Chap. 5: Identification of main human pathogenic bacteria

Learning objectives

- Step by step identifying procedures
- Aerobic gram-positive and gram-negative cocci
- Facultative anaerobic gram-negative bacilli
- Microaerophile gram-negative bacilli
- Aerobic gram-negative bacilli
- Anaerobic cocci gram-positive
- Spirochetes
- Mycobacterium
- Intracellular bacteria
- Others....
1/ step by step identifying procedures

a) samples managements

- To diagnose a disease, it is often necessary to obtain a sample of material that may contain the pathogenic microorganism.

- Samples must be taken more aseptically than possible and specifically those from aseptic sites.

- The sample container should be labeled with the patient’s name, room number (if hospitalized), date, time, and medications being taken.

- Samples must be transported to the laboratory immediately for to be managed. Indeed, delay in transport may result in the growth of some microorganisms, and their toxic product may kill other organism.
### Microbiology Requisition

<table>
<thead>
<tr>
<th>Gram Stain Report</th>
<th>Source of Specimen</th>
<th>Test(s) Requested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram POS. cocci, groups</td>
<td>Blood</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Gram POS. cocci, pairs/chain</td>
<td>CSF</td>
<td>Routine culture: Gram stain, anaerobic culture, susceptibility testing. Throat swabs done for Gp A Strept.</td>
</tr>
<tr>
<td>Gram POS. rods</td>
<td>Throat</td>
<td>Legionella culture</td>
</tr>
<tr>
<td>Gram NEG. cocci</td>
<td>Sputum, expectorated</td>
<td>Bartonella (Rochalimaea)</td>
</tr>
<tr>
<td>Gram NEG. rods</td>
<td>Other Respiratory: (Describe)</td>
<td>Blood Culture</td>
</tr>
<tr>
<td>Gram NEG. cocco/bacilli</td>
<td>URINE, Clean Catch Midstream</td>
<td>Other Non-Routine Cultures</td>
</tr>
<tr>
<td>Yeast</td>
<td>URINE, Indwelling Catheter</td>
<td>E. coli 0157:H7</td>
</tr>
<tr>
<td>Other</td>
<td>URINE, Straight Catheter</td>
<td>Vibrio</td>
</tr>
<tr>
<td></td>
<td>URINE, Entire First Morning</td>
<td>Yersinia</td>
</tr>
<tr>
<td></td>
<td>URINE, Other: (Describe)</td>
<td>H. ducreyi</td>
</tr>
<tr>
<td></td>
<td>STOOL</td>
<td>B. pertussis</td>
</tr>
<tr>
<td></td>
<td>GU (Specify Source): _vag.</td>
<td>Screening Cultures</td>
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<tr>
<td></td>
<td>ABSCESS (Specify Source)</td>
<td>Gonococci</td>
</tr>
<tr>
<td></td>
<td>TISSUE (Specify Source)</td>
<td>Group B Strept</td>
</tr>
<tr>
<td></td>
<td>ULCER (Specify Source)</td>
<td>Group A Strept</td>
</tr>
<tr>
<td></td>
<td>WOUND (Specify Source)</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>STERILIZER TEST</td>
<td>Acid-fast bacilli</td>
</tr>
</tbody>
</table>

- **Bacterial**
- **Fungal**
- **Viral**
- **Parasitology**
- **Toxin Assay**
- **Direct (Antigen Detection)**
- **Special**

*filled out by one person
*filled out by different person

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**Figure 10.7**: A clinical microbiology lab report form. In health care, morphology and differential diagnosis are vital for identifying the causative organism.
Pathogens tend to be fastidious and die if not kept in optimum environmental conditions.

In laboratory, samples from infected tissues are cultured on differential, usual or selective media in an attempt to isolate and identify any pathogen or organism that are not normally found in association with that tissue.

In laboratories, health workers including students, whose activities involves contact with patients or with blood or other body fluids, must take universal precautions and adhere to guidelines that will minimize the risk of transmitting HIV and AIDS, and the transmission of all nosocomial infections.

They must involve good laboratory practices and be concern on biosafety issues.
b) methods for identifying bacteria

Procedures commonly used are:

- Specimen processing
- Morphological characteristics determination, aided by various staining
- Biochemical testing
- Serological tests
- Antimicrobial susceptibility profiles
- Molecular biology tests
- Phage typing
Processing specimens for recovery of bacteria deals with:

= proper specimen selection, collection and transport
= rejection of not correctly collected or transported specimen
= that specimen must be representative of the disease process.
= dilution, concentration, homogenization or decontamination of certain specimens
= use of usual, differential, enriched or selective medium for the isolation of bacteria
= culture incubation at optimal temperature (normally at 35 to 37º C) and in a variety of atmospheric conditions including: CO2-enrichment, aerobic, microaerophilic and anaerobic.
Morphological characteristics are useful in the identification of microorganisms, especially when aided by differential staining techniques. We can use:

= direct non-staining slide smear; for example to examine fresh urinary collection and quantify on a Mallassez slide the number of pathogen and cells in it

= darkfield microscope for spirochetes

= smear staining with Gram, methylene blue, Giemsa, Ziehl-Neelsen (for acid-fast bacteria), direct fluorescence or Vago stain, Chinese ink for negative-staining of the capsule
Urinary collection

Light microscope

Staphylococcus gram-positive cocci

arranged in heap

Vago stain of spirochetes
Malassez cell

Giemsa stain of spirochetes

Chinese ink and Gram negative-staining of capsules
There are many sizes and shapes among bacteria; most bacteria range from 0.2-2 um in diameter and from 2-8 um in length.

- **Bacillus (rodlike), coccus (spherical or ovoid) and spiral (corkscrew or curved)** are the most common shapes, but some are star-shaped or square.
Biochemical testing

= the presence of various enzymes, as determined by biochemical tests is used in identifying microorganisms such as:

= *Staphylococcus aureus* is alone **coagulase-positive** among all species of the genus

= *Streptococcus pyogenes* is a β hemolytic (that it completely hemolyse red blood cell of the blood agar) while *Streptococcus pneumoniae* doesn’t completely hemolyse so is α hemolytic

= most facultative anaerobic enteric gram-negative bacilli have the same shape (pink rod like) and must be identified by biochemical tests

Serological tests, involving the reactions of the microorganisms with specific antibodies, are usefully in determining the identity of strains and species.

= Indirect Immuno fluorescence, ELISA, Western-Blotting, agglutination or hemagglutination are some currently used serological assays
Figure 10.10 A slide agglutination test. (a) In a positive test, the red blood cells clump together. (b) In a negative test, the red blood cells remain separate.

TECHNIQUE ELISA = IMMUNODETECTION DES ANTICORPS

1. Sérum patient (Anticorps) → fixation spécifique des anticorps
   LAVAGES

2. Antigène viral
   → antiglobulines marquées par une enzyme
   LAVAGES

3. Addition du substrat de l'enzyme → réaction colorée
If Lyme disease is suspected in a patient, a sample of the patient's serum is taken. Electrophoresis is used to separate proteins in the serum sample. Each band consists of many molecules of a particular protein (antigen). The bands are not visible at this point.

The bands are transferred to a nitrocellulose filter by blotting.

The filter with proteins (antigens) is positioned exactly as it was on the gel. The filter is then washed with antibodies against a particular antigen. The antibodies are tagged with a dye so that they are visible when they combine with their specific antigen (shown here in purple).

The test is read. If the tagged antibodies stick to the filter, evidence of the presence of the microorganism in question—in this case, *Borrelia burgdorferi*—has been found in the patient’s serum.

**Figure 10.12** The Western blot. Proteins separated by electrophoresis can be detected by their reactions with antibodies.

Name two diseases that may be diagnosed by Western blotting.
**Antibacterial susceptibility tests**

=antibacterial susceptibility testing may be performed reliably by either *dilution* or *diffusion method*

=dilution susceptibility testing methods are used to determine the minimal inhibitory concentration or MIC, usually in microgram per milliliter, of an antimicrobial agent required to inhibit or kill a microorganism.

=MIC can be usefully for evaluating relative degrees of susceptibility of bacteria to various antimicrobial agents and for comparing the relative activities of drugs against various species

=disk diffusion method of susceptibility testing allows categorization of bacteria isolates as *susceptible (or sensitive), resistant or intermediate* to a variety of antimicrobial agent.
The three interpretative categories are defined as follows.

**susceptible** indicates that an infection caused by the tested microorganism may be appropriately treated with the usually recommended dose of antibiotic.

**intermediate** indicates that the isolate may be inhibited by attainable concentration of certain drugs (e.g., beta-lactams) if higher dosages can be used safely or if the infection involves a body site in which the drug is physiological concentrated (e.g., the urinary tract).

**resistant isolates** are not inhibited by the concentration of antimicrobial agent normally achievable with the recommended dose and/or yield results that fall within a range in which specific resistance mechanisms are likely to be present.
Measurement of the CMI which is 4mg/l

E-test is performed on a plastic-coated strip contains a gradient of antibiotic concentrations, and the MIC can be read from a scale printed on the strip.
Resistant strain to ticarcillin and amoxicillin by synthesizing of penicilinases

Sensitive strain to β lactamines
Molecular biology tests are now commonly used such as:

- PCR for polymerase chain reaction that increase the amount of bacterial DNA to levels that can be detected and quantified
- Southern-Blotting, probes hybridization assays of the bacterial DNA
- Bacterial GC% determination by sequencing of the bacterial DNA

Phage typing which is the identification of bacterial species or strains by determining their susceptibility to various phages

According with disease and available laboratory facilities, diagnostics of infectious disease involve direct or indirect (serological) procedures.
PCR: Polymerase Chain Reaction

30 - 40 cycles of 3 steps:

**Step 1**: denaturation
- 1 minute 94 °C

**Step 2**: annealing
- 45 seconds 54 °C
- forward and reverse primers !!!

**Step 3**: extension
- 2 minutes 72 °C
- only dNTP's

(Andy Vierstraete 1999)
2/ Aerobic gram-positive and gram-negative cocci

- Most gram-positive cocci of medical importance are members of the genera *Staphylococcus* and *Streptococcus- Enterococcus*

- Gram-positive cocci are grouped into two families of:
  - *Micrococcaceae*;
  - *Streptococcaceae*.

- *Micrococcaceae* are strict aerobes or aero - anaerobes and catalase +.
- *Sterptococcaceae* are aero - anaerobes and catalase -.
In tube catalase-positive test

On slide catalase-positive test
### a) Classification of gram-positive cocci

<table>
<thead>
<tr>
<th>Positive Catalase</th>
<th>Family of the Micrococcaceae</th>
<th>Bacteria oxydative or strict aerobic = Bacteria fermentative or aero - anaerobic</th>
<th><em>Micrococcus</em> genera = <em>Staphylococcus</em> genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Catalase</td>
<td>Family of Streptococcaceae</td>
<td>Bacteria fermentative or aero- anaerobic</td>
<td><em>Streptococcus</em> genera and <em>Enterococcus</em></td>
</tr>
</tbody>
</table>

= Members of the genus *Staphylococcus* are gram-positive cocci (0.5 to 1.5 um in diameter), non-motile, non-spore forming, and usually non encapsulated that occur irregular grapelike clusters.

They have a DNA G+C content of 30 to 39 mol%
members of the genus *Micrococcus* are aerobic gram-positive cocci (1 to 1.8 um in diameter), occurring mostly in pairs or tetrads. Micrococci are widespread in nature and are commonly found on the skin of human and other mammals. Micrococci are found less frequently than staphylococci and are generally regarded as saprophytes rather than as opportunistic pathogens. Members of the genus *Micrococcus* have a G+C content within the range of 66 to 75 mol%.

members of the genus *Streptococcus* are gram-positive cocci, catalase-negative facultative aerobic bacteria forming chains or in pairs (*Streptococcus pneumoniae*) spherical or ovoid cells less than 2 um in diameter.
b) *Staphylococcus* genera

- Members of this genera are facultative anaerobes, but they usually grow much better aerobically on usual media or selective ones.

- All species of this genera appear as spherical Gram + (violet) cocci arranged in heap or grapelike clusters.

- Three main species are of medical importance:
  - *Staphylococcus aureus*, named for its yellow-pigmented colonies (*aureus* means gold), is the most important.
  - *Staphylococcus epidermidis*, is applied now to the predominant species from human skin microbiota.
  - *Staphylococcus saprophyticus* has a predilection for causing urinary tract infections in young, sexually active women.
For almost all clinical purposes, bacteria of *Staphylococcus* genera can be divide into those that produce *coagulase*, an enzyme that coagulates (clots) fibrin in blood, and those that do not: *coagulase-positive* and *coagulase-negative* strains.
- **Coagulase-negative strains**, mainly from *Staphylococcus epidermidis*, are very common on the skin, where they may represent 90% of the normal microbiota.

- Those coagulase-negative strains are generally pathogenic only when the skin barrier is broken or is invaded by medical procedures, such as the insertion or removal of catheters.

- *Staphylococcus saprophiticus* are mainly responsible of young ladies urinary tract infections and cystitis.

- *Staphylococcus aureus* is the most pathogenic of the staphylococci. Typically, it forms golden yellow colonies. Almost all pathogenic strains of *S. aureus* are *coagulase-positive*. They produce leukocidin, which destroys phagocytic leukocytes, and *exfoliative toxin*, which is responsible for scalded skin syndrome. They also produce *enterotoxin* which affects the gastrointestinal tract and is responsible for food intoxications.
some strains cause a toxic shock syndrome when a staphylococcal toxin **TSST-1** (for toxic shock syndrome **toxin-1**) enters in the bloodstream

- *Staphylococcus aureus Methicillin Resistant* or *SAMR* strains are main strains involving in nosocomial infectious

- At one time, this organism was almost uniformly extremely susceptible to penicillin, but now only about 10% of *S. aureus* strains are sensitive. Except SAMR strains, normally it’s sensitive to oxacillin, cloxacillin, methicillin and cephalosporin
Before any treatment we must test its susceptibility to antimicrobial agents

- For isolation of *S. aureus* we can use **selective medium of Chapman** and all colonies on this media must be coagulase-positive that is the discrimination test for *S. aureus* identification

- *S. aureus* is causative agent of many infectious diseases as showed on the following figure

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Staphylococcus aureus ‘s diseases and syndromes
Yellow colonies of *S. aureus*

White colonies of *S. epidermidis*

Coagulase tests
c) Members of genus Streptococcus

- Members of the genus *Streptococcus* are spherical or ovoid (cocci) gram-positive bacteria.

- Streptococci typically appear in chains that can contain a few as 4-6 cocci or many as 50 or more.

- One species, *Streptococcus pneumoniae*, is usually found only in pairs such as diplococcus surrounded by a capsule. This species is the causative agent of pneumonia and bacterial purulent meningitis.

- Streptococci do not use oxygen, although most are aerotolerant. A few may be obligatory anaerobic.

- Some strains of *Streptococcus pneumoniae* and certain viridans species require elevated (5%) CO2 level for growth.
Streptococci are nutritionally fastidious, with variable nutritional requirements, and growth on complex media is enhanced by the addition of blood or serum.

The traditional rules of streptococcal taxonomy, hemolytic reaction and Lancefield serological test can still be used as a first step in identification of clinical isolates.

One basis of classification and identification of streptococci is their action on blood agar:

- **alpha hemolytic** species reduce hemoglobin to methemoglobin that causes a greenish zone to surround the colony. They are, also called “viridans”
- **beta hemolytic** produces an hemolysin that forms, around colonies, a clear zone of hemolyse on blood agar
- **gamma hemolytic** species have no apparent effect on red blood cells. These species are non-hemolytic.
Gram-positive encapsulated diplococcus:

*Streptococcus pneumonia*
Antigens of *Streptococcus pyogenes* of the A group Lancefield classification based on the polysaccharide C surrounded the cell wall
Four criterions are used to identify streptococci species;

- direct detection of streptococci by gram stain that is most useful

- hemolytic reaction: alpha, beta, or gamma

- direct detection of streptococcal antigen mainly of Lancefield classification. Antigen are detected by direct agglutination, latex agglutination assays and enzyme immunoassay methods


- various physiological tests
Species of medical importance are:

1/ Beta hemolytic, bacitracin susceptibility (an antibiotic), PYR test positive (which determine the activity of pyrrolidonyl aminopeptidase an enzyme), large colony-forming with Lancefield group A antigen are include in the species of *Streptococcus pyogenes* and represent one of the impressive human pathogen.

*Streptococcus pyogenes* causes a wide array of serious infections including pharyngitidis, respiratory infection, skin (impetigo, erysipelas) and soft tissues infections, endocarditis, meningitis, puerperal sepsis and arthritis.
β hemolyse colonies

On left sensitive bacteria to bacitracine; on rigth resistant bacteria ones

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2/ Beta hemolytic streptococci, which hydrolyze hippurate, are positive-CAMP-test, classified in Lancefield's group B involve *Streptococcus agalactiae*. Those species are an important cause of serious neonatal infection characterized by sepsis and meningitis. They Colonization of the maternal genital tract is associated with colonization of infants and risk of neonatal disease.

CAMP test showing wing-shaped hemolysis of group B streptococci accentuated by a beta-hemolytic strain of *Staphylococcus aureus* runs vertically.
3/ *Streptococcus pneumoniae* is an alpha-hemolytic streptococci.

- It’s an important agent of community-acquired pneumonia. Other pneumococcal infections include otitis media, sinusitis, meningitis, and endocarditis.

- Oropharyngial carriage of pneumococci is common and contributes to the difficulty in interpreting the significance of pneumococci in cultures of expectorated sputum.
4/ Others medical important non-beta hemolytic *Streptococci* are:

*[viridans streptococci]* that are normal inhabitants of the oral cavity, gastrointestinal tract, and female genital tract. Among them, *S. sanguis*, *S. mitis*, *S. oralis* cause endocarditis especially in patients with prosthetic valves

*S. bovis (Lancefield group D)* which are agents of endocarditis

- For Identification of non-beta-hemolytic Streptococci (alpha and non-hemolytic or gamma) table below summarizes the physiological characteristics
<table>
<thead>
<tr>
<th><strong>Sreptococcus species</strong></th>
<th>Optochin Susceptibility</th>
<th>Bile soluble</th>
<th>Growth on Bile esculin agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/ <em>S. pneumoniae</em></td>
<td>+</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>2/ Viridans streptococci</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>3/ <em>S. bovis</em></td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td></td>
<td>_</td>
<td>+</td>
</tr>
</tbody>
</table>
(k) Solubilité des pneumocoques dans les sels biliaires. Les tubes de gauche et au centre contiennent une culture de pneumocoques *(Streptococcus pneumoniae)*. Le tube de droite contient un streptocoque α. 0,1 ml de désoxycholate a été ajouté aux tubes du centre et de droite, et 0,1 ml d’eau distillée au tube de gauche. Les pneumocoques au centre sont solubles dans les acides biliaires, puisque la suspension est claire. Les bactéries dans le tube de droite ne sont pas solubles dans les sels biliaires, puisque la suspension est trouble.

(q) Sensibilité à l’optochine. Un disque chargé d’optochine sur une gélose au sang inhibe spécifiquement la croissance des pneumocoques. Le test à l’optochine pour *Streptococcus pneumoniae* est révélé par la zone d’inhibition.

Bile solubility of *S. pneumonia*  

*S. pneumonia* inhibitor zone around Optochine
d/ genus of *Enterococcus*

- The enterococci are catalase-negative gram-positive cocci that occur single, in pairs, and in short chain.

- All strains grow in broth containing 6.5% of NaCl and hydrolyze esculin in presence of 40% bile salt (bili esculin medium).

- *Enterococcus faecalis* and *Enterococcus faecium* are commonly found in the gastrointestinal tracts of human and that are 80 to 90% of clinical isolates of enterococci. Those species are implicated in approximately 10% of urinary tract infections and 16% of nosocomial infections caused mainly by multiresistant *Enterococcus*.

- Because of acquired resistance to penicillins, vancomycin and high level to aminoglycoside has been describe for a variety of enterococci species, in vitro susceptibility testing should be done before treatment.
Identification algorithm of gram-positive cocci.

Criterions are:

1/ aerobio or fermentation
2/ catalase-positive or negative
3/ coagulase
4/ sensitivity to optichine and bile
5/ growth on bile esculine
6/ hemolyse α β γ
7/ CAMP test or hydrolyze of hippurate
8/ sensitivity to bacitracine
9/ resistance to novobiocine
members of genus Neisseria

- Most *Neisseria* species are non-pathogenic and are normal inhabitants only of the oro and nasopharyngeal mucous membranes of human
- Among *Neisseria* species only *Neisseria meningitidis* and *Neisseria gonorrhoeae* are considered pathogen:
  - *Neisseria gonorrhoeae* is causative agent of gonorrhea;
  - *Neisseria meningitidis* causative agent of outbreaks of bacterial meningitis and of meningococcemia; but it’s normally carried on naso-pharyngeal mucosa

- *Neisseria* species are non endospore-forming gram-negative diplococci with adjacent sides flattened to give a characteristic kidney or coffee bean appearance. All species are aerobic, oxidase positive and catalase positive
**Gram-negative cocci identification tests**

Criteria are:

1. aerobic or anaerobic
2. culture on Thayer-Martin selective medium
3. ONPG test
4. carbohydrate metabolizing
Figure 36.6 suite
Neisseria Gram-negative diplococcus with adjacent sides flattened

Neisseria meningitis
*N. gonorrhoeae* 's acute urethritis with copious purulent discharge

Intracellular diplococcus gram-negative: *N. gonorrhoeae*
Meningococci are subgrouped into 13 serological groups on the basis of outer membrane protein antigens. Strains belonging to groups A, B, C, Y, and W135 are most frequently implicated in systemic disease.

In sub-Saharan Africa, outbreaks of meningococcal group A and C disease are common; in North America and West Europe, group B and C are commonly associated with sporadic meningitis.

Vaccines against group C and A strains are available.
Species of *Neisseria* are differentiated by their ability to produce catalase, to produce acid from a few carbohydrates and to reduce nitrate and nitrite.

Specimens for isolation of *Neisseria gonorrhoeae* may be obtained from the genitourinary tract, urine, anal area, oropharynx and conjunctiva.

Specimens for the isolation of *meningococci* may include CSF, blood, petechial aspiration, sputum or transtracheal aspirates, and nasopharyngeal swabs.

Those *Neisseria* species are highly susceptible to temperature extremes and desiccation that specimens for isolation should be immediately transported to the laboratory.

We use selective media for isolation of *N. meningitidis* and *N. gonorrhoeae* such as Thayer-Martin medium and NYC medium that contain vancomycin to inhibit gram-positive bacteria, colistine to inhibit gram-negative bacteria the commensally *Neisseria* species, amphotericine B an antifungal agent, and trimethoprim lactate to inhibit swarming *Proteus species*. 

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Antimicrobial resistance to penicillin and to tetracycline is now widespread among strains of *N. gonorrhoeae*. Before treatment we must know strain antibiotic susceptibility.

**Recommendations of NCCLS of USA** are that a β-lactamase production may be determined by a highly sensitive nitrocefin test.

Although penicillin G has remained effective for the treatment of meningococcal infections, some strains exhibit resistance to penicillin and tetracycline.

So we always must know drug susceptibility of isolated strains involving in meningococcal infections although penicillins remain first line of meningitis antibiotic treatment with appropriate dosages.

In front of epidemics we always must vaccinate the population at risk by appropriated vaccine available for mainly group C or A.
3/ facultative anaerobic gram-negative rods

a) Enterobacteriaceae family

- This family includes a group of bacteria that inhabit the intestinal tracts of human and animals that they are commonly called enterics.

- Many of them cause disease of the gastrointestinal tract and others organs. Some species are permanent residents, other are found in only a fraction of the population, and still others are present only as agents of disease condition.

Definitions: Enterics are facultative anaerobic gram-negative rods, active fermenter of glucose and others carbohydrates. Enterics include motile as non motile species; those that are motile have peritrichous flagella. They are non-endospore-forming, some of them have a capsule.
Non-encapsulated, rod gram-negative bacilli

Bacilli surrounded by peritriouch flagella around all the bacterial cell

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Among the important genera included as enterics are *Escherichia*, *Salmonella*, *Shigella*, *Yersinia*, *Klebsiella*, *Serratia*, *Proteus*, and *Enterobacter*

Because of the clinical importance of enterics, there are many techniques for their isolation and identification.

While species of enterics can’t be morphologically distinguished, that all are gram-negative rods, biochemical tests are especially important in clinical laboratory work.

Enterics can also be distinguished from each other according to antigen present to their surfaces; that is by serology.

An identification key, using metabolic characteristics, and a modern tool using 15 biochemical tests are shown in figures below.
Figure 10.9 One type of rapid identification method for bacteria: Enterotube™ II from Becton Dickinson. 1 One tube containing media for 15 tests is inoculated. 2 After incubation, the tube is observed for results. 3 The value for each positive test is circled, and the numbers from each group of tests are added to give each portion of the I.D. value. 4 Comparing the resultant I.D. value with a computerized listing shows that the organism in the tube is Enterobacter cloacae. This example shows results for a typical strain of E. cloacae; however, other strains may produce different test results, which are listed in the Atypical Test Results column. The V-P test is used to confirm an identification.

In rapid identification methods, test results are weighted according to their relative importance.
Somatic antigen O and flagella antigen H of enteric bacteria

Agglutinations assays in tubes or on slides
Figure 10.8 The use of metabolic characteristics to identify selected genera of enteric bacteria.

Even closely related bacteria can usually be separated into distinct species by biochemical testing.
API E of Biomerieux* with 20 biochemical tests for enterics identification
## Some enteric’s discriminatory metabolic characteristics

<table>
<thead>
<tr>
<th>Genera</th>
<th>Glucose fermentation</th>
<th>Lactose fermentation</th>
<th>motility</th>
<th>desaminase</th>
<th>urease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia</strong></td>
<td>+ with gas production</td>
<td>+</td>
<td>+/_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td>+ with gas production</td>
<td>_</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><strong>Shigella</strong></td>
<td>+ without gas production</td>
<td>_</td>
<td>-</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><strong>Yersinia</strong></td>
<td>+ without gas production</td>
<td>_</td>
<td>+ at 25º C</td>
<td>_</td>
<td>_ ((+))</td>
</tr>
<tr>
<td><strong>Proteus</strong></td>
<td>+ with gas production</td>
<td>_</td>
<td>+++++</td>
<td>+++++</td>
<td>+</td>
</tr>
</tbody>
</table>
To isolate gram-negative rods we need selective medium such as MacConkey, Hecktoen, Salmonella-Shigella media etc.. on which specimen cultures should be attended

Pure colony species on those media will be submitted to a set of biochemical tests for identification

*Enteric* species exhibit intrinsic resistance to antibiotics with a narrow spectrum such as penicillin G,V and M, oxacillin, vancomycin, erythromycin, pristinamycin or clindamycin

They are also often resistant to large broad spectrum antibiotics, that should be a plasmidic acquired resistance.

Before treatment, in vitro antibiotics susceptibility testing would be helpful
Some biochemical tests

Tube 1: non-inoculated
Tube 3: lactose and sucrose fermentation with gas production
Tube 4: production of H2S
(b) La réaction de Voges-Proskauer. Les bactéries VP-positives produisent de l’acétone ou de l’acétyléméthylcarbinol, qui donnent une couleur rouge avec les réactifs (à gauche) ; une réaction VP-négative est montrée à droite. Utilisée pour différencier les Bacillus des bactéries entériques ; p. ex., E. coli est UP négatif et Enterobacter aerogenes est UP positif.

Réduction du nitrate. Après 24 à 48 heures d’incubation, du nitrate est ajouté aux cultures en tubes. Le tube de gauche montre l'ormation de gaz (réaction positive de réduction du nitrate) ; le milieu de couleur rouge, indique une réduction du nitrate nitrite ; à droite, une culture de contrôle négative. Utilisé pour des bactéries Gram-négatives.

=On left Voges-Proskauer positive production of acetone (red color) reaction

=in tube 2 nitrate reductase reaction (red color)

=on right V-P- negative reaction

=in tube 3 negative control
(h) **Test de l'indole.** Certaines bactéries transforment le tryptophane en indole. La présence d'indole est mise en évidence par l'addition du réactif de Kovacs. L'apparition d'une couleur rouge à la surface indique un test indole positif (à gauche) et la couleur jaune-orange est une réponse négative pour l'indole (à droite). Utilisé pour séparer les bactéries entériques comme *E. coli* (indole positif) de *Enterobacter* (indole négatif).

(on left indole formation from tryptophan) that means apparition of red color

after Kovac’s solution addition = on right yellow-orange color indicates negative reaction

(j) **Test de désamination de la phénylalanine.** Lorsqu'on ajoute une solution de chlorure ferrique10% à une gélose inclinée contenant de la phénylalanine, l'apparition d'une couleur vert foncé indique la présence de l'enzyme (test positif à droite). Le tube de gauche est un contrôle, qui n'a pas été inoculé et le tube au milieu montre une réponse négative. Utilisé pour différencier les membres du groupe *Proteus* (*Proteus vulgaris* est positif) de *E. coli* (négatif).

= on left non inoculated

= in center negative reaction

= on right desaminase of phenylalanine with green color
(m) Réactions de fermentation. Le tube de gauche montre la production d’acides (couleur jaune) et de gaz dans la cloche de Durham. Le tube au centre indique que le sucre n’est pas utilisé, ne produisant ni acide ni gaz. Le tube de droite montre une production d’acide moindre que dans le tube de gauche. Utilisé pour séparer les bactéries entériques.

= on left carbohydrate fermentation with acid and gas production
= in center any metabolic reaction
= on right less acid production
B) Genus *Escherichia*

- Type specie is *Escherichia coli* or *E. coli* that is mostly isolated from human specimens. It is a part of bowel flora of healthy individual; however certain strains may cause extraintestinal and intestinal infection in immunocompromised as well as healthy individuals.

- Some strains of *E. coli* cause urinary tract infection, bacteremia, meningitis and diarrheal disease.

- Some of them are specifically enteropathogenic and produce responsible enterotoxin of symptoms and diseases.
**Definitions:** *Escherichia coli* is the only specie of *Enterobacteriaceae* which ferment lactose (lactose +). It’s a motile non-endosporing Gram-negative bacilli which ferment glucose with production of gas, metabolize tryptophane into indole (indole +) but can’t use citrate of Simons as source of carbon (citrate of Simons -) and hasn't nor phenylalanine deaminase (APP-) neither an urease (urea-)

**Isolation of** *E. coli* **on** selective Maconkey plate media, show red or pink colonies which had, init lactose, fermented. Those colonies will be picked out for further biochemical testing
Pathogenicity of different pathogenic strains of *Escherichia coli*

fermented lactose red colonies
=on left indole formation from tryptophane with after addition of Kovac’s solution apparition of red color (E. Coli)
=on right yellow-orange color indicates negative reaction (Enterobacter)

(h) Test de l’indole. Certaines bactéries transforment le tryptophane en indole. La présence d’indole est mise en évidence par l’addition du réactif de Kovacs. L’apparition d’une couleur rouge à la surface indique un test indole positif (à gauche) et la couleur jaune-orange est une réponse négative pour l’indole (à droite). Utilisé pour séparer les bactéries entériques comme E. coli (indole positif) de Enterobacter (indole négatif).
There are at least three categories of recognized diarrheagenic *E. coli*:

1/*Enterotoxigenic E. coli (ETEC)*, which produce heat-labile (LT) enterotoxin or heat-stable (ST) enterotoxin or both LT and ST, is an important cause of diarrhea in developing country among young children and travelers

2/*Enteropathogenic E. coli (EPEC)* which was associated, in the early 1960, with, in USA’s outbreaks of infantile diarrhea in hospital nurseries. EPEC are now a common cause of infantile diarrhea in developing countries
3/Enteroinvasive *E. coli* strains invade cells of colon and produce a generally watery but occasionally bloody diarrhea by a pathway similarly to that of *Shigella*

- Those diarrheagenic *E. coli* are generally identified only during outbreaks. Rehydration is the main treatment aided by an appropriate antibiotic which reduce severity and duration of symptoms

- Antibiotics susceptibility determination may be helpful, that most outbreak strains associated are antibiotics multiresistant
C) Genus *Shigella*

- This genus is composed by non-motile bacteria, non-forming gas from fermentable carbohydrates that conform to the definition of the family *Enterobacteriaceae*

- *Shigella* are causative agents of bacillary dysentery. They cause bloody diarrhea (dysentery) and non-bloody diarrhea

- There are four, containing many serotypes, serogroups of *Shigella* that serologic identification can be performed by slides agglutination with polyvalent somatic (O) antigen grouping sera

  - *serogroup A of Shigella dysenteriae* with ten serotypes
  - *serogroup B of Shigella flexneri* with six serotypes
  - *serogroup C of Shigella boydii* with ten serotypes
  - *serogroup D of Shigella sonnei* with only one serotype
All four groups of Shigella can cause dysentery, but *Shigella dysenteriae 1* has been associated with a particularly severe form of illness though to be related to its production of Shiga toxin.

In the developing world, the most prevalent serogroups are *Shigella flexneri and Shigella dysenteriae 1*, the latter being the most frequent cause of epidemic dysentery.

*Shigella* strains are isolated from bloody diarrhea on selective medium such as MacConkey, Hecktoen or SS. Therefore we picked up pure cultured colony strains for by biochemical testing identification.
Definitions: Shigella strains are non motile, lactose -, ferment glucose without gas forming and don’t use citrate as source of carbon and don’t produce H2S but has a lysine decarboxylase

Shigellose, illness cause by Shigella, is must common in situations in which hygiene is limited

Most transmission is by person-to-person spread or also caused by ingestion of fecally contaminated food or water

Shigella infections are often treated with antimicrobial agents. Because of the widespread antimicrobial resistance among Shigella strains, all isolates should undergo antibiotic’s susceptibility testing

Reporting of susceptibility results to the clinician is particularly important for Shigella dysenteriea 1 isolates that cause the mostly severe dysenteriea
D) Genus *Salmonella*

- The genus *Salmonella* is composed of motile bacteria that conform to the definition of the family *Enterobacteriaceae*.

- Two species are currently recognized in the genus *Salmonella*:
  - *Salmonella enterica* with six subspecies
  - *Salmonella bongori* (formerly subspecies V)

- The type species is *Salmonella enterica* subsp. *enterica* which is designed *subspecie 1*.

- This subspecie 1 is serotyped according to their O (somatic) antigens, Vi (capsular) antigens and H (flagellar) antigens into 2,400 serotypes or serovars which are classified in the epidemiological *Kauffman-White Classification*. 
Somatic O antigen and flagellar H antigen

Capsular K (Vi) antigen

Kauffman-White classification

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**Salmonella enterica subspecie 1** is the causative agent of typhoid fever (*Salmonella serotype Typhi* belongs to this subspecie 1)

- Typhoid fever is transmitted through person-to-person contact or fecally contaminated food or water. It typically presents with sustained debilitating high fever and headaches, without diarrhea

- Typhoid fever is a serious bloodstream infection common in the developing world

- Strains of *Salmonella* serotypes *Paratyphi A*, *Paratyphi B* and *Paratyphi C* are responsible of a syndrome similar to typhoid fever named “paratyphoid fever”

- Strains of nontyphoidal *Salmonella* usually cause *salmonellosis* that is an intestinal infection often lasts 1 week or longer and is accompanied by diarrhea, fever, and abdominal cramps
Pathogenicity of *Salmonella* serotype *Thyphi* strains

Prof Dr MUHIRWA Gregoire MD, PHM, PhD
Clinical laboratories should consider that an isolate is confirmed as *Salmonella* when both determination of O serogroup and biochemical identification have been completed.

The approach most commonly used for determining O antigens is to initially test the isolates by slide agglutination in antisera against O Group. Antisera contain antibodies against O antigens.

Differential or selective media are used to isolate *Salmonella* from fecal and blood specimens as Maconkey (MAC), eosin methylene blue (EMB), SS for Salmonella-Shigella media, mainly to obtain a pure culture which will be further tested biochemically by a complete set of tests.
SS selective medium before (on left) and after (on right) isolation of lactose negative and producer of H2S colonies of *Salmonella* strains
In developing countries, typhoid fever is frequently diagnosed solely on clinical grounds, while isolation of the causative organism should be necessary for a definitive diagnosis.

- **Salmonella** serotype Typhi is more frequently isolated from blood cultures than from fecal specimens
- Blood cultures are positive for 80% of typhoid patients during the first week of fever but show decreasing positive results thereafter. So, alternatively, fecal specimens are used to isolate **Salmonella** serotype Typhi

- Some biochemical test useful for differentiating of **Salmonella** are: glucose fermentation with production of gas, production of H2S, indole-, Citrate of Simons +, motility +, urease –, Lysine decarboxylase+, lactose –, ONPG – (o nitrophenyl β D-galactopyranoside)

- **The Widal test** which is the most commonly used method for the serodiagnostic of **Salmonella** enteric fever, measures agglutinating antibodies to the O and H antigen of **Salmonella** serotypes Typhi, Paratyphi A, B and C
Unfortunately, Widal test may produce false-negative and false-positive reactions and does not provide a definitive diagnosis of individual cases of infections.

Standard diagnostic must use biochemical tests on isolates from bloody and/or fecal specimens

Antimicrobial therapy is not recommended for uncomplicated salmonellosis or *Salmonella* gastroenteritis.

In contrast, treatment with appropriate antimicrobial agent can be crucial for patients with invasive *Salmonella* and typhoidal and paratyphi infections and the susceptibilities of these isolates should be tested as soon as possible.

Indeed, recent reports have noted an increasing level of resistance to one or more antimicrobial agents particularly in *Salmonella* serotypes Typhi strains.
E) Genus Proteus- Morganella-Providencia

- **Proteus, Morganella and Providencia** traditionally have been included in the tribe of *Proteeae* of the family *Enterobacteriaceae*.

- **Definitions:** those genus have, especially, a *phenylalanine desaminase* and an active *urease*. They are very motile, invading rapidly all the plate medium surface. They grow well on non-selective as well on moderately selective media for isolation of *Enterobacteriaceae*.

- Up 10% of all uncomplicated urinary tract infections are caused by *Proteus mirabilis* which is the clinical most important specie. With *Morganella morganii*, they are often isolated from infected wounds, respiratory tract specimens and blood.

- They express a high frequency of aminoglycide resistance. Newer cephalosporins and quinolones are usually effective, depending on the results of antibiotic susceptibility testing.
(j) Test de désamination de la phénylalanine. Lorsqu'on ajoute une solution de chlorure ferrique 10% à une gélose inclinée contenant de la phénylalanine, l'apparition d'une couleur vert foncé indique la présence de l'enzyme (test positif à droite). Le tube de gauche est un contrôle, qui n'a pas été inoculé et le tube au milieu montre une réponse négative. Utilisé pour différencier les membres du groupe Proteus (Proteus vulgaris est positif) de E. coli (négalif).

= on left non inoculated
= in center negative reaction
= on right desaminase of phenylalanine with green color (Proteus)

(r) Production d'uréase. Gélose inclinée à l'urée de Christensen. Des bactéries uréase-positives hydrolysent l'urée en ammoniac, qui fait virer l'indicateur de pH, le rouge de phénol, vers le rouge-violet. De gauche à droite : contrôle négatif pas inoculé, réaction positive retardée (plus de 24 h), réaction positive précoce (moins de 4 h), réaction négative. Utilisée pour séparer les bactéries entériques.

Tube1: negative control
Tube3: active urease with red-violet color (Proteus)
f) genus Yersinia

Three species are of medical importance:

- **Yersinia pestis** causative agent of plague pandemics, which natural reservoir are rodents. Transmission occurs mainly via fleas of rodents, but occasionally by direct contact.

  Major epidemics and outbreaks in Tanzania (1991), RDC (1992 to 1993), Peru (1993 to 1994), India (1994), and Madagascar (1995) have demonstrated that plague is far from being eradicated.

- Infections due to **Yersinia enterocolitica** or **Yersinia pseudotuberculosis** may be acquired by ingestion of contaminated food and water. Both species have affinity for the intestinal lymphoid tissue and probably penetrate into the ileal mucosa via the M cells for causing intestinal yersiniosis which presents three clinical forms: enteritis, terminal ileitis or mesenteric lymphadenitis causing “pseudoappendicitis” and septicemia.
**Definitions:** Members of the genus *Yersinia* are non-forming spore gram-negative or variable rod-shaped bacteria. Except *Yersinia pestis*, which is nonmotile, the other species are motile at 22 to 30º C but not to 37º C. Glucose is fermentatively utilized without forming gas and with the exception of *Y. pestis*, urease is produced at 25 to 28º C.

**g) Klebsiella, Enterobacter, Citrobacter and Serratia**

All four genera are well-recognized nosocomial pathogen; common agents of nosocomial urinary tract, wound and bloodstream infections as well as causes of hospital-acquired pneumonia.
Definitions: All four genera belong to the family Enterobacteriaceae and are gram-negative facultative anaerobic rods or coccobacilli. Species are generally V.P. + or Voges-Proskauer + (which produce acetoin), don’t produce indole from tryptophane, ferment most sugar, nonmotile and some (Enterobacter) have mucoid colonies with encapsulated cells.

Emerging antimicrobial resistance among members of these genera is becoming increasingly worrisome. Presence of extended-spectrum β-lactamase (ESBL) was detected. As demonstrated by multicenter studies, resistance varies from hospital to hospital and regionally within any country. Therefore, at each institute must be established and monitored susceptibility profiles relevant for its patient population.
La réaction de Voges-Proskauer. Les bactéries VP-positives produisent de l’acétylénol, qui donnent une couleur rouge avec les réactifs (à gauche); une réaction VP-négative est montrée à droite. Utilisée pour différencier les Bacillus des bactéries entériques; p. ex., E. coli est VP négatif et Enterobacter aerogenes est VP positif.

Test de l’indole. Certaines bactéries transforment le tryptophane en indole. La présence d’indole est mise en évidence par l’addition du réactif de Kovacs. L’apparition d’une couleur rouge à la surface indique un test indole positif (à gauche) et la couleur jaune-orange est une réponse négative pour l’indole (à droite). Utilisé pour séparer les bactéries entériques comme E. coli (indole positif) de Enterobacter (indole négatif).

(on left positive-Voges-Proskauer with production of acetone (red color for Enterobacter)
=on right negative V-P

(on left indole formation from tryptophane with after addition of Kovac’s solution apparition of red color
=on right yellow-orange color indicates negative reaction of Enterobacter)
Summarize about enteric genera and species we deal with:

- **E. coli** urinary tract infections and enteropathogenic strains
- **Shigella** different serotypes bacillary dysenteriea causative agents
- Typhoidal and para-typhoidal *Salmonella* causative agents of typhoid and para-typhoidal fever strains and *salmonellosis* non-typhoidal strains
- Genus *Proteus-Morganella Providencia* species providers of very active desaminase and urease enzymes causative agents of urinary tract infections and wounds
- **Yersinia pestis** causative agent of non yet eradicated plague
- **Enterobacter-Serratia**
4) Genus Vibrio

- It belongs to the family *Vibrionaceae* and is the most important genus of that family.

- **Definitions:** *members of the genus are gram-negative facultative anaerobic rods that are frequently slightly curved.* *Genus Vibrio* is *oxidase positive and ferments glucose.* All *Vibrio* species grow to higher densities in nutrient broth or nutrient agar when 6.5% of NaCl is added; those species also well grow on usual medium.

- One important pathogen is *Vibrio cholerae* the causative agent of cholera. *Vibrio parahaemolyticus* causes a less serious form of gastroenteritis.
Cholera symptoms result from the action of **cholera toxin or CT**, a heat-labile enterotoxin, oligomeric protein produce only by two serotypes *Vibrio cholerae O1 and O 139 Bengal*.

Although *V. cholerae* has 155 somatic (O) antigens, and all these have been characterized.

CT activates adenylate cyclase, producing increased levels of cyclic AMP and resulting in the hypersecretion of water and electrolytes by infected enterocytes. The outpouring of water and electrolytes into the lumen of the intestine cause copious purging resulting in the severe dehydration of classical cholera gravis.

Cholera symptoms are an increase in peristalsis followed by loose stools, which rapidly progress to the watery, mucus-flecked, ”rice-water” stools characteristic of cholera. Vomiting often occurs in the early stages of cholera.
CT or cholera toxin’s exacerbation of the adenylase-cyclase pathway with resulting in loss of water and electrolyte by enterocytes
In cholera gravis, the rate of fluid loss quickly reaches 500 to 1,000 ml/h and can reach 20l/day.

Immediate medical care of rehydration and electrolyte replacement therapy is required to manage dehydration, electrolyte imbalance, hypovolemic shock, hypoglycemia and metabolic acidosis.

Moderate cases of cholera can usually be managed with oral rehydration therapy (ORT) when they are no more vomiting; that’s sterilized water containing electrolytes and some glucose.

While cholera gravis requires rapid intravenous rehydration with Ringer’ lactate to which potassium chloride has been added. Hypoglycemia requires the addition of intravenous glucose.

When patient is clinical stabilized and vomiting has subsided, antimicrobial therapy of tetracyclines first choice, may reduce the duration of diarrhea and the period of excretion of V. cholerae.
Suspect specimen should be transported into alkaline peptone broth.

To isolate pure *V. cholera* colonies, we use a selective medium **TCBS (containing thiosulfate, citrate, bile, sapharose)** on which *V. cholera* colonies are **yellow ones** that will be picked out for oxidase testing. *V. cholerae species are oxidase-positive ones from yellow colonies**

*V. cholerae O1* is divided into **the classical** and **El Tor biogroups or biotypes** for epidemiologic purposes. The classical biogroup of *V. cholerae O 1* is negative by the Voges-Proskauer test, while the El Tor is positive and represent the biogroup of last cholera outbreaks causative agent that had spread from Nepal still to Africa countries.
Positive-Oxidase test is also detected after application of a spot of colony-strains of *Vibrio cholerae*, *picket out from yellow colonies on a TCBS selective medium*, on paper containing tetramethyl-p-phenylenediamine involving in purple color developing
*V. Cholerae* can be subtyped further to subtypes O 1 *Ogawa*, *Hiroshima* and *Inaba* on the basis of minor O antigens, referred to O factor A, B, and C. Strains which produce:

- A and B, O factors belong to **subtype O1 Ogawa**
- A and C, O factors belong to **subtype O1 Inaba**
- A, B, and C, O factors belong to **subtype O1 Hiroshima**

Reports of increasing resistance among *V. cholerae* strains isolated during epidemics indicate that antimicrobial susceptibilities may need to be monitored before antibiotic treatment. But generally, *V. cholerae* strains are susceptibility to tetracyclines and phenicoles; theses antibiotics are used for prophylaxis.

*Cholera subunits and inactivated vaccines* are available used for travelers (from no-endemic to endemic regions) and for risked people during outbreak.
5/ aerobic gram-negative bacilli

A) genus Pseudomonas

- *Pseudomonas sp* are aerobic, non-spore-forming, gram-negative active rods which are straight or slight curved.

- Clinical isolates are oxidase positive, and catalase positive and grow on MacConkey agar appearing as lactose nonfermenters.

- Colonies of *P. aeruginosa* are usually spreading and flat, and have a metallic sheen when pigments pyoverdin or pyocyanin are produce. This specie grow at 42º C.
**Pseudomonas spp.** have a worldwide distribution, are found in water, soil and on plant including fruits and vegetables.

Because of their ability to survive in aqueous environments, particularly **Pseudomonas aeruginosa** have become problematic in hospital environment. It has been found in a variety of aqueous solution including disinfectants, ointment, soaps, irrigation fluids, eye drops, and dialysis fluids and equipments.

Community-acquired *P. aeruginosa* infections in nonimmunocompromised individuals tend to be localized and frequently associated with contaminated water or solution. It concern infections such as folliculites, osteomyelitis of the calcaneus in children, eye infection usually following minor trauma to the cornea, endocarditis in intravenous-drug users.
Community-associated isolates of *P. aeruginosa* are usually susceptible to the antipseudomonal penicillins (ticarcillin and piperacillin) aminoglycosides (gentamycin, tobramycin, amikacin) ciprofloxacin, cefoperazone, ceftazidime, meropenem and imipenem

*Pseudomonas aeruginosa* is major cause of nosocomial infections; such as nosocomial respiratory tract infections, urinary tract infections, wound infections, peritonitis in patients on chronic ambulatory peritoneal dialysis, and bacteremia

Wound infections due to *Pseudomonas aeruginosa* are particularly troublesome in burn patients

Nosocomial acquired *P. aeruginosa* tend to be resistant to multiple classes of antimicrobial agent
b) genus Haemophylus

1/ Haemophylus species are normal residents of upper respiratory tract of humans and animals.

Those species are oxidase positive, pleomorphic, gram-negative bacilli. Most species exhibit fastidious nutritional requirements and grow in laboratory only when provided with contained growth-factors nutrient-rich media.

2/ Haemophylus influenzae is non-hemolytic, and needs factor V (nicotinamid) and X (hemetine) for growing.

The major virulence factor of Haemophylus influenzae strains causing invasive infection is the capsule. Encapsulated strains belong to one of the six serotypes (serotype a to f).
Haemophilus influenzae only
growing around V and X growth-factors
Strains of *Haemophylus influenzae type b* cause bacterial meningitis and epiglottitis, pericarditis, pneumonia, septic arthritis, osteomyelitis and facial cellulitis in children less than 5 years.

W, H. O. provides to developing countries a licensed *H. influenzae type b* conjugated vaccine which is administered at the age of 2 months.

*3/Haemophylus ducreyi* needs factor X for growing, and causes chancroid, a sexually transmitted disease characterized by shallow genital ulceration that may be accompanied by inguinal lymphadenopathies.
6/ Endospore-forming Gram-positive Rods

- The two important gram-positive rod-shaped genera are *Clostridium* and *Bacillus*

1/ *Bacillus anthracis* cause anthrax, a disease of cattle, sheep, and horses that can be transmitted to the human

- Bacillus anthracis is non-motile facultative anaerobe which form a endospore in presence of oxygen.
- The endospore are centrally located
- This bacteria is one of the bacterial ward candidates

2/ *Bacillus cereus* cause food-borne illness, diarrheal type caused by a heat-labile enterotoxic complex or an emetic type caused by a heat-stable enterotoxin

3/ *Bacillus thuringiensis* is probably the best-known microbial insect pathogen. When it sporulates, it produce intracellular crystals of glycoprotein toxin that quickly causes paralysis of the insect’s gut when ingested.
Genus Clostridium include obligately anaerobic rods with central or subterminal heat resistant spore that swell the cells.

Diseases associated with clostridia include:
- tetanus caused by Clostridium tetani which form a terminal spore
- botulism caused by Clostridium botulinum with a subterminal spore
- gas gangrene by Clostridium perfringes which is also the cause of a common form of foodborne diarrhea

Clostridium tetani can be isolated from a variety of source, including soil, and the intestinal contents of numerous animal species. The clinical syndrome known as tetanus is an extremely dramatic illness produce by the action of a potent neurotoxin, tetanospasmine, which is elaborated by C. tetani.
Toxin is elaborated at the site of apparent minor trauma and rapidly binds to neural tissue, provoking a characteristic paralysis and spasms.

Tetanus is largely a disease of nonimmunized animals and human, since an effective vaccine toxoid has been in use for many years. Vaccine toxoid is an formalin inactivated *C. tetani* toxin.

Terminal *Clostridiun tetani* endospore.
6/Clostridium botulinum is widely distributed in soil and aquatic habitats throughout the world.

This specie has the ability to produce the most lethal poison known, namely, botulinum neurotoxin. Formed endospore is a subterminal one

They are seven antigenic toxin types (A through G), determined by serologic toxin neutralization test. Types A, B, E, and F are the principal cause of botulism in humans.
Neurotoxin, produced by *C. botulinum*, enters the bloodstream from the site where it was produced or absorbed and binds irreversibly at the neuromuscular junctions of motor neurons to prevent the release of acetylcholine that establishes an acute flaccid paralysis.

**7/Clostridium perfringens**

Clostridial myonecrosis or gas gangrene is a toxin-mediated breakdown of muscle tissue associated with growth of *Clostridium perfringens*.

*C. perfringens* type-A strains cause food-borne illness, the food vehicle is almost always an improperly cooked meal. This pathogen produces a variety of biological active proteins, or toxins, that play an important role in pathogenicity. On the basis of mouse lethality assays and specific neutralization, five toxin types (types A through E) of *C. perfringens* has been identified.
8/\textit{Clostridium difficile}, the major cause of antibiotic-associated diarrhea and pseudomembranous colitis, is also the most frequently identified cause of hospital-acquired diarrhea.

Patients with bowel stasis, those who have had bowel surgery, and those with no known risk factors can also develop \textit{C difficile}-induced gastrointestinal disease.

\textit{C. difficile} produce at least three potential virulence factors:
= an enterotoxin A
= a cytotoxin B
= a substance that inhibits bowel motility
Spirochetes are helical and have axial filaments under their outer sheath that enable them to move by a corkscrew-like rotation.
The Spirochetes include a number of important pathogenic bacteria from:

- genus Treponema which includes
  a) Treponema pallidum subsp pallidum causative agent of the Syphilis
  b) Treponema pallidum subsp endemicum Bejel’s causative agent
  c) Treponema pallidum subsp pertenue causative agent of Pian
  d) Treponema pallidum subsp carateum causative agent of Pinta or Carate

Bejel, Pian and Pinta are nonvenereal treponematosis

- genus Borrelia
- genus Leptospira
=genus *Borellia* whose arthropod-bore-members (ticks of lice) *Borellia recurrentis, Borellia hispanica and Borellia duttoni* cause relapsing fever, and *Borellia burgdorferi* is the causative agent of the *Lyme disease*

=vectors are from genus *Ornithodoros (in Africa), Argas (in America) and Ixodes (in Europe)*

- **Relapsing fever** in humans is a febrile, septicemic disease with sudden onset after an incubation period of 2 to 5 days. Fever persist for 3 to 7 days and is followed by an afebrile interval of several days to several weeks.

- Relapses may occur as a result of antigenic variations in the causative borreliae.
During acute phase of relapsing fever, large number of borreliae may circulate in peripheral blood and can be detected by light or dark-field microscopy.

- Giemsa, May-Grunwald, Wright are used to stain bloody thin- and thick-drop films for examination by conventional light microscopy.

Giemsa staining of *Borellia*
Vectors of *Borrelia*: a) *Ornithodoros* in Africa

b) *Argas*

c) *Ixodes*

FIGURE 1 Three genera of ticks that are vectors of *Borrelia*. (A) *Ornithodoros*; (B) *Argas*; (C) *Ixodes.*
genus Leptospira whose bacteria species are excreted in the urine of such animals as dogs, rats, and swine. Human disease or Leptospirosis usually spread from water contaminated

Members of the genus Leptospira consist of spiral-shaped, flexible helical bacteria rods with more than 18 coils per cells with hooked end

Electronic micrograph of Leptospira spiral-shaped with hooked end
Pathogenic serovars are assigned to the species *Leptospira interrogans*.

*Leptospirosis* is a zoonosis with a worldwide distribution. Infection usually result of a direct or indirect exposure to the urine of infected animal.

Leptospirosis exhibits a great variety of clinical manifestations ranging form a mild self-limited febrile illness (most patients) to a fulminating fatal illness associated with hepatorenal feature named Weil’s disease.
Blood, CSF, and urine are the specimen of choice for the recovery of leptospires from patients with leptospirosis.

Controlled clinical trials support the therapeutic efficacies of doxycycline, and penicillin.
Treponema pallidum

Suspension of Treponema pallidum are best visualized by dark-field microscopy or seen by phase contrast microscopy.

Treponema pallidum's view by dark-field microscopy

Morphologically, Treponema pallidum has 6 to 14 tiny regular helices per cell; fresh preparation of the organism exhibit rapid rotation about the axis.
Syphilis is still a common sexually transmitted disease in many areas of the world, despite the availability of effective therapy. Transmission of venereal or nonvenereal treponematoses occurs through direct contact with primary and secondary lesions which contain large number of microorganisms and are infectious.

For the laboratory diagnosis of syphilis, each stage of the disease has a particular testing requirement. Because T. pallidum cannot be readily cultured, others laboratory methods for the identification of the infection have been developed.

The current tests for syphilis fall into three categories:
= **direct microscopic examination** used when lesions are present
= **nontreponemal tests** used for screening antibodies nonspecific to syphilis antigens*
= **specific treponemal antibodies testing** that are confirmatory tests

Prof Dr MUHIRWA Gregoire MD, PHM, PhD,
During the primary stage of unique or multiple chancre with regional lymphadenopathy, *dark-field microscopy or the direct fluorescence antibody (DFA)* of serous fluids of lesions are the best chosen examination methods.

Humoral antibodies against syphilis usually do not appear until 1 to 4 weeks after the chancre. So serologic test are not usefully for diagnosis of the first stage of syphilis.

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By the secondary stage of syphilis the organism has invaded every organ of the body and virtually all the body fluids. At this stage all serology tests (standard nontreponemal and treponemal serology confirmatory tests) for syphilis are reactive.

Nontreponemal assays detect reagine or cardiolipid which are not specific antigens of *T. pallidum*

Treponemal assays react which specific *T. pallidum antigens*

The currently useful tests are:

- *dark-field and/or contrast phase microscopy, DFA* for direct fluorescent assay examinations
- *VDRL, RPR* are nontreponemal agglutination assays for screening
- *TPHA, FTA-abs, ELISA, Nelson test, Immunoblotting* that are treponemal tests for confirmatory or referral assays
- **VDRL**, usually used for syphilis screening, means Venereal Disease Laboratory Research, an agglutination assay.

- **RPR** for Rapid Plasma Reagene test is an agglutination assay.

- **TPHA** for Treponema Pallidum Hemagglutination assay, a hemagglutination assay.

- **FTA-abs** for Fluorescent Treponemal Antibodies absorption assays a fluorescent assay.

- **ELISA**, Enzyme Linked Immuno Sorbent Assay.

- **Nelson test** was the referral test of immobilization of treponemes.

- **Immunoblotting** use treponemal antigens blotting on a sheet. That’s now the currently referral test.
Syphilis nontreponemal and treponemal assays
In routine procedure general laboratories use **VDRL and RPR for screening completed by a TPHA or ELISA confirmatory assay**

The standard serologic tests for syphilis are uniformly reactive with yaws, pinta, and nonvenereal syphilis (bejel)

Syphilis infection still stay sensitive to Penicillin and its derivatives such as benzathine penicillin that are the recommended therapy
9/ Mycobacteria

- The genus *Mycobacterium* include obligate parasites, saprophytes, and opportunistic pathogens.

- Most species are free in soil and water, such as the *non tuberculous mycobacterium, (NTM)*; but the major ecological niche of others such as *Mycobacterium tuberculosis complex* and *Mycobacterium leprae* is disease tissue of humans and other warm-blooded animals.

- *Mycobacterium tuberculosis complex* includes the species *M. tuberculosis* *M. bovis*, *M. africanum*,

- *Mycobacterium bovis* causes tuberculosis in cattle, that is transmitted by milk contaminated to humans and others primates and causes pulmonary and extra-pulmonary infections.
Mycobacterium africanum is a cause of human tuberculosis in tropical Africa. It may represent an intermediate form between *M. tuberculosis* and *M. bovis*.

*Mycobacterium tuberculosis* is the Koch tubercule bacilli that in 1886 was established causative agent of tuberculosis.

*Mycobacterium tuberculosis* is carried in airborne particles, known as droplet nuclei, that are generated when patients with pulmonary tuberculosis cough, speak and sneeze. Infection occurs when susceptible person (only 5% of infected) inhales the droplet nuclei which then reach pulmonary alveoli, where the organisms are engulfed by alveolar macrophages and may spread throughout the body.
(a) Tuberculose primaire (peau−, rayons X−)

(b) Hypersensibilité retardée et immunité cellulaire (peau +, rayons X−)

(c) Tuberculose disseminée.
Extension directe de la tuberculose primaire.
Dissémination dans tout le corps.
De nombreux petits tubercules peuvent se former (tuberculose miliare). 
Observée le plus souvent chez les enfants ou des personnes débilitées, est souvent fatale.
Peu ou pas d'hypersensibilité.
Méfaire progressivement systémique et déces. (peau+, rayons X+)

(d) Tuberculose latente.
L'issue la plus fréquente.
La plupart des individus restent dans cet état pendant toute leur vie et n'en souffrent pas.
Elle peut même offrir une protection contre la réinfection.
Des millions de cas aux États-Unis aujourd'hui.
Des tubercules apparaissent précocement dans 5% des cas. 
(peau+, rayons X−)

(e) Tuberculose active.
Une extension lentement progressive de tubercules avec érosion dans les conduits à air et les vaisseaux sanguins.
Ces individus sont infectieux et la mort s'ensuit en l'absence de traitement. 
(peau+, rayons X+, crachat+)
However, some bacilli remain viable but dormant for many years after the initial infection, so patients are *latently infected* with a *positive purified protein derivative (PPD) skin test* or *positive tuberculin test*.

Intradermal injection of an amount of PPD (0.1ug or 5 tuberculin units) results in positive or negative reaction. More than 10mm of induration is a positive reaction when measured 48 hrs latter.

Injection of mycobacteria antigens can differentiate between infected (actively or latently) and non-infected people.
The only evidence of infection with mycobacteria in most patients is a lifelong positive tuberculin-test reaction and radiographic evidence of calcification of the initial active foci in the lungs or other organ.

Tuberculin-test has now been used commonly and is the best standardized test.

A positive PPD reaction usually develops 3 to 4 weeks after infection.

Patients infected with *M. tuberculosis* may not show a response to tuberculin skin test if they are anergic particularly true in HIV-co infected patients thus that’s limitation of test using.

Reactivity to intradermal injected lepromin, which is an antigen prepared from inactivated *M. leprae*, is valuable for confirming the clinical diagnosis of tuberculoid leprosy.

This test is not useful for identifying patients with lepromatous leprosy because these patients are anergic to the antigen.
Other clinical manifestations of *M. tuberculosis infection* can include *cervical adenitis, skin infections, pericarditis, synovitis and meningitis* which are *extra-pulmonary tuberculosis infections*.

*Human Immunodeficiency Virus (HIV) infection* is the greatest known risk factor for the progression of latent infection to active tuberculosis that is the most frequently AIDS opportunistic infection in Africa.

Combined HIV and tuberculosis infections, especially in association with drug resistance, have caused outbreaks of pulmonary tuberculosis with extremely high mortality rate for the first time in 1990 at New-York. Now that clinical situation is commonly encounter among HIV co-infected world widely.
Mycobacterium leprae or Hansen bacilli causes a chronic debilitating, granulomatous disease called Leprosy. The principal manifestations of the disease include anesthetic skin lesions and peripheral neuropathy with peripheral nerve thickening. Two referral clinical situations are tuberculosis or lepromatous leprosy.

Among NTM, the including two species, *M. avium* and *M. intercellularea*, Mycobacterium avium complex or MAC is ubiquitous in nature and most common NTM which causes human infections.

The greatest increase in MAC pulmonary infections during the three past decades has been in patients with AIDS as opportunistic infection.
The bacteria:

Definitions: Mycobacteria are aerobic, non-spore-forming, non-motile, slightly curved or straight bacilli, sometimes with branching. Filamentous or mycelium-like growth may occur but easily fragments into rods or coccoid elements.

The organisms have cell walls with a high lipid content, including waxes having characteristic mycolic acids with long branched chains.

The high lipid content of the cell wall excludes the usual aniline dyes. So mycobacteria are nor readily stained by Gram’s method but are considered gram positive.
Special staining procedures are used such as **Ziehl-Neelsen** and direct fluorescence auramine staining.

When Ziehl-Neelsen staining is applied, once stained mycobacteria are not easily decolorized even with acid-alcohol decolorizer.

This resistance to decolorization by acid-alcohol is termed **acid-fastness**, so mycobacteria are **acid-fast-bacteria or AFB**.

Thoses stainings are usually used for all acid-fast microorganisms such as species of genus *Nocardia* and NTM (Nontuberculous Mycocabacteria) as MAC (*Mycobacterium avium complex*) an opportunistic bacterium involved in SIDA.
In Ziehl-Neelsen staining procedure, the red dye carbolfushine is applied to a fixed smear, and the slide is gently heated for several minutes. Heating enhances penetration and retention of the dye.

Then the slide is cooled and washed with water.

The smear is next treated with acid-alcohol, a decolorizer which remove the red stain from bacteria that are not acid-fast.

The acid-fast microorganisms retain the red color because the carbolfuchsin is more soluble in the cell wall waxes than in the acid-alcohol. In non acid-fast bacteria, the carbolfuchsin is rapidly removed during decolorization, leaving the cells colorless.
- the smear is then stained with a methylene blue counterstain. Finally, acid-fast cells appear red, non acid-fast cells appear blue after application of the counterstain.
Growth for mycobacteria is slow to very slow, with generation times ranging by species from 2 to more than 20 h for *M. leprae*.

Easily visible colonies may be produced after 2 days to 8 weeks of incubation under optimal conditions (optimal temperature from 30 to almost 45º C) depending upon the species.

L-J for Lowenstein_Jensen medium with on it buff flat rough no-pigmented colonies of *Mycobacterium tuberculosis*

*L-J medium is a egg-based medium containing malachite*
- *Mycobacterium leprae* has not been cultured outside of living cells.

- The laboratory diagnosis of mycobacterial diseases depends on the detection and recovery of AFB or acid-alcohol fast bacteria from clinical respiratory or non-respiratory specimens.

- *Sputum*, both expectorated and induced, is the principal specimen obtained for the diagnostic of pulmonary tuberculosis: we can also use stomach’s aspirates that contain swallowed expectorations.

- Preferentially early-morning specimen, from a deep, productive cough should be collected on at least three consecutive days for smear films that may be stained.
Specific usually used staining methods are Ziehl-Neelsen and auramine direct fluorescence

- Only two AFB smear-positive specimens are needed for initial evaluation of pulmonary mycobacteriosis.

- Tissue, normally sterile body fluids as CFS, urine and gastric aspirates are other commonly submitted specimen.

- Concentration of mycobacteria in body fluids is usually low. A variety of techniques is used to concentrate mycobacterial from CSF and other body fluids, but nor digestion either decontamination procedures aren’t performed because these sites don’t contain any microbiota flora.

- Only on sputum specimens, that always contain a microbiota flora, digestion, homogenization and decontamination procedures are attended.
Because mycobacteria are slow growing and require long incubation times, a variety of nonmycobacterial microorganisms can overgrow cultures of specimens obtained from nonsterile sites (upper respiratory tract).

Appropriate *digestion* and *decontamination procedures*, *culture media*, and *condition of incubation* must be selected to facilitate optimal recovery of mycobacteria.

Many different media are available to use for recovery of mycobacteria and include selective and nonselective medium.

Solid media may be *egg based* such as *Lowenstein-Jensen* and/or *Colestos medium* that is the most commonly used in clinical laboratories*.
- Egg-based and agar based media all contain malachite green, a dye that suppresses the growth of contaminated bacteria.

- On transparent agar-base media colonies may be observed in 10 to 12 days, in contrast to 18 to 24 days with opaque egg-based media. Those agar-base media refer to Middlebrook medium 7H10 and 7H11 formulations.

- Agar-base media can be used for antibiotic susceptibility testing.

- The radiometric BACTEC AFB system allows the detection before 7 days for NTM and to 9-14 days for M. tuberculosis. This system has significantly improved the recovery rates and times of mycobacteria from respiratory secretions and other specimens.
The **BACTEC TB** system may be used for antimicrobial susceptibility testing

A natural division occurs between *slow grower (M. tuberculosis complex)* requiring more than 7 days to produce easily seen colonies on solid media and *rapid grower* requiring less than 7 days under comparable condition (most of the NTM)

Colony morphology varies among the species ranging from *smooth* to *rough*, and from *pigmented* to *non-pigmented*

Colonies of some species are regularly or variably *yellow, orange* Those of *M. tuberculosis* complex are *buff, flat, rough spreading to irregular periphery* on Middlebrook 7H10 agar
(t) Milieu de Lowenstein-Jensen. Des colonies de *Mycobacterium tuberculosis* nodulaires et non pigmentées.
Some species require light to form pigment as *the NTM photochromogen* and other form pigment in either the light or the dark, as *the NTM scotochromogen*.

* RUYON classification contains four groups of NTM;
  - slow grower photochromogen, *M. kansai* and *M. marinum*
  - slow grower scotochromogen *M. scrofulaceum, M. acquae* and *M. xenopi*
  - slow grower non-chromogen *M. avium-intercellularea complex* and *M. ulcerans*
  - rapid grower pigmented or not *M. fortuitum, M. abcessus* and *M. chelonae*
After a mycobacterial isolate has been assigned to a preliminary subgroup based on growth characteristics (rate of growth pigmentation, photoreactivity) it traditionally has been definitively identified to species or complex level by conventional biochemical tests.

Three main biochemical tests identifies isolates of *Mycobacterium tuberculosis*:

- **positive-Niacin accumulation test**; *M. bovis* are niacin-negative
- **positive-nitrate reductase test**; *M. bovis* can not reduce nitrate
- **positive-68°C catalase test**; catalase of *M bovis* is inactive at 68°C

Since specimens usually contain only a small number of tubercle bacilli, **PCR** may amplify microorganism nucleic acid that may be detected or quantified. Commercial nucleic amplification assay are available for detection of both *M. tuberculosis* and **MAC** nucleic acid.
The treatment of tuberculosis should include at least two drugs on which the organism is susceptible. This multitherapy is necessary to avoid emergence of resistance to antimycobacterial agents. CDC recommends that initial isolates from all patients be tested for drug susceptibility.

The first-line drugs in the treatment of tuberculosis are isoniazid (INH), rifampin, pyrazinamide, ethambutol and streptomycin.

Multiresistance involves in resistance to INH and rifampin.

WHO had established and recommended the DOTS in developing countries for reducing emergence of drug resistance. DOTS means direct observed treatment short within drugs are taken in front of a medical worker each days during six months.
Vaccination with attenuate *M. bovis*, the bacilli Calmette-Guerin (BCG), is commonly used in countries where tuberculosis is endemic (developing countries) and responsible for causing significant morbidity and mortality.

This practice can lead to a significant reduction in the incidence of tuberculosis if administered to people when they are young (it is less effective in adults).

Unfortunately, BCG immunization cannot be used in immunocompromised patients (e.g. HIV-infected patients).

An additional problem with BCG vaccination is that positive skin test reactivity develops in all immunized patients thus skin testing cannot be used to detect previous exposure to *M. tuberculosis*.
10) Obligate intracellular bacteria

- Genera *Rickettsia* and *Chlamydia* include endothelium (for *Rickettsia*) or epithelium (for *Chlamydia*) obligate intracellular bacteria

**A/Chlamydia**

- *Chlamydia* are non-motile, gram-negative, obligate intracellular bacteria.

- They replicate within the cytoplasm of host cells, forming characteristic inclusions that can be seen by light microscopy for diagnosis.
They have size of the biggest virus but differ from them by possessing both DNA and RNA and have cell wall quite similar in structure to those of gram-negative.

They are susceptible to many broad-spectrum antibiotic mainly to tetracyclines, posses a number of enzymes and expresses a lot of specific antigens, and have a restricted metabolism.

There are three species of medical importance:

1. *Chlamydia trachomatis* includes the organisms causing trachoma, inclusion conjunctivitis, lymphogranuloma venereum (LGV), and genital tract diseases. *Chlamydia trachomatis* also causes pneumonia in infants and occasionally in immunocompromised hosts, and is associated with oligoarthritis (Reiter syndrome).

The three biovar within the species are *trachoma, LGV, murine*.
1. *Chlamydia trachomatis* strains are sensitive to the action of sulfonamides and produce a glycogen-like material within the inclusion vacuole that stains with iodine for diagnosis.

2. *Chlamydia psittaci* strains infect many avian species and mammals, causing such diseases as *psittacosis, ornithosis*. Humans are infected secondarily from animals and develop *pneumonia* or *systemic infections*, including *endocarditis*.

3. *Chlamydia pneumonia* appears to be exclusively human pathogen. It has been identified as the cause of a variety of respiratory tract diseases: *pharyngitis, sinusitis, bronchitis and pneumonia*. 
The recommended procedure for *Chlamydia* isolation is *cell culture* by inoculation of clinical specimen into *cycloheximide-treated Mc Coy cells* or other appropriate cells.

*Use of fluorescein-conjugated monoclonal antibodies* represents the most sensitive method for detection of *C. trachomatis* inclusions in cell culture and also allows early detection of inclusions.

*Cytological testing with Giemsa stain* to detect inclusions is particularly useful in diagnosing acute inclusion conjunctivitis of the newborn.

The *tetracyclines* and *macrolides* have generally been the mainstays of therapy for infections due to *Chlamydia*.
**B/ Rickettsia**

Species of *Rickettsia* are small obligate intracellular, reside free in the cytoplasm of their host cell, and are found in an arthropod host for at least a part of their live cycle, where they are maintained by transovarian transmission and/or cycle involving horizontal transmission to mammalian hosts.

1/ *Rickettsia prowazekii* causes **primary louse-borne typhus** worldwide distributed disease that is transmitted to human by infected louse feces rubbed into broken skin or mucous membranes or inhaled aerosol.

It causes also **Brill-Zinsser disease** that is a recrudescence years after primary attack of louse-born typhus.
2/ *Rickettsia typhi* causes *murine typhi* a worldwide distributed illness transmitted by infected flea feces rubbed into broken skin or mucous membranes or as aerosol.

3/ *Rickettsia rickettsii* causative agent of *Rock Mountain spotted fever* is transmitted by a tick bite.

4/ *Rickettsia conori* causes *Boutonneuse fever* in southern Europe, Africa, Middle East, and is transmitted by Tick bite.

5/ *Orientalis tsustugamushi* transmitted by a Chigger bite, causes the *Scrub typhus* which geographic distribution concern Unites States, Japon, eastern Asia, northern Australia and west and southwest Pacific.
**Rickettsia isolation** is performed in only a few laboratories by cell culture isolation methods except for isolation of *O. stustugamushi* by intraperitoneal inoculation of mice.

Identification is performed on isolated strains by *indirect immunofluorescence* or by molecular methods such as *PCR*.

**Seroologic tests** for the diagnostic of rickettsia infections include indirect immunofluorescence assay, ELISA, *Poteus vulgaris O19 strains agglutination*.

Data supporting the use of *doxycycline or another tetracycline antibiotic* as the drug of choice of the treatment of infections caused by *Rickettsia and O. stustugamushi*.
11/ others bacteria...

A/ genus Brucella

- **Definitions:** *Brucella* species are aerobic strain faintly gram-negative cocci or short rods (tiny coccobacilli) arranged single or, less frequently in pairs, short chains, or small groups that grow on blood agar, are oxidase-positive, urea-positive nonmotile and nitrate reductase-positive.

- Cells are nonmotile and do not produce flagella, nor capsule neither spore are not produce.

- *Brucellosis* is a zoonotic disease so domestic animals serve as the reservoir.

- **1/ Brucella abortus** commonly infects cattle but has been isolated from affected, horse, American bison and yak. It causes abortion by female of those domestics animals.
2/ *Brucella melitensis*, a more virulent species, naturally infects not only sheep and goats but also alpaga and camel causing also abortion by female

3/ *Brucella suis* most commonly infects swine.

Transmission to humans of brucellosis can be the result of

*Ingestion* of contaminated animal products such as unpasteurized milk and milk products *(including cow, goat, and camel milk)* and meat.

*Direct contact* via skin and mucous membranes *(including the conjunctiva)*

*Inhalation* when handling infected animals in abattoirs or cultures of *Brucella ssp.* In laboratories
Brucellosis is one of the most commonly reported bacterial infection acquired in laboratories within aérosolisation is the primary mechanism of transmission.

Clinically human brucellosis may present acutely, subacutely or as localized disease, and systemic illness.

Cultures of blood and bone marrow aspirates with prolonged incubation (more than 48 h.) are performed for isolation of Brucella ssp., handling those types of cultures requiring biological safety level 3 precautions.

Tube agglutination test with Brucella antiserum confirm cultures.
Treatment of brucellosis requires combination antibiotic therapy for a prolonged period such as doxycycline for 45 days and streptomycin intramuscularly for the first 14 days

**B/ Campylobacter-Helicobacter**

**Definitions:** *Campylobacter jeuni* and *coli* are gram-negative, curved, S-shaped, or spiral rods motile, non-spore-forming. They are generally microaerophile; however some strain grow aerobically or anaerobically
In developing countries, *Campylobacter* is frequently isolated from individuals who may or may not have diarrheal disease. Most symptomatic infections occur in infancy and early childhood.

*C. jejuni* and *C. coli* are the most common *Campylobacter* species associated with diarrheal illness and are clinically and bacteriologically indistinguishable.

Identification of *Campylobacter* requires such as characteristics:
- gram-negative S-shaped rods
- oxidase-positive colonies
- growth on selective media incubated at 42ºC under microaerobic conditions.
Early therapy of *Campylobacter* infection with *erythromycin* or *Ciprofloxacin* is effective in eliminating the organism from stools and may also reduce the duration of symptoms associated with infection.

**C/ Helicobacter**

Persons infected with *Helicobacter pylori* may develop acute gastritis (abdominal pain, nausea, and vomiting) within two weeks following infection.

*Helicobacter pylori* as *Warren and Marshall* (their Australian discover) first suggest, has been associated with peptic ulcer disease and cancer of the human gastrointestinal tract.
- *H. pylori infection* has been associated with more than **90% of duodenal ulcers** and **the majority of gastric ulcers**

- **Definitions:** *Helicobacter pylori* are gastric spiral-shaped, gram-negative, non-spore-forming rods, motile with multiple monopolar sheathed flagella. It is catalase producer, has a nitrate reductase and a very active urease but don’t grow at 42º C to distinct it from *Campylobacter*

- Both fecal-oral and oral-oral modes of interhuman transmission are likely to represent the principal route of dissemination of *Helicobacter Pylori*

- *H. pylori* is usually diagnosed by nonculture methods such as **histology, serology** or **urease testing**
*H. pylori* produce a large amount of extracellular *urease*

Multiple specimen from the gastric angle *fresh biopsy tissue* should be submitted for *rapid urease testing* on a *reagent strip* or in a *agar gel-based rapid urease*.

A pH indicator in the strip or the gel causes a color change if urease is present and enables the diagnostic of *H. pylori*

*Urea breath test (UTB)* is an noninvasive, without endoscopy, urease testing by human breath specimen. In this test, a solution containing *isotopically labeled urea* (13C* or 14C*) is consumed by the patient. *Isotopically labeled carbon dioxide* formed by *H. pylori* urease activity in the stomach is absorbed into the bloodstream and exhaled in the breath sample collected 30 minutes after ingestion that may be detected by gas isotope ratio spectrometry( 13C*dioxide)
Commercial ELISA detecting anti-*H. pylori serum IgG* are the serological test of choice for primary screening of patients with uncomplicated infections.

Multiple regimens are required to attain successful cure rate for *H. pylori* infection.

*Tetracycline-metronidazole-bismuth* for 14 days, *is* one of the most successful treatment regimens.
d/ Corynebacterium diphteriae

C. diphteriae causes throat diphteriea, cutaneous diphtheria, endocarditis and pharyngitis

Definitions:
The severe systemic effects of C. diphteriae are caused by the C. diphteriae exotoxin which is encoded by a bacteriophage carrying the tox gene.
Gram staining of corynebacteria shows slightly curved gram-positive rods with side not parallel and sometimes slightly wider ends giving some of the bacteria a typical club shape.
From fluid media, they are arranged as single cells, in pairs in V forms, in palisades, or in clusters with a so-called Chinese letter appearance. They are nonmotile and always catalase positive.
Main manifestation of diphtheria is an upper respiratory tract infection with a nasopharyngeal adherent membrane which occasionally lead to obstruction and causes asphyxia.

Due to immunization programs with a toxoid (formalin treated C diphtheriae toxin) the disease has nearly disappeared in countries with high socioeconomic standards.

Moreover, since beginning of the 1990s, diphtheria has reemerged in states of the former Soviet Union and is still endemic in some subtropical and tropical countries.

The diagnosis of diphtheria is primary a clinical one.
Gardnerella

*Gardnerella vaginalis* is a bacterium that causes one of the common forms of vaginitis, inflammation of vaginal mucosa.

That unique specie of the genus can cause *bacterial vaginosis*, *endometritis, postpartum sepsis*.

**Definitions:**

- *Gram stains* show thin gram-positive rods or coccobacilli. *Catalase* is not produced, and cells are nonmotile and has a slow fermentative metabolism.

- *Gold standard of diagnosis of bacterial vaginosis* isn’t the culture of specimens expected infected by *G. vaginalis* since it can also be recovered from healthy women, but *direct examination* of the vaginal discharge to detect the typical “fishy” *trimethylamine odor*, which is enhanced after alkalinization with 10% KOH.
The typical smear of vaginal discharge from bacterial vaginosis patients shows “clues cells” or bacteria covering epithelial cells margins, together with mix flora consisting of large numbers of small gram-negative and gram-variable (G. vaginalis) rods and coccobacilli, whereas lactobacilli are almost absent.

- Metronidazole is the drug of choice both for the local therapy of bacterial vaginosis and for systemic therapy of extravaginal infections caused by bacterial vaginosis associated flora.
“clue” cell with *G. vaginalis* covering epithelial cell margin
How to recovery a bacteria from a collected specimen?

1/ collect appropriate specimens and reject inappropriate ones
2/ transport rapidly collected specimens to laboratory for testing
3/ direct and/or staining smears must be prepared from specimens and examine for first orientation
4/ culture specimens on usual, enriched or selective medium to isolate pure colonies of suspected bacteria
5/ from pure isolated colonies identify suspected bacteria accordingly morphological, physiological, biochemical characteristics
6/ attend, if necessary, serological testing or indirect diagnostic which document, in patients serum, presence of specific antibodies
7/ study identified bacteria susceptibility to commonly used antibiotics
Thank you for your attention!