



2. LÉKAŘSKÁ FAKULTA
UNIVERZITA KARLOVA



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Národní institut
virologie a bakteriologie

G+ rods: Corynebacteria, Listeria, Clostridia and bacilli

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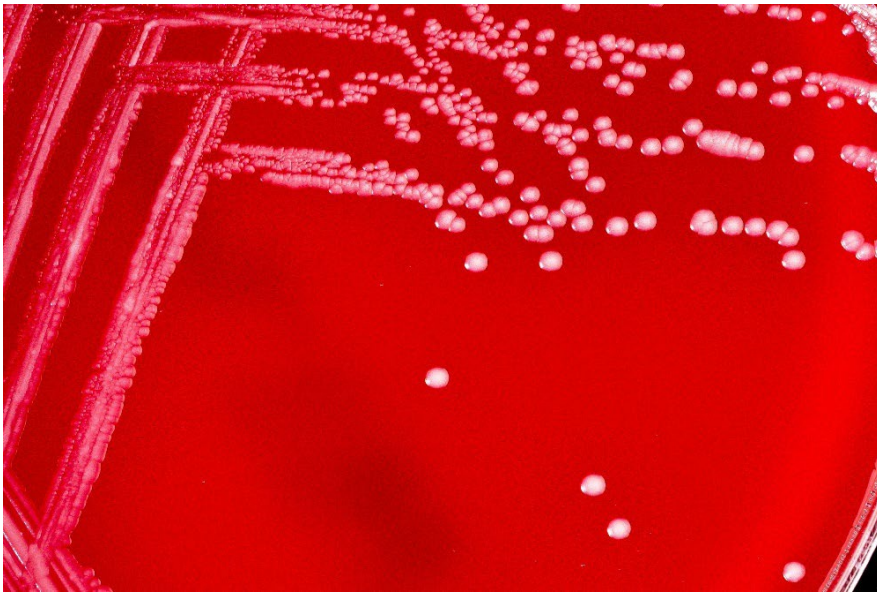
Microbiology I.
Practical sessions

Listeria and Corynebacteria

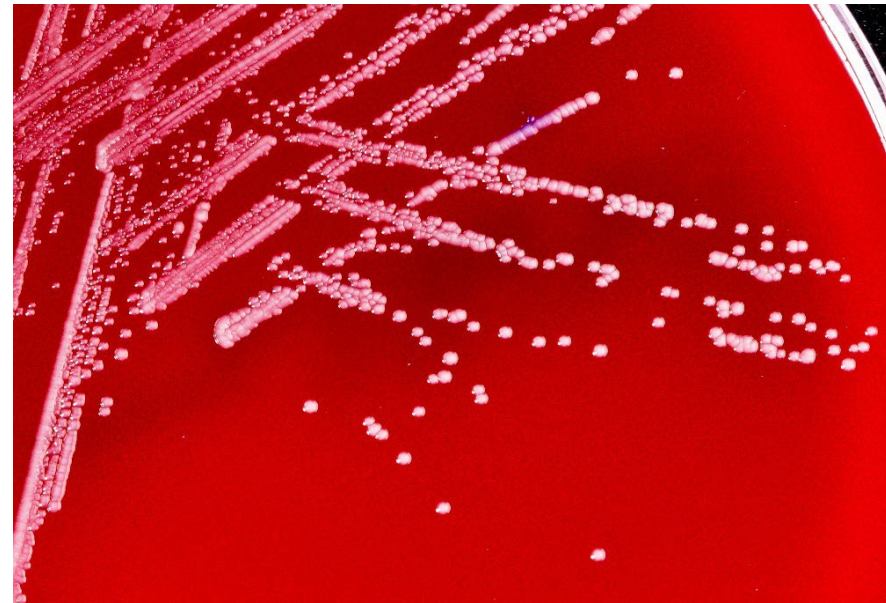
What are they?

Gram-positive, non-spore-forming rods

- **Grow on agar** – greyish colonies, β -haemolysis
- **Biochemically** – catalase-positive, coagulase-negative



Listeria monocytogenes



Corynebacterium diphtheriae

LISTERIOSIS

- *Listeria monocytogenes*
- **Source:** beef, sheep, fish and poultry products
 - **High-risk foods:** soft ripened cheeses and unpasteurised dairy products, pâtés, soft salamis, chilled fish and other products intended for direct consumption
- **Transmission:** faecal-oral (inadequate cooking)
 - Resistance to cold and NaCl!
- **ID:** 7–10 days



LISTERIOSIS

- **Source:** products from cattle, sheep, fish and birds
- **Transmission:** faecal-oral (poor heat treatment)
- **ID:** 7–10 days

A.



B.



C.



Listerióza

Listerióza dospělých

Afebrilní gastroenteritida

Mladí, zdraví

Samoúzdravné onemocnění

Listeriová sepsa a meningitida

U IK pacientů

Těžké onemocnění

Listerióza novorozenců

Nemoc s časným nástupem

Early-onset / vrozená listerióza

Transplacentárně

Projevy:

Sepsa

Granulomatosis infantiseptica

Velmi těžké onemocnění (letalita až 100%)

Late-onset

Porodní cesty, ruce personálu

Projevy:

Sepsa

Purulentní meningitida

Velmi těžké onemocnění

DIAGNOSIS

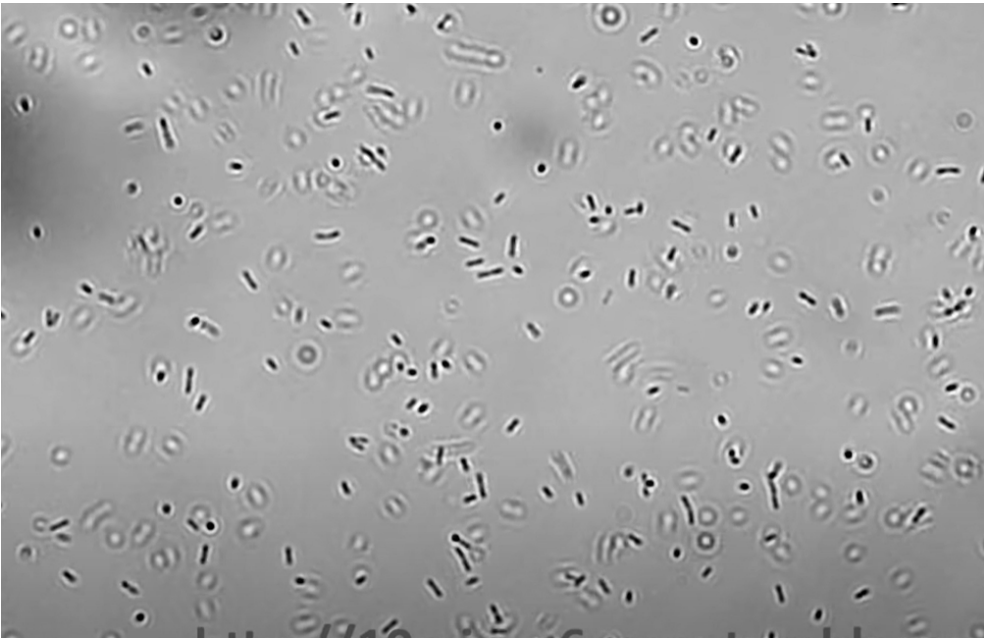
- **Material:**

- HMK, cerebrospinal fluid
- amniotic fluid, placental swabs
- Swabs from the birth canal

- **Methods:**

- Microscopy
- Culture + mass spectrometry
- PCR

Microscopy



Native preparation: motile rods – whirling motion, ‘somersaults and flips’.

[Video](#)

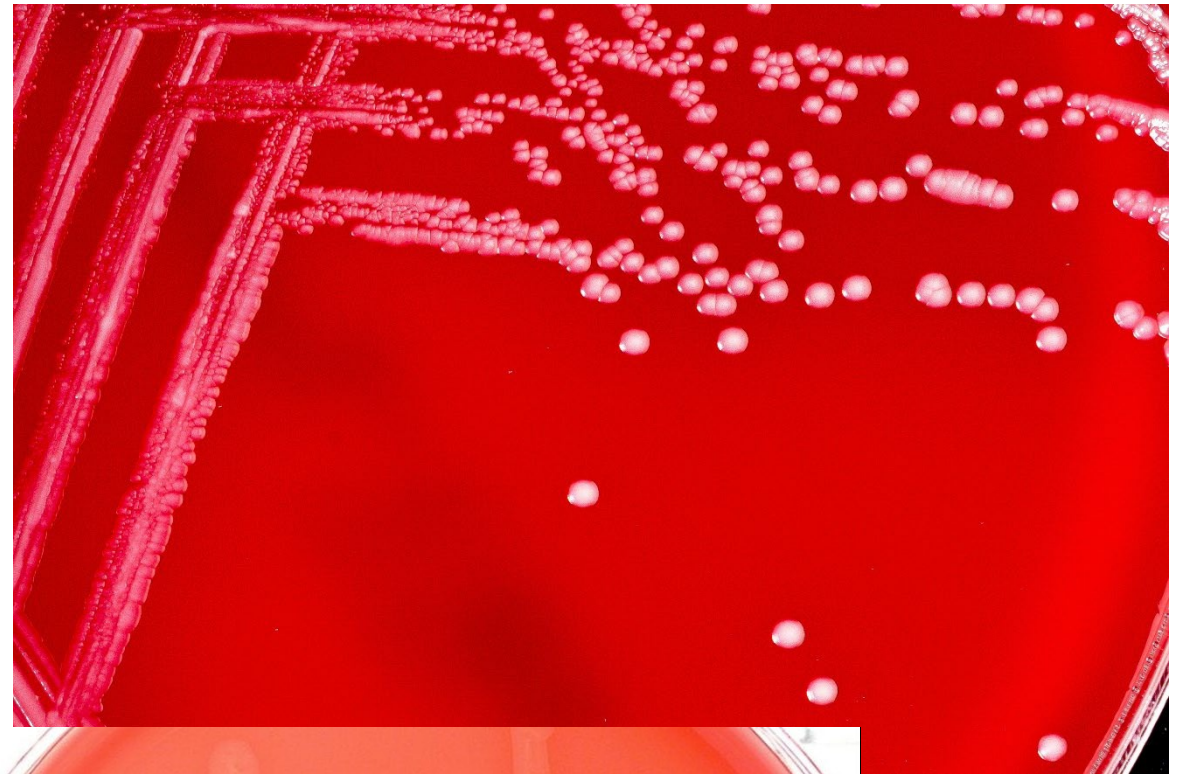


Gram: G+ rods in HMK, cerebrospinal fluid

Image sources on microbe-canvas.com

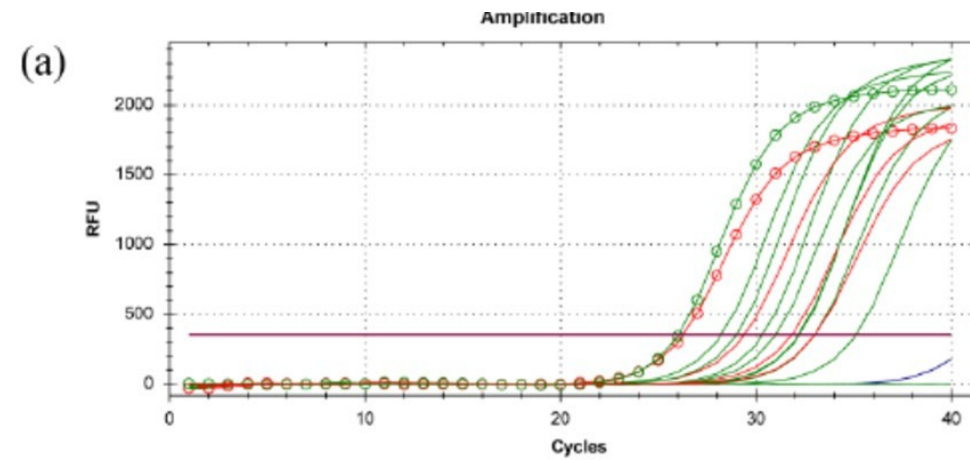
Cultivation

- **Facultatively anaerobic**
- **Growth on KA**
 - Small, smooth, greyish colonies
 - Narrow zone of haemolysis
 - ! Also grows on MacConkey
- **Biochemical tests**
 - CAMP positive
 - Catalase positive (therefore not GBS)



PCR

- **Rapid diagnosis from cerebrospinal fluid**
- Bacterial meningitis panel



Antibiotic treatment

- Cephalosporins of all generations are primarily resistant
- **First-line treatment: ampicillin** (possibly with gentamicin)

DIPHTHERIA

- **Source:** an infected person
- **Mode of transmission:** airborne, droplets / contact with skin lesions in the cutaneous form
- **At-risk population:** young children
- **Localisation:** primarily HCD
- **ID:** 2–6 days



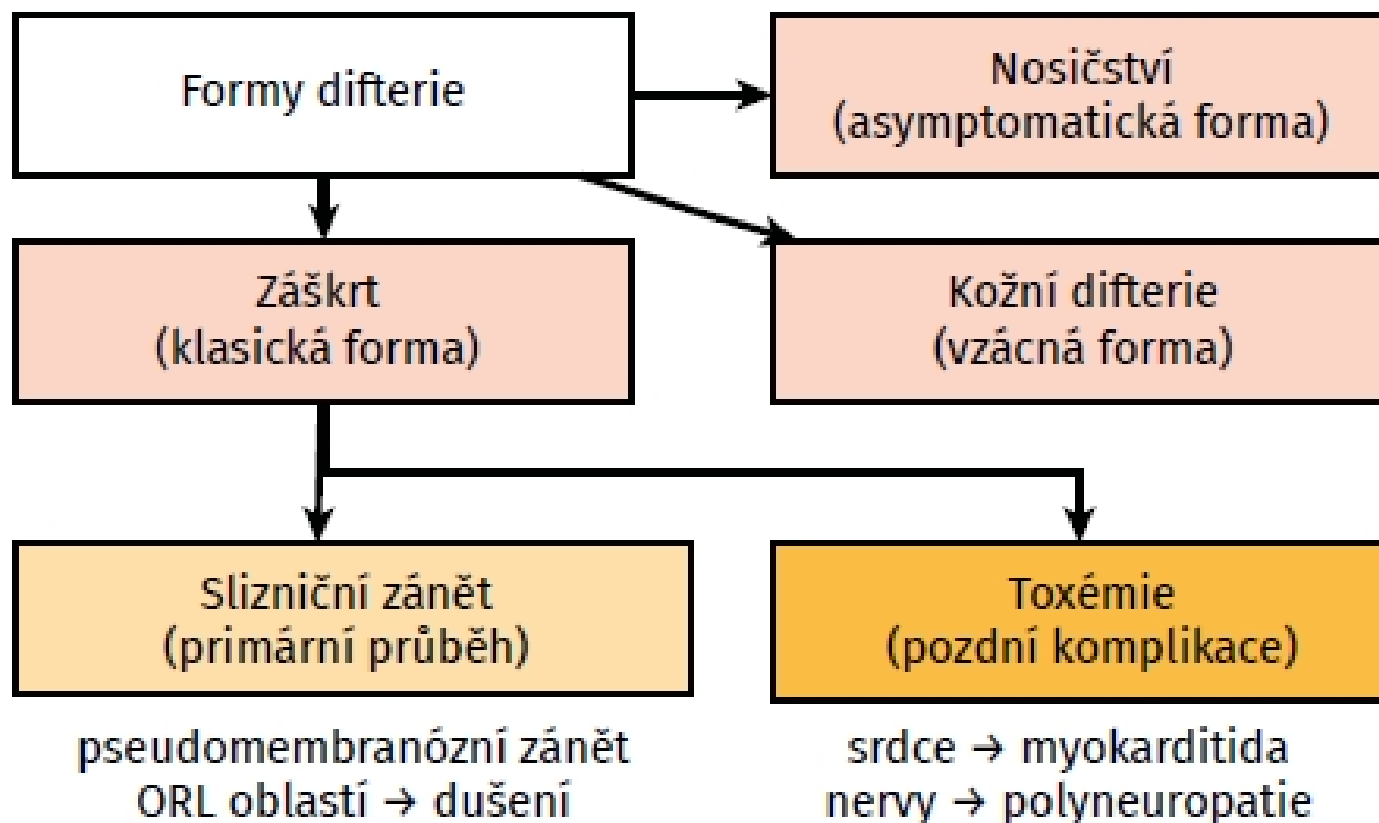
Throat swab, choking, diphtheria
“croup” (secondary suffocating cough)



Fibrinous inflammation with pseudomembrane formation (membrane) in the upper respiratory tract.

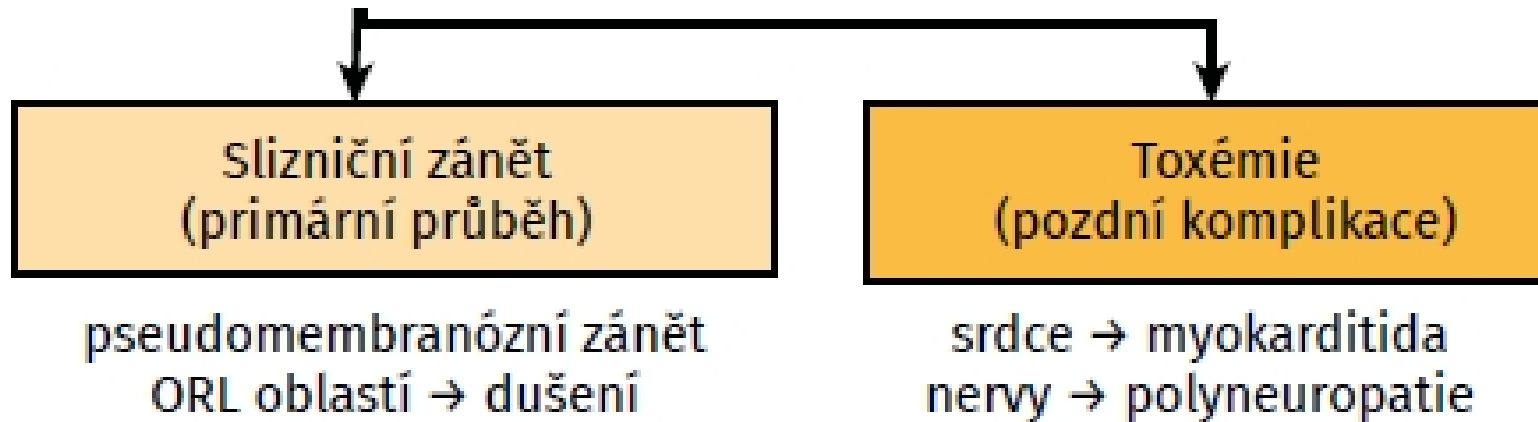
DIPHTHERIA

- Prevalence: developing countries
- Mortality rate up to 20% of cases



OBR. 62 FORMY DIFTERIE

DIAGNOSIS



- **Specimen:**

- Throat or nasopharyngeal swab

- **Methods:**

- Culture + MALDI-TOF MS
- Microscopy

- **Sample:**

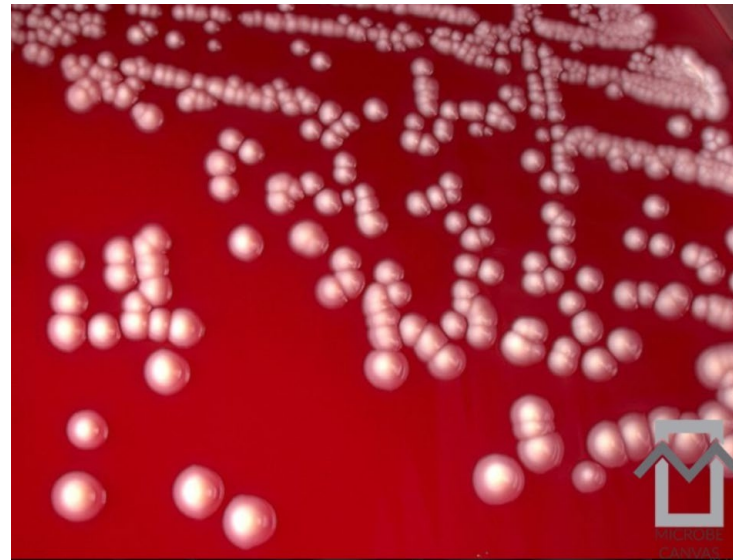
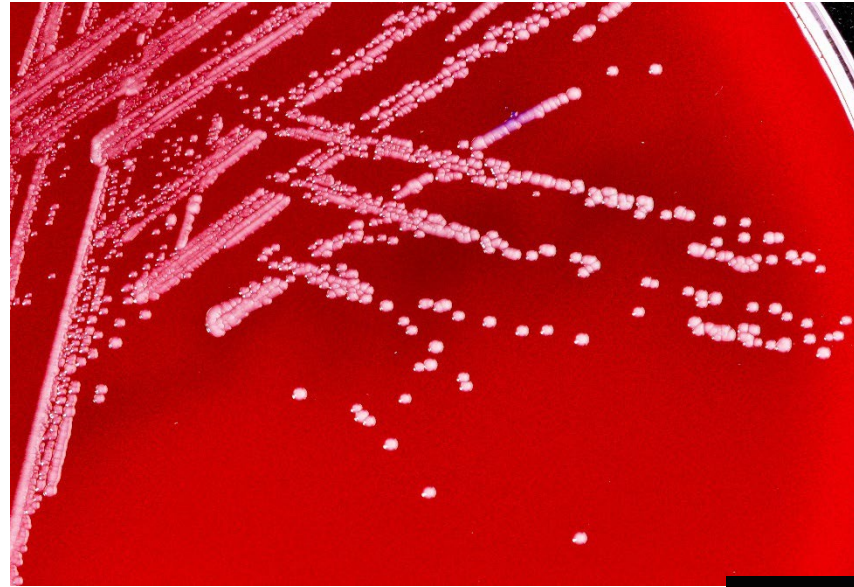
- Grown colony

- **Methods:**

- PCR

Cultivation

- **Facultatively anaerobic**
- **Growth on KA**
 - Small, greyish, translucent colonies
 - Small zone of haemolysis
 - Does not grow at all on MacConkey
 - Clauberg and Tinsdal soil
- **Biochemical tests**
 - Haemolysis
 - Catalase positive
 - CAMP negative



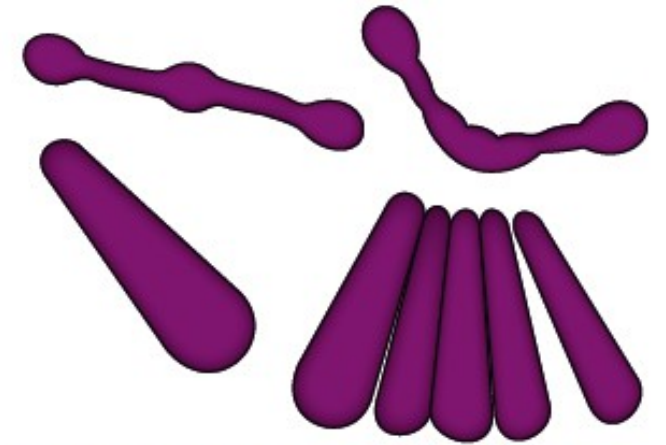
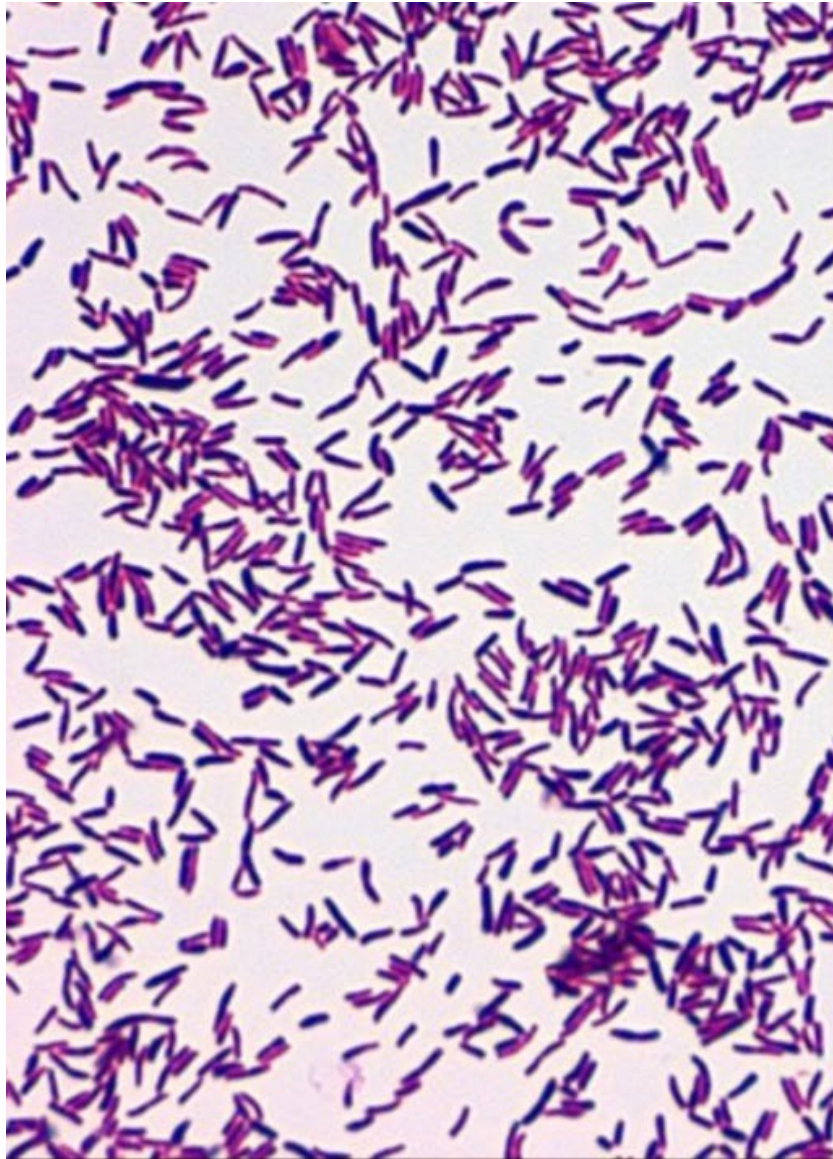
Blood agar



Tinsdale's medium

Microscopy

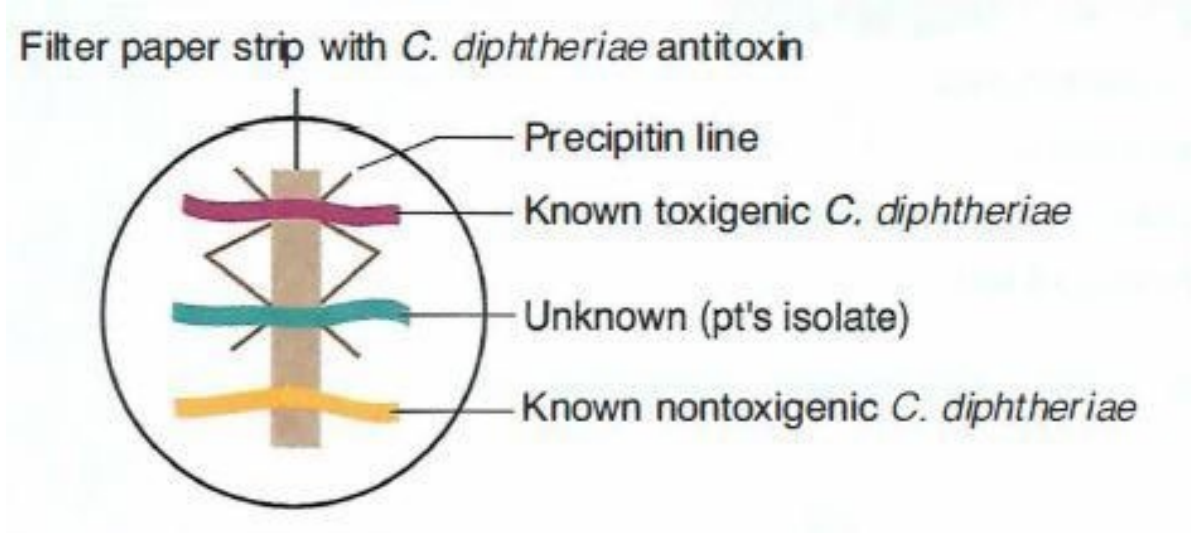
- Irregular G+ rods
- “Raven’s wing” shape



OBR. 61 MORFOLOGIE CORYNEBACTERIUM
DIPHTheriaE



Elek's test and PCR



PCR for the genes: *tox*, *Diph_rpoB*, and *CUP_rpoB*

Antibiotic treatment

- However, diphtheria antitoxin is causal
- The first-line antibiotic is **PNC G**
- An alternative is clindamycin

Prevention

- Vaccination is carried out with diphtheria toxoid

TAB. 120 SCHÉMA POVINNÉHO OČKOVÁNÍ DĚTÍ DLE OČKOVACÍHO KALENDÁŘE PLATNÉHO OD 1. 1. 2018			
Rok	Měsíc	Očkování proti difterii, tetanu, pertusi, hemofilovým nákazám typu b, viru hepatitidy B a poliomyelitidě	Očkování proti spalničkám, příušnicím a zarděnkám
1	2	Hexavakcína – 1. dávka (DiTePe + Hib + HBV + IPV) (od 9. týdne)	
	4	Hexavakcína – 2. dávka (DiTePe + Hib + HBV + IPV) (měsíc po první dávce)	
	11–13	Hexavakcína – 3. dávka (DiTePe + Hib + HBV + IPV)	
2	13–18		MMR vakcína – 1. dávka
5–6		Přeočkování proti DiTePe	MMR vakcína – 2. dávka
10–11		Přeočkování proti DiTePe + IPV	
25		Přeočkování proti Te (poté každých 10–15 let)	

Cutaneous Corynebacteria

- Normal skin microbiota (the vast majority)
- Exception: ***Corynebacterium striatum***
 - possible cause of wound infections

Arcanobacterium haemolyticum

Arcanobacterium haemolyticum

- Meaning:
 - Commensal of the skin and mucous membranes (diphtheria)
 - Tonsillopharyngitis, wound infections
- Reverse CAMP test
- Treatment: macrolides

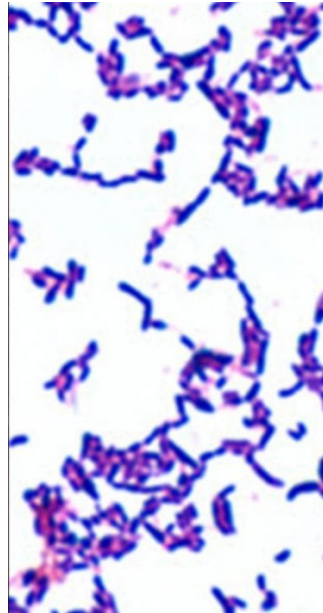




FIG. 1. Close-up view of a positive reverse CAMP reaction. The test organism, *Clostridium perfringens*, is streaked down the centre of the plate. *Streptococcus agalactiae* (group B) is streaked at right angles to the *Clostridium perfringens*. A positive reverse CAMP result for *Clostridium perfringens* is shown by the arrow-shaped zone of enhanced haemolysis pointing towards *Clostridium perfringens*. (Anne Hanson, University of Maine, Orono, ME)

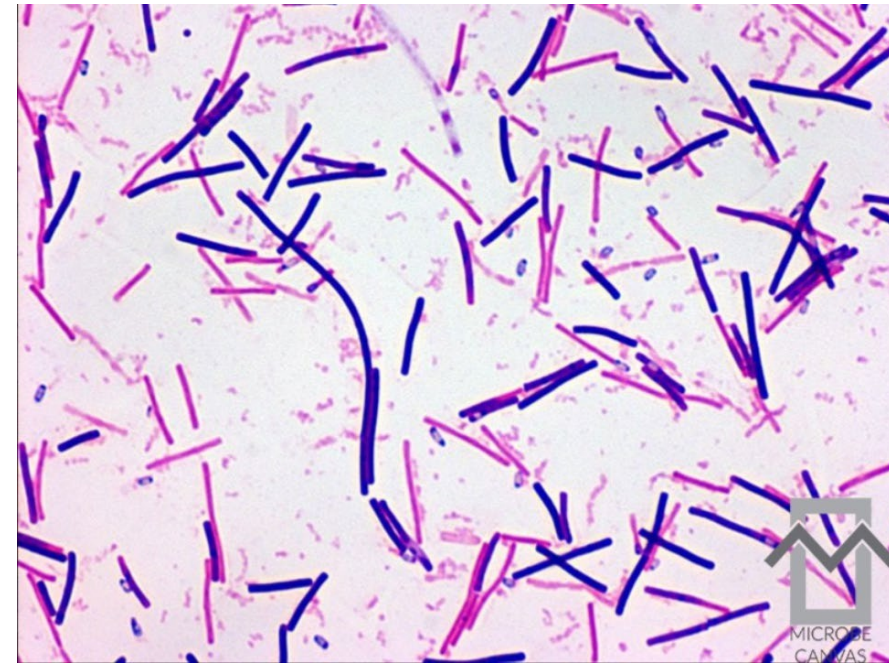
Clostridia

Clostridium spp.

- G+ spore-forming rods, anaerobic
- Species:
 - *Clostridioides (Clostridium) difficile*
 - *Clostridium perfringens, novyi, septicum*
 - *Clostridium botulinum*
 - *Clostridium tetani*

Clostridioides difficile

- G+ spore-forming anaerobic rod
- Virulence factors: toxins
 - Toxin A – enterotoxin
 - Toxin B – cytotoxin
 - binary toxin – ?
- Non-toxigenic strains, controversial findings in children



CLOSTRIDIAL COLITIS (CDI)

- Post-antibiotic diarrhoea
- Pseudomembranous colitis as a consequence of antibiotic therapy



DIAGNOSTICS

- Sample collection?
 - **Diarrhoea**
 - **NO SWAB**
- Certificate
 - Antigen detection (GDH antigen, toxin A, toxin B)
 - Immunochromatography, EIA (enzyme immunoassay)
 - Extended incubation, selective medium
 - PCR

THERAPY

Episode 1
CDI

- Vancomycin **p.o.**

Episode 2
CDI

- Fidaxomicin

Recurrence
recurrence

- Fecal microbiota transplantation

Prevention = Rational antibiotic therapy

!!! Germination of spores in alcohol



Histotoxic Clostridia

- *C. perfringens, novyi, septicum, histolyticum*
- Infections of wounds and soft tissues (including necrotising fasciitis and gas gangrene – Clostridial myonecrosis)
- Necrotising enteritis, enterotoxigenesis
- Sample collection
 - Pus, tissue → microscopy, culture
 - Enterotoxigenesis: antigen (toxin) detection
- Surgical treatment, antibiotics: penicillin G, clindamycin



FIG. 1. Close-up view of a positive reverse CAMP reaction. The test organism, *Clostridium perfringens*, is streaked down the centre of the plate. *Streptococcus agalactiae* (group B) is streaked at right angles to the *Clostridium perfringens*. A positive reverse CAMP result for *Clostridium perfringens* is shown by the arrow-shaped zone of enhanced haemolysis pointing towards *Clostridium perfringens*. (Anne Hanson, University of Maine, Orono, ME)

C. tetani

- Tetanospasmin – blocks the release of glycine and GABA
- Tetanolysin
- Culture and antigen detection unreliable, especially clinical presentation
- Vaccination (active immunisation)
 - Unvaccinated → complete vaccination
 - Last dose administered ≥ 5 years ago → booster
- Tetanus immunoglobulin (passive immunisation)
 - Unvaccinated patients, patients with incomplete vaccination
- Antibiotic therapy: penicillin G, metronidazole
- Compulsory vaccination (toxoid)

C. botulinum

- Botulinum toxin – inhibition of acetylcholine release at the neuromuscular junction
- Antigen detection (botulinum toxin), culture (wound, stool), PCR
- Antibotulinum serum; penicillin in cases of early botulism

Bacillus sp.

Bacillus sp.

- **Basic characteristics:**

- **Gram-positive**, rod-shaped bacteria
 - Form **endospores** – highly resistant to adverse conditions
- **Facultatively aerobic** or strictly aerobic
- Commonly found in **soil**, water and the digestive tract

- **Clinical significance:**

- ***B. anthracis***: a serious, sometimes fatal disease; risk of misuse as a **bioterrorist agent**
- ***B. cereus***: a common cause of **food poisoning** – poorly stored rice, pasta, etc.
- **A common contaminant** in cultures – it is essential to distinguish between colonisation and infection!



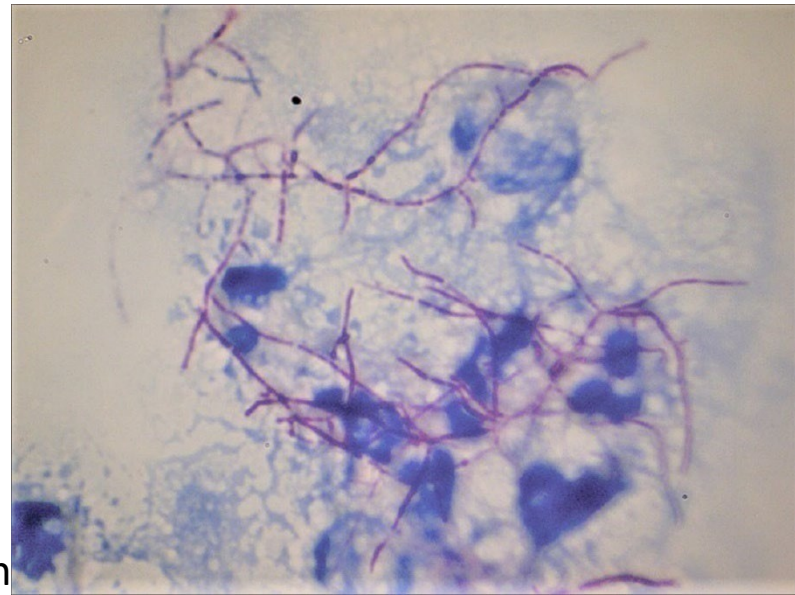
Representatives

Species	Significance / Disease
<i>B. anthracis</i>	Causative agent of anthrax
<i>B. cereus</i>	Diarrhoeal and emetic forms of food poisoning
<i>B. subtilis</i>	Model organism, biotechnology
<i>B. thuringiensis</i>	Biological insecticide (toxin production)

Nocardia, Actinomycetes

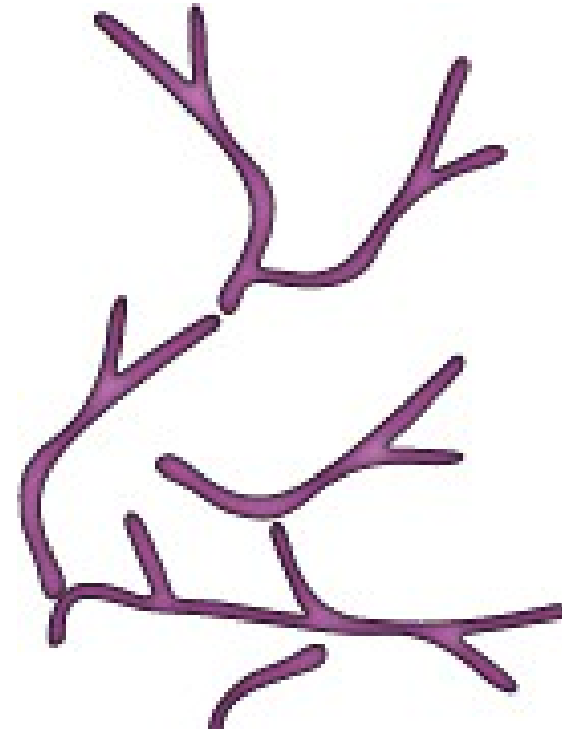
Nocardia

- *Nocardia asteroides*
 - G+ rods to filaments, aerobic
 - In BS mycolic acids
 - Staining: 'weakly acid-resistant' – distinguishing from actin
 - IC survival
 - Ubiquitous occurrence
- Opportunistic pathogen (in immunocompromised individuals)
 - Pneumonia, lung abscesses, skin infections (abscesses, cellulitis, etc.), meningitis, brain abscess (haematogenous spread)
- Diagnosis
 - Sampling: samples from DDC, tissue, CSF
 - Microscopy, extended culture (up to 1 week)
- Treatment
 - Cotrimoxazole

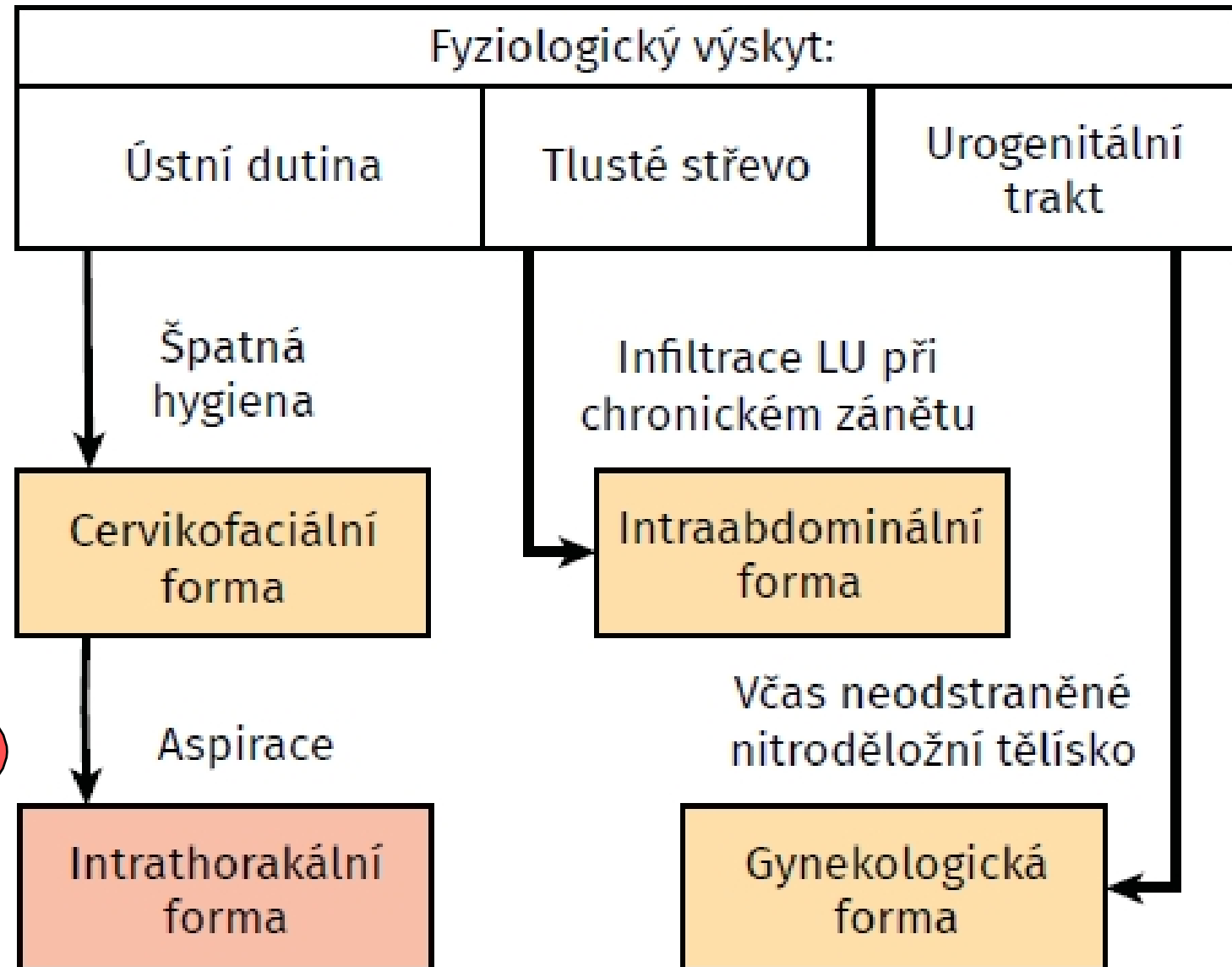


Actinomyces spp.

- Species: *A. israeli*, *A. naeslundii*, *A. odontolyticus*, *A. viscosus*.
- A common component of the microbiota of the upper respiratory tract, the large intestine and the female genital tract
- Contains mycolic acids
- **Pathogenicity:**
 - **Actinomycosis** – formation of abscesses
 - Risk of mediastinitis in the intrathoracic form



- Diphtheroid rods with branched growth
- Long incubation period (up to 14 days)



Risk of
mediastinitis

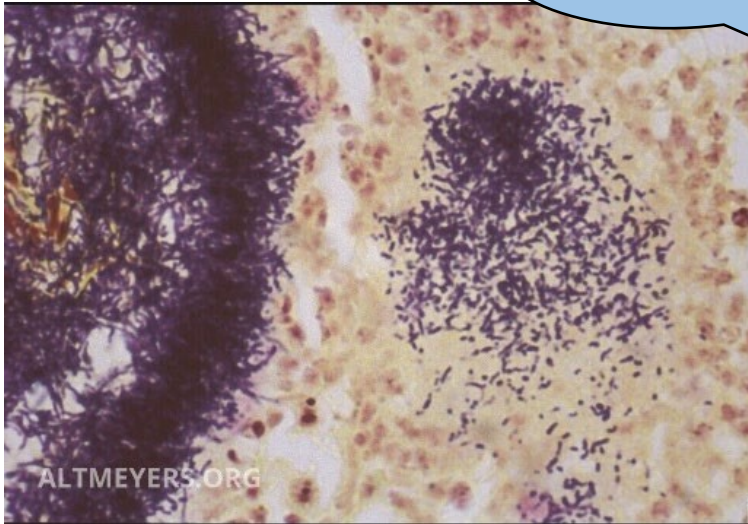
Contents of the abscess/exudate

Intrauterine devices

Druses

Microscopy

Gram stain



Culture

Schaedler's agar for 10–14 days
under anaerobic conditions

Identification

MALDI-TOF MS

Antibiotic treatment

- Penicillin G
- Clindamycin
- Topical and tetracyclines

Resistance

- Metronidazole