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دکتر فاطمه ریاحی زانیانی

دکترای تخصصی باکتری شناسی پزشکی

(عضو هیات علمی دانشگاه علوم پزشکی دزفول)

دانشگاه علوم پزشکی دزفول
معاونت آموزشی
مرکز مطالعات و توسعه آموزش پزشکی (EDC)

به نام خدا

تشخیص آزمایشگاهی مقاومت های آنتی بیوتیکی

اهداف:

- مروری بر آنتی بیوتیک ها و روش های انجام آنتی بیوگرام
- آشنایی با تشخیص آزمایشگاهی بتالاکتامازها، MRSA و وانکومایسین آگار اسکرینینگ در استافیلوکوک ها
- آشنایی با تشخیص آزمایشگاهی مقاومت القایی به کلیندامایسین
- آشنایی با تشخیص آزمایشگاهی بتالاکتامازهای وسیع الطیف
- آشنایی با تشخیص آزمایشگاهی کارباپنمازها
- آشنایی با تشخیص آزمایشگاهی مقاومت به کلیستین

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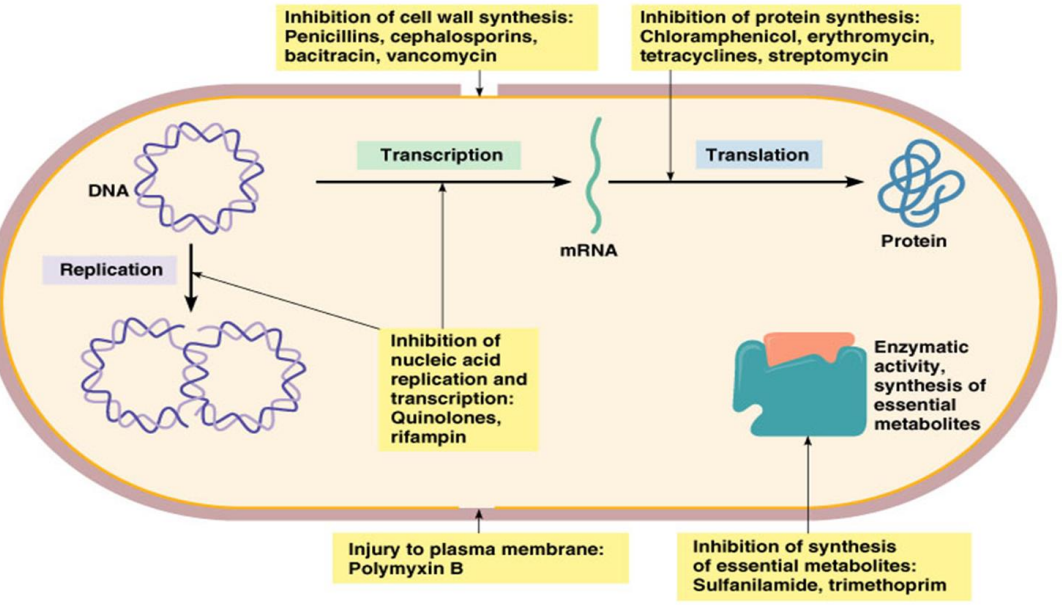
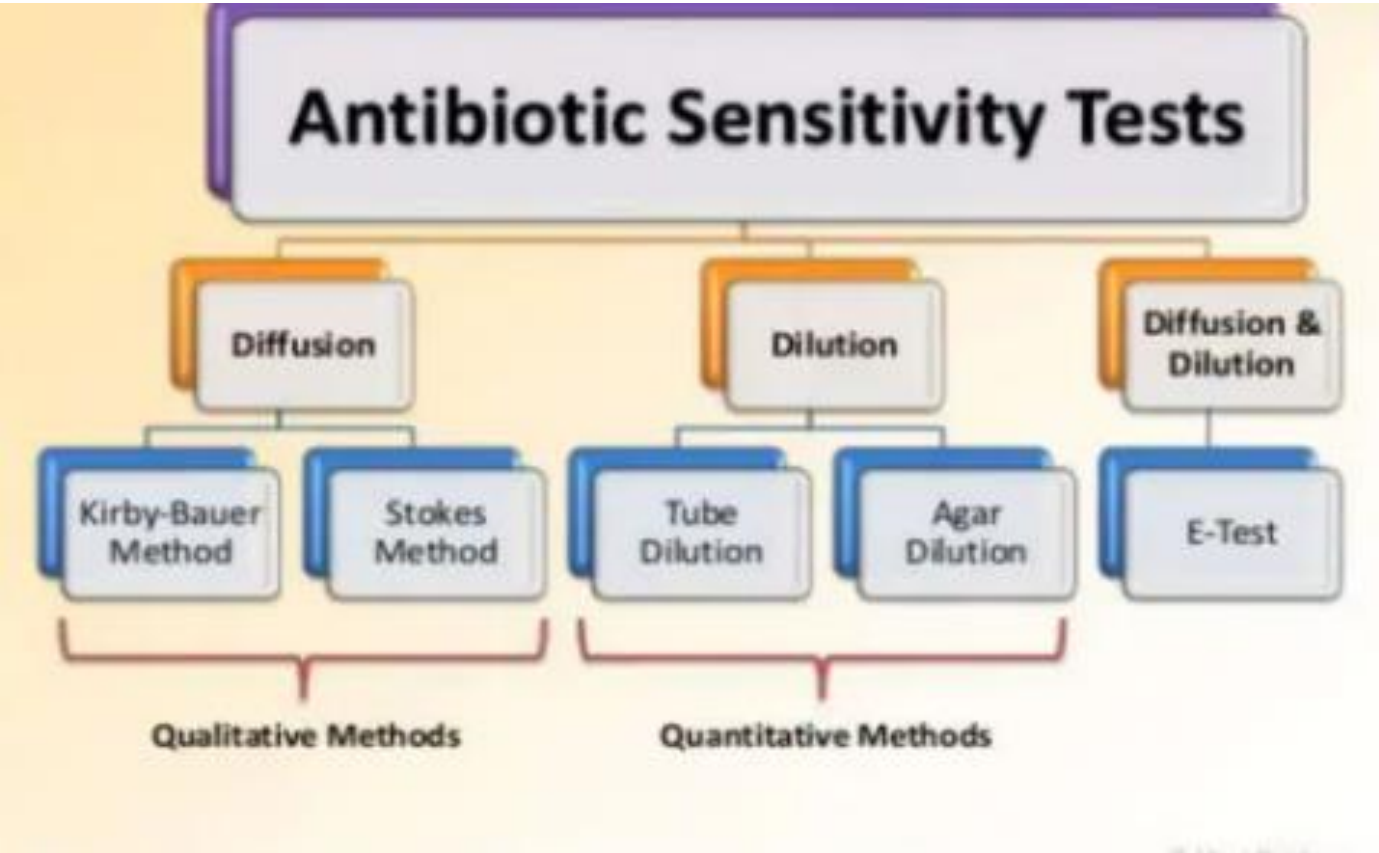
آنتی بیوتیک ها

مکانیسم اثر داروهای ضد میکروبی

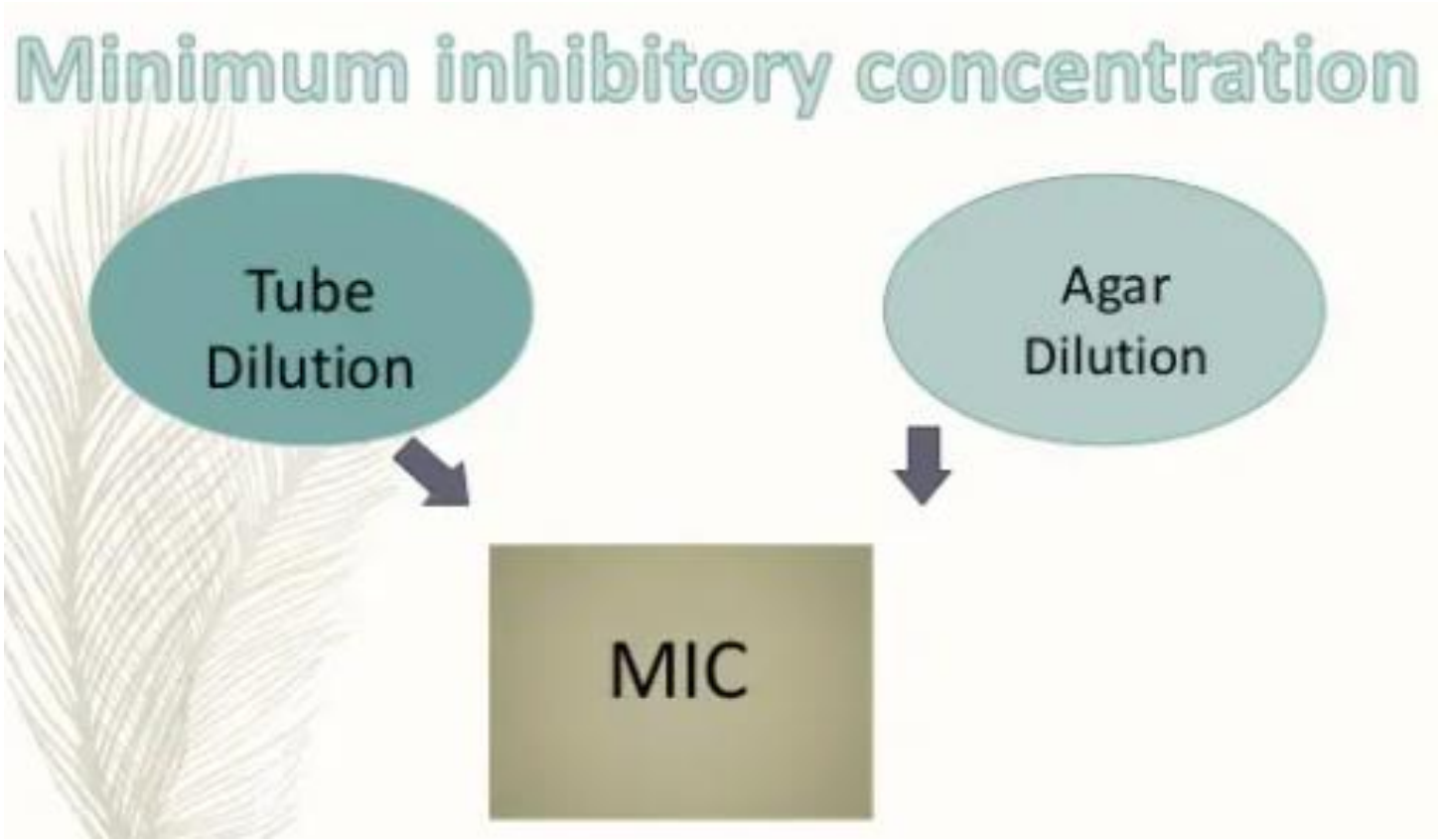
- اختلال در سنتز دیواره سلولی (پپتیدوگلیکان و ....)
- اختلال در غشای خارجی
- آسیب به غشاء سیتوپلاسمی
- مهار سنتز پروتئین
- مهار سنتز اسیدهای نوکلئیک
- غیر فعال نمودن آنزیم های کلیدی (آنتی متابولیت ها)



روش های تست حساسیت آنتی بیوتیکی (AST)



آگاردایلوشن و براث دایلوشن



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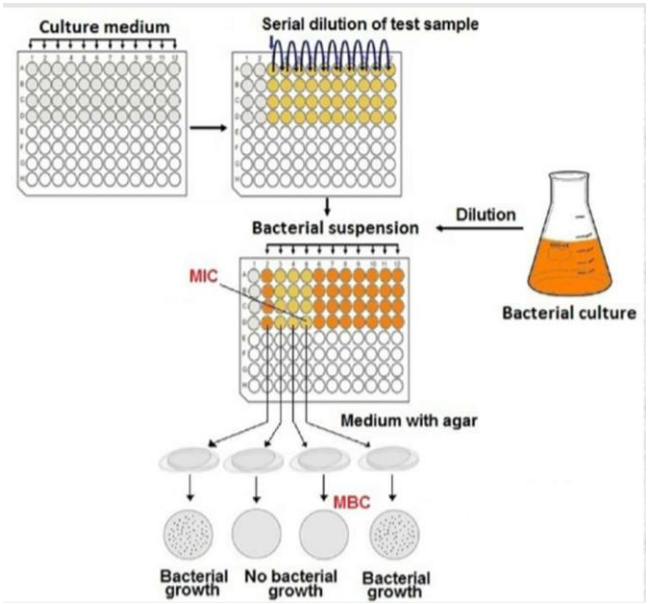

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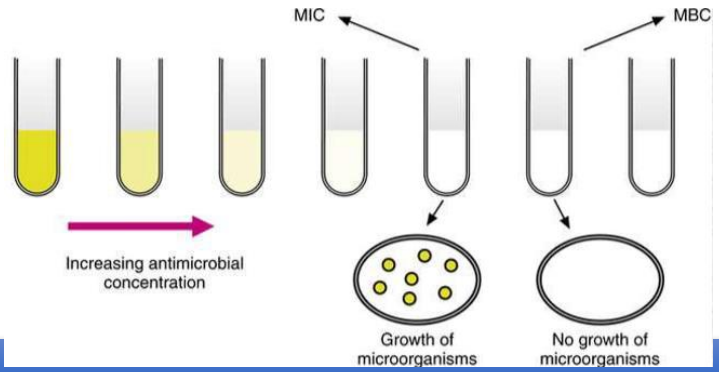
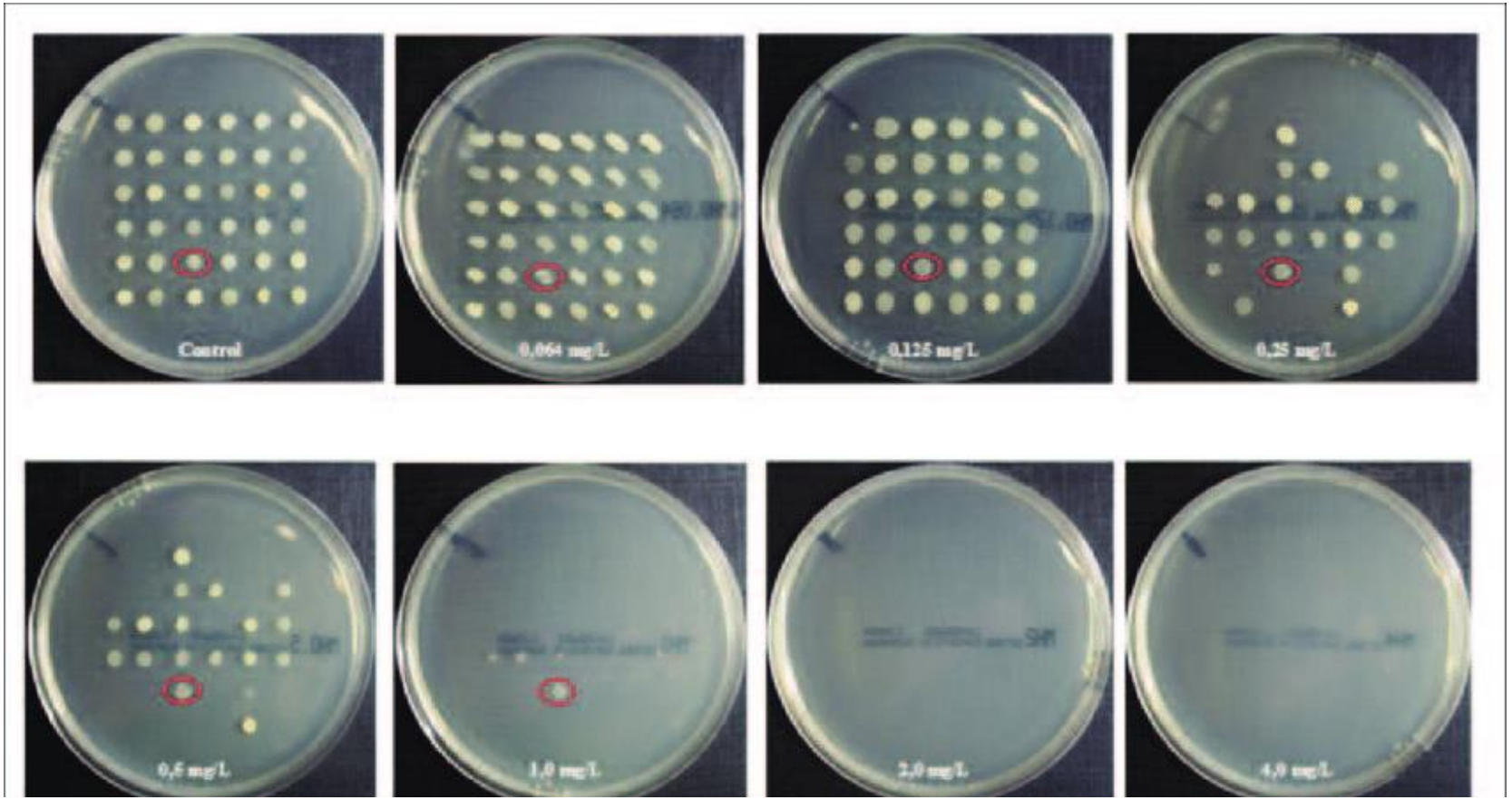


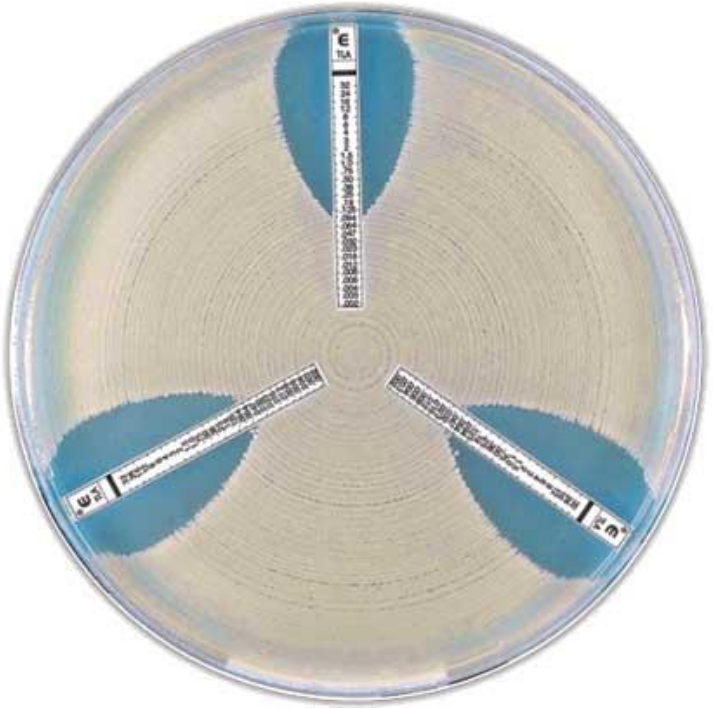
آگاردایلوشن



E-TEST

Epsilometer test

- Quantitative method of antibiotic sensitivity testing
- Applies both dilution of antibiotic and diffusion of antibiotic into the medium
- Combines the principles of disk diffusion and agar dilution methods.



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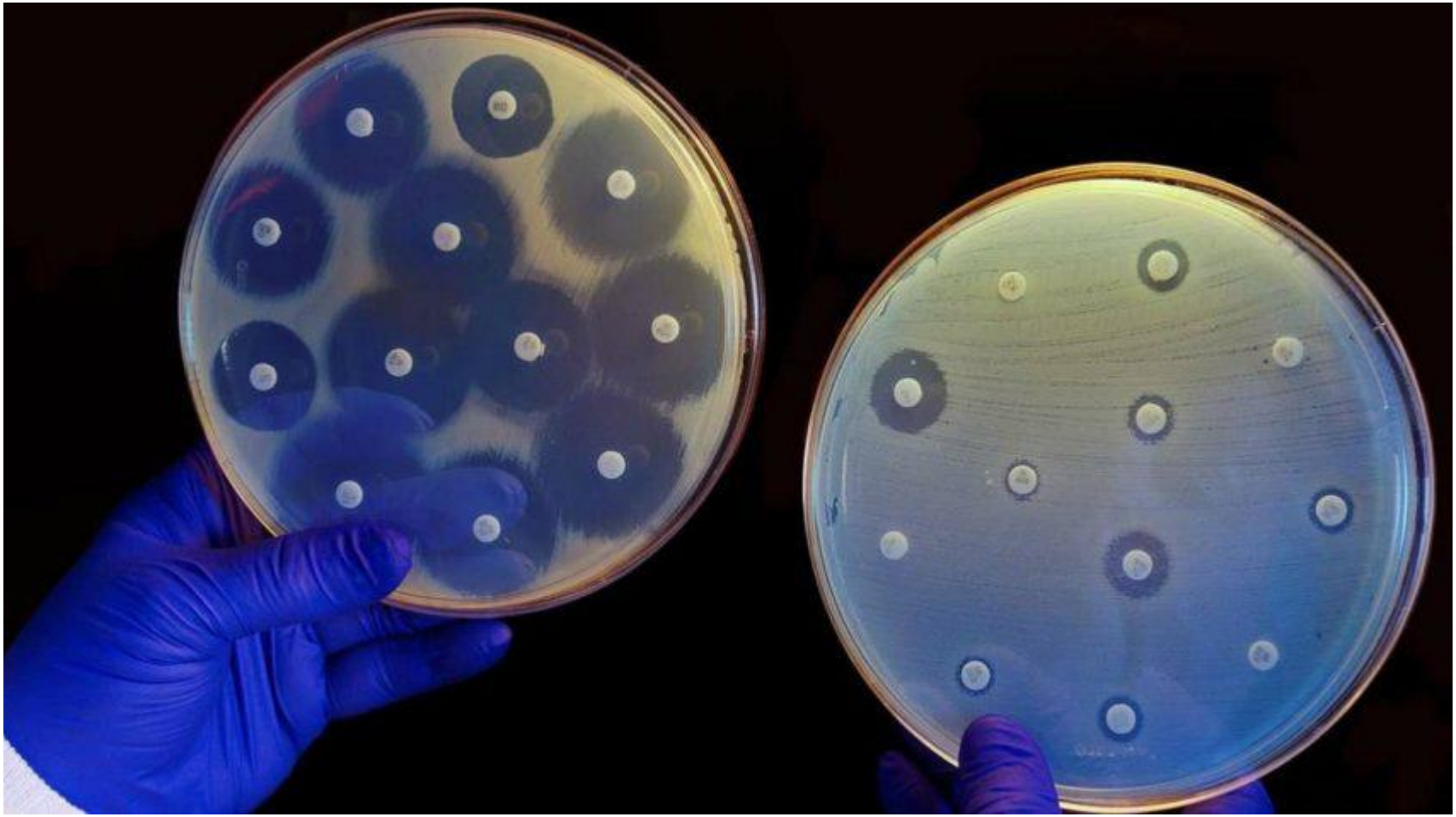

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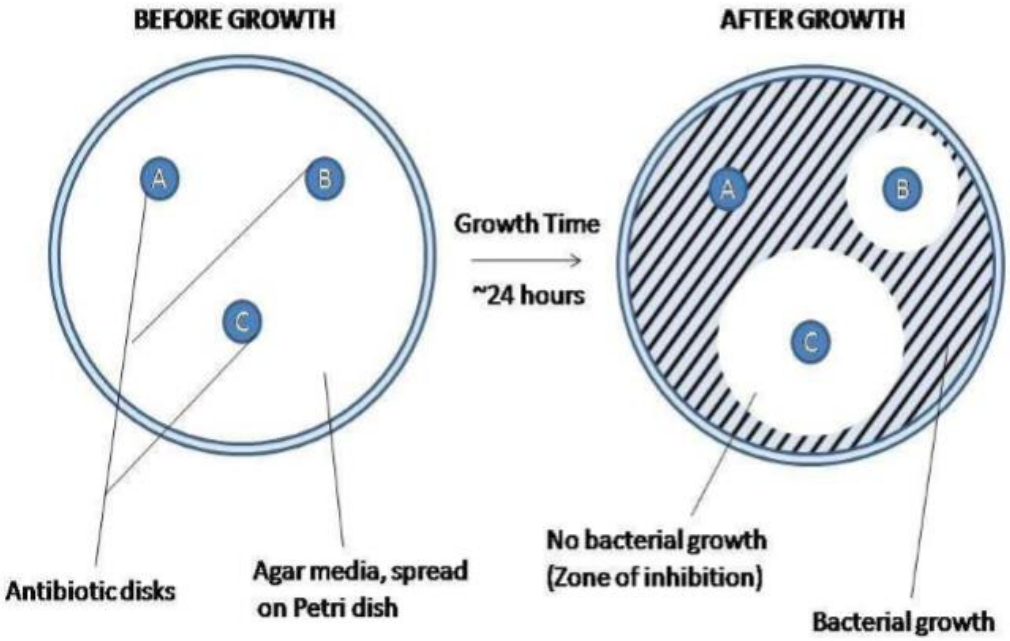
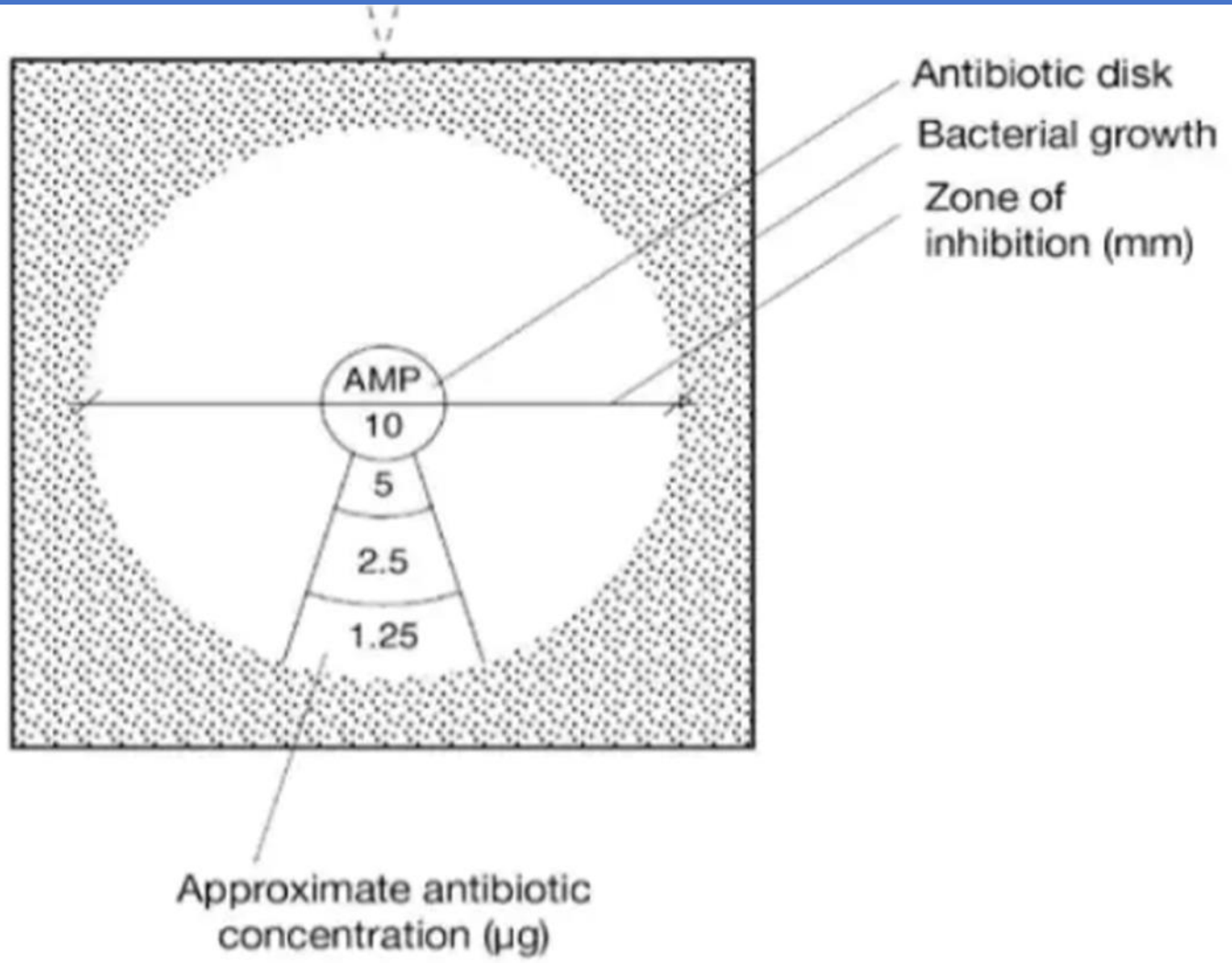


- محیط کشت
- باکتری
- دیسک آنتی بیوتیک


Antibiotic disk
Bacterial growth
Zone of inhibition (mm)
AMP
10
5
2.5
1.25
Approximate antibiotic concentration (µg)

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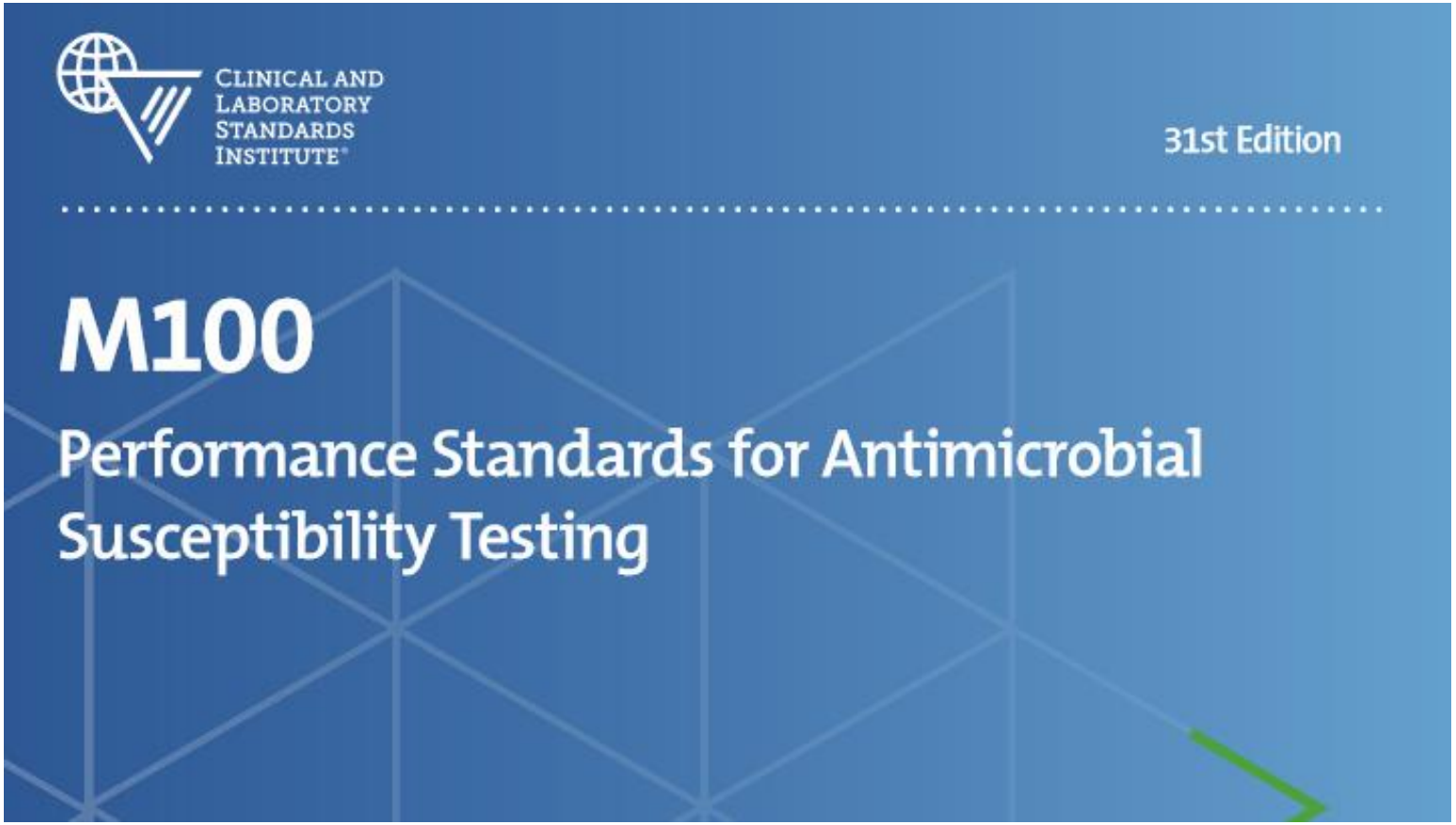
آشنایی با CLSI M100



تولید بتالاکتاماز در استافیلوکوک اورئوس

Table 3F. Test for Detection of β-Lactamase Production in *Staphylococcus* spp.

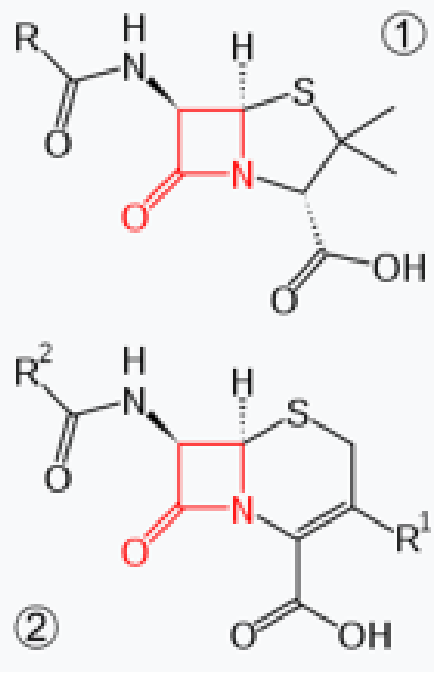
Test	β-Lactamase Production	
Test method	Disk diffusion (penicillin zone-edge test)	Nitrocefin-based test
Organism group	*S. aureus* with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm[a]	*Staphylococcus* spp.[a,b] with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm
Medium	MHA	N/A
Antimicrobial concentration	10 units penicillin disk	N/A
Inoculum	Standard disk diffusion procedure	Induced growth (ie, growth taken from the zone margin surrounding a penicillin or cefoxitin disk test on either MHA or a blood agar plate after 16-18 hours of incubation)
Incubation conditions	35°C ± 2°C; ambient air	Room temperature
Incubation length	16-18 hours	Up to 1 hour for nitrocefin-based test or follow manufacturer's directions
Results	Sharp zone edge ("cliff") = β-lactamase positive (see Figure 1 below this table) Fuzzy zone edge ("beach") = β-lactamase negative (see Figure 2 below this table)	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive.
Additional testing and reporting	β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.	Nitrocefin-based tests can be used for *S. aureus*, but negative results should be confirmed with the penicillin zone-edge test before reporting penicillin as susceptible. β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.
QC recommendations - routine[c]	*S. aureus* ATCC®[d] 25923 for routine QC of penicillin disk to include examination of zone-edge test (fuzzy edge = "beach")	
QC recommendations - lot/shipment[e]		*S. aureus* ATCC® 29213 - positive *S. aureus* ATCC® 25923 - negative (or see local regulations and manufacturers' recommendations)
QC recommendations - supplemental[f]	*S. aureus* ATCC® 29213 - positive penicillin zone-edge test (sharp edge = "cliff")	

Abbreviations: ATCC®, American Type Culture Collection; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; N/A, not applicable; QC, quality control.

مهارکننده های سنتز دیواره سلولی

- **بتالاکتام ها:**

بتالاکتام ها			مثال
پنی سیلین ها	پنی سیلین های طبیعی		پنی سیلین G پنی سیلین V،
	پنی سیلین های نیمه مصنوعی	پنی سیلین های مقاوم به بتالاکتاماز	کلوکز اسیلین ،دی کلوکز اسیلین ،فلو کلوکز اسیلین ،متی سیلین ،نفسیلین ،آکزاسیلین،
	مشتقات پنی سیلین (موثر بر گرم منفی و گرم مثبت ها)		آموکسی سیلین، آمپی سیلین، تی کارسیلین ،مزلوسیلین ،پی پراسیلین ،کاربنی سیلین ،آزلوسیلین
	پنی سیلین ها + مهارکننده های بتالاکتاماز		مهارکننده های بتالاکتاماز : تازوباکتام ،سولباکتام ،کلاونیک اسید مثال: آموکسی سیلین + کلاوونیک اسید = کوآموکسی کلاو
	کارباپنم		ایمی پنم و مروپنم
	منوباکتام		آزتروتام
سفالوسپورین	نسل اول		سفرادین ،سفاپرین ،سفالوتین ،سفالکسین،سفپوزیل،سفازولین،سفادوروکسیل
	نسل دوم		سفاکلر ،سفامندل،سفورانید،سفوراکسیم،سفوکسیتین،سفوتتان،سفپروزیل ،سفمتازول، سفوینسید
	نسل سوم		سفیکسیم ،سفوپرازون،سفوتاکسیم، سفپودوکسیم، سفتازیدیم،سفتیزوکسیم ،سفتریاکسون، سفتی بوتن،سفدینیر
	نسل چهارم		سفپیم سفپیروم



تولید بتالاکتاماز در استافیلوکوک اورئوس

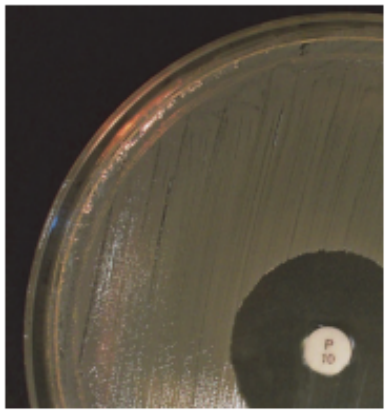


Figure 1. Positive Penicillin Disk Zone-Edge Test for ß-Lactamase Detection. The zone edge is sharp or like a "cliff" indicating ß-lactamase production.

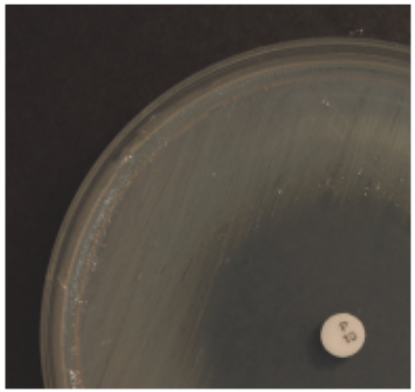


Figure 2. Negative Penicillin Disk Zone-Edge Test for ß-Lactamase Detection. The zone edge is fuzzy or like a "beach," indicating no ß-lactamase production.

تولید بتالاکتاماز در استافیلوکوک اورئوس

Footnotes

a. The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus*. The penicillin zone-edge test is recommended if only one test is used for β-lactamase detection. However, some laboratories may choose to perform a nitrocefin-based test first and, if this test is positive, report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, the penicillin zone-edge test should be performed before reporting the isolate as penicillin susceptible in cases in which penicillin may be used for therapy (eg, endocarditis).[1,2]

b. For *S. lugdunensis*, tests for β-lactamase detection are not necessary because isolates producing a β-lactamase will test penicillin resistant (MIC > 0.12 µg/mL and zone diameters < 29 mm). If a laboratory is using a method other than the CLSI disk diffusion or MIC reference methods and is unsure if the method can reliably detect penicillin resistance with contemporary isolates of *S. lugdunensis*, the laboratory should perform an induced nitrocefin assay or other CLSI reference method on isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible.

تشخیص مقاومت به متی سیلین در گونه های مختلف استافیلوکوک ها

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

تشخیص MRSA

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]	*S. aureus* ATCC®[f] 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®[c] 29213 - susceptible (≤ 1 colony; with each test day)
QC recommendations - lot/shipment[g]	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-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®[ef] 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[g]	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.

VRSA , VRE

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

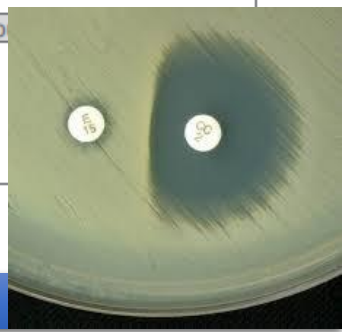
Screen Test	Vancomycin MIC ≥ 8 µg/mL	
Test method	Agar dilution	Agar dilution
Organism group	*S. aureus*	*Enterococcus* spp.
Medium	BHI agar	BHI[a] agar
Antimicrobial concentration	6 µg/mL vancomycin	6 µg/mL vancomycin
Inoculum	Colony suspension to obtain 0.5 McFarland turbidity Preferably, using a micropipette, 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®[c] 29212 - susceptible	*E. faecalis* ATCC® 29212 - susceptible
QC recommendations - lot/shipment[d]	*E. faecalis* ATCC® 51299 - resistant	*E. faecalis* ATCC® 51299 - resistant

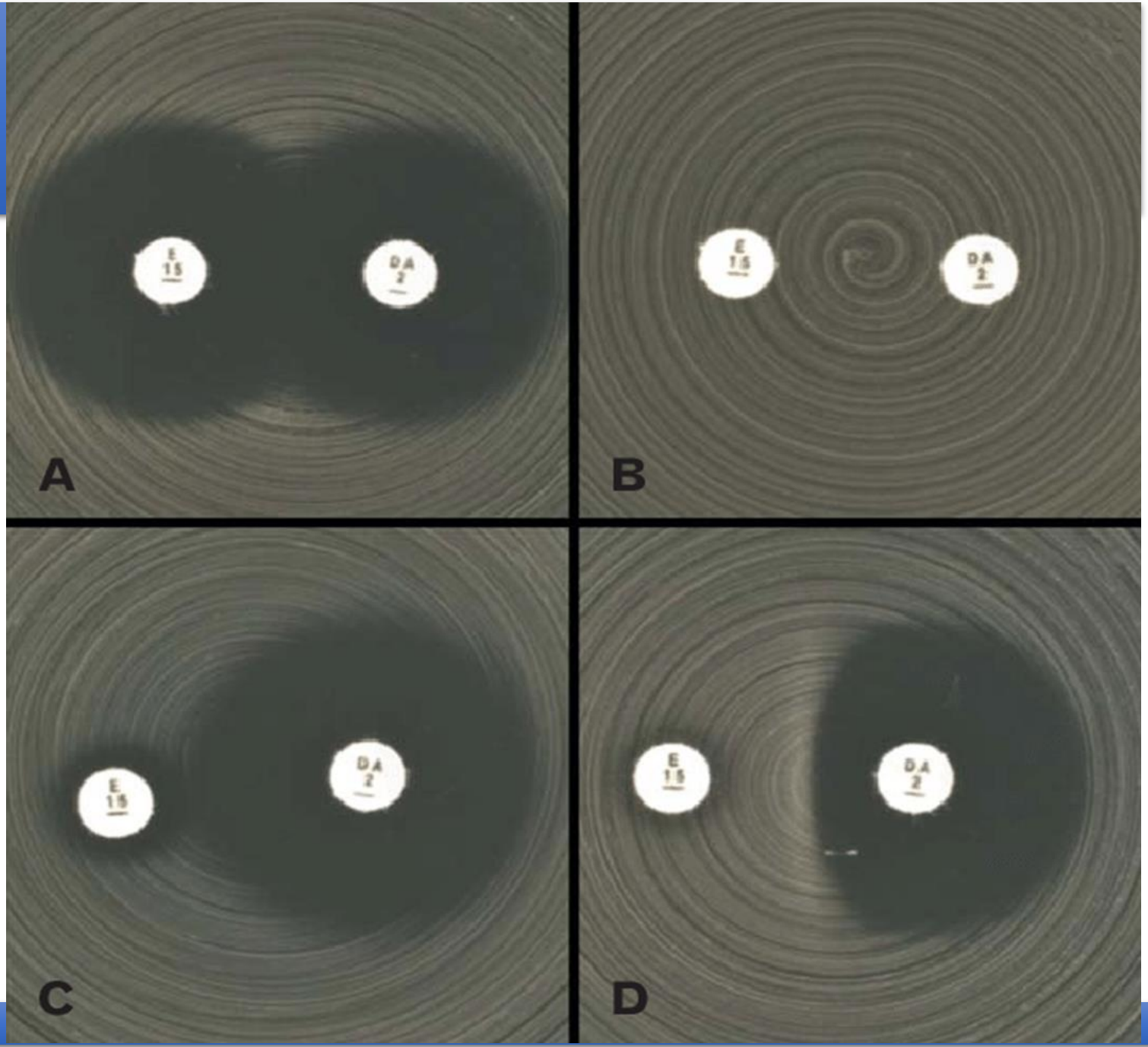
Abbreviations: ATCC®, American Type Culture Collection; BHI, brain heart infusion; MIC, minimal inhibitory concentration; QC, quality control.

Inducible Clindamycin Resistance

Table 3I. Test for Detecting Inducible Clindamycin Resistance in *Staphylococcus spp.*, *Streptococcus pneumoniae*, and *Streptococcus* spp. ß-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 ß-hemolytic *Streptococcus* spp.	All *Staphylococcus* spp.[c]	*S. pneumoniae* and ß-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 ho
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.	

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آشنایی با CLSI M100

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]			*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)	

تشخیص آزمایشگاهی ESBL

Table 3A. Tests for Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis*

NOTE: Following evaluation of PK/PD properties, limited clinical data, and MIC distributions, revised breakpoints for cefazolin, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and aztreonam were published in January 2010 (M100-S20) and are listed in Table 2A. Cefuroxime (parenteral) was also evaluated; however, no change in breakpoints was necessary with the dosage. When using the current breakpoints, routine ESBL testing is no longer necessary before reporting results (ie, it is no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant). However, ESBL testing may still be useful for epidemiological or infection prevention purposes. For laboratories that have not implemented the current breakpoints, ESBL testing should be performed as described in this table.

Breakpoints for drugs with limited availability in many countries (eg, moxalactam, cefonicid, cefamandole, and cefoperazone) were not evaluated. If considering use of these drugs for *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, or *Proteus mirabilis*, ESBL testing should be performed. If isolates test ESBL positive, the results for moxalactam, cefonicid, cefamandole, and cefoperazone should be reported as resistant.

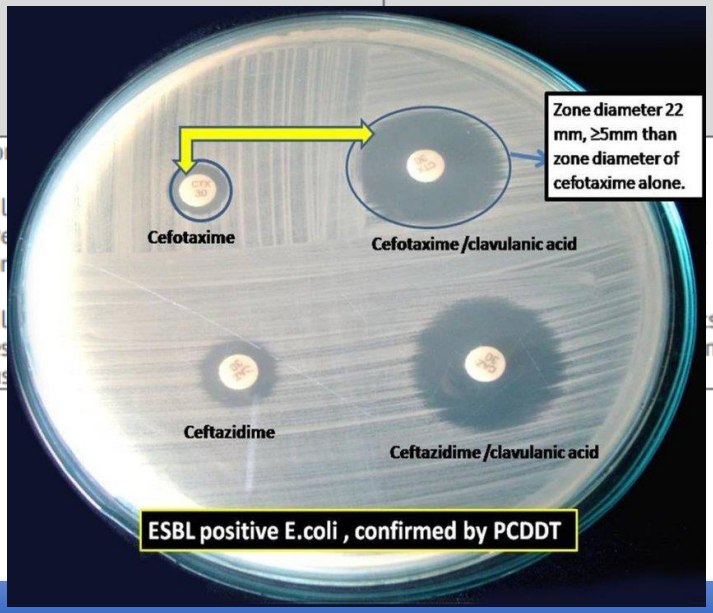
تشخیص آزمایشگاهی ESBL

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Medium	MHA	CAMHB	MHA	CAMHB
Antimicrobial concentration	For *K. pneumoniae*, *K. oxytoca*, and *E. coli*: Cefpodoxime 10 µg or Ceftazidime 30 µg or Aztreonam 30 µg or Cefotaxime 30 µg or Ceftriaxone 30 µg For *P. mirabilis*: Cefpodoxime 10 µg or Ceftazidime 30 µg or Cefotaxime 30 µg (Testing more than one antimicrobial agent improves the sensitivity of ESBL detection.)	For *K. pneumoniae*, *K. oxytoca*, and *E. coli*: Cefpodoxime 4 µg/mL or Ceftazidime 1 µg/mL or Aztreonam 1 µg/mL or Cefotaxime 1 µg/mL or Ceftriaxone 1 µg/mL For *P. mirabilis*: Cefpodoxime 1 µg/mL or Ceftazidime 1 µg/mL or Cefotaxime 1 µg/mL (Testing more than one antimicrobial agent improves the sensitivity of ESBL detection.)	Ceftazidime 30 µg Ceftazidime-clavulanate[a] 30/10 µg and Cefotaxime 30 µg Cefotaxime-clavulanate 30/10 µg (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)	Ceftazidime 0.25-128 µg/mL Ceftazidime-clavulanate 0.25/4-128/4 µg/mL and Cefotaxime 0.25-64 µg/mL Cefotaxime-clavulanate 0.25/4-64/4 µg/mL (Testing necessitates using both cefotaxime and ceftazidime, alone and in combination with clavulanate.)
Inoculum	Standard disk diffusion procedure	Standard broth dilution procedure	Standard disk diffusion procedure	Standard broth dilution procedure
Incubation conditions	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air	35°C±2°C; ambient air
Incubation length	16-18 hours	16-20 hours	16-18 hours	16-20 hours

تشخیص آزمایشگاهی ESBL

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
Results	For *K. pneumoniae*, *K. oxytoca*, and *E. coli*:	Growth at or above the concentrations listed may indicate ESBL production (ie, for *E. coli*, *K. pneumoniae*, and *K. oxytoca*, MIC ≥8 µg/mL for cefpodoxime or MIC ≥2 µg/mL for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for *P. mirabilis*, MIC ≥2 µg/mL for cefpodoxime, ceftazidime, or cefotaxime).	A ≥5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL (eg, ceftazidime zone = 16; ceftazidime-clavulanate zone = 21).	A ≥3 2-fold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone = ESBL (eg, ceftazidime MIC = 8 µg/mL; ceftazidime-clavulanate MIC = 1 µg/mL).
	Cefpodoxime zone ≤17 mm Ceftazidime zone ≤22 mm Aztreonam zone ≤27 mm Cefotaxime zone ≤27 mm Ceftriaxone zone ≤25 mm			
	For *P. mirabilis*:			
	Cefpodoxime zone ≤22 mm Ceftazidime zone ≤22 mm Cefotaxime zone ≤27 mm			
	Zones above may indicate ESBL production.			
Reporting			Fo If l bre for If l tes sus	s, m



تشخیص آزمایشگاهی ESBL

Table 3A. (Continued)

Test	Criteria for Performance of ESBL Test		ESBL Test	
Test method	Disk diffusion	Broth microdilution	Disk diffusion	Broth microdilution
QC recommendations	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily).	When testing antimicrobial agents used for ESBL detection, *K. pneumoniae* ATCC® 700603 is provided as a supplemental QC strain (eg, for training, competence assessment, or test evaluation). Either strain, *K. pneumoniae* ATCC® 700603 or *E. coli* ATCC® 25922, may then be used for routine QC (eg, weekly or daily).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be used for routine QC (eg, weekly or daily).	When performing the ESBL test, *K. pneumoniae* ATCC® 700603 and *E. coli* ATCC® 25922 should be tested routinely (eg, weekly or daily).
	E. coli ATCC® 25922 (see acceptable QC ranges in Table 4A-1)	*E. coli* ATCC® 25922 = no growth (see acceptable QC ranges listed in Table 5A-1)	**Acceptable QC:** *E. coli* ATCC® 25922: ≤2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.	**Acceptable QC:** *E. coli* ATCC® 25922: < 3 2-fold concentration decrease in MIC for antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.
	K. pneumoniae ATCC® 700603: Cefpodoxime zone 9-16 mm Ceftazidime zone 10-18 mm Aztreonam zone 10-16 mm Cefotaxime zone 17-25 mm Ceftriaxone zone 16-24 mm	*K. pneumoniae* ATCC® 700603 = Growth: Cefpodoxime MIC ≥8 µg/mL Ceftazidime MIC ≥2 µg/mL Aztreonam MIC ≥2 µg/mL Cefotaxime MIC ≥2 µg/mL Ceftriaxone MIC ≥2 µg/mL	*K. pneumoniae* ATCC® 700603: ≥5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone; ≥3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone.	*K. pneumoniae* ATCC® 700603: ≥3 2-fold concentration decrease in MIC for an antimicrobial agent tested in combination with clavulanate vs the MIC of the agent when tested alone.

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; ESBL, extended-spectrum β-lactamase; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic-pharmacodynamic; QC, quality control.

تشخیص آزمایشگاهی کاربا‌پنمازها

کاربا‌پنماز

- در ناحیه فعال یا سرین دارند: serine carbapenamase
- یا فلز روی:

۲.٤.۳.۱۱ متالوبتالاکتامازها(Metallo-β-lactamases)

متالوبتالاکتامازها، کاربا‌پنمازهایی هستند که برای فعالیت نیاز به روی(Zinc) دارند و توسط موادی نظیر اتیلن دیامین ترا استیک اسید(EDTA) که به روی وصل می‌شود، مهار می‌گردند. *استنوترووفوموناس مالتوفیلیا*، *باسیلوس آنتراسیس* و برخی از سویه‌های *باکتروئیدس فراژیلیس*، متالوبتالاکتاماز کروموزومی تولیدمی‌کنند. سایر متالوآنزیم‌ها ممکن‌است روی عناصر ژنتیکی متحرک حمل گردند و می‌توانند در گونه‌های *اسینتوباکتر*، *سودوموناس آئروژینوزا*، *سراشیا مارسسنس* و *کلبسیلا پنومونیه* رخ دهند.

تشخیص آزمایشگاهی کاربا‌پنمازها

Introduction to Tables 3B and 3C. Tests for Carbapenemases in Enterobacterales and *Pseudomonas aeruginosa*

Institutional infection prevention procedures or epidemiological investigations may necessitate identification of carbapenemase-producing Enterobacterales and *P. aeruginosa*. Such testing is not currently recommended for routine use.

Carbapenemase-producing isolates of Enterobacterales usually test intermediate or resistant to one or more carbapenems using the current breakpoints as listed in Table 2A (**NOTE:** Testing not susceptible to ertapenem is often the most sensitive indicator of carbapenemase production) and usually test resistant to one or more agents in cephalosporin subclass III (eg, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone). However, some isolates that produce carbapenemases such as SME or IMI often test susceptible to these cephalosporins.

Laboratories using Enterobacterales MIC breakpoints for carbapenems described in M100-S20 (January 2010) should perform the CarbaNP test, mCIM, eCIM, and/or a molecular assay (refer to Tables 3B and 3C for methods) when isolates of Enterobacterales are suspicious for carbapenemase production based on imipenem or meropenem MICs 2-4 µg/mL or ertapenem MIC 2 µg/mL (refer to Tables 3B-1 and 3C-1 for guidance on reporting). After implementing the current breakpoints, these additional tests may not need to be performed other than for epidemiological or infection prevention purposes (ie, it is no longer necessary to edit results for the carbapenems to resistant if a carbapenemase producer is detected).

تشخیص آزمایشگاهی کاربا‌پنمازها

Introduction to Tables 3B and 3C. (Continued)

	Tests Used for Epidemiological or Infection Prevention-Related Testing			
	CarbaNP (Table 3B)	mCIM (Table 3C)	mCIM With eCIM (Table 3C)	Other (eg, molecular assays)
Organisms	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems	Enterobacterales that are positive by mCIM	Enterobacterales and *P. aeruginosa* that are not susceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by CarbaNP or mCIM.
Strengths	Rapid	No special reagents or media necessary	No special reagents or media necessary	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected.	Requires overnight incubation	Requires overnight incubation	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

Abbreviations: eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method, MIC, minimal inhibitory concentration.

تشخیص آزمایشگاهی کاربا‌پنمازها

Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in Enterobacterales and *Pseudomonas aeruginosa*[1-6]

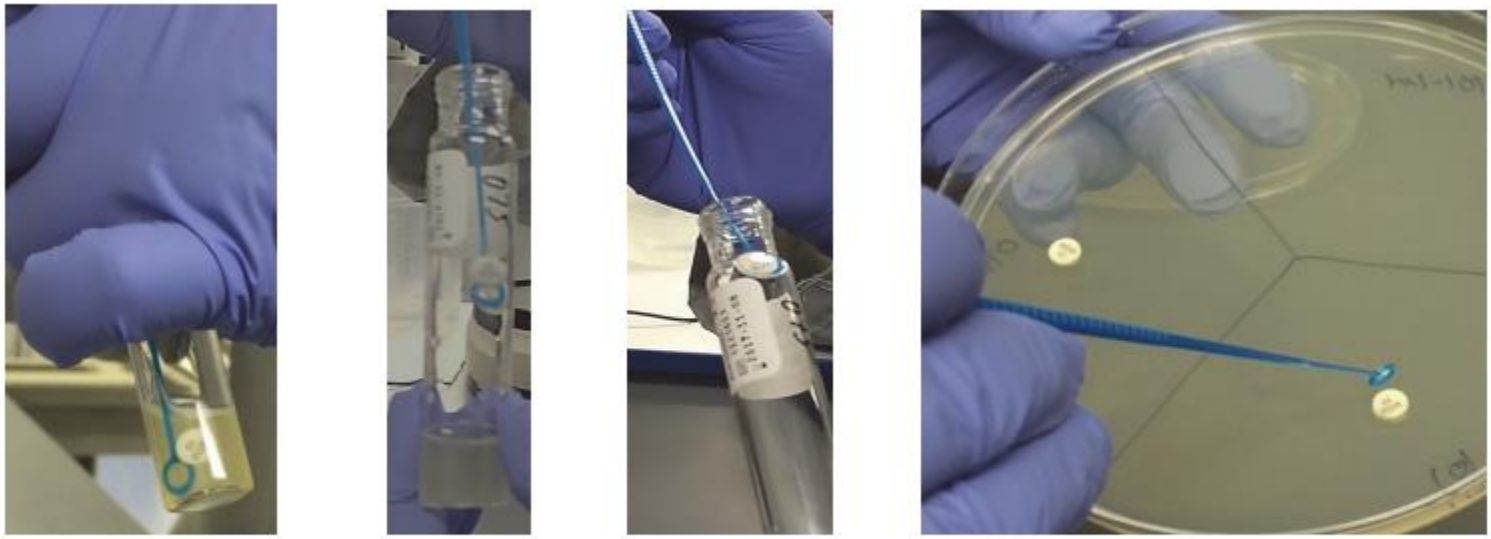
NOTE: If using FORMER MIC breakpoints for carbapenems described in M100-S20 (January 2010), please refer to modifications in Table 3C-1.

Test	mCIM Only or in Conjunction With eCIM
When to perform this test:	For epidemiological or infection prevention purposes. **NOTE:** No change in the interpretation of carbapenem susceptibility test results is necessary for mCIM positive and/or eCIM results. mCIM with or without eCIM testing is not currently recommended for routine use. • mCIM is used for detecting carbapenemases in Enterobacterales and *P. aeruginosa* whereas eCIM is used together with mCIM to differentiate metallo-β-lactamases from serine carbapenemases in Enterobacterales. • mCIM can be performed alone; however, eCIM must be performed together with mCIM. • eCIM is valid only if mCIM is positive.
Test method	Meropenem disk inactivation
Test reagents and materials	• TSB (2 mL aliquots) • Meropenem disks (10 µg) • 1-µL and 10-µL inoculation loops • Nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0-5.0 mL aliquots) • MHA plates (100 mm or 150 mm) • Meropenem-susceptible indicator strain - *E. coli* (ATCC® 25922) • 0.5 M EDTA (only for eCIM)

تشخیص آزمایشگاهی کاربا پنمازها

Test	mCIM Only or in Conjunction With eCIM
Test procedure: mCIM	1. For each isolate to be tested, emulsify a 1-µL loopful of bacteria for Enterobacterales or 10-µL loopful of bacteria for *P. aeruginosa* from an overnight blood agar plate in 2 mL TSB. 2. Vortex for 10-15 seconds. 3. Add a 10-µg meropenem disk to each tube using sterile forceps or a single disk dispenser. Ensure the entire disk is immersed in the suspension. 4. Incubate at 35°C ± 2°C in ambient air for 4 hours ± 15 minutes. 5. Just before or immediately following completion of the TSB-meropenem disk suspension incubation, prepare a 0.5 McFarland suspension (using the colony suspension method) of *E. coli* ATCC® 25922 in nutrient broth or saline. 6. Inoculate an MHA plate with *E. coli* ATCC® 25922 as for the routine disk diffusion procedure (see M02[4]) making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes. Allow the plates to dry for 3-10 minutes before adding the meropenem disks. 7. Remove the meropenem disk from each TSB-meropenem disk suspension using a 10-µL loop by placing the flat side of the loop against the flat edge of the disk and using surface tension to pull the disk out of the liquid. Carefully drag and press the loop along the inside edge of the tube to expel excess liquid from the disk. Continue using the loop to remove the disk from the tube and then place it on the MHA plate previously inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. Disk capacity: 4 disks on a 100 mm MHA plate; 8 disks on a 150 mm MHA plate (see Figure 1). 8. Invert and incubate the MHA plates at 35°C ± 2°C in ambient air for 18-24 hours. 9. Following incubation, measure the zones of inhibition as for the routine disk diffusion method (see M02[4]).
Test procedure: eCIM for Enterobacterales only; optional	1. For each isolate, label a second 2-mL TSB tube for the eCIM test. 2. Add 20 µL of the 0.5 M EDTA to the 2-mL TSB tube to obtain a final concentration of 5 mM EDTA. 3. Follow steps 1 through 9 above as for mCIM procedure. Process the mCIM and eCIM tubes in parallel. 4. Place the meropenem disks from the mCIM and eCIM tubes on the same MHA plate inoculated with the meropenem-susceptible *E. coli* ATCC® 25922 indicator strain. **NOTE:** Additional QC is needed for the eCIM test (see QC recommendations).

تشخیص آزمایشگاهی کاربا‌پنمازها



A B C D

Figure 1. Procedure for Placing Meropenem Disks for the mCIM. Remove the meropenem disk with a 10-µL loop (A) and drag the loop against the inside edge of the tube to expel any excess liquid (B). Use the same loop to remove the disk from the tube (C) and place it on the MHA plate (D) previously inoculated with the meropenem-susceptible *E. coli* (ATCC® 25922) indicator strain.

تشخیص آزمایشگاهی کاربا‌پنمازها

Test	mCIM Only or in Conjunction With eCIM
Test interpretation	For additional explanations, refer to Figures 2A, 2B, and 3A through 3D, as well as the notes section below. mCIM • Carbapenemase positive (see Figures 2A and 2B): – Zone diameter of 6-15 mm or presence of pinpoint colonies within a 16-18 mm zone – If the test isolate produces a carbapenemase, the meropenem in the disk will be hydrolyzed and there will be no inhibition or limited growth inhibition of the meropenem-susceptible *E. coli* ATCC® 25922. • Carbapenemase negative (see Figure 2A): – Zone diameter of ≥ 19 mm (clear zone) – If the test isolate does not produce carbapenemase, the meropenem in the disk will not be hydrolyzed and will inhibit growth of the meropenem-susceptible *E. coli* ATCC® 25922. • Carbapenemase indeterminate: – Zone diameter of 16-18 mm – Zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone – The presence or absence of a carbapenemase cannot be confirmed. eCIM - Interpret only when mCIM test is positive • Metallo-ß-lactamase positive: – A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (eg, mCIM = 6 mm; eCIM = 15 mm; zone diameter difference = 9 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figures 3B and 3C). – If the test isolate produces a metallo-ß-lactamase, the activity of the carbapenemase will be inhibited in the presence of EDTA such that the meropenem in the disk will not be hydrolyzed as efficiently as in the tube without EDTA. The result is inhibition of the meropenem-susceptible *E. coli* and an increase in the zone diameter for the eCIM zone diameter compared with the mCIM zone diameter. • Metallo-ß-lactamase negative: – A ≤ 4-mm increase in zone diameter for the eCIM vs zone diameter of mCIM (eg, mCIM = 6 mm; eCIM = 8 mm; zone diameter difference = 2 mm). For only the eCIM test, ignore pinpoint colonies within any zone of inhibition (see Figure 3D). – If the test isolate produces a serine carbapenemase, the activity of the carbapenemase will not be affected by the presence of EDTA and there will be no or marginal (≤ 4 mm) increase in zone diameter in the presence of EDTA compared with the mCIM zone diameter.

تشخیص آزمایشگاهی کاربا پنمازها

Figure 2A. mCIM Results for QC Strains: Negative Control *K. pneumoniae* ATCC® BAA-1706™ (A) and Positive Control *K. pneumoniae* ATCC® BAA-1705™ (B). **NOTE:** A narrow ring of growth around the meropenem disk as seen with the negative control (A) results from carryover of the test organism in the TSB and should be ignored.

تشخیص آزمایشگاهی کاربا پنمازها

Figure 2B. mCIM Test Interpretation

- Result: positive mCIM
- Report: carbapenemase detected

NOTE: A narrow ring of growth around the meropenem disk results from carryover of the test organism in the TSB and should be ignored.

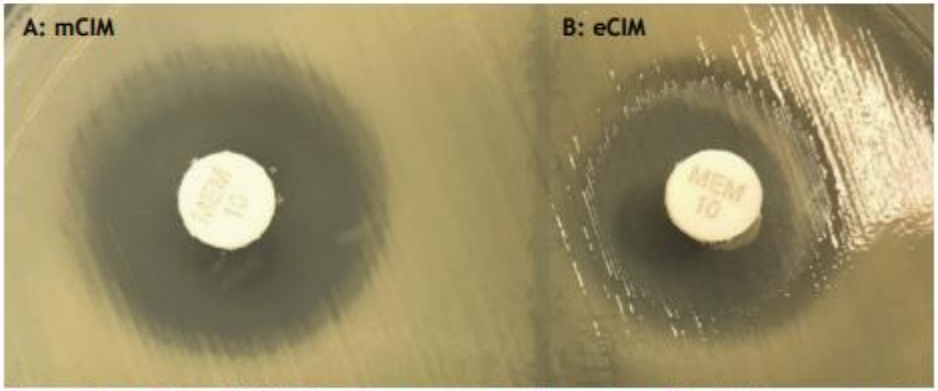


Figure 3A. mCIM and eCIM Test Interpretation: Negative mCIM. "A" shows an mCIM negative result (zone diameter = 20 mm) and "B" shows an eCIM invalid result. Do not interpret the eCIM result when the mCIM is negative as the isolate is negative for carbapenemase production.

- Result: negative for carbapenemase production
- Report: carbapenemase not detected

تشخیص آزمایشگاهی کاربا‌پنمازها

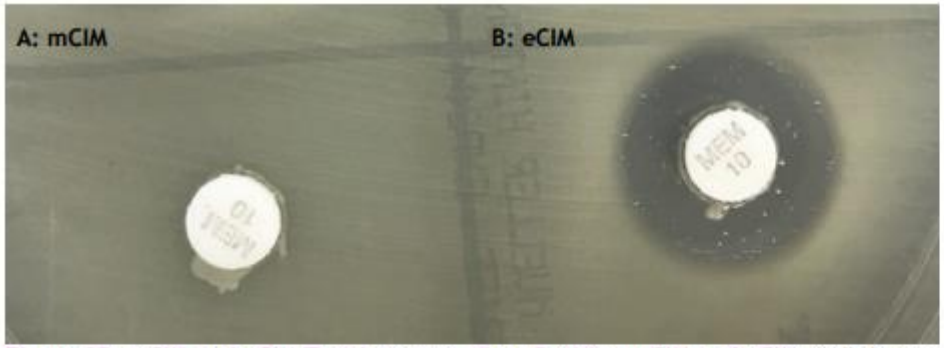


Figure 3B. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter of 6 mm) and "B" shows an eCIM positive result (zone diameter = 15 mm with pinpoint colonies throughout the zone of inhibition). **NOTE:** The pinpoint colonies throughout the zone of inhibition are ignored when measuring the zone for the eCIM test. A ≥ 5-mm increase in zone diameter for eCIM vs zone diameter for mCIM (15 mm – 6 mm = 9 mm) demonstrates the inhibition of the metallo-B-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-B-lactamase detected

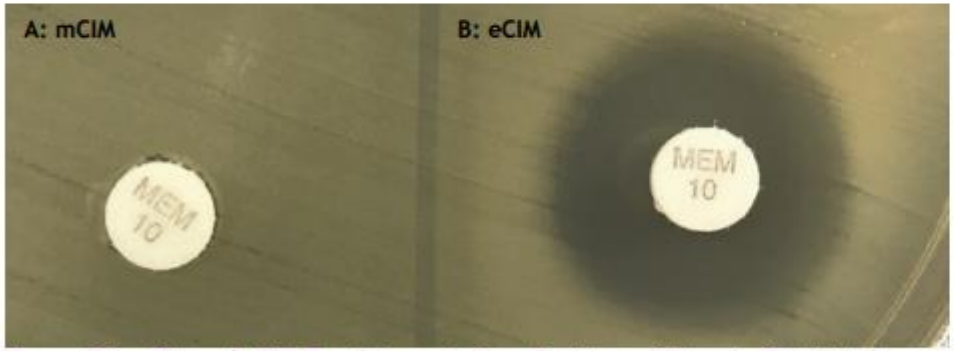


Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm – 6 mm = 13 mm) demonstrates the inhibition of the metallo-B-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-B-lactamase detected

تشخیص آزمایشگاهی کاربا‌پنمازها

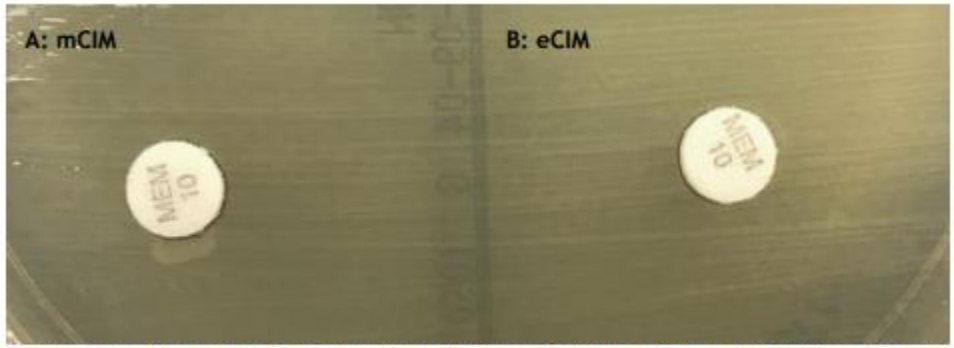


Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.

- Result: positive mCIM and negative eCIM
- Report: serine carbapenemase detected

تشخیص آزمایشگاهی کارباپنمازها

Test	mCIM Only or in Conjunction With eCIM

Reporting

mCIM Only

mCIM Result	eCIM Result	Report
Negative	Not set up	Carbapenemase not detected
Positive	Not set up	Carbapenemase detected
Indeterminate	Not set up	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

mCIM and eCIM Combination Test

mCIM Result	eCIM Result	Report
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected
Positive	Positive	Metallo-β-lactamase detected
Indeterminate	Do not interpret	Testing inconclusive for the presence of carbapenemase. Call laboratory to discuss.[a]

[a] If indeterminate results are obtained on repeat testing, consider performing a different phenotypic test for carbapenemase detection (ie, CarbaNP), a test for carbapenemase genes or send isolate to a referral laboratory for further testing.

If both a serine carbapenemase and a metallo-β-lactamase are co-produced by one organism, differentiation between enzymes will not be possible and false-negative eCIM results may occur.

تشخیص آزمایشگاهی کاربا‌پنمازها

Test	mCIM Only or in Conjunction With eCIM
NOTES	• For mCIM indeterminate results: – Check test isolate and *E. coli* ATCC® 25922 indicator strain for purity. – Check meropenem disk integrity by confirming acceptable results were obtained when disks were subjected to routine disk diffusion test QC. – Repeat the mCIM and/or eCIM for test isolate and QC strains. • mCIM only: For some tests, pinpoint colonies of the indicator organism (*E. coli* ATCC® 25922) may be observed within the zone of inhibition. If the colonies are present within a 6- to 18-mm zone of inhibition, the test should be considered carbapenemase positive. If colonies are present within a ≥ 19-mm zone, the test should be considered indeterminant. • eCIM only: Ignore pinpoint colonies within any zone of inhibition. Interpret results strictly based on the difference in zone diameters between the mCIM and eCIM tests. • mCIM negative and eCIM positive results should not occur. If this happens, perform checks as indicated in the first bullet above. If the repeat tests are the same, consider the tests invalid. • CLSI has currently standardized mCIM for Enterobacterales with a 1-µL loopful of bacteria and *P. aeruginosa* 10-µL loopful of bacteria only.
QC recommendations	Test positive and negative QC strains each day of testing (refer to Figures 2A and 2B for examples of positive and negative QC results).

QC Strain	Organism Characteristic	Expected Result
K. pneumoniae ATCC® BAA-1705™	KPC positive Serine carbapenemase producer	mCIM positive eCIM negative
K. pneumoniae ATCC® BAA-1706™	Carbapenemase negative	mCIM negative
K. pneumoniae ATCC® BAA-2146™[a]	NDM positive Metallo-B-lactamase producer	mCIM positive eCIM positive

[a] eCIM positive control; to be set up only when the eCIM test is performed.

In addition, perform QC of meropenem disks and test media daily or weekly following the routine disk diffusion QC procedure, and handle disks as described in M02.[4] Alternatively, perform QC of meropenem disks with each run by removing a disk from the cartridge of disks used for the run and placing it on the MHA plate inoculated with *E. coli* ATCC® 25922; incubate as above.

Abbreviations: ATCC®, American Type Culture Collection; eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSB, trypticase soy broth.

تشخیص آزمایشگاهی مقاومت به کلیستین

Table 3D. Tests for Colistin Resistance for Enterobacterales and *Pseudomonas aeruginosa*

The polymyxins (colistin and polymyxin B) are antimicrobial agents of last resort for treating multidrug-resistant infections. Clinical and PK/PD data suggest that these agents have limited clinical efficacy. Alternative agents are strongly preferred. If these agents are not available, knowledge of the colistin MIC may be helpful to inform treatment decisions.

For colistin, broth microdilution, broth disk elution and agar dilution MIC methods are acceptable. Broth microdilution is the only approved method for polymyxin B. Disk diffusion and gradient diffusion methods should not be performed.

Colistin and polymyxin B are considered equivalent agents, so MICs obtained from testing colistin predict MICs to polymyxin B and vice versa. At this time, CLSI has not evaluated polymyxin B testing methods, and the procedures below should not be adapted to polymyxin B. The methods below were evaluated for *Acinetobacter* spp. by CLSI and found to yield inaccurate results.

These methods were established with limited disk and/or media manufacturers and are considered provisional until additional data are evaluated by CLSI and shown to meet CLSI document M23[1] guidelines.

تشخیص آزمایشگاهی مقاومت به کلیستین

Test	Colistin Broth Disk Elution	Colistin Agar Test
Approved organisms	Enterobacterales and *Pseudomonas aeruginosa*	Enterobacterales and *P. aeruginosa*
Strengths	No special reagents or media necessary	Ability to test up to 10 isolates at one time
Limitations	Hands-on time and cost	Requires special media (colistin agar plate)
When to perform this test	Testing multidrug-resistant isolates for clinical or infection prevention purposes	Testing multidrug-resistant isolates for clinical or infection prevention purposes
Test method	Tube dilution using colistin disk as the colistin source	Agar dilution: slight variation of method described in M07[2] (ie, different inoculum and different approach to interpreting results)
Organism group	Enterobacterales and *P. aeruginosa*	Enterobacterales and *P. aeruginosa*
Medium	CAMHB (10-mL tubes)	MHA (20 mL in 100-mm Petri plate)[a]
Antimicrobial concentration	10-µg colistin **sulfate** disks Final concentration: 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin	**Colistin sulfate** Final concentration: 0 µg/mL (growth control), 1 µg/mL, 2 µg/mL, and 4 µg/mL colistin[a]
Inoculum	1. Using a loop or swab, pick 3-5 colonies from a fresh (18-24 hours) nonselective agar plate and transfer to sterile saline (4-5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard.	1. Using a loop or swab, pick 3-5 colonies from a fresh (18-24 hours) nonselective agar plate and transfer to sterile saline (4-5 mL). 2. Adjust turbidity to equivalent of a 0.5 McFarland turbidity standard. 3. Dilute the standardized inoculum 1:10 in saline.

تشخیص آزمایشگاهی مقاومت به کلیستین

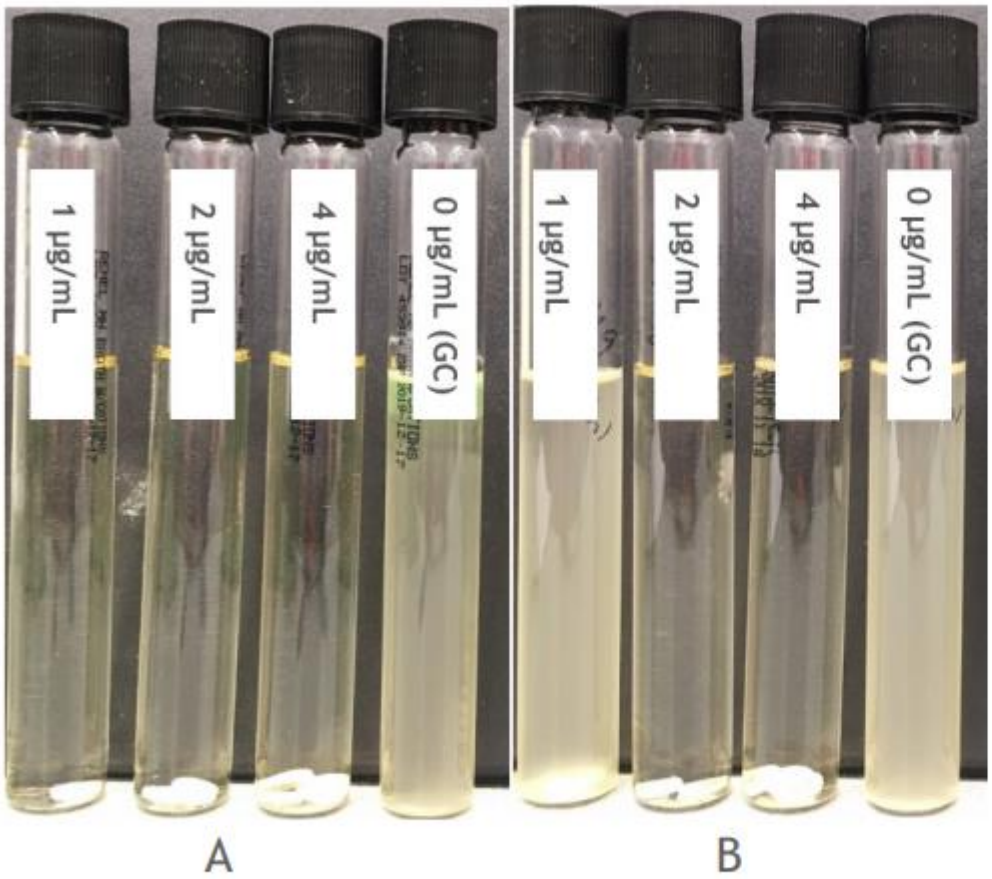
Test	Colistin Broth Disk Elution	Colistin Agar Test
Test procedure	1. Let the CAMHB tubes (10 mL) and colistin disks warm to room temperature. 2. Label 4 tubes of CAMHB for each isolate to be tested with 1, 2, and 4 µg/mL and control (see Figure 1). 3. Using aseptic technique, carefully add: • 1 colistin disk to the tube labeled "1 µg/mL" • 2 colistin disks to tube labeled "2 µg/mL" • 4 colistin disks to the tube labeled "4 µg/mL" 4. Gently vortex the tubes with the added disk and let the colistin elute from the disks for at least 30 minutes but no longer than 60 minutes at room temperature. 5. Prepare the standardized inoculum. 6. Add 50 µL standardized inoculum to the control and 1-, 2-, and 4-µg/mL tubes to attain a final inoculum concentration of approximately 7.5×10^5 CFU/mL. 7. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 8. Cap the tubes tightly and vortex each inoculated tube on slow speed to mix. Slow speed is suggested to prevent colistin from sticking to the cap and glass surface above the meniscus of liquid. 9. Loosen the caps slightly before incubation. 10. Incubate the tubes and purity plate.	1. Divide each colistin agar plate with increasingly doubled dilutions of colistin in up to 10 parts, with a marker to test up to 10 isolates per plate. Label each part with the appropriate isolate number (see Figure 2). 2. Using a pipette or a 10-µL loop, streak 10 µL of the 1:10 dilution onto the appropriate part of each colistin agar plate. 3. Using a 10-µL loop, subculture from the original inoculum tube to a blood agar plate as a purity check. 4. Incubate the colistin agar plates and purity plate.
Incubation conditions	33 to 35°C; ambient air	33 to 35°C; ambient air
Incubation length	16-20 hours	16-20 hours

تشخیص آزمایشگاهی مقاومت به کلیستین

Test	Colistin Broth Disk Elution	Colistin Agar Test
Results	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the growth control tube, which must demonstrate obvious turbidity for the test to be valid. NOTE: Some *P. aeruginosa* isolates may grow only near the meniscus. 3. Read the MIC as the lowest concentration that completely inhibits growth of the test isolate. (See Figure 1 for examples.) For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant	1. Examine the purity plate to ensure inoculum was pure. 2. Examine the growth control plate, which must demonstrate confluent growth for the test to be valid. 3. Examine the colistin plates carefully with transmitted light for colony or light film of growth. 4. Read the MIC as the lowest colistin agar plate concentration that completely inhibits growth of the test isolate (eg, even 1 colony would be considered growth). See Figure 2 for examples. For Enterobacterales and *P. aeruginosa:* • ≤ 2 µg/mL = intermediate • ≥ 4 µg/mL = resistant
Additional testing and reporting	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the tubes • Error inoculating the tubes	If there is an inconsistent growth pattern (eg, no growth in 2 µg/mL but growth at 1 µg/mL and 4 µg/mL), repeat the test. An inconsistent growth pattern may occur as a result of: • Contamination at higher dilutions • Heteroresistance • Improper concentrations of antimicrobial agent in the colistin agar plates • Error inoculating the plates
QC recommendations - routine[b]	*Escherichia coli* AR Bank #0349 *mcr-1* (≤ 1-4 µg/mL, with a target of 2 µg/mL)[c] and *P. aeruginosa* ATCC®[d] 27853 (≤ 1-4 µg/mL)	*E. coli* AR Bank #0349 *mcr-1* (≤ 1-4 µg/mL, with a target of 2 µg/mL)[c] and *P. aeruginosa* ATCC® 27853 (≤ 1-4 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CFU, colony-forming unit(s); MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic; QC, quality control.

تشخیص آزمایشگاهی مقاومت به کلیستین



Abbreviation: GC, growth control.

Figure 1. Colistin Broth Disk Elution. Results for routine QC strain *P. aeruginosa* ATCC® 27853 with an MIC ≤ 1 µg/mL (A) and supplemental QC strain *E. coli* AR Bank #0349 *mcr-1* with an MIC 2 µg/mL (B).

آشنایی با CLSI M100

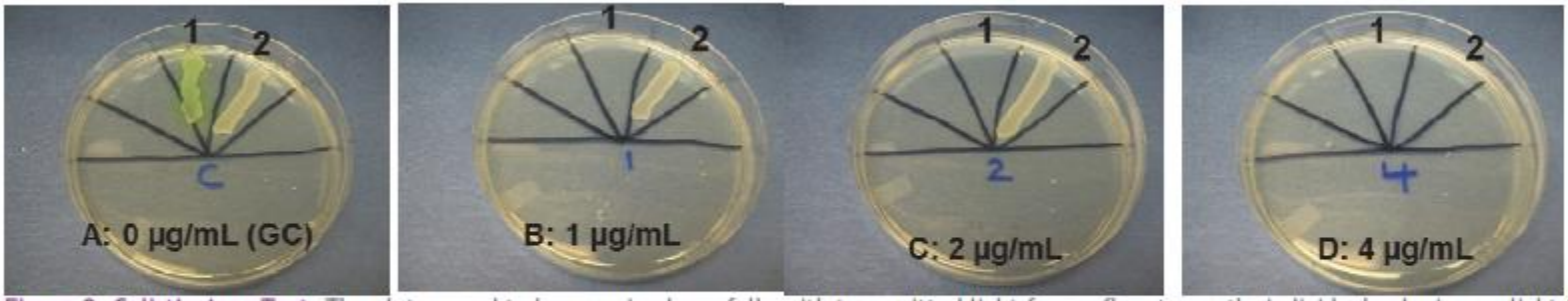


Figure 2. Colistin Agar Test. The plates need to be examined carefully with transmitted light for confluent growth, individual colonies, or light film of growth to determine the MIC. Colistin agar test results for routine QC strain *P. aeruginosa* ATCC® 27853 (position 1) with an MIC ≤ 1 µg/mL and for supplemental QC strain *E. coli* AR Bank #0349 *mcr-1* (position 2) with an MIC 4 µg/mL. The plates shown contain 0 µg/mL (control) (A), 1 µg/mL (B), 2 µg/mL (C), and 4 µg/mL (D) colistin.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