HAEMOPHILUS & BORDETELLA

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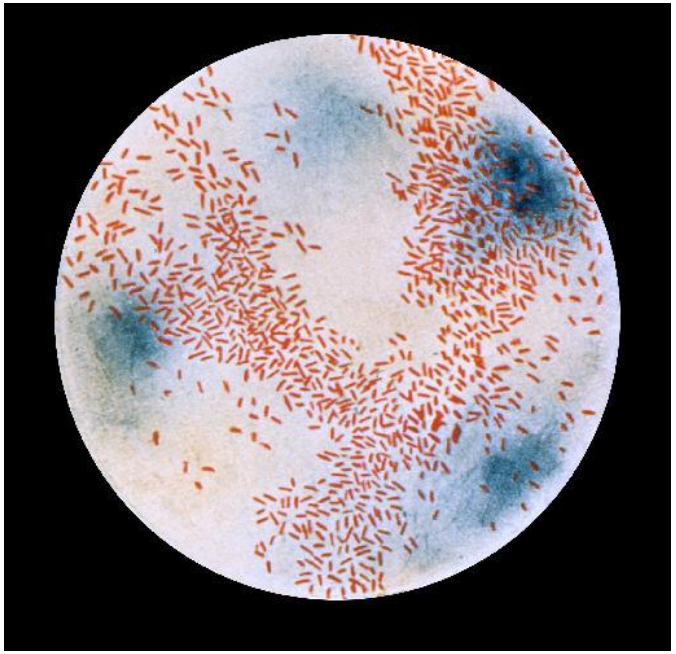
CONTENTS
(Description Headings)

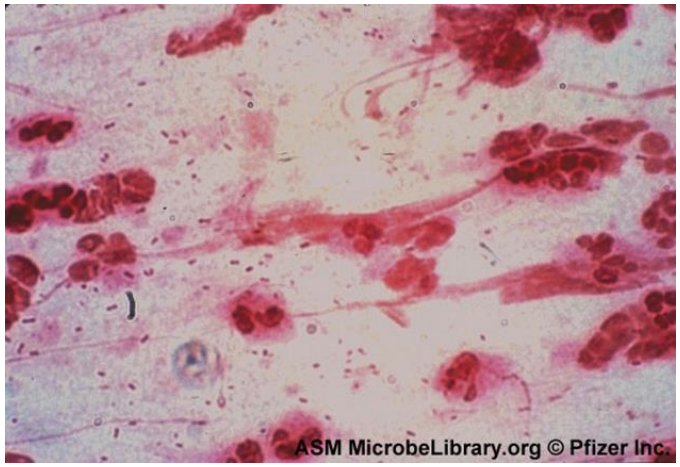
- List the general characteristics within the genus *Haemophilus*
- Describe the difference in the typeable and nontypeable categories of *Haemophilus*, their virulence factors, and the disease they cause
- Describe the isolation requirements necessary for optimal recovery of *Haemophilus*
  - Explain the satellite phenomenon and the chemical basis for the phenomenon
  - List the X and V factor requirements for *H. influenzae, H. parainfluenzae*, and *H. ducreyi*

Haemophilus spp.

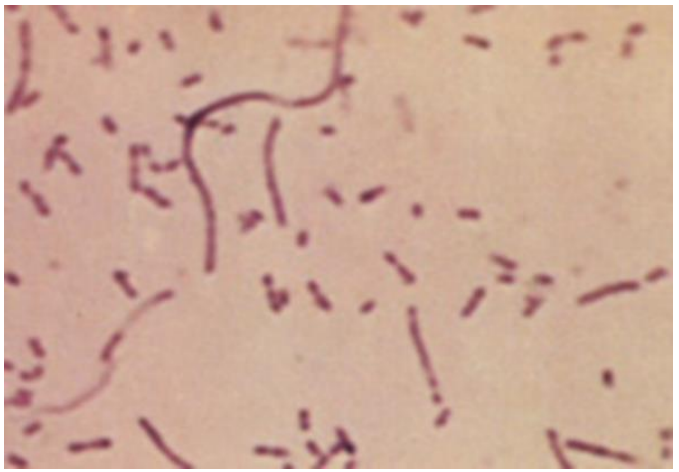
- Family: Pasteurellaceae
- Non-motile
- Facultative anaerobic
- Gram-negative rods
  - Sometimes pleomorphic
- Small

Pfeiffer's bacillus (*Haemophilus influenza*) under the microscope. Source: http://pathmicro.med.sc.edu/Infectious%20Disease/H...





CNS fluid sample



Sputum sample

Haemophilus species of clinical importance

1. ***H. influenzae***
   - Type b is an important human pathogen
2. ***H. ducreyi***
   - Sexually transmitted pathogen (chancroid)
3. **Other *Haemophilus* spp. are normal flora**
   - Sometimes can cause opportunistic infections
   - *H. aegyptius* – Pink eye (purulent conjunctivitis)
   - *H. parainfluenzae* – Pneumonia & endocarditis
   - *H. aphrophilus* – Pneumonia & endocarditis

Physiology and Structure

- Typical gram-negative rod
  - Lipopolysaccharide with endotoxin activity
- Polysaccharide capsule
  - Not all strains
    - Encapsulated
    - Non-encapsulated (nontypeable) strains
  - Six antigenic serotypes: (a-f)

For identification & determination of causative agent

- Eight biotypes (I-VIII)

  According to the;
  - Indole production
  - Urease activity
  - Ornithine decarboxylase activity

For epidemiologic purposes

Pathogenesis & Immunity

***H. parainfluenzae* &**
***H. influenzae* (nonencapsulated)**

- colonize the upper respiratory tract
- can spread locally
  - Otitis media
  - Sinusitis
  - Bronchitis
  - Pneumonia
- disseminated disease, relatively uncommon

H. influenzae
(encapsulated - serotype b)

- uncommon in the upper respiratory tract
- a common cause of disease in unvaccinated children
  - meningitis,
  - epiglottitis [obstructive laryngitis]
  - cellulitis

Pathogenesis & Immunity

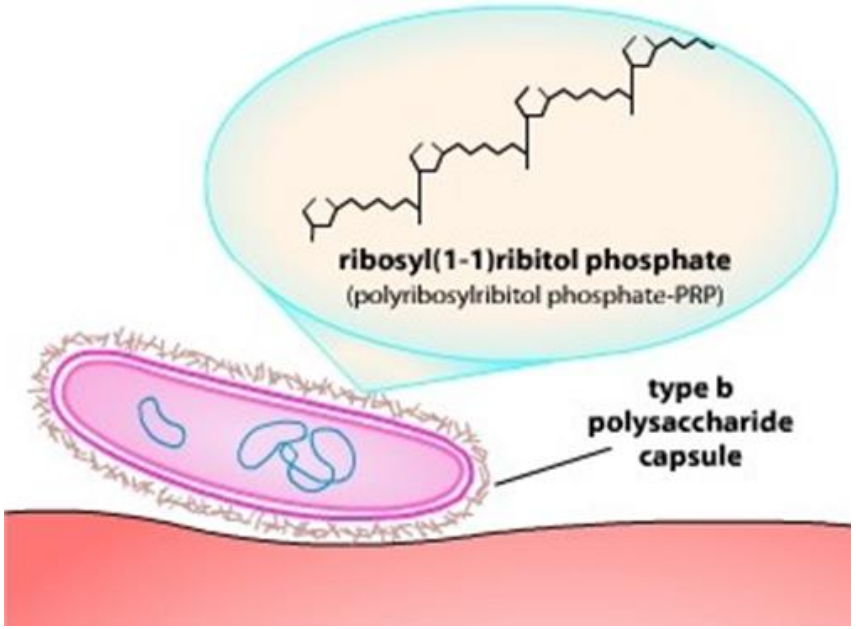
- Pili and nonpilus adhesins
  - mediate colonization of the oropharynx
- Cell wall components (LOS)
  - impair ciliary function
  - leading to damage of the respiratory epithelium

- *The nonencapsulated strains can spread locally*

- *The major virulence factor in H. influenzae type b is **the antiphagocytic polysaccharide capsule.***
- *In the absence of specific opsonic antibodies directed against the polysaccharide capsule, the bacteria can enter the blood.*

Pathogenesis & Immunity

- **Capsule**
  - ribose, ribitol, and phosphate (polyribitol phosphate [PRP])
- Antibodies (against the capsule)
  - stimulate bacterial phagocytosis
- Antibodies develop because of;
  - natural infection,
  - vaccination with purified PRP,
  - the passive transfer of maternal antibodies
- **IgA1 proteases**
  - facilitate colonization on mucosal surfaces



https://microbeonline.com/virulence-factors-haemophilus-influenzae/

Epidemiology

- Nonencapsulated strains of *H. influenzae* are commonly found in the upper respiratory tract; while, encapsulated strains are detectable only in small numbers (and only when highly selective culture methods are used)

- Transmitted from person to person by the respiratory route (encapsulated strains)
  - Other *Haemophilus* strains likely arise endogenously as a person's own microbiota gains access to a normally sterile site

- *H. influenzae* type b disease can be prevented by administration of *Haemophilus* b conjugate vaccine to children

Laboratory Diagnosis

Specimens

- Expectorated sputum
- Other types of respiratory specimens
  - Pus, blood, and spinal fluid for smears and cultures depending on the source of the infection

- Sinusitis or otitis ⟶ needle aspiration
- Pneumonia ⟶ sputum, blood

Laboratory Diagnosis

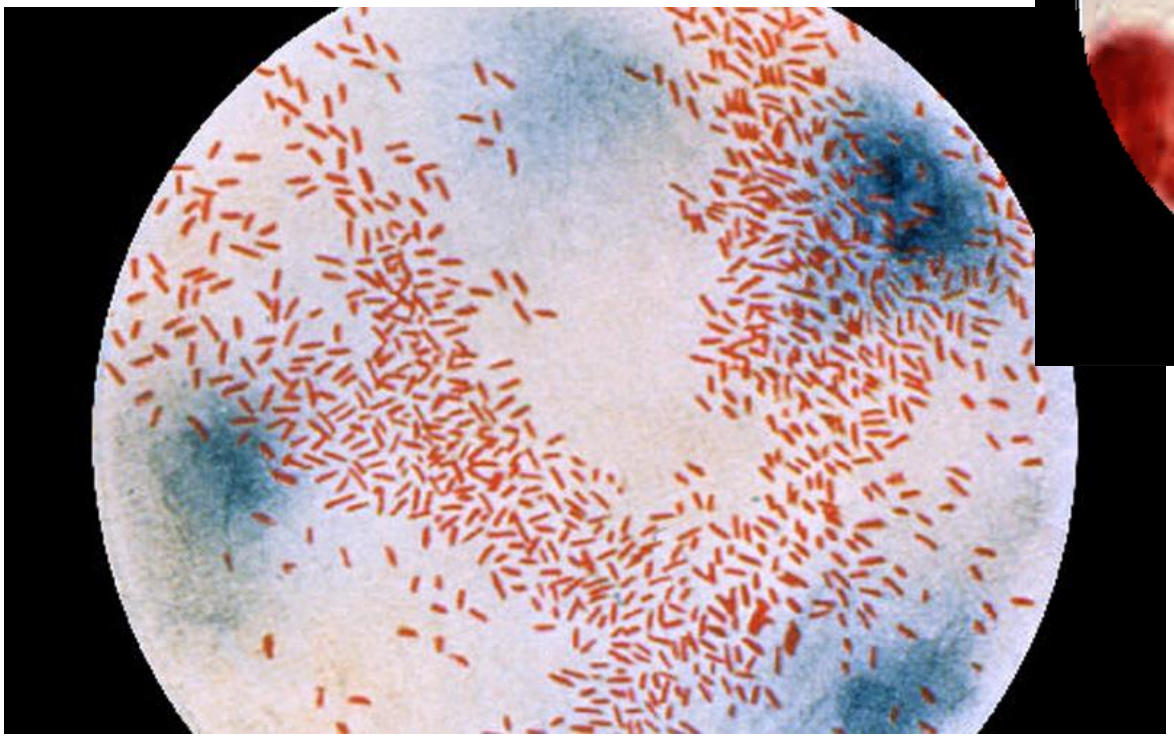
Specimens

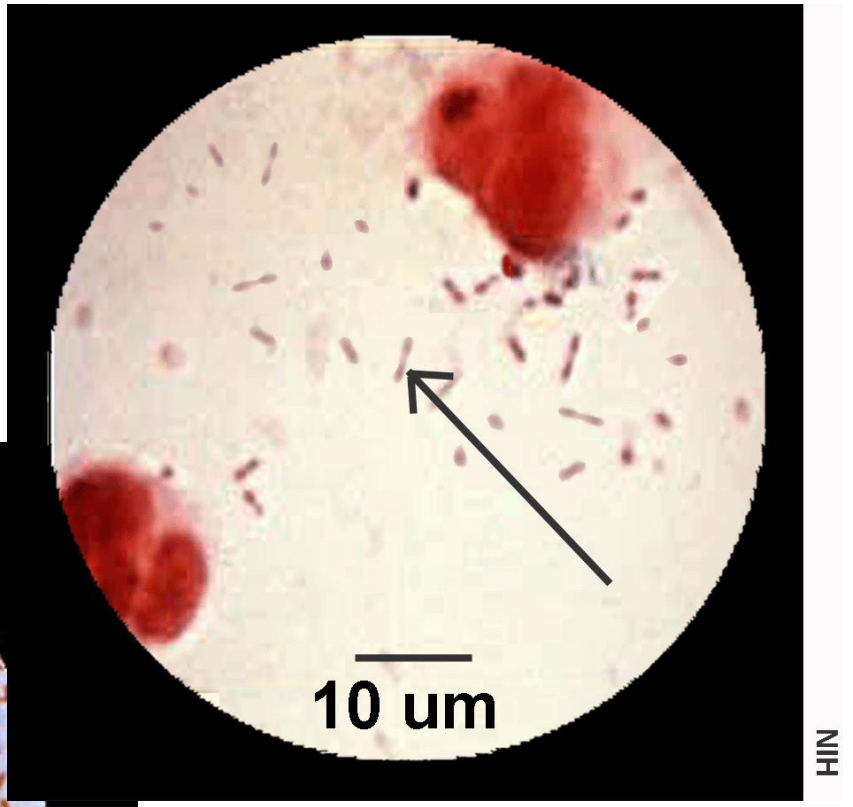
- Susceptible to drying & temperature extremes
  - Should be inoculated to the appropriate media immediately
- Susceptible to contamination
  - Lower respiratory specimen should be collected by bronchioalveolar lavage

Laboratory Diagnosis

Microscopy

- Sensitive & specific
  - Gram stain procedure must be carried out carefully



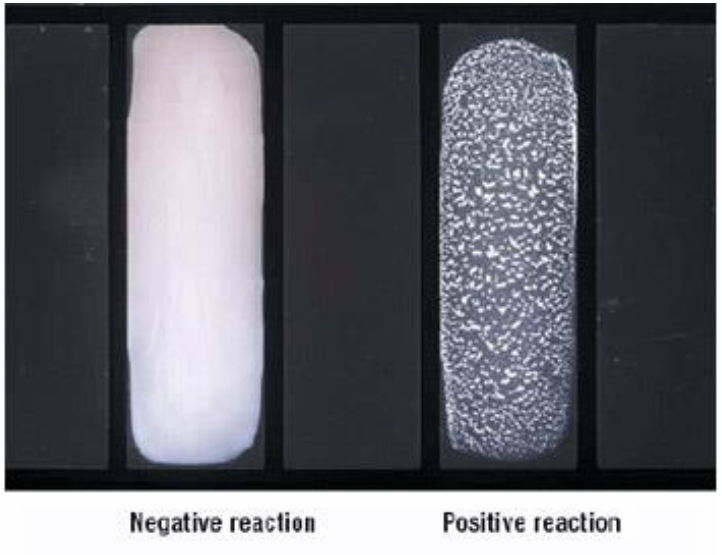


https://www.cdc.gov/hi-disease/about/photos.html
https://courses.cit.cornell.edu/biomi290/microscopycases/sars/documents/BacLIB.htm

Laboratory Diagnosis

Antigen detection

- Rapid and sensitive
  - Particle agglutination test
  - Agglutination occurs if PRP is present
  - Antigen can be detected in CSF and urine



http://www.medical-labs.net/haemophilus-influenzae-b-detection-using-rapid-latex-agglutination-test-2639/

Laboratory Diagnosis

Culture

The growth of most species of *Haemophilus* requires supplementation of media with one or both of the following growth-stimulating factors:

1. Hemin (also called X factor for “unknown factor”)
2. Nicotinamide adenine dinucleotide (NAD; also called V factor for “vitamin”)

Species	X factor	V factor
H.influenzae	+	+
H.heamolyticus	+	+
H.parainfluenzae	-	+
H.paraheamolyticus	-	+
H.ducreyi	+	-
H.aphrophilus	+	-

Laboratory Diagnosis

Culture

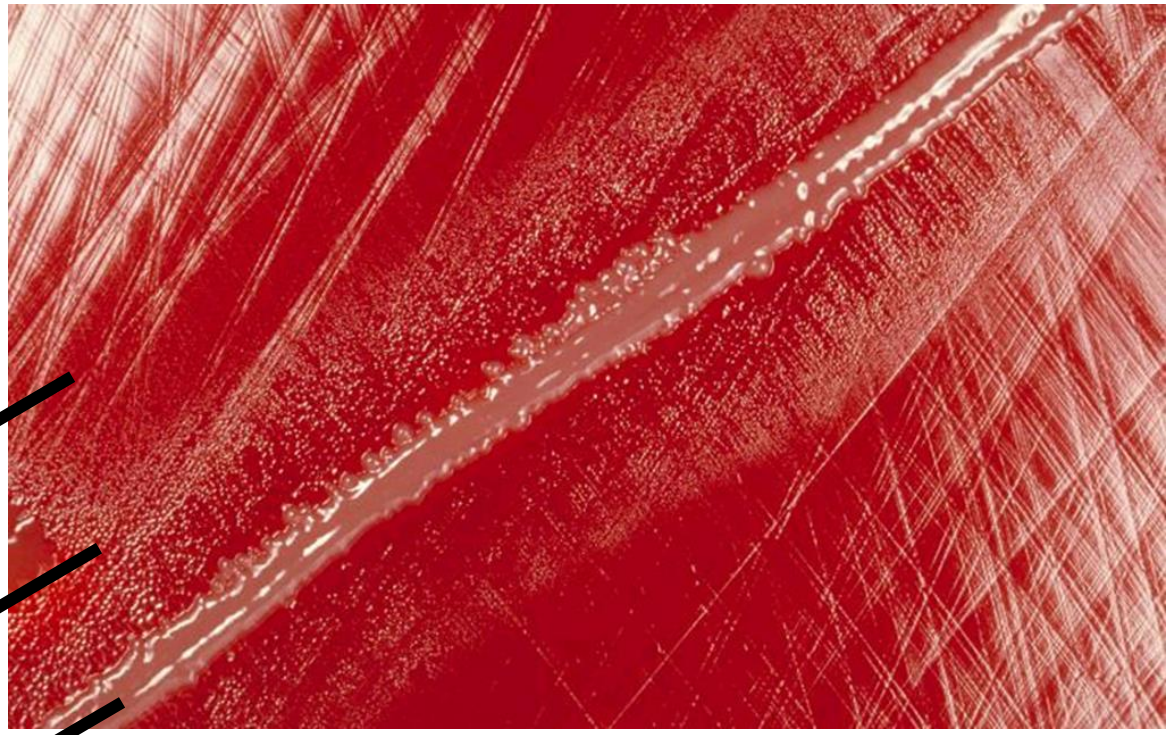
- Chocolate agar
  - at 35-37°C in a 5% CO_2
  - Contains **NAD (V factor)**
  - Contains **Hemin (X factor)**
  - 1- to 2-mm, smooth, opaque colonies after 24 hours of incubation

https://microbeonline.com/chocolate-agar-composition-uses-colony-characteristics/

Laboratory Diagnosis

Culture

- Satellite phenomenon



https://www.cdc.gov/hi-disease/about/photos.html

Treatment

- Broad-spectrum cephalosporins
- Amoxicillin
  - If susceptible; approximately 30% of strains are resistant
- Cephalosporin
- Azithromycin
- Doxycycline
- Fluoroquinolone
  - Routine susceptibility testing of clinical isolates as a guide to therapy may not be necessary (for now!)

Prevention

- Active immunization with purified capsular PRP
  - At 2-4-6. months & a rapel at between 12-15 months

- CDC recommends **Hib vaccination** for:
  - All children younger than 5 years old
  - Unvaccinated older children and adults with certain medical conditions
  - People who receive a bone marrow transplant

https://www.cdc.gov/vaccines/vpd/hib/index.html

Control

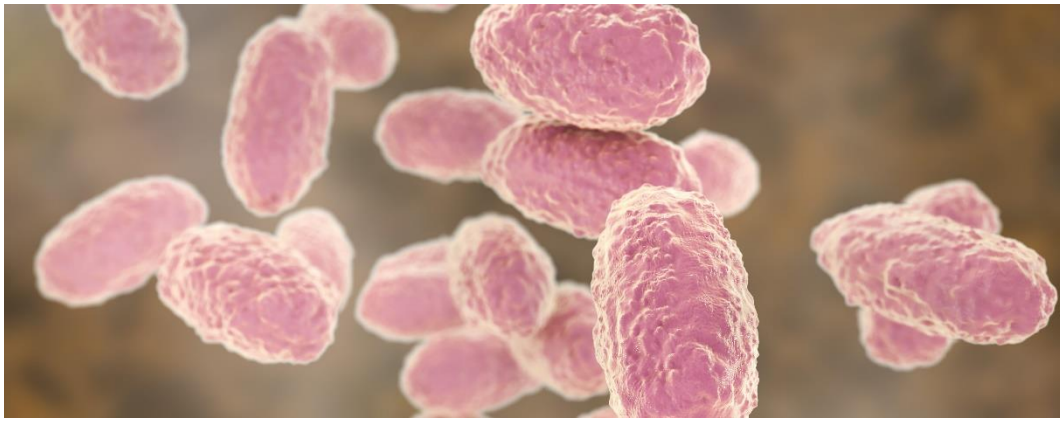
- Antibiotic chemoprophylaxis is used to eliminate the carriage of *H. influenzae* type b in children at high risk for disease
  - e.g., children <2 years in a family or day-care center where systemic disease is documented
- Rifampin prophylaxis has been used in these settings

CONTENTS
(Description Headings)

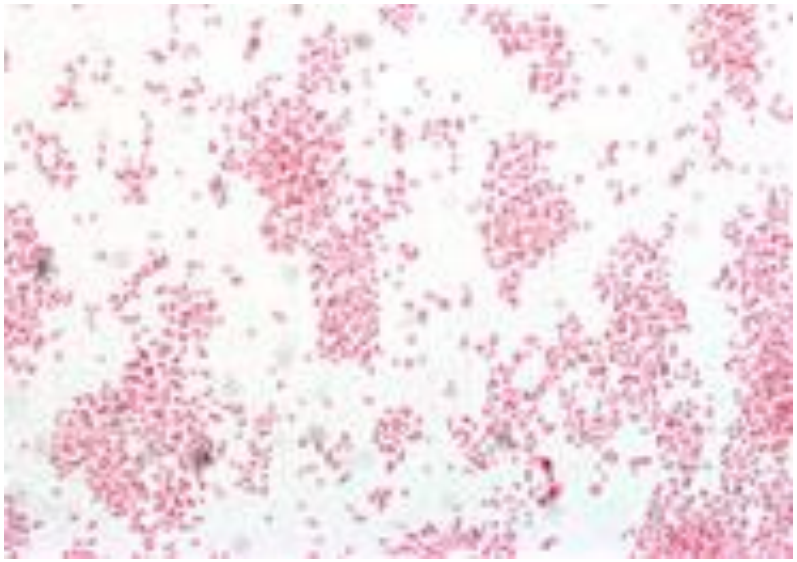
- Describe the general characteristics of the *Bordetella* spp.
- Describe the proper collection and transport of specimens for the detection of *B. pertussis*
- Describe the optimal conditions for culturing *B. pertussis*, including specimens of choice for optimal recovery
- Explain the limitations of direct fluorescent antibody (DFA) and polymerase chain reaction (PCR) methods for detecting *B. pertussis*, including assay specificity and sensitivity

Bordetella spp.

- Extremely small
  - 0.2 to 0.5 × 1 μm
- Strictly aerobic,
- Gram-negative
- Coccobacillus
- Non-motile
- Oxidize amino acids
- Don’t ferment carbohydrates



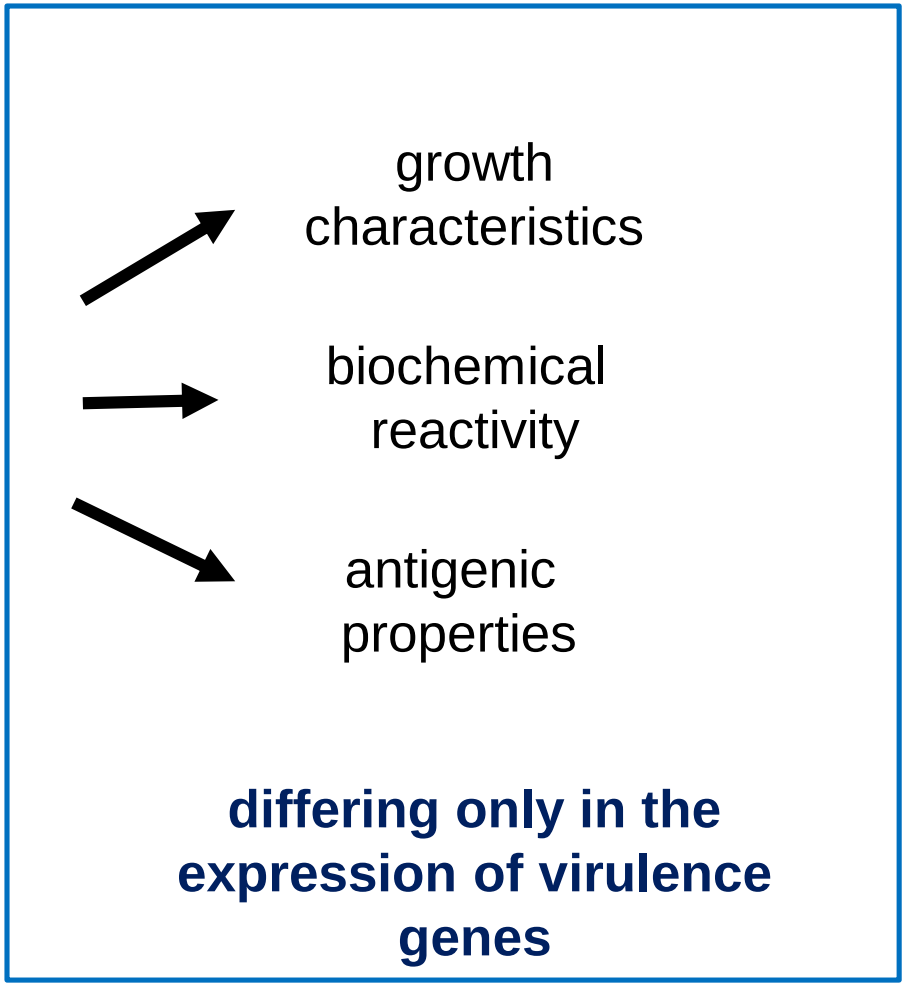
https://www.hygiene-in-practice.com/pathogen/bordetella-pertussis/



https://www.cdc.gov/pertussis/clinical/disease-specifics.html

Bordetella spp.

- *Bordetella pertussis*
  - pertussis or whooping cough
- *Bordetella parapertussis*
  - a milder form of pertussis
- *Bordetella bronchiseptica*
  - respiratory disease in dogs, swine, laboratory animals, and occasionally human



Bordetella pertussis

Pathogenesis & Immunity

- Exposure to the organism
- Bacterial attachment to the ciliated epithelial cells of the respiratory tract
- Proliferation of the bacteria
- Production of localized tissue damage and systemic toxicity

Bordetella pertussis

Pathogenesis & Immunity

Virulence factor	Biologic effect	
Adhesins		
Filamentous hemagglutinin	Required to binding to sulfated glycoproteins on membranes of ciliated cells in trachea	These adhesins also bind to CR3, a glycoprotein receptor on the surface of macrophages
Pertactin	As with filamentous hemagglutinin	
Fimbriae	Bind to mammalian cells; role in disease is unknown but stimulate humoral immunity	
Pertussis toxin	S2 subunit binds to glycolipid on surface of ciliated respiratory cells; S3 subunit binds to ganglioside on surface of phagocytic cells	

Bordetella pertussis

Pathogenesis & Immunity

Virulence factor	Biologic effect
Toxins	
Pertussis toxin	S1 subunit inactivates G1α, the membrane surface protein that controls adenylate cyclase activity; uncontrolled expression leads to increased cyclic adenoside monophosphate levels; toxin inhibits phagocytic killing and monocyte migration
Adenylate cyclase/ hemolysin toxin	Increases intracellular level of adenylate cyclase; inhibits phagocytic killing and monocyte migration

Bordetella pertussis

Pathogenesis & Immunity

Virulence factor	Biologic effect
Toxins	
Dermonecrotic toxin	Causes dose-dependent skin lesions or fatal reactions in experimental animal model; role in disease is unknown
Tracheal cytotoxin	A peptidoglycan fragment that kills ciliated respiratory cells and stimulates the release of interleukin-1 (fever)
Lipopolysaccharide	Two distinct lipopolysaccharide molecules with either lipid A or lipid X; activates alternate complement pathway and stimulates cytokine release; role in disease is unknown

Bordetella pertussis

Epidemiology

- Human reservoir host
- Worldwide distribution
- Children younger than 1 year are at greatest risk for infection, but disease is now most common in older children and young adults
- Nonvaccinated individuals are at greatest risk for disease
- Disease spreads person-to-person by infectious aerosols

Bordetella pertussis

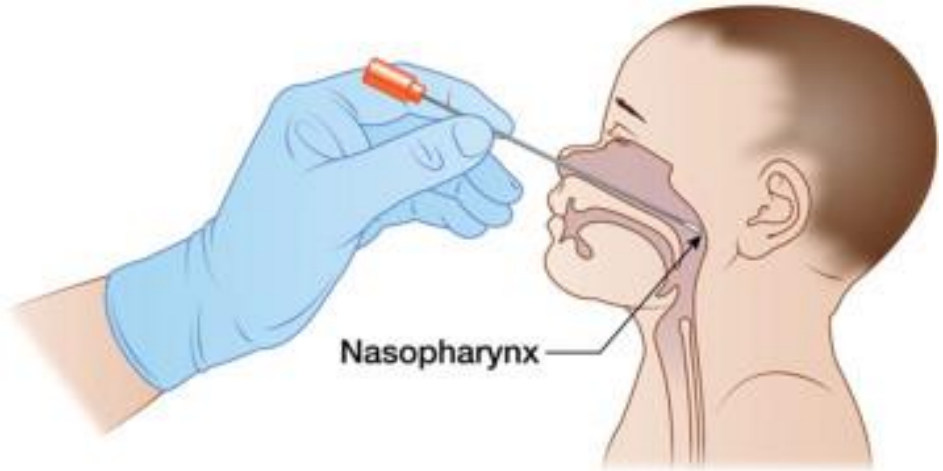
Laboratory Diagnosis

- Specimen Collection and Transport
- Microscopy
- Nucleic Acid–Based Tests
- Culture
- Identification
- Antibody Detection

Bordetella pertussis

Laboratory Diagnosis

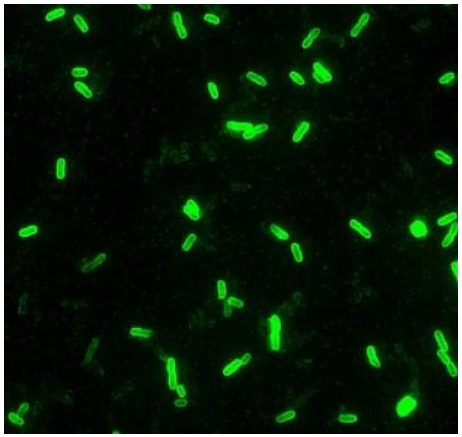
- Specimen Collection and Transport
  - optimal diagnostic specimen: **nasopharyngeal aspirate**
  - sensitive to drying
  - should be placed in a suitable transport medium



Bordetella pertussis

Laboratory Diagnosis

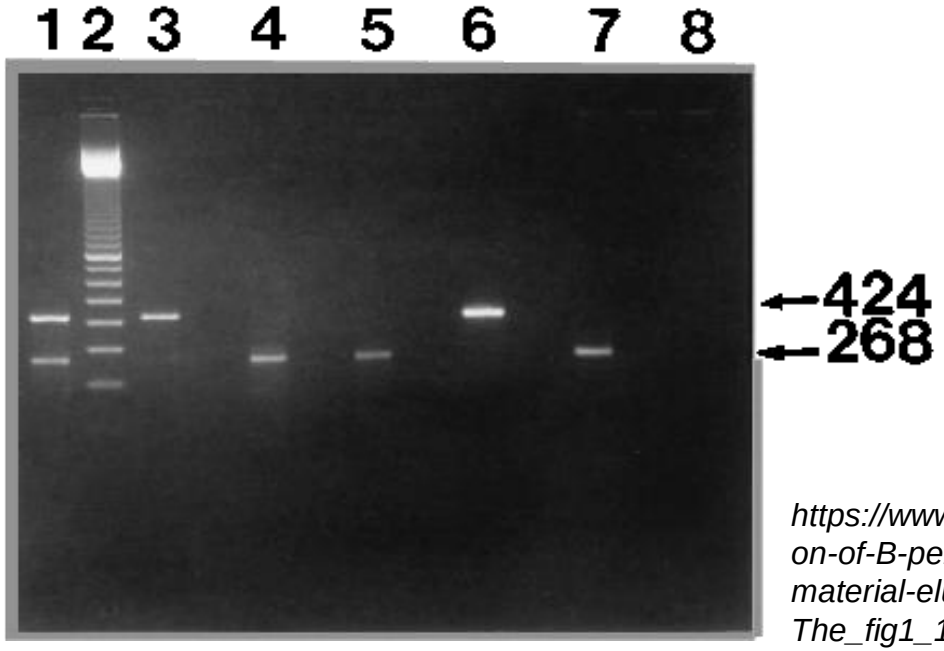
- Microscopy
  - a direct fluorescent antibody procedure using either monoclonal or polyclonal antibodies can be used
  - insensitive and nonspecific
  - PCR and/or culture should also be performed



Bordetella pertussis

Laboratory Diagnosis

- Nucleic Acid–Based Tests
  - the most sensitive and specific tests



https://www.researchgate.net/figure/Detection-of-B-pertussis-DNA-by-PCR-analysis-of-material-eluted-from-slides-The_fig1_14624876

Bordetella pertussis

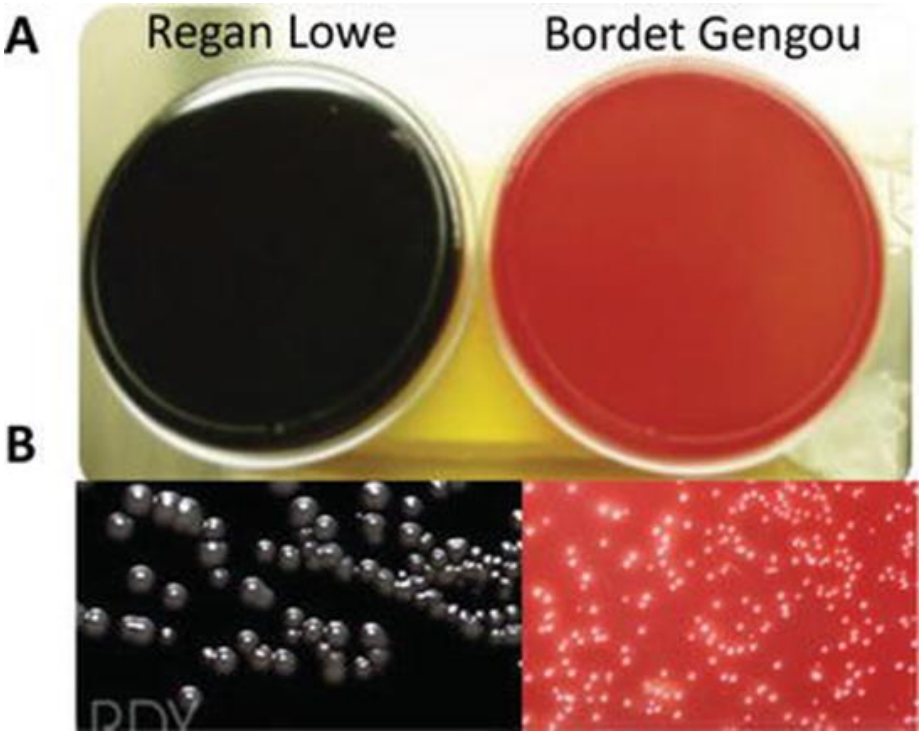
Laboratory Diagnosis

- Culture
  - specific but insensitive
  - affected by;
    - patient factors (i.e., stage of illness, use of antibiotics)
    - the quality of the specimen
    - transport conditions
    - culture methods

Bordetella pertussis

Laboratory Diagnosis

- Culture
  - Bordet-Gengou medium
  - Regan Lowe charcoal medium
    - supplemented with glycerol, peptones, and horse blood
    - should be incubated at 35°C, in a humidified chamber, and for 7 to 12 days



https://www.intechopen.com/books/pertussis-disease-control-and-challenges/update-on-epidemiology-diagnosis-and-treatment-of-pertussis

Bordetella pertussis

Laboratory Diagnosis

- Antibody Detection
  - Detection of IgG or IgA can be used as a confirmatory test
  - Enzyme-linked immunosorbent assay (ELISA) tests have been developed to detect IgA, IgM, and IgG antibodies against pertussis toxin, filamentous hemagglutinin, pertactin, and fimbriae

Bordetella pertussis

Treatment, Prevention, and Control

- Supportive
  - with nursing supervision during the paroxysmal and convalescent stages of the illness
- Macrolides (i.e., erythromycin, azithromycin, clarithromycin)
- Azithromycin
- Clarithromycin
- Trimethoprim-sulfamethoxazole
- Fluoroquinolones

THE END

THANKS FOR LISTENING ☺

References

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