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PEARLS OF LABORATORY MEDICINE

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Title Antimicrobial Susceptibility Testing (AST) of Commonly Encountered Gram Positive Bacteria

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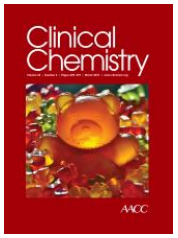

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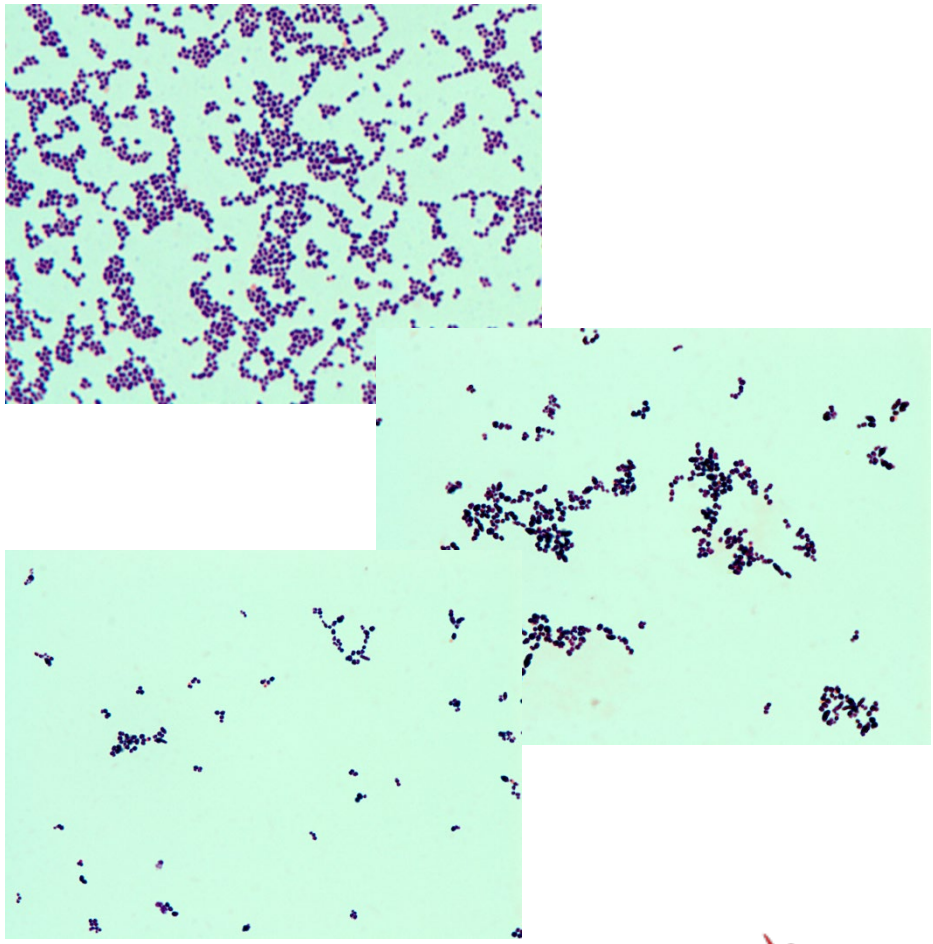
CCTC Clinical Chemistry Trainee Council

Objectives

- Describe testing for penicillin susceptibility in *S. aureus*
- Discuss methods for detection of MRSA
- Discuss concerns related to vancomycin and *S. aureus* including VISA and VRSA
- Explain testing for inducible clindamycin resistance in *S. aureus*
- Discuss penicillin and ampicillin resistance in ~~the~~ enterococci
- Explain methods for detection of vancomycin-resistant enterococci (VRE)
- Describe high level aminoglycoside resistance and synergy testing in enterococci.

Gram Positive Pathogens

- **Staphylococci**
  - *S. aureus*
  - *Coagulase-negative staphylococci*
- **Enterococci**
  - *E. faecalis*
  - *E faecium*
- Streptococci
  - *S. pyogenes*
  - *S. agalactiae*



Staphylococcus aureus Antimicrobial Therapy and Susceptibility Testing

Agents commonly used to treat *S. aureus* infections:

- MSSA - Oxacillin/nafcillin; narrow-spectrum cephalosporin; ß-lac/ß-lac inhibitor combination; carbapenem; macrolide; clindamycin; linezolid; daptomycin; fluoroquinolone
- MRSA (mild to moderate infection) – trimethoprim-sulfamethoxazole; doxycycline; clindamycin (D zone test negative)
- MRSA (serious infection) – vancomycin; linezolid; daptomycin

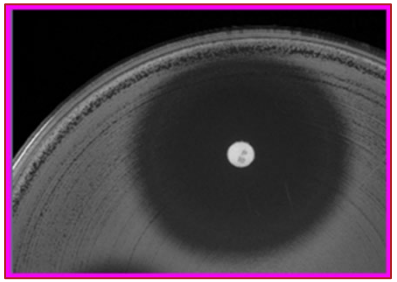
AST Concerns

- Penicillin
  - Zone Edge Test
- MRSA
  - Oxacillin
  - PBP2a
  - *mecA*
- Inducible Clindamycin
- Vancomycin

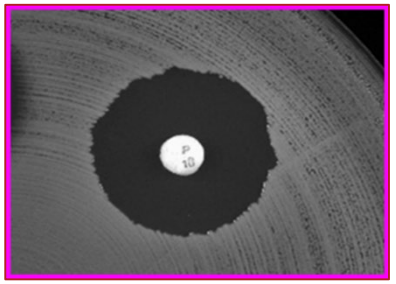
Penicillin Testing for *S. aureus*

- Most *S. aureus* are penicillin resistant
- Penicillin resistance due to β-lactamase (*blaZ)*
- *S. aureus* may test penicillin susceptible by disk diffusion or MIC but produce β-lactamase
  - Requires test for β-lactamase
  - Penicillin zone edge test

- Routine disk diffusion test with penicillin 10 µg disk
- Observe zone edge around penicillin disk



Fuzzy "Beach", β-lactamase negative, Penicillin - S

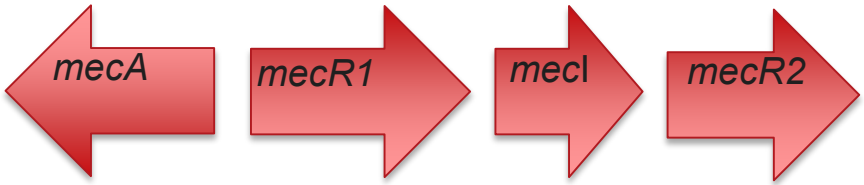


Sharp "Cliff", β-lactamase positive, Penicillin - R

Methicillin Resistant *S. aureus* (MRSA)

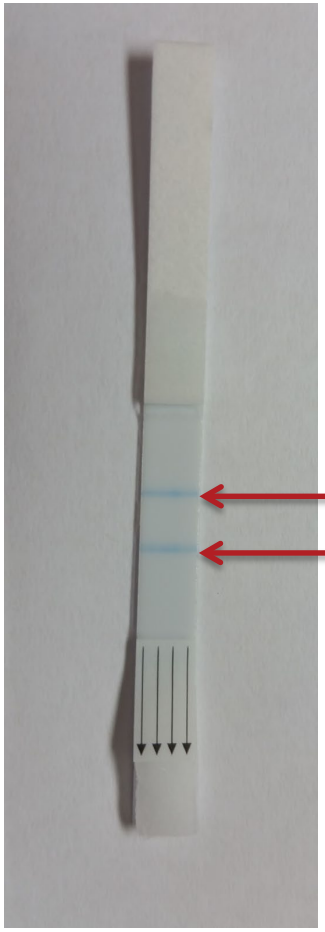
- First identified in 1959 and are now found worldwide
- Methicillin was replaced by oxacillin and nafcillin
- **MRSA are considered resistant to all β-lactam antimicrobial agents with the exception of ceftaroline, a newer β-lactam agent**
- Two varieties hospital (HA) and community acquired (CA)

- Resistance conferred by an altered penicillin binding protein (PBP)
- *mecA* (PBP2a) mediated is most common



PBP2a Testing and *S. aureus* (Testing for MRSA)

- Rapid detection of PBP2a from isolated colonies
- Different types of assays
  - Lateral Flow
  - Latex Agglutination
- Rely on monoclonal antibodies

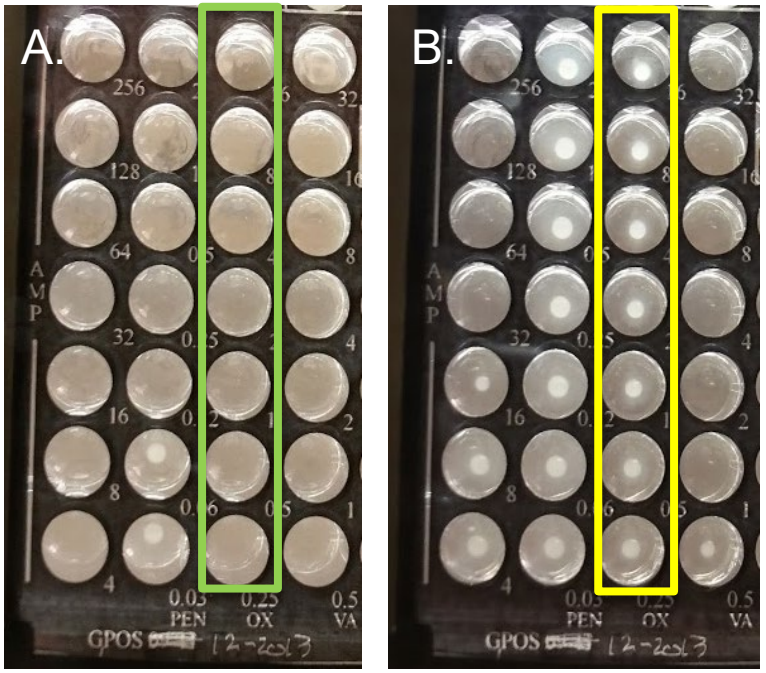


PBP2a Positive!

Oxacillin / Cefoxitin and *S. aureus* (Testing for MRSA)

- Test oxacillin MIC and/or cefoxitin disk or MIC
- Cefoxitin is used as a surrogate; test cefoxitin but report oxacillin
- Cefoxitin MIC breakpoints:
  - S ≤ 4 µg/ml
  - R ≥ 8 µg/ml
- Oxacillin MIC breakpoints:
  - S ≤ 2 µg/ml
  - R ≥ 4 µg/ml

Oxacillin Broth Microdilution MIC Test



MSSA **MRSA**

mecA Testing and *S. aureus* (Testing for MRSA)

- Molecular methods available for detection of *mecA*
  - PCR
  - Probes
- Advantages of molecular methods
  - Faster turn around time
  - Testing from patient specimen
- Disadvantages
  - Higher cost
  - Will not detect novel PBPs

Vancomycin Testing for *S. aureus*

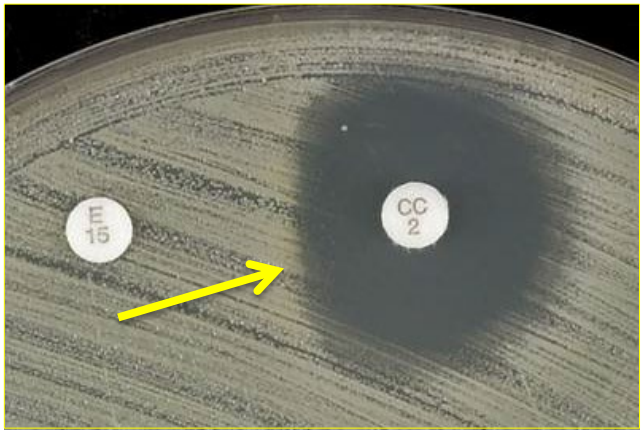
Vancomycin CLSI Breakpoints for *S. aureus*

Susceptible	Intermediate	Resistant
≤2 µg/ml	4-8 µg/ml	≥ 16 µg/ml
VSSA	VISA	VRSA

- Vancomycin-Susceptible *S. aureus* (VSSA)
- Vancomycin-Intermediate *S. aureus* (VISA)
  - Thicker cell wall, some metabolic changes
  - Uncommon but prevalence is rising
- Vancomycin-Resistant *S. aureus* (VRSA)
  - Modification to peptidoglycan
  - Only 14 cases since 2002
  - MRSA

Inducible Clindamycin Resistance Testing for *S. aureus*

- *S. aureus* isolates
  - Erythromycin R and
  - Clindamycin S or I
- Use erythromycin and clindamycin disks
- Inducible resistance produces a “D-Zone”
- Report as clindamycin resistant



A.

B.

S. aureus AST – Important Notes

- Oxacillin/cefoxitin are the only β-lactam agents routinely tested in most labs
  - Penicillin often tested but rarely prescribed
  - Deduce results for other anti-staphylococcal β-lactams from penicillin and oxacillin/cefoxitin results:
    - MSSA (oxacillin/cefoxitin susceptible) – susceptible to all β-lactams except ampicillin/amoxicillin/penicillin
    - MRSA (oxacillin/cefoxitin resistant) - resistant to all β-lactams except ceftaroline
- MRSA are more resistant than MSSA to non-β-lactam antimicrobial agents

Enterococcus spp. Antimicrobial Therapy and Susceptibility Testing

Agents commonly used to treat enterococcal infections:

- Serious infections – ampicillin, penicillin, vancomycin, (combination with aminoglycoside for endocarditis)
- Uncomplicated urinary tract infection – ampicillin, amoxicillin, nitrofurantoin

AST Concerns

- Penicillin and Ampicillin (species differences)
- Vancomycin High Level Aminoglycoside Resistance (HLAR)

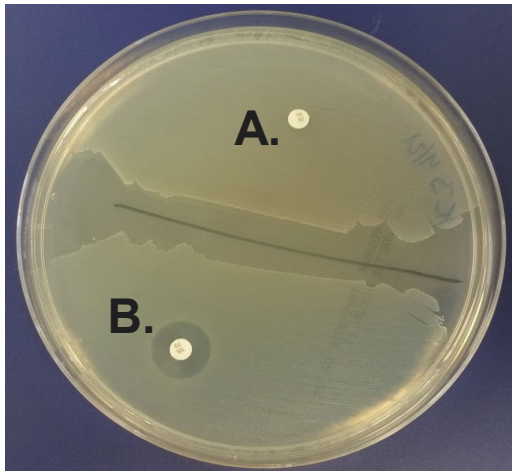
Penicillin and Ampicillin Testing for *Enterococcus* spp.

- Resistance due to an altered penicillin binding protein
- Most *E. faecium* are resistant to ampicillin and penicillin
- Most *E. faecalis* are susceptible to ampicillin and penicillin

	Zone Diameter (mm)			MIC (µg/ml)		
	S	**I**	**R**	**S**	**I**	**R**
Penicillin	≥15	-	≤14	≤8	-	≥16
Ampicillin	≥17	-	≤16	≤8	-	≥16

Vancomycin Testing for *Enterococcus* spp.

- Enterococci can acquire resistance to vancomycin
- Mostly in *E. faecium* but can occur in *E. faecalis*
  - *VanA* MIC 64 - >1,000 µg/ml
  - *VanB* MIC 4 - 1,024 µg/ml
- Disk diffusion or broth dilution methods



	S	I	R
Zone (mm)	≥17	15-16	≤14
MIC (µg/ml)	≤4	8-16	≥32

High Level Aminoglycoside Resistance (HLAR) in *Enterococcus* spp.

- Serious enterococcal infections (e.g. endocarditis) require combination therapy
  - Cell wall active agent (ampicillin, penicillin or vancomycin) plus aminoglycoside (e.g., gentamicin) results in “synergistic” interaction
  - Cell wall active agent allows aminoglycoside to penetrate the cell wall
- Enterococci can acquire high level aminoglycoside resistance
- Isolate must be susceptible to both cell wall active agent and aminoglycoside for a “synergistic” interaction

Synergy Testing for High Level Aminoglycoside Resistance (HLAR) in *Enterococcus* spp.

- Test high concentrations of gentamicin and streptomycin
- Growth at high concentration indicates high level aminoglycoside resistance
- Susceptibility to high level aminogylcosides and cell wall active agents suggests synergy can be achieved

	#1	#2	#3	#4
Ampicillin	S	S	R	S
Gentamicin 500 µg/ml	S	R	S	S
Streptomycin 1000 µg/ml	S	R	S	R

- Isolate #1 – synergy likely with ampicillin plus gentamicin or streptomycin
- Isolate #2, #3 – no synergy with ampicillin plus gentamicin or streptomycin
- Isolate #4 – synergy likely with ampicillin plus gentamicin but NOT with ampicillin plus streptomycin

Enterococci AST – Important Notes

- Enterococci are intrinsically resistant to cephalosporins, aminoglycosides, clindamycin, trimethoprim-sulfmethoxazole
- Enterococci can readily acquire resistance to ampicillin, penicillin, vancomycin and high levels of aminoglycosides and present therapeutic challenges due to few therapeutic options
  - Options - daptomycin, linezolid, quinupristin-dalfopristin (*E. faecium* only)

References

1. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin resistant enterococci. 2000;13:686-706.
2. CLSI. Performance Standards for antimicrobial susceptibility testing; twenty-sixth informational supplement. CLSI Document M100-S26 Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
3. Gardete S, Tomasz A. Mechanisms of vancomycin resistance in *Staphylococcus aureus.* J Clin Invest 2014;124:2836-40.
4. Howden BP, Davies JK, Johnson PDR, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 2010;23:99-139.
5. Limbago BM, Swenson JM. Special phenotypic methods for detecting antibacterial resistance. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW, editors. Manual of clinical microbiology. 11th ed. Washington (D.C.): ASM Press;2015. p1286-1313.
6. Liu C, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. Clin. Infect. Dis. 2011;52:1-37.
7. Rivera AM, Boucher HW, Current concepts in antimicrobial therapy against select gram positive organisms: methicillin resistant *Staphylococcus aureus*, penicillin resistant pneumococci, and vancomycin resistant enterococci. Mayo Clin Proc 2011;86:1230-40.

Disclosures/Potential Conflicts of Interest

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