

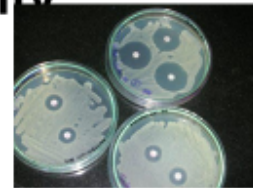
## Microbiology practical – week 13

### Antibiotics susceptibility testing

# Antibiotic sensitivity

- is a term used to describe the susceptibility of bacteria to antibiotics.
- Antibiotic susceptibility testing (AST) - to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*.
- Ideal antibiotic therapy - determination of the aetiological agent and its relevant antibiotic sensitivity.
- Empiric treatment - is started before laboratory microbiological reports are available - when treatment should not be delayed (due to the seriousness of the disease).

## Disk diffusion antibiotic sensitivity testing

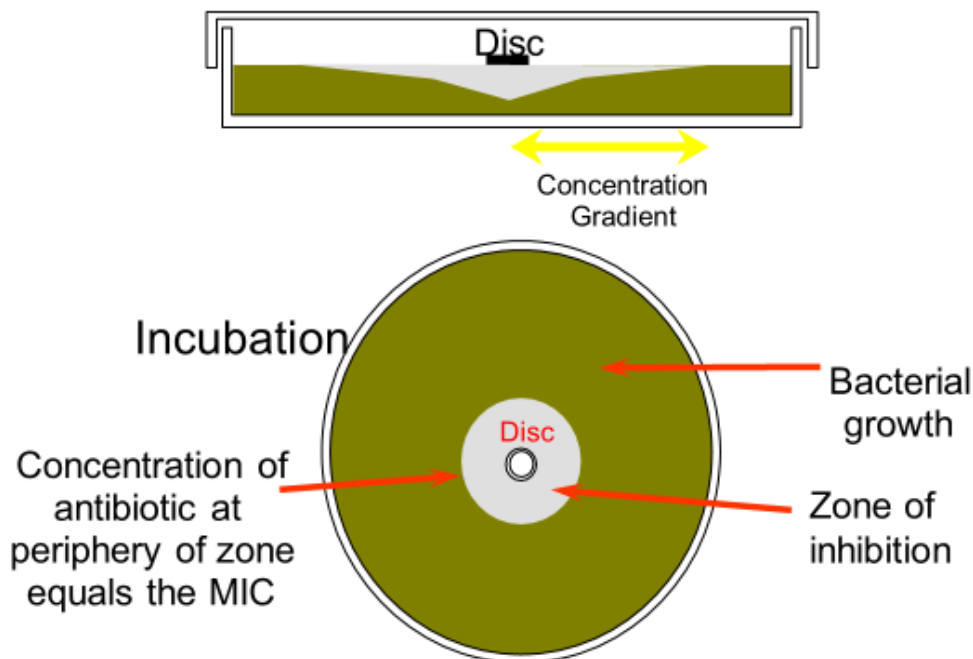


- is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics.
- A known quantity of bacteria are grown on agar plates in the presence of thin wafers containing relevant antibiotics.
- If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the wafer where bacteria are not capable of growing (called a zone of inhibition).

## Disk diffusion antibiotic sensitivity testing

- The test is performed under standardized conditions and standard zones of inhibition have been established for each antibiotic.
- **If the zone of inhibition is equal to or greater** than the standard, the organism is considered to be **sensitive** to the antibiotic.
- **If the zone of inhibition is less** than the standard, the organism is considered to be **resistant**.

### Diffusion of antibiotic from a paper disc

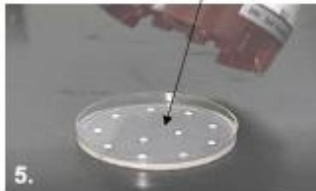


## Disk diffusion antibiotic sensitivity testing



- Placing antibiotic disk on agar plates streaked with bacterial suspension

[www.oid.f3.cuni.cz/mikrobiol](http://www.oid.f3.cuni.cz/mikrobiol)



### Procedure of Disc diffusion test:

1. Prepare the inoculum from the primary culture plate by touching with a loop the tops of each of 3 – 5 colonies, of similar appearance, of the organism to be tested
2. transfer this growth to a tube of saline
3. Inoculate the plates by dipping a sterile swab into the inoculum.
4. Streak the swab all over the surface of the medium three times
5. Place the appropriate antimicrobial-impregnated disks on the surface of the agar
6. The plates should be placed in an incubator at **35-37 °C** within 30 minutes of preparation
7. After overnight incubation, the diameter of each zone (including the diameter of the disc) should be measured and recorded in mm. The results should then be interpreted according to the **antimicrobial susceptibility interpretation chart**.

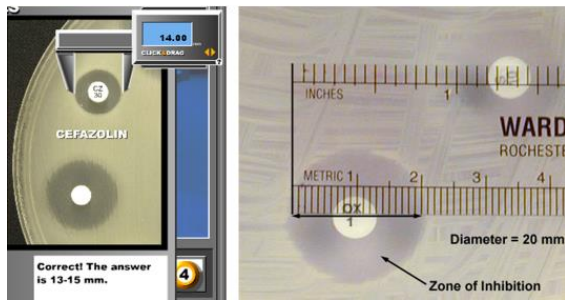


TABLE 1

Details of the antibiotics that were used in the study to test for antibiotic resistance

Group	Antibiotic	Abbreviation	Generally accepted antibiotic disc concentrations ( $\mu\text{g}$ )	Inhibition zone (mm)		
				Resistant	Intermediate resistant	Susceptible
Aminoglycosides	Streptomycin	S	10	$\leq 11$	12 – 14	$\geq 15$
Macrolides	Erythromycin	E	15	$\leq 13$	14 – 22	$\geq 23$
Tetracyclines	Oxytetracycline	OT	30	$\leq 14$	15 – 18	$\geq 19$
Beta-lactams	Ampicillin	AP	10	$\leq 11$	12 – 14	$\geq 15$
	Penicillin G	PG	10	$\leq 20$	21 – 28	$\geq 29$
	Methicillin	MT	5	$\leq 9$	10 – 13	$\geq 14$
Glycopeptides	Vancomycin	V	30	$\leq 9$	10 – 11	$\geq 12$
	Nitrofurantoin	NI	300	$\leq 14$	15 – 18	$\geq 19$
Sulphonamides	Sulphamethoxazole	Smx	300	$\leq 10$	11 – 15	$\geq 16$

Source: The concentration used as well as the inhibition zone measurements were according to the National Committee on Clinical Laboratory Standards<sup>23</sup>

Note: The abbreviations are as they appeared on the antibiotic discs.

## MIC

- The basic quantitative measures of the *in vitro* activity of antibiotics are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).
- The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (*i.e.* colonies on a plate or turbidity in broth culture) under standard conditions.
- The MBC is the lowest concentration of the antibiotic that kills 99.9% of the original inoculum in a given time. Figure 1 illustrates how to determine the MIC of an antibiotic.

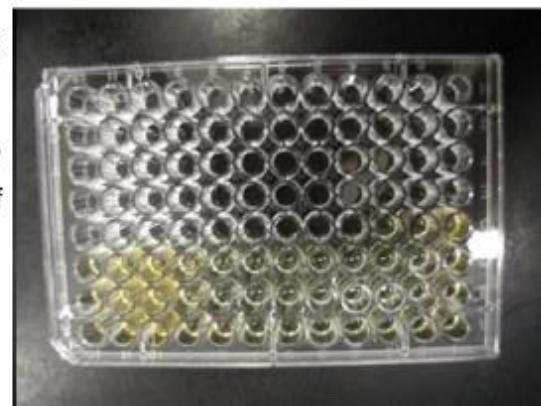
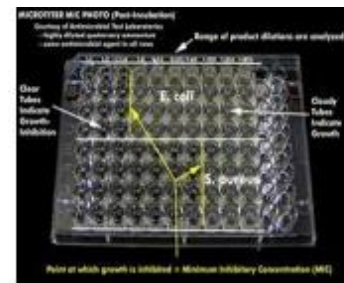


# MIC

- To interpret the susceptibility test results, the breakpoint, a discriminating concentration, has been used to define isolates as susceptible, intermediate or resistant
- Breakpoints are estimated in a variety of ways, the most widely used being the minimal inhibitory concentration (MIC), which is the lowest concentration that completely inhibits microbial growth

## MIC – quantitative test

- A pure culture of a single microorganism is grown in Mueller-Hinton broth
- a volume of the standardized inoculum is added to each dilution vessel
- The inoculated, serially diluted antimicrobial agent is incubated at an appropriate temperature for the test organism for a pre-set period, usually 18 hours.
- After incubation, the series of dilution vessels is observed for microbial growth, usually indicated by turbidity and/or a pellet of microorganisms in the bottom of the vessel.
- **The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.**



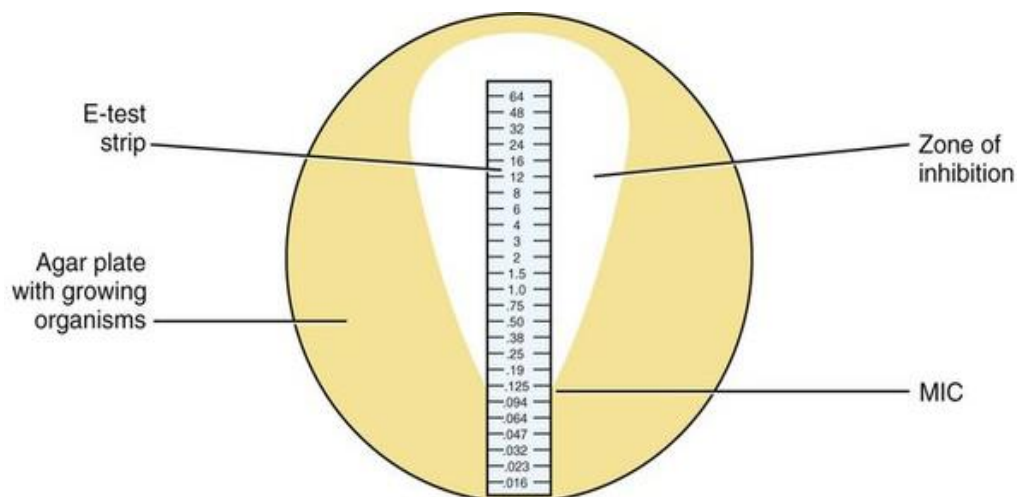
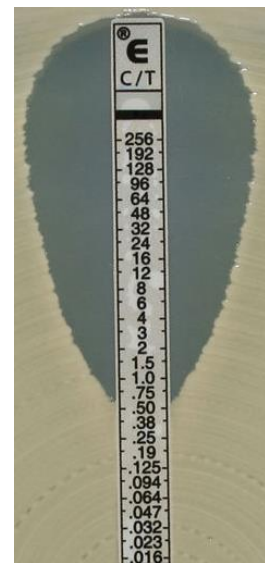
## E test (Epsilonometer test) – quantitative test

- test used to determine whether or not a specific strain of bacterium is susceptible to the action of a specific antibiotic.
- The principle of the epsilonometer test was first described in 1988

### E test - Principle



- The E test is basically an agar diffusion method.
- rectangular strip that has been impregnated with the drug to be studied.
- bacteria is spread and grown on an agar plate
- the E test strip is laid on top;
- the drug diffuses out into the agar, producing an exponential gradient of the drug to be tested. There is an exponential scale printed on the strip.
- After 24 hours of incubation, an elliptical zone of inhibition is produced and the point at which the ellipse meets the strip gives a reading for the minimum inhibitory concentration (MIC) of the drug.



### SOURCES:

[https://www.ifmed.uniba.sk/fileadmin/ilf/Pracoviska/ustav-mikrobiologie-a-imunologie/VLa/7. LS\\_ang\\_Pract\\_ATB\\_sensitivity\\_testing.pdf](https://www.ifmed.uniba.sk/fileadmin/ilf/Pracoviska/ustav-mikrobiologie-a-imunologie/VLa/7. LS_ang_Pract_ATB_sensitivity_testing.pdf)

<https://www.biomerieux-diagnostics.com/etest-ceftolozane-tazobactam>

<https://microbeonline.com/antimicrobial-susceptibility-testing-procedure-modified-kirby-bauer-method/>