Module II Gram Positive Cocci

Lesson 1 Staphylococcus

Lesson 2 Streptococcus

LESSON 1

GRAM POSITIVE COCCI

G. STAPHYLOCOCCI

- Belongs to F. Micrococcaceae
- Staphylococci are Gram-positive cocci, approximately 1 *um* in diameter, that tend to occur in irregular clusters resembling bunches of grapes.
- The name derives from the Greek words *staphyle* and *kokkos* for a 'bunch of grapes' and a 'berry' respectively.
- At least 30 *Stapylococcus* species occur aas commensals on skin and mucous membranes; some may act as opportunistic pathogens causing pyogenic infections.
- Most staphylococci are facultative anaerobes and catalase-positive.
- They are non-motile, oxidase-negative and do not form spores.
- Two species, *S. aureus* subsp. *anaerobius* and *S. saccharolyticus* are anaerobic and catalase-negative.
- The coagulase-positive *S. aureus* subsp. *aureus* and *S. intermedius,* and the coagulase-variable *S. hyicus* are important pathogens of domestic animals.
- Coagulase production correlates with pathogenicity

Key points:

- ✓ Gram-positive cocci in clusters resembling bunches of grapes.
- ✓ Grow on non-enriched media
- ✓ Moderately-sized white or golden colonies
- ✓ Colonies of *S. aureus* and *S. intermedius* produce double hemolysis.
- ✓ Facultative anaerobes, non-motile, catalase-positive
- ✓ Commensals on mucous membranes and skin
- ✓ Coagulase production correlates with pathogenicity
- ✓ Comparatively stable in the environment
- ✓ Capable of resisting dehydration for long periods, they are relatively heat-resistant, and they tolerate the ordinary disinfectants.
- ✓ Cause pyogenic infections.

Differentiation of Gram-positive cocci

Characteristic	***Staphylococcus*** spp.	*Micrococcus* spp.	***Streptococcus*** and *Enterococcus* spp.
Appearance in stained smears	Irregular clusters	Packets of four	Chains
Coagulase production	±	-	-
Catalase production	+	+	-
Oxidase production	-	+	-
O-F test[a]	F	O	F
Bacitracin disc (0.04 units)	Resistant	Susceptible	Resistant

A Oxidation-fermentation test, O oxidative, F fermentative

Distnguishing features of coagulase-positive staphylococci

Species	Colony color	Hemolysis on sheep blood agar	Coagulase production Tube test	Coagulase production Slide test	Acetoin production	Maltose utilization[a]
S. aureus	Golden yellow[b]	+	+	+	+	+
S. intermedius	White	+	+	v	-	±
S. hyicus	White	-	v	-	-	-
S. aureus subsp *anaerobius*[c]	White	+	+	-	-	na
S. delphini	White	+	+	-	-	na
S. schleiferi subsp *ciagulans*	White	+	+	-	+	na

a 1% maltose in purple agar base
b bovine and human strains only
c anaerobic
na not available

+ over 90% strains positive
- over 90% strains negative
± poor utilization
v variable reactions

Differentiation of S. aureus from S. epidermidis

Characteristic	*S. aureus*	*S. epidermidis*
1. Coagulase	+	-
2. Mannitol (anaerobically)	Acid	-
3. DNA'se	+	-
4. Biotin requirement	-	+
5. Cell wall constituents		
❖ Ribitol	+	-
❖ Glycerol	-	+
❖ Protein A	+	-
6. Alpha hemolysin	+	-

Pathogenesis and Pathogenicity

- Because staphylocci are pyogenic bacteria, they often cause suppurative lesions.
- Minor trauma or immunosuppression may predispose to the development of infection.
- There are virulence factors that are encoded in the staphylococcal genome that of pathogenic significance (see table below).
- Structural features including capsular polysaccharide, techoic acids and protein A interfere with opsonization and subsequent phagocytosis.
- Its cell wall proteins, which bind to fibronectin and fibrinogen, may facilitate bacterial attachment to tissue damaged by the toxic factors elaborated by the organisms.
- Production of coagulase by staphylococci is an important indicator of pathogenicity.

- Additional markers for pathogenicity are DNAse activity and protein A production.

Toxins and enzymes (virulence factors) produced by *S. aureus*

Toxin	Action
1. alpha hemolysin	Hemolysis (sheep, rabbit); vasoconstriction and dermonecrosis. The major toxin in gangrenous mastitis. It causes spasm of smooth muscle and is necrotizing and potentially lethal
2. B hemolysin	Hot-cold hemolysis (sheep). A sphingomyelinase which damages cell membranes.
3. G hemolysin	Weak hemolysis
4. d hemolysin	Dermonecrosis; destruction of WBC
5. leukocidin	Leukocytic degranulation
6. enterotoxin	Heat-stable toxins associated with staphylococcal food poisoning in man. Emesis and/or diarrhea.
7. Toxic shock syndrome toxins (TSST)	Induce excessive lymphokine production, resulting in tissue damage. Bovine and human strains of *S. aureus* produce TSST-1. Sheep and goat strains produce a variant of this toxin. The significance of these toxins in animals is unclear.
8. Exfoliative toxins	Responsible for desquamation in staphylococcal scalded skin syndrome in man.
Enzymes	
9. coagulase (free)	Clots purified fibrinogen in the presence of CRF (coagulase reacting factor) from prothrombin
10.Hyaluronidase (spreading factor)	Hydrolyzes hyaluronic acid in intracellular ground substance.
11.DNAse	Hydrolyzes DNA
12.Fibrinolysin (staphylokinase)	Dissolves clots by activation of plasminogen
13. Lipase	Hydrolyzes bactericidal lipids of skin
14. Protease	Hydrolyzes protein
15. Lipase, esterases, elastases, staphylokinase, deoxyribonuclease, phospholipase	Enzymes which contribute to virulence.

Diagnostic Procedures

- Exudative epidermitis in piglets and tick pyemia of lambs are the clinical conditions of domestic animals specifically attributable to pathogenic staphylococci.
- Gram-stained smears of pus or other suitable specimens may reveal typical staphylococcal clusters.

- Specimens are cultured on blood agar, selective blood agar and MacConkey agar and incubated aerobically at 37_oC for 24-48 hours.
- Identification criteria for isolates:
  - Colonial characteristics
  - Presence or absence of hemolysis
  - Catalase production
  - Coagulase production
  - Biochemical profile
- Phage typing

Disease caused by *S. aureus* and *S. epidermedius*

1. Horses – botryomycosis – usually begins, after castration, in the stump of the spermatic cord → infected cord becomes greatly enlarged and sclerotic → small pockets of pus are found and there is mass of new-formed tissue and in the pus, small granules resembling those of actinomycosis are found.
2. Bovine – mastitis → sheep, goats, mares, sows, cats, and mink
3. Tick pyemia of lambs – associated with heavy infestations of lambs by the sheep tick *Ixodes ricinus* → acute septicemia or bacteremia is often produced with toxemia, which rapidly kills the lamb.
4. Canine pyoderma – S. aureus is the major bacterial pathogen of the canine skin, ectoparasitism, and matted, dirty hair may predispose to its invading the superficial and deeper layers of the skin.
5. S. aureus also causes external eye disease and urinary tract infections in the dog.
6. Purulent synovitis in poultry → S. auerus causes lameness, swellings on the feet, and occasionally spondylitis (inflammation of vertebrae).

Treatment

- Penicillin
- Lincomycin, erythromycin and chloramphenicol → have good penetration into abscesses and minimal binding to purulent material.

Main disease caused by pathogenic staphylocci

Species	Host/s	Diseases
Staphylococcus aureus	Many animal species	Abscesses and suppurative conditions. Infection can be systemic. Notorious for infections following surgery.
	Cattle	Mastitis: subclinical, chronic, acute, peracute or gangrenous. Udder impetigo: small pustules often at base of teats.
	Sheep	Mastitis: acute, peracute or gangrenous. Tick pyemia of lambs (2-5 weeks old): associated with heavy tick infestation

		(Ixodes ricinus). Periorbital eczema (dermatitis): infections of abrasions, often due to insufficient feed-trough space. Staphylococcal dermatitis: predisposed to by scratches fro vegetation such as thistles.
	Goats	Mastitis: subacute or peracute Staphylococcal dermatitis
	Pigs	Mastitis: acute, subacute and chronic (botryomycosis) Necrotizing staphylococcal endometritis. Udder impetigo: after abrasions from teeth of piglets.
	Horses	Mastitis: acute Scirrhous cord / Botryomycosis (spermatic cord) after castration.
	Rabbits	Exudative dermatitis in neonates. Abscesses, conjunctivitis and pyemic conditions.
	Poultry	'Bumble-foot': pyogranulomatous process of subcutaneous tissue of foot that can involve the joints. Staphylococcal arthritis and septicemia in turkeys. Omphalitis (although more commonly caused by E. coli)
	Dogs, cats	Suppurative conditions similar to those listed *S. intermidicus*
S. aureus subsp. anaerobius	Sheep	Lesions similar to those of caseous lymphadenitis (*Corynebacterium pseudotuberculosis*)
S. intermedius	Dogs, cats	Canine (feline) pyoderma (juvenile and adult). Chronic and recurrent pyoderma is a complex syndrome possibly involving cell mediated hypersensitivity, endocrine antibiotic disorders and a genetic predisposition. Responds poorly to antibiotic therapy alone. Staphylococcal pustular dermatitis occurs in neonates or in adults under conditions of poor hygiene. Responds readily to antibiotic therapy. Pyometra

		Otitis externa (together with other pathogens). Infections involving respiratory tract, bones, joints, wounds, eyelids and conjunctiva.
	Horses, cattle	Rare infections in these species.
S. hyicus	Pigs	Exudative epidermitis (greasy pig disease), usually in pigs under 7 weeks old. There is systemic involvement and the condition can be fatal. Septic poluarthritis
	Cattle	Rare cases of mastitis.
S. delphini	dolphins	Suppurative skin lesions
S. schleiferi subsp. *coagulans*	Dogs	Otitis externa

Coagulase-negative Staphylococci isolated from animals

Species	Host/Source
S. arlettae	Goats / nares Poultry / skin
S. capitis	Cattle / milk
S. caprae	Goats / skin
S. caseolyticus	Cattle / milk, milk products
S. chromogenes	Cattle / milk Pigs, poultry / skin
S. cohnii	Cattle / milk
S. epidermidis	Cattle / milk Dogs, horses / wound infections
S. equorum	Horses / skin
S. felis	Cats / otitis externa, skin infections
S. gallinarum	Poultry / skin infections
S. haemolyticus	Cattle / milk
S. hominis	Cattle / milk
S. lentus	Pigs, sheep, goats / skin infections
S. saprophyticus	Cats / skin
S. sciuri	Cats and other animals / skin infections
S. simulans	Cattle / milk Dogs, cats, pigs / skin
S. vitulinus	Cattle, sheep, pigs / skin
S. warneri	Cattle / milk
S. xylosus	Cattle, sheep / milk Cats, poultry pigs, horses / skin

Review Questions:

1. Give the three 3 species of Staphylococcus that cause disease in animals.
2. Give at least five significant morphological, cultural and biochemical characteristics that will differentiate Staphylococcus from other bacteria.
3. Differentiate *S. aureus* from *S. epidermicus*.
4. Enumerate the virulence factors produced by Staphylococcus and give their mechanisms of action.
5. What are the factors that aid in the penetration and lesion production of Staphylococcus?
6. What are the important factors that involved in staphylococcal survival, multiplication, and lesion production after initial production

LESSON 2

STREPTOCOCCI

- The streptococci are a group of bacteria that infect many animal species, causing suppurative conditions such as mastitis, metritis, polyarthritis and meningitis.
- The group contains the genera *Streptococcus, Enterococcus,* and *Peptostreptococcus.*
- Most pathogenic species are in the genus *Streptococcus.*
- These organisms are Gram-opositive cocci, approximately 1.0 *u*m in diameter, which form chains of different lengths.
- *Streptococcus* species are catalase-negative, facultative anaerobes, which are non-motile.
  - o They are fastidious bacteria and require the addition of blood or serum to culture media.
  - o *Streptococcus pneumoniae* (pneumococcus) occurs as slightly pear-shaped cocci in pairs. Pathogenic strains have thick capsules and produce mucoid colonies.
- *Enterococcus* species are enteric streptococci which are found in the intestinal tract of animals and man.
  - o They are opportunistic pathogens and differ from the *Streptococcus* species in:
    a. They tolerate bile salts and grow on MacConkey agar as red, pin-point colonies.
    b. Some isolates are motile
- *Peptostreptococcus indolicus* is an anaerobic streptococcus, which is etiologically implicated in bovine 'summer mastitis' in association with *Arcanobacterium pyogenes.*

Key points:

- ✓ Gram-positive cocci in chains
- ✓ Fastidious, requiring enriched media
- ✓ Small, usually hemolytic, transluscent colonies
- ✓ Catalase-negative
- ✓ Facultative anaerobes, usually non-motile
- ✓ Commensals on mucous membranes (upper respiratory tract,lower genital tract)
- ✓ Susceptible to desiccation
- ✓ Cause pyogenic infections

Pathogenesis and Pathogenicity

- Pyogenic stre[ptococci are associated with abscess formation, other suppurative conditions and septicemias.
- Beta-hemolytic streptococci are generally more pathogenic than those producing alpha-hemolysis.
- Virulence factors include enzymes and exotoxins such as streptolysins (hemolysins), hyaluronidase, DNase, NADase, streptokinase and proteases.

The specific action and significance of some of these factors are poorly understood.

- Polysaccharide capsules, which are major virulence factors of *S. pyogenes*, . *S. pneumoniae* and some strains of *S. equi*, are antiphagocytic.
- The cell wall M proteins of *S. pyogenes*, . *equi*, and *S. porcinus* are also antiphagocytic.
- In the absence of antiphagocytic factors, these bacteria are rapidly killed by phagocytes.

Diagnostic Procedures

- History, clinical signs and pathology may be indicative of certain streptococcal infections such as strangles.
- Streptococci are highly susceptible to desiccation and specimens should be cultured promptly.
- PCR – sensitive technique for detecting *S. equi.*
- Chains of Gram-positive cocci be demonstrable in smears of specimens.
- Specimens should be cultured on blood agar, slective blood agar and MacConkey agar; incubated aerobically at 37°C for 24-48 hours.
- Identification criteria for isolates:
  - Small, transluscent colonies, some of ehich may be mucoid.
  - Type of hemolysis on blood agar
  - Chains of Gram-positive cocci
  - No growth on MacConkey agar with the exception of *E. faecalis*
  - Negative catalase test
  - Lancefield grouping
  - Biochemical test profile

GENUS STREPTOCOCCUS

- Recent changes in nomenclature

Lancefield group	Previous Name	Present Name
C	*Streptococcus equisimilis*	*S. dysgalactiae subsp. equisimilis*
C	*Streptococcus equi*	*S. equi subsp. equi*
C	*S. zooepidermicus*	*S. equi subsp. zooepidermicus*
D	*S. faecalis*	*Enterococcus faecalis*
D	*S. faecium*	*Enterococcus faecium*
D	*S. durans*	*Enterococcus durnas*
Q	*S. avium*	*Enterococcus avium*
N	*S. lactis*	*Lactococcus lactis*

- Classification of Streptococcus according to Sherman:
  1. pyogenic group – include most of the pathogenic species.
  2. viridans group – chiefly characterized by the production of alpha hemolysis or a greenish discoloration around colonies on blood agar.
  3. Lactic group - composed of strains associated with milk and having the ability to produce lactic acid in this substrate.
  4. enterococcus group – composed of strains that resemble S. faecalis, intestinal inhabitant.

  *** This is no longer used in its entirety, but the tolerance tests he used to establish his primary divisions are still useful in classification.
- Classification according to Bergey's Manual
  - Based partly on tolerance test and partly on biochemical nehavior; serology.
  - Serologic test done by Lancefield.
  - This correlates with that of Sherman.
  - Based on the composition of antigen/protein.
  1. Group A – unknown
  2. Group B – contain the majority of animal pathogens; cell wall has rhamnose-glucosamine.
  3. Group C – animal pathogens also; cell wall has rhamnose-N-acetyl-galactosamine polysaccharide.
  4. Group D – antigen is teichoic acid
  5. Group E – unknown
  6. Group N – antigen is teichoic acid.

Morphology and Staining Reactions

- Chains of cocci can be short or very long depending on species differences and the medium on which the culture is growing.
- Typical chain formation is best seen in fluid media; on solid media the chains become so entangled that they are difficult top distinguish.
- Theindividual cells of streptococcus are seldom perfectly spherical.
- Sometimes the cells are flattened from side to side, more often they are elongated.
- Can be pleomorphic on primary isolation that they can be mistaken for short rods.
- Spores are never formed and, with rare exceptions, the organisms are nonmotile.
- Some species form definite capsules when developing in tissues or in culture media containing blood serum.
- Some strains show 2 mucoid or matt-type of colony formation rather than the more usual smooth (glossy) or rough forms.

Cultural and Biochemical Features of Streptococcus

- Horse-meat infusion agar is the best medium for isolation of animal strains of Streptococcus.
- All Streptococcus produce small, delicate, transluscent colonies about 1mm in diameter on solid media.
- The surface of the growth is smooth and glistening, and the margins of individual colonies are perfectly circular.
- Deep colonies in agar usually are lenticular in shape.
- In softer media, colonies can be globular and barely visible to the naked eye.
- In broth, they produce long chains that quickly settle, resulting in a fluffy sediment at the bottom of the tube.
- All streptococci grow well in milk, and most strains produce lactic acid in the substrate.
- They are unique among aerobic bacteria in that they are likely to synthesize heme compounds but cannot synthesize cytochromes and are incapable of oxidative phosphorylation by means of a cytochrome mediated electron-transport chain → Because of this the cytochrome inhibitor sodium azide is used in media for the selective isolation of Streptococcus from contaminated specimens.
- Catalse negative and ferment sugars to dextro-rotatory lactic acid.
- Only a few streptococcus and none of the pathogenic types produce gas.
- The temperature growth range of Streptococcus varies from below 10°C to above 45°C.
- Many of the pathogenic group completely lyze horse erythrocytes.

Lance field group	**Species**	**Hem olysis**	**Host/s**	**Disease**	**Natural habitat (if known)**
A	*S. pyogenes*	B	Humans	Scarlet fever, septic sore throat, puerperal fever, erysipelas, abscesses and rheumatic fever	Human upper respirator y system
			Cattle	Mastitis (rare)	
			Foals	Lymphangitis	
B	*S. agalactiae*	B (a,y)	Cattle, sheep and goats	Chronic mastitis	Milk ducts
			Humans and dogs	Neonatal septicemia	Maternal vagina
			Cats	Kidney and uterine infections	

C	*S. dysgalactiae*	A (B,y)	Cattle	Acute mastitis	Buccal cavity and genitalia
			Lambs	Polyarthritis	
	S. dysgalactiae subsp. equisimilis	B	Horses	Abscesses, endometritis and mastitis	Skin and vagina
			Pigs, cattle, dogs and birds	Various suppurative conditions	
	S. equi subsp. equi	B	Horses	Strangles, genital and suppurative conditions, mastitis and purpura hemorrhagica	Equine tonsils
	S. equi subsp. zooepidermicus	B	Horses	Mastitis, abortion, secondary pneumonia and navel infections	Vagina and skin
			Cattle	Metritis and mastitis	
			Pigs	Septicemia and arthritis in 1-3 week-old piglets	Skin and mucous membranes of sows
			Poultry	Septicemia and vegetative endocarditis	
			Lambs	Pericarditis and pneumonia	
D	*Enterococcus fecalis* *E. faecium* *E. durans*	A (B,y)	Many species	Opportunistic infections such as septicemia in chickens, bovine mastitis, endocarditis in cattle and lambs, and urinary-tract infections in dogs	Intestinal tract of many animals
	S. equines *S. bovis*	a	Many species	Opportunistic infections	Intestinal tract of many animals
E (P,U,V)	*S. porcinus*	B	Pigs	Jowl abscesses and lymphadenitis	Mucus membrane
G	*S. canis*	B	Carnivores	Neonatal septicemia. Genital, skin and wound infections	Genital tract and anal mucosa
			Cattle	Occasional mastitis	
N	*Lactococcus lactis*	a	Cattle	Unknown pathogenicity	Milk, plants and

					tonsils of pigs fed on whey
Q	*Enterococcus avium*	a, y	Many species	Unknown pathogenicity	Feces of birds and mammals
R (D)	*S. suis type 2*	a	Pigs (weaning to 6 months)	Maningitis and arthritis	Tonsils and nasal cavity
			Humans	Meningitis and septicemia	Pigs
S (D)	*S. suis type 1*	a(B)	Pigs (2-4 weeks old)	Meningitis, arthritis, pneumonia and septicemia	Tonsils and nasal cavity
Ungroupable	*S. uberis*	a (y)	Cattle	Mastitis	Skin, vagina and tonsils
	S. pneumoniae	a	Humans and primates	Pneumonia. Septicemia and meningitis	Upper respiratory tract
			Guinea pigs and rat colonies	Pneumonia (outbreaks can occur)	

Epizootiology and Pathogenesis (S. agalactiae)

- Their habitat is largely confined to the mammary gland.
- Infection is spread between cows by the milker's hands or by contaminated milking equipment.
- The organism enters through the end of the teat, and colonization of the gland is possibly favored by adhesion to the epithelium of the gland sinuses.
- Multiplication of the organism on the surface of the teat and duct sinuses results in a slowly progressively inflammation and fibrosis of adjoining areas of the gland → therefore disease develops gradually.
- Older animals are more frequently affected.
- Excessive stripping can exacerbate the disease.
- The milk becomes altered in varying degrees, sometimes showing little or no abnormality and sometimes showing flakes, stringy masses of fibrin, blood, and thick purulent material.
- The inflammation in the udder causes the formation of new interstitial tissue, which changes the normally soft gland into hard lump.
- The milk from affected quarters becomes alkaline and leukocyte count increases.
- In animals with advanced cases the secretion is much reduced in volume and is thin and watery.

Diagnosis

- Blood dextrose agar containing B toxin allows presumptive recognition of colonies → be confirmed by biochemical tests.

Treatment

- Penicillin, ampicillin, erythromycin, chloramphenicol, cephalosporins and tetracycline

Review Questions:

1. What are the recent changes in nomenclature of Streptococcus?
2. Give the classification of Streptococcus according to Sherman
3. What are the species of Streptococcus that cause disease in animals.
4. Give at least five significant morphological, cultural and biochemical characteristics that will differentiate Streptococcus from other bacteria.
5. Enumerate the virulence factors produced by Staphylococcus and give their mechanisms of action.