

HAEMOPHILUS & BORDETELLA

- Gram Negative, pleomorphic forms, non-motile, non-spore forming
- Require enriched media for growth
- *H. influenzae* type b is an important human pathogen. causing upper respiratory tract infection, meningitis in children
- *H. duceryi* a sexually transmitted human pathogen.
- *Bordetella pertussis* causing whooping cough-
- other haemophilus species are among normal flora

Morphology & Identification

- Coccoid-bacilli shape occurring in pairs or short chain can be isolated from acute infection & rich medium 6-8 hrs old.
- Longer, liced bacteria &/or pleomorphic shape can be isolated from 6-18 hrs old culture which have a definite capsule.
- Capsule is the antigen used for typing; type b is consist of polyribose-ribitol phosphare (PRP)

CULTURE OF *H. influenzae*

- On Chocolate agar: flat , grayish-brown colonies with 1-2 mm can be seen after 24 hrs of incubation.
- Growth can be enhanced by addition of Iso VitaleX in media
- *H. influenzae* does not grow on sheep blood agar except around staphylococci colonies.

GROWTH CHARACTERS

- Identification of *H. influenzae* depends in part upon: 1) the needs for the growth factors V & X. Factor V can be replaced by Nicotine amide Adenine Dinucleotide (NAD) & Factor X acts physiologically as haemin. 2) Capsule existence or absence.* TRANSFORMATION which occasionally leads to transfer DNA from one species to another. Am & Chloramph. Resistnace is control by genes on transmissible plasmid.

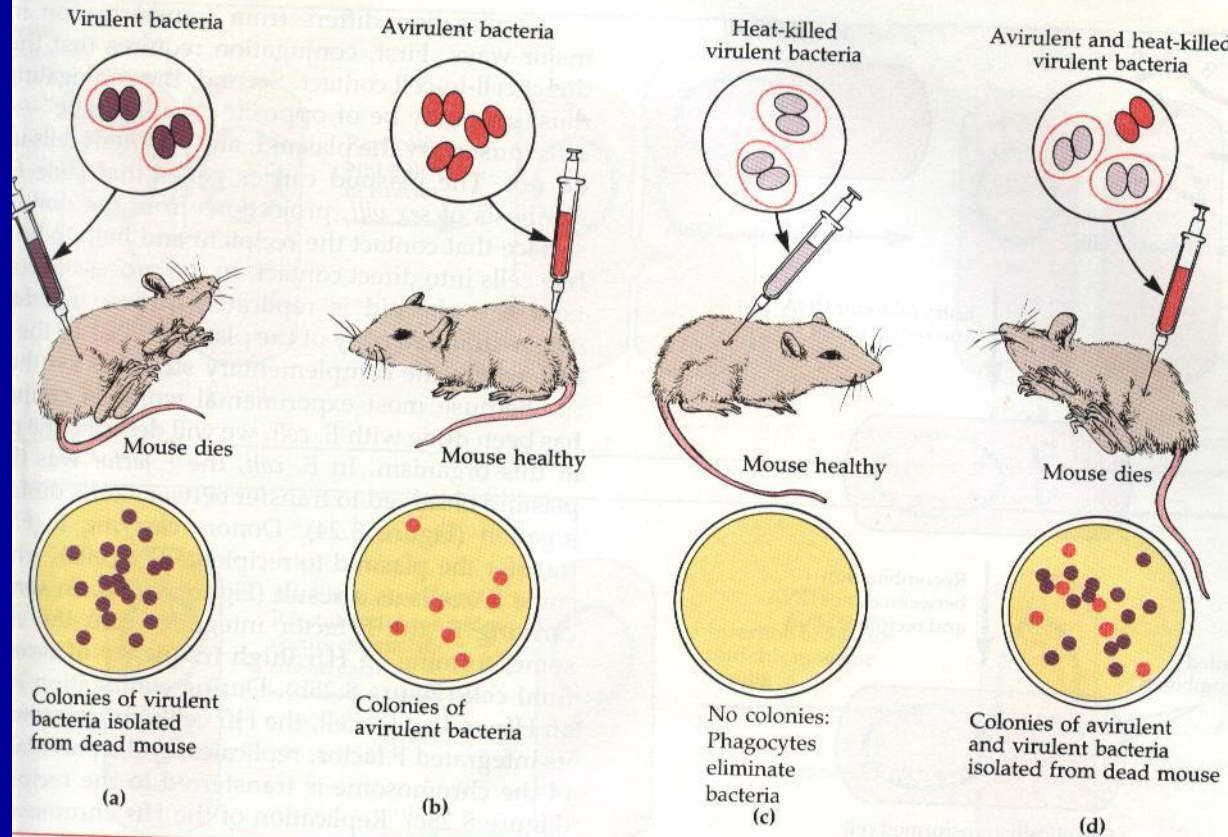


FIGURE 8.22 Transformation. Griffith's experiment demonstrating genetic transformation. Some material from the heat-killed virulent bacteria transformed the living avirulent bacteria into virulent bacteria, which killed the mouse. Avirulent bacteria lack capsules and are readily destroyed by the host; therefore, few show up as colonies on the medium in (b) and (d).

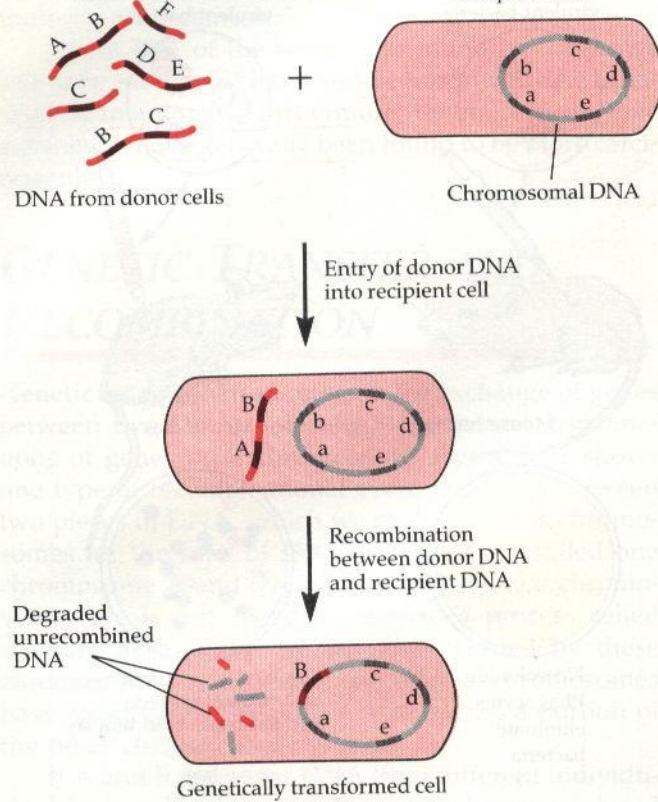


FIGURE 8.23 Mechanism of genetic transformation.

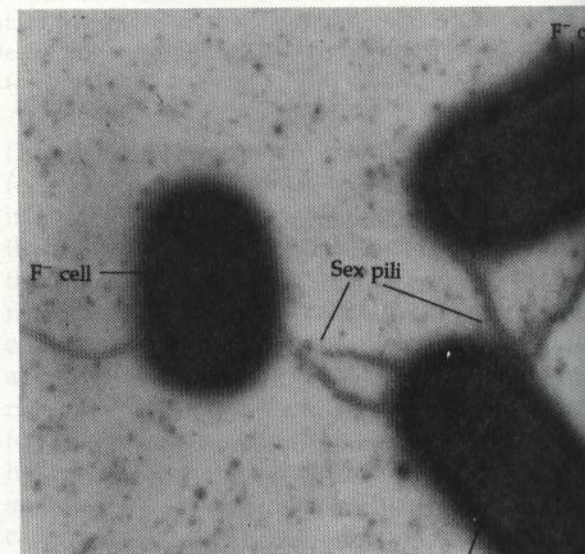
ferred to the recipient, it is still a very large molecule that must pass through the recipient cell wall and membrane. When a recipient cell is in a physiological state in which it can take up the donor DNA, it is said to be **competent**. Competence may be related to alterations in the cell wall that make it permeable to the large DNA molecule.

The well-understood and widely used bacterium *E. coli* is not naturally competent for transformation. However, a simple laboratory treatment enables *E. coli* to readily take up DNA. The discovery of this treatment has enabled *E. coli* to be used for genetic engineering, as will be discussed in Chapter 9.

Conjugation in Bacteria

major ways. First, conjugation requires that the direct cell-to-cell contact. Second, the conjugating must generally be of opposite "mating type"—cells must carry the plasmid, and recipient cells do not. The plasmid carries genes that code for synthesis of *sex pili*, projections from the donor surface that contact the recipient and help to bring two cells into direct contact. In the process of conjugation, the plasmid is replicated during transfer of a single-stranded copy of the plasmid DNA to the recipient, where the complementary strand is synthesized.

Because most experimental work on conjugation has been done with *E. coli*, we will describe the process in this organism. In *E. coli*, the *F factor* was the plasmid observed to transfer between cells during conjugation (Figure 8.24). Donors carrying *F* (F^+ cells) transfer the plasmid to recipients (F^- cells), which become F^+ cells as a result (Figure 8.25a). In some cases, carrying *F*, the *F factor* integrates into the chromosome, forming an **Hfr (high frequency of recombination) cell** (Figure 8.25b). During conjugation between an Hfr and an F^- cell, the Hfr cell's chromosome, with its integrated *F factor*, replicates, and a parental copy of the chromosome is transferred to the recipient (Figure 8.25c). Replication of the Hfr chromosome begins within the *F factor*, and a small piece of the



Pathogenesis

- Non-capsulated *H. influenzae* is part of the normal flora , produce no endotoxins or exotoxins. Its capsule is antiphagocytic **Polyribos phosphate(PRP) is the major viriolent factor.**
- **Type b causes also, meningitis pneumonia, epiglottitis, cellulitis & septic atrithritis.**
- **Non-typeable may cause ch bronchitis, otitis media, sinusitis & conjunctivitis following *the breakdown of* normal defense mechanism.**

Clinical Findings

- *H. influenzae* & *pneucoccus* inter through the RT. They are two of the most Common etiologic agents of bacterial otitis media. •
- Before the use of conjugate vaccine, it was the most common cause of meningitis in children of 5 months to 5 years where •
- Obstructive layngotracheitis with swollen cherry-red epiglottis may develops which require prompts of childen •

TREATMENT

- Mortality of untreated *H.influanze* may reach 90%. Most strains are susceptible •
Ampicillin. However, about 25% are producing B- •
lactamase under the control
of a transmissible plasmids. •
- Cephalosporins is almost used w/o •
resistance. •
- Cefotaxime given intravenously with excellent results. •
- Late complications of meningitis leading to •
accumulation of local fluid require surgical drainage.

Epidemiology & Prevention

- Respiratory route is the way of transmission. •
- Type b disease can be prevented by •
 - administration of conjugate vaccine (CV) to •
 - children. CV can be prepared by :
 - a- mutantans of *C. diphtheriae*(CRM197) •
 - toxins or b) Neisseria meningitis outer •*
 - membrane complex.*
- *By this procedure 95% of meningitis can be •*
- reduced in children.*

BORDETELLAE

- 1- *B. pertussis* causes whooping cough •
- 2- *B. parapertussis* causes similar disease •
- 3- *B. bronchiseptica* causes diseases in •
animals.
- 4- *B. avium* causes turkey coriza & not •
known to infect humans.

BORDETELLA PERTUSSIS

- Is a capsulated minute gram negative •
coccobacilli similar to *H. influenzae*. With •
touidine blue stain. •
- Requires enriched media for growth •
(potato-blood-glycerol agar)+ pencilline G •
0.5ug/mL. Also charcoal media of •
Legionella penumophila is preferable.
Moist environment is required for
incubation for 3-7 days at 35-37 C.

Sugar Fermentation

- Glucose & lactose are fermented by •
B. pertussis at strictly aerobic environment with production of acid but no gas. It does not require X & V.
- The virulent *B. pertussis* is showing hemolysis on blood agar at its virulent phase.

Clinical Diagnosis

- Catarrhal inflammation with mild coughing & sneezing appeared after 2 weeks of incubation period. •
 - Patient is highly infectious due to the large number of contaminated droplets. •
 - The cough develops its explosive character of “whoop upon inhalation” during the paroxysmal stage. •
- The whoop occurs in infants & paroxysmal coughing occurs in older children & adults. •

Clinical Diagnosis(Cont.)

- WBC count is high(16,000 – 30,000/uL •
- Prolong coughing (4-6 weeks) in adult. •
- These signs & symptoms may be •
confused with infections of adenovirus
And/or Chlamydia pneumoniae. •

LAB. DIAGNOSIS

- 1- Direct Test: Specimens of nasal •
pharyngeal swabs, saline nasal wash and •
droplets from coughing can be tested •
directly by fluorescent antibodies and/or •
culture . False - & false + are expected •
& The sensitivity of the test is about 50%. •
- 2-Culture: growing colonies will be •
identified by Immunofluorescence staining •
Or by slide agglutination with specific Abs. •

LAB. DIAGNOSIS(Cont.)

- 3- Polymerase Chain Reaction (PCR) is the most sensitive method of diagnosis. •
- 4- Serological Test is not recommended •
Because a rise of agglutinating or precipitating antibodies does not occur until the third week of illness. •

IMMUNITY

- Recovery or immunization helps in making the second infection is mild if occur but the re-infection may occur years later in adults will be sever. i.e. the antibodies may prevent attachment of *B. pertussis* to cilia of the respiratory epithelium.

TREATMENT & PREVENTION

- 1-Erythromycin during catarrhal stage •
- 2- Treatment after paroxysmal stage has •
a very little effect. •
- 3-Oxygen inhalation & sedation may •
prevent anoxic damage to the brain.

QUIZ

How you cure & prevent the spreading of the following diseases:

1- Leptosirosis

a- treatment:

b- prevention

2- Lyme disease

a- traetment

B- prevention

3- Meningitis caused by *H. influenzae*

A- treatment

b- prevention