ENTEROCOCCI

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OBJECTIVES

- Discuss basic antimicrobial susceptibility principles and resistance mechanisms for *Enterococcus*
- Describe issues surrounding AST of enterococci
  - Vancomycin
  - Aminoglycosides and synergy testing
- Discuss antimicrobial susceptibility testing and reporting strategies

ENTEROCOCCI BACKGROUND

- Enterococci are intrinsically resistant to a number of agents and are extremely adept at acquiring antimicrobial resistance
- Infections typically present in immunocompromised individuals further hindering treatment strategies
- Persist in the hospital environment allowing for opportunities for transmission
- Common colonizers of the GI tract
- In the U.S., approximately 30% of enterococcal isolates are resistant to vancomycin

CASE

- 46-year old male inpatient becomes febrile while receiving vancomycin for MRSA bacteremia.
- Gram stain = GPC
- Verigene BC-GP test = *E. faecium*, but no resistance determinants were detected (i.e. negative for *vanA* and *vanB*).
- The patient remained on vancomycin and Gram negative coverage was added while awaiting culture results. Two days later, the isolate is identified as confirmed as *E. faecium* and phenotypic susceptibility testing reveals the isolate is resistant to vancomycin (MIC >64). What happened?

VANCOMYCIN RESISTANCE GENES

	VanA	VanB	VanM	VanD	VanE	VanC
Vancomycin	≥64	≥4	>256	64-128	8-32	2-32
Teicoplanin	R	S	S-R	S-R	S	S
Transferable	Yes	Yes	Yes	No	No	No
Notable	VRE	VRE	VRE			*E. gallinarum* *E. casseliflavus*

Others: VanD, VanG, VanL, VanN; table adapted from O'Driscoll and Crank

- Determination of specific resistance gene (often) unnecessary
- Vancomycin and teicoplanin MICs can be helpful in differentiating VRE from enterococci that are vancomycin-resistant

O'Driscoll T & Crank CW, Infect & Drug Resist, 2015

MOLECULAR DETECTION OF RESISTANCE

- Numerous platforms available with that primarily target *vanA* and *vanB*
- Performance of direct from positive blood assays is well established as highly sensitive (~95%)

MOLECULAR DETECTION OF RESISTANCE

- Achilles heel of a molecular assay is the target
  - Commercial assays are designed to detect known, characterized resistance markers and typically only the most common resistance determinants are included
- Real or perceived "misses" (i.e. true false negative vs mechanism other than one targeted) by molecular platforms when genotypically tests negative but phenotypically positive

Abbott AN & Fang FC, Manual of Clinical Microbiology, 11th Ed., 2015

RESOLUTION OF DISCORDANT GENOTYPIC AND PHENOTYPIC RESULTS

- Discordant results must be evaluated
- Possible sources:
  - Missed *vanA* or *vanB* by molecular platform
    - Martinez et. al. described less than desirable performance for detection of *vanA* (sources: false negative possibly due to inhibitory substance and *vanA* gene detected by Verigene (and confirmed with other molecular assay) with phenotypic susceptibility. Others have reported more favorable performance characteristics.
  - Incorrect vancomycin resistance by phenotypic analysis
  - Mechanism other than *vanA* or *vanB*

Martinez RM et. al., JCM, 2014

THE OTHER VANS

- Case: phenotypic susceptibility confirmed, ran isolate on another molecular platform and remained *vanA* negative. This case scenario would fit for van gene other than *vanA* or *vanB*
  - *VanM* – extremely rare, reported in Singapore and China
- Lack of routine monitoring of the van genes in circulation
  - Not all laboratories routinely test teicoplanin
  - Not all laboratories utilize molecular assays that target *vanA* and *vanB*
- As molecular detection continues to expand, so will recognition of these other van genes

Teo JWP et. al., JCM, 2011

VRE SCREENING

- Unlike blood culture assays, VRE surveillance by PCR has struggled
- Poor positive predictive value associated with *vanB* results
  - Numerous reports of *vanB* in non-enterococci (e.g. *Clostridium*, *Eggerthella, Ruminococcus*, others)
  - Leads to high number of false positives
- Use of PCR has allowed for detection of previously unrecognized phenotypes

Mak A et. al., JCM, 2009
Bourdon N et. al., Diagn Microbiol Infect Dis, 2010

WHAT ABOUT THE REVERSE PROBLEM?

- Genotypic resistance detected but phenotypically susceptible
- Termed Vancomycin Variable Enterococci (VVE) harbor "silent" resistance which may result in VSE to VRE transition while on therapy
- Ontario outbreak (2012) with >95 patients in 13 hospitals positive for vanA-carrying vancomycin-susceptible isolates

	PCR (vanA)	Agar (6ug/ml vanc)	Broth microdilution
Result	Pos	Growth/No Growth	S

- VVE strains pose a significant challenge for diagnostic tests used for VRE detection

VANCOMYCIN VARIABLE ENTEROCOCCI

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Resistance Hot Topic!

Vancomycin-Variable Enterococci: An Unrecognized Threat?

- Treatment of VVE with vancomycin could lead to treatment failure
- When identified, isolates should be reported as vancomycin resistant even if phenotypically susceptible

VANCOMYCIN-DEPENDENT ENTEROCOCCI

- Will not grow *in vitro* in the absence of vancomycin
- Detected during surveillance or by molecular methods

Tambyah PA et. al., Emerg Infect Dis, 2004

PHENOTYPIC TESTING CONSIDERATIONS

- Disk diffusion or Etest plates should be held for a full 24 hours and zones examined using transmitted light
  - Enhancement of growth that allows for better detection of intermediate resistance at 24 hrs compared to 18 hrs
  - Growth may be hazy in which transmitted light aids in recognition of inner growth
- Commercial systems vs Etest
  - Vancomycin MIC determination is somewhat method dependent; however, categorical agreement typically high
  - Etests generally produce vancomycin MICs that are 1 $\log_2$ concentration higher than the commercial systems

REPORTING CONSIDERATIONS

- Correct reporting of VRE for infection control purposes
  - Primarily interested in isolates carrying *vanA* or *vanB*
- Be aware of discrepancies between molecular and phenotypic methods (may be surveillance and infection isolates) and attempt to resolve

AMINOGLYCOSIDES VERSUS ENTEROCOCCUS

THERAPEUTIC DILEMMAS

- Enterococci are intrinsically resistant or exhibit inherent reduced susceptibility to a number of GP agents
- Although ampicillin is the preferred therapy for ampicillin-susceptible enterococcal infections, MICs against enterococci are typically elevated
- Furthermore, enterococci are resistant to clinically achievable concentrations of aminoglycosides (monotherapy)
- For serious enterococcal infections, combination of a cell wall agent (e.g. ampicillin) plus aminoglycoside results in bactericidal synergism

SYNERGY MECHANISM

- Beta-lactams (ampicillin) are structurally similar to the building blocks of the cell wall (pentapeptides)
- Aminoglycosides inhibit protein synthesis, so they must enter the cell in order to work
- Cell wall agent (e.g. beta-lactam or glycopeptide) allows penetration of the aminoglycoside into the cell → synergy
- In the lab, a disk containing high levels of the aminoglycoside are used to test for synergy of the agent when used in combination with a cell wall drug

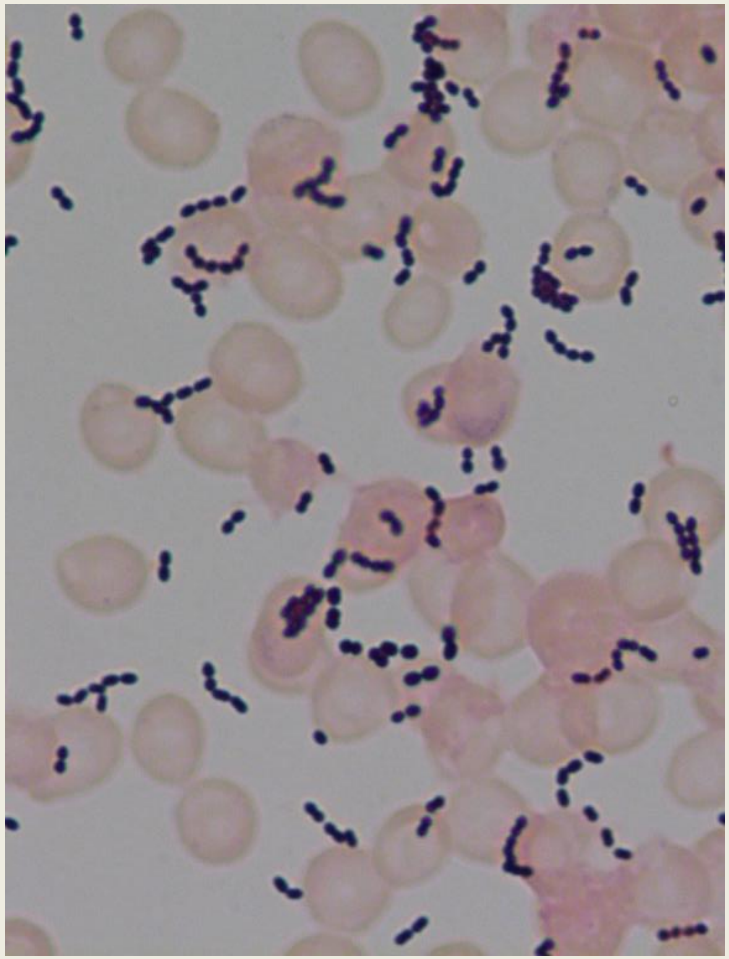
HLAR DETECTION

- Screening test for high level aminoglycoside resistance (HLAR) can be performed by disk diffusion, broth microdilution, or agar dilution
- Both gentamicin and streptomycin should be considered independently as one cannot predict the other
- Other aminoglycosides are considered inferior and should not be tested

Agent	Standard disk content*	HLAR disk content
Gentamicin	10 µg	**120 µg**
Streptomycin	10 µg	**300 µg**

*Do not use standard disk content for testing enterococci

CONSIDERATIONS



- Unnecessary to test and/or report HLAR on isolates other than those from blood cultures or specimens submitted for evaluation of endocarditis
- Other select cases: CSF – enterococcal meningitis
  - No reported mortality benefit with combination therapy

AMINOGLYCOSIDE REPORTING

- For enterococci, aminoglycoside results should NEVER be reported as susceptible or resistant
- Rather indicate “Synergy” or “No Synergy”
  - SYN vs SYN-R
- Use of comments:
  - Synergy is achievable with gentamicin and susceptible cell wall agent.
  - Gentamicin is synergistic with a cell wall agent that is also susceptible.

WHAT IF RESISTANT TO ALL CELL WALL AGENTS TESTED?

E. faecium

Agent	Interp
Penicillin	R
Ampicillin	R
Vancomycin	R
Daptomycin	R
HL Gentamicin	SYN
HL Streptomycin	SYN

- Gentamicin is synergistic with a cell wall agent that is also susceptible

REPORTING WHEN RESISTANT TO CELL WALL AGENTS

- Confirm susceptibility
- Knowing the MICs for cell wall agents will be helpful
  - Strains of *E. faecium* with ampicillin MICs of ≤64 ug/ml may respond to high-dose ampicillin therapy in combination with gentamicin
  - Combination therapy with two cell wall agents is gaining in popularity
- Report aminoglycoside synergy even if the cell wall agents are reported as resistant

Murray BE, NEJM, 2000

S. PNEUMONIAE

OBJECTIVES

- Discuss basic antimicrobial susceptibility principles and resistance mechanisms for *S. pneumoniae* versus penicillin and ceftriaxone
- Discuss antimicrobial susceptibility testing and reporting strategies

BACKGROUND

- Common inhabitants of the upper respiratory tract and can be isolated from the nasopharynx of 5-90% of the population
- Most infections occur by direct extension (e.g. pneumonia) or by hematogenous spread (e.g. meningitis, peritonitis, bacteremia)
- Meningitis and non-meningitis breakpoints exist for the common beta-lactams used to treat pneumococci (penicillin, cefepime, cefotaxime, ceftriaxone)

BACKGROUND: PENICILLIN BREAKPOINTS

- Generally speaking, breakpoints are established based upon physiologically achievable concentrations
  - Somewhat biased toward blood concentrations
  - Penicillin breakpoints historically based on CSF concentrations
- Penicillin penetrates poorly into the CNS; higher concentrations are achieved in fluids other than CSF
- CLSI incorporated meningitis and non-meningitis breakpoints in 2008 allowing penicillin to remain in the arsenal for treatment of community acquired pneumonia

Weinstein MP et. al., CID, 2009

BETA-LACTAM BREAKPOINTS

Agent	Non-Meningitis (MIC)			Meningitis (MIC)		
	S	I	R	S	I	R
Penicillin (p)	≤2	4	≥8	≤0.06	-	≥0.12
Cefotaxime	≤1	2	≥4	≤0.5	1	≥2
Ceftriaxone	≤1	2	≥4	≤0.5	1	≥2
Cefepime	≤1	2	≥4	≤0.5	1	≥2

- Reliable disk diffusion breakpoints do not yet exist. In vitro activity is best determined by a MIC-based method.

BETA-LACTAM CLSI COMMENTS

- Amoxicillin, ampicillin, cefepime, cefotaxime, ceftriaxone, cefuroxime, ertapenem, imipenem, and meropenem may be used to treat pneumococcal infections; however, reliable disk diffusion susceptibility tests for these agents do not yet exist.
  - Cefepime – non-FDA-approved indication
  - Meropenem > imipenem (seizure risk)
- For *S. pneumoniae* isolated from the CSF penicillin and cefotaxime, ceftriaxone, or meropenem should be tested by a reliable MIC method and reported routinely. Such isolates can also be tested against vancomycin using the MIC or disk method.

DECIPHERING THE M100

Table 2G. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	I	R	MIC Interpretive Criteria (µg/mL) S	I	R	Comments
PENICILLINS									
(4) For nonmeningitis isolates, a penicillin MIC of ≤0.06 µg/mL (or oxacillin zone ≥20 mm) can predict susceptibility to the following β-lactams: ampicillin (oral or parenteral), a[illegible]cillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozil, ceftaroline, ceftizoxime, ceftriaxone, cefu[illegible], doripenem, ertapenem, imipenem, loracarbef, meropenem, and penicillin (oral or parenteral). See comment (3).									
A	Penicillin	1 µg oxacillin	≥20	–	–	–	–	–	(5) Isolates of pneumococci with oxacillin zone sizes of ≥ 20 mm are susceptible (MIC ≤ 0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for those isolates with oxacillin zone diameters of ≤ 19 mm, because zones of ≤ 19 mm occur with penicillin-resistant, intermediate, or certain susceptible strains. For isolates with oxacillin zones ≤ 19 mm, do not report penicillin as resistant without performing a penicillin MIC test.
A	Penicillin parenteral (nonmeningitis)	–	–	–	–	≤2	4	≥8	(6) *Rx*: Doses of intravenous penicillin of at least 2 million units every four hours in adults with normal renal function (12 million units per day) can be used to treat nonmeningeal pneumococcal infections due to strains with penicillin MICs ≤ 2 µg/mL. Strains with an intermediate MIC of 4 µg/mL may require penicillin doses of 18 to 24 million units per day. (7) For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
A	Penicillin parenteral (meningitis)	–	–	–	–	≤0.06	–	≥0.12	(8) *Rx*: Use of penicillin in meningitis requires therapy with maximum doses of intravenous penicillin (eg, at least 3 million units every four hours in adults with normal renal function). (9) For CSF isolates, report only meningitis interpretations.
A	Penicillin (oral penicillin V)	–	–	–	–	≤0.06	0.12–1	≥2	(10) Interpretations for oral penicillin may be reported for isolates other than those from CSF.

PARENTERAL BREAKPOINTS

Penicillin parenteral (nonmeningitis)	–	–	–	–	≤2	4	≥8
Penicillin parenteral (meningitis)	–	–	–	–	≤0.06	–	≥0.12

(6) *Rx*: Doses of intravenous penicillin of at least 2 million units every four hours in adults with normal renal function (12 million units per day) can be used to treat nonmeningeal pneumococcal infections due to strains with penicillin MICs ≤ 2 µg/mL. Strains with an intermediate MIC of 4 µg/mL may require penicillin doses of 18 to 24 million units per day.

(7) For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.

(8) *Rx*: Use of penicillin in meningitis requires therapy with maximum doses of intravenous penicillin (eg, at least 3 million units every four hours in adults with normal renal function).

(9) For CSF isolates, report only meningitis interpretations.

- Automated instruments use these breakpoints for interpretation
- Provide dosing information if possible

REPORTING PENICILLIN MIC RESULTS – NONMENINGITIS (NON-CSF, NON-CNS)

Agent	MIC	Interp
Penicillin (parenteral - meningitis)	1	R
Penicillin (parenteral - nonmeningitis)	1	S
Penicillin (oral)	1	I

- Why include interpretations for the oral formulation of penicillin?
  - Penicillin V is the treatment of choice for pneumococcal pneumonia
- Be aware of what antibiotics practitioners preferentially prescribe (formulary agents, local practices)

REPORTING PENICILLIN MIC RESULTS – MENINGITIS (CSF, CNS)

Agent	MIC	Interp
Penicillin (parenteral - meningitis)	1	R

- Only report meningitis interpretations
- Consider options for providing dosing information

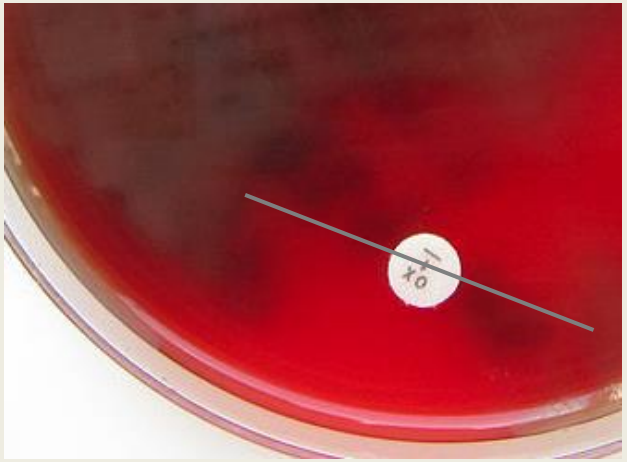
PENICILLIN DISK DIFFUSION

Penicillin	1 µg oxacillin	≥ 20	–		–		–		–

(5) Isolates of pneumococci with oxacillin zone sizes of ≥ 20 mm are susceptible (MIC ≤ 0.06 µg/mL) to penicillin. Penicillin and cefotaxime, ceftriaxone, or meropenem MICs should be determined for those isolates with oxacillin zone diameters of ≤ 19 mm, because zones of ≤ 19 mm occur with penicillin-resistant, intermediate, or certain susceptible strains. For isolates with oxacillin zones ≤ 19 mm, do not report penicillin as resistant without performing a penicillin MIC test.

- For use with nonmeningitis isolates only

PENICILLIN DISK DIFFUSION



- Blood isolate
- Oxacillin zone = 19 mm
- What next?

- For nonmeningitis isolates, a zone of ≤19 mm may correspond to susceptible, intermediate, or resistant MICs
- **Perform a penicillin MIC**
- Do not report penicillin as resistant without performing a MIC test

PENICILLIN DISK DIFFUSION

- Blood isolate
- Oxacillin zone = **20 mm**
- What next?

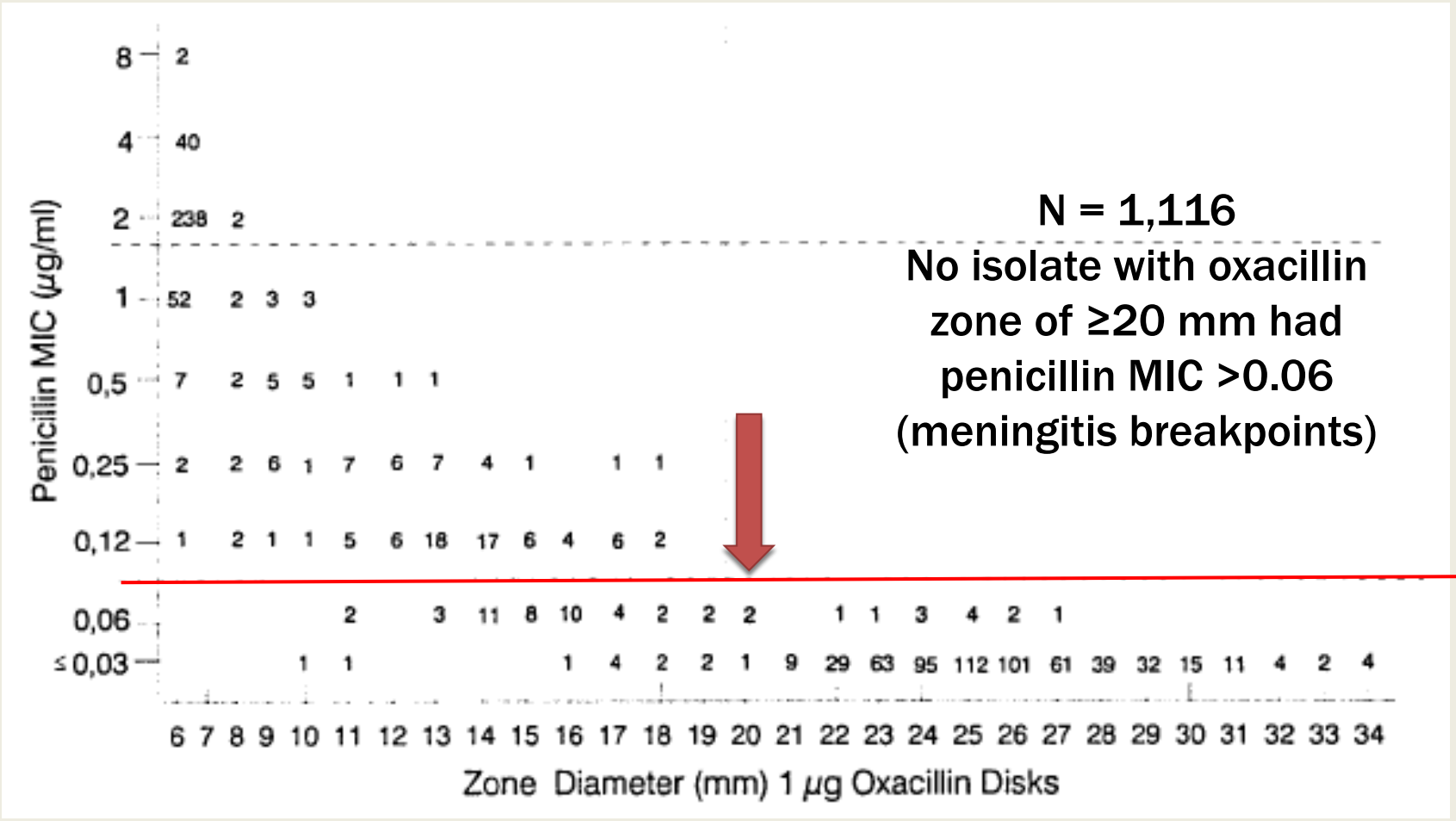
- For nonmeningitis isolates, oxacillin zone ≥20 mm is susceptible and corresponds to a penicillin MIC 0.06 µg/ml (which would be susceptible using both meningitis and nonmeningitis breakpoints)

REPORTING OF PENICILLIN SUSCEPTIBILITY- NONMENINGITIS ISOLATE

- Per comment (4), one can report as susceptible to penicillin and can be used to predict susceptibility for several agents (e.g. cefotaxime, ceftriaxone, meropenem) for NONMENINGITIS isolates
- What about comment (7) "For all isolates other than CSF, report interpretations for both meningitis and nonmeningitis"?

Agent	Result (interpretation)
Penicillin (parenteral - meningitis)	S
Penicillin (parenteral - nonmeningitis)	S
Penicllin (oral)	S
Ceftriaxone (meningitis)	S
Ceftriaxone (nonmeningitis)	S

OXACILLIN DISK FOR PREDICTING PENICILLIN SUSCEPTIBILITY

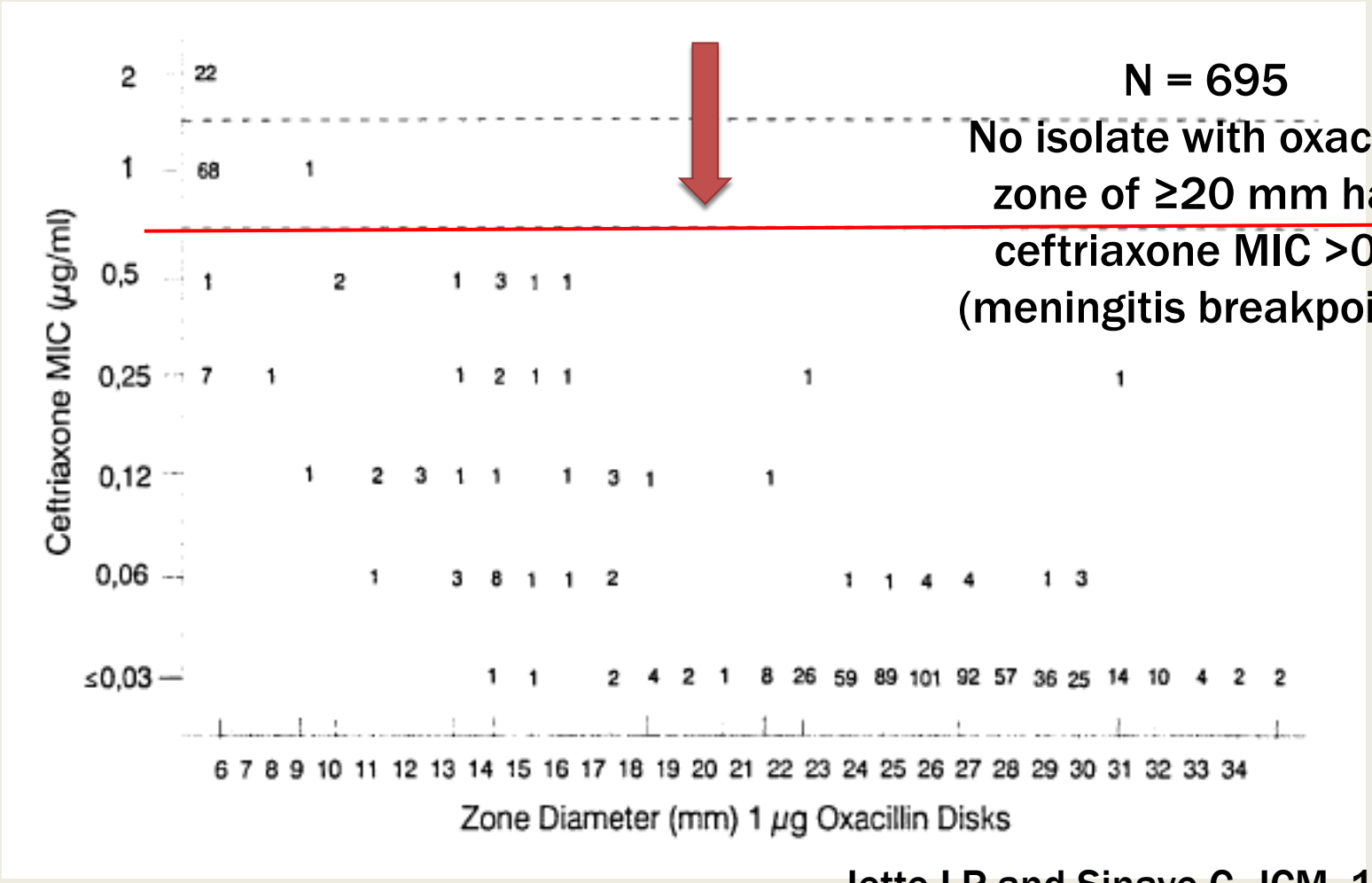


N = 1,116

No isolate with oxacillin zone of ≥20 mm had penicillin MIC >0.06 (meningitis breakpoints)

Jette LP and Sinave C, JCM, 1999

OXACILLIN DISK FOR PREDICTING CEFTRIAXONE SUSCEPTIBILITY



N = 695

No isolate with oxacillin zone of ≥20 mm had ceftriaxone MIC >0.5 (meningitis breakpoints)

Jette LP and Sinave C, JCM, 1999

DECIPHERING THE M100

Amoxicillin (nonmeningitis)	–	–	–	–	≤2	4	≥8	
Amoxicillin-clavulanate (nonmeningitis)					≤2/1	4/2	≥8/4	
RENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)								
Cefepime (meningitis)	–	–	–	–	≤0.5	1	≥2	(11) In the United States, for CSF isolates, report only nonmeningitis interpretations. There is not an FDA–approved indication for the use of cefepime for meningitis in the United States.
Cefepime (nonmeningitis)	–	–	–	–	≤1	2	≥4	(12) In the United States, only report interpretations for nonmeningitis and include the nonmeningitis notation on the report.
Cefotaxime (meningitis)	–	–	–	–	≤0.5	1	≥2	(13) For CSF isolates, report only meningitis interpretations.
Ceftriaxone (meningitis)	–	–	–	–	≤0.5	1	≥2	(14) *Rx*: Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See comment (3).
Cefotaxime (nonmeningitis)	–	–	–	–	≤1	2	≥4	(15) For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
Ceftriaxone (nonmeningitis)	–	–	–	–	≤1	2	≥4	
Ceftaroline (nonmeningitis)	30 µg	≥26	–	–	≤0.5	–	–	(16) Interpretive criteria are based on a dosage regimen of 600 mg every 12 h.
Cefuroxime (parenteral)	–	–	–	–	≤0.5	1	≥2	

- Cefepime only nonmeningitis interpretations in U.S.
- Cefotaxime and/or ceftriaxone meningitis and nonmeningitis interpretations should be reported

REPORTING CEPHALOSPORIN RESULTS - NONMENINGITIS (NON-CSF, NON-CNS)

Agent	MIC	Interp
Cefotataxime (parenteral - meningitis)	1	I
Cefotaxime (parenteral - nonmeningitis)	1	S
Ceftriaxone (parenteral - meningitis)	1	I
Ceftriaxone (parenteral - nonmeningitis)	1	S

- May not be necessary to report both cefotaxime and ceftriaxone

REPORTING CEPHALOSPORIN RESULTS - MENINGITIS (CSF, CNS)

Agent	MIC	Interp
Cefotataxime (parenteral - meningitis)	1	I
Ceftriaxone (parenteral - meningitis)	1	I

- May not be necessary to report both cefotaxime and ceftriaxone
- May consider comment providing recommended dosing (CLSI indicates “maximum doses,” confer with pharmacy about recommendation)
- May consider reporting of other agents with or without interpretations (e.g. cefepime, ceftaroline)

CEFTAROLINE FOR S. PNEUMONIAE MENINGITIS

- Small case series (4 patients) indicated that ceftaroline may be a viable treatment option for *S. pneumoniae* meningitis
  - 3 out of 4 patients were successfully treated
  - Potential agent for penicillin-resistant isolates
- Thus far, very limited number of clinical descriptions of ceftaroline for meningitis
  - Case by case basis with pharmacy/ID involvement

Sakoulas G et. al., AAC, 2015