

M45-A3 (AST of Infrequently Isolated or Fastidious Bacteria) Working Group

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M45 Update

- Approved Guideline – May 2006
- 2nd Edition – August 2010
- 3rd Edition – Draft completion for vote Aug 2014;
Publication May 2015
- Conference calls (5) and face-to-face meetings (2)
 - August 29
 - December 10
 - Jan 12 (3 h at CLSI)
 - March 6
 - April 16
 - June 3
 - June 29 (3 h at CLSI)

M45 Revision Plan

For all organisms/tables:

- Review literature and update current list of references for the Table being updated or created. Determine if any current references should be deleted
- Contact individuals who may have unpublished data
- Determine if method, QC ranges, and breakpoints are appropriate
- For each new organism, determine if existing CLSI methods can be used
- Determine if a limited amount of supplemental testing would be useful for the proposed changes or new additions (eg, growth studies, broth microdilution, disk diffusion)
- Update Supplemental Information section of table

Table 4. *Campylobacter jejuni/coli* (Tom Fritsche, Erika Matuschek, Sonya Bodeis-Jones)

- Disk diffusion media & incubation:
 - Reassessed media using MHA, MHA-5% sheep blood and HTM
 - Confirmed MHA-5% sheep blood growth is best
 - Determined 42°C & 24 h incubation is best (delete 36-37°C for 48 h)
- Disk diffusion breakpoints
 - Adding S, I breakpoints for ciprofloxacin, erythromycin
 - Adding S, I, R breakpoints for tetracycline
 - Developed by testing 307 isolates: *C. jejuni* (N=206), *C. coli* (N=101)
 - 205 retail meat (120 *C. jejuni*, 85 *C. coli*)
 - 102 human (86 *C. jejuni*, 16 *C. coli*).
 - MIC-zone diameter regression analysis: no VM or major errors, and only 1-1.4% minor errors
- Adding statement: “Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline.”

Table 5: *Corynebacterium* spp. & Coryneforms^{a,b,c}

- ^aCoryneforms include the genera *Arcanobacterium*, **Arthrobacter**, *Brevibacterium*, *Cellulomonas*, **Cellulosimicrobium**, *Dermabacter*, *Leifsonia*, *Microbacterium*, *Oerskovia*, **Truperella** and *Turicella*.

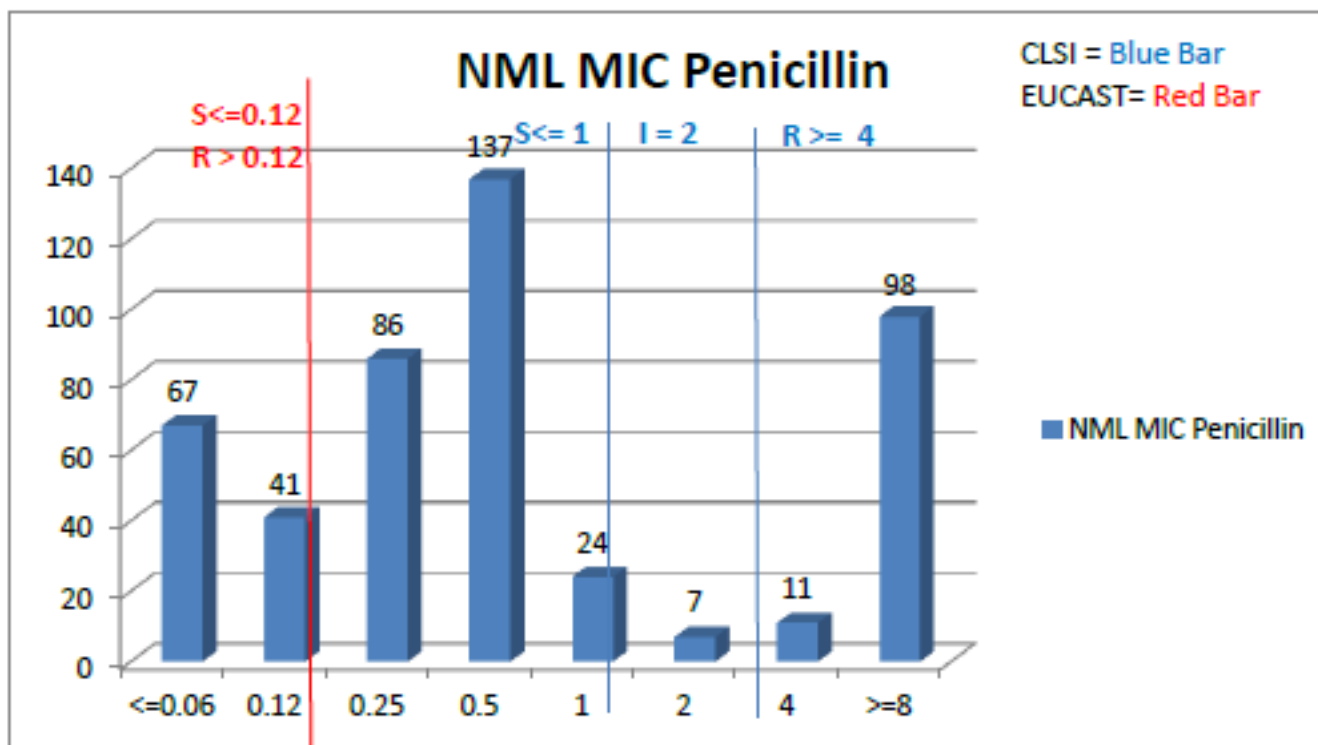
Note that several strains of lipophilic *Corynebacterium* species (*C. kroppenstedtii*, *C. tuberculostearicum*), 1 strain each of *Microbacterium hatanonsis* and *M. oleivorans*, did not grow well enough to be interpreted using these criteria (unpublished observations from 1 member of the working group). *Rothia dentocariosa* strains generally grow poorly under these conditions and should be tested anaerobically.

Table 5: *Corynebacterium* spp. & Coryneforms^{a,b,c}

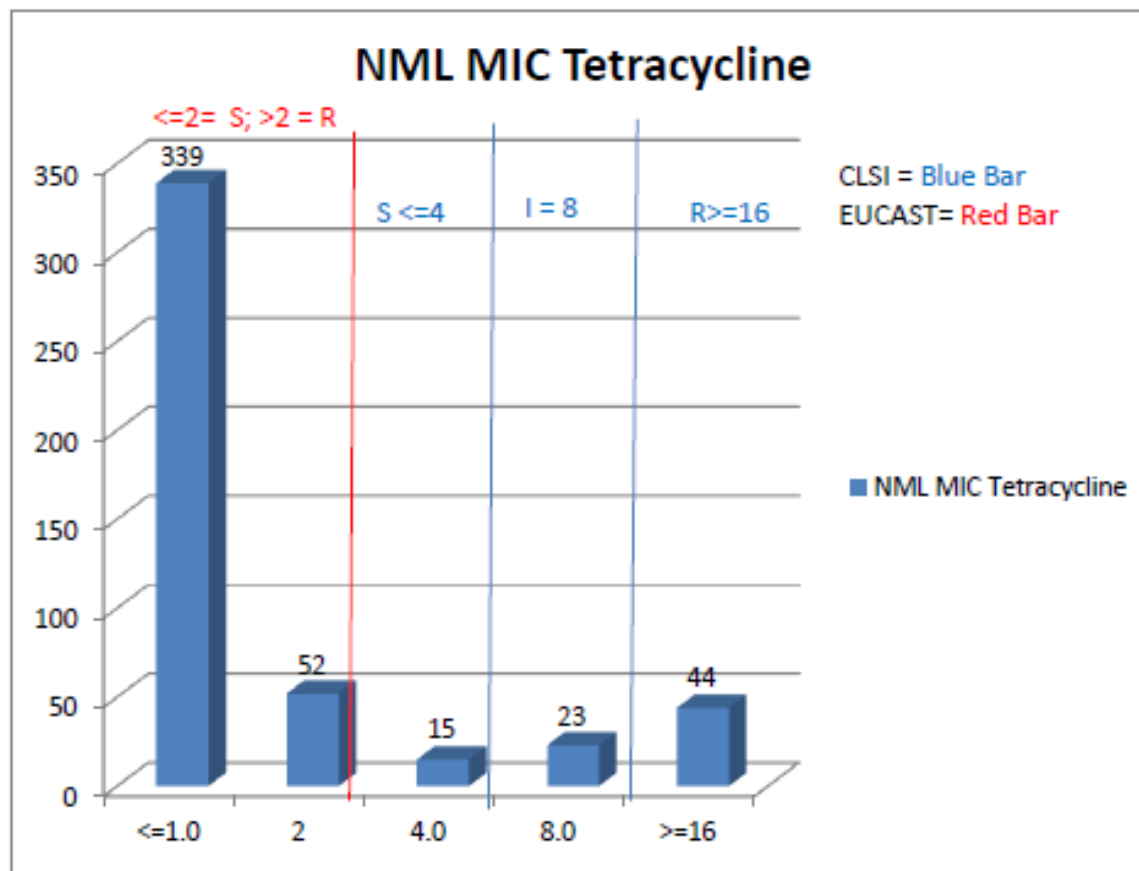
- ^bThe following are very rare human pathogens with little to no supportive AST data described in the literature, but clinicians may consider applying these criteria to the following coryneform genera: *Auritidibacter*, *Curtobacterium*, *Exiguobacterium*, *Helcobacillus*, *Janibacter*, *Knoellia* and *Pseudoclavibacter* (unpublished observations from 1 member of the working group)
- ^cThe following asporogenous, Gram positive bacilli have been tested and have provided reproducible data using these methods and criteria but are not considered as 'coryneforms'. This includes aerotolerant strains of several genera typically considered as anaerobic: *Actinomyces* identifiable as *A. bovis*, *A. europaeus*, *A. graevenitzii*, *A. johnsonii*, *A. naeslundii*, *A. neuii*, *A. odontolyticus*, *A. oris* group, *A. radingae*, *A. turicensis*, *A. urogenitalis*, *A. viscosus*, as well as aerotolerant *Bifidobacterium*, such as *B. scardovii* and *B. tsurumense*, and some *Varibaculum* isolates. Other *Actinomyces* species (including *A. dentalis* and *A. israelii*), as well as *Actinobaculum* isolates, should be tested using anaerobic protocols. (unpublished observations from 1 member of the working group)

Corynebacterium spp. & Coryneforms

Reviewed breakpoints for penicillin, gentamicin, tetracycline, trim-sulfa (since these all different from EUCAST); no changes will be made.



Corynebacterium spp. & Coryneforms



Corynebacterium spp. & Coryneforms

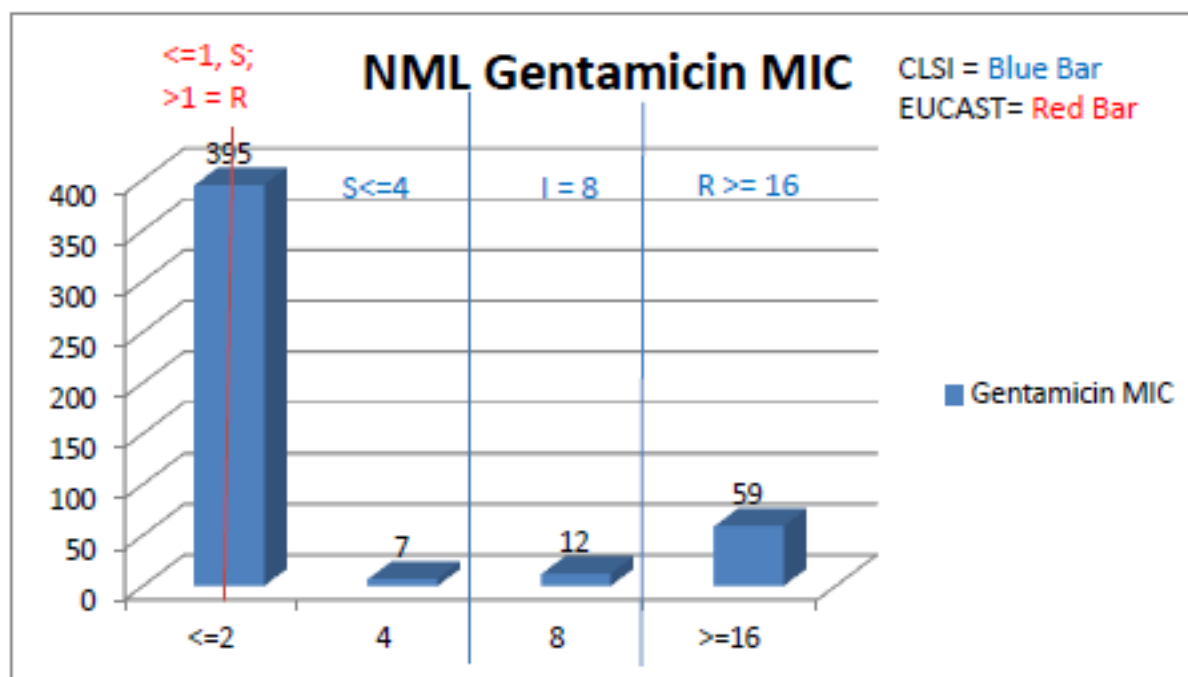


Table 8. *Helicobacter pylori*

- Peggy Kohner performed growth studies and confirmed that aged sheep blood for agar dilution results in better growth than fresh sheep blood: “Aged blood was shown to provide superior growth to blood plates prepared with fresh blood.”
- Add footnote: “*In vitro* resistance to metronidazole under these test conditions does not reliably predict *in vivo* treatment failure; therefore, testing for metronidazole is not recommended.”

New Table: *Lactococcus* spp.

<u>Source</u>	<u># Isolates</u>	<u>Species</u>	<u>Method</u>
ARUP (2009-2012)	54	-	CAMHB-LHB (Sensititre custom)
Mayo Clinic	29	L. garvieae (12) L. lactis (16)	MHA-LHB Agar dilution 20-24 h in CO ₂
UCLA (2005-2013)	4	L. lactis (1)	CAMHB-2.5% LHB (In-house BMD)
Walther et al. (bovine milk - mastitis)	72	L. lactis (41) L. garvieae (31)	MHB-5%LHB (Sensititre custom) Walther et al., Vet Microbiol 2008;131:348-57
Florez et al (EUCAST)	89	L. lactis (89)	Etest MH, 48 h, Florez et al. Intl J Probiotics & Prebiotics 2008; 3:249-56.
Total	250		

New Table: *Lactococcus* spp.

Testing Conditions Medium: CAMHB with LHB (2.5% to 5% v/v) Inoculum: Direct colony suspension, equivalent to a 0.5 McFarland standard Incubation: 35 °C; ambient air; 20 to 24 hours	Minimal QC Recommendations <i>Streptococcus pneumoniae</i> ATCC® 49619	Agents to Consider for Primary Testing Penicillin or ampicillin Ceftriaxone Vancomycin Erythromycin Clindamycin
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General Comments

Growth characteristics on routine media: often fastidious; requires blood-supplemented media for adequate growth; ambient air; 20 to 24 hours.

Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria			Comments
		S	I	R	
PENICILLINS					
	Penicillin	≤ 1	2	≥ 4	(2) Therapy of serious infections such as <u>endocarditis</u> often involves combined therapy with an <u>aminoglycoside</u> .
	<u>Ampicillin</u>	≤ 1	2	≥ 4	
CEPHEMS					
	<u>Ceftriaxone</u>	≤ 1	2	≥ 4	See comment (2).
CARBAPENEMS					
	<u>Meropenem</u>	≤ 0.25	0.5	≥ 1	
GLYCOPEPTIDES					
	<u>Vancomycin</u>	≤ 2	-	-	See comment (2).
TETRACYCLINES					
	Tetracycline	≤ 2	4	≥ 8	
MACROLIDES					
	Erythromycin	≤ 0.5	1-4	≥ 8	
LINCOSAMIDES					
	<u>Clindamycin</u>	≤ 0.5	1-2	≥ 4	
FLUOROQUINOLONES					
	<u>Levofloxacin</u>	≤ 2	4	≥ 8	
FOLATE PATHWAY INHIBITORS					
	Trimethoprim-sulfamethoxazole	≤ 2/38	-	≥ 4/76	

New Table: *Lactococcus* spp.

Resistance:

Lactococcus garvieae are intrinsically resistant to clindamycin. Resistance to clindamycin and erythromycin mediated by *ermB* has been reported in veterinary isolates of *Lactococcus lactis* subsp. *lactis*. Tetracycline resistance attributed to *tetM* or *tetS* has been detected in *L. lactis* subsp. *lactis* and *L. garvieae*.

Reasons for Testing/Not Testing:

Testing of isolates from normally sterile body sources (eg, blood cultures, deep tissue) may be warranted.

Derivation of Interpretive Criteria:

Interpretive criteria for penicillin and ampicillin are based primarily on MIC distributions. Interpretive criteria for vancomycin, clindamycin, erythromycin, and trimethoprim-sulfamethoxazole are adapted from those for *Staphylococcus* spp.; meropenem interpretive criteria are adapted from those for *Streptococcus pneumoniae*; and interpretive criteria for all other antimicrobial agents are adapted from those for viridans group streptococci, as published in CLSI document M100. In addition to MIC data provided by clinical laboratories, key citations used in derivation of interpretive breakpoints include....

New Table: *Micrococcus* spp.

<u>Source</u>	<u># Isolates</u>	<u>Species</u>	<u>Method</u>
ARUP (2009-2012)	134	-	CAMHB (Sensititre custom)
Mayo Clinic,	263	<i>M. luteus</i> (183) <i>M. lylae</i> (3)	MH agar (Agar dilution)
UCLA (2005-2013)	27	-	CAMHB (In-house BMD)
Iowa (2009- 2013)	46	-	CAMHB (Sensititre GPALL2F)
Total	470		

New Table: *Micrococcus* spp.^a

Table ____ *Micrococcus* spp.—Information and Interpretive Criteria for Broth Microdilution Susceptibility Testing

Testing Conditions Medium: CAMHB Inoculum: Direct colony suspension, equivalent to a 0.5 McFarland standard Incubation: 35 °C; ambient air; 20 to 24 hours	Minimal QC Recommendations <i>Staphylococcus aureus</i> ATCC® 29213	Agents to Consider for Primary Testing Penicillin Vancomycin
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General Comments

- (1) Growth characteristics on routine media: nonfastidious; grows well on BAP; ambient air; 16 to 20 hours.



Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria			Comments
		S	I	R	
PENICILLINS					
	Penicillin	≤ 0.12	-	≥ 0.25	
GLYCOPEPTIDES					
	Vancomycin	≤ 2	-	-	
MACROLIDES					
	Erythromycin	≤ 0.5	1-4	≥ 8	
LINCOSAMIDES					
	Clindamycin	≤ 0.5	1-2	≥ 4	

New Table: *Micrococcus* spp.^a

^aAlthough there is little supportive AST data, application of these criteria to organisms formerly included in the *Micrococcus* genus (ie, *Kocuria*, *Nesterenkonia*, *Dermacoccus*, *Kytococcus* spp) may be considered.

Resistance:

Micrococcus spp. with resistance to β -lactams and erythromycin have been reported.

Reasons for Testing/Not Testing:

Micrococcus spp. often represent contaminating bacteria in cultures. Testing of isolates from patients with multiple positive blood cultures or implanted prosthetic devices may be warranted.

Derivation of Interpretive Criteria:

Interpretive criteria are adapted from those for *Staphylococcus* spp. as published in CLSI document M100.3 In addition to MIC data provided by clinical laboratories, key citations used in derivation of interpretive breakpoints include references.....

New Table: *Rothia mucilaginosa*

<u>Source</u>	<u># Isolates</u>	<u>Method</u>
ARUP (2009-2012)	134	CAMHB-LHB (Sensititre custom)
Mayo Clinic	30	MHA-LHB Agar dilution, CO ₂ 20-24 h incubation (another 63 isolates [$\sim 2/3$] with inadequate growth on control plate)
UCLA (2005-2013)	25	CAMHB (In-house BMD)
Total	189	

New Table: *Rothia mucilaginosa*

Table ____ *Rothia mucilaginosa* —Information and Interpretive Criteria for Broth Microdilution Susceptibility Testing

Testing Conditions Medium: CAMHB with LHB (2.5% to 5% v/v) Inoculum: Direct colony suspension, equivalent to a 0.5 McFarland standard Incubation: 35 °C; ambient air; 20 to 24 hours	Minimal QC Recommendations <i>Streptococcus pneumoniae</i> ATCC® 49619	Agents to Consider for Primary Testing Penicillin Vancomycin
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General Comments

- (1) Growth characteristics on routine media: often fastidious; requires blood-supplemented media for adequate growth; ambient air; 20 to 24 hours.

Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria			Comments
		S	I	R	
PENICILLINS					
	Penicillin	≤ 1	2	≥ 4	
GLYCOPEPTIDES					
	Vancomycin	≤ 2	-	-	
MACROLIDES					
	Erythromycin	≤ 0.5	1-4	≥ 8	
LINCOSAMIDES					
	Clindamycin	≤ 0.5	1-2	≥ 4	
FLUOROQUINOLONES					
	Levofloxacin	≤ 1	2	≥ 4	
FOLATE PATHWAY INHIBITORS					
	Trimethoprim-sulfamethoxazole	≤ 2/38	-	≥ 4/76	

Supplemental Information

New Table: *Rothia mucilaginosa*

Resistance:

Resistance to β -lactams, clindamycin, erythromycin, and fluoroquinolones has been reported.

Reasons for Testing/Not Testing:

Testing of isolates from normally sterile body sources (eg, blood cultures, deep tissue) may be warranted.

Derivation of Interpretive Criteria:

Interpretive criteria for penicillin are based primarily on MIC distributions. All other interpretive criteria are adapted from those for *Staphylococcus* spp. as published in CLSI document M100.3 In addition to MIC data provided by clinical laboratories, key citations used in derivation of interpretive breakpoints include references....

M45-A3 (Part 2)
June 30, 2014

Organism Group	Addition/Modification for M45-A4
<i>Aeromonas</i> spp.	<ul style="list-style-type: none"> • Move <i>Plesiomonas shigelloides</i> to M100 (Enterobacteriaceae) • Harmonize β-lactam breakpoints with those of Enterobacteriaceae in M100 (include dosage information)
<i>Bacillus</i> spp. (not <i>B. anthracis</i>)	<ul style="list-style-type: none"> • Tentative - add comment that Table applies to closely related genera (now identifiable with MALDI): Brevibacillus, Cohnella, Lysinibacillus, Paenibacillus, Solibacillus and Sporolactobacillus • Add breakpoints for meropenem (≤ 4 S, 8 I, ≥ 16 R $\mu\text{g/ml}$; same as imipenem)

Organism Group	Addition/Modification for M45-A4
HACEK	<ul style="list-style-type: none"> • Growth studies in various broths to include CAMHB-5% LHB, HTM, BB-5% LHB + Vit K and hemin (BB), EUCAST's MH-F, suggests: <ul style="list-style-type: none"> – MH-F > HTM > CAMHB-5% LHB for <i>Aggregatibacter</i> – HTM > MH-F > CAMHB-5% LHB for <i>Cardiobacterium</i> – BB > CAMHB-5% LHB for <i>Eikenella</i> • Representative MIC studies in progress
<i>Lactobacillus</i> spp.	<ul style="list-style-type: none"> • Tentative - meropenem MICs are 2-3 dilutions greater than imipenem MICs <ul style="list-style-type: none"> – Add meropenem breakpoints (≤ 1 S, 2 I, ≥ 4 R $\mu\text{g/ml}$)

Organism Group	Addition/Modification for M45-A4
<i>Listeria monocytogenes</i>	<ul style="list-style-type: none"> • Change T-S breakpoint to “S only” ($\leq 0.5/9.5$ $\mu\text{g/ml}$) • Add meropenem “S only” breakpoint ($\leq 0.25\mu\text{g/ml}$)
<i>Moraxella catarrhalis</i>	<ul style="list-style-type: none"> • Remove cefaclor breakpoints
<i>Vibrio</i> spp.	<ul style="list-style-type: none"> • Harmonize β-lactam breakpoints with those of Enterobacteriaceae in M100 (include dosage information)

Organism Group	Addition/Modification for M45-A4
<i>Aerococcus</i> spp.	<ul style="list-style-type: none"> • Adapted from viridans streptococci • Includes <i>Aerococcus urinae</i>, <i>Aerococcus viridans</i> and <i>Aerococcus sanguinocola</i> • CAMHB with LHB (2.5% to 5% v/v); CO₂ • Testing isolates from normally sterile sources (blood cultures) may be warranted • “<i>A. urinae</i> are intrinsically R to TMP-SMX but may test S in vitro”
<i>Gemella</i> spp.	<ul style="list-style-type: none"> • Adapted from viridans streptococci (and <i>Abiotrophia/Granulicatella</i>) • CAMHB with LHB (2.5% to 5% v/v); ambient air • Testing isolates from normally sterile sources (blood cultures) may be warranted