Evaluation of the activity of cefiderocol and other traditional and new antibiotics

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PAHO Pan American Health Organization
World Health Organization Americas

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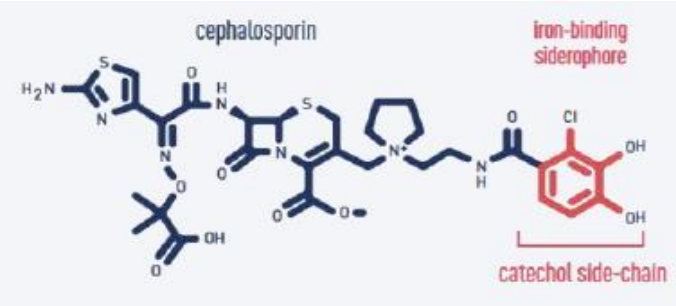
Cefiderocol



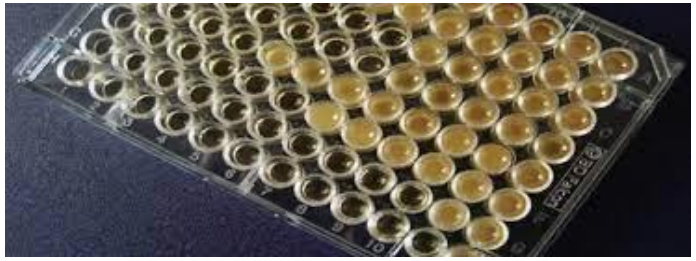
- A novel siderophore cephalosporin.
- Uses the bacterial iron transport system to access their periplasmic compartment.
- Antibacterial activity against a large variety of multidrug-resistant Gram-negatives, including carbapenem-resistant Enterobacterales, *P. aeruginosa* and *A. baumannii*.
- There is some evidence of acquired resistance to cefiderocol; nevertheless, it seems that the potential for resistance acquisition remains low.

Cefiderocol – Antimicrobial susceptibility testing

Reference methods:

BROTH MICRODILUTION (BMD)

Iron-depleted cation-adjusted Mueller-Hinton broth (0.01 - 0.03 µg/mL).



DISK DIFUSION 30 µg

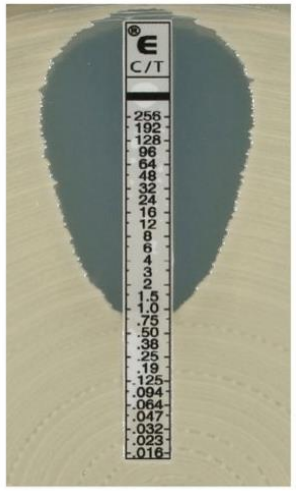
Standard Mueller-Hinton agar.

Cefiderocol – Antimicrobial susceptibility testing

Commercial methods:

GRADIENT STRIPS

- MIC test strips (Liofilchem)
- Etest® (bioMérieux)



COMMERCIAL BMD PANELS

- Sensititre® (ThermoFisher) – temporarily unavailable
- UMIC® (Bruker)
- ComASP® (Liofilchem)

Challenges for the cefiderocol AST

- Unique mechanism of action - iron-depleted state (ID-MHBCA).
- BMD and disk diffusion methods have presented limited reliability for *A. baumannii* - Trailing
- Differences in interpretative criteria established by the CLSI and EUCAST breakpoints:

	MIC breakpoints (mg/L)					**Disk diffusion (mm) - 30 µg**					
	CLSI			EUCAST		CLSI			EUCAST		
Organism	S	I	R	S	R	S	I	R	S	R	ATU
Enterobacterales	≤4	8	≥16	≤2	>2	≥16	9-15	≤8	≥22	<22	18-22
P. aeruginosa	≤4	8	≥16	≤2	>2	≥18	13-17	≤12	≥22	<22	14-22
A. baumannii	≤4	8	≥16	≤2	>2	≥15	-	-	Note[A]	-	-

A. Zone diameters of ≥17 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L.

Warning issued concerning cefiderocol susceptibility testing

Cefiderocol MIC by broth microdilution with Sensititre panels:

- ThermoFisher has issued a warning against all current batches of MH broth when used together with any freeze dried Sensititre BMD panel for cefiderocol.
- Temporarily unavailable to purchase - Risk of false susceptible results.

SENSITITRE™ EUMDROXF PLATE FORMAT

Plate Code: **EUMDROXF** Date: **9/15/2020**

	1	2	3	4	5	6	7	8	9	10	11	12
A	AZT 1	AZT 2	AZT 4	AZT 8	AZT 16	AZT 32	C/T 0.25/4	C/T 0.5/4	C/T 1/4	C/T 2/4	C/T 4/4	C/T 8/4
B	COL 0.5	COL 1	COL 2	COL 4	COL 8	COL 16	P/T4 4/4	P/T4 8/4	P/T4 16/4	P/T4 32/4	TGC 0.5	TGC 1
C	IMI 1	IMI 2	IMI 4	IMI 8	IMR 0.06/4	IMR 0.12/4	IMR 0.25/4	IMR 0.5/4	IMR 1/4	IMR 2/4	IMR 4/4	IMR 8/4
D	FEP 1	FEP 2	FEP 4	FEP 8	FEP 16	CZA 0.25/4	CZA 0.5/4	CZA 1/4	CZA 2/4	CZA 4/4	CZA 8/4	CZA 16/4
E	AMI 2	AMI 4	AMI 8	AMI 16	AMI 32	ERV 0.008	ERV 0.015	ERV 0.03	ERV 0.06	ERV 0.12	ERV 0.25	ERV 0.5
F	FDC 0.03	FDC 0.06	FDC 0.12	FDC 0.25	FDC 0.5	FDC 1	FDC 2	FDC 4	FDC 8	FOS+ 16	FOS+ 32	FOS+ 64
G	MERO 0.12	MERO 0.25	MERO 0.5	MERO 1	MERO 2	MERO 4	MERO 8	MERO 16	TOB 0.5	TOB 1	TOB 2	TOB 4
H	MEV 0.06/8	MEV 0.12/8	MEV 0.25/8	MEV 0.5/8	MEV 1/8	MEV 2/8	MEV 4/8	MEV 8/8	MEV 16/8	POS	POS	POS

ANTIMICROBICS

AZT	Aztreonam
COL	Colistin
IMI	Imipenem
FEP	Cefepime
AMI	Amikacin
FDC	Cefiderocol
MERO	Meropenem
MEV	Meropenem / Vaborbactam Constant 8
IMR	Imipenem / Relebactam Constant 4
CZA	Ceftazidime / Avibactam Constant 4
ERV	Eravacycline
C/T	Ceftolozane / Tazobactam Constant 4
P/T4	Piperacillin / Tazobactam Constant 4
TOB	Tobramycin
FOS+	Fosfomycin+glucose-6-phosphate
POS	Positive Control
TGC	Tigecycline

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

AST of bacteria

- Organization
- Consultations
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- Expert rules and expected phenotypes
- Resistance mechanisms
- Guidance documents
- SOP
- MIC and zone distributions and ECOFFs
- AST of bacteria
  - IVDR
  - Media preparation
  - MIC determination

EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST Development Laboratories, from time to time discover products (disks, media batches, gradient tests, devices and/or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page.

We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question.

Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice. Use the EUCAST subject related contact form.

10. Cefiderocol MIC by broth microdilution on freeze dried panels from Thermofisher - the manufacturer has issued a warning against all current batches of MH broth when used together with any freeze dried Sensititre BMD panel.

The following plates are listed as containing cefiderocol, MDRGNX2F, CAN2MSTF, GNARUM6F, THAMDR1F, CMP3QLN, DEURUB1, SWEEDL1, EUSHION8, FRCNRP4 and the manufacturer issues the following recommendation: "If you have bought any of these lots, then please ignore results for Cefiderocol". EUCAST recommends, If uncertain confirm directly with the manufacturer (January, 2022),

12. Cefiderocol: EUCAST has evaluated (August 2022) commercially available tests and all have problems with accuracy, reproducibility, bias and/or for some, with skipped wells. For this reason, it is difficult to resolve the susceptibility interpretation of isolates in the ATU (area of technical uncertainty) in routine testing. Considering the current limitations and the need for testing in multiresistant bacteria, these are the recommendations of EUCAST until the issue of confirmatory MIC determination is solved.

- Disk diffusion. Laboratories are recommended to start testing cefiderocol with disk diffusion. Resistance to beta-lactam agents is increasing and therapeutic alternatives are few. Disk diffusion, when correctly performed and calibrated using quality material and recommended quality control guidelines, is predictive of susceptibility and resistance outside the ATU.
  Template zone diameter distributions for relevant species against which to calibrate in-hou
- Inside the ATU, and as long as there is no alternative method to resolve interpretative uncertainties (eg MIC-
  laboratory), EUCAST
  zone diameter breakp
- However, as with all A
  quality of materials us
  investigate all product
  manufacturers of disks
  those that are not mer

For cefiderocol, using a cross-over protocol, disks from three manufacturers and Mueller-Hinton media from five manufacturers were evaluated.

If care is taken to ensure that the EUCAST QC criteria for the two strains, E. coli ATCC 25922 and P. aeruginosa ATCC 27853, are met, i.e. with the mean of at least 5 repeated tests is within +/-1 mm of the target values, disk diffusion using EUCAST breakpoints performed well. When the mean value was more than +/-1 mm from the target, an increasing proportion of results were erroneous. This was particularly problematic for *Pseudomonas aeruginosa*.

Among the evaluated cefiderocol 30 µg disks (Liofilchem, Mast and Oxoid) and Muller-Hinton agars (BBL, bioMérieux, Bio-Rad, Liofilchem and Oxoid), disks from Oxoid and MH agar from Bio-Rad produced larger than acceptable zone diameters for both QC strains and clinical isolates. Combining Oxoid disks with Bio-Rad MH agar increased the problem further.

Further work to elucidate the problems surrounding cefiderocol susceptibility testing is ongoing. Two commercial products currently under investigation, are UMIC (Bruker) and ComASP (Liofilchem).

The issue has been discussed by the EUCAST Steering Committe, January 2023, and it was decided that issues have not yet been resolved and that the warning should remain.

A EUCAST evaluation is ongoing and results will be made available as soon as testing is completed and an analysis by the committee performed.

UMIC® (Bruker)

J Antimicrob Chemother
https://doi.org/10.1093/jac/dkad149

Journal of Antimicrobial Chemotherapy

Performance evaluation of the UMIC® Cefiderocol to determine MIC in Gram-negative bacteria

Laurent Dortet [1,2,3]†, Claudia Niccolai[4,5]†, Niels Pfennigwerth [6]†, Stefanie Frisch[7], Camille Gonzalez[1,2], Alberto Antonelli[4,5], Tommaso Giani[4,5], Robert Hoenings[7], Soeren Gatermann[6]†, Gian Maria Rossolini[4,5]† and Thierry Naas [1,2,3]*†

EA = 90.8%
CA = 90.1%
n = 283 isolates

ComASP® (Liofilchem)

antibiotics

MDPI

Article

Disc Diffusion and ComASP® Cefiderocol Microdilution Panel to Overcome the Challenge of Cefiderocol Susceptibility Testing in Clinical Laboratory Routine

Gabriele Bianco [1,2,*], Matteo Boattini [1,2,3], Sara Comini [1,2], Giuliana Banche [2], Rossana Cavallo [1,2] and Cristina Costa [1,2]

EA = 84%
CA = 94%
n = 50 isolates

Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Objective

To evaluate the susceptibility of cefiderocol and other active drugs in CRO of Enterobacterales, *A. baumannii* complex and *P. aeruginosa* in Latin American and Caribbean countries in 2022.

Specific Objectives

- To describe the antimicrobial susceptibility patterns of CRO to cefiderocol and other active drugs.
- To correlate MIC with disk diffusion zone diameters.
- To evaluate the reproducibility of BMD and disk diffusion techniques.
- To describe the demographic distribution of patients with CRO, stratified by pathogen in Latin America and the Caribbean.

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Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Inclusion Criteria

- CRO isolates of Enterobacterales, *A. baumannii* complex and *P. aeruginosa* from bloodstream, urinary and respiratory tract samples collected betweem January 1, 2022 and December 31, 2022.
  - 1st consecutive CRO per person during the study period.
  - All strains must have their resistance mechanisms determined.
  - Only samples from patients whose demographic data are available.

- All isolates will be shipped to the corresponding NRL where their identities will be confirmed using traditional methods.

Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Distribution of isolates per country

LATIN AMERICA (16)

Argentina (80)
Brazil (300)
Chile (30)
Colombia (90)
Costa Rica (30)
Cuba (30)
Ecuador (30)
El Salvador (30)
Guatemala (30)
Mexico (200)
Nicaragua (30)
Panama (30)
Paraguay (30)
Peru (60)
Uruguay (30)
Venezuela (50)

THE CARIBBEAN (9)

Barbados (30)
Belize (30)
Dominica (10)
Guyana (20)
Haiti (10)
St. Vincent and The Grenadines (30)
Trinidad and Tobago (30)
Bahamas (30)
Jamaica (30)

Total of 1300 isolates

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Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Expected Results

- Current epidemiology of carbapenem resistance in GNB in LAC after more than 2 years of COVID-19 pandemic.
- Local epidemiological information about antibiotic activity to advice national authorities for introduction of alternative treatment options – EQUITY.
- Regional data to inform Strategic Fund about regional needs.
- Increase the capacity for diagnosis and characterization of organisms resistant to carbapenems in LAC.
- Study about performance of CLSI and EUCAST breakpoints for ceftazidime/avibactam in GNB.

Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Antimicrobial susceptibility testing

- Broth microdilution:
  - Sensititre® panel (ThermoFisher)
  - UMIC® panel (Bruker)
  - Other methods
- Disk diffusion according to NRL (CLSI, EUCAST) guidelines
  - Cefiderocol 30 µg
  - Ceftazidime/avibactam (30/20 µg and 10/4 µg)
- Characterization of CROs in charge of the countries.

- Cefiderocol
- Aztreonam
- Colistin
- Imipenem
- Cefepime
- Amikacin
- Meropenem
- Eravacycline
- Tobramycin
- Fosfomycin
- Tigecycline

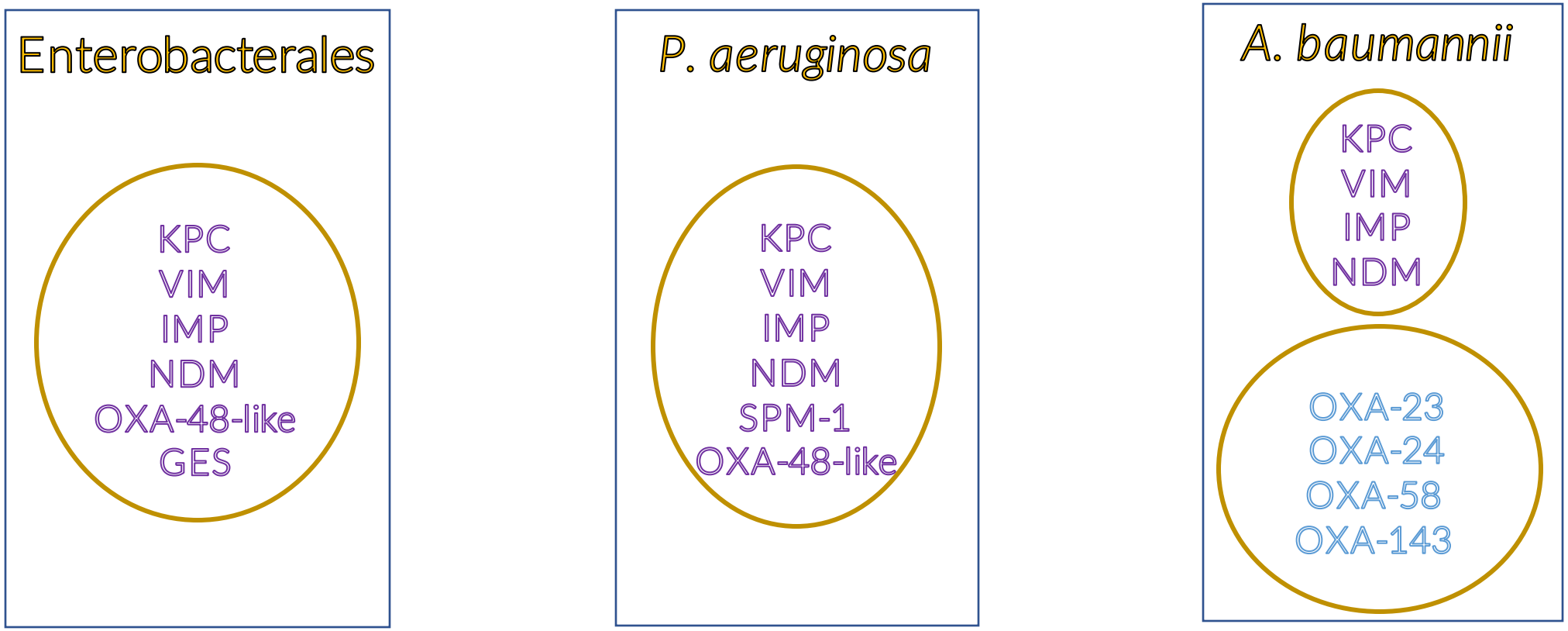
- Ceftazidime/avibactam
- Ceftolozane/tazobactam
- Imipenem/relebactam
- Meropenem/vaborbactam
- Piperacillin /tazobactam

- Ampicillin/sulbactam*
- Gentamicin*
- Ciprofloxacin or levofloxacin*
- Trimethoprim/sulfametoxazol*

*Each NRL will use routine laboratory methodology to determine the MIC.

Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Molecular characterization of carbapenemases



Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Distribution of reagents provided by PAHO for antimicrobial susceptibility testing

- ATCC strains
- Cefiderocol antibiotic discs
- CAZ-AVI antibiotic discs (30/20 µg and 10/4 µg)
- Mueller-Hinton agar
- UMIc ® BMD panels – cefiderocol
- Sensititre® BMD panels – other drugs
  - Sensititre sterile water
  - Sensititre Mueller-Hinton broth

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Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Distribution of reagents provided by PAHO for molecular characterization of carbapenemases

- Primers, positive controls and PCR protocol will be supplied to the countries that do not have them in their laboratory routine.
  - KPC, NDM, OXA-48, IMP, VIM, GES and SPM-1

    (Multiplex qPCR for Enterobacterales and *P. aeruginosa*)

  - OXAs (OXA-23, OXA-24, OXA-58, OXA-143)

    (Multiplex qPCR for *A. baumannii* complex)

  - NG-Test CARBA 5 (NG Biotech®)

Surveillance protocol for cefiderocol and other drugs susceptibility in carbapenem-resistant organisms in Latin America and the Caribbean

Data collection and analysis

- Develop data collection form in an electronic data collection tool (RedCap).
- Description of the antimicrobial susceptibility patterns of CRO to cefiderocol and other active drugs.
- The number of isolates carrying each carbapenemase gene/total CRO, isolated in the period.
- Correlation of cefiderocol MICs with DD zone diameters and evaluation of the reproducibility of BMD and DD techniques.
- Development of a final report, which will be submitted to a peer review journal as a manuscript.

Shionogi evaluation of cefiderocol susceptibility testing by broth microdilution and disk diffusion

Broth Microdilution

Methodology

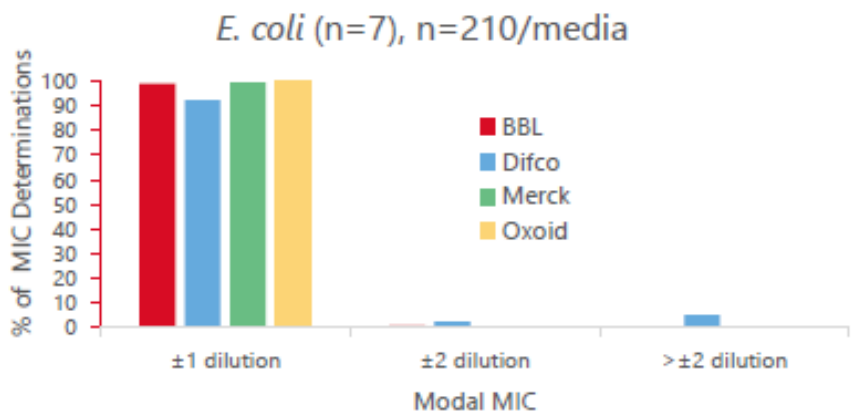
- MIC determinations by broth microdilution for 85 isolates with MIC values "validated" with *in vivo* efficacy experiments using ID-CAMHB.
  - 4 different brands (1 lot for each brand) of CAMHB: BD BBL, BD Difco, Oxoid, and Merck.
  - Perform testing over 3 days (three separate inoculum).
  - 10 replicates* per isolate per media per day (same inoculum).
  - Total of 30 or 9* MIC determinations for each brand of CAMHB (120 or 36* total per strain).
- Preparation of ID-CAMHB
  - Stir CAMHB with Chelex100 (analytical grade, 100–200 mesh, sodium form) for 6 hours.
  - Filter and replenish medium with Ca2+ (22.5 mg/L), Mg2+ (11.25 mg/L) and Zn2+ (0.65 mg/L)#.
  - Adjust pH to 7.2 -7.4 if needed.
  - Confirm final iron concentration ≤0.03 mg/L (VISOCOLOR HE Iron).
- 7 *E. coli*, 17 *K. pneumoniae*, 14 *P. aeruginosa* and 47 *A. baumannii*.
  - Historical cefiderocol MIC ranges from 0.25 - 64 µg/mL.
- Bacterial inoculum (0.5 McFarland) controlled by nephelometer.
- Assessed reproducibility of MIC determinations (mode ±1 dilution) amongst and across media.

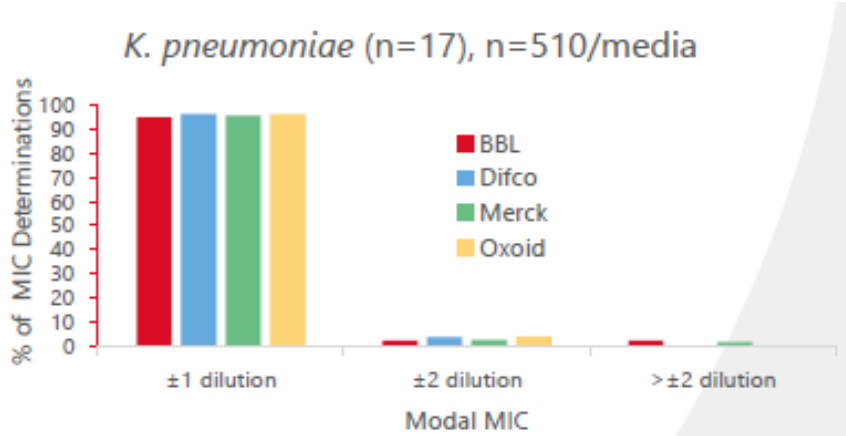
With permission from SHIONOGI

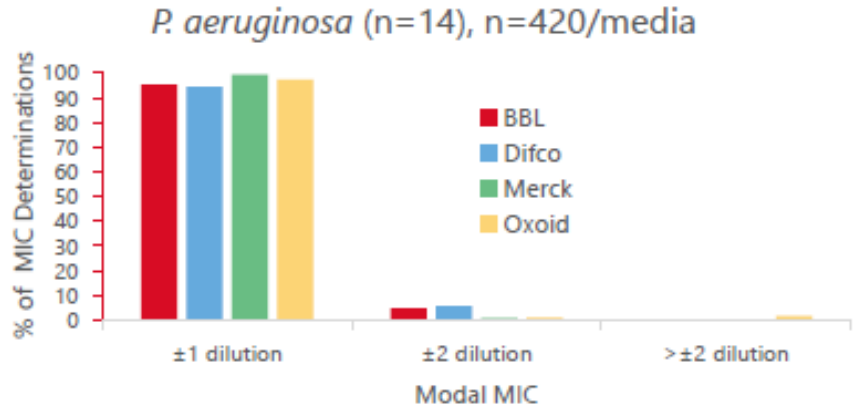
*32 *A. baumannii* isolates were tested in triplicate; #CaCl2, MgCl2, and ZnSO4 were used.

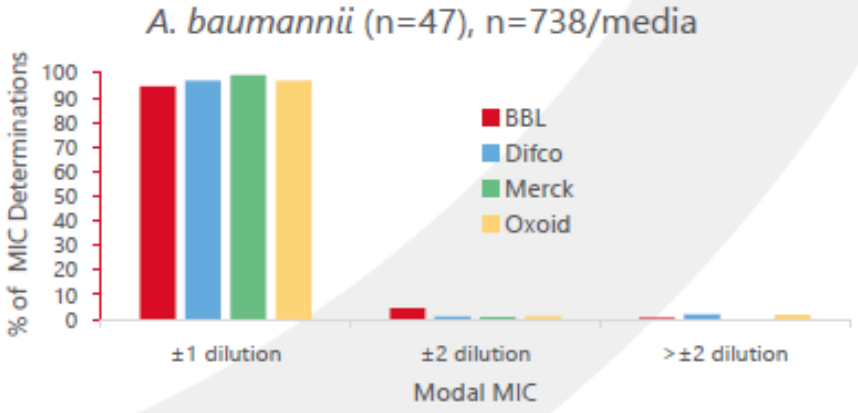
Broth Microdilution Reproducibility

- Highly reproducible MIC values were obtained for each media.
  - >92% of MIC determinations were within ±1 dilution of modal MIC values.









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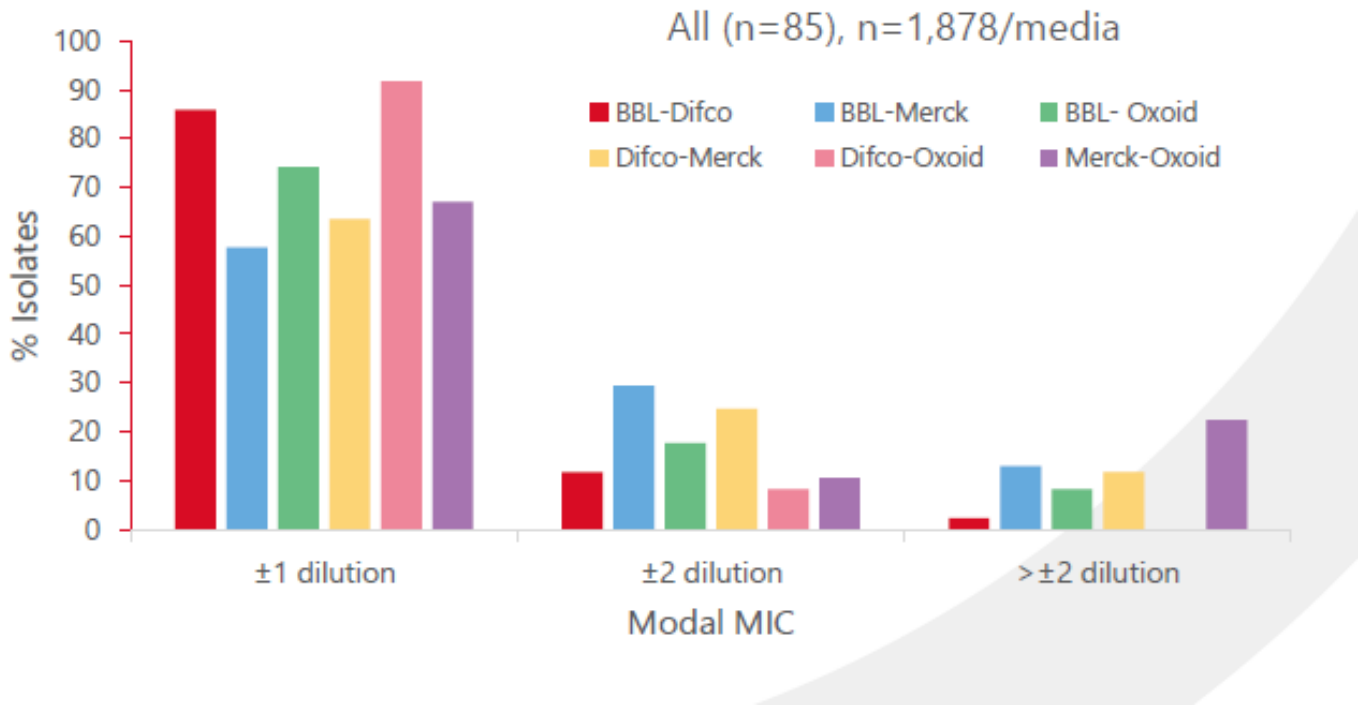
Broth Microdilution Media Comparisons

- MIC values across different media show variability.
  - BBL-Difco and Difco-Oxoid show the best correlation, with 86% and 92% of the isolates showing modal MIC values within ±1-fold dilution.
  - BBL-Difco, BBL-Oxoid, and Difco-Oxoid showed modal MIC vales within ±2-fold dilution for >90% of isolates.



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Media Cation Content

- Same (low) iron content across different media.
- MIC differences between media cannot be attributed to differences in iron content.

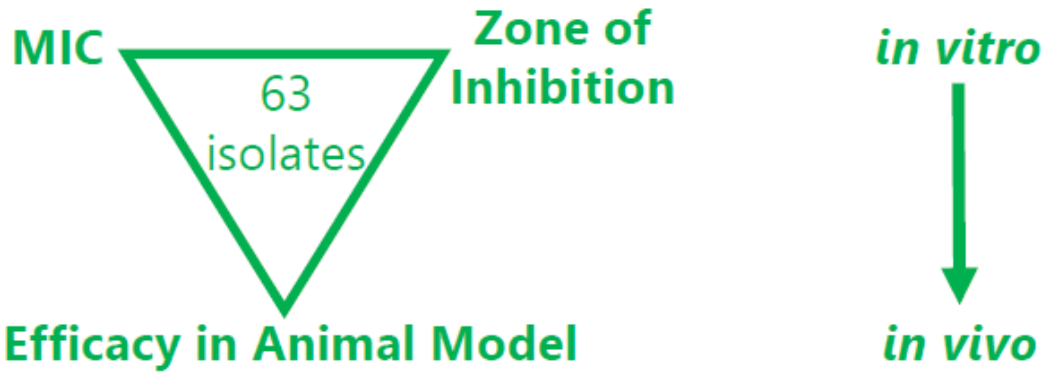
Cation content of media (adjusted for cations) after 6-hour Chelex treatment

Medium	Fe (mg/L) Visocolor	Fe (mg/L) ICP-MS	Mg (mg/L) ICP-MS	Ca (mg/L) ICP-MS	Zn (mg/L) ICP-MS
BBL	≤0.01	0.017	11	24	0.57
Difco	≤0.01	0.022	11	24	0.56
Oxoid	0.01-0.02	0.031	11	24	0.56
Merck	≤0.01	0.025	11	23	0.58

ICP-MS, Inductively coupled plasma mass spectrometry

Correlation of in vitro MIC to in vivo Efficacy

- Isolates tested in mouse thigh infection model using humanized dose of cefiderocol[1]
  - 5 *E. coli*, 5 *K. pneumoniae*, 12 *P. aeruginosa*, and 41 *A. baumannii* (n=63).
- Correlate MIC with in vivo outcome
  - Susceptible isolates (MIC ≤4 µg/ml) should result in CFU reduction.
  - Intermediate and resistant isolates (MIC >4 µg/ml) should not result in CFU decrease.



[1]Monogue et. Al. AAC 2017

in vitro S/I/R by Media Compared to in vivo Response

- MIC values generated in ID-CAMHB from BD BBL and Difco correlate best with in vivo efficacy results.

Media	MIC ≤4 µg/mL (S)		MIC ≥8 µg/mL (I/R)		Agreement of *in vivo* CFU response with *in vitro* S/I/R
	CFU reduction *in vivo*	CFU increase *in vivo*	CFU reduction *in vivo*	CFU increase *in vivo*	
Historical (BBL)*	36/38 (95%)	2/38 (5%)	1/25 (4%)	24/25 (96%)	60/63 (95%)
BBL	36/40 (90%)	4/40 (10%)	1/23 (4%)	22/23 (96%)	58/63 (92%)
DIFCO	34/36 (94%)	2/36 (6%)	3/27 (11%)	24/27 (89%)	58/63 (92%)
MERCK	28/30 (93%)	2/30 (7%)	9/33 (27%)	24/33 (72%)	52/63 (83%)
OXOID	31/33 (94%)	2/33 (6%)	6/30 (20%)	24/30 (80%)	55/63 (87%)

* Historical data were used to determine breakpoints

Conclusions

- New procedure to generate ID-CAMHB resulted in reproducible MIC values for each ID-CAMHB.
- Differences in MIC values were observed across the different ID-CAMHB.
  - Differences cannot be attributed to differences in iron content.
- MIC values generated with ID-CAMHB from BD-BBL and Difco correlated the best with the *in vivo* pharmacology response.
  - BD-BBL and Difco ID-CAMHB recommended for use in cefiderocol broth microdilution susceptibility testing.
- Enterobacterales and *P. aeruginosa* showed clear endpoints, while trailing was observed with some *A. baumannii* isolates for all media.
  - Trailing complicates determination of clear MIC endpoint and improved reading guidance with example pictures are recommended to include in M100 to improve consistency across laboratories.
- Set of isolates available for testing to third parties for validation.

Current M100 Reading Guidelines for cefiderocol broth microdilution

Appendix I. (Continued)

I3 Determining Broth Microdilution End Points

The steps for reading and interpreting broth microdilution end points for cefiderocol when tested with ID-CAMHB are listed below.

Step	Action	Comments
1	Read the MIC as the lowest concentration of antimicrobial agent that completely inhibits organism growth in the tubes or microdilution wells as detected by the unaided eye.	See step 2 for exceptions. Viewing devices intended to facilitate reading microdilution tests and recording results may be used as long as there is no compromise in the ability to discern growth in the wells.
2	Compare the amount of growth in the wells containing the cefiderocol with the amount of growth in the growth-control well containing ID-CAMHB (no antimicrobial agent).	For a test to be considered valid, acceptable growth (definite turbidity or button) must occur in the growth-control well (see Figure I1). Trailing may occur in some organisms (eg, *Acinetobacter* spp.) and should be ignored when a tiny button or light or faint turbidity relative to the growth control may be observed. Read the MIC as the first well in which growth is significantly reduced (see Figure I2).
3	Interpret the results.	Refer to the appropriate portion of Tables 2 for breakpoints.

Abbreviations: ID-CAMHB, iron-depleted cation-adjusted Mueller-Hinton broth; MIC, minimal inhibitory concentration.

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

Footnote

a. Ensure all reagents (eg, deionized water to prepare acid and base and cation solutions) have been verified as having an iron content of ≤ 0.03 mg/L.

Appendix I. (Continued)

Figure I1. Cefiderocol Test With a Clear End Point. The cefiderocol concentrations in wells G1 to G12 are 0.03 to 64 µg/mL. Row G shows the cefiderocol MIC at 0.5 µg/mL in well G5 (red circle). The growth-control well is H11 (black box).

Figure I2. Cefiderocol Test With a Trailing End Point. The cefiderocol concentrations in wells A1 to A12 are 0.03 to 64 µg/mL. Row A shows the cefiderocol MIC at 0.25 µg/mL in well A4 (red circle). The growth control well is B11 (black box).

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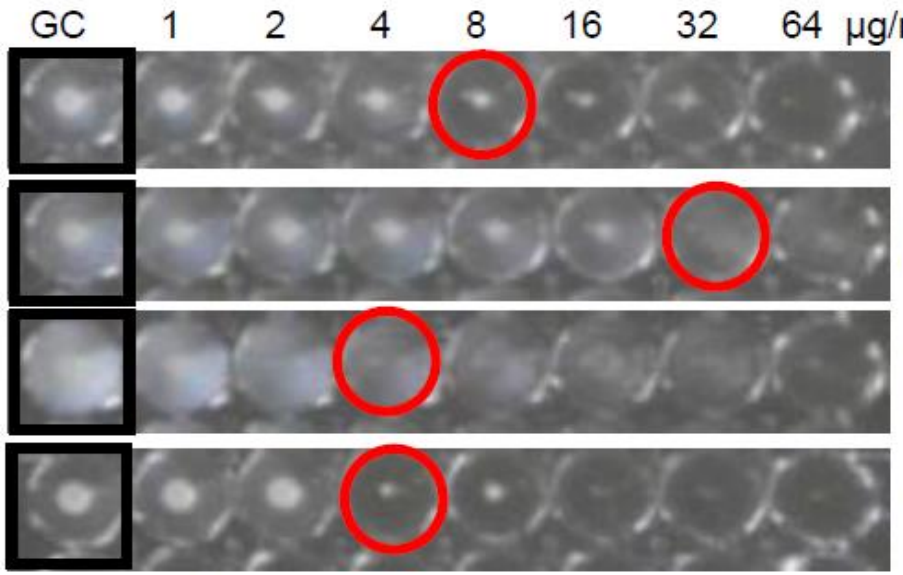
Proposed M100 Reading Guidelines

- The positive control should show strong growth in the form of a button of >2 mm or heavy turbidity.
- The MIC of cefiderocol is read as the first well in which the reduction of growth corresponds to a button of <1 mm or is replaced by the presence of light haze/faint turbidity.



MIC is read at first well corresponding to a button of <1 mm; 8 µg/mL

MIC is read at the first well corresponding by the presence of light haze/faint turbidity; 32 µg/mL

MIC is read at the first well corresponding by the presence of light haze/faint turbidity; 4 µg/mL

MIC is read at first well corresponding to a button of <1 mm; 4 µg/mL

Examples of *A. baumannii* growth patterns. GC (black box) is positive control showing strong growth with either a button of >2 mm or heavy turbidity. MIC is indicated by red circle

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Shionogi evaluation of cefiderocol susceptibility testing by broth microdilution and disk diffusion

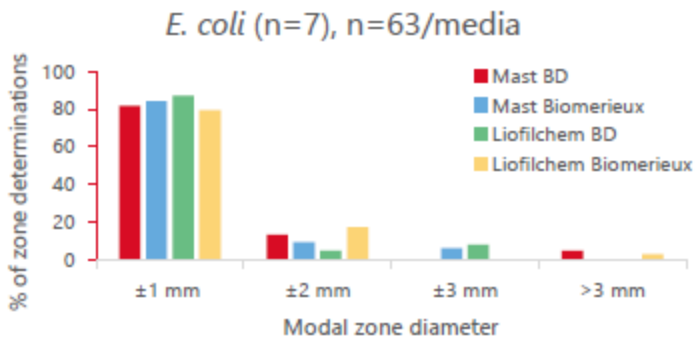
Disk diffusion

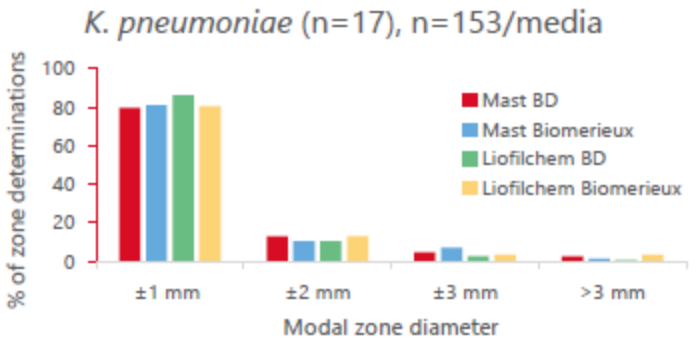
Methodology

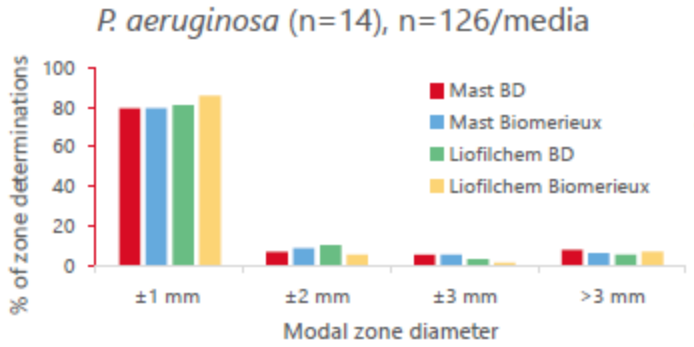
- Zone diameter determination by disk diffusion.
  - 2 different brands of disks: MAST/Hardy and Liofilchem.
  - 2 different brands (1 lot for each brand) of Mueller-Hinton Agar: BD BBL and BioMerieux.
  - Perform testing over 3 days (three separate inoculum).
  - 3 replicates per isolate per media (same inoculum).
  - Total of 18 readings for each disk (36 total per strain).
- Inner and outer disk zones were determined.
- Bacterial inoculum (0.5 McFarland) controlled by nephelometer.
  - Same inoculum was also used for broth microdilution.
- Assessed reproducibility of zone diameters across disks and media.
- Assessed categorical agreement with broth microdilution.

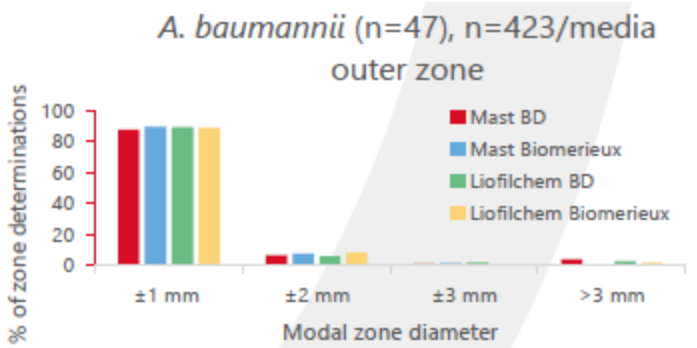
Disk Diffusion Reproducibility

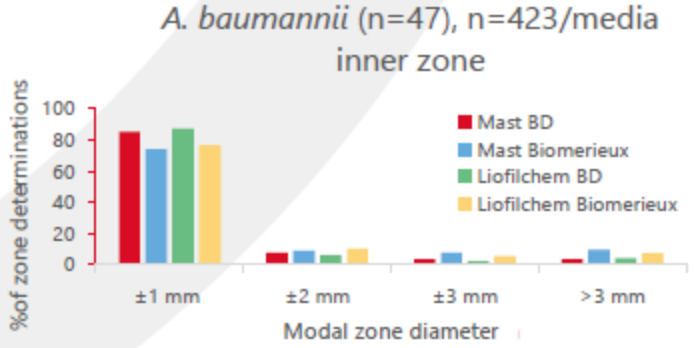
- Similar reproducibility for disks and media.
- Majority of inhibition zones within ±2 mm (>90%) of modal zone.
  - *P. aeruginosa* and *A. baumannii* Biomerieux inner zones slightly lower (>85%).











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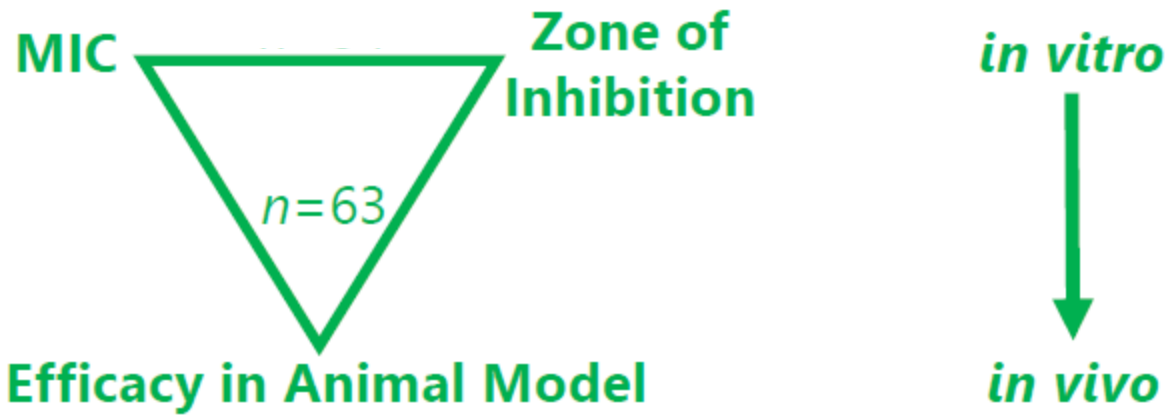
Correlation of in vitro Zone of Inhibition to in vivo Efficacy

- Isolates tested in mouse thigh infection model using humanized dose of cefiderocol.
  - 5 E. *coli*, 5 *K. pneumoniae*, 12 *P. aeruginosa*, and 41 *A. baumannii* (n=63).
- Correlate MIC with in vivo outcome.
  - Susceptible isolates (MIC ≤4 µg/ml) should result in CFU reduction.
  - Intermediate and resistant isolates (MIC >4 µg/ml) should not result in CFU decrease.



Categorical Agreement for *E. coli*, *K. pneumoniae* and *P. aeruginosa* (n=38)*

- None of the media-disk combinations yielded >90% categorical agreement.
- None disk-media combination presented VME.
- Liofilchem-BD showed highest categorical agreement.
- All disk-media combinations are “overcalling” intermediate/resistance.

Media-Disk	Categorical Agreement	Minor Error	Major Error	Very Major Error
Mast BD	72%	21.1%	7.9%	0
Mast Biomerieux	72%	26.3%	2.6%	0
Liofilchem BD	84%	13.2%	2.6%	0
Liofilchem Biomerieux	74%	26.3%	0	0

Mast BD

		DD		
		S	I	R
BMD (BBL)	S	24	7	3
	I	0	2	0
	R	0	1	1

Mast Biomerieux

		DD		
		S	I	R
BMD (BBL)	S	25	8	1
	I	0	1	1
	R	0	1	1

Liofilchem BD

		DD		
		S	I	R
BMD (BBL)	S	29	4	1
	I	0	2	0
	R	0	1	1

Liofilchem Biomerieux

		DD		
		S	I	R
BMD (BBL)	S	26	8	0
	I	0	1	1
	R	0	1	1

*Additional analysis ongoing.

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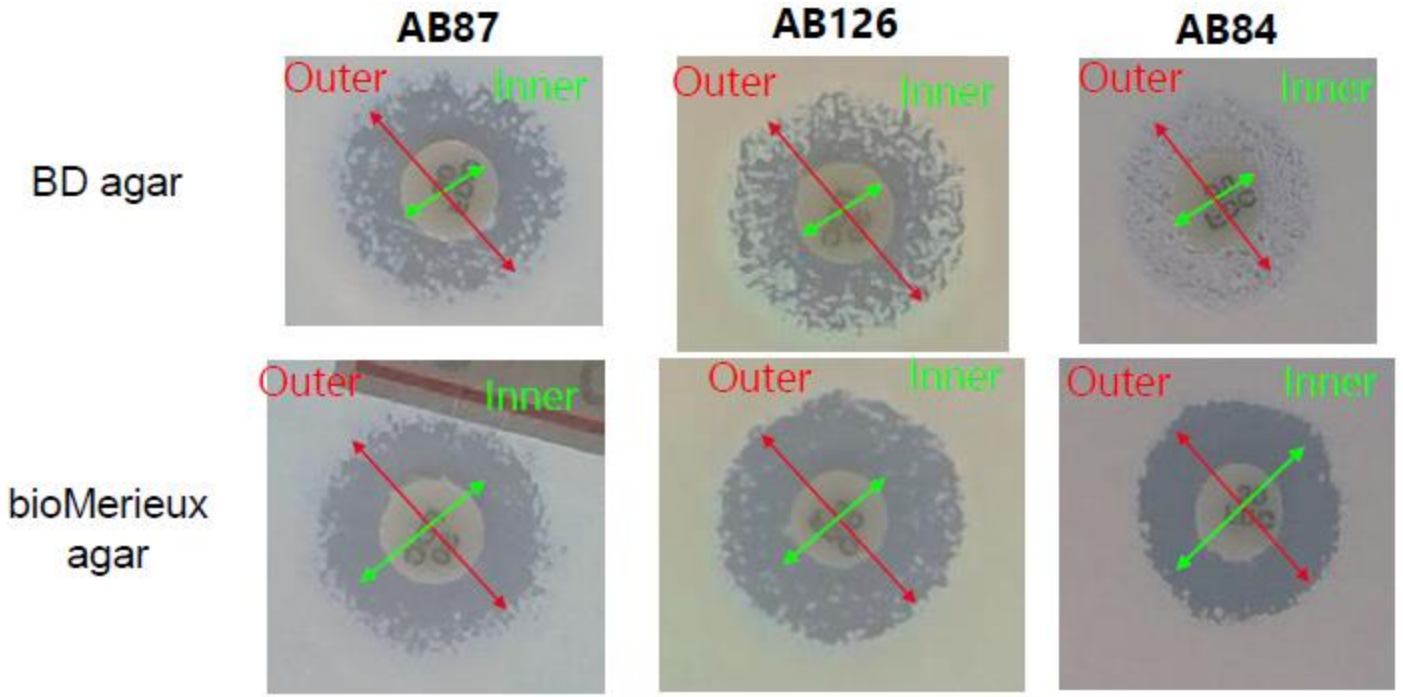
in vitro S/I/R by Media Compared to in vivo Response *E. coli*, *K. pneumoniae* and *P. aeruginosa* (n=22)

- Liofilchem BD data showed best correlation with in vivo efficacy data.
- All susceptible isolates presented CFU reduction in vivo.
- All disk media combinations are "overcalling" resistance, in line with categorical agreement.

Media	Inhibition zone S		Inhibition zone I/R		Agreement of *in vivo* CFU response with *in vitro* S/I/R
	CFU reduction *in vivo*	CFU increase *in vivo*	CFU reduction *in vivo*	CFU increase *in vivo*	
Mast BD	14/14 (100%)	0/14 (0%)	5/8 (62.5%)	3/8 (37.5%)	17/22 (77.3%)
Mast Biomerieux	15/15 (100%)	0/15 (0%)	4/7 (57.1%)	3/7 (42.9%)	18/22 (81.8%)
Liofilchem BD	16/16 (100%)	0/16 (0%)	3/6 (50%)	3/6 (50%)	19/22 (86.4%)
Liofilchem Biomerieux	15/15 (100%)	0/15 (0%)	4/7 (57.1%)	3/7 (42.9%)	18/22 (81.8%)

Disk Zone Determinations for *A. baumannii*

- With *A. baumannii* microcolonies were observed within inhibition zone for some isolates.
  - In general, more colonies appeared in the inhibition zone on BD agar compared to bioMerieux agar.
- Outer and inner diameters were measured and categorical agreement with MIC values and correlation with in vivo efficacy data were determined to assess which zone would provide best correlation.



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Categorical Agreement for *A. baumannii* (n=46)

- Categorical agreement >90% for all media/disk combinations when outer zones are read, except Mast Biomerieux (85%), but with very major errors.
- Categorical agreement for inner zones is slightly lower, but very major errors are avoided.

Media-Disk	Zone	Categorical Agreement	Minor Error	Major Error	Very Major Error
Mast BD	Outer	93%	2.2%	0	4.3%
	Inner	80%	0	19.6%	0
Mast Biomerieux	Outer	85%	4.3%	0	10.9%
	Inner	85%	0	15.2%	0
Liofilchem BD	Outer	96%	2.2%	0	2.2%
	Inner	83%	0	17.4%	0
Liofilchem Biomerieux	Outer	93%	2.2%	0	4.3%
	Inner	85%	0	15.2%	0

Conclusions

- Inhibition zones could be easily determined for Enterobacterales and *P. aeruginosa;* for few isolates, colonies appeared in the inhibition zones or showed less turbid zones around clear zone.
  - Categorical agreement with BBL MIC values remained below 90% for all disk-media combinations.
    - Liofilchem BD showed highest categorical agreement (84%).
    - Should zone cut-offs and/or "zone reading" to be revisited?

- *A. baumannii* isolates with high cefiderocol MIC values showed variability in inhibition zones due to the appearance of colonies within the inhibition zone, which was poorly reproducible.
  - Outer zones were more reproducible and resulted in better categorical agreement, but these introduced very major errors and labeled isolates that showed no response *in vivo* as susceptible.

- Difficult to identify preferred media disk combination and use of inner or outer zones of inhibition.

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Journal of Antimicrobial Chemotherapy

Rapid cefiderocol NP test for detection of cefiderocol susceptibility/ resistance in Enterobacterales

Patrice Nordmann[1,2,3,4], Maxime Bouvier[1,2], Laurent Poirel[1,2,3] and Mustafa Sadek[1,5]*

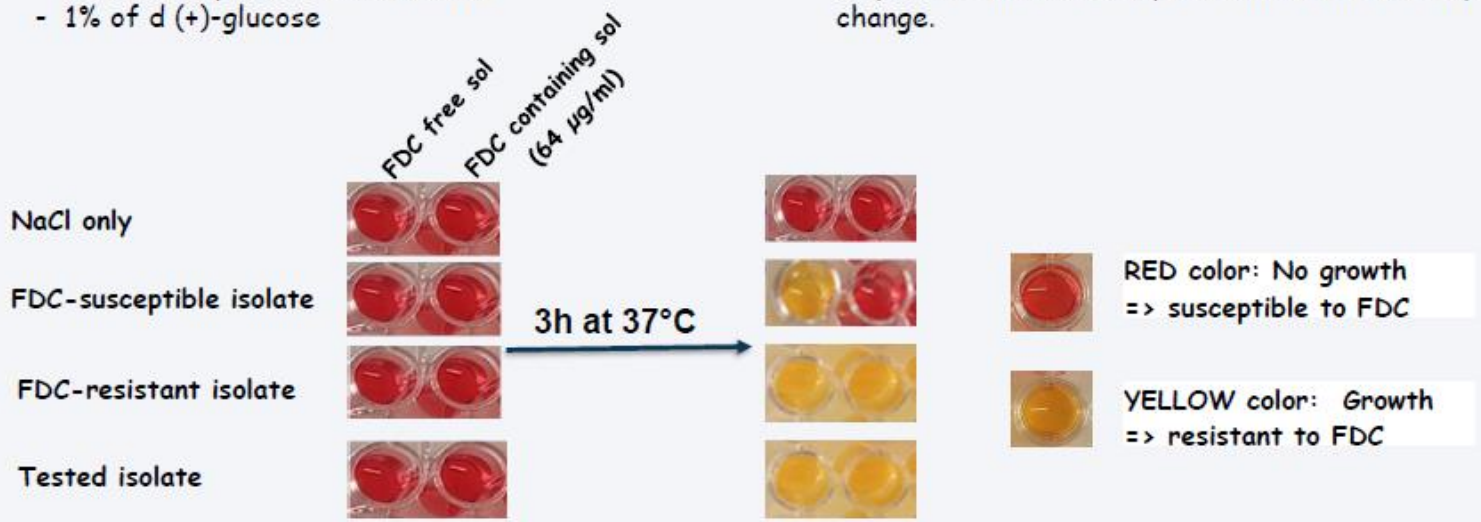
➢ Rapid Cefiderocol NP Test

- Based on glucose metabolization when bacterial growth occurs in the presence of a defined concentration of FDC (64 µg/ml). Bacterial growth is visually detectable by a red or orange/yellow color change of red phenol, a pH indicator. The results are obtained within 3 h.

➢ Rapid Cefiderocol NP solution

- Iron-depleted cation-adjusted Mueller-Hinton broth
- (ID-MHBCA)
- 0.005% of phenol red indicator
- 1% of d (+)-glucose

- The pH of the solution was adjusted to 7.5
- The solution must be pre-warmed at 37°C before use to prevent growth delay and therefore a delayed color change.



PREPARATION AND CONSERVATION OF 1 LITER OF Iron-depleted cation-adjusted Mueller-Hinton broth (ID-MHBCA):

1. Mix 25 g of CAMHB Powder in 1 liter of distilled water
2. Add 100g of Chelex Resin and mix for 2h
3. Filter (0.2 µM)
4. Adjust pH to 7.3
5. Add 22.5 mg of $CaCl_2$, 11.25 mg $MgCl_2$ and 0.56 mg of $ZnSO_4$
6. Filter (0.2 µM)

Vacuum-filtering →

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Bacteriology

Reproducibility of broth microdilution MICs for the novel siderophore cephalosporin, cefiderocol, determined using iron-depleted cation-adjusted Mueller-Hinton broth

Meredith A. Hackel [a,*], Masakatsu Tsuji [b], Yoshinori Yamano [c], Roger Echols [d], James A. Karlowsky [e], Daniel F. Sahm [a]

PREPARATION AND CONSERVATION OF THE NP SOLUTION:

1. For 1 liter of NP solution, prepared in a glass bottle:

- 990 ml of iron-depleted cation-adjusted Mueller-Hinton broth (ID-MHBCA)
- 50 mg of red phenol (0.005%)
- Adjust pH to 7.5 very precisely by adding some drops of HCl 1M

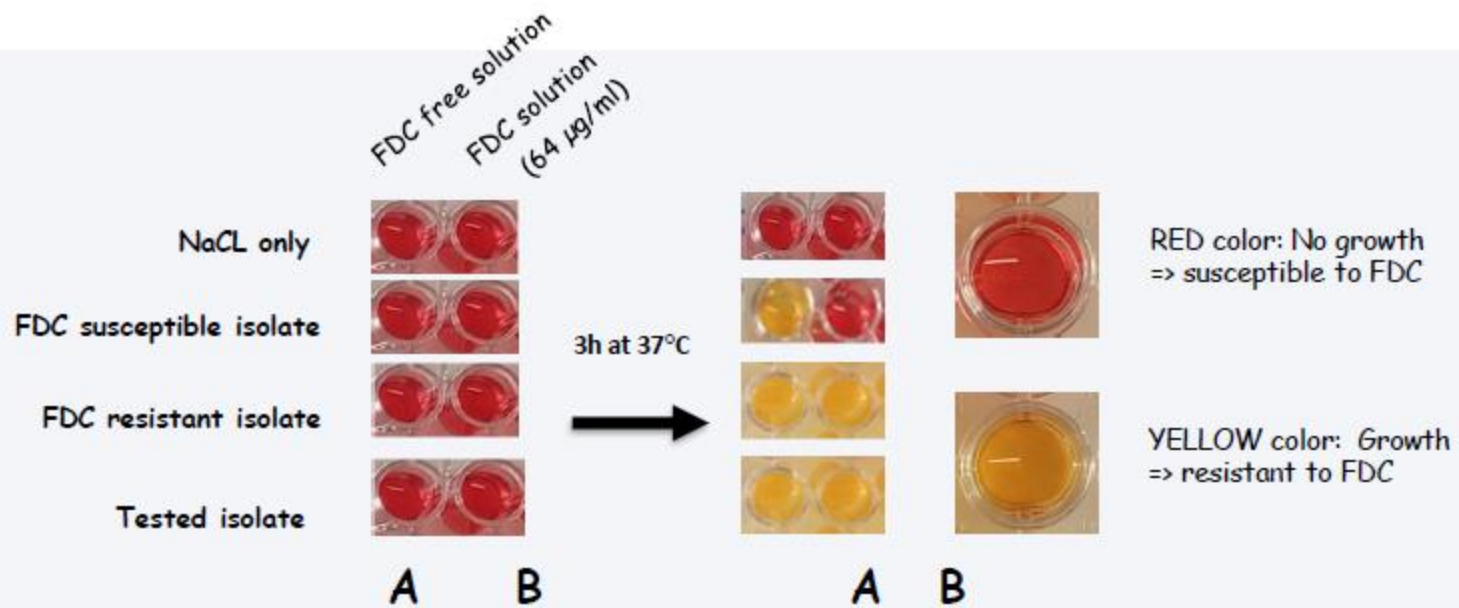
2. Autoclave at 121°C during 15 min, then let it cooling down at room temperature
3. Then, add 10 ml of D(+) glucose 10% previously sterilised by filtration
4. Keep at 4°C

STABILITY OF REAGENTS OVER TIME:

- The medium (without glucose added) can be easily stored over time at room temperature.
- Add glucose extemporaneously.

EXTEMPORANEOUS PREPARATION OF THE RAPID CEFIDEROCOL SOLUTION:

1. Prepare a solution containing cefiderocol at 85.3 mg/L (=> to get a final concentration at 64 mg/L in the testing well)
2. In a 96 wells plate, distribute 150 µL of NP solution in column A, and 150 µL of NP solution with cefiderocol at 85.3 mg/L in Column B
3. Pre-warm the 96-well microplate at 37°C
4. Prepare a 0.5 McFarland inoculum of the bacteria to be tested
5. Add 50 µL of this sample in Columns A and B



EVALUATION

- 72 enterobacterales isolates from world Wide origin and various clinical sources
  - *E. coli* (22 resistant, 15 susceptible)
  - *K. pneumoniae* (10 resistant, 9 susceptible)
  - *E. cloacae* (8 resistant, 7 susceptible)
  - *K. aerogenes* (susceptible)
- The reference broth microdilution using ID-MHBCA was considered as the standard for the comparison with the results obtained with the rapid Cefiderocol NP test.
- All experiments were repeated in triplicate in separate experiments

RAPID CEFIDEROCOL NP TEST

- **Rapid: within 3 h.**
- **Sensitivity: 98 %**
- **Specificity: 91%**
- **Easy to perform**
- **No special reading equipment**

- This new test is user-friendly, sensitive and specific, and easy to implement in routine microbiology laboratories.
- The use of such rapid accurate test may also contribute to improve the rapid implementation empirical treatment and infection control measures

Price: <$0.1 per isolate

Limitations of the Rapid Cefiderocol NP Test, and perspectives

Problems with non-fermenters

- The test did not give satisfactory results when testing *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* or *Pseudomonas aeruginosa* isolates (color change insufficient, likely due to a too-limited acidification of the medium, likely resulting from the lack of metabolization of glucose)

- Ongoing work is being performed to develop a test based on a different detection principle in order to be useful for non-fermenting bacteria (promising results already achieved)

PILOT PROJECT: evaluation of the Rapid Cefiderocol NP test

- A possible alternative as an AST for cefiderocol.
- Clinical screening.
  - Rapid result on the activity of cefiderocol to improve the rapid implementation of empirical treatment.

- 3 countries from the ReLAVRA+.
- Epidemiology to evaluate the method.
- All materials will be provided by PAHO (cefiderocol, Chelex)
- Possibility to extend to all other LAC countries.

Thank you!

•

¡Gracias!

•

Obrigada!

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