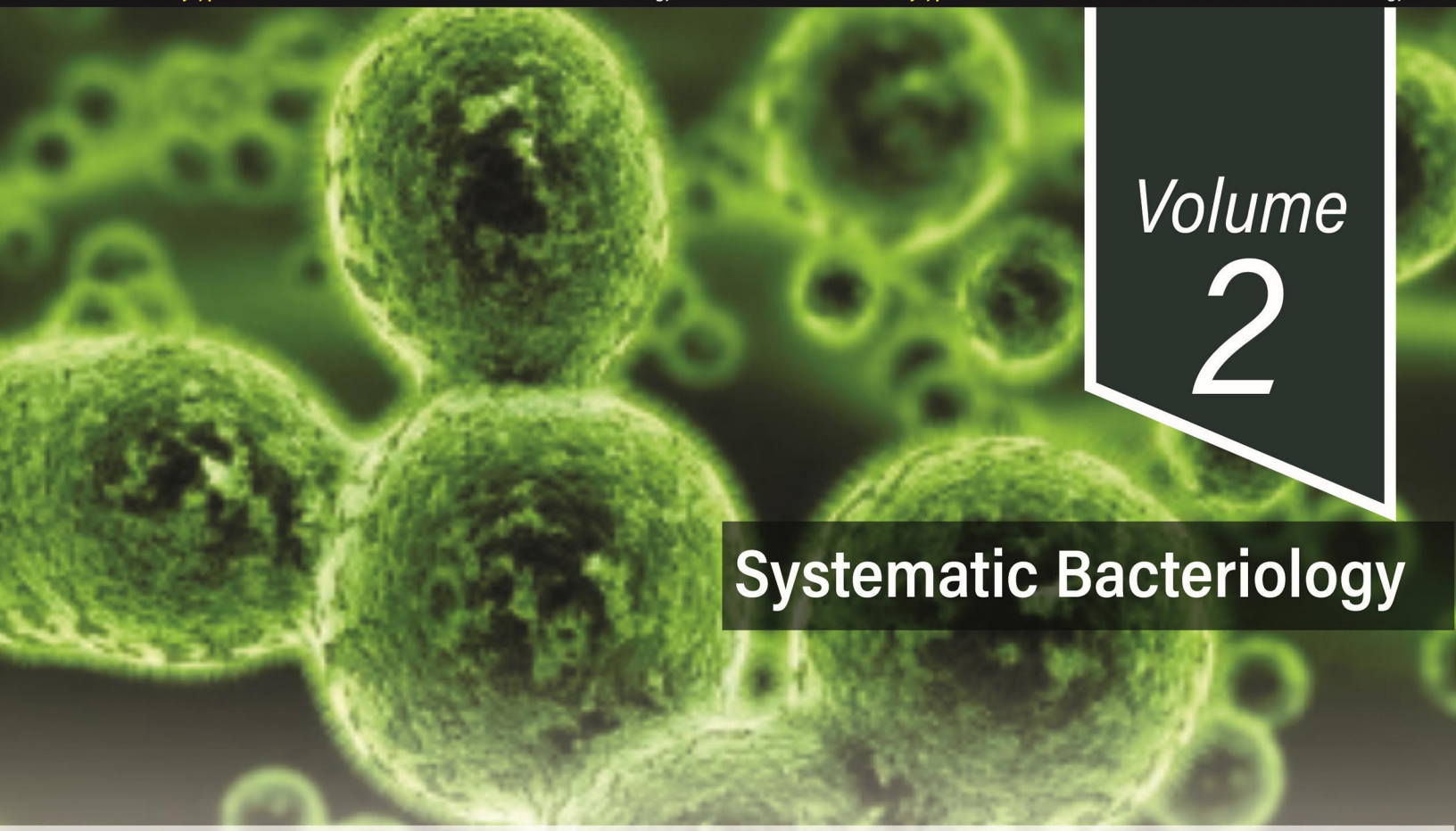

Volume 2

Systematic Bacteriology

Essentials of MICROBIOLOGY for Postgraduates

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JAYPEE

Contents

CHAPTER

3 Gram-negative Cocci: Neisseria and Moraxella catarrhalis

INTRODUCTION AND TAXONOMY[1, 2]

Gram-negative cocci include *Neisseria, Moraxella catarrhalis,* and *Veillonella* (the latter is a non-sporing anaerobe described in Chapter 26).

Genus Neisseria

Neisseria species belong to the family Neisseriaceae, order Neisseriales, and class β-Proteobacteria **(Table 3.1)**.

- The family Neisseriaceae also includes some clinically important non-fermenting gram-negative bacilli of the genera *Chromobacterium, Eikenella,* and *Kingella*, which are discussed in different chapters.
- Members of the genus *Neisseria* are catalase and oxidase-positive, non-motile, gram-negative diplococci.
- Two species are pathogenic to humans—(1) *N. meningitidis* (causes pyogenic meningitis) and (2) *N. gonorrhoeae* (causes gonorrhea), both differ from each other in various aspects **(Table 3.2)**.
- Other species are commensals of genital tract or oral cavity, such as *N. lactamica, N. flavescens, N. mucosa, N. sicca, N. subflava*, etc.

Genus Moraxella

Moraxella species are classified in the family Moraxellaceae, order Moraxellales and class γ-Proteobacteria **(Table 3.1)**.

- It comprises >20 species, with *M. catarrhalis* being the most clinically significant species recovered from human specimens.
- *M. catarrhalis* is a gram-negative diplococcus similar to *Neisseria* species, but the other *Moraxella* species that are gram-negative coccobacilli, discussed in Chapter 14.4.

Table 3.1: Taxonomic classification of the family Neisseriaceae and Moraxellaceae.[1, 2]

Class	Order	Family	Genera
β-Proteobacteria	Neisseriales	Neisseriaceae	*Neisseria, Allysiella, Aquaspirillum, Chromobacterium, Eikenella, Kingella, Simonsiella,* and *Vitreoscilla*
γ-Proteobacteria	Moraxellales	Moraxellaceae	*Acinetobacter, Moraxella*

Table 3.2: Differences between *Neisseria meningitidis* and *Neisseria gonorrhoeae.*[4]

N. meningitidis	*N. gonorrhoeae*
Capsulated	Noncapsulated
Lens-shaped/half-moon-shaped (diplococci with adjacent sides flattened)	Kidney-shaped (diplococci with adjacent sides concave)
Ferments glucose and maltose	Ferments only glucose
Rarely have plasmids	Usually possess plasmids, coding for drug-resistant genes
Exist in both intra- and extracellular forms	Predominantly exist in intracellular form
Colony—circular	Colony—varies in size with irregular margin
Habitat—nasopharynx	Habitat—genital tract (urethra, cervix), rarely pharynx

3.1 Neisseria meningitidis (Meningococcus)

INTRODUCTION AND HISTORY[3]

Neisseria meningitidis (the meningococcus) is an obligate human pathogen, often found as commensal in the nasopharynx of many healthy adolescents, but can cause invasive disease in susceptible individuals—called epidemic cerebrospinal fever, presenting as acute bacterial meningitis and/or mild bacteremia to devastating septicemia. They are

capsulated gram-negative diplococci with adjacent sides flattened.

- **First report:** The first epidemic of meningococcal disease was reported from Geneva in 1805; since then, several outbreaks have been occurring till World War II, the majority from Africa.
- **Naming:** In 1884, Italian pathologists Ettore Marchiafava and Angelo Celli first described intracellular oval cocci in cerebrospinal fluid (CSF).
  - Anton Weichselbaum (1887) was the first to isolate this organism in CSF and named it *Diplococcus intracellularis meningitidis* because of its presence inside neutrophils from cerebrospinal fluid (CSF).
  - The name was later changed to *Neisseria meningitidis* after the German scientist and clinician, Albert Neisser who discovered the causative agent (*N. gonorrhoeae*) of gonorrhea.
  - Meningococcus is derived from the Greek words meninx (membrane) + kokkos (berry).
- **Carrier state:** Kiefer (1896) and Albrecht Ghon (1901) found that healthy persons were nasopharyngeal carriers of the meningococcus.
- **Serum therapy:** Following an epidemic in New York (1907), intrathecal equine serum therapy was introduced by Flexner for the treatment of meningococcal meningitis, which resulted in reducing mortality from 85% to 40%.
- **Serogroups:** Between the 1940–1950s, Branhan defined meningococcal serogroups based on capsular polysaccharides and developed the internationally standardized nomenclature A, B, and C, which was later expanded in the 1960s by the work of Slaterus to include other serogroups.

VIRULENCE FACTORS

N. meningitidis possesses several virulence factors, of which the most important is the capsule. Schematic diagram of the meningococcal cell envelope is given in **Figure 3.1**.

Capsular Polysaccharide[5, 6]

It is the principal virulence factor of meningococci; protects the bacteria from complement-mediated phagocytosis.

- **Serogroups:** Based on the antigenic nature of the capsule, meningococci can be typed into 13 serogroups [A–E, H, I, K, L, W–Z]; among which only 6 serogroups—**A, B, C, X, Y, and W** (formerly W135)—account for the majority of cases of invasive disease.[3]
- Other capsular serogroups and noncapsulated meningococci (16% of isolates are not capsulated) commonly colonize the nasopharynx of asymptomatic carriers and are rarely associated with invasive disease.[5]
- Capsule expression is not a prerequisite for colonization. Indeed, adherence and subsequent transmigration across the epithelial cell barrier are enhanced in the absence of the capsule. Only following invasion, the capsule becomes an essential virulence factor that protects the organism against phagocytosis and complement-mediated killing.[3]
- **Structure:** Capsules show structural and antigenic heterogeneity.
  - The serogroup A capsule is composed of mannosamine phosphate, while that of serogroups B, C, Y, and W contains sialic acid (i.e. N-acetylneuraminic acid), but differ from each other in the linkage.
  - For example, the group B and C capsules are composed of homopolymers of N-acetylneuraminic acid with α-2,8 and α-2,9 linkages, respectively.
  - This minor difference in structure leads to markedly different immunological properties—while the group B capsule is poorly immunogenic, the group C capsule mounts a strong immune response.
- **Regulation of expression:** Capsule is encoded by a genetic island of a 30-kb region, called the island of horizontal transfer **(IHT-A1)**.
  - In addition to capsular biosynthesis, **IHT-A1** also helps in other capsule-related functions such as its acetylation, assembly, and transport to the cell surface, and protection of the capsule from degradation.
  - Evolutionary divergence in the genes of this island resulted in capsular heterogeneity among serogroups, which was the key factor behind the emergence of invasive meningococcal disease.
  - In *N. gonorrhoeae,* commensal *Neisseria* species, and pharyngeal carrier strains of *N. meningitidis*, the island has been lost or has not been acquired.
- **Phase variation (on↔off):** Meningococci are capable of turning the capsule on and off, and modifying both structure (e.g. acetylation) and amount of capsule expressed. When capsular expression is turned off, it increases the affinity between bacterial and host surfaces, facilitating colonization. Turning the capsule off also helps the organism to escape from the host immune response mounted against the capsule.
- **Capsular switching:** This is another mechanism seen in meningococci, which is different from phase variation (on↔off) of the capsule.
  - *Definition:* Capsular switching refers to the change of a strain from one capsular serogroup to another by acquiring the capsular gene by horizontal gene transfer.
  - *New serogroup:* Isolates which has undergone capsular switching will express a different capsular serogroup, but retain the genetic serotype, serosubtype, and immunotype of the original strain, as these typing methods are based on various non-capsular antigens.
  - *Immunologic escape:* Capsular switching helps the strain to escape from the immune response mounted against the original serogroup of the given strain.
  - *Vaccine failure:* When a vaccine of a particular serogroup is administrated, the isolates of that serogroup can undergo capsular switching acquiring a new capsule, thus escaping from the immune response established by the vaccine. This mechanism has the potential to initiate an outbreak, which can be sustained over a period of time, despite the vaccination program is ongoing.

Outer Membrane Proteins[5, 6]

They are porin proteins present beneath the capsule, embedded in the outer membrane which are involved in the

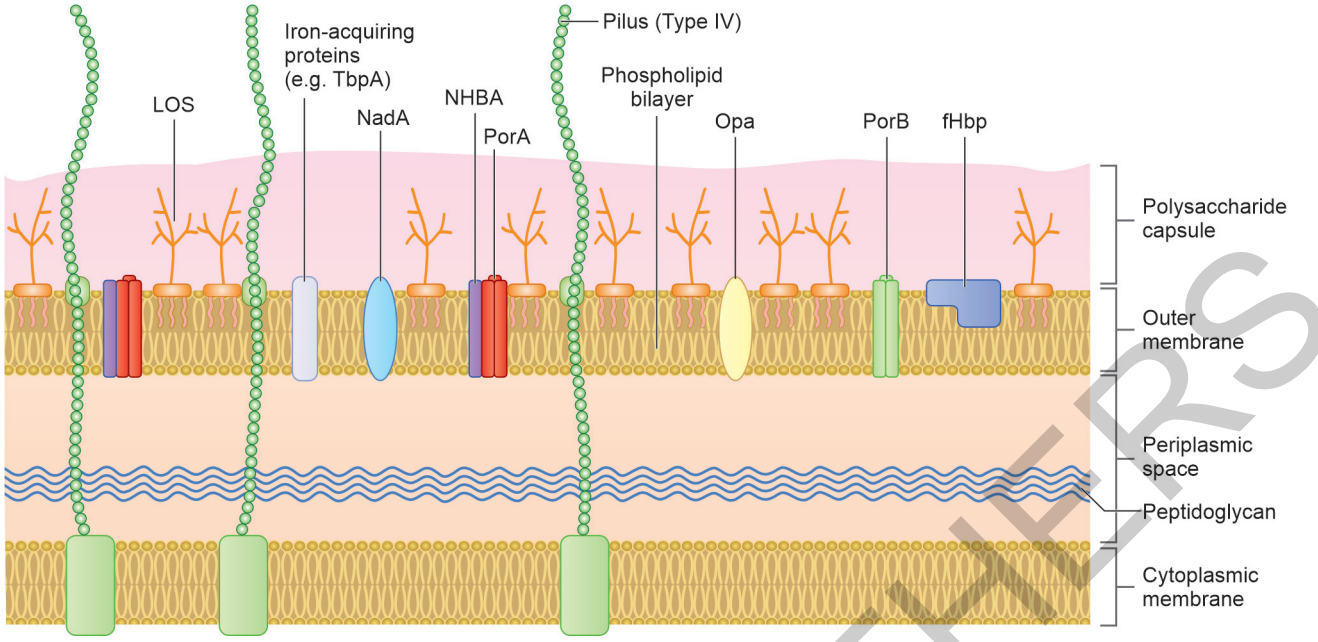


Fig. 3.1: Schematic diagram of the meningococcal cell envelope.[5]
(LOS: lipooligosaccharide; Opa: opacity proteins; NHBA: neisserial heparin binding antigen; NadA: neisserial adhesin A; fHbp: factor H binding protein; TbpA: transferrin binding protein)

host-cell interactions and remain as targets for bactericidal antibodies. They are of two types—PorA and PorB; both show antigenic variability.

- **PorB:** It is the major outer membrane porin, that induces Ca^{+2} influx, and activates Toll-like receptor 2 (TLR2) and cell apoptosis. PorB is responsible for serotyping of meningococci.
- **PorA:** It is a major target of the meningococcal outer membrane vesicle (OMV) vaccine, which is one of the components of the MenB vaccine. PorA is responsible for the serosubtyping of meningococci.

Lipooligosaccharide and Endotoxin[3, 7]

Lipooligosaccharide (LOS) of meningococci differs from a typical lipopolysaccharide (LPS) of gram-negative bacteria by—lacking O-side chains and having a distinct type of lipid A (endotoxin). LOS can mimic the 'I' human blood group antigens.

- **Functions:** LOS is an important virulence factor, facilitates host cell damage, meningococcal attachment, and invasion of host cells, and evades complement-mediated killing.
- **Mechanism:** Endotoxin binds to CD14 molecules on the host cell surface, in association with Toll-like receptor-4 (TLR4). This in turn activates the endothelial cells by inducing the release of various inflammatory mediators, such as chemokines and cytokines.
- **Serum or CSF levels** of endotoxin are directly correlated with the severity of meningococcal sepsis or meningitis.
- LOS also **downregulates the genes** involved in oxidative phosphorylation and mitochondrial function in human cells.
- The **immunotyping** of meningococci is based on the LOS antigen.

Type IV Pili[3, 7]

Type IV pili of meningococci are complex surface appendages requiring at least 23 proteins (e.g. PilE, PilC, secretin, PilT, PilQ) for their biogenesis and function.

- **Structure:** Pili are anchored in the outer membrane and radiate through an oligomeric ring, extending up to several thousand nanometers from the bacterial surface.
- **Adhesion:** Pilus is the organ of adhesion, facilitating meningococcal attachment and anchoring to the human epithelial or endothelial cells.
- **Other functions of pili** include remolding of the cytoskeleton, facilitating aggregation and microcolony formation, DNA transformation, and twitching motility.

Other Virulence Factors[3]

- **IgA proteases:** They cleave mucosal IgA1 (but not IgA2), facilitating entry and colonization. They also cleave Lamp1 which is the major integral membrane glycoprotein of lysosomes that results in enhanced intracellular survival of the organism.
- **Iron-acquiring proteins:** As iron is necessary for survival, colonization, and infection, meningococci scavenge iron from human proteins by expressing various iron-acquiring proteins, such as:
  - Transferrin binding protein (TbpA and TbpB)
  - Lactoferrin binding protein (HbpA and HbpB)
  - Hemoglobin binding protein (HmbR)
  - Hemoglobin-haptoglobin binding protein (HpnB)
  - Ferric enterobactin receptor (FetA).
- **Factor H binding protein (fHbp):** It is a lipoprotein involved in meningococcal resistance to complement-mediated killing. It is used as a target in the MenB vaccine.
- **Other adhesins:** Meningococci also express various other virulence factors that help in adhesion such as:

- Neisserial adhesin A (NadA): It also mediates cell invasion.
- Neisserial heparin binding antigen (NHBA): It is also involved in protection against complement-mediated killing.
- Opacity proteins (Opa and Opc).

Genetic Islands[3]

Different meningococcal strains possess various large genetic **island of horizontal transfer** (IHT)—IHT-A1, IHT-A2, IHT-B, IHT-C, IHT-E. These islands encompass several important genes, that are responsible for virulence or survival of the bacteria. For example:

- IHT-A1 contains the gene coding for capsular polysaccharide.
- IHT-A2 locus encodes an ABC transporter.
- IHT-C locus encodes genes for several toxin homologs, a bacteriophage, and various potential virulence proteins.

Genotyping[3, 8]

Molecular typing is now the preferred approach for identifying the relatedness of the strains isolated during outbreaks.

- Various techniques have been used for molecular typing including PCR, pulsed-field gel electrophoresis (PFGE), multilocus enzyme electrophoresis (MLEE), and multilocus sequence typing (MLST).
- Currently, MLST is the **gold standard** molecular typing method, which classifies meningococcal strains into different STs (sequence types) based upon polymorphisms in seven housekeeping genes.
- While many of the STs are linked to meningococcal serogroups, some STs which are independent of the serogroups have also been identified.
- Certain STs are over-expressed in invasive strains (e.g. ST-11) compared to carriage strains and so have been termed hyperinvasive lineages.

Clonal Complexes[3, 8]

Groups of genetically closely related meningococci are grouped into clonal complexes (CC).

- Currently, 12 genomic CC types cause most endemic and epidemic invasive diseases worldwide.
  - ST-5 and ST-7 (serogroup A)
  - ST-41/44, ST-32, ST-18, ST-269, ST-8, and ST-35 (serogroup B)
  - ST-11 (serogroups C or W)
  - ST-23 and ST-167 (serogroup Y)
  - ST-181 (serogroup X).
- While few clonal complexes are nearly exclusively restricted to strains of a single serogroup (e.g. ST-5 to serogroup A), certain other complexes (e.g. ST-11) are found to be associated with multiple serogroups.

PATHOGENESIS[3, 5, 6]

Humans are the only natural host for meningococci. The most common source of infection is nasopharyngeal carriers (mainly children). The mode of transmission is by droplet inhalation and the portal of entry is nasopharynx.

Pathogenesis of meningococcal disease involves adhesion and colonization, invasion into the bloodstream, followed by invasion into the meninges.

Adhesion and Colonization[3, 5, 6]

The most common site of meningococcal colonization is the human nasopharynx, as it provides the optimal conditions for its survival such as a temperature of 34°C, 3–4% CO_2, and high humidity (75–80%).

- **Receptor interaction:** Colonization of the nasopharynx involves a series of interactions of meningococcal adhesins such as type IV pili (key adhesin) and others such as Opa proteins, NadA, and NHBA with their receptors present on the epithelial mucosa such as carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) and integrins.
- The IgA1 protease produced by meningococcus degrades mucosal IgA and thereby facilitating colonization.
- **Capsule turning off:** During colonization, the capsular expression is usually turned off, thus increasing the affinity between bacterial and host surfaces, facilitating colonization.
- **Factor contributing to invasion:** While a majority of infections remain carriage, the invasion occurs in a few cases. The factors that attribute to invasion are:
  - *Serogroup of the strain:* Certain strains (e.g. ST-11) are more invasive and express virulence factors more efficiently.
  - *Host factors:* Certain host factors such as smoking, underlying viral and *Mycoplasma* infection of the respiratory tract or genetic factors such as complement deficiency, etc. facilitate invasion (*See* epidemiology).
  - *Environmental factors* such as low humidity or dusty wind or dry weather, etc. will facilitate invasion.

Invasion into Bloodstream[3, 5, 6]

Invasion through the mucosa into the bloodstream occurs rarely, usually within a few days (1–14 days) of the acquisition of an invasive strain by a susceptible individual. Although meningococcus is a facultative intracellular pathogen, it remains extracellular in the bloodstream.

- Receptor interaction at the nasopharynx induces epithelial actin polymerization and plaque formation which helps in the internalization of meningococci within epithelial cells.
- **Capsule turning on:** Following the invasion, the capsule plays an important role. Capsule expression results in meningococcal disaggregation from the mucosal surfaces and spread into the bloodstream. Capsule also protects the organism against phagocytosis and complement-mediated killing.
- **Entry into endothelial cell:** Type IV pili adhere to endothelial cells, which leads to the formation of microcolonies on endothelium, the activation of signaling pathways in endothelial cells, and the formation of cytoskeletal plaques resulting in the entry of meningococci into endothelial cells.
- **Membrane protrusions:** Meningococci continue to proliferate in the bloodstream during which they release

blebs of the outer membrane containing OMPs and LOS (endotoxin).

- **Inflammatory cascade:** Meningococcal endotoxin binds to endothelium surface-bound CD14 in association with TLR4, which results in the release of various inflammatory mediators like tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, IL-8, IL-10, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein-3α (MIP-3α), MCP-5, plasminogen-activator inhibitor 1 (PAI-1), and leukemia inhibitory factor. The soluble CD14-bound endotoxin acts as a mediator of endothelial activation.
- **Endothelial injury:** The inflammatory mediators induce endothelial injury which in turn leads to increased vascular permeability (attributed to loss of glycosaminoglycans and endothelial proteins), with subsequent gross proteinuria.
  - *Capillary leak syndrome:* Leakage of fluid and electrolytes from capillaries into the tissues leads to hypovolemia, tissue edema, and pulmonary edema.
  - *Decreased cardiac output:* Hypovolemia results in compensatory vasoconstriction and tachycardia, eventually reducing the cardiac output.
- **Intravascular thrombosis:** Endothelial activation results in the upregulation of tissue factors on the endothelium that leads to the activation of procoagulant pathways.
  - Multiple anticoagulant pathways are downregulated through the loss of endothelial thrombomodulin and protein C receptors and result in decreased levels of antithrombin III, protein C, protein S, and tissue factor pathway inhibitor.
  - Thrombolysis is also profoundly impaired in meningococcal sepsis through the release of high levels of PAI-1.
  - All these are responsible for disseminated intravascular coagulation.
- **Meningococcal shock:** A combination of factors such as capillary leak syndrome, myocardial depression, and decreased perfusion of tissues can cause widespread organ dysfunction, including renal impairment.

Invasion into Meninges[3, 5, 6]

Meningeal invasion by *N. meningitidis* is the most severe and life-threatening step of meningococcal pathogenesis.

- **Spread:** Meningococci reach the meninges either by: (1) hematogenous route causing septicemia (most common); (2) from the nasopharynx by direct spread along the olfactory nerve through cribriform plate; or (3) rarely through the conjunctiva.
- **Crossing the blood-brain barrier:** *N. meningitidis* binds to endothelial receptors (e.g. CD147 and β2-adrenergic receptors) on brain microvascular endothelial cells. Meningococci induce damage to brain microvascular endothelium through activation of β2-adrenoceptor leading to actin polymerization (causing cytoskeletal rearrangement), membrane protrusions, and plaque formation, as discussed before. This results in the breakdown of tight junctions between endothelial cells, through which the meningococci cross the blood-brain barrier and gain access to the subarachnoid space.
- **Inflammatory cascade:** Once in the CSF, *N. meningitidis* replicates and releases LOS endotoxin, which triggers a strong pro-inflammatory response (via TNF-α, IL-1β, and IL-6). The inflammation leads to increased BBB permeability, brain edema, and neutrophil infiltration, resulting in the clinical symptoms of meningitis.
- **Changes in CSF:** These include cellular, biochemical, and hydrodynamic changes in the CSF.
  - Increased CSF secretion and impaired CSF absorption lead to the accumulation of CSF, raised intracranial pressure, and reduced cerebral blood flow.
  - Increase in CSF lactate indicates brain tissue hypoxia.
- **Neuronal injury:** Due to shock-induced hypoperfusion, vasculitis, and DIC, brain tissue hypoxia occurs, which results in neuronal injury and varying degrees of brain tissue damage.

EPIDEMIOLOGY[5]

Worldwide, nearly 5 lakh cases of meningococcal disease occur each year with mortality of about 10%.[5] However, the cases have been declining recently as a result of vaccination programs. Six serogroups A, B, C, W, X, and Y—cause most diseases worldwide **(Table 3.3)**.

Epidemiological Patterns[9, 10]

Several patterns of invasive disease are seen, ranging from sporadic infection to endemic, hyperendemic, and explosive epidemics. The definition of epidemiological pattern would vary depending on the prevalence of the disease.

- **Outbreak:** A meningococcal disease outbreak occurs when multiple cases of the same serogroup (type) happen in a population over a short period. The number of cases, above which the outbreak can be declared depends on the population size and underlying endemicity, e.g. in areas with low endemicity, an outbreak can be considered even with two cases.[9]
- **Endemicity:** Countries can be classified into three groups based on the endemicity of the meningococcal disease.[10]
  - Countries with high endemic rates: >10 cases/lakh population/year and/or ≥1 epidemic over the last 20 years.
  - Countries with moderate endemic rates: 2–10 cases/lakh population/year.

Table 3.3: Serogroup distribution of invasive meningococcal disease.[18]

World regions	Most frequent	Less frequent
Sub-Saharan Africa	C, W, X	–
Southern Africa	B, W	C
North America	B, C, Y	W
South America	B, C, W	–
Europe	B, W	C, Y
Fareast Asia	B, W	C, Y
Central Asia	A, W	B
Australia	B	W

Source: World Health Organization, 2020[18]

- Countries with low endemic rates: <2 cases/lakh population/year.

❖ **Epidemic:** For the African meningitis belt, WHO defines the meningococcal disease epidemic as >100 cases/lakh population.[10]

❖ **Hyperendemic:** The disease is said to be hyperendemic when it reaches up to 1,000 cases/lakh population.[9]

Global Situation[9, 11-15]

Meningococcal meningitis occurs globally as an endemic illness. Globally, nearly 5 lakh cases with 50,000 deaths used to occur annually earlier. However, the cases dropped down significantly in the recent years (5,985 cases in 2023).[13]

❖ **African meningitis belt:** The sub-Saharan region of Africa—called the African meningitis belt is the most prevalent area for meningococcal infections. It involves 26 countries, stretching from Ethiopia in the East to Senegal in the West **(Fig. 3.2)**. The most affected countries in the region are Burkina Faso, Chad, Ethiopia, and Niger.[9]

❖ **Incidence (Africa):** Meningococcal disease is hyperendemic to the meningitis belt region, and causes periodic epidemics during the dry season (December–June) which sometimes reaches up to 1,000 cases/lakh population.[9]

❖ **Incidence (rest of World):** In contrast, in the other parts of the world such as Europe, Australia, South America, and Asia, the disease is reported in the range from 0.12 to 3 cases/lakh population/year.[9] Serogroup distribution of invasive meningococcal disease in different areas of the world is enlisted in **Table 3.3**.

❖ **Group A:** Historically, outbreaks in the meningitis belt were primarily due to serogroup A (80–85%), with large-scale epidemics occurring every 5–12 years. However, with the introduction of the meningococcal vaccine in this region in 2010, the proportion of serogroup A cases has declined dramatically.[9]

❖ **Recent outbreaks** in the meningitis belt have primarily been due to **serogroups C and W**, although serogroup X outbreaks are also reported.[9] Between 2010 and 2017, 65% of outbreaks reported in the meningitis belt were caused by group C, followed by group W (35%).[5]

❖ **Serogroup B** is the major cause of prolonged outbreaks, hyperendemic disease, and endemic (sporadic) meningococcal disease, especially in infants and young children in developed countries.[3] Surprisingly, serogroup B is quite rare in sub-Saharan Africa but common in South Africa and other parts of the world **(Table 3.3)**.[3]

❖ **Latest outbreak in Africa:**[16] In 2021, there was an outbreak of meningococcal meningitis reported from the Democratic Republic of the Congo, with a total of 2662 cases and 205 deaths. There is another small outbreak reported from the Zinder Region, southeast of Niger from November 2022 to January 2023.

❖ The **Hajj pilgrimage** to Saudi Arabia has also been associated with outbreaks of meningococcal disease in returning pilgrims and their contacts. Group W (formerly

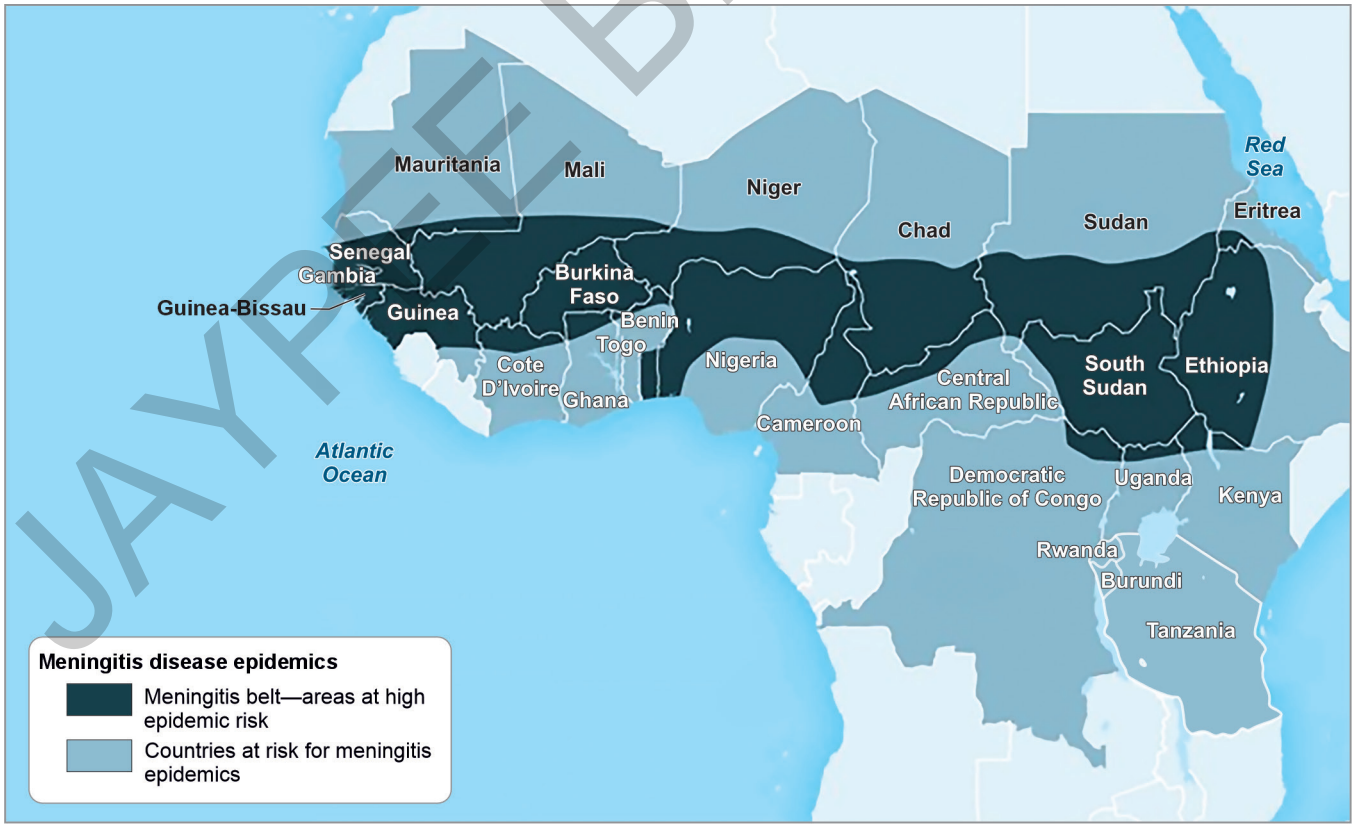


Fig. 3.2: African meningitis belt.

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

W 135) of clonal complex ST-11 caused the global outbreak in 2000/2001 in the Hajj pilgrimage.[3]

- **Florida outbreak:** There is a small outbreak of meningococcal meningitis reported from Florida, USA (2021–2023), mainly among gay and bisexual men.[15]
- **India:** The burden of meningococcal disease in India is underestimated, largely because of underreporting. According to a meta-analysis, meningococcus is the third most common cause of acute bacterial meningitis in children <5 years in India, and accounts for 1.9% of all cases regardless of age.[17]
  - The disease remains endemic in India, with major outbreaks reported in Delhi (1966, 1986, 2005–2008), Madhya Pradesh (1989), Odisha (1989), Andhra Pradesh (1989), Meghalaya (2008–2009), and Tripura (2009) over the last 25 years.[13]
  - Majority of cases in India are caused almost exclusively by serogroup A; however, serogroups B, C, W, and Y have also been documented.
  - The National Health Profile published by the Central Bureau of Health Intelligence (CBHI) in India shows that the estimated burden of meningococcal meningitis in India is:[14]
    - For the year 2020: 28,682 cases and 47 deaths (highest burden from West Bengal).
    - For the year 2021: 2,153 cases and 35 deaths (highest burden from Andhra Pradesh).
    - For the year 2022: 1,234 cases and 8 deaths (highest burden from Odisha, Rajasthan, Telangana and Meghalaya).

Predisposing Factors

Factors associated with increased susceptibility to meningococcal infection include:

- **Age group:**[9] Meningitis is common in early childhood (3 months to 5 years) with a second peak occurring in adolescents (15–25 years of age).
  - In meningitis belt countries, high rates of disease are seen in people up to age 30 years, and the highest rates are in children and adolescents aged 5–14 years.
  - In contrast, outside the meningitis belt, the disease is more common among infants and adolescents.
  - Children are at increased risk because of the absence of specific adaptive immunity in combination with very close contact with colonized individuals, including parents.
- **Risk factors that promote colonization** include: Overcrowding and semi-closed communities, such as schools,[3] military and refugee camps, and travelers (Hajj pilgrims).
- **Risk for travelers** is highest in people visiting the meningitis belt countries who have prolonged contact with local populations during an epidemic.[9]
- **Seasonality:** Meningococcal infections are common in winter and spring (cold and dry climate) and low humid atmosphere.[5]
- **Normal flora** of the upper respiratory tract also influences meningococcal colonization, e.g. the presence of *N. lactamica* is negatively correlated with meningococcal carriage.[3]
- **Smoking:** Both active and passive exposure to tobacco smoke damage the nasopharyngeal epithelium.[3]
- **Viral and *Mycoplasma*** infection of the respiratory tract or the dry season: They either increase the expression of adhesion molecules in the nasopharynx, promoting adhesion, or facilitate meningococcal invasion of the bloodstream.
- **Genetic factors** that are associated with increased disease susceptibility are:[3, 5]
  - **Complement deficiency**, mainly terminal complement components (C5–9), properdin, or factor D, or eculizumab therapy—a terminal complement inhibitor.
  - **Hyposplenism:** Spleen is the site of the destruction of capsulated organisms. Therefore, functional or structural defects in the spleen would increase the susceptibility to these organisms.
  - Hypogammaglobulinemia: Absence of specific antibody leads to increased susceptibility.
  - Polymorphisms in genes coding for the Fcγ-receptor II (CD32), Fcγ-receptor III (CD16), mannose-binding lectin (MBL) protein, Toll-like receptor (TLR)4 have been associated with increased risk for meningococcal disease.
  - Genetic variation in the β2-adrenoceptor increases susceptibility to meningococcal meningitis.

CLINICAL MANIFESTATIONS

Asymptomatic colonization is the most common presentation. Symptomatic individuals show several manifestations of which meningitis and septicemia are the most important.

Rashes[3, 5]

A non-blanching rash (petechial or purpuric) develops in more than 80% of the cases **(Fig. 3.3)**.

- Rashes represent subcutaneous hemorrhage, which occurs due to occlusion of small vessels and endothelial necrosis in the skin showing neutrophilic infiltrate.
- The pin-head shaped (<2 mm) **petechial lesions** are formed initially, which become larger to form **purpuric**

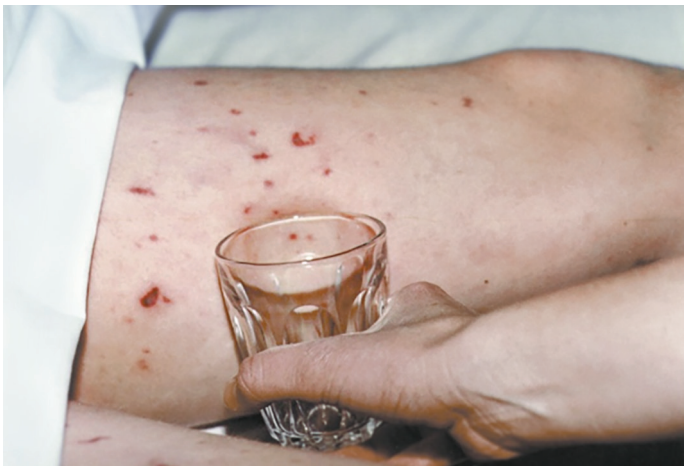


Fig. 3.3: Non-blanching rashes, that do not disappear with pressure detected by 'glass test'.

Source: National Health Service (NHS) (*with permission*).

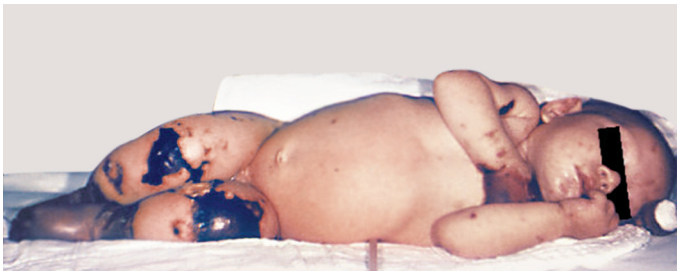


Fig. 3.4: Child with gangrene of lower extremities due to meningococcemia.

Source: Public Health Image Library, ID#/1335/Mr Gust/Centers for Disease Control and Prevention (CDC), Atlanta (*with permission*).

lesions (>2 mm), that can further coalesce to form larger **ecchymotic lesions** of >10 mm size. In the most severe cases, large purpuric lesions with skin necrosis are formed called **purpura fulminans (Fig. 3.4)**.

- ❖ However, rashes may be absent in early illness, and some patients may not develop a rash.
- ❖ Viral infections need to be ruled out as they are more common to cause a petechial or purpuric rash.

Septicemia[3, 5]

Meningococcemia (septicemia with shock) is seen in 20% of cases. It is attributed to endotoxin-induced endothelial injury leading to increased vascular permeability and intravascular thrombosis.

- ❖ Starts as early nonspecific symptoms, then develop rashes, and rarely purpura fulminans in severe cases.
- ❖ Subsequently **progresses to shock** (manifested by tachycardia, poor peripheral perfusion, tachypnea, and oliguria), and multiorgan failure.
- ❖ **Waterhouse–Friderichsen syndrome:** It is a severe form of fulminant meningococcemia, characterized by large purpuric rashes (purpura fulminans), shock, disseminated intravascular coagulation (DIC), bilateral adrenal hemorrhage and multiorgan failure.
- ❖ Patients usually have very high levels of **bacteremia** (10^5–10^8 CFU/mL) and **endotoxemia** [up to 10^3 endotoxin units (EU)/mL].
- ❖ In **fulminant meningococcemia**, the time window between the progression from initial symptoms to death can be as low as a few hours.
- ❖ The **mortality rate** is high (25–40%), but can be reduced to 10% with early aggressive management.
- ❖ Mild or transient bacteremia without sepsis can be a rare presentation, seen in <5% of cases.

Acute Bacterial Meningitis[3, 5]

It is the most common presentation of invasive meningococcal disease, seen in 40–65% of cases. It commonly affects young children (3–5 years of age).

- ❖ **Older children** present with fever, vomiting, headache, altered mental status, meningeal signs (neck stiffness, Kernig or Brudzinski sign), and photophobia—similar to any other bacterial meningitis, except for the presence of rashes.
- ❖ **Infants** usually present with fever and irritability and may have a bulging fontanelle; may not have the classic signs of meningitis, such as neck stiffness and photophobia.
- ❖ Focal neurologic signs, seizures, subdural empyema, etc. are less common than in meningitis due to other bacterial agents such as pneumococcus.
- ❖ Up to 40% of meningitis patients also co-present with septicemia.

Chronic Meningococcemia[3, 5]

It occurs rarely and is characterized by repeated episodes of petechial rash, fever, arthritis, and splenomegaly.

- ❖ It is commonly seen in conditions such as complement deficiencies, inadequate sulfonamide therapy, etc.
- ❖ Isolates from these patients are shown to have *lpxL1* gene mutation, leading to a reduced inflammatory response to endotoxin.

Postmeningococcal Reactive Disease[3, 5]

Immune complexes (made up of capsular antigens and their antibodies) develop 4–10 days later, leading to manifestations like arthritis, rash, iritis, pericarditis, polyserositis, and fever. This condition usually resolves without any sequelae.

Less Common Presentations[3, 5]

- ❖ **Respiratory infections:** Primary pneumonia can be seen up to 10%, especially with serogroup Y, and in adults. Pharyngitis can also be seen. But focal infections such as epiglottitis, sinusitis, and otitis media are very rare.
- ❖ **Septic arthritis** (2%): It is often monoarticular (knee or ankle). It is seen more in young adults and has been associated with ST-11 strains of serogroup C and W.[3]
- ❖ **Purulent pericarditis:** Seen more in adolescents and adults and has been predominantly caused by ST-11 strains of serogroup C and W.
- ❖ **Conjunctivitis:** Occurs rarely, mainly in children. It is characterized by unilateral hyperacute purulent exudate and edema. Transmission to the eye occurs via direct contact or droplet spread. It can rarely progress to panophthalmitis, therefore needs systemic therapy with intravenous antibiotics and chemoprophylaxis for contacts.
- ❖ **Urethritis, and proctitis:** Transmission through orogenital sex has been postulated. There was a urethritis outbreak in Florida, USA due to a specific group C, ST-11.2 clade; mainly affecting gay and bisexual men.
- ❖ **Others:** gastroenteritis, necrotizing fasciitis, etc.

Complications[5]

Mortality is high (>10%), even with treatment; reaches up to 50% when untreated or treatment is delayed. More than 10% of the survivors suffer from severe sequelae.

- ❖ **Scarring:** Necrosis of purpuric skin lesions leading to scarring is the most common complication (10% of cases), for which skin grafting may be necessary.
  - ■ The most often affected area is the lower limbs, followed by the upper limbs. On average, 13% of the skin surface area is involved.

Fig. 3.5: Baby Charlotte from New Zealand, a survivor of meningococcal disease after undergoing amputations of limbs, she was the face of the vaccination campaign in the country.

Source: Wikipedia/http://babycharlotte.co.nz (*with permission*).

- Amputation may be required (rarely, in 1–2% of cases) if the tissue viability is severely compromised due to peripheral ischemia **(Fig. 3.5)**.

❖ **Neurologic complications** may be seen in 5–7% of cases such as hearing loss, psychological disorders, poorer neurologic development, etc.

❖ **Serogroup:** Group C and W (ST-11 clone) are reported to have a higher frequency of complications and even mortality, compared to other groups.

❖ Death is commonly attributed to hypovolemic shock (in meningococcemia), or rarely raised intracranial pressure (in meningococcal meningitis).

LABORATORY DIAGNOSIS

Specimen Collection[4]

Important specimens include CSF, blood and skin scrapings from petechial rashes from cases and nasopharyngeal swabs from carriers.

❖ Specimens are collected in sterile containers and transported immediately without any delay.

❖ **CSF** should be processed immediately. It should *never be refrigerated* as suspected agents of meningitis, such as meningococci and *Haemophilus influenzae* may die on refrigeration.

❖ **Blood culture:** Multiple blood cultures (2–3 sets) should be collected in the brain–heart infusion broth, or automated systems such as BacT/Alert.

❖ Nasopharyngeal swabs, pus, or scrapings from rashes should be carried in transport media (such as **Stuart's medium**).

CSF Examination[4]

For bacteriological examination, the CSF is divided into the following three portions:

❖ **First portion** of CSF is centrifuged.
- The supernatant is used for capsular antigen detection and biochemical analysis (reveals elevated CSF pressure, increased protein content, and decreased glucose content).
- The sediment is used for direct Gram staining.

❖ **Second portion of CSF:** It is directly inoculated onto an enriched media, such as blood agar and chocolate agar.

❖ **Third portion of CSF:** It is inoculated into an enriched broth, such as BHI broth, incubated till granular turbidity is produced, and then subcultured onto blood agar and chocolate agar.

Gram Stain[3, 19]

Meningococci appear as gram-negative diplococci (0.6 × 0.8 µm in size[3]) with adjacent sides flattened (**lens** or **half-moon-shaped**), present inside the polymorphs, often extracellular also.[19] This presumptive diagnosis helps to start empirical antibiotics **(Fig. 3.6)**.

❖ Sometimes, meningococci may tend to resist decolorization.[19]

❖ If the patient is on antimicrobials, the sensitivity of Gram stain falls considerably.

❖ The sensitivity of Gram stain improves by techniques such as heaped smear, making smear from pellets from centrifuged specimens, or cytocentrifugation.

Direct Antigen Detection[19]

Latex agglutination test (LAT) for capsular polysaccharides has been used for the detection of *N. meningitidis* in CSF, serum, and also urine samples, especially in patients who received prior antibiotics.

❖ Using specific antibodies coated with latex beads, the capsular polysaccharide antigens can reliably be identified in the specimen.

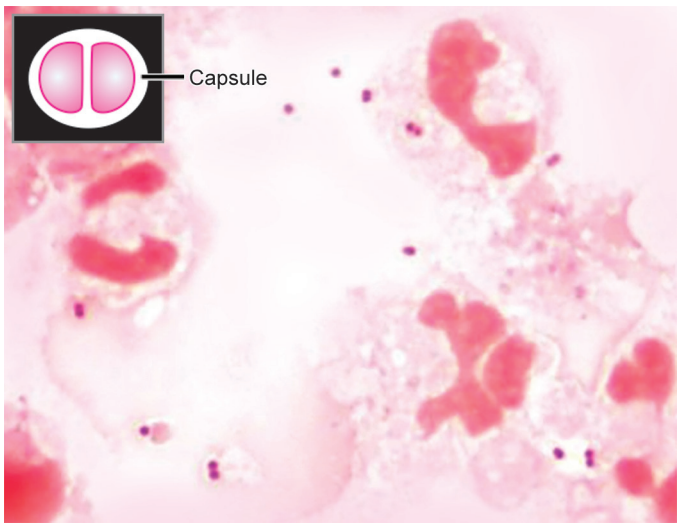


Fig. 3.6: Meningococci in CSF smear (gram-negative diplococci, lens-shaped).

Source: Microman/Wikipedia (*with permission*).

- **Examples** of commercially available LAT are:
  - Pastorex meningitis test (Biorad Pvt. Ltd.): It contains latex reagents specific for *N. meningitidis* A, C, Y/W, and B/*E. coli* K1.
  - Wellcogen kit test (Oxoid Ltd.): It contains latex reagents specific for *N. meningitidis* A/C/Y/W and *N. meningitidis* B/*E. coli* K1.
  - In addition, both the above kits contain latex reagents specific for the capsule of pneumococcus, *H. influenzae* type b, and *S. agalactiae*.
- This test is **not reliable for serogroup B** due to the poor immunogenicity of its capsule. More so, LAT for serogroup B can cross-react with K1 *E. coli* capsular polysaccharide, which is another important cause of meningitis in young infants.
- LAT is of questionable clinical usefulness compared with Gram staining and should therefore not be used as a substitute for microscopy.[19]
- LAT is less sensitive and should be replaced by molecular assays when possible.[5]
- Use of LAT can be restricted to laboratories or epidemiologic settings where a molecular assay is not readily available.[3]

Culture[1, 19, 20]

N. meningitidis is fastidious and grows better when incubated in an increased CO_2 (3–7%) atmosphere. Plates should be incubated for up to 72 hours before negative results.

- **Sheep blood agar:** Colonies are smooth, round, moist, gray to white about 1 mm in size after 18 hours of incubation **(Fig. 3.7)**. Heavily encapsulated strains produce mucoid colonies. Blood agar beneath the colonies may display a gray-green discoloration. Young cultures have a smooth consistency, while older cultures become gummy due to autolysis.
- **Chocolate agar:** Same as blood agar, but colonies are more opaque than blood agar.
- **Selective media:**[19] They are used for nasopharyngeal swabs to suppress the growth of normal flora. The following selective media are used, where meningococci produce medium-to-large bluish-gray mucoid colonies.
  - **Thayer–Martin medium:** It is chocolate agar with added vancomycin, colistin, and nystatin. It inhibits commensal *Neisseria* species. The modifications of this media are as follows:
    - **Modified Thayer–Martin medium:** It contains trimethoprim in addition, which inhibits the swarming of *Proteus.*
    - **Martin–Lewis (ML) agar:** It contains anisomycin instead of nystatin, which has increased activity against *Candida albicans.*
    - **GC–Lect agar:** It additionally contains lincomycin which inhibits *Capnocytophaga* species and vancomycin-resistant gram-positive contaminants.
  - **New York City (NYC) agar (Fig. 3.8):** It is a peptone-corn starch agar containing yeast dialysate, citrated horse plasma, and lysed horse RBC, with antibiotics vancomycin, colistin, amphotericin B, and trimethoprim.

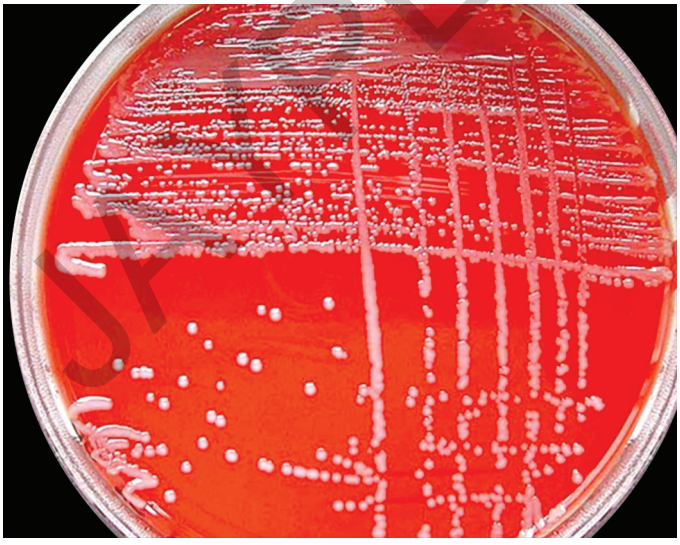


Fig. 3.7: Colonies of meningococci on blood agar.

Source: Erasmus MC—Microbe Canvas, www.microbe-canvas.com (*with permission*).

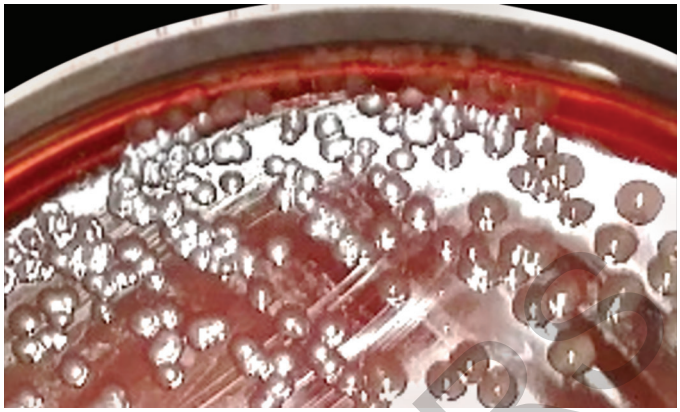


Fig. 3.8: Colony of meningococci on New York City agar.

Source: Xishan/Wikipedia (*with permission*).

Culture Smear and Motility Testing[19]

Gram stain must be performed on suspected *N. meningitidis* colonies to confirm the presence of uniform gram-negative diplococci.

- Consistent results are obtained when Gram stain is performed on freshly isolated colonies before autolytic processes appear.
- Gram-negative coccobacilli such as *Moraxella, Acinetobacter,* and *Kingella* may resemble meningococci in microscopy, therefore should be subjected to further biochemical tests for differentiation.
- Motility testing by hanging drop reveals nonmotile cocci.

Biochemical Identification

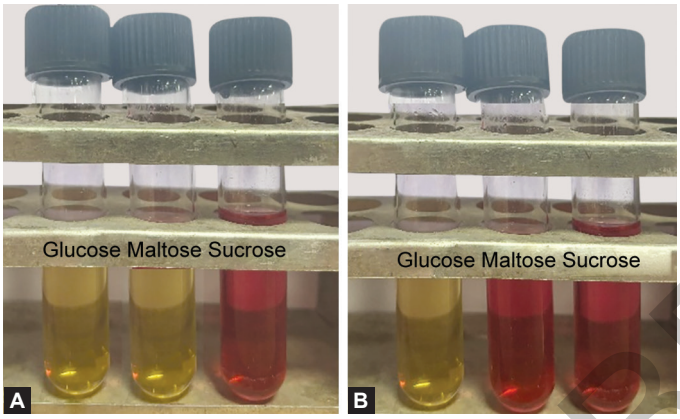
Meningococci are positive for both catalase and oxidase test. Certain additional biochemical tests are carried out to differentiate meningococci from other commensal *Neisseria* species.

Rapid Carbohydrate Utilization Test[1, 19]

Previously, a fermentation method employing cysteine tryptic digest semisolid agar (CTA) was used, giving results

in 24 hours. Now it is replaced by the rapid carbohydrate utilization test (RCUT), which demonstrates acid production from carbohydrates by oxidation (in 4 hours) but not fermentation.

- **RCUT procedure:** Heavy suspension of the test isolate is made in a small volume of balanced phosphate-buffered saline solution containing phenol red indicator added with the test carbohydrate. The tubes are incubated in a non-CO_2 incubator or water bath for 4 hours.
- **Result:** The development of yellow color by 4 hours indicates the particular carbohydrate is utilized **(Figs. 3.9A and B)**.
- **Commercial RCUT kits** are also available using microtiter plates, e.g. CarboFerm *Neisseria* Kit (Hardy Diagnostics), which can differentiate various *Neisseria* and *Moraxella* spp. **(Fig. 3.10)**.



Figs. 3.9A and B: Rapid carbohydrate utilization test (RCUT): (A) Meningococcus fermenting glucose, maltose, but not sucrose; (B) Gonococcus fermenting only glucose.

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

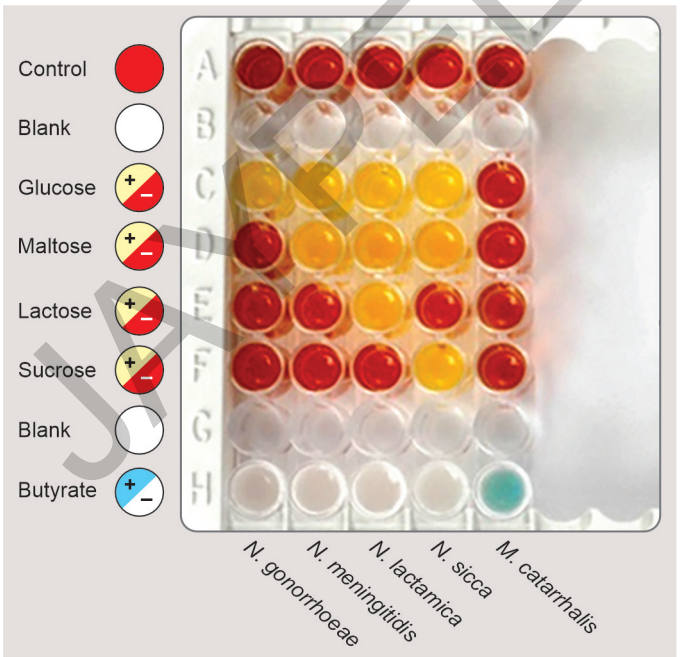


Fig. 3.10: *CarboFerm Neisseria* RCUT Kit (Hardy Diagnostics).

- **Differentiation:** Meningococci utilize both glucose and maltose but not sucrose, in contrast to gonococci which utilize only glucose.

Chromogenic Enzyme Substrate Tests[1, 19]

The enzymatic identification systems use specific biochemical substrates that, after hydrolysis by bacterial enzymes, yield a colored end product (e.g. a yellow nitrophenol or nitroaniline product).

- **Kits:** The commercially available kits are the NET test (Thermo Scientific, **Fig. 3.11**) and GonoCheckII (TCS Biosciences).
- These kits can simultaneously differentiate four organisms—*N. gonorrhoeae, N. meningitidis, N. lactamica,* and *Moraxella catarrhalis,* within 30 minutes of incubation.
- **Enzymes:** The enzymatic activities that are detected in these systems include **(Fig. 3.11)**:
  - γ-glutamyl aminopeptidase: Specific for *N. meningitidis* (yellow color)
  - β-galactosidase: Specific for *N. lactamica* (blue color)
  - Prolyl-iminopeptidase: Specific for gonococcus (red color)
  - Butyrate esterase: Specific for *Moraxella catarrhalis* (colorless).
- **Selective media:** Chromogenic tests should be used to identify suspected meningococci recovered from selective media but not from blood agar and chocolate agar.
- **Test limitations:** Rarely, isolates of meningococci (carrier) may lack γ-glutamyl aminopeptidase activity. Similarly, some strains of meningococci may also produce prolyl-iminopeptidase.

Automated Methods of Identification[1, 19]

MALDI-TOF MS[22, 23]

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI TOF-MS) is a promising tool for the rapid identification of *Neisseria* species.

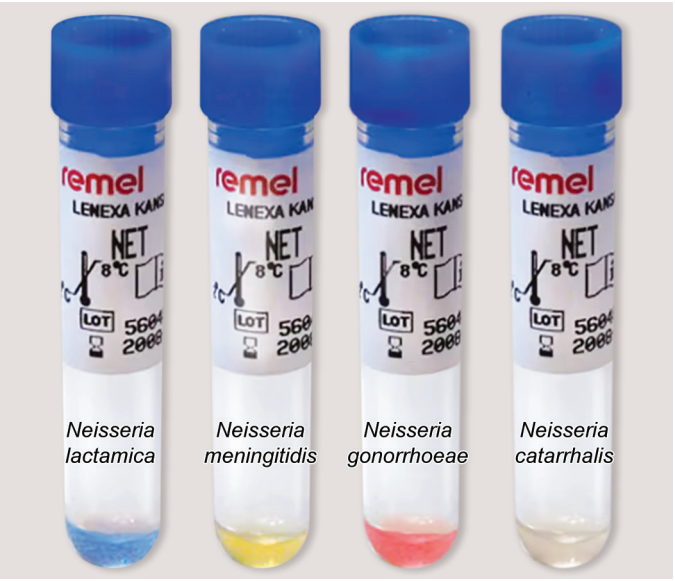


Fig. 3.11: NET test.

Source: Thermo Scientific.

- Studies have reported that the mass spectra profiles are sufficiently different to allow species identification of pathogenic *Neisseria* isolates.[19]
- However, there have been occasional reports of misidentification of commensal *Neisseria* species as *N. meningitidis.*[20]
- Both VITEK MS and Bruker biotyper can be used to identify more than 12 *Neisseria* species.

Other Automated Systems[24]

VITEK2, Phoenix, and MicroScan Walkaway systems can also be used to identify meningococcus. VITEK2 NH (*Neisseria-Haemophilus*) card can identify various species of *Neisseria* such as *N. meningitidis, N. gonorrhoeae, N. lactamica, N sicca, N. elongata,* and *N. weaveri.* RapID NH system (Thermo Scientific) can identify *Neisseria* species and other fastidious gram-negative organisms including *Haemophilus*. They use modified conventional tests (e.g. acid production from carbohydrates, urease, indole, ornithine decarboxylase) and chromogenic substrates and helps in identification of the organism within 2- to 6-hour.

Serology[6, 19]

The primary role of serological tests is to assess the antibody response in individuals following vaccination and for seroepidemiological purposes.

- A rise of **serogroup-specific titer** post-vaccination compared to baseline can be used as an indicator of successful vaccination.
- However, serology is **not useful for active infection** as antibodies develop later in the convalescent phase. More so, it is also *not useful to ascertain the retrospective diagnosis* of a suspected culture/PCR-negative invasive disease, since asymptomatic carriage can also elicit antibody titers.
- **Serum bactericidal assay:** It is regarded as the best surrogate test for assessing vaccine protection across all serogroups. It uses complement (from a baby rabbit or human) to determine the bactericidal antibody titer of serum (of a vaccinated person) when mixed with an inoculum of *N. meningitidis.*[19, 21]
- **ELISA format or bead assays** are also available to detect IgG antibodies in serum against serogroups A, C, W, and Y.[19] ELISA using crude preparation of outer membrane vesicle antigen is also available.[6]

Molecular Diagnosis[19]

Molecular assays such as PCR are highly sensitive, detect even a few bacteria in CSF, detect earlier than culture, and also help in serogroup identification. It also has a significant role in **culture-independent diagnosis**—in situations where there is a high chance of culture turning negative (e.g. prior antibiotic administration or poor transport conditions).

- **Real-time PCR** is even more sensitive, and specific, takes less time, and quantitative.
- **Multiplex PCR** (e.g. BioFire FilmArray) and multiplex real-time PCR can be used for the simultaneous detection of common agents of pyogenic meningitis.
- **Common genes** targeted include *siaD, porA, porB*, *fetA*, *IS1106*, *ctrA, crgA,* and *sodC*.
  - *siaD* (polysialyl-transferase gene) can be used for serogrouping of B, C, W, and Y.
  - *porA* and *porB* (porins), *fetA*, and housekeeping genes allow culture-independent typing.
  - *IS1106* may show false-positive results; therefore, not to be used as a single target for routine screening.
  - *ctrA* (capsule transport gene) can be negative rarely in strains not harboring the capsule locus.
  - *crgA* is a gene involved in adhesion.
  - *sodC* is a Cu-Zn superoxide dismutase gene.

Typing[3, 8]

Typing of the strain isolated in culture helps to determine the relatedness between the strains isolated from different cases of a geographical area, which can further assist in establishing an outbreak. It also helps to identify clonal complexes (CC), and strains with the potential to cause outbreaks.

- **Slide agglutination serogrouping (SASG)** test is the traditional test done for serogrouping of meningococcal invasive isolates by using appropriate antisera. It uses commercial polyclonal antibody reagents raised against prototypical *N. meningitidis* isolates representing the important disease-associated serogroups. Subjective reading of the end-result with naked eye is the limitation of this method.[25]
- **Other serogrouping methods** use capsule-specific monoclonal antibodies, which include dot blot assays, whole-cell ELISAs and flow cytometry.
- **Genotyping:** PCR-based approaches are available to target *ctrA* gene (capsule transport), as well as serogroup-specific genes for capsule biosynthesis.
  - **Serogroup-specific real-time PCR** targeting the capsule biosynthesis genes *sacB* (group A), *siaD* (group B or C), *synG* (group W), *xcbB* (group X), and *synF* (group Y) have also been used to detect the genetic capsule type.
  - **MLST:** Currently, multilocus sequence typing (MLST) is the **gold standard** molecular typing method, which classifies meningococcal strains into different STs (sequence types) based upon polymorphisms in seven housekeeping genes. It is now the preferred approach for identifying clonal complexes (CC), closely related strains, and strains with the potential to cause an outbreak.

Antimicrobial Susceptibility Testing[26, 27]

Antimicrobial susceptibility testing (AST) for *N. meningitidis* is not routinely performed in many clinical settings, as it requires a **biosafety cabinet** (BSL 2 or 3 set up, based on the nature of work). Manipulation of cultures outside a BSC is associated with an increased risk of contracting laboratory-acquired infection. If a BSC is unavailable, manipulation of these isolates should be minimized, limited to Gram staining or serogrouping using phenolized saline.

Salient Features

This is probably the first microbiology book in India written exclusively for postgraduate students. This book is the Volume 2, covering—Systematic Bacteriology.

- **Sections:** Book is divided into five sections—gram-positive and gram-negative cocci, gram-positive bacilli, gram-negative bacilli, anaerobic bacteria, and other groups of bacteria.
- **Figures:** More than 1,000 figures have been incorporated, which include—(i) schematic diagrams representing geographical distributions of various infectious diseases across the world, depicting the microdetails of complex pathogenesis, etc., and (ii) photographs of microscopy and culture findings of several bacterial pathogens (including infrequently encountered bacteria), most of which are from the collection of the author's own department, representing an analog of a **bacteriology atlas**.
- **Tables:** More than 500 tables have been incorporated to enable quick comparison between different entities (e.g., bacterial species, biochemical reactions, diagnostic methods). Summarizing key concepts in a table reinforces learning and improves retention.
- **Taxonomy and classification** of every bacterial pathogen has have been adapted from standard reference sources such as National Center for Biotechnology Information (NCBI) Taxonomy and Genome Taxonomy Database. The latest updates in the taxonomy have been incorporated such as change of name from *Borrelia burgdorferi* to *Borreliella burgdorferi*, updates in the family composition of Order Enterobacterales, and so on.
- **Pathogenesis** of most of the bacterial pathogens has been adapted from Mandell's 9th edition, Harrison's 21st edition, and review articles of reputed journals such as Nature Microbiology, Oxford, Frontiers in Microbiology, etc. A large number of flowcharts, line diagrams, and illustrations have been incorporated to simplify the understanding of pathogenesis.
- **Epidemiology** of most of the bacterial diseases has been referenced from Mandell's 9th edition and Harrison's 21st edition, Park's Preventive and Social Medicine 27th edition, CDC's and WHO's websites, and review articles of reputed journals. Indian data on epidemiology of various bacterial diseases has been adapted from National Health Profile 2023, 18th issue, Ministry of Health & Family Welfare, Government of India, and also from Integrated Disease Surveillance Program (IDSP), India.
- **Clinical manifestation part** of most of the infectious diseases (bacterial) has been referenced from Mandell's 9th edition and Harrison's 21st edition. Also, infectious disease experts were consulted and practical important information from their experience have has been incorporated.
- **Laboratory diagnosis of infectious diseases** has been referenced from standard citations such as Bailey & Scott's 15th edition, Koneman's 7th edition, Topley & Wilson's Microbiology, 10th edition, Mackie & McCartney, 14th edition, Gillespie's 18th edition, Murray's (ASM), 13th edition, Wadsworth Anaerobic Bacteriology Manual, and review articles of reputed journals, manufacturer's product specific literatures, updates from Clinical and Laboratory Standards Institute (CLSI), and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines.
- **Treatment part** has been referenced from Mandell's 9th edition and Harrison's 21st edition, and The Sanford Guide to Antimicrobial Therapy. Antimicrobial resistance of the concerned microbial pathogens to the various drugs has been incorporated in every chapter with a detailed note of on the mechanism of resistance, detection methods, and current status of AMR in India.
- **Prevention part:** Type of infection control measures to be followed in specific infections has been adapted from Guideline for Isolation Precautions, CDC and author's textbook on *Essentials of Hospital Infection Control*. Vaccine updates have been referenced from Mandell's 9th edition and Harrison's 21st edition, as well as from CDC's and from WHO's latest updates.
- **Most features of author's popular MBBS book has have been maintained in this book:**
  - Concise, bulleted format and to-the-point text—easy to read during examination
  - Simple and lucid language—makes the understanding easy
  - Separate highlighted boxes—for important topics for quick review.

Apurba S Sastry MD (JIPMER) DNB MNAMS PDCR has done his MBBS from MKCG Medical College, Berhampur, Odisha, India, and residency from the prestigious institute, JIPMER, Puducherry, India. He is currently working as an Additional Professor, Department of Microbiology, in the same institute. He is also the Hospital Infection Control Officer and Antimicrobial Stewardship Lead (Microbiology) of JIPMER. He is the course coordinator of the two most popular workshops of India—"National Workshop on Hospital Infection Control", and "National Workshop on Antimicrobial Stewardship", both conducted annually by HICP, JIPMER; in which more than 1,000 practitioners across India have been trained. He has developed "Clinical Microbiology Reporting Software" based on CLSI and EUCAST guidelines, which will be revolutionary in upgrading the diagnostic stewardship to the next level. He has guided and co-guided more than 60 student dissertations and contributed more than 100 research publications. He is passionate about teaching and has been a popular teacher among the students.

Sarumathi D MD DNB MNAMS CIC PDF-HIC, has done her graduation from Madras Medical College, Chennai, Tamil Nadu, India, and postgraduation and fellowship in Hospital Infection Control from JIPMER, Puducherry, India. Currently, she is working as Assistant Professor, Department of Microbiology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India. She has contributed more than 40 research publications and 5 book chapters. She has undergone NABL internal auditor's training course. She is the co-editor of the book "Essentials of Antimicrobial Stewardship, 1st edition (2023)".

Dr Apurba Sastry and his team have also authored several other popular books which are extremely appreciated among faculty and students:

1. Essentials of Medical Microbiology, 5th edition, for MBBS
2. Essentials of Hospital Infection Control, 1st edition
3. Essentials of Antimicrobial Stewardship, 1st edition
4. Essentials of Medical Parasitology, 2nd edition
5. Review of Microbiology and Immunology, 9th edition, for PG entrance examination
6. Essentials of Practical Microbiology, 2nd edition, for MBBS
7. Essentials of Applied Microbiology for Nurses, 1st edition
8. Microbiology for FMGE, 2nd edition, for foreign medical graduate entrance examination
9. Essentials of Microbiology—Organism based, 1st edition, for BScMLT and other allied health science courses

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