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TITLE: Antimicrobial Susceptibility Testing (AST) of Commonly Encountered Gram Positive Bacteria**.**

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Slide 1:

Hello, my name is Kurt Jerke. I am a Post-Doctoral Fellow at the University of California Los Angeles. Welcome to this Pearl of Laboratory Medicine on "Antimicrobial Susceptibility Testing (AST) of Commonly Encountered Gram Positive Bacteria."

Slide 2:

Here are the objectives for today's presentation. At the end of this Pearl you should have a better understanding of antimicrobial susceptibility testing in *Staphylococcus aureus* and the enterococci, particularly with regards to MRSA and VRE.

Slide 3:

The Gram positives comprise a diverse group of bacteria many of which can be human pathogens. Today we will focus our discussion on *Staphylococcus* aureus and the enterococci. *Staphylococcus* aureus is capable of causing a variety of diseases in humans and is considered to be a pathogen. The enterococci are part of the normal human gut flora; however, they are adept opportunistic pathogens as well. Both *Staphylococcus* aureus and the enterococci are associated with the development of multidrug-resistant strains. The streptococci can also be human pathogens, this includes the Group B streptococci, *Streptococcus pneumoniae*, and *Streptococcus*

pyogenes. The beta-hemolytic streptococci however are generally susceptible to penicillin and therefore susceptibility testing is not needed except under certain conditions such as a patient who is allergic to penicillin. Increasing penicillin MICs have been observed in *S. pneumoniae* and while penicillin is a recommended treatment option resistance remains a concern.

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The antimicrobials that are used to treat *Staphylococcus* aureus vary depending on whether the isolate is methicillin sensitive (MSSA) or methicillin resistant (MRSA) and how serious the infection is. There are a number of drugs that can be used to treat MSSA including oxacillin and cephalosporins, however the options for treating a mild case of MRSA are more limited and treatment of serious cases of MRSA, such as bacteremia and endocarditis are limited to a handful of drugs. More specific treatment guidance can be found in the IDSA guidelines provided in the references. The list on this slide shows some of the more common tests that are used when testing susceptibilities in S. aureus. While most of these tests look at cell wall active agents we will also discuss inducible resistance with clindamycin which prevents protein synthesis. I would like to mention here that all of the methods described in the following slides are based on recommendations found in the antimicrobial susceptibility testing documents published by the Clinical and Laboratory Standards Institute or CLSI.

Slide 5:

Greater than 90% of *Staphylococcus* aureus are resistant to penicillin due to the production of penicillinase, a type of β-lactamase, encoded by the *blaZ* gene. Penicillin is therefore not widely used for treatment of staphylococcal infections. There are conditions such as osteomyelitis or endocarditis where penicillin might be considered if the isolate is confirmed as penicillin susceptible. Occasional isolates of *S. aureus* test susceptible to penicillin with MICs less than or equal to 0.12 micrograms per milliliter or zones of inhibition greater than or equal to 29 mm, however some of these isolates produce β-lactamase. If penicillin is used in these cases, treatment will likely fail. To

address this concern, performance of the penicillin Zone Edge Test is recommended to confirm penicillin susceptibility prior to reporting the result. A routine penicillin disk diffusion test is performed and the edge of the zone of inhibition is examined. If the edge of the zone has a fuzzy appearance, or what is sometimes referred to as a "beach", the isolate is β-lactamase negative and susceptible to penicillin. In contrast if the edge is very sharp and more closely resembles a "Cliff", the isolate is considered β-lactamase positive and resistant to penicillin. Examples of a penicillin resistant and a penicillin susceptible strain of *S. aureus* are shown in the pictures to the right. Please keep in mind that this test is for *S. aureus* only, β-lactamase testing of coagulase-negative staphylococci is done by alternative methods.

Slide 6:

Methicillin resistant *S. aureus*, or MRSA, is an organism that I imagine most of you have heard of. Methicillin resistant strains of *S. aureus* were first identified in 1959, at the time methicillin, which is a semisynthetic penicillin that is resistant to degradation by penicillinase, was used to treat infections with penicillin resistant organisms. *S. aureus* strains that were resistant to methicillin began to emerge and were called MRSA. Today methicillin is no longer used, having been replaced by oxacillin or sometimes naficillin, but the naming convention has stuck. When we talk about MRSA we differentiate between infections that were acquired in a health care setting from those acquired in the community, such as locker rooms. Functionally the health care acquired strains tend to have resistance to multiple antibiotics while community acquired strains are less resistant, although this trend may be changing. Oxacillin resistance is conferred by the *mecA* gene which encodes an altered penicillin binding protein called PBP2a. Oxacillin is able to bind to the wild type penicillin binding protein, inhibiting its function and preventing cell wall synthesis. Oxacillin cannot bind PBP2a, as a result cells with the *mecA* gene can synthesize an intact cell wall in the presence of oxacillin. The figure below shows the entire operon which includes the regulatory genes *mecR1*, *mecR2* and *mecI.* It is important to remember that MRSA is considered to be resistant

to all β-lactam drugs with the exception of ceftaroline, a β-lactam agent that was introduced in the USA in 2010.

Slide 7:

Early detection of methicillin resistance in colonies identified as *S. aureus* can provide important information to help guide antimicrobial therapy particularly in cases of serious infections such as those from sterile body sites. Conventional antimicrobial testing provides a comprehensive picture of the organism's resistance, however the typical turn-around time is about twenty-four hours. Rapid assays on isolated colonies of *S. aureus*, or on *S. aureus* grown in broth can detect MRSA in minutes and are therefore valuable tools for managing infections. The image on the right shows a lateral flow assay that detects the PBP2a protein using a monoclonal antibody. The test is performed on a suspension made from an isolated colony of *S. aureus*. If PBP2a is present in the specimen a blue line will appear at the test line within five minutes after the strip is inoculated. A control line is included as a quality control and must be present for the assay to be considered valid. In this example the tested isolate would be considered PBP2a positive since both the test line and the control line are visible. Once these results are noted, the *S. aureus* can be reported as MRSA.

Slide 8:

Oxacillin resistance in *S. aureus* can be detected by testing oxacillin or by using cefoxitin as a surrogate. Although oxacillin or nafcillin are the drugs that are used clinically, cefoxitin has been shown to have greater sensitivity than oxacillin for in vitro testing as cefoxitin is a more potent inducer of *mecA* expression resulting in more easily interpretable results. Either disk diffusion or broth microdilution MIC methods can be used for testing cefoxitin against *S. aureus*, however, oxacillin is only reliably tested by MIC methods. If the cefoxitin MIC is less than or equal to 4 micrograms per milliliter the isolate is considered to be susceptible to oxacillin. However, if the isolate is able to grow at cefoxitin concentrations greater than or equal to 8 micrograms per milliliter the

isolate is reported as being resistant to oxacillin. The image on the right shows two different isolates being tested using oxacillin broth microdilution. The bracketed wells show seven two-fold dilutions from 0.25 to 16 micrograms per milliliter of oxacillin. The growth of the isolate shown in panel A. is inhibited at all the concentrations of oxacillin tested, including 0.25 micrograms. The MIC is less than or equal to 0.25 micrograms per milliliter and the isolate would therefore be considered susceptible to oxacillin or MSSA. In contrast the isolate shown in panel B. Is able to grow at all seven concentrations of oxacillin as shown by the button of precipitated cell material and the MIC is greater than 16 micrograms per milliliter. This is greater than the 4 micrograms per milliliter resistant breakpoint, and the isolate would therefore be considered resistant to oxacillin and classified as an MRSA.

Slide 9:

The detection of the *mecA* gene using molecular methods is another option available to laboratories for MRSA detection. There are a variety of FDA cleared *mecA* assays currently available. In general, these assays are either PCR based or use a nucleic acid probe for the detection of *mecA.* The main advantage of molecular testing is a much shorter turn-around time, usually less than twenty four hours, as opposed to thirty six hours plus for culture based detection methods. Many of these assays can be run directly from the patient specimen, significantly reducing the time required. A disadvantage to the molecular assays, aside from the higher cost, is that they may not detect novel PBP genotypes, such as *mecC*, should they emerge. In either case the results of molecular test should be confirmed by culture based methods either disc diffusion or MICs.

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Vancomycin is currently the drug of choice for treating MRSA infections as most *S. aureus* are still susceptible. The emergence of resistance to vancomycin is therefore a significant concern. The table below shows the current CLSI breakpoints for determining vancomycin resistance in *S. aureus*. It is important to note that only MIC

methods can reliably predict vancomycin resistance and disk diffusion is not used. Isolates that are inhibited by 2 micrograms per milliliter or less of vancomycin are classified as susceptible or VSSA, those with an MIC between 4 and 8 micrograms per milliliter are intermediate, or VISA. Isolates with an MIC of 16 micrograms per milliliter or greater are resistant and are known as VRSA. Fortunately, the isolation of VRSA from clinical specimens has occurred only a handful of times. Since 2002 only 14 confirmed VRSA cases have occurred in the United States . Resistance to vancomycin results from a modification to the cell wall that prevents binding of the drug. This modification is mediated by the *vanA* gene. The VISA phenotype is thought to result from a thickening of the cell wall which reduces the penetration of the drug. Vancomycin Intermediate *S. aureus* are of concern because the elevated MIC for vancomycin is correlated with poor outcomes when vancomycin is used for treating MRSA infections. Although VISA is still relatively rare the prevalence of reported cases in the United States has been rising.

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In situations where a *S. aureus* isolate tests resistant to erythromycin and susceptible or intermediate to clindamycin it is important to check for inducible clindamycin resistance. The D-zone test is one method that can be used. A 15-µg erythromycin disk is placed adjacent to a 2-µg clindamycin disk about 20 mm apart. An example of this is shown in image A. If the cells have inducible clindamycin resistance, the erythromycin will induce them to turn on their clindamycin resistance mechanism. This becomes apparent in the area where both erythromycin and clindamycin have diffused into the media and results in a D shaped zone of inhibition. In contrast the isolate shown in image B. is resistant to erythromycin and susceptible to clindamycin, without clindamycin induction as evidenced by no distortion of the zone. When inducible clindamycin resistance is observed, the isolate should be reported as clindamycin resistant because it has been shown that clindamycin would probably not be effective for treatment of infections due to *S. aureus* with inducible clindamycin resistance. The concern is that an inducible strain may become resistant to clindamycin without requiring induction.

Slide 12:

Here are somethings to keep in mind when doing susceptibility testing with *Staphylococcus* aureus*.* Oxacillin and cefoxitin are the β-lactams that are routinely tested, although labs may test for penicillin it is rarely prescribed. The results from oxacillin or cefoxitin testing can be used to predict susceptibility to other β-lactams. In the case of MRSA they should be considered resistant to all other β-lactams except ceftaroline. It is also important to point out that MRSA are very often more resistant to other antibiotics than are strains of MSSA.

Slide 13:

We are now going to move on and talk a little bit about susceptibility testing in the enterococci. While the enterococci are considered to be normal flora they can also be associated with serious infections such as endocarditis as well as a cause of urinary tract infection. Shown here are some special considerations for susceptibility testing in the enterococci that we will be discussing today, differences in susceptibility between *E. faecium* and *E. faecalis,* vancomycin resistance and high level aminoglycoside resistance.

Slide 14:

Resistance in the enterococci is the result of an altered penicillin binding protein. *E. faecium* is generally resistant to penicillin and ampicillin in contrast to *E. faecalis* which is usually susceptible to these agents. The table below shows the current CLSI breakpoints for ampicillin and for penicillin, by both disk diffusion and dilution methods.

Slide 15:

Resistance to vancomycin can be acquired and is more typically associated with *E. faceium.* Vancomycin resistance can be conferred by either the *vanA* or *vanB* genes. The MIC may vary depending on the gene that is present with *vanA* resulting in somewhat higher MIC values. Susceptibility testing can be done by either MIC or disc

diffusion methods. In the table below the CLSI breakpoints are included for both the disc diffusion as well the MICs. In cases where the zone has a diameter of less than or equal to 14 millimeter the isolate is considered to be resistant to vancomycin and therefore is a VRE, however if the zone is equal to or greater than 17 millimeters then the isolate is susceptible to vancomycin and is not VRE. The image on the right shows two different isolates of *E. faecium*. The isolate shown in A. is resistant to vancomycin as the culture grows right up to the disc. In contrast isolate B has a zone of inhibition of 18 millimeters and is susceptible to vancomycin.

Slide 16:

I briefly wanted to discuss the concept of synergy as it pertains to the enterococci. As previously mentioned, the enterococci are less susceptibile to penicillin and ampicillin, as a result the effect of these drugs may be bacteriostatic and not bactericidal. The enterococci also have intrinsic low level resistance to the aminoglycosides meaning that this class of drug is largely ineffective as well. The recommended therapy for treating serious enterococcal infections calls for the combination of cell wall active agents like ampicillin combined with an aminoglycoside. The idea is that the β-lactam can weaken the cell wall, this enables the aminoglycoside to better penetrate and exert a bactericidal effect. While neither class of drug is effective as monotherapy for serious enterococcal infections, the combination can be effective through a process called synergy. However, for synergy to work the isolate cannot have acquired high level resistance to the aminoglycosides or resistance to the cell wall active agents.

Slide 17

Although synergy refers to the combined effect of multiple antibiotics given together, in this case an aminoglycoside and a cell wall active agent, the antibiotics are tested separately. Susceptibility can be measured by either disc diffusion or by dilution methods; however higher concentrations of the aminoglycosides are used. Disc diffusion requires disc concentrations of 120 micrograms for gentamicin and 300 micrograms for streptomycin while the MICs are set at 500 micrograms per milliliter for

gentamicin and 1000 micrograms per milliliter for streptomycin. The table on the right provides a few examples. Synergy would be expected to work in case one as the isolate is susceptible to ampicillin as well high level streptomycin and gentamicin. In cases two and three synergy cannot be achieved; in case four synergy can be achieved as the isolate is susceptible to ampicillin, even though it is resistant to high level streptomycin it is susceptible to high level gentamicin.

Slide 18:

Here are some important points to remember about susceptibility testing and the enterococci. The enterococci are intrinsically resistant to a number of antimicrobials. In addition to this intrinsic resistance they can acquire resistance to many antimicrobials, such as vancomycin. As a result, treatment of serious infections with enterococci can be difficult as only a limited number of antimicrobials may be available.

Slide 19: References

Here are the references that were used for this talk.

Slide 20: Disclosures

Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Antimicrobial Susceptibility Testing (AST) of Commonly Encountered Gram Positive Bacteria**."