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Haemophilus Influenzae Surveillance and Its Clinical Laboratory Diagnosis

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ABSTRACT

Haemophilus influenzae is an aerobic pleomorphic gram-negative coccobacillus that requires both X and V factors for growth. It grows poorly, if at all, on ordinary blood agar unless streaked with Staph. aureus. It grows well on chocolate agar. Because this medium is often not used in culturing specimens from adults and because the organism may be overgrown by other bacteria, the frequency of H. influenzae infections has undoubtedly been seriously underestimated. This is aggravated by the failure of many physicians to obtain blood cultures in suspected bacterial infections and the failure of many laboratories to subculture them routinely onto chocolate agar. H. influenzae, along with Streptococcus pneumoniae, is a major factor in acute sinusitis. It is probably the most frequent etiologic agent of acute epiglottitis. It is probably a common, but commonly unrecognized, cause of bacterial pneumonia, where it has a distinctive appearance on Gram stain. It is unusual in adult meningitis, but should particularly be considered in alcoholics; in those with recent or remote head trauma, especially with cerebrospinal fluid rhinorrhea; in patients with splenectomies and those with primary or secondary hypogammaglobulinemia. It may rarely cause a wide variety of other infections in adults, including purulent pericarditis, endocarditis, septic arthritis, obstetrical and gynecologic infections, urinary and biliary tract infections, and cellulitis. Antimicrobial susceptibility testing is somewhat capricious in part from the marked effect of inoculum size in some circumstances. In vitro and in vivo results support the use of ampicillin, unless the organism produces beta- lactamase. Alternatives in minor infections include tetracycline, erythromycin, and sulfamethoxazole-trimethoprim. For serious infections chloramphenicol is the best choice if the organism is ampicillin-resistant or the patient is penicillin- allergic.

INTRODUCTION

The genus haemophilus contain small, non motile, non sporing, oxidase positive, pleomorphic, Gram negative bacilli that are parasitic on human beings and animals. They are characterized by their requirement of one or both of two accessory growth factors (x and v) present in blood.

Haemophilus influenzae is the first free living organism whose complete genome has been sequenced.

Pfeiffer in 1892 observed that a small Gram negative bacillus was constantly present in the sputum of patient from the influenza pandemic of 1882 -92 and mistakenly proposed this as a causative agent of human influenza . This came to be known as the influenza bacillus or (pfeiffer's bacillus) , later renamed haemophilus influenzae .

Haemophilus influenzae Clinical case

A six month old child was brought to the pediatrics outpatient department with the chief complaint of fever , inability to feed and seizures since the morning . On examination the child was found to have altered sensorium and neck rigidity A lumbar puncture was carried out and CSF sent for cytology , biochemistry and microbiological examination. The cytology and biochemistry results were suggestive of pyogenic meningitis. microscopic examination revealed the presence of polymorphs along with gram negative coccobacilli antigen detection by latex agglutionation was positive for haemophilus influenzae type b and the culture on the next day showed a growth on chocolate agar identified as H. Influenza the child responded to treatment with ceftriaxone.

HAEMOPHILUS INFLUENZAE MORPHOLOGY

Haemophilus influenzae is a small (1.0 × 0.3 Micron meter) , gram negative , non motile , non sporing bacillus , exhibiting considerably pleomorphism . In sputum, it usually occurs as clusters of cocco bacillary forms , while in csf from meningitis cases , long , bacillary and filamentous forms predominate . Cells from young cultures (18-24hours) are usually coccobacillary, while older cultures are distinctly pleomorphic. Strains isolated from acute infections are often capsulated.Cultural Characteristics of Haemophilus Influenzae

H. Influenzae needs fastidious growth requirements. Factors X and V are essential for growth. X is hemin heat stable and porphyrins for the synthesis of cytochromes. V factor is coenzyme nicotinamide adenine dinucleotide (NAD) phosphate acts as a hydrogen acceptor. It grows well on chocolate agar and it is a non-selective, enriched growth medium that is the lysed blood agar. The name of agar is for its color when the red blood cells (RBCs) lysis gives the medium a chocolate-brown color without having chocolate products. It uses for the isolation of fastidious bacteria, such as haemophilus *influenzae* when

incubated at 35-37°C in a 5% CO_2 incubator. *Haemophilus influenzae* shows opaque cream to gray colonies.*H. influenzae* in a Gram stain of a sputum sample appears as Gram-negative coccobacilli. *Haemophilus influenzae* requires X and V factors for growth and therefore, it grows only around the paper disk that has been impregnated with X and V factors on non-selective and non-enriched media like nutrient agar or MHA. There is no bacterial growth around the disk that only contains either X or V factor but may be in between X and V disks as shown above picture. It also shows satellitism.

Resistance

It is a delicate organism and it is readily killed by heating at 55°C for 30 minutes. Refrigeration, drying, and disinfectants destroy the organisms. For long-term preservation, the culture may be lyophilized.

Antigenic Structures of Haemophilus Influenzae

It contains 3 major surface antigens and they are-

1. Capsular polysaccharide
2. Outer membrane proteins (OMP)
3. Lipopolysaccharides (LPS)

Pathogenicity

A human pathogen can produce invasive and non-invasive lesions. The prominent organism is producing meningitis. It can produce laryngoepiglottitis, conjunctivitis, bacteremia, pneumonia, arthritis, endocarditis and pericarditis. Most strains are opportunistic pathogens. Most strains of *H. influenzae* are opportunistic pathogens; that is, they usually live in their host without causing disease, but cause problems only when other factors (such as a viral infection or reduced immune function) create an opportunity.

Laboratory Diagnosis of Haemophilus Influenzae

Specimens: It depends on the site of infection, the following specimens may be collected-

- Sputum
- Csf
- Throat Swab
- Pus

Aspirates from joints, middle ears, or sinuses

Collection and Transport of specimens

Specimens should be collected in sterile conditions and under all aseptic conditions.

Microscopy

Gram stain : Gram-stained smear of CSF in meningitis shows pleomorphic long and filamentous form predominate while in sputum coccobacillary forms.

Immunofluorescence and quellung reaction: For a direct demonstration of H. influenzae after mixing with specific tube b antiserum.

Antigen detection: Type b capsular antigen can also be detected in patient serum, CSF, urine, or pus by following methods-

MATERIAL METHODS

Sputum culture

Colony Morphology and Staining

Serotyping: It may be performed with type-specific antisera as shown abovepicture.

Treatment

Following antimicrobial drugs are useful to treat and they are-

- Cefotaxime
- Ceftazidime
- Ampicillin
- Contrimixazole
- Amoxycillin with Clavulanate
- Clarithromycin

Epidemiology and Prevention

1. Similar to Pneumococci
2. The infection enters through the respiratory tract
3. Immunity is type-specific
4. HIB is protected by the PRP vaccine
5. Poorly immunogenic in children below 2 years

6. Rifampicin can be given for 4 days and prevents secondary infectionand eradicates carrier state.

RESULTS In conclusion, Total no of samples taken = 83 Total no of positive patients = 24 Total no of negative patients = 59 Positive percentag=28.9% Negative percentage = 71.08%

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