Topic PZ03: Some more Gram-positive bacteria (enterococci, listeriae, corynebacteria, bacilli)

To study: *Enterococcus, Listeria, Corynebacterium, Bacillus* (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification

Table for major results of Task 1 to Task 5 (to be filled step by step):

Strain		K	L	M	N	P	Q	R	S
Gram stain – Task 1									
Task 2 Colonies on the blood agar	Size								
	Colour								
	Shape								
	Profile								
	Agar changes								
	Other								
Catalase test Task 3a									
Slanetz-Bartley medium – Task 3b									
Bile-aesculin medium – Task 3c									
Arabinose test Task 4a									
MALDI TOF Task 4b									
Growth in refrigerator Task 5a									
FINAL CONCLUSION*									

**In Bacillus and Listeria, write genus name only. Species level diagnostics would require further tests that could not be performed in our practicals.*

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them and write your results in the table. Do not forget to write important details ("rods in palisades", "robust, spore forming rods" etc.). To avoid confusion, label the slides using a wax pencil. For remaining tasks, only Gram-positive bacteria are going to be studied.

Task 2: Morphology of colonies of G+ cocci and bacilli

Describe the colonies as usually. Do not describe colonies of bacteria proven not to be G+ cocci or bacilli.
In the strains microscopically found to be Gram-positive rods try to guess to which genus the bacterium might belong, according to the following description:
Bacillus – large, flat, dry, felt-like colonies, "spreading" through the agar surface, sometimes with a massive haemolysis, sometimes with no haemolysis at all. Microscopically very robust rods, sometimes with central or subterminal endospores, that may, but not necessarily, be larger than the diameter of the rod.
Listeria – colourless to greyish colonies, very similar to those of *Enterococcus*, with or without haemolysis, microscopically tinier than *Bacillus*, not arranged in palisades, rather in short chains.
Corynebacterium (and related genera) – greyish or whitish colonies, similar to those of *Staphylococcus* but sometimes considerably smaller, usually nonhaemolytic, microscopically rather smaller rods than in the previous genera, but club-shaped and arranged in palisades.

Task 3: Several common biochemical and culture tests

a) Catalase test

Perform catalase test for all the strains proven to be G+.

Note: Catalase testing of G+ rods might seem useless, as *Listeria, Corynebacterium* and *Bacillus* are catalase positive. Nevertheless, some other coryneform rods (e. g. *Arcanobacterium*) are catalase negative and that is why catalase testing is recommended to be performed.

b) Growth on Slanetz-Bartley medium

On your plate, the same strains as in Task 1 are cultivated in sectors. Positive strains should be not only growing, but also of pink to maroon colour of colonies. *Enterococcus* is the only G+ bacterium growing on this medium. Write your result in the table.

c) Growth on bile-aesculin medium

Unlike the Slanetz-Bartley medium, bile-aesculin medium enables not only the growth of *Enterococcus* (allowing the diagnostics of this genus among G+ cocci), but also *Listeria* (diagnostic among G+ bacilli). In a positive case, you see black colonies. Write your result in the table.

Task 4: Differentiation of enterococci

a) Arabinose test for species determination of two most common enterococci

Examine the two strains proven to be enterococci in the previous tasks. Observe the test tubes with the result of the arabinose test. Yellow colour means positiveness (typical for *Enterococcus faecium*) and green colour means negativity (typical for *Enterococcus faecalis*).

b) MALDI-TOF for more precise diagnostics of enterococci and coryneform rods

Although arabinose test is considered sufficient for common cases, in some situations we need diagnostics based on more signs and able to detect more than two species of enterococci. Similarly, the term "coryneform rods" is usually sufficient level of diagnostics of corynebacteria and related bacteria (especially If it an isolate from skin), but not for important isolates (e. g. from the bloodstream).
Both for enterococci and coryneform rods, it is possible to use some biochemical tests. The test for Enterococcus is made also in Czechia („EN-COCCUStest by Erba Lachema), but there is no Lachema-produced test for coryneforms, so it would be necessary to use other tests (e. g. API® Coryne by Bio-Mérieux).
Recently, it is also possible to replace both of them by MALDI-TOF.
Read the results of MALDI-TOF for three of your strains (two enterococci and one coryneform rod).
Write the results in the table and answer to following question:
To what level the strain is diagnosed by means of MALDI-TOF? Underline, what is valid:

First enterococcus, that means strain ___ (write the letter) is diagnosed to the level:

Sure species identification – likely species identification – genus identification – not identified

Second enterococcus, that means strain ___ (write the letter) is diagnosed to the level:

Sure species identification – likely species identification – genus identification – not identified

The coryneform rod, that means strain ___ (write the letter) is diagnosed to the level:

Sure species identification – likely species identification – genus identification – not identified

Task 5: More methods for diagnostics of *Listeria*

a) Growth of listeriae at 4 °C

Observe a plate with blood agar where the strains of Gram-positive rods were inoculated, and the plates were then cultivated at refrigerator temperature. Write the results (*does grow – does not grow*) in the main table.

b) Demonstration of *Listeria monocytogenes* growth on a chromogenic medium

Examine the appearance of listerial growth on a chromogenic medium. The medium is specific for this species. In medical microbiology, we do not use the chromogenic media for *Listeria* very often; however, it plays an important role in food industry.

Result: On the medium called_____*L. monocytogenes* has_____-coloured colonies.

Task 6: Enterococci and G+ rods and their relations to antibiotics

Task 6a: Antibiotic susceptibility tests in enterococci and selected G+ rods

On your table, you will find diffusion disc tests for strains found to be *Enterococcus faecalis* and *Listeria* sp. There is no test for *Enterococcus faecium* – majority of clinical isolates come from stool, so there is no need to perform antibiotic susceptibility testing. Nevertheless, UTI isolates are also not uncommon (see Task 6b). There is also no test for *Corynebacterium* sp. – let us suppose that our strain is a skin isolate, i. e. it is a part of normal microflora. And finally, there is no test for *Bacillus* sp. – the findings of this genus are usually interpreted as environmental contamination and thus not tested.

Interpret the strains as susceptible (S), intermediary (I) or resistant (R) to given antibiotics.

Strain →							
Antibiotic	Susceptible if	Inter-mediate if	Resistant if	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation
Ampicillin AMP	≥ 10 mm	8–9 mm	< 8 mm				
Nitrofurantoin F	≥ 15 mm		< 15 mm				
Vancomycin VA	≥ 12 mm		< 12 mm				
Tetracycline* TE	≥ 19 mm	15–18 mm	< 15 mm				
Q. + D.** QD	≥ 22 mm	20–22 mm	< 20 mm				
Gentamicin CN***	≥ 8 mm		< 8 mm				

**result is also valid for doxycycline*
***quinupristin + dalfopristin, combination of two streptogramin antibiotics*
****only suitable for enterococci if combined with beta-lactamic antibiotics*

Task 6b: Demonstration of antibiotic susceptibility test for *Enterococcus faecium*

On the side table you can see a test for *E. faecium*. Write the name of antibiotic that is used as drug of choice for *E. faecalis* infections, but cannot be used for *E. faecium* because of primary resistance: _____

Task 6c: Demonstration of a VRE strain

On the side table or in the slideshow you can also see a VRE strain. Using your memory and/or protocols from spring semester, write what that abbreviation stands for: _____

Task 7: Demonstration of Elek test

The principle of the Elek test is precipitation between the toxin of a toxic strain and the antitoxin from a paper strip with the antiserum. Both the toxin and the antitoxin diffuse through the agar plate. Students of dental medicine do not perform this test.