



29

HAEMOPHILUS

29.1 INTRODUCTION

The genus *Haemophilus* contains small, nonmotile, nonsporing, oxidase positive, pleomorphic, gram negative bacilli that are parasitic on human beings or animals. *Haemophilus* means blood loving organisms.



OBJECTIVES

After reading this lesson you will be able to:

- describe the morphology of *Haemophilus Influenzae*
- discuss the cultural characteristics
- describe the pathogenesis of *Haemophilus influenza*
- explain the laboratory diagnosis

29.2 MORPHOLOGY

H. influenzae is a small gram negative, nonmotile, nonsporing bacillus exhibiting pleomorphism. In sputum it usually occurs as clusters of coccobacillary forms. The bacilli are relatively difficult to stain. Staining for 5-15 minutes with Leoffler's methylene blue or dilute carbol fuchsin gives good results.

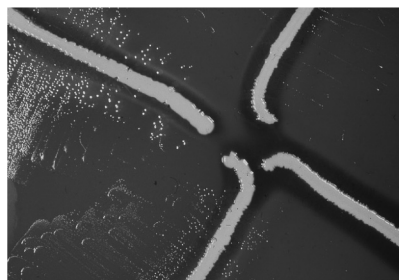


Fig. 29.1



Notes

29.3 CULTURAL CHARACTERISTICS

The bacillus has fastidious growth requirement. Growth factors namely X & V, present in blood are essential for growth. It is aerobic but grows anaerobically also. The optimum temperature is 37°C and does not grow below 20°C. When staphylococcus aureus is streaked across a plate of blood agar on which a specimen containing H influenza has been inoculated after overnight incubation, the colonies of H. influenzae will be large & well developed alongside the streak of staphylococcus and smaller farther away. This phenomenon is called satellitism and demonstrates the dependence of H. influenzae on v factor which is high near staphylococcal growth. This is a routine test in clinical bacteriology for identification of H. influenzae

When blood agar is heated to 80-90°C, or boiled for a few minutes, the V factor is released from within erythrocytes & hence these media are superior to blood agar for growing H. influenzae. Clear transparent media may be prepared by boiling & filtering a mixture of blood & nutrient broth or by adding a peptic agar is best for primary isolation of H. influenzae and gives a copious growth. Capsulated strains produce translucent colonies with a distinctive iridescence on Levinthal's agar.



INTEXT QUESTIONS 29.1

1. H. influenzae is small gram bacilli
2. H. influenzae exhibits characteristic
3. Hemophilus means organism
4. staining is commonly used in identification of Haemophilus
5. phenomenon is seen in Haemophilus
6. Streaks of enhances the growth of haemophilus

29.4 BIOCHEMICAL REACTIONS

Glucose and xylose are fermented with acid production but not lactose, sucrose and mannitol. Catalase and oxidase reactions are positive. Nitrates are reduced to nitrites.

29.5 RESISTANCE

H. influenzae is a delicate bacterium, destroyed by heating at 55°C for 30 minutes and refrigeration at 0-4°C, drying and disinfectants. In culture, the cells

die within two or three days due to autolysis. Cultures may be preserved for about a month on chocolate agar slopes in screw capped bottles.

29.6 ANTIGENIC PROPERTIES

Capsular polysaccharide, outer membrane protein and lipooligosaccharide are the major surface antigens. The major antigenic determinant of capsulated strains is the capsular polysaccharide based on which H. influenzae strains have been classified with six capsular types a to f. Capsular typing is of medical importance as major acute invasive infections belong to type b. The type capsular polysaccharide has a unique chemical structure, containing the pentose sugars ribose and ribitol instead of the hexoses and hexosamines as in the other five serotypes. H. Influenzae strains lacking a capsule cannot be typed and are called nontypable strains. Next to Hib, the nontypable strains are the most relevant in clinical infections.



Notes

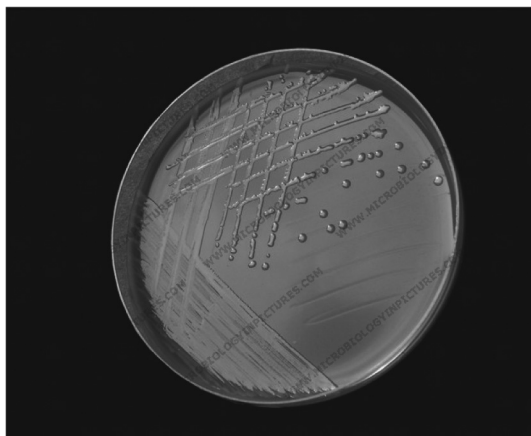


Fig. 29.2: Haemophilus influenzae



INTEXT QUESTIONS 29.2

1., & are the major surface antigens of haemophilus
2. Common infections caused by invasive haemophilus are, &
3. Common infections caused by non invasive haemophilus are, &
4. Capsulated stains caused infections



Notes

29.7 PATHOGENECITY

H. influenzae is an exclusively human pathogen. Diseases due to *H. influenzae* are invasive and noninvasive. In invasive the bacillus acts as a primary pathogen, causing acute invasive infections. The bacilli spread through blood, being protected from phagocytes by their capsule. Haemophilus meningitis is the most important infection in this group, and others are laryngoepiglottitis, conjunctivitis, Bacteremia, pneumonia, arthritis, endocarditis and pericarditis. These are most commonly seen in children and mostly capsulated and type b antigen strain.

In noninvasive the bacillus spreads by local invasion along mucosal surfaces and causes secondary or superadded infections, usually of the respiratory tract. These are otitis media, sinusitis and exacerbations of chronic bronchitis and bronchiectasis. These are usually seen in adults and are often caused by noncapsulated strains.

29.8 LABORATORY DIAGNOSIS

In meningitis, presence of pleomorphic in CSF, and Gram-negative bacilli that do not stain well are suspicion of *H. influenzae* infection. The capsular polysaccharide antigen may be present in the CSF in meningitis and in urine in systemic infection. Its demonstration by latex particle agglutination or CIE is useful in diagnosis.

For isolation, CSF should be plated promptly on blood agar or chocolate agar and incubated in an environment of 5-10 percent of CO₂ and high humidity. The specimen should not be refrigerated before inoculation as the bacillus is very sensitive to low temperatures. A strain of staphylococcus should be streaked across the plate. After overnight incubation at 37°C, small opaque colonies appear that show satellitism. Iridescence may be demonstrated on Levinthal's medium. Typing may be done if antisera are available.



WHAT HAVE YOU LEARNT

- Haemophilus means blood loving organisms.
- Haemophilus are non-motile, non-sporing, oxidase positive, pleomorphic, gram negative bacilli
- Haemophilus can be Stained with Leoffler's methylene blue or dilute carbol fuchsin
- Growth factors namely X & V, present in blood are essential for growth
- They demonstrate satellitism



TERMINAL QUESTIONS

1. What is the morphology of haemophilus
2. What are the cultural characteristics of haemophilus
3. Describe the laboratory diagnosis of haemophilus



ANSWERS TO INTEXT QUESTIONS

29.1

1. Negative
2. Pleomorphism
3. Blood loving
4. Leoffler's methylene blue
5. Satellitism
6. Staphylococcus aureus

29.2

1. Capsular polysaccharide, outer membrane protein and lipooligosaccharide
2. Haemophilus meningitis, laryngoepiglottitis, conjunctivitis, Bacteremia
3. Otitis media, sinusitis and exacerbations of chronic bronchitis and bronchiectasis
4. Invasive infections

