Determination of allelic diversity in the *mec* operon of methicillin-resistant *Staphylococcus aureus* in Wisconsin

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ICEID 2002

Background

- Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major bacterial pathogen, because it is resistant to a variety of antibiotics and causes nosocomial infections worldwide

- It is a Gram positive coccus organism. It is also catalase and coagulase positive
- It is associated with a wide range of infections ranging from skin, to bacteremia to endocarditis

- It's epidemiology seems to be changing: from primarily a hospital acquired pathogen to a community acquired pathogen in recent years

- MRSAs have acquired methicillin resistance after acquisition of 30 to 50 kb of DNA called *mec* DNA from other species
- The *mec* DNA primarily consists of *mecA*, the structural gene, and two other adjoining regulatory genes *mecI* and *mecR1* besides additional *mec* associated DNA
- *mecA* codes for a penicillin binding protein called PBP2a that binds to beta lactam antibiotics with low affinity
  - Expression of *mecA* is essential for intrinsic methicillin resistance in MRSA

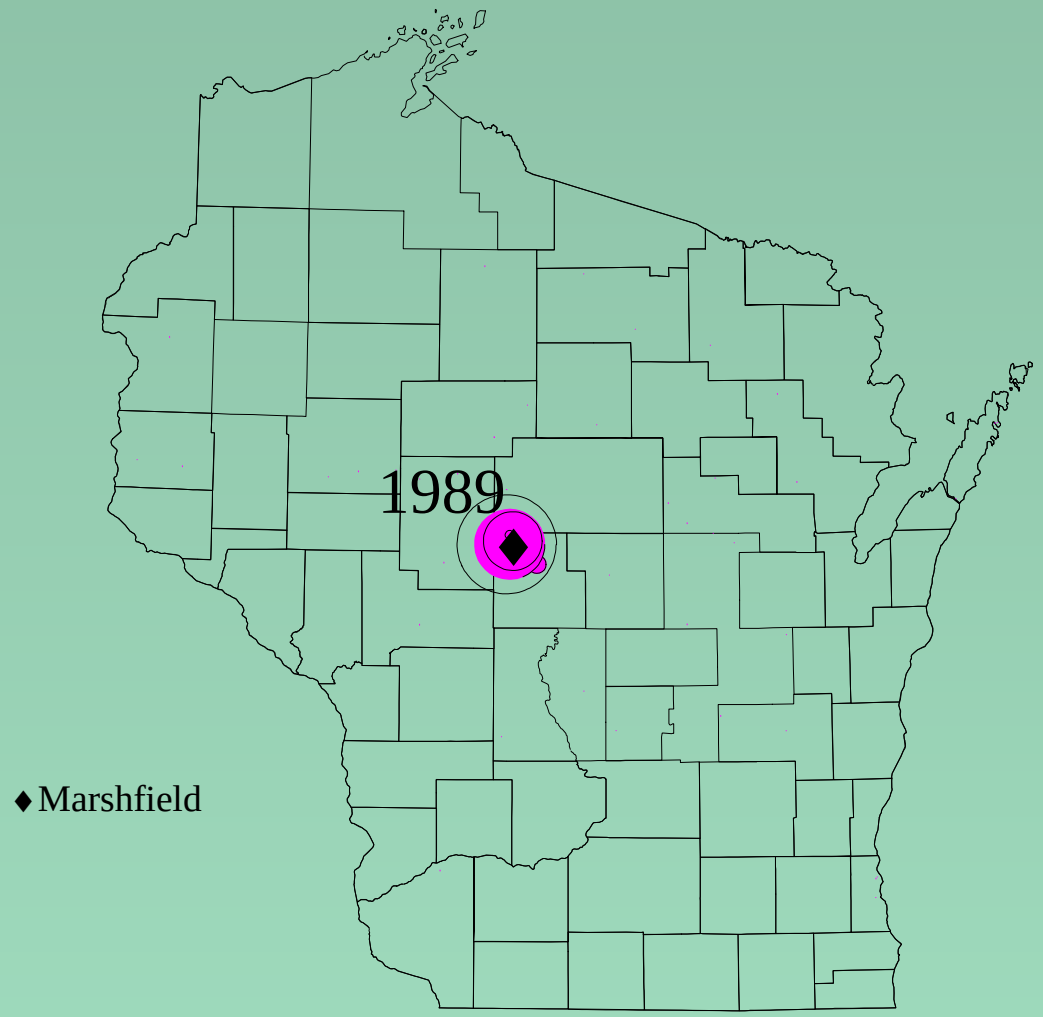
- MecI is a transcriptional repressor of *mecA* and a mutation in *mecI* gene leads to constitutive expression of *mecA*

- MecR1 is a transmembrane sensor-transducer protein that can sense the presence of beta lactam antibiotics in the extracellular environment

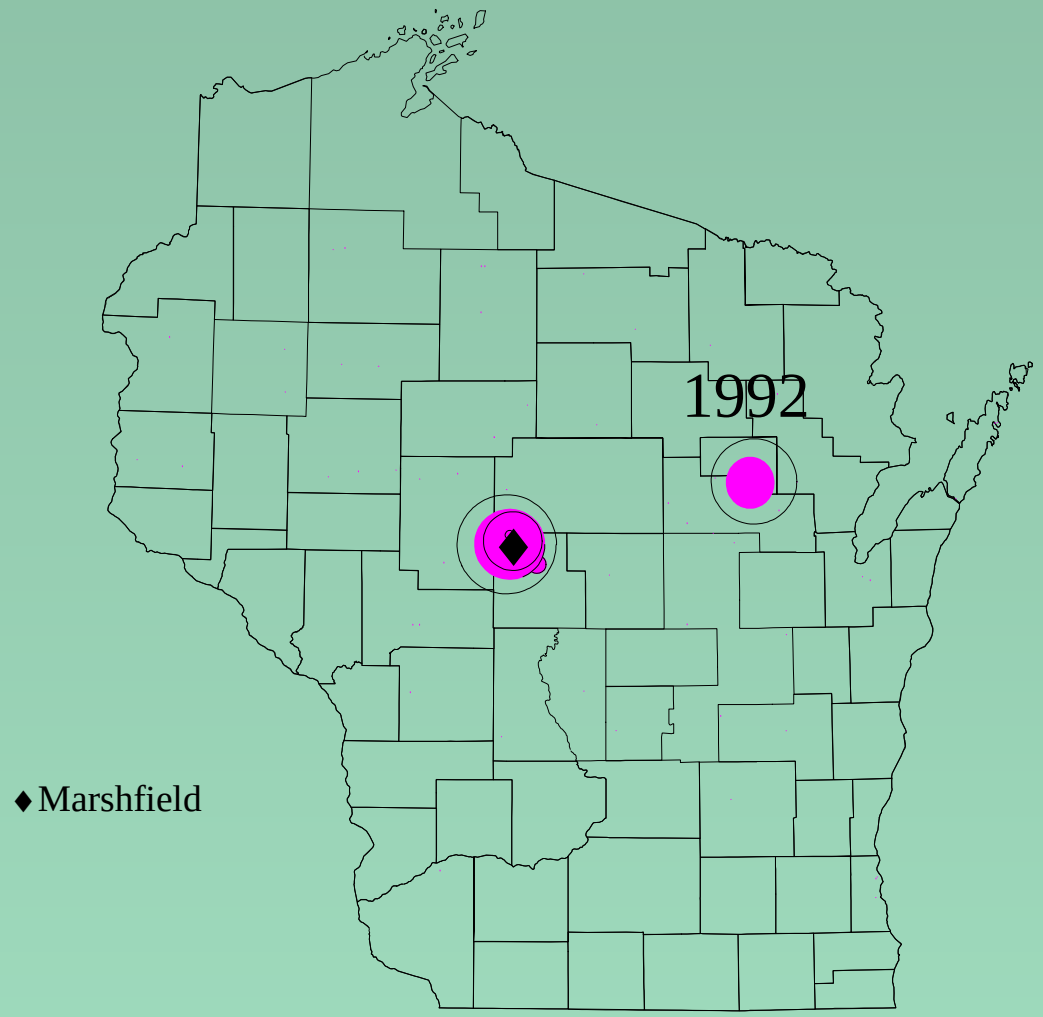
- It has been shown previously by Dot Blot and limited sequencing experiments that some clinical isolates of MRSA contain deletions of *mecI* and partial deletion of *mecR1*

Brief history of this project

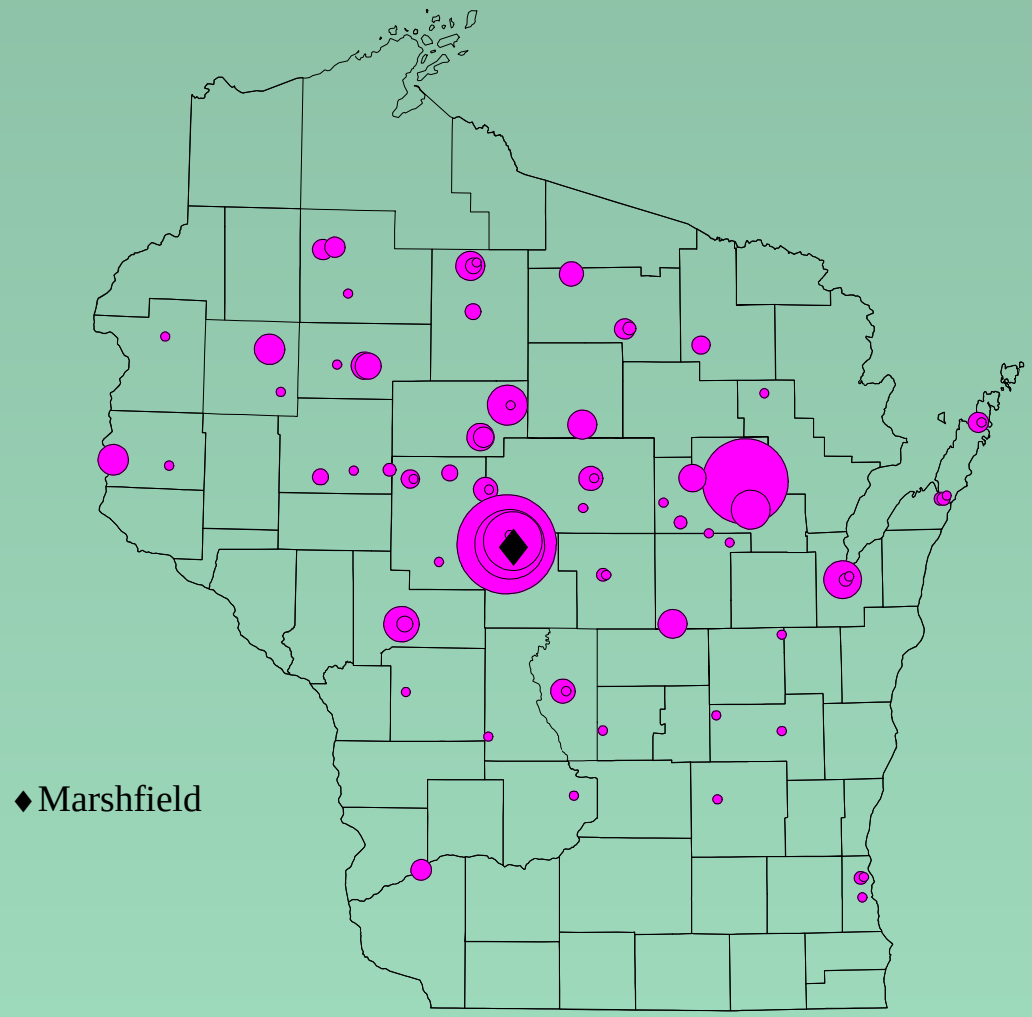
In central Wisconsin MRSA was delayed compared to large academic medical centers and hospitals in coastal US cities



Map of Wisconsin showing location of Marshfield laboratories where in 1989 first MRSA from central WI was isolated



In 1992, an Outbreak of MRSA occurred in a Native American Community



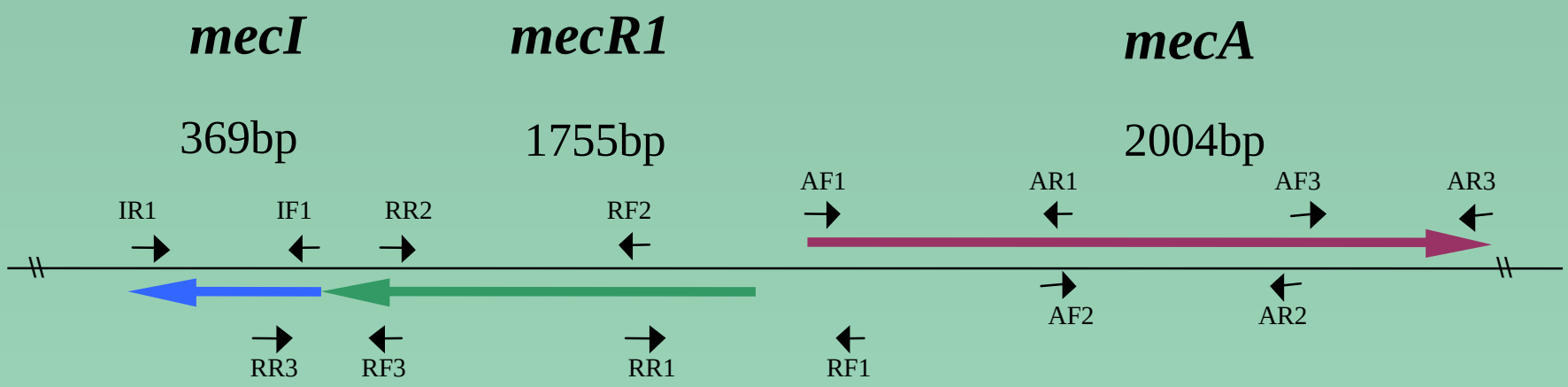
Locations where MRSAs were isolated from 1989-1999

- Our goal was to determine the allelic diversity in the *mec* operon from a select group of MRSA isolates collected over a 10-year period : 1989 to 1999 from Wisconsin
- How do mutations in the *mec* operon of these isolates correlate with
  - oxacillin MICs
  - antibiograms
  - and PFGE profiles?

Methods

- PFGE profiles were determined after *sma*I digestion of the chromosomal DNA
- The PFGE patterns were analyzed with Multianalyst Fingerprinting plus software (BioRad)
- Antibiograms for seven drugs (erythromycin, gentamycin, rifampin,ciprofloxacin, clindamycin, tetracycline, and trimethoprim-sulfamethoxazole were determined by the Vitek System
- Oxacillin MIC was determined by E-test method

Methods continued: PCR and Sequencing



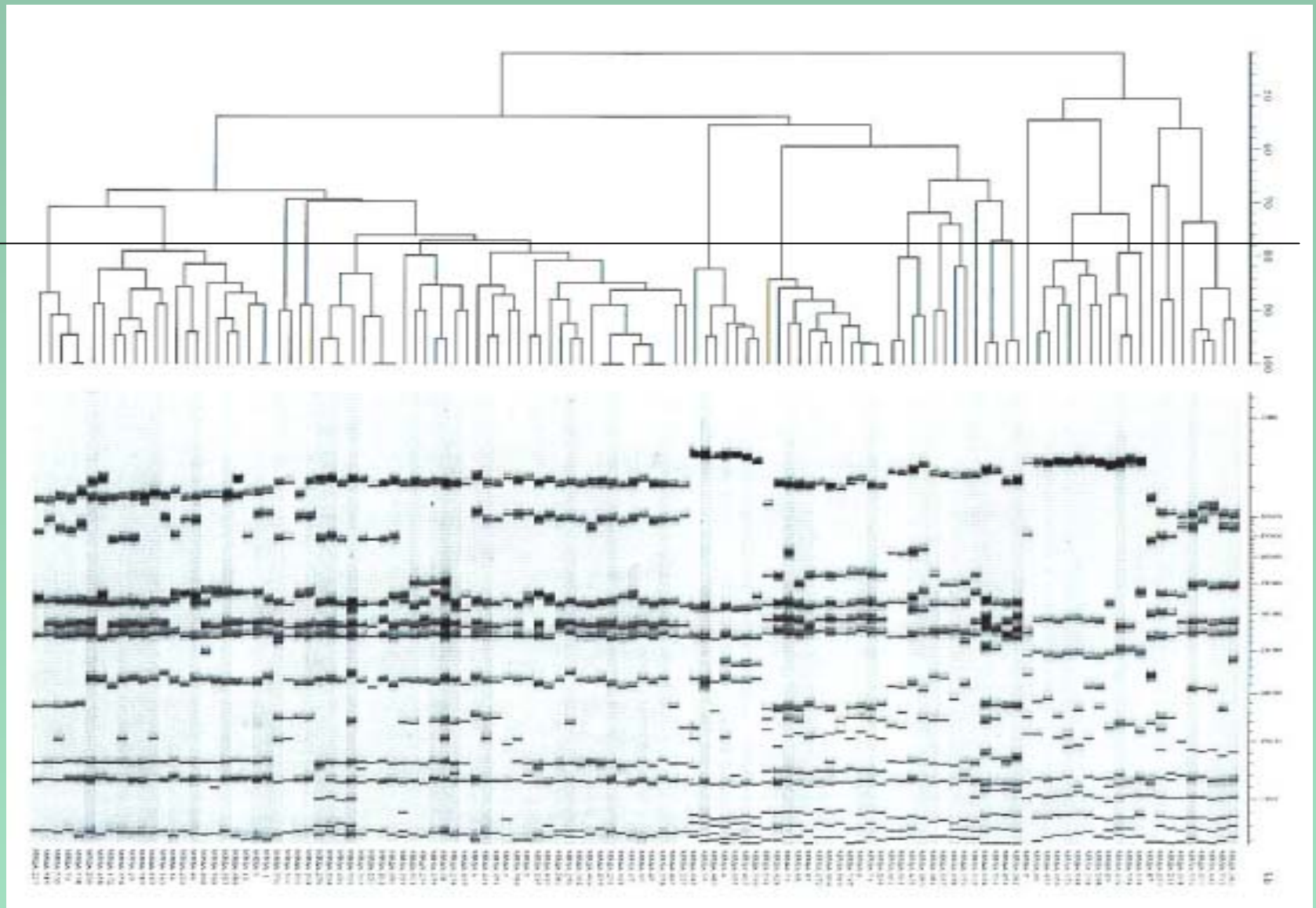
- Schematic representation of *mec* genes and location of PCR primers
- Amplification was done by colony PCR method

Results

- Of the 316 MRSA isolates studied, 23 PFGE clonal groups were identified of which 7 isolates (3.2%) had unique fingerprints
- Fifteen different susceptibility patterns were identified
- The largest antibiogram group had 124 isolates with resistance to three drugs (cip, clin, and ery) in addition to oxacillin

MRSA Pulsed-field Gel Electrophoresis (PFGE) Dendrogram

Stemper *et al.* (unpublished)



Results Continued

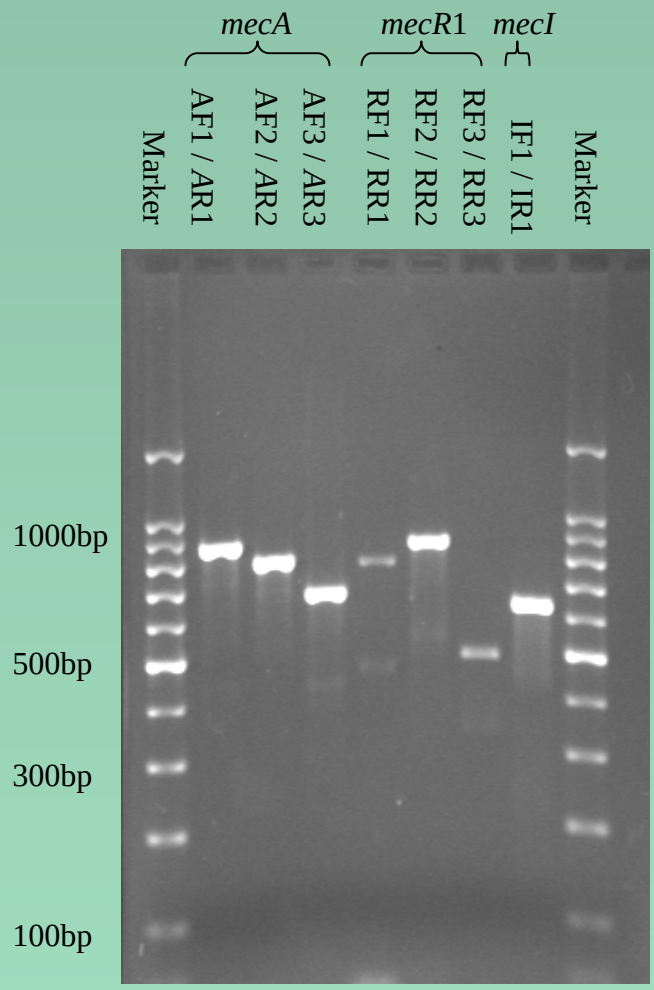
- Fifteen different susceptibility patterns were identified
- The largest antibiogram group had 124 isolates with resistance to three drugs (cip, clin, and ery) in addition to oxacillin

Antibiogram types detected in the MRSA isolates from Wisconsin.

Antibiogram Type	Resistant To [a]
2	Cip, Clin, Ery
3	Cip, Cli, Ery, Tet, Sxt
4	Cip, Clin, Ery, Tet
5	Cip, Clin, Ery, Sxt
8	Cip
9	Cip, Ery
11	Clin, Ery, Tet
12	Clin, Ery
16	Ery, Tet
18	Ery
19	[none]
20	Tet
22	Cip, Clin, Ery, Tet, Gent, Rif
23	Cip, Clin, Ery, Rif
24	Cip, Clin, Ery, Tet, Sxt, Gent, Rif

[a] Cip, Ciprofloxacin; Clin, Clindamycin; Ery, erythromycin; Tet, tetracycline; Sxt, trimetheprim sulfamethoxazole; Gent, Gentamycin; Rif, Rifampin

PCR Results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mecR1
mecI
Marker
AF1 / AR1
AF2 / AR2
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RF1 / RR1
RF2 / RR2
RF3 / RR3
IF1 / IR1
Marker
1000bp
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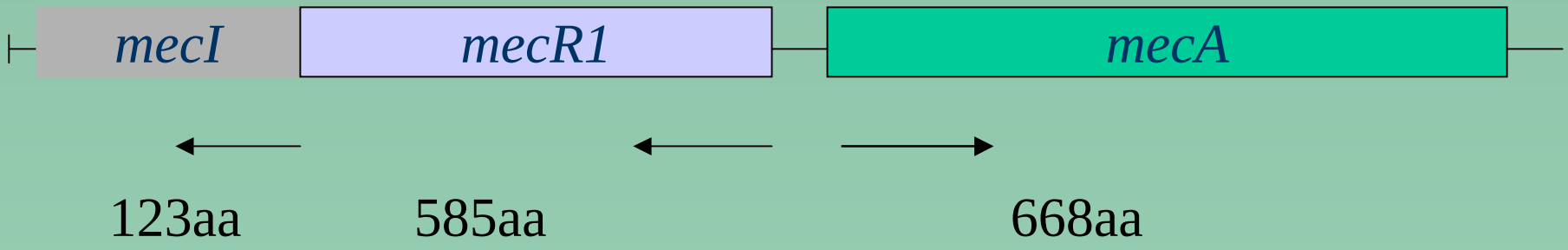
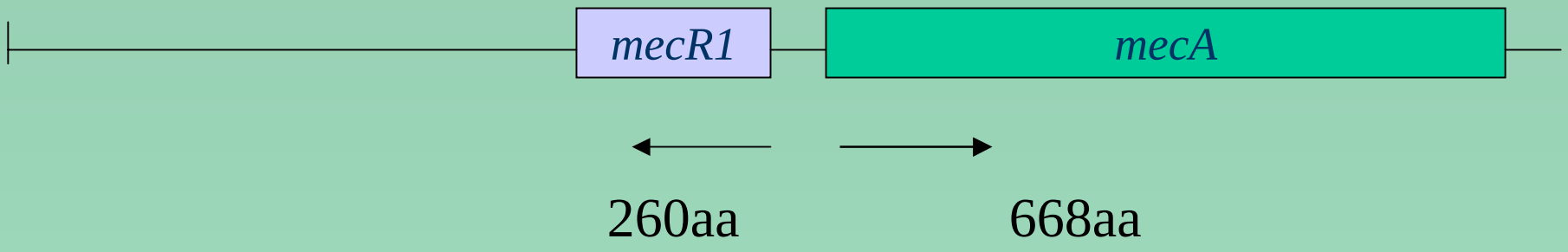
100bp

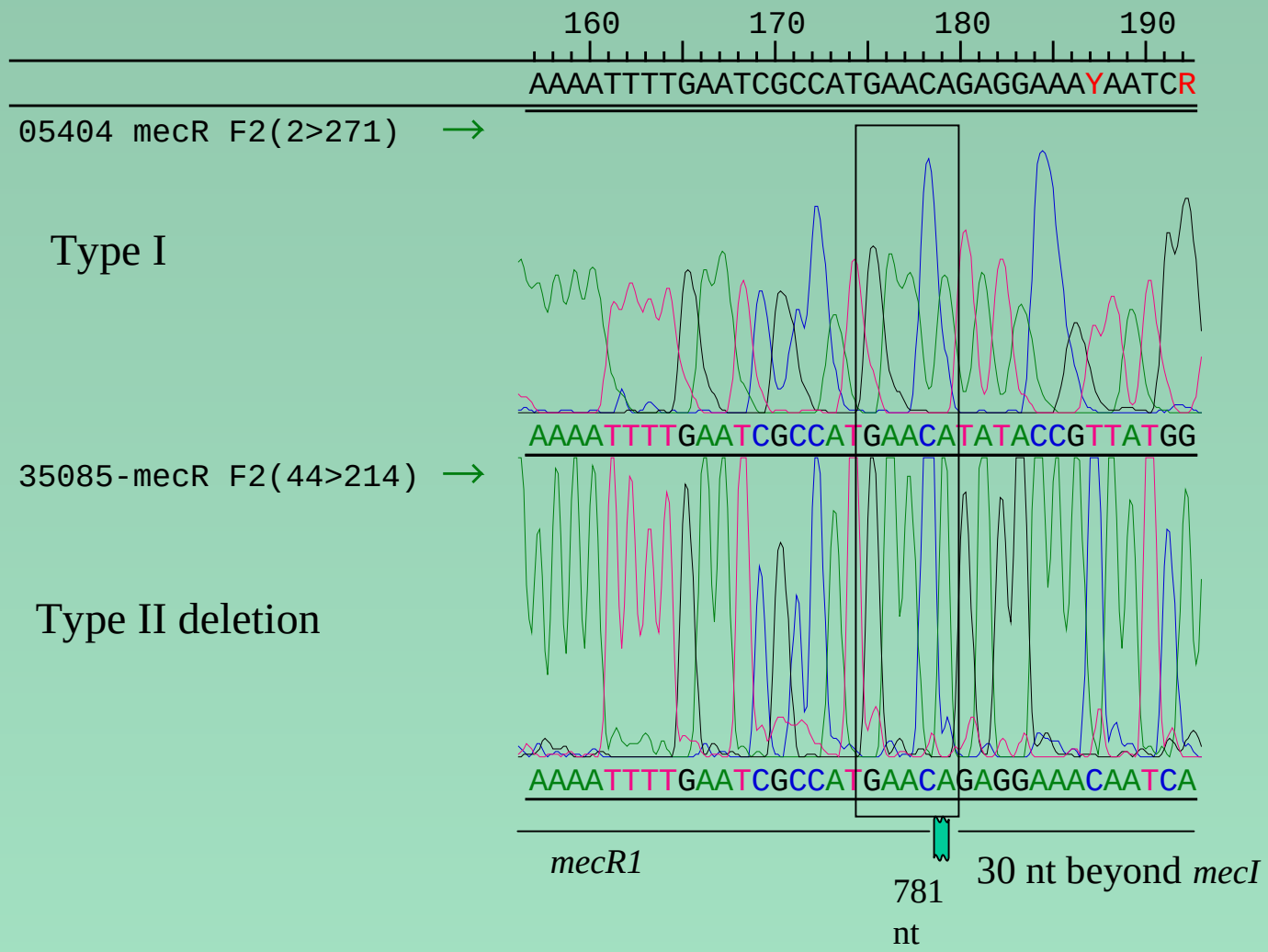
Type I



Type II



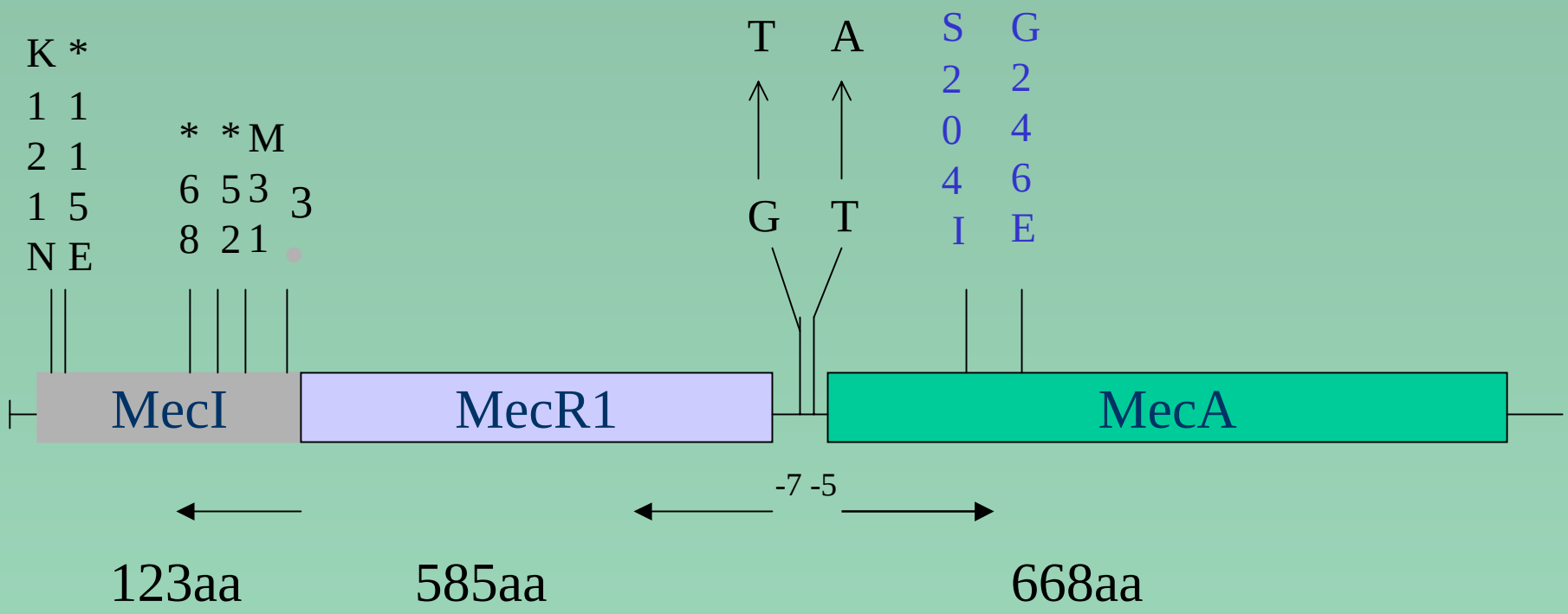
mecR1 Deletion Junction



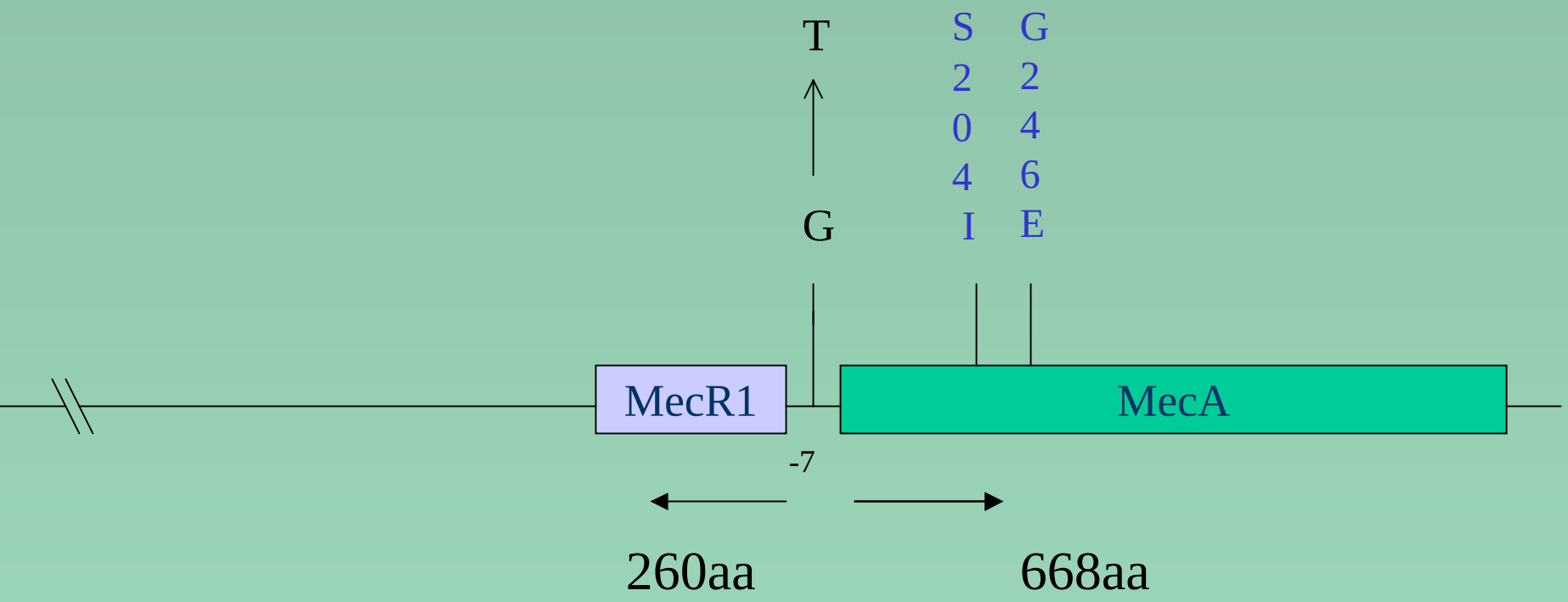
Summary of the *mec* DNA types

- At the gene level we found two types of *mec* DNA: *mec* Type I and *mec* Type II
- Type I had all three *mec* genes intact
- Type II had a deletion of 1344 bp
- Due to this deletion entire *mecI* gene and 2/3rd of *mecR1* was lost
- The deletion junction was identified

Type I


K * 1 1 2 1 1 5 N E
* * M 6 5 3 8 2 1 3
T A G T
S G 2 2 0 4 4 6 I E
MecI
MecR1
MecA
-7 -5
123aa
585aa
668aa

Type II



Summary of major mutations

- ***mecA mutations***
  - 33% of the isolates had –7 upstream G to T change in *mecA*
  - At codon 246: Glutamic acid → to Glycine
- ***mecI mutations***
  - 12.5% had Asparagine → Lysine change at codon 121
  - Mutations that could lead to truncation of MecI at amino acids 55, 68, and 115
- ***mecR mutations***
  - *mecR1* has a synonymous change at codon 583: Glu → Glu

Correlation between mutations in *mec* genes and oxacillin MICs

Major Mutations	% of Isolates	Oxacillin MIC ≤96 μg/ml (%)	Oxacillin MIC ≥ 96 μg/ml (%) = high resistance	Resistant to	Major Clonal Group & (%)
mec type II	20	33	66	52% to Ery	Clonal group 2 (65%)
mecA ups -7 G to T	33	37	63	67% to Ery	Clonal group 2 (81%)
mecA 246 Glu -> to Gly	96	23	77	38% to Clin, Cip, Ery	Clonal group 7 (35%)
mecI 121 Asn to Lys	13	84	16	80% to Ery	Clonal group 2 (90%)
mecR1 Glu-> to Glu	7.5	13	87	79% to Clin, Cip, Ery	Clonal group 4 (71%)

Conclusions

- It appears that
  - i) deletion of *mecI and partial deletion of mecR1*
  - ii) mutation in *mecA* promoter/operator sequences
  - iii) mutations in *mecA or mecR1* genes

  contributes to high level of resistance against oxacillin in 2/3rd of the MRSA isolates from Wisconsin

- The oxacillin resistance in remaining 1/3rd of the isolates is probably influenced by other *mecA* regulatory elements such as *blaI-blaR1*

Conclusions continued

- Other genes such as *femA*, *femB*, *etc*. (factor essential for methicillin resistance) might play a role as well

Future studies

- We intend to determine the presence or absence of *blaI-blaR1* genes in those 1/3rd of isolates
- Sequence *blaI-blaR1* from a select number of isolates to see if mutations in these genes will help explain the discrepancies noted in oxacillin resistance in *S. aureus*

- Marshfield Medical Research Foundation
  - Jennifer Conradt
  - Mary Stemper
  - Kurt Reed
- Baylor College of Medicine, Houston,TX
  - Srinivas Ramaswamy
  - Richard Reich
  - Edward Graviss

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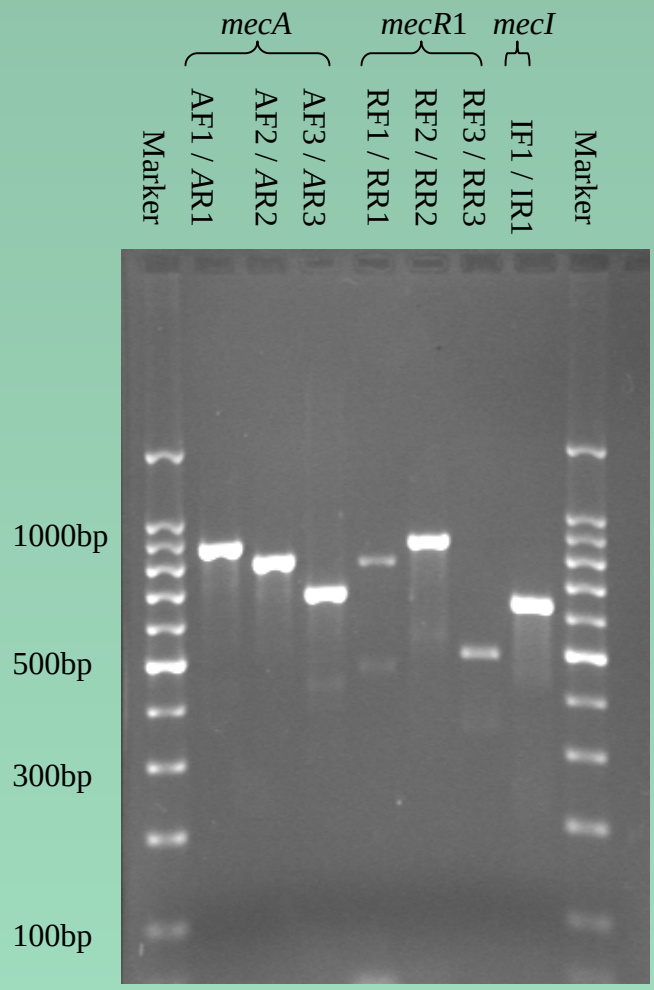
mecA
mecR1
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AF1 / AR1
AF2 / AR2
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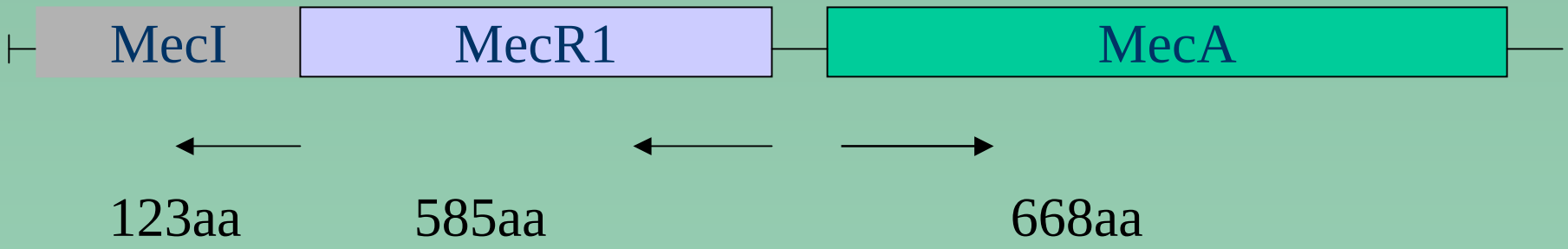
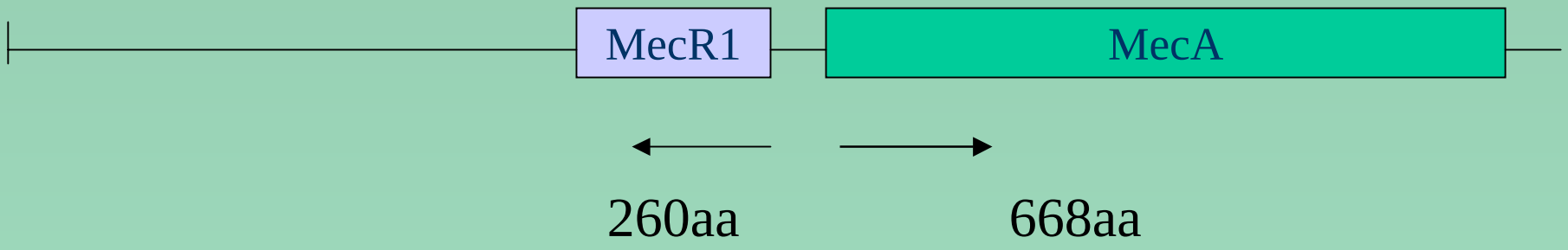
IF1 / IR1
Marker
1000bp
500bp
300bp
100bp

- Two types of mec operon in Wisconsin MRSA isolates
  - Type I is intact with all three genes
  - Type II has a deletion of 1344 bp
    - Loss of *mecI* and 2/3rd of *mecR1*
- Mutations in *mecA* promoter/operator sequences, *mec* genes

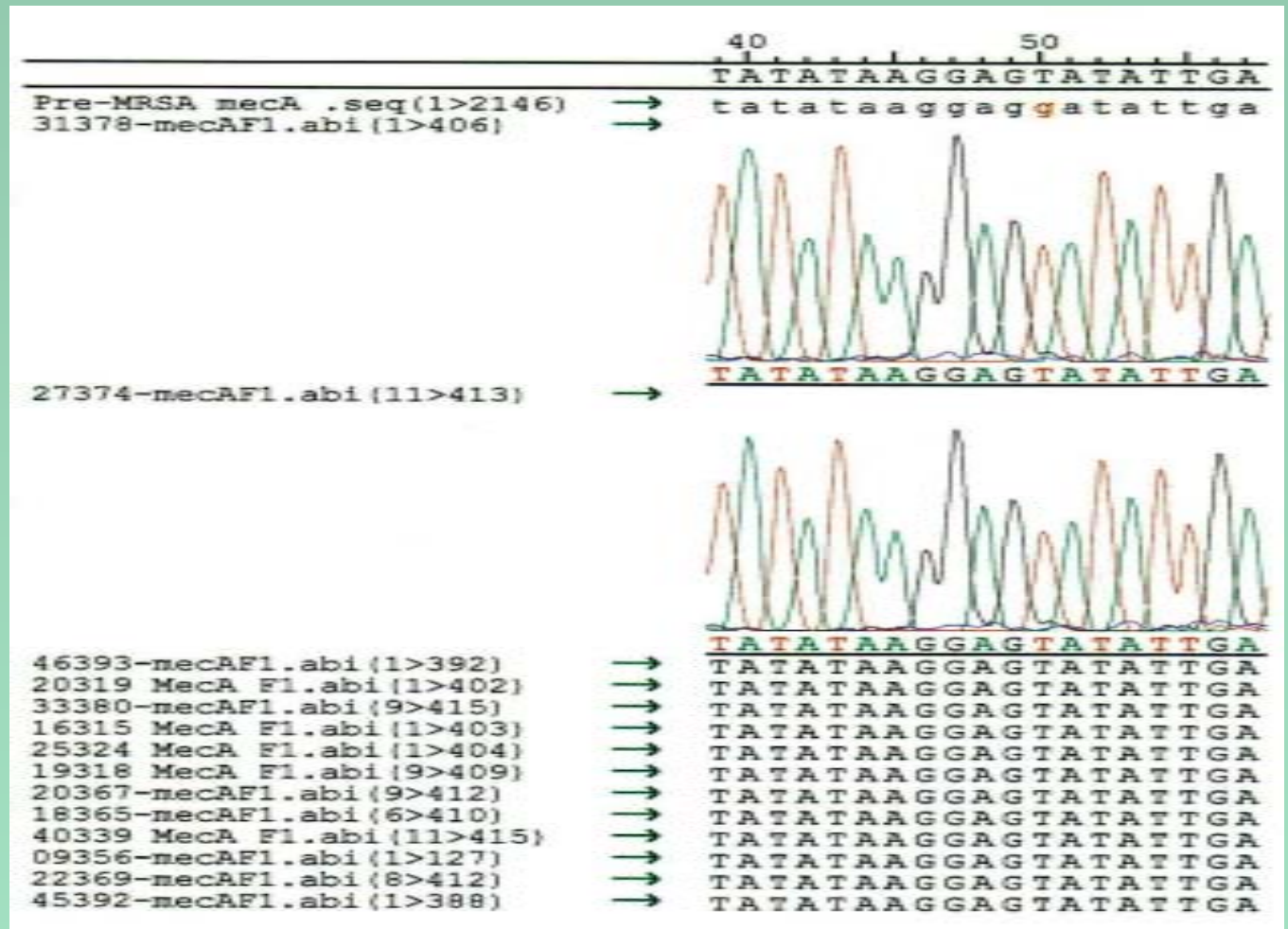
Type I

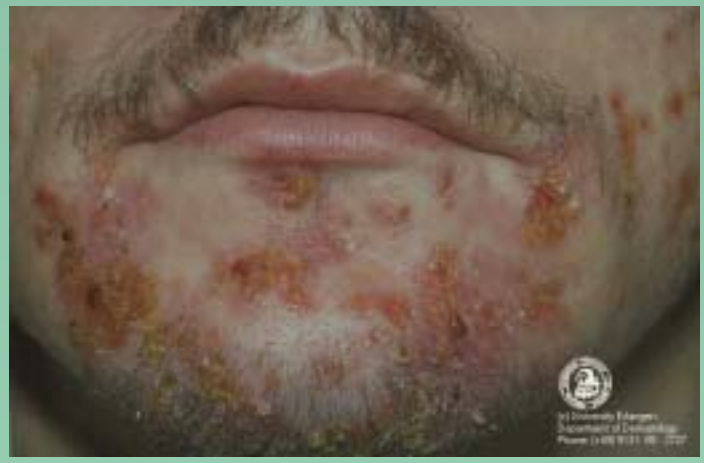


Type II

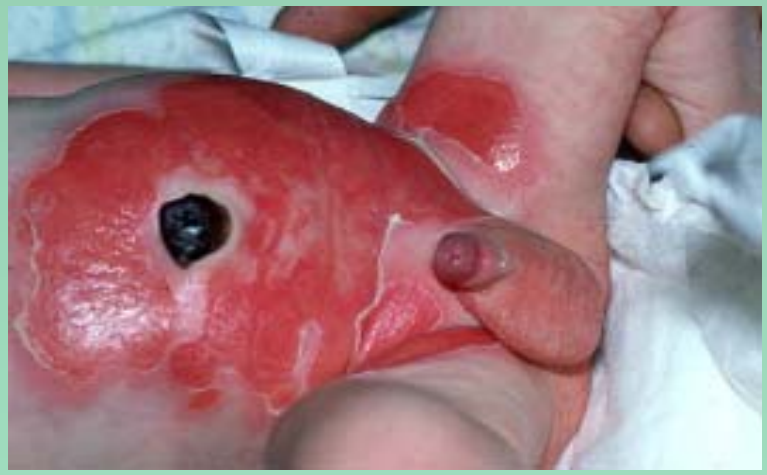


Results

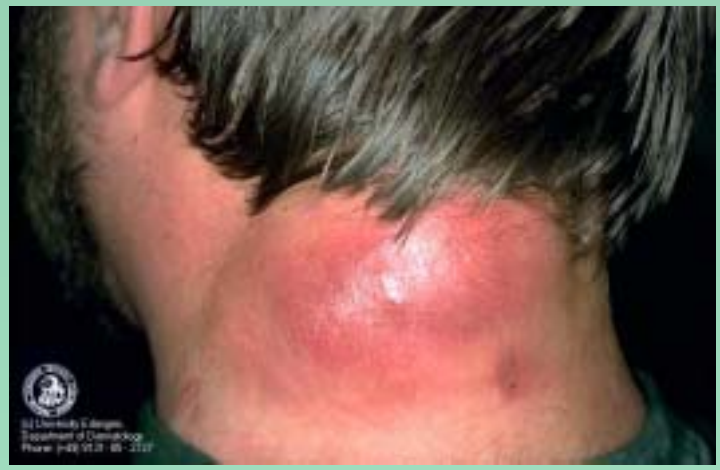

40
50
TATATAAGGAGTATATTGA
Pre-MRSA mecA .seq(1>2146)
31378-mecAF1.abi(1>406)
tatataaggaggatattga
TATATAAGGAGTATATTGA
27374-mecAF1.abi(11>413)
TATATAAGGAGTATATTGA
46393-mecAF1.abi(1>392)
20319 MecA F1.abi(1>402)
33380-mecAF1.abi(9>415)
16315 MecA F1.abi(1>403)
25324 MecA F1.abi(1>404)
19318 MecA F1.abi(9>409)
20367-mecAF1.abi(9>412)
18365-mecAF1.abi(6>410)
40339 MecA F1.abi(11>415)
09356-mecAF1.abi(1>127)
22369-mecAF1.abi(8>412)
45392-mecAF1.abi(1>388)
TATATAAGGAGTATATTGA



Staphylococcus aureus impetigo

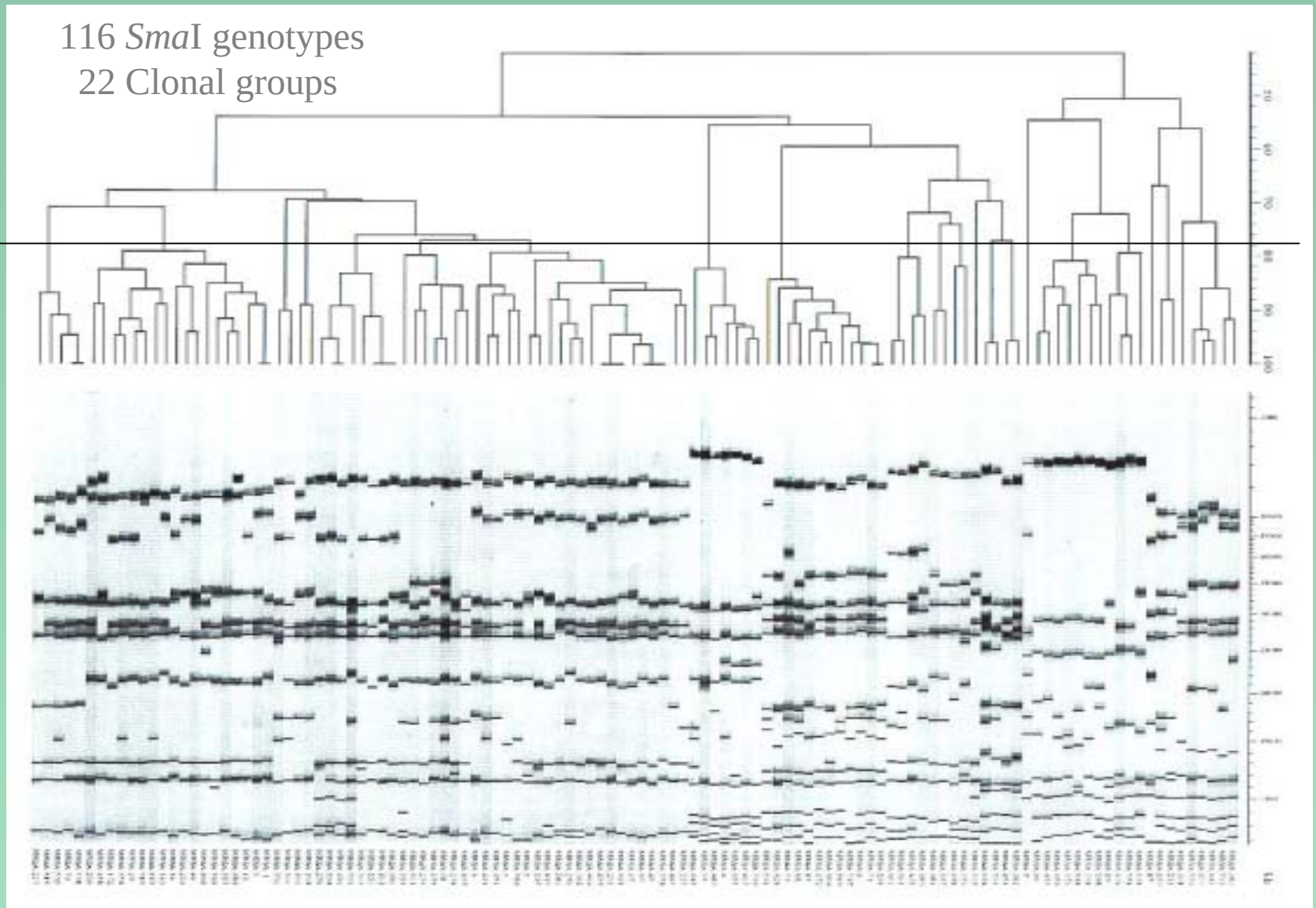


Staphylococcal scalded-skin



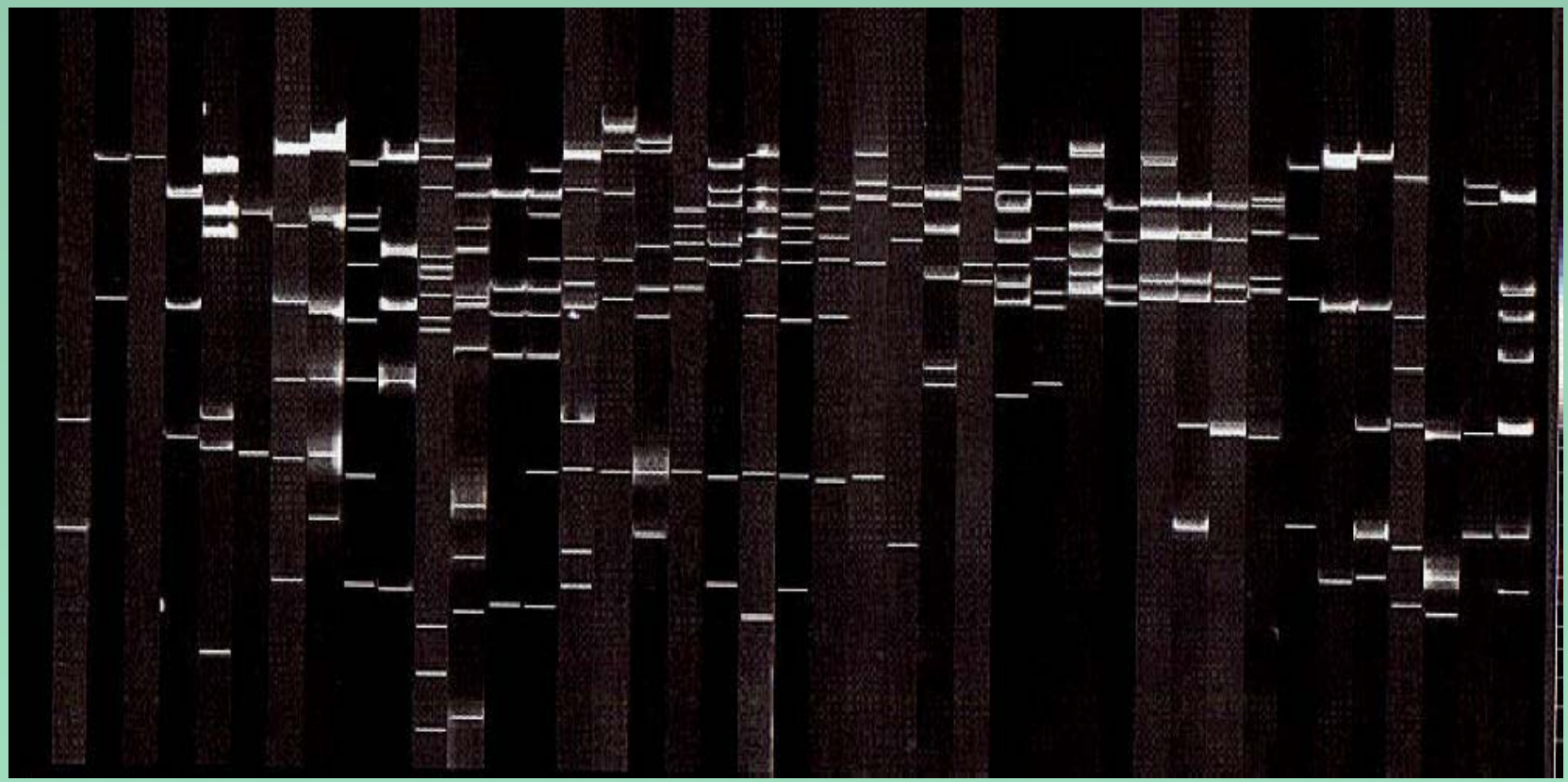
Staphylococcus aureus carbuncle

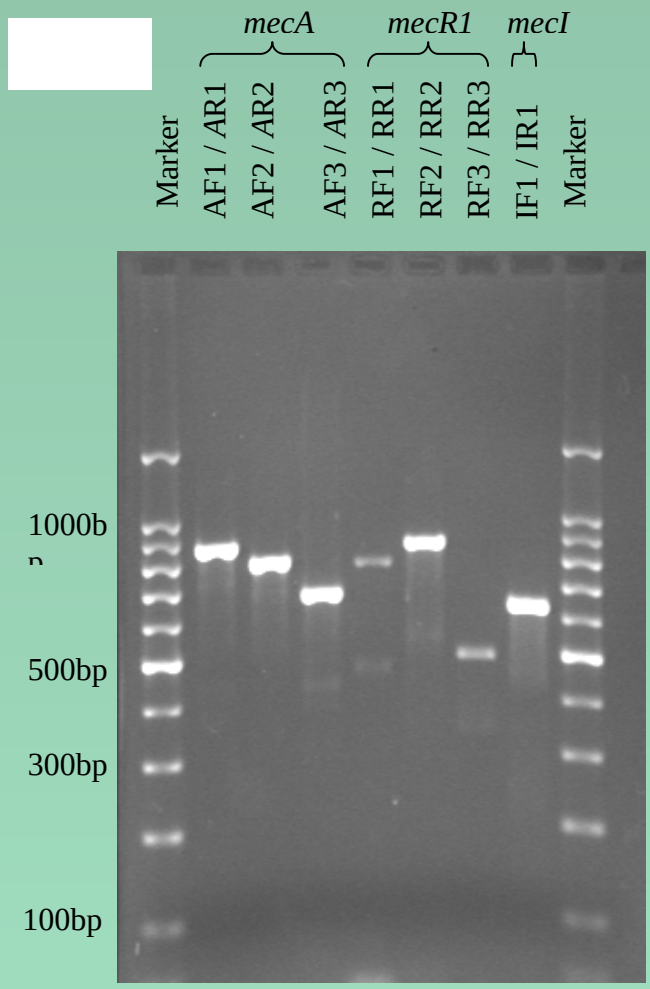
MRSA Pulsed-field Gel Electrophoresis (PFGE) Dendrogram



MRSA Plasmid Library

42 unique *Eco*RI restriction digest patterns

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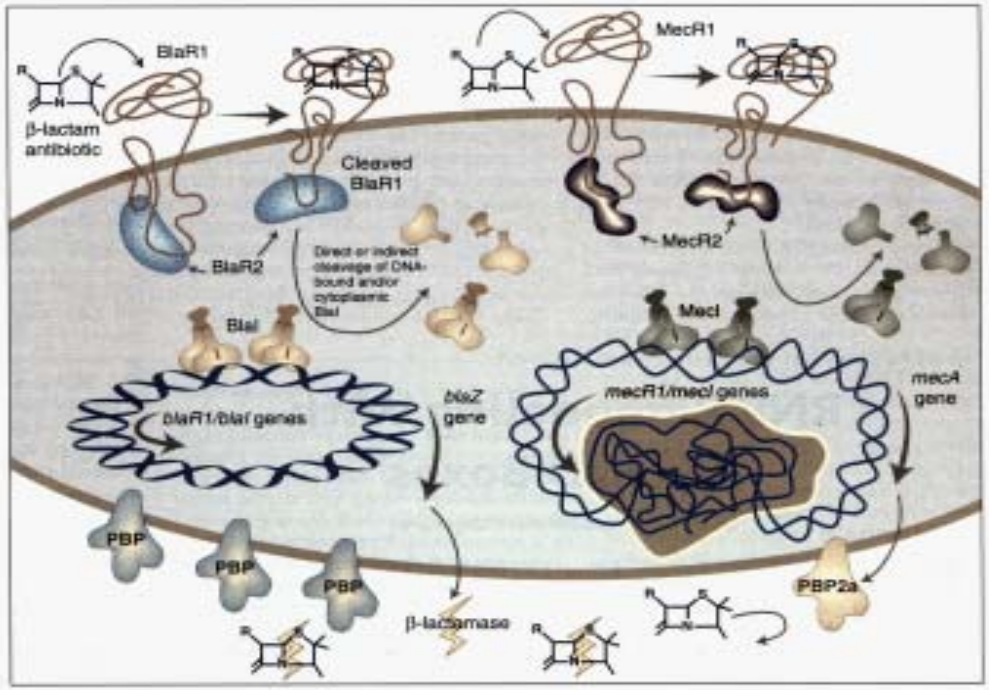
Signaling Antibiotic Resistance in Staphylococci



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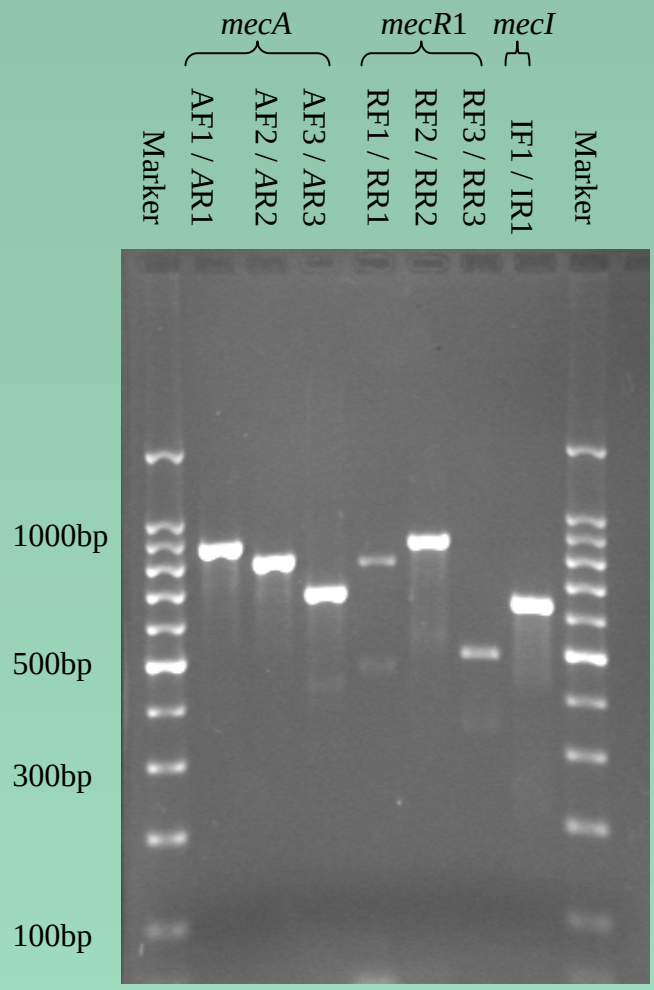
iversity, Richmond,
her@hsc.vcu.edu,

Regulation of β-lactam resistance. Two related pathways regulate resistance to β-lactam antibiotics in staphylococci. (**Left**) Production of β-lactamase is regulated by the sensor-transducer BlaR1 and the repressor BlaI, which blocks transcription of the β-lactamase gene, *blaZ*. When a β-lactam antibiotic binds to the extracellular sensor domain of BlaR1, the cytoplasmic transducer domain is proteolytically cleaved. The transducer is then free to cleave and inactivate the BlaI repressor, and transcription of *blaZ* ensues. (**Right**) The *mecA* gene encodes PBP2a, which binds β-lactam antibiotics with low affinity. Expression of *mecA* is regulated by a similar sensor-transducer and repressor system. The BlaI and MecI repressors regulate production of the β-lactamase and PBP2a genes in similar ways, but their sensor-transducers are not interchangeable. BlaR2 (blue oval) and MecR2 (purple oval) are hypothetical accessory molecules that may be required for the sensor-transducers to interact with their repressors.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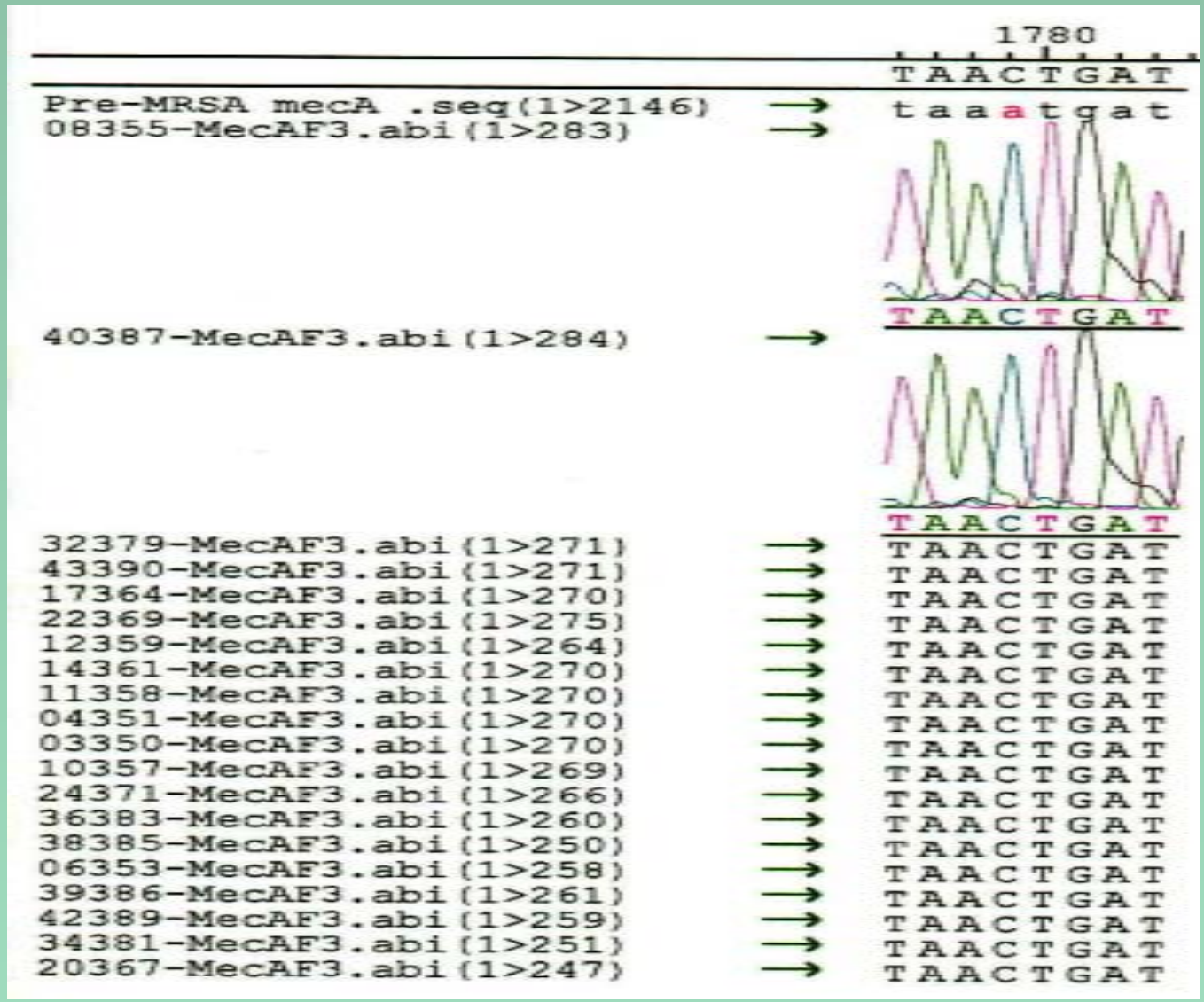
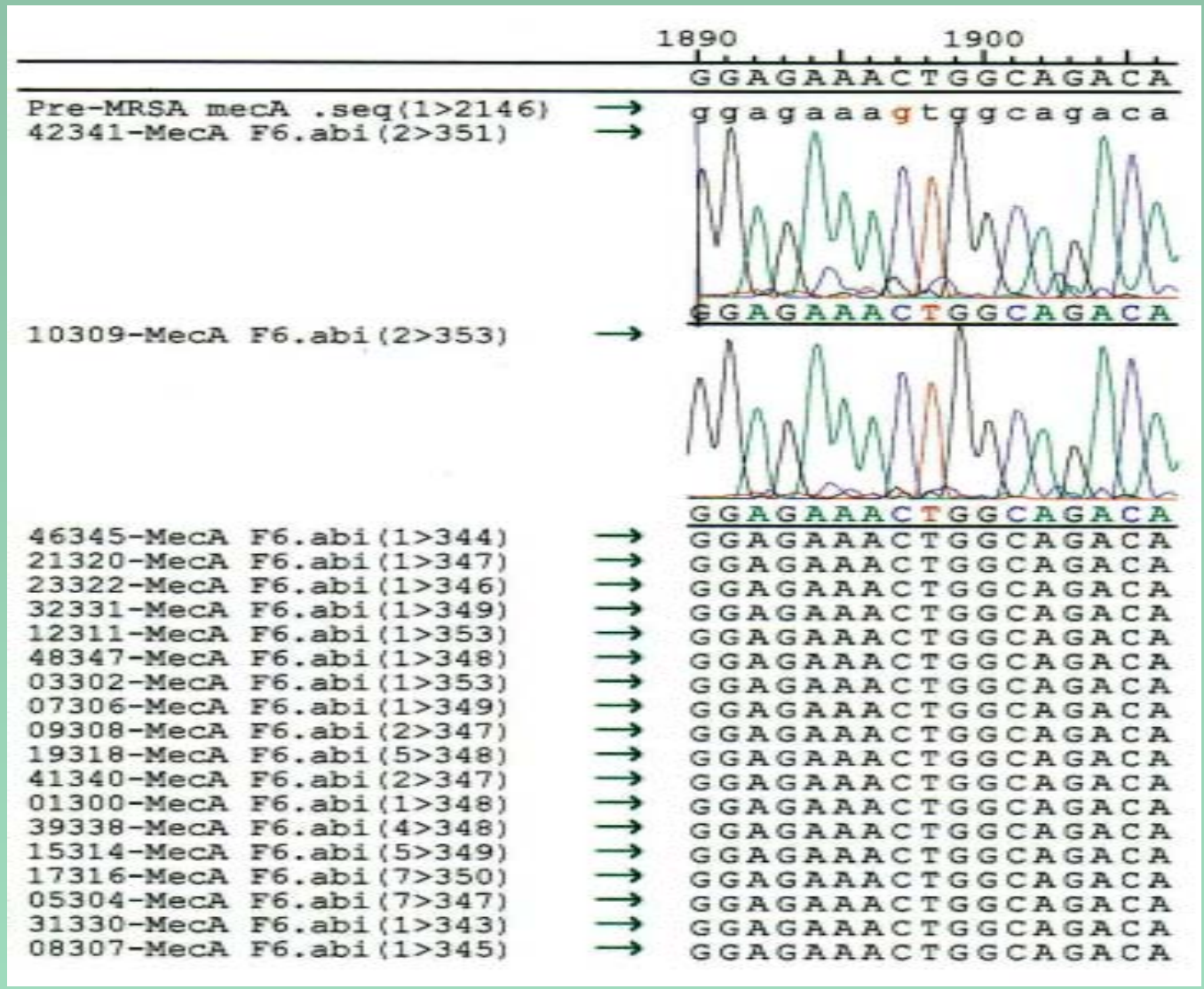


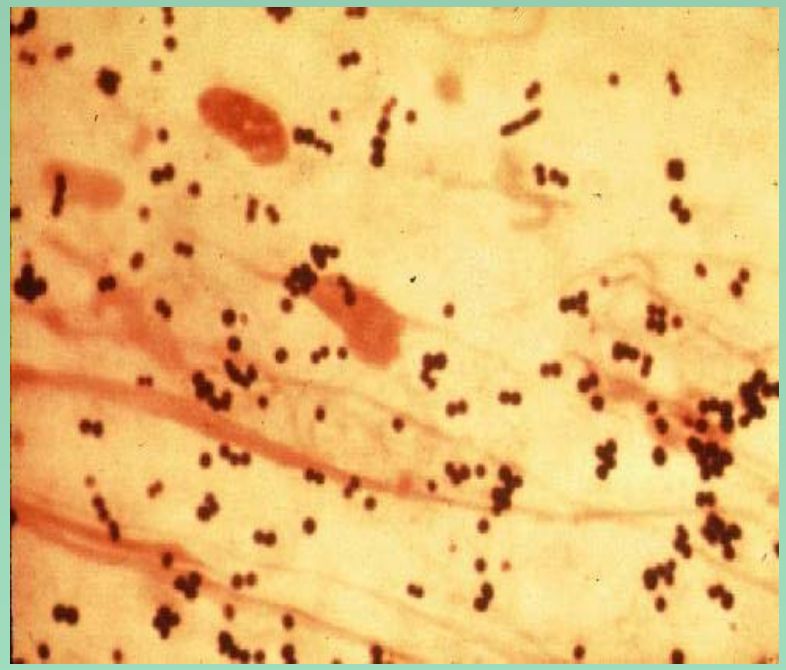
Codon 572 A**A**T --> A**C**T; Asn--> Thr



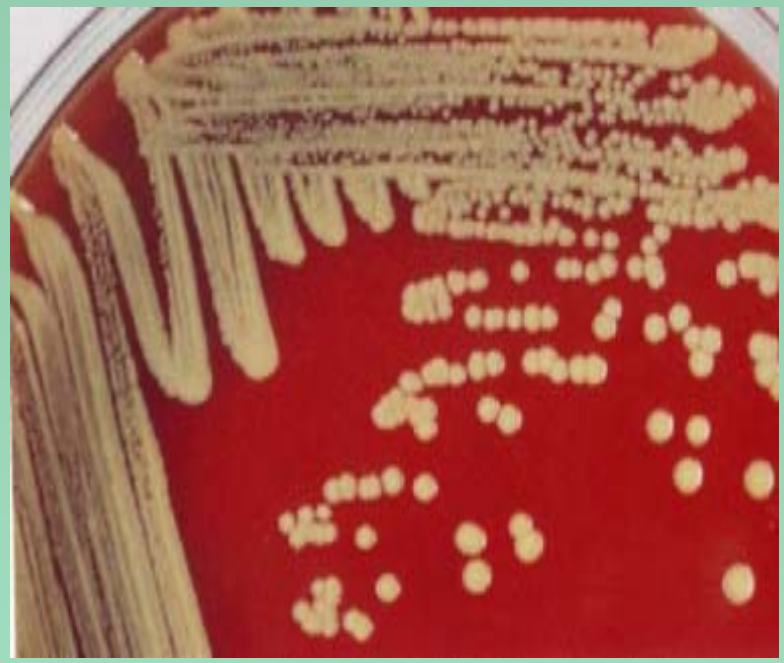
Codon 610 AGT --> ACT; Ser --> Thr

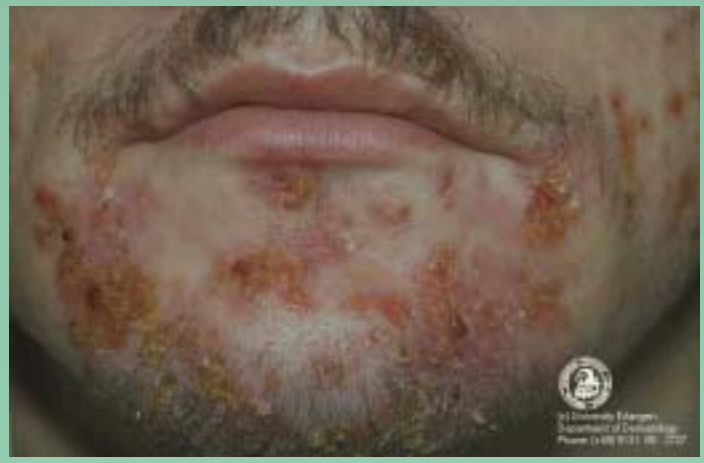
Staphylococcus aureus

Gram stain



Colony morphology





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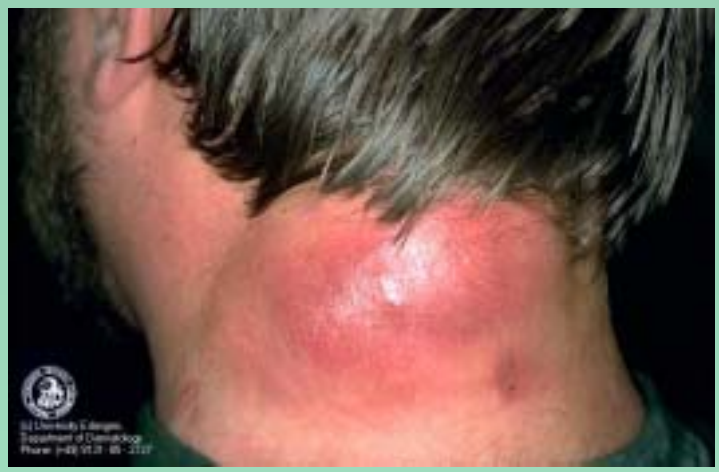

Staphylococcus aureus carbuncle

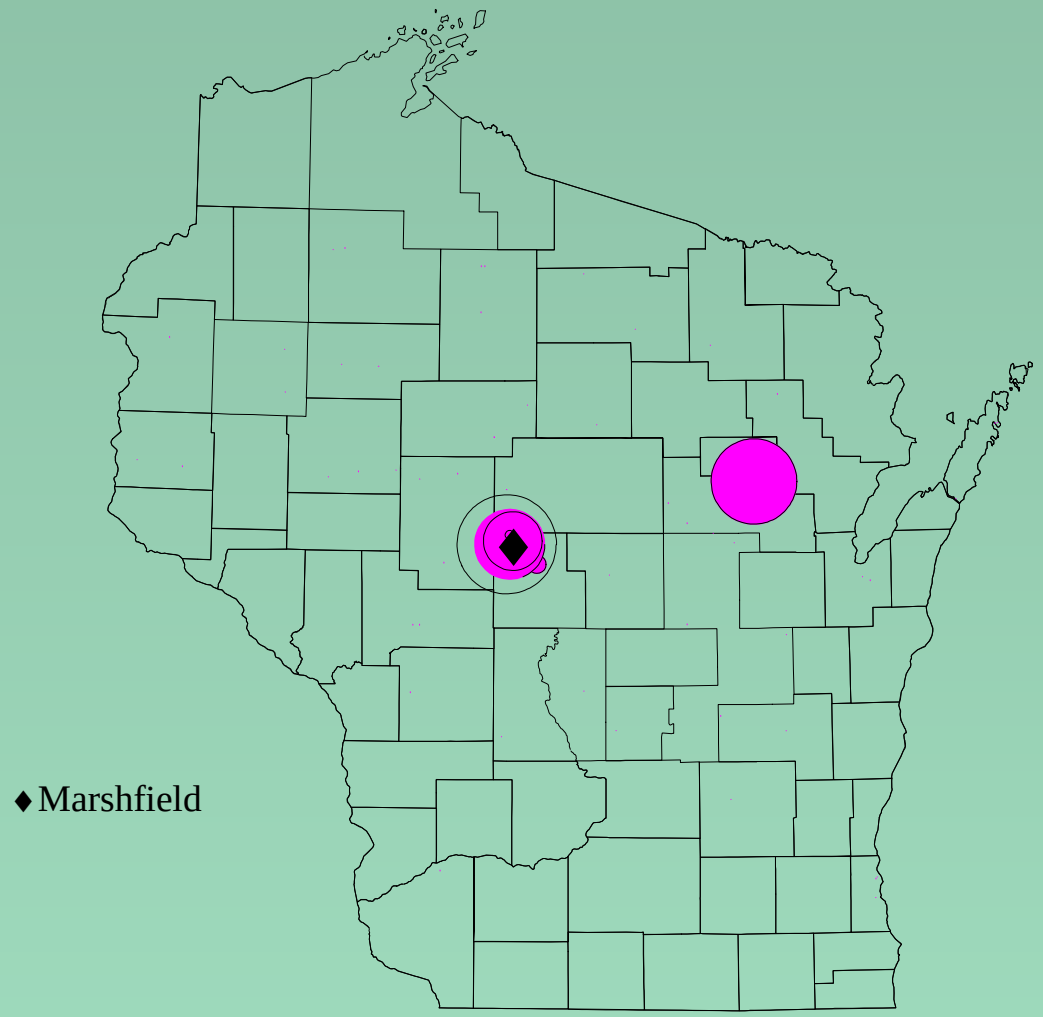

Figure 1. Map of Wisconsin showing the locations where MRSAs were isolated.