٢. CLOSTRIDIUM SPECIES Large gram-positive rods Produces bulging spores, C. perfringens rarely produce spores. Size, shape and location of endospores used for species differentiation Anaerobic/ some are aerotolerant Catalase and oxidase negative Motile by means of peritrichous falgella, except C. perfringens Require enriched media for growth They are toxigenic. They are non-capsulated except C. perfrigens fermentative

٣. Colony forms Colonial morphology is of limited value for differentiating most clostridial species. Colonies of C. perfringens are surrounded by a characteristic double-zone of .haemolysis

٤. Growth characteristics ferment a variety of sugars many can digest proteins. Milk is turned acid by some and digested by others Saccharolytic or proteolytic

٥. Antigenic characteristics clostridia share some antigens (somatic) possess specific soluble antigens (exotoxins)permit grouping by precipitin tests

٦. Diseases Categorized into three major groups based on toxin activity Neurotoxic clostridium Histotoxic clostridia C. tetani C. botulinum Enterotoxemic and Enteropathogenic clostridia C. perfringens type A-E C. difficile localized lesion in liver and muscle C. chauvoei C. septicum C.novyi type A C. perfringens type A C. sordelli C. haemolyticum C. novyi type B

٧. Detection and differentiation of clostridia Blood agar enriched with yeast extract, vitamin K and haemin is suitable for the culture of clostridia under anaerobic condition Demonstration of histotoxic clostridia in tissues - PCR-based techniques - Fluorescent antibody techniques ELISA can be used for toxin detection. Note: They are often derived from the normal flora. oxygen sensitive, isolation takes several days or longer

٨. Neurotoxic clostridia Clostridium tetani- agent of tetanus Morphology • long thin gram- ”positive organism • round terminal spore gives drumstick appearance or “tennis racket

٩. Tetanus Calf with tetanus following castration. Note the rigid limbs due to muscle spasm

١٠. Morphology and physiology • motile by peritrichous flagella • grow on blood agar or cooked meat medium with swarming • beta-hemolysis exhibited on blood agar • spores resist boiling for 20 minutes • Strict anaerobe (vegetative cell is extremely O2 sensitive)

١١. Clostridium tetani Antigenic Structure Depending uponFlagella (H), somatic (0), ten serotypes . Single antigenic toxin characterizes all strains (tetanospasmin) Abs to neurotoxin of any serotype can neutralize neurotoxin produced by others (cross-reaction)

١٢. Pathogenicity Determinants • play a role in local infection only in conjunction with other bacteria that create suitable environment for their multiplication locally. Produce exotoxin that act in sites faraway from site of infection • Plasmid-mediated neurotoxin (tetanospasmin), heat-labile, systemic-acting exotoxin • Heat- stable hemolysin (tetanolysin) of unknown significance

١٣. Mode of Action toxin binds to peripheral nerve endings, internalize, and travel via retrograde intra-axonal transport to the spinal cord it interferes with the activity of the inhibitory interneurons blocks release of inhibitory neurotransmitters continuous stimulation by excitatory transmitters spastic paralysis

١٤. Mechanism of Action of Tetanus Toxin

١٥. Notes locked jaw/tetanus muscle spasms (spastic paralysis) (lockjaw), cardiac arrhythmias, fluctuations in blood pressure Infect wounds Toxin produced in wounds and act systemically Toxin production in regulated by genes encoded in plasmids One antigenic type of toxin Toxin causes synaptic spasms Treated by antitoxin Prevented by toxoid

١٦. Lab Identification Isolation of C. tetani must rest on production of toxin production and its neutralization by specific antitoxin.

١٧. C. botulinum: botulism- agent of botulism, a rare, but severe (lethal) neuroparalytic disease Morphology and Physiology • Fastidious, strictly anaerobic G+ bacilli • Motile by peritrichous flagella • Oval, Subterminal endospores • proteolytic and non-proteolytic • preformed toxin in canned foods, carcasses, decaying vegetation etc • most potent biological toxin known

١٨. Antigenic Structure Seven antigenically distinct botulinum toxins (types A, B, C, D, E, F,and G) somatic antigens - heat stable and heat labile spore antigens - more specific

١٩. Pathogenicity Determinants • Phage-mediated (regulated by genomes), systemic-acting, neurotoxin (botulinum toxin = botulin) released at cell lysis • Lethal foodborne intoxication with Toxins A through G (neurotoxins) Mode of Action of botulin toxins - binds specific receptors on peripheral cholinergic nerve endings(neuromuscular junctions), blocks release of acetylcholine (excitatory neurotransmitter), blocking muscle stimulation & resulting in flaccid paralysis

٢٠. Mechanism of Action of Botulinum Toxin

٢١. Flaccid paralysis An animal with botulism exhibiting paralysis and excess salivation due to impaired swallowing

٢٢. Lab Identification microscopic detection or Cx (culture) are often (few organisms and slow growing) toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA Disease is treated by antitoxin, prevented by toxoids

٢٣. Enterotoxaemia clostridia: toxins (α,β,ε,ι) C. perfrigens type A – E C. perfringens or C. welchii Morphology and Physiology • large wide G+ rods • non-motile, capsulated • Rapid spreading growth on blood agar • grow at temperature of 20-50°C (optimum 45°C) and pH of 5.5-8.0

٢٤. Clostridium perfringens Cultural and biochemical properties It grows on most ordinary media Produces double zone of hemolysis on sheep blood agar, inner narrow zone of complete hemolysis caused by theta (θ) toxin, and wider zone of incomplete hemolysis caused by alpha (α) toxin In litmus milk, ferment lactose (acid production),color changed from blue to red. Coagulation of milk casein by acid, and the clotted milk is disrupted by vigorous gas production stormy reaction

٢٥. Reaction on Litmus Milk

٢٦. Pathogenicity Determinants C. perfringens produces 12 distinct exotoxins and enterotoxin • four major lethal toxins to lab animals 1) alpha () is common to all types of C. perfringens, is lecithinase C, phospholipase, split lecithin and sphingomyelin; disrupt cell membrane phospholipid, hemolysin, and necrotizing effect 1) beta () 2) epsilon () have lethal and necrotizing 3) iota () properties

٢٧. Minor toxins gamma (γ) eta () delta(), lethal and hemolytic theta(), oxygen labile, lethal and hemolytic (genetically related to streptolysin O) kappa(), collagenase lambda(), proteinase and gelatinase mu(), hyaluronidase nu(ν)toxin deoxyribonuclase (DNAse) Have only minor lethal actions

٢٨. Types of C. perfringens On the bases of the four major toxins C. welchii are differentiated into five types

٢٩. Enterotoxaemia clostridia: toxins (α,β,ε,ι) C. perfringens type A – E Type A (α toxin): necrotic enteritis in chicken, necrotizing enterocolitis in pigs, canine haemorrhagic gastroenteritis Type B (α, β(major), ε): lamb dysentery; haemorrhagic enteritis in calves and foals Type C (α, β (major)): struck in adult sheep, necrotic enteritis in chickens, haemorrhagic enteritis in neonatal piglets, sudden death in goats and feedlot cattle Type D (α, ε (major)): pulpy kidney in sheep, enterotoxaemia in calves, adult goats and kids Type E (α and ι(major)): haemorrhagic infection in calves, enteritis in rabbits

٣٠. Pathogenesis of C. perfringens A- Enterotoxemia (types A-E) Transmitted by ingestion Type A found in human and animal GIT and in soil Types B to E found mostly in animal GIT & soil

٣١. Type A In human food poisoning, vegetation occurs in cooked meat, ingested and sporulation occur in GUT In dog & cat vegetation, and sporulation and toxin production in intestine Upon sporulation, heat-labile enterotoxin is produced that increase permeability of epithelial cell and cytolysis which lead to diarrhea necrotic enteritis in broiler chickens

٣٢. Type B - beta toxin cause hemorrhagic enteritis in small intestine - produce severe disease in newborn, because β toxin is inactivated by trypsin

٣٣. Type C - cause hemorrhagic enterotoxemia in neonate animals - cause fatal toxemia- bacteremia in adult sheep “struck” - beta toxin and other factors are involved

٣٤. Type D Produce overeating disease, pulpy kidney in older lambs ε toxin produced in intestine activated by proteases ↑intestinal permeability→ enter blood → damage to vascular endothelium→ edema

٣٥. Laboratory diagnosis 1) Gram staining of content of small intestine→ large no. of G + rod 2) Culture → rapid growth property → stormy reaction → double zone of hemolysis → Nagler reaction 3) Animal inoculation for demonstration of toxins in vivo from specimens or culture 4) PCR

٣٦. .Clostridium perfringens cultivated on blood agar

٣٧. Nagler reaction: C. perfringens phospholipase causes turbidity around the colonies on egg-yolk

٣٨. C. difficile It causes chronic diarrhoea in dogs and haemorrhagic enterocolitis in newborn foals In human, cause antibiotic-associated diarrhea, pseudomembranous colitis following administration of broad spectrum antibiotic (like clindamycin or third generation cephalosporin), chemotherapy, nonsteroidal anti-inflammatory drug that alter gut normal flora and allow C. difficile to grow

٣٩. Determinant of pathogenicity Production of two exotoxins Exotoxin A which is enterotoxin cause fluid loss Exotoxin B is cytotoxin

٤٠. Lab diagnosis Culture on CCFA (cycloserine, cefoxitin, fructose agar) Toxin detection using -golden hamster ovary (CHO) cells - Enzyme immuno assay (EIA) Toxin gene detection by PCR

٤١. histotoxic clostridia: they produce toxins (α,β,γ,δ toxins) C. perfingens type A (α toxin): cause myonecrosis & gas gangrene in human and domestic animals Pathogenesis: in human cause anaerobic cellulitis, gas gangrene alone or with other bacteria Alpha toxin cause cell and tissue damage(membranolytic activity) Other toxins produced by C.perfringens also involved in destruction process Lead to necrotizing cellulitis, myonecrosis with edema and hemorrhage In animals, the disease is rare, but usually occurs in injection sites deep in muscle (horses)

٤٢. Summery

٤٣. histotoxic clostridia C. novyi General properties - G+, motile, noncapsulated, spore forming large, oval highly resistant spore - strict anaerobe - three distinct types A, B, & C - C. haemolyticum may be added as type D

٤٤. C. novyi type A Alpha toxin is the major toxin produced by type A, it has lethal and necrotizing activity Disease: bighead in young rams, wound infection Gas gangrene in human Found in soil and intestine

٤٥. C. novyi type B (α, β) 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

٤٦. C. haemolyticum(β) toxin Resembles C.novyi type B, except it produce no α toxin and much more β toxin (has lecithinase activity) bacillary haemoglobinuria in cattle and occasionally in sheep Liver fluke infection may induce germination of spores in liver macrophages

٤٧. C. chauvoei Produce typical subterminal spores Strict anaerobe Inhabits intestine, liver and other tissues Cause blackleg disease in cattle and sheep a disease of young cattle from 6 months to 2 years old (well-fed cattle) Usually, lesions occur in hind limb muscle

٤٨. Pathogenesis Skeletal muscle is seeded with dormant spores from intestine (ingestion), conditions favoring spore germination include, trauma or bruising The toxins α toxin (necrotizing), β,γ,δ produced may involved in disease process that characterized by emphysematous necrotizing myositis The affected area is hot, swollen and painful. Gas builds up in the muscle. There is rapid progression to gangrene. Lameness is

٤٩. C. septicum The leading clostridium in wound infections in farm animals Short, stout, and pleomorphic G+ rod Colonies on blood agar are circular with rhizoid or irregular margins Produce β-hemolysis on blood agar Found in soil, and intestine of human and animal Acquired by wound infection or ingestion

٥٠. pathogenesis The organism cause malignant edema in domestic animal which is associated with contaminated wound infection Production of toxins, especially α (oxygen stable hemolysin) toxin may be involved in disease process Hemorrhage, edema and necrosis, spread rapidly along facial planes from point of infection The initial crepitant swelling is painful, then become anesthetic and cold

٥١. pathogenesis Braxy (Scots) or bradsot (Danish) is a fatal disease of sheep, associated with ingestion of frozen or cold feed→ hemorrhagic necrotic abomasitis

٥٢. C. sordellii Cause myositis is cattle, sheep, horses Produce numerous toxins, including hemolytic and lethal toxins

٥٣. A positive C. chauvoei FAT smear

٥٤. Lab Identification 1) Fluorescent antibody techniques (microscopic examination) 2) Culture: double zoon of hemolysis, and Nagler reaction for perfringens type A 3) PCR