Antibacterial agents

Antibiotic susceptibility testing

- **Aim: investigate the antibiotic susceptibility and resistance of pathogenic bacteria to antibiotics and determine the group of applicable antibiotics.**

Group of tested antibiotics depends on
- the pathogenic bacterium and its generic resistance
- localization of infection and penetration of the drug (e.g. penetration to CSF)
- nosocomial vs. community acquired infection

- **Appropriate method should be chosen for evaluation of antibiotic susceptibility (e.g. disk diffusion method cannot be used for anaerobic bacteria).**

Definitions

Minimum inhibitory concentration (MIC)
the lowest concentration of an antibiotic that effectively inhibits the growth of the bacterial strain isolated from the patient
(the smaller the better!)

- **MIC range**
  minimum and maximum MIC values determined in different strains of a bacterium species
- **MIC 50**
  the MIC which inhibits the growth of 50% of the strains tested
- **MIC 90**
  MIC that inhibits the growth of 90% of the strains tested

Based on the MIC range, MIC50 and MIC90, bacterial strains can be enrolled to the following categories according to their MIC values:

- **Susceptible**
  (If the MIC of the isolate is lower than a certain breakpoint value)
- **Resistant**
  (If the MIC of the isolate is higher than an other breakpoint value)
- **Intermediate**
  (If the MIC of the isolate is between the 2 breakpoint values)

Table III. Summary of breakpoint recommendations (concentration in mg/L)

	Group 1		Group 2	
	Staphylococci, streptococci, *M. catarrhalis*, *H. influenzae*		Enterobacteriaceae, *Pseudomonas* spp.	
	susceptible ≤	resistant ≥	susceptible ≤	resistant ≥
5.1.1 Penicillins	0.12	0.25	-	-
5.1.1.1. benzyl penicillin[a]				
5.1.1.2 Penicillinase-resistant penicillins				
flucloxacillin	4	8	-	-
methicillin	4	8	-	-
oxacillin	2	4	-	-
5.1.1.3 Broad-spectrum penicillins				
amoxycillin	1	2	8	16
ampicillin	1	2	8	16
co-amoxiclav	1	2	8	16
5.1.1.4 Anti-pseudomonas penicillins				
piperacillin ± tazobactam	2	4	16	32
ticarcillin ± clavulanate	2	4	16	32[b]/128[b]
5.1.2 Cephalosporins, cephamycins & other ß-lactams				
cefaclor	1	2	1	2
cefadroxil	1	2	-	-
cefepime	2	4	1	2
cefixime	1	2	1	2
cefodizime	2	4	2	4
cefotaxime	1	2	1	2
cefotetan	4	8	4	8
cefoxitin	4	8	4	8
cefoperazone	4	8	4	8
cefpirome	1	2	1	2
cefpodoxime	1	2	1	2
cefprozil	-	-	-	-
ceftazidime	2	4	2[b]/8[b]	4[b]/16[b]
ceftibuten	1	2	1	2
ceftriaxone	1	2	1	2
cefuroxime iv	1	2	8	32
cefuroxime po	1	2	1	2
cephalexin	2	4	2	4

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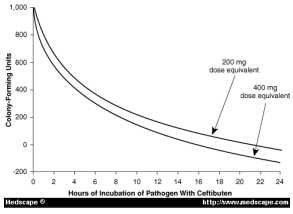
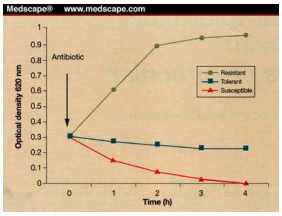
Minimum bactericidal concentration (MBC)
the lowest concentration of an antibiotic that effectively kills at least 99.9% the bacterial isolate

Bactericidal agent
MBC is equal or close to the MIC

Bacteriostatic agent
MBC is significantly higher than the MIC

- **Serum bactericidal titre (SBT)**
  the highest dilution of the patient's serum (CSF), in which the antibiotic kills the bacterium isolated from the patient
- **Time-kill curves**
  describe the kinetics of antimicrobial effect
  show the change in the number of viable bacterium cells as a function of time
  describe the interactions between different antibiotics (antagonism, synergism, or additive interaction)





Interactions of antibiotics

- Synergism
  the effect of the two drugs together is significantly higher than the sum of the effects of the two drugs used separately
  (penicillin + gentamicin)
- Antagonism
  the effect of the two drugs together is lower than the sum of the activities of the two drugs used separately
  (penicillin + tetracycline)
- Indifference
  the two drugs have additive effect when used together

Quantitative methods
Agar dilution method

- determination of minimum inhibitory concentration
- two-fold serial dilutions (decreasing concentration) of a given antibiotic in agar media
- standard inoculum of bacterium strain is inoculated onto the agar medium
- MIC is the lowest concentration of the antimicrobial drug at which no growth of bacteria can be seen
- expensive and time-consuming

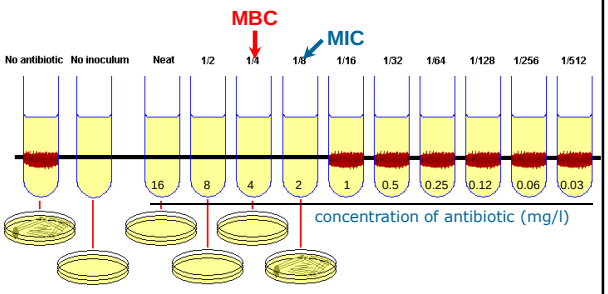
Vancomycin sensitivity testing in MRSA isolates

Ann. Trop. Med. Publ. Health (2012) 5: 178-180.

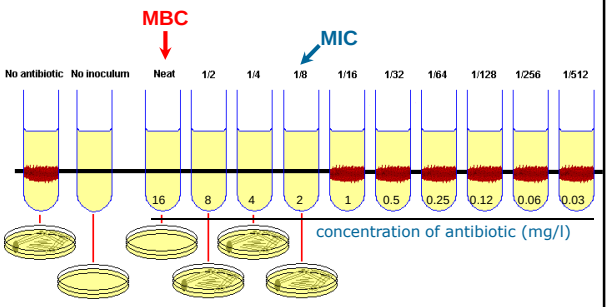
Quantitative methods
Broth dilution method

- application: MIC, MBC, SBT testing
- two-fold serial dilution of the antibacterial drug in liquid medium
- standard inoculum of isolated bacterium strain is inoculated into the broth
- MIC – the lowest concentration of antibiotic that prevents the visible growth of the microorganism
- microdilution (100-200 µl)
- macrodilution (1 ml)
- expensive and time-consuming method

Bactericidal antibiotic

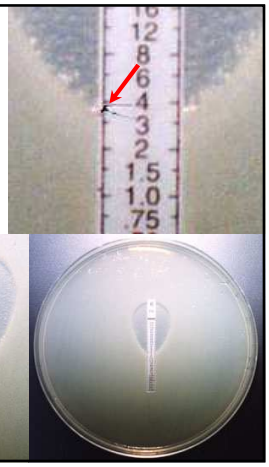


Bacteriostatic antibiotic



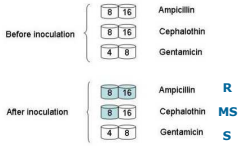
Quantitative methods E test

- MIC
- gradient of increasing concentrations of test antibiotic is incorporated into a plastic-coated strip
- strip is placed on agar surface onto which test organism has been streaked
- antibiotic diffuses into the agar medium according to the concentration gradient
- elliptic inhibition zone
- intersection of lowest point of elliptical zone of growth inhibition and gradient strip is interpreted as minimal inhibitory concentration
- easy to use, but expensive



Semi-quantitative methods Breakpoint method

- Antimicrobial effect is examined at only two concentrations, at the susceptible and the resistant breakpoints.
- It can be tested on solid medium and in broth containing critical concentrations of antibiotic.
- If there is no growth at either concentrations, the microbe is susceptible.
- If there is no growth at the higher concentration, but there is growth at the lower concentration, the microbe is intermediate.
- If growth can be seen at both concentrations, the microbe is resistant.

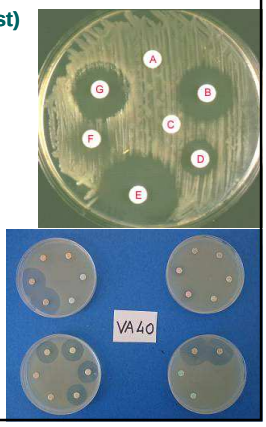


Semi-quantitative methods Resistance screening

- Similar to the breakpoint method but only one agar plate is used with the higher breakpoint concentration.
- If bacterial growth can be seen, the microbe is suspected to be resistant, but this should be confirmed by MIC tests.
- Is useful in heteroresistance detection and to identify aminoglycoside resistance of enterococci.

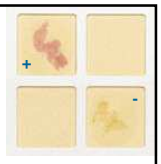
Semi-quantitative methods Disk diffusion test (Kirby-Bauer test)

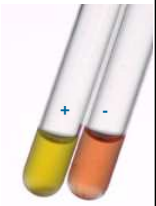
- Antibiotic-impregnated disks are placed onto the inoculated agar surface
- Incubation at 35-37°C for 18-24h.
- Antibiotics diffuse into the medium.
- If the bacterium is susceptible, zone of inhibition will be formed around the disks.
- The size of zones depends on:
  - Concentration of the antibiotic.
  - The effect of the antibiotic against microbe.
  - The diffusion ability of the antibiotic in the medium.
- The size of the zone of inhibition is compared to standards to determine the susceptibility of the microbe to the drug.
- Not useful in anaerobic infections, slowly growing microbes and fungi, but cheap and easy to perform.



Detection of β-lactamases

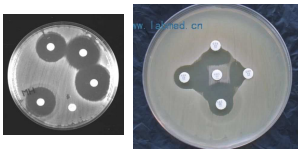
- It is important if it is more reliable than culturing methods (N. gonorrhoeae).
- The absence of detectable β-lactamase activity does not mean that the strain is susceptible to β-lactams.
- Different methods:
  - Nitrocefin test: N. is a cephalosporin and if the lactam ring is hydrolyzed by the β-lactamase of the bacterium, colour change from yellow to red can be seen. Haemophilus, Neisseria, Moraxella.
  - Iodometric test: if the β-lactam ring is hydrolyzed, reduction of iodine can be detected with starch.
  - Acidimetric test: in case of β-lactam hydrolysis, pH decreases because of carboxyl groups, which can be detected with an indicator





Detection of extended spectrum B-lactamases

- ESBL: is able to hydrolyze 3rd generation cephalosporins.
- ESBLs can be inhibited by β-lactamase inhibitors in vitro. So the cephalosporins will be effective in the presence of the inhibitors in vitro (but not in vivo).



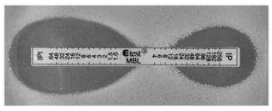
Disk diffusion method

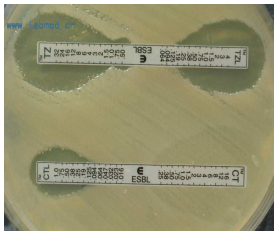
- uses a disk only with cephalosporin and an other with cephalosporin and clavulanic acid on the same inoculated solid medium
- test is positive, if the difference between the inhibitory zones is more than 5 mm

Double disk method

- uses a disk impregnated with cephalosporin, and an other with clavulanic acid and the disks are 2 cms apart
- the inhibitory zone stretches toward the disk containing clavulanic acid.

- **In case of E-test, one end is impregnated only with cephalosporin, and the other with cephalosporin and clavulanic acid.**





PBP2a detection of methicillin resistant Staphylococcus aureus

- **Using monoclonal antibodies in latex agglutination test.**

Direct detection of resistance genes can be done by PCR