دانشگاه اصفهان

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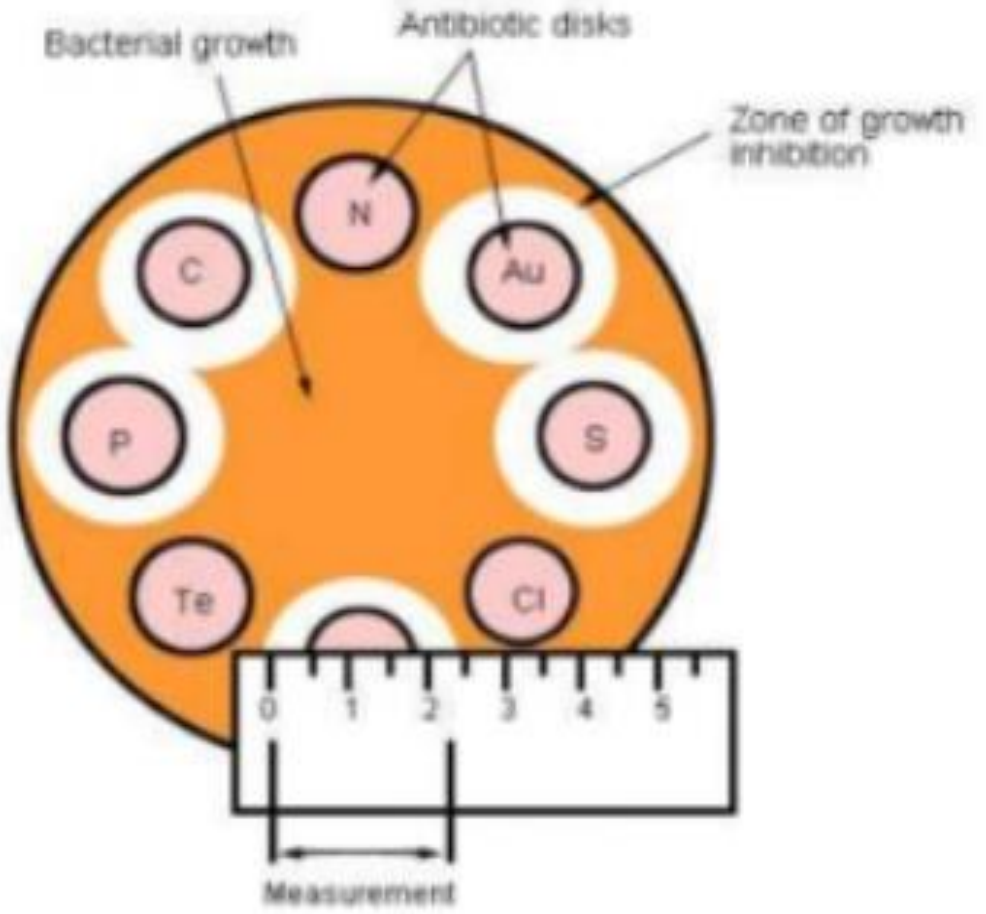
آزمایشگاه باکتری شناسایی ۱

انجام آنتی بیوگرام به منظور تعیین حساسیت استافیلوکوکسی جدا شده از بینی نسبت به آنتی بیوتیک های مختلف و بررسی نتایج آنتی بیوگرام و تعیین حساسیت و مقاومت سویه ها بر اساس جداول CLSI

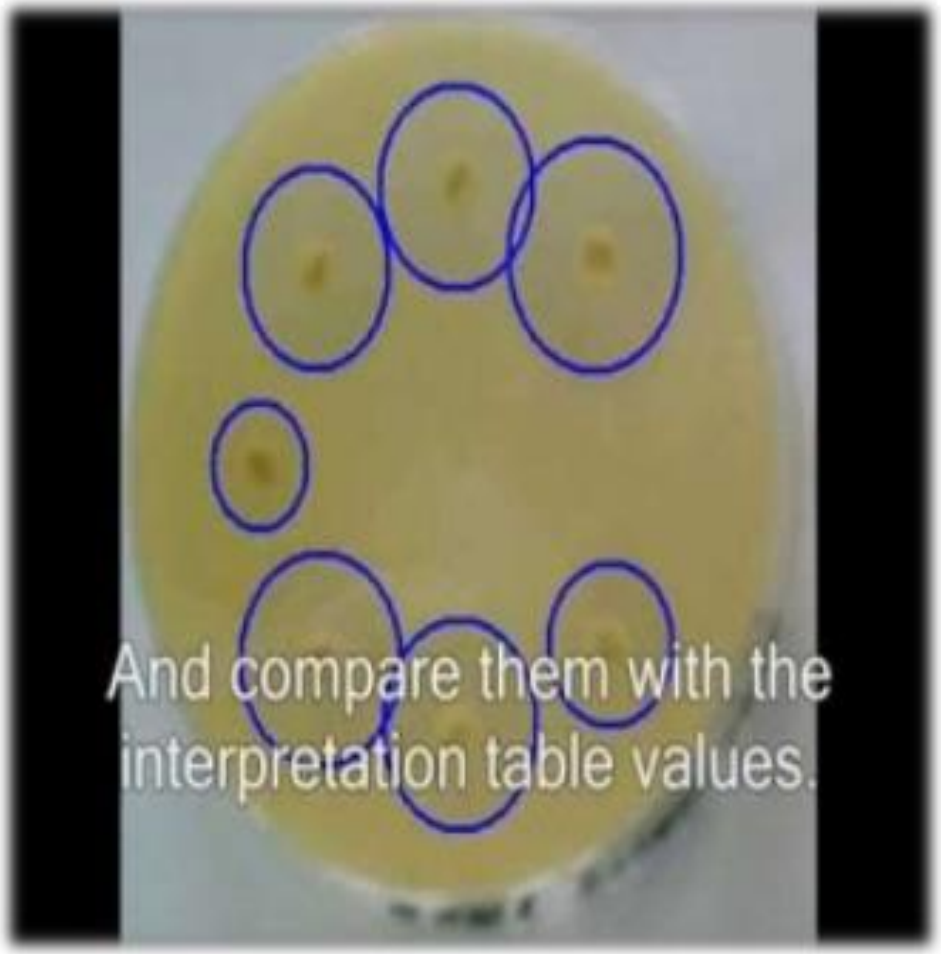
What is a Antibiogram

Antibiogram

- Microbiology The profile of an organism's susceptibility/resistance to a panel of antibiotics, which can be used to determine genetic relatedness of various bacteria Cf Molecular strain typing

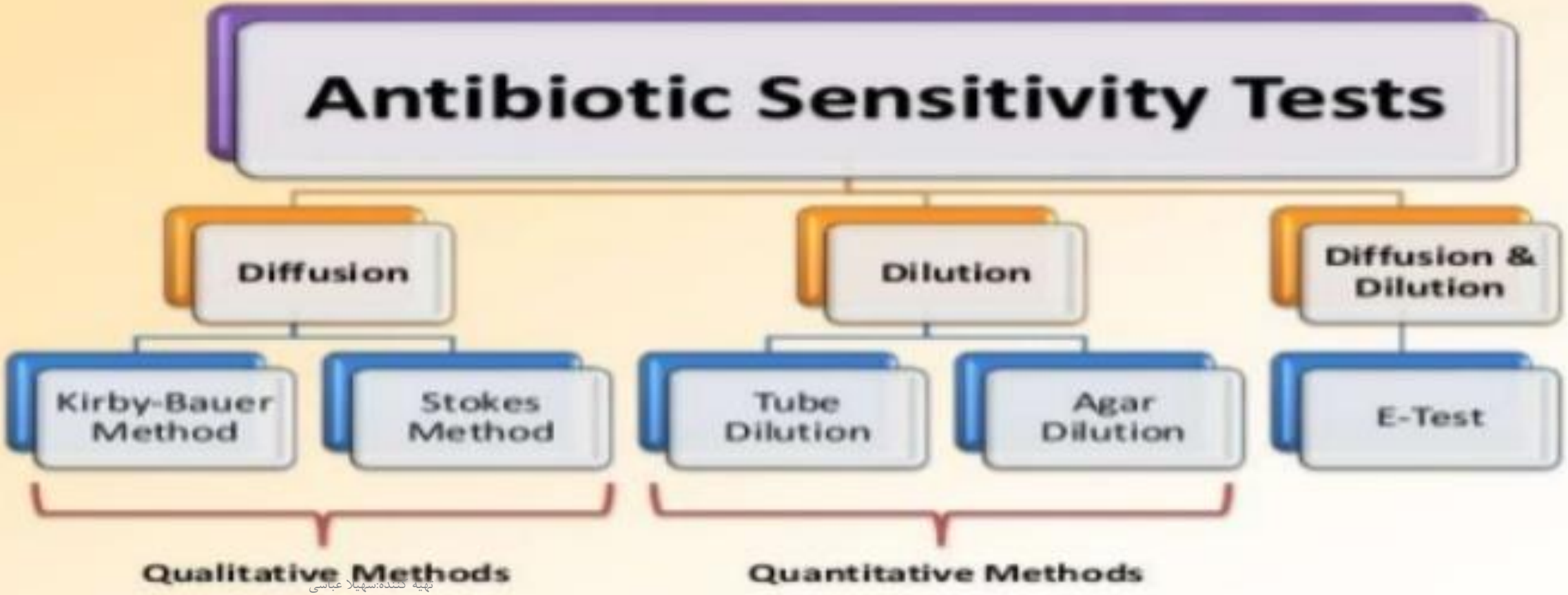


It also Means



- **antibiogram (an'·tē·bī··ō·gram),**
- A method of testing the efficacy of antibiotics by introducing an antibiotic into the middle of a bacteria-laden petri dish. A clear zone indicates the bactericidal activity. The greater the diameter of the zone, the higher the efficacy of the antibiotic.

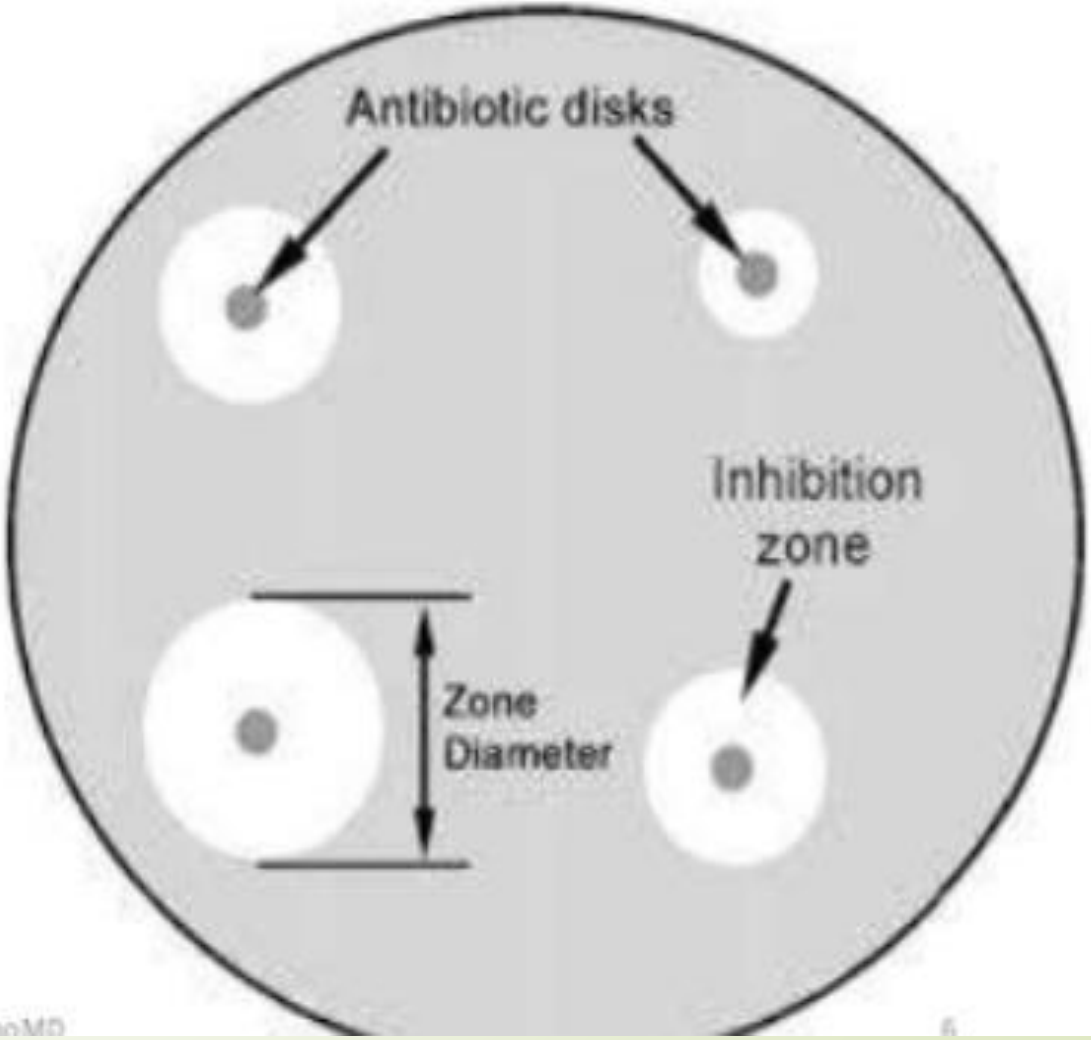
Different methods to Test Antibiotic Sensitivity patterns



تهیه کننده:سهیلا عباسی

Once a culture is established, there are two possible ways to get an antibiogram:

- A semi-quantitative way based on diffusion **(Kirby-Bauer method);** small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture in the petri dish. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible.

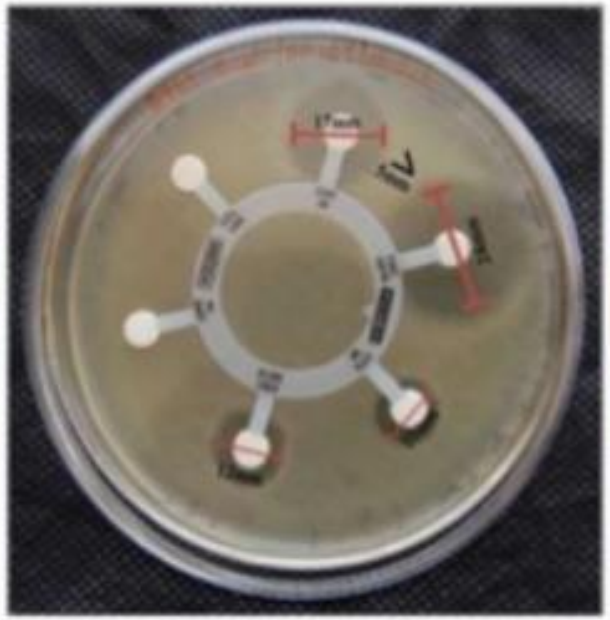


Antibiograms create the Vitro Sensitivity and Resistance patterns of the Antibiotics used in Clinical practice

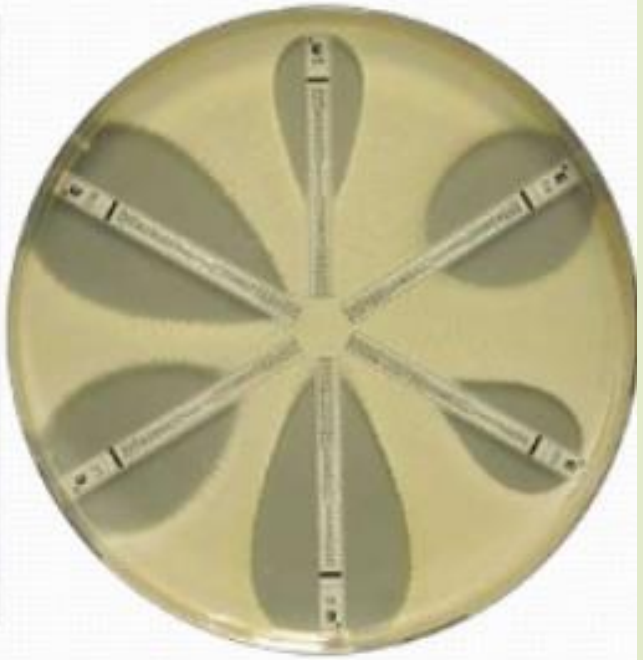
- An antibiogram is the result of an antibiotic sensitivity test, a laboratory test for the sensitivity of an isolated bacterial strain to different antibiotics. It is by definition an in vitro sensitivity, but the correlation of in vitro to in vivo sensitivity is often high enough for the test to be clinically useful.

1- Disc Diffusion method (Manual labs)

Disc Diffusion



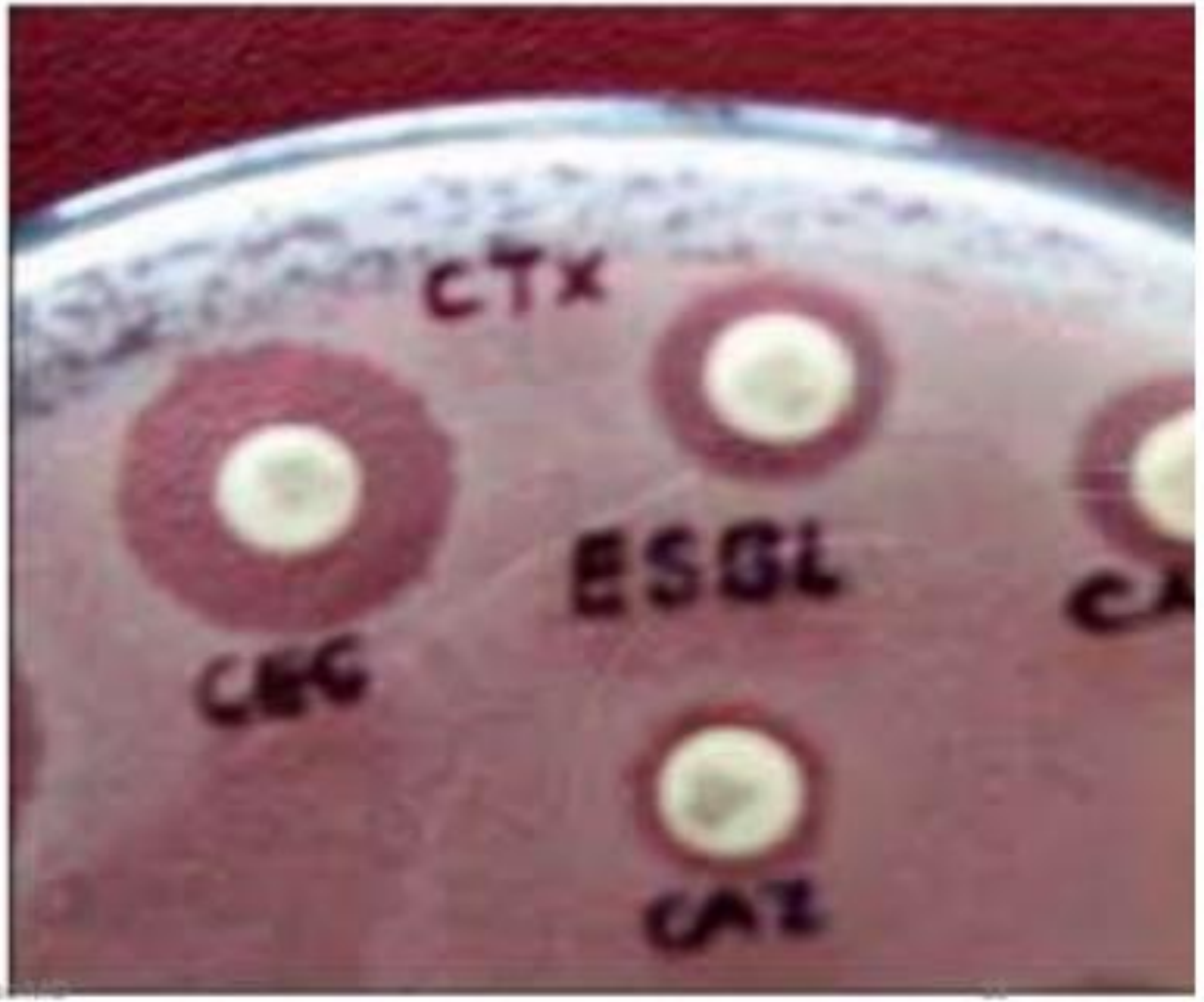
Ring Disc Diffusion



E- Test.

Kirby-Bauer method

- Semi-quantitative way based on diffusion (Kirby-Bauer method); small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow



Disk diffusion

step 1 Preparation

تهیه سوسپانسیون باکتری

آماده سازی محیط کشت

انتخاب کردن دیسک کاغذی

step 2 Lawn culture

سواپ استریل که به سوسپانسون آمیخته شده است را به صورت چمنی بر روی آگار کشت دهید

step 3 Disk Diffusion

دیسک های آنتی بیوتیک را روی آگار قرار دهید

step 4 Measure

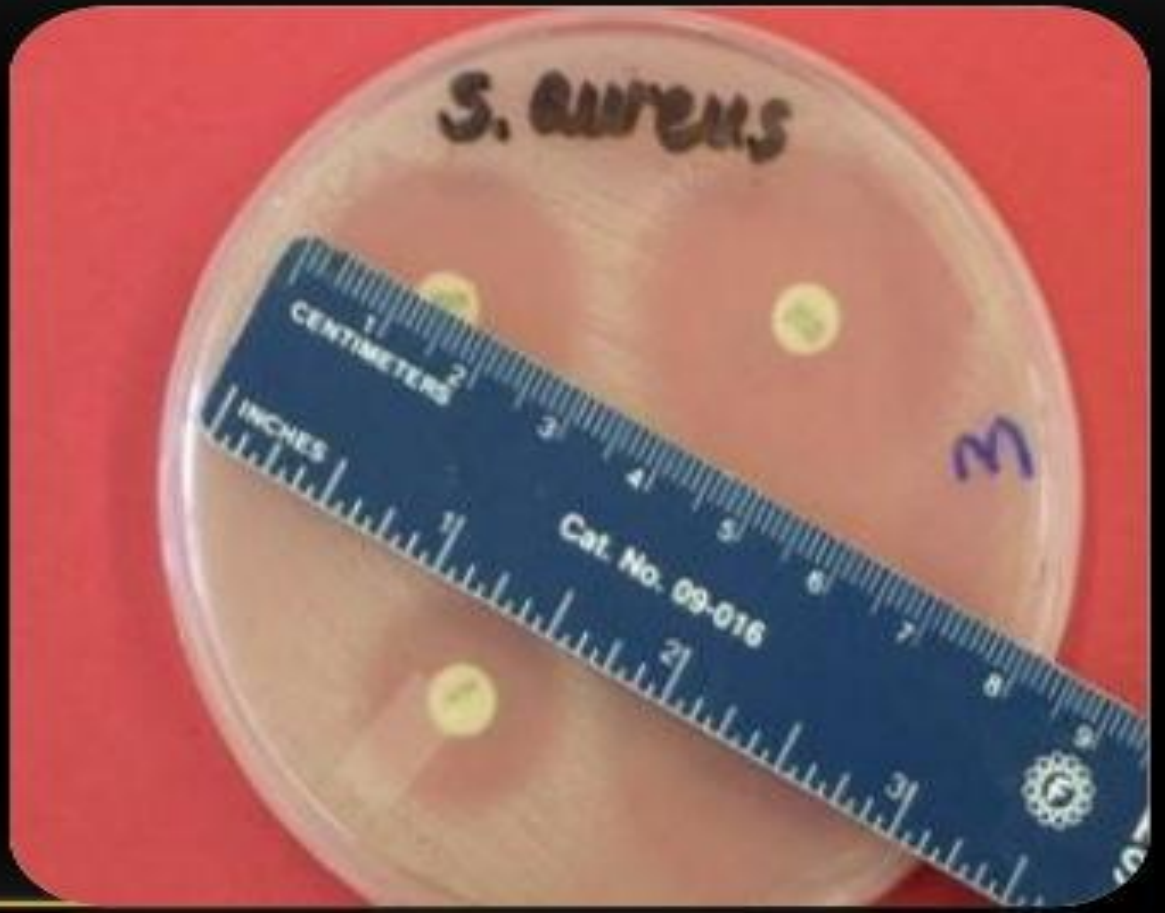
منطقه هاله آنتی بیوتیک را اندازه گیری کنید


TOBRAMYCIN
TOB 10
AMIKACIN
AN 30
P7


Antimicrobial Susceptibility Test Discs

DISC DIFFUSION METHOD

- Place the appropriate drug-impregnated disc on the surface of the inoculated agar plate
- Invert the plates and incubate them at 35 °C, o/n (18-24 h)
- **Measure the diameters of inhibition zone in mm**



Clip slide

Understanding about the Inhibition of the bacterial growth

- Since the concentration of the antibiotic was the highest at the centre, and the lowest at the edge of this zone, the diameter is suggestive for the Minimum Inhibitory Concentration (conversion of the diameter in millimetre to the MIC, in µg/ml, is based on known linear regression curves).

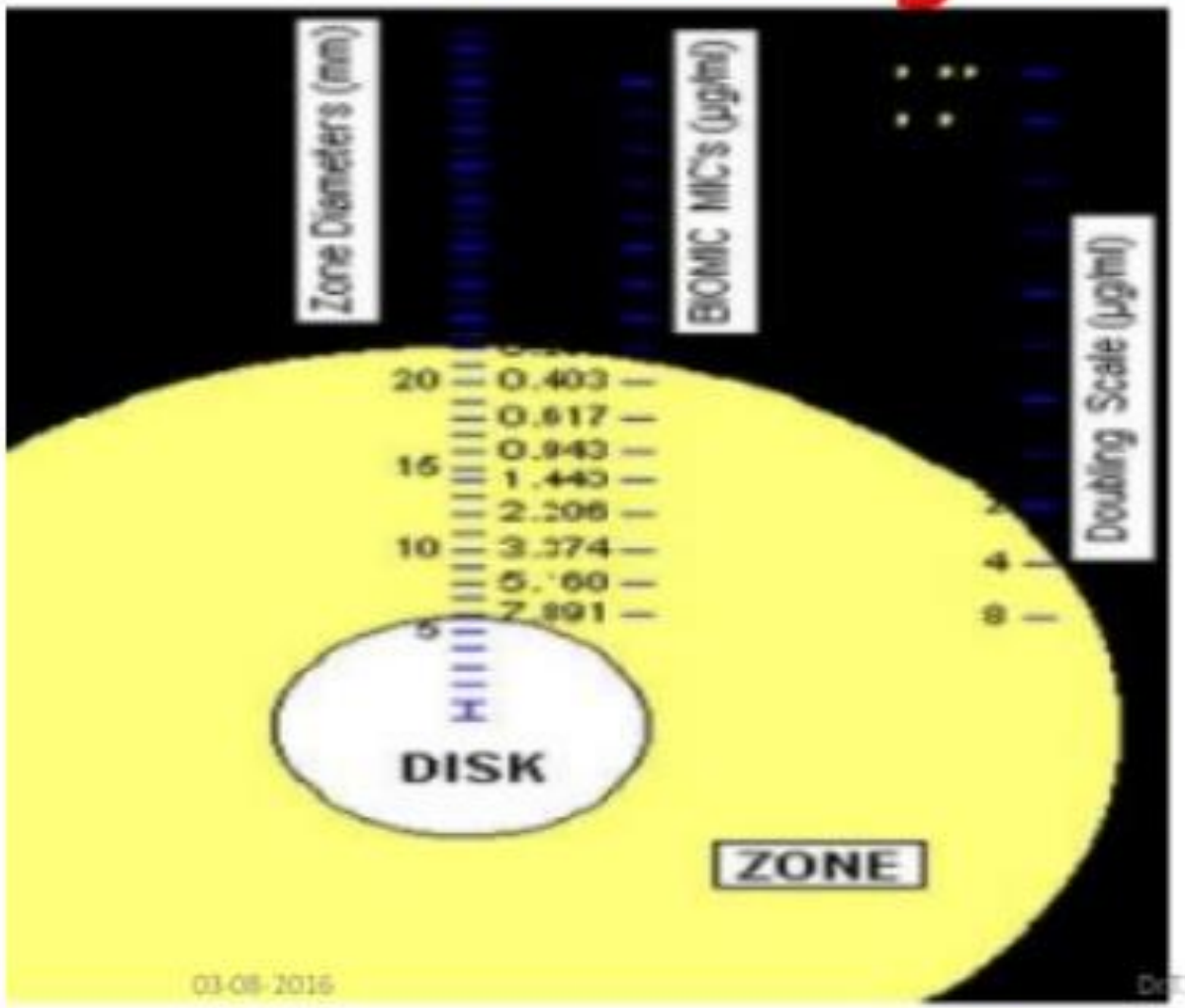
Interpreting an antibiogram

- The correct interpretation of the antibiogram will be of interest to Microbiologists and laboratory technicians alike. Standardized methods are established and can be found in the WHO manuals.

Basic Interpretation of Zone Sizes

- Therefore, it is often assumed that the larger the diameter of the zone of inhibition, the more potent the antimicrobial

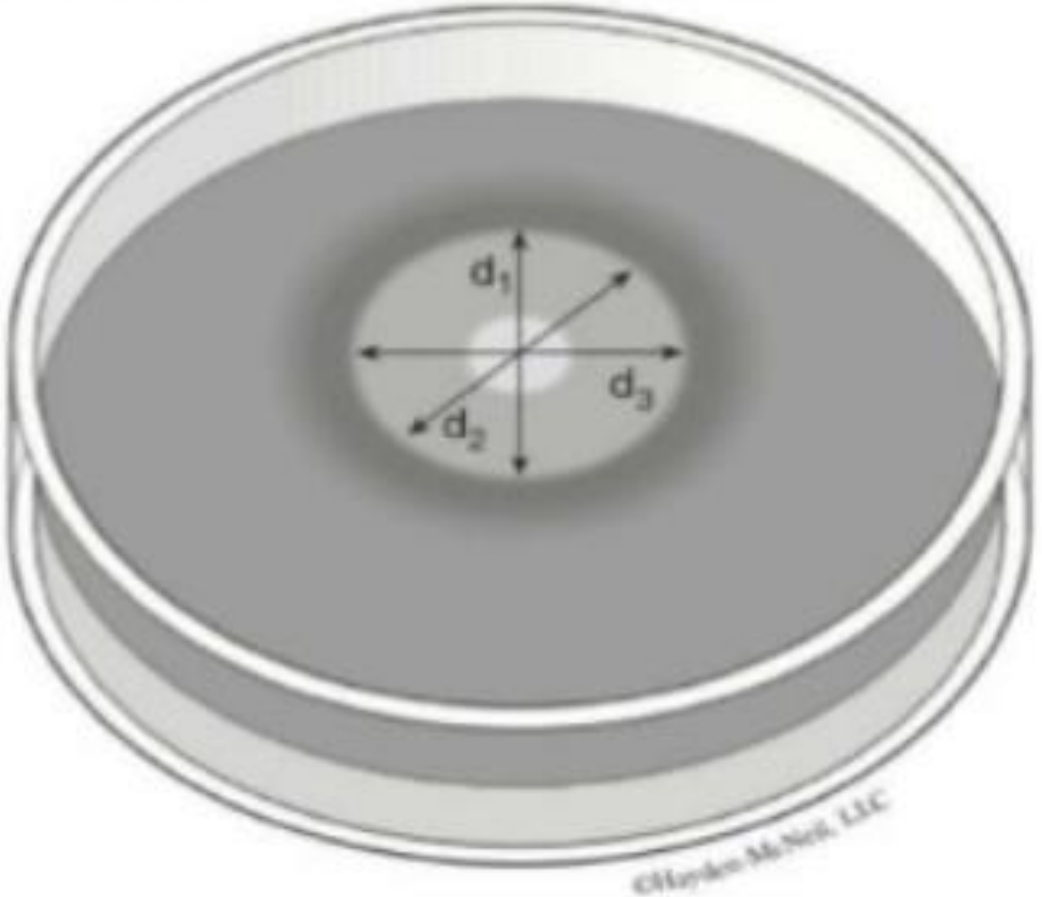
Time too matters in optimal Reading of Antibiograms



- Also, the length of time allowed for the process to occur can greatly influence the diameter of the zone of inhibition as the longer diffusion is allowed to take place the higher the concentrations at any given point in the gradient will be.

Formulating the Concentration of Antibiotic and relevance of zones depend on

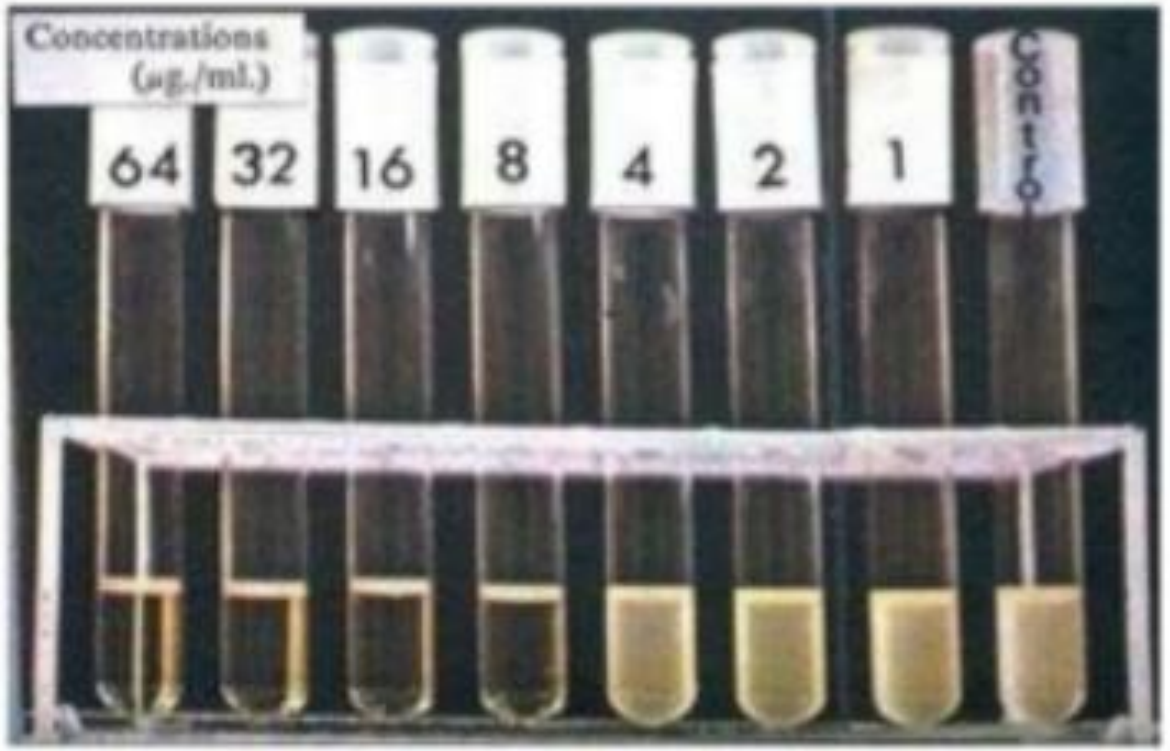
- Comparing zone diameters to minimum inhibitory concentrations (MICs) of a large number of isolates, including those with known mechanisms of resistance relevant to the particular class of drug



Minimal Inhibiting Concentration.

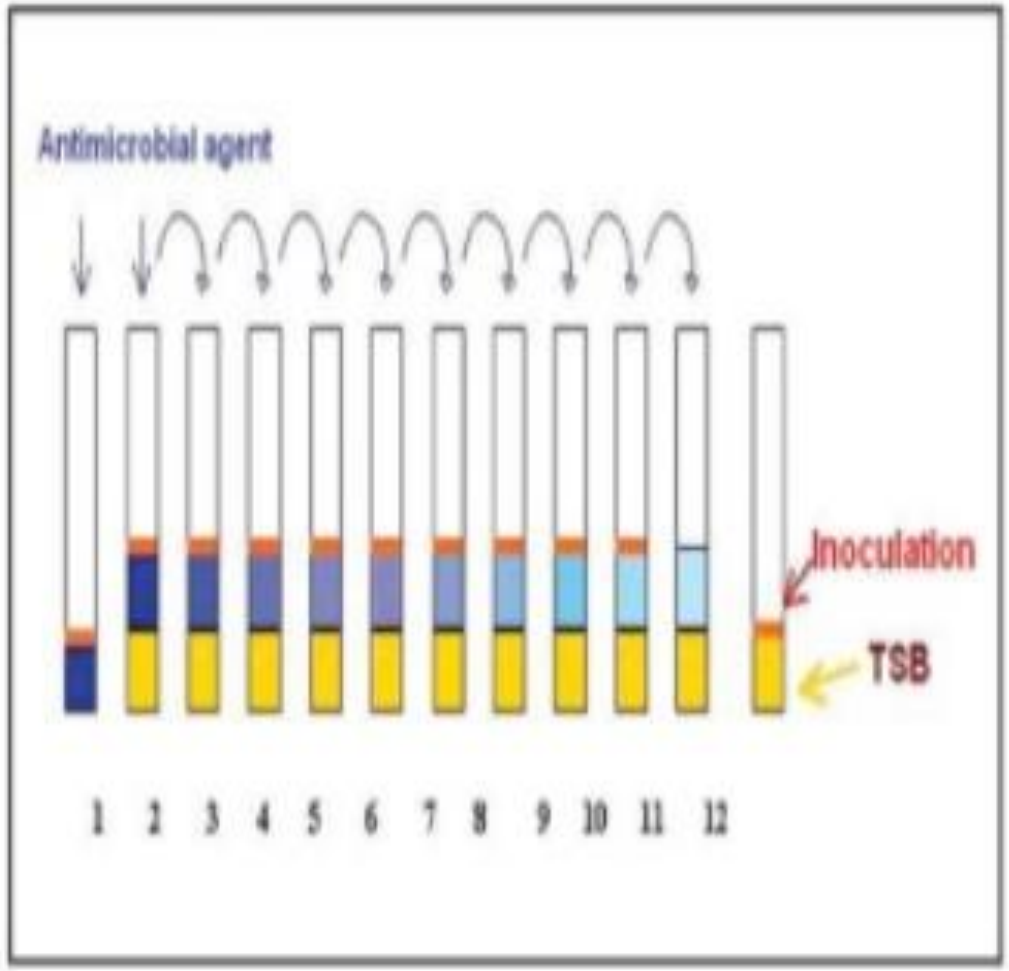
- A quantitative way based on dilution: a dilution series of antibiotics is established (this is a series of reaction vials with progressively lower concentrations of antibiotic substance). The last vial in which no bacteria grow contains the antibiotic at the Minimal Inhibiting Concentration.

Broth dilution method



Minimal Inhibiting Concentration

- Once the MIC is calculated, it can be compared to known values for a given bacterium and antibiotic: e.g. a MIC > 0,06 µg/ml may be interpreted as a penicillin-resistant Streptococcus pneumoniae. Such information may be useful to the clinician, who can change the empirical treatment, to a more custom-tailored treatment that is directed only at the causative bacterium.



What is Minimal Inhibiting Concentration

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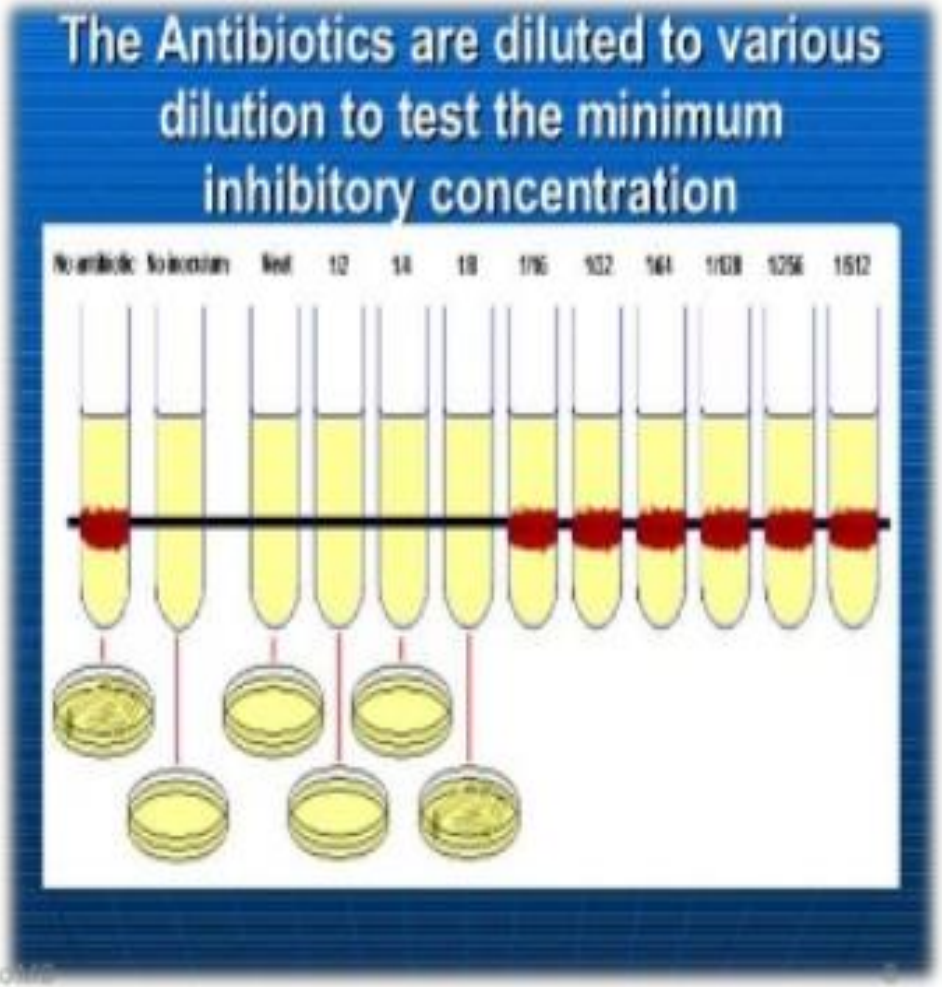
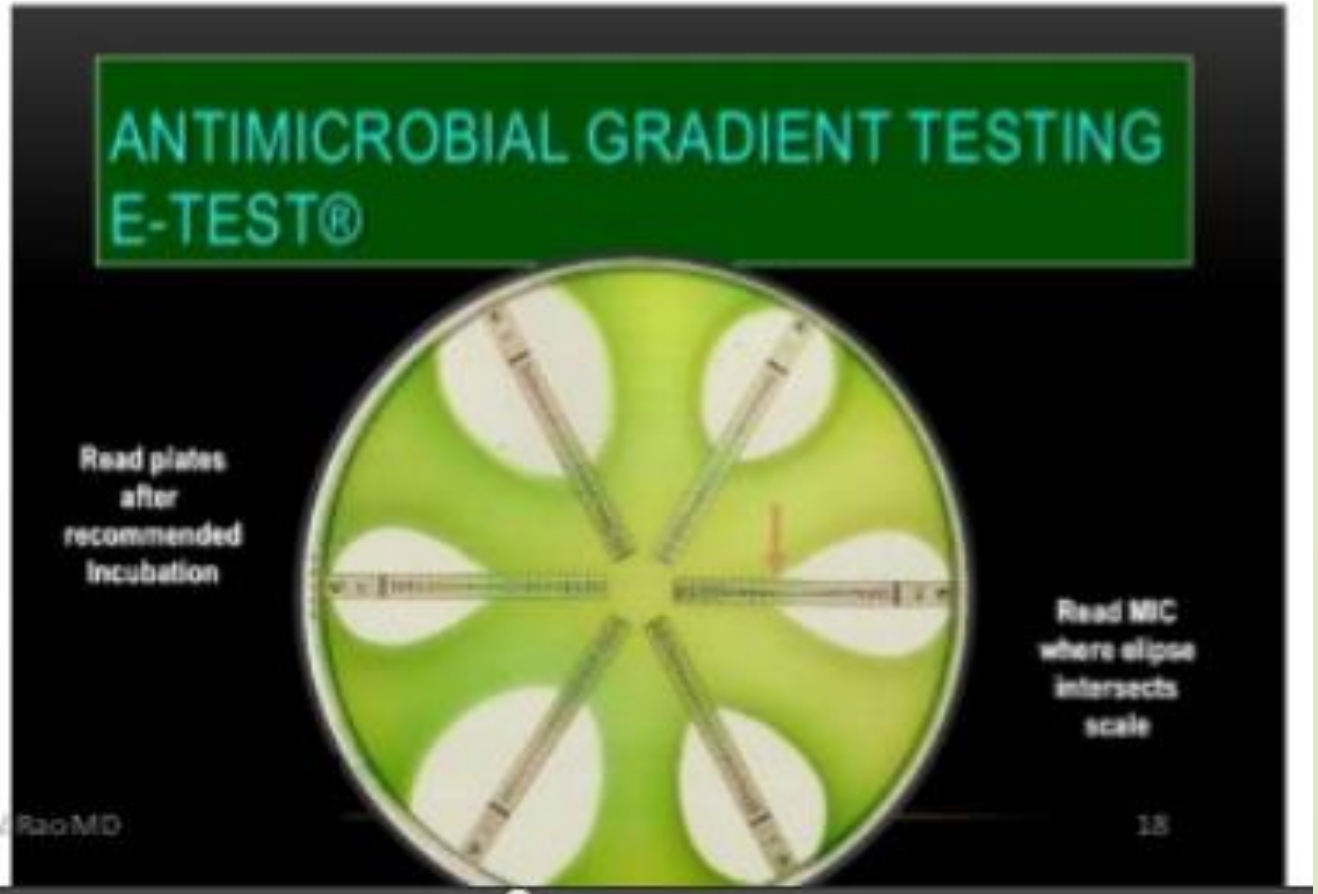


MIC Estimation will help the Clinicians in prescription

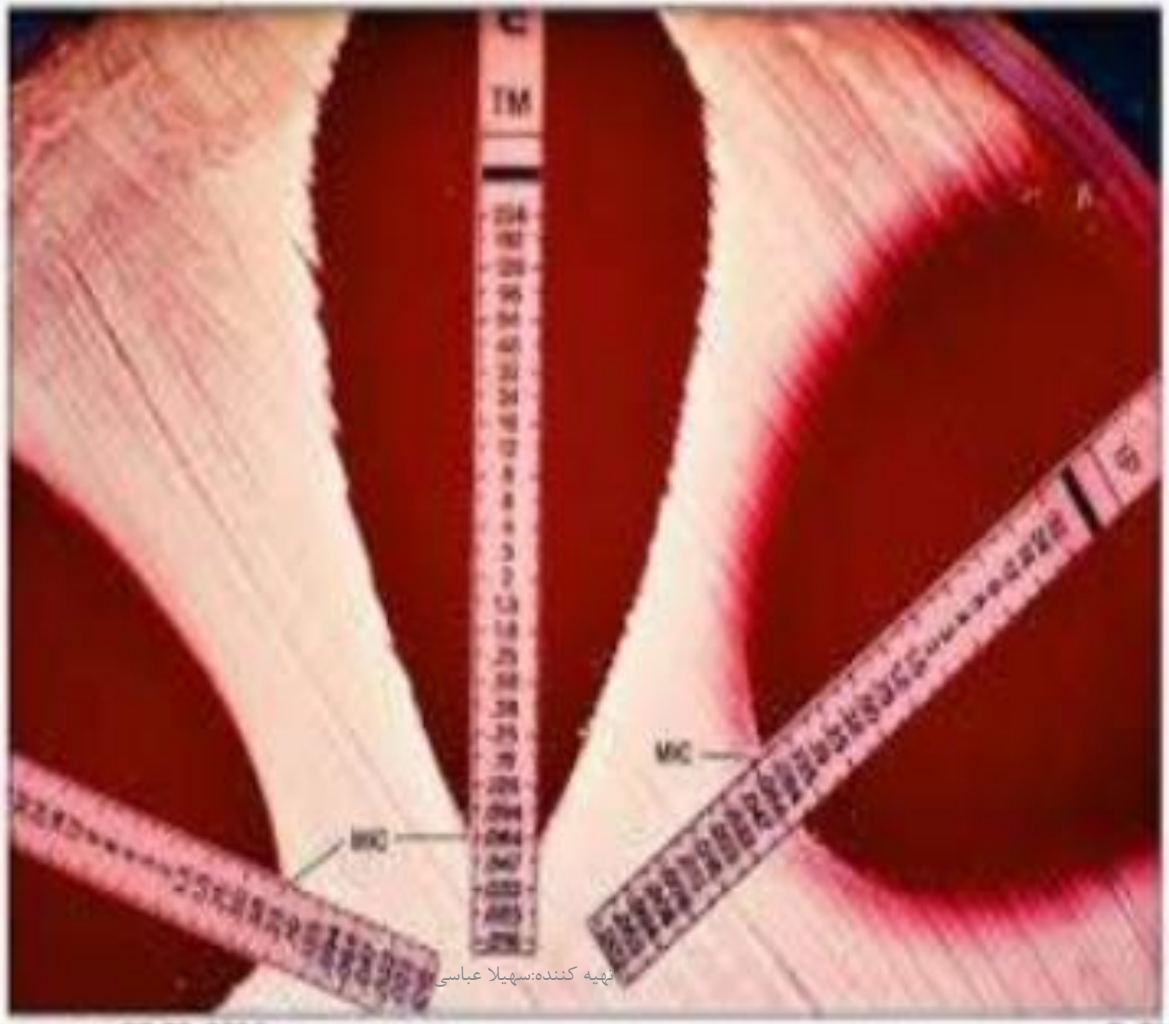
- Once the MIC is calculated, it can be compared to know values for a given bacterium and antibiotic: e.g. a MIC > 0,06µg/ml may be interpreted as a penicillin-resistant Streptococcus pneumoniae. Such information may be useful to the clinician, who can change the empirical treatment, to a more custom-tailored treatment that is directed only

E-Test is Epsilometer test

- Etest, (previously known as Epsilometer test) manufactured by bioMérieux, is a manual in vitro diagnostic device used by laboratories to determine the MIC (Minimum Inhibitory Concentration) and whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antimicrobial.



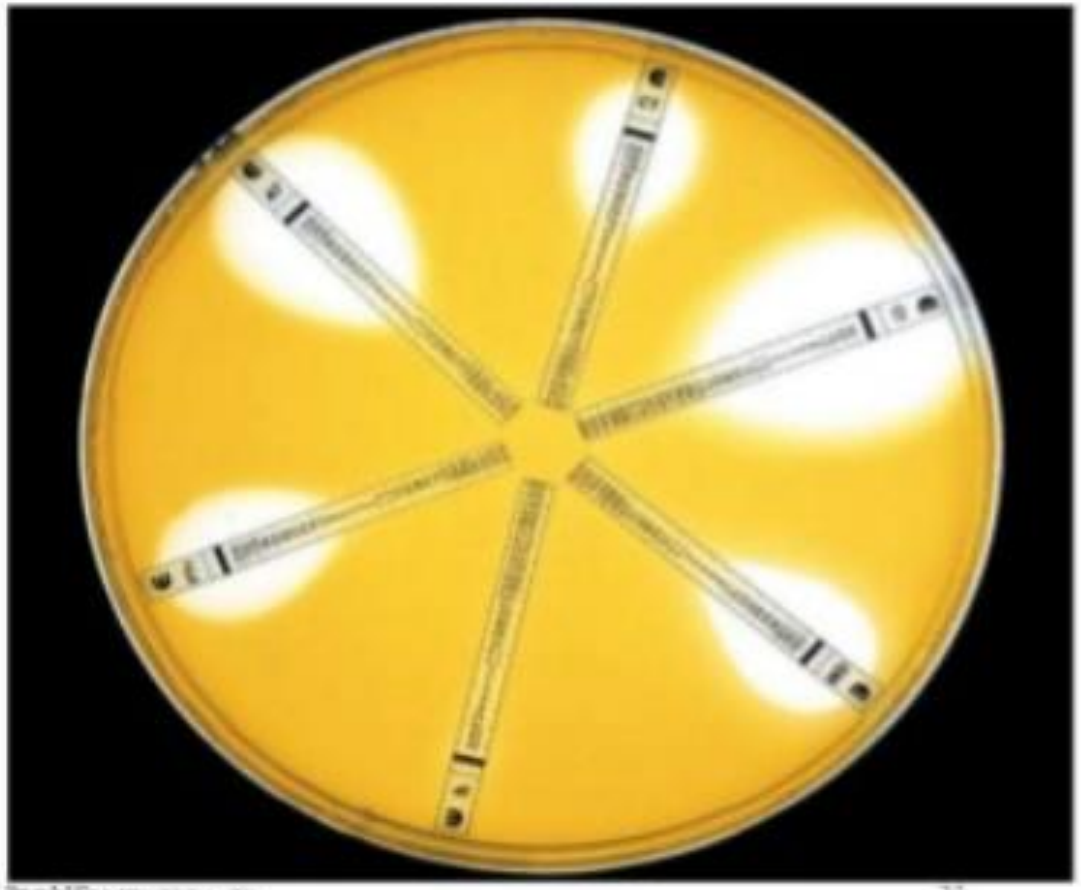
E-Test



- This type of test is most commonly used in healthcare settings to help guiding physicians in treatment of patients by indicating what concentration of antimicrobial would successfully treat an infection

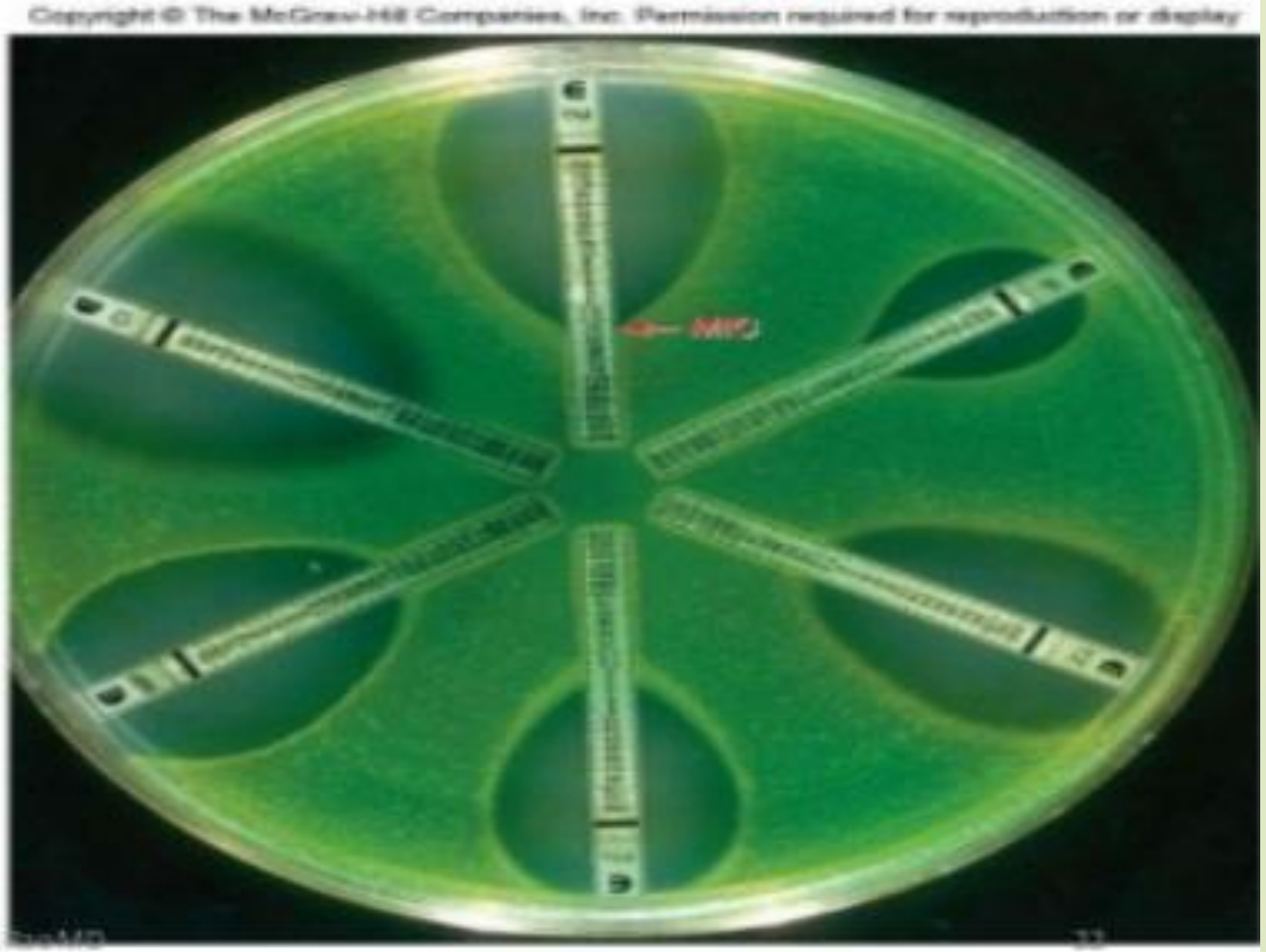
Intended use of E Test

- Etest is a quantitative technique for determining the antimicrobial susceptibility (AST) and MIC (in µg/mL) of Gram-negative and Gram-positive aerobic bacteria such as Enterobacteriaceae, Pseudomonas, Staphylococcus, and Enterococcus species and fastidious bacteria, such as anaerobes, N. gonorhoeae, S. pneumoniae, Streptococcus and Haemophilius species



Factors influencing the interpretation of an antibiogram

- Based on this reasoning, the diffusion method is sometimes mistakenly interpreted as a quantitative method. The more potent an antimicrobial compound, the less concentrated it need be, and consequently at points further from the disc with consequently lower concentrations, microbial growth will still be inhibited



Steps in Antibiogram Creation

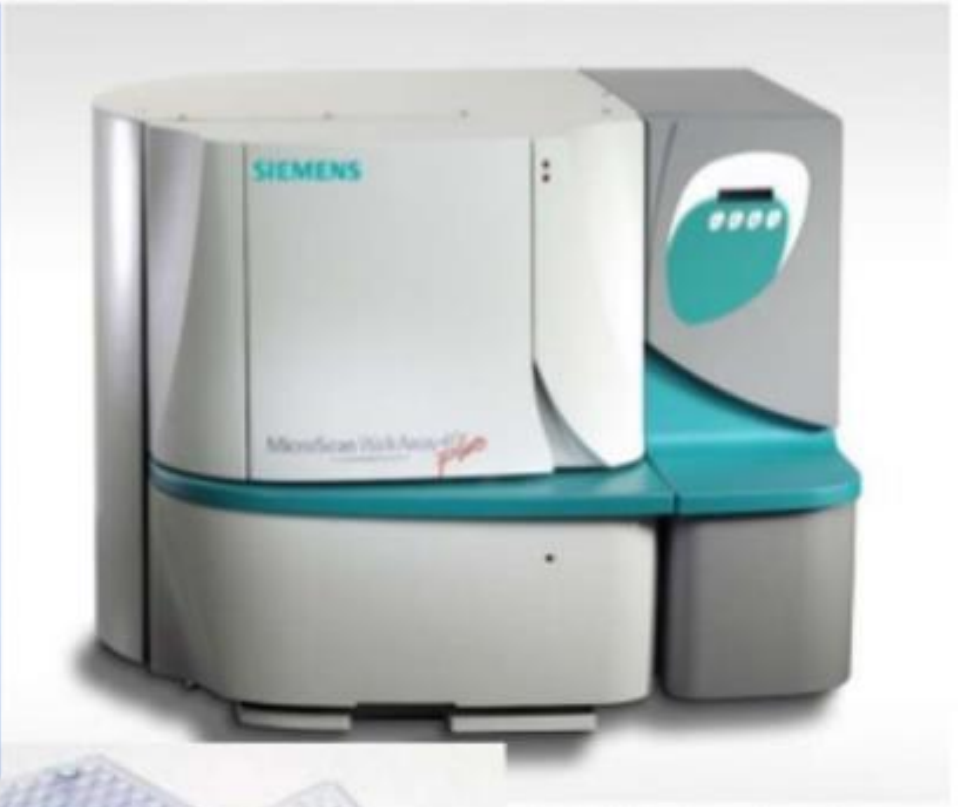
- Ensure a process of identification, preferably as part of the day-to-day function of data verification, of new patterns of resistance during or after treatment,since the first isolate per patient is insensitive to second or later isolates which may develop resistance

	Antimicrobial Agents[A]									
	Am	C	Cip	Gm	K	NA	N	S	G	Te	Va
Staphylococcus aureus	R[B]	I	R	S	R	R	R	S	S	S	S
Pseudomonas aeruginosa	R	R	I	I	R	R	R	R	R	R	R
Enterococcus faecalis	S	R	R	R	R	R	R	R	R	R	R

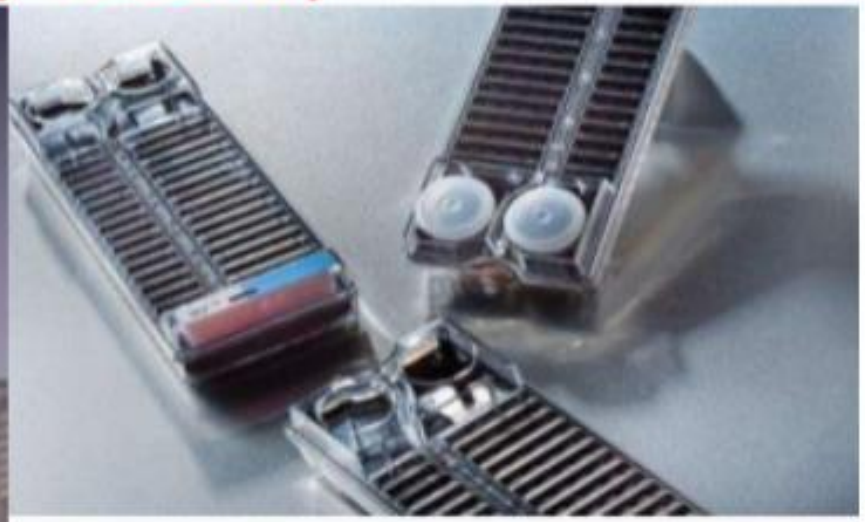
Dissemination and Use of Antibiogram for Education: After the Antibiogram

- An overlooked aspect of antibiogram development and surveillance is the decision of what to do with the antibiogram data and analyses – how are recommendations to be conveyed to prescribers, how is education to be conducted, and how will impacts of the education be assessed. Not infrequently, the development and publication of the antibiogram marks the endpoint of the process

2- Broth Microdilution (Microscan)

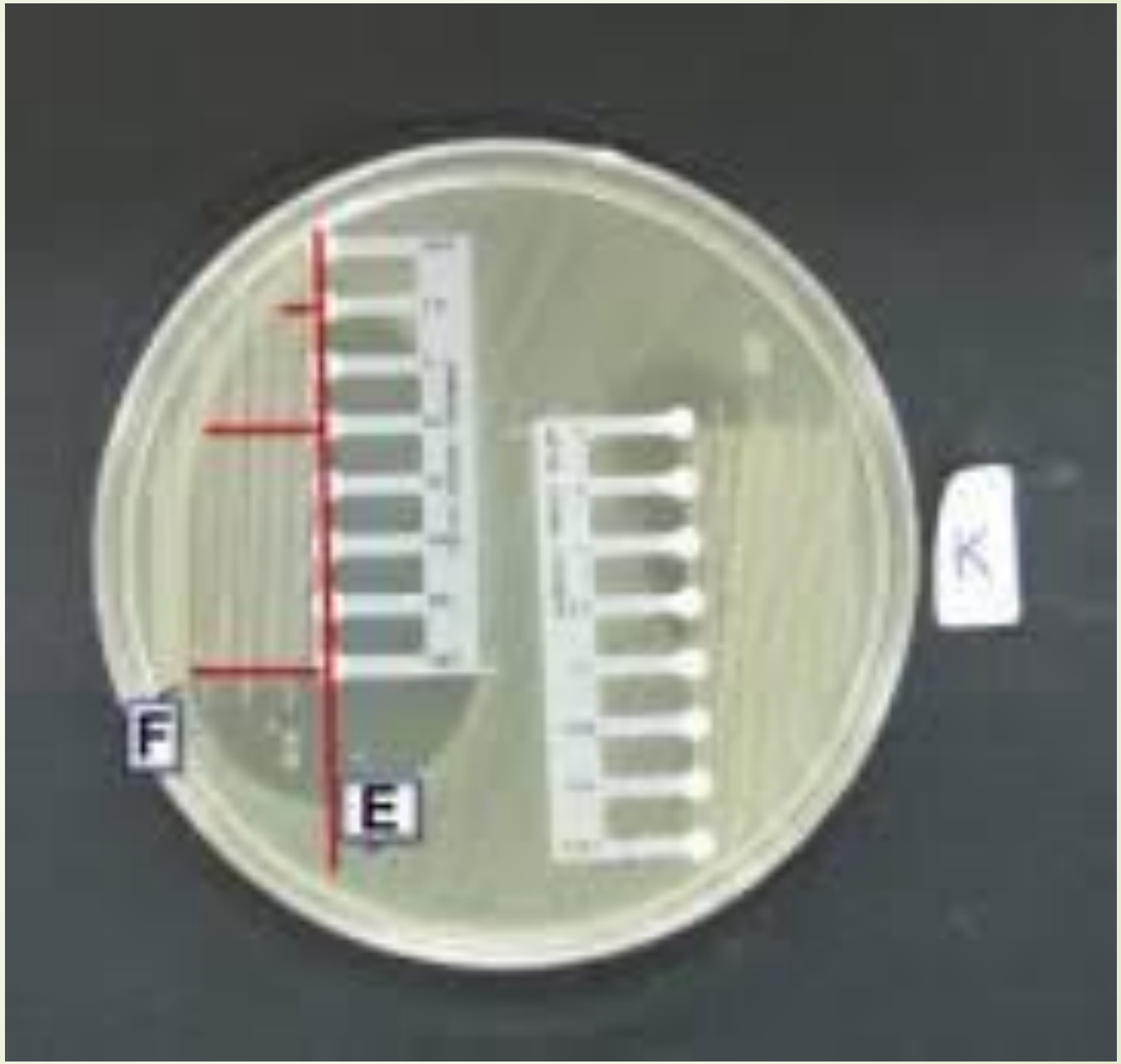


2- Broth Microdilution (Phoenix)



Presenting Emerging Resistance Trends:

- Presentation of resistant organisms over years can be presented in tables or graphs e.g.:
- *MRSA (S. aureus – %S for oxacillin - inpatient and outpatient);*
- *VRE (Enterococcus spp. – %S to vancomycin isolates from* sterile body sites);
- *E. coli – %S to trimethoprim-sulfamethoxazole (urine isolates) and fluoroquinolones;*
- *ESBLs (K. pneumoniae and E. coli – % of isolates that produce ESBLs);*
- *P. aeruginosa – %S to fluoroquinolones and/or imipenem.*


F
E
K

جدول ۱- MIC آنتی بیوتیک های مورد بررسی برای *استافیلوکوکوس ارئوس* با روش شانه ای

نام آنتی بوتیک	غلظت شانه ها (µg)	استاندارد تفسیر نتایج			نتیجه	
		حساس	متوسط	مقاوم	MIC	حساسیت
آمیکاسین	۰/۰۰۱-٤	۲	-	٤	۰/٥	حساس
آموکسی سیلین	۰/۰۰۱-۸	۲	٤	۸	۰/٥	حساس
کلرآمفنیکل	۰/۰۰۱-۳۲	۸	۱٦	۳۲	>۳۲	مقاوم
کوتریموکسازول	۰/۰۰۱-٤	۲	-	٤	>٤	مقاوم
جنتامایسین	۰/۰۰۱-۱٦	٤	۸	۱٦	۱	حساس
نالیدیکسیک اسید	۰/۰۱-۲٤۰	۱٦	-	۳۲	>۳۲	مقاوم
نیتروفورانتوئین	۰/۰۱-۲٤۰	۳۲	٦٤	۱۲۸	۱۰	حساس
اکساسیلین	۲-۲٥٦	۲	۸-٤	۱٦	>٦٤	مقاوم

آنتی بیوتیک	حساس	نیمه حساس	مقاوم
اریترومایسین	۲۰	۱۵	۶۵
امپی سیلین	۲۷	۸	۶۵
اموکسی سیلین	۱۳	۷	۸۰
امیکاسین	۷۴	۱۳	۱۳
پنی سیلین	۲۰	۰	۸۰
تتراسیکلین	۷۳	۲۷	۰
تری متوپریم	۴۰	۵۳	۷
جنتامایسین	۸۶	۱۴	۰
سفالوتین	۲۷	۰	۷۳
سفتیزوکسیم	۸	۴۰	۵۲
کانامایسین	۸۰	۲۰	۰
کاربنی سیلین	۲۷	۲۰	۵۳
کلرامفنیکل	۴۰	۷	۵۳
کلیستین	۷۳	۲۰	۷
نالیدیکسیک اسید	۱۳	۲۷	۶۰
نیتروفورانتوئین	۱۳	۲۷	۶۰
ونکومایسین	۴۰	۷	۵۳

SYMBOL	Antimicrobial Agent	Disk Content (µg)	S	I	R	Zone Diameter Interpretive Criteria (nearest whole mm)							
						Escherichia Coli ATCC 25922	Staphylococcus aureus ATCC 25923	Pseudomonas Aeruginosa ATCC 27853	Escherichia Coli ATCC 35218	Haemophilus Influenzae ATCC 49247	Haemophilus Influenzae ATCC 49766	Neisseria Gonorrhoeae ATCC 49226	Streptococcus Pneumoniae ATCC 49619
AM	**Ampicillin**	*10*	≥		≤	**15-22**	**27-35**	-	**6**	**13-21**	-	-	**30-36**
	Enterobacteriaceae		17	14-16	13								
	Enterococcus SPP.		17	-	16								
	Streptococcus SPP. (beta hemolytic) *Refer to CLSI 2019 M100, page 89*		24	-	-								
	Streptococcus SPP. (Viridans)		-	-	-								
	Neisseria meningitidis		-	-	-								
	Haemophilus influenza & parainfluenza		22	19-21	18								
AN	**Amikacin**	*30*				**19-26**	**20-26**	**18-26**	-	-	-	-	-
	Enterobacteriaceae		17	15-16	14								
	Other Non-Enterobacteriaceae		-	-	-								

Thank you