

Methicillin / β -lactam resistance in *S. aureus*

Resistance towards penicillinase stable penicillins, cephalosporins and carbapenems in *S. aureus* (MRSA) is somewhat complex

So lets take it piece for piece

**The good thing is for almost all the items
Cefoxitin disk diffusion is the answer 😊**

Methicillin / β -lactam resistance in *S. aureus*

- Resistance mechanisms
 - *mec* encoded production of an extra PBP - PBP2'/PBP2a/b/c
 - Enzymatic degeneration (BORSA)
 - Modified PBPs (MODSA)



***mec* encoded resistance**

- *mec* encoded resistance
 - The mechanism of epidemiological significance!!
 - R to all β -lactam antibiotics except 5th generation cephalosporins (ceftaroline and ceftobiprole)
- Four *mec* genes described in staphylococci, macrococcus and Mammaliicoccus sciuri (former *S. sciuri* group) - 9 subtypes
 - ***mecA*** (*mecA*, A1 and A2)
 - *mecB* – only described in one isolate of *S. aureus* (located on a plasmid) and in *macrococcus*
 - ***mecC*** (*mecC*, C1, C2 and C3)
 - *mecD* – only found in macrococcus and not in *S. aureus* (resistant to 5th gen cephalosporins)



mecA/C encoded resistance

- *mec* genes is foreign to staphylococci
 - introduction pathway is not fully elucidated but involve mammaliicoccus and macrococcus
- *mec* gene complex
 - *mecA* genes
 - regulatory genes *mecI* – *mecR1/R2*
 - IS431 / IS1272
- 5 types A, B, C1, C2 and E
 - type D only in *S. caprae*
 - Type E do not have an IS element



SSCmec

- *mecA and mecC gene complex* is situated in the Staphylococcal cassette chromosome mec (SSCmec)
- SSCmec consist of:
 - *Mec gene complex*
 - cassette chromosome recombinase (*ccr*) gene complex
 - *ccrA, ccrB and ccrC*
 - Joining regions
 - J1, J2 and J3
- SSCmec is always situated in the *OriC* region just downstream of *orfX*

SSCmec

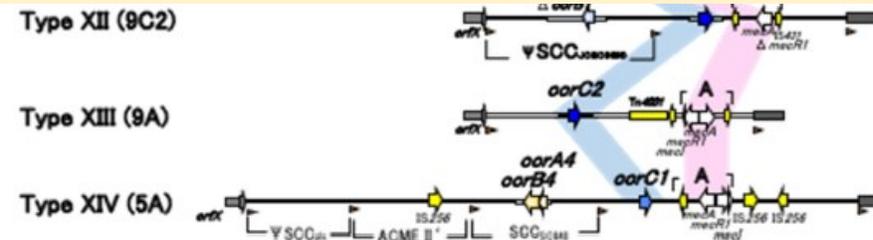


SSCmec types are based on

- Recent study by Huang et al. used datamining to examine the NCBI database to screen for novel SSCmec types
 - 12 novel *ccr* genes (6 *ccrA*, 3 *ccrB* and 3 *ccrC*)
 - *ccrA*1-14, *ccrB*1-12 and *ccrC*1-5
 - 12 novel *ccr* gene complexes – i.e a total of 22 different *ccr* complexes
 - **10 novel SSCmec types - i.e. a total 25 different SSCmec types**

Huang, J. inf Dis, 2024, 231-8

Uehara, Antibiotics, 2022
IWG-SSCmec, AAC 2009
<https://www.sccmec.org>
Wang JAC, 2022





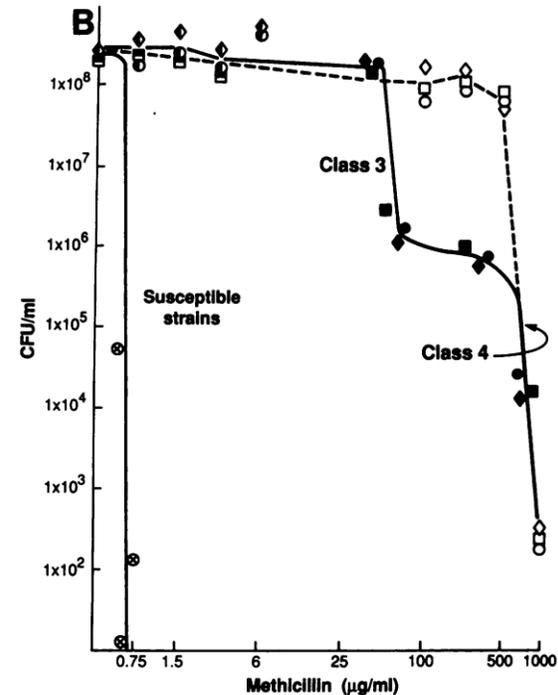
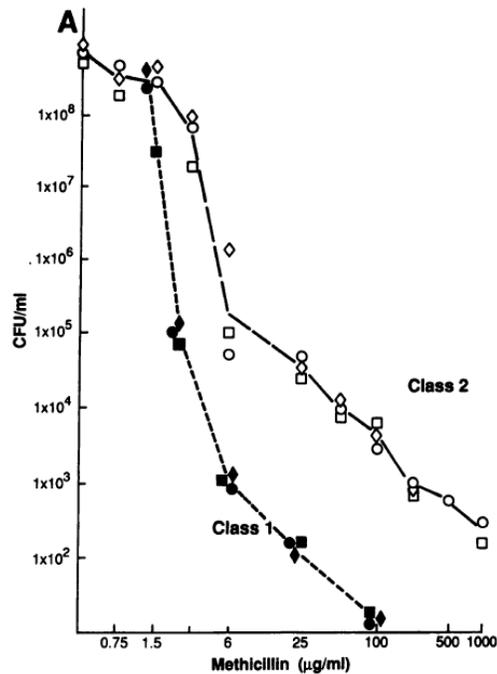
Additional genetic elements involved in the expression of the MRSA phenotype

- *blaZ* regulatory genes *blaI* - *blaR1*
- Genes / regulator systems involved in cell wall precursor metabolism as well as physiological processes including
 - *femA/B/C*
 - *GlnR*
 - ClpXP system
 - GdpP
 - RNA polymerase (*RPoB/C*)
 - AGR system
 - *SarA*
 - *Stk1/stp1*
 - *FtsH* protease
 - Stringent response - *rel* gene



mecA/C mediated resistance - Resistance types

- Most *S. aureus* express a heterogeneous resistance profile with only a part of the cells expressing high level resistance
 - Noted already by Jevons in her first paper describing MRSA
 - Thomasz et al. showed in 1991 that *S. aureus* could be categorized into as heteroresistance type I-III or homogeneous resistance (type IV)





Hetero resistance- stringent response

- Heteroresistance type is probably related to the **stringent response**
 - controlled by the *rel* gene
 - regulating the cell level of (p)ppGpp (Guanosine tetra and pentaphosphates) - “alamone” / master regulator of most bacteriological processes





Other mechanisms of methicillin resistance

- Degradation due to hyper production of penicillinase
 - Low level resistance (Oxacillin 4-16 mg/L) - “Borderline resistant *S. aureus*” - (BORSA)
 - Very little evidence on the clinical implication for these isolates!
- **In practice, those resistant to cefoxitin should be treated as MRSA**
- Modified PBPs (MODSA)
 - Low / High level resistance (4 - >256 mg/L)
 - PBP4 overproduction, PBP4 + GdpP alterations
 - **In practice, those resistant to cefoxitin should be treated as MRSA**



OS-MRSA

- Oxacillin susceptible *mecA* positive *S. aureus* i.e. isolates with oxacillin < 4 mg/L
- Nomenclature is used both for
 - Heteroresistant MRSA class 1 Characteristic of the individual isolate
 - regulated by the stringent response of the isolate
 - *mecA* positive isolates with mutations in the *mec*, in the regulatory genes and auxiliary genes
 - Multiple mechanisms and multiple mechanisms of restoration of activity during treatment = Resistant - even for the same mutations!!



OS-MRSA - HETERORESISTANT MRSA CLASS 1

- Characteristic of the individual isolate
 - Regulated by the stringent response of the isolate
 - Population analysis reveal resistant subpopulations
 - Cefoxitin screen positive (cefox R)
 - Oxacillin/ β -lactam exposure will reveal selection of resistant mutants
- *mecC* isolates has higher affinity to oxacillin than cefoxitin i.e. MIC for oxacillin is lower
 - Oxa S – cefox R (MIC) is quite common in *mecC* isolates

Implication for clinical practice – MRSA
Cefoxitin DD detect these isolates = R



OS-MRSA – MUTATIONS

- *mecA* positive isolates with mutations in *mec*, in regulatory genes, auxiliary genes, or
- Population analysis will not show a resistant population
- Cefoxitin screen negative (cefox S)
- Oxacillin/ Oxacillin/ β -lactam exposure **may** (will eventually?) lead to selection of resistant mutants

Clinical Practice -

You will only find them if you test both with genotypic and phenotypic methods

Importance is uncertain

Report as MRSA if found



Conclusions

- The genetics of methicillin resistance is highly complex
 - very closely associated to the epidemiology of MRSA
- Literature on MRSA is to some extent complicated by lack of consensus on definitions and nomenclature
- The Stringent response has in recent studies be shown to be involved in both tolerance and heterogeneity
- OS-MRSA represent a challenge for the clinical laboratories

**Clinical practice – Use cefoxitin disc diffusion as your primary method
if R report and treat as MRSA**

- Consider periodically to check that OS-MRSA is not part of your local epidemiology



Thank you



Questions?

