

## ***ESCHERICHIA COLI***

### *E. coli* diseases (enteric and extraintestinal)

- Enteric colibacillosis
- Colisepticaemia
- Oedema disease in pigs
- Post-weaning diarrhoea in pigs
- Coliform mastitis
- Urinogenital tract infection

### Other diagnostic procedure

- Serology/serotyping
- PCR
- Toxin detection
  - Cytotoxicity
  - Loop ligation test
  - Sereny test (invasiveness)
- Animal inoculation

### *Salmonella* diseases

- Septicaemic salmonellosis
- Enteric salmonellosis
- Fowl typhoids
- Pullorum (bacillary white diarrhoea)
- Ipuman infection
- Abortion in cattle

### Diagnosis

#### Sample from Suspected Animals

- Tissue
- Faeces:
  - Inoculation into enrichment broth e.g. selenite F, rappapoort, Tetrathionate (37°C for 48 hours aerobically)

- Subculture at 24 and 48 hours onto MacConkey agar, brilliant green and xylose-lysine-deoxycholate
- Direct inoculation: MacConkey agar, brilliant green and xylose-lysine-deoxycholate (37°C for 24 hours aerobically)
  - Suspicious colonies
  - Inoculation of TSI agar and lysine decarboxylase broth
  - Typical salmonella reactions
  - Serological confirmation with polyvalent antisera
  - Definitive serotyping into specific 'O' and 'H' antisera
  - Biotyping or Phagotyping

### *E. coli*

Commensal

Opportunistic

Enteric

Extraintestinal:

Urogenital (uropathogenic), cystitis

Avian

Mastitis

Pyometria (dogs and cats)

Septiceamic (endotoxin): cystitis mainly in bitches

Virulence factors of *E. coli*

CNF: Cytotoxic Necrotizing Factor

EPEC: Adhesins, K88 pigs, and K99 calves and lambs for colonization, heat stable enterotoxins (ST), and heat labile enterotoxins (LT)

Diarrhoea in neonatal piglets, calves, lambs, post-weaning diarrhoea in pigs

EPEC: A/E factor, intimin, haemolysin, destruction of microvilli, shedding of enterocytes, stunting of villi malabsorption, diarrhoea in piglets, lambs, pigs

VTEC: VT1, VT2, VT2e, damage to vasculature in intestine and other locations, oedema disease in pigs, haemolytic colitis in calves, post-weaning diarrhoea in pigs, haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC) in humans

Necrotoxicogenic: CNF1 and CNF2 (Cytotoxic Necrotizing Factor). Damage to enterocytes and blood vessels, HC in cattle, enteritis in piglets and calves, diarrhoea in rabbits, dysentery in horses

### ***BACILLUS SPECIES***

- *Bacillus species* are large Gram-positive rods about 10.0 µm in length
- They produce endospores
- They appear singly, in pairs or in long chains
- They are aerobic or facultative anaerobes
- *Bacillus species* are catalase-positive and oxidase negative
- They are motile except *B. anthracis* and *B. mycoides*
- Most species are saprophytes but often contaminate clinical specimen and laboratory media
- *B. species* can tolerate extreme adverse conditions such as high temperature and desiccation because of their endospores
- *B. anthracis* produces capsule

#### Diseases

##### *B. anthracis*

Cattle and sheep: total peracute or acute septicaemic anthrax

Pigs: subacute anthrax with oedematous swelling in pharyngeal region, intestinal form with higher mortality is less common

Horses: subacute anthrax with localised oedema, septicaemia with enteritis and colic

Human: skin, pulmonary and intestinal forms of anthrax

#### *B. cereus*

Cattle: mastitis

Human: food poisoning, eye infection

#### *B. licheniformis*

Cattle, sheep: sporadic abortion

#### Diagnosis:

- Ability to produce catalase and grow aerobically distinguish *B. species* from *Clostridium spp*
- *Bacillus species* are differentiated based on colonial characteristics, biochemical test and genetic composition
- Colonial characteristics:
  - *B. anthracis* colonies are up to 5mm in diameter, flat, dry, greyish and with a 'ground-glass' appearance after 48 hours incubation. At low magnification, curled outgrowth from the edge of the colony impart a characteristic 'medusa head' appearance. Isolates are rarely haemolytic. When present, haemolysis is weak
  - *B. cereus*: colonies are similar to those of *B. anthracis* but slightly larger with a greenish tinge. The majority of strains produce a wide zone of complete haemolysis around the colonies
  - *B. licheniformis*: colonies are dull, rough, wrinkled and strongly adherent to the agar. Characteristic hair-like outgrowth are produced from streaks of the organisms on agar media

#### **Distinguishing features of *B. anthracis* and *B. cereus***

Feature	<i>B. anthracis</i>	<i>B. cereus</i>
Motility	Non-motile	Motile
Appearance on sheep blood agar	Non-haemolytic	Haemolytic
Susceptibility to penicillin	Susceptible	Resistant
Lecithinase activity on egg yolk agar	Weak and slow	Strong and rapid
Effect of gamma phage	Lysis	Lysis rare
Pathogenicity for animals	Death in 24 -48 hours	No effect

#### Diagnosis of Anthrax:

- History of sudden death
- Pathology: carcass is bloated, putrefies rapidly and no rigor mortis
- Collect peripheral blood and make smear
- Stains smear with polychrome methylene blue
- *B. anthracis* appears as blue-staining rods with square-end surrounded by pink capsules
- Culture on blood and MacConkey agar
- Incubate aerobically at 37<sup>0</sup>C for 24 to 48 hours
- Study colony morphology
- No growth on MacConkey agar
- Study microscopic appearance
- Do biochemical test
- Conduct pathogenicity test
- Do Ascoli test:
  - o A thermoprecipitation test
  - o Detects *B. anthracis* antigen
  - o A ring precipitation or gel diffusion test with *B. anthracis* antiserum
- Other tests: agar gel immunodiffusion, CFT, ELISA, IFT and PCR

## ***CLOSTRIDIUM SPECIES***

- Large gram-positive rods
- Produces endospores. *C. perfringens* rarely produce spores
- Anaerobic
- Catalase and oxidase negative
- Motile except *C. perfringens*
- Require enriched media for growth
- Size, shape and location of endospores used for species differentiation
- They are toxigenic. They are non-capsulated except *C. perfringens*

*C. perfringens*: large wide rods. Rarely form endospores in-vitro

*C. tetani*: thin rods. Characteristically produce terminal endospores (drumstick appearance)

*C. chauvoei*: medium-size rods. Produce lemon shaped endospores

### Diseases:

Categorised into three major groups based on toxin activity

- Neurotoxic clostridium: *C. tetani*, *C. botulinum*
- Histotoxic clostridia: 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*
- Enterotoxigenic clostridia: *C. perfringens type A-E*
- Less important groups
  - *C. piliforme*: spore-forming, filamentous gram-negative intracellular pathogens (atypical member of the clostridia). Has not been cultured artificially on media
    - Grows only in tissue culture and fertile egg
    - Causes Tyzzer's disease (a severe disease causing hepatic necrosis) in laboratory animals foals rarely in calves, dogs and cats
  - *C. difficile*: chronic diarrhoea in dogs and haemorrhagic enterocolitis in newborn foals
  - *C. spiroforme*: enteritis in rabbits
  - *C. colinum*: enteritis in birds
- Neurotoxic clostridia:

- i. *C. tetani*: locked jaw/tetanus
  - Infect wounds
  - Terminal endospores
  - Toxin produced in wounds
  - Toxin production is regulated by genes encoded in plasmids
  - One antigenic type of toxin (tetanus plamin)
  - Toxin causes synaptic spasms
  - Prevented by toxoid
  - Treated by antitoxin
- ii. *C. botulinum*: botulism
  - subterminal endospores
  - preformed toxin in canned foods, carcasses, decaying vegetation etc.
  - toxin production regulated by genomes
  - eight antigenically distinct toxins (A-G)
  - toxins inhibit neuromuscular transmission
  - produces flaccid paralysis
  - most potent biological toxin known
  - prevented by toxoids, treated by antitoxin
- histotoxic clostridia: they produce toxins ( $\alpha, \beta, \gamma, \delta$  toxins)
  - *C. chauvoei* ( $\alpha, \beta, \gamma, \delta$ ): blackleg in cattle and sheep.
  - *C. septicum* ( $\alpha, \beta, \gamma, \delta$ ): malignant oedema in cattle, pig and sheep. Braxy (abomastitis) in sheep and occasionally calves
  - *C. noryi* type A ( $\alpha$ ): big head in young rams, wound infection
  - *C. sordell* ( $\alpha, \beta$ ): myositis in cattle, sheep, horses, abomastitis in lambs
  - *C. novyi* type B ( $\alpha, \beta$ ), infectious necrotic hepatitis (black disease) in sheep and occasionally in cattle
  - *C. haemolyticum* ( $\beta$ ): bacillary haemoglobinuria in cattle and occasionally in sheep

- *C. perfringens* type A ( $\alpha$ ): necrotic enteritis in chicken, necrotizing enterocolitis in pigs, gas gangrene.
- Enterotoxaemia clostridia: toxins ( $\alpha, \beta, \epsilon, \iota$ ) *C. perfringens* type A – E
  - Type A ( $\alpha$  toxin): necrotic enteritis in chicken, necrotizing enterocolitis in pigs, canine haemorrhagic gastroenteritis
  - Type B ( $\alpha, \beta$  (major),  $\epsilon, \iota$ ): lamb dysentery; haemorrhagic enteritis in calves and foals
  - Type C ( $\alpha, \beta$  (major)): struck in adult sheep, necrotic enteritis in chickens, haemorrhagic enteritis in neonatal piglets, sudden death in goats and feedlot cattle
  - Type D ( $\alpha, \epsilon$  (major)): pulpy kidney in sheep, enterotoxaemia in calves, adult goats and kids
  - Type E ( $\alpha$  and  $\iota$  (major)): haemorrhagic infection in calves, enteritis in rabbits

### Diagnosis

- Clostridia are fastidious and anaerobic
- Samples are collected from live or recently dead animals
- Tissues or exudates for culture should be placed in anaerobic transport media
- Samples should be cultured promptly
- Ideal medium is blood agar enriched with yeast extract, vitamin K and haemin
- Robertson cooked medium is used for anaerobic enrichment
- Media should be freshly prepared or pre-reduced to ensure absence of oxygen
- Test for toxin production in laboratory animals. Toxin neutralisation by antitoxin.
- Cultured plates are incubated in anaerobic jar containing hydrogen supplemented with 5 to 10% carbon dioxide
- Identification and differentiation among *C. species* are based on colonial morphology, biochemical tests, toxin neutralization methods and gas-liquid chromatography for profiling organic acids
- Fluorescent antibody techniques, immunoassay such as ELISA and molecular technique like PCR are of diagnostic importance



## Special Features

*C. tetani* produces filmy growth on blood agar with narrow zone of haemolysis. Prevent swarming by using 4% agar (stiff) or sodium azide

*C. perfringens*: produces double zone of haemolysis on blood agar (narrow zone of incomplete haemolysis and wide zone of partial haemolysis). Produces marked opalescence on egg yolk medium because of the lecithinase action of alpha toxin (Nagler reaction).

CAMP test positive with *Streptococcus agalactiae*

*C. novyi type A*: give a characteristic 'pearly layer' on egg yolk medium due to the lipase it produces. It is also Nagler's reaction

Principle of Nagler's reaction: lecithinase action on lecithin in egg yolk leading to the opacity due to insoluble fatty acid accumulation

## Tutorial questions

1. list the steps involved in bacterial infection
2. what is virulence
3. how do bacteria obtain iron from the host
4. List three bacteria that can survive in macrophages
5. what is the significance of coagulase production by bacteria
6. list five difference between exotoxins and endotoxins
7. what is the causative agent in tick pyaemia in lamb
8. what selective medium will you use for the isolation of staphylococcus
9. list three differences between staphylococci and streptococci
10. what is lancefield grouping