Diagnostic Microbiology

Lecture: 9
Diagnosis of Bacterial Infections

Clinical Diagnosis

Non-Microbiological investigations

Hematology
Biochemistry

Take the Correct Specimen
Lab & Package the Specimen up Correctly

Appropriate Transport & Storage of Specimen
Bacterial identification flow chart

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Cocci

Gram (+)

Catalase (+), irregular clusters, tetrads

- Strictly aerobic: Micrococcus
- Facultative anaerobic: Staphylococcus Planococcus

Gram (-)

Catalase (-), pairs, chain arrangement

- Aerobic, oxidase (+), catalase (+): Neisseria Branhamella Moraxella
- Anaerobic, oxidase (-), catalase (-): Veillonella

Rods

Gram (+)

Sporeformer

- Acid-fast: Bacillus Clostridium
- Not acid-fast: Mycobacterium Nocardia Lactobacillus Listeria

Non-sporeformer

- Regular: Corynebacterium Propionibacterium
- Pleomorphic: Pseudomonas Alcaligenes

Gram (-)

Aerobic oxidase (+)

- Facultative anaerobic, oxidase (-) (ferment glucose): Escherichia Enterobacter Citrobacter Proteus Salmonella Erwinia

Motile: Shigella Klebsiella

Nonmotile:
Diagnosis of Bacterial infections

**COLLECTION OF SPECIMENS (Sample):**

1. Determine agents of the disease.
2. Choose the appropriate specimen.
3. Obtain specimen properly, avoiding contamination.
4. Transport quickly to lab.
5. Store properly
6. Provide all information needed by lab. Staff.
**BACTERIOLOGICAL Diagnostic METHODS:**

**Isolate identification by:**

- Microscopical examination
- Specimen culture
- Naked eye examination (Culture characteristics)
- Growth on selective media
- Biochemical reactions.
- Serological tests to detect antibodies or antigens
- Sensitivity testing of isolate.
- Isolate typing for epidemiological studies, e.g.: phage typing.

**It should be:**
Sensitive and specific
- Rapid
- Easy to perform, not labor intensive
- Data easy to interpret
- Widely available
- Cost effective
- Automation high-throughput analysis
- Upload of the results
Traditional bacterial identification

**Phenotypic identification** (gram stain- culture characteristics- antibiogram- biochemical methods- fully or partly automated identification methods (Vitek, Phoenix,...)

- **Cultivation** (Pure cultures- Unique characteristics- Highly related species cannot be phenotypically differentiated- Corresponding databases are often limited, less accurate identification)

In the last 15 years molecular and chemotaxonomic methods have proven beneficial in overcoming some of these limitations

- **Identification of bacteria**: Comparison of microbial identification methods
  - traditional methods
  - molecular methods
  - chemotaxonomic methods
  - Database identifications

- **Molecular methods** (DNA and RNA present in all bacteria- whole genome- Sensitive and specific- Directly on specimen samples (blood, sputum,...)- PCR, PFGE, fingerprint methods- Ribotyping, microarrays, oligonucleotide probes,...)

- **Chemotaxonomic methods** (Classify organisms based on differences and similarities in chemical markers (cell wall constituents, lipids, whole cell proteins- Chemotaxonomic fingerprints- SDS-PAGE of whole cell proteins, spectroscopy)
microscopy
unstained or stained with e.g. Gram stain

culture
Stain Decolorize Counter stain
identification by biochemical or serological tests on pure growth from single colony

sensitivities
on plates or in broth by disc diffusion methods, breakpoints or MICs

Sero diagnosis
DNA technologies
**BLOOD CULTURE:**

1. **Diseases suspected:**
   - septiceamia, endocarditis, osteomyelitis, meningitis, pneumonia, enteric fever, brucellosis, etc.

2. **Organisms suspected:**
   - *Staphylococcus aureus*, *Streptococcus pneumoniae*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Brucella*, etc.

3. **Collect at least:**
   - 10 ml. blood every 24 hours, because bacteria in blood are scanty and intermittent.

4. **Collect blood:**
   - aseptically to avoid contaminants.

5. **The blood culture bottle obtained from the lab. must contain**
   - 100 ml. of a suitable growth medium, e.g.: brain-heart infusion broth.

6. **In the lab.** blood culture bottles are checked daily for turbidity up to 21 days.
7. Bottle is subcultured after 24 hrs., 72 hrs, one week, 2 weeks, and 3 weeks.

   Standard media used for subculture are: blood agar, MacConkey, chocolate agar, Sabouraud agar, etc.

8-Identification of isolate is by standard methods, and sensitivity tests are performed.

9. If no growth after three weeks, discard bottle. Bottles are automatically tested every 10 minutes. Positive results are tagged for quick processing. Negative bottles can be batch-scanned out of the system and unloaded at the end of protocol.
THROAT CULTURE:

1. Mainly used to isolate β-haemolytic *Streptococcus pyogenes* that cause pharyngitis. Requested to diagnose diphtheria, gonorrhea, & candidosis.

2. Swab posterior pharynx, tonsils, & tonsillar fossae.

3. Swab is inoculated on B.A. and bacitracin disc is added. Then incubate for 18-24 hrs at 37°C.

4. Colonies of Lancefield group (A) *S. pyogenes* are β-haemolytic and bacitracin sensitive.
1. Performed to diagnose pneumonia, Tuberculosis (TB), lung abscess.
2. Sputum must be real not saliva.
3. Gram stain will show if it is saliva or not. Good sputum shows (25) leucocytes and less than (10) epithelial cells per 100x field.
4. If the patient cannot cough you may choose:
   a) Induction of sputum.
   b) Bronchial lavage.
   c) Lung biopsy.
5. Do gram stain to assess cause of pneumonia (large numbers of organisms).
6. Culture is made on Blood agar and other selective media. Identify by serological and biochemical tests.
7. Mycoplasma is diagnosed by antibody rise on serology. TB is diagnosed by acid fast stain and culture on selective media (Lowenstein Jensen medium).
SPINAL FLUID CULTURE:

1. CSF is collected to diagnose meningitis, encephalitis, brain abscess.

2. Causes of meningitis are:
   (3 encapsulated organisms) *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*.

3. Send specimen immediately to laboratory. Gram stain may give a presumptive diagnosis.

4. Identification is made by antisera and capsule swelling reaction, and immunofluorescence.

5. Culture is on Blood Agar & chocolate agar. Incubate plates at 35°C in 5% CO₂.

6. *Mycobacterium tuberculosis* (causes TB) & *Cryptococcus neoformans* (yeast) cause subacute meningitis. Ziehl Neelsen stain (acid fast stain) is made to identify *M. tuberculosis*. *Cryp. neoformans* capsule may be detected by India Ink staining.

7. Heamatin (X-factor) and NAD (V-factor) may help in identification of *H. influenzae*.

8. Serological tests (latex agglutination) are used to identify organism causing meningitis.
**STOOL CULTURE:**

1. Pathogenic organisms are  
   Shigella, Salmonella, Campylobacter.

2. Stool general may reveal:  
   a) Leukocytes and pus cells by methylene blue stain.  
   b) Gram stain is not performed.

3. Culture on MacConkey & Eosin-methylene blue, & other selective media. Identify by biochemical reactions and antisera. Widal test is made for enteric fever.

4. *Campylobacter jejuni* is cultured on selective Skirrow agar at 42°C in 10% CO₂.
URINE CULTURE:

1. Performed to diagnose pyelonephritis & cystitis.
   Organisms isolated are:
   **E. coli, Proteus, Enterobacter, Enterococcus faecalis, Pseudomonas, Klebsiella**

2. Midstream, morning urine sample is collected after washing external orifices. Catheterization may also be used for urine collection.

3. If there is delay culture urine within one hour after collection, or store at 4°C for no more than 18 hrs.

4. Bacterial urine counts are made by inoculating the sample on MacConkey agar using a (1µl) loop. Then multiply number of colonies by 1000 (10³).

@ Count interpretation:
  a) For symptomatics:
     significant count is \(100 \times 10^3\) (100,000) colonies/ml.
  b) For asymptomatics:
     significant count is only 100 colonies /ml.
GENITAL TRACT CULTURE:

1. Performed to diagnose gonorrhea caused by *Neisseria gonorrhoeae*, using culture and microscopical examination.

2. Discharge is swabbed from urethra, cervix, & anal canal. It is inoculated quickly on Thayer-Martin chocolate agar, or transported in transgrow or Stuart media (media for transport *N. gonorrhoeae & N. meningitides*).

3. *N. gonorrhoeae* is identified microscopically as gram negative intracellular, diplococci within the pus (neutrophil) cell.

4. *Chlamydia trachomatis* cultured on yolk sac of chick embryo or human tissue culture.

5. Syphilis caused by *Treponema pallidum* is seen by Dark Field microscopy of a chancre fluid. Syphilis is diagnosed by non-specific or specific serological tests.
WOUND AND ABSCESS CULTURES:

1. Abscess is caused by *Bacteroides, Staphylococcus aurous, Streptococcus pyogenes*. Wound infections are due to *Clostridium perfringes, S. aurous, Pasteurella multocida*.

2. Swab is transported immediately to lab. in thioglycolate.

Several aerobic and anaerobic media are inoculated.