Notes

14

STAPHYLOCCOUS

14.1 INRODUCTION

Staphylococci are gram positive cocci that occur in groups in cluster. They are ubiquitous and most common cause of localized lesions in human beings. They develop resistance to pencillin and other antibiotics

OBJECTIVES

After reading this lesson, you will be able to:

- classify staphylococcus
- describe the morphology of staphylococcus
- discuss the characteristics of staphylococcus
- describe the laboratory diagnosis of staphylococcus

Staphylocci was first observed in human by Von Recklinghausen. Sir Alexander Oysten established the causative role of coccus in abscesses and other lesions. He named in staphylococcus which means, staphylo – bunches of grapes, kokkos means a berry because of the grape like clusters in cultures. Staphylococcae strains from pyogenic lesions produce yellow colonies and white colonies from normal skin.

Classification

1. *Staphylococcus aureus* – gives positive coagulase-test, ferments mannitol and mostly pathogenic

2. *Staphylococcus epidermidis* contains coagulase negative non ferments with mannitol and mostly nonpathogenic

14.2 STAPHYLOCOCCUS AUREUS

A. Morphology

They are spherical in shape which are approximately 1µm in diameter arranged in grape like clusters. These are non-motile and non-sporing. They are uniformly Gram Positive

Fig. 14.1

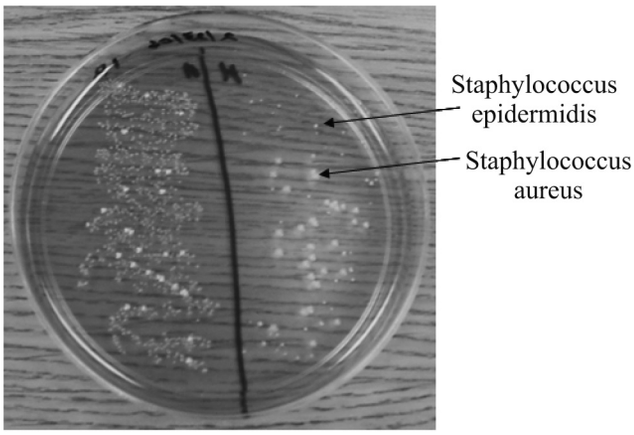


Fig. 14.2

B. Cultural characteristics

They grow readily on ordinary media with temperature ranging from 10-42°C, optimum being 37°C with pH of 7.4 – 7.6 and they are aerobes

On nutrient agar, the colonies are large (2-4 diameter) circular, convex, smooth, opaque and easily emulsifiable. Most strains produce pigment optimally at 22°C and in aerobic cultures which is enhanced by adding 1% glycerol monacetate or milk in the medium. Colonies on blood agar are similar to that of nutrient agent.

Several selective media containing (8-10% NaCl) like salt-milk agar, salt broth, Lithium chloride and tellurite helps in isolating S.aeures from specimen of faeces

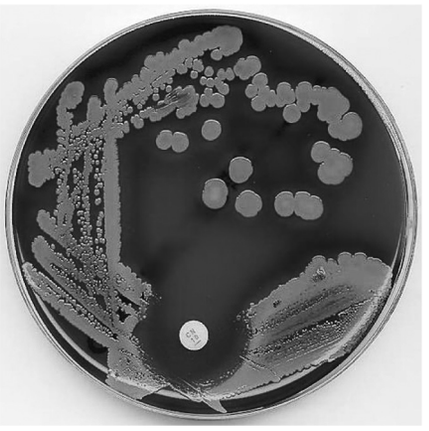


Fig. 14.3

C. Biochemical reactions

They ferment many sugars producing acid but not gases. S.auere ferments mannitol mostly. They are Catalast positive, reduces nitrates to nitrites

Characteristics
Coagulase positive
Greater biochemical activity, ferment mannitol
Produce clear hemolysis on blood agar
Produce a golden yellow pigment
Liquefy gelatin
Produce phosphatase

D. Resistance

They are more resistant nonsporing bacteria. They retain their viability for 3-6 months. Staphylococci may withstand 60^{o}c for minutes, with thermal death point

of 62°C for 30 minutes. Heat resistant strains may grow even at high temperatures as 45°C. Most strains grow in the presence of 10% NaCl and some even in 15% NaCl

Staphylococci were uniformly sensitive to penicillin and some strains produce pencillinase. Pencillinase resistant are of three types namely

- Produce beta lactamase (pencillinase) which inactivates penicillin by splitting the beta lactam ring. Staphylococci produce four types of pencilinases A to D & hospital stains are usually type A pencillinase
- Changes the bacterial surface receptors reducing binding of beta lactam antibiotics to cells. This also covers beta lactamase resistant pencillins such as Methicillin and Cloxacillin. They are called Methicillin Resistant Staphylococcus Aureus (MRSA). As methicillin is a unstable drug cloxacillin is used for sensitivity testing
- Development of tolerance to pencillin, by which the bacterium is only inhibited but not killed
- Staphylococci shows resistances to all clinical useful antibiotics like erythromycin, tetracycline, aminoglycosides and hence vancomycin is found useful

INTEXT QUESTIONS 14.1

1. Staphylococci are gram ..... cocci
2. Staphylococci are facultative .....
3. Staphylococci occur in .....
4. Staphylococci produce ..... colour colonies in pyogenic lesions
5. Staphylococcus aureus are coagulase ..... and ..... mannitol
6. Staphylococcus epidermidis are coagulase ..... and ..... mannitol

14.3 PATHOGENICITY AND VIRULENCE

Staphylococci produce two types of disease infections and intoxication

The virulence factors include

(i) Cell associated polymers – cell wall polysaccharide offeres rigidity and structural integrity to bacterial cell

(ii) Cell surface proteins

Protein A present on S.aureus strains induces platelet damage and hypersensitivity. Protein A binds to Fc terminal of IgG molecule, leaving fab

Notes

region free to combine with its specific antigen. Protein A bearing staphylococci coated with any IgG antiserum will be agglutinated if mixed with its corresponding antigen. This is known as coagulation.

Clumping factor

Surface protein, bound coagulase is responsible for slide coagulase test. When a saline suspension of S.aureus is mixed on a slide with a drop of human plasma the cocci are clumped. Slide coagulase test is routinely used for identification of S. aureus

(iii) Extracellular enzymes

Lipases-lipd hydrolases helps S.aureus infect the skin and subcutenous tissues. Hyaluronidase breaks down the connective tissue. Staphylokinase helps in initiating and spread of infection.

Nuclease a heat stable nuclease is a characteristic feature of *Staphylococcus aureus*

Protein receptors, Staphylococci possess receptors for many mammalian proteins such as fibronectin, fibrinogen, IgG and C1q. these facilitate staphylococcal adhesion to host cells and tissues.

(iv) Toxins

Cytolytic toxins are membrane active substance consisting of heamolysin namely Alpha hemolysin, Beta, Gamma, and Delta & Leucocidin.

Enterotoxin

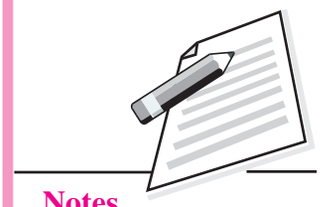
This is responsible for manifestations of Staphlococcal food poisoning like nausea, vomiting and diarrhea. The toxin is heat stable resisting at 100^{o}c for 10-40 minutes. Nearly 2/3 strains frowing in carbohydrate & protein secrete toxins. Meat, fish, milk and milk products are common items of source of infection. The source of infection is usually food handlers who are carriers. The illness is usually self limiting.

Toxic Shock syndrome Toxin (TSST)

Toxic Shock syndrome Toxin is a positively fatal multisystem disease presented with fever, hypotension, myalgia, vomiting, diarrhea, mucosal hyperemia and an erythematous rash.

Exfoliative (epidemolytic) toxin

This causes staphylococcal scalded skin syndrome (SSSS), a exfoliative skin disease in which the outer layer of the epidermis gets separated from the



underlying tissues. The severe form of the disease is known as Ritter's disease in the newborn and toxic epidermal necrolysis in older patients. Milder forms are pemphigus neonatorum and bullous impetigo.

INTEXT QUESTIONS 14.2

1. Virulence factors of staphylococci are ....., ..., ... & .....
2. ..... causes staphylococcal food poisoning
3. ..... toxin causes staphylococcal scalded skin syndrome
4. Severe form of staphylococcal scalded skin syndrome is ..... in children

14.4 STAPHYLOCOCCAL DISEASE

Staphylococcal infections are among the most common of bacterial infections and range from the trivial to the fatal. They are characteristically localized pyogenic lesions, in contrast to the spreading nature of streptococcal infection. Common staphylococcal infections are as follows

Region	Infections
Skin and soft tissue	Folliculitis furuncle(boil), abscess, wound infection, carbuncle, impetigo, paronychia
Muscloskeletal	Osteomyelitis, arthritis, bursitis, pyomyositis
Respiratory	Tonsillitis, pyaryngitis, sinusitis, otitis, bronchopneumonia, lung abcess, empyema, rarely pneumonia
Central nervous system	Abscess, meningitis, intracranial thrombophlebitis
Endovascular	Bacteremia, septicemia, pyemia, endocarditis
Urinary	Instrumentation, implants and bacteria related Bacteremia

Bacteriophage typing

Staphylococci may be typed, based on their susceptibility to bacteriophages and the typing is done in pattern method. The strain is inoculated on a plate of nutrient agar to form a lawn culture. After drying, the phages are applied over marked squares in a fixed dose. After overnight incubation, the culture will be observed to be lysed by some phages but not by others. The phage type of the

Notes

strain is expressed by the designations of all the phages that lyse it. Phage typing is of great importance in epidemiological studies of staphylococcal infections.

Laboratory Diagnosis

Specimen Collection

The specimens to be collected depend on the type of lesion, like pus from suppurative lesions, sputum from respiratory infection. In case of food poisoning, feces and the remains of suspected food should be collected. For detection of carriers, nasal swab is the usual specimen. Swabs from perineum, pieces of hair and umbilical stump are taken.

Direct Microscopy

Direct microscopy with Gram stained smears is useful in the case of pus, where cocci in clusters may seen. Diagnosis may be readily made by culture. The specimens are plated on blood agar. Staphylococcal colonies appear after overnight incubation. Specimens where staphylococci are expected to be scanty and outnumbered by other bacteria, selective media like Ludlams or salt-milk agar or Robertson's cooked meat medium containing 10 percent sodium chloride may be used for inoculation. Smears are examined and coagulase test done when staphylococci are isolated

Biochemical Test

The coagulase test can be done using two methods, tube and slide. The tube coagulase test detects free coagulase. About 0.1ml of a young broth culture or agar culture suspension of the isolate is added to about 0.5ml of human or rabbit plasma in a narrow test tube. EDTA, oxalate or heparin may be used as the anticoagulant for preparing the plasma. The tubes are incubated in water bath at 37^{o}c for 3- 6 hours. If positive, the plasma clots and does not flow when the tube is tilted.

The slide test detecting bound coagulase is much simpler and usually gives results parallel with the tube test. When there is divergence, the tube test will be the deciding factor. For the slide test, the isolate is emulsified in a drop of saline on a slide. After checking for absence of autoagglitination, a drop of human or rabbit plasma is added and mixed. Prompt clumping of the cocci indicated a positive test. Positive and negative controls also are set up. Antibiotic sensitivity tests should be performed as a guide to treatment.

Coagulase Negative Staphylococci

Coagulase negative staphylococci constitute a major component of the normal flora of the human body, whereas some like staph epidermidis, staph haemolyticus and staph saprophyticus cause disease. Staph epidermidis is a normal flora of the

skin but may cause disease when the host defences are compromised. It commonly causes stitch abcesses, and may grow on foreign bodies such as artificial heart valves, intravascular catheters and prosthetic appliances causing bacteremia.

Staph saprophyticus is also a normal flora present on normal skin and periurethral area and can cause urinary tract infection in sexually active young women.

Characteristics	Staph aureus	Staph epidermidis	Staph saprophyticus
Coagulase	+	-	-
Novobiocin sensitivity	S	S	R
Acid from mannitol Anaerobically	+	-	-
Phosphatase	+	+	-
S – Sensitive R – Resistant			

INTEXT QUESTIONS 14.3

1. ..... typing is of great importance in epidemiological studies of staphylococcal infections
2. ..... medium is used for inoculation of staphylococcal infections
3. ..... coagulose test detects free coagulase
4. ..... coagualose test detects bound coagulase

WHAT YOU HAVE LEARNT

- Staphylococcus are spherical shaped, nonmotile, and facultative anaerobes
- Positive to catalase test by Gram stain,
- Coagulase positive are staphylococcus aureus and coagulase negative staphylococcus saprophyticus, staphylococcus epidermidis.
- Staphylococci are susceptible to penicillinase resistant penicillins such as methicillin and cloxacillin and to aminoglycosides and macrolides. Methicillin resistant staphylococcus aureus cause nosocomial infections.

TERMINAL QUESTIONS

1. Describe the morphological characteristics of Staphylococcus
2. Discuss the laboratory diagnosis of Staphylococcus
3. Explain the pathogenecity of Staphylococcus
4. Describe Methicillin Resistant Staphylococcus Aureus

ANSWERS TO INTEXT QUESTION

14.1

1. Positive
2. Anaerobes
3. Clusters
4. Yellow
5. Positive & ferments
6. Negative & non-ferments

14.2

1. Cell polymers, cell surface protein, toxins & extracellular enzymes
2. Enterotoxin
3. Exfoliative
4. Ritter's disease

14.3

1. Phage
2. Robertson's cooked meat
3. Tube
4. Slide