The Challenge of Antimicrobial Susceptibility Testing in EQAP Round 46

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Overview of Changes (Continued)

Section/Table	Changes
Tables 2. (Continued)	
Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 94) **Revised:** • Daptomycin reporting comment (p. 101) • Quinupristin-dalfopristin reporting comment (p. 102)
Table 2D. Zone Diameter and MIC Breakpoints for *Enterococcus* spp.	**Added:** • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 106) **Revised:** • Dalbavancin and daptomycin (*E. faecium* only) reporting comment (p. 109) • Erythromycin and fosfomycin reporting comments (p. 110) • Quinupristin-dalfopristin and tedizolid reporting comments (p. 111)
Table 2E. Zone Diameter and MIC Breakpoints for *Haemophilus influenzae* and *Haemophilus parainfluenzae*	**Added:** • MH-F agar as a medium for disk diffusion to the testing conditions box for *H. influenzae* (p. 112) • MH-F broth as a medium for broth dilution to the testing conditions box for *H. influenzae* (p. 112) • General comment regarding antimicrobial agents that should be considered for testing and reporting (p. 112) • General comment regarding the use of MH-F broth vs HTM broth in MIC testing (p. 113) • General comment regarding the use of MH-F agar broth vs HTM broth in disk diffusion testing (p. 113) **Revised:** • Routine QC recommendations box to clarify media for each QC strain (p. 112) • Ceftolozane-tazobactam reporting comment (p. 115)

Suggested Groupings

از سال 2023 در مستند CLSI گروه بندی آنتی بیوتیک ها بر اساس گروههای A و B و C و O حذف شده و گروه بندی جدید تحت عنوان تی یر Tierجایگزین روش قدیمی شده است.

Table 1H. *Staphylococcus* spp.

Tier 1: Antimicrobial agents that are appropriate for routine, primary testing and reporting	Tier 2: Antimicrobial agents that are appropriate for routine, primary testing but may be reported following cascade reporting rules established at each institution	Tier 3: Antimicrobial agents that are appropriate for routine, primary testing in institutions that serve patients at high risk for MDROs but should only be reported following cascade reporting rules established at each institution	Tier 4: Antimicrobial agents that may warrant testing and reporting by clinician request if antimicrobial agents in other tiers are not optimal because of various factors
Azithromycin or clarithromycin or erythromycin[a]			
Clindamycin[a]			
Oxacillin[b,c,d,e] Cefoxitin[b,c,d] (surrogate for oxacillin)		Ceftaroline[f]	
Doxycycline Minocycline[a] Tetracycline[g]			
Trimethoprim-sulfamethoxazole			
Vancomycin[h]			
	Penicillin [b,i]		
	Daptomycin[h,j]		
	Linezolid	Tedizolid[f]	
		Rifampin[h,k]	
		Lefamulin[a,f]	
			Ciprofloxacin or levofloxacin Moxifloxacin
			Dalbavancin[f,h]
			Oritavancin[f,h]
			Telavancin[f,h]
			Gentamicin[l]
Urine Only			
Nitrofurantoin			

Abbreviations: MDRO, multidrug-resistant organism; MIC, minimal inhibitory concentration.

Footnotes

a. Not routinely reported on organisms isolated from the urinary tract.

b. Penicillin-susceptible staphylococci are also susceptible to other ß-lactam agents with established clinical efficacy for staphylococcal infections. Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available ß-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of ß-lactam antimicrobial agents may be determined from testing only penicillin and either cefoxitin or oxacillin. Routine testing of other ß-lactam agents, except ceftaroline, is not advised.

c. If a penicillinase-stable penicillin is tested, oxacillin is the preferred agent, and results can be applied to the other penicillinase-stable penicillins (refer to Glossary I). Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods, as described in Tables 2C, 3G-1, and 3G-2.

d. See oxacillin and cefoxitin comments in Table 2C for using cefoxitin as a surrogate test for oxacillin.

e. For *S. aureus*, *S. lugdunensis*, and other *Staphylococcus* spp. (except *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*), only MIC testing, not disk diffusion testing, is acceptable; see exceptions in Table 2C.

f. For *S. aureus* only, including methicillin (oxacillin)-resistant *S. aureus*.

g. Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.

h. MIC testing only; disk diffusion test is unreliable.

i. If penicillin is tested, report results when confirmed susceptible (see Table 2C comment [11], and Table 3F).

j. Not routinely reported on organisms isolated from the respiratory tract.

k. *Rx*: Rifampin should not be used alone for antimicrobial therapy.

l. For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.

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

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در برنامه کشوری مهار مقاومت میکروبی
بر اساس راهنمای سازمان جهانی بهداشت
(GLASS)

ویرایش **هفتم**

سال ۱۴۰۲

کمیته تخصصی میکروب شناسی
آزمایشگاه مرجع سلامت
وزارت بهداشت، درمان و آموزش پزشکی

ویرایش هفتم این سند جایگزین ویرایش قبلی (ویرایش ششم – سال ۱۴۰۱) می باشد. تغییرات عمده در ویرایش هفتم این سند در جدول زیر فهرست شده است. تغییرات کوچک یا ویراستاری و توضیحات، با حروف پررنگ نوشته شده است.

Overview of Changes

Table	Changes
Escherichia coli	Added: • Levofloxacin disk diffusion breakpoints (page 6) Revised: • Gentamicin, and amikacin disk diffusion breakpoints (page 6)
Klebsiella pneumonia	Added: • Levofloxacin disk diffusion breakpoints (page 10) Revised: • Gentamicin, and amikacin disk diffusion breakpoints (page 9)
Salmonella spp.	Added: • Levofloxacin MIC breakpoints (page 12) • Imipenem, meropenem and tetracycline disk diffusion breakpoints (page 12)
Shigella spp.	Added: • Levofloxacin, imipenem, meropenem and tetracycline disk diffusion breakpoints (pages 13-14)
Tests for Extended-Spectrum β-Lactamases in *Escherichia coli, Klebsiella pneumonia, Salmonella* spp. *and Shigella* spp.	Added: • Note (page 15)
Pseudomonas aeruginosa	Revised: • Piperacillin-tazobactam and tobramycin disk diffusion breakpoints (pages 17-18) • Urine designation for amikacin (page 18) Deleted: • Gentamicin disk diffusion breakpoints
Acinetobacter spp.	Added: • Comment (d) (page 19)
Staphylococcus aureus	Added: • Levofloxacin disk diffusion breakpoints (page 23)
Enterococcus spp.	Added: • Levofloxacin disk diffusion breakpoints (page 25)

Staphylococcus aureus					
Antimicrobial Agent	**Disk Content**	**Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm**			**Comments**
		S	**I**	**R**	
PENICILLINASE-LABILE PENICILLINS					
Penicillin	10 units	≥ 29	-	≤ 28	(a) Penicillin should be used to test the susceptibility of all staphylococci to all penicillinase-labile penicillins. Penicillin-resistant strains of staphylococci produce β-lactamase. Perform test(s) to detect β-lactamase production on staphylococci for which the penicillin MICs are ≤ 0.12 µg/mL or zone diameters ≥ 29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for β-lactamase production may appear negative by β-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and β-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *blaZ* β-lactamase gene may be considered. See Table 3F, Pages **186-187**. (b) For methicillin (oxacillin)-resistant staphylococci report penicillin as resistant or do not report.

***Staphylococcus aureus* (continued)**					
PENICILLINASE-STABLE PENICILLINS					
Oxacillin (Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*.)	30 µg Cefoxitin (surrogate test for oxacillin)	≥ 22 (cefoxitin)	-	≤ 21 (cefoxitin)	(a) Cefoxitin is tested as a surrogate for oxacillin for some species of *Staphylococcus*. Isolates that test resistant by cefoxitin or oxacillin, when using the appropriate test method for the species, should be reported as methicillin (oxacillin) resistant. If testing only cefoxitin, report as methicillin (oxacillin) susceptible or resistant based on the cefoxitin result. Isolates that test either *mecA* negative or PBP2a negative or cefoxitin susceptible should be reported as methicillin (oxacillin) susceptible. (b) For isolates of *S. aureus* that do not grow well on CAMHB* or unsupplemented MHA (eg, small-colony variants), testing on other media (eg, BMHA) does not reliably detect *mecA*-mediated resistance. Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 hours incubation in 5% CO_2) or *mecA* should be done. *Cation Adgusted Mueller Hinton Agar

***Staphylococcus aureus* (continued)**					
GLYCOPEPTIDES					
Vancomycin	-	-	-	-	(a) For *S. aureus,* vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. (b) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin susceptible, -intermediate, and -resistant isolates of *Staphylococcus* spp. other than *S. aureus* all of which give similar size zones of inhibition. (c) Send any *S. aureus* for which the vancomycin is ≥ 8 µg/mL to a reference laboratory. **Interpretive Categories and MIC Breakpoints, µg/mL** **S** ≤ 2 / **I** 4-8 / **R** ≥ 16
Teicoplanin (Optional) (Investigation)	-	-	-	-	**Interpretive Categories and MIC Breakpoints, µg/mL** **S** ≤ 8 / **I** 16 / **R** ≥ 32
TETRACYCLINES					
Doxycycline	30 µg	≥ 16	13-15	≤ 12	
MACROLIDES					
Erythromycin	15 µg	≥ 23	14-22	≤ 13	Not routinely reported on organisms isolated from the urinary tract.
FLUOROQUINOLONES					
Ciprofloxacin **or** **levofloxacin**	5 µg **5 µg**	≥ 21 **≥ 19**	16–20 **16-18**	≤ 15 **≤ 15**	*Staphylococcus* spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within three to four days after initiation of therapy. Testing of repeat isolates may be warranted.

Staphylococcus aureus (continued)					
NITROFURANTOINS					
Nitrofurantoin	300 µg	≥ 17	15-16	≤ 14	Report only on organisms isolated from the urinary tract.
FOLATE PATHWAY INHIBITORS					
Trimethoprim-sulfamethoxazole	1.25/ 23.75 µg	≥ 16	11-15	≤ 10	
LINCOSAMIDES					
Clindamycin	2 µg	≥ 21	15-20	≤ 14	(a) Not routinely reported on organisms isolated from the urinary tract. (b) For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR* by disk diffusion using the D-zone test or by broth microdilution is required befor reporting clindamycin (See Table 3I, Pages **196-198**). (c) D-zone test: 15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart. Report isolates with ICR as "clindamycin resistant" (See Table 3I, Pages **196-198**). *ICR: Inducible clindamycin resistance
ANSAMYCINS					
Rifampin	5 µg	≥ 20	17-19	≤ 16	*Rx*: should not be used alone for antimicrobial therapy.

Table 2C. Zone Diameter and MIC Breakpoints for *Staphylococcus* spp.

Testing Conditions

Medium:	Disk diffusion: MHA Broth dilution: CAMHB; CAMHB + 2% NaCl for oxacillin; CAMHB supplemented to 50 µg/mL calcium for daptomycin. Agar dilution: MHA; MHA + 2% NaCl for oxacillin. **NOTE:** Agar dilution has not been validated for daptomycin.
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35°C ± 2°C; ambient air Disk diffusion: 16–18 hours; 24 hours (for cefoxitin when testing *Staphylococcus* spp., except *S. aureus*, *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*) Dilution methods: 16–20 hours; 24 hours for oxacillin and vancomycin Testing at temperatures above 35°C may not detect MRS.

Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)

Disk diffusion:
S. aureus ATCC®[a] 25923

Dilution methods:
S. aureus ATCC® 29213

Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of β-lactam combination agents.

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) Refer to Table 1H for antimicrobial agents that should be considered for testing and reporting by microbiology laboratories.

(2) 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,[1] 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 (see the *M02 Disk Diffusion Reading Guide*[2]). Hold the Petri plate a few inches above a black background illuminated with reflected light, except for linezolid, which should be read with transmitted light (plate held up to light source). 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. For linezolid, any discernible growth within the zone of inhibition is indicative of resistance to the respective agent.

(3) *S. aureus* complex consists of the coagulase-positive species *S. aureus*, *Staphylococcus argenteus*, and *Staphylococcus schweitzeri*. If *S. argenteus* is identified by MALDI-TOF MS or sequencing, it is recommended that it be reported as "*S. aureus* complex (*S. argenteus*)," and *S. aureus* phenotypic testing method recommendations, breakpoints, and interpretive categories should be used. Human infections with *S. schweitzeri* have yet to be reported.[3]

Table 2C. Staphylococcus spp. (Continued)

(4) For staphylococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[4] Figures 3 and 4). With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, read the end point at the concentration in which there is ≥ 80% reduction in growth compared with the control (see M07,[4] Figure 5).

(5) Routine testing of urine isolates of *Staphylococcus saprophyticus* is not advised, because infections respond to concentrations achieved in urine of antimicrobial agents commonly used to treat acute, uncomplicated UTIs (eg, nitrofurantoin, trimethoprim - sulfamethoxazole, or a fluoroquinolone).

(6) Historically, resistance to the penicillinase-stable penicillins (see Glossary I) has been referred to as "methicillin resistance" or "oxacillin resistance." MRSA are strains of *S. aureus* that express *mecA*, *mecC*, or another mechanism of methicillin (oxacillin) resistance, such as changes in affinity of penicillin-binding proteins for oxacillin (modified *S. aureus* strains).

(7) Most methicillin (oxacillin) resistance is mediated by *mecA*, encoding PBP2a (also called PBP2'). Tests for *mecA* and PBP2a are the most definitive tests for detection of methicillin (oxacillin) resistance for *Staphylococcus* spp. Isolates that test positive for *mecA* or PBP2a or resistant by any of the recommended phenotypic methods should be reported as methicillin (oxacillin) resistant (see Appendix H and the table below).

Detection of methicillin (oxacillin) resistance in staphylococci is achieved by using specific methods as listed in Table 2C and further described in Tables 3G-1 and 3G-2.

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

(8) MRS, as defined by cefoxitin or oxacillin testing, as appropriate to the species, are considered resistant to other β-lactam agents, ie, penicillins, β-lactam combination agents, cephems (with the exception of ceftaroline), and carbapenems. This is because most cases of documented MRS infections have responded poorly to β-lactam therapy or because convincing clinical data that document clinical efficacy for those agents have not been presented.

(9) For tests for β-lactamase production, methicillin (oxacillin) resistance and *mecA*-mediated methicillin (oxacillin) resistance using cefoxitin, reduced susceptibility to vancomycin, ICR, and high-level mupirocin resistance (*S. aureus* only), refer to Tables 3F, 3G-1, 3G-2, 3H, and 3J, respectively.

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

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

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-LABILE PENICILLINS											
(10) Penicillin-susceptible staphylococci are susceptible to other β-lactam agents with established clinical efficacy for staphylococcal infections (including both penicillinase-labile and penicillinase-stable agents; see Glossary I). Penicillin-resistant staphylococci are resistant to penicillinase-labile penicillins. **(11)** Penicillin should be used to test the susceptibility of all staphylococci to penicillinase-labile penicillins (see Glossary I). Penicillin-resistant strains of staphylococci produce β-lactamase. Perform a test(s) to detect β-lactamase production on staphylococci for which the penicillin MICs are ≤0.12 µg/mL or zone diameters ≥29 mm before reporting the isolate as penicillin susceptible. Rare isolates of staphylococci that contain genes for β-lactamase production may appear negative by β-lactamase tests. Consequently, for serious infections requiring penicillin therapy, laboratories should perform MIC tests and β-lactamase testing on all subsequent isolates from the same patient. PCR testing of the isolate for the *blaZ* β-lactamase gene may be considered. See Table 3F.										
Penicillin	All staphylococci	10 units	≥29	-	-	≤28	≤0.12	-	-	≥0.25	**(12)** For MRS, report penicillin as resistant or do not report.
PENICILLINASE-STABLE PENICILLINS											
(13) Cefoxitin is tested as a surrogate for oxacillin for some species of *Staphylococcus*. Isolates that test resistant by cefoxitin or oxacillin, when using the appropriate test method for the species, should be reported as methicillin (oxacillin) resistant. If testing only cefoxitin, report as methicillin (oxacillin) susceptible or resistant based on the cefoxitin result. **(14)** Oxacillin (or cefoxitin) results can be applied to the other penicillinase-stable penicillins (cloxacillin, dicloxacillin, methicillin, and nafcillin). For agents with established clinical efficacy and considering site of infection and appropriate dosing, methicillin (oxacillin)-susceptible staphylococci can be considered susceptible to: • β-lactam combination agents (amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam) • Oral cephems (cefaclor, cefdinir, cephalexin, cefpodoxime, cefprozil, cefuroxime, loracarbef) • Parenteral cephems including cephalosporins I, II, III, and IV (cefamandole, cefazolin, cefepime, cefmetazole, cefonicid, cefoperazone, cefotaxime, cefotetan, ceftizoxime, ceftriaxone, cefuroxime, ceftaroline, moxalactam) • Carbapenems (doripenem, ertapenem, imipenem, meropenem) Methicillin (oxacillin)-resistant staphylococci are resistant to all currently available β-lactam antimicrobial agents, with the exception of ceftaroline. Thus, susceptibility or resistance to a wide array of β-lactam antimicrobial agents may be deduced from testing only penicillin and either cefoxitin or oxacillin. Testing of other β-lactam agents, except ceftaroline, is not advised. See general comments (7) and (8). Additional explanation on the use of cefoxitin for prediction of *mecA*-mediated methicillin (oxacillin) resistance can be found in Subchapter 3.12 of M07[4] and Subchapter 3.9 of M02.[1]										

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

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)											
Oxacillin	*S. aureus* and *S. lugdunensis*	-	-	-	-	-	≤2 (oxacillin)	-	-	≥4 (oxacillin)	**(15)** Oxacillin disk testing is not reliable for *S. aureus* and *S. lugdunensis*. **(16)** For isolates of *S. aureus* that do not grow well on CAMHB or unsupplemented MHA (eg, small-colony variants), testing on other media (eg, BMHA) does not reliably detect *mecA*-mediated resistance. Testing for PBP2a using induced growth (ie, growth taken from the zone margin surrounding a cefoxitin disk on either BMHA or a blood agar plate after 24 hours incubation in 5% CO_2) or *mecA* should be done. See general comments (7) and (8) and comments (10), (13), and (14).
		30 µg cefoxitin (surrogate test for oxacillin)	≥22	-	-	≤21	≤4 (cefoxitin)	-	-	≥8 (cefoxitin)	
Oxacillin	*S. epidermidis*	1 µg oxacillin	≥18 (oxacillin)	-	-	≤17 (oxacillin)	≤0.5 (oxacillin)	-	-	≥1 (oxacillin)	See general comments (7) and (8) and comments (10), (13), and (14). **(17)** Cefoxitin MIC testing is not reliable for detecting *mecA*-mediated resistance in *S. epidermidis*.
		30 µg cefoxitin (surrogate test for oxacillin)	≥25 (cefoxitin)	-	-	≤24 (cefoxitin)	-	-	-	-	
	S. pseudintermedius and *S. schleiferi*	1 µg oxacillin	≥18	-	-	≤17	≤0.5	-	-	≥1	**(18)** Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius* and *S. schleiferi*. See general comments (7) and (8) and comments (10), (13), and (14).

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

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
PENICILLINASE-STABLE PENICILLINS (Continued)											
Oxacillin	*Staphylococcus* spp., except: *S. aureus* *S. lugdunensis* *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	30 µg cefoxitin (surrogate test for oxacillin)	≥ 25 (cefoxitin)	-	-	≤ 24 (cefoxitin)	≤ 0.5 (oxacillin)	-	-	≥ 1 (oxacillin)	**(19)** Oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 1-2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 1-2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible. See general comments (7) and (8) and comments (10), (13), and (14).
CEPHEMS (PARENTERAL)											
Ceftaroline	*S. aureus*, including MRSA	30 µg	≥ 25	20-24		≤ 19	≤ 1	2-4	-	≥ 8	**(20)** The breakpoint for susceptible is based on a dosage regimen of 600 mg administered every 12 h. **(21)** The breakpoint for SDD is based on a dosage of 600 mg every 8 h administered over 2 h.

Organism	Phenotypic Methods for Detection of Methicillin (Oxacillin)-Resistant *Staphylococcus* spp.				
	Cefoxitin MIC	Cefoxitin disk diffusion	Oxacillin MIC	Oxacillin disk diffusion	Oxacillin salt agar
S. aureus	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	Yes (24 h)
S. lugdunensis	Yes (16-20 h)	Yes (16-18 h)	Yes (24 h)	No	No
S. epidermidis	No	Yes (24 h)	Yes (24 h)	Yes (16-18 h)	No
S. pseudintermedius	No	No	Yes (24 h)	Yes (16-18 h)	No
S. schleiferi	No	No	Yes (24 h)	Yes (16-18 h)	No
Staphylococcus spp. (not listed above or not identified to the species level)	No	Yes[a] (24 h)	Yes[a] (24 h)	No	No

Table 3G-1. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]		Detecting *mecA*-Mediated Resistance Using Oxacillin	Detecting *mecA*-mediated Resistance Using Oxacillin Salt Agar for *S. aureus* Only
Test method	Disk diffusion	Broth microdilution	Broth microdilution and agar dilution	Agar dilution for *S. aureus*
Medium	MHA	CAMHB	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)	MHA with 4% NaCl
Antimicrobial concentration	30-µg cefoxitin disk	4 µg/mL cefoxitin	2 µg/mL oxacillin	6 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard broth microdilution procedure	Standard broth microdilution procedure or standard agar dilution procedure	Colony suspension to obtain 0.5 McFarland turbidity Using a 1-µL loop that was dipped in the suspension, spot an area 10-15 mm in diameter. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot a similar area or streak an entire quadrant.
Incubation conditions	33 to 35°C; ambient air[c]			
Incubation length	16-18 hours	16-20 hours	24 hours (may be reported after 18 hours, if resistant)	24 hours; read with transmitted light
Results	≤ 21 mm = positive for *mecA*-mediated resistance ≥ 22 mm = negative for *mecA*-mediated resistance	≥ 8 µg/mL = positive for *mecA*-mediated resistance ≤ 4 µg/mL = negative for *mecA*-mediated resistance	≥4 µg/mL = positive for *mecA*-mediated resistance ≤ 2 µg/mL = negative for *mecA*-mediated resistance	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = positive for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]			
QC recommendations – routine[e,f]	*S. aureus* ATCC®[g] 25923 - *mecA* negative (zone 23-29 mm)	*S. aureus* ATCC® 29213 - *mecA* negative (MIC 1-4 µg/mL)	*S. aureus* ATCC® 29213 - *mecA* negative (MIC 0.12-0.5 µg/mL)	*S. aureus* ATCC® 29213 - susceptible (≤ 1 colony; with each test day)
QC recommendations – lot/shipment[h]	N/A	*S. aureus* ATCC® 43300 - *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 - *mecA* positive (MIC ≥ 8 µg/mL)	*S. aureus* ATCC® 43300 - *mecA* positive (>1 colony)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-1. (Continued)

Footnotes

a. Including members of the *S. aureus* complex (see Table 2C, comment [3]).

b. Cefoxitin is used as a surrogate test for detecting *mecA*-mediated methicillin (oxacillin) resistance.

c. Testing at temperatures above 35°C may not detect MRS.

d. Testing of other β-lactam agents, except ceftaroline, is not advised.

e. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[2])

f. Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

h. QC Recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3G-1

1. CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute; 2018.
2. CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard M07. Clinical and Laboratory Standards Institute; 2018.

Table 3G-2. Test for Detecting Methicillin (Oxacillin) Resistance in *Staphylococcus* spp. Except *Staphylococcus aureus*[a] and *Staphylococcus lugdunensis*

Test	Detecting *mecA*-Mediated Resistance Using Cefoxitin[b]	Detecting *mecA*-Mediated Resistance Using Oxacillin	
Test method	Disk diffusion	Disk diffusion	Broth microdilution and agar dilution
Organism group	*Staphylococcus* spp. except: *S. aureus* (refer to Table 3G-1) *S. lugdunensis* (refer to Table 3G-1) *S. pseudintermedius* (not recommended) *S. schleiferi* (not recommended)	Testing is only indicated for the species listed below: *S. epidermidis* *S. pseudintermedius* *S. schleiferi*	*Staphylococcus* spp. except: *S. aureus* (refer to Table 3G-1) *S. lugdunensis* (refer to Table 3G-1)
Medium	MHA	MHA	CAMHB with 2% NaCl (broth microdilution) MHA with 2% NaCl (agar dilution)
Antimicrobial concentration	30 µg cefoxitin disk	1-µg oxacillin disk	0.5 µg/mL oxacillin
Inoculum	Standard disk diffusion procedure	Standard disk diffusion procedure	Standard broth microdilution procedure or standard agar dilution procedure
Incubation conditions	33 to 35°C; ambient air[c]		
Incubation length	24 hours (may be reported after 18 hours, if resistant)	16-18 hours	24 hours (may be reported after 18 hours, if resistant)
Results	≤ 24 mm = positive for *mecA*-mediated resistance ≥ 25 mm = negative for *mecA*-mediated resistance	≤ 17 mm = positive for *mecA*-mediated resistance ≥ 18 mm = negative for *mecA*-mediated resistance	≥ 1 µg/mL = positive for *mecA*-mediated resistance ≤ 0.5 µg/mL = negative for *mecA*-mediated resistance
Additional testing and reporting	Isolates that test positive for *mecA*-mediated resistance should be reported as methicillin (oxacillin) (not cefoxitin) resistant; other β-lactam agents, except ceftaroline, should be reported as resistant or should not be reported.[d]		
			For *Staphylococcus* spp., excluding *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*, oxacillin MIC breakpoints may overcall resistance, and some isolates for which the oxacillin MICs are 1-2 µg/mL may be *mecA* negative. Isolates from serious infections for which oxacillin MICs are 1-2 µg/mL may be tested for *mecA* or for PBP2a. Isolates that test *mecA* or PBP2a negative should be reported as methicillin (oxacillin) susceptible.
QC recommendations - routine[e]	*S. aureus* ATCC® 25923 - *mecA* negative (zone 23-29 mm)	*S. aureus* ATCC® 25923 - *mecA* negative (zone 18-24 mm)	*S. aureus* ATCC® 29213 - *mecA* negative (MIC 0.12-0.5 µg/mL)
QC recommendations - lot/shipment[f]	N/A	*S. aureus* ATCC® 43300 - *mecA* positive (zone ≤ 24 mm)	*S. aureus* ATCC® 43300 - *mecA* positive (MIC ≥ 8 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant *Staphylococcus* spp.; N/A, not applicable.

Table 3G-2. (Continued)

Footnotes

a. Including members of the *S. aureus* complex (see Table 2C, general comment [3]).

b. Cefoxitin is tested as a surrogate for detecting *mecA*-mediated methicillin (oxacillin) resistance; however, recent data suggest that the cefoxitin disk diffusion test may not perform reliably for all species (eg, *S. haemolyticus*).[1]

c. Testing at temperatures above 35°C may not detect MRS.

d. Testing of other β-lactam agents, except ceftaroline, is not advised.

e. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[2] and M07[3])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

g. QC Recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

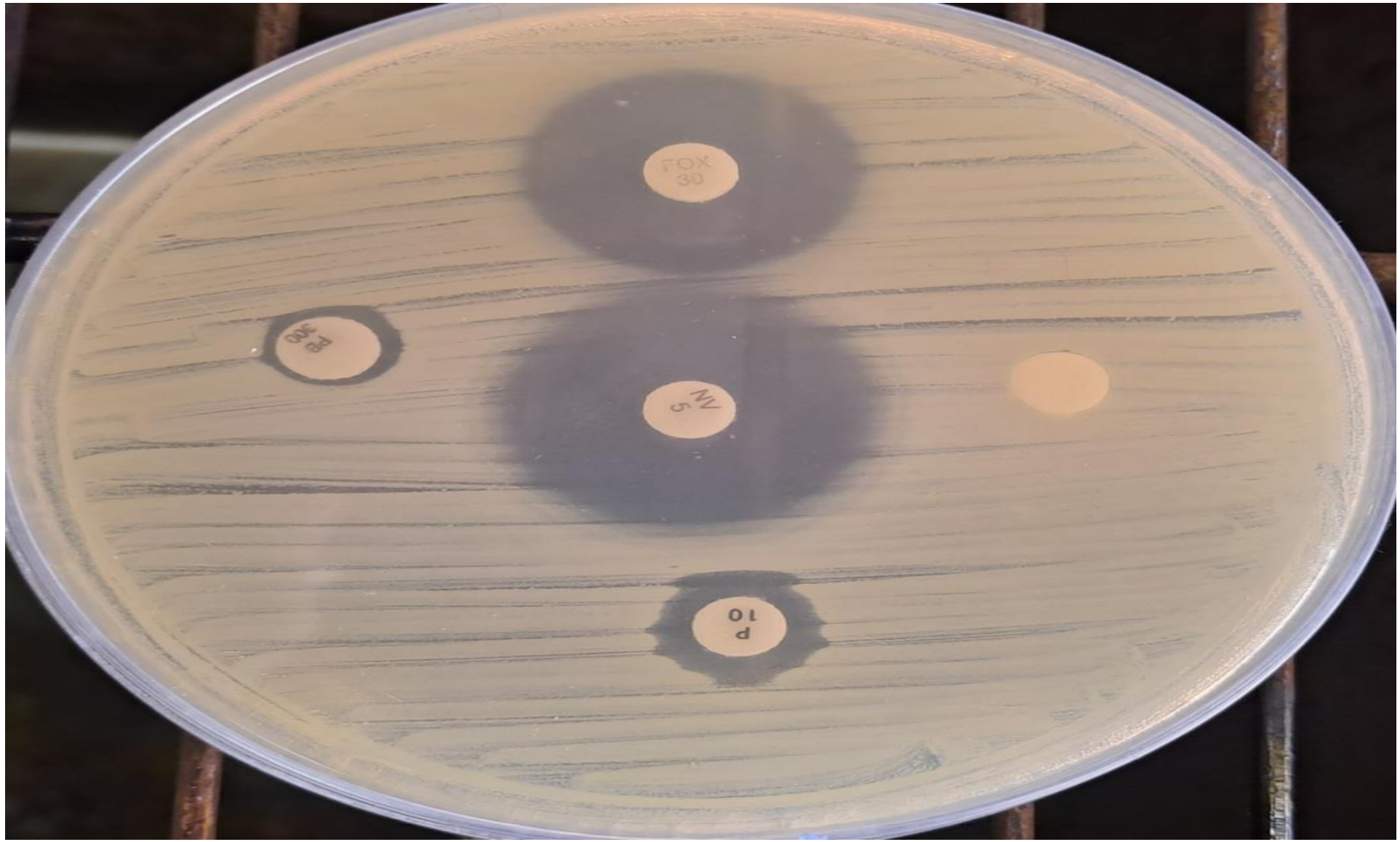
References for Table 3G-2

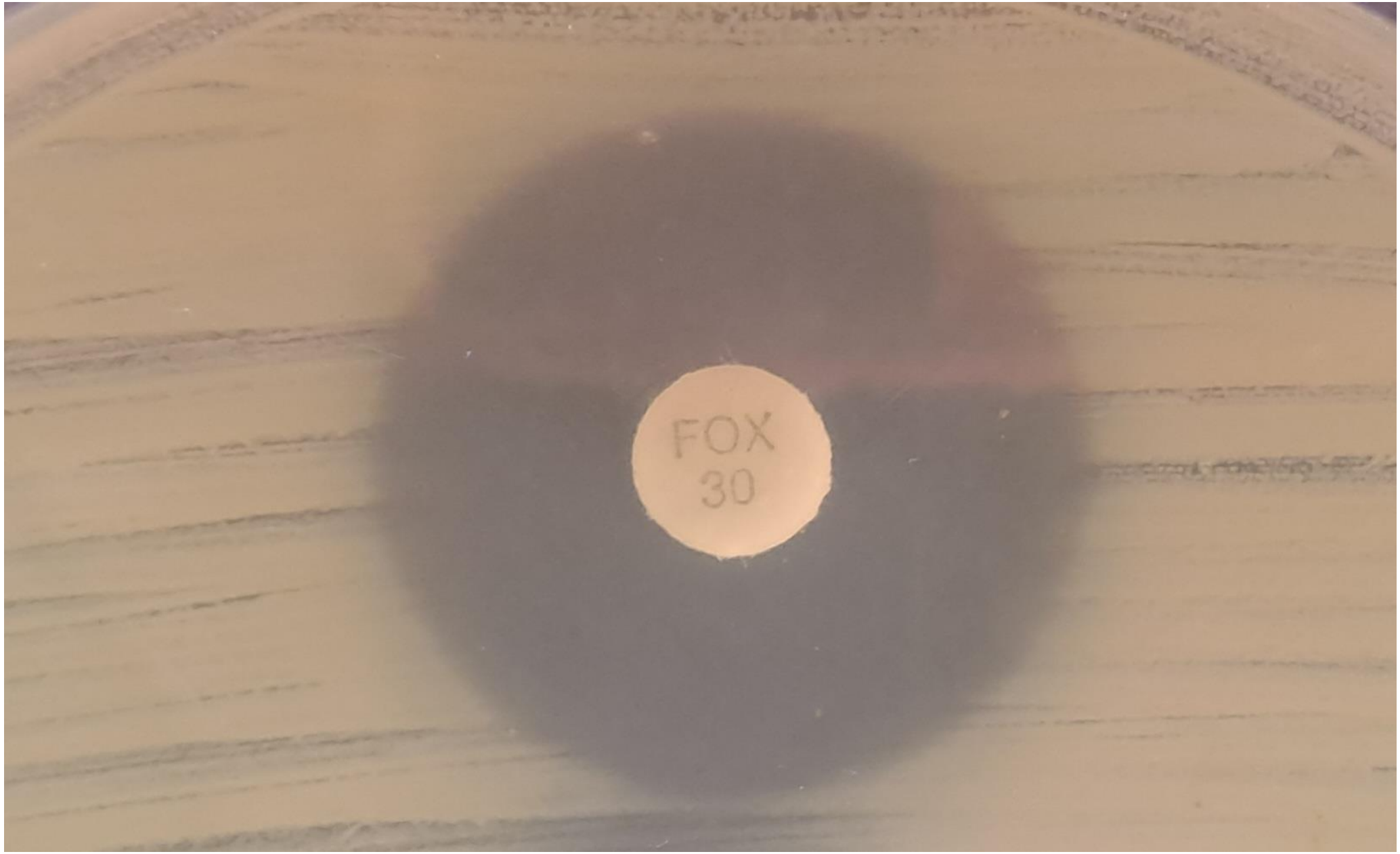
1 Humphries RM, Magnano P, Burnham CA, et al. Evaluation of surrogate tests for the presence of *mecA*-mediated methicillin resistance in *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus capitis* and *Staphylococcus warneri*. *J Clin Microbiol*. 2020;59(1):e02290-20.

2 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests*. 13th ed. CLSI standard *M02*. Clinical and Laboratory Standards Institute; 2018.

3 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 11th ed. CLSI standard *M07*. Clinical and Laboratory Standards Institute; 2018.

بررسی حساسیت به دیسک Cefoxitin جهت جداسازی سویه های MRSA

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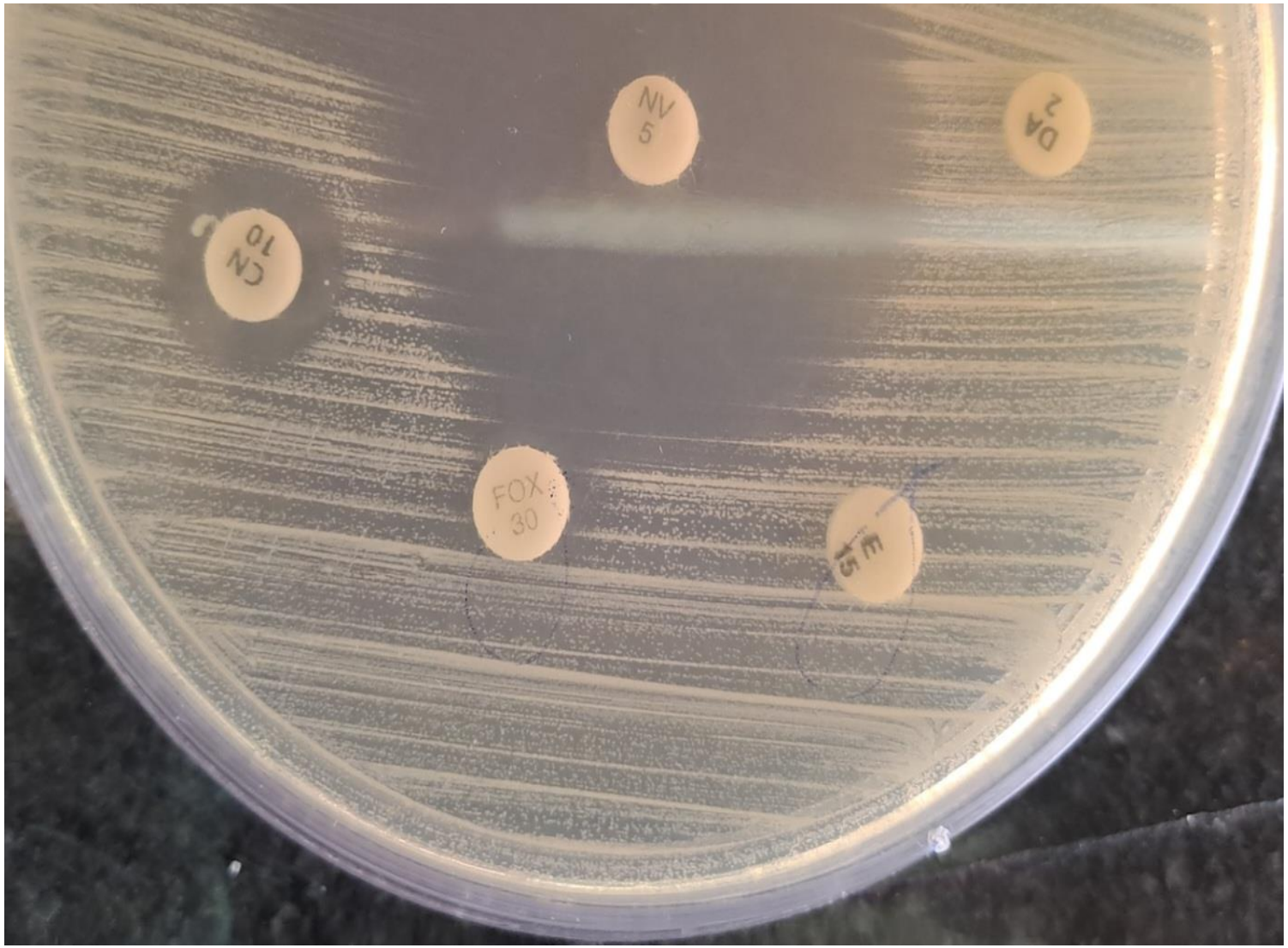
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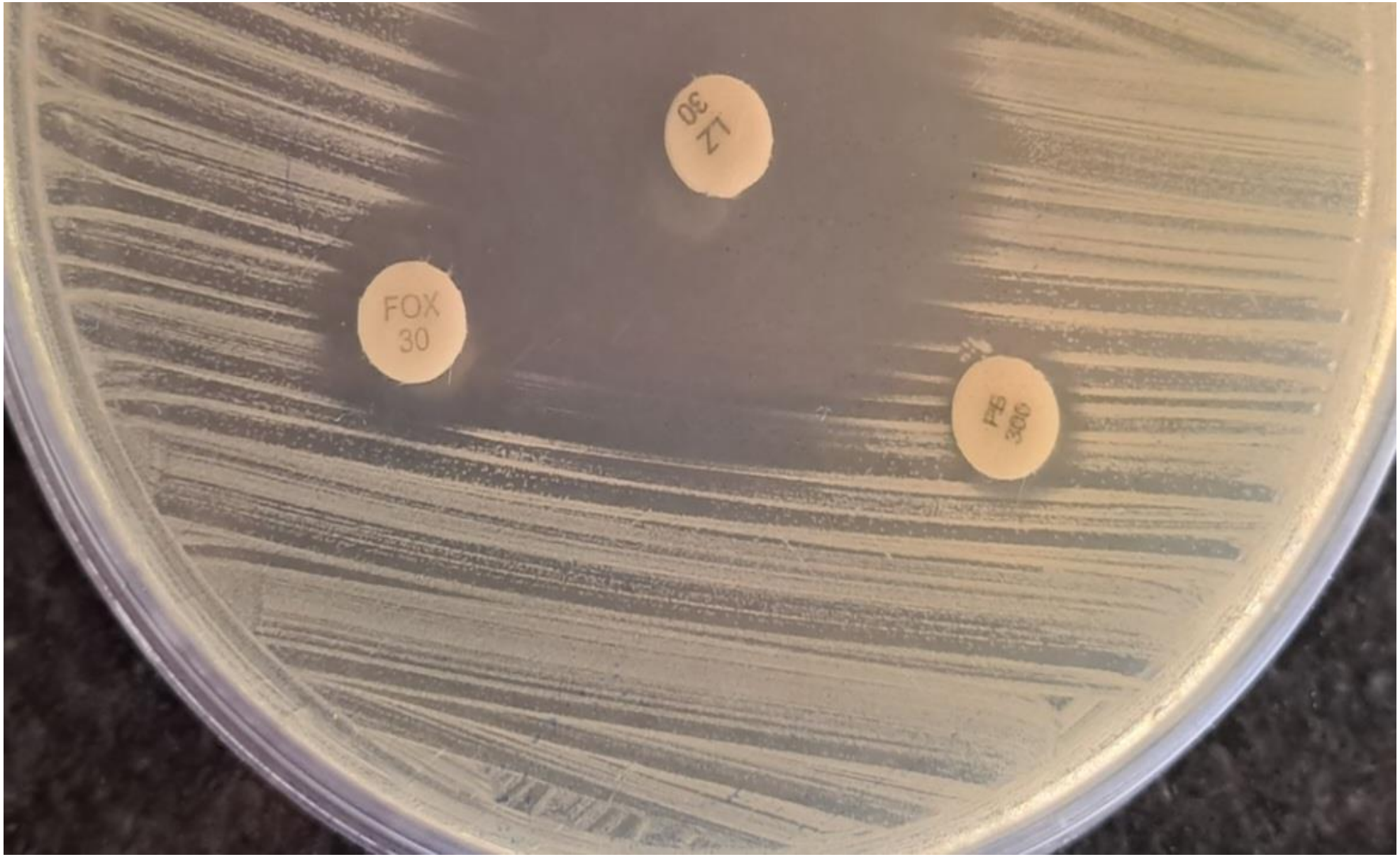
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Table 2C. *Staphylococcus* spp. (Continued)

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
GLYCOPEPTIDES											
(22) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of *Staphylococcus* spp. other than *S. aureus*, all of which give similar size zones of inhibition.											
Vancomycin	*S. aureus*, including MRSA	-	-	-	-	-	≤2	-	4–8	≥16	**(23)** For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy. **(24)** Send any *S. aureus* for which the vancomycin is ≥8 µg/mL to a referral laboratory. See Appendix A. Also refer to Table 3G-1 for *S. aureus*, Subchapter 3.12 in M07,[4] and Subchapter 3.9 in M02.[1]
	Staphylococcus spp. other than *S. aureus*	-	-	-	-	-	≤4	-	8–16	≥32	**(25)** Send any *Staphylococcus* spp. other than *S. aureus* for which the vancomycin MIC is ≥32 µg/mL to a referral laboratory. See Appendix A. See also Subchapter 3.12 in M07[4] and Subchapter 3.9 in M02.[1]
LIPOGLYCOPEPTIDES											
Dalbavancin	*S. aureus*, including MRSA	-	-	-	-	-	≤0.25	-	-	-	**(26)** Breakpoints are based on a dosage regimen of 1500 mg (single dose) or 1000 mg (two doses) IV administered over 30 minutes followed one week later by 500 mg IV administered over 30 minutes.
Oritavancin		-	-	-	-	-	≤0.12	-	-	-	**(27)** Breakpoints are based on a dosage regimen of 1200 mg IV administered once.
Telavancin		-	-	-	-	-	≤0.12	-	-	-	**(28)** Breakpoints are based on a dosage regimen of 10 mg/kg administered every 24 h.
Teicoplanin **(Inv.)**	All staphylococci	-	-	-	-	-	≤8	-	16	≥32	

Table 3H. Vancomycin Agar Screen for *Staphylococcus aureus* and *Enterococcus* spp.

Screen Test	Vancomycin MIC ≥ 8 µg/mL	
Test method	Agar dilution	Agar dilution
Organism group	*S. aureus*	*Enterococcus* spp.
Medium	BHI agar	BHI[a] agar
Antimicrobial concentration	6 µg/mL vancomycin	6 µg/mL vancomycin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Preferably, using a micropipette, spot a 10-µL drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.	1–10 µL of a 0.5 McFarland suspension spotted onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation length	24 hours	24 hours
Results	Examine carefully with transmitted light for >1 colony or light film of growth. >1 colony = presumptive reduced susceptibility to vancomycin	>1 colony = presumptive vancomycin resistance
Additional testing and reporting	Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S. aureus* that grow on BHI-vancomycin screening agar. Testing on BHI-vancomycin screening agar does not reliably detect all vancomycin-intermediate *S. aureus* strains. Some strains for which the vancomycin MICs are 4 µg/mL will fail to grow.	Perform vancomycin MIC on *Enterococcus* spp. that grow on BHI-vancomycin screening agar and test for motility and pigment production to distinguish species with acquired resistance (eg, *vanA* and *vanB*) from those with intrinsic, intermediate-level resistance to vancomycin (eg, *vanC*), such as *Enterococcus gallinarum* and *Enterococcus casseliflavus*, which often grow on the vancomycin screen plate. In contrast to other enterococci, *E. casseliflavus* and *E. gallinarum* with vancomycin MICs of 8–16 µg/mL (intermediate) differ from vancomycin-resistant enterococci for infection prevention purposes.
QC recommendations – routine[b]	*E. faecalis* ATCC® 29212 – susceptible	*E. faecalis* ATCC® 29212 – susceptible
QC recommendations – lot/shipment[d]	*E. faecalis* ATCC® 51299 – resistant	*E. faecalis* ATCC® 51299 – resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; MIC, minimal inhibitory concentration; QC, quality control.

Table 3H. (Continued)

Footnotes

a. Even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably with BHI media.

b. QC recommendations – routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapter 4.7.2.3 in M02[1] and M07[2])
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

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

d. QC recommendations – lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

References for Table 3H

1 CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests.* 13th ed. CLSI standard *M02*. Clinical and Laboratory Standards Institute; 2018.

2 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.* 11th ed. CLSI standard *M07*. Clinical and Laboratory Standards Institute; 2018.

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

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
LIPOPEPTIDES											
Daptomycin	All staphylococci	-	-	-	-	-	≤1	-	-	-	(29) **Not routinely** reported **on organisms** isolated from the respiratory tract.
AMINOGLYCOSIDES											
(30) For staphylococci that test susceptible, gentamicin is used only in combination with other active agents that test susceptible.											
Gentamicin	All staphylococci	10 µg	≥15	-	13-14	≤12	≤4	-	8	≥16	
MACROLIDES											
(31) Not routinely reported on organisms isolated from the urinary tract.											
Azithromycin	All staphylococci	15 µg	≥18	-	14-17	≤13	≤2	-	4	≥8	
or clarithromycin or erythromycin		15 µg	≥18		14-17	≤13	≤2		4	≥8	
		15 µg	≥23		14-22	≤13	≤0.5		1-4	≥8	
Dirithromycin*		15 µg	≥19	-	16-18	≤15	≤2	-	4	≥8	
TETRACYCLINES											
(32) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.											
Tetracycline	All staphylococci	30 µg	≥19	-	15-18	≤14	≤4	-	8	≥16	
Doxycycline		30 µg	≥16	-	13-15	≤12	≤4	-	8	≥16	
Minocycline		30 µg	≥19	-	15-18	≤14	≤4	-	8	≥16	See comment (31).
FLUOROQUINOLONES											
(33) *Staphylococcus* spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.											
Ciprofloxacin or levofloxacin	All staphylococci	5 µg	≥21	-	16-20	≤15	≤1	-	2	≥4	
Moxifloxacin		5 µg	≥19	-	16-18	≤15	≤1	-	2	≥4	
		5 µg	≥24	-	21-23	≤20	≤0.5		1	≥2	
Enoxacin* **(U)**[b]		10 µg	≥18	-	15-17	≤14	≤2	-	4	≥8	
Gatifloxacin*		5 µg	≥23	-	20-22	≤19	≤0.5	-	1	≥2	
Grepafloxacin*		5 µg	≥18	-	15-17	≤14	≤1	-	2	≥4	
Lomefloxacin*		10 µg	≥22	-	19-21	≤18	≤2	-	4	≥8	
Norfloxacin* **(U)**[b]		10 µg	≥17	-	13-16	≤12	≤4	-	8	≥16	
Ofloxacin*		5 µg	≥18	-	15-17	≤14	≤1	-	2	≥4	
Sparfloxacin*		5 µg	≥19	-	16-18	≤15	≤0.5	-	1	≥2	
Fleroxacin **(Inv.)**		5 µg	≥19	-	16-18	≤15	≤2	-	4	≥8	

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

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
NITROFURANS											
Nitrofurantoin **(U)**[b]	All staphylococci	300 µg	≥17	-	15-16	≤14	≤32	-	64	≥128	
LINCOSAMIDES											
Clindamycin	All staphylococci	2 µg	≥21	-	15-20	≤14	≤0.5	-	1-2	≥4	**(34)** For isolates that test erythromycin resistant and clindamycin susceptible or intermediate, testing for ICR by disk diffusion using the D-zone test or by broth microdilution is required before reporting clindamycin (see Table 3I, Subchapter 3.9 in M02,[1] and Subchapter 3.12 in M07[4]). See comment (31).
FOLATE PATHWAY ANTAGONISTS											
Trimethoprim-sulfamethoxazole	All staphylococci	1.25/23.75 µg	≥16	-	11-15	≤10	≤2/38	-	-	≥4/76	
Sulfonamides **(U)**[b]	All staphylococci	250 or 300 µg	≥17	-	13-16	≤12	≤256	-	-	≥512	**(35)** Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
Trimethoprim **(U)**[b]	All staphylococci	5 µg	≥16	-	11-15	≤10	≤8	-	-	≥16	
PHENICOLS											
Chloramphenicol*	All staphylococci	30 µg	≥18	-	13-17	≤12	≤8	-	16	≥32	See comment (31).
ANSAMYCINS											
Rifampin	All staphylococci	5 µg	≥20	-	17-19	≤16	≤1	-	2	≥4	**(36)** ***Rx:*** Rifampin should not be used alone for antimicrobial therapy.
STREPTOGRAMINS											
Quinupristin-dalfopristin*	*S. aureus*	15 µg	≥19	-	16-18	≤15	≤1	-	2	≥4	**(37) Report only on** methicillin (oxacillin)-susceptible *S. aureus*.

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

Antimicrobial Agent	*Staphylococcus* spp. Indications	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm				Interpretive Categories and MIC Breakpoints, µg/mL				Comments
			S	SDD	I	R	S	SDD	I	R	
OXAZOLIDINONES											
(38) *S. aureus* that test susceptible to linezolid by MIC are also considered susceptible to tedizolid. However, some organisms that test resistant to linezolid may be susceptible to tedizolid.											
Linezolid	All staphylococci	30 µg	≥21	-	-	≤20	≤4	-	-	≥8	**(39)** When testing linezolid, disk diffusion zones should be examined using transmitted light. Organisms with resistant results by disk diffusion should be confirmed using an MIC method.
Tedizolid	*S. aureus*, including MRSA	-	-	-	-	-	≤0.5	-	1	≥2	**(40)** Breakpoints are based on a dosage regimen of 200 mg administered every 24 h.
PLEUROMUTILINS											
Lefamulin	*S. aureus*, including MRSA	20 µg	≥23	-	-	-	≤0.25	-	-	-	**(41)** The breakpoints for susceptible are based on a dosage regimen of 150 mg IV or 600 mg orally administered every 12 h. **(42)** Not routinely reported on organisms isolated from the urinary tract.

Abbreviations: ATCC®, American Type Culture Collection; BMHA, blood Mueller-Hinton agar; CAMHB, cation-adjusted Mueller-Hinton broth; I, intermediate; ICR, inducible clindamycin resistance; **Inv., investigational agent;** IV, intravenous; MALDI-TOF MS; matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin (oxacillin)-resistant staphylococci; MRSA, methicillin (oxacillin)-resistant *S. aureus;* PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; QC, quality control; R, resistant; S, susceptible; SDD, susceptible-dose dependent; **U, urine;** UTI, urinary tract infection.
Symbol: *, designation for "Other" agents that are not included in Tables 1 but have established clinical breakpoints.

Footnotes

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

b. Report only on organisms isolated from the urinary tract.

Table 3I. Tests for Detecting Inducible Clindamycin Resistance in *Staphylococcus* spp., *Streptococcus pneumoniae*, and *Streptococcus* spp. B-Hemolytic Group[a,b]

Test	ICR			
Test method	Disk Diffusion (D-zone test)		Broth Microdilution	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and B-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and B-hemolytic *Streptococcus* spp.
Medium	MHA or blood agar purity plate used with MIC tests	MHA supplemented with sheep blood (5% v/v) or TSA supplemented with sheep blood (5% v/v)	CAMHB	CAMHB with LHB (2.5% to 5% v/v)
Antimicrobial concentration	15-µg erythromycin and 2-µg clindamycin disks spaced 15-26 mm apart	15-µg erythromycin and 2-µg clindamycin disks spaced 12 mm apart	4 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well	1 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well
Inoculum	Standard disk diffusion procedure or heavily inoculated area of purity plate	Standard disk diffusion procedure	Standard broth microdilution procedure	
Incubation conditions	35°C ± 2°C; ambient air	35°C ± 2°C; 5% CO_2	35°C ± 2°C; ambient air	
Incubation length	16-18 hours	20-24 hours	18-24 hours	20-24 hours
Results	Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = ICR. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.		Any growth = ICR. No growth = no ICR.	

Table 3I. (Continued)

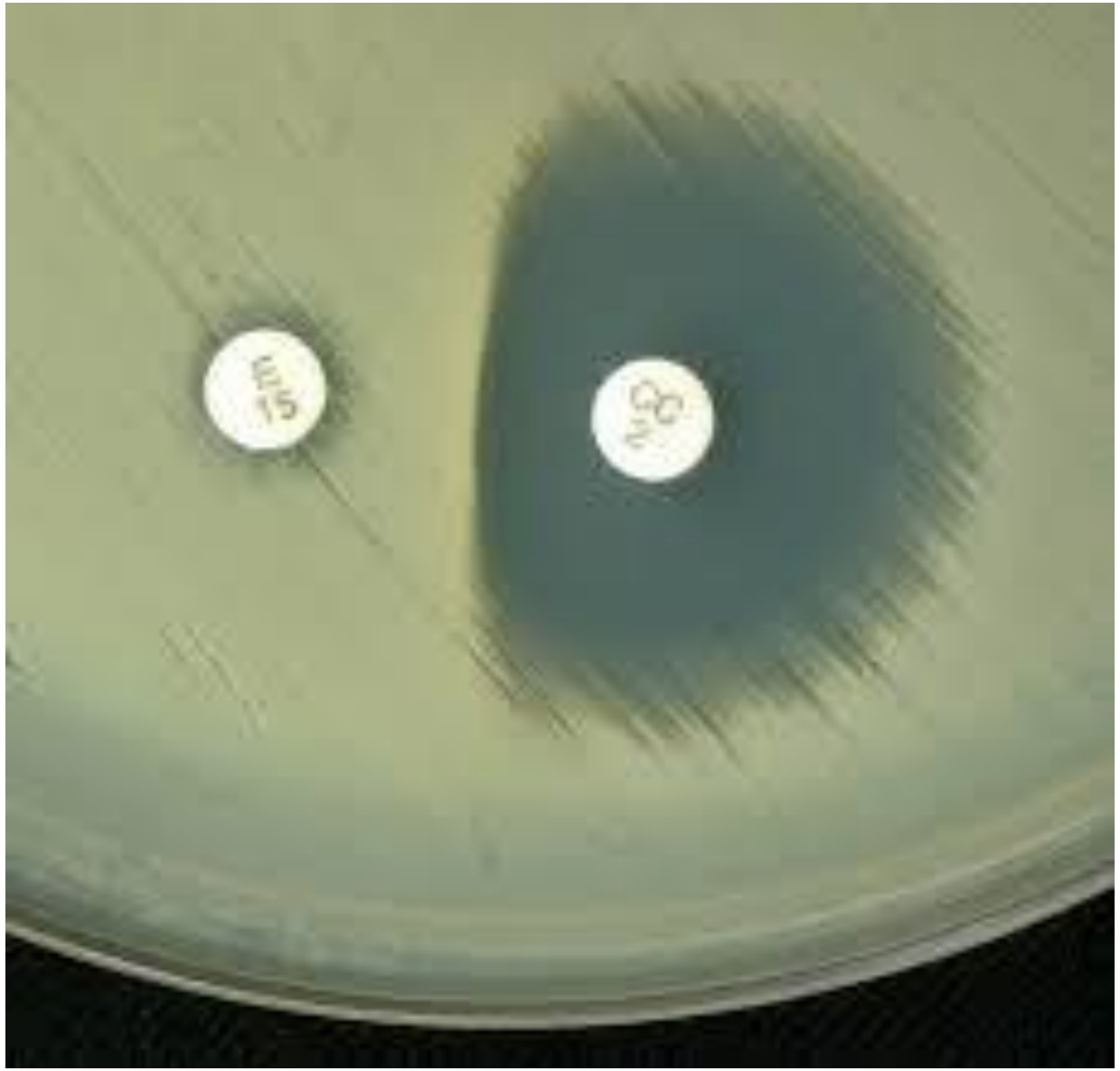
Test	ICR			
Test method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	All *Staphylococcus* spp.	*S. pneumoniae* and B-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and B-hemolytic *Streptococcus* spp.
Additional testing and reporting	Report isolates with ICR as "clindamycin resistant." The following comment may be included with the report: "This isolate is presumed to be resistant based on detection of ICR, as determined by testing clindamycin in combination with erythromycin."			
QC recommendations – routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of erythromycin and clindamycin disks	*S. pneumoniae* ATCC® 49619 for routine QC of erythromycin and clindamycin disks	*S. aureus* ATCC® BAA-976™ or *S. aureus* ATCC® 29213 – no growth	*S. pneumoniae* ATCC® 49619 or *S. aureus* ATCC® BAA-976™ – no growth
QC recommendations – lot/shipment[e]	**Perform QC according to standard disk diffusion QC procedures per M02[1] (eg, daily or weekly)**		*S. aureus* ATCC® BAA-977™ – growth	
QC recommendations – supplemental[f]	*S. aureus* ATCC® BAA-976™ (D-zone test negative) *S. aureus* ATCC® BAA-977™ (D-zone test positive) Use of unsupplemented MHA is acceptable for these strains.		*S. aureus* ATCC® BAA-976™ (no growth) *S. aureus* ATCC® BAA-977™ (growth)	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ICR, inducible clindamycin resistance; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

Footnotes

a. Antimicrobial susceptibility testing of B-hemolytic streptococci does not need to be performed routinely (see general comment [5] in Table 2H-1). When susceptibility testing is clinically indicated, test for ICR in strains that are erythromycin resistant and clindamycin susceptible or intermediate.

b. In accordance with 2010 guidance from the Centers for Disease Control and Prevention, colonizing isolates of group B streptococci from penicillin-allergic pregnant women should be tested for clindamycin (including ICR) (see comment [16] in Table 2H-1).[2] For isolates that test susceptible to clindamycin (with erythromycin induction), consider adding the following comment to the patient's report: "This group B *Streptococcus* does not demonstrate inducible clindamycin resistance as determined by testing clindamycin in combination with erythromycin."



D-Zone Test

کامنت گزارش به پزشک

Inducible Resistant to Clindamycin

آنالیز نتایج آنتی بیوگرام باکتری M2

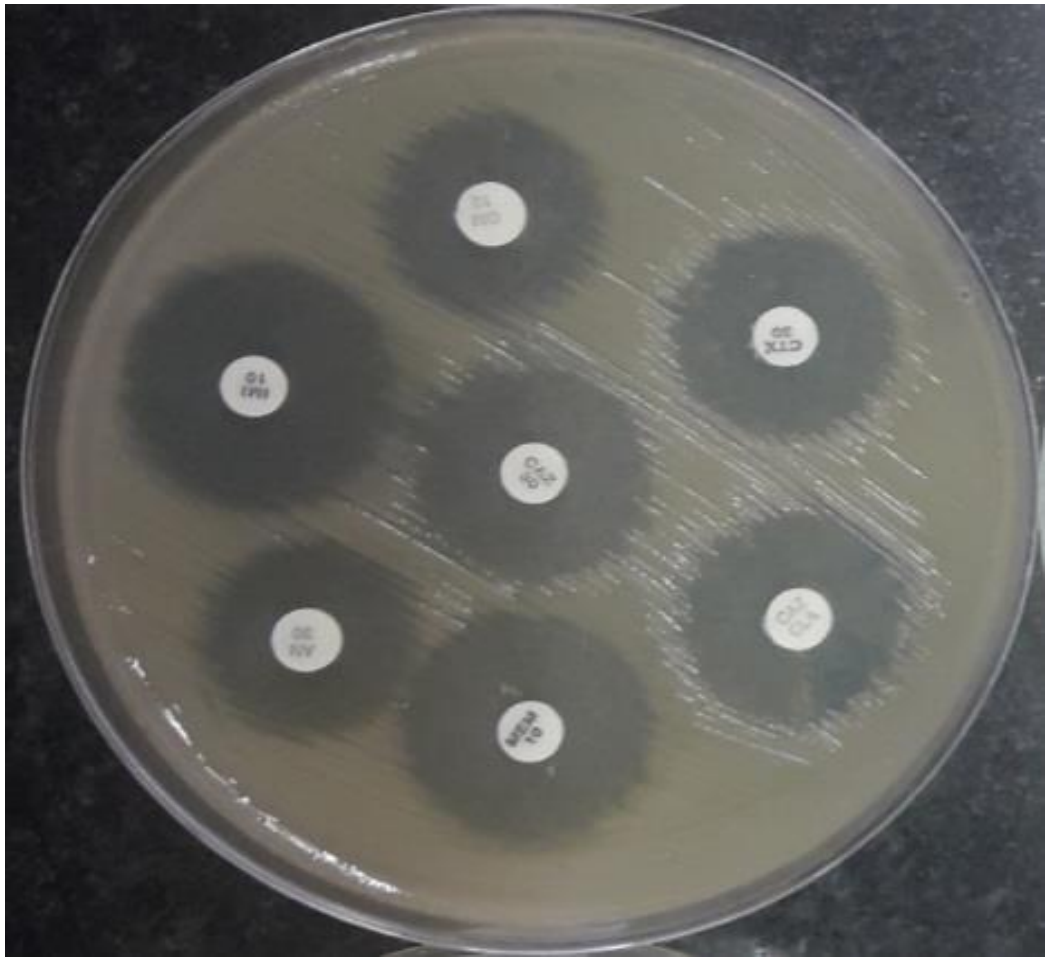
نام دیسک	تعداد استفاده	درصد استفاده
Cefazolin	125	1.13
Cefepime	28	0.25
Cefixime	164	1.48
Cefotaxime	108	0.97
Amikacin	251	2.26
Ceftazidime	67	0.6
Ceftizoxime	47	0.42
Ceftriaxon	190	1.71

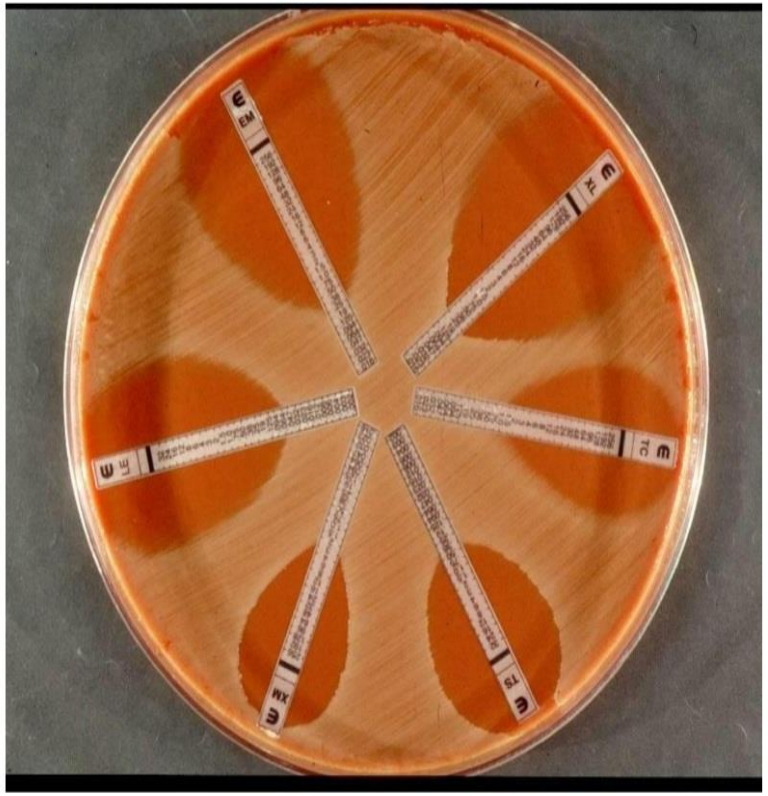
نام دیسک	تعداد استفاده	درصد استفاده
Cephalexin	75	0.68
Cephalothin	54	0.49
Imipenem	96	0.87
Meropenem	37	0.33
Nalidixic Acid	93	0.84
Nitrofurantoin	523	0.472
Vancomycin	343	3.10
Oxacillin	199	1.80
Methicillin	14	0.13
Cloxacillin	30	0.27

نام دیسک	تعداد استفاده	درصد استفاده	R	I	S
Cefoxitin	448	4.04	402*	9	33
Ciprofloxacin	1281	11.56	34	75	1163*
Clindamycin	670	6.05	654*	12	1
Doxycycline	272	2.45	260*	8	3
Erythromycin	717	6.47	695*	7	13
Gentamicin	1061	9.57	75	117	863*
Levofloxacin	201	1.81	4	5	192*
Linezolid	107	0.97	2	1	104*

نام دیسک	تعداد استفاده	درصد استفاده	R	I	S
Penicillin	802	7.24	790*	5	4
Rifampin	96	0.87	1	2	93*
Tetracycline	483	4.36	468*	2	13
Trimethoprim Sulfamethoxazole	1225	11.05	39	43	1137*
Vancomycin (E-Test)	95	0.86	17	16	60*

Anti susceptibility testing



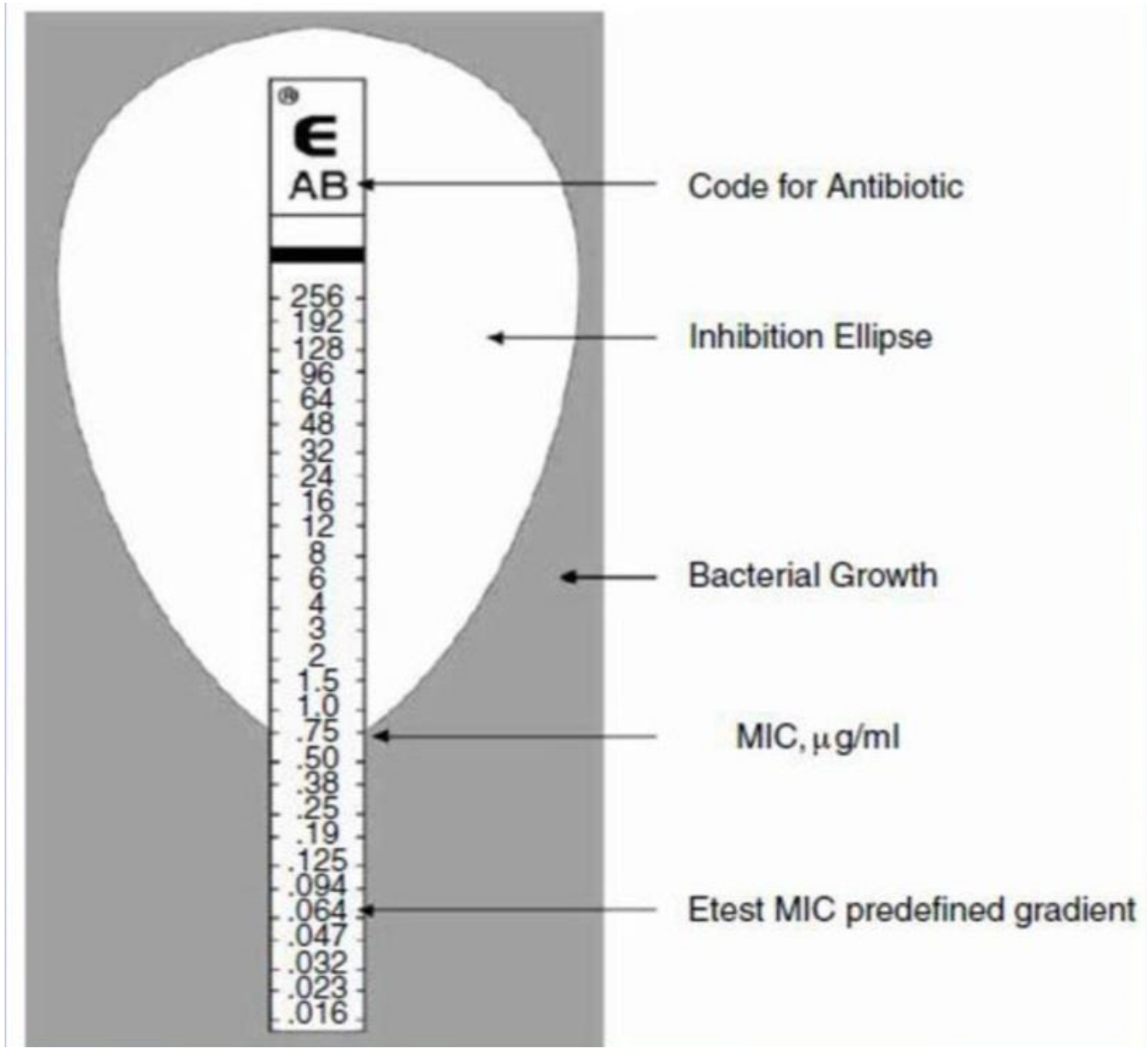


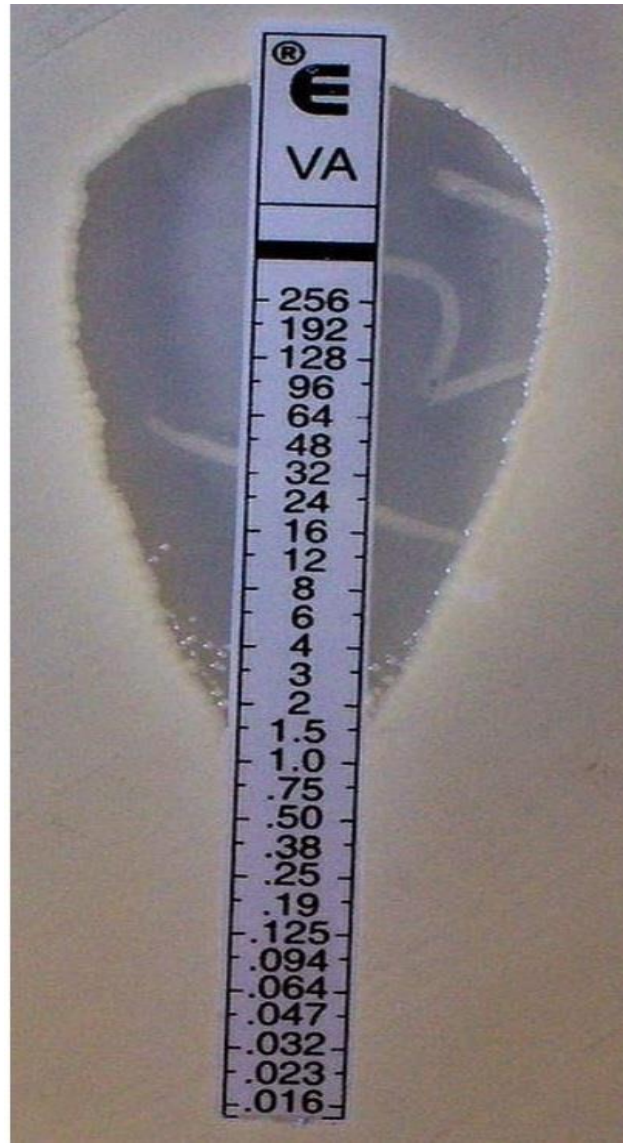
روش های متداول تعیین حساسیت آنتی بیوتیکی

دیسک دیفیوژن (کربی بائر)

براث میکرودایلوشن MIC

E –test


AB
256
192
128
96
64
48
32
24
16
12
8
6
4
3
2
1.5
1.0
.75
.50
.38
.25
.19
.125
.094
.064
.047
.032
.023
.016
Code for Antibiotic
Inhibition Ellipse
Bacterial Growth
MIC, μg/ml
Etest MIC predefined gradient


VA
256
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.75
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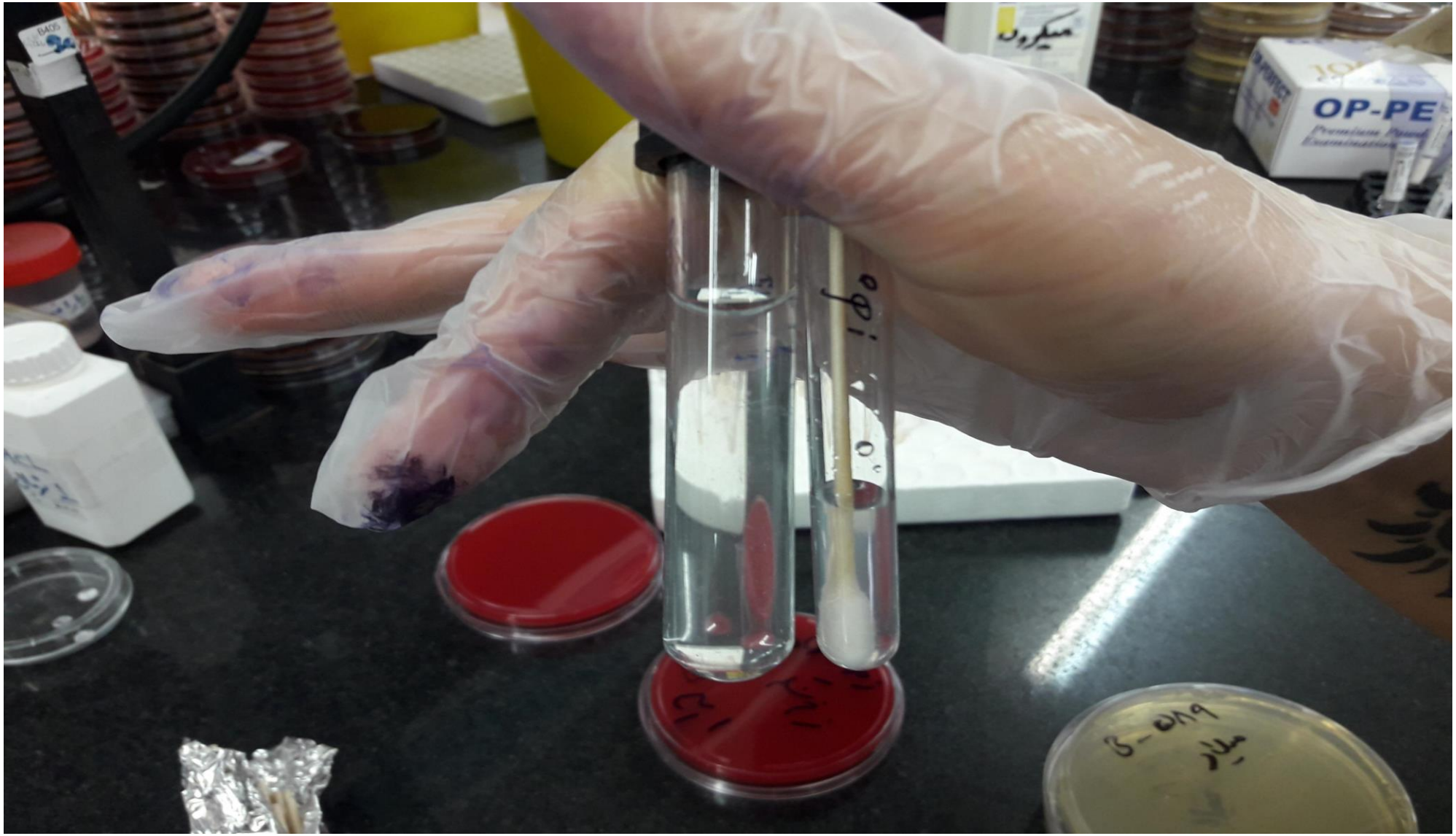
.19
.125
.094
.064
.047
.032
.023
.016

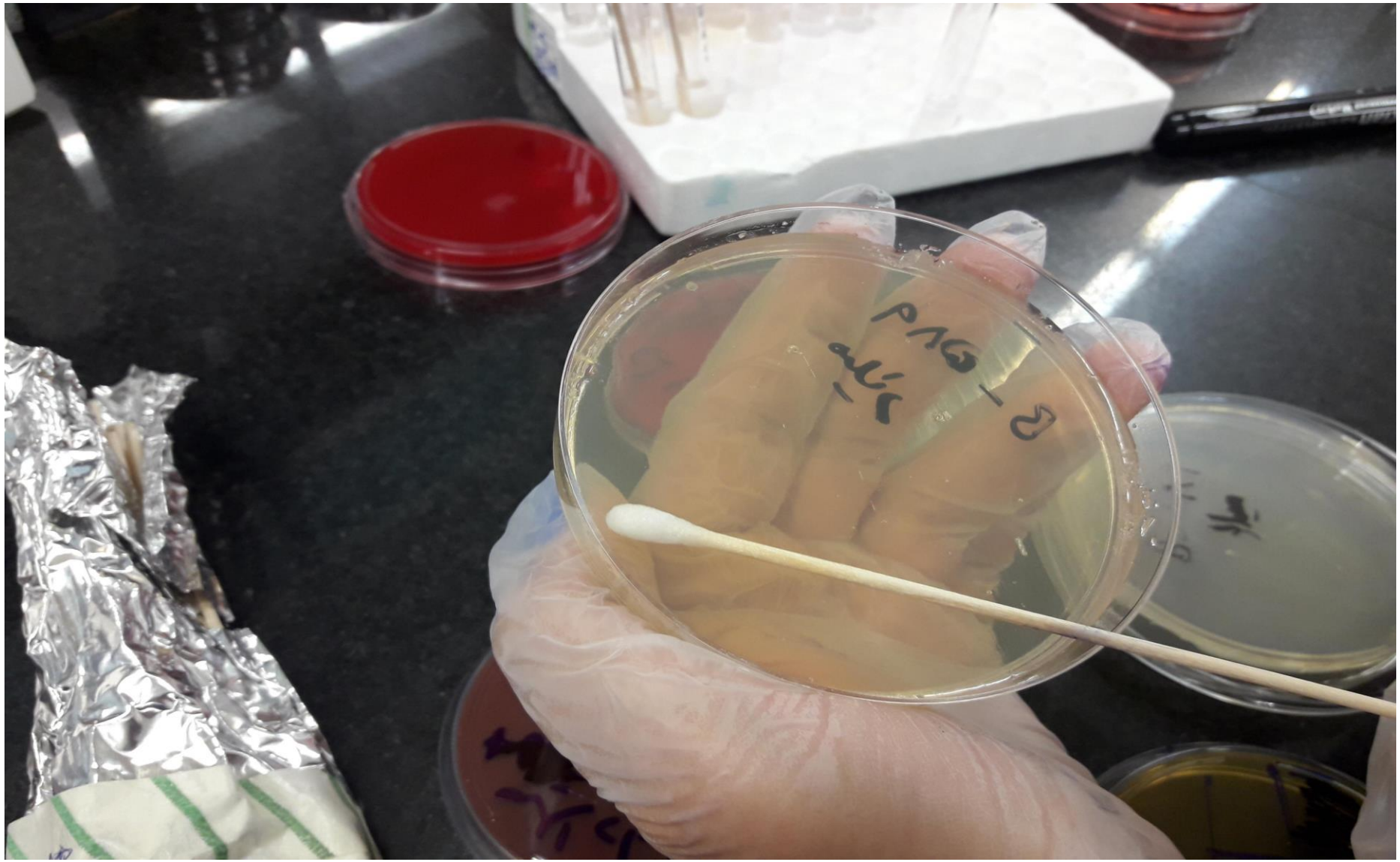
Disk diffusion method

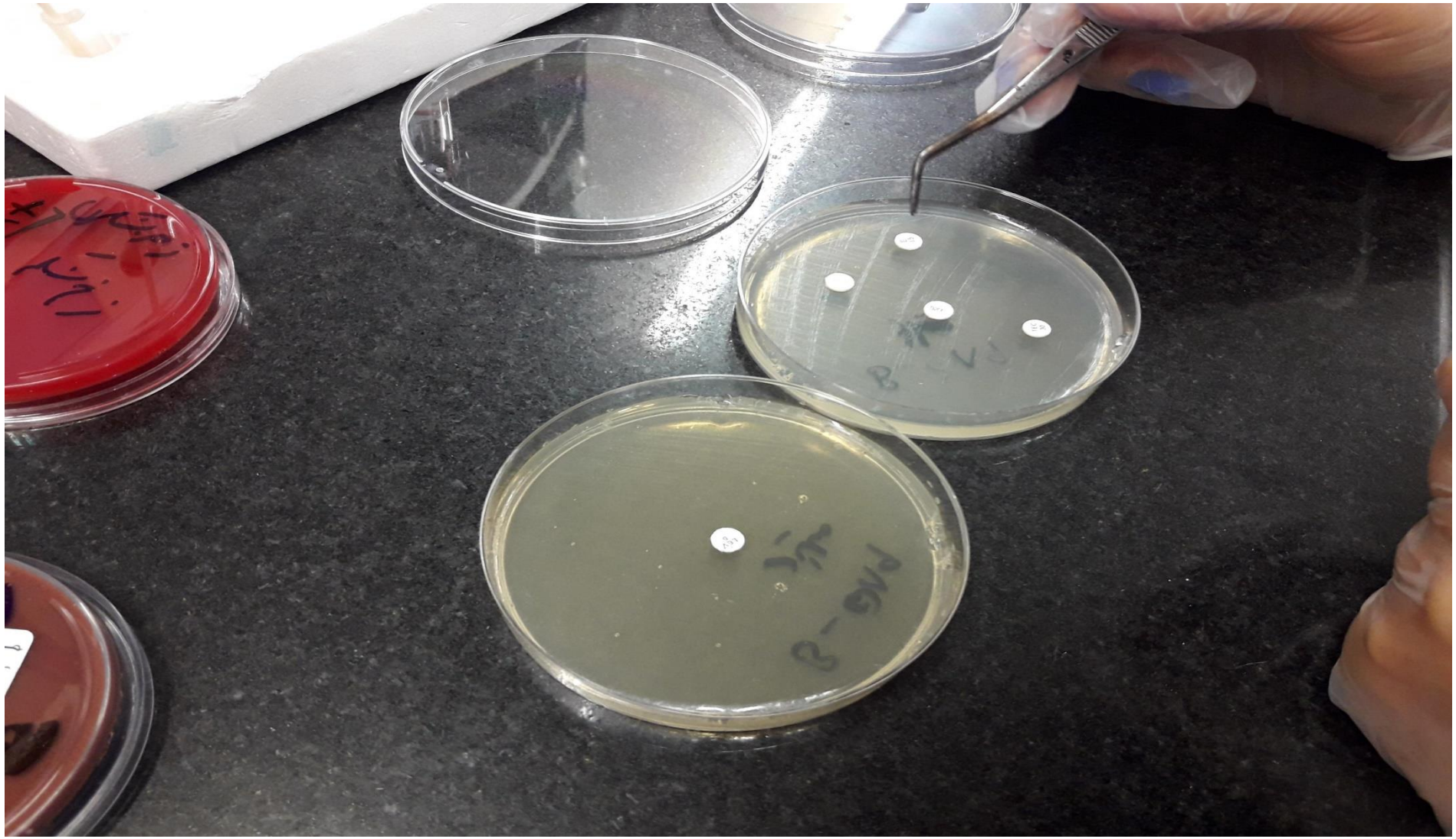
- ❖ استفاده از محیط های کشت ، مواد مصرفی و دیسک های آنتی بیوتیک کنترل کیفی شده
- ❖ تهیه اسمیر جهت رنگ آمیزی گرم و مشاهده میکروسکوپی
- ❖ استفاده از کلنی های کشت تازه و خالص باکتری
- ❖ تهیه سوسپانسیون میکروبی
- ❖ استفاده از استاندارد نیم مک فارلند
- ❖رعایت فواصل زمانی مراحل انجام تست (۱۵- ۱۵ -۱۵)

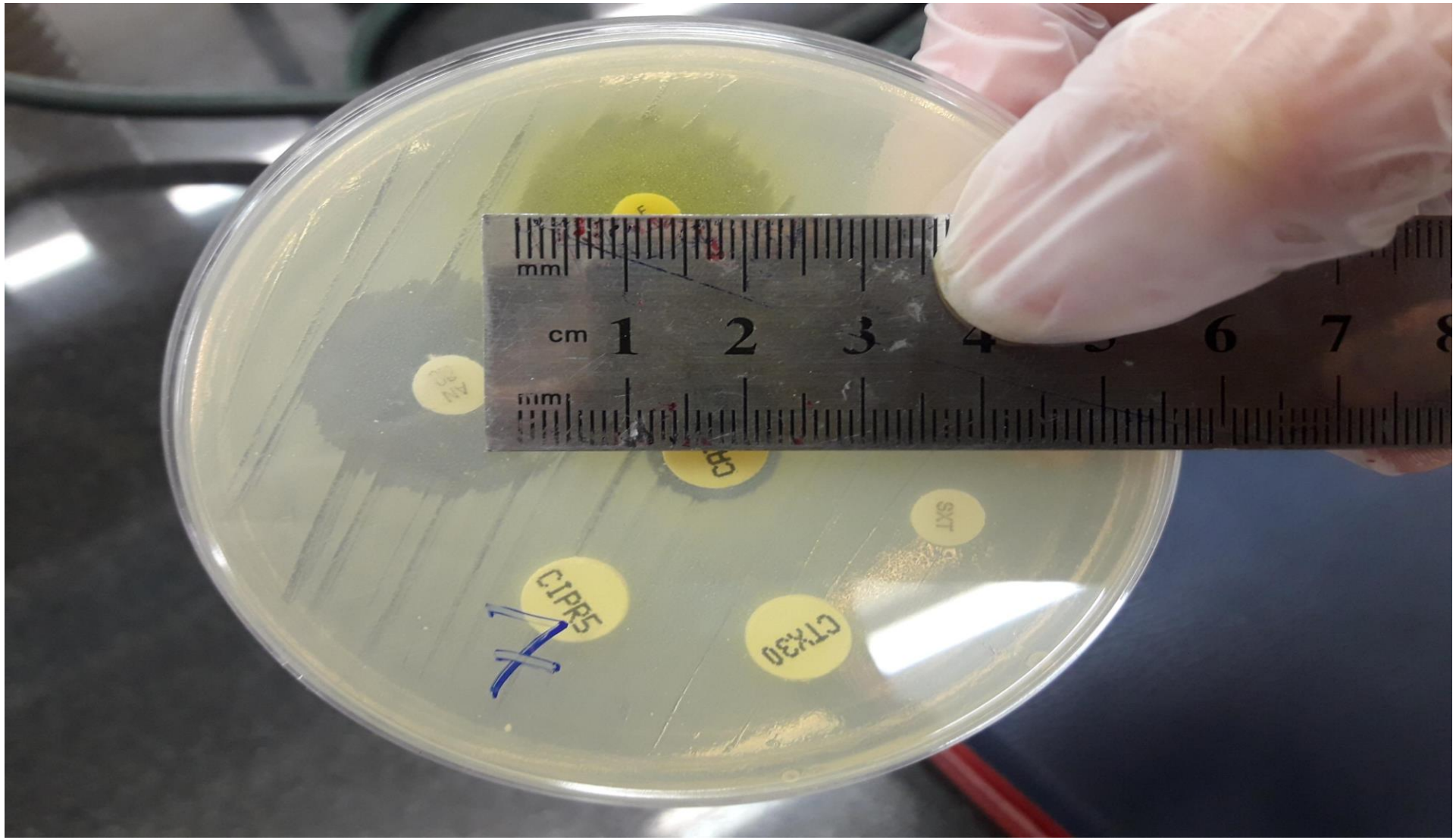
- دمای ذخیره سازی دیسک های آنتی بیوتیک حتما طبق توصیه کمپانی سازنده صورت گیرد .(درب یخچال،کارباپنم ها)
- انتخاب دیسک های آنتی بیوتیک حتما بر اساس نوع باکتری ، محل و منشا جداسازی باکتری و بر اساس جدول استاندارد CLSI باید صورت گیرد .
- برای مراکز درمانی بیمارستانی بهتر است پنل آنتی بیوتیک های هر گروه از باکتریها به اصطلاح بومی سازی شود. یعنی تلفیقی از آنتی بیوتیک های منتخب جدول CLSI و آنچه پزشکان و تیم کنترل عفونت بیمارستان، بیشتر از آن دسته داروها برای درمان بیماران استفاده می کنند.

McFarland Standards


OP-PE






mm
cm 1 2 3 4 6 7 8
mm
SXT
CIPR5
CTX30

مقاومت های دارویی در باکتری Staph aureus

کدهای مقاومت آنتی بیوتیکی	
MRSA	Methicillin Resistant Staph aureus
MSSA	Methicillin Sensitive Staph aureus
MRS	Methicillin Resistant Staph
VSSA	Vancomycin Sensitive Staph aureus
VISA	Vancomycin Intermediate Staph aureus
VRSA	Vancomycin Resistant Staph aureus

با آرزوی موفقیت...