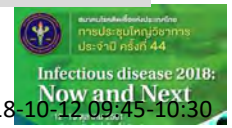
A Year in Clinical Microbiology

Rongpong Plongla, MD MSc D(ABMM)

Division of Infectious Diseases, Department of Medicine
Faculty of Medicine, Chulalongkorn University
King Chulalongkorn Memorial Hospital, Thai Red Cross Society
Rongpong.P@chula.ac.th



2018-10-12 09:45-10:30

Disclosure

- None

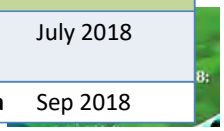
Outline

- Bacteriology
- Mycobacteriology
- Mycology
- Virology
- Parasitology
- Emerging and re-emerging pathogen

- Diagnostic test evaluation and test utility
- Molecular microbiology: syndromic panel and next-generation sequencing
- Susceptibility testing and mechanism of resistance

CLSI

		Release
M58, 1st ed.	**Methods for the identification of cultured microorganisms using MALDI-TOF MS**	April 2017
M60, 1st ed.	**Performance standards for antifungal susceptibility of yeasts**	Nov 2017
M61, 1st ed.	**Performance standards for antifungal susceptibility testing of filamentous fungi**	Nov 2017
M27, 4th ed.	**Reference method for broth dilution antifungal susceptibility testing of yeasts**	Nov 2017
M38, 3rd ed.	**Reference method for broth dilution antifungal susceptibility testing of filamentous fungi**	Nov 2017
M59, 2nd ed.	**Epidemiological cutoff values for antifungal susceptibility testing: *Aspergillus, Cryptococcus***	Jan 2018
MM18, 2nd ed.	**Interpretive criteria for identification of bacteria and fungi by targeted DNA sequencing**	July 2018
M48, 2nd ed.	**Laboratory detection and identification of Mycobacteria**	Sep 2018



MM18, 2nd ed.
July 2018
Replaces MM18-A

Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing

Microorganism or Group[1]	Appropriateness of 16S rRNA (V1–V3 Region ≈ 500 bp)	Comments for 16S rRNA	Alternative DNA Targets	Indications for Identification to Species and Recommendations for Resolution[2]
***Streptococcus mitis* group**[64,82-101] *S. mitis* *S. sanguinis* *S. parasanguinis* *S. pseudopneumoniae* *S. pneumoniae* **Also includes:** *S. gordonii* *S. cristatus* *S. oralis* *S. infantis* *S. peroris* *S. australis* *S. sinensis* *S. orisratti* *S. oligofermentans* *S. massiliensis* *S. tigurinus* *S. dentisani*	Resolution to genus and group, with poor resolution to species. *S. mitis*, *S. pneumoniae*, and *S. pseudopneumoniae* cannot be distinguished. The rest of the group may be differentiated with the help of mismatches at position ≈200 bp.	Exclude *S. pneumoniae* and report as *S. mitis* group. High variability observed for *S. sanguinis* and *S. parasanguinis*. If additional speciation is needed for clinical or epidemiological purposes, an alternate DNA target should be sequenced.	The *rpoB* gene provides better resolution to species. The *tuf*, *dnaJ*, DNA gyrase subunit B (*gyrB*), *groEL*, *recN*, and *rnpB* genes provide better resolution to species but have been used primarily for research studies.	Phenotypic methods needed to identify *S. pneumoniae* (eg, optochin, bile solubility). MALDI-TOF MS aids in genus and *S. mitis* group species resolution, except for *S. tigurinus*. MALDI-TOF MS may also misidentify atypical *S. pneumoniae* and nonpneumococcal *S. mitis* group isolates.

CLINICAL AND LABORATORY STANDARDS INSTITUTE

28th Edition

M100

M02, 13th ed: Disk diffusion 2018
M07, 11th ed: MIC method 2018
M11-A8: anaerobic MIC method 2012

Performance Standards for Antimicrobial Susceptibility Testing

Major Changes: CLSI M100, 28th ed.

- New breakpoints
  - Ceftazidime-avibactam
    - DD and MIC *Enterobacteriaceae* and *P. aeruginosa*
  - Ceftolozane-tazobactam
    - DD for *Enterobacteriaceae*

    (already have DD and MIC for *P. aeruginosa* and MIC for *Enterobacteriaceae*)
  - Meropenem-varbobactam, Delafloxacin: not yet (FDA breakpoint)
  - Dalbavancin MIC
- Revised breakpoints
  - Piperacillin-tazobactam for anaerobes
- Updated antibiogram for anaerobes
- ***Staphylococcus* spp.**
  - ***S. schleiferi***
- **Carbapenemase testing**
- QC
- ECVs

Previous method for detection of methicillin resistance in staphylococci

	Oxacillin MIC	Cefoxitin MIC	Cefoxitin Disk	Oxacillin Salt Agar Screen
S. aureus	Yes	Yes	Yes	Yes
S. lugdunensis	Yes	Yes	Yes	No
CoNS (xS.lug)	Yes*	No	Yes	No

	Oxacillin MIC (µg/ml) S	I	R
CoNS	≤0.25	-	≥0.5
SA/S.lug	≤2	-	≥4

→ * Oxacillin MIC may overcall resistance for some species other than *S. epidermidis*

MICs in the 0.5-2.0 µg/mL range may lack *mecA*
→ test *mecA*, PBP2a or cefoxitin disk

M100S 26th Ed.

Cefoxitin is much better inducer of *mecA* than is oxacillin

Staphylococcus pseudintermedius and S. schleiferi

S. pseudintermedius

- Veterinary staphylococcus
- Positive tube coagulase, PYR

Breakpoint	CA	VME (%)	ME (%)
Cefoxitin disk (*S.aureus* BP)	76%	76%	0%
Cefoxitin disk (CoNS BP)	90%	30%	0%
Cefoxitin MIC (*S.aureus* BP)	71%	89%	0%
Oxacillin Disk or MIC (CoNs BP)	99%	0%	1.2%

Wu MT. JCM2016;54:535-542.

S. schleiferi

- Colinizes skin and ears of dogs and cats, zoonosis; Positive PYR
- *Subsp. coagulans*: tube coagulase positive
- Subsp. *scheifeiri*: clumping factor positive

Breakpoint	VME (%)	ME (%)
Cefoxitin disk	64-72%	0%
Cefoxitin MIC	60%	0%
Oxacillin Disk or MIC	0%	1.2%

Huse HK. JCM2018;56:e01653-17.

Summary: Susceptibility testing for CoNS – need species identification

Oxacillin (For *S. pseudintermedius* and *S. schleiferi*)	1 µg oxacillin	≥18	-	≤17	≤0.25	-	≥0.5	**(15)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* and *S. schleiferi*.
Oxacillin (For CoNS except *S. lugdunensis, S. pseudintermedius, and S. schleiferi*)	-	-	-	-	≤0.25 (oxacillin) Oxacillin MIC	-	≥0.5 (oxacillin)	**(16)** ***S. epidermidis*** **isolates with oxacillin MIC ≥0.5 µg/mL should be reported as oxacillin resistant. However, oxacillin MIC breakpoints may overcall resistance for some CoNS, because some non-*S. epidermidis* strains for which the oxacillin MICs are 0.5–2 µg/mL lack *mecA*. Non-*S. epidermidis* isolates from serious infections with MICs in this range may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as oxacillin susceptible.** [Cefoxitin disc removed] See general comment (5) and comments (8), (11), and (13).
	30 µg cefoxitin (surrogate test for oxacillin)	≥25	-	≤24	-	-	-	

Susceptibility testing of *S. aureus* What *mec* you crazy?

MRSA that are not *mecA* mediated

BORSA: Mediated by overexpression of *blaZ*

mecC (formerly mecA$_{LGA251}$)* : PBP2a encoding-*mecA* homologue in human and bovine MRSA; UK, Denmark and Ireland; 70% homology to *mecA*, carry on type XI SCC*mec

Oxacillin	Cefoxitin	Mechanism	Prevalence	Report as OX:
S	S	None	Common	S
R	R	*mecA*	Common	R
S	R	*mecC*	Uncommon	R
S	R	*mecA* (low level expression)	Uncommon	R
R	S	PBP changes or hyperproduction of beta-lactamase (BORSA)	Rare	R

Laurent. EID 2012;18:1163
Garcia-Alvarez. Lancet ID 2011; 11:595.

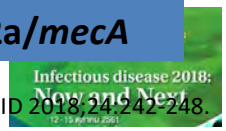
Plasmid-Encoded Transferable *mecB*-Mediated Methicillin Resistance in *Staphylococcus aureus*

Karsten Becker, Sarah van Alen, Evgeny A. Idelevich, Nina Schleimer, Jochen Seggewiß, Alexander Mellmann, Ursula Kaspar, Georg Peters

- **1 case, cefoxitin-based nasal screening, Germany**
- **Negative for *mecA/ mecC* and SCC*mec-orfX* junction region**
- ***mecB (formerly mecA$_M$)*: plasmid borne, negative PBP2a, previously found in *Macrococcus***
  - **Plasmid harbors *aacA-aphD, aphA, aadK, ermB, tetS***
- **60% homolog to *mecA***
- **68.7% homolog to *mecD* in *Macrococcus caseolyticus, which* elevated MIC to ceftaroline**
- **Cefoxitin MIC 32 mg/L; Oxacillin MIC 4 mg/L**

Clue for *mecB/ mecC*: Elevated cefoxitin MIC values compared to oxacillin in the absence of PBP2a/*mecA*

EID 2018;24:242-248.



Detection of Carbapenemases

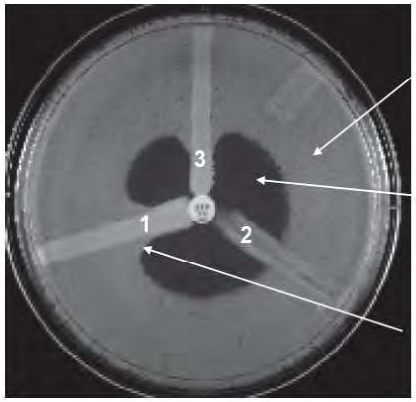
- Carbapenmase testing M100, 28th ed.
  - mCIM test for *P. aeruginosa* (already used in *Enterobacteriaceae*)
  - eCIM to differentiate serine carbapenemase from metallo-beta-lactamases in *Enterobacteriaceae*
  - Deleted Modified Hodge Test
  - Deleted *Acinetobacter* spp. recommendation for CarbaNP
    - Currently no reliable phenotypic carbapenemase test for *A. baumannii*

A. baumannii	Sens	Spec
mCIM	79.8%	52.9%
CarbaNP	18.8%	100%

Simner PJ. JCM2018;56:e01369-17.

If using current breakpoint: perform carbapenemase testing for **Epidemiological and infection control purpose, if requested

Modified Hodge test



E. coli ATCC® 25922

Inhibition of *E. coli* ATCC® 25922 by ertapenem

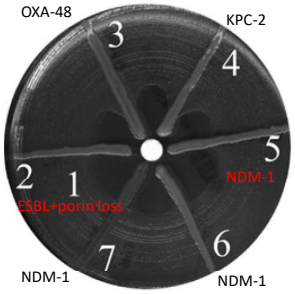
Enhanced growth of *E. coli* ATCC® 25922. Carbapenemase produced by *K. pneumoniae* ATCC® BAA-1705 inactivated ertapenem that diffused into the media. Thus, there is no longer sufficient ertapenem here to inhibit *E. coli* ATCC® 25922 and an indentation of the zone is noted.

Simple to perform
No special reagents or media required

Clinical and Laboratory Standards Institute. M100-S25: Performance Standards for Antimicrobial Susceptibility Testing

MHT: Limitations

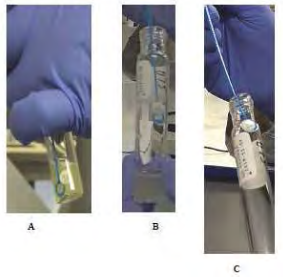
- **Often difficult to interpret**
  Long turnaround time
- **Not all carbapenemase-producing isolates are MHT positive: False negative**
  - **High level of sensitivity (>90%) and specificity (>90%) in detecting KPC-type carbapenemases**
  - **Sensitivity of 11% for NDM-type carbapenemases**
- **MHT-positive results may be encountered in isolates with carbapenem resistances other than carbapenemase production: False positive**
  - **ESBL or AmpC enzymes coupled with porin loss**
- **Only applies to *Enterobacteriaceae***

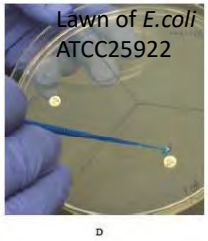


Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. J Clin Microbiol. 2012

Modified carbapenem inactivation method (mCIM) for *Enterobacteriaceae* and *P.aeruginosa*

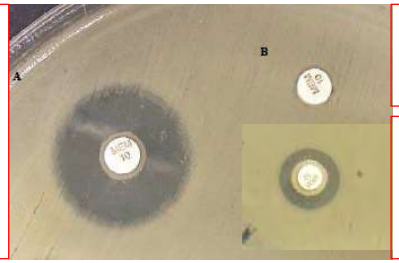
- **1 µL bacteria (10 µL for *P.aeruginosa*) from an overnight blood agar plate in 2 mL TSB (instead of water)**
- **10 µg meropenem disc**
- **35±2°C in ambient air 4h ±15min (instead of 2h)**
- **Place disc on MHA with *E.coli* ATCC 25922 0.5 McFarland**
- **Incubate 35±2°C in ambient air 18-24h**





Negative: Inhibition zone ≥ 19mm

NB: ignore carryover of test organism in the TSB



Positive: Inhibition zone 6-15 mm or presence of colonies within a 16-18 mm zone

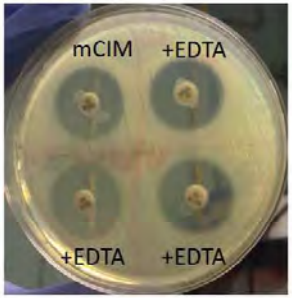
Indeterminate: Inhibition zone 16-18 mm
- Check test isolate and *E.coli* ATCC 25922 for purity, and check meropenem disk QC
- Repeat; if indeterminate, molecular study

Clinical and Laboratory Standards Institute. M100-S27: Performance Standards for Antimicrobial Susceptibility Testing

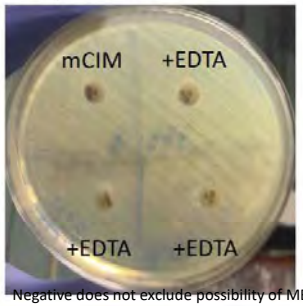
Inhibitor-enhanced mCIM (imCIM), eCIM

- Differentiate between serine and MBL carbapenemases (Perform in conjunction with mCIM)
- Add EDTA to a second tube - to determine if carbapenemase activity is inhibited by EDTA 5mM

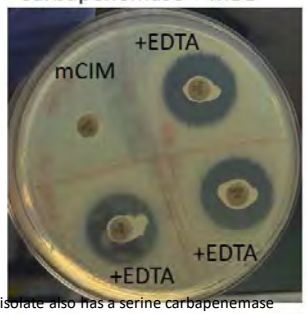
K. pneumoniae ATCC 1706
Non-CP-CRE



K. pneumoniae ATCC 1705
Carbapenemase + KPC



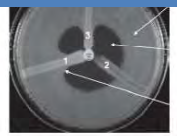
K. pneumoniae ATCC 1705
Carbapenemase + MBL



Negative does not exclude possibility of MBL if isolate also has a serine carbapenemase (eg, OXA-181 +NDM)

Only for *Enterobacteriaceae*: sensitivity 99%, specificity 94%

Summary: CLSI endorsed methods for carbapenemase detection



	Tests Used for Epidemiological or Infection Control–Related Testing			
	MHT (Table 3B)	Carba NP (Table 3C)	mCIM (Table 3D)	Other (eg, molecular assays)
Organisms	*Enterobacteriaceae* that are not susceptible to one or more carbapenems	*Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. that are not susceptible to one or more carbapenems	*Enterobacteriaceae* that are not susceptible to one or more carbapenems	*Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by MHT or Carba NP
Strengths	Simple t… No speci… …s or media necessa…	Rapid	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	False-… …can occu… …roduce ESB… …pC… co… …h por… False-negative results are occasionally noted (eg, some isolates producing NDM	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected.	Only applies to *Enterobacteriaceae* Requires overnight incubation	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

+/- eCIM only for *Enterobacteriaceae*

Removed in 2018

Further applies to *Pseudomonas* (10ul loop) 2018

Colistin Susceptibility The Search Continues …

- **CLSI M100, 28th ed**: The only approved MIC method for testing is **broth microdilution.**
- Disk diffusion and gradient diffusion methods should not be performed
  - **Disc diffusion: 23% very major errors in one study; poor diffusion in the agar**

Colistin Etest Studies (select)					
Organism	N	*vs.*	Results		Reference
			VME	ME	
A. baumannii	115 (22-R)	BMD	1.7%	0.0%	Arroyo, JCM 2005;43:903.
A. baumannii *P. aeruginosa*	58 (0-R) 47 (15-R)	AD	0.0% 11%	1.9% 30%	Tan, CMI 2007;14:539.
P. aeruginosa	64 (12-R)	AD	8.3%	50%	Goldstein, JAC 2007;59:1039
A. baumannii *P. aeruginosa* *K. pneumoniae*	27 (8-R) 60 (9-R) 20 (8-R)	BMD	50% 0% 25%	7.8%	Hindler, JCM 2013;51:1678

Breakpoints and ECVs: CLSI/EUCAST Joint Working Group

	MIC (µg/mL)			Zone (mm)		
BREAKPOINTS	S	I	R	S	I	R
• *P. aeruginosa*	≤2	-	≥4		None	
• *A. baumannii complex*	≤2	-	≥4		None	
• *Enterobacteriaceae*	Insufficient clinical and PK/PD data					

EPIDEMIOLOCICAL CUTOFF VALUE (ECV)	Wild-type	Non-wild-type	
• *E. coli*	≤2	≥4	None
• *K. pneumoniae*			None
• *Enterobacter cloacae*			None
• *R (E.) aerogenes*			None
• *Raoultella ornithinolytica*			None

CLSIM100S27. Jan 2017

Testing Methods

- **Molecule is large and has a propensity to adsorb to testing surfaces**
- **ISO standard broth microdilution (20776-1), without surfactant**
  - **RUO; no FDA-cleared device**
  - **Cation-adjusted MHB**
  - **No additives may be included: polysorbate-80→lower MIC**
  - **Trays must be made of plain polystyrene and not treated in any way before use (, but colistin binds less efficiently to the glass tubes than the polysterene plastics)**
  - **Sulphate salt of polymyxins must be used**

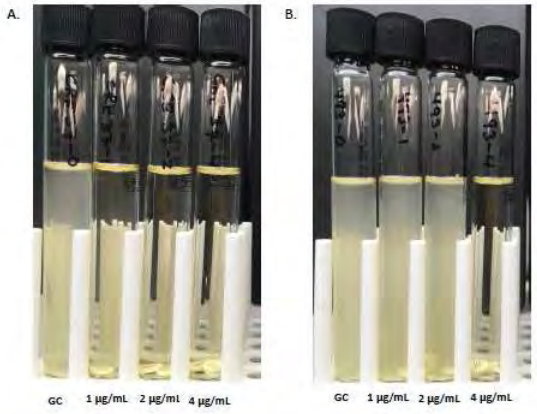
Method	Manufacturer	Regulatory Status	Notes
Disk diffusion	BD	RUO	Not recommended by CLSI / EUCAST Must confirm "S" per package insert
Gradient diffusion	Etest (bioMerieux) MTS (Liofilchem)	RUO	Not recommended by CLSI / EUCAST
Sensititre	ThermoFisher	RUO	Broth microdilution custom panel
MicroScan colistin well on dried GN ID panel	Beckman Coulter	RUO for AST	Some preliminary data to suggest may work, but not for *A. baumannii*

RUO, research use only

CLSIM23A4:
Sader HS, et al. DMID 2012;74:412-14.
Lo-Ten-Fo JR, et al. AAC 2007;51:3726-30.

Two-Site Evaluation of the Colistin Broth Disk Elution Test to Determine Colistin *In Vitro* Activity Against Gram-Negative Bacilli

Patricia J. Simner[1], Yehudit Bergman[1], Marisol Trejo[2], Ava A. Roberts[1], Remy Marayan[1], Tsigereda Tekle[1], Shelley Campeau[3], Abida Kazmi[1], Drew Bell[1], Shawna Lewis[1], Pranita D. Tamma[4], Romney Humphries[3] and Janet A. Hindler[2]



- 10 mL CA-MHB + 0, 1, 2, and 4 colistin 10 µg disks [RT 30 min]+
- 50 µL of 0.5 McFarland ($7.5x10^5$ CFU/mL),
- 16-20h at 35°C in ambient air

JCM 2018.doi:101.1128/JCM.01163-18

Colistin Broth Disk Elution Test

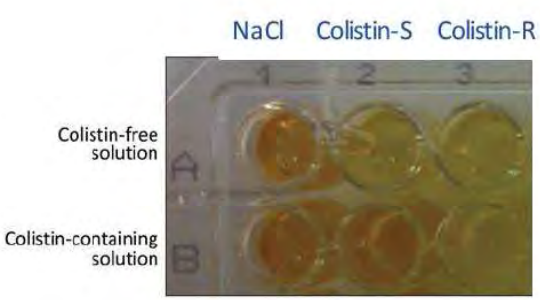
Isolates	N	BMD Results S or WT (N)	BMD Results R or NWT (N)	CA (%)	EA (%)	VME (%)	ME (%)
Site 1							
Acinetobacter baumannii	12	5	7	100	100	0	0
Pseudomonas aeruginosa	20	18	2	100	100	0	0
Enterobacteriaceae	24	10	14	100	100	0	0
Site 2							
Retrospective CRE	65	58	7	100	97[a]	0	0
A. baumannii	12	12	0	100	100	0	0
P. aeruginosa	14	14	0	100	100	0	0
Prospective CRE	19	17	2	100	100	0	0
Both Sites							
mcr-1 E. coli[b]	6	0	6	50	100	50	0
Overall	172	134	38	98	99	8	0

S: Susceptible, R: Resistant, WT: wild-type, NWT: non-wild-type; CA: categorical agreement; EA: essential agreement; VME: very major error; ME: major error
[a] 1 *Citrobacter freundii* had a MIC of ≤0.25 µg/mL by BMD and 2 µg/mL by CBDE and 1 *Enterobacter cloacae* had a MIC of 0.5 µg/mL by BMD and 2 µg/mL CBDE
[b] 3 *mcr-1 E. coli* had MICs of 4 µg/mL by BMD and 2 µg/mL by CBDE. These results were reproduced at the 2 sites.

- 172 isolates:
  - *A. baumannii, P. aeruginosa, Enterobacteriaceae*
  - 6 *mcr-1 E.coli*
- VMES: *mcr-1* with MIC 2 of 2 mg/L by CBDE and 4 mg/L by BMD
- MIC ≥2 mg/L by CBD should be confirmed by reference method and evaluate for *mcr* gene

JCM 2018.doi:101.1128/JCM.01163-18

Rapid polymyxin NP test for *Enterobacteriaceae*



- CA-MHB + 10% glucose + phenol red
- Added 3.75 µg/ml colistin
- Positive reaction = growth (yellow)
- Negative reaction = no growth (orange)
- 2 hours to perform

200 Enterobacteriaceae evaluated (135 colistin-NWT)
- 99.3% sensitivity vs. BMD
- 95.4% specificity vs. BMD
- 7/7 *mcr-1* isolates gave positive results

Nordmann 2016 EID 22:1038

Evaluation of the Rapid Polymyxin NP test for detection of colistin susceptibility in Enterobacteriaceae isolated from Thai patients

Sakda Yainoy [a], Monchanok Hiranphan [a], Thanawat Phuadraksa [a], Warawan Eiamphungporn [a], Surapee Tiengrim [a], Visanu Thamlikitkul [b,*]

[a] Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand
[b] Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Bacterial species	Sensitivity, %	Specificity, %	PPV, %	NPV, %
All	100	95.9	98.3	100
E. coli	100	91.7	86.6	100
K. pneumoniae	100	97.0	99.0	100
E. aerogenes	100	100	100	100

Ref.	Isolates	S	R	Isolates with MIC 0.5-2 mg/L	Sens, %	Spec, %
Nordmann, 2016	200	65	135	9	99.3	95.4
Jayol, 2018	123	40	83	≤25	98.8	97.5
Poirel, 2018	105	35	70	0	100	100
Current	339	94	245	92	100	95.9

DMID 2018;92:102-106.

A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates

Patrice Nordmann,[a,b] Aurélie Jayol,[a] Laurent Poirel[a]

Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg,[a] and HFR-Hôpital Cantonal,[b] Fribourg, Switzerland

- ELITechGroup, France
- From culture and from swab
- Good sensitivity and specificity
- Lowest detection limit 10^3-10^4 CFU/mL

Compound	Stock solution (mg/ml)	Quantity or vol to add[a]	Final concn[b]
EMB agar powder		15 g	3.75%
Distilled water		400 ml	
Colistin sulfate	20 In water in glass tubes	70 µl	3.5
Daptomycin	20 In water	200 µl	10
Amphotericin B	20 In D-(+)-glucose 10%	100 µl	5

[a] The volume of 400 ml of SuperPolymyxin medium was for, i.e., 20 plates.
[b] Concentrations are in micrograms per milliliter unless noted otherwise.

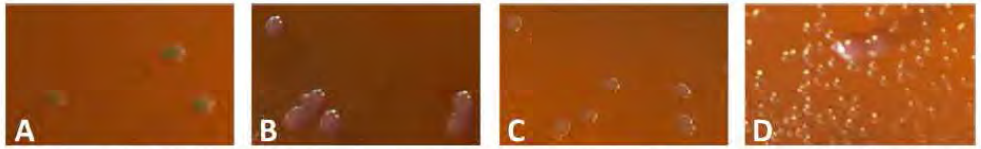


FIG 1 Polymyxin-resistant lactose-positive *E. coli* (A), polymyxin-resistant *K. pneumoniae* (B), polymyxin-resistant lactose-negative *E. coli* (C), and a mix of a heavy inoculum of *P. mirabilis* and a low inoculum of polymyxin-resistant *K. pneumoniae* (C) growing on the SuperPolymyxin medium.

Nordmann P. JCM 2016;54:1395-1399.
Jayol A. DMID 2018;92:95-101.

Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.

E. Matuschek*, J. Åhman, C. Webster, G. Kahlmeter

Essential and categorical agreements for colistin MIC tests for 75 Gram-negative bacteria with MICs on frozen broth microdilution panels as reference

	Organism	*E. coli* and *K. pneumoniae* (*n*=32)	*P. aeruginosa* (*n*=21)	*Acinetobacter* spp. (*n*=22)	All isolates (*n*=75)
	Colistin reference MIC range (mg/L)	0.25–32	0.25–128	0.5–32	0.25–128
% Essential agreement (EA)[a]	Sensititre custom plate[b]	96	100	91	96
	MICRONAUT-S	97	100	91	96
	MICRONAUT MIC-Strip	97	100	100	99
	SensiTest[c]	96	93	71	88
	UMIC[d]	91	75	77	82
Gradient diffusion	Etest, Oxoid MH	84	62	59	71
Gradient diffusion	Etest, BBL MH	63	52	4.5	43
Gradient diffusion	Etest, MHE	75	43	9.1	47
Gradient diffusion	MTS, Oxoid MH	59	57	41	53
Gradient diffusion	MTS, BBL MH	75	57	59	65
% Categorical agreement (CA)[a]	Sensititre custom plate	97	95	91	95
	MICRONAUT-S	94	86	86	89
	MICRONAUT MIC-Strip	94	91	86	91
	SensiTest	94	91	82	89
	UMIC	94	91	91	92
Gradient diffusion	Etest, Oxoid MH	94	71	73	81
Gradient diffusion	Etest, BBL MH	94	67	68	79
Gradient diffusion	Etest, MHE	94	76	82	85
Gradient diffusion	MTS, Oxoid MH	81	71	82	79
Gradient diffusion	MTS, BBL MH	84	71	68	76

CMI 2018;24:865-870.

Colistin and Polymyxin B Susceptibility Testing for Carbapenem-Resistant and *mcr*-Positive *Enterobacteriaceae*: Comparison of Sensititre, MicroScan, Vitek 2, and Etest with Broth Microdilution

Ka Lip Chew,[a] My-Van La,[b] Raymond T. P. Lin,[a,c] Jeanette W. P. Teo[a]

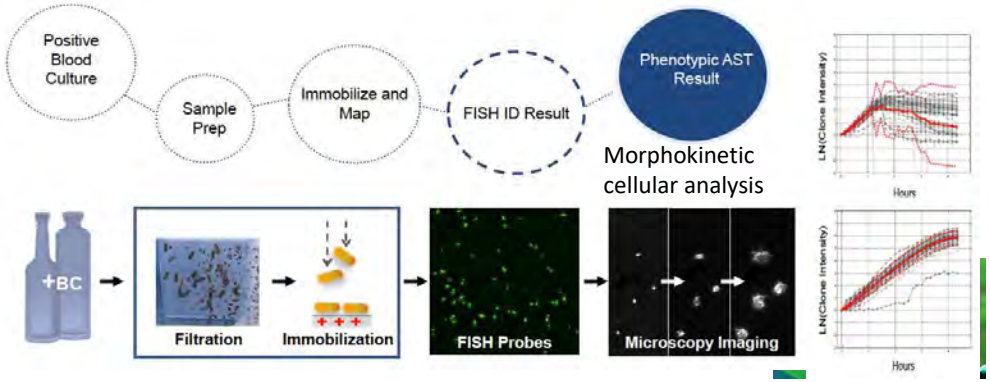
Department of Laboratory Medicine, Division of Microbiology, National University Hospital, Singapore, Republic of Singapore[a]; Department of Laboratory Medicine, Changi General Hospital, Republic of Singapore[b]; Ministry of Health, National Public Health Laboratory, Singapore, Republic of Singapore[c]

Drug	Method	No. of isolates that were susceptible	No. of isolates that were resistant	No. (%) of isolates exhibiting EA	No. (%) of isolates exhibiting CA	No. of isolates exhibiting VMEs (%)	No. of isolates exhibiting MEs (%)
Colistin	Vitek 2	60	16	71 (93.4)[b]	67 (88.2)	9 (36.0)	0 (0)
	Sensititre	46	30	68 (89.5)	69 (90.1)[b]	1 (4)	6 (11.8)
	Etest	51	25	57 (75.0)	70 (92.1)[b]	3 (12.0)	3 (5.9)
	MicroScan	44	32	NA[d]	67 (88.2)	1 (4)	8 (15.8)

JCM 2017;55(9):2609-1616.

Accelerate Pheno System

- **US FDA-cleared, 2017**
- **Time to ID ~90 min**
- **Time to phenotypic AST ~ 7h**
- **Automated, 3 min set up time**



Accelerate Pheno System

Identification Probes

Gram-Positive	Gram-Negative
S. aureus	*E. coli*
S. lugdunensis	*Klebsiella* spp.
CoNS spp.	*Enterobacter* spp.
E. faecalis	Proteus spp.
E. faecium	Citrobacter spp.
Streptococcus spp.	*S. marcescens*
	P. aeruginosa
	A. baumannii

Fungi

- *C. albicans*
- *C. glabrata*

Universal Probes

- Universal Bacteria
- Universal Eukaryotic
- Acridine Orange Stain

Antibiotics

Gram-Positive	Gram-Negative
Ampicillin	Amp-Sulbac
Ceftaroline	Pip-Tazo
Doxycycline[1]	Cefepime
Erythromycin	Ceftazidime
TMP-SMX[1]	Ceftriaxone
Daptomycin	Ertapenem
Linezolid	Meropenem
Vancomycin	Amikacin
	Gentamicin
	Tobramycin
	Ciprofloxacin
	Aztreonam
	Colistin[1]

Resistance

- MRSA (Cefoxitin)
- MLSb (Ery-Clind)

[1]RUO

Evaluation of the Accelerate Pheno System for Fast Identification and Antimicrobial Susceptibility Testing from Positive Blood Cultures in Bloodstream Infections Caused by Gram-Negative Pathogens

Matthias Marschal,[a,b] Johanna Bachmaier,[a] Ingo Autenrieth,[a,b] Philipp Oberhettinger,[a,b] Matthias Willmann,[a,b] Silke Peter[a,b]

- April 2016-Oct 2016
- German tertiary care academic medical center
- 115 episodes of positive blood culture bottles with Gram-negative rods on initial Gram stain

	Accelerate Pheno	Conventional Methods
Median time to identification after start of blood culture processing (h) [IQR] MALDI-TOF MS from colony	3.58 [2.75-4.38]	31.07 [28.82-46.55]
Median Time to AST after start of blood culture processing (h) [IQR] Vitek 2	8.88 [8.1-9.67]	49.27 [46.0-49.8]
Median duration of system run (h) [IQR]	6.65 [6.63-6.67]	N/A

- Ward E. JCM2018- Ped
- Pancholi P. JCM2018-USA vs. Vitek2 for ID and BMD/DD
- Pantel A. JAC2018- spiked MDR-GN

- Correct identification 102 of 115 samples, **including 10 polymicrobial**
- No ID for 8 specimens with off-panel organisms
- AST reported for 95 of 104 samples
  - Overall categorical agreement 96.4%, 2.3% major errors, 1.0% very major errors
- Major errors most common with ampicillin-sulbactam and piperacillin-tazobactam

Marschal M. JCM 2017;55:2116-2126.

Accelerate Pheno System: Concerns

- Decrease performance in AST for *Pseudomonas and* beta-lactam in *Enterobacteriales* (piperacillin/tazobactam, ceftazidime)

Descours G. Eur J Clin Microbiol Infect Dis 2018;371:573-1583.
Charnot-Katsikas A. JCM 2018;56:e001166-17.

- False-positive Gram-positive

Lutgring JD. JCM2018;56:e01672-17.

- Clinical impact and cost-effectiveness?