CASE 2

Case 2

- 56 yr old presenting to ED in septic shock
  - Fever 103°F
  - Markedly tachypneic
- History of present illness:
  - 2d of fevers/chills
  - 2d of shortness of breath
- Past medical history:
  - Kidney transplant 6 yrs ago, on renal dialysis
  - AV fistula placement 3 yrs ago

Case 2

- Microbiology Cultures
  - 2 sets of blood cx
    - 2/2 *S. aureus*
  - MRSA Nasal Screen by PCR
    - Positive

- Results Timeline
  - Day 1- Positive blood cx
    - » GPR on GS
    - » MRSA Detected by PCR
  - Day 2- Growth on culture plates
    - » *S. aureus*
  - Day 3- MIC available for *S. aureus*
    - » Discordant molecular and MIC
  - Day 4-5- Repeat testing/re-isolation

Case 2

Molecular AR

From Positive Blood Cx	
mecA	Detected
SCCmec	Detected

Clinical Lab Results

MIC Testing

Antimicrobial	MIC µg/mL
Cefoxitin Screen	S
Oxacillin	≤ 0.5 S
Erythromycin	≥ 8 R
Clindamycin	≤ 0.25 S
Vancomycin	1 S
InCR	Negative

From a Lab Director's Perspective

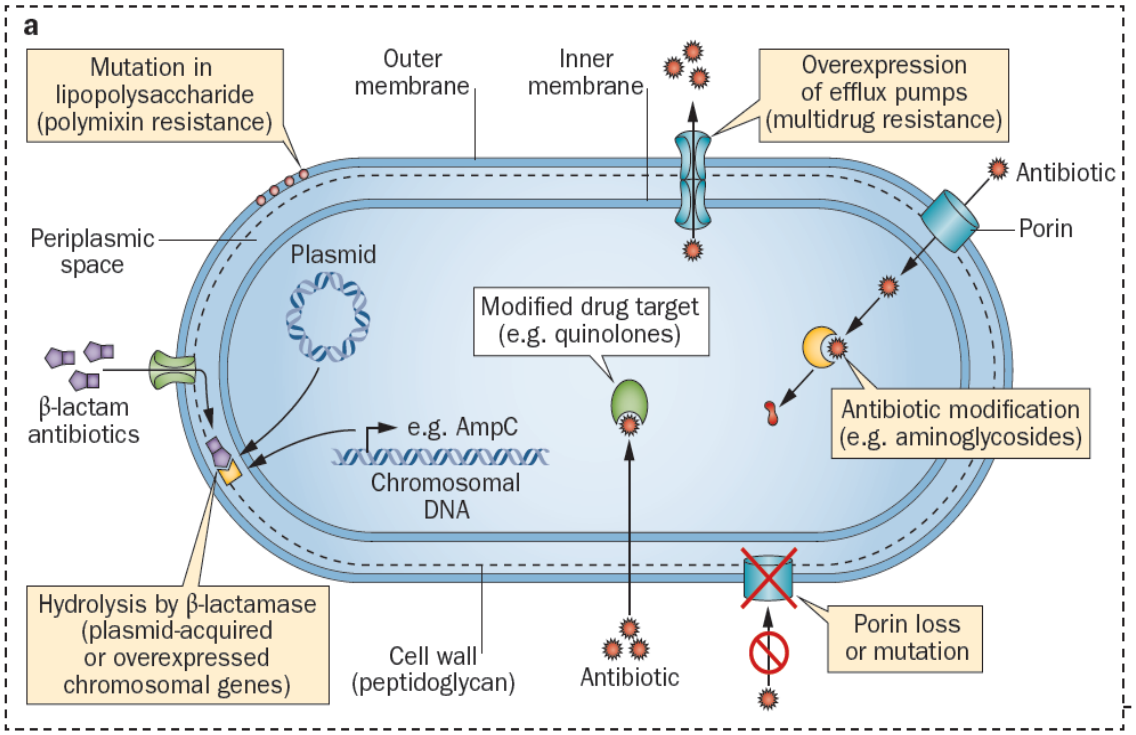
3 Different Scenarios Encountered:

1. Genotype correlates with phenotype – Woohoo!

2. Detection of a AMR resistance marker with a susceptible AST profile

3. Lack of detection AMR resistance marker and a resistant AST profile

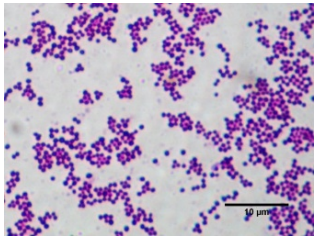
Mechanisms of β-lactam Resistance



1. β-lactamase enzymes- mostly Gram-negatives
2. Efflux pumps – actively pump the drug out
3. Porin mutations – preventing the drug from coming in
4. Altered penicillin binding proteins – altered target – mostly Gram-positives

The Interface Between Lab & Clinicians

Molecular Panel: *mecA* & *Staphylococcus aureus* detected by NAT



Oxacillin MIC:
≤0.5 µg/ml
Susceptible

Day 0 — Collection and incubation of the blood culture bottles

Day 1 — Blood culture flags positive

Day 2 — Growth and confirmation of *S. aureus*

Day 3 — AST results

Staphylococcus aureus
in Anaerobic Bottle

	S. aureus	
ANTIBIOTICS	MIC	INTRP
Oxacillin	0.5	S
Vancomycin	<=0.5	S

BACTERIOLOGY

Bac Blood Cult * FINAL 05/12/19

Gram stain positive for Gram Positive Cocci in Clusters
Critical action value called to and read back by G Sell
05/10/2019 09:29
Identified as Staphylococcus aureus, methicillin resistant by the Nucleic Acid Microarray Test.

"Phenotypic antimicrobial susceptibility testing results do not match the genotypic antimicrobial resistance gene results for oxacillin. Infectious diseases consult may be warranted."

What Are the Possibilities?

- Lack of expression of *mecA* in *S. aureus*
- Heteroresistance
- Mixed with a coagulase-negative staphylococci or another *S. aureus* harboring *mecA*
- False-positive *mecA* detection? Cross-reactivity? Or exogenous nucleic acid?
- Issues with the method/instrument

What Do You Do When Genotype and Phenotype Don't Agree?

Table H1. (Continued)

Indication	Target(s)	Method	Specimen Type	Results		Suggestions for Resolution	Consider reporting as[a]:	Comments[b]
				Genotype or Predicted Phenotype	Observed Colony Phenotype (if tested)			
Detection of methicillin resistance in *S. aureus* (Continued)	SCC*mec*-*orfX* junctional regions and *mecA* and/or other targets	NAAT	Blood culture broth, surveillance specimen	SCC*mec* AND *mecA* or other target detected	Cefoxitin R	N/A	If tested, report phenotypic result as found (methicillin R) and consider reporting molecular result per institutional protocol.	3–6
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin S	N/A	If tested, report phenotypic result as found (methicillin S) and consider reporting molecular result per institutional protocol.	3–6
				SCC*mec* AND *mecA* or other target detected	Cefoxitin S	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT if available. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R.	2
				SCC*mec* AND *mecA* or other target not detected	Cefoxitin R	Confirm isolate identification, repeat AST and consider *mecA* colony NAAT if available. If mixed culture, test isolates individually	If discrepancy is not resolved by suggested testing, report as methicillin R.	3, 11

Cefoxitin Disk to Uncover the Culprit

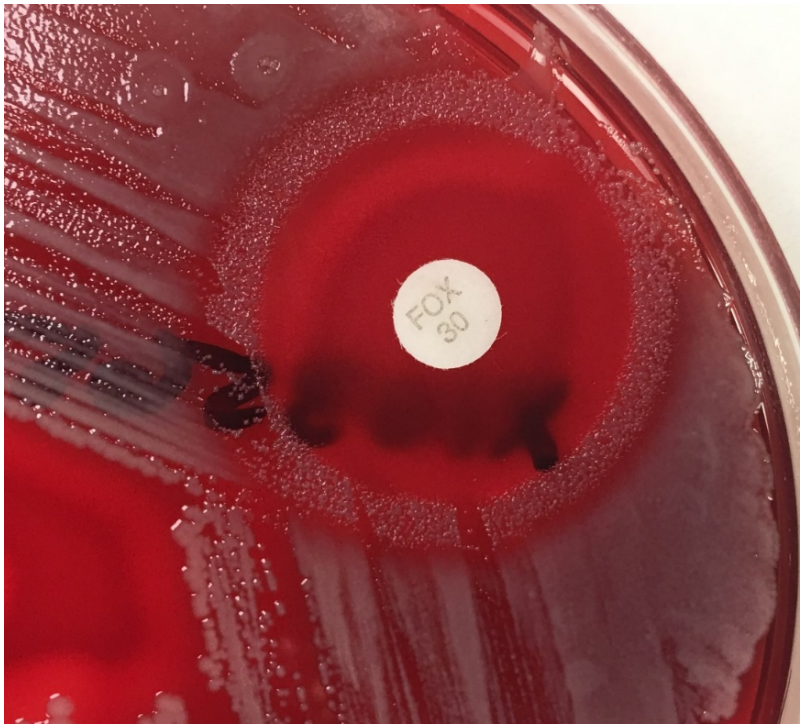


Image courtesy of Raquel Martinez

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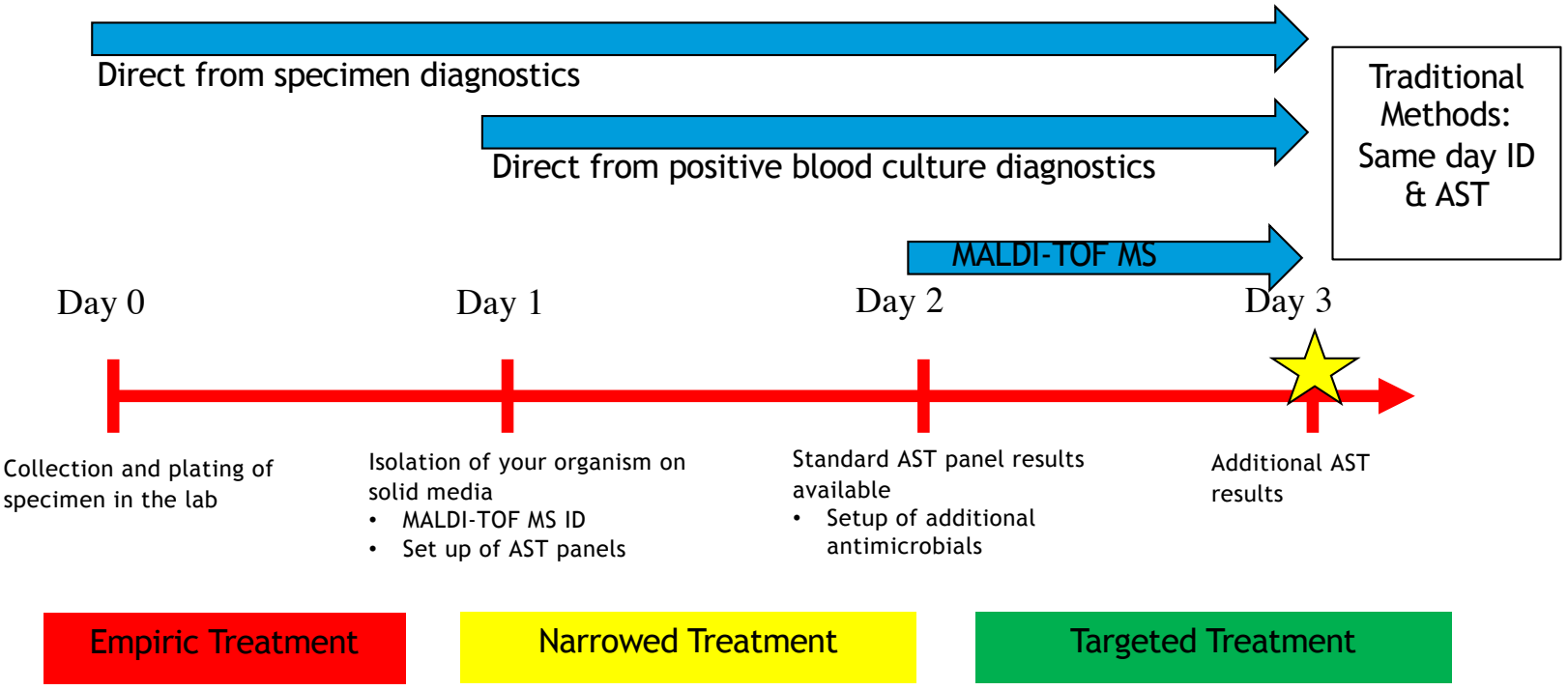
Importance & Reliance on Antibiograms Grow!



Average TAT: 2-3 days

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Available & Forthcoming AMR Detection Methods

Source	Test	AMR genes	TAT (hr)	FDA Status
Whole blood	T2 Resistance	*mecA/C*, *vanA/B*, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-23/OXA-48-like}$, bla_{CMY}, bla_{DHA}	3-5	
+ Blood Cultures	Xpert MRSA/SA BC	*mecA*	1	✓
	Biofire BC-ID	*mecA*, *vanA/B*, bla_{KPC}	1	✓
	Verigene BC-GP & BC-GN	*mecA*, *vanA/B*, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA}	2.5	✓
	Genmark BCID-GP & -GN	*mecA/C*, *vanA/B*, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-23/OXA-48-like}$	1.5	✓
Respiratory	Biofire RP & RP2	None	1	✓
	Biofire Panel Pneumonia	*mecA/C*, *MREJ vanA/B*, bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}	1	✓
	Curetis Unyvero HPN	Expanded panel	4-5	✓
Urine	OpGen Acuitas ARM Gene Panel u5.47	Expanded panel	3	
Isolates	Xpert Carba-R	bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{OXA-48-like}$		✓
	WGS	Comprehensive (known AMR)		???

Combining AMR Testing With The Antibiogram

Organism	No. Strains	%S									
		CLI	DAP	DOX	ERY	LNZ	OXA	PEN	RIF	SXT	VAN
All *S. aureus*	244	80	99	98	50	100	52%	13	98	96	100
Oxacillin-resistant *S. aureus* (MRSA)	126	44	99	96	4	100	0	0	95	94	100
Oxacillin-susceptible *S. aureus* (MSSA)	118	97	100	99	72	100	100	18	99	97	100
S. aureus & *mecA* PCR*	231						53%				

Demonstrates high concordance between molecular and phenotypic methods for prediction of MRSA by *mecA*

M39-A5, CLSI, Coming Soon!

Case 2---What do you do with this result?

Molecular AR

From Positive Blood Cx	
mecA	Detected
SCCmec	Detected

Clinical Lab Results

MIC Testing

Antimicrobial	MIC µg/mL
Cefoxitin Screen	S
Oxacillin	≤ 0.5 S
Erythromycin	≥ 8 R
Clindamycin	≤ 0.25 S
Vancomycin	1 S
InCR	Negative

Try to sort out if it is OS-MRSA versus *mecA*-"MSSA"

The lab says even the PBP2a phenotypic test is negative

Dicloxacillin works better than nothing for OS-MRSA in a murine thigh model

Isolate	PFGE type	SCC*mec* type	Oxacillin MIC (µg/ml)	Highest oxacillin concn (µg/ml) at which cell growth occurred	Avg log CFU ± SD (%) per g thigh tissue			Susceptibility status defined by Vitek 2[c]	
					Untreated	Treated	*P* (treated vs untreated)	OXA	VAN
1306	Ia	IV	0.5	32	6.55 (8.6)	4.71 (9.7)	<0.001	R	S
1326	Ib	IV	0.25	0.5	6.6 (6.5)	4.50 (4.3)	<0.001	S	S
1552	II	IV	1	64	6.25 (10.3)	3.75 (9.1)	<0.001	R	S
4666	Ic	IV	1	1	6.53 (8.3)	3.72 (10.4)	<0.001	S	S
6083	Ic	IV	6	128	7.45 (11.2)	5.02 (6.2)	<0.001	R	S
2712	III	ND	256	>128	6.32 (3.6)	6.25 (8.8)	NS[e]	R	S
29213	IV	NA	0.125	0.5	6.70 (6.7)	1.18 (12.4)	<0.001	S	S

Ikonomidis et al. AAC 2008 52(11):3905-8

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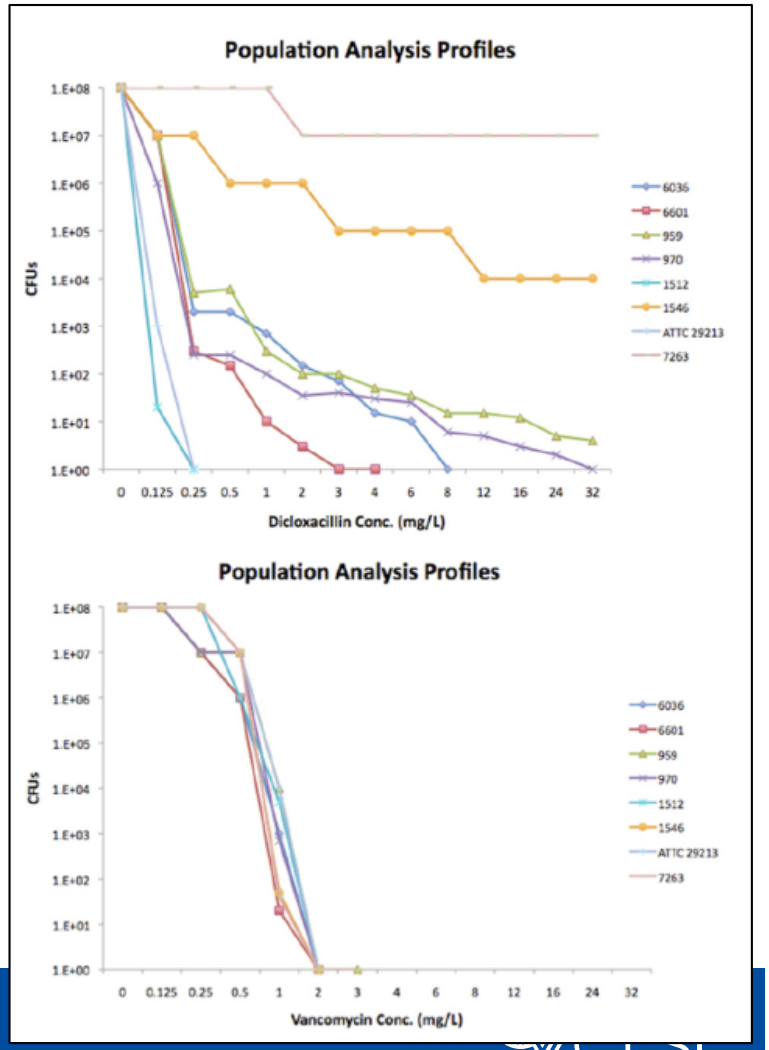
Treating OS-MRSA with dicloxacillin does not always work as well as vancomycin

Compared 15 OS-MRSA (mecA+/PBP2a+) isolates in time-kill and murine thigh

MICs of oxacillin =0.25-1µg/mL (one MRSA with MIC 256 µg/mL)

7 of the OS-MRSA had significantly less killing with the dicloxacillin

Success of 67% versus 75% diclox versus vanco

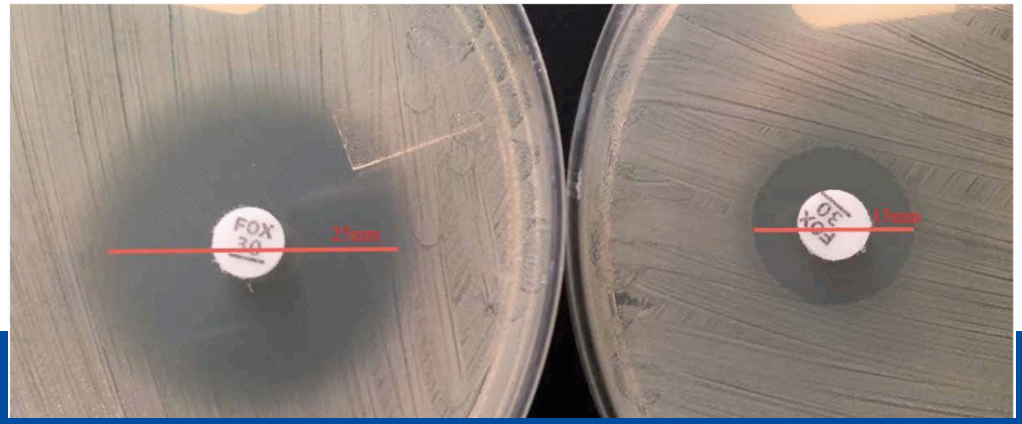


Labrou M et al AAC 2012 56(6):3388-91

My approach is mecA “MSSA”

- Patient high risk for endovascular focus and complicated bacteremia
- Very little in the literature around what to do besides treat as MRSA
- What is the cause of the “false positive” *mecA*

*mec*A-positive-
”MSSA”
After overnight induction with cefoxitin S➔R



Tenover FC & Tickler IA. Clin Micro News (2015) 37(10):79-84

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Antimicrobial exposure across multiple *mecA*-MSSA PCR positive caused reversion to resistance by frequent point mutation which restored the resistant phenotype

		Before cefoxitin selection			After cefoxitin selection		
Isolate #	SCC*mec* Type	Oxacillin MIC (µg/ml)	Cefoxitin screen (µg/ml)	PBP2a	Oxacillin MIC (µg/ml)	Cefoxitin screen (µg/ml)	PBP2a
CRG2382	IV	0.5	≤4	Negative	>2	>4	Positive
CRG2383	IV	0.5	≤4	Negative	>2	>4	Positive
CRG2935	IV	≤0.25	≤4	Negative	>2	>4	Positive
CRG2937	II	≤0.25	≤4	Negative	>2	>4	Positive
CRG2939	II	≤0.25	≤4	Negative	1	>4	Positive
CRG2941	II	≤0.25	≤4	Negative	>2	>4	Positive
CRG2943	IV	≤0.25	≤4	Weak positive	>2	>4	Positive

Table adapted from publication RV Goering et al. AAC 2019. doi:10.1128/AAC.00558-19

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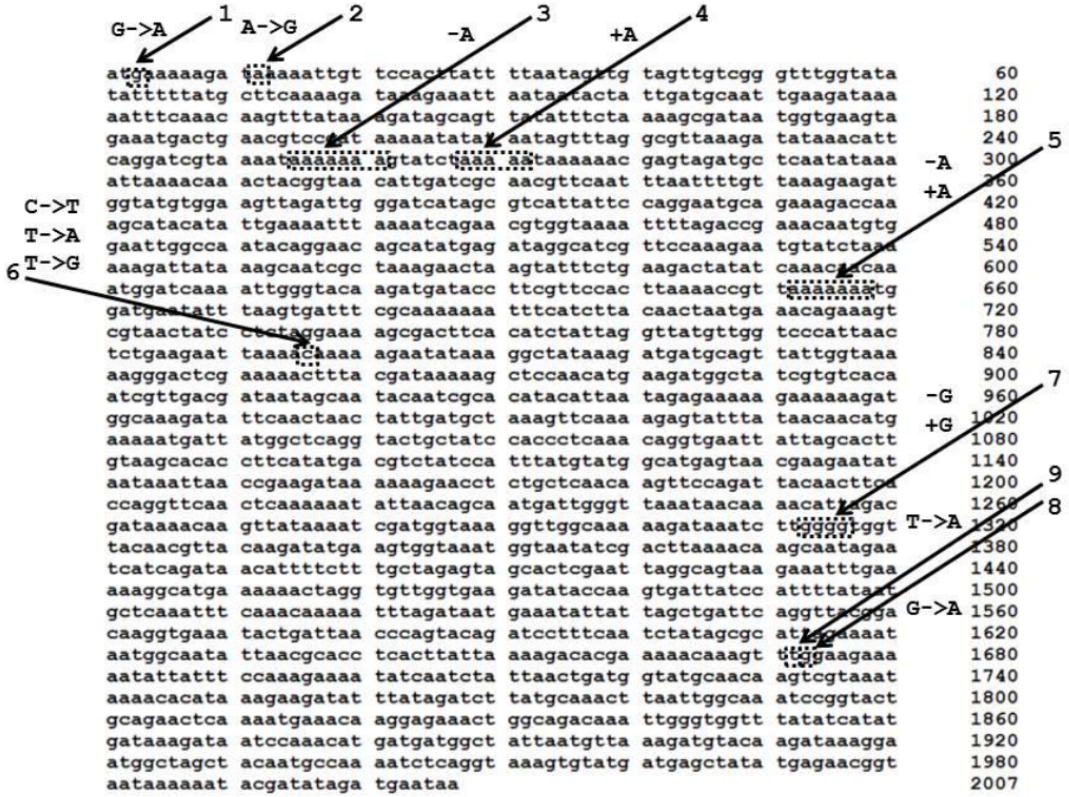
PBP2a mutations reverted after exposure/selection with cefoxitin

	OS-MRSA		MRSA revertant		
Isolate	**Relevant *mecA* sequence**	**Result**	**Relevant *mecA* sequence**	**Result**	**Position(s) on *mecA* sequence map**
CRG2382[a]	C->T@ nucleotide 796	stop codon replaces glutamine	T->A @ nucleotide 796	lysine replaces stop codon	6
CRG2383[a]	C->T@ nucleotide 796	stop codon replaces glutamine	T->G @ nucleotide 796	glutamic acid replaces stop codon	6
CRG2935	G->A@ nucleotide 1673	stop codon replaces tryptophan	T->A @ nucleotide 1672	lysine replaces stop codon	8, 9
CRG2937	G->A@ nucleotide 3	stop codon replaces methionine	A->G @ nucleotide 12	new methionine created (only first 3 aa's lost)	1, 2
CRG2939	loss of A @ nucleotide 255-261	reading frame shift produces stop codon	insertion of A @ nucleotide 268-272	reading frame restored	3, 4
CRG2941	loss of A @ nucleotide 662-668	reading frame shift produces stop codon	insertion of A @ nucleotide 662-668	reading frame restored	5
CRG2943	loss of G @ nucleotide 692-695	reading frame shift produces stop codon	insertion of G @ nucleotide 692-695	reading frame restored	7

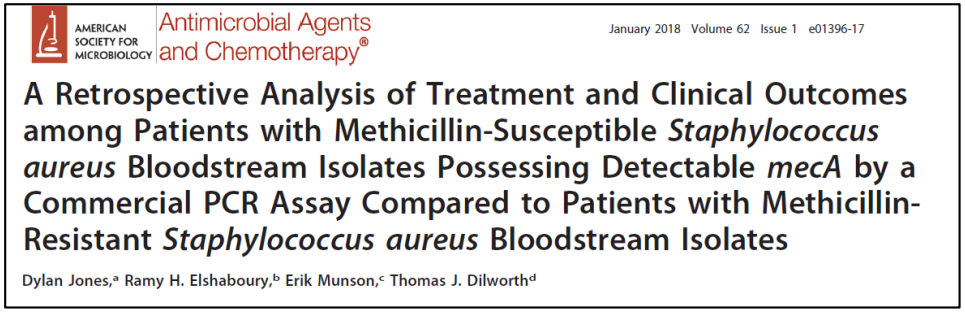
[a] Isolates obtained from different patients during different time periods (7)



RV Goering et al. AAC 2019. doi:10.1128/AAC.00558-19

Clinical outcomes are hard to find beyond case reports of failure?

- Retrospective matched case series
- 17 *mecA*-MSSA (positive PCR, oxacillin-S* and cefoxitin disk-S)
- 17 *mecA*-MRSA
- Matched on age, primary bacteremia, source of bacteremia



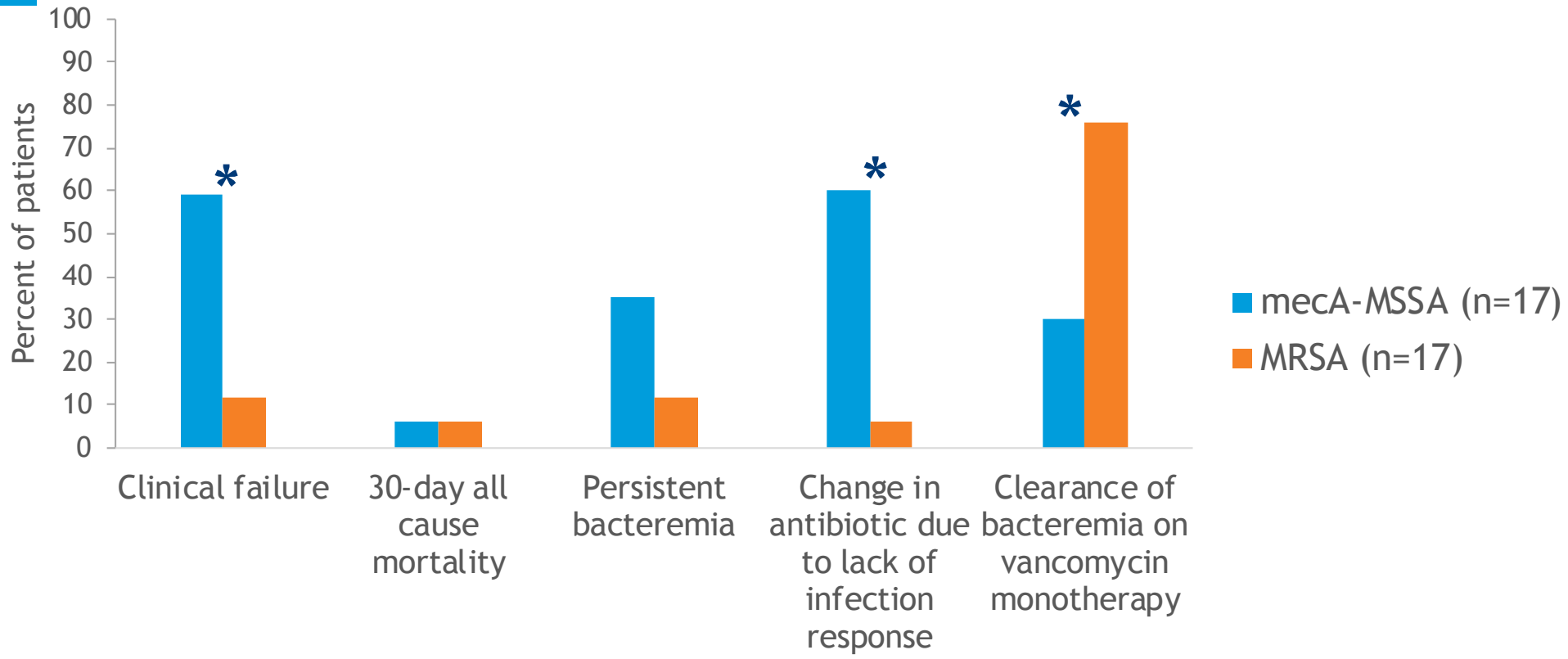
All with vancomycin ≤1µg/mL

All *mecA*-MSSA were reported as such

Clinical failure composite end-point

*VITEK2 Oxacillin≤2µg/mL

Even though anti-MRSA therapy was primarily used *mecA*-MSSA did not respond as quickly as MRSA



*p≤0.05

Jones D et al. AAC 2018 62:e01396-17

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Clinical summary-Trust the genotype?

- With the mutation reversion data and the worse outcome data (although not robust) would favor treating invasive bacteremia as a MRSA which is high risk for failure

- Would use vancomycin or ceftaroline or both depending on the clinical picture

Case 2: Public Health Surveillance - MRSA

HAIC MRSA Isolates Overview: 2005 - 2016

- Emerging Infections Program (EIP)
  - Active Bacterial Core Surveillance (ABCs)
    - Invasive bacterial pathogens of PH importance (MRSA)
  - Healthcare-Associated Infections – Community Interface (HAIC)
    - Invasive *S. aureus* (MRSA/MSSA) Infection Tracking

M. Karlsson and A. Gargis; CDC

Case 2: Public Health Surveillance - MRSA

HAIC MRSA Isolates Overview: 2005 – 2016

- Almost 12,000 MRSA isolates collected and characterized
  - Molecular strain typing
  - Reference antimicrobial susceptibility testing
  - Toxin testing (PVL and TSST-1)
  - SCCmec cassette typing

M. Karlsson and A. Gargis; CDC

Case 2: Public Health Surveillance – MRSA

HAIC MRSA Isolates Overview: 2005 – 2016

- State participation has varied from year to year (3-9)
  - 2005: 8 states collected
  - 2016: 3 states collected
- Method for strain typing has changed over the years
  - 2005-2012: PFGE/Inferred PFGE
  - 2013-2016: spa typing and inferred Clonal Complex (CC)
- WGS on majority of EIP isolates (start with 2017 isolates)
  - Will allow for ability to detect emerging AR mechanisms and study genotypic/phenotypic relationships.

M. Karlsson and A. Gargis; CDC

Case 2: What If…..

Molecular AR

From Positive Blood Cx	
mecA	NOT Detected

Clinical Lab Results

MIC Testing

Antimicrobial	MIC µg/mL
Cefoxitin Screen	R
Oxacillin	1.0 S
Erythromycin	≥ 8 R
Clindamycin	≤ 0.25 S
Vancomycin	1 S
InCR	Negative

Case 2: Public Health Surveillance – MRSA

Molecular Detection of *mecA*

- Nucleic acid amplification tests, such as PCR, can be used for direct detection of *mecA*, the most common gene mediating oxacillin resistance in staphylococci
  - *mecA* PCR tests will not detect novel resistance mechanisms, such as *mecC*
  - Mechanisms of oxacillin resistance other than *mecA* are rare
- Since 2005, no *mecC*- harboring MRSA have been identified among EIP isolates
  - ID of phenotypically resistant, but *mecA*-negative MRSA would indicate the potential presence of *mecC*, and WGS would be conducted.

M. Karlsson and A. Gargis; CDC

Case 2: Background Information - *mecC*

- First described in 2011, following the WGS of a phenotypically resistant, but *mecA*-negative MRSA strain from bovine mastitis in England
- ***mecC*** shares ~70% homology with *mecA*
  - not detected by *mecA*-based PCR or PBP2a slide agglutination
  - Commercial and in-house PCR assays must be modified to allow simultaneous detection of *mecA* and *mecC* MRSA
- ***mecC*** is encoded within a SCC*mec* element that is distinct from SCC*mec* types encoding *mecA*

Sharon J. Peacock and Gavin K. Paterson. Mechanisms of Methicillin Resistance in *Staphylococcus aureus*. Annual Review of Biochemistry 2015 84:1, 577-601

CLSI

Case 2: Background Information - *mecC*

- Described throughout Europe and in a wide range of host animal species (1)
  - Farm and wildlife animals are reservoirs for *mecC*-harboring MRSA
- ***mecC* MRSA strains are relatively rare**; prevalence rate among MRSA (1):
  - 0.06% in Germany
  - 0.46% in England
  - 2.8% in Denmark in 2011, having increased since 2009
- Found predominantly in CC130 and ST425
- While overall prevalence is low, *mecC* prevalence may be underestimated because of its misidentification as methicillin-susceptible *S. aureus* (MSSA) due to its borderline resistant phenotype (2).

(1) Sharon J. Peacock and Gavin K. Paterson. Mechanisms of Methicillin Resistance in *Staphylococcus aureus*. Annual Review of Biochemistry 2015 84:1, 577-601

(2) Kriegeskorte A. et al., Comparison of Different Phenotypic Approaches To Screen and Detect mecC-Harboring Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol. 2017 Dec 26;56(1).

Case 2: Background Information - *mecC*

- PBP2a$_{mecC}$ has a higher affinity for oxacillin than for cefoxitin, whereas PBP2a$_{mecA}$ shows less difference between the two β-lactams
- *mecC* MRSA typically displays an unusual profile of **susceptibility to oxacillin** and **resistance to cefoxitin**:
  - When tested using the Vitek 2 system, this profile had a sensitivity of 88.7% and a specificity of 99.5% for the identification of *mecC* MRSA isolates (1)
  - A 2018 study also found the phenotypic resistance pattern most frequently observed by AST devices for isolates with the *mecC* genotype was "**cefoxitin resistance/oxacillin susceptibility**," ranging from 54.1% (Phoenix) and 83.8% (Vitek 2) to 92.8% (WalkAway), (2)

(1) Cartwright E.J.P. Use of Vitek 2 antimicrobial susceptibility profile to identify mecC in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 2013;51:2732–2734

(2) Kriegeskorte A. et al., Comparison of Different Phenotypic Approaches To Screen and Detect mecC-Harboring Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol. 2017 Dec 26;56(1).

Case 2: What If…Think Possible *mecC*-Confirm

Molecular AR

From Positive Blood Cx	
mecA	NOT Detected

Clinical Lab Results

MIC Testing

Antimicrobial	MIC µg/mL
Cefoxitin Screen	R
Oxacillin	1.0 S
Erythromycin	≥ 8 R
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