Lab class no. 3 – Determination of sensitivity of microorganisms to antimicrobial compounds

(in pairs)

Aims:

- *Disc diffusion test*
- *Microdilution method - determination of MIC*
- *Catalase test*

Material

Densitometer, prepared dish with Nutrient broth agar (from 1st lab class), liquid Nutrient broth, automatic pipettes, sterile P. dishes for multichannel automatic pipette, sterile tips, solution of chloramphenicol, sterile paper discs, sterile tweezers, microbiological tubes, microtitration plates, plastic bacteriological loop, vortex, hydrogen peroxide solution 3%

Working procedure

1. Preparation of inoculum (suspension) of *E. coli*:

- use one colony from cross scattering; suitable density is 0,5 McFarland, measure with densitometer

2. Disc diffusion test:

- on bottom side of Petri dish mark spots for discs with concentrations of chloramphenicol
- transfer 1 ml of bacterial suspension to; aspirate surplus liquid
- place 3 discs on agar using sterile tweezers (in advance, add to discs SLOWLY using pipette 10 µl of prepared chloramphenicol solutions):

  1st disc 3000 µg/mL sol. chloramphenicol (amount in disc is 30 µg)

  2nd disc 1500 µg/mL sol. chloramphenicol (amount in disc 15 µg)

  3rd disc 750 µg/mL sol. chloramphenicol (amount in disc 7,5 µg)

  4th disc Nutrient medium

3. Quantitative assay using microdilution method (determination of MIC):

- 3 pairs of students will have one microtitration plate and gradually pair after pair will prepare their 4 columns:

	1st pair				2nd pair				3rd pair				
	1	2	3	4	5	6	7	8	9	10	11	12	final conc.
A													32 µg/ml
B													16 µg/ml
C													8 µg/ml
D													4 µg/ml
E													2 µg/ml
F													1 µg/ml
G													0.5 µg/ml
H													CTRL

- first add to your wells the liquid Nutrient broth medium: row A 180 µl
  rows B to G 100 µl
  row H 100 µl
- to wells in row A add 20 µl of chloramphenicol stock solution 180 µg/ml, mix by pipetting and to row B transfer 100 µl - proceed until row G (do not forget to take 100 µl out from wells in row G - so the volume is the same in all wells)
- now pour prepared inoculum of *E. coli* (0,5 McFarland) to bath, dip the tips of multichannel pipette to inoculum and transfer to wells, proceed from CTRL to highest concentration
- let the plate incubate at 37°C for 24 hrs; plates will be kept in fridge until next class.

4. Catalase test:

Transfer the bacterial culture with sterile loop to a drop of hydrogen peroxide solution on a glass-slide. Positive result is confirmed by vigorous bubbling.

5. Microscopy – permanent preparations

Protocol no. 3 will contain:

- what is McFarland optical density scale? and how can we determine it?
- procedure of disc diffusion test, in next class measure diameter of inhibition zones (is *E. coli* sensitive or resistant?, drawing or photo)
- procedure of determination of MIC, in next class see the result (is *E. coli* sensitive or resistant?, drawing or photo)
- describe chloramphenicol (bactericidal or -static ATB, mechanism of action, usage,...)
- drawing of photo of permanent preparations - describe: cocci, bacilli, G+, G-, aerobic, anaerobic, normal microbiota or not, where we can find them (skin, GIT, …), disease which they can cause