LECTURE 11 (2022-2023)

Laboratory Diagnosis of Respiratory Tract Infections

Respiratory tract infections divided in to:

1- Upper Respiratory tract infections

2- Lower Respiratory tract infections

Upper respiratory tract infections

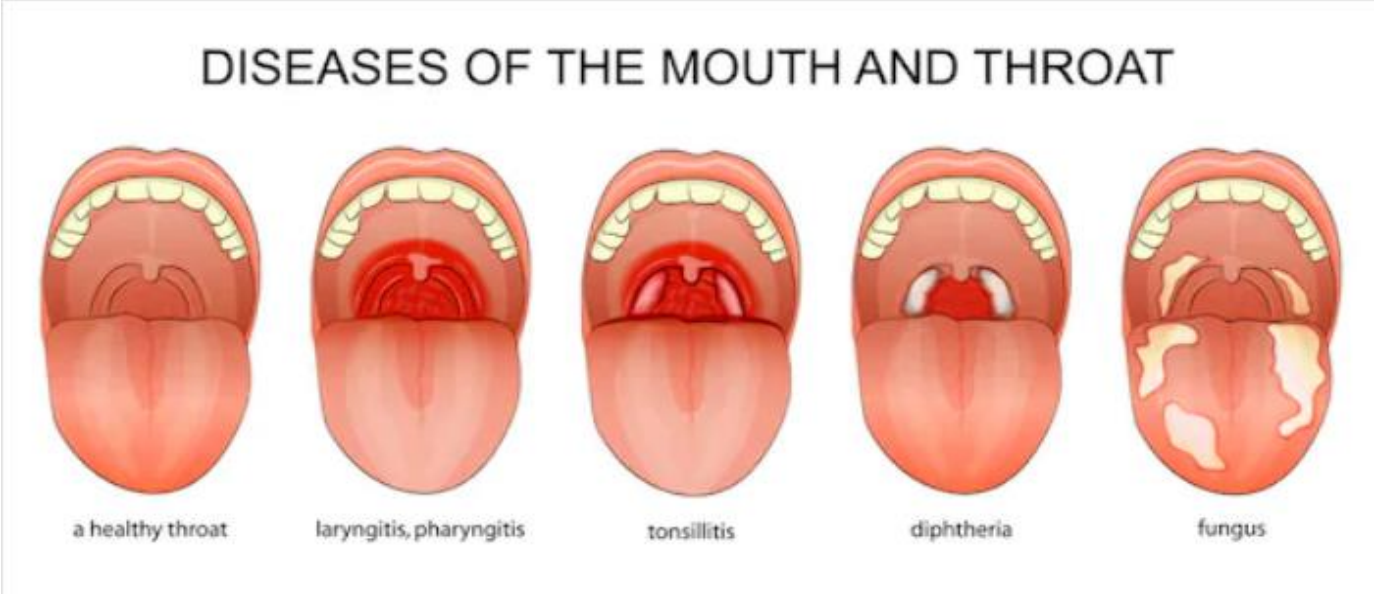
The upper respiratory tract extends from the larynx to the nostrils and comprises the oropharynx and the nasopharynx together with the communicating cavities, the sinuses and the middle ear. The upper respiratory tract can be the site of several types of infection:

— pharyngitis, sometimes involving tonsillitis, and giving rise to a “sore throat”
— nasopharyngitis
— otitis media
— sinusitis
— epiglottitis

Bacterial infections:

Pharyngitis

*Streptococcus pyogenes*is by far the most frequent causeof bacterial pharyngitis and tonsillitis. This infection is particularly prevalent in young children (5–12 years). When streptococcal pharyngitis is associated with a characteristic skin rash, the patient is said to have scarlet fever. In infants, a streptococcal throat infection may often involve the nasopharynx and be accompanied by a purulent nasal discharge.



Diphtheria

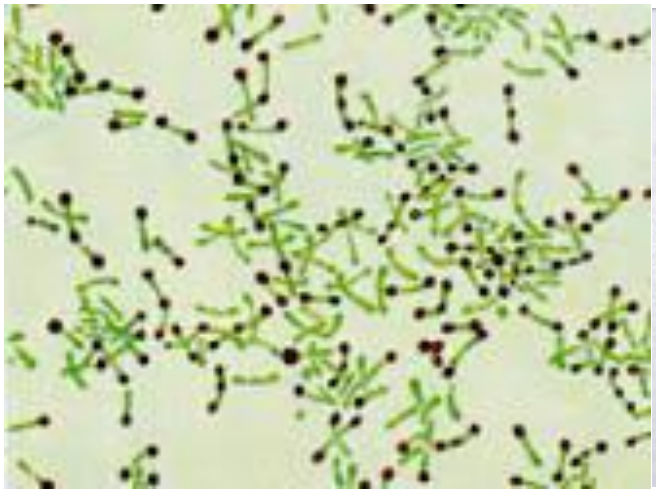
*Corynebacterium diphtheriae*is the cause of diphtheria, Diphtheria is a serious disease. *C. diphtheria* causes a typical form of infection, characterized by a greyish-white membrane at the site of infection (pharynx, tonsils, or larynx).

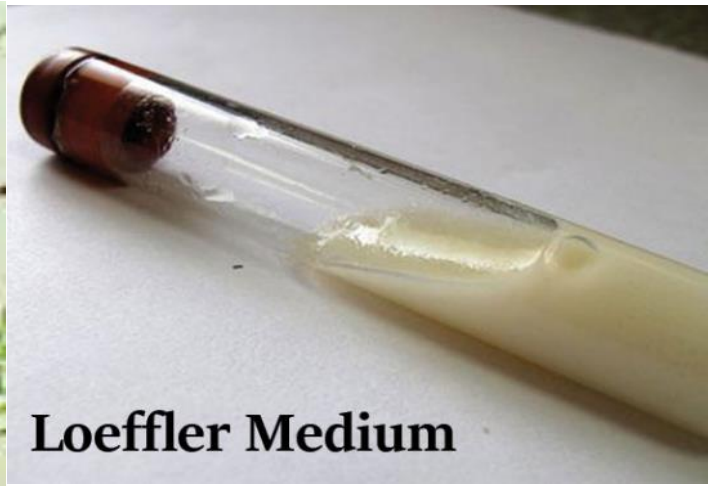
Culture for *Corynebacterium diphtheriae*

Although the diphtheria bacillus grows well on ordinary blood agar, growth is improved by inoculating one or two special media:

Löffler coagulated serum or Dorset egg medium. Both of these media give abundant growth of the diphtheria bacillus after overnight incubation. Moreover, the cellular morphology of the bacilli is more "typical": irregularly stained, short to long, slightly curved rods, showing metachromatic granules, and these bacteria arranged like Chinese letters.

Metachromatic granules are more apparent after staining with **Albert stain** by which the bacillus bacteria stained green while the metachromatic granules stained brown.





Gonococcal pharyngitis

Culture of throat swabs for gonococci should be done on specific request from the clinician, using the appropriate selective medium (Thayer–Martin medium).

Whooping Cough (Pertussis)

Whooping cough is the common name for **pertussis,** The disease is caused by *Bordetella pertussis***,** a tiny, encapsulated, strictly aerobic, Gram-negative rod. These organisms do not tolerate drying or sunlight and die quickly outside the host. laboratory diagnosis include culturing of nasopharyngeal swabs on a selective media (Bordet-Gengou medium).

Lower respiratory tract infections

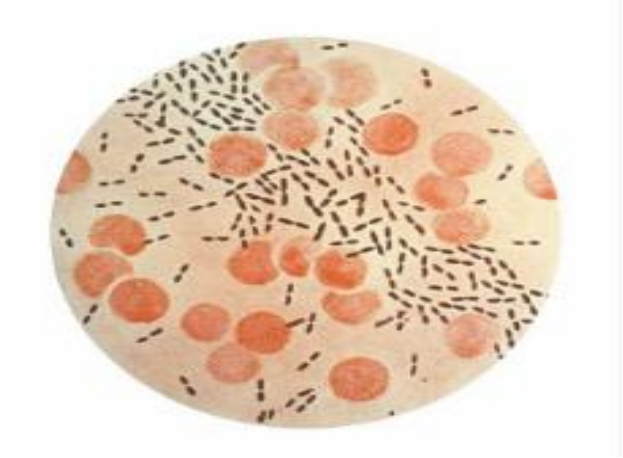
Lower respiratory tract infections (LRTI) are infections occurring below the level of the larynx, i.e. in the trachea, the bronchi, or in the lung tissue (tracheitis, bronchitis, lung abscess, pneumonia).

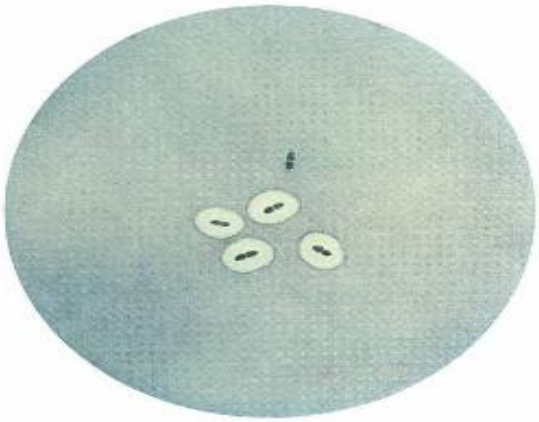
The most common infections

- **Pneumonia**

Causative agent of Pneumonia is *Streptococcus pneumoniae,* the pneumococcus, is a Gram- positive diplococcus. The most striking characteristic of *S. pneumoniae* is its thick polysaccharide capsule, which is responsible for the organism's virulence. This infection is nearly always caused by *S. pneumoniae*. A rare cause of a rather similar form of pneumonia is *Klebsiella pneumoniae.*

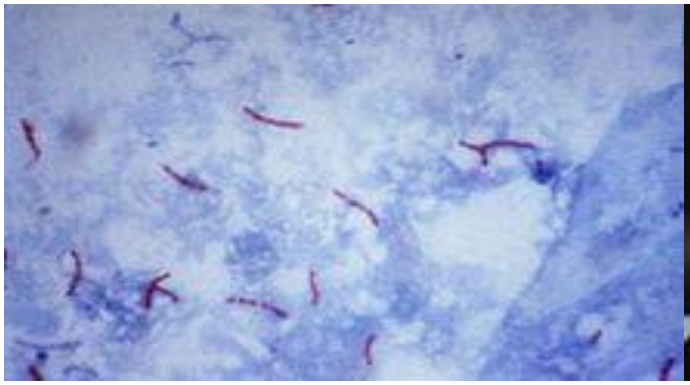
Other Gram-negative rods also can cause pneumonia, especially if host defenses are impaired.





- **Pulmonary tuberculosis**

The sputum of patients with pulmonary tuberculosis should be examined microscopically for acid-fast stained smear (Ziehl–Neelsen) to detect immediately any patients who have acid-fast bacteria in their sputum1. After the smear has been stained, the sputum should be treated by a decontamination procedure in order to kill as many of the non mycobacterial organisms as possible and to leave the tubercle bacilli viable and thus suitable for culture on Löwenstein–Jensen medium.



Collection of sputum specimens

- The sputum should be collected in a sterile wide-mouthed container with a secure, tight-fitting cover and sent to the laboratory.
- Label the specimen with the patient's name, the date and time collected.
- Send the specimen to the laboratory immediately.
- Refrigerate the specimen if a delay of greater than one to two hours is anticipated.

Microscopic examination

A portion of the purulent or mucopurulent sputum should be used for the preparation of a Gram-stained smear. Gram-stained smear may provide guidance to the clinician in the choice of antimicrobial chemotherapy.

Sputum Culture

Sputum or phlegm is the mucousy substance secreted by cells in the lower airways (bronchi and bronchioles) of the respiratory tract. It differs from saliva, which is produced higher up, in the mouth. Sputum can be any color including clear, white, yellow, green, pink or red and blood tinged with different medical conditions.

In addition to containing dead cells, foreign debris that is inhaled into the lung, and at times, bacteria, sputum contains white blood cells and other immune cells that protect the airway from infections.

By using a sterile loop sputum inoculate on to the various culture plates. A suggested routine set of culture media is as follows:

• Blood agar, with a streak of *S. aureus* to facilitate satellite growth of *H. influenzae*, and with an optochin disc placed in the middle of the secondary streaking,

• Chocolate agar.

• MacConkey agar.

The blood agar and chocolate agar plates are incubated at 36–37 C in an atmosphere containing CO2 (e.g. in a candle jar) and the MacConkey plate is incubated in air.

- Sabouraud dextrose agar used for the isolation of fungi.

Laboratory Diagnosis of Sexually Transmitted Diseases

The laboratory diagnosis of STDs is related to the sex of the patient, although some infections are common to both sexes like gonorrhea, syphilis and chlamydial infection but there are differences in the symptoms, the sites and methods of specimens collection in these infections.

Genital infections and STDs in women

These include:

1- **Vaginitis :**

Is caused by a limited number of infectious agents include:

- *Trichomons vaginalis*

Trichomoniasis:is an infection of urogenital tract and the most common site of infection is the urethra and vagina in women, it is caused by the single-celled protozoan parasite *Trichomons vaginalis* which classically produce a copious, frothy yellow or yellow-green discharge.

- *Candida albicans*

Vulvovaginal *Candidia*sis: is caused by *Candida albicans, squamous epithelial cells of vaginal is invaded and inflamed causing vaginal discharges and pain.* Discharge is typically more thick than trichomoniasis and curd like.

2- **Bacterial Vaginosis:**is caused by a number of infectious agents include:

- *Gardnerella vaginalis*
- *Peptococcoccus*
- Mycoplasma

3- **Cervisitis with or without Urethritis:** is caused by gonococci or *Chlamidiatrachomatis*

4- **Uterine sepsis:** is caused by **S.** *pyogenes, S. aureus, Clostridium* and *Mycoplasma*

5- **Genital ulceration**: is caused by *T. pallidum, Haemophilus ducreyi* and *Chlamidia*

6- **Tuberculosis of uterus:**is caused by *Mycobacterium tuberculosis*

7- **Viruses:** is caused by viruses like Cytomegalo virus, Herpes

Genital infections and STDs in men

The infections in men are mostly caused by the same organisms as in women, include:

1- **Urethritis:**

In men *C. trachomatis* causes urethritis lead to epididymitis and prostatitis.

2- **Prostatitis:** caused by gonococci or Chlamydia

3- **Ulceration:** caused by Herpes simplex virus, *T. pallidum, Haemophilusducreyi*and *Chlamydia***.**

Collection of specimen in men:

Cleanse around the urethral opening using a swab moistened with sterile physiological saline. When culture is indicated collect a sample of pus on a sterile cotton-wool swab.

Collection of Sample in women*:*

Endocervical canal for isolation *N. gonorrhoeae:*

Use a sterile vaginal speculum to examine the cervix and collected the specimen:

Pass a sterile cotton wool swab 20-30 mm into the endocervical canal and gently rotate the swab against the endocervical wall to obtain a specimen

Collection of vaginal discharge to detect *T. vginalis*, *C. albicans*,*G. vaginalis*:

Two preparations are required:

1. Wet preparation to detect motile *T.vaginalis*. Use a sterile swab to collect a specimen from the vagina.
2. Dry smear for Gram staining to detect *Candida* and examine for clue cells
   Gram positive cells and pseudohyphae of *C.albicans*

Collection of specimen to detect *T. pallidum*:

1. Wearing protective rubber gloves, cleanse around ulcer(chancre) using a swab moistened with physiological saline
2. Gently squeeze the lesion to obtain serous fluid collect a drop on a cover glass
3. Immediately deliver the preparation to laboratory for examination by dark-field microscopy

Culture the specimen

Different culture media used including:

a) Thayer Martin medium. For isolation of *N. gonorrhoeae* and incubate in moist carbon dioxide enriched atmosphere at 35 – 37 C for up to 48 hr. Thayer Martin medium contains the antibiotics (Vancomycin, Colistin, Nastatin).
b) Blood agar (aerobic and anaerobic)
c) MacConkey agar
d) Cooked meat medium. When puerperal sepsis or septic abortion is suspected. Incubated specimen in cooked meat medium and incubate at 35 -37 C and then sub culturing as indicated 24 hr,48 hr, 72 hr
e) Chocolate agar
f) Sabaroued agar. For *Candida* isolation

PH of discharge:

The normal reaction of vaginal discharge (puberty to menopause) is PH 3-3.5

- This to indicate the following:
- *T.vaginalis*: yellow-green purulent discharge with PH over 5
- *C.albicans*: White odorless discharge with PH below 5
- *G.vaginalis*: Grey offensive smelling (fishy ammoniacal smell) thin discharge with PH over 5

- ***Gram stain to examine:***
- Pus cells containing Gram negative diplococci or pus cells have been damaged and the organism seen lying outside the pus cells that could be *N. gonorrhoeae*
- Large G+ve yeast cells and Pseudohyphae that could *be C. albicans*

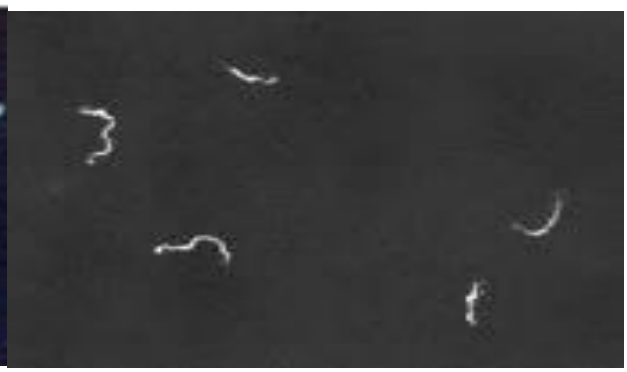
- **Smear from a patient with suspected puerperal sepsis or septic abortion, looked especially among pus cells for:**

- *Large G+ve rods with straight ends –C. perfringens*
- *G+ve Streptococci –S. pyogenes*
- *G+ve cocci- Staph. aureus*

- **Wet (saline) preparation to detect *T. vaginalis*:**

- To detect motile *T. vaginalis* trophozoites.


- **Dark field preparation to detect motile *T. palladium***

Urinary Tract Infection (UTI)

Urinary Tract Infection (UTI) is a bacterial infection that affects any part of the urinary tract. The main causal agent is *Escherichia coli*. The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Women are more prone to UTIs than men.

Factors that increase female susceptibility to UTI:

- Short length of the urethra
- Urethral contamination by rectal pathogens
- Introital & vestibular colonization by pathogenic bacteria
- Decreased urethral resistance after menopause

Urine culture

The urine samples routinely culture on Blood agar and MacConkey agar and now culture on **Cystine Lactose electrolyte-deficient (CLED) agar**.

Incubate the plate aerobically at 35 – 37Cº overnight.

CLED agar is now used by most laboratories to isolate urinary pathogens because it gives consistent results and allows the growth of both Gram negative and Gram positive pathogens. (the indicator in CLED agar is bromothymol blue and therefore lactose fermenting colonies appear yellow)..

Possible pathogens:

❑ **Bacteria:**

➢ **Gram positive:**

- *Staphylococcus saprophyticus*
- *Staphylococcus aureus*
- *Haemolytic streptococci*

➢ **Gram Negative:**

- *E. coli (commonest about 60 – 90 % of UTI)*
- *Proteus species(usually in hospitalized patient & with renal stones)*
- *Pseudomonas aeruginosa*
- *Klebsiella strains*

❑ **Fungi:**

- *Candida species(usually in hospitalized patient, in diabetic patient& immunosuppression*

❑ **Parasite:** *Schistosoma haematobium*

Gastrointestinal Tract Infections (GTI)

Enteric bacterial infections, causing diarrhoea, dysentery, and enteric fevers are important health problems throughout the world. Diarrhoeal infections are second only to cardiovascular diseases as a cause of death, and they are the leading cause of childhood death.

Etiological agents

The etiological agents which causing gastrointestinal tract infections divided in to:

1- Bacterial infections :

- The genus *Salmonella* cause gastroenteritis and typhoid fever
- *Shigella* spp. are the main cause of bacterial bacillary dysentery
- diarrhoea-producing *Escherichia coli*
- *Vibrio cholerae* cause Cholera,
- *Campylobacter jejuni*
- *Clostridium difficile* is the primary cause of enteric disease related to antimicrobial therapy. It produces a broad spectrum of diseases ranging from mild diarrhoea to potentially fatal pseudomembranous colitis.

2- **Viral diarrheas:** Rotavirus is a major cause of diarrheal disease in children.

3- **Parasitic diarrheas:** *Entamoeba histolytica* and *Giardia lamblia* can cause of diarrheal disease.

Media for enteric pathogens

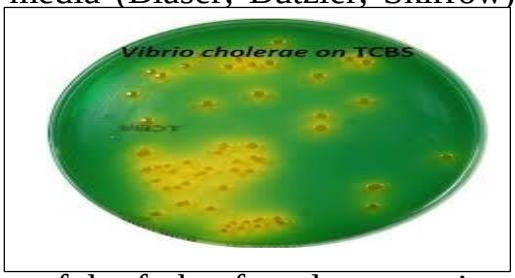
For *Shigella* spp., *Salmonella* spp. and *Y. enterocolitica*, MacConkey agar with crystal violet is recommended as a general purpose medium.

Xylose–lysine–deoxycholate (XLD) agar is recommended for the isolation of *Shigella* and *Salmonella*. Hektoen enteric agar (HEA) or *Salmonella–Shigella* (SS) agar are suitable alternatives.

For *Campylobacter* spp. there are several selective media (Blaser, Butzler, Skirrow) containing different antimicrobial supplements used.

Thiosulfate citrate bile salts sucrose (TCBS) agar is selective for *V. cholerae.*

Cefoxitin–cycloserine–fructose agar (CCFA) is selective for *Clostridium difficile*.



After inoculation of these media with one loopful of the faecal suspension, incubate the agar plates. Incubate the plates for the isolation of *Salmonella, Shigella* and *Yersinia* spp. and *V. cholerae* at 35 C in anaerobic incubator (without CO2), the plates for *Campylobacter* spp. at 42 C in an microaerophilic atmosphere with 10% CO2, and the plates for *Clostridium difficile* at 35 ∞C in an anaerobic atmosphere.

Purulent exudates, burns, wounds and abscesses

One of the most commonly observed infectious disease processes is the production of a purulent (sometimes seropurulent) exudate as the result of bacterial invasion of a cavity, tissue, or organ of the body.

A smear for Gram-staining and examination should be made for every specimen

Culture

All specimens of wounds, burns, pus or exudate should preferably be inoculated onto aminimum of two culture media:

— A blood agar plate for the isolation of staphylococci, streptococci and *Clostridium*

— A MacConkey agar plate for the isolation of Gram-negative rods;

All organisms isolated from wounds, pus, or exudates should be considered significant and efforts made to identify them.

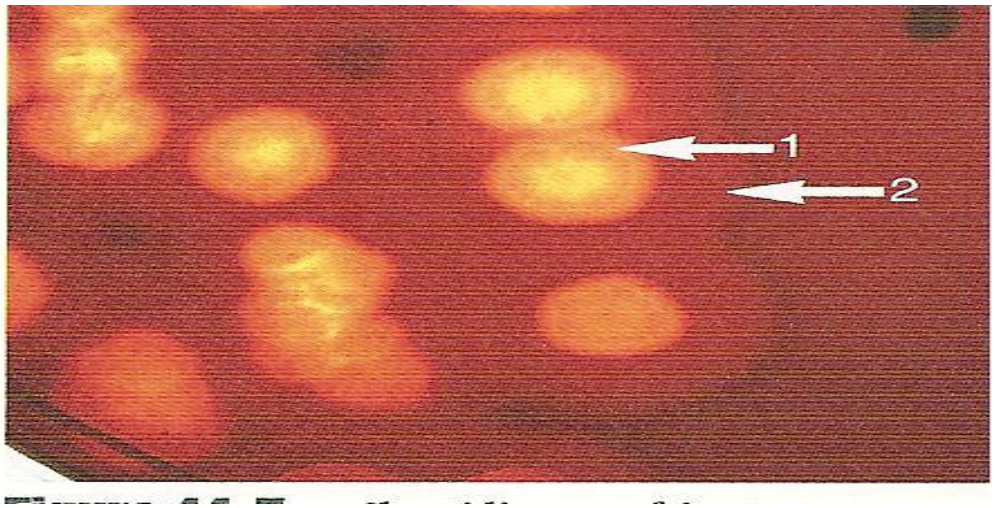
Wound swabs

Most common pathogens found in wound swabs

- Clostridium
- Candida
- Staphylococcus aureus
- Streptococcus
- Escherichia coli
- Fusobacterium
- Klebsiella
- Enterobacter
- Enterococci
- Peptostreptococcus
- Proteus
- Pseudomonas
- Bacteroides

The clinically most significant species is *Clostridium perfringens*. It is commonly associated with gas gangrene.

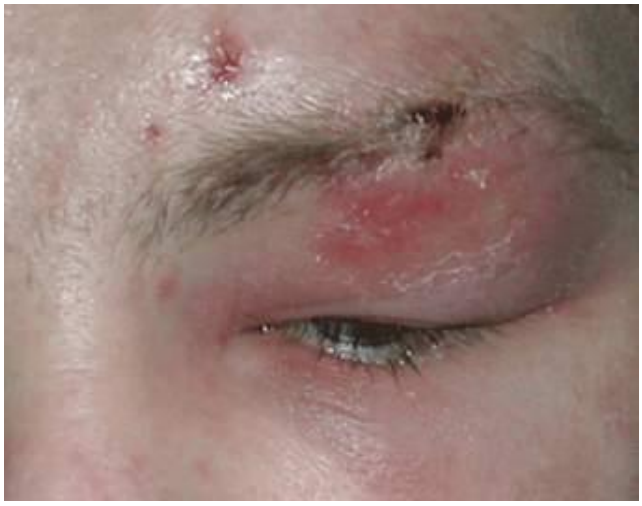
C. perfringens grows rapidly in anaerobic broth with the production of abundant gas. On anaerobic blood agar, colonies of moderate size (2–3 mm) are seen after 48 hours. **Most strains produce a double zone of haemolysis**: an inner zone of complete clear haemolysis, and an outer zone of partial haemolysis.



Clostridium perfringens

Eye and Ear infections

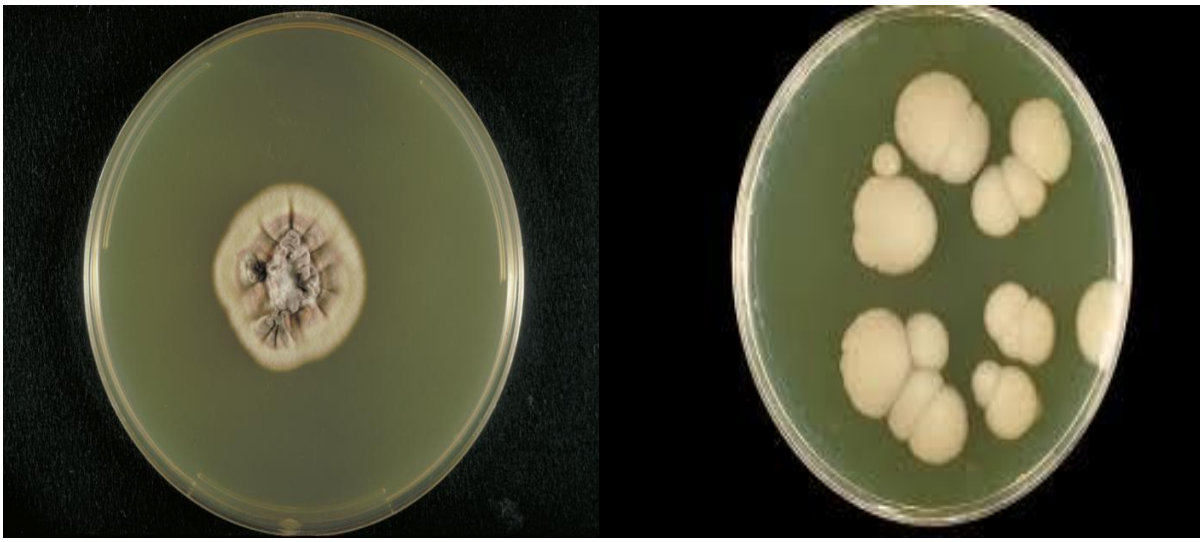
Ocular infection can be caused by bacteria, viruses, or chlamydia and can be detected by culture**.** Cotton swab will be used to collect the specimen from infected eye.



The culture of ear swab is a lab test. This test checks for germs that can cause infection. The sample taken for this test can contain fluid, pus, wax, or blood from the ear. Cotton swab will be used to collect the specimen from inside the outer ear canal. In some cases, a sample is collected from the middle ear during ear surgery

Specimens of eye and ear should be inoculated on to a minimum culture media:

— Blood agar plate for the isolation of staphylococci and streptococci.
— MacConkey agar plate for the isolation of Gram-negative bacteria.
— Chocolate agar plate for the isolation of *Neisseria*.
— Sabouraud dextrose agar plate for the isolation of fungi.



Sabouraud dextrose agar plate

Antibiotic susceptibility tests

Sensitivity (susceptibility) testing is used to select effective antimicrobial drugs. The standardized disc-diffusion method (Kirby–Bauer) is used.

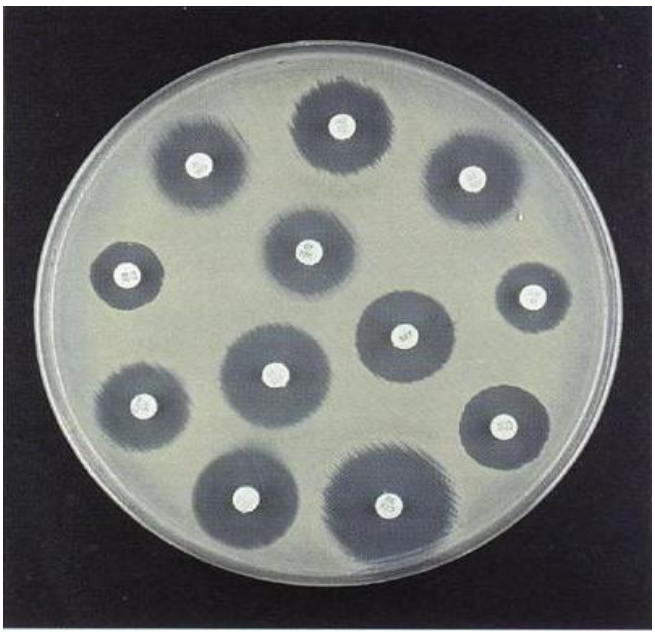
Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial sensitivity. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of sensitivity testing agar (Mueller–Hinton agar for most bacteria and blood agar for some bacteria) which uniformly inoculated with the test organism.

The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited. Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

- All strains of streptococci (such as *S. pneumoniae*) should be tested on blood agar for susceptibility.
- All Gram-negative rods and staphylococci were tested on Mueller hinton for susceptibility.
- Strains of *H. influenzae*and Neisseria should be tested for susceptibility using chocolate agar.

Also now automated susceptibility testing by VITEK 2 system have been used, which uses a new fluorescence-based technology to detect the susceptibility of bacterial isolates toward antibiotics. VITEK 2 system was evaluated for the identification and susceptibility testing of gram-negative and positive clinical isolates.

Blood agar plate



Mueller–Hinton agar plate