

## **Clostridium spp.**

**Dr. Sahar Mahdi 2020-2021**

Phylum: firmicutes

Class: clostridia

Order :clostridiales

Family: clostridiaceae

Genus ; Clostridium

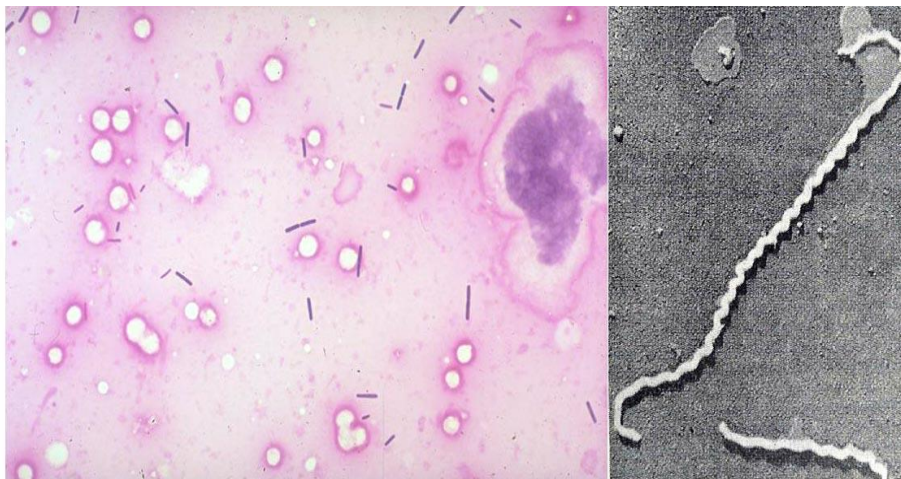
Organisms of this genus are anaerobic or microaerophilic Gram +ve rods, sporforming fermentative and motile except *C.perfringens* .

They are present normally in soil and intestines of animals and humans , most species are pathogenic under certain conditions.

### **1- *C.chauvoei***

A- The cause of black or black quarter in sheep and cattle .

B- cigar-shaped rod , non –encapsulated, forms spores which are oval, wide and central or subterminal.



***Clostridium chauvoei* in a Gram-stained smear and the scanning EM**



Black leg in cattle

***2-C- septicum***

A- The cause of malignant oedema in horses, sheep and cattle and gangrenous dermatitis in chickens, in addition of being one of the causes of gas gangrenes in man and animals.

B- Dose not form capsules and can produce acid and gas in litmus milk.



Malignant oedema in horses

### 3- *C. novyi*

A- the cause of gas gangrene in cattle , sheep and human, also big head disease in rams(especially *C.novyi* type A), and black disease or infectious hepatitis)in sheep and cattle (especially *C. novyi* type B).

B- motile under anaerobic conditions, and may show pleomorphic forms as it is a strict anaerobe and glucose or freshly-prepared blood for the enhancement of its growth.

C- when grown on egg-yolk medium , the colonies of type A,E and D will produce an opalescent zone of precipitation (naglar reaction ) due to the action of Lecithinase enzyme (produced by the organisms on egg-yolk.

D- spores may survive heating to 95°C for 15 min, but are killed in moist heat at 120 °C for 5 min.

## *C. novyi* type B ( $\alpha$ , $\beta$ )



- infectious necrotic hepatitis (black disease) in sheep and occasionally in cattle
- Dormant Spores germinate as secondary infection to liver fluke infestation that cause anaerobic condition



#### 4- *C. tetani*

A- Causes tetanus in many human and animals , with the spores being distributed everywhere.

B- Organisms are slender rods, motile (most strains). Gram+ve, and the form large and terminal spores with characteristic tennis racket appearance

C- Many are strict anaerobes growing at 14 to 44 °C.

D- Toxigenic strains may produce tetanolysin and tetanospasmin which may cause a condition characterized by rigidity of jaw, neck, limbs and trunk. This condition depends entirely on germination of spores into bacilli and the elaboration of toxins

C- used the fildes technique for cultured

**Clostridium tetani** grows on ordinary media.

Growth can be improved by blood and serum.

Because of marked tendency to **swarm** over the surface of the agar, surface colonies are difficult to obtain.

After incubating anaerobically for 24 hours, if the water of condensation at the bottom of a slope of nutrient agar is inoculated with a mixed cell culture, subcultures from the top of the tube will yield a pure growth of tetanus bacillus.

This is called **Fildes technique**.



*Clostridium tetani*



Rigidity of jaw, neck, limbs and trunk

### **5- *C. botulinum***

A- This organism and its spores are well distributed in soil, vegetables. Fruits and pastures.

B- strict anaerobe, may grow within 20-35°C and dose not ferment lactose.

C- it may cause food poisoning in man and animals after ingestion of performed toxin which is excreted in the foodstuff

under suitable conditions . These toxins are extremely lethal, especially type A toxin



Poisoning signs may involve paralysis of the hind limbs, muscles of the mouth, pharynx and neck, ending in coma and death.

#### 6- *Clostridium difficile*:

is gram-positive, a spore-forming, toxin-producing, anaerobic bacterium that causes antibiotic-associated colitis. It colonizes the human intestinal tract after the normal gut flora has been altered by antibiotic therapy. *C. difficile* infection is one of the most common healthcare-associated infections and a significant cause of morbidity and mortality among older adult hospitalized patients.

Man cause pseudomembranous colitis in human and ileocolitis in laboratory animals.

It is of minor importance in veterinary public health.

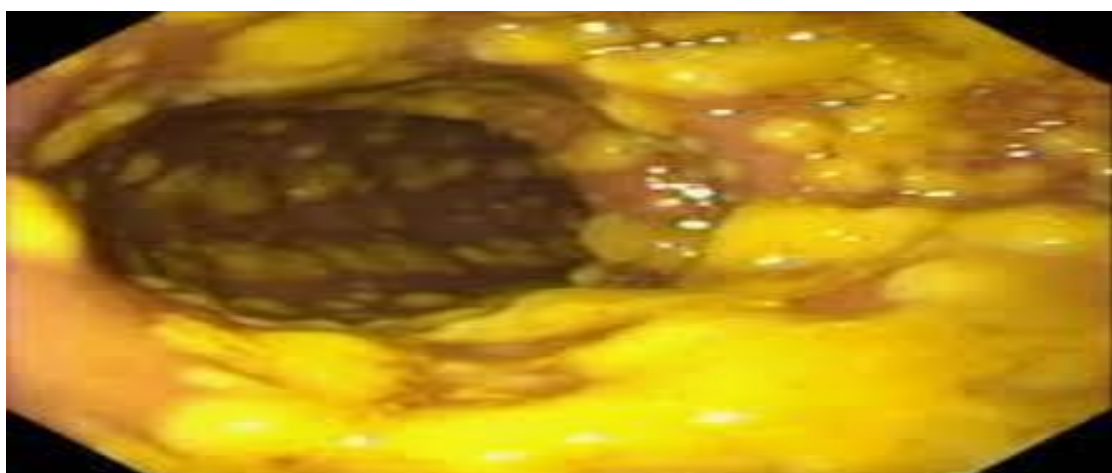
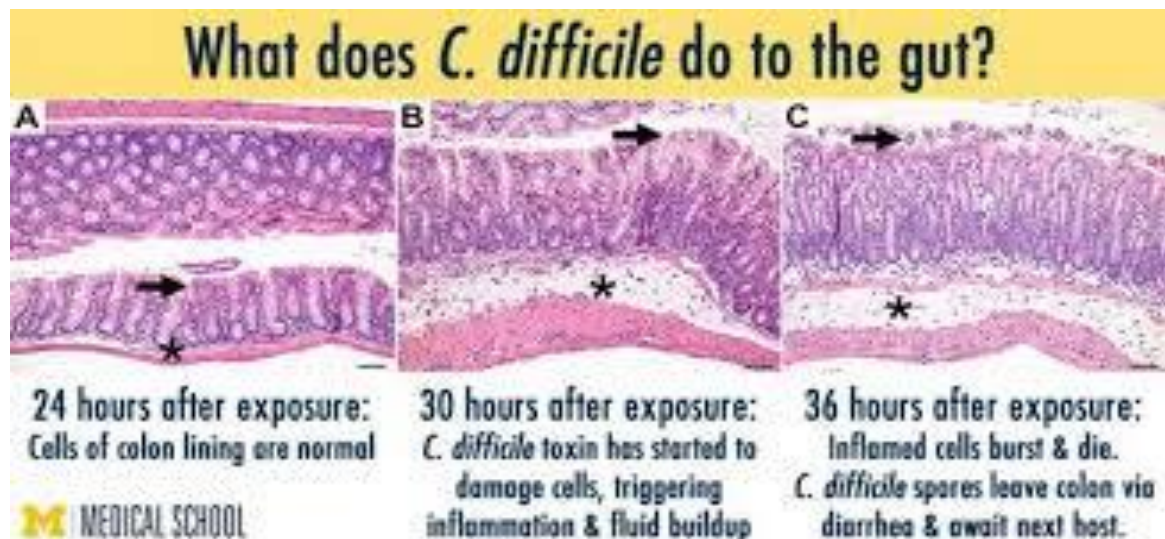
This organism may be a normal constituent of the fecal flora of health infants; however, it is rare and transient in the intestine of the healthy adult.

Non-hemolytic and non-proteolytic.

### **Diarrhea with colitis**

Watery diarrhea is the cardinal symptom of

*C. difficile*-associated diarrhea (CDAD) with colitis. Other manifestations include lower abdominal pain and cramping, low-grade fever, nausea, anorexia, and leukocytosis. Diarrhea may be associated with mucus or occult blood.



*Clostridium difficile*

## 7- *Clostridium perfringens* (or *welchii*)

A- the cause of gas gangrene in human and animals plus other important Enterotoxaemic infections in sheep and other animals.

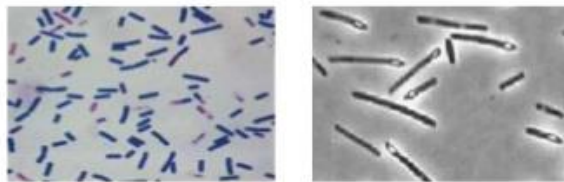
B- different strains produce several toxins which can be differentiated by neutralization test . On this basis *C. perfringens* is classified into type A, B, C, D, E, each of which causes a certain disease.

C- the organism is found in soil and intestines of healthy animals and human , but under certain encouraging conditions may reverse to become pathogenic and cause disease.

### 1. *Clostridium perfringens* (*Cl. welchii*)

#### Morphology

- Large Gram-positive bacilli with straight, parallel sides & slightly rounded ends.
- Measure 4-6x1 $\mu$ m in size, occurring singly or in chains
- Pleomorphic, capsulated & non-motile.
- Spores are **central or sub terminal**. **Spores are rarely seen in culture media or material from pathogenic lesions**, a characteristic morphologic feature



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Morphology and cultural characteristics of clostridia:

1-rod-shaped, occurs singly or in pairs , forms capsules especially in smears from infected tissues, non-motile, gram+ve, and forms oval, central or subterminal spores.

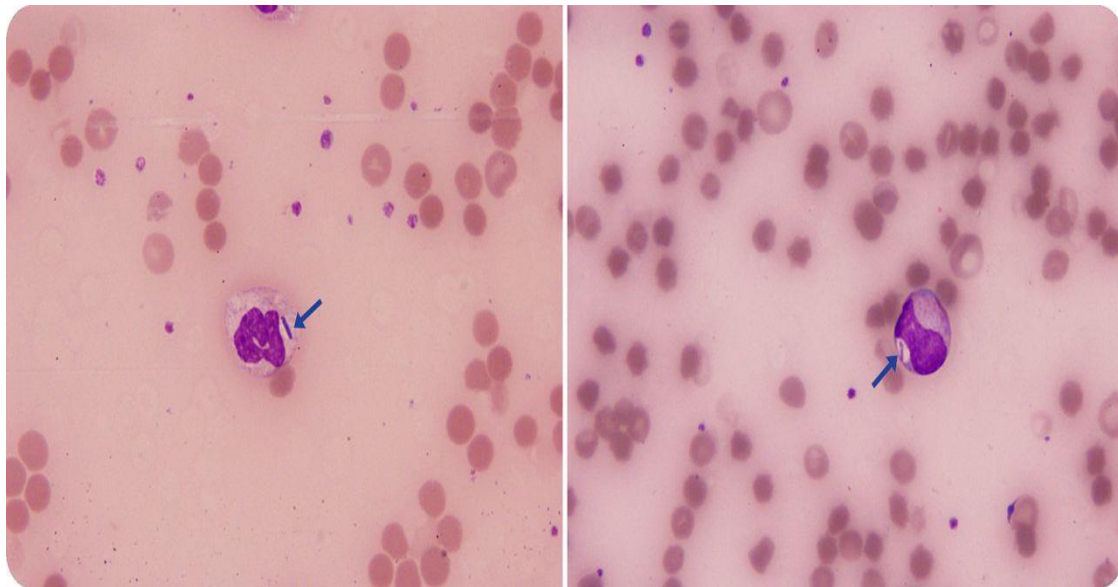


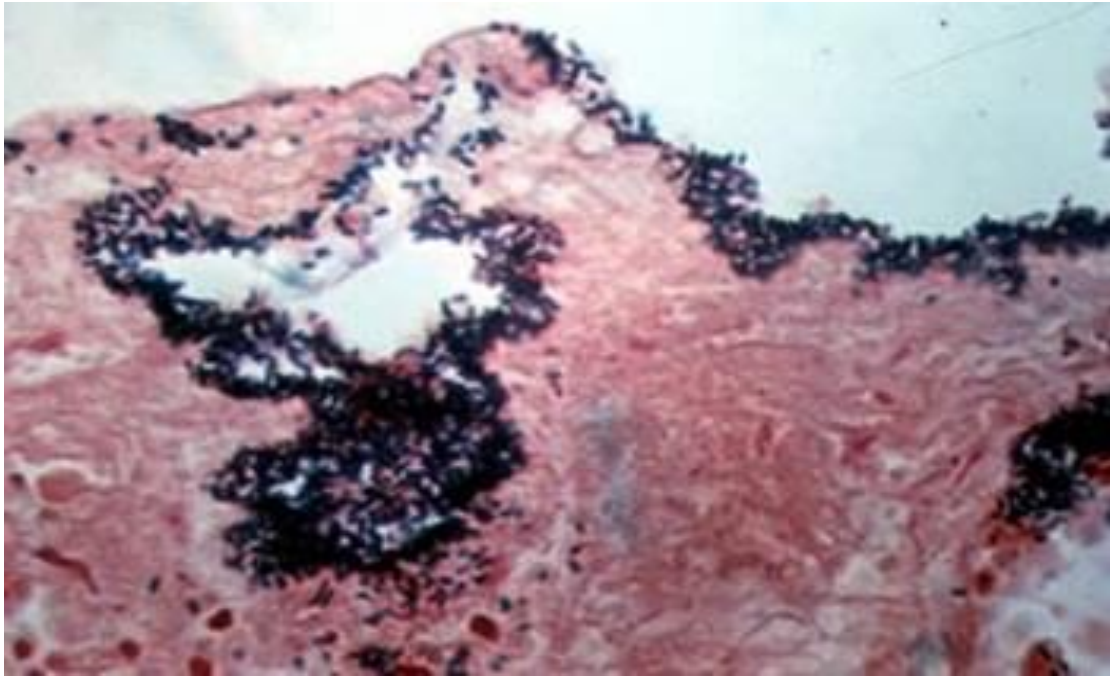
2- grow well on ordinary media at 37-47 °C, and growth is enhanced by the presence of glucose or blood.

3- on blood agar, the round colonies of smooth strains are surrounded by a narrow zone of  $\beta$ - haemolysis, and a wider zone of  $\alpha$  -haemolysis. This feature will become very distinct when the plates are stored at a lower temperature.

4- On media containing human serum or filtered egg-yolk, the organisms produce a marked opalescence caused by  $\alpha$  toxin (lecithinase) and this phenomenon is called : Nagler or lecithinase reaction .

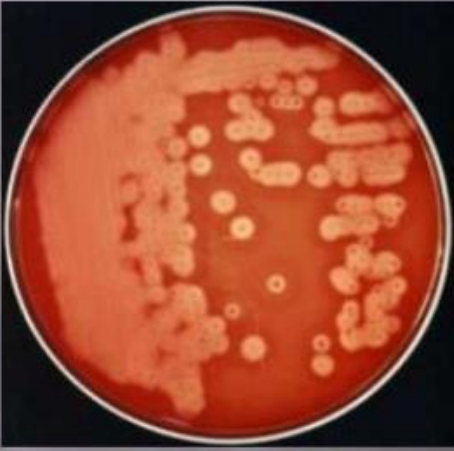
5- Enterotoxaemic infections are characterized by the presence of very large numbers of *C. perfringens* in the intestines , clearly demonstrate by gram's stain smear prepared (immediately after death of the animal from the intestinal contents or lesions in the intestinal wall).



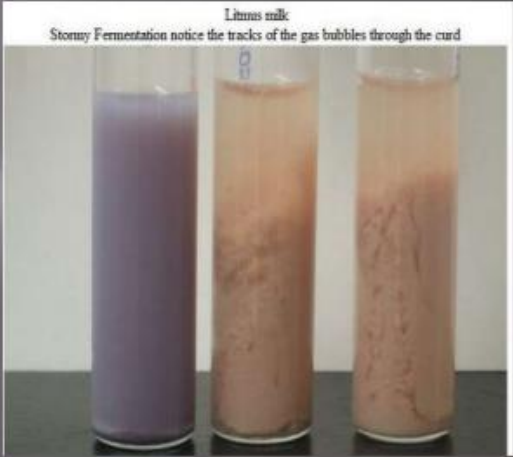


C. perfringens in tissue

***Clostridium. perfringens***  
***Culture & identification***



B – Haemolysis  
On blood agar media



Litmus milk  
Stormy Fermentation notice the tracks of the gas bubbles through the curd

1= Control, 2 and 3= "Stormy clot fermentation"  
*Clostridium perfringens* will produce acid and gases

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6- fresh samples from an infected animal are cultured onto blood agar, incubated anaerobically for 24-48 hr. Another alternative

is to inoculate the sample into Cooked meat broth (freed of oxygen by preheating at 100°C in a water bath for few minutes then cooled and cultured and then subcultured onto blood agar.

## Media used for Cultivation

- Liquid medium for cultivation cooked meat broth
- Thiglyclolate broth
- CMB contain unsaturated fatty acids which take up oxygen
- Proteolytic medium turns the medium black and Saccharolytic medium turn the meat pink



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### Main important toxins of *C. perfringens*

Type of <i>C. perfringens</i>	major lethal toxins produced
A	alpha
B	alpha, beta and epsilon
C	alpha and beta
D	alpha and epsilon
E	alpha and iota

A- alpha toxin is a major lethal toxin

B- beta is a major lethal and necrotizing toxin

C- Epsilon toxin is a major lethal and necrotizing toxin

D- iota toxin is a major lethal and necrotizing prototoxin.

## Diagnosis of clostridia infections

A- direct examination : making smear from the intestinal contents and staining them with grams stain to check for large numbers of G<sup>+</sup>ve rods.

B- isolation , either by direct cultivation into media like thioglycollate or cooked meat broths or by streaking onto agar media, like blood or SPS(sulphite-polymyxin-sulphadiazine) agar.

C- identification: by different techniques, like fluorescent antibody and gas chromatography.

D- laboratory animal inoculation.

E- biochemical identification: using the following tests:

- Heamolysis on blood agar
- Shape and location of spore
- Gelatin liquification
- Nitret reduction
- Motility
- Litmus milk
- Indole
- H<sub>2</sub>S productions
- Urease
- Starch hydrolysis
- Sluggish double zone of heamolysis (  $\beta$  and  $\alpha$  )

- **Antigenic Structure:**
- Flagella (H), somatic (O), and spore antigens.
- **Pathogenicity Determinants:**
- play a role in local infection only in conjunction with other bacteria that create suitable environment for their invasion
- systemic-acting, **plasmid-mediated A-B neurotoxin (tetanospasmin)** produced intracellularly
- Mode of Action --- one of most poisonous substances
- binds gangliosides in synaptic membranes (synapses of neuronal cells) and **blocks release of inhibitory neurotransmitters**, continuous stimulation by excitatory transmitters
- muscle spasms (spastic paralysis) (trismus (**lockjaw**), risus sardonicus, opisthotonus), cardiac arrhythmias, fluctuations in blood pressure.