Part 3. Bacterial Infections

SECTION OUTLINE

1. Gram-positive and Gram-negative Cocci Infections: *Staphylococcus*, *Streptococcus*, Pneumococcus, *Enterococcus* and *Neisseria*

2. Gram-positive Bacilli Infections: *Corynebacterium* and *Bacillus*

3. Anaerobic Infections

4. Mycobacteria Infections

5. Gram-negative Bacilli Infections-I: Enterobacterales and *Vibrio*

6. Gram-negative Bacilli Infections-II: Nonfermenters, Fastidious and Others

7. Miscellaneous Bacterial Infections

ANTIBIOTIC RESISTANCE

HANDLE ANTIBIOTICS WITH CARE

Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause. This is compromising our ability to treat infectious diseases and undermining many advances in medicine.

We must handle antibiotics with care so they remain effective for as long as possible.

WHAT HEALTH WORKERS CAN DO

1. **Prevent infections** by ensuring your hands, instruments and environment are clean
2. Keep your patients' **vaccinations** up to date
3. If you think a patient might need antibiotics, where possible, **test to confirm** and find out which one
4. Only prescribe and dispense antibiotics when they are **truly needed**
5. Prescribe and dispense the **right antibiotic at the right dose for the right duration**

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#AntibioticResistance

World Health Organization

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Chapter 12. Gram-positive and Gram-negative Cocci Infections

CHAPTER PREVIEW

Gram-positive cocci

- *Staphylococcus*
- *Streptococcus*
- *Enterococcus*

Gram-negative cocci

- Meningococcus
- Gonococcus

GRAM-POSITIVE COCCI INFECTIONS

Gram-positive cocci that are of human importance include:

- *Staphylococcus*: *S. aureus* and coagulase-negative staphylococci (CoNS)
- *Streptococcus*: #-hemolytic streptococci, Viridans streptococci, *S. pneumoniae* (pneumococcus)
- *Enterococcus*: *E. faecalis* and *E. faecium.*

The gram-positive cocci infecting man can be differentiated from each other by various laboratory findings *(Table 12.1)*.

STAPHYLOCOCCUS

Staphylococci can be classified into *S. aureus*, the most pathogenic species to man and other species called coagulase-negative staphylococci (CoNS) that are less pathogenic to man.

Staphylococcus aureus

Staphylococcus aureus is the most common gram-positive cocci infecting humans. It can cause both community and nosocomial acquired infections that may range from relatively milder skin and soft tissue infections to life-threatening systemic infections.

Virulence Factors

The pathogenic potential of *S. aureus* is due to the expression of several virulence factors which include:

- **Toxins** such as hemolysins, exfoliative toxin, enterotoxin, and toxic shock syndrome toxin
- **Extracellular enzymes** such as coagulase, heat-stable thermonuclease, staphylokinase, hyaluronidase, etc.
- **Cell wall-associated factors** such as peptidoglycan layer, teichoic acid, clumping factor, and protein A.

Clinical Manifestations

The clinical spectrum of *S. aureus* includes *(Figs. 12.1A and B)*:

- Skin and soft tissue infections such as folliculitis, furuncle, cellulitis, abscess, impetigo, etc.
- Musculoskeletal infections such as osteomyelitis, septic arthritis, and pyomyositis
- Respiratory tract infections such as pneumonia
- Bacteremia, sepsis, and infective endocarditis
- Urinary tract infections (UTI).

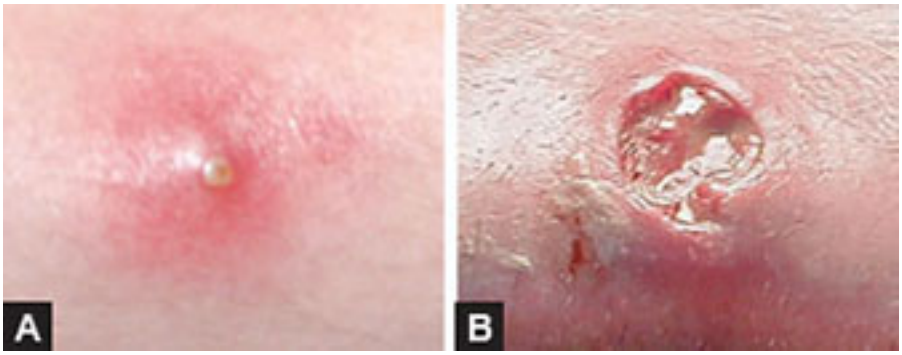
Toxin-mediated infections: *S. aureus* can also cause several toxin-mediated diseases such as:

Table 12.1. Differentiating features of laboratory findings of various gram-positive cocci infecting man.

Organism	***Catalase***	***Gram-positive cocci***	***Culture finding on blood agar***	***Other tests***
S. aureus	Positive	Arranged in clusters	Pinhead-shaped colonies with a narrow zone of complete (β) hemolysis and golden yellow pigmentation	Coagulase test: positive
CoNS	Positive	Arranged in clusters	Pinhead-shaped colonies without hemolysis and no pigmentation	Coagulase test: negative
#-hemolytic streptococci	Negative	Arranged in short chains	Pinpoint colonies with a wide zone of complete (β) hemolysis	*S. pyogenes:* Bacitracin (S), CAMP test negative *S. agalactiae:* Bacitracin (R), CAMP test positive
Viridans streptococci	Negative	Arranged in long chains	Minute #-hemolytic (green-colored) colonies	Bile solubility test: negative Inulin: not fermented Optochin: resistant
S. pneumoniae	Negative	Arranged in pairs, lanceolate-shaped	Draughtsman-shaped or carom coin-shaped	Bile solubility test: positive

Organism	*Catalase*	*Gram-positive cocci*	*Culture finding on blood agar*	*Other tests*
			colonies with partial (α) hemolysis	Inulin: fermented Optochin: sensitive
Enterococcus	Negative	Arranged in pairs, spectacle eye-shaped	Small, translucent, non-hemolytic colonies	Bile esculin test: positive *E. faecalis*: Arabinose not fermented *E. faecium*: Arabinose fermented
Abbreviation: CoNS, coagulase-negative staphylococci; S, sensitive; R, resistant; CAMP test, Christie–Atkins–Munch-Peterson test.				

Figs. 12.1A and B. *A.* Staphylococcal folliculitis; *B.* Staphylococcal abscess (ruptured).



Source: Centers for Disease Control and Prevention (CDC), Atlanta *(with permission)*.

- **Scalded skin syndrome:** Mediated by exfoliative toxin (or epidermolytic toxin), characterized by localized tender blisters and bullae formation and exfoliation of the skin
- **Food poisoning:** Mediated by enterotoxin. It is a *preformed toxin* (i.e. secreted in food before consumption) so that it can act rapidly. As a result, the *incubation period is short* (1–6 hours)
- **Toxic shock syndrome:** Mediated by toxic shock syndrome toxin (TSS)
  - It is common among women using vaginal tampons during menstruation
  - The toxin is absorbed into circulation to cause a potentially fatal multisystem disease with erythematous rashes.

HAI: Overall, *S. aureus* is a leading cause of healthcare-associated infections (HAI). The hospital staff are the potential carriers of *S. aureus*. Hospital strains are often multidrug-resistant, spread to patients either from hospital staff/other patients/environment or also from patients' skin flora.

Laboratory Diagnosis

The various specimens collected depend on the nature of the lesion such as pus, wound swab, sputum, midstream urine, and blood.

- **Direct smear microscopy:** Reveals gram-positive cocci in clusters and pus cells *(Fig. 12.2A)*

- **Culture:** Incubation at 37°C for 24h reveals the following growth:
  - Nutrient agar—produces golden yellow-pigmented colonies
  - Blood agar—shows pin-head colonies with a narrow zone of complete (β) hemolysis *(Fig. 12.3)*
  - Selective media such as mannitol salt agar—produces yellow colonies.
- **Culture smear microscopy** from the colonies reveals gram-positive cocci in clusters *(Fig. 12.2B)*
- **Biochemical identification:** Various tests which help in the identification of *S. aureus* are:
  - Catalase test—positive (differentiates staphylococci from streptococci)
  - Coagulase test—positive (differentiates *S. aureus* from CoNS). Two test formats are available: slide coagulase and tube coagulase tests *(Figs. 12.4A to C)*
  - Protein A detection (differentiates *S. aureus* from CoNS).
- **Automated identification systems** such as VITEK and MALDI-TOF can be performed for rapid and accurate identification of *S. aureus*
- **Antimicrobial susceptibility testing** can be performed by disk diffusion method (on Mueller–Hinton agar) or MIC-based method (VITEK).

TREATMENT

S. aureus

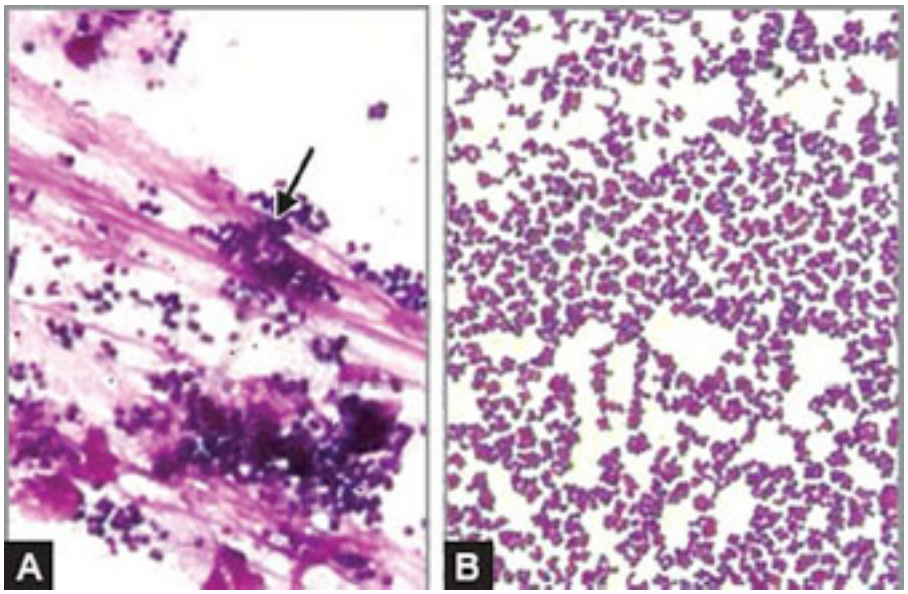
S. aureus is primarily treated by antistaphylococcal penicillins such as cloxacillin.

However, for MRSA infections (see below), vancomycin is the drug of choice. Others include clindamycin, doxycycline, co-trimoxazole, or linezolid, etc.

MRSA

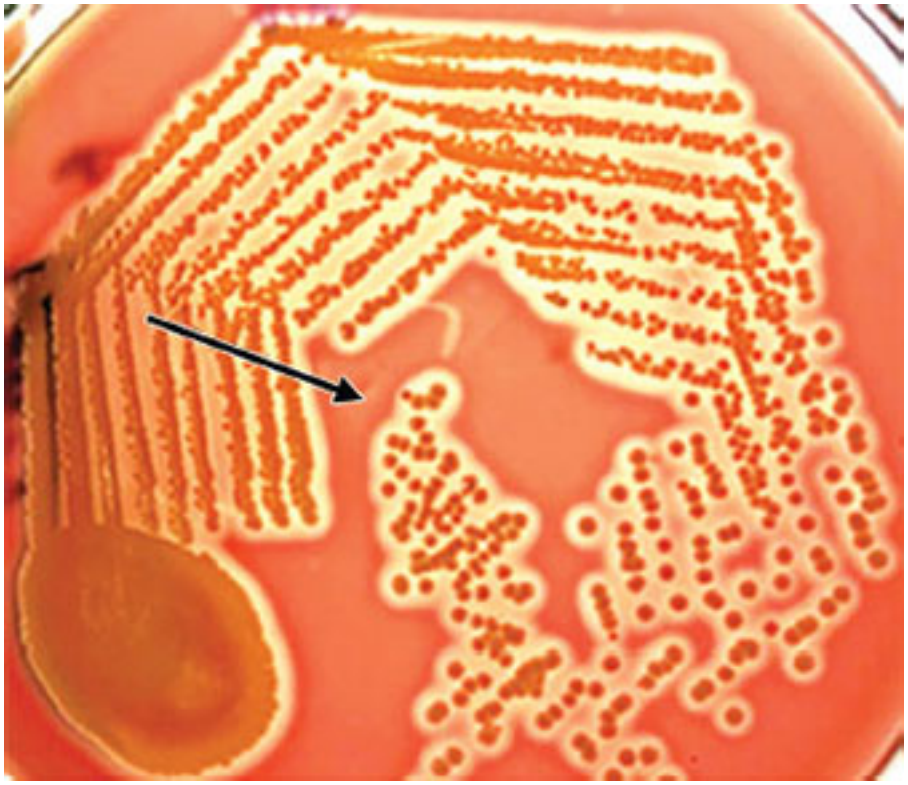
Methicillin-resistant *S. aureus* (MRSA) is a resistant phenotype, that has been increasingly reported over the last few decades. It shows resistance to all #-lactam antimicrobials and thus possesses a great therapeutic challenge. It is widespread in hospital settings, causing several outbreaks.

Figs. 12.2A and B. *A.* Direct smear: arrow showing gram-positive cocci in clusters with pus cells; *B.* Culture smear showing gram-positive cocci in clusters.



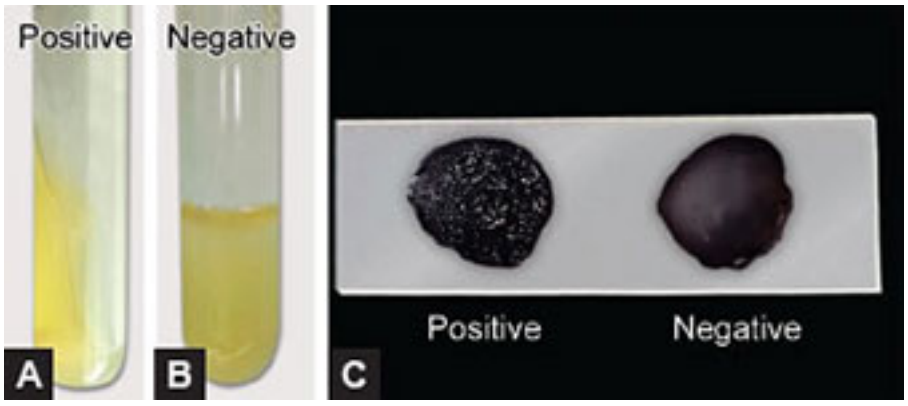
Source: Department of Microbiology, JIPMER, Puducherry *(with permission)*.

Fig. 12.3. Colonies of *S. aureus:* Blood agar—arrow shows a narrow zone of beta-hemolysis surrounding the colonies.



Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry *(with permission)*.

Figs. 12.4A to C. Coagulase test: *A.* Tube coagulase test (positive); *B.* Tube coagulase test (negative); *C.* Slide showing coagulase test.



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- **Mechanism:** Mediated by a gene called *mecA gene,* which alters penicillin-binding protein (PBP) present on *S. aureus* cell wall to PBP2a.

  # PBP is an essential protein needed for the cell wall synthesis of bacteria. #-lactam drugs bind and inhibit this protein, thereby inhibiting the cell wall synthesis

  # The altered PBP2a of MRSA strains have less affinity for #-lactam antibiotics; hence, MRSA strains are resistant to all #-lactam antibiotics
- # **Epidemiology:** MRSA rate is very high, accounting for 30-40% of *S. aureus* infections in India
- # **Detection:** By performing a susceptibility test for oxacillin or cefoxitin
- # **Treatment:** Vancomycin or linezolid are recommended for serious infections, whereas doxycycline or cotrimoxazole can be given for nonlife-threatening infections.

Control Measures

Prevention of spread of *S. aureus* infections in hospitals involves:

- Ensure proper *infection control measures* such as hand hygiene (most efficient way to prevent hospital spread), isolation of the patients and all other measures of *contact precautions* (described in detail in Chapter 38)
  - **Screening of MRSA carriers** among hospital staff should be done when there is an outbreak
  - **Treatment of carriers** is done by use of topical 2% mupirocin (for nasal carriers) and chlorhexidine body bath (for skin carriers)
  - **Stoppage of antibiotic misuse** in hospitals.

Coagulase-negative Staphylococci (CoNS)

Other species of *Staphylococcus* do not produce coagulase enzyme and are called as coagulase-negative staphylococci (CoNS).

- They are usually harmless skin commensals and rarely pathogenic to man
- They are less virulent than *S. aureus* and may cause infections in immunocompromised patients, infections in prosthetic devices associated, and surgical site infections
- *S. epidermidis* is the most common CoNS infecting man
- Others include—*S. saprophyticus, S. lugdunensis, S. schleiferi,* and *S. haemolyticus*.

STREPTOCOCCUS

Streptococci can be classified based on the pattern of hemolysis they produce on blood agar.

- α *or partial hemolysis*: Greenish discoloration surrounding the colonies; e.g. Viridans streptococci and *S. pneumoniae*
- β **or complete hemolysis:** Yellowish discoloration surrounding the colonies; e.g. #-hemolytic streptococci such as *S. pyogenes* and *S. agalactiae*
- γ **hemolysis:** No hemolysis surrounding the colonies; e.g. *Enterococcus* (now it is separated from the genus *Streptococcus)*.

Lancefield grouping: The #-hemolytic streptococci are further classified based on the C-carbohydrate antigen in the cell wall into 20 serological groups. The majority of streptococci causing human infection include—group A streptococci *(S. pyogenes)* and group B streptococci *(S. agalactiae)*.

Streptococcus pyogenes

Streptococcus pyogenes (group A *Streptococcus)* is one of the leading cause of pyogenic infections in humans.

Virulence Factors

The virulence factors of *S. pyogenes* include:

- Cell wall antigens such as C-carbohydrate antigens, M protein, capsule, etc.
- Toxins such as streptococcal pyrogenic exotoxin and hemolysins
- Various enzymes such as streptokinase, streptodornase, hyaluronidase, etc.

Clinical Manifestations

Streptococcus pyogenes is associated with a variety of suppurative and non-suppurative manifestations.

- **Suppurative manifestations** include:
  - Sore throat (pharyngitis): *S. pyogenes* is the most common bacterial cause of pharyngitis in children
  - Superficial skin and soft-tissue infections: Such as impetigo, cellulitis, and erysipelas
  - Necrotizing fasciitis: Involves extensive necrosis of subcutaneous tissue, fascia, and muscles
  - Bacteremia

- Toxic shock syndrome (mediated by streptococcal pyrogenic exotoxin)
- Puerperal sepsis, following vaginal delivery.

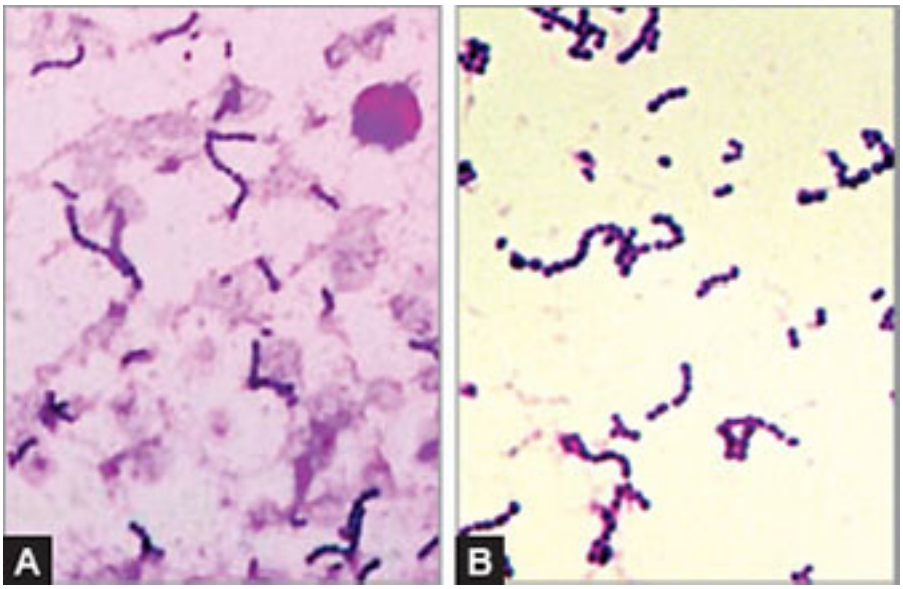
- **Non-suppurative manifestations** are acute rheumatic fever (affecting the heart) and post-streptococcal glomerulonephritis (affecting the kidney). The underlying pathogenesis is due to *molecular mimicry*; where, the antibodies produced against previous streptococcal infections cross-react with human tissues (heart or kidneys) to produce lesions.

Laboratory Diagnosis

The specimen to be collected depends on the site of the infection. Common specimens are pus, throat swab, blood, etc.

- **Direct smear microscopy:** Reveals pus cells with gram-positive cocci in short chains *(Fig. 12.5A)*

Figs. 12.5A and B. Streptococci: *A.* In Gram-stained smear of pus; *B.* In culture smear showing gram-positive cocci in short chains.



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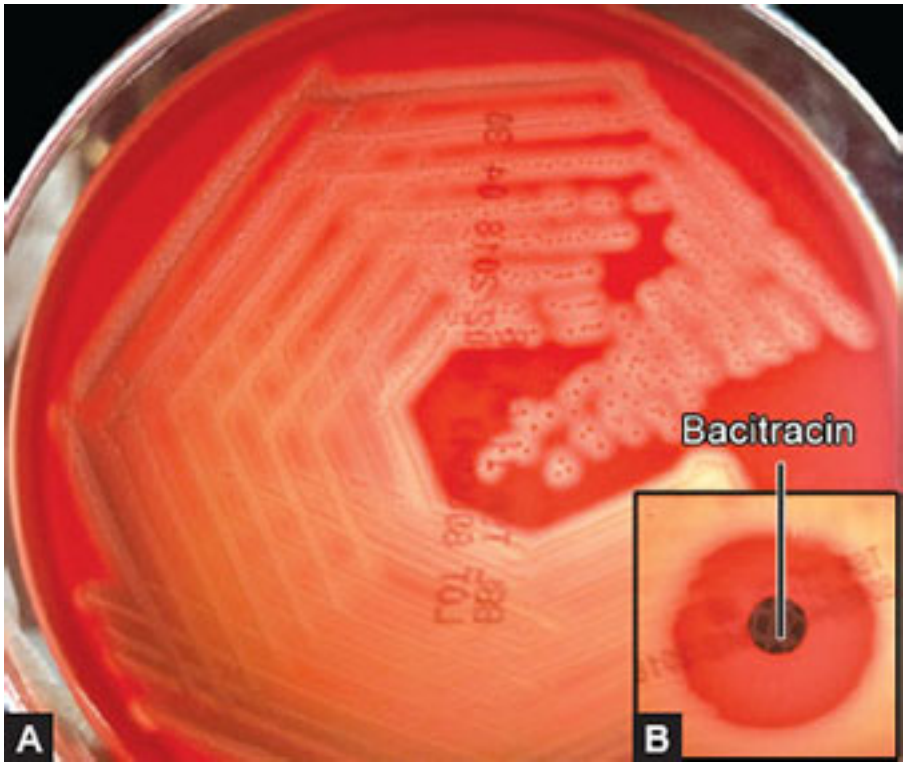
Figs. 12.6A and B. *Streptococcus pyogenes: A.* Growth on blood agar with a wide zone of beta-hemolysis around the pinpoint colonies; *B.* Bacitracin sensitive.



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- **Culture** on blood agar reveals pinpoint colonies with a wide zone of #-hemolysis *(Fig. 12.6A)*
- **Culture smear** microscopy from the colonies reveals gram-positive cocci in short chains *(Fig. 12.5B)*
- **Biochemical identification:** Various tests which help in the identification of *S. pyogenes* are:
  - Catalase negative
  - Susceptible to bacitracin *(Fig. 12.6B)*
  - CAMP (Christie, Atkins, and Munch-Peterson) test: Negative.
- **Lancefield grouping** shows group A *Streptococcus*
- **Automated ID systems** such as VITEK and MALDI-TOF can be performed for rapid and accurate identification of *S. pyogenes*
- **Serology:** ASO (anti-streptolysin O) antibodies and anti-DNase B antibodies are elevated
- **Antimicrobial susceptibility testing** can be performed by disk diffusion method (on Mueller–Hinton blood agar) or MIC-based method (VITEK).

TREATMENT

S. pyogenes

The infections caused by *S. pyogenes* are primarily treated by penicillin. Erythromycin can be given in case of penicillin allergy.

Streptococcus agalactiae

Streptococcus agalactiae colonizes the female genital tract and therefore the infection is common in neonates and in pregnancy.

- It has been recognized as a major cause of neonatal sepsis and meningitis
- Infections in pregnancy can lead to peripartum fever, endometritis, and puerperal sepsis
- Similar to *S. pyogenes*, it also produces #-hemolytic pinpoint colonies, gram-positive cocci in chains, and catalase negative
- But it differs from *S. pyogenes*, being bacitracin resistant, CAMP test positive and Lancefield grouping showing group B *Streptococcus*
- Penicillin/ampicillin plus gentamicin are the drug of choice for all *S. agalactiae* infections.

Viridans streptococci

Viridans streptococci are commensals of the mouth and upper respiratory tract.

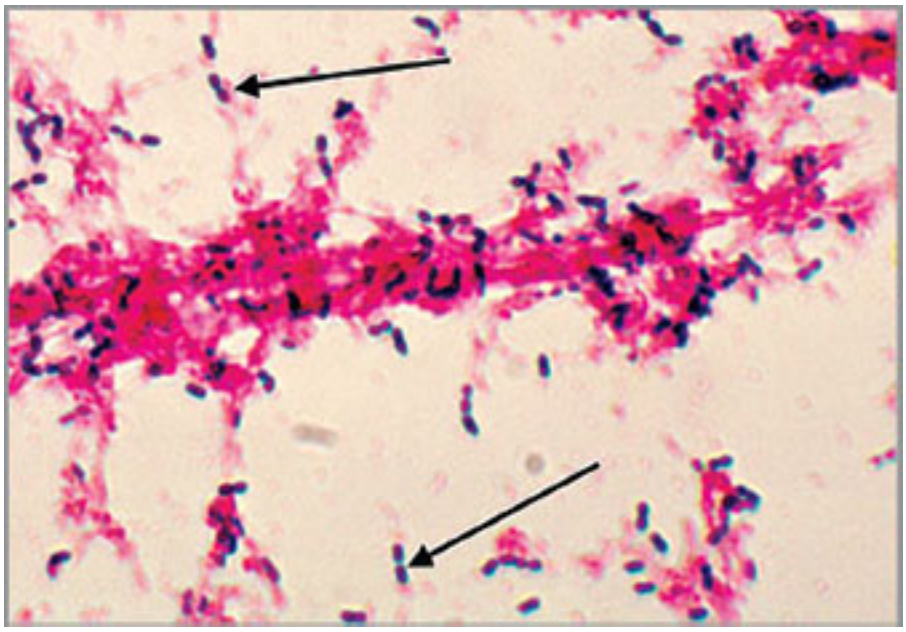
- However, occasionally they can cause infections such as dental caries, subacute bacterial endocarditis and suppurative infections
- They appear as long chains of gram-positive cocci and produce minute #-hemolytic colonies on blood agar
- They are usually susceptible to penicillin and vancomycin.

Streptococcus pneumoniae (Pneumococcus)

Streptococcus pneumoniae, commonly referred to as pneumococcus is the leading cause of lobar pneumonia, otitis media in children, and meningitis in all ages.

- **Clinical manifestations:** *S. pneumoniae* can cause both invasive infections such as lobar pneumonia, bloodstream infection, pyogenic meningitis, septic arthritis and non-invasive infections such as otitis media and sinusitis
- **Risk factors** for pneumococcal infection include—children less than two years, splenectomy, underlying comorbid conditions (e.g. chronic lung, kidney, and liver disease), etc.

Fig. 12.7. Pneumococci in Gram stained smear of sputum [lanceolate shaped gram-positive cocci in pair surrounded by clear halo (capsule)].



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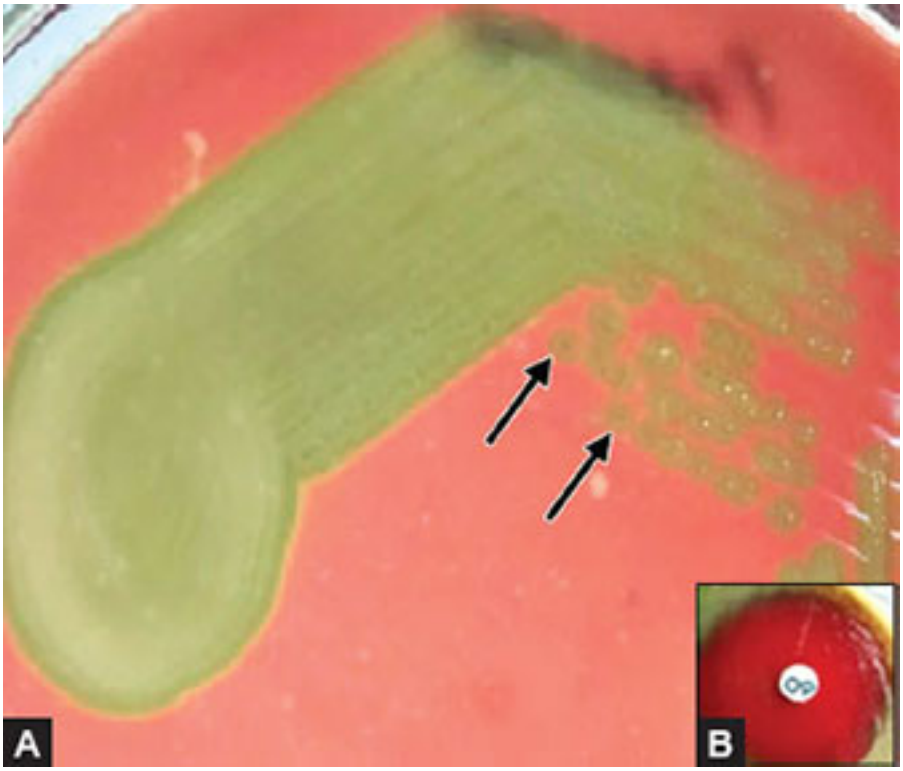
- **Laboratory diagnosis:** Clinical specimens include CSF, blood, sputum, etc. depending on the system involved
  - *Gram stain:* Pneumococci appear as capsulated gram-positive cocci in pair, lanceolate-shaped *(Fig. 12.7)*
  - *Antigen detection:* Detection of capsular antigens in CSF by latex agglutination test
  - *Culture:* On blood agar, pneumococci produce characteristic draughtsman or carom coin-shaped #-hemolytic colonies *(Fig. 12.8A)* and on chocolate agar, it produces greenish discoloration (bleaching effect)
  - *Identification:* Pneumococcus shows—(i) a positive bile solubility test, (ii) susceptibility to optochin *(Fig. 12.8B)***,** and (iii) positive for inulin fermentation. Automated ID systems (e.g. MALDI-TOF or VITEK) can also be used for identification
  - **Antimicrobial susceptibility testing:** It can be performed by disk diffusion method (on Mueller–Hinton blood agar) or MIC-based method (VITEK).
- **Treatment:** Meningitis and bacteremia cases respond well to penicillin-G.

Figs. 12.8A and B. Properties of pneumococci: *A.* #-hemolytic draughtsman-shaped colonies on blood agar; *B.* Sensitive to optochin.



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Ceftriaxone or vancomycin can be given in case of penicillin resistance

- Meningitis cases require early treatment as are associated with high fatality
- Pneumonia cases can be treated with oral amoxicillin or levofloxacin or IV ceftriaxone.

- **Infection control measures** such as droplet precautions should be followed in hospitals (refer Chapter 38)
- **Vaccine**: There are two vaccines available for pneumococcus:
  - 23-valent pneumococcal polysaccharide vaccine (PPSV23)—given to high-risk adults such to old age, immunodeficiency, splenic dysfunction, etc., but not to children. It is less immunogenic and provides short-term immunity
  - Pneumococcal conjugate vaccine (PCV13)—given both to children and high-risk adults. It is more immunogenic and provides longer immunity.

ENTEROCOCCUS

Enterococci are part of the normal flora of the human intestine. *E. faecalis* and *E. faecium* are the common species infecting man.

- **Clinical manifestations:** Enterococci can cause various infections ranging from UTI, chronic prostatitis, bacteremia, endocarditis, and intra-abdominal infections

- **Laboratory diagnosis**: Enterococci have the following laboratory features.
  - *Gram stain:* They appear oval-shaped gram-positive cocci in pairs; at an angle to each other
  - *Culture:* Produce non-hemolytic translucent colonies on blood agar
  - *Identification:* Enterococci show a positive bile esculin hydrolysis test. *E. faecalis* and *E. faecium* are differentiated by the arabinose test. Automated ID systems can also be used for identification
  - **Antimicrobial susceptibility testing:** It can be performed by disk diffusion method (on Mueller–Hinton agar) or MIC-based method (VITEK).
- **Treatment**: Enterococci can be treated with ampicillin ± gentamicin, vancomycin, and fosfomycin.

GRAM-NEGATIVE COCCI INFECTIONS

Gram-negative cocci that are pathogenic to man are *Neisseria meningitidis* and *Neisseria gonorrhoeae.*

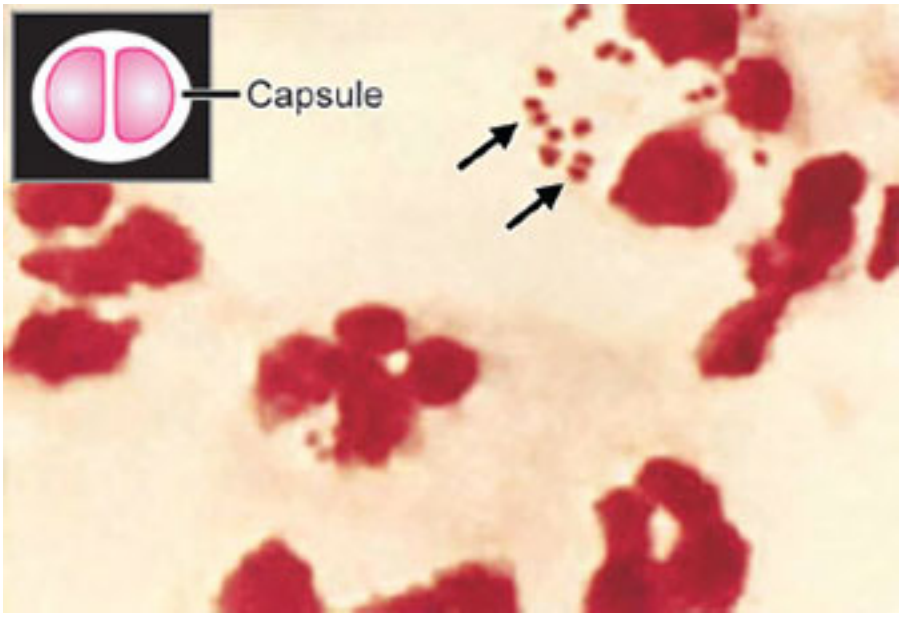
NEISSERIA MENINGITIDIS

N. meningitidis (or meningococci) are capsulated gram-negative diplococci— one of the important causes of pyogenic meningitis.

- **Virulence factors:** Pathogenesis of meningococcal infection is due to the expression of several virulence factors
  - Important virulence factors are—polysaccharide capsule, endotoxin, and outer membrane proteins
  - Based on the capsule, meningococci can be typed into several serotypes
  - Serotypes A, B, C, X, Y and W135 cause invasive disease.
- **Clinical manifestations:** Meningococcus is transmitted by droplet inhalation
  - The majority of infections result in a nasopharyngeal carriage
  - In susceptible children, it spreads through the hematogenous route to CNS to cause pyogenic meningitis
  - Systemic spread can cause fatal septicemia and complication such as Waterhouse–Friderichsen syndrome—characterized by adrenal hemorrhage, disseminated intravascular coagulation, purpuric rashes, and shock.
- **Epidemiology**: Meningococcus causes several patterns of invasive disease ranging from sporadic infection, to endemic, and explosive epidemics
  - The serogroups distribution varies among various regions of the world
  - The sub-Saharan belt of Africa is the most prevalent area.
- **Laboratory diagnosis**: Useful specimens are CSF and blood for cases and nasopharyngeal swab for carriers
  - *Gram stain* reveals gram-negative diplococci, capsulated, lens-shaped *(Fig. 12.9)*
  - *Useful culture media* are—blood agar and chocolate agar (for CSF specimen), blood culture bottles (for blood), and Thayer Martin media (for nasopharyngeal swab)
  - *Identification:* It is catalase and oxidase-positive, ferments glucose and maltose.

- **Treatment:** Third-generation cephalosporins such as ceftriaxone are the drug of choice. Meningitis cases are associated with high fatality and therefore warrant early treatment
- **Vaccines:** Capsular polysaccharide vaccine and conjugated capsular vaccine are available for meningococcus
- **Infection control measures** such as droplet precautions should be followed in hospitals (refer Chapter 38).

Fig. 12.9. Meningococci in CSF smear (gram-negative diplococci, lens-shaped) (arrows showing).



Source: Centers for Disease Control and Prevention (CDC), Atlanta *(with permission)*.

NEISSERIA GONORRHOEAE

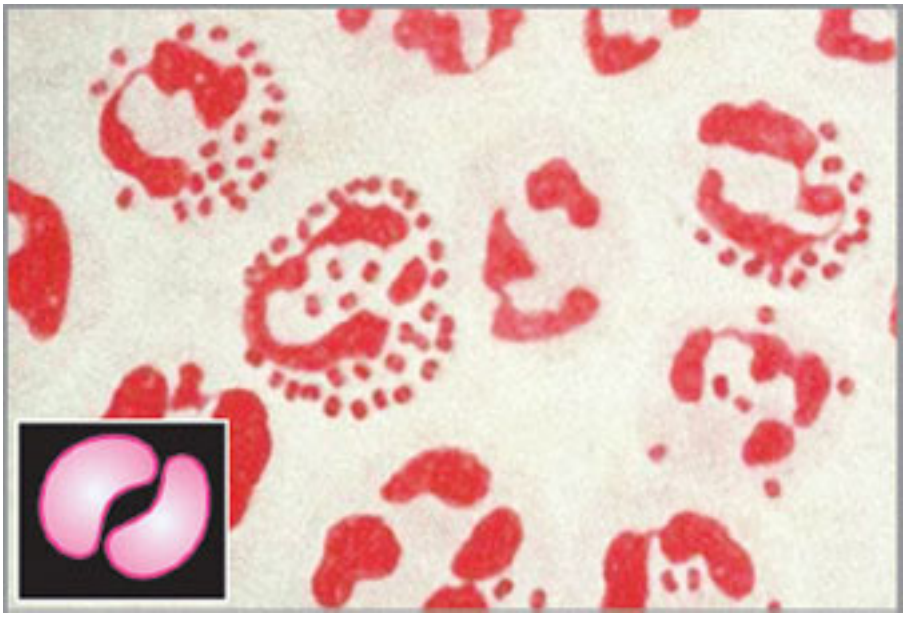
Neisseria gonorrhoeae causes a sexually transmitted infection (STI), known as 'gonorrhea'.

- **Virulence factors** of *N. gonorrhoeae* that mediate pathogenesis include—pili (helps in adhesion) and outer membrane protein
- **Clinical manifestations:** Gonorrhea commonly manifests as:
  - In males: Acute urethritis, characterized by purulent urethral discharge
  - In females: Mucopurulent cervicitis is the most common presentation
  - In both sexes: Anorectal and pharyngeal gonorrhea
  - Among neonates: Transmission during delivery from the maternal genital tract to the baby can cause conjunctivitis *(ophthalmia neonatorum)* in the newborn
  - Disseminated gonococcal infection: Presents as polyarthritis, and endocarditis.
- **Laboratory diagnosis**: Urethral swabs (for males) and endocervical swabs (for females) are the ideal specimens. Specimens should be collected in charcoal-coated swabs (Stuart's transport medium)
  - They appear gram-negative intracellular kidney-shaped diplococci *(Fig. 12.10)*

- For culture, selective media such as Thayer-Martin medium and modified New York City medium are used
- It is oxidase-positive, and ferments only glucose, but not maltose.

- **Treatment:** Third-generation cephalosporin such as ceftriaxone is the drug of choice. Both sexual partners should be treated.

Fig. 12.10. Gonococcus (gram-negative diplococci, kidney-shaped).



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EXPECTED QUESTIONS

1. I. Write an essay on:

1. Discuss the manifestations, laboratory diagnosis, and treatment of staphylococcal infections.

2. II. Write short notes on:

1. Infections are caused by *S. pyogenes*.
2. Laboratory diagnosis and treatment of pneumococcal infections.
3. Methicillin-resistant *Staphylococcus aureus* (MRSA).
4. Meningococcal meningitis.
5. Clinical manifestations and laboratory diagnosis of gonorrhea.
6. Write briefly on clinical spectrum and laboratory diagnosis of enterococci infections.

3. III. Multiple Choice Questions (MCQs):

1. **Scalded skin syndrome is mediated by:**

   a. Hemolysin

b. Coagulase

c. Enterotoxin

d. Exfoliative toxin

2. **All of the above can be given for the** *treatment of MRSA, except:*

a. Meropenem

b. Vancomycin

c. Cotrimoxazole

d. Linezolid

3. **Streptococcus pyogenes can be differentiated from S. agalactiae by testing susceptibility to:**

a. Optochin

b. Bacitracin

c. Polymyxin

d. Novobiocin

4. **Pneumococcus** *can be identified by testing susceptibility to:*

a. Polymyxin

b. Novobiocin

c. Optochin

d. Bacitracin

5. **Carrom coin appearance of colonies is seen for:**

a. *S. pyogenes*

b. Viridans streptococci

c. *S. agalactiae*

d. *S. pneumoniae*

6. **Serotyping of meningococci are based on:**

a. Outer membrane proteins

b. Endotoxin

c. Capsular polysaccharide

d. Transferrin binding proteins

7. **Which of the following is the property of enterococci?**

a. Bacitracin sensitive

b. CAMP positive

c. Bile esculin hydrolysis

d. Optochin sensitive

Answers

1. d	**2.** a	**3.** b	**4.** c	**5.** d	**6.** c	**7.** c