14th year!

2017 CLSI药敏试验（AST）更新

February 1 & 2, 2017

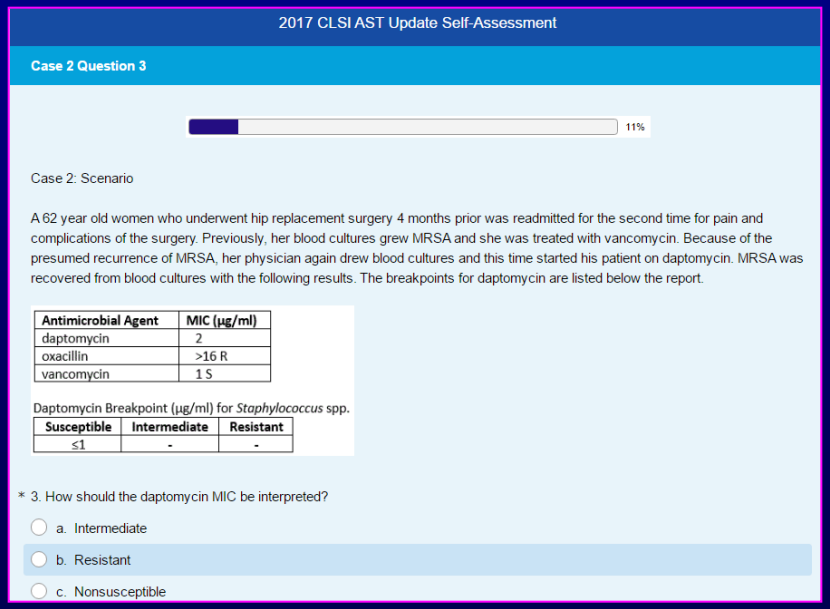
Janet A. Hindler, MCLS MT(ASCP) F(AAM)
Sr. Specialist, Clinical Microbiology
UCLA Health System, Los Angeles, CA
jhindler@ucla.edu

Audrey N. Schuetz, MD, MPH, D(ABMM)
Mayo Clinic
Rochester, Minnesota, USA
schuetz.audrey@mayo.edu

本演讲目标

- CLSI M100 27th 版本中的主要变化
- 设计在临床实践中实施新的推荐内容的策略
- 开发通知临床工作人员药敏试验和报告中重大变化的沟通策略

请查看这个网络研讨会的自我评估！


2017 CLSI AST Update Self-Assessment

Case 2 Question 3

11%

Case 2: Scenario

A 62 year old women who underwent hip replacement surgery 4 months prior was readmitted for the second time for pain and complications of the surgery. Previously, her blood cultures grew MRSA and she was treated with vancomycin. Because of the presumed recurrence of MRSA, her physician again drew blood cultures and this time started his patient on daptomycin. MRSA was recovered from blood cultures with the following results. The breakpoints for daptomycin are listed below the report.

Antimicrobial Agent	MIC (µg/ml)
daptomycin	2
oxacillin	>16 R
vancomycin	1 S

Daptomycin Breakpoint (µg/ml) for *Staphylococcus* spp.

Susceptible	Intermediate	Resistant
≤1	-	-

* 3. How should the daptomycin MIC be interpreted?

a. Intermediate

b. Resistant

c. Nonsusceptible

CLSI 药敏分会
扩大工作组 (ORWG)

- 培训大家药敏试验操作和推荐
- 提供资源帮助大家理解和实施CLSI药敏试验推荐
  - 每年2次教育简报
  - 网络研讨会
  - CLSI会议中的教育培训
  - 与其他组织的合作 (例如, APHL, ASM, CAP)
  - …更多!

2017年6月2日星期五上午7:30-8:30
专家会议: “抗菌药物耐药性”

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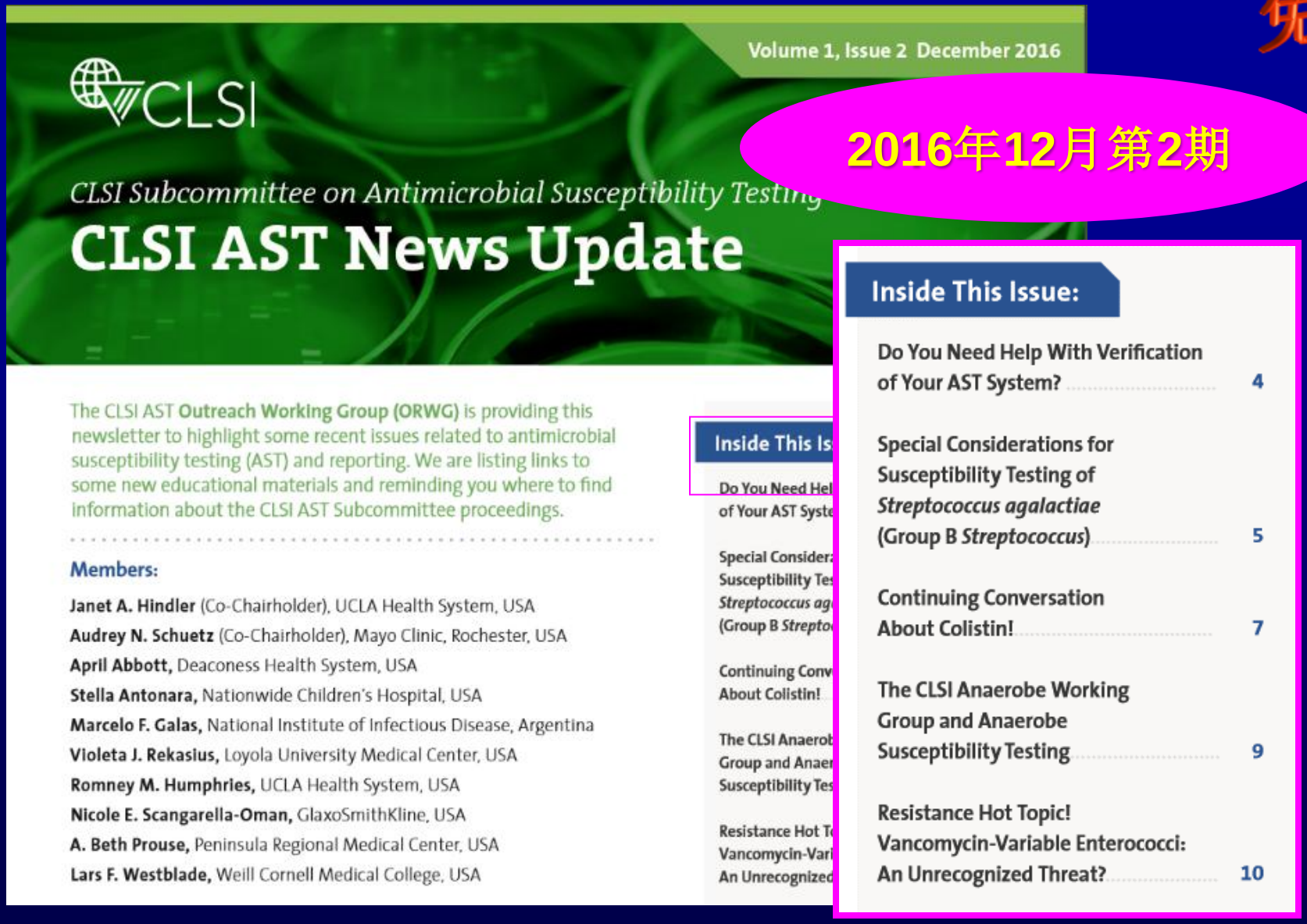

Volume 1, Issue 2 December 2016

CLSI

CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

2016年12月 第2期

The CLSI AST **Outreach Working Group (ORWG)** is providing this newsletter to highlight some recent issues related to antimicrobial susceptibility testing (AST) and reporting. We are listing links to some new educational materials and reminding you where to find information about the CLSI AST Subcommittee proceedings.

Members:

Janet A. Hindler (Co-Chairholder), UCLA Health System, USA
Audrey N. Schuetz (Co-Chairholder), Mayo Clinic, Rochester, USA
April Abbott, Deaconess Health System, USA
Stella Antonara, Nationwide Children's Hospital, USA
Marcelo F. Galas, National Institute of Infectious Disease, Argentina
Violeta J. Rekasius, Loyola University Medical Center, USA
Romney M. Humphries, UCLA Health System, USA
Nicole E. Scangarella-Oman, GlaxoSmithKline, USA
A. Beth Prouse, Peninsula Regional Medical Center, USA
Lars F. Westblade, Weill Cornell Medical College, USA

Inside This Issue:

查看ORWG新闻更新链接.....

验证协议,表格,问与答（Q&A）

Verification of Revised Ertapenem, Imipenem and Meropenem Breakpoints for Enterobacteriaceae on AST System XYZ

Verification Performed: _____(DDMMYY) to _____(DDMMYY)

Test Impleme

I. Purpose
Verify per
breakpoin

	A	B	C	D	E	F	G	H	I	J	K	L
1	Isolate #	Date / Tech	Organism	Ertapenem			Imipenem			Meropenem		
2				Reference	Test System		Reference	Test System		Reference	Test System	
3				S, I, R	MIC	S, I, R	S, I, R	MIC	S, I, R	S, I, R	MIC	S, I, R
4	1											
5	2											

抗菌药物技术纲要

Antimicrobial Resistance – β-Lactams

	Mode of action	Gram-positive Note if drugs covers MRSA, VRE			Gram-negative Note if drug can be used for CRE/CPO			Anaerobes		Atypicals (no cell wall)	Resistance and resistance mechanisms	Clinical and other notes/body site specificity
		Staphylo-cocci	Strepto-cocci	Entero-cocci	Entero-bacteriaceae	*P. aerug.*	Other	Gram-positive	Gram-negative			
β-Lactams	Bind to at least two penicillin-binding proteins (PBPs), inhibiting transpeptidation, the last step of bacterial cell wall synthesis.									N	Gram-positive cocci: (1) Production of β-lactamases that hydrolyze the β-lactam bond in penicillins and some cephems, rendering the drug inactive; (2) Modified PBPs with decreased affinity for the	Serious and occasionally fatal hypersensitivity (anaphylactoid) reactions may occur. The frequency of these reactions is greater for penicillins, followed by

2017年春天即将发布

2017年1月CLSI 药敏试验标准

新!

M100 27th 版 表格 (2017)[1]

与以下….一起使用

M02-A12 纸片扩散法 (2015)[2]

M07-A10 MIC法 (2015)[2]

M11-A8 厌氧菌MIC法 (2012)

[1] M100每年至少更新1次

[2] M02, M07每3年更新

Recently Published

CLSI's Newest Products

QMS03-ED4 | TRAINING AND COMPETENCE ASSESSMENT, 4TH EDITION

M100-S27 | PERFORMANCE STANDARDS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING, 27TH EDITION

Upcoming Webinar

14th Annual CLSI/APHL Antimicrobial Susceptibility Testing Update

— Learn about the changes in M100S 27th edition.

— Gain tips to help you easily implement the new recommendations into your protocols.

— Receive a self-study tool that can be accessed by you and your staff

LEARN MORE»

Presenters:
Janet A. Hindler and Audrey Schuetz, MD

Dates:
Wednesday, February 1, 2017 | 1:00-2:30 PM EST

Thursday, February 2, 2017 | 3:00-4:30 PM EST

ASM-CLSI Webinar Series

An ASM-CLSI Webinar Series on Antimicrobial Susceptibility Testing: Fundamentals of Susceptibility Testing, Reporting, and Test Validation

The purpose of this P.A.C.E.®-accredited five-part webinar series is to provide the fundamentals of antimicrobial susceptibility testing (AST) in the clinical microbiology laboratory.

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M100 Resources

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December 2016 AST News Update

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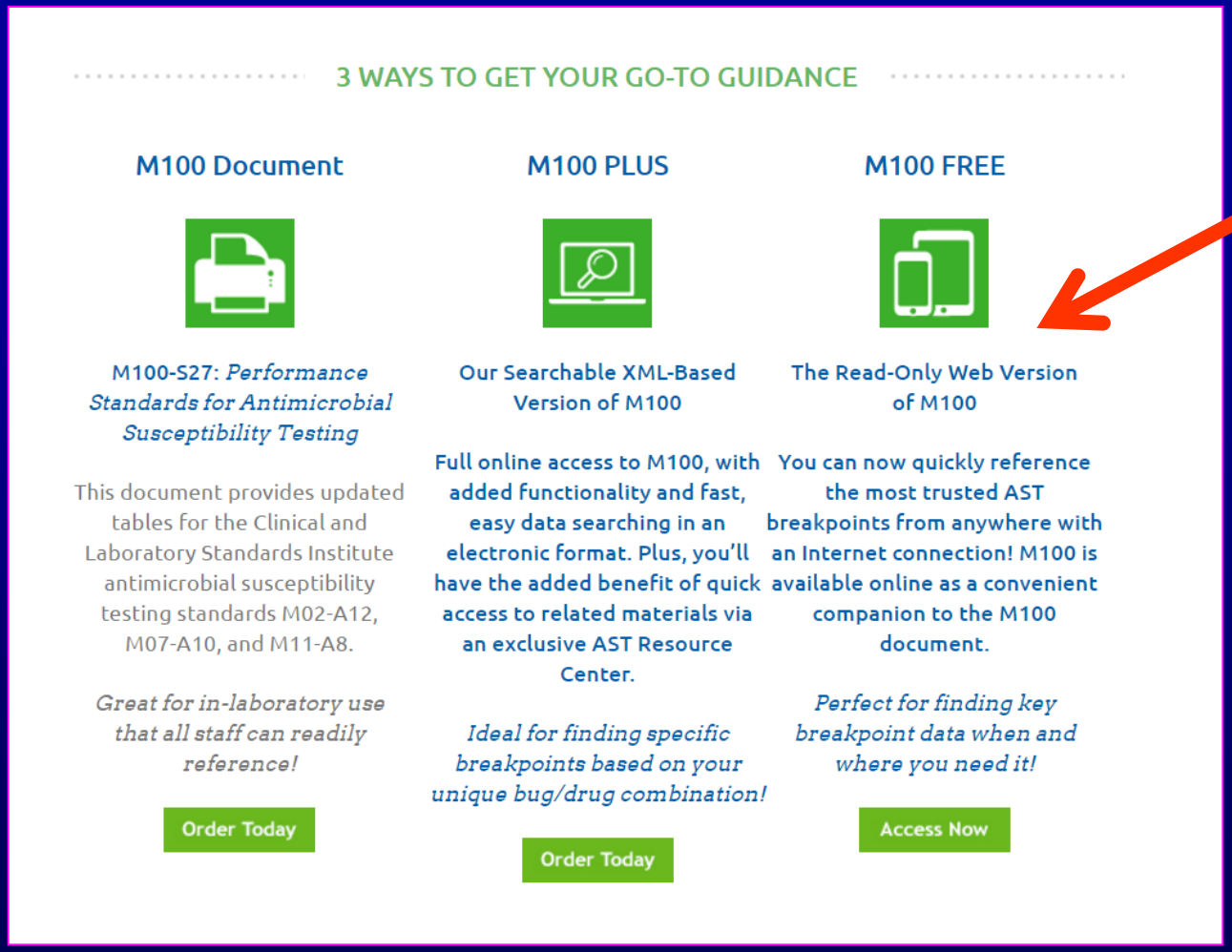

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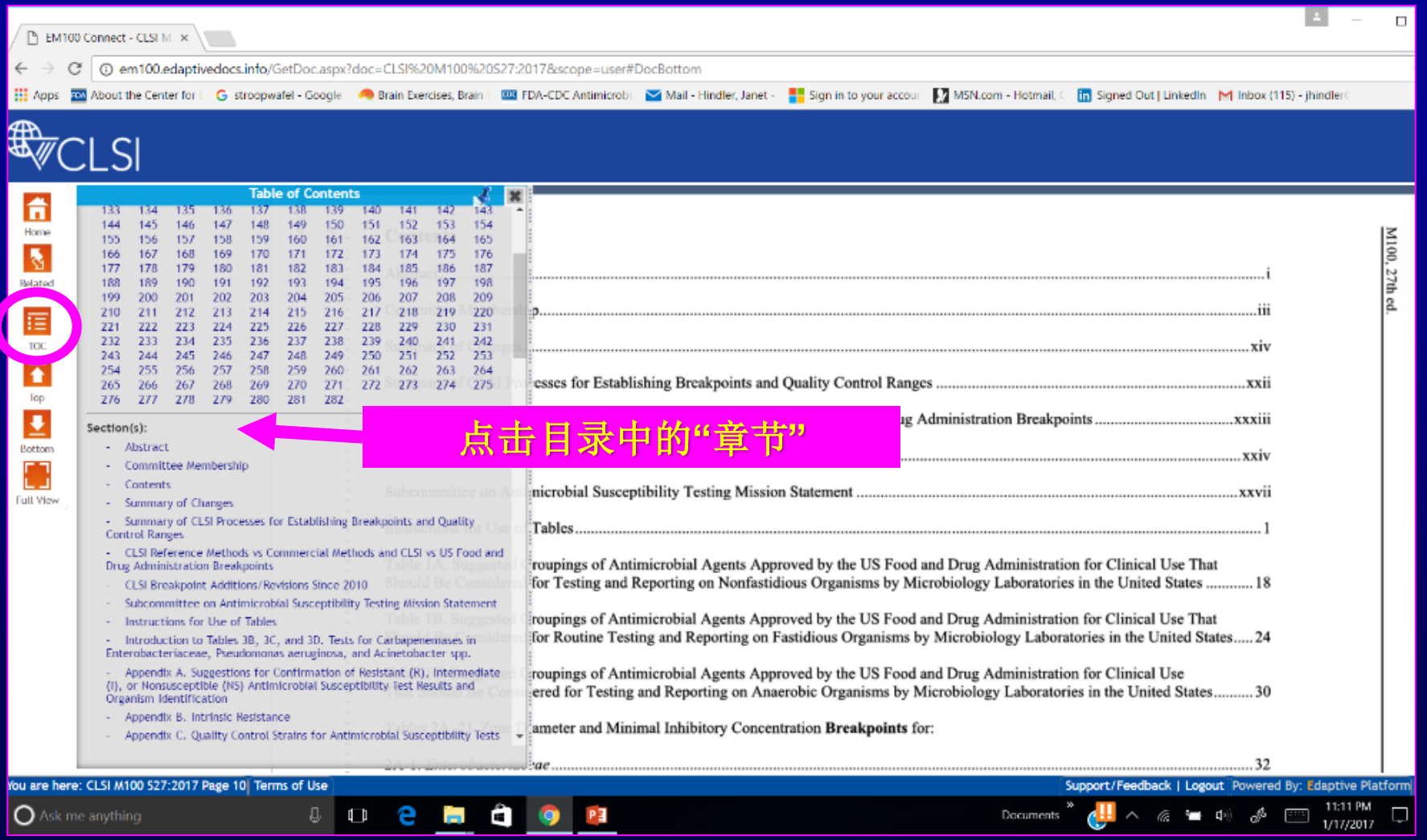
http://clsi.org/m100/

M100导航“e”版本....


EM100 Connect - CLSI M
em100.edaptivedocs.info/GetDoc.aspx?doc=CLSI%20M100%20S27:2017&scope=user#DocBottom
CLSI
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- CLSI Reference Methods vs Commercial Methods and CLSI vs US Food and Drug Administration Breakpoints
- CLSI Breakpoint Additions/Revisions Since 2010
- Subcommittee on Antimicrobial Susceptibility Testing Mission Statement
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点击目录中的“章节”
M100, 27th ed.
You are here: CLSI M100 S27:2017 Page 10
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11:11 PM
1/17/2017

“旧版本” CLSI M100折点归档

Breakpoints Eliminated from CLSI M100 Since 2010

Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter BPs (nearest whole mm) S	I	R	Interpretive Categories and MIC BPs (µg/mL) S	I	R	M100 Ed where BPs were last listed/Comments	Rationale
Enterobacteriaceae									
Nalidixic acid	30 µg	≥ 19	14–18	≤ 13	≤ 16	–	≥ 32	M100-S26 Deleted for *Salmonella* spp. only	Nalidixic acid does not perform reliably in predicting susceptibility to fluoroquinolones that might be used for treatment of *Salmonella* infections. It has been shown to produce both false resistant and false susceptible results.[1,2]
Cephalothin (surrogate test for uncomplicated UTI)	30 µg	≥ 18	15–17	≤ 14	≤ 8	16	≥ 32	M100-S25	Cefazolin has been shown to be a more reliable surrogate than cephalothin for predicting results for oral cephalosporins that might be used for treatment of uncomplicated UTIs.
Cefazolin	30 µg	≥ 18	15–17	≤ 14	–	–	–	M100-S20	When cefazolin MIC breakpoints were revised, these disk diffusion BPs were no longer valid. (Disk diffusion BPs to correlate with the new cefazolin MIC BPs have not yet been established.)
Ticarcillin	75 µg	≥ 20	15–19	≤ 14	≤ 16	32–64	≥ 128	M100-S26	This agent is no longer available.
Pseudomonas aeruginosa									
Cefoperazone	75 µg	≥ 21	16–20	≤ 15	≤ 16	32	≥ 64	M100-S21	These agents are no longer available or have limited indications for *P. aeruginosa*.
Cefotaxime	30 µg	≥ 23	15–22	≤ 14	≤ 8	16–32	≥ 64		
Ceftriaxone	30 µg	≥ 21	14–20	≤ 13	≤ 8	16–32	≥ 64		
Ceftizoxime	30 µg	≥ 20	15–19	≤ 14	≤ 8	16–32	≥ 64		
Moxalactam	30 µg	≥ 23	15–22	≤ 14	≤ 8	16–32	≥ 64		
Ticarcillin								M100-S26	
Acinetobacter spp.									
Mezlocillin	10 µg	≥ 15	12–14	≤ 11	≤ 8	16	≥ 32	M100-S26	This agent is no longer available

很快在CLSI网站上发布!

新!

2017年主要变化

通用

- 仅仅使用术语“折点”-不使用“解释标准”
- 继续删除过时的药物/重新定位老药

澄清的测试/报告:

- 铜绿假单胞菌和鲍曼不动杆菌对粘菌素/多粘菌素
- 非典型（生长不良）金黄色葡萄球菌
- 凝固酶阴性葡萄球菌和苯唑西林

流行病学界值（ECVs）:

- 扩充定义/讨论
- 增加以下ECVs:
  - 粘菌素 – 肠杆菌科
  - 阿奇霉素 – *淋病奈瑟菌*

新!

2017年主要变化 (续)

新方法

- 用于肠杆菌科碳青霉烯酶检测的mCIM

质量控制 / 质量保证

- 修改的纸片扩散法质控范围:
  - 头孢吡肟 - 铜绿假单胞菌 ATCC 27853
  - 美罗培南 - 大肠埃希菌 ATCC 25922
- 修改的MIC质控范围:
  - 美罗培南 - 铜绿假单胞菌 ATCC 27853
  - 泰地唑胺 – 金黄色葡萄球菌 ATCC 29213
- 增加一些正在研发中的新药物的质控范围 (还没有用于人)
- 扩充的MIC问题排除指南

增加*脆弱拟杆菌*建议

删除*摩根摩根菌*对四环素天然耐药

Summary of Changes

The following are additions or changes unless otherwise noted as a ***"deletion."***

CLSI Breakpoint Additions/Revisions Since 2010
Added colistin breakpoint changes.

CLSI Epidemiological Cutoff Value Addition Since 2015
Added new table for epidemiological cutoff values.

Subcommittee on Antimicrobial Susceptibility Testing Mission Statement
Added epidemiological cutoff values.

Instructions for Use of Tables
Revised results reporting definitions to harmonize with CLSI library of susceptibility testing documents (p. 1).

Routine, Supplemental, Screening, Surrogate Agent, and Equivalent Agent Testing to Determine Susceptibility and Resistance to Antimicrobial Agents
Supplemental Tests – Optional: Added modified carbapenem inactivation method.

Tables 1A, 1B, 1C – Drugs Recommended for Testing and Reporting
Deleted from Tables 1A, 1B, and/or 1C – cefuroxime (parenteral) and gemifloxacin.

Deleted from Table 1A – norfloxacin.

Staphylococcus spp. and *Enterococcus* spp.:
Moved ortivancin and televancin to Test/Report Group C (p. 19).

Haemophilus influenzae and *Haemophilus parainfluenzae*:
Moved ciprofloxacin, levofloxacin, and moxifloxacin to Test/Report Group B (p. 24).

Moved trimethoprim-sulfamethoxazole to Test/Report Group C (p. 25).

Clarified reporting of results for isolates of *H. influenzae* from CSF (p. 26).

Clarified antimicrobial agents used as empiric therapy for respiratory tract infections due to *Haemophilus* spp. (p. 26).

变化概要

M100 27th ed. p. xiv-xxi.

通用变化

新!

扩充的定义/描述:

- “折点和解释分类”
- “流行病学界值”

II. Breakpoints and Interpretive Category Definitions

A. Breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, nonsusceptible, or resistant; NOTE 1: MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; NOTE 2: Because breakpoints are based on pharmacologically and clinically rich datasets using *in vitro* and *in vivo* data, they are considered to be robust predictors of likely clinical outcome; NOTE 3: Also known as “clinical breakpoint”; NOTE 4: See interpretive category.

B. Interpretive category – category derived from microbiology characteristics, pharmacokinetic/pharmacodynamics parameters, and clinical outcome data, when available; NOTE 1: MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; NOTE 2: See breakpoint; EXAMPLE: MIC or zone diameter value breakpoints or interpretive categories are established per CLSI document M23 for categories of susceptible, intermediate, and resistant (and susceptible-dose dependent and nonsusceptible, when appropriate).

Example:

Interpretive Category	Breakpoints*	
	MIC (µg/mL)	Zone Diameter (mm)
Susceptible	≤4	≥20
Susceptible-Dose Dependent	8–16	15–19
Intermediate	8–16	15–19
Resistant	≥32	≤14
Nonsusceptible	>4	<20

*Formerly “interpretive criteria.”

Susceptible, susceptible-dose dependent, intermediate, resistant, or no
follows:

III. Epidemiological Cutoff Value

A. Definition

Epidemiological cutoff value (ECV) – the minimal inhibitory concentration (MIC) value or zone diameter value that separates microbial populations into those with and without acquired and/or mutational resistance based on their phenotypes (wild-type [WT] or non-wild-type [NWT]). The ECV defines the upper limit of susceptibility for the wild-type population of isolates.

Example:

Interpretive Category	ECVs	
	MIC (µg/mL)	Zone Diameter (mm)
Wild-type	≤4	≥20
Non-wild-type	≥8	≤19

B. ECV Interpretive Categories:

- Wild-Type (WT) – a category defined by an ECV value that describes isolates with no mechanisms of acquired resistance or reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.
- Non-Wild-Type (NWT) – a category defined by an ECV that describes isolates with presumed or known mechanisms of acquired resistance and reduced susceptibility for the antimicrobial (antifungal) agent being evaluated.

CLSI M100 27th ed. pp 3-7.

折点 / 解释分类定义

II. Breakpoints and Interpretive Category Definitions

A. Breakpoint – minimal inhibitory concentration (MIC) or zone diameter value used to categorize an organism as susceptible, susceptible-dose dependent, intermediate, nonsusceptible, or resistant; NOTE 1: MIC or zone diameter values generated by a susceptibility test can be interpreted based upon established breakpoints; NOTE 2: Because breakpoints are based on pharmacologically and clinically rich datasets using *in vitro* and *in vivo* data, they are considered to be robust predictors of likely clinical outcome; NOTE 3: Also known as "clinical breakpoint"; NOTE 4: See interpretive category.

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"折点 - …. 因为折点基于体外和体内丰富的药理和临床数据，所以可认为对临床结局具有强有力的指示作用;
注3: 也称为"临床折点" ….."

"解释分类–分类基于
•微生物特征, 例如MIC分布
•PK/PD参数
•临床结局数据（当可获得时）….."

CLSI M100 27th ed. p 3.

2016使用的“折点”或“解释标准”

Table 2A-1. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	SDD	I	R	MIC Interpretive Criteria (µg/mL) S	SDD	I	R	Comments
PENICILLINS											
A	Ampicillin	10 µg	≥17	–	14–16	≤13	≤8	–	16	≥32	(4) Results of ampicillin testing can be used to predict results for amoxicillin. See comment (2).

2017仅使用“折点”

Table 2A-1. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm) S	SDD	I	R	Interpretive Categories and MIC Breakpoints (µg/mL) S	SDD	I	R	Comments
PENICILLINS											
A	Ampicillin	10 µg	≥17	–	14–16	≤13	≤8	–	16	≥32	(4) Results of ampicillin testing can be used to predict results for amoxicillin. See general comment (2).

“敏感”折点

“耐药”折点

为什么变化? 简化!

定义
“解释分类”

抗生素	纸片含量	解释分类和抑菌圈直径折点 (最近的整数毫米)				解释分类和MIC 折点 (μg/mL)			
		S	SDD	I	R	S	SDD	I	R
氨苄西林[1]	10 μg	≥ 17	-	14-16	≤ 13	≤ 8	-	16	≥ 32

[1] 肠杆菌科

解释分类:

- **敏感**
- **剂量依赖敏感**
- **中介**
- **耐药**
- **非敏感**

CLSI M100 27th ed. pp 3-6.

“非敏感”解释分类

- 由于不存在或很少存在耐药菌株，仅仅指定了敏感折点.
- 一株分离菌被解释为非敏感不一定意味着这株细菌存在耐药机制.可能是这株菌的MIC高于敏感折点，但缺乏耐药机制，可能是野生株，仅仅在设定敏感折点之后出现而已.

- 当描述细菌/药物中介和耐药分类的时候不能使用“非敏感”项，分离株在“中介”或“耐药”分类中，可以被称为“不敏感（not susceptible）”而不是“非敏感（nonsusceptible）.”

新!

完整定义见…CLSI M100 27th ed. pp 4-5.

“非敏感 (Nonsusceptible)” vs. “不敏感 (Not Susceptible)” 阐述

抗菌药物	纸片含量	解释分类和抑菌圈直径折点 (最近的整数毫米)				解释分类和MIC 折点 (μg/mL)			
		S	SDD	I	R	S	SDD	I	R
氨苄西林[1]	10 μg	≥17	-	14-16	≤13	≤8	-	16	≥32
万古霉素[2]	30 μg	≥17	-	-	-	≤1	-	-	-

[1] 肠杆菌科
[2] *肺炎链球菌t*

CLSI M100 27th ed. pp 33, 81.

- ***大肠埃希菌*MIC 16, 32, 64….等 μg/ml对氨苄西林“不敏感”**
- ***肺炎链球菌*MIC 2 μg/ml 对万古霉素“非敏感” (需要确认)**
- **与“不敏感”结果相比, “非敏感” 是对MIC或抑菌圈直径的解释, 并且在患者报告中报告**

附录B. 天然耐药

B1. 肠杆菌科

菌种	四环素类	替加环素
摩根摩根菌		R
奇异变形杆菌	R	R
彭氏变形杆菌	R	R
普通变形杆菌	R	R
雷氏普罗维登斯菌	R	R
斯氏普罗维登斯菌	R	R

B1. *Enterobacteriaceae*

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Piperacillin	Ticarcillin	Cephalosporin I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline			
Citrobacter freundii	R	R	R			R	R	R						
Citrobacter koseri	R			R	R									
Enterobacter aerogenes	R	R	R			R	R	R						
Enterobacter cloacae complex	R	R	R			R	R	R						
Escherichia coli	There is no intrinsic resistance to β-lactams in this organism.													
Escherichia hermannii	R				R									
Hafnia alvei	R	R	R			R	R							
Klebsiella pneumoniae	R				R									
Morganella morganii	R	R				R		R	*		R	R	R	
Proteus mirabilis	There is no intrinsic resistance to penicillins and cephalosporins in this organism.								*	R	R	R	R	
Proteus penneri	R					R		R		R	R	R	R	
Proteus vulgaris	R					R		R		R	R	R	R	
Providencia rettgeri	R	R				R			*	R	R	R	R	
Providencia stuartii	R	R				R			*	R	R	R	R	†
	There is no intrinsic resistance to β-lactams in these organisms; refer to													

CLSI M100 27th ed. p. 210.

流行病学界值
ECVs

新!

CLSI流行病学界值列表 – 自2015年以来增加/修订

CLSI Epidemiological Cutoff Value Additions/Revisions Since 2015

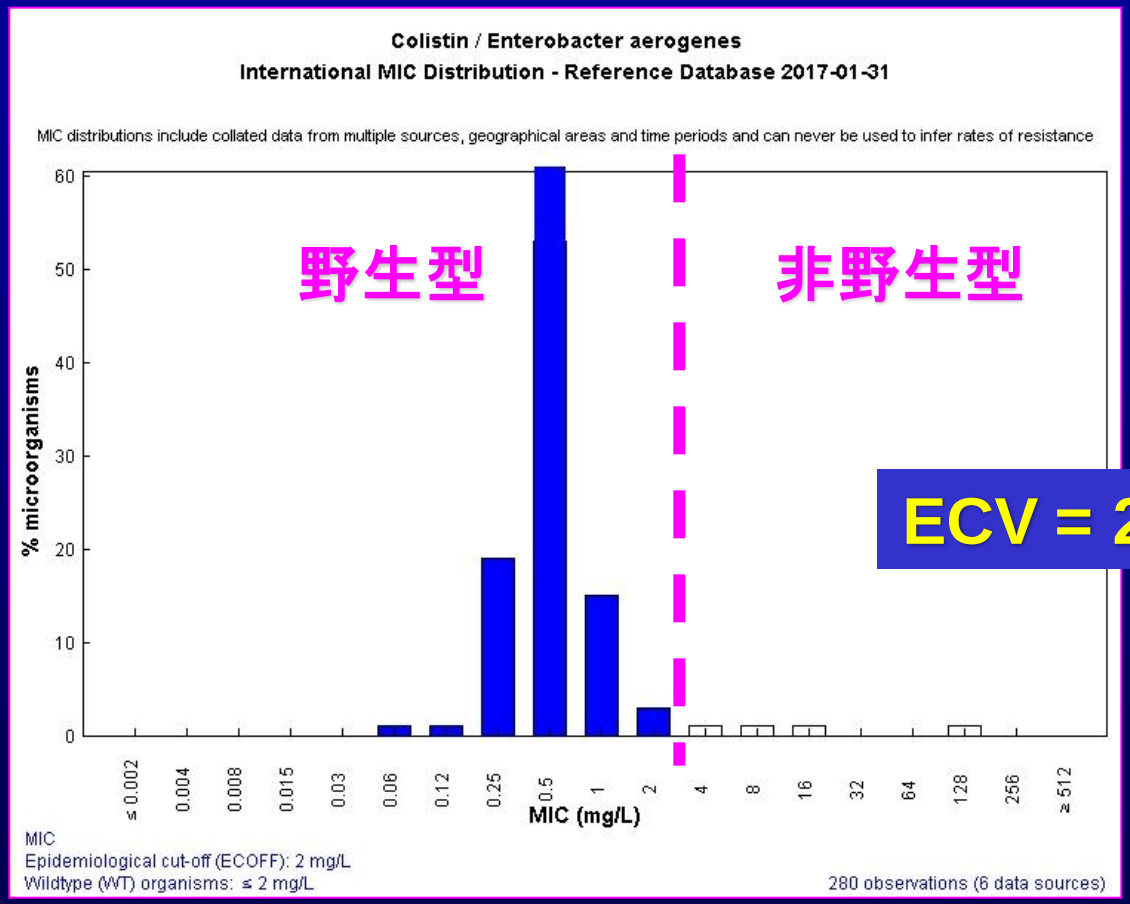
Antimicrobial Agent	Date of Addition (M100 edition)	Comments
Enterobacteriaceae		
Azithromycin	January 2016 (M100S, 26th ed.)	For use with *S. flexneri* and *S. sonnei*.
Colistin	January 2017 (M100, 27th ed.)	For use with *E. aerogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae*, and *R. ornithinolytica*.
Neisseria gonorrheoae		
Azithromycin	January 2017 (M100, 27th ed.)	
Anaerobes		
Vancomycin	January 2015 (M100-S25)	For use with *P. acnes*.

定义 (CLSI M100 27th ed):

- ECV =将微生物群体区分成不获得耐药或无突变耐药的野生型(WT)、获得耐药或有突变耐药的非野生型(NWT)的MIC值或抑菌圈直径.
- ECV定义为野生型菌株群体敏感性的上限.

CLSI M100 27th ed. p. xxvi & 6.

粘菌素 / 产气肠杆菌



ECV = 2 µg/ml

EUCAST.org

野生型 vs. 非野生型

- 野生型 (WT) –抗菌药物（包括抗真菌药物）评估中将未获得耐药机制或无敏感性下降菌株的ECV值定义为野生型.

- 非野生型 (NWT) –抗菌药物（包括抗真菌药物）评估中将认为或已知获得耐药机制或存在敏感性下降菌株的ECV值定义为非野生型.

CLSI M100 27th ed. p. 6.

临床折点vs.
流行病学界值 (ECVs)

- 折点 (临床的) = 预测患者治疗成功可能性的MIC值
- ECVs = 表示耐药出现的MIC值
  - 基于菌株表型（MICs）将菌群分成获得突变“耐药”和不获得突变“耐药”.
  - 种特异性
  - 可用于监测研究

临床折点基于:
- MIC分布
- PK/PD数据
- 临床结局数据

ECVs基于:
- 仅仅MIC分布

Appendix G. Epidemiological Cutoff Values

CLSI M100 27th ed. pp 234.

1. Q: What are epidemiological cutoff values (ECVs)?

A: ECVs are minimal inhibitory concentration (MIC) values that separate bacterial populations into those with and without acquired and/or mutational resistance mechanisms based on their phenotypes (MICs). ECVs are based solely on *in vitro* data. The term "wild-type" (WT) is used to describe strains with MIC values at or below the ECV that are presumed not to possess acquired and/or mutational resistance mechanisms, while the term "non-wild-type" (NWT) is used to describe strains with MIC values above the ECV that are presumed to possess acquired and/or mutational resistance mechanisms. ECVs are principally used to signal the emergence and evolution of NWT strains. They are not the same as clinical breakpoints. The ECV is defined as the MIC value that best defines the estimated upper end of the WT population.

2. Q: How are ECVs determined?

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

1st Edition

M57

真菌ECVs

Principles and Procedures for the Development of Epidemiological Cutoff Values for Antifungal Susceptibility Testing

ECVs = ECOFFs

流行病学界值

ECVs更多资料

www.clsi.org

Turnidge J et al. 2006. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. Clin Microbiol Infect. 12:418-25.

当前CLSI ECVs

抗菌药物	菌种	ECV (μg/ml)	
		WT	NWT
阿奇霉素	*淋病奈瑟菌*	≤1	≥2
阿奇霉素	*福氏志贺菌* *宋内氏志贺菌*	≤8 ≤16	≥16 ≥32
粘菌素	*产气肠杆菌, 阴沟肠杆菌, 大肠埃希菌, 肺炎克雷伯菌, 解鸟氨酸拉乌尔菌*	≤2	≥4
万古霉素	*痤疮丙酸杆菌*	≤2	≥4

WT, 野生型; NWT, 非野生型

CLSI M100 27th ed. pp. 40,42,76,100.

ECVs用于如下药物/细菌组合:

- **没有充足的临床和/或PK/PD数据建立临床折点**
- **某种药物对某种细菌具有重要的临床和/或公共卫生意义**

粘菌素 / 多粘菌素B

粘菌素 / 多粘菌素B

- 联合CLSI-EUCAST多粘菌素折点标准化工作组:
  - 参考试验方法 (包括质控)
  - 结果解释
  - 一直强调粘菌素 (vs. 多粘菌素B)


Clinical Microbiology
NEWSLETTER
CMN
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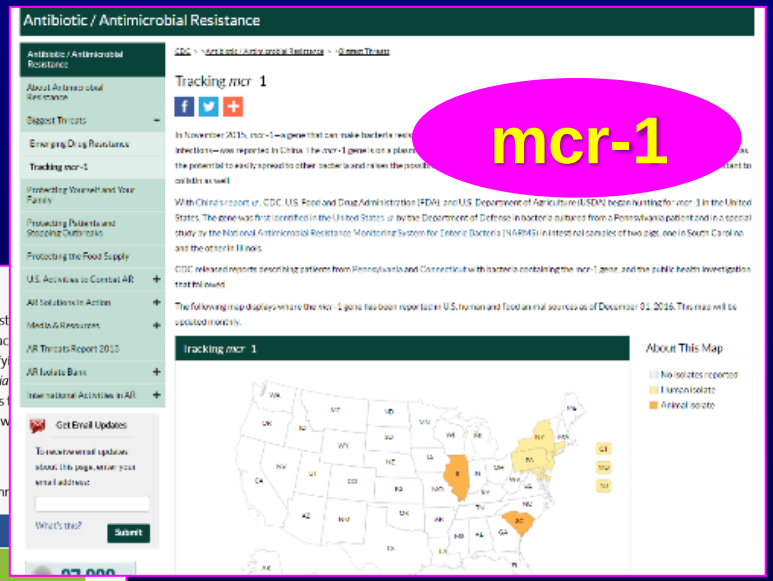
CMN
Vol. 38, No. 9
May 1, 2016
www.cmnewsletter.com
IN THIS ISSUE
Polymyxin Susceptibility Testing: a Cold Case Reopened


Continuing Conversation About Colistin!
CLSI ORWG 新闻
Breakpoints and ECVs
The CLSI/EUCAST Joint Working Group recommended the following clinical breakpoints, which were approved by the CLSI AST Subcomm
Organism	Susceptible	Resistant
Acinetobacter spp.	≤2 µg/mL	≥4 µg/mL
Pseudomonas aeruginosa	≤2 µg/mL	≥4 µg/mL


Antibiotic / Antimicrobial Resistance
Tracking *mcr* 1
mcr-1
Tracking *mcr* 1
About This Map

粘菌素 / 多粘菌素B
联合工作组最终推荐

新!

- 修订铜绿假单胞菌折点 (删除粘菌素 "I")
- 强调 *不动杆菌* 折点 仅仅用于 *鲍曼不动杆菌* 复合体
- 增加鲍曼不动杆菌和铜绿假单胞菌治疗评论

  "粘菌素(甲磺酸盐)通常给予负荷剂量及最大的推荐剂量，并且与其它抗菌药物联合，特别对于正常或超常肾功能患者."
- 删除 "非肠杆菌科"粘菌素/多粘菌素B折点(表 2B-5)
- 取消纸片扩散法 折点
- 对选定的肠杆菌科菌种建立ECV
- 强调不用梯度扩散法试验, 可能不能可靠的检测耐药性.

CLSI AST SC Meeting, June 2016.

粘菌素
2017 CLSI 折点 / ECV

New

菌种	MIC折点 (μg/ml)			抑菌圈直径折点 (mm)		
	敏感	中介	耐药	敏感	中介	耐药
鲍曼不动杆菌复合体	≤2	-	≥4	none		
铜绿假单胞菌	≤2	-	≥4	none		
肠杆菌科	没有足够的临床和PK/PD数据去建立 “折点”					

菌种	ECV (μg/ml)	
	WT	NWT
肠杆菌[1]	≤2	≥4

[1]*产气肠杆菌, 阴沟肠杆菌, 大肠埃希菌, 肺炎克雷伯菌*, 和解鸟氨酸拉乌尔菌

CLSI M100 27th ed. pp. 40, 43, 47.

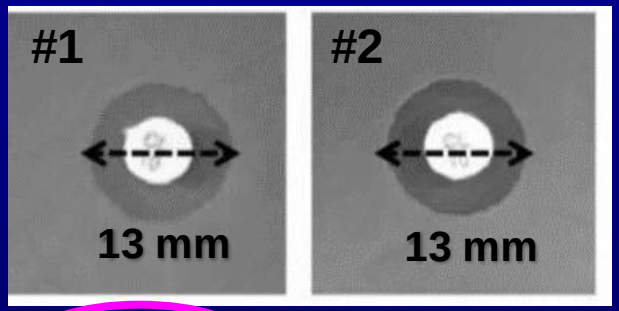
粘菌素 / 多粘菌素B
之前的纸片扩散法折点

菌种	抑菌圈直径 (mm)		
	敏感	中介	耐药
不动杆菌属	none		
铜绿假单胞菌	≥11[1]	-	≤10
	≥12[2]	-	≤11

[1] 粘菌素
[2] 多粘菌素B

CLSI M100 26th ed.

铜绿假单胞菌 #1 & #2



>4 “R”　　≤0.25 “S”

粘菌素 MIC μg/ml
Jerke et al. 2016. CMN. 38:69.

纸片扩散法区分度差!
取消纸片扩散法折点.

粘菌素 / 多粘菌素B
2017 CLSI 折点

铜绿假单胞菌

		抑菌圈直径 (mm)			MIC (μg/ml)			
		S	I	R	S	I	R	
LIPOPEPTIDES								
O	Colistin	–	–	–	–	≤ 2	–	≥ 4
O	Polymyxin B	–	–	–	–	≤2		

(15) Colistin (methanesulfonate) should generally be administered with a loading dose and at the maximum recommended

(15) Colistin (methanesulfonate) should generally be administered with a loading dose and at the maximum recommended doses, in combination with other agents.

New

多粘菌素B折点 – 2017无变化!

菌种	MIC折点 (μg/ml)		
	敏感	中介	耐药
*鲍曼不动杆菌*复合体	≤2	-	≥4
铜绿假单胞菌	≤2	4	≥8

CLSI M100 27th ed. pp. 40, 43, 47.

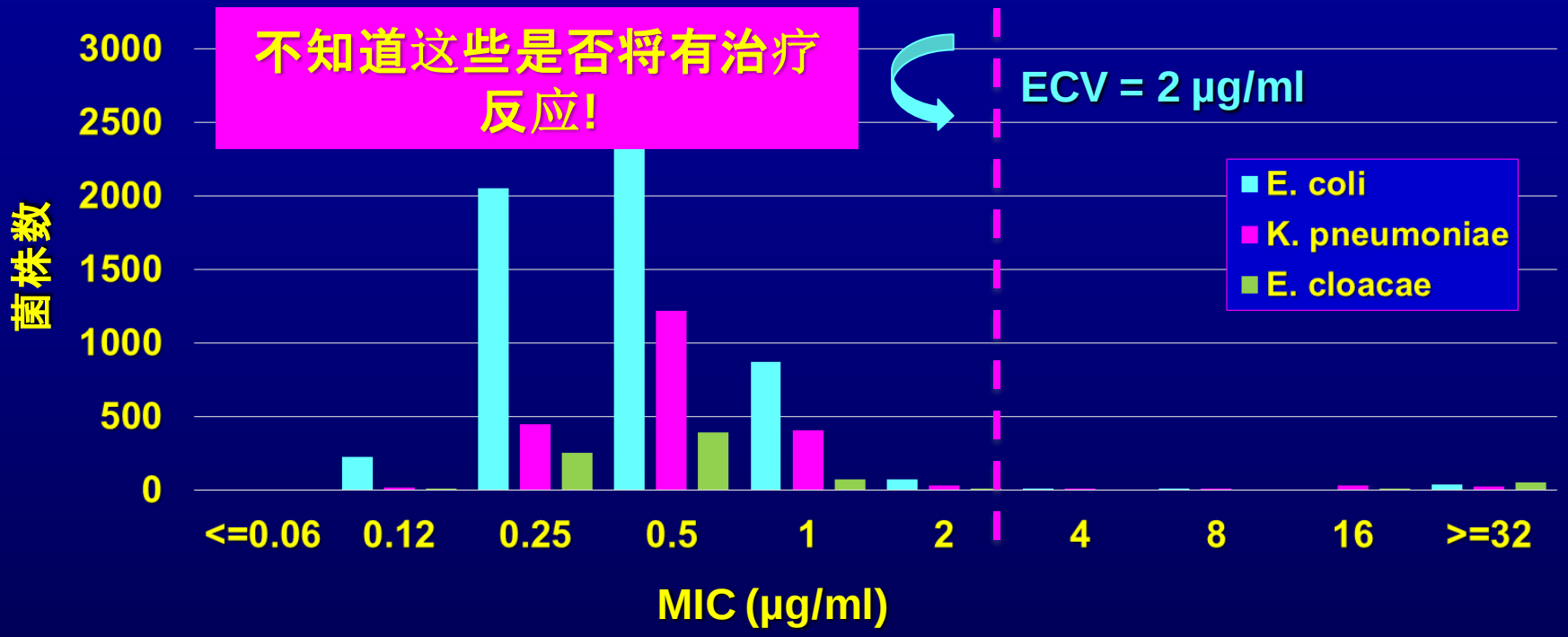
标本：肺泡灌洗液
诊断：肺炎
铜绿假单胞菌

带有可选评论的最终报告 (举例)

	MIC (µg/ml)
阿米卡星	>32 R
头孢吡肟	>32 R
环丙沙星	>4 R
粘菌素	1.0 S*
庆大霉素	>16 R
美罗培南	>8 R
哌拉西林-他唑巴坦	>128/4 R
妥布霉素	>16 R

*“应Dr. Jones的要求报告粘菌素. 粘菌素（甲磺酸盐）通常给予负荷剂量及最大的推荐剂量，并且与其它抗菌药物联合. 感染性疾病咨询建议".

粘菌素 vs. 肠杆菌科
建立ECV



也检测了*产气肠杆菌, 解鸟氨酸拉乌尔菌*.

来自EUCAST website. www.eucast.org

表 2A-2. 肠杆菌科ECVs

新!

Table 2A-2. Epidemiological Cutoff Values for *Enterobacteriaceae*

General Comments

"Caution": ECVs for disk diffusion and MICs are not to be reported as susceptible, intermediate, or resistant. The ECVs should not be used as clinical breakpoints, but are provided for informational purposes. Refer to Appendix G for an explanation of ECVs.

(1) ECVs are MIC values that separate bacterial populations into those with and without acquired and/or mutational resistance mechanisms based on their phenotypes (MICs). ECVs must not be interpreted and reported as susceptible, intermediate, or resistant. Refer to Appendix G for further information and an explanation of ECVs.

(2) ECVs listed below are only applicable to the species indicated. Currently there are insufficient data to support their use with other species.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	Disk Content	Zone Diameter ECV (mm)		MIC ECV (µg/mL)		Comments
		WT	NWT	WT	NWT	
Azithromycin[1-5]	15 µg	≥16	≤15	≤8	≥16	For use with *S. flexneri*. **See Table 2A-1 for azithromycin and *Salmonella* spp.**
	–	–	–	≤16	≥32	For use with *S. sonnei*.
Colistin	**–**	**–**	**–**	**≤2**	**≥4**	**For use with *E. aerogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae* and *R. ornitholytica*.**

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

CLSI M100 27th ed. pp. 40.

标本：血
诊断：败血症
肺炎克雷伯菌

带有评论的最终报告 (举例)

	MIC (µg/ml)
阿米卡星	>32 R
头孢吡肟	>32 R
头孢曲松	>32 R
环丙沙星	>4 R
厄他培南	>8 R
庆大霉素	>16 R
亚胺培南	>8 R
美罗培南	>8 R
哌拉西林-他唑巴坦	>128/4 R
妥布霉素	>16 R
粘菌素	≤0.5 *

*“应Dr. Jones要求报告粘菌素 . 没有既定的*肺炎克雷伯菌*粘菌素解释标准 . 这株菌的MIC低于或等于在无突变或获得粘菌素耐药肺炎克雷伯菌分离株中观察到的MIC值; 这个结果和粘菌素治疗结局之间的相关性未知. 感染性疾病咨询建议".

Adapted from comment used at U of PA (with permission).

淋病奈瑟菌

CLSI ECV
淋病奈瑟菌-阿奇霉素

淋病奈瑟菌耐药逐渐发展

磺胺类 → 青霉素 → 四环素类 → 氟喹诺酮类

宫颈，尿道和直肠的无并发症淋球菌感染

头孢曲松250 mg 肌注 + 阿奇霉素 1 mg 口服

www.cdc.gov/std/tg2015/gonorrhea.htm

- 三代头孢菌素敏感性下降
  - 少见菌株头孢曲松“耐药”（日本，法国，西班牙）
- 阿奇霉素
  - 需要监测耐药出现
  - 临床折点缺少临床和PK/PD证据

CLSI AST SC Meeting, June 2016.

表2F-2. 淋病奈瑟菌 ECVs

新!

Table 2F-2. Epidemiological Cutoff Values for *Neisseria gonorrhoeae*

General Comments

"Caution": ECVs for MIC are not to be reported as susceptible, intermediate, or resistant. The ECVs should not be used as clinical breakpoints, but are provided for informational purposes. Refer to Appendix G for an explanation of ECVs.

(1) ECVs are MIC values that separate bacterial populations into those with and without acquired and/or mutational resistance mechanisms based on their phenotypes (MICs). ECVs must not be interpreted and reported as susceptible, intermediate, or resistant. Refer to Appendix G for further information and an explanation of ECVs.

(2) ECVs listed below are only applicable to the species indicated. Currently there are insufficient data to support their use with other species.

NOTE: Information in boldface type is new or modified since the previous edition.

Antimicrobial Agent	MIC ECV (µg/mL)		Comments
	WT	NWT	
Azithromycin	≤1	≥2	For use with *N. gonorrhoeae*.

Abbreviations: ECV, epidemiological cutoff value; MIC, minimal inhibitory concentration; NWT, non-wild-type; WT, wild-type.

References for Table 2F-2

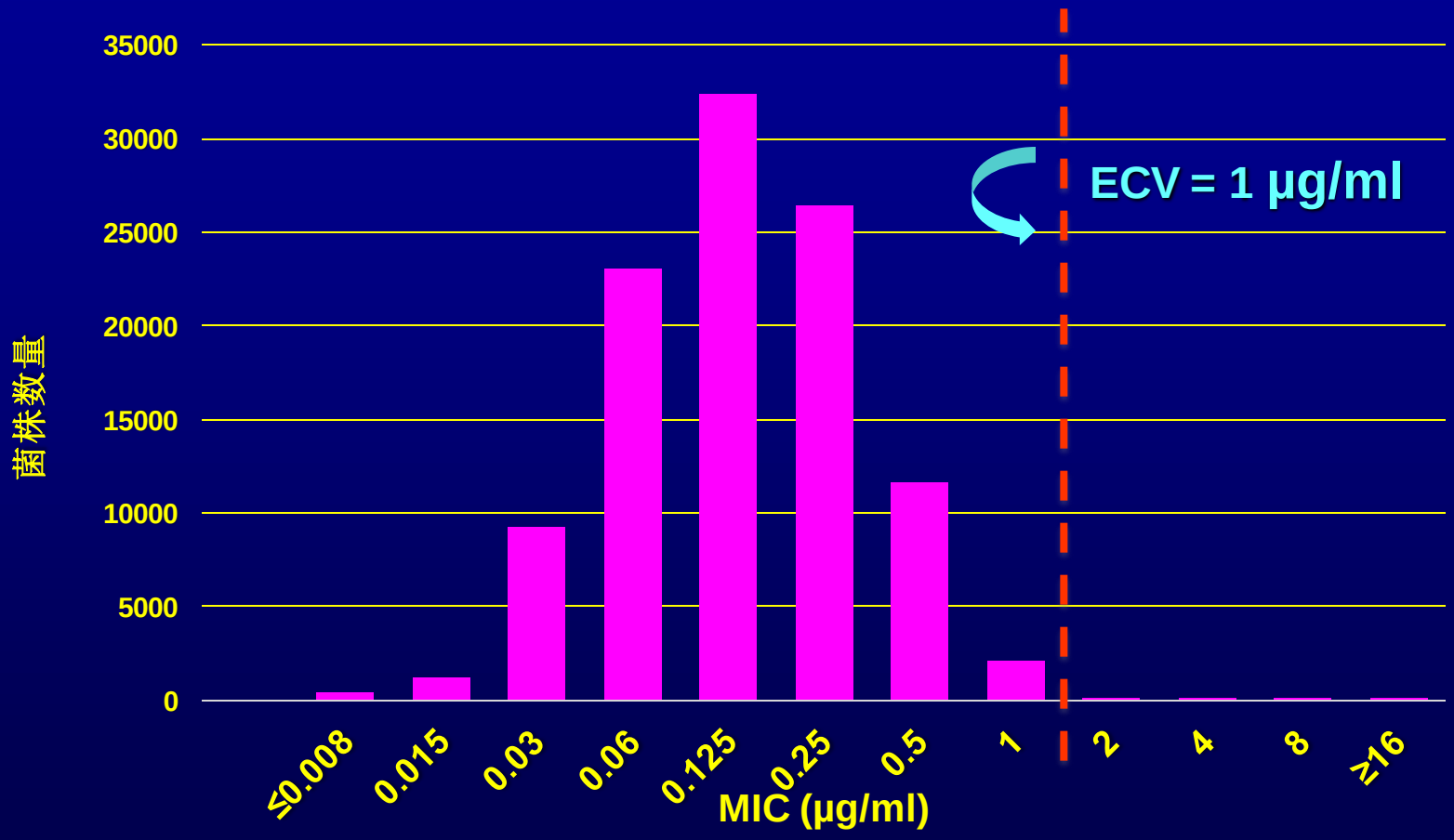
1 Chisholm SA, Dave J, Ison CA. High-level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. *Antimicrob Agents Chemother.* 2010;54(9):3812-3816.

2 Demczuk W, Martin I, Peterson S, et al. Genomic epidemiology and molecular resistance mechanisms of azithromycin-resistant *Neisseria gonorrhoeae* in Canada from 1997 to 2014. *J Clin Microbiol.* 2016;54(5):1304-1313.

3 Grad YH, Harris SR, Kirkcaldy RD, et al. Genomic epidemiology of gonococcal resistance to extended spectrum cephalosporins, macrolides, and fluoroquinolones in the United States, 2000-2013. *J Infect Dis.* 2016;214(10):1579-1587.

CLSI M100 27th ed. pp. 76.

淋病奈瑟菌- 阿奇霉素 MIC 分布 (1992-2012)



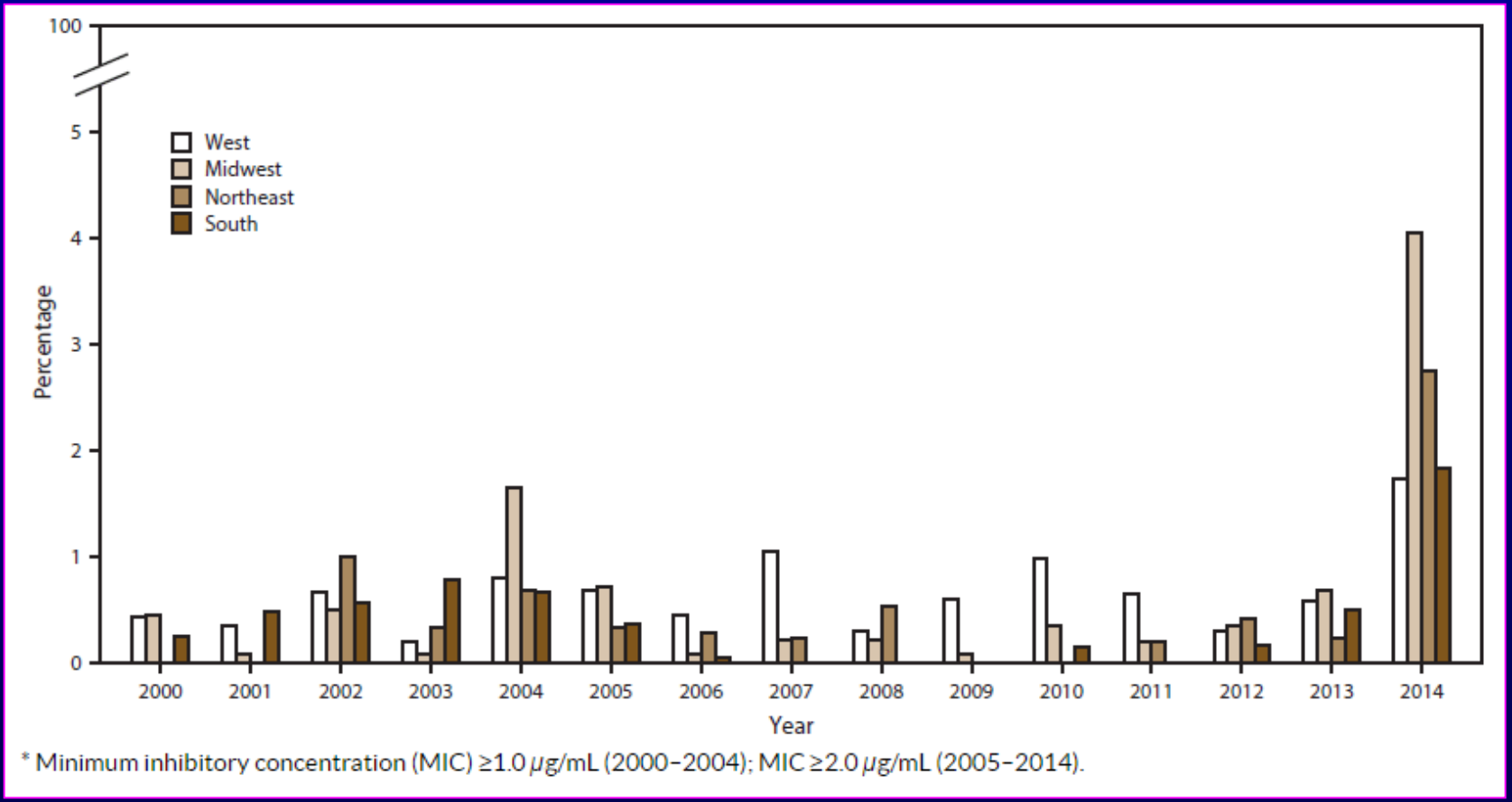
CLSI AST SC Meeting, June 2016.

淋病奈瑟菌- 阿奇霉素ECV数据支持…

- 微生物学分布 (MIC) 数据
  - 淋球菌监测项目(GISP)
    http://www.cdc.gov/std/GISP/
- 2009-2010年236株GISP收集菌株测序
  - 27 株阿奇霉素MIC ≥2 µg/ml
    - 25/27 菌株在23S rRNA 4个位点中，含有≥2 位点C2611T突变
    - 209 株 阿奇霉素 MIC <2 µg/ml (相当于≤1)
    - 所有菌株在4个位点含有野生型23S rRNA
    - Grad Y et al. 2014. Lancet ID. 14:220-26.

CLSI AST SC Meeting, June 2016.

图 3. 阿奇霉素敏感性降低的尿路淋病奈瑟菌菌株百分比,* 依地区和年份—淋球菌监测项目，美国，2000 – 2014



MMWR. July 15, 2016 / 65(7);1–19.

碳青霉烯耐药的肠杆菌科细菌(CRE)

- mCIM (改良碳青霉烯类灭活试验)

何时需要检测肠杆菌科细菌中的碳青霉烯酶？

- 常规病人检测?
  - 不需要 ，使用更新的折点时
  - 使用目前的折点所得到的结果足够指导临床治疗
- 感染控制?
  - 需要，怀疑暴发时
  - 需要，感控要求时
- 流行病学研究?
  - 需要，但通常不在临床实验室水平(有时公共卫生部门要求)

用于流行病学或感染控制相关的试验

Introduction to Tables 3B, 3C, and 3D. (Continued)

	Tests Used for Epidemiological or Infection Control–Related Testi			
	MHT (Table 3B)	**Carba NP (Table 3C)**	**mCIM (Table 3D)**	**Other (eg, molecular assays)**
Organisms	*Enterobacteriaceae* that are not susceptible to one or more carbapenems	*Enterobacteriaceae, P. aeruginosa,* and *Acinetobacter* spp. that are not susceptible to one or more carbapenems	***Enterobacteriaceae* that are not susceptible to one or more carbapenems**	*Enterobacteriaceae, P. aeruginosa,* and *Acinetobacter* spp. that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by MHT or Carba NP
Strengths	Simple to perform No special reagents or media necessary	Rapid	**No special reagents or media necessary**	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	False-positive results can occur in isolates that produce ESBL or AmpC enzymes coupled with porin loss. False-negative results are occasionally noted (eg, some isolates producing NDM carbapenemase). Only applies to *Enterobacteriaceae*.	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapene (eg, OXA-type, chromosomally en not consistently de	**Only applies to *Enterobacteriaceae*** **Requires overnight incubation**	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase s not targeted.

Abbreviations: ESBL, extended-spectrum β-lactamase; MIC, minimal inhibitory concentration; MHT, modified Hodge test; NDM, New Delhi metallo-β-lactamase.

新！

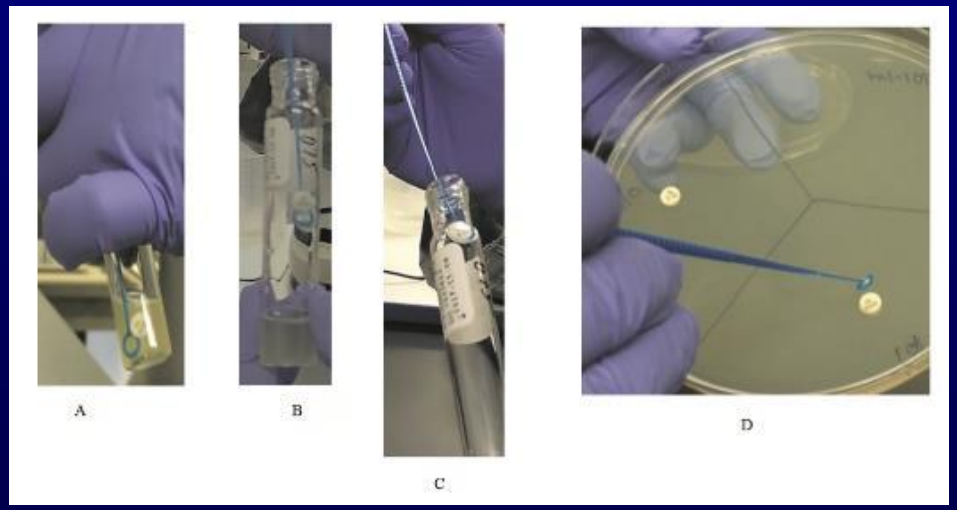
mCIM – 3个CLSI表型试验中最有效

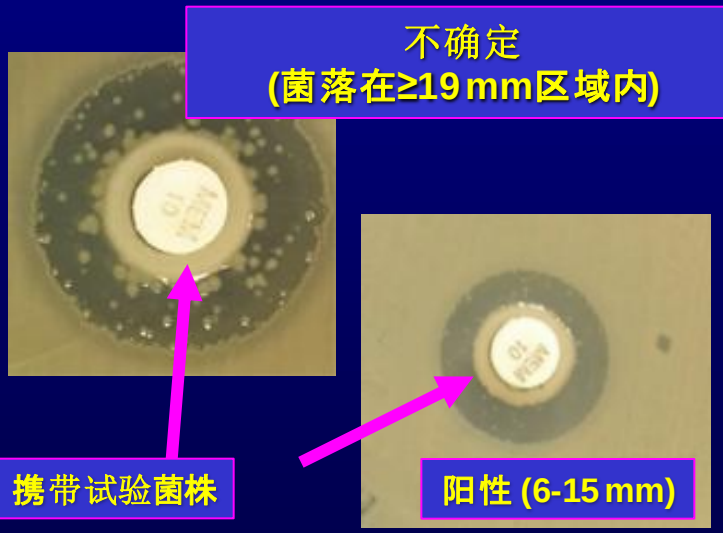
CLSI M100 27th ed. pp. 107.

mCIM 试验

基本步骤…

1. 挑取试验菌株(1 µl 接种环)
2. 悬于肉汤加美罗培南纸片(10 µg)
3. 孵育4 h
4. *E. coli* ATCC 25922 接种MHA平板，用于纸片扩散法
5. 悬液中取出美罗培南纸片
6. 美罗培南纸片放在*E. coli* 密涂平板上
7. 孵育过夜
8. 读抑菌圈





应使用哪种方法检测碳青霉烯酶?

碳青霉烯酶检测敏感性

机制	MHT[1]	CarbaNP[2]	CIM[3, 4]
KPC	98%	100%	100%
Oxa-48-like	93%	38.5%	100%
MBL	12%	100%	96-100%

1 Doyle et al. 2012. JCM 50: 3877-80.
2 Osterblad et al . 2014. AAC 58:7553-6.
3 Tijet et al. 2016. JAC 71: 274-6.
4 Van der Zwaluw et al. 2015. PLoS One 10: e0123690.

没有哪个表型试验能够检测所有碳青霉烯酶

CDC CRE 监测的定义

以前

“I” 或“R” 对
- 多利培南 或
- 美罗培南 或
- 亚胺培南

以及

“R” 对所有广谱头孢菌素试验（头孢曲松，头孢他啶，头孢噻肟）

目前

“R” 对
- 厄他培南 或
- 多利培南 或
- 亚胺培南 或
- 美罗培南

或

产 碳青霉烯酶

- 高敏感性，而且没有明确的定义
- 非碳青霉烯酶机制（如 ampC，ESBL）可能导致只对厄他培南耐药

https://www.cdc.gov/hai/organisms/cre/definition.html or
Google! CDC CRE Definition

碳青霉烯耐药的肠杆菌科细菌 (CRE)
基于纸片扩散法或 MIC 结果

碳青霉烯酶试验阳性

产碳青霉烯酶CRE (CP-CRE)

增加流行病学关注

碳青霉烯酶试验阴性

CRE

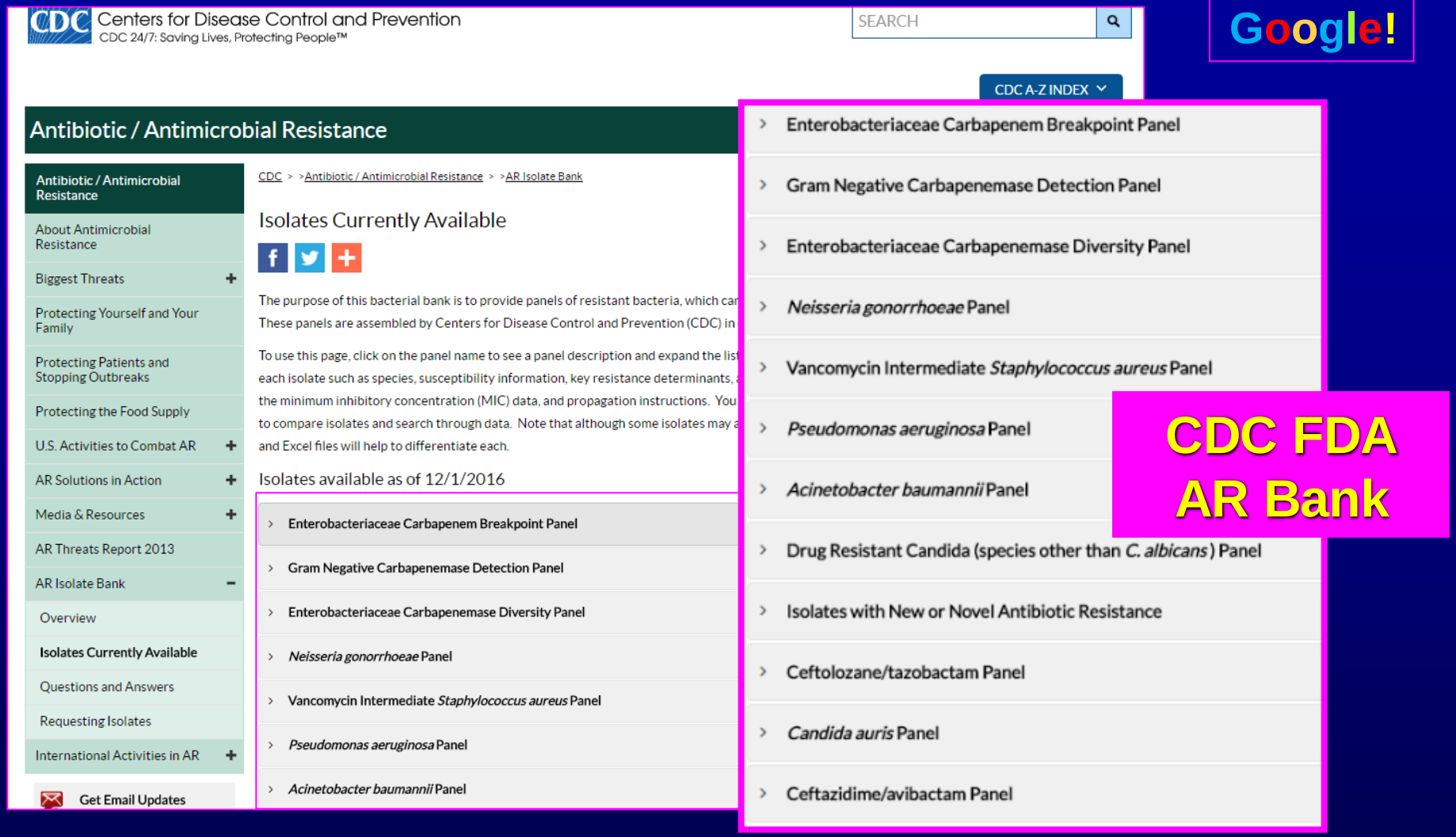
经常出现：

厄他培南	R
多利培南	S
亚胺培南	S
美罗培南	S

目前鉴定产碳青霉烯酶的肠杆菌科细菌的CDC定义的使用联用和不联用碳青霉烯酶辅助试验(Cepheid Xpert or CIM)[1]

试验	敏感性	特异性
使用目前的定义不联用辅助试验:		
	98.9%	15.2%
增加碳青霉烯酶检测结果:		
Cepheid Xpert Carba-R	98.9%	100%
CIM [3]	98.9%	100%

[1] N=125 选择菌株，含有各种碳青霉烯酶
[2] R 对 厄他培南 或多利培南或亚胺培南或美罗培南
3 与CLSI mCIM 试验有微小差别
UCLA 提交出版



Google!

CDC FDA AR Bank

含有多种耐药机制菌株的有效性！

沙门氏菌属

沙门氏菌属
喹诺酮类检测

- 现状…
  - 环丙沙星, 左氧氟沙星是治疗沙门氏菌的合理选择
  - 使用专门的“沙门氏菌属折点”
  - 培氟沙星 纸片效果好 ….美国没有培氟沙星纸片
  - 2017 淘汰 萘啶酸 “替代” 检测

新!

如何检测见… CLSI M100 27th ed pp. 32.

(2) When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. In addition, for extraintestinal isolates of *Salmonella* spp., a third-generation cephalosporin should be tested and reported, and chloramphenicol may be tested and reported if requested. Susceptibility testing is indicated for typhoidal *Salmonella* (*S.* Typhi and *Salmonella* Paratyphi A–C) isolated from extraintestinal and intestinal sources. Routine susceptibility testing is not indicated for nontyphoidal *Salmonella* spp. isolated from intestinal sources. In contrast, susceptibility testing is indicated for all *Shigella* isolates.

沙门氏菌属
喹诺酮类检测

- **检测方法:**
  - MIC 方法 (优先)
    - 一些商品化系统不能检测到足够低
    - 梯度扩散 (Etest)
  - 纸片扩散法

Etest and disk testing reference….
Deak, E., et al. 2015. J Clin Microbiol. 53:298.

病原体	环丙沙星					
	纸片扩散法(mm)			MIC (μg/ml)		
	S	I	R	S	I	R
沙门氏菌属	≥31	21-30	≤20	≤0.06	0.12-0.5	≥1
其他肠杆菌科细菌	≥21	16-20	≤15	≤1	2	≥4

CLSI M100 27th ed. pp. 37-38.

伤寒沙门氏菌
阿奇霉素折点

Table 2A-1. *Enterobacteriaceae* (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm)				Interpretive Categories and MIC Breakpoints (µg/mL)				Comments
			S	SDD	I	R	S	SDD	I	R	
AMINOGLYCOSIDES (Continued)											
O	Netilmicin	30 µg	≥15	–	13–14	≤12	≤8	–	16	≥32	
O	Streptomycin	10 µg	≥15	–	12–14	≤11	–	–	–	–	(32) There are no MIC interpretive standards.
MACROLIDES											
Inv.	Azithromycin	15 µg	≥13	–	–	≤12	≤16	–	–	≥32	(33) *Salmonella* Typhi only: **breakpoints** are based on MIC distribution data **and** limited clinical data. For *Shigella flexneri* and *Shigella sonnei* see Table

- **阿奇霉素**“经验用药”治疗沙门氏菌，志贺氏菌和其他胃肠炎 (指征外)
- **活性**归因于 细胞内浓度 中性粒细胞 和巨噬细胞(>100 倍血清浓度)

(33) 仅伤寒沙门氏菌 折点基于MIC分布和有限的临床数据. 福氏志贺菌和宋内志贺菌见表 2A-2.

New!

CLSI M100 27th ed. p. 37.

葡萄球菌属

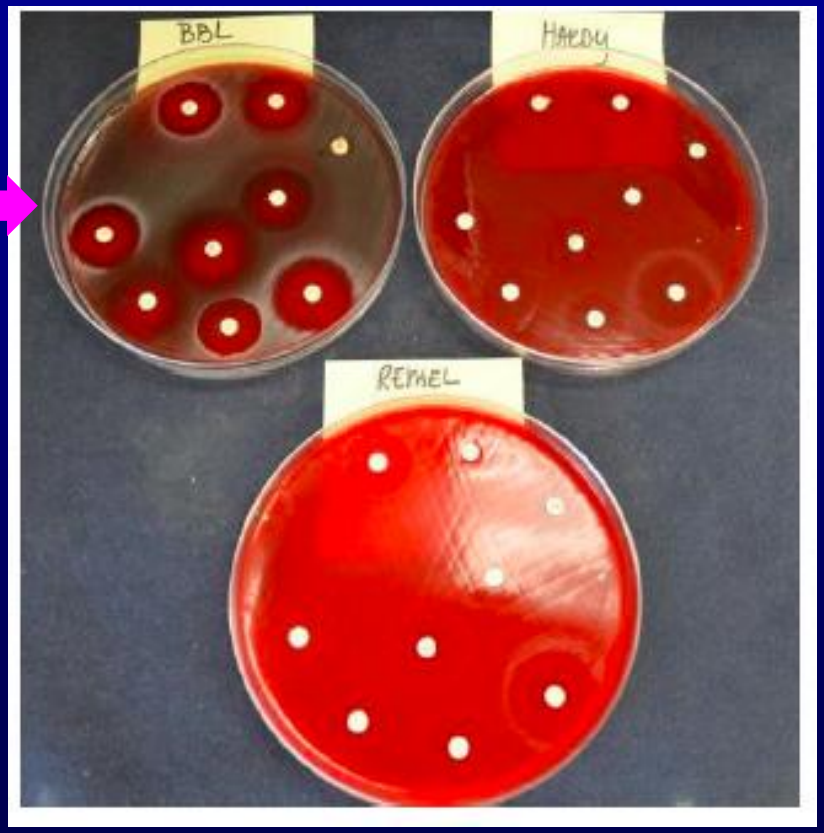
“非典型”*金黄色葡萄球菌*
(小菌落变异体)

- 定义:床旁检测标准，菌株在未校正MHA或离子校正MHB上不生长，普通血平皿生长
  - 连续重复培养时恢复正常菌落
  - 囊性纤维化病人，整形外科病人可见
- 需要药敏试验(esp. MSSA / MRSA)
- 能够在商品化MHA加5%羊血(BMHA)上做纸片扩散法吗?
- CLSI 研究
- 3 个实验室
  - 37 菌株; 多种形态

CLSI AST SC Meeting, June 2016.

影响非典型金葡菌在BMHA上生长能力的来源...

CLSI研究只使用BBL BMHA f; 其他平板生长差检测多种药物; 只分析头孢西丁



CLSI AST SC Meeting, June 2016.

非典型金黄色葡萄球菌 [1]
头孢西丁 30 μg 纸片扩散法 血MH琼脂平板

mecA PCR	24 hours		48 hours	
	头孢西丁-R	头孢西丁-S	头孢西丁-R	头孢西丁-S
阳性	117	10	119	8
阴性	0	44	0	44

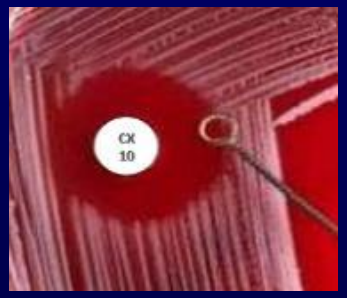
[1] 171 种形态被检测, 3 实验室
24h和48h时一些假敏感结果

CLSI AST SC Meeting, June 2016.

非典型金黄色葡萄球菌
商品化 PBP2a 检测

商品化检测	No. [1]	敏感性 (%)		特异性 (%)	
		不产	产	不产	产
Alere PBP2a LFA	54	90	96	100	100
Oxoid PBP2a Latex	43	85.7	95.9	87.5	100

[1] 被检测形态; 一些菌株在BAP上有多种形态



产PBP2a样本

Miller et al. 2017. J Clin Microbiol. 55:485.

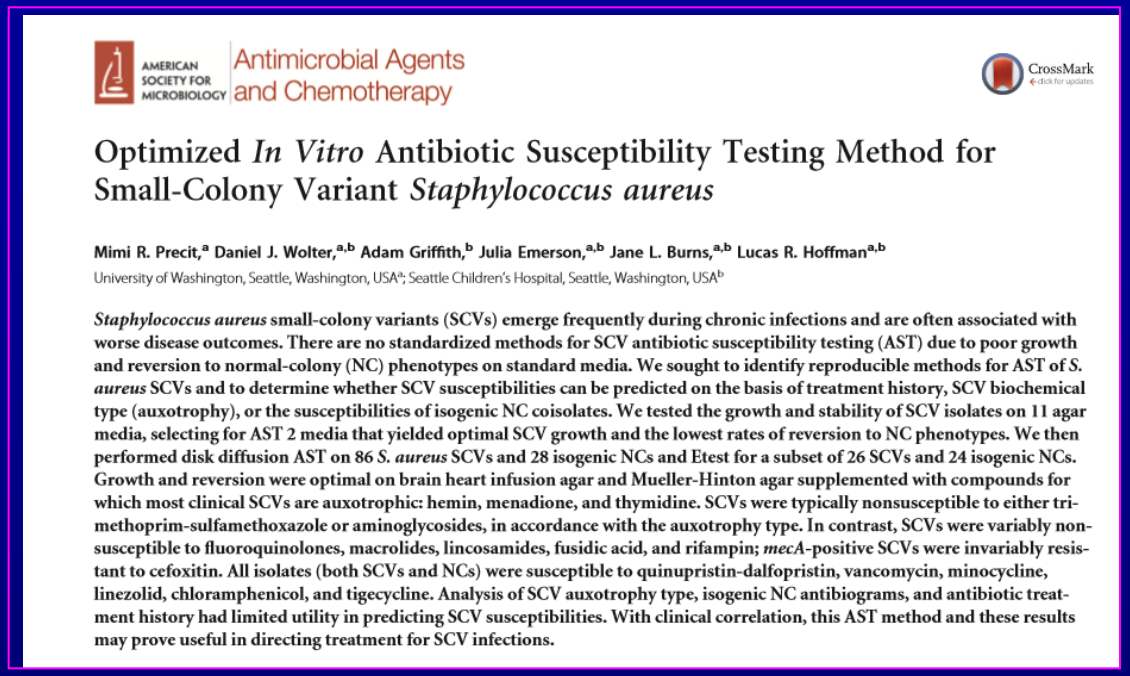
“非典型”金黄色葡萄球菌 CLSI M100 27th ed.内容

新!

“(14) 头孢西丁 MIC 和纸片扩散法在非CAMHB 或 未校正 的MHA 平板上不能可靠地检测*mecA*-介导的 在这些平板上不生长的金葡菌耐药(如, 小菌落变异).

诱导生长检测PBP2a (如, 在头孢西丁纸片边缘选取，接种BMHA或血琼脂平板，5% CO_2 24小时孵育或检测mecA)

CLSI M100 27th ed. pp. 59.



AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

CrossMark

Optimized *In Vitro* Antibiotic Susceptibility Testing Method for Small-Colony Variant *Staphylococcus aureus*

Mimi R. Precit,[a] Daniel J. Wolter,[a,b] Adam Griffith,[b] Julia Emerson,[a,b] Jane L. Burns,[a,b] Lucas R. Hoffman[a,b]

University of Washington, Seattle, Washington, USA[a]; Seattle Children's Hospital, Seattle, Washington, USA[b]

Staphylococcus aureus **small-colony variants (SCVs) emerge frequently during chronic infections and are often associated with worse disease outcomes. There are no standardized methods for SCV antibiotic susceptibility testing (AST) due to poor growth and reversion to normal-colony (NC) phenotypes on standard media. We sought to identify reproducible methods for AST of *S. aureus* SCVs and to determine whether SCV susceptibilities can be predicted on the basis of treatment history, SCV biochemical type (auxotrophy), or the susceptibilities of isogenic NC coisolates. We tested the growth and stability of SCV isolates on 11 agar media, selecting for AST 2 media that yielded optimal SCV growth and the lowest rates of reversion to NC phenotypes. We then performed disk diffusion AST on 86 *S. aureus* SCVs and 28 isogenic NCs and Etest for a subset of 26 SCVs and 24 isogenic NCs. Growth and reversion were optimal on brain heart infusion agar and Mueller-Hinton agar supplemented with compounds for which most clinical SCVs are auxotrophic: hemin, menadione, and thymidine. SCVs were typically nonsusceptible to either trimethoprim-sulfamethoxazole or aminoglycosides, in accordance with the auxotrophy type. In contrast, SCVs were variably nonsusceptible to fluoroquinolones, macrolides, lincosamides, fusidic acid, and rifampin; *mecA*-positive SCVs were invariably resistant to cefoxitin. All isolates (both SCVs and NCs) were susceptible to quinupristin-dalfopristin, vancomycin, minocycline, linezolid, chloramphenicol, and tigecycline. Analysis of SCV auxotrophy type, isogenic NC antibiograms, and antibiotic treatment history had limited utility in predicting SCV susceptibilities. With clinical correlation, this AST method and these results may prove useful in directing treatment for SCV infections.**

囊性纤维化病人来源的非典型金葡

Precit et al. 2016. Antimicrob Agents Chemother. 60:1725.

- **多数生长需要氯高铁血红素,甲萘醌和/或 胸苷**
- **纸片扩散法 –推荐特定琼脂，不能商品化获得**
- **头孢西丁有效(囊性纤维化菌株)**

凝固酶阴性葡萄球菌和苯唑西林

Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm) S	I	R	Interpretive Categories and MIC Breakpoints (µg/mL) S	I	R	Comments
PENICILLINASE-STABLE PENICILLINS (Continued)									
A	Oxacillin (For CoNS except *S. lugdunensis* and *S. pseudintermedius*)	–	–	–	–	≤0.25 (oxacillin)	–	≥0.5 (oxacillin)	For use with CoNS except *S. lugdunensis* and *S. pseudintermedius*. **(15)** Oxacillin MIC **breakpoints** may overcall resistance for some CoNS, because some non–*S. epidermidis* strains for which the oxacillin MICs are 0.5–2 µg/mL lack *mecA*. For serious infections with CoNS other than *S. epidermidis*, testing for *mecA* or for PBP 2a or with cefoxitin disk diffusion may be appropriate for strains for which the oxacillin MICs are 0.5–2 µg/mL. **Isolates that test either *mecA* negative or PBP2a negative or cefoxitin susceptible should be reported as oxacillin susceptible.**
		30 µg cefoxitin							(8),

15) 苯唑西林MIC折点可能高估了一些凝固酶阴性葡萄球菌的耐药, 因为一些青霉素MIC 0.5–2 µg/mL的非表皮葡萄球菌无*mecA*. *对于非表葡的凝固酶阴性葡萄球菌引起的严重感染*, 检测*mecA* 或PBP 2a或头孢西丁纸片扩散法对于青霉素MIC 0.5–2 µg/mL的菌株可能是适当的..菌株检测mecA阴性或PBP2a阴性或头孢西丁敏感应报告苯唑西林敏感

CLSI M100 27th ed. pp. 60.

报告策略
凝固酶阴性葡萄球菌苯唑西林MIC结果*

*检测无菌部位引起感染的非表皮葡萄球菌菌株

苯唑西林 MIC (μg/ml)		
S	I	R
≤0.25	-	≥0.5

草绿色链球菌

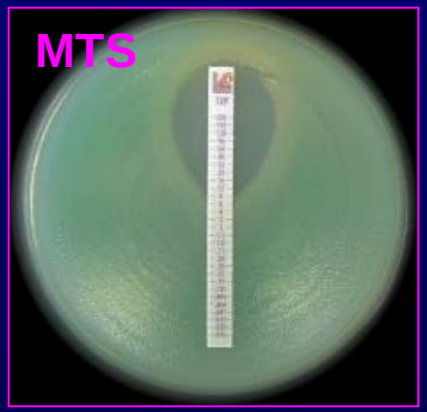
草绿色链球菌
青霉素

Table 2H-2. *Streptococcus* spp. Viridans Group (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm) S	I	R	Interpretive Categories and MIC Breakpoints (µg/mL) S	I	R	Comments
PENICILLINS									
A	Penicillin	–	–	–	–	≤0.12	0.25–2	≥4	(5) Viridans streptococci isolated from normally sterile body sites (eg, CSF, blood, bone) should be tested for penicillin susceptibility using an MIC method.
A	Ampicillin					≤0.25	0.5–4	≥8	
									(6) A penicillin MIC of ≤0.125 µg/mL is the same as a penicillin MIC of ≤ 0.12 µg/mL and both should be interpreted as

- 梯度扩散条的最先发行

(6) 青霉素 MIC of ≤0.125 µg/mL 等同于青霉素 MIC ≤0.12 µg/mL，都应解释为敏感. 实验室应将MIC ≤0.125 µg/mL 报告为 ≤0.12 µg/mL.



New!

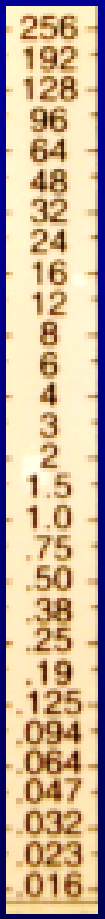
CLSI M100 27th ed. pp. 89.

MIC (μg/ml)	Round to:	Interpret Penicillin	
		Spneu	Svir
4	4	R	R
3	4	R	R
2	2	R	I
1.5	2	R	I
1	1	I	I
0.75	1	I	I
0.5	0.5	I	I
0.38	0.5	I	I
0.25	0.25	I	I
0.19	0.25	I	I
0.125	0.12	I	S
0.094	0.12	I	S
0.064	0.06	S	S

Etest 和Liofilchem MTS 包括浓度“在 两倍稀释度之间”….必须“舍去最后一位小数并且进位至两倍稀释度…然后解释!

例: 青霉素

肺炎链球菌		
Susc	Int	Res
≤0.06	0.12-1	≥2
草绿色链球菌		
≤0.12	0.25-2	≥4



“取舍” MIC 值

新!

Actual two-fold concentration (µg/ml)	Round to:
128	128
64	64
32	32
16	16
8	8
4	4
2	2
1	1
0.5	0.5
0.25	0.25

Actual two-fold concentration (µg/ml)	Round to:
0.125	0.12
0.0625	0.06
0.03125	0.03
0.015625	0.016
0.0078125	0.008
0.0039063	0.004
0.0019531	0.002

CLSI M100 27th ed. pp. 198.

AHA Scientific Statement

Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications

A Scientific Statement for Healthcare Professionals From the American Heart Association

Endorsed by the Infectious Diseases Society of America

Larry M. Baddour, MD, FAHA, Chair; Walter R. Wilson, MD; Arnold S. Bayer, MD; Vance G. Fowler, Jr, MD, MHS; Imad M. Tleyjeh, MD, MSc; Michael J. Rybak, PharmD, MPH; Bruno Barsic, MD, PhD; Peter B. Lockhart, DDS; Michael H. Gewitz, MD, FAHA; Matthew E. Levison, MD; Ann F
James M. Steckelberg, MD; Robert S. Baltimore, MD; Anne M
Patrick O'Gara, MD, FAHA; Kathryn A. Taubert, PhD, FAHA; on bel
Association Committee on Rheumatic Fever, Endocarditis, and Kawasak
Cardiovascular Disease in the Young, Council on Clinical Cardiology,
Surgery and Anesthesia, and Stroke Council

Background—Infective endocarditis is a potentially lethal disease that has undergon
pathogen. The epidemiology of infective endocarditis has become more comple
associated factors that predispose to infection. Moreover, changes in pathogen preva
staphylococcal origin, have affected outcomes, which have not improved despite me

Methods and Results—This statement updates the 2005 iteration, both of which were
Association under the auspices of the Committee on Rheumatic Fever, Endocarditis,
Cardiovascular Disease of the Young. It includes an evidenced-based system for diagno
used by the American College of Cardiology and the American Heart Association fo

Conclusions—Infective endocarditis is a complex disease, and patients with this disease generally require management by a team of physicians and allied health providers with a variety of areas of expertise. The recommendations provided in this document are intended to assist in the management of this uncommon but potentially deadly infection. The clinical variability and complexity in infective endocarditis, however, dictate that these recommendations be used to support and not supplant

Overview of VGS, *Streptococcus gallolyticus* (Formerly Known as *Streptococcus bovis*), *Abiotrophia defectiva*, and Granulicatella Species

VGS are common pathogenic agents in community-acquired NVE in patients who are not IDUs. The taxonomy of VGS is evolving. The species that most commonly cause IE are *S sanguis*, *S oralis (mitis)*, *S salivarius*, *S mutans*, and *Gemella morbillorum* (formerly called *S morbillorum*). Members of the *S anginosus* group (*S intermedius*, *anginosus*, and *constellatus*) also have been referred to as the

Baddour et al. 2015. Circulation. 132:DOI: 10.1161.

草绿色链球菌成人人工瓣膜心内膜炎治疗: 基于青霉素MIC的推荐

青霉素 MIC (μg/ml)	建议的治疗*
≤0.12	青霉素 或头孢曲松 +/- 庆大霉素
>0.12 to <0.5	青霉素 + 庆大霉素 或单独头孢曲松
≥0.5	青霉素 或头孢曲松 + 庆大霉素

*万古霉素用于β-内酰胺类不耐受的病人

Baddour et al. 2015. Circulation. 132:1435.

Liofilchem MTS

头孢洛扎他唑巴坦
达巴万星
万古霉素
美罗培南 – 刚批准!

Google!

目前, 美国限制性获得经销商很快出现

何时使用bioMerieux Etest 或 Liofilchem MTS…

- 了解FDA-取消 vs 科研用(RUO) 的意义
- 准确依照制造商的推荐
  - 回顾要求的琼脂培养基类型, 培养基悬浮和密度以及孵育要求
  - 回顾特定的阅读指导 (如, 边界清晰vs 边界模糊)
- 病人检测使用前证实性能 (CLIA 493.12)

Great resources
Google!

Etest Reading Guide

流感嗜血杆菌
副流感嗜血杆菌

	Haemophilus influenzae[d] and *Haemophilus parainfluenzae*
GROUP A PRIMARY TEST AND REPORT	Ampicillin[d,f]
GROUP B OPTIONAL PRIMARY TEST REPORT SELECTIVELY	Ampicillin-sulbactam
	Cefotaxime[d] or ceftazidime[d] or ceftriaxone[d]
	Ciprofloxacin or levofloxacin or moxifloxacin
	Meropenem[d]
GROUP C SUPPLEMENTAL REPORT SELECTIVELY	Azithromycin[e] Clarithromycin[e]
	Aztreonam
	Amoxicillin-clavulanate[e]
	Cefaclor[e] Cefprozil[e]
	Cefdinir[e] or cefixime[e] or cefpodoxime[e]
	Ceftaroline[g]
	Cefuroxime[e]
	Chloramphenicol[c]
	Ertapenem or imipenem
	Rifampin[h]
	Tetracycline[b]
	Trimethoprim-sulfamethoxazole

流感嗜血杆菌
副流感嗜血杆菌

建议测试/报告药物的变化

- 复方磺胺 由 Group A 到 C
- 环丙沙星/左氧沙星/莫西沙星 由 Group C 到 B
- 头孢呋辛 由 Group B 到 C

一些实验室:

- 仅对重要分离株进行 β-内酰胺酶 试验
- 也对脑脊液分离株进行纸片扩散或MIC试验

CLSI M100 27th ed. pp. 24-25.

流感嗜血杆菌
副流感嗜血杆菌(续)

d. 对于脑脊液分离的*流感嗜血杆菌*, 仅报告氨苄西林试验结果、三代头孢菌素和美罗培南。*

e. 阿莫西林-克拉维酸，阿奇霉素，头孢克洛，头孢地尼，头孢克肟，头孢泊肟，头孢丙烯，头孢呋辛和克拉霉素用于嗜血杆菌属引起的呼吸道感染的经验性治疗。这些抗生素的敏感性试验结果通常对于个别患者的管理不是必要的

* 三代头孢菌素和美罗培南的高MIC分离株很罕见
Skaare et al. 2014. Eurosurveillance. 19(49):pii=20986.

CLSI M100 27th ed. pp. 26.

厌氧菌

附录 A. 建议确认的耐药(R)、中介(I)和不敏感(NS)的药敏试验结果与菌株鉴定结果

新!

Appendix A. (Continued)

Organism or Organism Group	Resistance Phenotype Detected[a]	Occurrence and Significance of Resistance and Actions to Take Following Confirmation of Results[a]		
		Category I	Category II	Category III
		Not reported or only rarely reported to date	Uncommon in most institutions	May be common, but is generally considered of epidemiological concern
Streptococcus, viridans group	Daptomycin – NS Ertapenem or meropenem – NS Linezolid – NS Oritavancin – NS Quinupristin-dalfopristin – I or R Tedizolid – NS Telavancin – NS Vancomycin – NS	x		
***Bacteroides fragilis* Group**	**Metronidazole – I or R**		x	
	Doripenem, ertapenem, imipenem, or		x	

***Bacteroides fragilis* Group**	**Metronidazole – I or R**		x	
	Doripenem, ertapenem, imipenem, or meropenem – I or R		x	

NOTE 2: An isolate that is interpreted as nonsusceptible does not necessarily mean that the isolate has a resistance mechanism. It is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution subsequent to the time the susceptible-only breakpoint is set.

NOTE 3: For strains yielding results in the "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed (see footnote "a").

CLSI M100 27th ed. Table 4A. p. 207.

厌氧菌的新生抗性

- 脆弱拟杆菌群增加的耐药性
- 碳青霉烯类
  - 甲硝唑(可以是质粒携带或染色体介导)

甲硝唑和碳青霉烯耐药的多形拟杆菌
- 腹内脓肿和脓胸

*Sadarangani SP et al. 2015. Antimicrob Agents Chemother. 59:4157

常见厌氧菌对特定抗生素的敏感率变化

细菌组	抗生素	敏感率	
		2007-2009	2010-2012
G+厌氧球菌	氨苄西林－舒巴坦	98%	88%
	莫西沙星	82%	63%
脆弱拟杆菌	甲硝唑	100%	96%
	美罗培南	97%	96%

Hastey CJ et al. 2016. Anaerobe. 42:27.

什么时候对厌氧菌进行药敏试验？

- *脆弱拟杆菌*
  - 一个最不敏感的厌氧菌
  - 考虑AST
- *厌氧菌进行AST的适应症*
  - 敏感药物的选择对于疾病的治疗很关键
  - 需要长期治疗
  - 从无菌部位分离
  - 经验性治疗后仍然存在感染
- *测试方法*
  - CLSI M11（方法）＋M100（折点，QC，药物测试/报告）
  - 其他选项
    - Etest
    - 送到参考实验室

Schuetz AN. Clin Infect Dis 2014; 59:698-705.

质量控制

QC 改变 / 增添

- 修改的 QC 范围:

抗生素	Zone (mm)		MIC (μg/ml)	
	大肠埃希菌 ATCC 25922	*铜绿假单胞菌* ATCC 27853	*铜绿假单胞菌* ATCC 27853	*金黄色葡萄球菌* ATCC 29213
头孢吡肟	no change	25–31	no change	no change
美罗培南	28–35	no change	0.12-1.0	no change
泰迪唑胺	-	-	-	0.12-1.0

- 更新 MIC 故障排除指南

Table 5G. MIC: Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range QC primarily with CAMHB for broth microdilution. Refer to M07-A10 (MIC), Chapter 4, Quality Control and Quality Assurance.

Out-of-range QC tests **are often the result of contamination or the use of an incorrect QC strain; corrective action should first include repeating the test with a pure culture of a freshly subcultured QC strain**. If the issue is unresolved, this troubleshooting guide should be consulted regarding additional suggestions for troubleshooting out-of-range QC results and unusual clinical isolate results. In addition, **see general corrective action outlined in M07 and notify manufacturers** of potential product problems.

CLSI M100 27th ed. Table 4A, 5A,5G.

MIC QC 故障排除指南

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/ Suggested Actions
不同的抗生素	*大肠埃希菌*ATCC® 35218 *肺炎克雷伯菌*ATCC® 700603	MIC 太低	编码β-内酰胺酶的质粒的自发丢失	参见关于QC微生物维护的一般性意见(1)。
不同的抗生素	任何	一个Qc结果失控，但是这个药不报告给临床(eg, 不在医院处方).	不适用	当药剂没有正常报告，如果有适当的控制以防止报告超出范围的抗生素，则不需要进行重复。

...这些是示例...表5G中还有一些其他更改

CLSI M100 27th ed. Table 5G. pp. 182-185.

新型抗生素

新型抗菌药物

通用名	商品名	Notes
头孢他啶-阿维巴坦	Avycaz	β-内酰胺/β-内酰胺酶抑制剂组合 CRE治疗（不含MBLs）
Ceftolozane-他唑巴坦	Zerbaxa	β-内酰胺/β-内酰胺酶抑制剂组合 治疗UTI，IAI（ESBL阳性），包括铜绿假单胞菌
达巴万星	Dalvance	脂糖肽 皮肤感染的治疗（2 IV剂量）
奥利万星	Orbactiv	脂糖肽 皮肤感染的治疗（1 IV剂量）
替拉万星	Vibativ	脂糖肽 肺炎治疗（一天一次给药）
泰地唑胺	Sivextro	恶唑烷酮 皮肤感染的治疗（PO，IV）

新型抗菌药物
检测方法

通用名	FDA 批准 AST	Notes
头孢他啶-阿维巴坦	纸片法 (BD, Hardy) Trek	使用FDA折点 CLSI折点将于2018年推出（与FDA折点相同）
Ceftolozane- 他唑巴坦	纸片法 (Hardy) Trek Liofilchem	CLSI折点 = FDA折点
达巴万星	Trek	纸片扩散法不是可接受的测试方法 不存在CLSI MIC折点
奥利万星 替拉万星	Trek	纸片扩散法不是可接受的测试方法
泰地唑胺	Trek	不存在CLSI纸片扩散法折点 FDA存在纸片扩散法折点

新型抗菌药物
何时进行试验?

Generic Name	Notes
头孢他啶-阿维巴坦	大多数需求是用于CRE（例如，KPC）；不适用于产NDM或其他MBL碳青霉烯酶的细菌 For RUO纸片/ Etest：avycazeval.com
Ceftolozane- 他唑巴坦	大多数需求是用于试图避免使用碳青霉烯时铜绿假单胞菌的治疗，或用于碳青霉烯耐药的铜绿假单胞菌 RUO 纸片/Etest: www.mist-ruo.com
达巴万星 奥利万星 替拉万星	通常规定经验性用于MRSA; 耐药率很低
泰地唑胺	通常规定经验性用于MRSA; 耐药率很低

QC 表中的新型抗菌药物

通用名	类别	给药途径	目标适应症	状态
美罗培南-vaborbactam	β-内酰胺/β-内酰胺酶抑制剂	静脉	cUTI MDR GNR (KPCs)	Phase 3
纳菲霉素	酮内酯	口服	CAP	Phase 2
培西加南	多肽	局部	糖尿病足	Phase 3

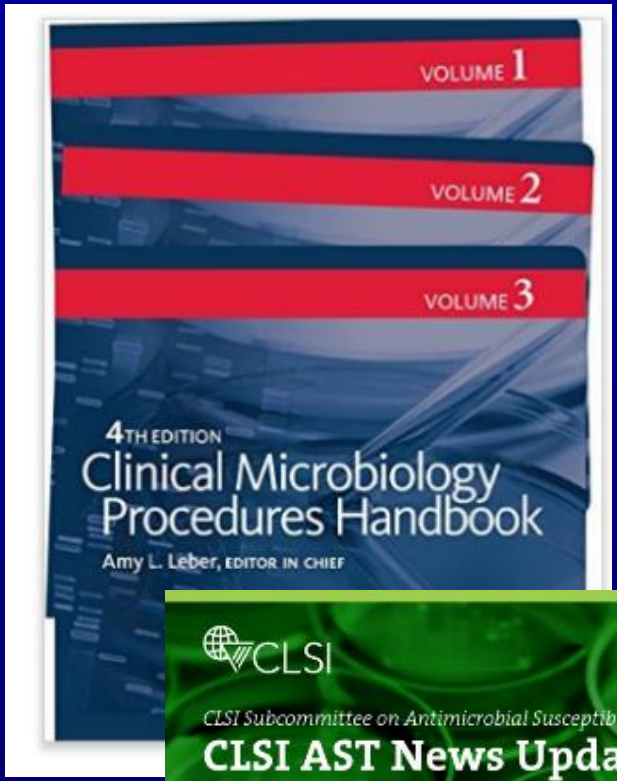
了解更多....

- 发展中的抗菌药物
  - Pew Trust

    http://www.pewtrusts.org/~/media/assets/2016/05/antibiotics-currently-in-clinical-development.pdf
- 21st 世纪治疗法
  - 更容易、更快的药物批准途径
  - 更快的医疗器械批准
  - 专注于持续研究

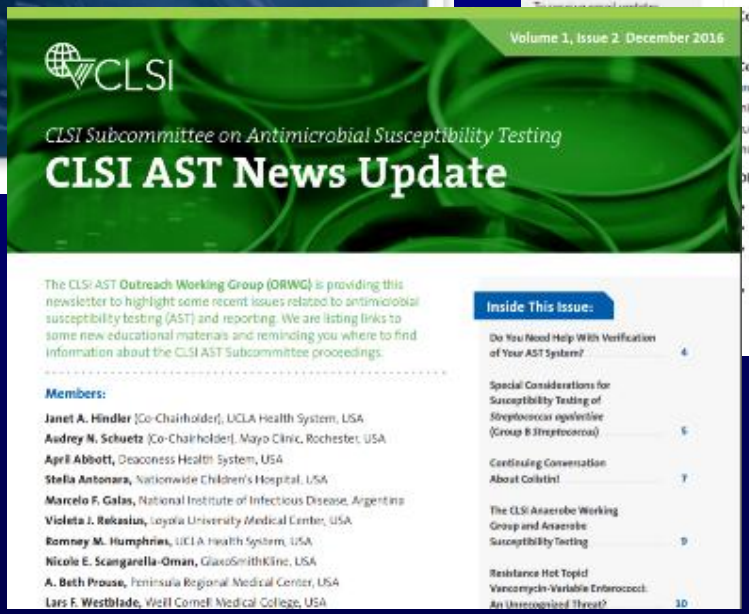
目前由CLSI AST小组委员会评估的一些附加主题

- 折点
  - 头孢他啶-阿维巴坦 - 2018公布折点（与FDA相同）
  - 达托霉素 - 对肠球菌重新进行评估
  - 环丙沙星和左氧氟沙星 - 对肠杆菌科重新进行评估
  - Ceftolozane-他唑巴坦 - 增加肠杆菌科的纸片扩散法折点
  - 苯唑西林/头孢西丁 - 研究其它CoNS（例如，施氏葡萄球菌）
- ECVs
  - 合并在M100的一个部分
  - 附加ECVs
- 方法
  - 铜绿假单胞菌的mCIM试验
  - 评估粘菌素/多粘菌素B的方法
  - 血培养物直接进行纸片扩散AST
- “组合”药物的质控
- 确定在新药物可用时支持AST可用性的方法
- 新M02 / M07文件2018



CDC MASTER计划
多级抗菌药物敏感性试验教育资源

“去上课”，并免费注册！

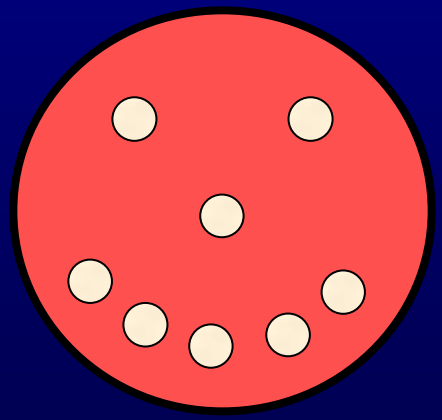


模块：
1.方法（纸片扩散，MIC，商业）
2.质量保证和质量控制
3.CLSI标准

谢谢!

….听过今天…请不要忘记进行自我评估

致谢:
APHL工作人员（特别是Denise Korzeniowski）
CLSI工作人员
CLSI AST外部工作组
CLSI AST小组委员会



以下摘要幻灯片不会被讨论，
并提供给参与者回顾。

总结 (1)

- CLSI每年一月更新 AST 表格 (M100).
- CLSI 每3年更新讲述如何进行参考纸片扩散法(M02)和参考MIC法(M07)试验的文件 (最近一次更新在2015).
- CLSI 文件的改变被总结在每个文件之前
- 以粗体显示的信息是与上一版本M100相比的新版本或修改版本
- 最近的解释标准（折点）添加/修订日期列在M100第27版（第xxiv-xxvi页）的前面

总结 (2)

- CLSI AST扩大工作组 (ORWG) 提供培训材料帮助大家更好理解AST和报告推荐.
- 在这里可以找到第2版ORWG 新闻更新(2016年12月):
  - http://clsi.org/wp-content/uploads/sites/14/2013/07/CLSI-News-Winter-2016.pdf
  - 点击第1期和第2期.
  - 查看新闻更新链接中的更多资料 (例如, 验证表格, PPT和更多内容!)
- 抗菌药物全面纲要和旧折点归档即将很快发布.
- 现在可以在CLSI网站上获取M100 免费版本!

总结 (3)

- CLSI不再在AST文件中使用术语“解释标准”; 将仅仅使用 “折点”.
- “解释分类” (S, SDD, I, R, NS)的定义已经稍加改写.
- “非敏感” 是只有“S”折点的药物/细菌组合解释分类.
- “不敏感” 用于描述菌株对于药物SDD, I, 或 R.
- 天然耐药表格(M100附录B)是非常有用 的; 摩根摩根菌修改为对替加环素天然耐药而对四环素类不是天然耐药.

总结 (4)

- 流行病学界值 (ECVs) 不同于临床折点，他们是:
  - 基于菌株表型(MICs)将菌群分成获得突变耐药机制和不获得突变耐药机制(野生型或非野生型).
  - 在只有体外MIC分布但无PK/PD和/或临床结局数据时建立.
  - 有助于监测耐药性出现.
  - 应仅仅在与患者临床医生讨论之后报告.
- 抗菌药物评估中将未获得耐药机制或无敏感性下降菌株的ECV值定义为野生型.
- 抗菌药物评估中将认为或已知获得耐药机制或存在敏感性下降菌株的ECV值定义为非野生型.

总结 (5)

- ECVs已经增加在下列情况:
  - *产气肠杆菌, 阴沟肠杆菌, 大肠埃希菌, 肺炎克雷伯菌*, 和解鸟氨酸拉乌尔菌对粘菌素
  - *淋病奈瑟菌*对阿奇霉素
- 粘菌素 / 多粘菌素B:
  - 纸片扩散法折点已经完全取消, 并且纸片扩散法不用于这些药物检测.
  - “其他非肠杆菌科” 折点已经取消.
  - 粘菌素“S” 和“R” MIC 折点用于 鲍曼不动杆菌复合体 .
  - 当报告鲍曼不动杆菌或铜绿假单胞菌粘菌素MICs时, 治疗评语应该考虑增加.
  - 鲍曼不动杆菌和铜绿假单胞菌多粘菌素MIC折点还没有变化, 也许随后会改变.
  - 当报告粘菌素ECVs时, 应考虑在患者报告中增加评语.

总结 (6)

- 当前CDC推荐治疗淋病的药物包括头孢曲松 + 阿奇霉素.
- 淋病奈瑟菌阿奇霉素ECVs能够进一步监测耐药性的出现.
- CRE耐药可能由于产碳青霉烯酶或产其他beta-内酰胺酶和外膜蛋白突变.
- 产碳青霉烯酶的CRE对于流行病学是至关重要的，因为编码这些酶的基因位于传播性强的质粒上 .
- 碳青霉烯酶常规试验对于病人治疗不是必须的，但对感控是有用的.

总结 (7)

- 改良碳青霉烯类灭活方法（mCIM）是一个实用的方法，对于临床实验室来讲是一个比MHT更可靠的表型方法．mCIM需要的试剂与常规纸片扩散法试验类似.
- 没有哪个表型试验 (MHT, CarbaNP or mCIM) 可以检测所有碳青霉烯酶.
- 分子试验检测特异的碳青霉烯酶基因，并且仅仅只能检测已知的基因.
- 当应用当前CDC关于CRE监测的定义，增加试验检测碳青霉烯酶可以提高识别产碳青霉烯酶菌株的特异性.

总结 (8)

- 纸片扩散法试验对于不典型（生长不良）*金黄色葡萄球菌*不可靠
  - 头孢西丁纸片扩散法试验产生假敏感结果.
  - 采用诱导生长商品化PBP2a试验或mecA PCR检测这些菌株中mecA-介导的MRSA是可靠的.
- 凝固酶阴性葡萄球菌苯唑西林MICs落在0.5-2.0 µg/ml之间时，mecA阴性、PBP2a阴性或头孢西丁纸片法“S”时应报告苯唑西林”S”.
- MIC 0.125 µg/ml应按0.12 µg/ml解释MIC.
- 准确的青霉素MIC对于草绿色链球菌引起的自体瓣膜心内膜炎成年患者尤其重要.

总结 (9)

- 单独的β-内酰胺酶试验对于大多数流感嗜血杆菌分离株是足够的;从CSF的分离菌株应考虑测试氨苄西林，三代头孢菌素和美罗培南。
- 脆弱拟杆菌群分离株对甲硝唑和/或碳青霉烯的耐药率正在增加。
  - 实验室应确认临床分离菌的此类结果
- 商业AST的可用性有限的情况仍然存在：
  - 头孢曲松-他唑巴坦
  - 头孢他啶-阿维巴坦
  - 达巴万星
  - 奥利万星
  - 特拉万星
  - 泰迪唑胺

总结 (10)

- 检查本网络研讨会的参考资料; 咨询当地药品代表或诊断仪器制造商，获得关于测试可用性和新型抗菌药物的最新信息。
- CLSI AST ORWG 欢迎建议我们如何改进与你沟通AST问题！

The End!