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MICROBIOLOGY AND INFECTIOUS DISEASES



EUCAST Workshop: Antimicrobial susceptibility testing with EUCAST breakpoints and methods

Susceptibility testing of infrequently isolated fastidious organisms

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FASTIDIOUS Bacteria and SUSCEPTIBILITY Testing

Fastidious: lat. *Fastidiosus* (*fastidium* = Loathing)

Organisms needing media supplemented with blood (or blood components) and possibly needing an atmosphere other than ambient air for satisfactory growth [...when performing susceptibility testing]

NOT TO BE CONSIDERED...

Fastidious & “Frequent” (...Traditionally well recognized bacteria):

Streptococcus pneumoniae

Streptococcus spp. other than *S. pneumoniae*

Neisseria gonorrhoeae -- *N. meningitidis*

Haemophilus influenzae -- *H. parainfluenzae*

Uncommon & Non-fastidious:

Bacillus spp.

[*Aeromonas* spp.]

Plesiomonas shigelloides

Vibrio spp.

FASTIDIOUS & UNCOMMON BACTERIA

LIMITATIONS FOR SUSCEPTIBILITY TESTING

- Infections may respond well (*Legionella* spp., *Bordetella* spp.) to the usually recommended drugs of choice
- Little microbiological, clinical and pharmacological information.
- Special media required: Technical difficulties
- Breakpoints often based on interpretive criteria defined for other organisms (published literature and/or personal experience)
- [Some organisms (i.e., *Bacillus*, *Corynebacterium*, *Lactobacillus*, *Leuconostoc*) can represent environmental bacteria or normal microbiota]

<i>Campylobacter coli</i> , <i>C. jejuni</i>	Fquinolone/Macrolide Resistance
<i>Corynebacterium jeikeium</i> , <i>C. urealyticum</i> , <i>C. striatum</i> , <i>C. amycolatum</i> ...	Multiresistance
<i>Erysipelothrix rhusiopathiae</i>	Glycopeptide Resistance
<i>Leuconostoc</i> spp, <i>Peidococcus</i> spp.	
[<i>Lactobacillus</i> spp.]	
“HACEK” group	Clavulanate-Inhibited β-lactamase
<i>Pasteurella</i> spp.	
<i>Moraxella catarrhalis</i>	
<i>H. pylori</i>	Metronidazole/ Macrolide/Fquinolone Resistance

M45-A2

Vol. 30 No. 18

Replaces M45-A

Vol. 26 No. 19

Methods for Antimicrobial Dilution and
Disk Susceptibility Testing of **Infrequently**
Isolated **or** **Fastidious** Bacteria; Approved
Guideline—Second Edition



2010

Organism	CLSI M45-A2	EUCAST 2014
<i>Abiotrophia, Granulicatella</i>	X	
<i>Corynebacterium</i> spp./Coryneform bacteria	X	[X]
<i>Erysipelothrix rhusiopathiae</i>	X	
Facultatively anaerobic <i>Lactobacillus</i> spp.	X	
<i>Leuconostoc</i> spp, <i>Pediococcus</i> spp.	X	
[<i>Listeria monocytogenes</i>]	X	X
<i>Campylobacter coli, C. jejuni</i>	X	X
<i>Aggregibacter-Cardiobacterium-Eikenella-Kingella</i> ["HACEK" group]	X	
[<i>Moraxella catarrhalis</i>]	X	X
<i>Pasteurella</i> spp.	X	X
<i>Helicobacter pylori</i>	X	X
<i>Brucella</i> spp.	X	
<i>Francisella tularensis</i>	X	

METHODOLOGY

Organism	COMMITTEE	METHOD	MEDIUM	ATMOS.	T°	TIME (h)
<i>Corynebacterium</i> spp.	EUCAST	DD	MH-F	5%CO ₂	35±1	18±2 // (40-48)
	CLSI M45-A	BMD	CAMHB+LHB	Ambient	35	[20]-24 (48)
<i>Listeria monocytogenes</i>	EUCAST	DD	MH-F	5%CO ₂	35±1	18±2
	CLSI M45-A	BMD	CAMHB+LHB	Ambient	35	[20]-24 (48)

METHODOLOGY

Organism	COMMITTEE	METHOD	MEDIUM	ATMOS.	T°	TIME (h)
<i>C. coli, C. jejuni</i>	EUCAST	DD	MH-FA	Microae.	41±1	24 // (40-48)
	CLSI M45-A2	DD BMD ^B	MHA-5%SB CAMHB+LHB	Microae.	36-37 // 42	48 // 24
<i>M. catarrhalis</i>	EUCAST	DD	MH-F	5%CO ₂	35±1	18±2
	CLSI M45-A2	DD BMD ^C	MHA CAMHB	5%CO ₂ Ambient	35	20-24
<i>Pasteurella</i> spp.	EUCAST	DD	MH-F	5%CO ₂	35±1	18±2
	CLSI M45-A2	DD ^D BMD ^C	MHA-5%SB CAMHB+LHB	Ambient	35	16-18 18-24

^APre-dried plates

^BAgar dilution (animal isolates)

^CNitrocefin assay for β -lactamase: A positive result predicts resistance to Penicillin/Ampicillin-Amoxycillin,
BUT... A negative result does not implies susceptibility to these agents (other mechanisms!)

^DWhen 5% CO₂ is required for growth, test by BMD

METHODOLOGY

Organism	COMMITTEE	METHOD	MEDIUM	ATMOS.	T°	TIME (h)
<i>Helicobacter pylori</i>	EUCAST	MIC method	Breakpoints based on ECOFFs			
	CLSI M45 A2	Agar Diluton	MHA-SB ^A	Microae.	35±2	72

^Aaged plates

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METHODOLOGY

Organism	COMMITTEE	METHOD	MEDIUM	ATMOS.	T°	TIME (h)
<i>Abiotrophia Granulicatella</i>	CLSI M45-A2	BMD	CAMHB+LHB +pyridoxal	Ambient	35	20-24
<i>E. rhusiopathiae</i>	CLSI M45-A2	BMD	CAMHB+LHB	Ambient	35	20-24
<i>Lactobacillus spp.</i>	CLSI M45-A2	BMD	CAMHB+LHB	5%CO ₂	35	24-48
<i>Leuconostoc spp.</i> <i>Pediococcus spp.</i>	CLSI M45-A2	BMD	CAMHB+LHB	Ambient	35	20-24

METHODOLOGY

Organism	COMMITTEE	METHOD	MEDIUM	ATMOS.	T°	TIME (h)
<i>HACEK</i>	CLSI M45-A2	BMD	CAMHB+LHB	5%CO ₂	35	24-48
<i>Brucella</i> spp.	CLSI M45-A2	BMD	Brucella broth (adj. pH)	[Ambient]	35(±2)	48
<i>Francisella</i> spp.	CLSI M45-A2	BMD	CAMHB+DGS	Ambient	35(±2)	48
<i>Yersinia pestis</i>	CLSI M45-A2	BMD	CAMHB	Ambient	35±2	24 (48)

BP-CORYNEBACTERIA	EUCAST 2014		CLSI M45-A2	
	S	R	S (<=)	R (>=)
<i>Penicillin</i>	0,12	0,12	1	4
<i>Cefepime, Cefotaxime Ceftriaxone</i>			1	4
<i>Imipenem</i>			4	16
<i>Meropenem</i>			4	16
<i>Vancomycin</i>	2	2	2	-
<i>Daptomycin</i>			1	-
<i>Gentamicin</i>	1	1	4	16
<i>Erythromycin</i>			0.5	2
<i>Clindamycin</i>	0.5	0.5		
<i>Ciprofloxacin</i>	1	1	1	4
<i>Moxifloxacin</i>	0.5	0.5		
<i>Doxycycline</i>			4	16
<i>Tetracycline</i>	2	2	4	16
<i>Cotrimoxazole</i>			2/38	4/76
<i>Rifampin</i>	0.06	0.5	1	4
<i>Quinupristin-Dalf.</i>			1	4
<i>Linezolid</i>	2	2	2	-

Gradient Diffusion Method for susceptibility testing of fastidious bacteria?

- An easy practical approach .
- Allows media supporting growth of the tested organism and different incubation conditions
- **Limitation: Not standardized by EUCAST/ CLSI**

Results should be interpreted with caution

A comment can be considered when preparing the clinical report

Other (unresolved) issues related to susceptibility testing of fastidious bacteria?

- Uncommon bacteria still to be considered by the CLSI (and EUCAST)
- Reliability (and usefulness) of data obtained with automated method or with a methodology different of the standardized one ((i.e. *C. striatum* etc.!)
- Intracellular bacteria
- Uncultivable bacteria
- ...

FINAL REMARKS

- Both CLSI and (to a lesser extent by the moment) EUCAST have defined technical conditions and evaluation criteria for susceptibility testing of “fastidious & uncommon” bacteria
- However, as for other organisms, different methods and breakpoints have been defined by both committees
- Multiple issues remain to be solved when considering susceptibility testing of fastidious organisms, and much more studies are needed before they can be reliably solved.