BACTERIAL PATHOGENICITY

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- Majority of bacteria are non-pathogenic saprophytes
- Bacteria which causes disease in humans and animals are small in number compared to those that dot not cause disease
- Bacteria that cause disease are said to be pathogenic
- The development and severity of bacterial infections are influenced by host-related determinants such as phy-biological status and immune competence
- Commensal bacteria can cause opportunistic infection in the host

Steps in bacterial infection

Route of bacterial entrance into the host: skin, mucus, membranes, teat canal and umbilicus

Steps in Bacterial Pathogenesis:

Adhesion to the host cells

Local proliferation or multiplication

Damage to the host tissue

Invasion

Dissemination

Tissue and host specificity

- Virulence of bacteria relates to the ability to invade and produce disease in a normal animal
- Ability to adhere: virulent pathogens often possess specific surface molecule which allow adherence to receptors on ost cells
- Adherence factors include: adhesions, fimbriae, intimin, invasion (all in gram-negative bacteria)
- Adherence factors in gram-positive bacteria: protein F (a fibrionectin-binding protein) is necessary for adherence of streptococci to respiratory epithelial-the coagulase of pathogenic staphylococci promotes adherence to fibrinogen-coated surfaces

- Capsule-like material in *Klebsiella pneumoniae* enhance its interaction with human intestinal cells
- In general, capsule are thought to hinder bacterial adherence to host cells
- Iron is essential for bacterial respiration
- Most iron in the animal host is bound by iron-binding proteins like lactoferrin and transferring, and therefore unavailable for the bacteria
- Pathogenic bacteria obtain iron from the host by producing iron-chelating compounds like siderophores which can remove iron from transferring and lactoferrin
- Other lyse erythrocytes to obtain iron from haemoglobin
- Bacterial multiplication, tissue invasion and avoidance of host defence mechanism

<u>Mechanism employed by bacteria for survival in the host</u>

- O antigen polysaccharide chain: length of polysaccharide chain hinders binding of the membrane attack complex of complement to the outer membrane of many gram-negative bacteria
- Capsular antigen: incorporation of sialic acid by some gram-negative bacteria has an inhibitory effect on complement activity
- Capsule production: antiphagocytic
- H-protein production: antiphagocytic activity e.g. *S. equi*
- Production of Fc-binding proteins: *Staphylococci* and *Streptococci* produce protein which bind to the Fc region of IgG and prevent interaction with the Fc receptor on membranes of phagocytes
- Production of leukotoxins: cytolysis of phagocytes by toxins produced by *Manheimia haemolytica, Actinobacillus species* and other pathogenic bacteria
- Interference with phagosome-lysosome fusion, allows the survival of pathogenic mycobacterium within phagocytes
- Escape from phagosomes: survival mechanism used by *Listeria monocytogenes* and *Rickettsiae*
- Resistance to oxidative damage: allows the survival of *Salmonella* and *Brucellae* within phagosytes

- Antigenic mimicry of the host antigens: adaptation of surface antigens by *Mycoplasma spp* to avoid recognition by the immune system
- Antigenic variation of surface antigens: permits survival of *Mycoplasma spp* and *Borreliae* despite the host's immune response to these pathogens
- Coagulase production: conversion of fibrinogen to fibrin by *Staphylococcus aureus* can isolate site of infection from effective immune response

Dissemination of bacteria in the host

- Avoidance of host defence mechanism is essential for successful invasion and dissemination of the pathogen
- Enzymes such as collagenases, lipases, hyaluronidases and fibrinolysin produced by bacterial pathogens facilitate breakdown of host tissue
- Bacteraemia is the transcient presence of the bacteria in the blood stream without replicating
- Septicaemia is the persistent presence of bacteria multiplying in the blood stream

Damage to host tissue and associated clinical signs

- Direct damage is caused by exotoxin and endotoxin production
- Indirect damage results from the activity of enzymes secreted by the bacteria and host immune response to infection

Comparison of exotoxins and endotoxins

Exotoxin	Endotoxin
Produced by live bacteria	Released during death and lysis of cells
Secreted actively	Component part of cell wall
Produced by both gram-positive and gram-negative bacteria	Produced by gram-negative bacteria
High molecular weight protein	Lipopolysaccharide complex containing lipid A, the toxic component
Heat-labile	Heat-stable
Potent toxins, usually with specific activity	Toxin with moderate non-specific

	generalised activity
Not pyrogenic	Potent pyrogens
Highly antigenic	Weakly antigenic
Readily converted to toxoid	Not amenable to toxoid production
Induced neutralizing antibodies	Neutralising antibodies not associated with natural exposure
Synthesis determined extra-chromosomally	Encoded by chromosome

STAPHYLOCOCCUS SPECIES

- Gram-positive bacteria
- Spherical (cocci) in shape
- About 1 μm in diameter
- Occur in irregular clusters
- Staphyle bunch of grapes
- Kokkos berry
- Common commensals on skin and mucous membrane
- Often cause pyogenic infections
- Oxidase-negative, catalyse-positive, non-motile, non-sporing facultative anaerobes
- Important animal pathogens include *S. aureus, S. intermedius*, *S. hyicus*
- Pathogenic species often produce coagulase
- *S. aureus and S. intermedius* are coagulase positive while *S. hycus* is coagulase variable

- Coagulase negative staphylococcus are of low virulence but may occasionally cause disease in animals and man

- Diseases in animals
  - Exudative epidermitis in piglets (greasy-pig diseases): *S. hyicus*
  - Tick pyaemia of lambs : *S. aureus*
  - Bovine staphylococcal mastitis: *S. aureus*
  - Botryomycosis (Scirrhous cord) (horse, pigs, cattle): *S. aureus*
  - Wound infection (most animals): *S. aureus, S. hyicus, S. intermidus*
  - Mastitis: *S. aureus, S. hyicus, S. intermedius*
  - Bumble foot, omphalitis in poultry: *S. aureus*
  - Pyoderma, otitis externa, cystitis, endometritis in dogs: *S. intermedius*
- Diagnosis
  - Sample collection: pus, exudates
  - Media: grow on non-enriched media
    - Nutrient agar, blood agar
  - Selective medium: mannitol salt agar (staphylococci can tolerate high concentration of NaCl). Mannitol salt agar contains 7-10% NaCl
  - P- agar for cultural differentiation of staphylococci
- Colonial characteristics
  - Colour: usually white, opaque and up to 4mm in diameter. Colonies of bovine and human strains of *S. aureus* are golden yelloe. Saprophytic staphylococcus may be pigmented

- Staphylococcus may produce haemolysis on sheep/ox blood agar. Types of haemolysis: alpha, beta, gamma, delta haemolysis
- *S. aureus and S. intermedius* produce double zones of narrow complete and wide incomplete haemolysis on blood agar
- *S. hyicus* is non-haemolytic

- Coagulase test: mix a suspension of staphylococcus isolate with rabbit plasma either on a slide or in a small tube. Coagulase convert fibrinogen to fibrin (strand/lumps)
  - Slide coagulase test detects the presence of bound coagulase or clumping factor within 1 to 2 minutes
  - Tube coagulase test detects free coagulase or staphylocoagulase secreted by bacteria. It is the definitive test for coagulase. The plasma clots within 24 hours of incubation at 37^0C
- Differentiation tests

  Differentiation of Gram-positive cocci

Organism	Appearance of stained smear	Coagulase	Catalase	Oxidase	O-F test	Bacitracin test
Staphylococcus spp	Irregular cluster	±	+	-	F	Resistant
Micrococcus spp	Packets of four	-	+	+	O	Susceptible
Streptococcus and enterococcus spp	Chain	-	-	-	F	Resistant

Purple agar: contains indicator-bromocresol purple, sugar (1% maltose)

Species	Colony colour	Haemolysis on sheep BA	Tube coagulase	Slide coagulase	Acetone production	Maltose utilization
S. aureus	Golden yellow	+	+	+	+	+

S. intermedius	White	+	+	V	-	±
S. hyicus	White	-	V	-	-	-

- Molecular procedure carried out in research and reference laboratories

Treatment: Penicillin and its derivatives

STREPTOCOCCUS SPECIES

- Gram-positive bacteria chain 1.0 μm in diameter
- Non-motile, non-sporing, oxidase negative, catalase-negative, facultative anaerobes
- Fastidious organism requiring enriched media for growth
- Pathogenic species cause suppurative conditions such as mastitis, metritis, polyarthritis and meningitis in animals
- *Enterococcus spp* are opportunitistic enteric *streptococci* found in intestinal tracts of animals and humans
- Unlike *Streptococcus spp, enterococci* can tolerate bile salt and therefore grow on MacConkey agar as red pinpoint colonies. Some streptococci are also motile
- Most *streptococcu spp* live as commensals on the mucosae of the upper respiratory tract and lower urogenital tract
- *Streptococci* are fragile and susceptible to desiccation

<u>Diseases:</u>

- Bovine streptococcal mastitis:
  - *i. S. agalactiae, B, β (α,γ)*

ii. *S. dysgalactiae* C, α (β, γ)

iii. *S. uberis* NA α (γ)

iv. *Enterococcus faecalis* D, α (β, γ)

v. *S. pyogenes* A, β

vi. *S. zooepidemiccos* C, β

- (i-iii): principal pathogens of mastitis
- (iv-vi) are less associated with mastitis

- Strangles in horses: *S. equi* C, β
- Abscess and other suppurative conditions and septicaemia in many species of animals

S. pyogenes (*A, β):* humans

S. canis (*G, β):* dogs

S. suis (*D, α (β)):* pigs

S. equisimilis (C, β)*:* horses

Diagnosis

- History, clinical signs and pathology may be indicative of streptococcal infection
- Samples are collected and cultured promptly: streptococcal are highly susceptible to desiccation. Samples include pus and exudates
- Samples can be placed in transport medium
- Stained smear of clinical samples may reveal gram-positive cocci in chains
- Samples should be cultured on blood agar and MacConkey agar
- Incubate agar plates aerobically at 37^0C for 24-48 hours
- Streptococcal colonies are small, translucent and some may be mucoid

- Differentiation of the streptococci:
    i. Type of haemolysis
    ii. Lancefield grouping
    iii. Biochemical testing

i. Type of haemolysis on blood agar
    - Beta-haemolysis: complete haemolysis of clear zones around colonies
    - Alpha-haemolysis: partial, incomplete haemolysis, greenish or hazy zones around colonies
    - Gamma-haemolysis: no observable changes in the blood agar around colonies

ii. Lancefield grouping: a serological method of classification based on the group-specific C-substance. Lancefield grouping test methods include:
    - Ring specification test
    - Extract C-substance by acid or heat from the *Streptococcus spp*
    - The extract (antigen) is layered over antisera of different specificities in narrow tubes placed in plasticine on slide
    - A positive reaction is indicated by the formation of a white ring of precipitate close to the interface of the two fluids within 30 minutes
    - Latex agglutination test: latex-coated group-specific antibodies are commercially available for the test. Antigen is extracted enzymatically from the *streptococcus spp* under test
    - Mix antiserum and antigen together on a slide
    - Positive reaction is indicated by agglutination

iii. Biochemical tests: oxidase, catalase, sugar fermentation tests. Biochemical tests are available commercially for rapid identification of streptococci

Biochemical differentiation of equine group C Streptococci

Species	Trehalose	Sorbitol	Lactose	Maltose
S. equi	-	-	-	+
S. zooepidemicus	-	+	+	+(-)
S. equisimilis	+	-	V	+

Differentiation of streptococci associated with mastitis:

S. pyogenes: Bacitracin sensitive (all group A)

S. agalactiae: CAMP test (Christie, Atkins, Munch and Petersen) positive with *S. aureus* and *Corynebacterium pseudotuberculosis (*all group B *Streptococci)*

S. uberis: Aesculin hydrolysis (black brown zones of discolouration around dark coloured colonies on Edward's medium).

S. pneumoniae: quellung reaction/capsule swelling test, bile solubility, Optorchin-sensitivity, *S. pneumoniae* appears a lancet-shaped organisms in pairs. It is capsulated

CAMP test: enhanced haemolysis (synergism) of staphylococcal beta-toxin or corynebacterial phospholipase D

Optorchin: ethylhydrocupreine hydrochloride

LISTERIA SPECIES

- Gram positive coccobacillary rods about 2μm in length
- Catalase positive, oxidase negative, facultative anaerobes
- There are six species in the genus, three of which are pathogenic
- *L. monocytogenes* is the most important species. It was first isolated from rabbits with septicaemia and monocytosis
- Can tolerate wide temperature (4^0C – 45^0C) and pH (5.5 – 9.6) ranges

Diseases

i. *Listeria monocytogenes*

Cattle, sheep, goats: encephalitis (neural form), abortion, septicaemia, endophthamitis.

Cattle: mastitis (rare).

Dogs, Cats, horses: abortion, encephalitis (rare)

Pigs: abortion, septicaemia, encephalitis

Birds: septicaemia

ii. *L. ivanovii*

Sheep, cattle: abortion

iii. *L. innocua*

sheep: meningoencephalitis

Diagnosis: (microbiological)

- Sample collection: cerebrospinal fluid, tissue from brain (medulla and pons), specimen from abortion cases: cotyledons, foetal abomasal contents, uterine discharges. Septicaemia: spleen, blood. Collect only fresh samples
- Smear from cotyledon or liver lesion may reveal several gram-positive coccobacillary bacteria
- Immunoflourescence using monoclonal antibodies gives rapid result
- Isolation
    - Inoculate sample onto blood agar, selective blood agar and MacConkey
    - Incubate aerobically at 37^0C for 24 to 48 hours

    - A cold enrichment procedure may be necessary for recovery of Listeria from clinical specimen
    - Inoculate a 10% suspension of sample into nutrient/enrichment broth
    - Keep the inoculated broth at 4^0C in a refrigerator
    - Subculture weekly from the broth onto blood agar for up to 12 weeks
- Two forms are formed on culture media; smooth and rough forms
    - Smooth: small, smooth, flat, more common (short filament and coccal forms, older culture)
    - Rough: young culture, entirely of long filament
- *L. monocytes* colonies are small, smooth and flat
- *L. monocytes* produces a blue-green colour with oblique illumination
- Colonies are surrounded by a narrow zone of complete haemolysis
- It is catalase positive. *Streptococci* and *Arcanobacterium pyogenes* have similar colonies but are catalase negative
- It is cAMP test positive with *staphylococcus aureus* but not with *Rhodocossus equi*
- It hydrolysis aesculin
- Produces a characteristic tumbling motility after incubation in broth at 25^0C for 2-4 hours
- Pathogenicity test in rabbit to confirm virulence: instil a broth culture into rabbit eye. Virulence strains induce keratoconjuctivitis. This is called Anton test
- *Listeria spp* are zoonotic

Laboratory methods for differentiating Listeria species

Listeria spp	Haemolysis on sheep blood agar	CAMP test		Acid production from sugar		
		S. aureus	*R. equi*	D-mannitol	L-rhamnose	D-xylose
L. monocytogenes	+	+	-	-	+	-
L. ivanovii	++	-	+	-	-	+
L. innocua	-	-	-	-	V	-
L. seeligeri	+	+	-	-	-	+
L. welshimeri	-	-	-	-	V	+

L. grayi	-	-	-	+	V	-

+ = positive reaction, - = negative reaction V = variable reaction

ERYSIPELOTHRIX SPECIES/ E. rhusiopathiae

- Gram positive slender rods which may be curved or straight
- Have tendency to form elongated filaments
- May appear in pairs or in groups
- Some thickened filaments are beaded with gram's staining
- Small rods (small form), filament (rough form)
- Both forms occur on culture media
- Smooth forms are isolated from acute infections
- Isolate from chronically infected animals from rough colonies
- Produce small colonies with incomplete haemolysis in 48 hours
- Grow over wide temperature and pH ranges
- Catalase negative
- Coagulase positive
- Non-motile, oxidase negative, facultative anaerobe
- Form H_2S along slab line in Triple Sugar Iron agar

Diseases

- *Erysipelothrix rhusiopathiae*
    - Pigs (swine erysipelas): septicaemia, diamond skin lesions, chronic arthritis, chronic valvular endocarditis, abortion. Almost 50% of healthy pigs harbour *E. rhusiopathiae* in tonsillar tissues
    - Sheep: polyarthritis in lambs, post dipping lameness, pneumonia, valvular endocarditis
    - Turkey (turkey erysipelas): septicaemia, arthritis, valvular endocartitis

Diagnosis

- Specimen: blood, liver, spleen, heart valves, synovial tissue. Organism rarely recovered from skin lesions or chronically affected joints

- Microscopic examination of specimen from acutely affected animals may reveal slender gram-positive rods
- Filamentous elements may be seen in samples of chronic valvular lesion
- Inoculate specimen into blood and MacConkey agar plates
- Incubate aerobically at 37^0C for 24 to 48 hours
- Selective media containing either sodium azide (0.1%) or crystal violet (0.001%) may be used for contaminated samples
- Non-haemolytic, pin-point colonies appear after incubation for 24 hours and after 48 hours, a narrow zone of greenish, incomplete haemolysis develops around the colonies
- Catalase-negative, coagulase-positive (as in staphylococcus), H_2S positive
- Serotyping for epidemiological studies
    - Virulence testing in laboratory animals. Because *E. rhusiopathiae* isolates vary in virulence, it is necessary to confirm virulence by intraperitoneal inoculation of mice or pigeons.
    - PCR for virulence detection