Can we stay positive, please?

Case studies of Gram-positive bacterial antimicrobial resistance

APHL ID Lab Con
Mimi Precit, PhD, D(ABMM), M(ASCP)
March 14th, 2023
Disclosures

• None
Case 1: Positively Perplexed by PBPs
Clinical Presentation

Presentation:
• 3-week-old neonate
• Nasal congestion and cough
• Vomiting
• Reduced feeding

On exam:
• Alert
• Intercostal retractions
• Bilateral wheezing
• Clear nasal discharge
• Febrile at 38°C

At birth:
• Uncomplicated vaginal birth
Microbiology studies ordered

- Molecular respiratory panel
  - No targets detected

- Cerebral spinal fluid (CSF) studies

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>3 WBCs/mm³</td>
<td>Normal</td>
</tr>
<tr>
<td>Glucose</td>
<td>45 mg/dl</td>
<td>Normal</td>
</tr>
<tr>
<td>Protein</td>
<td>58 mg/dl</td>
<td>Normal</td>
</tr>
<tr>
<td>Gram stain</td>
<td>No organisms seen</td>
<td>Unremarkable</td>
</tr>
<tr>
<td>Culture</td>
<td>No Growth</td>
<td>Unremarkable</td>
</tr>
</tbody>
</table>

- Blood cultures
  - Pediatric aerobic blood culture bottle flagged positive at 19 hours
Blood culture results

Bottle Gram stain

Next day: subculture to blood agar grew the following organism

Based on the Gram-stain the tech also placed an optochin (P) disk, organism inhibition observed
Streptococcus pneumoniae
**S. pneumoniae** susceptibility results

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

In *S. pneumoniae*, primary mechanism(s) of beta-lactam resistance mediated by penicillin binding proteins (PBPs) **NOT** beta-lactamases.

Patient was treated with vancomycin and recovered.
The ABC’s of the PBPs
The ABC’s of the PBPs

• Classified into Class A, B, or C based on architecture and corresponding enzyme activity/domains

• Key enzymatic process: amino acid cross-linking through transpeptidase activity in final steps of peptidoglycan synthesis
The ABC’s of the PBPs

- Classified into Class A, B, or C based on architecture and corresponding enzyme activity/domains
- Key enzymatic process: amino acid cross-linking through transpeptidase activity in final steps of peptidoglycan synthesis
The ABC’s of the PBPs

- Classified into Class A, B, or C based on architecture and corresponding enzyme activity/domains

- Key enzymatic process: amino acid cross-linking through transpeptidase activity in final steps of peptidoglycan synthesis
PBP transpeptidase substrate and the beta-lactams

Bind in place of the normal substrate arresting cell wall synthesis resulting in cell death
Make way for the famous (acquired) PBPs

Sure get’s a lot of attention huh...

*S. pneumoniae*

Methicillin resistant *Staphylococcus aureus* (MRSA)

PBP2a *(mecA)*

PBP2c *(mecC)*
How many penicillin binding proteins have been described in *S. pneumoniae*?

A) 5  
B) 12  
C) 6  
D) 23
How many penicillin binding proteins have been described in *S. pneumoniae*?

<table>
<thead>
<tr>
<th>Class</th>
<th>PBP</th>
<th>Enzyme activity</th>
<th>Requirement for SPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PBP1a, PBP1b, PBP2a</td>
<td>Bi-functional: transglycosylase and transpeptidase</td>
<td>Non-essential</td>
</tr>
<tr>
<td>B</td>
<td>PBP2x, PBP2b</td>
<td>Mono-functional: transpeptidase</td>
<td>Individually essential</td>
</tr>
<tr>
<td>C</td>
<td>PBP3</td>
<td>D,D-carboxypeptidase activity</td>
<td>Non-essential</td>
</tr>
</tbody>
</table>

A) 5
B) 12
C) 6
D) 23
How many penicillin binding proteins have been described in *S. pneumoniae*?

<table>
<thead>
<tr>
<th>Class</th>
<th>PBPs</th>
<th>Enzyme activity</th>
<th>Requirement for SPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>PBP1a, PBP1b, PBP2a</td>
<td>Bi-functional: transglycosylase and transpeptidase</td>
<td>Non-essential</td>
</tr>
<tr>
<td>B</td>
<td>PBP2x, PBP2b</td>
<td>Mono-functional: transpeptidase</td>
<td>Individually essential</td>
</tr>
<tr>
<td>C</td>
<td>PBP3</td>
<td>D,D-carboxypeptidase activity</td>
<td>Non-essential</td>
</tr>
</tbody>
</table>

Main PBPs responsible for conferring beta-lactam resistance in *S. pneumoniae*
Primary mechanisms of beta-lactam resistance in *S. pneumoniae* are gene modifications affecting the PBP transpeptidase domain

- Acquisition of point mutations

- PBP gene recombination following interspecies horizontal gene transfer with other viridans group streptococci
Beta-lactam resistant *S. pneumoniae* is a WHO priority pathogen

**Priority 1: CRITICAL**

*Acinetobacter baumannii*, carbapenem-resistant  
*Pseudomonas aeruginosa*, carbapenem-resistant  
*Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant

**Priority 2: HIGH**

*Enterococcus faecium*, vancomycin-resistant  
*Staphylococcus aureus*, methicillin-resistant, vancomycin intermediate and resistant  
*Helicobacter pylori*, clarithromycin-resistant  
*Campylobacter*, fluoroquinolone-resistant  
*Salmonella* spp., fluoroquinolone-resistant  
*Nesteria gonorrhoeae*, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

**Priority 3: MEDIUM**

*Streptococcus pneumoniae*, penicillin-non-susceptible  
*Haemophilus influenzae*, ampicillin-resistant  
*Shigella* spp., fluoroquinolone-resistant

- *S. pneumoniae* is a vaccine preventable, offers protection against severe infection  
  - Our patient was too young to be vaccinated (6 weeks minimum age)  
  - Different vaccine formulations  
  - Pediatric and adult schedules

https://www.cdc.gov/vaccines/vpd/pneumo/index.html
ARLN S. pneumoniae initiative

- Request penicillin resistant isolates from sterile sites
- Associate serotypes with antibiotic resistance
- Detect and understand vaccine escape strains
- Inform treatment guidelines and vaccine formulations
Summary of Case 1

• Beta-lactam resistant *S. pneumoniae* is an important pathogen that can cause invasive disease, most often in pediatric and elderly patients
  o Vaccination is recommended

• The primary mechanism(s) of beta-lactam resistance in *S. pneumoniae* is mediated by PBP transpeptidase domain modifications

• The WHO, CDC, and APHL recognized drug resistant *S. pneumoniae* as a public health threat
Case 2: All aboard the roundworm bus!
Clinical Presentation

Presentation:
• 69 y/o male
• Currently hospitalized due to complications with recent splenectomy
• Day 45 of hospitalization showed signs of altered mental status and headache

On exam:
• Febrile, at 39.4C
• Nuchal rigidity
• Photophobia
• Agitated and disoriented

Past Medical History:
• Diabetes (on insulin)
• Autoimmune hemolytic anemia (on long term steroid therapy, 6 years)
• Recently diagnosed with *Strongyloides stercoralis* hyper infection
Microbiology studies ordered

• Blood cultures
  • 4 sets: No Growth

• CSF studies

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>289 WBCs/mm³</td>
</tr>
<tr>
<td>Glucose</td>
<td>18 mg/dl</td>
</tr>
<tr>
<td>Protein</td>
<td>195 mg/dl</td>
</tr>
<tr>
<td>Gram stain</td>
<td>No organisms seen, 4+ WBCs</td>
</tr>
</tbody>
</table>

Consistent with bacterial meningitis

Patient placed empirically on ampicillin, ceftazidime, and vancomycin
CSF culture results

After 48 hours this organism was seen on blood agar

Catalase negative
PYR+
LAP+
Esculin+

Colony Gram stain

StrepQuick™

PYR add developer
LAP add developer
ESC no developer
Enterococcus

E. faecium
Stongyloides stercoralis

- Filariform larvae transported to lungs and coughed up and swallowed
- Eggs in intestines hatch
- Rhabditiform larvae excreted in stool
- Penetrate skin
- Adult female in intestine
- Rhabditiform larvae develop into filariform, penetrate directly into bowel
- Eggs dispersed in environment
- Develop into adults
- Rhabditiform larvae excreted in stool

= Infective Stage
= Diagnostic Stage
Stongyloides stercoralis

- Filariform larvae transported to lungs and coughed up and swallowed
- Eggs in intestines hatch
- Rhabditiform larvae excreted in stool
- Eggs dispersed in environment
- Develop into adults
- Rhabditiform larvae penetrate skin
- Adult female in intestine
- Filariform larvae develop into Rhabditiform, penetrate directly into bowel
- Eggs in intestines hatch

[Diagram showing life cycle of Stongyloides stercoralis with arrows indicating movement and stages]
**Strongyloides stercoralis**

- Eggs dispersed in environment
- Eggs in intestines hatch
- Rhabditiform larvae excreted in stool
- Rhabditiform larvae develop into Filariform, penetrate directly into bowel
- Adult female in intestine
- In setting of immunosuppression can result in very high worm burden
- Filariform larvae transported to lungs and coughed up and swallowed
- Filariform larvae penetrate skin
- Eggs in intestines hatch
- Rhabditiform larvae developed into filariform larvae
- Development of adults
- Eggs dispersed in environment

**Note:**
- ▲ = Infective Stage
- ▲ = Diagnostic Stage
Filariform larvae penetrate bowel and enter the circulatory system. With them they bring commensal gastrointestinal (GI) bacteria, enteric Gram-negative rods, and enterococci. GI organisms can establish infection in the central nervous system.
**E. faecium** isolate susceptibility results

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Quinupristin/Dalfopristin</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

Recall: Patient on ampicillin, vancomycin, and ceftazidime (NO effective therapy)
E. faecium isolate susceptibility results

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Quinupristin/Dalfopristin</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

Patient treated with Linezolid for 28 days and recovered

Mechanisms of intrinsic and acquired resistance in enterococci are a complex alphabet soup...
Enterococci are naturally multi-drug resistant organisms, care must be taken in selecting treatment.
## Cell wall related mechanisms of resistance in enterococci

<table>
<thead>
<tr>
<th>Site of resistance</th>
<th>Strategy</th>
<th>Antibiotic</th>
<th>Primary gene or gene product</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall</td>
<td>PBP (enzyme) modification</td>
<td>Beta-lactams</td>
<td><em>pbp5</em></td>
<td>Low-affinity PBPs allow peptidoglycan synthesis in the presence of β-lactams</td>
</tr>
<tr>
<td></td>
<td>Drug inactivation</td>
<td>Beta-lactams</td>
<td><em>blaZ</em></td>
<td>β-Lactamase. Low-level constitutive production may be missed on routine laboratory screening</td>
</tr>
<tr>
<td></td>
<td>Alternate pathway</td>
<td>Beta-lactams/glycopeptides</td>
<td>LDT$_{fm}$</td>
<td>L,D-Transpeptidation only observed <em>in vitro</em></td>
</tr>
<tr>
<td></td>
<td>Cell signaling</td>
<td>Cephalosporins</td>
<td><em>croRS</em> <em>ireK</em></td>
<td>Mutations in these pathways sensitize enterococci to cephalosporins</td>
</tr>
</tbody>
</table>
|                    | Altered target    | Glycopeptides              | *van* operons               | • *vanA/B* – Acquired genes  
• Intrinsic low-level resistance in *E. gallinarium* and *E. casseliflavus vanC*                                               |
<table>
<thead>
<tr>
<th>Site of resistance</th>
<th>Strategy</th>
<th>Antibiotic</th>
<th>Primary gene or gene product</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell wall</strong></td>
<td>PBP (enzyme) modification</td>
<td>Beta-lactams</td>
<td><em>pbp5</em></td>
<td>Low-affinity PBPs allow peptidoglycan synthesis in the presence of β-lactams</td>
</tr>
<tr>
<td></td>
<td>Drug inactivation</td>
<td>Beta-lactams</td>
<td><em>blaZ</em></td>
<td>Low-level constitutive β-Lactamase production may be missed on routine laboratory screening</td>
</tr>
<tr>
<td></td>
<td>Alternate pathway</td>
<td>Beta-lactams/glycopeptides</td>
<td>LDT$_{fm}$</td>
<td>L,D-Transpeptidation only observed <em>in vitro</em></td>
</tr>
<tr>
<td></td>
<td>Cell signaling</td>
<td>Cephalosporins</td>
<td><em>croRS</em> <em>ireK</em></td>
<td>Mutations in these pathways sensitize enterococci to cephalosporins</td>
</tr>
<tr>
<td></td>
<td>Altered target</td>
<td>Glycopeptides</td>
<td><em>van</em> operons</td>
<td>• <em>vanA/B</em> – Acquired genes&lt;br&gt;• Intrinsic low-level resistance in <em>E. gallinarium</em> and <em>E. casseliflavus vanC</em></td>
</tr>
</tbody>
</table>
# Cell wall related mechanisms of resistance in enterococci

<table>
<thead>
<tr>
<th>Site of resistance</th>
<th>Strategy</th>
<th>Antibiotic</th>
<th>Primary gene or gene product</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall</td>
<td>PBP (enzyme)</td>
<td>Beta-lactams</td>
<td><em>pbp5</em></td>
<td>Low-affinity PBPs allow peptidoglycan synthesis in the presence of β-lactams</td>
</tr>
<tr>
<td></td>
<td>modification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drug inactivation</td>
<td>Beta-lactams</td>
<td><em>blaZ</em></td>
<td>β-Lactamase. Low-level constitutive production may be missed on routine laboratory screening</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternate pathway</td>
<td>Beta-lactams/glycopeptides</td>
<td>LDT&lt;sub&gt;fm&lt;/sub&gt;</td>
<td>L,D-Transpeptidation only observed <em>in vitro</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cell signaling</td>
<td>Cephalosporins</td>
<td><em>croRS</em></td>
<td>Mutations in these pathways sensitize enterococci to cephalosporins</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>ireK</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Altered target</td>
<td>Glycopeptides</td>
<td><em>van operons</em></td>
<td>• <em>vanA/B</em> – Acquired genes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Intrinsic low-level resistance in <em>E. gallinarium</em> and <em>E. casseliflavus</em> vanC Vancomycin resistance</td>
</tr>
</tbody>
</table>
VanA/B mediated Vancomycin resistance in enterococci
VanA/B mediated Vancomycin resistance in enterococci

VanA or B activity confers modifications to from D-Ala-D-Ala → D-Ala-D-Lac in the peptidoglycan cell wall.

Vancomycin no longer binds.

Plasmid-borne, acquired resistance mechanism
## MORE mechanisms of resistance in enterococci

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Genes described</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daptomycin</td>
<td>LiaFSR, YycFG Cls, GdpD</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>AAC(6’)-li, APH(3’)-IIa, aadA/ANT(3’), AAC(6’)-Ie/APH (2’)-Ia, efmM</td>
</tr>
<tr>
<td>Quinupristin/Dalfopristin</td>
<td>vatD and vatE, vgbA and vgbB, isa, msrC, eatA</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>tetM, tetO, tetS, tetK, tetL</td>
</tr>
<tr>
<td>Quinolones</td>
<td>gyrA, parC, norA, qnr</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>rpoB</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>Intrinsic – use exogenous folate</td>
</tr>
</tbody>
</table>
Which of the following newer-ish drugs have CLSI recognized enterococci breakpoints

A) Dalbavancin

B) Eravacycline

C) Delafloxacin

D) Omadacycline
Which of the following newer-ish drugs have CLSI recognized enterococci breakpoints

A) Dalbavancin

B) Eravacycline (FDA and EUCAST)

C) Delafloxacin (FDA)

D) Omadacycline (FDA)
New-ish drugs with activity against enterococci

<table>
<thead>
<tr>
<th>Drug</th>
<th>CLSI</th>
<th>FDA</th>
<th>EUCAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telavancin</td>
<td>MIC(^a,b)</td>
<td>CLSI</td>
<td>-</td>
</tr>
<tr>
<td>Oritavancin</td>
<td>MIC(^a,b)</td>
<td>CLSI</td>
<td>-</td>
</tr>
<tr>
<td>Dalbavancin</td>
<td>MIC(^a,b)</td>
<td>CLSI</td>
<td>-</td>
</tr>
<tr>
<td>Tedizolid</td>
<td>MIC(^a,e)</td>
<td>CLSI</td>
<td>-</td>
</tr>
<tr>
<td>Delafloxacin</td>
<td>-</td>
<td>MIC(^e,f)</td>
<td>DD(^e,f)</td>
</tr>
<tr>
<td>Omadacycline</td>
<td>-</td>
<td>MIC(^e,f)</td>
<td>DD(^e,f)</td>
</tr>
<tr>
<td>Eravacycline</td>
<td>-</td>
<td>MIC(^a,c)</td>
<td>MIC(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Only a susceptible breakpoint is published

\(^b\)Breakpoint applies to vancomycin-susceptible *Enterococcus faecalis* isolates only

\(^c\)Breakpoint applies to *Enterococcus faecalis* and *Enterococcus faecium* only

\(^d\)Breakpoints differ between *Enterococcus faecalis* versus *Enterococcus faecium*

\(^e\)Breakpoint applies to *Enterococcus faecalis* isolates only

\(^f\)Breakpoint applies to acute skin/skin structure infections only
Vancomycin resistant *E. faecium* WHO Priority Pathogen

**Priority 1: CRITICAL**

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*\(^\ast\), carbapenem-resistant, 3\(^{rd}\) generation cephalosporin-resistant

**Priority 2: HIGH**

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter*, fluoroquinolone-resistant
- *Salmonella* spp., fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, 3\(^{rd}\) generation cephalosporin-resistant, fluoroquinolone-resistant

**Priority 3: MEDIUM**

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant
Summary of Case 2

- Multidrug resistant enterococci can present a treatment challenge

- The mechanisms of intrinsic and acquired resistance in enterococci are numerous and complex

- There are more novel, less commonly used antimicrobials with activity against the enterococci but there are limitations in their use

- The WHO recognizes vancomycin resistant *E. faecium* as a high priority public health threat
Case 3: Well aren’t you looking DAP-R
Clinical Presentation

Presentation:
- 78 y/o male hemodialysis patient
- Drainage, erythema, swelling at permanent hemodialysis catheter site
- Fever

On exam:
- Febrile, at 39C
- Tachycardic
- Hypertensive

Past Medical History:
- Diabetes (on insulin)
- Renal failure on dialysis
- Chronic coronary artery disease
Microbiology studies ordered

- Peripheral blood cultures
  - 2 of 2 sets flagged positive within 24 hours of incubation

Next day: subculture to blood agar grew the following organism

- Catalase +
- Staphaurex +
- PBP2a + lateral flow
Methicillin Resistant
*Staphylococcus aureus*
Susceptibility Profile

<table>
<thead>
<tr>
<th>Drug</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Susceptible (MIC 2 ug/mL)</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>Susceptible (MIC 0.5 ug/mL)</td>
</tr>
</tbody>
</table>

Susceptible, but elevated vancomycin MICs (e.g., 2 μg/ml) in some older studies demonstrated association with worse clinical outcomes.

Patient had catheter replaced, was treated with long course of Daptomycin, improved and was discharged.

Tenover et al. CID. 2007
Musta et al. JCM. 2009
Soriano et al. CID 2008
Takesue et al. J Infect Chemother. 2010
Presentation:
• ~1 month later returned to ED
• Respiratory distress
• Purulent drainage and swelling at catheter site
• Fever
Microbiology studies ordered

- Peripheral blood cultures
  - 3 of 3 sets flagged positive

Next day: subculture to blood agar grew the following organism

Catalase +
Staphaurex +
PBP2a +
lateral flow
Methicillin Resistant
*Staphylococcus aureus*
New susceptibility profile after daptomycin therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Old Result</th>
<th>New Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vancomycin (VANC)</td>
<td>Susceptible (MIC 2 ug/mL)</td>
<td>Intermediate (MIC 4 ug/mL)</td>
</tr>
<tr>
<td>Daptomycin (DAP)</td>
<td>Susceptible (MIC 0.5 ug/mL)</td>
<td>Non-susceptible (MIC 8 ug/mL)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Linezolid</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>
New susceptibility profile after daptomycin therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Old Result</th>
<th>New Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vancomycin (VANC)</td>
<td>Susceptible (MIC 2 ug/mL)</td>
<td>Intermediate (MIC 4 ug/mL)</td>
</tr>
<tr>
<td>Daptomycin (DAP)</td>
<td>Susceptible (MIC 0.5 ug/mL)</td>
<td>Non-susceptible (MIC 8 ug/mL)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Linezolid</td>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

Likely the same MRSA, difference in colony morphology explained by fitness cost observed in multi-drug resistant isolates. Poor source control of catheter site.

Li et al. Frontier Micro. 2017
New susceptibility profile after daptomycin therapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Old Result</th>
<th>New Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vancomycin (VANC)</td>
<td>Susceptible (MIC 2 ug/mL)</td>
<td>Intermediate (MIC 4 ug/mL)</td>
</tr>
<tr>
<td>Daptomycin (DAP)</td>
<td>Susceptible (MIC 0.5 ug/mL)</td>
<td>Non-susceptible (MIC 8 ug/mL)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Linezolid</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>N/A</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

Reduced susceptibility to both vancomycin and daptomycin following therapy....
Daptomycin mechanism of action

Humphries. CMR. 2013
Daptomycin mechanism of action
Mechanism of resistance

• Following exposure to DAP the cell membrane presumably loses the anionic surface, resulting in net positive charge.
Proposed primary mechanism of resistance

- Following exposure to DAP the cell membrane presumably loses the anionic surface, resulting in net positive charge.

- DAP+CA^{2+} no longer binds the same way, effectively repelled.
Proposed primary mechanism of resistance

- Following exposure to DAP, the cell membrane presumably loses its anionic surface, resulting in a net positive charge.

- DAP+C\text{A}^{2+} no longer binds the same way, effectively repelled.
Which of the following has been described to play a role in daptomycin resistance in MRSA?

A) \textit{erm}

B) \textit{blaZ+mecC}

C) \textit{mprF}

D) \textit{rpoB}
Which of the following has been described to play a role in daptomycin resistance in MRSA?

A) \textit{erm} (macrolide)

B) \textit{blaZ+mecC} (beta-lactams)

C) \textit{mprF}

D) \textit{rpoB} (rifampin)
What about that elevated VANC?
What about that elevated VANC?

Vancomycin intermediate *S. aureus* (VISA)

Several genes described:
- *walK*
- *clpP*
- *graSR*
- *vraSR*
- *msrR*
- *rpoB*

Cell-wall thickening

Mediated by the *vanA* cluster from *Enterococcus*

Vancomycin resistant *S. aureus* (VRSA)

Daptomycin non-susceptible isolates have been shown to have increased cell wall thickening.

MRSA with elevated MICs to VANC is a WHO priority pathogen

**Priority 1: CRITICAL**
- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant

**Priority 2: HIGH**
- *Enterococcus faecium*, vancomycin-resistant
- **Staphylococcus aureus**, methicillin-resistant, vancomycin intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter*, fluoroquinolone-resistant
- *Salmonella spp.*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

- Daptomycin resistance and/or vancomycin resistance are uncommon clinical findings

**Priority 3: MEDIUM**
- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella spp.*, fluoroquinolone-resistant

- If encountered
  - Confirm ID
  - Confirm pure isolate was tested
  - Retest
  - Send out for confirmation

Markwart et al. Front Microbiol. 2021 PMID: 34135877
Summary of Case 3

- MRSA with reduced susceptibility to vancomycin and/or daptomycin are rare but important pathogens.

- Daptomycin resistance is typically seen following treatment with the drug in conjunction with an uncontrolled source.
  - Catheter associated

- The WHO recognizes vancomycin intermediate and resistant MRSA as a high priority public health threat.
Thank you for your attention!

mimi.precit@providence.org
EXTRA Slides
<table>
<thead>
<tr>
<th>Case</th>
<th>General</th>
<th>History</th>
<th>Examination</th>
<th>Vitals</th>
<th>Laboratory Results</th>
<th>Antibiotics</th>
<th>Culture</th>
<th>Susceptibility</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CA: 20</td>
<td>G: M</td>
<td>Maternal: Fever 37.7°C; iv Ampicillin 40 min before delivery. Infant: Nasal congestion and cough for 1 wk; retractions and tachypnea, vomiting with cough anorexia for 1 day. Exposure to pneumonia and upper respiratory infection</td>
<td>Alert, intercostal retractions, bilateral wheezing, clear nasal discharge</td>
<td>T = 37.1°C P = 156/min</td>
<td>CBC: WBC = 3, S = 27%, L = 6%, M = 9%, O = 3% CSF: WBC = 3, M = 100%, G = 45 mg/dl, P = 48 mg/dl</td>
<td>iv ampicillin/Cefotax for 2 days iv Cefotax for 8 days</td>
<td>Blood: + CSF: − Urine: −</td>
<td>Pen-resistant Cefotax − intermediate</td>
</tr>
</tbody>
</table>
Clinical resistance to penicillin in Streptococcus pneumoniae was first reported by researchers in Boston in 1965; subsequently, this phenomenon was reported from Australia (1967) and South Africa (1977). Since these early reports, penicillin resistance has been encountered with increasing frequency in strains of S. pneumoniae from around the world. In South Africa strains resistant to penicillin and chloramphenicol as well as multiresistant strains have been isolated. Similar patterns of resistance have been reported from Spain. Preliminary evidence points to a high prevalence of resistant pneumococci in Hungary, other countries of Eastern Europe, and some countries in other areas of Europe, notably France. In the United States most reports of resistant pneumococci come from Alaska and the South, but resistance is increasing in other states and in Canada. Pneumococcal resistance has also been described in Zambia, Japan, Malaysia, Pakistan, Bangladesh, Chile, and Brazil; information from other African, Asian, and South American countries is not available. The rising prevalence of penicillin-resistant pneumococci worldwide mandates selective susceptibility testing and epidemiological investigations during outbreaks.
https://www.cdc.gov/mmwr/preview/mmwrhtml/00040449.htm

• https://pubmed.ncbi.nlm.nih.gov/8994784/