Genotypic and Phenotypic Susceptibility Tests and Clinical Correlation

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อายุรแพทย์ที่ปรึกษาหน่วยโรคติดเชื้อ และห้องปฏิบัติการจุลชีววิทยา

โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ

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Clinical Microbiology Laboratory

- **Identification (ID)**
  - **Causative pathogens**
- **Antimicrobial susceptibility tests (AST)***
  - **Phenotypic vs Genotypic**
- **Antimicrobial resistance (AMR)**
  - **Chromosomal vs plasmid resistance**
  - **Intrinsic vs acquired resistance**

Basic and conventional biochemical methods

Advance and molecular identification methods

Antimicrobial Susceptibility Tests

- Basic principle
- Antimicrobial Susceptibility Tests
  - Phenotypic test
  - Genotypic test
- Clinical correlation
- Summary

- **Update CLSI 2017 = October 15, 2017 (Dr. Surapee, Dr. Nuntra)**

- I have no conflict interest to be disclosure

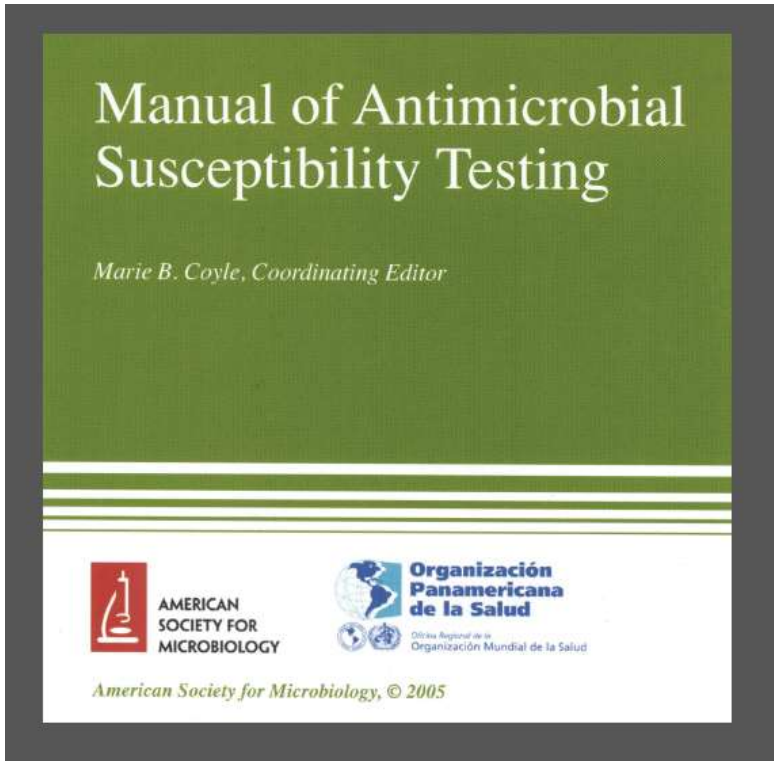
Antimicrobial Susceptibility Tests

- Standardized, reproducible methods for assessing antibiotic activity
- Routine tests (manual and automated methods) ~ Phenotypic >>>> Genotypic
- Guideline for performing the tests and breakpoints interpretation (CLSI/ EUCAST)
- Specialized tests for specific applications

ESBL, CRE confirmation tests

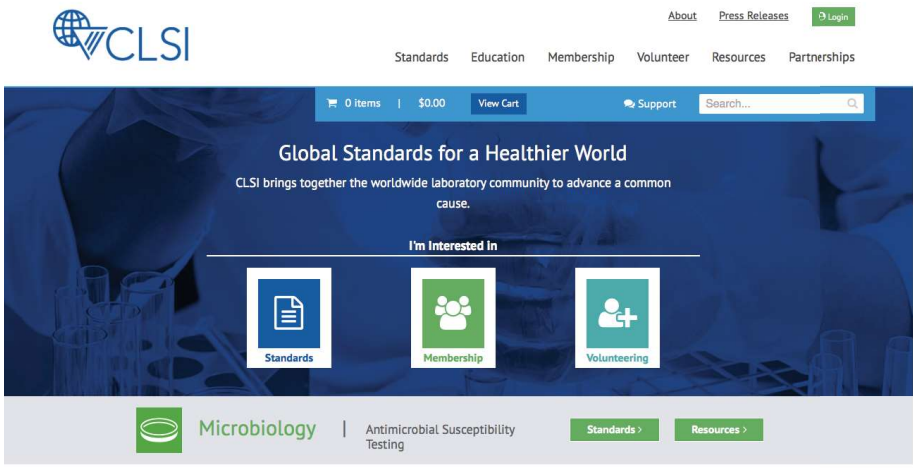
Methicillin resistance in Staphylococcus (mecA test)

Inducible clindamycin resistance (D test)



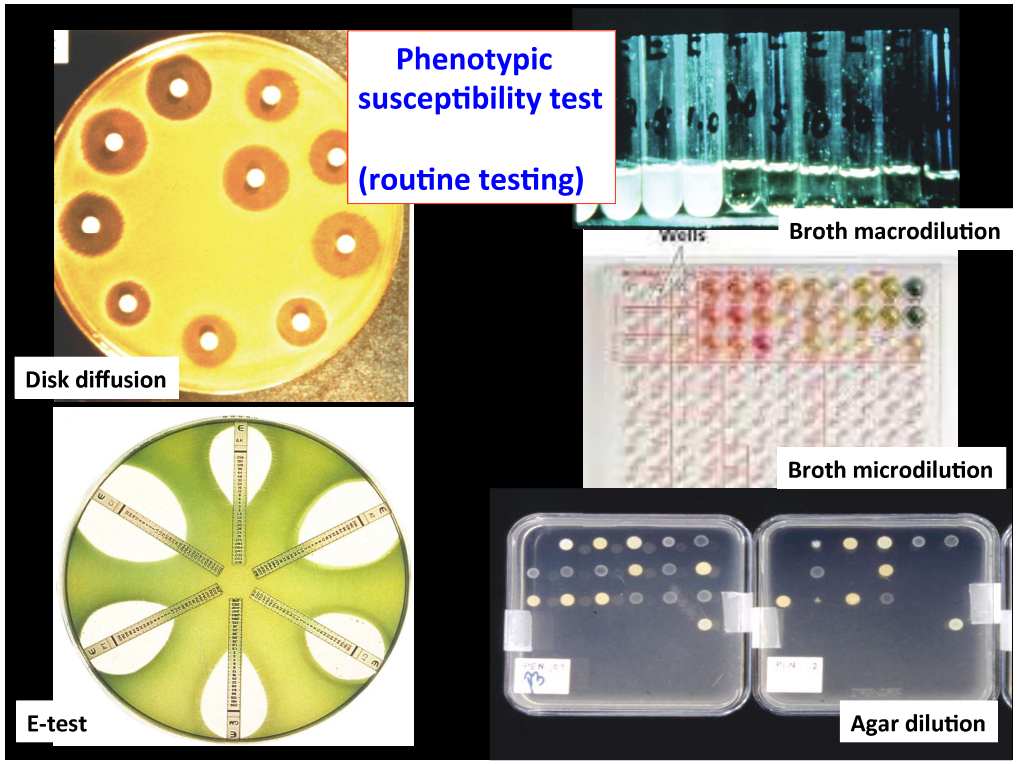
Clinical breakpoints
vs
MIC distributions

Clinical and Laboratory Standards Institute



CLSI Documents - Examples

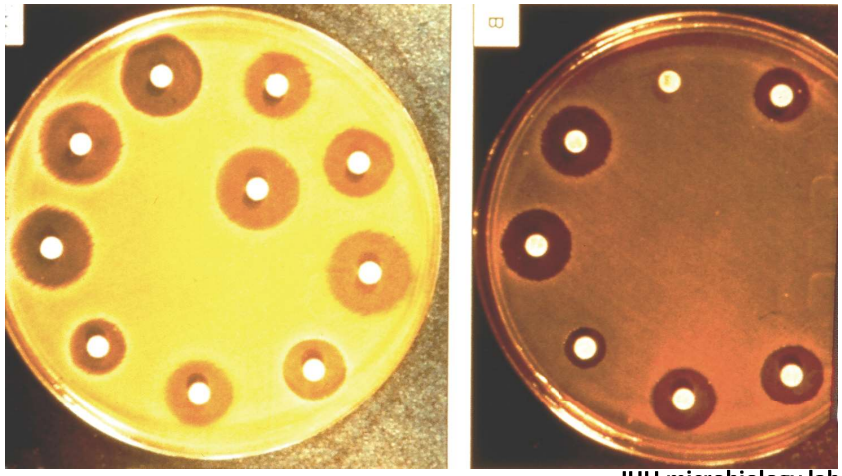
- **M2** – Performance Standards for Antimicrobial Disk Susceptibility Tests
- **M7** – Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically
- **M11** – Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria
- **M24** – Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes
- **M27** – Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts
- **M33** - Antiviral Susceptibility Testing: Herpes Simplex Virus
- **M44** – Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts
- **M100** – Performance Standards for Antimicrobial Susceptibility Testing



1. Disk Diffusion

Kirby-Bauer Method

Antibiotic susceptibility testing in which disks containing various antibiotics are placed on a plate swabbed with the organism.
Zones of inhibition are measured to determine whether the organism is susceptible, intermediate or resistant.
(Based on interpretation guideline, CLSI (USA), EUCAST)



JHU microbiology laboratory

Figure 4.1—Selecting well-isolated colonies for the inoculum

Figure 4.2—Standardizing the inoculum

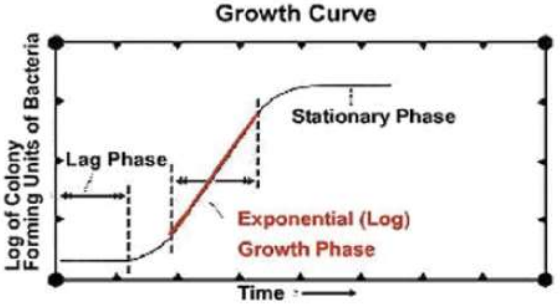


Figure 4.3—Plot of log phase growth in broth

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test

GOOD TO rule-out mix infection (not pure colonies)

Figure 4.10–Double zone of inhibition

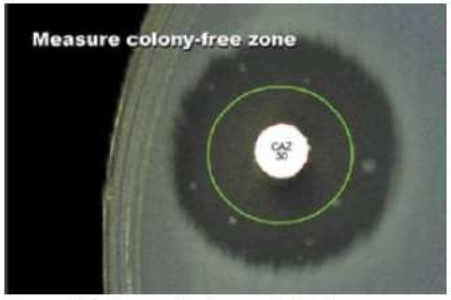


Figure 4.11–(zone with inner colonies)

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test

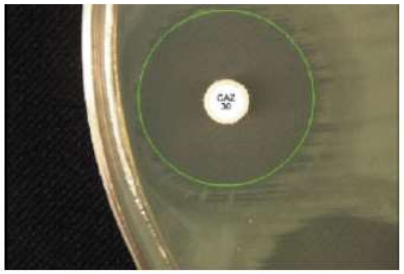


Figure 4.12–Feathered zone around CAZ disk

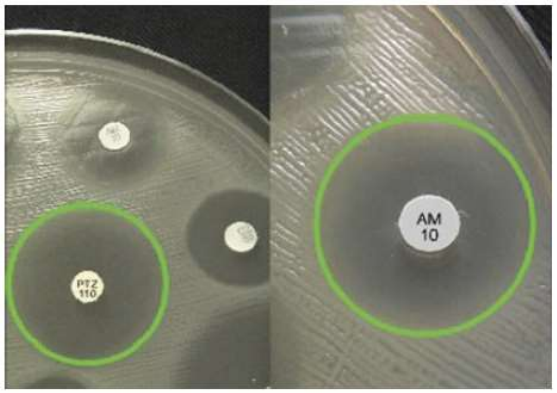


Figure 4.13–Zones with swarming *P. mirabilis*

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test

Figure 4.15—Heterogeneous resistance to oxacillin

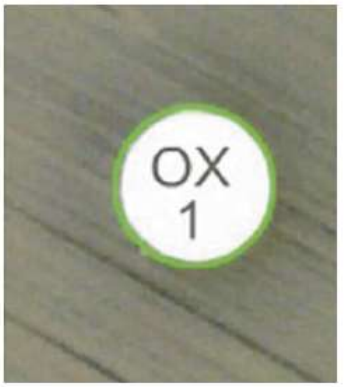


Figure 4.16—Homogeneous resistance to oxacillin

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test

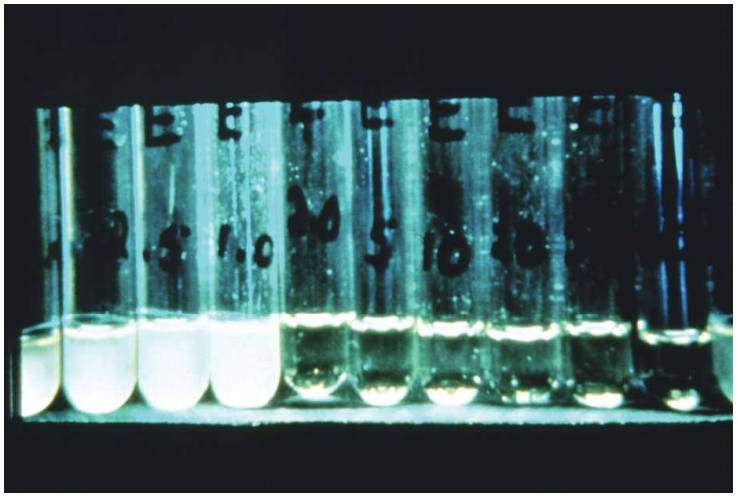
Variable factors

Review the variables listed below that must be controlled in performance of the disk diffusion test.

- Media composition
- Media pH
- Agar depth
- Concentration of inoculum
- Inoculation procedure
- Antimicrobial concentration in disk
- Disk storage

2. Tube Dilution Method

The first tube in which there is no visible growth is the MIC level of the antibiotic for the organism tested.



Antibiotic Concentration Low ----------High

MIC test

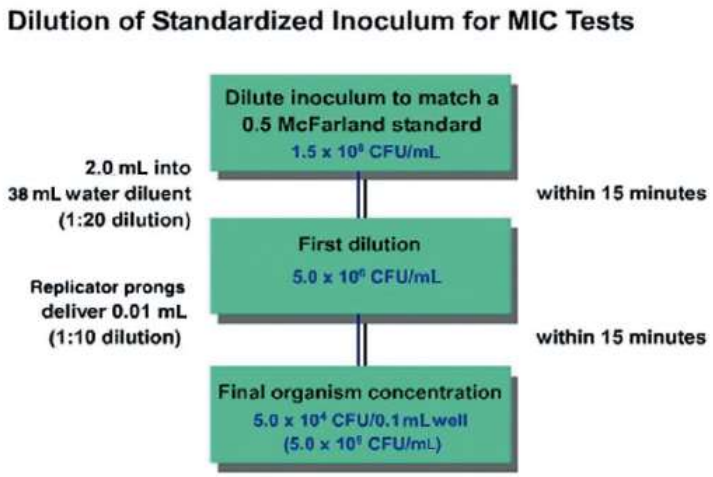
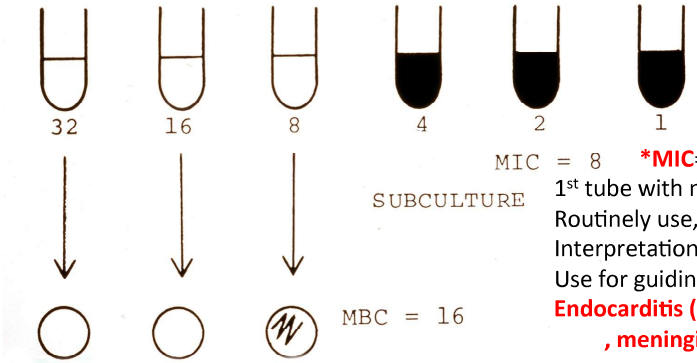


Figure 5.1—Dilution scheme for preparing a standardized inoculum for MIC tests

Illustration of the difference between MIC, minimum inhibitory concentration, and MBC, minimum bactericidal concentration, of an antibiotic.



*MIC=
1st tube with no visible growth
Routinely use, CLSI and EUCAST Interpretation
Use for guiding treatment*
Endocarditis (*Enterococcus, S. bovis*), meningitis (*S. pneumoniae*)
MDR bacteria;
MRSA
ESBL/CRE

MBC = 99% bacterial growth inhibition
(Currently use only for research, previously use for endocarditis treatment)

Broth microdilution: manual or commercial system ie MicroScan, TREK panels Phoenix system, VITEK2 system --- Give MIC interpretation

MIC test

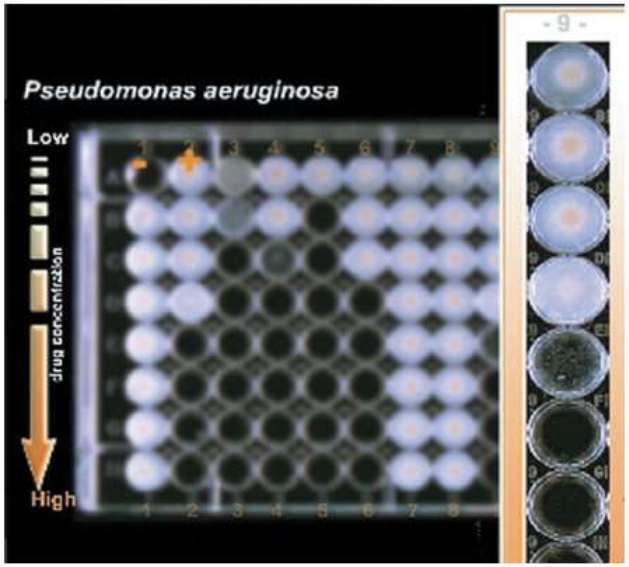


Figure 5.2—An MIC microtiter plate

MIC Test

The number of dilutions and range of concentrations tested may vary among broth microdilution MIC panels for different antimicrobial agents.

The range of concentrations tested should encompass the interpretive breakpoints and the anticipated MIC of the quality control organism.

Generally 6–8 dilutions are tested for a "full range" MIC test.

Panels that include only those concentrations that define the breakpoint (typically only 2 or 3 dilutions) are called breakpoint panels. Breakpoint panels are often difficult to quality control because the QC results are typically above or below the concentrations on the panel.

The table below shows the interpretive categories for ampicillin. For the breakpoint panel, only three concentrations are tested and these represent susceptible, intermediate and resistant interpretations.

Full range versus breakpoint MIC panels

Full Range MIC (mcg/mL)	Breakpoint MIC (mcg/mL)	Interpretation
0.5	–	
1.0	–	Susceptible
2.0	–	
4.0	–	
8.0	8.0	
16.0	16.0	Intermediate
32.0	32.0	Resistant

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M100

Performance Standards for Antimicrobial Susceptibility Testing

Interpretive Criteria

Interpretive criteria are the MIC or zone diameter values used to indicate susceptible, intermediate, and resistant breakpoints.

Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)		
		S	I	R	S	I	R
X	30 µg	≥20	15–19	≤14	≤4	8–16	≥32
Y	—	—	—	—	≤1	2	≥4
Z	10 µg	≥16	—	—	≤1	—	—

For example, for antimicrobial agent X with interpretive criteria in the table above, the susceptible breakpoint is 4 µg/mL or 20 mm and the resistant breakpoint is 32 µg/mL or 14 mm.

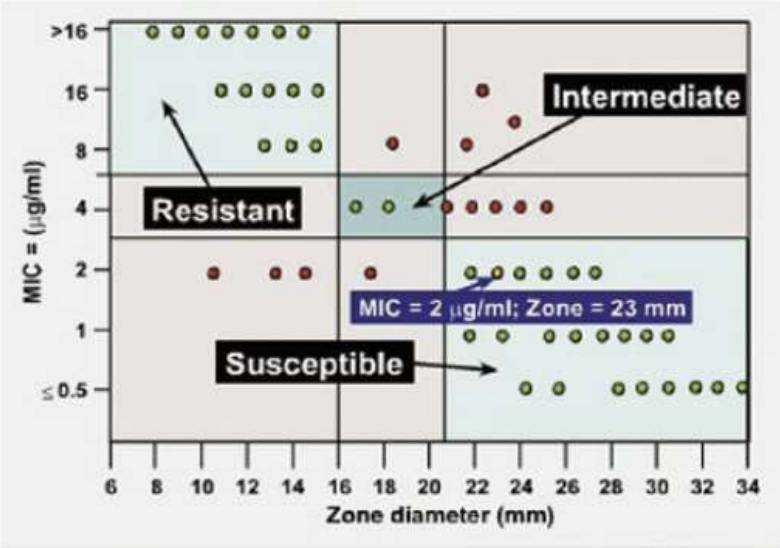


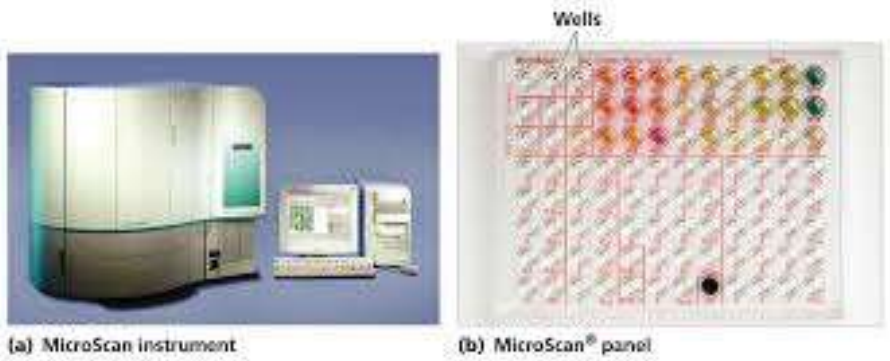
Figure 3.2—Scattergram

Scattergrams (also known as scatterplots) are used to establish MIC and disk diffusion interpretive criteria that also are called breakpoints. The scattergram represents results from MIC tests and disk diffusion tests of many strains with a hypothetical antimicrobial "X."

- Breakpoints are established by taking the following steps:
  - Several hundred isolates are tested by the standard NCCLS disk diffusion and MIC methods. The MIC and corresponding zone diameter is plotted for each isolate. In this scatterplot, each dot represents results from testing one or more isolates.
- Next, **MIC breakpoints** are established following analysis of:
  - The distribution of MICs
  - Pharmacokinetic and pharmacodynamic properties of the antimicrobial agent (basically, how the antimicrobial agent is distributed and works in the patient)
  - Clinical data correlating individual MIC results with patient outcomes
- Then the **Disk Diffusion breakpoints** are established by:
  - Examining the scattergram to determine the zone measurements that best correlate with the resistant, intermediate, and susceptible MIC breakpoints
  - The number of "outliers" (red dots) is counted to calculate the percent of isolates that demonstrate disagreement between the disk diffusion and the MIC interpretations. For the interpretative criteria to be acceptable, the percentage of errors cannot exceed preset limits established by the FDA and NCCLS.

Automated biochemical identification and susceptibility method

MicroScan system (Walkaway system)
VITEK 2 system
BD Phoenix system
Sensititer



(a) MicroScan instrument (b) MicroScan® panel

MicroScan

VITEK 2™ — technology

Identification and AST cards (pH changes)

Routine ID method at TUH since September 2015

Nonfermenters → *Elizabethkingae, Ekinella, Chryseobacterium, Salphingomonas, Roseomonas, Methylobacterium etc*

Beta-hemolytic streptococci → GAS*: S. pyogenes*,

GBS: *S. agalactiae,* Group D strep → *S. gallolyticus, Enterococcus* spp.

Calculated MIC TAT 6-8 hrs

Pro: Faster for common bacteria, size of machine

Con: Indeterminate for special pathogens (not good for yeast), need to refrigerate ID/AST cards

BD Phoenix™ AP Workflow

BD Phoenix™ M50

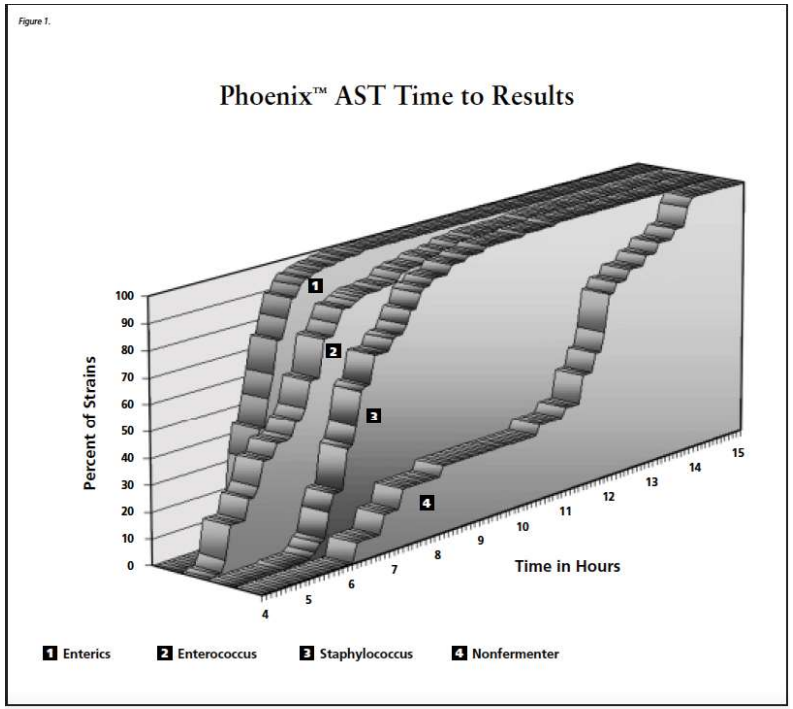
BD Phoenix™ M50 Automated Microbiology System , with its unique technology for susceptibility testing, provides high accuracy combined with rapid time to result. Because MRSA, VRE and Extended Spectrum beta-lactamase (ESBL) are the most important resistance markers linked to HAIs, the performance of the laboratory's ID-AST system to detect these mechanisms is critical.

Fluorescent detection + Semi-calculated MIC TAT 6-8hrs

Pro: Faster(common bacteria), confirmation tests, accurate MIC

Con: Indeterminate for special bacteria

size of machine/ package?



Example of comparison test

As presented at the 106th General Meeting of the American Society for Microbiology (ASM), Orlando, FL, 2006.

Direct Comparison of Antimicrobial Susceptibility Testing by the BD Phoenix, bioMérieux VITEK 2, and Disk Diffusion Test Methods as Compared to Results Generated by the CLSI Broth Microdilution Test

J. H. Jorgensen, S. A. Crawford, M. Masterson, M. K. Mansell, M. L. McElmeel, and L. C. Fulcher

University of Texas Health Science Center and University Hospital • San Antonio, Texas 78229

RESULTS

Table 2. Overall error rates and category agreement for all antimicrobial agents by organism group

Organism Group	Phoenix				VITEK 2				Disk Diffusion			
	VM	M	m	CA	VM	M	m	CA	VM	M	m	CA
Enterobacteriaceae	6	5	55	1490	12	2	60	1482	10	4	93	1449
Pseudomonas spp.	3	1	17	459	6	0	28	460	1	2	24	358
Non-*Enterobacteriaceae*	1	0	15	172	7	1	23	160	0	0	1	111
S. aureus	1	0	10	395	1	0	8	397	0	0	9	397
CNS	1	28	4	285	1	2	3	312	1	0	4	314
Enterococcus spp.	1	2	3	276	2	0	4	276	0	0	2	124
Total	13	36	104	3077	29	5	126	3087	12	6	133	2753
Frequency %	1.2%	1.8%	3.2%	95.3%	2.7%	0.2%	3.9%	95.1%	1.4%	0.2%	4.5%	94.7%

VM = Very Major Error, M = Major Error, m = Minor Error, CA = Category Agreement

Table 5. Time required for generation of susceptibility results

Gram-negatives	Phoenix	VITEK 2
Enterobacteriaceae	11:58:11[a]	7:35:31[a]
Pseudomonas spp.	15:39:04[a]	11:45:53[a]
Non-*Enterobacteriaceae*	12:32:24[a]	9:06:06[a]
Gram-positives	**Phoenix**	**VITEK 2**
S. aureus	13:02:21[b]	7:05:29[b]
CNS	14:43:36[b]	9:32:11[b]
Enterococcus spp.	12:22:21[b]	9:38:00[b]

[a] $p<0.05$; [b] $p<0.05$

Table 6. Comparison of MICs generated by instrument and reference methods

Organism Group	Total # MICs	% On scale MICs	% Strains with on scale MICs within +/- 1 dilution of Reference		
			# strains on scale	# strains +/-1	%EA[a]
Enterobacteriaceae					
Phoenix	1683	13.5%	228	186	81.6
Vitek 2	1989	13.4%	266	206	77.4
Pseudomonas spp.					
Phoenix	385	30.9%	119	116	97.5
Vitek 2	550	24.9%	137	131	95.6
Non *Enterobacteriaceae*					
Phoenix	192	13.0%	25	23	92.0
Vitek 2	384	19.3%	74	61	82.4
S. aureus					
Phoenix	558	17.6%	98	96	98.0
Vitek 2	496	8.3%	41	39	95.1
CNS					
Phoenix	294	23.8%	70	60	85.7
Vitek 2	392	18.9%	74	70	94.6
Enterococcus spp.					
Phoenix	354	20.9%	74	71	95.0
Vitek 2	472	19.9%	94	84	89.3
Overall Average					
Phoenix					**91.5**
Vitek 2					**89.1**

[a] Percent of MICs that agree within 1 twofold dilution

Pro

Good for Yeast (Candida AST)

MTB/NTM

Colistin AST?

Con

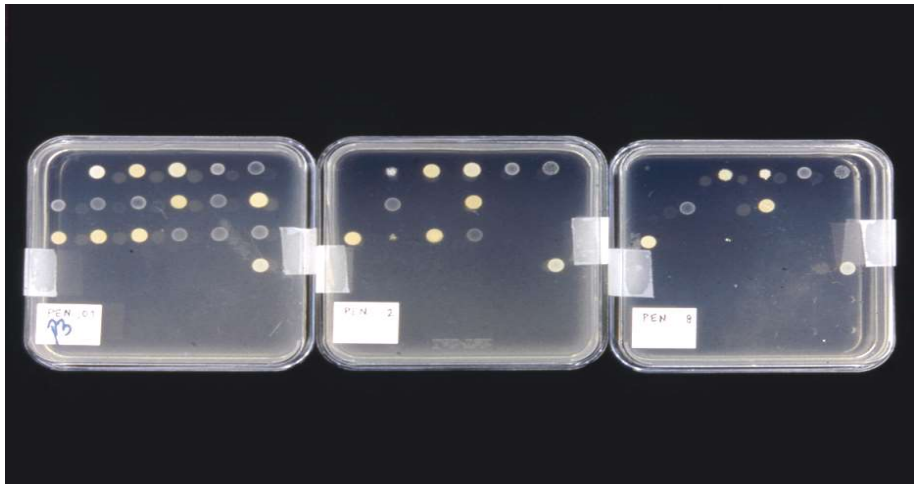
Not automated system

3. Agar Dilution Method

Formerly used at JHH, this method measures MICs of antibiotics by comparing growth of colonies on plates of increasing antibiotic concentrations.

Labor intensive/ rarely use in routine labs

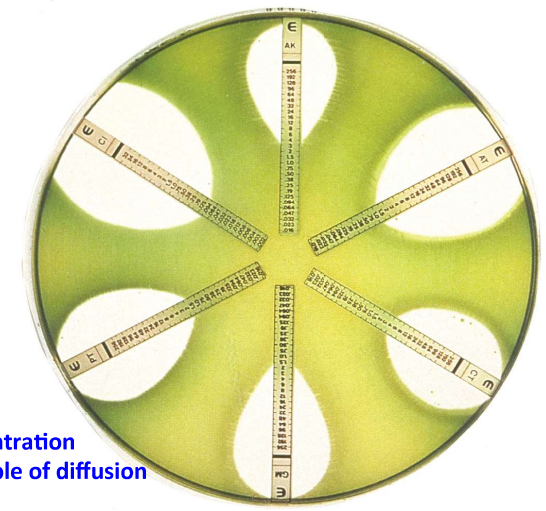
For research: Standard for Anaerobic bacteria AST, *N. gonorrhea* AST



JHU microbiology laboratory

4. E-test Method

Each strip is impregnated with increasing concentrations of a different antibiotic ; strips are placed on a plate swabbed with the organism to be tested, and incubated overnight. The MIC level for each antibiotic is at the line crossed on the strip where the organism is inhibited from growing.



Note

E-test/ Gradient concentration may have variable of diffusion as disk diffusion

E= elliptical shape

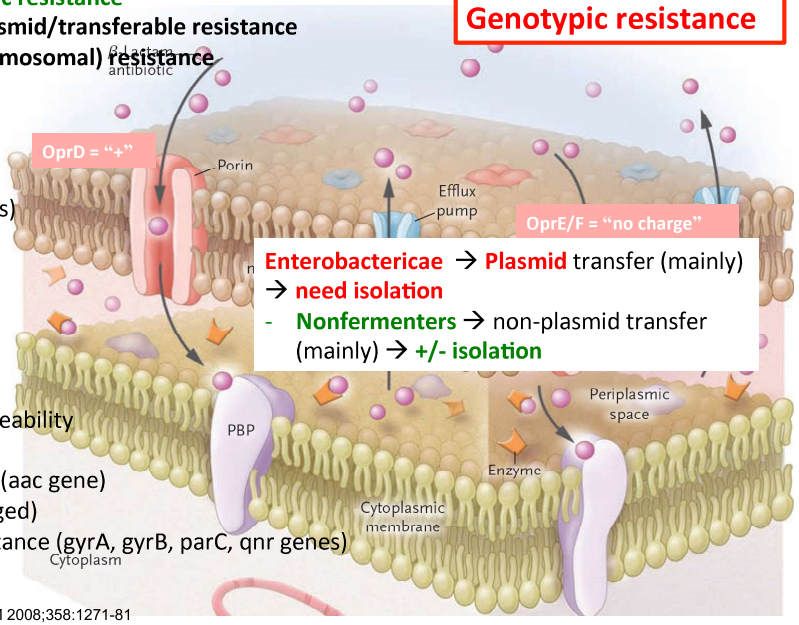
JHU microbiology laboratory

Mechanism of GN resistance
- **Enzymatic resistance**
  Non-enzymatic resistance
- **Acquired (plasmid/transferable resistance**
  Intrinsic (chromosomal) resistance

Phenotypic resistance
vs
Genotypic resistance

Enzymatic
(hydrolytic enz)
betalactamase
(several bla genes)

Non-enzymatic
-Efflux pump
-Porin change
(oprD gene)
-Decreased
membrane permeability
(omp gene)
-Aminoglycoside (aac gene)
(target site changed)
-Quinolone resistance (gyrA, gyrB, parC, qnr genes)



Enterobactericae → **Plasmid** transfer (mainly) → **need isolation**
- **Nonfermenters** → non-plasmid transfer (mainly) → **+/- isolation**

AAC 1999;43(2):424-7 NEJM 2008;358:1271-81

CRE vs CPE (CP-CRE)

- **Definition**

- **CRE** = Carbapenem Resistance Enterobacteriaceae

CDC 2015 definition

Resistance to imipenem, meropenem, doripenem or ertapenem OR documentation that the isolate produce carbapenemase

- **CP-CRE** = Carbapenem-Producing Enterobacteriaceae

→ plasmid transferable gene (carbapenemase)

→ → Infection control implementation needed

CDC; Healthcare associate infection

CRE vs CPE (CP-CRE)

Variability in geographic distribution

USA: KPC (most common), MBLs such as NDM-1

Asia: Less KPC, more MBLs (NDM-1)

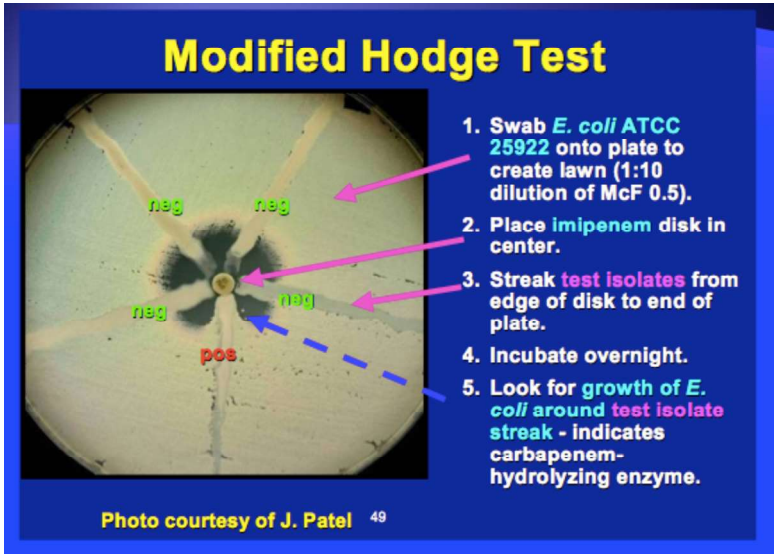
Novel pathogen = MCR-1 (colistin-R) in China

(Recently found in the USA)

Europe: Greece VIMs

South America: KPC, MBLs

Lanman D et al J Clin Microbiol 43:5639-41



CRE confirmation test

No need to perform if use new CLSI breakpoints (2010)

KPC *Providencia stuartii*

15 minutes, Orange

30 minutes, Light Orange

45 minutes, Dark Yellow

60 minutes, Yellow

120 minutes, Yellow

Comparison of a Novel, Rapid Chromogenic Biochemical Assay, the Carba NP Test, with the Modified Hodge Test for Detection of Carbapenemase-Producing Gram-Negative Bacilli

Shawn Vasoo,[a] Scott A. Cunningham,[a] Peggy C. Kohner,[a] Patricia J. Simner,[a] Jayawant N. Mandrekar,[b] Karen Lolans,[c] Mary K. Hayden,[c,d] Robin Patel[a,e]

JCM, Sep 2013

The CIM (Carbapenemase inactivation method) a new phenotypic Test to assess Carbapenemase activity

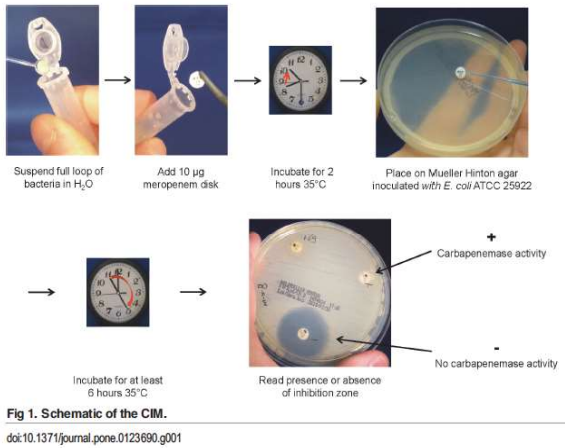
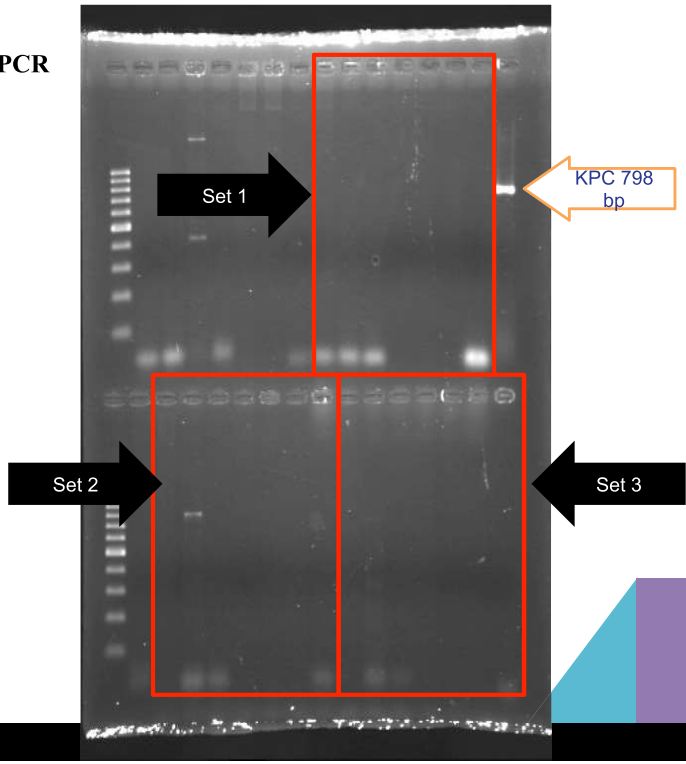


Fig 1. Schematic of the CIM.

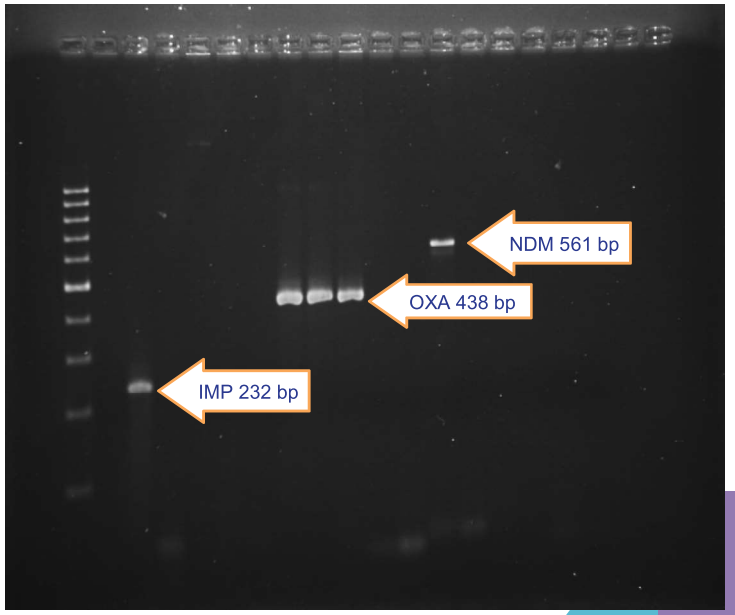
doi:10.1371/journal.pone.0123690.g001

van der Zwaluw K, de Haan A, Pluister GN, et al. The Carbapenem Inactivation Method (CIM), a simple and low-cost alternative for the Carba NP Test to assess phenotypic carbapenemase activity in Gram-Negative rods. PLoS ONE 2015; 10(3): e0123690.

ผลการทำ PCR



ผลการทำ PCR



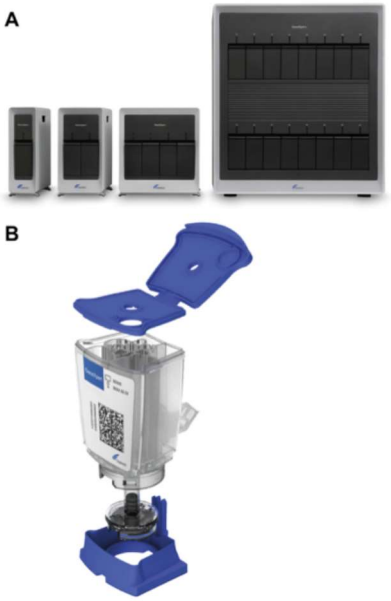


Fig. 1. Cepheid GenXpert system. (*A*) Instruments with 1- to 16-cartridge capacity. (*B*) Exploded view of GenXpert cartridge. (*Courtesy of* Cepheid, Sunnyvale, CA; with permission.)

Cepheid
GeneXpert

CRE stool
Screening

KPC
NDM

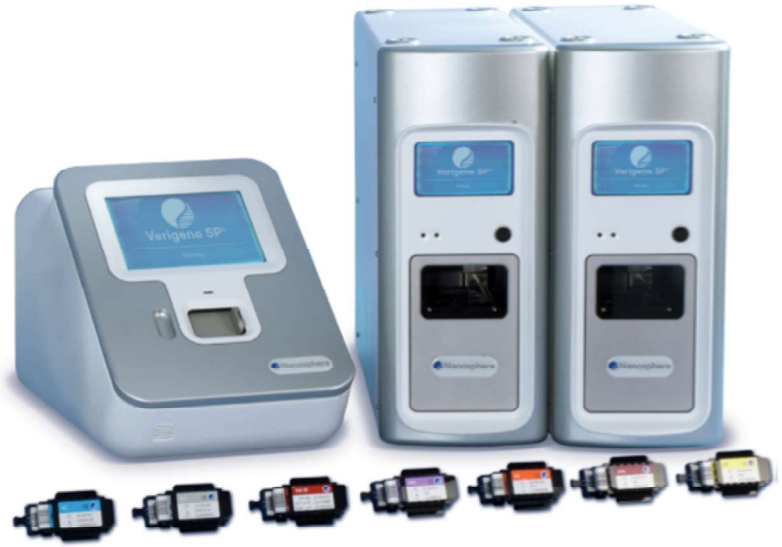
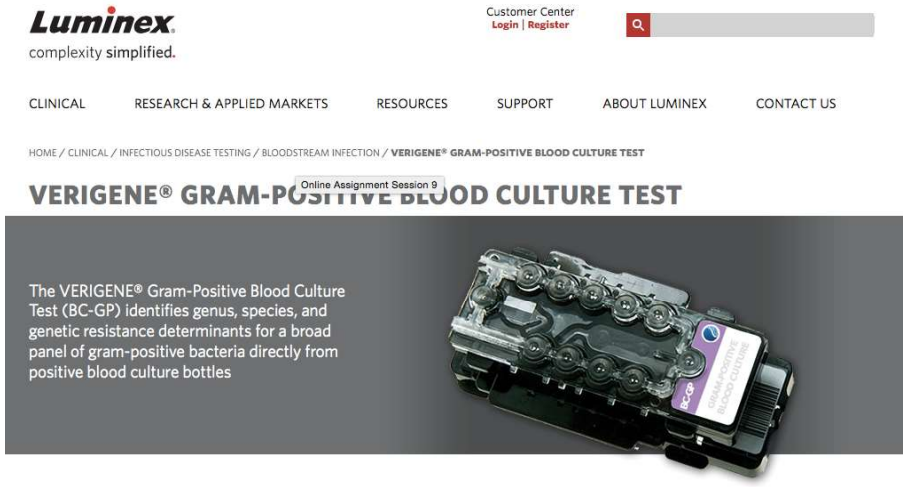


Fig. 3. Nanosphere Verigene system. Verigene processor on the right, reader and cartridges on the left. Instrument footprints in inches: Processor, 7.6 width × 18.7 height × 22.9 depth; reader, 11.7 width × 12.4 height × 20.5 depth. Processors are stackable. No computer is required for operation. (*Courtesy of* Nanosphere, Northbrook, IL; with permission.)



Gram-Positive Blood Culture Test Specifications

Targets	U.S./FDA-Cleared	Outside U.S.
Species		
Staphylococcus aureus	▪	▪
Staphylococcus epidermidis	▪	▪
Staphylococcus lugdunensis	▪	▪
Streptococcus anginosus Group	▪	▪
Streptococcus agalactiae	▪	▪
Streptococcus pneumoniae	▪	▪
Streptococcus pyogenes	▪	▪
Enterococcus faecalis	▪	▪
Enterococcus faecium	▪	▪

Genus		
Staphylococcus spp.	▪	▪
Streptococcus spp.	▪	▪
Micrococcus spp.		▪
Listeria spp.	▪	▪
Resistance		
mecA (methicillin)	▪	▪
vanA (vancomycin)	▪	▪
vanB (vancomycin)	▪	▪

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HOME / CLINICAL / INFECTIOUS DISEASE TESTING / BLOODSTREAM INFECTION / **VERIGENE® GRAM-NEGATIVE BLOOD CULTURE TEST**

VERIGENE® GRAM-NEGATIVE BLOOD CULTURE TEST

The VERIGENE® Gram-Negative Blood Culture Test (BC-GN) identifies genus, species, and genetic resistance determinants for a broad panel of gram-negative bacteria directly from positive blood culture bottles

Gram-Negative Blood Culture Test Specifications

Targets	U.S./FDA-Cleared	Outside U.S.
Species		
*Escherichia coli**	▪	▪
Klebsiella pneumoniae	▪	▪
Klebsiella oxytoca	▪	▪
Pseudomonas aeruginosa	▪	▪
Serratia marcescens		▪
Genus		
Acinetobacter spp.	▪	▪
Citrobacter spp.	▪	▪
Enterobacter spp.	▪	▪
Proteus spp.	▪	▪

Resistance		
CTX-M (ESBL)	▪	▪
IMP (carbapenemase)	▪	▪
KPC (carbapenemase)	▪	▪
NDM (carbapenemase)	▪	▪
OXA (carbapenemase)	▪	▪
VIM (carbapenemase)	▪	▪

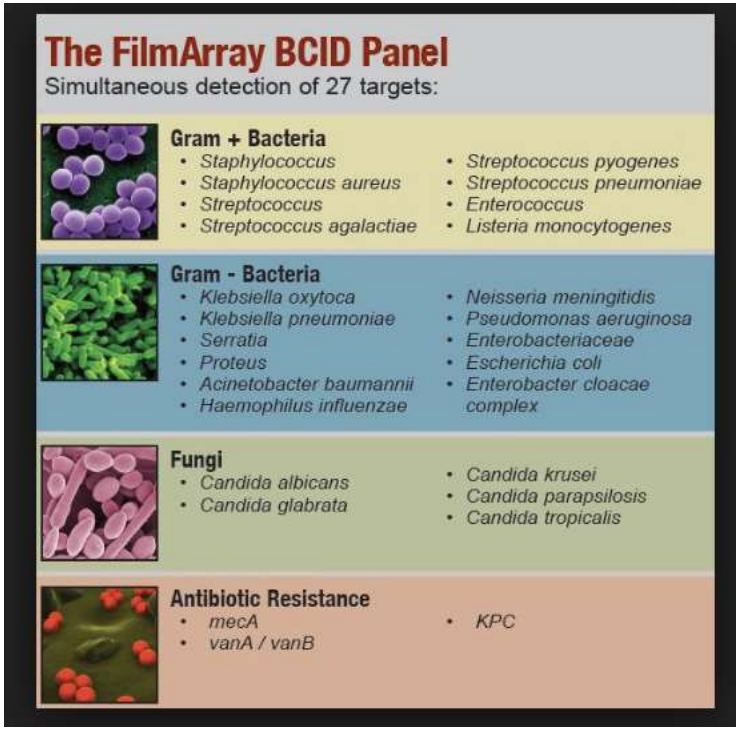
* BC-GN will not distinguish *Escherichia coli* from *Shigella* spp. (*S. dysenteriae, S. flexneri, S. boydii,* and *S. sonnei*).

Caution!
Gram-negative bacteria resistance mechanism
= genotypic (enzymatic) and non genotypic (non-enzymatic)

Fig. 6. BioFire FilmArray respiratory panel assay. (*A*) FilmArray RP Pouch. (*B*) FilmArray instrument and pouch. Instrument footprint in inches: 10.00 width × 6.5 height × 15.5 depth. A computer is required for operation. (*Courtesy of* BioFire Diagnostics Inc, Salt Lake City, UT; with permission.)

- **Biofire Filmarray**

Positive **Blood panel** (Multiplex PCR) (1 h result)



MIC vs Disk Diffusion Test

Interpretative criteria/guideline: CLSI, EUCAST

Treatment guideline: research data, patient care

The interpretive errors with our hypothetical disk diffusion test are categorized as follows:

Error Category	MIC	Disk Diffusion
Very Major (false susceptible)	R	S
Major (false resistant)	S	R
Minor	S or R	I
Minor	I	S or R

For antimicrobial agent "X," the following interpretive criteria were derived:

Method	Susceptible	Intermediate	Resistant
Disk Diffusion (mm)	≥21	17–20	≤16
MIC (mcg/mL)	≤2	4	≥8

Specimen source: Wound drainage
Results: *Pseudomonas aeruginosa*

Drug	Susceptibility
Ceftazidime	S
Ciprofloxacin	R
Gentamicin	S
Imipenem	S
Piperacillin	S
Tobramycin	S

Do you see any difference between gentamicin and tobramycin?
Now view the MIC report, do you see any difference between gentamicin and tobramycin?

Drug	Interpretation	MIC
Ceftazidime	S	<0.5
Ciprofloxacin	R	>4
Gentamicin	S	4
Imipenem	S	<0.5
Piperacillin	S	<8
Tobramycin	S	0.5

MIC vs Disk Diffusion Test

Patient dose not response with Gentamicin. Why?

Note:
MIC Tobramycin is lower than Gentamicin (all susceptible)
In vitro data
Tobramycin is a better choice.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

New Jan 2017 (CLSI: M100S27)

27th Edition

M100

Performance Standards for Antimicrobial Susceptibility Testing

Interpretive Criteria

Interpretive criteria are the MIC or zone diameter values used to indicate susceptible, intermediate, and resistant breakpoints.

Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)		
		S	I	R	S	I	R
X	30 µg	≥20	15–19	≤14	≤4	8–16	≥32
Y	—	—	—	—	≤1	2	≥4
Z	10 µg	≥16	—	—	≤1	—	—

For example, for antimicrobial agent X with interpretive criteria in the table above, the susceptible breakpoint is 4 µg/mL or 20 mm and the resistant breakpoint is 32 µg/mL or 14 mm.

Contents

Table 2B-5. Minimal Inhibitory Concentration Breakpoints (µg/mL) for Other Non-*Enterobacteriaceae* (Refer to General Comment 1)

Testing Conditions

Medium: Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35°C ± 2°C; ambient air; 16–20 hours

Routine QC Recommendations (See Tables 4A and 5A for acceptable QC ranges.)

Escherichia coli ATCC® 25922 (for chloramphenicol, tetracyclines, sulfonamides, and trimethoprim-sulfamethoxazole)
Pseudomonas aeruginosa ATCC® 27853
Escherichia coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Other non-*Enterobacteriaceae* include *Pseudomonas* spp. (not *P. aeruginosa*) and other nonfastidious, glucose-nonfermenting, gram-negative bacilli, but exclude *P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia*, *B. mallei*, *B. pseudomallei*, and *Stenotrophomonas maltophilia*. Refer to Tables 2B-2, 2B-3, and 2B-4 for testing of *Acinetobacter* spp., *B. cepacia* complex, and *S. maltophilia*, respectively, and CLSI document M45 for testing of *Burkholderia mallei*, *B. pseudomallei*, *Aeromonas* spp., and *Vibrio* spp.

(2) For other non-*Enterobacteriaceae*, the disk diffusion method has not been systematically studied. Therefore, for this organism group, disk diffusion testing is not recommended.

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

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									
O	Piperacillin	–	–	–	–	≤16	32–64	≥128	
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS									
B	Piperacillin-tazobactam	–	–	–	–	≤16/4	32/4–64/4	≥128/4	
O	Ticarcillin-clavulanate	–	–	–	–	≤16/2	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
A	Ceftazidime	–	–	–	–	≤8	16	≥32	
B	Cefepime	–	–	–	–	≤8	16	≥32	
C	Cefotaxime	–	–	–	–	≤8	16–32	≥64	
C	Ceftriaxone	–	–	–	–	≤8	16–32	≥64	
O	Cefoperazone	–	–	–	–	≤16	32	≥64	
O	Ceftizoxime	–	–	–	–	≤8	16–32	≥64	
O	Moxalactam	–	–	–	–	≤8	16–32	≥64	

Non-Enterobacteriacae (not *Acinetobacter, Pseudomonas*): No disk diffusion breakpoint

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
MONOBACTAMS											
C	Aztreonam	30 µg	≥21	–	18–20	≤17	≤4	–	8	≥16	**(25) Breakpoints** are based on a dosage regimen of 1 g every 8 h. See comment (8).
CARBAPENEMS											
(26) Following evaluation of PK-PD properties, limited clinical data, and MIC distributions that include recently described carbapenemase-producing strains, revised **breakpoints** for carbapenems were first published in June 2010 (M100-S20-U) and are listed below. Because of limited treatment options for infections caused by organisms with carbapenem MICs or zone diameters in the intermediate range, clinicians may wish to design carbapenem dosage regimens that use maximum recommended doses and possibly prolonged intravenous infusion regimens, as has been reported in the literature.[1-4] Consultation with an infectious diseases practitioner is recommended for isolates for which the carbapenem MICs or zone diameter results from disk diffusion testing are in the intermediate or resistant ranges.										
Laboratories using *Enterobacteriaceae* MIC **breakpoints** for carbapenems described in M100-S20 (January 2010) should perform the MHT, the Carba NP test, **mCIM**, and/or a molecular assay when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL (refer to Tables 3B, 3C, **and 3D**). After implementation of the current **breakpoints**, **these additional tests do** not need to be performed other than for epidemiological or infection control purposes (refer to Table 3B).										
The following information is provided as background on carbapenemases in *Enterobacteriaceae* that are largely responsible for MICs and zone diameters in the intermediate and resistant ranges, and thus the rationale for setting revised carbapenem breakpoints:										
• The clinical effectiveness of carbapenem treatment of infections produced by isolates for which the carbapenem MIC or disk diffusion test results are within the intermediate range is uncertain due to lack of controlled clinical studies.										
Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (eg, MICs in the intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated imipenem MICs by mechanisms other than production of carbapenemases.											
B	Doripenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(27) Breakpoints** are based on a dosage regimen of 500 mg every 8 h.
B	Ertapenem	10 µg	≥22	–	19–21	≤18	≤0.5	–	1	≥2	**(28) Breakpoints** are based on a dosage regimen of 1 g every 24 h.
B	Imipenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(29) Breakpoints** are based on a dosage regimen of 500 mg every 6 h or 1 g every 8 h.
B	Meropenem	10 µg	≥23	–	20–22	≤19	≤1	–	2	≥4	**(30) Breakpoints** are based on a dosage regimen of 1 g every 8 h.
AMINOGLYCOSIDES											
(31) WARNING: For *Salmonella* spp. and *Shigella* spp., aminoglycosides may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.											
A	Gentamicin	10 µg	≥15	–	13–14	≤12	≤4	–	8	≥16	
A	Tobramycin	10 µg	≥15	–	13–14	≤12	≤4	–	8	≥16	
B	Amikacin	30 µg	≥17	–	15–16	≤14	≤16	–	32	≥64	
O	Kanamycin	30 µg	≥18	–	14–17	≤13	≤16	–	32	≥64	

Using CLSI 2010 breakpoint: no need for ESBL/ CRE confirmation test

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(CLSI: M100S27)

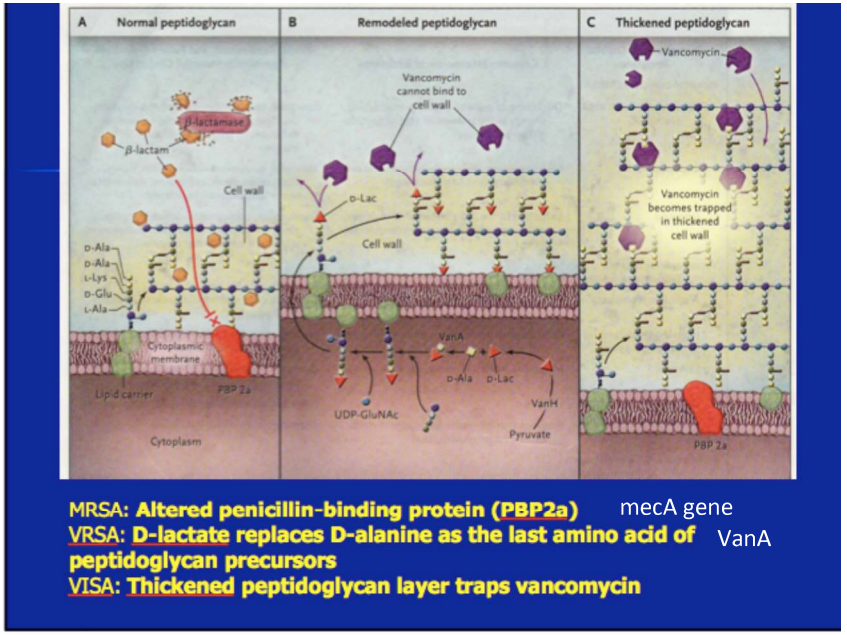
Table 2C. *Staphylococcus* spp. (Continued)

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
GLYCOPEPTIDES									
(19) For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.									
B	Vancomycin (For *S. aureus*)	–	–	–	–	≤2	4–8	≥16	For use with *S. aureus*. **(20)** MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of CoNS, all of which give similar size zones of inhibition. **(21)** Send any *S. aureus* for which the vancomycin is ≥ 8 µg/mL to a reference laboratory. See Appendix A. Also refer to Table 3G for *S. aureus*, Subchapter 3.13.1.7 in M07-A10, and Subchapter 3.9.1.7 in M02-A12.
B	Vancomycin (For CoNS)	–	–	–	–	≤4	8–16	≥32	For use with CoNS. See comment **(20)**. **(22)** Send any CoNS for which the vancomycin MIC is ≥ 32 µg/mL to a reference laboratory. See Appendix A. See also Subchapter 3.13.1.7 in M07-A10, and Subchapter 3.9.1.7 in M02-A12.
Inv.	Teicoplanin	–	–	–	–	≤8	16	≥32	
LIPOGLYCOPEPTIDES									
C	Oritavancin	–	–	–	–	≤0.12	–	–	See comment **(17)**.
C	Telavancin	–	–	–	–	≤0.12	–	–	See comment **(17)**.
LIPOPEPTIDES									
B	Daptomycin	–	–	–	–	≤1	–	–	**(23)** Daptomycin should not be reported for isolates from the respiratory tract.

For Use With M02-A12 and M07-A10

M100, 27th ed.

MRSA: Vancomycin test = MIC only
No disk diffusion breakpoint



MRSA: **Altered penicillin-binding protein (PBP2a)** mecA gene
VRSA: **D-lactate replaces D-alanine as the last amino acid of peptidoglycan precursors** VanA
VISA: **Thickened peptidoglycan layer traps vancomycin**

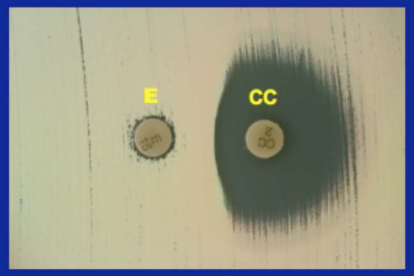
NEJM-review, IDSA Guideline for MRSA treatment, Endocarditis treatment MSSA vs MRSA
Definition, Mechanism of resistance, CA-MRSA vs HA-MRSA (USA type, Scc type)

Vancomycin Resistance in *S. aureus*

Strain	Definition	Genetic event	Mechanism/ Significance
Pen Resistance		Penicillinase	Enzyme Modification
MRSA	Meth/Ox resistance	*mec*A	PBP2a ***Normal Cell wall***
Vanc suscept *S. aureus*-VSSA	**MIC ≤ 2 µg/mL**	-	
Vanco-intermediate *S. aureus* (VISA)	MIC 4-8 µg/mL	Unknown; ? *vraSR* & *graSR* mutation Cui *AAC* 2009; 53:1231	***-Thickened cell wall*** - increased vanco binding
Vanco-resistant *S. aureus* (VRSA)	MIC ≥ 16 µg/mL	**vanA from VR *E. faecalis***	***Remodeled Cell Wall*** D-ala-D-ala to D-ala-D-lactate

Vancomycin MIC creeping (MIC > 1) → more treatment failure
→ Vancomycin should not be avoid.
Use: Linezolid/ Daptomycin/ Ceftaroline
Endocarditis, Severe Pneumonia, Severe Skin infection, Osteomyelitis
Need to monitor Vancomycin MIC and vancomycin trough level (drug level)

Resistance due to MLS_B



-Perform on all erythro-R, clinda- S *S. aureus* isolates
-Treatment failures have occurred

D- test: Micro labs report should be….
Erythromycin-Resistance
Clindamycin-Resistance
(Macrolide-inducible clindamycin resistance)

New Jan 2017 (CLSI: M100S27)

Table 2G. *Streptococcus pneumoniae* (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									
(5) 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), ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefepime, cefotaxime, cefpodoxime, cefprozil, ceftaroline, ceftizoxime, ceftriaxone, cefuroxime, doripenem, ertapenem, imipenem, loracarbef, meropenem. See general comment (4).									
A	Penicillin	1 µg oxacillin	≥20	–	–	–	–	–	**(6)** 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	**(7)** ***Rx:*** Doses of intravenous penicillin of at least 2 million units every 4 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 necessitate penicillin doses of 18–24 million units per day. **(8)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
A	Penicillin parenteral (meningitis)	–	–	–	–	≤0.06	–	≥0.12	**(9)** ***Rx:*** Use of penicillin in meningitis requires therapy with maximum doses of intravenous penicillin (eg, at least 3 million units every 4 hours in adults with normal renal function). **(10)** For CSF isolates, report only meningitis interpretations. **See General Comment (4).**
A	Penicillin (oral penicillin V)	–	–	–	–	≤0.06	0.12–1	≥2	**(11)** Interpretations for oral penicillin may be reported for isolates other than those from CSF.

***S. pneumoniae*: Disk diffusion breakpoint only for Penicillin susceptible (zone of inhibition more than 20mm)**

For Use With M02-A12 and M07-A10
M100, 27th ed.

New Jan 2017 (CLSI: M100S27)

Table 2G. *Streptococcus pneumoniae* (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 (Continued)									
C	Amoxicillin (nonmeningitis)	–	–	–	–	≤2	4	≥8	
C	Amoxicillin-clavulanate (nonmeningitis)					≤2/1	4/2	≥8/4	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
See comment (5).									
O	Cefepime (meningitis)	–	–	–	–	≤0.5	1	≥2	**(12)** 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.
B	Cefepime (nonmeningitis)	–	–	–	–	≤1	2	≥4	**(13)** In the United States, only report interpretations for nonmeningitis and include the nonmeningitis notation on the report.
B	Cefotaxime (meningitis)	–	–	–	–	≤0.5	1	≥2	**(14)** For CSF isolates, report only meningitis interpretations. **(15)** ***Rx:*** Use of cefotaxime or ceftriaxone in meningitis requires therapy with maximum doses. See general comment (4).
B	Ceftriaxone (meningitis)	–	–	–	–	≤0.5	1	≥2	
B	Cefotaxime (nonmeningitis)	–	–	–	–	≤1	2	≥4	**(16)** For all isolates other than those from CSF, report interpretations for both meningitis and nonmeningitis.
B	Ceftriaxone (nonmeningitis)	–	–	–	–	≤1	2	≥4	
C	Ceftaroline (nonmeningitis)	30 µg	≥26	–	–	≤0.5	–	–	**(17)** Breakpoints are based on a dosage regimen of 600 mg every 12 h.
C	Cefuroxime (parenteral)	–	–	–	–	≤0.5	1	≥2	
CEPHEMS (ORAL)									See comment (5).
C	Cefuroxime (oral)	–	–	–	–	≤1	2	≥4	
O	Cefaclor	–	–	–	–	≤1	2	≥4	
O	Cefdinir	–	–	–	–	≤0.5	1	≥2	
O	Cefpodoxime	–	–	–	–	≤0.5	1	≥2	
O	Cefprozil	–	–	–	–	≤2	4	≥8	
O	Loracarbef	–	–	–	–	≤2	4	≥8	

***S. pneumoniae*: Disk diffusion breakpoint only for Penicillin susceptible (zone of inhibition more than 20mm)**

M100, 27th ed.
For Use With M02-A12 and M07-A10

New Jan 2017 (CLSI: M100S27)

Table 2G. *Streptococcus pneumoniae* (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
CARBAPENEMS									
See comment (5).									
B	Meropenem	–	–	–	–	≤0.25	0.5	≥1	See general comment (4) and comment (6).
C	Ertapenem	–	–	–	–	≤1	2	≥4	
C	Imipenem	–	–	–	–	≤0.12	0.25–0.5	≥1	
O	Doripenem	–	–	–	–	≤1	–	–	
GLYCOPEPTIDES									
B	Vancomycin	30 µg	≥17	–	–	≤1	–	–	See general comment (4).
MACROLIDES									
(18) Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin. **(19)** Not routinely reported for organisms isolated from the urinary tract.									
A	Erythromycin	15 µg	≥21	16–20	≤15	≤0.25	0.5	≥1	
O	Azithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Clarithromycin	15 µg	≥21	17–20	≤16	≤0.25	0.5	≥1	
O	Dirithromycin	15 µg	≥18	14–17	≤13	≤0.5	1	≥2	
O	Telithromycin	15 µg	≥19	16–18	≤15	≤1	2	≥4	
TETRACYCLINES									
(20) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline.									
B	Tetracycline	30 µg	≥28	25–27	≤24	≤1	2	≥4	
B	Doxycycline	30 µg	≥28	25–27	≤24	≤0.25	0.5	≥1	
FLUOROQUINOLONES									
B	Gemifloxacin	5 µg	≥23	20–22	≤19	≤0.12	0.25	≥0.5	**(21)** *S. pneumoniae* isolates susceptible to levofloxacin are predictably susceptible to gemifloxacin and moxifloxacin. However, *S. pneumoniae* susceptible to gemifloxacin or moxifloxacin cannot be assumed to be susceptible to levofloxacin.
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
B	Moxifloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Gatifloxacin	5 µg	≥21	18–20	≤17	≤1	2	≥4	
O	Ofloxacin	5 µg	≥16	13–15	≤12	≤2	4	≥8	
O	Sparfloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
FOLATE PATHWAY INHIBITORS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥19	16–18	≤15	≤0.5/9.5	1/19–2/38	≥4/76	
PHENICOLS									
C	Chloramphenicol	30 µg	≥21	–	≤20	≤4	–	≥8	See comment (19).
ANSAMYCINS									
C	Rifampin	5 µg	≥19	17–18	≤16	≤1	2	≥4	**(22)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.

***S. pneumoniae*: Disk diffusion breakpoint only for Penicillin susceptible (zone of inhibition more than 20mm)**

For Use With M02-A12 and M07-A10
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Sanford Guideline (Infectious Disease Treatment GL)

Viridans strep, *S. bovis (S. gallolyticus)* **endocarditis**

- **Pen G MIC <0.12 mcg/mL = Susceptible**

→ Pen G or Ceftriaxone x 4 weeks

→ shorten duration = Pen G or Ceftriaxone PLUS gentamicin x 2 wks

- **Pen G MIC > 0.12 to < 0.5 = Intermediate resistance**

→ Pen G or Ceftriaxone x 4 weeks PLUS gentamicin x 2 weeks

→ Vancomycin x 4 weeks

- **Pen G MIC > 0.5 = Resistance**

→ Pen G (or Ampicillin) PLUS gentamicin x 4-6 weeks

→ Vancomycin PLUS gentamicin x 4-6 weeks

Other indication for MIC monitoring/use

***Enterococcus Endocarditis, *S. pneumoniae* meningitis Osteomyelitis, Endovascular infection, treatment failure etc.**

****MDR pathogen: MRSA ESBL/CRE**

ESBLs treatment

- **Severe infection/ bacteremia**
- Recommended to use **Carbapenems**
- Fosfomycin?
- Aminoglycoside (source of infection)
- **UTIs, de-escalation therapy**
- Depend on susceptibility profiles
- **Pip/tazo (if low MIC)?**
- Newer version quinolones? (if no bacteremia)
- Fosfomycin
- Aminoglycoside etc
- **Isolation the patient if possible (or cohorting the patient)**
- **In Thailand, may not need for isolation (high prevalence)**

Tamma PD et al. Carbapenem therapy is associated with improved survival compared with piperacillin-tazobactam for patients with extended-spectrum β-lactamase bacteremia. Clin Infect Dis. 2015;60(9):1319-25.
Perez F, Bonomo RA Editorial Commentary: Bloodstream Infection Caused by Extended-Spectrum β-Lactamase–Producing Gram-Negative Bacteria: How to Define the Best Treatment Regimen? Clin Infect Dis. 2015;60(9):1326-29

Treatment Options for Carbapenem-Resistant *Enterobacteriaceae* Infections

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ID consultation

Combination therapy with
Colistin/ polymixin B/ Aminoglycoside/ Tigecycline etc.
Carbapenem high dose/ prolong infusion
(need to check MIC)**

Room Isolation (Plasmid transferable)

Keywords. carbapenemases; carbapenem-resistant *Ente*
treatment.

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DOI: 10.1093/ofid/ofv050

Colistin and polymyxin B susceptibility testing for carbapenem-resistant and *mcr*-positive *Enterobacteriaceae*: Comparison of Sensititre, Microscan, Vitek 2, and Etest with broth microdilution

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Running title: Colistin and polymyxin B susceptibility testing

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Sensitivity of colistin in *mcr-1* detection (N=21)

Table 2. Sensitivity of colistin and polymyxin B susceptibility testing methods for the detection of *mcr-1* positive isolat

Drug	Method	Susceptibility with breakpoint of ≤2mg/L (%)	Susceptibility with breakpoint of ≤1mg/L (%)
Colistin	BMD	71.4	90.5
	Sensititre	100	100.0
	Vitek 2	42.9	95.2
	E-test	76.2	95.2
	Microscan	100	N/A
Polymyxin B	BMD	81.0	85.7
	Sensititre	95.2	100.0
	Vitek 2	95.2	100.0
	E-test	66.7	95.2

N/A: Not applicable as lowest MIC interpretation possible for Microscan (colistin) is 2 mg/L.

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Table3: Performance characteristics of colistin and polymyxin B susceptibility testing methods in comparison to broth microdilution (BMD)

Drug	Method	Susceptible	Resistant	EA n (%)	CA n (%)	VME n (%)	ME n (%)	Spearman's coefficient
Colistin	BMD	51	25		N/A			
	Vitek2	60	16	71 (93.4%)#	67 (88.2%)	9 (36.0%)	0 (0%)	0.873*
	Sensititre	46	30	68 (89.5%)	69 (90.1%)#	1 (4%)	6 (11.8%)	0.863*
	E-test	51	25	57(75.0%)	70 (92.1%)#	3 (12.0%)	3 (5.9%)	0.600*
	Microscan	44	32	N/A**	67 (88.2%)	1 (4%)	8 (15.8%)	N/A**
Polymyxin B	BMD	49	27		N/A			
	Vitek2	47	29	73 (96.1%)#	72 (94.7%)#	1 (3.7%)	3 (6.1%)	0.917*
	Sensititre	47	29	73 (96.1%)#	72 (94.7%)#	1 (3.7%)	3 (6.1%)	0.877*
	E-test	53	23	38 (48.7%)	68 (89.5%)	6 (26.1%)	1 (1.9%)	0.534*

BMD: Broth Microdilution, EA: Essential agreement, CA: categorical agreement VME: Very major error, ME: Major error
N/A: Not applicable
Spearman's coefficient indicates concordance of MIC against BMD.
#Indicates that testing method-drug combination meets CLSI M52 recommendations for acceptable EA or CA performance
*p-value <0.001
**EA and Spearman's correlation coefficient not determined for Microscan due to narrow MIC range

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Rapid polymyxin NP

TAT 2 h

Sensitivity 99.3%
Specificity 95.4%

From
135 colistin R isolates
and 65 cosistin S isolates

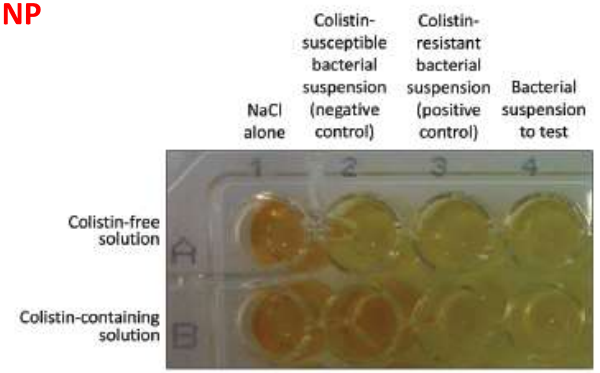

Figure. Representative results of the rapid polymyxin NP [Nordmann/Poirel] test. Noninoculated wells are shown as controls (first column). The rapid polymyxin NP test was performed with a reference colistin-susceptible isolate (second column) and with a reference colistin-resistant isolate (third column) in a reaction medium without (upper row) and with (lower row) colistin. The tested isolate grew in the presence (and absence) of colistin (wells B4 and A4, respectively) and was therefore reported to be colistin-resistant.

Rapid Detection of Polymyxin Resistance in *Enterobacteriaceae*

Patrice Nordmann, Aurélie Jayol, Laurent Poirel

For identification of polymyxin resistance in *Enterobacteriaceae*, we developed a rapid test that detects glucose metabolization associated with bacterial growth in the presence of a defined concentration of colistin or polymyxin B. Formation of acid metabolites is evidenced by a color change (orange to yellow) of a pH indicator (red phenol). To evaluate the test, we used bacterial colonies of 135 isolates expressing various mechanisms of colistin resistance (intrinsic, chromosomally encoded, and plasmid-mediated MCR-1) and 65 colistin-susceptible isolates. Sensitivity and specificity were 99.3% and 95.4%, respectively, compared with the standard broth microdilution method. This new test is inexpensive, easy to perform, sensitive, specific, and can be completed in <2 hours. It could be useful in countries facing endemic spread of carbapenemase producers and for which polymyxins are last-resort drugs.

Among the most clinically significant multidrug-resistant bacteria are carbapenemase-producing *Enterobacteriaceae*. Because these bacteria usually remain susceptible to polymyxins, an old class of antimicrobial drugs two-component systems or alterations of the *mgrB* gene) (*6*). A recent report revealed that addition of phosphoethanolamine may also be plasmid mediated through the *mcr-1* gene, which confers the first known plasmid-mediated resistance to colistin in isolates from humans and animals (*7*). More recently, the *mcr-1* gene was identified in several plasmid backbones, mostly in *Escherichia coli* (*8–10*). There is therefore a need for a test that enables rapid detection of polymyxin resistance in *Enterobacteriaceae* and that may contribute to its containment.

We developed a test (the rapid polymyxin NP [Nordmann/Poirel] test) that detects bacterial growth in the presence of a defined concentration of a polymyxin. Bacterial growth detection (or absence) is based on carbohydrate metabolism (*11*). Acid formation associated with carbohydrate metabolism in *Enterobacteriaceae* can be observed through the color change of a pH indicator. This test is rapid (<2 h) and easy to perform.

Materials and Methods