

Clinical Microbiology 2: Antimicrobial Susceptibility Tests

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13 March 2018

IDAT Week 2018



Antimicrobial Susceptibility Tests

- Basic principle
- Antimicrobial Susceptibility Tests (AST)
 - Phenotypic test
 - Genotypic test
- Clinical correlation and clinical application
- Summary

- I have no conflict interest to be disclosure



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

- 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)



Manual of Antimicrobial Susceptibility Testing

Marie B. Coyle, Coordinating Editor



AMERICAN
SOCIETY FOR
MICROBIOLOGY



Organización
Panamericana
de la Salud
Oficina Regional de la
Organización Mundial de la Salud

American Society for Microbiology, © 2005

CLSI Documents - Examples

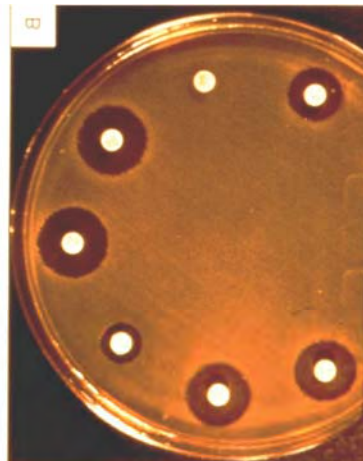
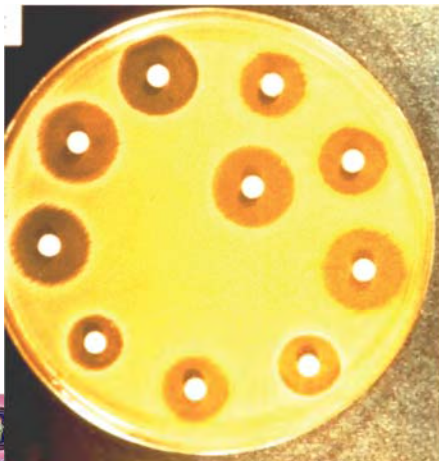
- M2 – Performance Standards for Antimicrobial Disk Susceptibility Tests
- M7 – Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically
- M11 – Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria
- M24 – Susceptibility Testing of Mycobacteria, Nocardiae, 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

Kirby-Bauer Method 1. Disk Diffusion

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, EUCAST)



JHU microbiology laboratory



Figure 4.1—Selecting well-isolated colonies for the inoculum



Figure 4.2—Standardizing the inoculum

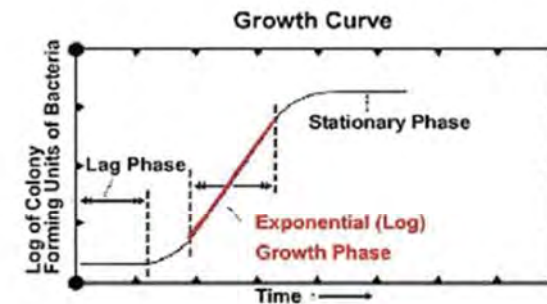


Figure 4.3—Plot of log phase growth in broth

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test



Figure 4.4—Removing excess liquid from the swab



Figure 4.5—Inoculation of the plate

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test



Figure 4.6—Applying disks with dispenser

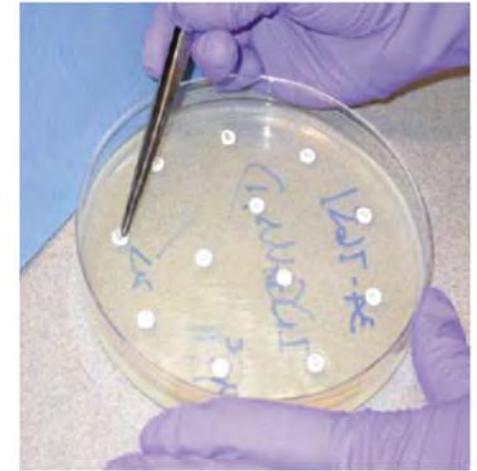


Figure 4.7—Tapping disks to ensure contact

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test



Figure 4.8—Reflected light is used to measure zones from back of plate



Figure 4.9—Measuring zones on a blood agar plate with lid off

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test



Figure 4.10—Double zone of inhibition



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



Figure 4.11—(zone with inner colonies)

Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test

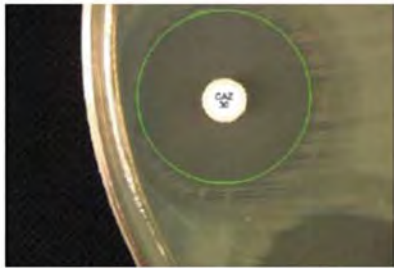


Figure 4.12—Feathered zone around CAZ disk

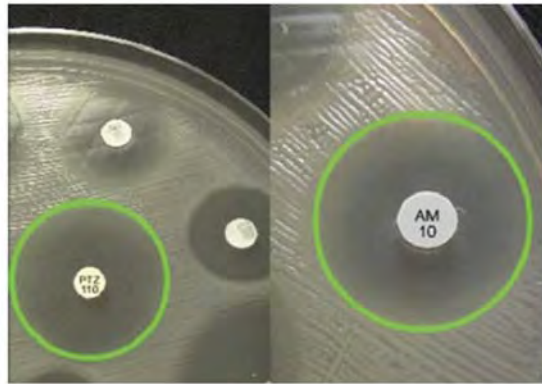


Figure 4.13—Zones with swarming *P. mirabilis*



Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test



Figure 4.15—Heterogeneous resistance to oxacillin

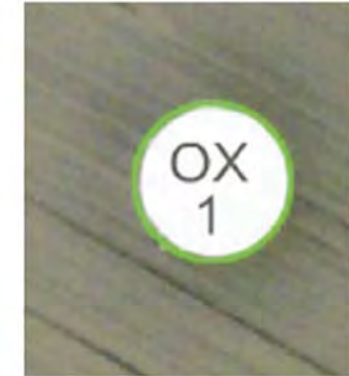


Figure 4.16—Homogeneous resistance to oxacillin



Manual of antimicrobial susceptibility testing, ASM, 2005

Disk Diffusion Test

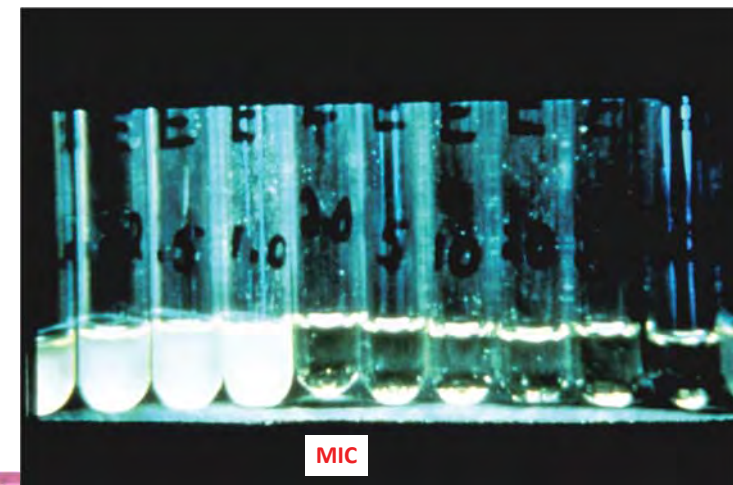
Variable factors

- 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

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MIC test

Dilution of Standardized Inoculum for MIC Tests

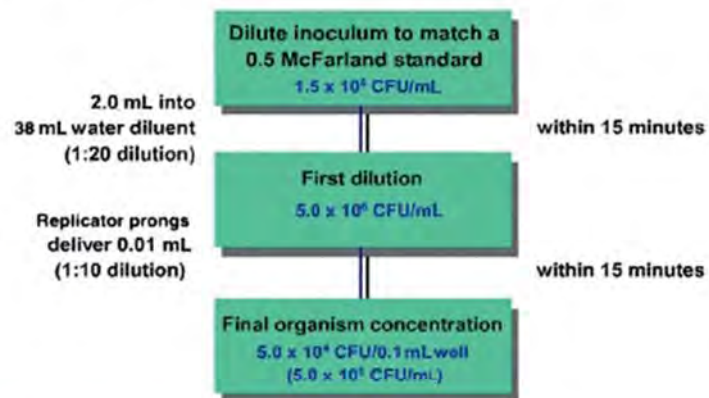
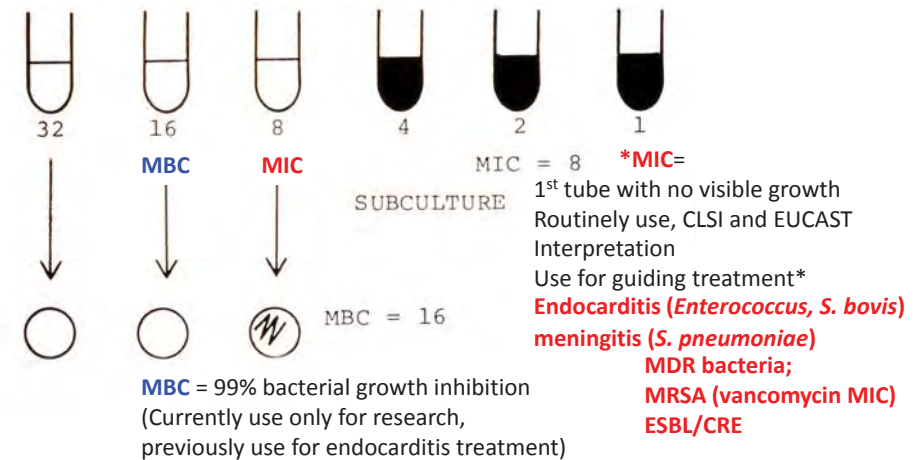


Figure 5.1—Dilution scheme for preparing a standardized inoculum for MIC tests

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



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

MIC test

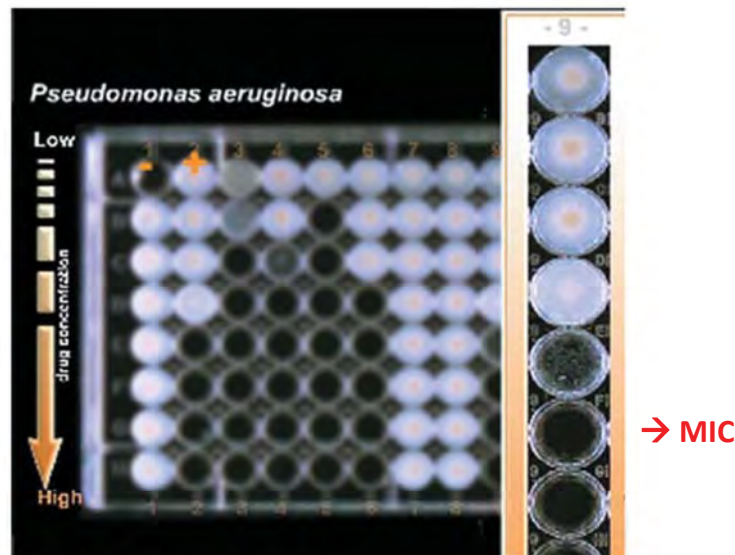


Figure 5.2—An MIC microtiter plate

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

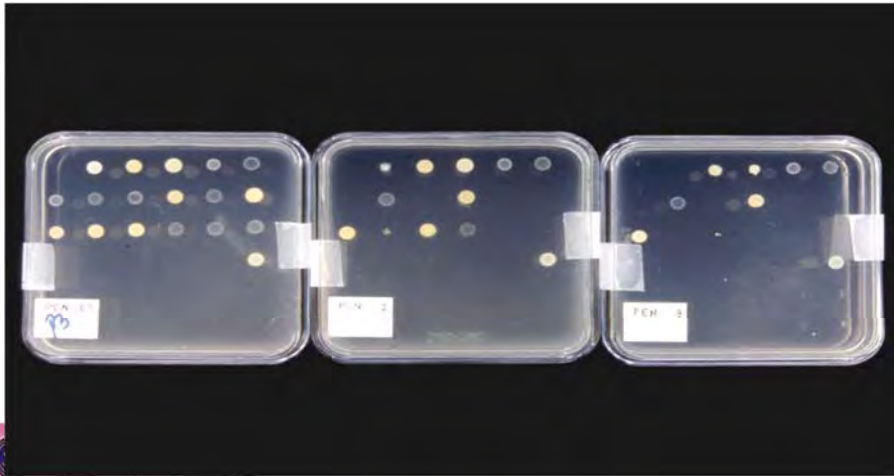
Full Range MIC (mcg/mL)	Breakpoint MIC (mcg/mL)	Interpretation
0.5	–	Susceptible
1.0	–	
2.0	–	
4.0	–	
8.0	8.0	
16.0	16.0	Intermediate
32.0	32.0	Resistant

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

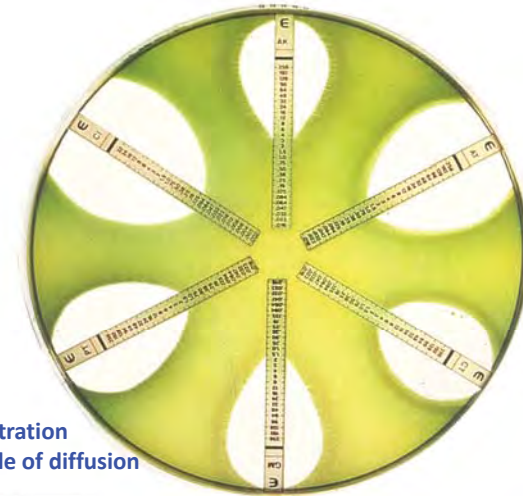
For research: Standard for Anaerobic bacteria AST, *N. gonorrhea* AST



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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

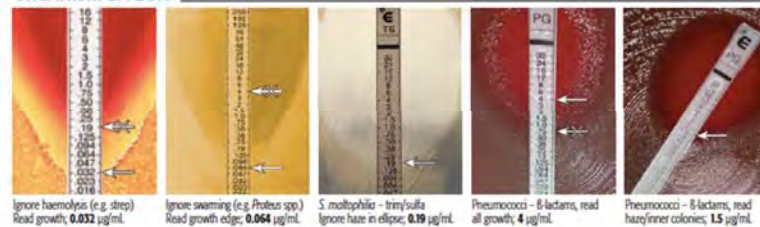
disk diffusion

E= elliptical shape

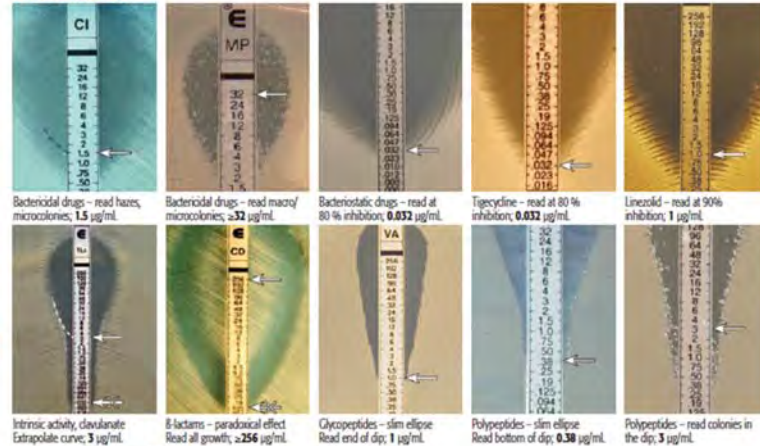
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Etest
For on-scale
MIC determination

ORGANISM EFFECTS



DRUG EFFECTS



AEROBIC BACTERIA

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE

New Jan 2018
(CLSI: M100S28)

28th 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.

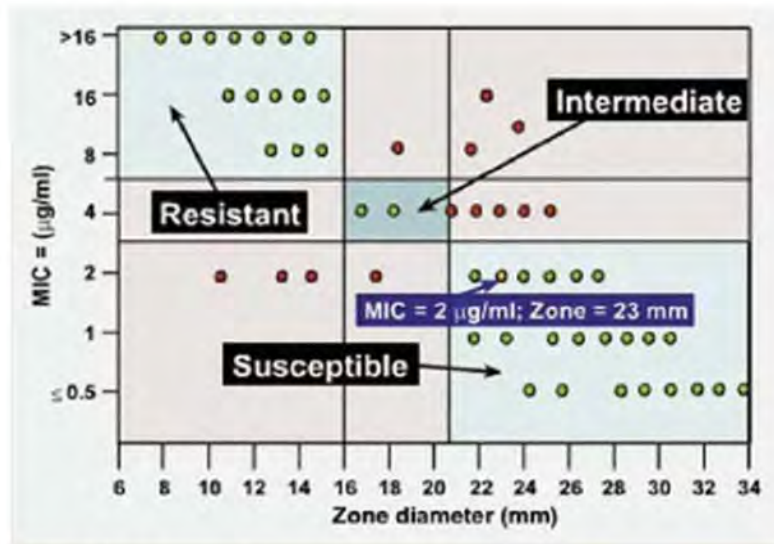


Figure 3.2—Scattergram

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."

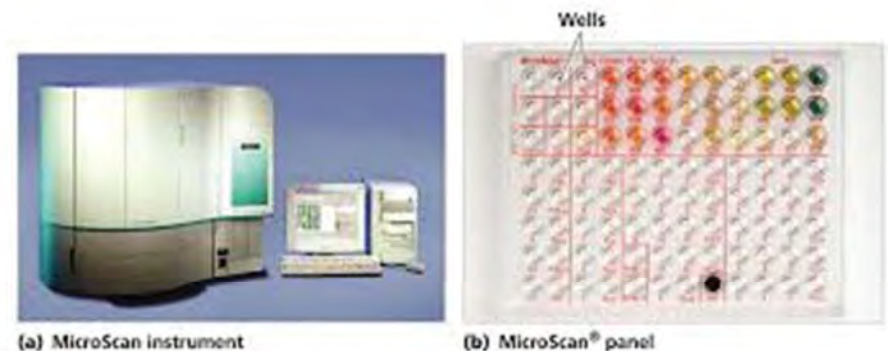
- Breakpoints are established by taking the following steps:
 - Several hundred isolates are tested by the standard NCCLS disk diffusion and MIC methods. The MIC and corresponding zone diameter is plotted for each isolate. In this scatterplot, each dot represents results from testing one or more isolates.
- 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 interpretive criteria to be acceptable, the percentage of errors cannot exceed preset limits established by the FDA and NCCLS.

What do you need from AST?

- Accurate results
(QA/QC, interpretation → identification)
- Short/ Appropriate TAT
- Clinical applicable
- Clinical impact for patient care
- Cost effectiveness

Automated biochemical identification and susceptibility method

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



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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™ 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 (CPE?), accurate MIC

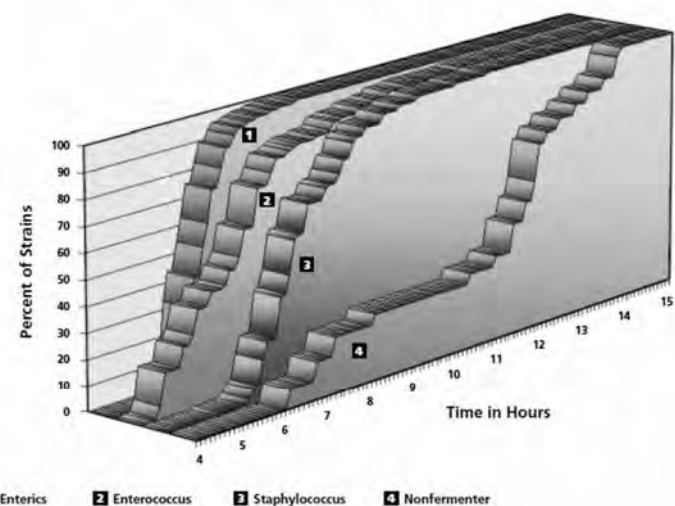
Con: Indeterminate for special bacteria
size of machine (better with M50)/ package?

BD Phoenix™ AP Workflow



Figure 1

Phoenix™ AST Time to Results



Comparison of BD Phoenix AP Workflow with Vitek 2[®]

Alan D. Junkins, Sophie S. Arbefeville, Wanita J. Howard, and Sandra S. Richter*

University of Iowa Carver College of Medicine, Iowa City, Iowa

Received 18 January 2010/Returned for modification 23 February 2010/Accepted 4 March 2010

TABLE 2. Comparison of Vitek 2 and BD Phoenix AP overall time requirements for batches of 14 organisms^a

Time	Mean time ± SD (s) for:		
	Vitek 2	Phoenix AP	Phoenix manual method
Hands-on time per batch	1,414.2 ± 103.7	1,252.5 ± 70.0 ^b	2,487.5
Wait time per batch	48.2 ± 46.0	398.1 ± 173.2	0
Total time per batch	1,464.4 ± 99.6	1,650.6 ± 187.6 ^c	2,487.5
Hands-on time per isolate	101.0	89.5	177.7

^a Sixteen batches of 14 organisms were set up on Vitek 2 and Phoenix AP; only two batches of 14 organisms were set up manually on the Phoenix system.

^b $P < 0.001$ compared to Vitek 2.

^c $P = 0.002$ compared to Vitek 2; $P = 0.162$ compared to the manual method.



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

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



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 $p < 0.05$



Thermo Scientific TREK Diagnostic Systems

Sensititre

The only FDA cleared broth microdilution plate for antifungal susceptibility testing

the New Energy in Automated Microbial Detection

Clinical:

If you have been utilizing the YO2V format, you should have received a letter in October 2010 announcing the discontinuance of this format, and the new YO2VD format. If you did not receive this announcement, please contact TREK Customer Service at this link. Please click here to view a copy of the announcement.

Sensititre® YeastOne® (Part #YO-2V)

- **Colorimetric alamarBlue agent** — Provides reliable, easy and consistent endpoint determination with visual reading or with SensiTouch®.
- **Four antifungal agents** — Yields low cost per test compared to traditional macrobroth dilution tests for *Candida* sp.
- **In vitro diagnostic label** — Allows technician to perform FDA cleared susceptibility tests in house.
- **Two tests per plate** — Allows end user to perform quality control on the same plate.
- **24-hour incubation** — Ensures quick and appropriate patient intervention.
- **Individual packaging** — Allows laboratory to test one plate at a time with no waste.
- **Inclusive on scale QC ranges** — Provides immediate quality assurance of testing methodology.
- **24 month, room temperature storage** — Eliminates inventory control concerns.

Pro
Good for
Yeast
(Candida AST)

MTB/NTM

Colistin AST?

Con
Not automated
system

Mechanism of GN resistance

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

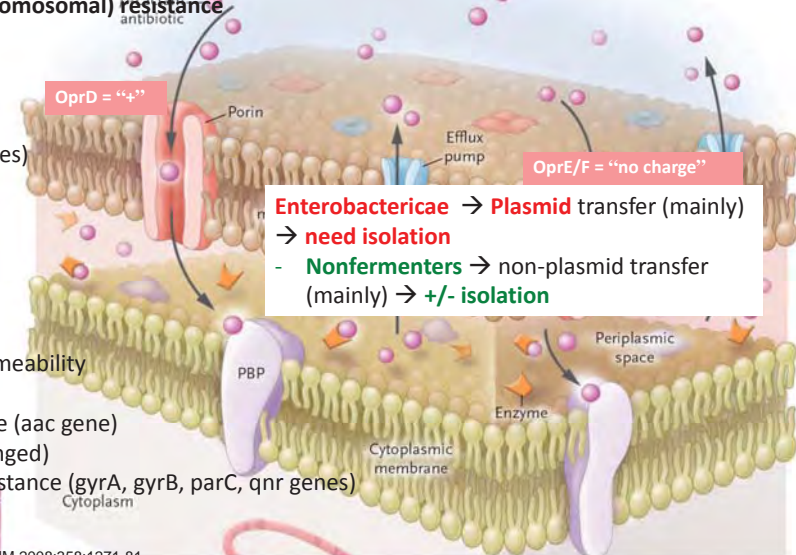
Enzymatic
(hydrolytic enz)
betalactamase
(several bla genes)

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)

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

Phenotypic resistance
VS
Genotypic resistance



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

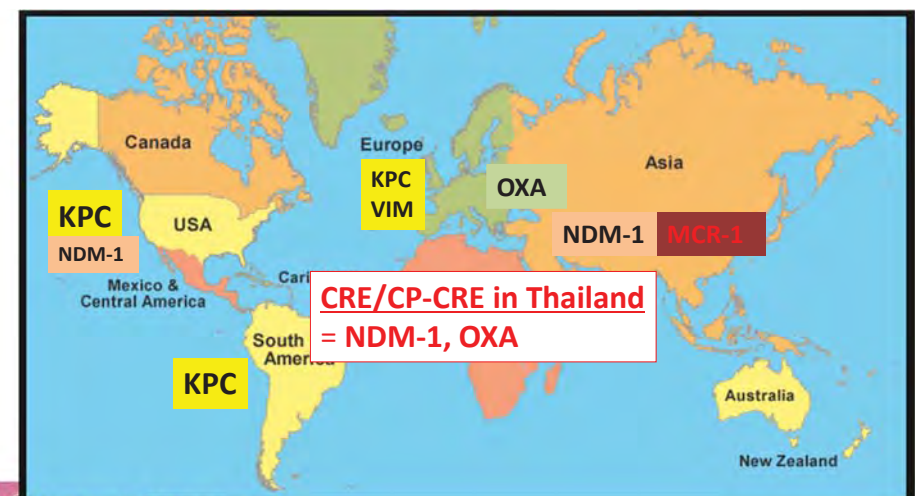
→ **plasmid transferable gene** (carbapenemase)

→ → **Infection control implementation needed**

CDC; Healthcare associated infection

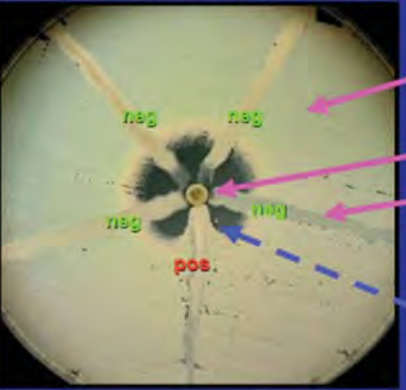
CRE vs CPE (CP-CRE)

Carbapenem resistant Enterobacteriaceae



Variable in **geographic distribution** (Genotypic resistance)

Modified Hodge Test



1. Swab *E. coli* ATCC 25922 onto plate to create lawn (1:10 dilution of McF 0.5).
2. Place imipenem disk in center.
3. Streak test isolates from edge of disk to end of plate.
4. Incubate overnight.
5. Look for growth of *E. coli* around test isolate streak - indicates carbapenem-hydrolyzing enzyme.

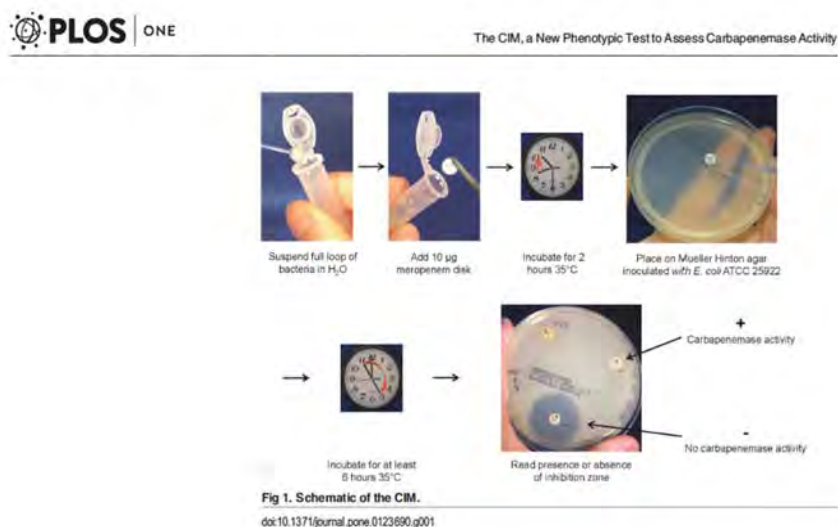
Photo courtesy of J. Patel 49

CRE confirmation test

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

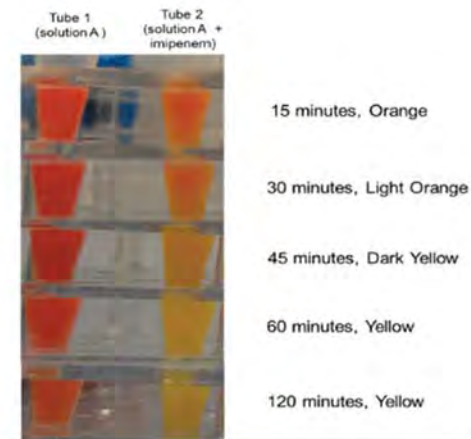


**The CIM (Carbapenemase inactivation method)
a new phenotypic Test to assess Carbapenemase activity**



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.

KPC *Providencia stuartii*



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

PCR Results (Manual in house test)

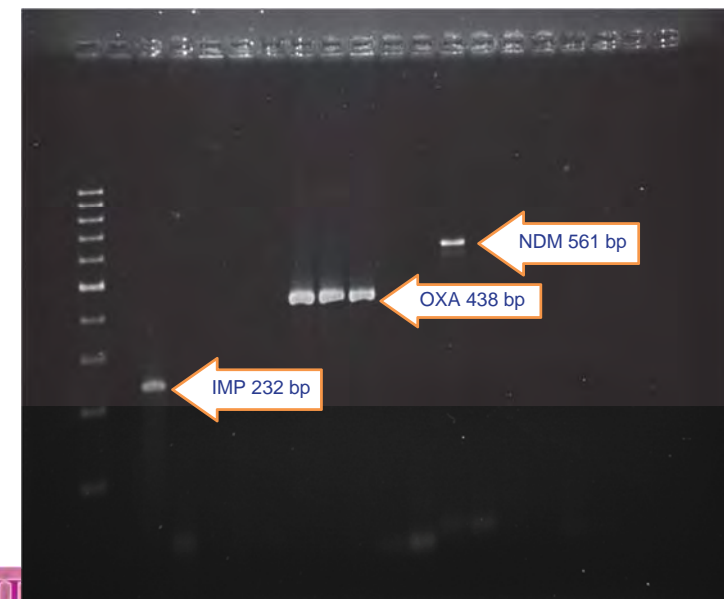




Fig. 1. Cepheid GenXpert system. (A) Instruments with 1- to 16-cartridge capacity. (B) Exploded view of GenXpert cartridge. (Courtesy of Cepheid, Sunnyvale, CA; with permission.)

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.)

Evaluation of the Verigene Gram-Positive Blood Culture Test (BC-GP)

C. Anderson*, K. Kaul* ‡, B. Voss*, and R.B. Thomson* ‡

ASM 2012

on, IL

*NorthS

Table 1. Verigene BCGP Identifiable Targets

Gram-Positive Blood Culture (BC-GP) Test			
Genus	Species	Gene target	
<i>tuf</i> gene	<i>Staphylococcus</i> spp.	• <i>Staphylococcus aureus</i>	<i>gyrB</i>
	<i>Streptococcus</i> spp.	• <i>Staphylococcus epidermidis</i>	<i>hsp60</i>
	<i>Micrococcus</i> spp.	• <i>Staphylococcus lugdunensis</i>	<i>sodA</i>
	<i>Listeria</i> spp.	• <i>Streptococcus pneumoniae</i>	<i>gyrB</i>
Resistance gene	<i>mecA</i>	• <i>Streptococcus anginosus</i> Group	<i>gyrB</i>
	<i>vanA</i>	• <i>Streptococcus agalactiae</i> (GBS)	<i>hsp60</i>
	<i>vanB</i>	• <i>Streptococcus pyogenes</i> (GAS)	<i>hsp60</i>
		• <i>Enterococcus faecalis</i>	<i>hsp60</i>
		• <i>Enterococcus faecium</i>	<i>hsp60</i>

Table 2. Successful Identification Rates of Bacterial Isolates

Isolated Organism	Verigene Result/Micro Lab Result
Staphylococcus	87/87 (100%)
Streptococcus	29/31 (93%)
Enterococcus	8/9 (89%)
Micrococcus	2/2 (100%)
Corynebacterium*	0/4 (0%)
Aerococcus*	0/1 (0%)
Bacillus sp*	0/1 (0%)
Lactobacillus*	0/1 (0%)
Total # of isolates	126/136 (93%)
Total # of identifiable isolates	126/129 (98%)

*Not an intended target of the Verigene BCGP Test

Verigene: BC-GP test
(Nanosphere)

blood c/s
multiplex PCR,
microarray:



Limitation

FDA cleared
Aerobic bottle
only

Mixed
infection
Mixed c/s

Luminex
complexity simplified.

Customer Center
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CLINICAL RESEARCH & APPLIED MARKETS RESOURCES SUPPORT ABOUT LUMINEX CONTACT US

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

VERIGENE® GRAM-POSITIVE BLOOD CULTURE TEST

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



Luminex
complexity simplified.

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CLINICAL RESEARCH & APPLIED MARKETS RESOURCES SUPPORT ABOUT LUMINEX CONTACT US

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.




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)



Gram-Negative Blood Culture Test Specifications

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



• Biofire
Filmarray

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

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

The FilmArray BCID Panel

Simultaneous detection of 27 targets:



Gram + Bacteria

- *Staphylococcus*
- *Staphylococcus aureus*
- *Streptococcus*
- *Streptococcus agalactiae*
- *Streptococcus pyogenes*
- *Streptococcus pneumoniae*
- *Enterococcus*
- *Listeria monocytogenes*



Gram - Bacteria

- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Serratia*
- *Proteus*
- *Acinetobacter baumannii*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Enterobacteriaceae*
- *Escherichia coli*
- *Enterobacter cloacae* complex



Fungi

- *Candida albicans*
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*



Antibiotic Resistance

- *mecA*
- *vanA / vanB*
- *KPC*

Antimicrobial Susceptibility Tests

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

MIC vs Disk Diffusion Test

Interpretative criteria/guideline: CLSI, EUCAST

Treatment guideline: research data, patient care

The interpretative 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.

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.

Table 2B-5
Other Non-Enterobacteriaceae
M07

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

Testing Conditions		Routine QC Recommendations (See Tables 4A and 5A for acceptable QC ranges.)	
Medium:	Broth dilution: CAMHB Agar dilution: MHA	<i>Escherichia coli</i> ATCC® 25922 (for chloramphenicol, tetracyclines, sulfonamides, and trimethoprim-sulfamethoxazole) <i>Pseudomonas aeruginosa</i> ATCC® 27853 <i>Escherichia coli</i> ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)	
Inoculum:	Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard	When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.	
Incubation:	35°C±2°C; ambient air; 16–20 hours		

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)			Interpretive Categories and MIC Breakpoints (µg/mL)			Comments
			S	I	R	S	I	R	
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	Ceftiofur	—	—	—	—	≤8	16–32	≥64	
O	Moxalactam	—	—	—	—	≤8	16–32	≥64	

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

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New Jan 2017
(CLSI: M100S27)

Week 2018

Table 2B-1
Burkholderia cepacia complex
M02 and M07

Table 2B-3. Zone Diameter and MIC Breakpoints for *Burkholderia cepacia* complex

Testing Conditions		Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)	
Medium:	Disk diffusion: MHA Broth dilution: CAMHB Agar dilution: MHA	<i>Escherichia coli</i> ATCC® 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamethoxazole) <i>Pseudomonas aeruginosa</i> ATCC® 27853	
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard	Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.	
Incubation:	35°C±2°C; ambient air; 20–24 hours, all methods	When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.	

ATCC® is a registered trademark of the American Type Culture Collection.

General Comment

- (1) For disk diffusion, test a maximum of 12 disks on a 150-mm plate and no more than 6 disks on a 100-mm plate; disks should be placed no less than 24 mm apart, center to center (see M02, Subchapter 3.6). Each zone diameter should be clearly measurable; overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

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

Caution!
No breakpoint for *Burkholderia pseudomallei* in CLSI (Meliodosis)
See CDC recommendation or reference articles

Week 2018

Melioidosis Diagnostic Workshop, 20131

Alex R. Hoffmaster, David AuCoin, Prasith Baccam, Henry C. Baggett, Rob Baird, Saithip Bhengsri, David D. Blaney, Paul J. Brett, Timothy J.G. Brooks, Katherine A. Brown, Narisara Chantrata, Allen C. Cheng, David A.B. Dance, Saskia Decuyper, Dawn Defenbaugh, Jay E. Gee, Raymond Houghton, Possawat Jorakate, Ganjana Lertmemongkolchai, Direk Limmathurotsakul, Toby L. Merlin, Chiranjay Mukhopadhyay, Robert Norton, Sharon J. Peacock, Dionne B. Rolim, Andrew J. Simpson, Ivo Steinmetz, Robyn A. Stoddard, Martha M. Stokes, David Sue, Apichai Tuanyok, Toni Whistler, Vanaporn Wuthiekanun, Henry T. Walke

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 21, No. 2, February 2015

Identification

Automated system:

Burkholderia pseudomallei misidentification is not uncommon.

AST:

No CLSI interpretative criteria

Disk diffusion: overall TMP-SMX resistance

MIC test is recommended.



Table 2A-1. Enterobacteriaceae MO2 and MO7

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	I	R	S	I	R			
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-S20-10) 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 erapenem 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 <i>Proteus</i> spp., <i>Providencia</i> spp., and <i>Morganella morganii</i> tend to be higher (i.e., 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	Erpopenem	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 <i>Salmonella</i> spp. and <i>Shigella</i> 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

Table 2. Common misconceptions and pitfalls in the identification of *Burkholderia pseudomallei* and diagnosis of melioidosis

Misconception or pitfall	Comments
Melioidosis is endemic only to some parts of Asia and northern Australia.	Melioidosis is reported in many regions of the world, including regions of Central and South America, various Pacific and Indian Ocean islands, and some countries in Africa.
Melioidosis is not endemic to the area because <i>B. pseudomallei</i> has never been reported from the microbiological facilities.	<i>B. pseudomallei</i> can be misidentified as another <i>Burkholderia</i> species, <i>Pseudomonas</i> spp., or other organisms, especially by laboratory staff unfamiliar with <i>B. pseudomallei</i> .
Melioidosis is only an acute, septic illness.	10%–15% of patients have chronic disease that may mimic other conditions, including tuberculosis.
Lifetime travel history to non-melioidosis-endemic areas is not taken.	Melioidosis may appear many years after exposure.
Do not provide treatment for melioidosis unless any diagnostic test is positive.	Melioidosis is often fatal, and treatment effective against <i>B. pseudomallei</i> should be provided immediately if melioidosis is suspected.
Throat swab and urine specimens should be collected only from patients with symptoms of pharyngitis or urinary tract infection. Culture is a sensitive method for diagnosing melioidosis.	Swabs of throat (anterior fauces) or urine may be positive in patients without focal symptoms.
Indirect hemagglutination assay is a reliable diagnostic test. <i>B. pseudomallei</i> can be a colonizing organism.	As with most infections, the sensitivity of culture depends on the quality of the specimen, and deep, occult sites of infection are also possible.
Selective media for <i>B. pseudomallei</i> are not necessary.	Sensitivity and specificity of indirect hemagglutination assay is poor.
The "safety pin" appearance is a reliable characteristic of gram-stained <i>B. pseudomallei</i> .	Although chronic infection after treatment has been described, isolation of <i>B. pseudomallei</i> from any body site should be regarded as indicative of disease.
Automated microbiology systems can reliably detect <i>B. pseudomallei</i> .	Sensitivity of culture is lower and the diagnosis would be missed for many patients if selective media are not used for specimens from nonsterile sites.
	<i>B. pseudomallei</i> usually stains unevenly but is not always bipolar, whereas other organisms such as <i>Escherichia coli</i> or <i>Klebsiella</i> spp. may appear bipolar with gram stain.
	Although these systems are generally reliable, misidentification is not uncommon, particularly in regions where few strains are included in phenotypic databases.



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

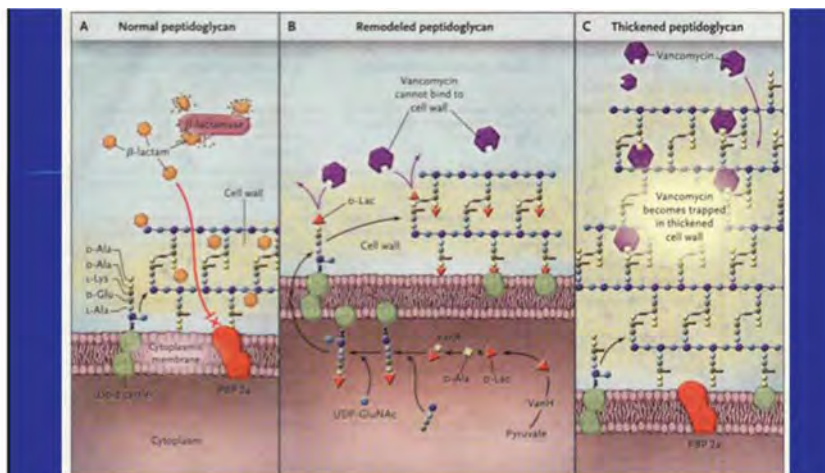
Table 20. *Staphylococcus* spp. (Continued)

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	I	R	S	I	R				
GLYCOPOLYPTIDES												
(19) For <i>S. aureus</i> , vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.												
B	Vancomycin (For <i>S. aureus</i>)	—	—	—	—	≤2	4–8	≥16	For use with <i>S. aureus</i> . (20) MIC tests should be performed to determine the susceptibility of all isolates of <i>Staphylococcus</i> to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of <i>S. aureus</i> 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 <i>S. aureus</i> for which the vancomycin MIC is ≥ 8 μg/mL to a reference laboratory. See Appendix A. Also refer to Table 3G for <i>S. aureus</i> , 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				
LIPOLYCOPEPTIDES												
C	Ornithan	—	—	—	—	≤0.12	—	—	See comment (17).			
C	Telavancin	—	—	—	—	≤0.12	—	—	See comment (17).			
LIPOTEPTIDES												
B	Daptomycin	—	—	—	—	≤1	—	—	(23) Daptomycin should not be reported for isolates from the respiratory tract.			

(CLSI: M100S27)

MRSA: Vancomycin test = MIC only
No disk diffusion breakpoint

Table 2C. *Staphylococcus* spp. M02 and M07



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

Vancomycin Resistance in *S. aureus*

Strain	Definition	Genetic event	Mechanism/Significance
Pen Resistance		Penicillinase	Enzyme Modification
MRSA	Meth/Ox resistance	<i>mecA</i>	PBP2a Normal Cell wall
Vanc suscept <i>S. aureus</i> -VSSA	MIC ≤ 2 $\mu\text{g/mL}$	-	
Vanco-intermediate <i>S. aureus</i> (VISA)	MIC 4-8 $\mu\text{g/mL}$	Unknown; ? <i>vraSR</i> & <i>graSR</i> mutation <small>Cui AIC 2009; 53:1231</small>	-Thickened cell wall - increased vanco binding
Vanco-resistant <i>S. aureus</i> (VRSA)	MIC \geq 16 $\mu\text{g/mL}$	<i>vanA</i> from <i>VR E. faecalis</i>	Remodeled Cell Wall D-ala-D-ala to D-ala-D-lactate

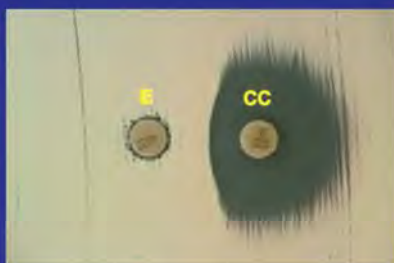
**Vancomycin MIC creeping (MIC > 1) → more treatment failure
 → Vancomycin should not be avoided.**

Use: Linezolid/ Daptomycin/ Ceftaroline

Endocarditis, Severe Pneumonia, Severe Skin infection, Osteomyelitis
 Need to monitor Vancomycin MIC and vancomycin trough level (drug level)

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

Resistance due to MLS_B



-Perform on all erythro-R, clinda- S *S. aureus* isolates
-Treatment failures have occurred
 Found on same *S. aureus* as *mecA* high % of

D- test: Micro labs report should be....

Erythromycin-Resistance

Clindamycin-Resistance

(Macrolide-inducible clindamycin resistance)

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

Update CLSI 2017

- **Colistin susceptibility test matter?**
- No disk diffusion test interpretation

Treatment Options for Carbapenem-Resistant *Enterobacteriaceae* Infections

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ID consultation

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

Room Isolation (Plasmid transferable)

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

ions due to carbapenem resistance is an emerging treatment for β -lactamase-resistant *Enterobacteriaceae*. Despite this, few remaining carbapenems, which are effective against these organisms, are available. Thus, current carbapenem resistance is a major public health concern.

April 2015. Associate Professor, University of Rhode Island, Kingston, RI

Open Forum Infectious Diseases

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A.baumannii/ *P.aeruginosa*

Antimicrobial agents	Inhibition zone (mm)		MIC ($\mu\text{g/mL}$)	
	S	R	S*	R
Colistin	-	-	≤ 2	≥ 4

***loading dose, maximum recommended dose, and combination with other agents**

A.baumannii/ P.aeruginosa

Antimicrobial agents	Inhibition zone / MIC (μg/mL)	S*	R
Colistin	-	≤ 2	≥ 4

Disc diffusion no interpretation

*loading dose, maximum recommended dose, and combination with other agents



Enterobacteriaceae: Colistin

Organization	MIC (μg/mL)	
	WT	NWT
CLSI 2017 (ECV)	≤ 2	≥ 4
EUCAST 2017 (Clinical breakpoint)	S ≤ 2	R > 2



Comparative methods for colistin Susceptibility tests

1. Broth microdilution (BMD)
2. Agar dilution (AD)
3. Agar gradient (Etest)
4. Disk diffusion (DD)



RESEARCH

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 metabolism 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.

two-component systems or alterations of the *mcrB* 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

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



Rapid polymyxin NP

TAT 2 h

Sensitivity 99.3%

Specificity 95.4%

From
135 colistin R isolates
and 65 colistin S isolates

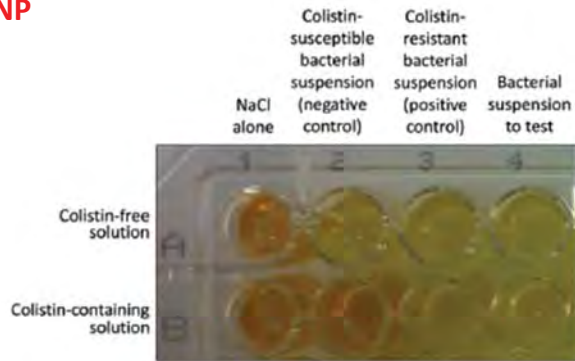


Figure. Representative results of the rapid polymyxin NP [Nordmann/Poirel] test. Noninoculated wells are shown as controls (first column). The rapid polymyxin NP test was performed with a reference colistin-susceptible isolate (second column) and with a reference colistin-resistant isolate (third column) 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.



Colistin susceptibility test

- BMD: gold standard
- AD: essential agreement with BMD
- DD and Etest: poor agreement with BMD

• Need clinical breakpoints
: MIC-PK/PD-Clinical outcomes



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