14. Corynebacteria and Listeria

14.1. GENERAL FEATURES

Corynebacteria are gram-positive, non-motile rods with metachromatic granules. Some are members of the normal flora (diphtheroids) and some are serious human pathogens (*C. diphtheriae, C. ulcerans*). Listeriae are gram-positive motile rods widespread in nature and cause serious infections especially in susceptible patients (*L. monocytogenes*).

14.2. VIRULENCE FACTORS & PATHOGENESIS

Virulence factors are genetic, biochemical, or structural features that enable an organism to cause disease.

Diphtheria toxin of the corynebacterium is a bi-component (A-B) exotoxin. Fragment B transports the toxic fragment A into the affected cell where it abruptly stops elongation of proteosynthesis. Structural genes of the exotoxin are located on lytic β phage so the strains of *C. diphtheriae* that do not have the phage do not produce the diphtheria toxin. *C. ulcerans* may also acquire this phage and cause serious infection.

Listeriolysin O of the listeriae lyses the membrane of phagolysosomes which allows the listeriae to escape into the cytoplasm of host cells. **ActA** is a listerial surface protein that induces host cell actin polymerization, which propels them into neighbouring cells, and thereby avoiding intercellular passage and host immune defence (fig. 1).

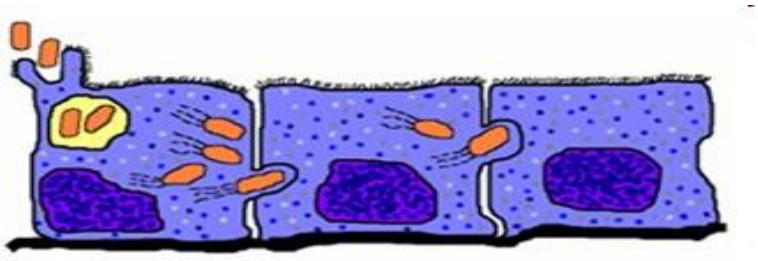


Fig. 1. Listerial cycle – listeriae (shown as brown rods) are engulfed by a cell (blue). Listeriae multiply and move because of actin proteins (dashed lines) which shoot them ahead. Actin is synthetised by the affected host cells (adapted A.W. Wilson, Bacterial pathogenesis, 2011)

14.3. INFECTIONS & EPIDEMIOLOGY

Corenybacterium diphtheriae. The infections are spread by droplets or by contact from a patient or a healthy carrier to a susceptible host. Locally, sore throat, exudative and pseudomembranous pharyngitis with regional lymphadenitis or cutaneous diphteria (non-healing ulcers) occur. Systemic disease proceeds when respiratory tract infection progresses presenting with generalized symptoms caused by absorption of the diphtheria exotoxin (necrosis and parenchymal degeneration in muscles, heart, kidney, neurons). Toxigenic *C. ulcerans* may also cause a similar disease to diphtheria, however it is isolated only very rarely.

***Listeria monocytogenes*.** The infections are associated with consumption of contaminated food products (e.g., soft cheese, milk, turkey, raw vegetables – *food borne disease*) or spread from mother transplacentally to foetuses or during delivery to the neonate; sporadic cases and epidemics occur throughout the year but peak in warmer

months. Only some categories of people are susceptible to the infection such as young, elderly and pregnant women, as well as patients with defects in cellular immunity.

14.5. TREATMENT, PREVENTION & CONTROL

Corenybacterium diphtheriae. Treatment: Combined therapy both eliminating the infectious process by antibiotics (e.g. penicillin, erythromycin) and neutralization of the circulating exotoxin by antitoxin is applied. An important note is that once the toxin is bound to a cell surface receptor it cannot be eliminated by the antitoxin.
Prevention: Immunization with toxoid, usually administered in DTP triple vaccine (tetanus toxoid and pertussis antigen is also included).
***Listeria monocytogenes*.** Treatment: penicillin or ampicillin, either alone or with gentamicin is the treatment of choice. Erythromycin can be used in patients allergic to penicillin. An important note is that the bacteria are naturally resistant to cephalosporins.
Prevention: because listeriae are ubiquitous and most infections are sporadic, prevention and control are difficult. People at high risk of the infection should avoid eating raw or partially cooked foods of animal origin, soft cheeses, and unwashed raw vegetables. A vaccine is not available.

14.6. LABORATORY DIAGNOSIS

The decision to treat diphtheria must be based on clinical observation, and must never be delayed!

a) Specimen collection: nasopharyngeal swabs (diphtheria), pus, and other clinical material dependent on the localization of the infection.
b) Microscopy: gram-positive club shaped rods (fig.1).

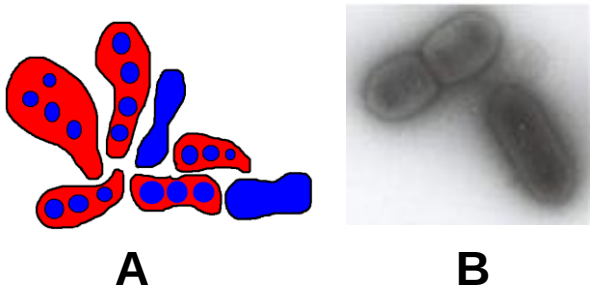


Fig. 1. Diagram of corynebacteria - club shaped rods arranged in „Chinese letters" with metachromatic granules. In some cases corynebacteria may resemble to gram-negative bacteria but they have dark granules (A); Electron microscopy of L. monocytogenes cells (B)

c) Culture: Corynebacteria and listeria grow on general enriched culture media (blood agar) as whitish or grey colonies. Only some biotypes of *C. diphtheriae* and *L. monocytogenes* have hemolytic properties. Hemolysis can sometimes be seen beneath colonies of *L. monocytogenes.* In case of suspicion of the occurrence of competitive flora in the sample, selective or diagnostic media can be used (tellurite agar, on which corynebacterial colonies have a specific colour).

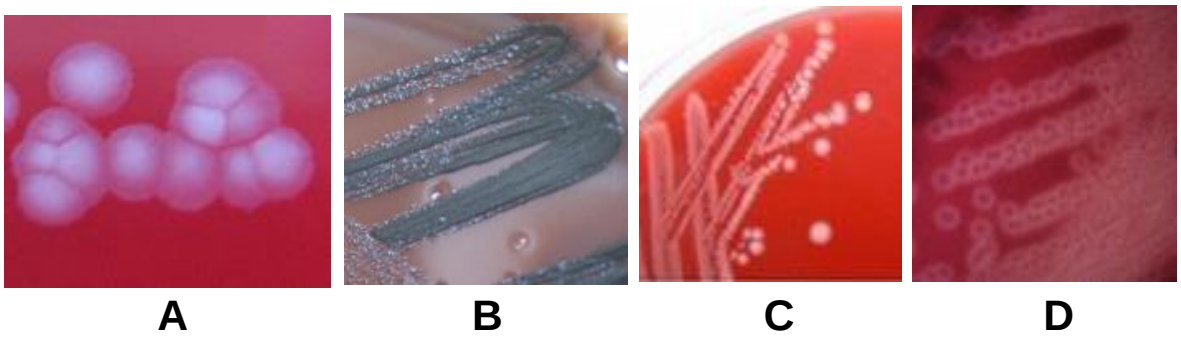


Fig. 2. A. Detail of matt colonies of commensal diphtheroids, B. Colonies of C. diphtheria on diagnostic tellurite agar, C. Colonies of L. monocytogenes where hemolysis is located beneath the colonies, D. Arcanobacterium (Corynebacterium) hemolyticum. The bacterial colonies in figure A,C,D are grown on blood agar.

d) Phenotypical identification: Focused on detection of specific biological properties of corynebacteria (microscopy, bacterial colonies, enzymatic detection, see also Chapter 6 – Identification).
Screening tests (preliminary identification): detection of some phenotypic properties are demonstrated in fig.3.

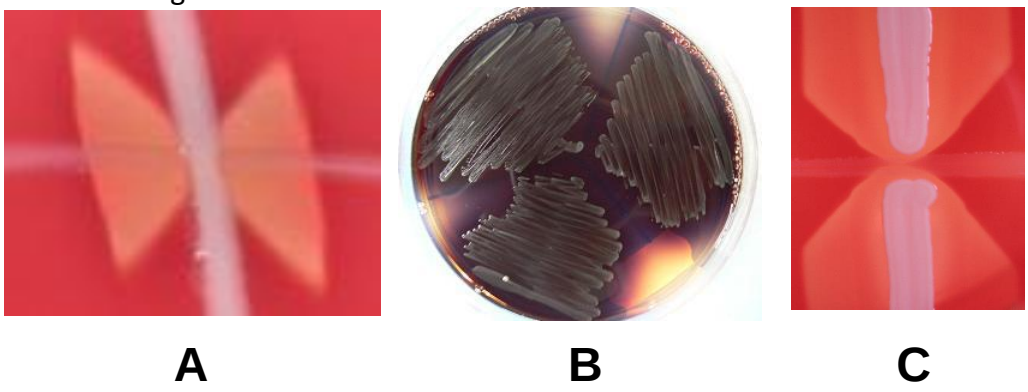


Fig. 3. A. positive CAMP test - L. monocytogenes lines is streaked horizontally and S. aureus vertically, incubated overnight, B. production of esculin by L. monocytogenes is indicated by black colour of the medium, C. positive reverse CAMP test reaction – Arcanobacterium haemolyticum streaked horizontally and S. aureus vertically and incubated overnight.

Biochemical identification. Multiple enzyme tests are performed to identify the bacteria. Positive and negative reactions are noted. The metabolic profiles of known species are compared with the strain being analysed. Key dichotomous method and numerical biochemical identification are used (see also chapter 6 – Identification).

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry - (MALDI – TOF MS): analyses molecular structure (mainly proteins) of an unknown microbial isolate, as the mass spectral pattern consisting of a number of structurally related mass spectral peaks, and comparing with a known patterns analyzing the isolate. The mass spectrometer first ionizes, then mass separates and finally detects time of the ions flight, thus producing a mass spektrum and comparing it with known mass spektra analyse the studied microorganism (see also chapter 6 - Identification).

Detection of diphteria toxin production by Elek test is striking the agar with the analyzed strain and with a negative and positive control. Then a strip of filter paper containing the

anti-toxin is placed over the plate. Precipiation lines will appear in the places where toxin production overlaps with anti-toxin (fig. 4).

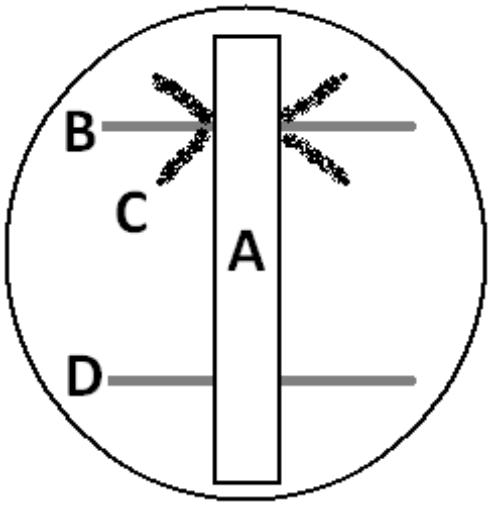
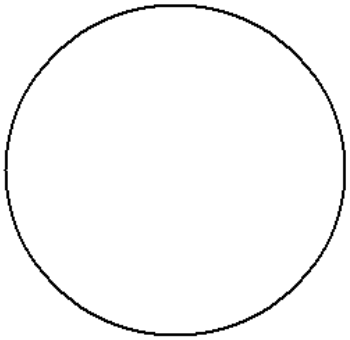


Fig.4. Detection of diphteria toxin production by Elek test . Filter paper with the antitoxin (A), strain of C. diphtheriae producing diphtheria toxin (B), precipitation lines (C), strain of C. diphtheriae not producing diphtheria toxin (D)

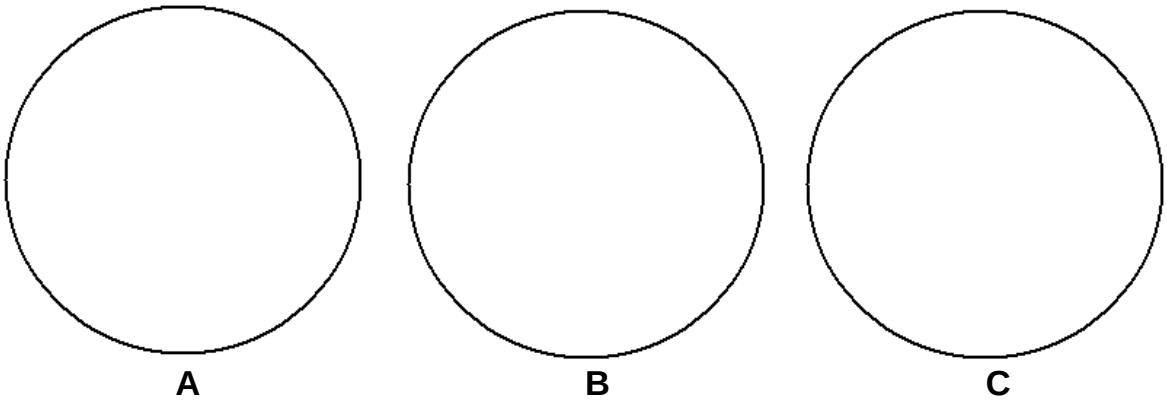
e) Genotypical identification There are several ways to identify a strain or species using genotype. For instance, comparing electrophoresed fragments allows visualisation of the restriction profile. Alternatively, homology of highly conserved regions, such as the 16S RNA gene, can assist identification of the species. (see also chapter 6 – Identification).
f) Susceptibility testing. Qualitative (disk diffusion method), quantitative methods or their combination (E-test) are used to test the susceptibility (see Chapter 5 – ATB testing).

14.7. PRACTICAL PART – CORYNEBACTERIA & LISTERIA

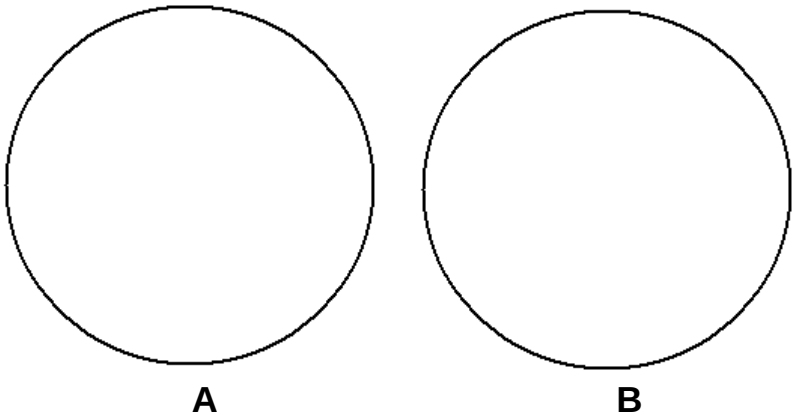
Exercise 1: MICROSCOPY: prepare a gram-stained smear of purulent material from an infection caused by *L. monocytogenes*. After drying, fixing and gram-staining draw the morphology of the colony that you could see in the microscope.



Exercise 2: MICROSCOPY: Stain hemolytic and nonhemolytic colonies of various biotypes of *C. diphtheriae*. After drying, fixing and gram-staining draw the morphology of the colony you that could see in the microscope. Do they vary in their cell morphology or not?



Exercise 3: *CULTURE OF C. DIPHTERIAE STRAIN ON TELLURITE DIAGNOSTIC MEDIA. Grow the clinical material collected from a pseudomebranous pharyngitis on a general enriched culture media (blood agar) and a diagnostic media. Draw and note the procedures in the space below.*



Exercise 4: *INTERPRET THE RESULT OF BIOCHEMICAL IDENTIFICATION AND SUSCEPTIBILITY TESTING OF VARIOUS SPECIES OF CORYNEBACTERIA AND LISTERIA. Using producer recommendation interpret the result of the biochemical identification of the analysed strain. Using standardized criteria interpret the result of the disk diffusion method or determine minimal inhibitory concentration of the antibiotics tested.*

14.8. LAB QUIZ

1. Specify virulence factors of *C. diphtheriae* and *L. monocytogenes*.
2. Specify the infections caused by *C. diphtheriae* . How should the infection be diagnosed and why?
3. Describe principles of combined treatment of *C.diphtheriae* infections.
4. Specify the direct detection methods used in diagnostics of *C. diphtheriae* and *L. monocytogenes.*
5. Specify how to prevent and control *C. diphtheriae* and *L. monocytogenes* infections.