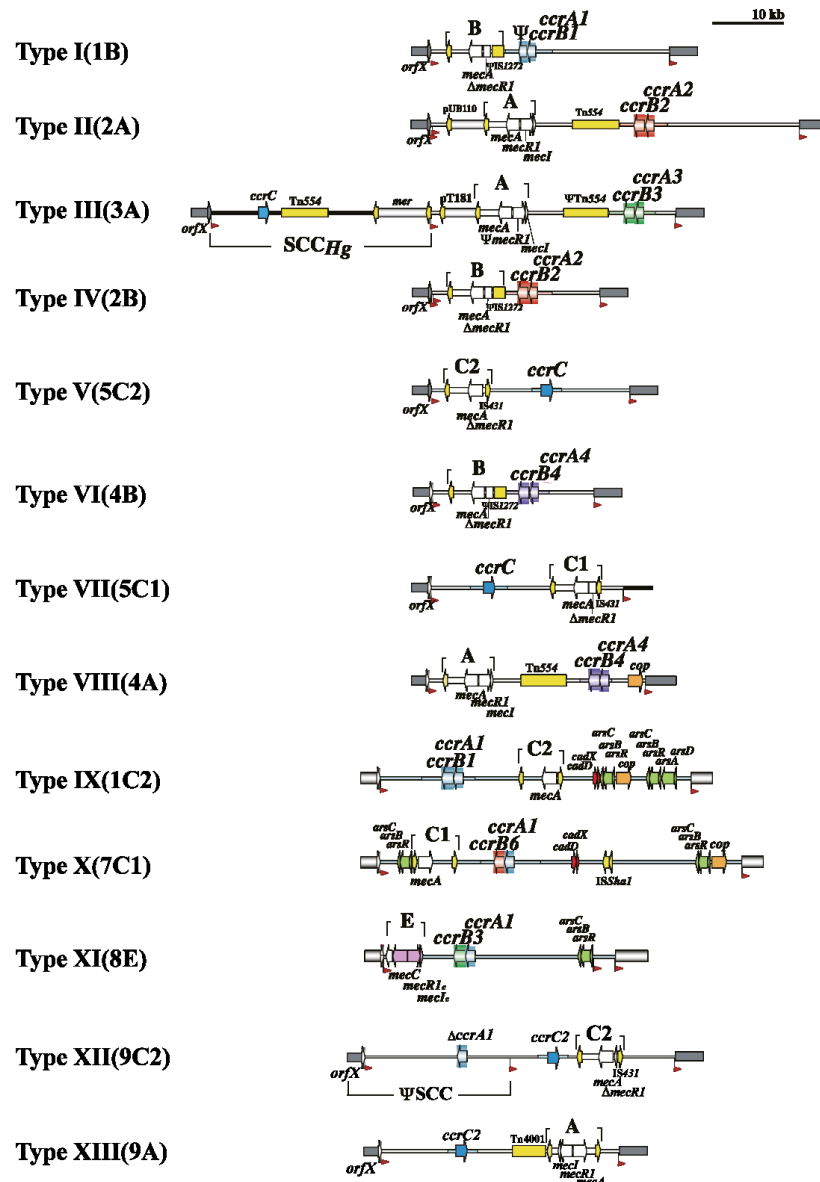
Type III(3A)
(85/2082)



The *SCCmec* element

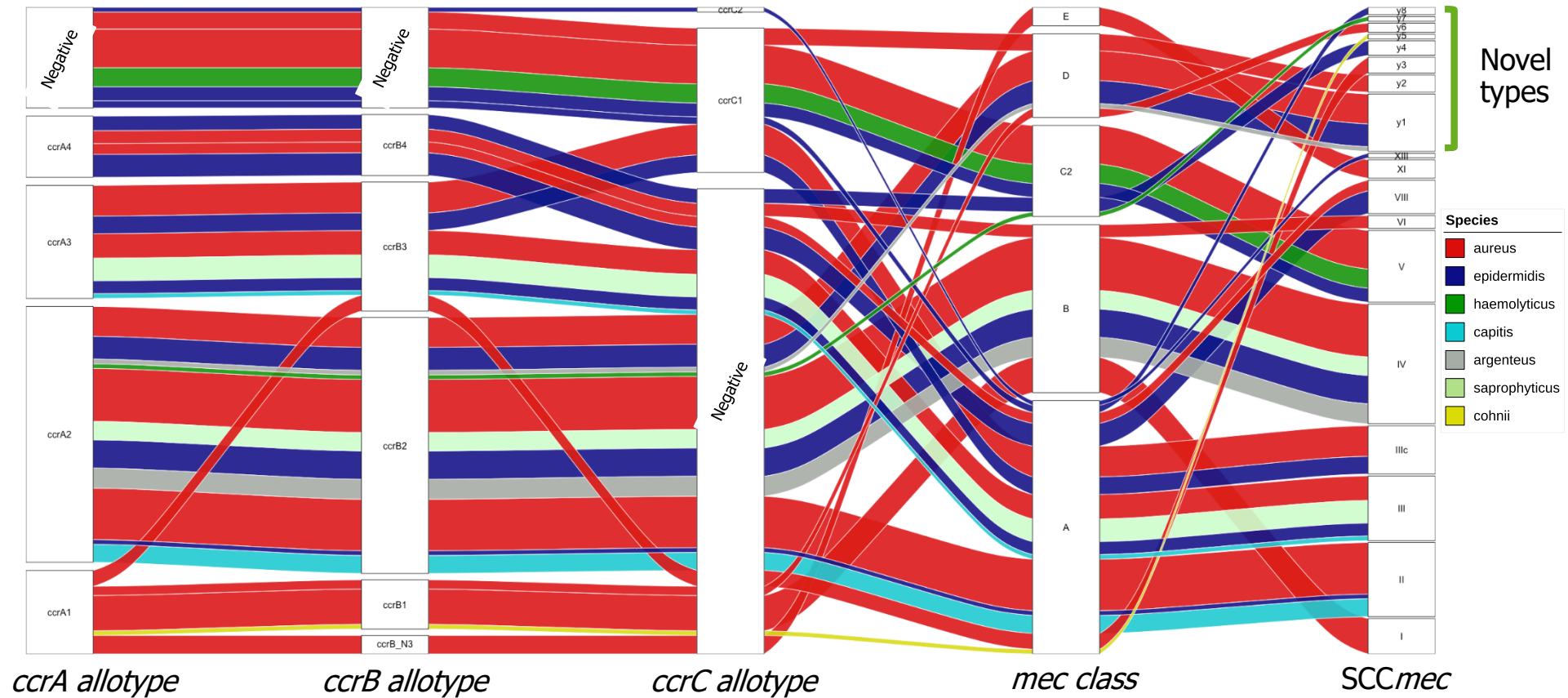
- Nomenclature
IWG-SCC

- 15 Types and many more subtypes (based on variations in the joining (J) regions)



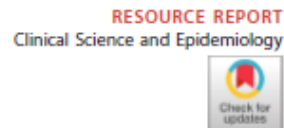
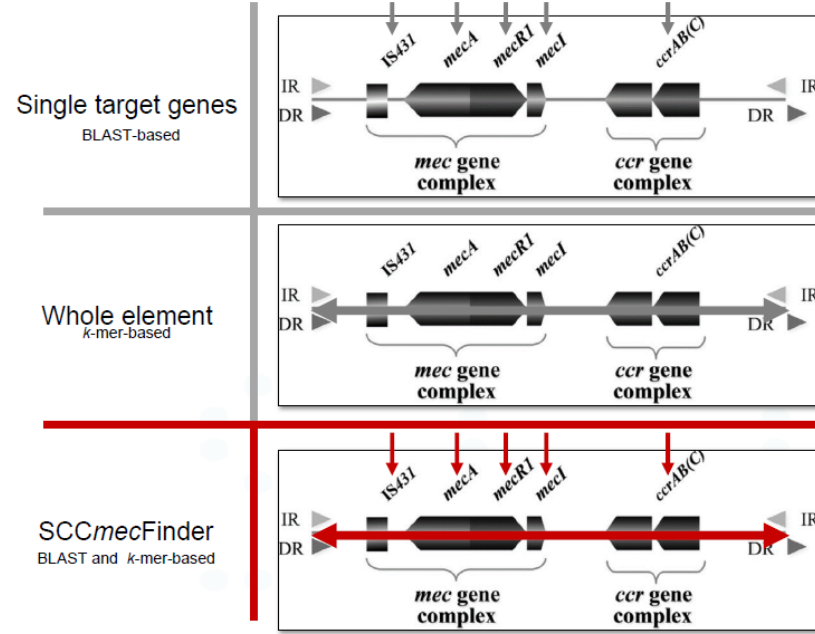
Composition and abundance of SCCmec across Staphylococci

Variability is near exclusive to the *ccr/mec* compositions



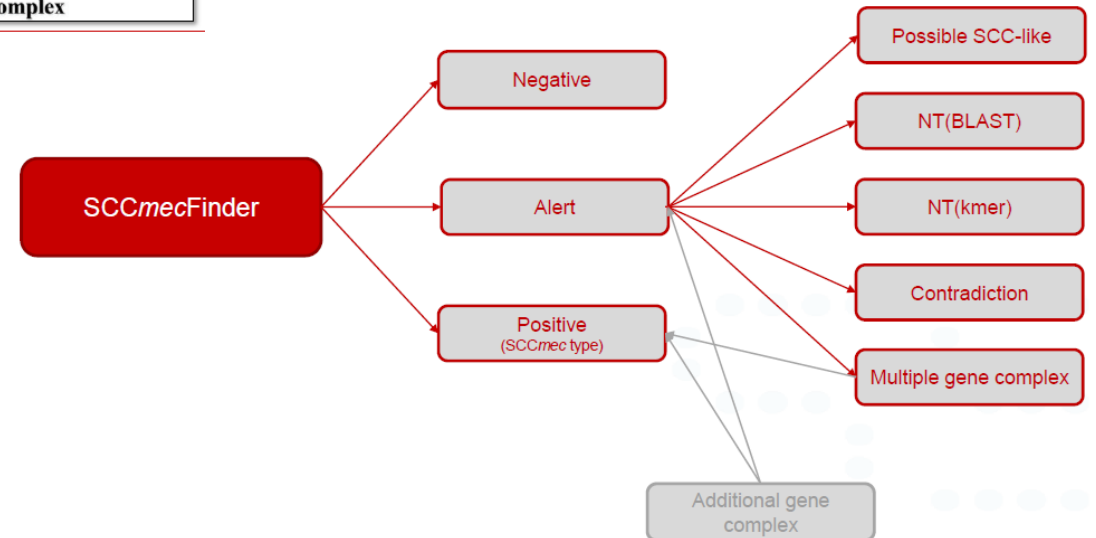
SCCmecFinder

- ❖ An *in silico* web-based tool
- ❖ Freely available
 - <https://cge.cbs.dtu.dk/services/SCCmecFinder/>
- ❖ Utilizes two different approaches
 - BLAST-based
 - *k*-mer-based
- ❖ Two databases
 - Single target genes
 - Whole SCC*mec* element (reference)
 - Whole SCC*mec* element (extended)

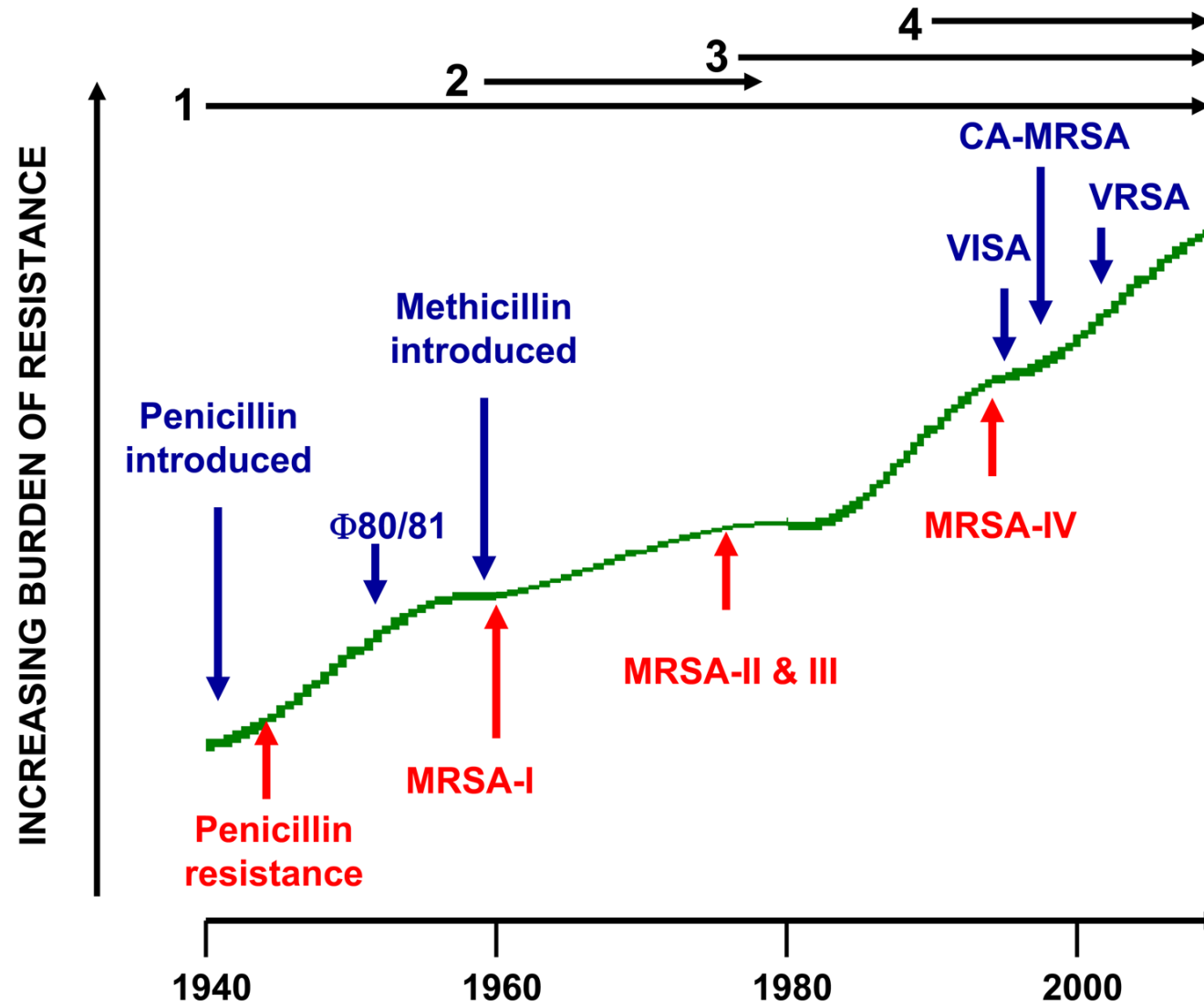


SCC*mec*Finder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome *mec* in *Staphylococcus aureus* Using Whole-Genome Sequence Data

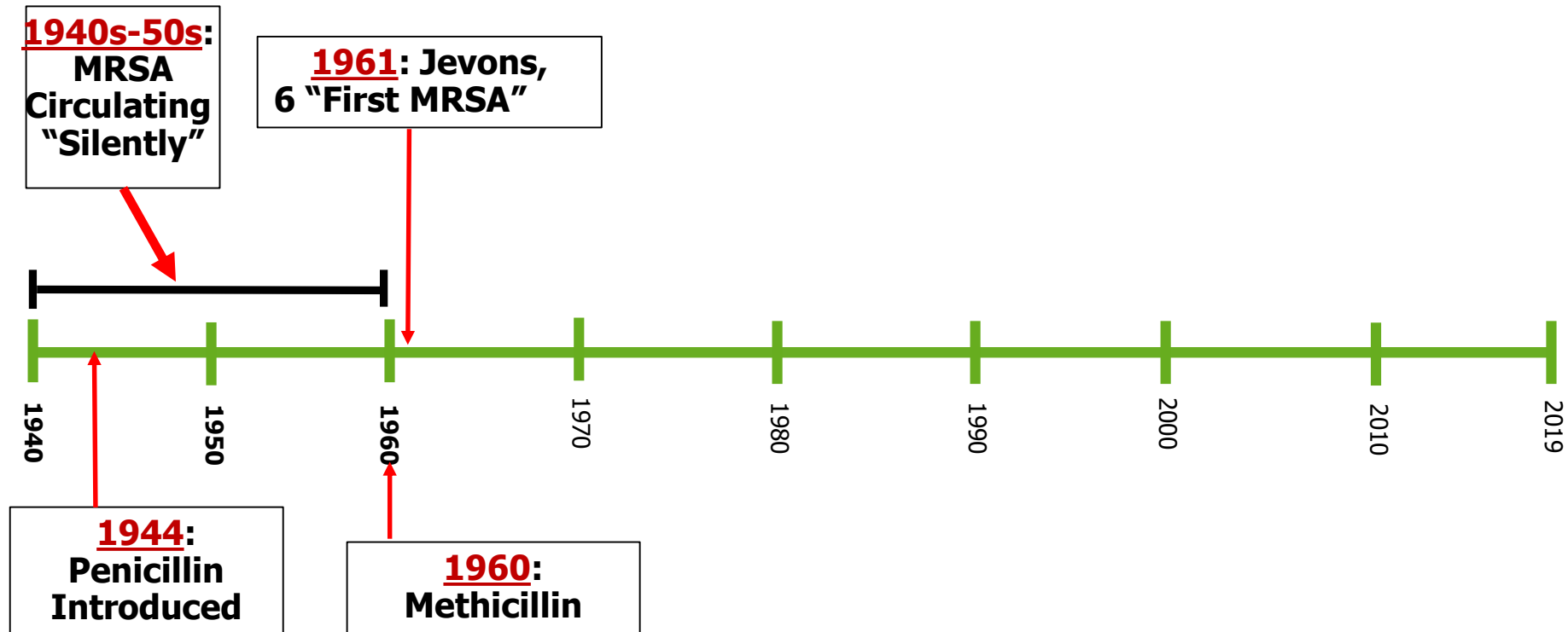
Hülya Kaya,^{a,b*} Henrik Hasman,^{a*} Jesper Larsen,^{a*} Marc Stegger,^{a*} Thor Bech Johannesen,^{a*} Rosa Lundbye Allesøe,^{c*} Camilla Koldbæk Lemvig,^{c*} Frank Møller Aarestrup,^{b*} Ole Lund,^{c*} Anders Rhod Larsen^{a*}



Evolution



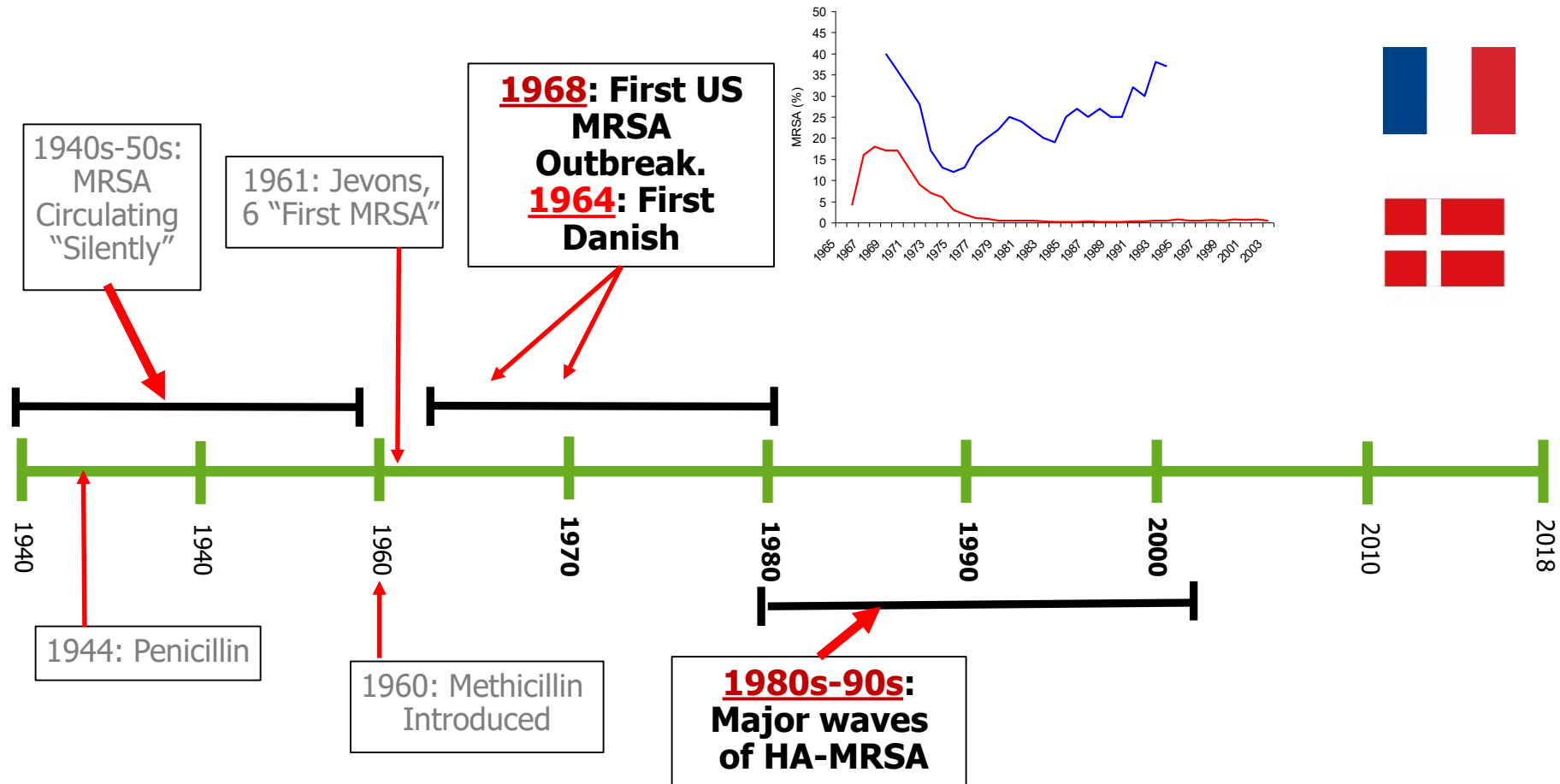
The first MRSA



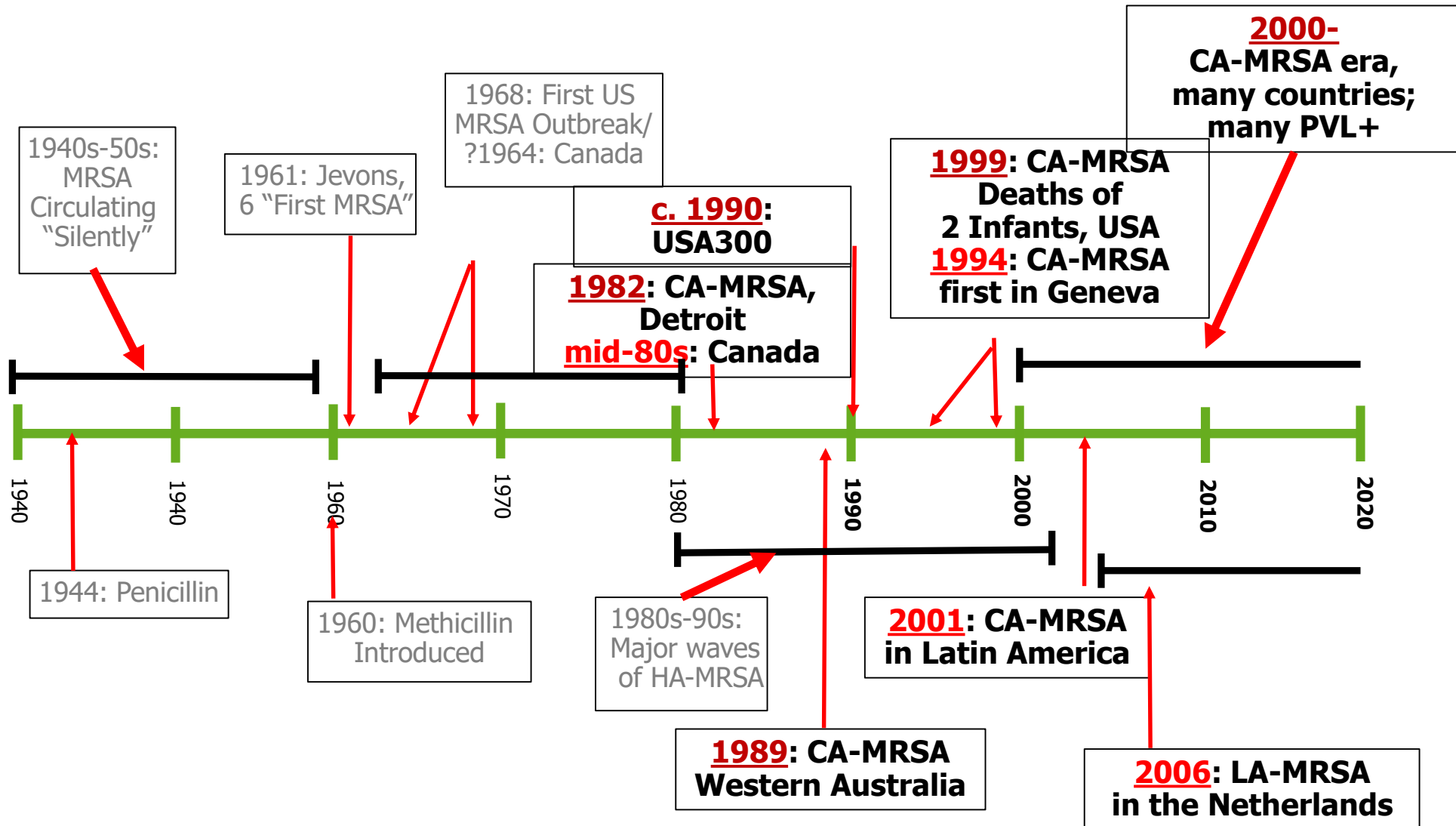
Hankins CP, et al. Genome Biol 2017;18:130; Chambers & DeLeo Nat Rev Microbiol 2009;7:629-41.

Modified from original slide, in courtesy of Michael Z. David, University of Pennsylvania

HA- MRSA: first wave

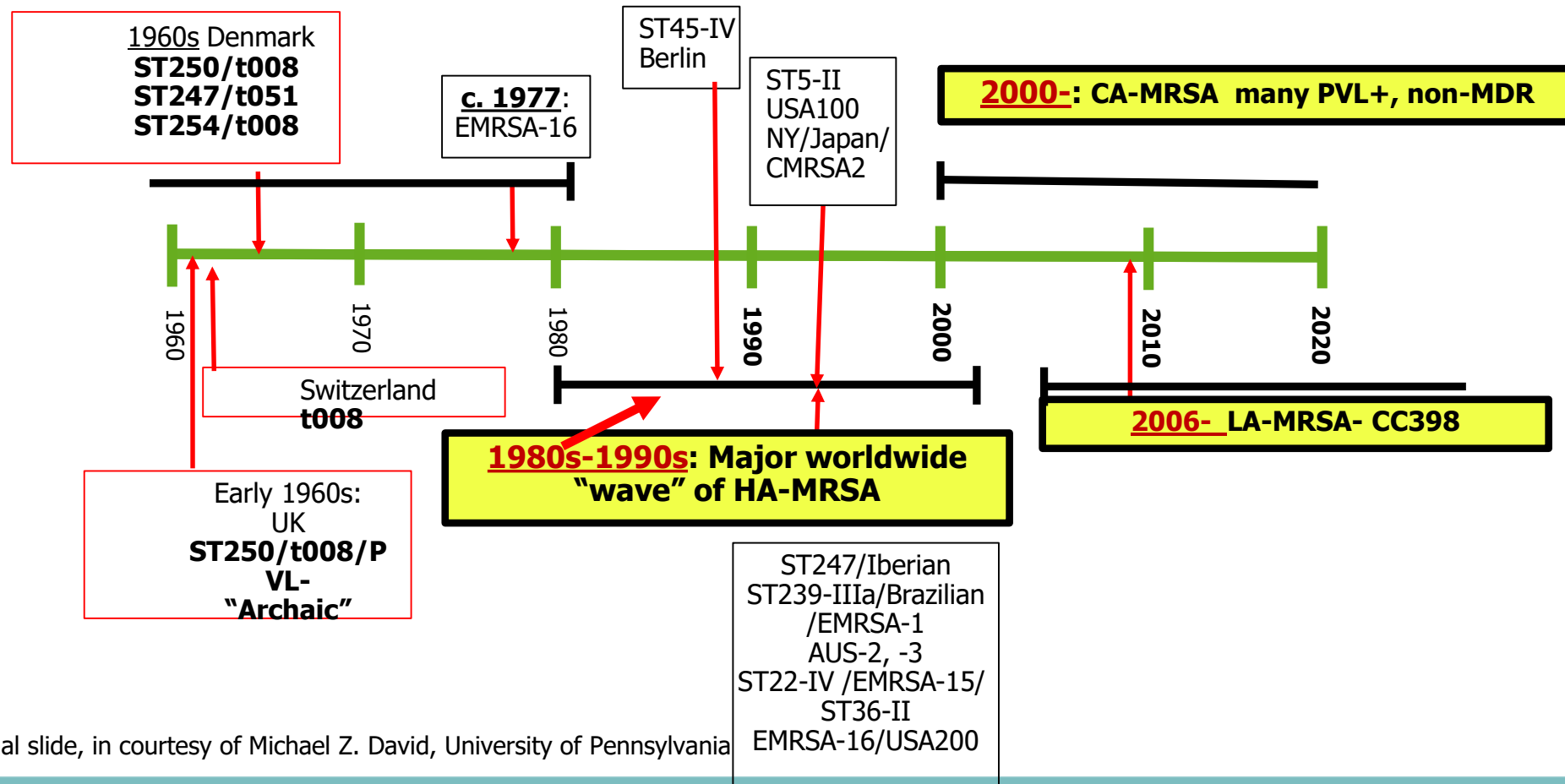


HA-, CA, and LA- MRSA waves



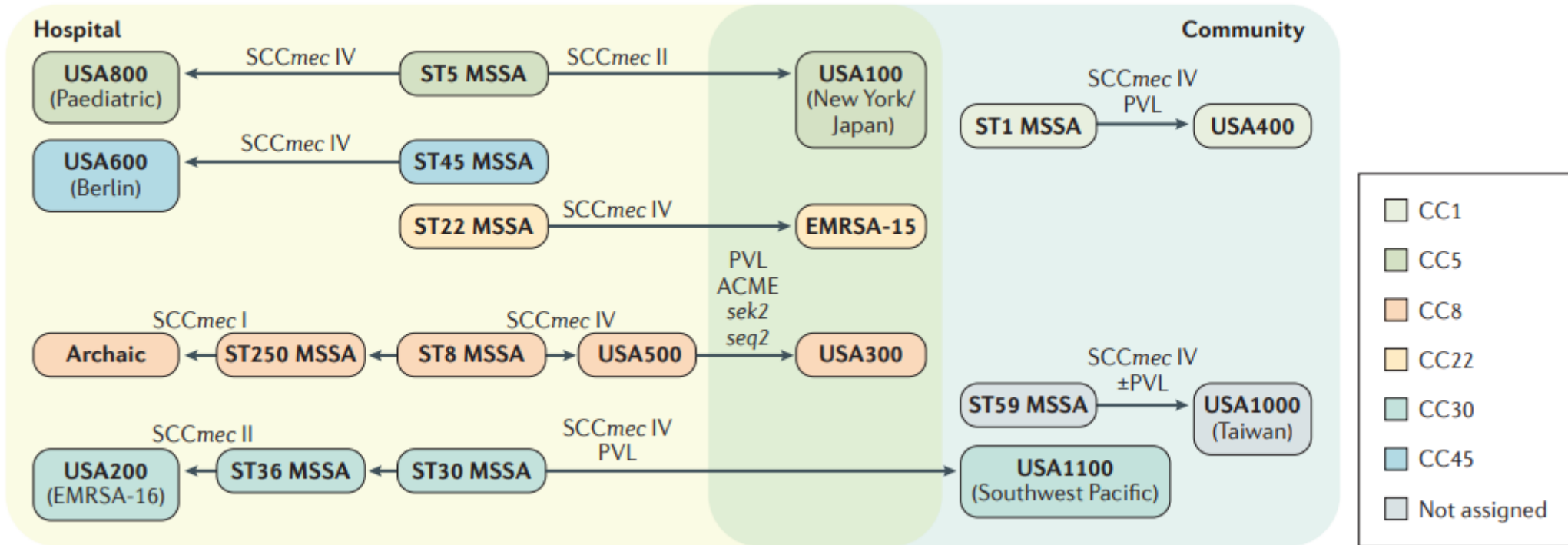
Modified from original slide, in courtesy of Michael Z. David, University of Pennsylvania

Timeline of major events in MRSA history



Modified from original slide, in courtesy of Michael Z. David, University of Pennsylvania

Most frequent MRSA clones



• Lee AS et.al. Nature reviews Disease Primers 2018 May 31;4:18033

Take home



Typing of MRSA consists of MLST (or *spa*) and SCC*mec* typing

Certain genetic characteristics determine the pathogenic potential and epidemiology of *S. aureus*

CGE online tools can be used to identify ST, SCC*mec*, virulence determinants and antimicrobial resistance genes in *S. aureus*

Acknowledgements

The creation of this training material was commissioned by ECDC to Statens Serum Institut (SSI) and Danish Technical University with the direct involvement of Andreas Petersen (SSI) , Hülya Kaya (SSI), Lauge Holm Sørensen (DTU) and Niamh Lacy Roberts (DTU)

T

Exercise