CLOSTRIDIA

Gram positive, anaerobic, spore forming bacilli

CLOSTRIDIA

- Pathogens
- Saprophytes
- Industrial impt.- Cl.acetobutylicum

CLOSTRIDIA

- Cl.perfringens- gas gangrene
- Cl.tetani- tetanus
- Cl.botulinum- food poisoning
- Cl.difficile- antibiotic associated colitis

CLOSTRIDIA

- Highly pleomorphic
- GPB of 3-8 x 0.4-1.2µm in size
- Long filaments & involution forms- common
- Spores- wider than bacillary body, resembling a spindle (*kloster*- spindle)
- Frequency of spore format^{N}- vary with diff spp.
- Shape & position of spores vary in diff. spp. helps in identification & classification

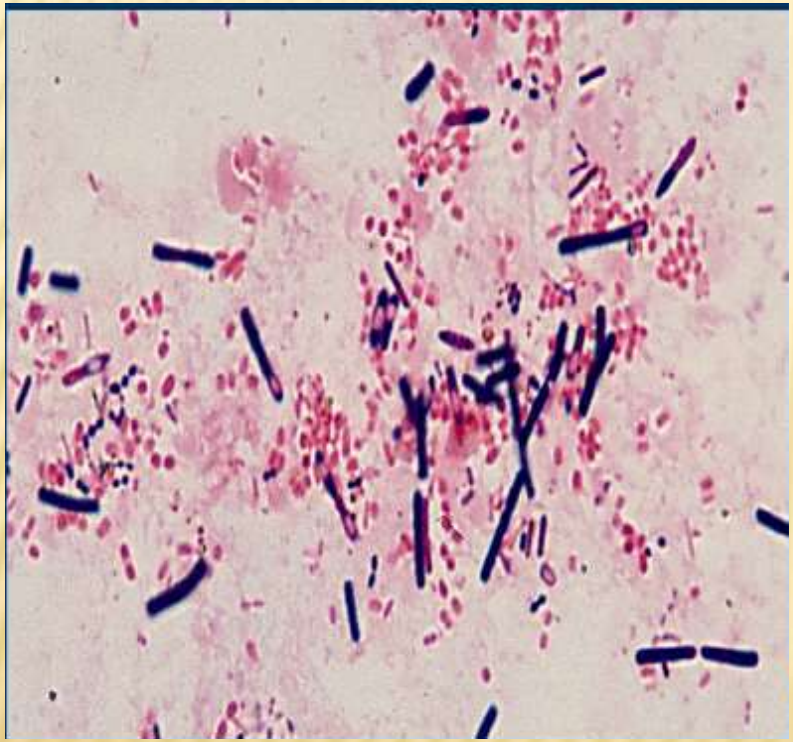
CLOSTRIDIA- SPORES

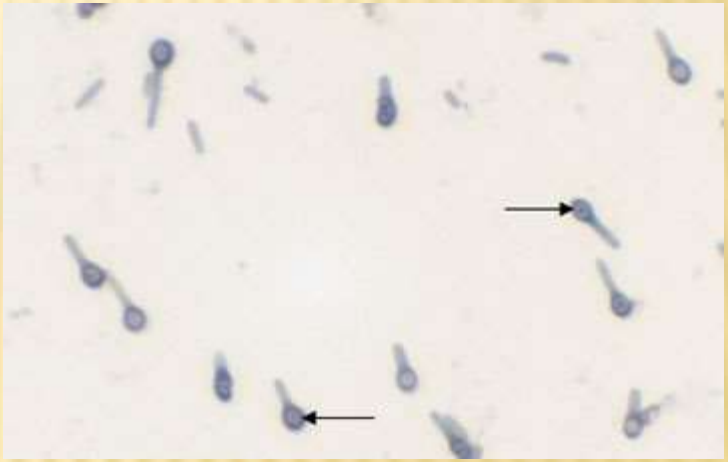
- Central/ equitoral- spindle shaped bacillus e.g. Cl.bifermentans
- Subterminal- club shaped bacillus e.g. Cl.perfringens/ Cl.welchii
- Oval & terminal- bacillus resembles tennis racket e.g. Cl.tertium
- Spherical & terminal- giving drumstick appearance e.g. Cl.tetani

CLOSTRIDIA

- Cl. perfringens

Cl. tetani





CLOSTRIDIA

- Easily stained, often Gram variable
- Sensitivity to O_2- varies in diff. spp.
- Provision of sufficient low redox potential (Eh) in the medium is impt. which is achieved by adding reducing substances
- A small conc. of CO_2 enhance growth
- Optm temp.- 37^oC, optm pH- 7-7.4
- Slow growth on solid media
- RCM- saccharolytic & proteolytic activities
- Litmus milk medium- product^N of acid, clot & gas

CLOSTRIDIA

- Spores exhibit a pronounced but variable resistance to heat, drying & disinfectants
- Sensitive to metro, peni, chephalo & chloramphenicol; less sensitive to tetra & resistant to aminoglycosides & quinolones
- Toxin mediated $inf^{N}s$, invasive power is limited e.g. botulisum d/t preformed toxin in food, tetanus d/t potent toxin but it is invasive & gas gangrene clostridia are toxigenic & invasive thus spread along the tissues causing septicemia

CLASSIFICATION- CLOSTRIDIA

Position of spores	Predominant proteolytic	Predominant saccharolytic	Proteolytic, but not saccharolytic	Saccharol ytic but not proteolytic
Central/ sub-terminal	Cl.bifermentans, Cl.botulinum (A, B, F), Cl.histolyticum	Cl.perfringens, Cl.septicum, Cl.novyi		Cl.fallax, Cl.botulinum (C,D,E)
Oval/ terminal		Cl.defficile		Cl.tertium
Spherical & terminal			Cl.tetani	

CLOSTRIDIA- PATHOGENIC SPP.

A. Gas Gangrene Group	
Established pathogens	Cl. perfringens/ Cl.welchii Cl. septicum, Cl.novyi
Less pathogenic	Cl. histolyticum, Cl.fallax
Doubtful pathogens	Cl.bifermentans, Cl.sporogenes
B. Tetanus	Cl. tetani
C. Food Poisoning	
Gastroenteritis	Cl. perfringens (Type A)
Necrotizing enteritis	Cl. perfringens (Type C)
Botulism	Cl.botulinum
D. Acute colitis	Cl. difficile

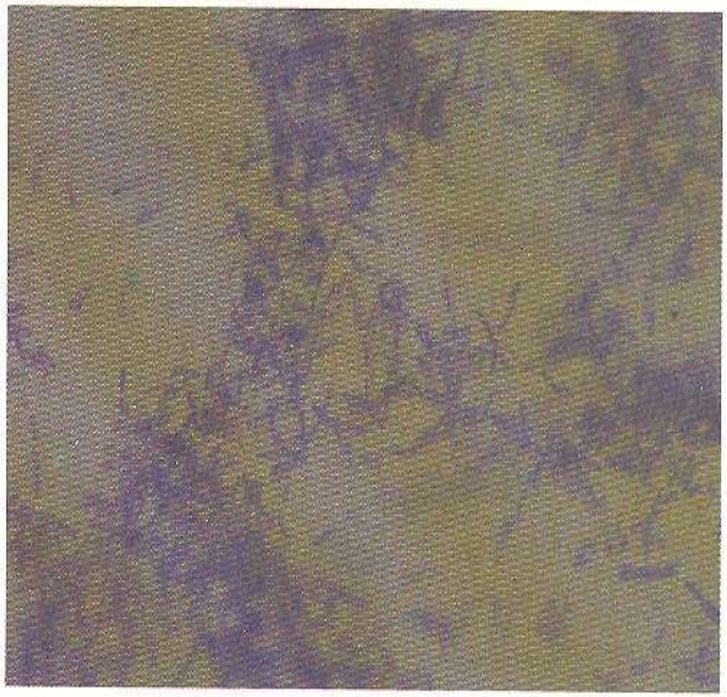
CL. PERFRINGES

- Originally cultivated by Achalme (1891)
- 1st described by Welch & Nuttall (1892), isolated from blood & organs of a cadaver
- Impt agent of gas gangrene, also cause food poisoning & necrotitic enteritis
- Normal habitat- large intestine
- Spores are found in soil, dust, air

CL. PERFRINGES- MORPHOLOGY

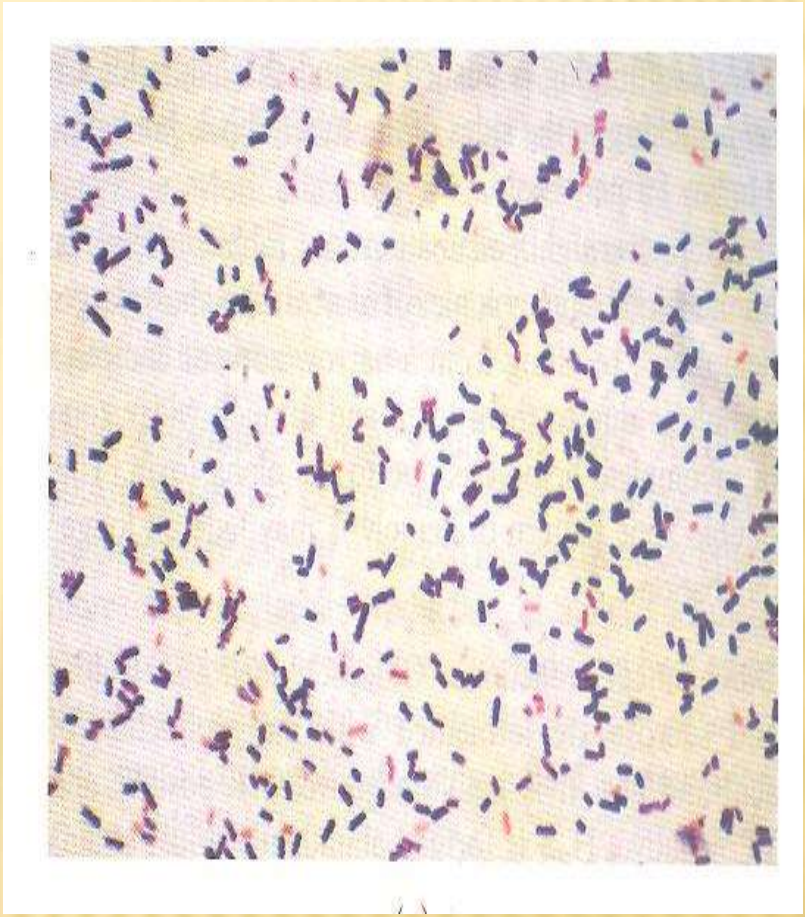
- Plump, GPB (4-6 x 1 µm) with straight, parallel sides, rounded/ truncated ends, capsulated, non-motile
- Spores are central/ subterminal and rarely seen in artificial culture or in material from pathological lesions
- ***Absence of spores is the characteristic morphological feature***

CL. PERFRINGES- MORPHOLOGY



(*b*)

Fig. 28.1 (a) and (b) Gram stain and spore stain of *Clostridium perfringens*



CL. PERFRINGES CONTD.

- Culture:- anaerobe but can grow under microaerophilic conditions.
- Temp. 20-50ºC, usually @37ºC (optm. 45ºC)
- pH- 5.5-8
- Culture media:- RCM- meat turn to pink, no digestion
  Blood agar– target hemolysis- double zone of hemolysis
- Stormy fermentation: In litmus milk, fermentation of lactose leads to formation of acid- indicated by the color change of litmus. The acid coagulates the casein & the clotted milk is disrupted due to the vigorous gas production. The paraffin plug is pushed up & shreds of clot are seen sticking to the sides of the tube

CL. PERFRINGES CONTD.

- Biochemical Reactions:-

L, G, Maltose, S– fermented with product^{N} of acid & gas

- Indole -ve, MR +Ve ,
- VP -ve H_2S +Ve

CL. PERFRINGENS CONTD.

Destruction of spores:- boiling for 5mins, food poisoning strains (Type A, C) resist boiling for 1-3hrs,
autoclaving @ 121ºC is lethal

Spores resistant to antiseptics & disinfectants

CL. PERFRINGENS CONTD.

- Classification- 5 types (A,B,C,D,E) based on toxin production
- Mainly depends on 4 major toxins
- Done by neutralizat^{N} by using sp. antitoxin

VIRULENCE FACTORS

- Toxins- 12 distinct toxins
- 4 are major toxins (α,β,ε & iota)
- Enzymes
- Biologically active soluble substances

TOXINS PRODUCED BY CL. PERFRINGENS

- Major toxins:-

I) Alpha toxin:- lethal, dermonecrotic, haemolytic, a phospholipidase (lecithinase C). Lecithinase in presence of Ca++ & Mg++ ions, splits lecithin into phosphoryl cholin & diglyseride which is seen as opalescence in serum or egg yolk agar. This is specifically neutralised by antitoxin. Play role in gas gangrene, wd infNs, septicemia & food poisoning

II) Beta:- lethal, necrotic. Play role in enteritis

III) Epsilon:- lethal, necrotic

IV) Iota:- lethal, necrotic

NAGLER'S REACTION

When Cl.perfringens is grown in a medium containing 6% agar, 5% Fildes peptic digest of sheep blood and 20% human serum with the antitoxin spread on one half of the pate, colonies on the other half without the antitoxin will be surrounded by a zone of opacity. No opacity around colonies with antitoxin d/t neutralisat^{N} of α-toxin- sp lecithinase effect

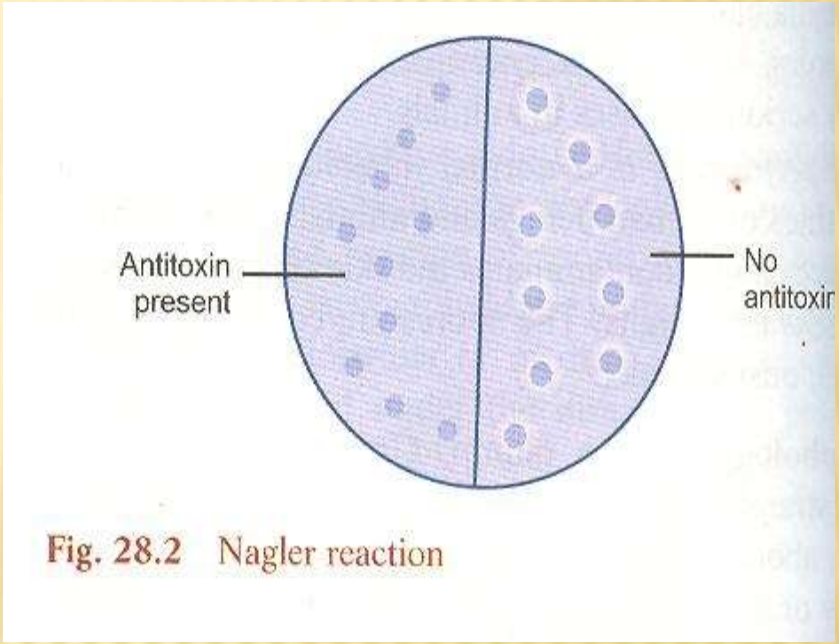


Fig. 28.2 Nagler reaction

TOXINS PRODUCED BY CL.PERFRINGENS

- Other toxins:-

Gamma- minor lethal action

Eta- minor lethal action

Delta- lethal & haemolytic actions

Theta- O_2 labile haemolysin, lethal & cytolytic

Kappa- a collagenase

Lambda- a proteinase & gelatinase

Mu-toxin- a hyaluronidase

Nu-toxin- a deoxyribonuclease

ENZYME & OTHER SOLUBLE SUBSTANCES

- Enzymes which destroy the blood gr. substances
- A neuraminidase
- A haemagglutinin
- A haemolysin
- A fibrinolysin
- Histamine
- A bursting factor- which has a specific action on muscle tissue, may be responsible for muscle lesions in gas gangrene
- A circulation factor

PATHOGENICITY

- Gas gangrene- Cl.perfringens type A
- Food poisoning- Cl.perfringens type A
- Gangrenous appendicitis- Cl.perfringens Type A
- Necerotising enterities- Cl.perfringens Type C
- Biliary tract infections- acute emphysematous cholecystitis, postcholecystectomy septicaemia
- Endogenous gas gangrene of intra- abdominal origin
- Brain abscess & meningitis
- Panophthalmitis
- Thoracic infections
- Urogenital infections

GAS GANGRENE

A rapidly spreading edematous myonecrosis, occuring characteristically in association with severe wounds of excessive muscle masses that have been contaminated with pathogenic clostridia, particularly with Cl.perfringens (Cl.welchii)

GAS GANGRENE

- Life threatening condition characterized by:
  1. Muscle necrosis with edema
  2. Sepsis
  3. Gas production- usually a mixture of hydrogen, carbon dioxide, nitrogen and oxygen
- Also called Malignant edema/ Anaerobic myositis/ Clostridial myonecrosis
- Can rapidly lead to septicemia, shock and death.
- Mostly follows
  - Trauma e.g. burns, crush injuries, battle wounds, open fractures, large muscle involvement e.g. thigh
  - Features relating to the wound e.g. contamination with dirt or sharpnel
  - Surgeries, Abortion (especially criminal abortion) and Caesarian section
  - Intramuscular injections

GAS GANGRENE CONTD.

- The mere presence of clostridia in wounds does not constitute gas gangrene
- MacLennan described 3 types of anaerobic wound infections:-

1) Simple wound contamination *with no invasion* of underlying tissues
2) *Anaerobic cellulitis*- clostridia invade the fascial planes with minimal toxin production and no invasion of muscle tissues
3) *Anaerobic myositis/gas gangrene*- most serious, clostridial invasion of healthy muscle tissues & abundant formation of exotoxins

GAS GANGRENE CONTD.

- Low O_2 tension is the most favorable condition for clostridia multiplication. This is achieved ideally in battle wounds, so this is the disease of war
- In civilian life- the disease generally follows road accidents or other type of injury involving crushing of large muscle tissues
- Rarely- follow surgical operations

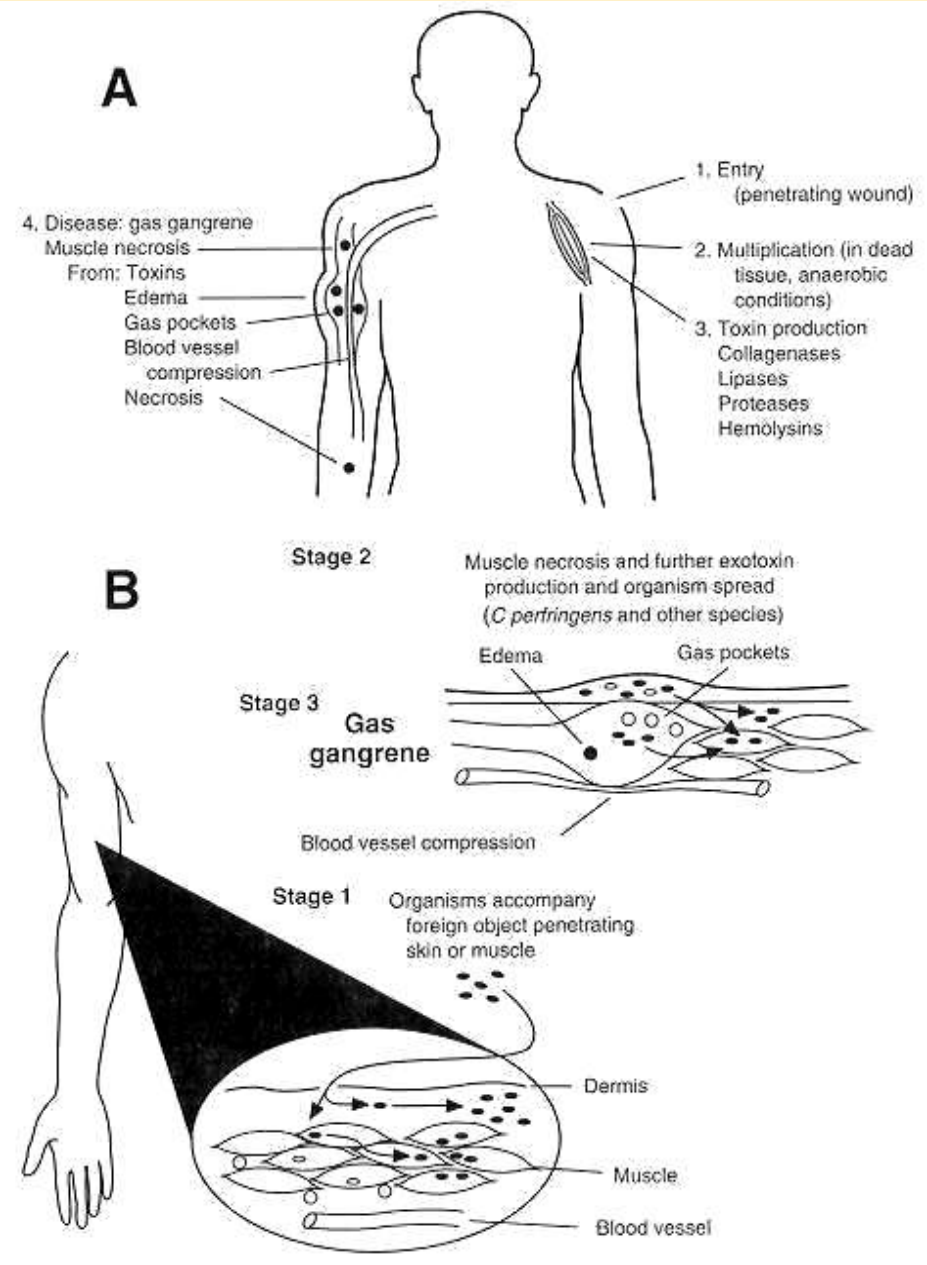
PATHOGENESIS

- Organisms enter the tissue by contamination of traumatized area from foreign particle such as soil, dust
- Presence of foreign body and facultative anaerobes- create anaerobic conditions
- Proliferating clostridia release necrotizing toxin & hyaluronidase causing devitalization of adjacent tissues & interfere the blood supply
- Tissue necrosis, severe toxemia & death
- Clinical features:-

- Swelling of infected tissue, crepitation in subcutaneous tissue & muscle, foul-smelling discharge, pain, necrosis
- fever, toxemia, shock, death

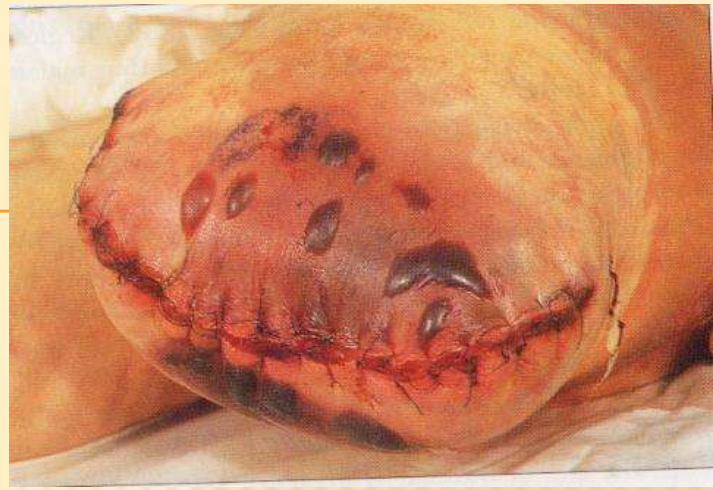
PATHOGENESIS OF GAS GANGRENE

- Entry of clostridia (spores) into the wounds along with implanted foreign particles such as soil, road dust, bits of clothing or sharpnel
- Anaerobic/ low oxygen tension environment
- Germination of spores
- Release of exotoxins (lecithinases, collagenases & hyaluronidases)
- Extensive tissue damage – edema, necrosis
- Gas production (crepitus)



Pathogenesis of Gas gangrene

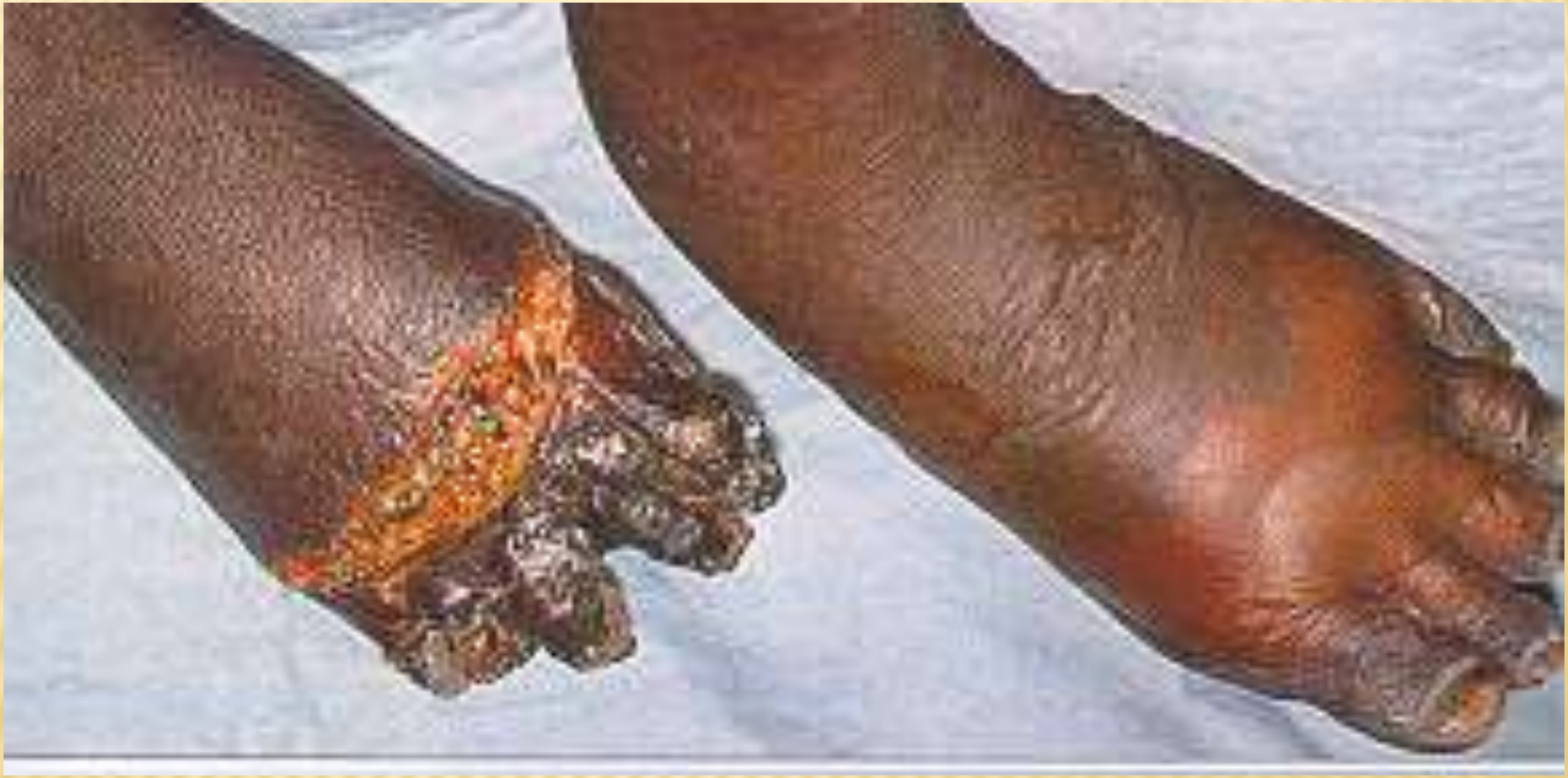
CLINICAL PRESENTATION



- Initially - no skin changes just pain
- Systemic symptoms e.g. fever, dehydration
- Once nerves damaged anaesthesia occurs
- Paralysis
- Skin changes - cellulitic progressing to dark purple; develop vesicles and bullae
- Subcut. air on palpation (may not be present early on)
- Foul smelling discharge
- Edema
- Necrotic or hemorrhagic tissue
- Patients may also present in septicaemic shock with tachycardia, hypotension, fever, stupor

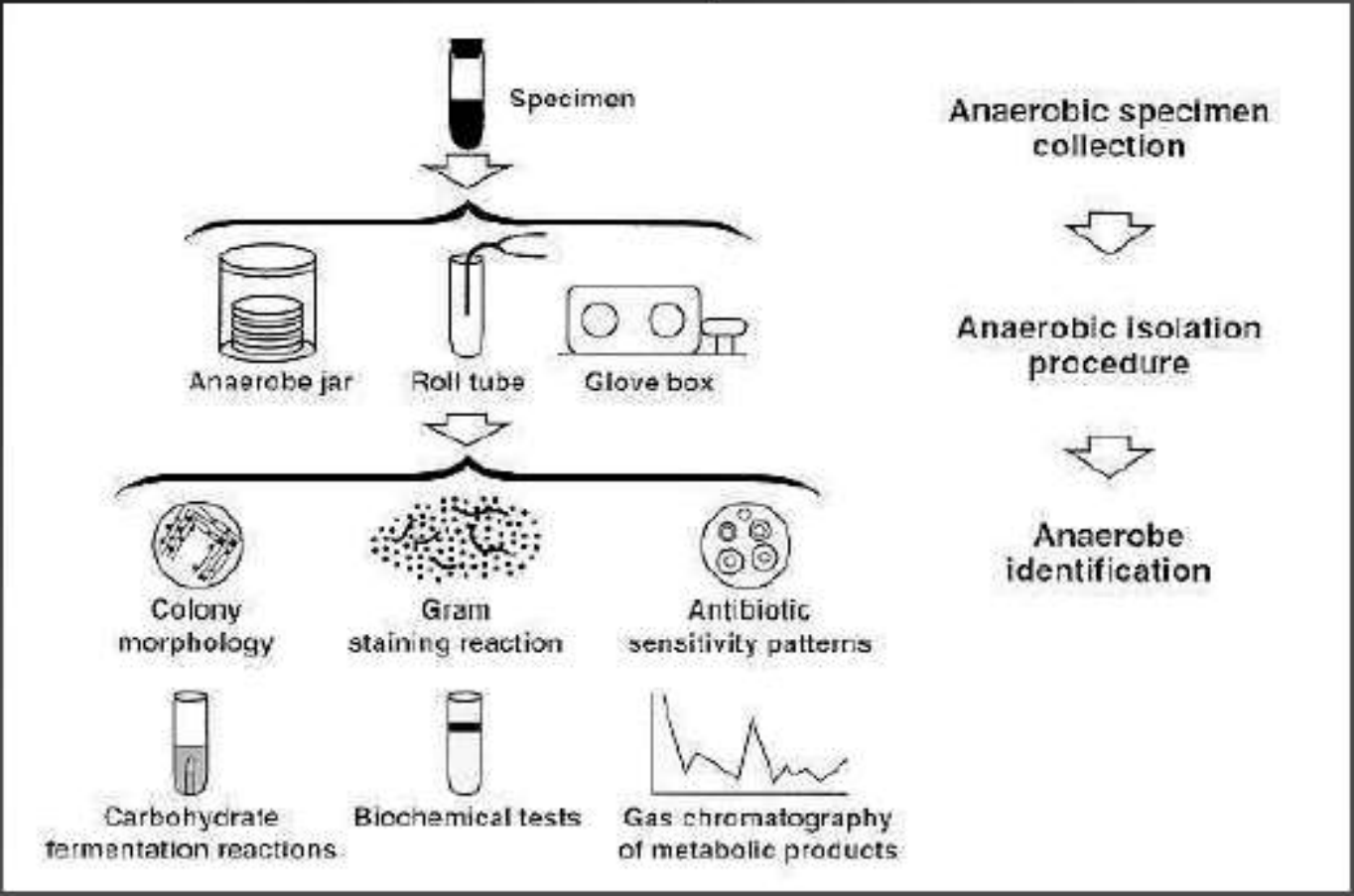
Fig. 28.4 Gas gangrene

Tissue Damage Caused by Microbial Enzymes of Clostridium perfringens



Severe gangrene caused by Clostridium perfringens.
Source: Tropical Medicine and Parasitology, 1997

ANAEROBES LABORATORY DIAGNOSIS



LABORATORY DIAGNOSIS

- Sample Collection:-

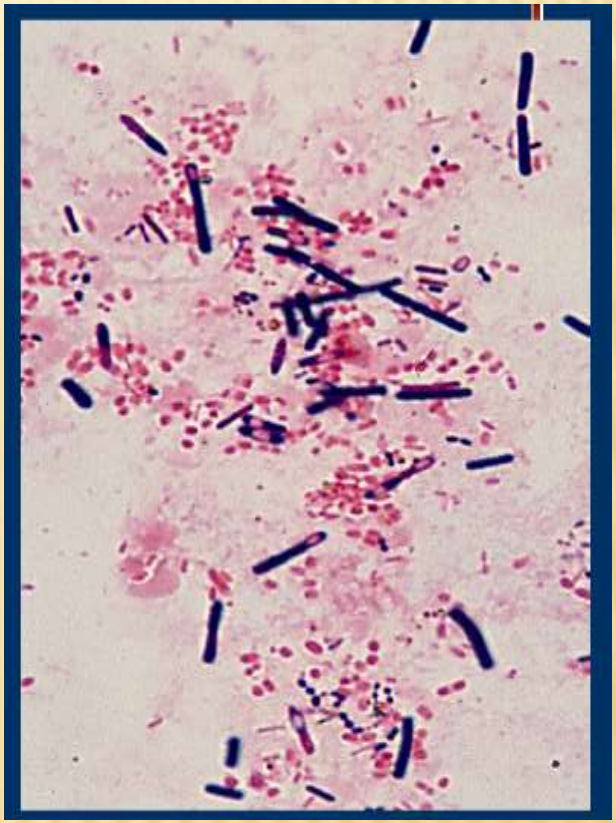
1) Films from the muscles at the edge of the affected area, from the tissue in the necrotic area & from the exudates in the deeper part of the wound
2) Exudates from the parts where infection appears to be most active & from the depths of the wound, to be collected with a capillary pipette or a swab
3) Necrotic tissue & muscle fragments

LABORATORY DIAGNOSIS CONTD.

- Methods:-
- Bacteriological– by Gram stain
  Culture- aerobic & anaerobic
- Fresh & heated Bl. agar,
- Serum/egg yolk medium- Nagler react^{N}
- RCM broth
- Blood culture
- Nagler's reaction- rapid detection of Cl.perfringens in clinical specimens

LABORATORY DIAGNOSIS CONTD.

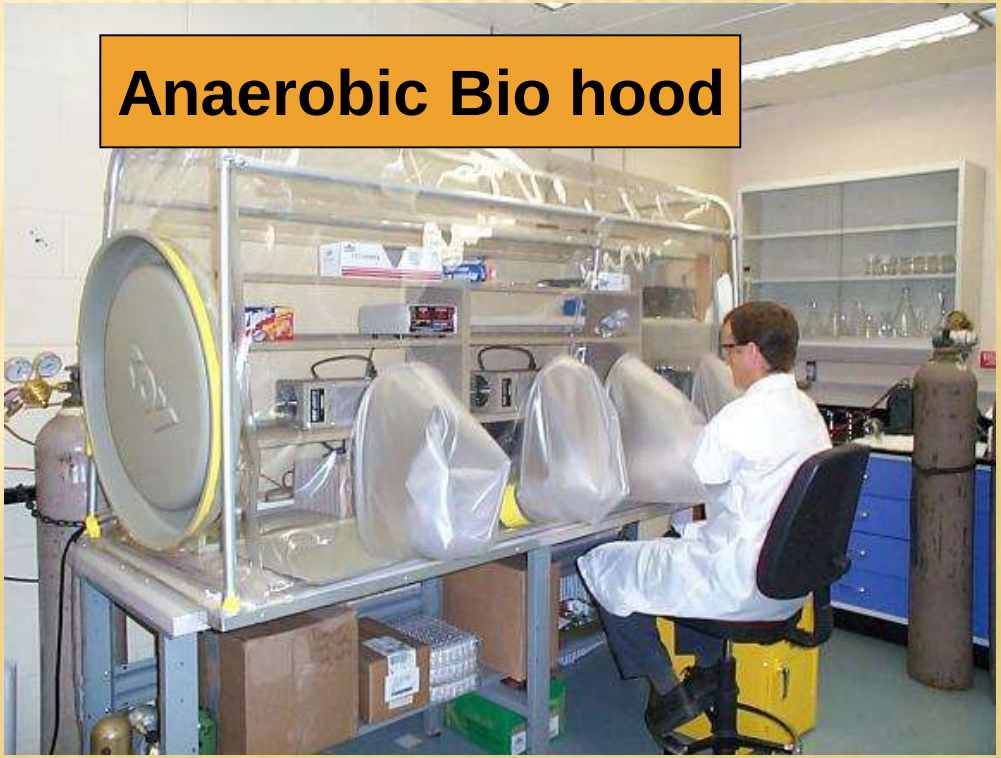
- Microscopy– scanty pus cells, regularly shaped (box-car shape) gram +ve bacilli with / without spores



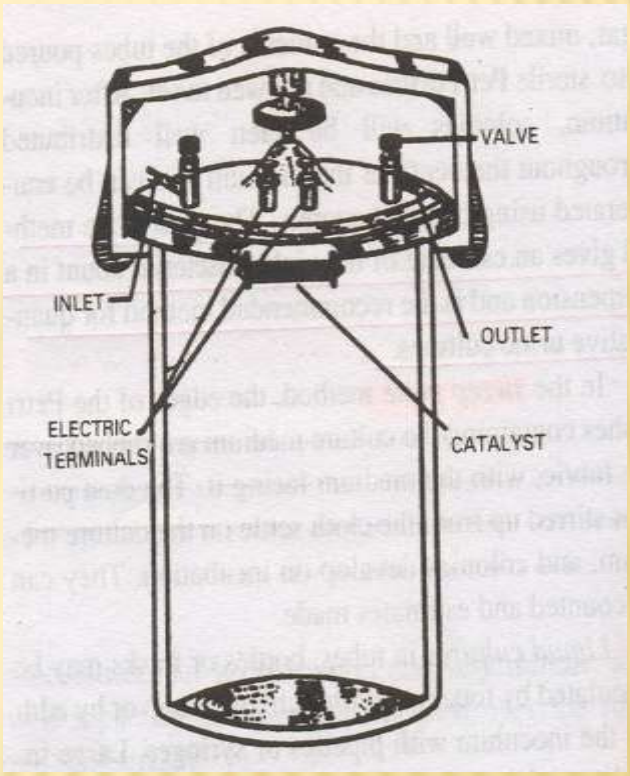
LABORATORY DIAGNOSIS

- Culture Media
  - Robertson cooked meat medium (RCM)
  - Media containing reducing substances like unsaturated fatty acids, ascorbic acid, thioglycollic acid, glutathione

- Culture methods
  - McIntosh-Fildes'
  - Gaspak method
  - Anaerobic Bio-hood
  - Deep butt culture



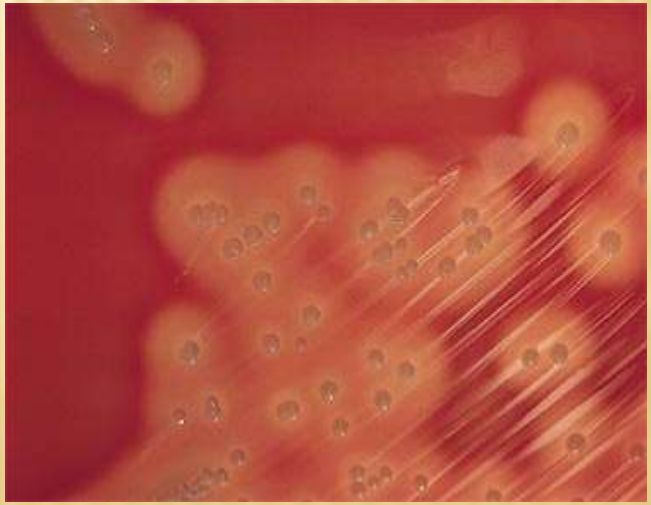
Gaspak



McIntosh-Fildes' anaerobic jar

LABORATORY DIAGNOSIS

- Cultural Characteristics
  1. RCM Broth – meat turns pink (saccharolytic reaction)
  2. Litmus milk test/ Stormy fermentation
  3. Target Hemolysis



LABORATORY DIAGNOSIS

Detection of Alpha toxin: “Nagler Reaction”

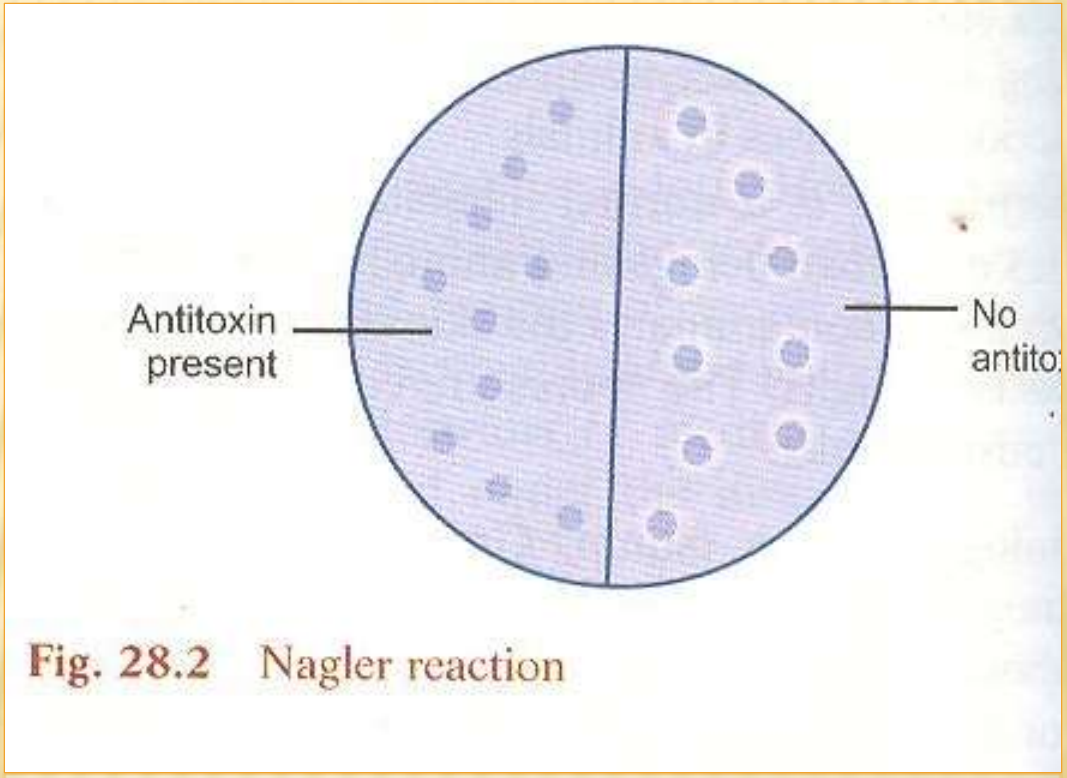


Fig. 28.2 Nagler reaction

PROPHYLAXIS & T/T

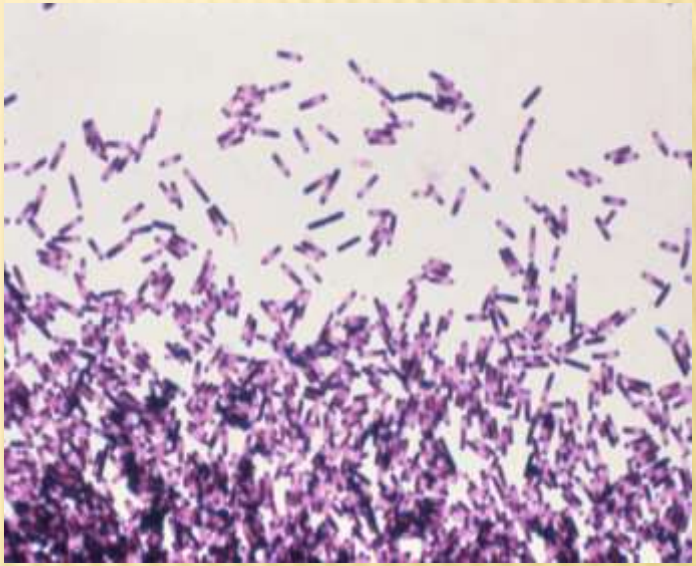
- Surgery
- Antibiotics - broad spectrum
- Passive immunisation- anti-gas gangrene Sr.(equine polyvalent antitoxin)

MANAGEMENT OF GAS GANGRENE

- Supportive therapy - e.g. analgesia, oxygen, intravenous fluids and good nourishment
- Surgical - radical debridement of necrotic tissue (may require amputation if limb involved)
- Antibiotics - these do not work alone as they are unable to penetrate the necrotic tissue. Cover gram negative, gram positive and anaerobes e.g. combination of penicillin, gentamicin and metronidazole
- Hyperbaric oxygen therapy - kills anaerobic *C. perfringens*; but efficacy not proven

CL. DIFFICILE

- Gram +ve bacilli with large, oval & terminal spores
- Normally present in the gut– 3% of healthy adults, 66% of infants



- Disease caused -
  - Pseudomembranous colitis- PMC
  - Antibiotic associated diarrhoea- AAD
  - Antibiotic associated colitis- AAC

PATHOGENESIS OF PMC

- Complication of oral antibiotic therapy –
  - Clindamycin
  - Lincomycin
  - Ampicillin
  - Tetracycline
  - Chloramphenicol
- Occurs due to alteration in normal gut flora and overgrowth of *Cl. difficile* –
  - 4-9 days after starting antibiotic therapy
  - Up to 6 weeks after discontinuation
- Person to person spread - spores shed in feces

RISK FACTORS

- Admission to intensive care unit
- Advanced age >65 years
- Antibiotic therapy (overuse)
- Prolonged hospital stay
- Immunosuppressive therapy
- Multiple and severe underlying diseases
- Recent surgical procedure
- Sharing a hospital room with a difficile-infected patient

Pseudo-membranous colitis (PMC)

Virulence factors

Enterotoxin - (Toxin A)

Cytotoxin - (Toxin B)

Diagnosis

Clinical suspicion

Culture of feces

Detection of toxin

Management

Discontinue antibiotics-

Ampi/Tetra/Clinda

Oral metronidazole

Oral vancomycin

PREVENTION

- Good personal hygiene
- Bathroom, kitchens & other areas to be cleaned regularly with detergents / disinfectants
- Isolation of patient with difficile diarrhoea