STAPHYLOCOCCI

What is to be studied?

- morphology, isolation, growth, colonial, biochemical and antigenic characters. Pathogenicity and diagnosis of bacterial and fungal diseases

INTRODUCTION

- **Staphyloccocci - derived from Greek "stapyle" (bunch of grapes), "kokkos"-berry**
- **Gram positive cocci arranged in clusters**
- **Hardy organisms surviving many non physiologic conditions**
- **Include a major human pathogen and skin commensals**

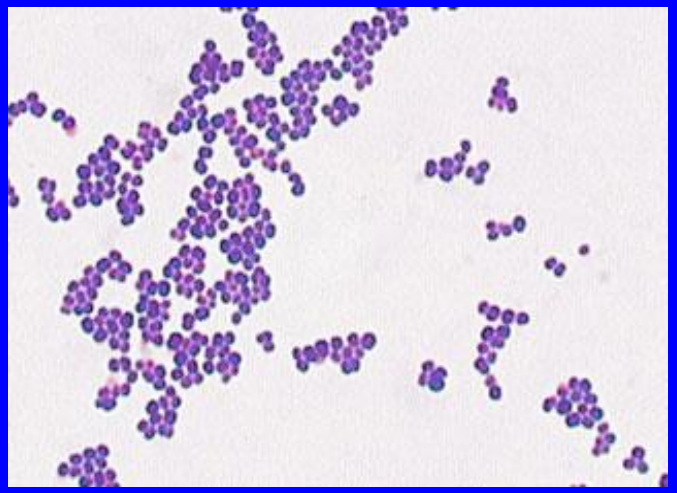
Morphology

- Gram positive, spherical cells arranged in clusters

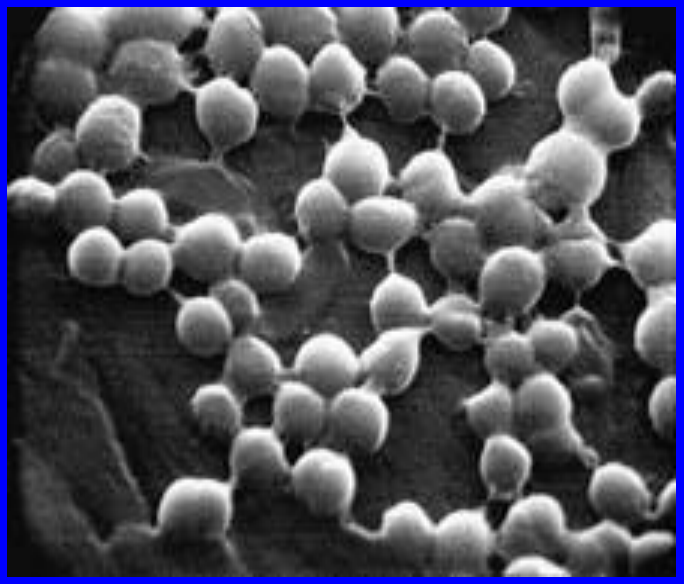
- Staphylococci are perfectly spherical cells about 1 micrometer in diameter. The staphylococci grow in clusters because the cells divide successively in three perpendicular planes with the sister cells remaining attached to one another following each successive division. Since the exact point of attachment of sister cells may not be within the divisional plane, and the cells may change position slightly while remaining attached, the result is formation of an irregular cluster of cells

Staphylococcus: General Characteristics

- Gram-positive spherical cells (0.5-1.5 μm) in singles, pairs, and clusters
- Appear as "bunches of grapes"



Gram-stained smear of staphylococci from colony



Scanning electron micrograph of staphylococci

Staphylococcus: General Characteristics

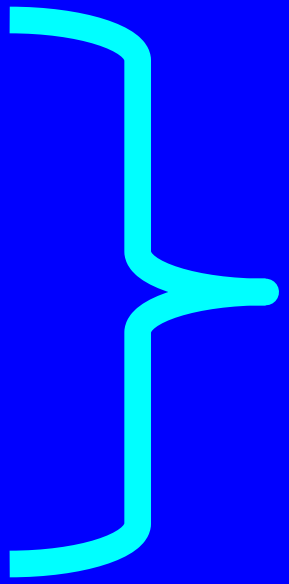
- Nonmotile
- Non–spore-forming
- Noncapsulated
- Catalase-producing

Genus *Staphylococcus*

- Approximately 33 species
- 14 to 17 species associated with humans
- Several veterinary pathogens
- Species initially differentiated by the coagulase test

Coagulase-Positive Staphylococci

- S. *aureus*
- *S. intermedius*
- *S. hyicus*
- *S. delphini*
- *S. schleiferi*



Human pathogen

Animal-associated species

Veterinary pathogens

Coagulase-Negative Staphylococci

- *S. epidermidis*
- *S. saprophyticus*
- *S. haemolyticus*
- *S. lugdunensis*
- *S. kloosii*
- *S. saccharolyticus*
- *S. simulans*
- *S. capitis*
- *S. caprae*
- *S. sciuri*
- *S. hominis*
- *S. schlieferi*
- *S. cohnii*
- *S. xylosus*

Clinically Significant Staphylococci: *Staphytoccus aureus*

- Habitat: anterior nares (carriers)
- Primary pathogen of the genus
- Produce superficial to systemic infections
- Mode of transmission: traumatic introduction
- Predisposing conditions
  - **Chronic infections**
  - **Indwelling devices**
  - **Skin injuries**
  - **Immune response defects**

Virulence Factors: Extracellular Enzymes

- Hemolysins: hemolyze RBCs
  - **Alpha: platelets/WBCs/tissue**
  - **Beta (hot/cold): sphingomyelin of RBCs**
  - **Gamma: host cell membranes**
  - **Delta: less lethal**
- Leukocidin (Panton-Valentine): kill PMNs
- Enterotoxins
  - **A/D: food poisoning**
  - **F: TSSAT**
  - **B: pseudomembranous enterocolitis**

Cultural characteristics

- *Staphylococcus aureus* forms a fairly large yellow colony on rich medium; *S. epidermidis* has a relatively small white colony. *S. aureus* is often hemolytic on blood agar; *S. epidermidis* is non hemolytic. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. Nearly all strains of *S. aureus* produce the enzyme coagulase: nearly all strains of *S. epidermidis* lack this enzyme. *S. aureus* should always be considered a potential pathogen; most strains of *S. epidermidis* are nonpathogenic and may even play a protective role in humans as normal flora. *Staphylococcus epidermidis* may be a pathogen in the hospital environment

CULTURE:

Media used :-

i) Non selective media: Nutrient agar,
Blood agar,
MacConkey's agar.

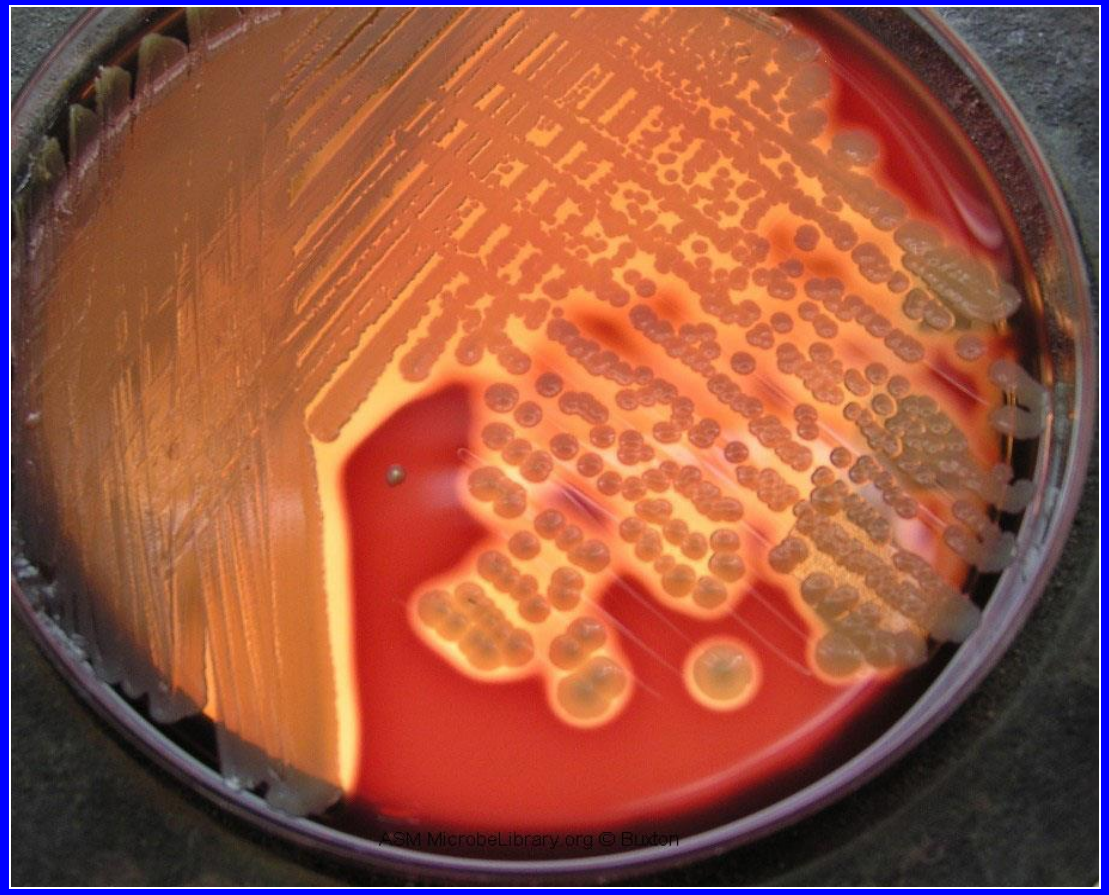
ii) Selective media: Salt-milk agar,
Ludlam's medium

Cultural Characteristics:

i) On nutrient agar- The colonies are large, circular, convex, smooth, shiny, opaque and easily emulsifiable. Most strains produce golden yellow pigments.

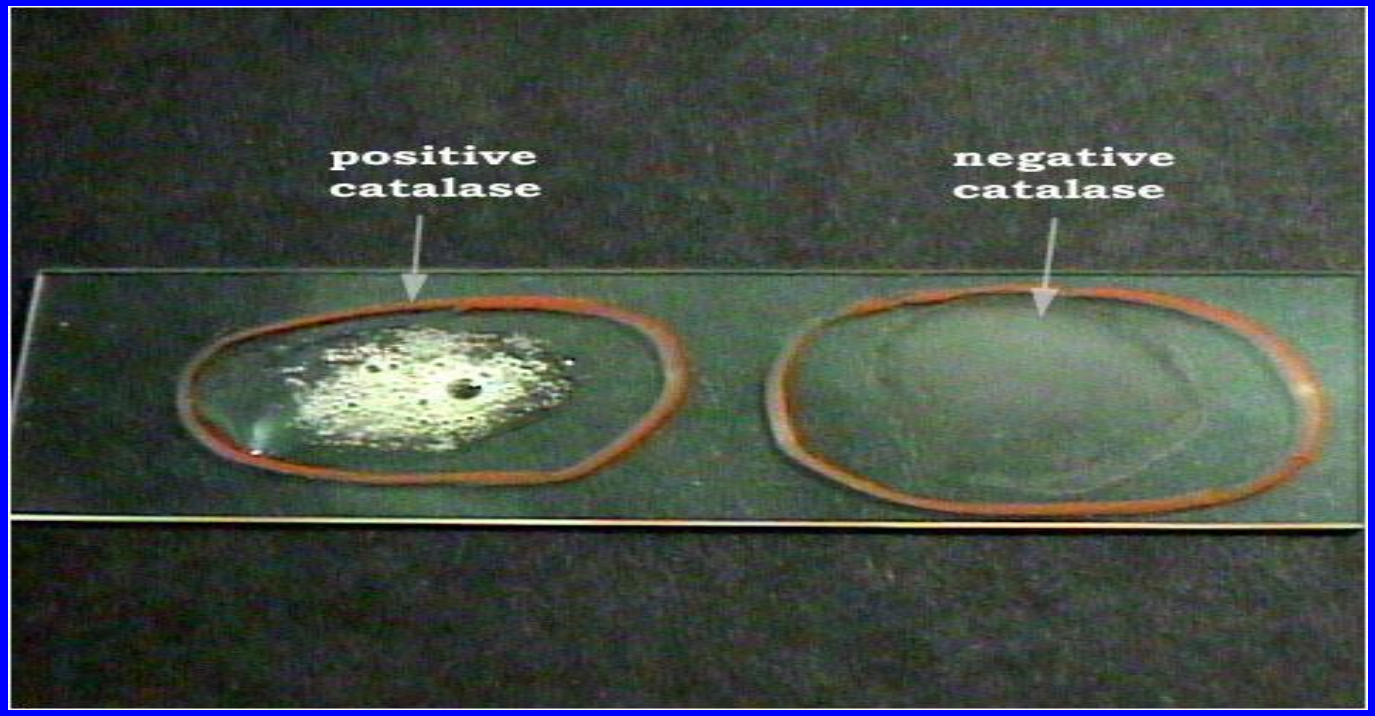
ii) On MacConkey's agar- The colonies are small & pink in colour.

iii) On blood agar- Most strains produce β-haemolytic colonies.



Biochemical reactions:

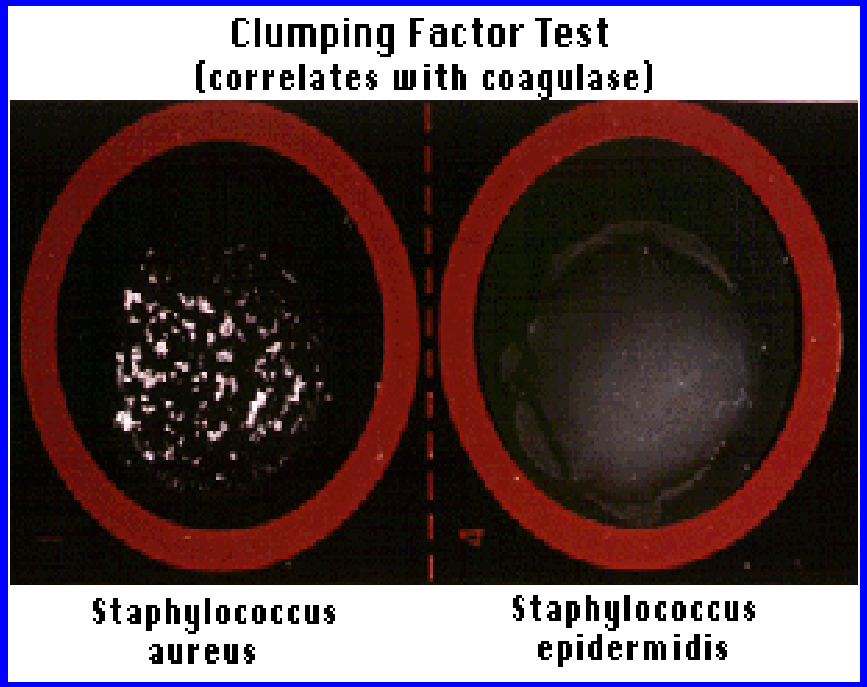
1) Catalase test- Positive.



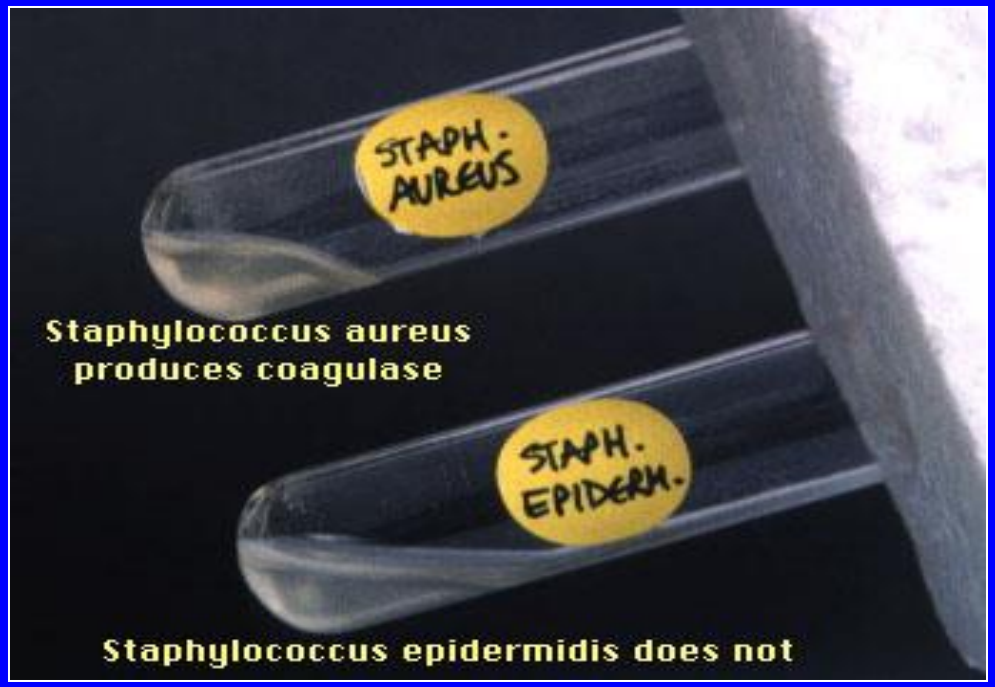
2) Coagulase test-

i) Slide coagulase test- Positive.

ii) Tube coagulase test- Positive.



SLIDE COAGULASE TEST



TUBE COAGULASE TEST

3) Reduces nitrate to nitrite.

4) Ferments mannitol anaerobically with acid only.

5) Urea hydrolysis test- Positive.

6) Gelatin liquefaction test- Positive.

7) Produces Lipase.

8) Produces Phosphatase.

9) Produces Thermostable nuclease.

Number of species

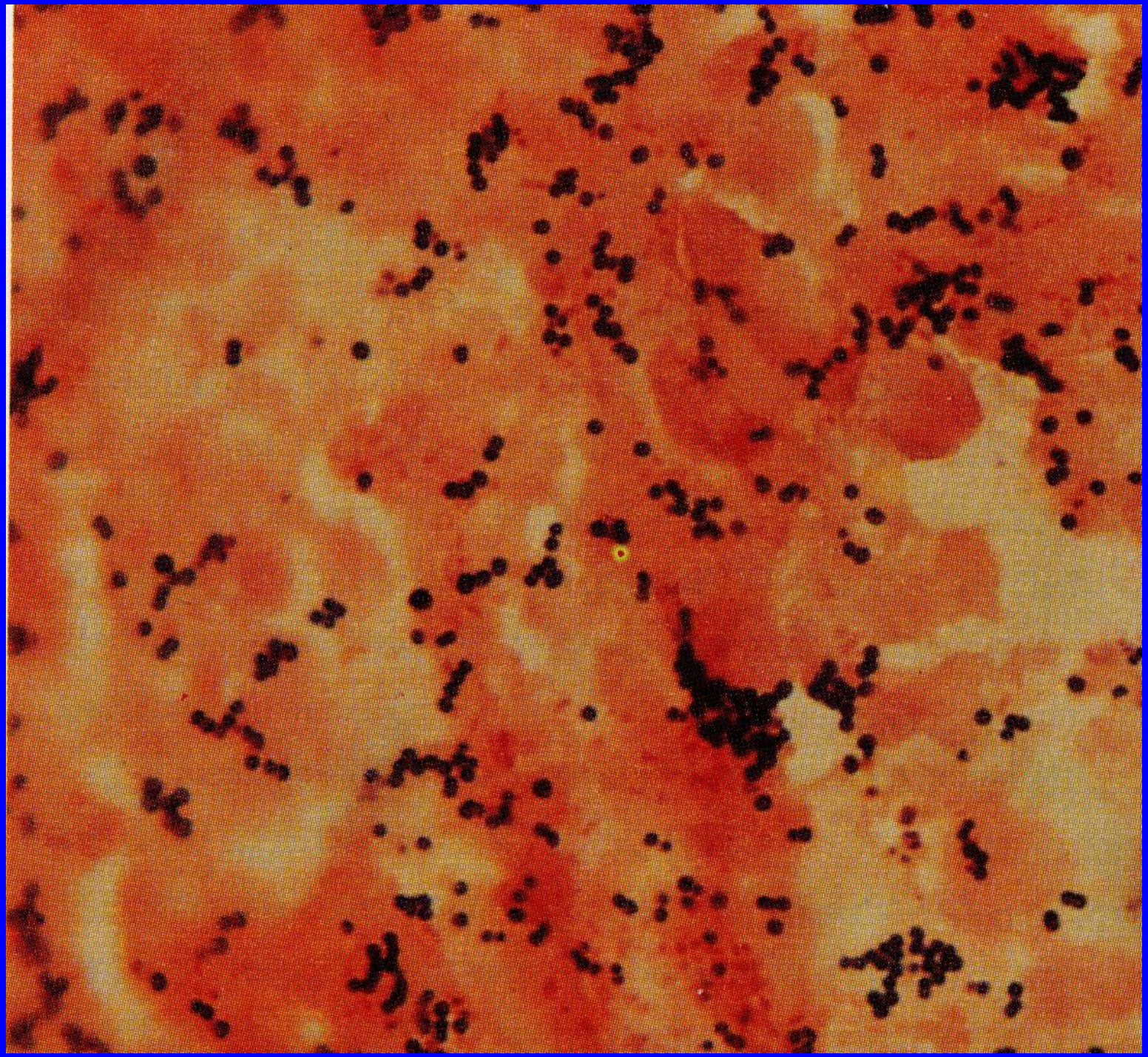
- Coagulase positive and coagulase negative
- Not host species specific

Coagulase positive:

- *S. aureus*-bovine, ovine, porcine, -mastitis, suppurative lesion
- *S.intermedius*: canine, equine, avian
- *S.schleiferi* subsp. *Coagulans*: canine
- *S. delphini*: in dolphin

Coagulase negative:

- *S.hyicus*
- *S.chromogenes*
- *S.simulans*
- *S.xylosus*
- *S.epidermidis*
- *S.gallinarum*
- *S.hemolyticus*



Stapylococcus and related organisms

***S. aureus*:** major pathogen for humans, may cause suppuration, abscess formation, scalded skin syndrome, toxic shock syndrome and food poisoning.

***S. epidermidis*:** may cause infection from prosthetic devices.

***S. saprophyticus*:** may cause urinary tract infections (UTI) in young women.

***S. haemolyticus*:** endocarditis, UTI, and opportunistic infections.

***Micrococcus* spp.:** opportunistic infections.

***Stomatococcus* spp.:** endocarditis, opportunistic infections.

***Alloiococcus otitidis*:** chronic middle ear infection.

Morphology and Identification

Staphylococci

Gram-positive cocci
(a bunch of grapes)

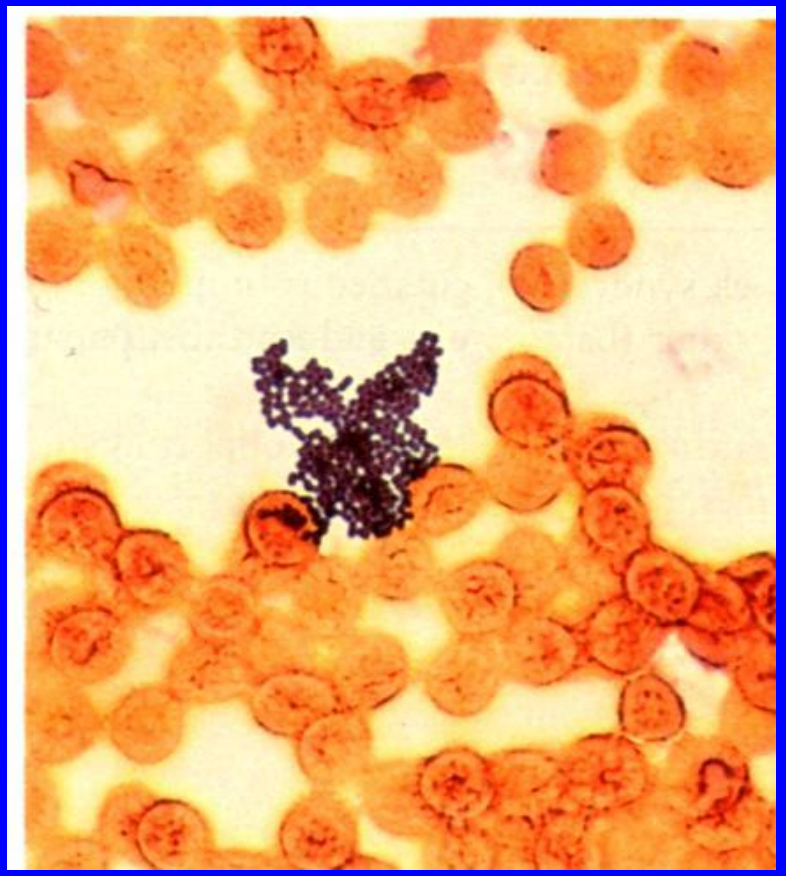
Nonmotile.

Grow readily on most bacteriological media; facultative anaerobic.

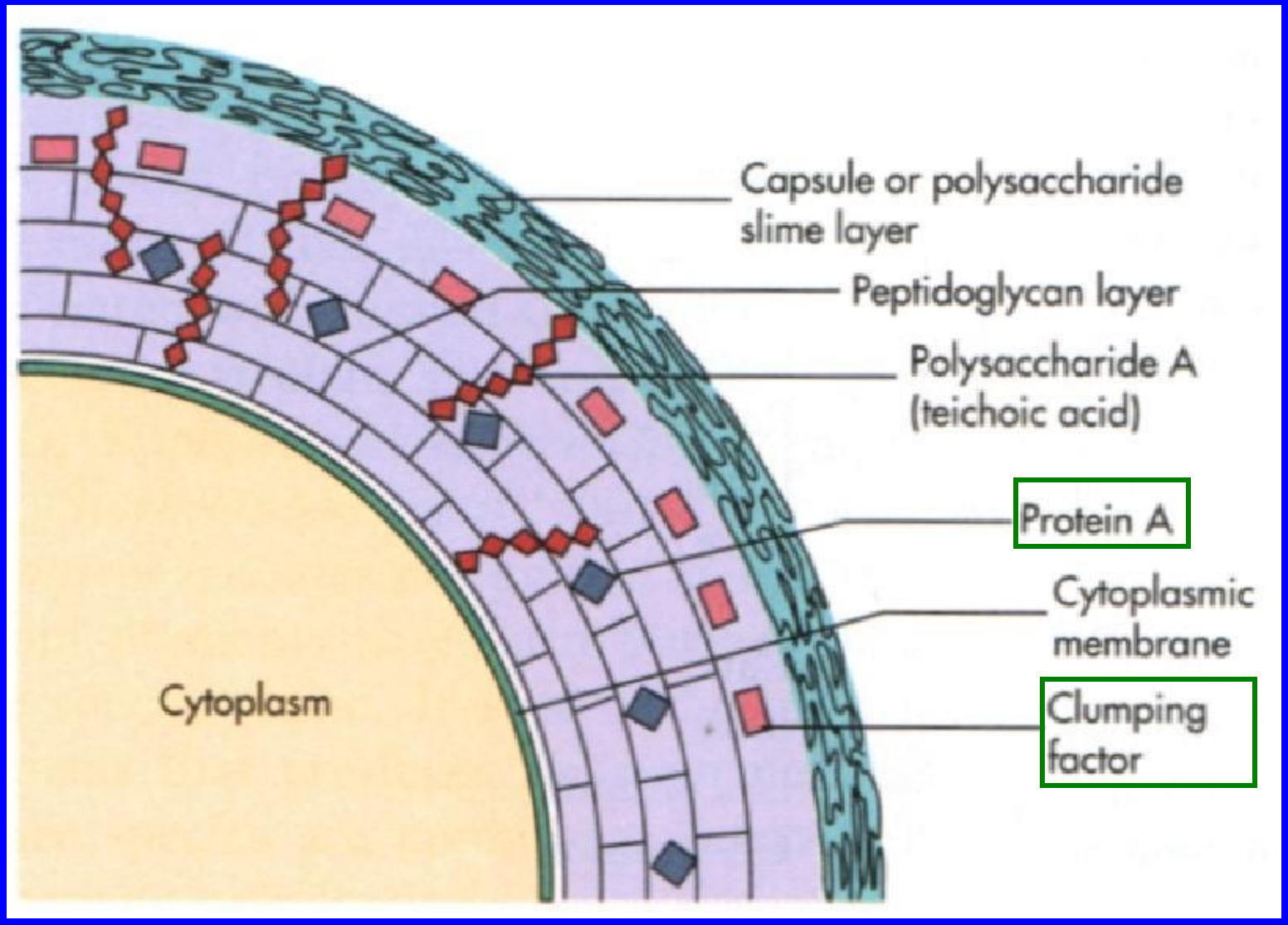
Grow most rapidly at 37 ^{o}C, but form carotenoid pigment best at room temperature under aerobic condition on solid medium.

Produce catalase.

Relatively resistant to drying, heat (40^{o}C) and 10% NaCl.



Structure of staphylococcal cell wall



Capsule

Not readily seen *in vitro*

At least 11 types in *S. aureus*

Inhibiting phagocytosis by polymorphonucleocytes

Slime layer

Loose-bound, water-soluble film

Facilitates bacterial adherence to tissues or foreign bodies and, consequently, biofilm formation (important for the pathogenesis of coagulase-negative staphylococci)

Peptidoglycan

Has endotoxin-like activity

- induces production of cytokines
- activates complement
- induces aggregation of polymorphonucleocytes

Teichoic acids and lipoteichoic acids

Bind covalently to peptidoglycan; species-specific; bind to fibronectin of host cells (adherence); antibodies may be found in systemic staphylococcal disease, particularly endocarditis.

Protein A: present on the surface of *S. aureus* strains, but not other species. Binds to the Fc portion of IgG except IgG3, preventing clearance of bacteria.

Coagulase (clumping factor)

Produced by most *S. aureus* on the cell wall surface; binds to fibrinogen and converts it to fibrin, resulting in aggregates of bacteria.

Coagulase-positive vs. coagulase-negative staphylococci

Other adhesins bind with collagen, elastin and fibronectin

Virulence factors:

These include

A) Cell associated factors

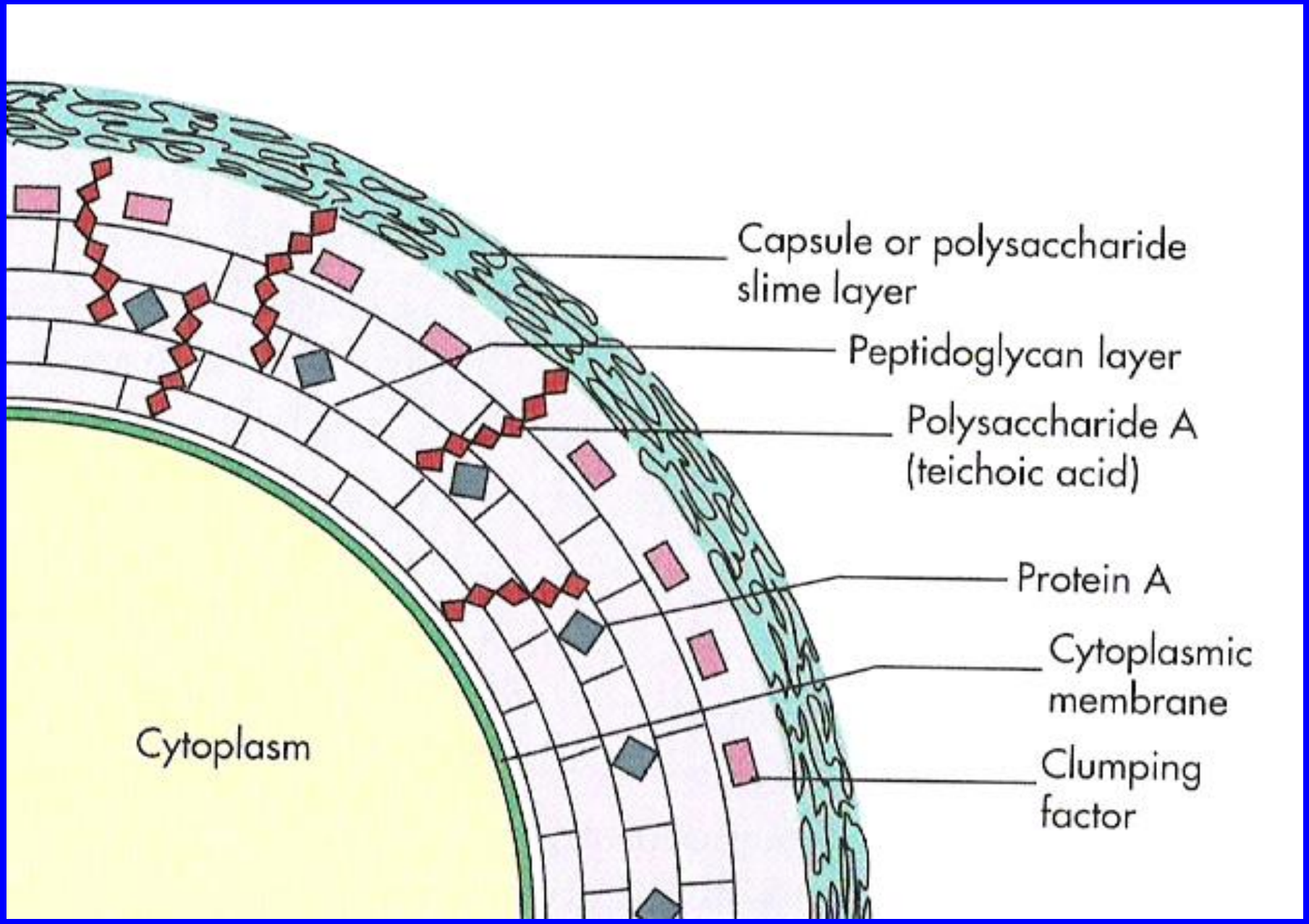
B) Extracellular factors

A) CELL ASSOCIATED FACTORS:

a) Cell associated polymers
b) Cell surface proteins

a) CELL ASSOCIATED POLYMERS
   1. Cell wall polysaccharide
   2. Teichoic acid
   3. Capsular polysaccharide

b) CELL SURFACE PROTEINS:
   1. Protein A
   2. Clumping factor (bound coagulase)



Structure of Staphylococcal cell wall

B) EXTRACELLULAR FACTORS

a) Enzymes

b) Toxins

a) Enzymes:

1. Free coagulase
2. Catalase
3. Lipase
4. Hyaluronidase
5. DNAase
6. Thermonuclease
7. Staphylokinase (Fibrinolysin)
8. Phosphatase

Enzymes

Coagulase: bound and free forms. May deposit fibrin on the surface of staphylococci and alter their ingestion by and destruction within the phagocytic cells (associated with invasiveness).

Fibrinolysin (staphylokinase): to dissolve fibrin clot.

Catalase: to remove H_2O_2.

Hyaluronidase: to facilitate spread of *S. aureus* in tissue.

Lipase: associated with superficial skin infection.

Nuclease: produced only by *S. aureus*.

Penicillinase

Pathogenesis and Immunity

S. *aureus* can produce diseases both through invasiveness and production of toxins.

Toxins

Cytotoxins

α-toxin: pore-forming , cytotoxic to many types of cells including muscle cells.

β-toxin: degrades sphingomyelin and is toxic for many kinds of cells, including human RBCs.

γ-toxin: bicomponent toxins, pore-forming.

δ-toxin: has detergent-like activity.

P-V leukocidin: similar to γ-toxin in structure, kills WBCs of many animals and release the lysosomal enzymes. Associated with severe pulmonary and cutaneous infections.

Toxins

Exfoliative (epidermolytic) toxins: proteases that split desmoglein 1 of the intercellular bridges in epidermis; produced by about 5-10% of *S. aureus*; causes the generalized desquamation of the staphylococcal scalded skin syndrome (SSSS).

Toxic shock syndrome toxin-1 (TSST-1): superantigen, associates with fever, shock, desquamative skin rash of toxic shock syndrome in humans.

Enterotoxins: superantigens, at least 10 (A, B, C1, C2, C3, D, E, G, H, and I) soluble toxins produced by about 50% of *S. aureus*.

Heat-stable (100^{o}C, 30 min.) and resistant to the gastric acid and gut enzymes.

Enterotoxins are produced in carbohydrate and protein foods.

Causing emesis, a characteristic of staphylococcal food poisoning.

b) Toxins:

1. Cytolytic toxins
   - i) Haemolysins
     - Alpha haemolysin
     - Beta haemolysin
     - Gamma haemolysin
     - Delta haemolysin
   - ii) Leucocidin (Panton-Valentine toxin)

2. Enterotoxin
3. Toxic shock syndrome toxin (TSST)
4. Exfoliative (epidermolytic toxin)
5. Ability to form biofilms

Pathogenesis and Immunity

***S. aureus* can produce diseases both through invasiveness and production of toxins.**

Toxins

Cytotoxins

α-toxin: pore-forming , cytotoxic to many types of cells including muscle cells.

β-toxin: degrades sphingomyelin and is toxic for many kinds of cells, including human RBCs.

γ-toxin: bicomponent toxins, pore-forming.

δ-toxin: has detergent-like activity.

P-V leukocidin: similar to γ-toxin in structure, kills WBCs of many animals and release the lysosomal enzymes. Associated with severe pulmonary and cutaneous infections.

Toxins (continued)

Exfoliative (epidermolytic) toxins: proteases that split desmoglein 1 of the intercellular bridges in epidermis; produced by about 5-10% of *S. aureus*; causes the generalized desquamation of the staphylococcal scalded skin syndrome (SSSS).

Toxic shock syndrome toxin-1 (TSST-1): superantigen, associates with fever, shock, desquamative skin rash of toxic shock syndrome in humans.

Enterotoxins: superantigens, at least 10 (A, B, C1, C2, C3, D, E, G, H, and I) soluble toxins produced by about 50% of *S. aureus*.

Heat-stable (100^{o}C, 30 min.) and resistant to the gastric acid and gut enzymes.

Enterotoxins are produced in carbohydrate and protein foods.

Causing emesis, a characteristic of staphylococcal food poisoning.

Enzymes

Coagulase: bound and free forms. May deposit fibrin on the surface of staphylococci and alter their ingestion by and destruction within the phagocytic cells (associated with invasiveness).

Fibrinolysin (staphylokinase): to dissolve fibrin clot.

Catalase: to remove H_2O_2.

Hyaluronidase: to facilitate spread of *S. aureus* in tissue.

Lipase: associated with superficial skin infection.

Nuclease: produced only by *S. aureus*.

Penicillinase

Epidemiology

Staphylococci can permanently (coagulase-negative strains) or transiently (*S. aureus*) colonize various areas of the human body, with the anterior nasopharynx as the most common colonization site for *S. aureus* in older children and adults (30% of healthy adults.)

Nasopharyngeal or skin carriers of *S. aureus* are responsible for many hospital infections.

S. aureus can be transmitted through direct personal contact or contact with contaminated fomites.

Areas at highest risk for severe infections: new born nursery, ICU, operating rooms and cancer chemotherapy wards.

Grouping for Clinical Purposes

- **1. Coagulase positive Staphylococci**
  - ***Staphylococcus aureus***
- **2. Coagulase negative Staphylococci**
  - ***Staphylococcus epidermidis***
  - ***Staphylococcus saprophyticus***

A. Staphylococcus aureus

- **Major human pathogen**
- **Habitat - part of normal flora in some humans and animals**
- **Source of organism - can be infected human host, carrier, fomite or environment**

Natural history of disease

- Many neonates, children, adults - intermittently colonised by S. aureus
- Usual sites - skin, nasopharynx, perineum
- Breach in mucosal barriers - can enter underlying tissue
- Characteristic abscesses
- Disease due to toxin production

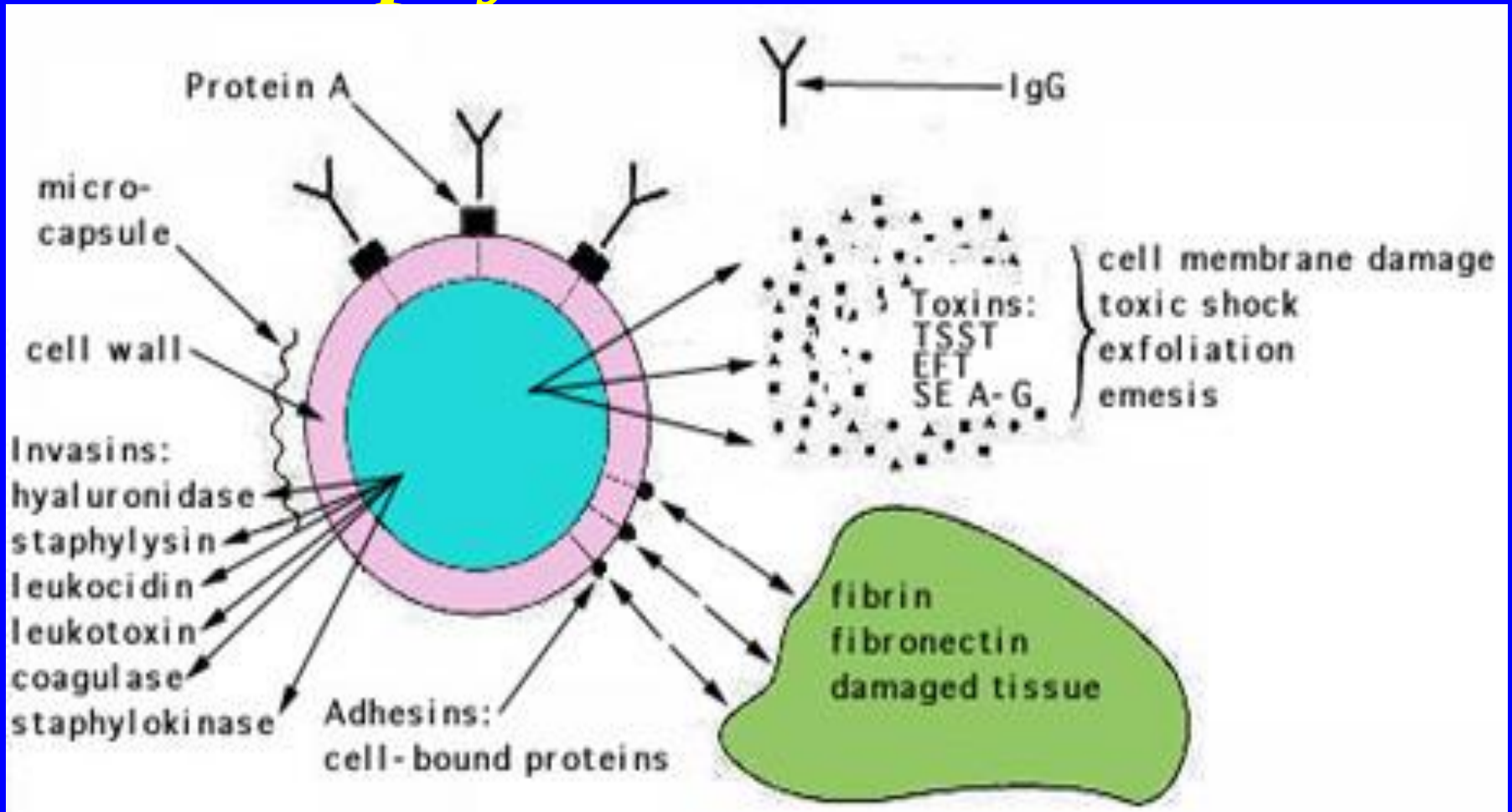
DISEASES

- Due to direct effect of organism
  - Local lesions of skin
  - Deep abscesses
  - Systemic infections
  - MASTITIS
- Toxin mediated
  - Food poisoning
  - toxic shock syndrome
  - Scalded skin syndrome

Factors predisposing to S. aureus infections

- Host factors
  - Breach in skin
  - Chemotaxis defects
  - Opsonisation defects
  - Neutrophil functional defects
  - Diabetes mellitus
  - Presence of foreign bodies
- Pathogen Factors
  - Catalase (counteracts host defences)
  - Coagulase
  - Hyaluronidase
  - Lipases (Imp. in disseminating infection)
  - B lactasamase(ass. With antibiotic resistance)

Virulence determinants of *Staphylococcus aureus*



SKIN LESIONS

- **Boils**
- **Styes**
- **Furuncles(infection of hair follicle)**
- **Carbancles (infection of several hair follicles)**
- **Wound infections(progressive appearance of swelling and pain in a surgical wound after about 2 days from the surgery)**
- **Impetigo(skin lesion with blisters that break and become covered with crusting exudate)**

Membrane-damaging toxins

- **alpha toxin (alpha-hemolysin)** The best characterized and most potent membrane-damaging toxin of *S. aureus* is alpha toxin. It is expressed as a monomer that binds to the membrane of susceptible cells. Subunits then oligomerize to form heptameric rings with a central pore through which cellular contents leak.

  In humans, platelets and monocytes are particularly sensitive to alpha toxin. Susceptible cells have a specific receptor for alpha toxin which allows the toxin to bind causing small pores through which monovalent cations can pass. The mode of action of alpha hemolysin is likely by osmotic lysis.

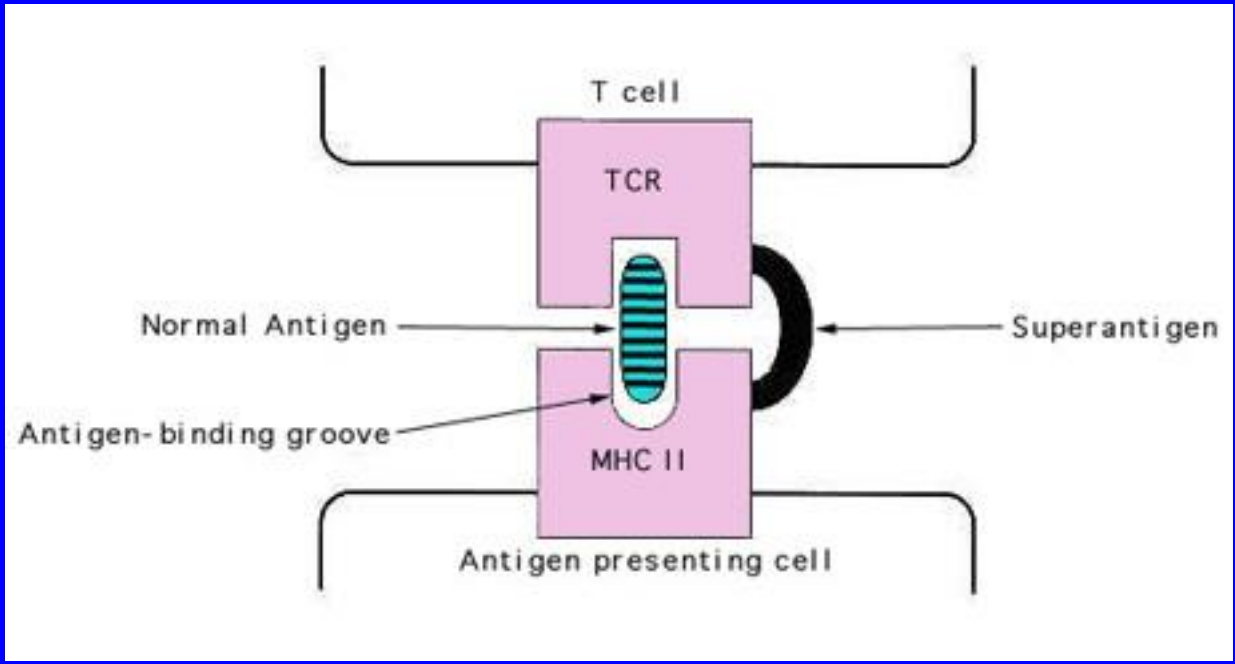
  ß-toxin is a sphingomyelinase which damages membranes rich in this lipid. The classical test for ß-toxin is lysis of sheep erythrocytes. The majority of human isolates of *S. aureus* do not express ß-toxin. A lysogenic bacteriophage is known to encode the toxin.

  delta toxin is a very small peptide toxin produced by most strains of *S. aureus*. It is also produced by *S. epidermidis*. The role of delta toxin in disease is unknown.

  Leukocidin is a multicomponent protein toxin produced as separate components which act together to damage membranes. Leukocidin forms a hetero-oligomeric transmembrane pore composed of four LukF and four LukS subunits, thereby forming an octameric pore in the affected membrane. Leukocidin is hemolytic, but less so than alpha hemolysin.

  Only 2% of all of *S. aureus* isolates express leukocidin, but nearly 90% of the strains isolated from severe dermonecrotic lesions express this toxin, which suggests that it is an important factor in necrotizing skin infections.

Superantigens and the non-specific stimulation of T cells. Superantigens bind directly to class II major histocompatibility complexes (MHC II) of antigen-presenting cells outside the normal antigen-binding groove. Up to one in five T cells may be activated. Cytokines are released in large amounts, causing the symptoms of toxic shock



DEEP ABSCESSSES

- Can be single or multiple
- Breast abscess can occur in 1-3% of nursing mothers in puerperiem
- Can produce mild to severe disease
- Other sites - kidney, brain from septic foci in blood

Systemic Infections

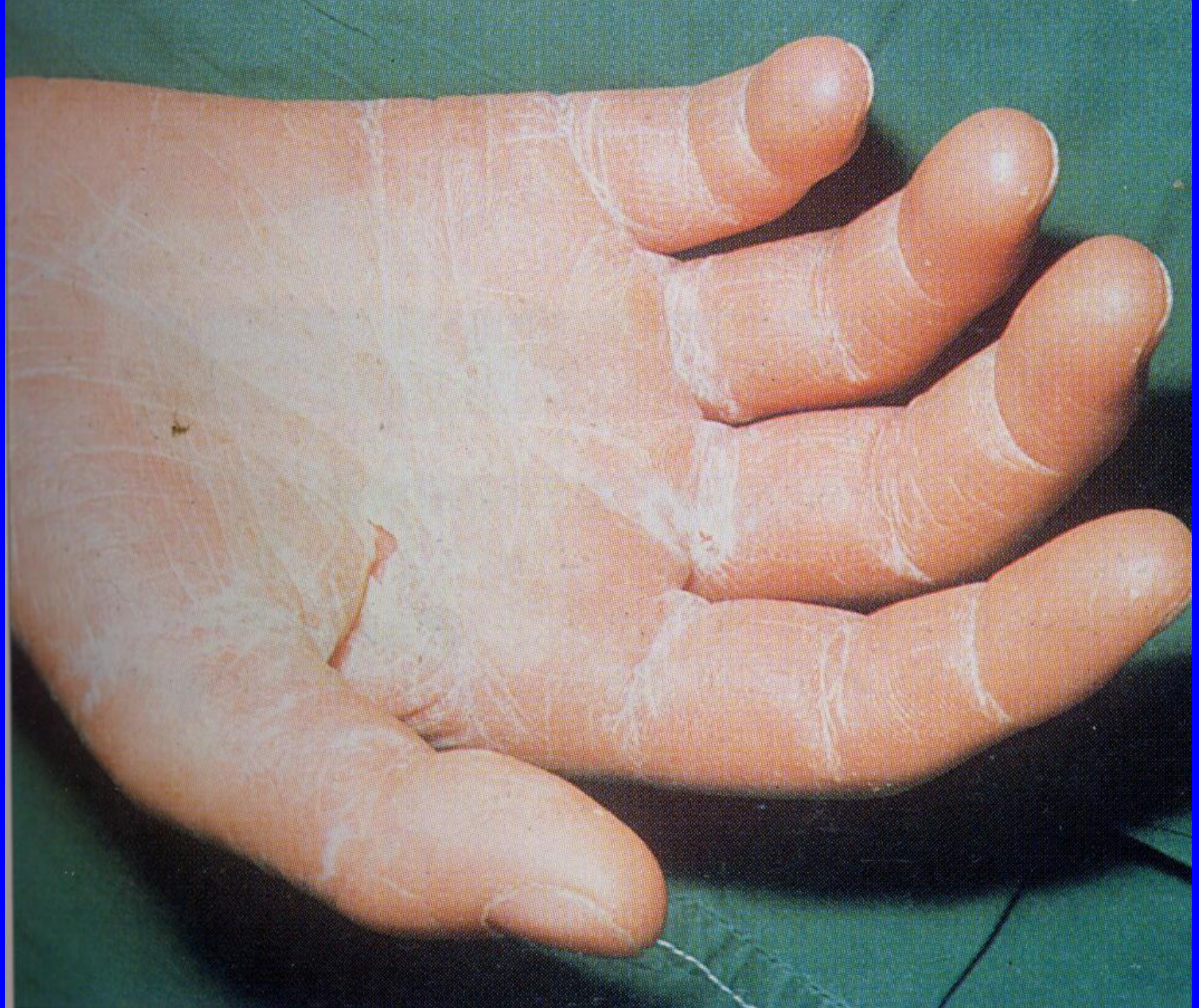
- 1. **With obvious focus**
  - **Osteomyelitis, septic arthritis**
- **2. No obvious focus**
  - **heart (infective endocarditis)**
  - **Brain(brain abscesses)**
- **3. Ass. With predisposing factors**
  - **multiple abscesses, septicaemia(IV drug users)**
  - **Staphylococcal pneumonia (Post viral)**

B. TOXIN MEDIATED DISEASES

- **1. Staphylococcal food poisoning**
  - **Due to production of entero toxins**
  - **heat stable entero toxin acts on gut**
  - **produces severe vomiting following a very short incubation period**
  - **Resolves on its own within about 24 hours**

2. Toxic shock syndrome

- **High fever, diarrhoea, shock and erythematous skin rash which desquamate**
- **Mediated via ‘toxic shock syndrome toxin’**
- **10% mortality rate**
- **Described in two groups of patients**
  - **ass. With young women using tampones during menstruation**
  - **Described in young children and men**



3. Scalded skin syndrome

- Disease of young children
- Mediated through minor Staphylococcal infection by ‘epidermolytic toxin’ producing strains
- Mild erythema and blistering of skin followed by shedding of sheets of epidermis
- Children are otherwise healthy and most eventually recover

Antibiotic sensitivity pattern

- Very variable and not predictable
- Very imp. In Pt. Management
- Mechanisms
  - 1.B lactamase production - plasmid mediated
    - Has made S. aureus resistant to penicillin group of antibiotics - 90% of S. aureus (Gp A)
    - B lactamase stable penicillins (cloxacillin, oxacillin, methicillin) used
  - 2. Alteration of penicillin binding proteins
    - (Chromosomal mediated)
    - Has made S. aureus resistant to B lactamase stable penicillins
    - 10-20% S. aureus Gp (B) GH Colombo/THP resistant to all Penicillins and Cephalasporins)
    - Vancomycin is the drug of choice

- **Tested in lab using methicillin**
- **Referred to as methicillin resistant S. aureus (MRSA)**
- **Emerging problem in the world**
- **In Sri Lanka prevalence varies from 20- 40% in hospitals**
- **Drug of choice - vancomycin**
- **In Japan emergence of VIRSA(vancomycin intermediate resistant S. aureus)**
- **No effective antibiotics discovered -We might have to discover**

Virulence Factors: Extracellular Enzymes

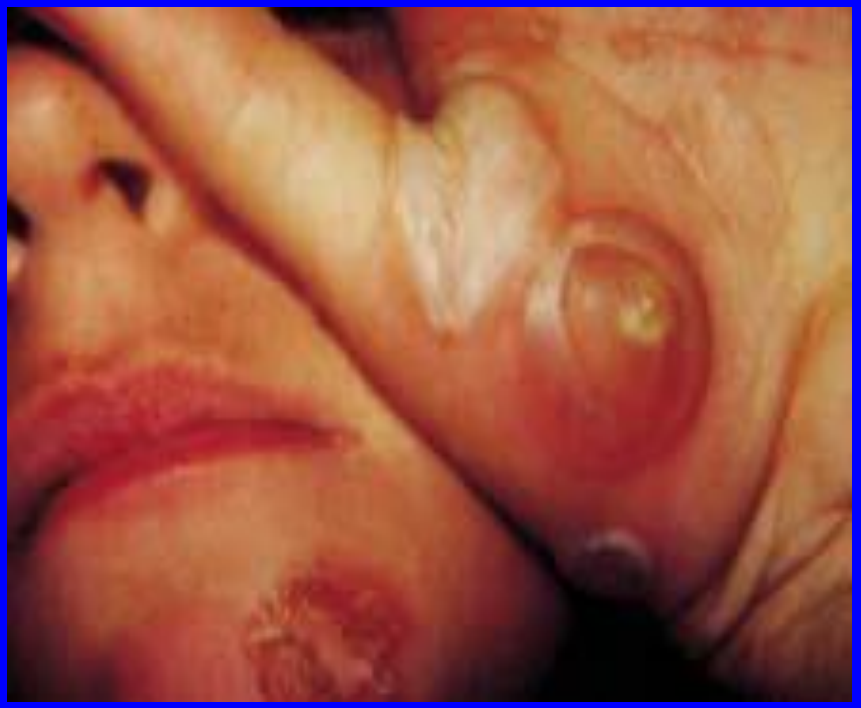
- Exfoliatin
  - **Epidermolytic toxin**
  - **Phage group II staphylococci**
  - **SSS or Ritters Disease**
- TSST-1: Toxic shock syndrome toxin-1
  - **Multisystem disease**
  - **High fever**
  - **Hypotension**
  - **Shock**

Virulence Factors: Extracellular Enzymes

- Hyaluronidase: connective tissue
- Staphylokinase: fibrinolysin
- Coagulase: virulence marker
- Lipase: allows colonization
- Penicillinase: confers resistance

Staphylococcus aureus: Clinical Infections

- Skin and wound
  - Impetigo
  - Furuncles
  - Carbuncles
  - Boils
  - Surgical wound infections
- Food poisoning
- Scalded skin syndrome



Bullous impetigo

Staphylococcus aureus: Clinical Infections

- Toxic shock syndrome
- Other infections
  - Respiratory (less often)
  - Bacteremia
  - Osteomyelitis

Coagulase-Negative Staphylococci

- Habitat: skin and mucous membranes
- Approximately 33 species
- Common human isolates
  - *S. epidermidis*
  - *S. saprophyticus*
  - *S. haemolyticus*

Coagulase-Negative Staphylococci: *Staphylococcus epidermidis*

- Habitat: skin and mucous membranes
- Cell wall: glycerol-teichoic acids
- Virulence factor: “slime”
- Mode of transmission: implantation of medical devices such as catheters, shunts, and prosthetic devices
- Infections are acquired nosocomially
- Serious infections among immunosuppressed patients may occur

Coagulase-Negative Staphylococci: *Staphylococcus saprophyticus*

- Habitat: skin and mucosal membranes of the genitourinary tract
- Common cause of urinary tract infections in young, sexually active females
- When present in urine cultures, may be found in low numbers, but significant

Other Gram-Positive Cocci

- Habitat: skin and mucous membranes
- Rarely implicated in infections
- *S. haemolyticus* associated with wound infections, bacteremia, and endocarditis
- *S. lugdunensis* and *S. schleiferi* are also found to be opportunists

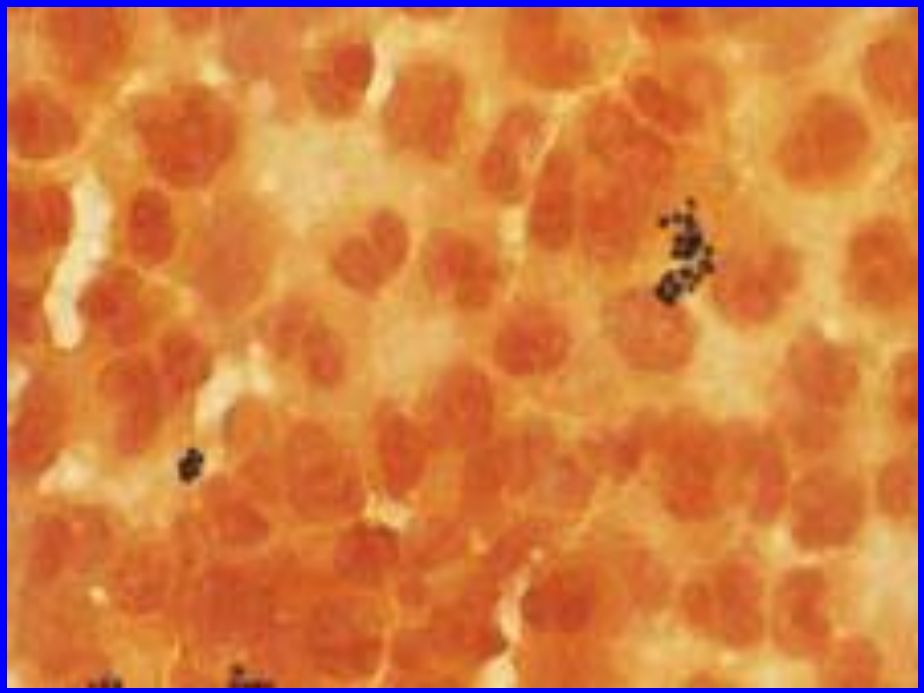
Laboratory Diagnosis: Specimen Collection and Handling

- Samples must be taken from the actual site of infection
- Prevent delay in transport of collected material from infected sites
- Transport in appropriate collection device that would prevent drying and minimize growth of contaminating organisms

Laboratory Diagnosis: Direct Smear Examination

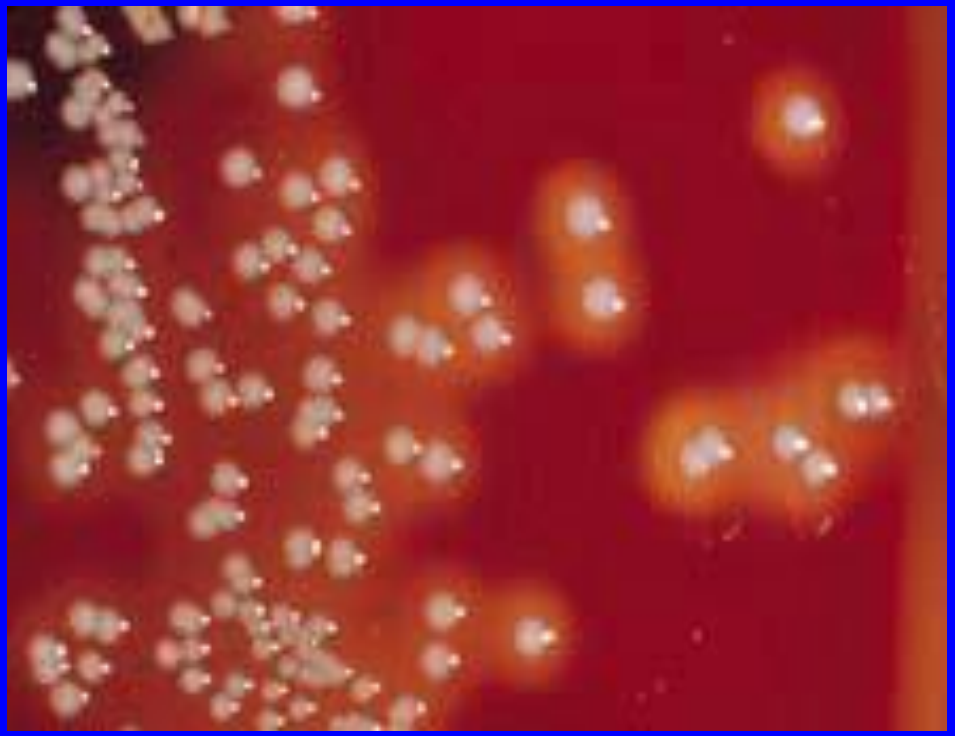
Microscopic Examination

- Gram-positive cocci
- pairs and clusters
- Numerous polymorphonuclear cells (PMNs)



Laboratory Diagnosis: Cultural Characteristics

- Colony morphology
  - Smooth, butyrous, white to yellow, creamy
  - *S. aureus* may produce hemolysis on blood agar



S. aureus

Laboratory Diagnosis: Cultural Characteristics

- Coagulase-negative staphylococci
  - Smooth, creamy, white
  - Small-to medium- sized, usually non-hemolytic
- *S. saprophyticus*
  - Smooth, creamy, may produce a yellow pigment

Identification Tests: Catalase

- Principle: tests for enzyme catalase

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

- Drop H_2O_2 onto smear
- Bubbling = POS (Most bacteria, O_2 generated)
- No bubbling = NEG (Streptococci and other lactic acid bacteria, no O_2 generated)

Identification Tests: Coagulase Test

- Detects enzyme coagulase
  - Cell-bound “clumping factor”
  - Extracellular enzyme “free coagulase”
- Two methods
  - Slide test
  - Tube test

Slide coagulase test detects clumping factor

DIAGNOSIS

- 1. In all pus forming lesions
  - Gram stain and culture of pus
- 2. In all systemic infections
  - Blood culture
- 3. In infections of other tissues
  - Culture of relevant tissue or exudate

2. Staphylococcus epidermidis

- Skin commensal
- Has predilection for plastic material
- Ass. With infection of IV lines, prosthetic heart valves, shunts
- Causes urinary tract infection in cathetarised patients
- Has variable ABS pattern
- Treatment should be aided with ABST

3. Stapylococcus saprophyticus

- **Skin commensal**
- **Imp. Cause of UTI in sexually active young women**
- **Usually sensitive to wide range of antibiotics**

Laboratory Diagnosis

Specimen: pus, sputum, blood, anterior nasal and perineal swabs, left-over food etc.

Smear: except for abscess material, gram stain of the smear is usually not informative.

Serology: antibodies against teichoic acid can be detected in patients with staphylococcal endocarditis, but not those with osteomyelitis or wound infection. Elevated antibody titers is an indication for prolonged antibiotic treatment.

Laboratory Diagnosis

Culture: blood agar plates. Use 7.5% NaCl to inhibit contaminants. Mannitol-salt agar can be used as a selective medium for *S. aureus*. Hemolysis and pigment production may not occur until several days later and are optimal at room temperature.

Identification: catalase test; coagulase test. Fluorescent in situ hybridization (FISH) with a *S. aureus*-specific DNA probe can be used for identification of this organism in clinical specimens.

Various subtyping methods (such as pulsed-field gel electrophoresis) are used for epidemiological purpose.

Identification Tests: Coagulase Test

- Detects enzyme coagulase
  - Cell-bound “clumping factor”
  - Extracellular enzyme “free coagulase”
- Two methods
  - Slide test
  - Tube test

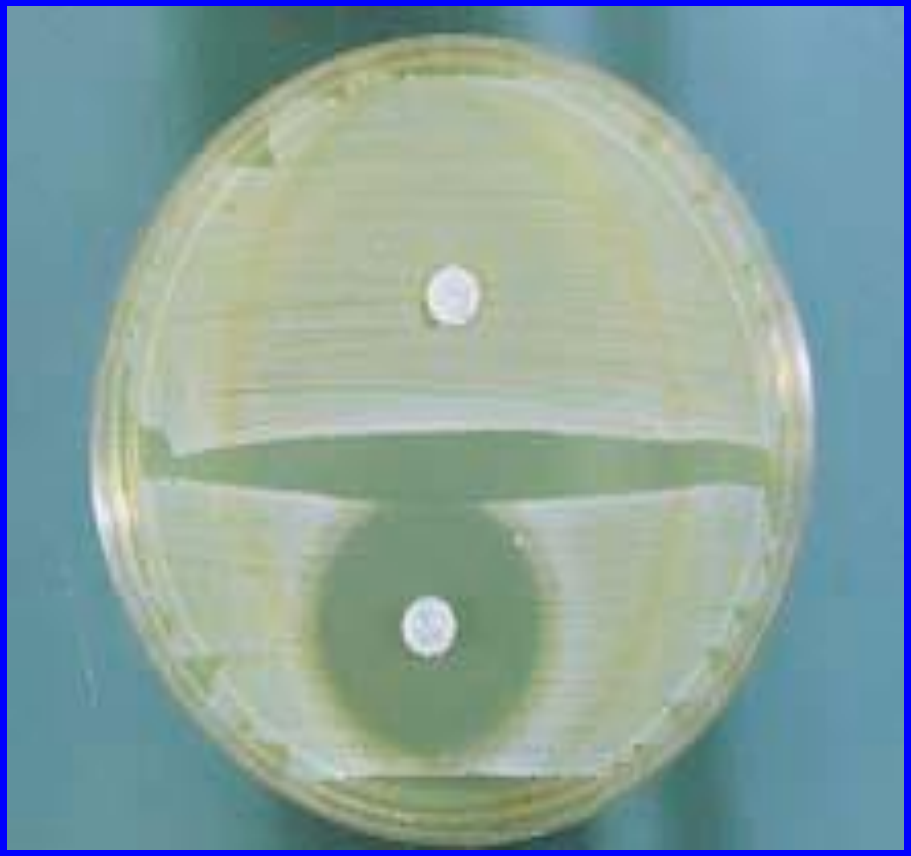
Slide coagulase test detects clumping factor

Identification Tests: Coagulase Test

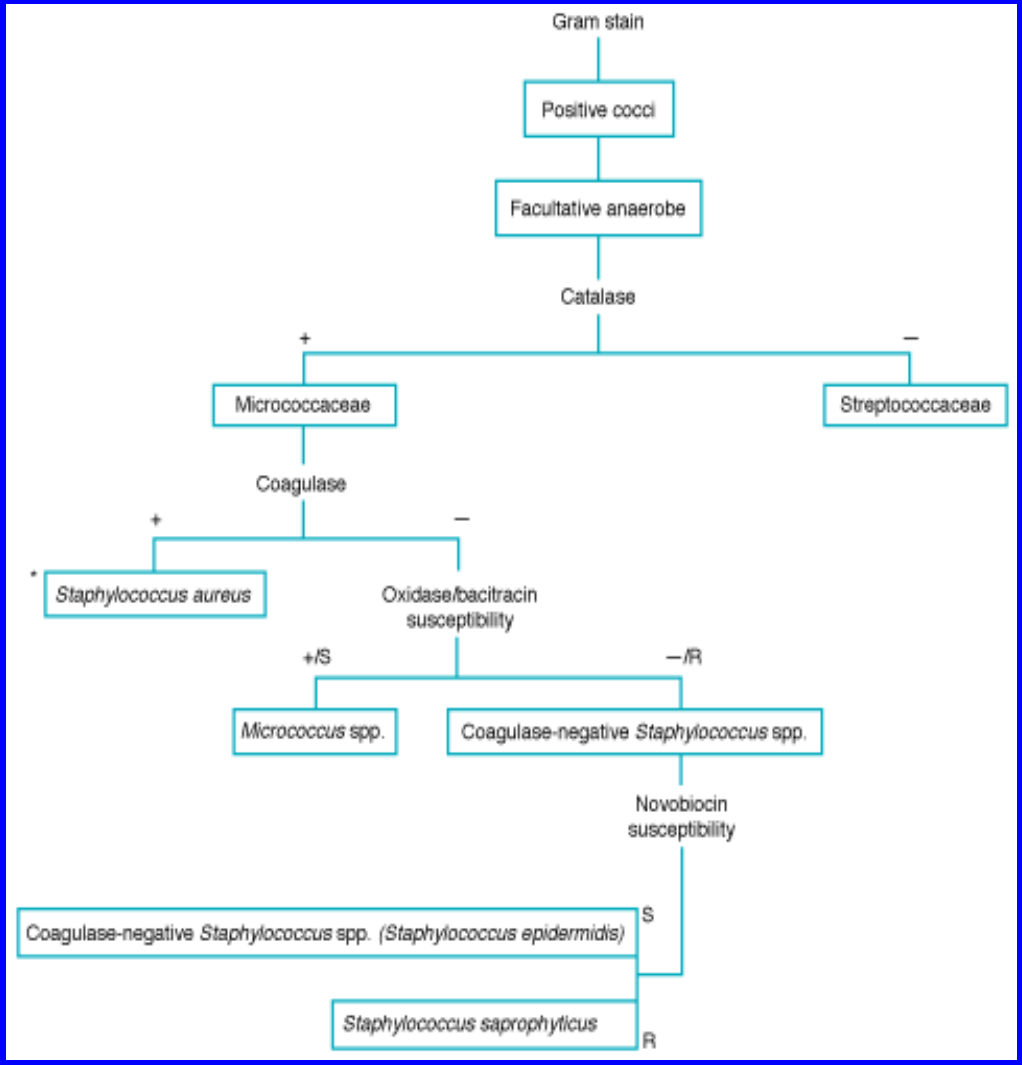
Tube test detects the extracellular enzyme "free coagulase"

Novobiocin Susceptibility Test

- Test to differentiate coagulase-negative staphylococci from *S.saprophyticus* from urine samples
  - *S. saprophyticus* is resistant (top)
  - Other CNS are susceptible



Schematic Diagram for Identifying Staphylococcal Species



Antimicrobial Susceptibility

- For non–beta-lactamase producing *S. aureus* (methicillin-susceptible)
  - Penicillinase-resistant synthetic penicillins (methicillin, nafcillin, oxacillin, dicloxacillin)
- For methicillin -resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis (MRSE)*
  - Vancomycin combined with rifampin or gentamicin
- Emergence of vancomycin resistance