GRAM-POSITIVE BACTERIAL PATHOGENS

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I. INTRODUCTION

Gram-positive pathogens possess various advantageous characteristics and mechanisms that allow them to adhere to, colonize, and attack host cells. They are capable of inducing a spectrum of illnesses, ranging from mild gastrointestinal distress and pharyngitis to severe pulmonary infections and nervous system-related diseases. Within this spectrum, a significant number progress to highly lethal acute infections, exhibiting case fatality rates as high as 80% in certain instances. This chapter will introduce to you the morphological, biochemical and molecular distinct properties, the culture conditions and frequently used culture media, as well as the diseases, treatments, and prevalence of most important gram-positive pathogens in the present clinical settings, including *Bacillus cereus, Clostridium difficile, Corynebacterium jeikeium, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae,* and *Streptococcus pyogenes.*

II. BACILLUS CEREUS

1. **Morphological Characteristics:** *Bacillus cereus (B. cereus)*, a Gram-positive rod- shape bacterium, displays a distinctive purple or blue hue owing to its robust peptidoglycan layer, which retains the stain during Gram staining. The name "cereus", which means waxy in Latin, comes from its large, feathery, and dull gray colonies appearance on blood agar plates. Its colonies are also granular, spreading with opaque, rough matted surfaces and irregular perimeters. Typically arranged in chains or pairs, although occasionally as solitary bacilli, *B. cereus* is encapsulated with a polysaccharide capsule which protects it from the environment and from being devoured by the host immune system. Under unfavorable conditions such as food scarcity, it can generate spores which can withstand high temperature for long periods of time. This pathogen has many long flagella which signifies its high motility in both swimming and swarming.
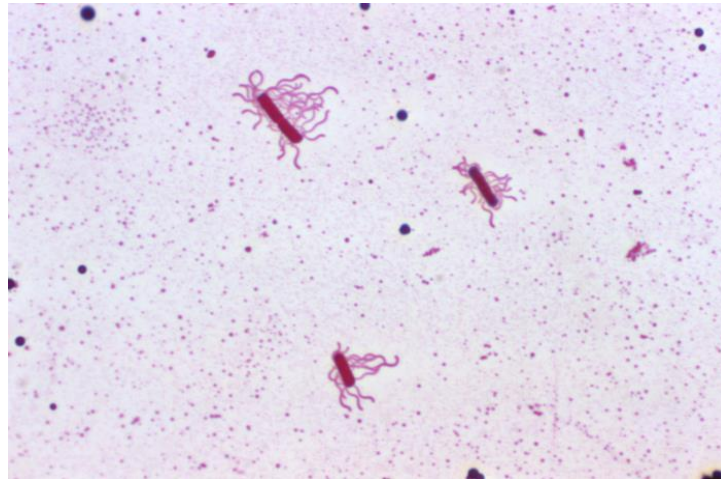
Figure 1: *Gram-stained Bacillus cereus photomicrograph showcases highly motile flagella. Leifson flagella stain method was used to achieve the optimal observation of the flagella.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Dr. William A. Clark, 1977
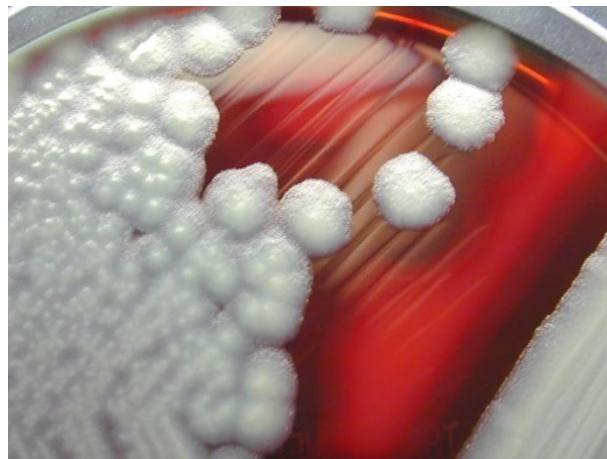

Figure 2: *Gray, dull and feathery colonies of Bacillus cereus on sheep blood agar medium (SBA). The name "cereus", which means wax-like, is based on the appearance of colonies.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC)/ Courtesy of Larry Stauffer, Oregon State Public Health Laboratory, 2002.

2. **Phenotypic and Biochemical Properties:** *B. cereus* is a facultative anaerobic bacterium that can grow in both oxygen-abundant and oxygen-deficient environments. Remarkably, it exhibits catalase activity to catalyze the decomposition of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). This reaction is important for protecting the bacterium from oxidative damage caused by hydrogen peroxide. Moreover, it is beta-hemolytic which can completely lyse red blood cells on blood agar plates. Notably, *B. cereus* produces a heat-stable toxin (enterotoxin 1) that causes food poisoning in reheated food, especially in reheated rice, particularly known as the "fried rice syndrome".

3. **Culture Condition and Media:** *B. cereus* is selectively cultured in mannitol yolk polymyxin B agar (MYPA) and polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA). The selectivity of the media depends on *B. cereus*'s distinct metabolic capabilities which are fermentation of mannitol and hydrolysis of egg yolk (Mayer, 2015). Another option is Brilliance Bacillus cereus Agar (BBC) which improves selectivity via the addition of trimethoprim (Mayer, 2015). BBC shows effectiveness in inhibiting background microflora in unpasteurized food samples and is considered to be a better option among MYPA, PEMBA and BBC (Chon et al., 2014).

4. **Molecular Identification:** Polymerase chain reaction (PCR) is used for the detection of *B. cereus* by amplification and sequencing of target genes such as 16S rRNA and virulence factor genes including non-haemolytic exotoxin genes *nheA/nheB/nheC*, hemolysin genes *hblC/hblD/hblA*, cereulide synthetase gene *cesB*, and cytotoxin K gene *cytK* (Liu et al., 2020). Moreover, MALDI-TOF mass spectrometry can reliably identify *B. cereus* and distinguish it from closely related species like *B. thuringiensis* based on the intensity profiles of small molecule biomarkers (Ha et al., 2019).

5. **Virulence Factors:** The virulence factors of *B. cereus* include a range of toxins and substances that contribute to its pathogenicity and ability to cause food poisoning and infections. One key virulence is Hemolysin BL (Hbl) - a protein known for its hemolytic, cytotoxic, dermonecrotic, and capillary permeability properties. Nonhemolytic Enterotoxins (Nhe) is a three-component enterotoxin, encoded by *nheA, nheB* and *nheC,* which are the major causation of foodborne infections in humans (Lindbäck et al., 2004). The mechanism of Nhe toxicity involves the formation of pore on cell membrane resulting in osmotic lysis. Other toxins of *B. cereus* include Cytotoxin K (CytK) - a cytotoxic protein that affects capillary permeability and cytotoxicity in the host; Enterotoxin FM (EntFM) which is linked to cytotoxicity and capillary permeability. These enterotoxins are heat labile. They can be produced while the pathogen is already within the intestine, triggers diarrhea and abdominal pain 6 to 15 hours after consumption.

   Emetic (vomiting) Toxin (Cereulide) which can cause deadly vomitting/nausea food poisoning (Owusu-Kwarteng et al., 2017). This toxin is heat stable. It is preformed in food by the bacteria and causes nausea and vomiting within 30 minutes to 6 hours post-ingestion. It is the reason *B. cereus* is famously known for inducing "fried rice syndrome", a syndrome where food poisoning is caused by reheating rice.

6. **Diseases and Treatments:** *B. cereus*, a foodborne pathogen, elaborates two distinct enterotoxins, resulting in two categories of gastrointestinal illness. The heat-labile proteinacous toxin is associated with diarrheal syndrome while the emetic syndrome, characterized by vomiting, is linked to cereulide. Beyond foodborne intoxication, *B. cereus* can also contribute to ocular, respiratory, and wound infections (McDowell et al., 2023).

7. **Prevalence:** The general prevalence of *B. cereus* is 23.746% (CI 95%). In compared to developed countries, *B. cereus* prevalence is higher in developing countries. Among all reported countries, Australia has the lowest rate of prevalence, while the United States is an exception, possessing the highest prevalence.

Besides, *B. cereus* prevalence depends on the types of food as well as the detection method. A study showed that this pathogen was found the highest in cereals and beans, the second highest in vegetables and dairy products. Diagnostic methods utilizing molecular techniques, such as polymerase chain reaction (PCR), detect a prevalence rate that is double that identified by traditional morphological and biochemical methods (Rahnama et al., 2023).

III. CLOSTRIDIUM DIFFICILE

1. **Morphological Characteristics:** *Clostridium difficile (C. difficile)* is a rod-shaped and spore-forming bacterium. The rods are usually straight or slightly curved. Individual cells of *C. difficile* can vary in size, but generally around 1.0 to 1.5 micrometers in width and 4.0 to 6.0 µm in length. This Gram-positive bacterium is also an anaerobe (Markovska et al., 2023).
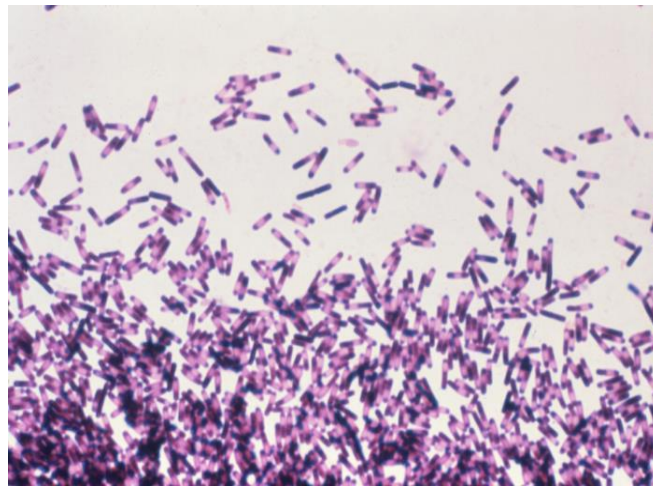

Figure 3: *This photomicrograph demonstrates the presence of abundant Clostridium difficile bacteria within an impression smear prepared on blood agar. The smear was incubated for 72 hours under anaerobic conditions to promote the growth of these oxygen-sensitive organisms.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Dr. Gilda Jones, 1980.

2. **Phenotypic and Biochemical Properties:** These bacteria achieve optimal growth on blood agar media incubated at 37°C in the absence of oxygen (anaerobic conditions). Notably, *C. difficile* lacks the enzymes catalase and superoxide dismutase, rendering it susceptible to oxidative stress.

3. **Culture Conditions:** *C. difficile* exhibits rapid growth at 37°C (human body temperature) under anaerobic conditions. For the selective and differential isolation of *C. difficile* from fecal samples, CCFA medium is employed. This specialized agar medium incorporates cycloserine, cefoxitin, fructose, and egg yolk. CCFA was found to be the most selective and sensitive medium to recover *C. difficile* (George et al., 1979).

4. **Identification Methods:** Using PCR method to detect *C. difficile* toxin gene. Due to its high sensitivity, PCR offers rapid confirmation of *C. difficile* toxin genes in fecal

samples. However, the potential for false-positive results can be effectively reduced by strategically testing only symptomatic individuals with a high likelihood of *C. difficile* infection (CDI). While PCR offers a rapid and sensitive approach, other diagnostic methods, such as gastrointestinal pathogen panels, cell cytotoxicity assays, and toxigenic stool cultures, may also be employed. Cytotoxicity assay, also known as the tissue culture assay, has traditionally been considered the gold standard for *C. difficile* diagnosis. To confirm the presence of *C. difficile* toxins, specific anti-toxin antibodies is used. It is a sensitive method to detect toxins, however the test results take 24 to 48 hours which hinder its application in clinical laboratories.

5. **Virulence Factors and Antibiotic Resistance Genes/Proteins:** The pathogenicity of *C. difficile* is primarily driven by the production of numerous exotoxins. The two primary toxins are TcdA, which has a size of 308 kDa, and TcdB, which has a size of 270 kDa. These toxins exhibit both enterotoxic and cytotoxic effects by functioning as glycosyltransferases that deactivate human Rho GTPases. This deactivation results in increased levels of proinflammatory cytokines (IL-1, TNFα, IL-8), infiltration of leukocytes, inflammation, secretion of fluids, depolymerization of actin filaments, disruption of tight junctions, apoptosis of cells, and damage to the gut epithelium. Pseudomembranous colitis, characterized by thick yellow fibrinous exudates, can occur in severe instances. Although TcdA and TcdB share 47% of their structural characteristics, they have separate processes and interact with different receptors. Based on tests conducted on human tissue, Toxin B is considered more potent, and monoclonal antibodies against TcdB are used in treatment, underscoring its significance (Markovska et al., 2023).

In addition to exotoxins, *C. difficile* generates enzymes, including collagenase, chondroitin sulfatase, and hyaluronidase which contribute to its virulence by disrupting tight junctions within the intestinal epithelium, leading to increased fluid loss and diarrhea. Additional elements that contribute to the ability of a pathogen to cause disease include surface proteins, specifically the S layer, and the production of durable spores. The formation of tough spores by *C. difficile* significantly enhances its environmental survival capabilities, allowing for prolonged persistence and potential transmission. Biofilm generation is a factor in the pathogenicity of *C. difficile*, although its mechanisms are not completely known. Biofilm formation is a significant contributor to *C. difficile* pathogenesis. Biofilms can modulate the host immune response, allowing the bacteria to evade clearance and potentially establish recurrent infections (Markovska et al., 2023).

6. **Diseases and Treatment:** *C. difficile* is spread in the form of dormant spores by the route of fecal-oral transmission. Once within the gastrointestinal system, these spores develop into active cells that can produce several toxins, leading to the development of severe illness and colitis (Edwards et al., 2013). *C. difficile* infection manifests with a constellation of symptoms, including frequent diarrhea (at least three loose stools daily), elevated body temperature, dehydration, potentially severe abdominal pain, loss of appetite, and nausea.

Treatment of *C. difficile* infections (CDIs) involves careful use of antibiotics, which are both a treatment and a risk factor for secondary infections. Standard therapy includes metronidazole and vancomycin, with vancomycin being more effective. Fidaxomicin is crucial for treating CDIs and preventing recurrences. For an initial infection, only

vancomycin or fidaxomicin are recommended, while metronidazole may be used in combination with vancomycin.

It is possible that CDIs are outocme of antibiotic treatment for other infections. If it is the case, antibiotics are considered to be stopped or switched to lower-risk options like macrolides, aminoglycosides, sulphonamides, vancomycin, or tetracyclines. Bezlotoxumab, a monoclonal antibody for preventing recurrent CDI that the FDA approved in 2016, is one of the new treatments being considered. Fecal microbiota transplantation (FMT) has shown a 75–90% success rate in preventing recurrences (Cheng & Fischer, 2023). Other emerging treatments involve toxin blockers like calcium aluminosilicate, human serum albumin, aspirin, human alpha-defensins, and ambroxol.

7. **Prevalence and Prevention:** *C. difficile* is the predominant causative agent in pseudomembranous colitis and contributes to a significant proportion (25-30%), of antibiotic-associated diarrhea cases (Biswas et al., 2023). Individuals most susceptible to CDI infection include elderly people, those residing in healthcare facilities, and patients who have undergone antibiotic therapy. In 2011, CDI killed nearly 500,000 people in the USA and about 29,000 people (Sandhu & McBride, 2018). CDI ranks as the most prevalent healthcare-associated infection within the United States, incurring substantial healthcare expenditures exceeding $4.8 billion.

Currently, there is no commercially available vaccination for *C. difficile*. Unfortunately, clinical trials investigating *C. difficile* toxin-based antigens yielded not so promising results. These vaccine candidates did not achieve adequate efficacy, as they were unable to impede the colonization and transmission of the pathogen amongst patients (Razim et al., 2023). To decrease CDI rates effectively, recommended approaches are utilizing antibiotics alongside infection control measures, including environmental disinfection, meticulous hand hygiene protocols, patient isolation practices, and the appropriate utilization of personal protective equipment (Gouliouris et al., 2011).

IV. CORYNEBACTERIUM JEIKEIUM

1. **Morphological Characteristics:** *Corynebacterium jeikeium* (*C. jeikeium*) is a rod-shaped, non-spore-forming, and non-motile, lacking the structures needed for movement. Individual cells vary in size but are generally around 0.5 to 1.5 micrometers in width and 2.0 to 6.0 µm in length. *Corynebacterium* cells may occur singly, in pairs, or V shaped or palisade arrangements. Some species may also form characteristic Chinese letter-shaped arrangements. Upon cultivation on blood agar, this Gram positive bacterium appear as small, grayish-discs colonies exhibiting a granular texture. These colonies are primarily translucent, although their central regions may appear opaque. Additionally, the colonies adopt a convex morphology with smooth, uninterrupted borders.

2. **Phenotypic and Biochemical Properties:** *C. jeikeium* can grow in both aerobic and facultatively anaerobic environments. Its metabolism can also shift to a fermentative pathway under specific conditions, producing lactic acid from carbohydrates. *C. jeikeium* is a fastidious pathogen, displaying slow growth even in enriched media. Other biochemical characteristics of *C. jeikeium* include non-hemolytic, urease-negative, and catalase positive.

3. **Culture Conditions:** Tellurite Blood Agar serves as a selective medium specifically designed to cultivate and isolate *Corynebacterium* species. All *Corynebacterium* strains require biotin as an essential nutrient for growth. Additionally, these bacteria also exhibit growth on a broader range of media, including Loeffler's medium, blood agar, and trypticase soy agar (TSA). *C. jeikeium* demonstrates optimal growth at a temperature of 37°C.

4. **Identification Methods:** For a rapid and accurate identification of *Corynebacterium* species within clinical settings, various biochemical test systems have been established. These systems often incorporate rapid 4-hour tests for the detection of urease, pyrazinamidase, catalase activity, and the ability to reduce nitrate. *C. jeikeium* is nitrate-negative, urease-negative, catalase-positive and pyrazinamidase-positive. For further identification, typing methods, whole genome sequencing, 16S rRNA gene (rDNA) sequence analysis can be used.

5. **Virulence Factors:** *C. jeikeium*'s virulence factors are probably degradative enzymes. These enzymes likely contribute to exogenous fatty acid metabolism by facilitating the breakdown of host tissue (Tauch et al., 2005). It is also able to form biofilms and resists to multiple antibiotics.

6. **Diseases and Treatment:** Residing on the human skin microbiome, *C. jeikeium* can become a problematic pathogen in hospital-acquired (nosocomial) infections, often displaying resistance to multiple antibiotic drugs. Several risk factors of *C. jeikeium* infection include extended hospitalizations, the use of broad-spectrum antibiotics, and damage to skin integrity. *C. jeikeium* is also the cause of skin and wound infections, catheter-associated infections, enteritis, meningitis, osteomyelitis, and many other infections in immunocompromised patients such as HIV (human immunodeficiency virus)-infected patients.

   Due to its intrinsic resistance to penicillins, cephalosporins, and aminoglycosides, treatment of *C. jeikeium* is a challenge. Fortunately, *C. jeikeium* remains susceptible to vancomycin, the most potent antibiotic agent and highly recommended for these infections. Besides, its susceptibility to quinolones, macrolides, tetracyclines, and rifampin exhibits significant variability depending on patients and their health records (Rezaei Bookani et al., 2017).

7. **Prevalence and Prevention:** *C. jeikeium* is responsible for most corynebacterial bacterermia (Yamamuro et al., 2021). Importantly, *C. jeikeium* infections pose a severe clinical threat, with an alarmingly high mortality rate of around 30% in infected patients. The reason for this high fatalility rate is thought to stem from its ability to form biofilm and resist to multiple antibiotics (Dowling & Koen, 2020). Preventing the nosocomial transmission of *C. jeikeium* depends on rigorous hand hygiene and strict adherence to aseptic techniques.

V. ENTEROCOCCUS FAECALIS

1. **Morphological Characteristics:** *Enterococcus faecalis (E. faecalis)* is a non-motile, non-spore-forming microorganism which is naturally found in the upper respiratory tract,

intestines, and mouths of healthy individuals. This Gram-positive bacterium displays as small, gray, and γ-hemolytic colonies on culture media.
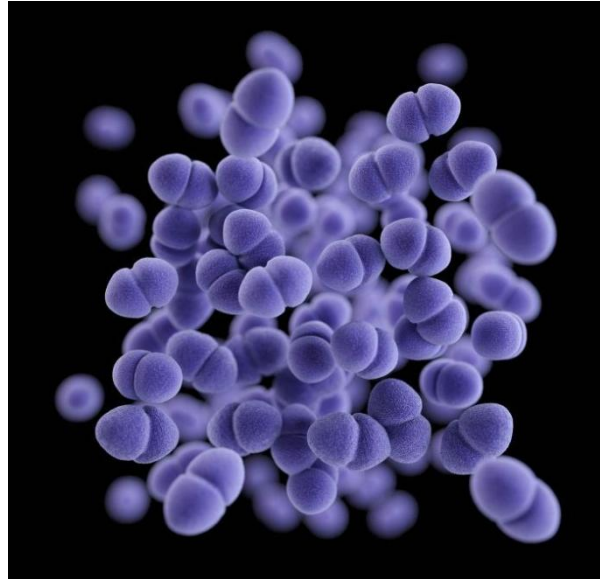

Figure 4: *A cluster of vancomycin-resistant Enterococcus (VRE) bacteria in a three-dimensional (3D) computer-generated format. The bacteria appear in pairs, known as diplococci.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Dan Higgins, 2013.

2. **Phenotypic and Biochemical Properties:** *E. faecalis* exhibits versatile biochemical characteristics. While it ferments glucose without generating gas and lacks a catalase reaction with hydrogen peroxide, it possesses the remarkable ability to catabolize a diverse range of energy sources. These substrates include glycerol, lactate, malate, citrate, arginine, agmatine, and numerous keto acids. *E. faecalis* demonstrates remarkable resilience in harsh environments. The bacterium can grow in highly alkaline conditions (pH up to 9.6) and elevated salt concentrations. It can resist to ethanol, heavy metals, bile salts, azide, detergents, and even desiccation. Furthermore, this microorganism can survive in high salt concentrations at 10 to 40°C and high pH at temperatures of 60 °C for 30 minutes (Morandi et al., 2005)

3. **Culture Conditions:** *E. faecalis* can grow on blood agar, bile esculin agar, brain heart infusion (BHI) agar/broth, and tryptic soy agar (TSA). Enterococcosel agar is a selective medium containing bile salts and sodium azide for isolating and differentiating *Enterococcus* species from other bacteria.

4. **Identification Methods:** In order to confirm the identity of *E. faecalis*, a PCR assay that detects internal segments of the *ddl* gene encoding for D-alanine-D-alanine ligase is utilized. The two primary clinically significant species, *E. faecalis* and *E. faecium* can also be distinguished using this marker (Dutka-Malen et al., 1995).

5. **Virulence Factors and Antibiotic Resistance Genes/Proteins:** *E. faecalis* poses a significant clinical challenge due to its emergence of multidrug resistance. This bacterium

further complicates treatment with ability to colonize and form biofilm. Biofilms are responsible for most infections and can exhibit up to 1000 times more resistance to antibiotics compared to planktonic status (M.-A. Kim et al., 2020). Enterococcal surface proteins play a key role in bacterial aggregation, promoting cell-to-cell adhesion. Aggregation substances, including cytosolin and gelatinase, are thought to facilitate the horizontal transfer of genetic material between *Enterococcus* cells. These factors help regulate adherence and inhibit competitive bacteria, allowing the bacteria to bind to target cells.

6. **Diseases and Treatment:** *Enterococci* have the potential to cause urinary tract infections, peritonitis, gastrointestinal tract infection and sepsis. *E. faecalis* is also recognized as a causative agent of infective endocarditis. This infection primarily affects individuals with previous heart issues or those who have undergone invasive medical operations.

   Enterococcus infections, including Vancomycin-resistant *Enterococci* (VRE) infections, cause a diverse array of symptoms based on where the infection occurs on the body. These infections can happen in the bloodstream (bacteremia), the urinary tract (urinary tract infections or UTIs), and wounds, particularly those associated with catheters or surgical procedures. Linezolid or daptomycin are used for treating VRE infections. Alternatively, assuming it is non-VRE, we have the option to provide either ampicillin or vancomycin.

7. **Prevalence and Prevention:** *Enterococci* have a broad distribution in the natural environment. They colonize a remarkably diverse range of ecological niches, including the oral cavity, gastrointestinal tract, and upper genital tract of humans and other mammals, as well as in animals such as reptiles, birds, and insects (Morandi et al., 2005). *Enterococci* have emerged as formidable nosocomial pathogens, implicated in a variety of healthcare-associated infections such as endocarditis, bacteremia, and urinary tract infections. In terms of enterococcal infections, *E. faecalis* is the cause of estimated 80–90% of cases, while *E. faecium* is responsible for the remaining cases (Sreeja et al., 2012).

Preventing infections caused by *E. faecalis*, particularly in healthcare settings, involves several strategies: infection control practices inclucing hand hygiene, environmental cleaning, and the use of personal protective equipment, antibiotic stewardship such as limiting the use of broad-spectrum antibiotics and following guidelines for appropriate antibiotic, aseptic insertion and maintenance like following strict aseptic techniques during insertion and maintenance of catheters and other invasive devices to prevent infections.

VI. LISTERIA MONOCYTOGENES

1. **Morphological Characteristics:** *Listeria monocytogenes (L. monocytogenes)* is a Gram-positive bacterium which appears purple or blue under microscopic observation. It is rod-shaped and cannot form spores. Notably, *L. monocytogenes* exhibits motility, showcasing a unique tumbling motility, motile at 22°C and non-motile at 35-37°C. At the optimal temperature of 22°C, its means of motility is flagella, however, at body temperature of around 37°C where flagella are no longer useful, it utilizes special filaments called actin rockets to move within host cells. Actin filaments form part of the cytoskeletal framework

supporting various cellular processes, and *Listeria* manipulates these filaments to move intracellularly and intercellularly, facilitating the rapid spread of infection.
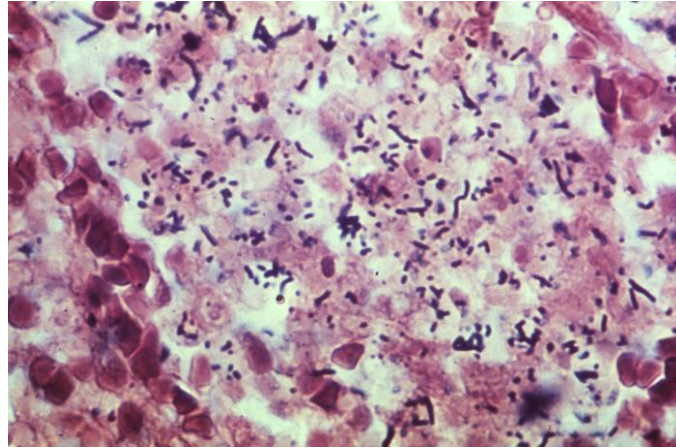

Figure 5: *Listeria monocytogenes isolated from a neonatal listeriosis patient's lung. Levaditi silver impregnation method was used to histochemically process the specimen.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Dr. Heinz Seeliger, 1965.
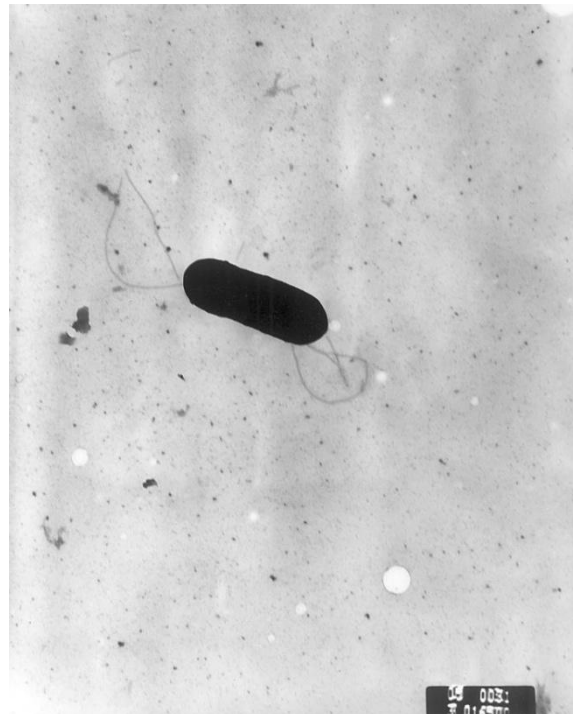

Figure 6: *Flagella of Listeria monocytogenes was observed under transmission electron microscopic (TEM) at very high magnification of 41,250X.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Elizabeth White, 2002.

2. **Phenotypic and Biochemical Properties:** *L. monocytogenes* exhibits catalase positivity, harboring the enzyme catalase. This enzyme facilitates the decomposition of hydrogen peroxide into harmless water and oxygen molecules, providing the bacterium with the ability to resist oxidative stress encountered within host environments. However, it is oxidase-negative, lacking the enzyme cytochrome c oxidase in its electron transport chain. Furthermore, *L. monocytogenes* exhibits beta-hemolytic activity, inducing complete erythrocyte lysis on blood agar plates. This results in the formation of a transparent halo encircling the bacterial colonies. Additionally, it is facultatively anaerobic, capable of thriving in both aerobic and anaerobic conditions, facilitating its adaptability to diverse environments, which contributes to its ability to infect a wide range of hosts and survive in various niches. These biochemical attributes contribute significantly to the pathogenicity and survival strategies of *L. monocytogenes*.

3. **Culture Condition and Media:** *L. monocytogenes* is an auxotrophic bacterium that requires the supplementation of seven essential amino acids including leucine, arginine, glutamine, valine, methionine, cysteine, and isoleucine and four auxiliary vitamins including riboflavin, thiamine, thioctic acid, and biotin for growth (Premaratne et al., 1991). Brain Heart Infusion (BHI) is a non-selective medium frequently used for cultivating *Listeria* species since it can offer these growth elements (Jones & D'Orazio, 2013). In addition, commonly recommended selective media for the cultivation of *L. monocytogenes* include PALCAM agar and Oxford agar. PALCAM agar consists of Columbia Blood Agar and its selectivity is achieved by the addition of polymyxin B, acriflavine, ceftazidime, and esculin. Following incubation at 37°C for 24-48 hours, *L. monocytogenes* colonies on PALCAM agar exhibit the appearance of opaque, grayish-green discs with a central jet-black coloration. Additionally, a surrounding black halo encircles the colony (Law et al., 2015). Oxford medium utilizes Columbia Blood Agar as its foundation, supplemented with several selective agents (lithium chloride, acriflavine, colistin sulfate, cycloheximide, cefotetan, and fosfomycin) to inhibit unwanted microbiota. Upon incubation at 37°C for 24 hours, *L. monocytogenes* colonies on Oxford agar exhibit a characteristic olive-green coloration with a surrounding black halo. After 48 hours, the colonies develop a central jet-black pigmentation. Additionally, the black halo intensifies, encompassing a broader zone (Law et al., 2015).

4. **Molecular Identification:** Rapid detection and identification of *L. monocytogenes* can be performed using PCR. Specific genes unique to the pathogen include internalin genes *inlA/inlB*, listeriolysin O precursor gene *hly*, phospholipase gene *plcA/plcB*, actin-assembly inducing protein precursor gene *actA* (Joseph et al., 2006). Along with PCR, enzyme-linked immunosorbent assay (ELISA) and DNA hybridization has also been used and showed equal sensitivity. In recent times, aside from DNA-based tests, RNA-targeting molecular techniques like Real-time quantitative polymerase chain reaction (RT-qPCR) and Nucleic acid-based sequence amplification (NASBA) have been created. These tests can be used for quantitative analysis for the purpose of providing a measure of cell viability (Gasanov et al., 2005).

5. **Virulence Factors:** One major characteristic of *L. monocytogenes* is the actin filaments, which are commonly referred to as "rocket tails" and especially helpful for propelling within host cell's cytoplasm in low temperature. Surface protein ActA is the virulence factor essential for inducing actin polymerization and forming actin comet tails in the pathogen (Coelho et al., 2019). Other surface-exposed proteins are internalin A and B

(InlA/InlB), with InlA primarily mediating entry into epithelial cells and InlB facilitating entry into many other cell types. These internalins also contribute to the traversal of anatomical barriers, such as the intestinal, blood-brain, and placental barriers, enabling the bacterium to cause systemic infections (Ireton et al., 2021). Listeriolysin O (LLO), a pore forming toxin, can break phagosome membranes, enable the pathogen to enter the cytosol, proliferate and minimize harm to its replicative environment within the host cell's cytosol. Activity of LLO is increased with the assistance of phospholipase PlcB, which regulates the presence of cholesterol in the lipid membrane (Petrišič et al., 2021).

6. **Diseases and Treatments:** *L. monocytogenes* is the causative agent of listeriosis, a foodborne illness contracted through the consumption of contaminated, unpasteurized dairy products and refrigerated meats. Notably, this bacterium grows at low temperatures, therefore improper storage of contaminated food in refrigerators can exacerbate the risk of infection. This paradoxical phenomenon of enhanced growth at cold temperatures is termed "cold enhancement". Listeriosis has a high mortality rate of 20-30% and affects most severely on pregnant women, adults above 65, infants, and immunocompromised patients. Infection in immunocompromised people can cause gastroenteritis with symptoms of watery diarrhea, fever, and abdominal cramps. In pregnancy, *L. monocytogenes* infection can cause abortion and premature delivery. Newborns infected at the time of delivery as well as immunocompromised individuals can develop meningitis, which usually accompanies other characteristic signs associated with meningitis (Jamshidi et al., 2009). Antibiotics used to treat Listeriosis are ampicillin and in some cases combined with gentamicin (Temple 7 Nahata, 2000).

7. **Prevalence:** Prevalence of *L. monocytogenes* infections and listeriosis in most industrialized countries has decreased over the past several decades due to improved sanitary methods. However, pregnant women still have a tendency of infection which occurs in 3.66% in women experiencing spontaneous abortion and 1.83% in women with normal deliveries (Ahmadi et al., 2022). Furthermore, in South-East Asia, the pathogen was identified to exist 16% in food, animal, and environmental sources (Jibo et al., 2022). Infection prevalence doubled during the dry season, reaching 22.2%, compared to the rainy season which is about 10.4% (Meza-Bone et al., 2023). The presence of *L. monocytogenes* in food products such as cheese and meat are from around 2% to more than 20% (Churchill et al., 2019; Meza-Bone et al., 2023).

VII. STAPHYLOCOCCUS AUREUS

1. **Morphological Characteristics:** *Staphylococcus aureus* (*S. aureus*) is coccal shaped, non-spore-forming and non-motilew Gram positive bacterium. It is facultatively anaerobic. Upon Gram staining and subsequent light microscopic examination, these bacteria frequently exhibit a clustered morphology resembling grapes. When cultivated on agar media that are rich in nutrients, *S. aureus* appears as colonies of considerable size, exhibiting either a golden or white pigmentation. This coloration stems from its carotenoids and pigments. This bacterium exhibits a versatile hemolytic profile on blood agar due to its production of a diverse array of hemolysins, including alpha, beta, gamma, and delta types. The size of individual cells can vary, but they are typically around 0.5 to 1.0 micrometers (μm) in diameter.
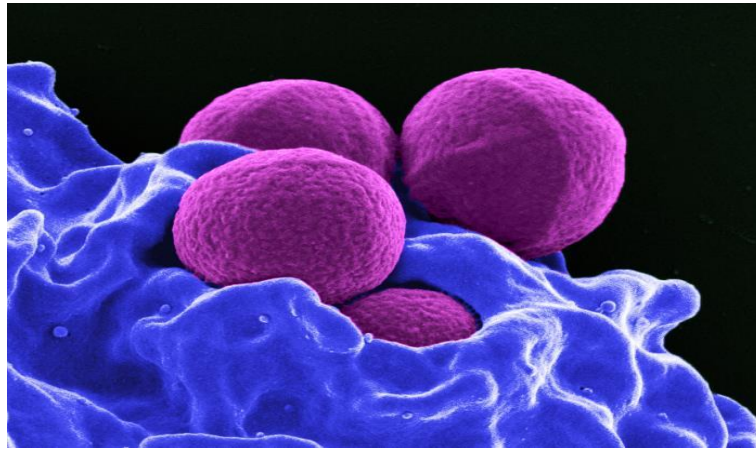
Figure 7: Four spherical, magenta-colored, methicillin-resistant Staphylococcus aureus (MRSA) bacteria undergoing phagocytosis by a blue-colored human neutrophil. Image was digitally colorized from a scanning electron microscopic (SEM) image.

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), National Institute of Allergy and Infectious Diseases (NIAID), 2011.

2. **Phenotypic and Biochemical Properties:** Some important biochemical features of *S. aureus* help to identify and comprehend its pathogenicity. These properties include catalase production and coagulase production (Cheung et al., 2021). Hemolysin production is one of the important features of *S. aureus*. This enzyme lyses erythrocytes inducing the formation of a transparent zone surrounding bacterial colonies on blood agar plates. Mannitol fermentation is another key feature, with *S. aureus* fermenting mannitol salt agar, resulting in acid production that turns the agar yellow. These biochemical characteristics contribute to *S. aureus* virulence and are also used for identification.

3. **Culture Conditions:** *S. aureus* exhibits a distinctive golden pigmentation due to the production of staphyloxanthin, a yellow pigment. This characteristic is evident when the organism is cultivated on a variety of rich media, including tryptic soy agar/broth (TSA/TSB), brain heart infusion (BHI) agar, and Luria-Bertani (LB) agar, at an optimal temperature of 37°C under aerobic conditions. Baird-Parker agar is a highly selective medium for *S. aureus*. On Baird-Parker agar supplemented with egg yolk and tellurite, *S. aureus* has the appearance of distinct black colonies encircled by a halo of precipitated lipids. This lipid precipitation is induced by the release of glycerol-ester hydrolases (lipases) (Rosenstein & Götz, 2000). The presence of tellurite and lithium chloride in Baird-Parker agar effectively suppresses the growth of most contaminating bacterial flora. Conversely, pyruvate and glycine act in synergy to selectively promote the proliferation of *S. aureus*.

4. **Identification Methods:** Initially, a Gram stain is performed as a preliminary step to provide guidance in the identification of *S. aureus*. This stain should reveal characteristic Gram-positive cocci clustered together. Subsequent isolation and cultivation can be performed on mannitol salt agar, a specialized medium containing 7.5% NaCl that promotes the growth of *S. aureus*. This growth leads to the formation of yellow colonies due to the fermentation of mannitol and the consequent decrease in the pH of the medium.

In addition, to definitively distinguish between *Staphylococcus* species, a battery of biochemical tests is performed. These tests exploit the unique metabolic capabilities of each species. The catalase test, universally positive for all staphylococci, confirms their genus membership. The coagulase test specifically identifies *S. aureus* by detecting its ability to form a fibrin clot. DNAse testing utilizes a specialized agar medium, and a clear zone surrounding the bacterial growth indicates a positive result. Lipase activity, another differentiating factor, is revealed by a characteristic yellow coloration and a rancid odor on the culture medium. Finally, the phosphatase test relies on a colorimetric indicator, producing a pink color if the enzyme is present. The coagulase test is specifically used to determine whether an infection is caused by *S. aureus.*

5. **Virulence Factors and Antibiotic Resistance Genes/Proteins:** *S. aureus* is a pathogen that produces various enzymes and toxins that enhance its pathogenicity and adaptability. These enzymes include coagulase, which converts fibrinogen to fibrin, hyaluronidase, which breaks down hyaluronic acid, deoxyribonuclease, which degrades DNA, lipase, staphylokinase, and beta-lactamase, which promote the spread of infections. Additionally, *S. aureus* secretes several exotoxins, each linked to specific pathologies. Superantigens, such as 25 identified staphylococcal enterotoxins (SEA - SEZ) and toxic shock syndrome toxin (TSST-1). These toxins have the potential to induce a severe illness known as toxic shock syndrome (TSS). This syndrome accompanies some symptoms, including fever, rash, hypotension (low blood pressure), shock, multi-organ dysfunction, and desquamation (peeling of the skin). Some strains also produce enterotoxins that cause gastroenteritis, diarrhea, abdominal pain, nausea, and vomiting. Furthermore, the activity of exfoliative toxins causes Staphylococcal scalded skin syndrome (SSSS), predominantly affecting infants and young children. *S. aureus* also produces other toxins that act on cell membranes, such as alpha, beta, and delta toxins, and bicomponent toxins like Panton-Valentine leukocidin (PVL). PVL has been implicated in the development of severe necrotizing pneumonia, particularly affecting children. These enzymes and toxins significantly contribute to the virulence and adaptability of *S. aureus*, making it a formidable pathogen.

S. aureus biofilms play a crucial role in the development of diseases, as they can enhance resistance to antibiotics and evade the immune system. The biofilm formed by *S. aureus* exhibits significant resistance to both antibiotic treatments and the immunological response of the host.
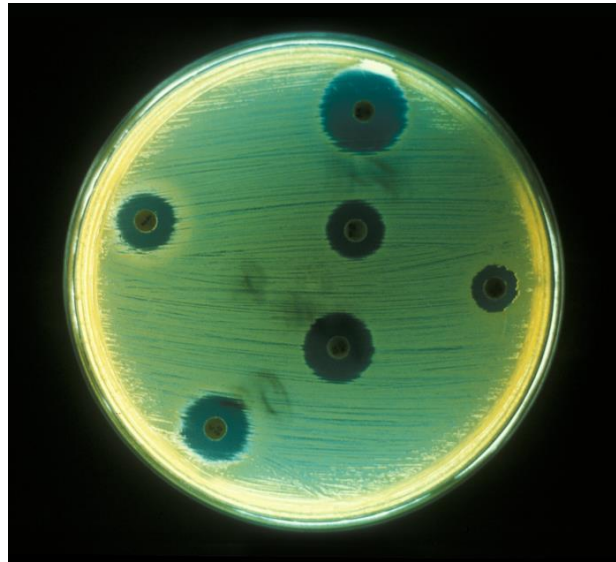
Figure 8: *This visualization portrays the facultative anaerobic Staphylococcus aureus on a Petri dish containing an unidentified culture medium. This culture was established to assess its antimicrobial susceptibility profile and was incubated under anaerobic conditions.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Don Stalons, 1972.

6. **Diseases and Treatment:** *S. aureus* colonizes the upper respiratory tract (nose and throat), hair, skin, and mucosal surfaces of healthy individuals. These commensal bacteria can, however, become opportunistic pathogens, causing a variety of skin and soft tissue infections, including abscesses and cellulitis. *S. aureus* can also induce severe infections, including pneumonia (lung infection) and bacteremia (bloodstream infection). Malaise, fever, shivers, and difficulty breathing are some of the symptoms of these infections.

Figure 9: *Streptococcal impetigo causes lesions on the volar surface of the patient's forearm. Streptococcal impetigo is a common dermatological condition most frequently caused by Staphylococcus aureus bacteria.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC),Dr. Herman Miranda, Univ.of Trujello, Peru; A. Chambers, 1964.

Infections involving *S. aureus* are treated with antibiotics. Penicillin is the preferred treatment for *S. aureus* infection in susceptible strains. However, in the majority of countries, penicillin resistance is exceedingly prevalent (>90%). The initial therapeutic approach typically involves the administration of a penicillinase-resistant beta-lactam antibiotic, such as oxacillin or flucloxacillin (both of which exhibit a mechanism of action similar to penicillin), or vancomycin. The specific choice is based on the prevailing patterns of antibiotic resistance within the local area. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of several strains of *S. aureus* that have developed resistance to the majority of β-lactam antibiotics. Vancomycin, a glycopeptide antibiotic, is often used to treat MRSA as a result.

7. **Prevalence and Prevention:** In 2019, *S. aureus* was identified as the second most prevalent pathogen causing deaths associated with antimicrobial resistance (Zhang et al., 2023). Developing a vaccine against *S.aureus* has been unsuccessful for over a decade. StaphVAX (Nabi Biopharmaceuticals, Rockville, MD) and V710 (Merck, Kenilworth, NJ) are notable examples. A critical challenge lies in identifying immunological markers that correlate with protective immunity against *S. aureus*.

VIII.STAPHYLOCOCCUS EPIDERMIDIS

1. **Morphological Characteristics:** *Staphylococcus epidermidis (S. epidermidis)* is a coagulase-negative, catalase-positive, and facultative anaerobic Gram- positive bacteria that forms clusters. *S. epidermidis* cells are in cocci shape which is around 0.5 to 1.5 micrometers in diameter. It is frequently isolated from human epithelium, especially from axillae, nares, and the head.
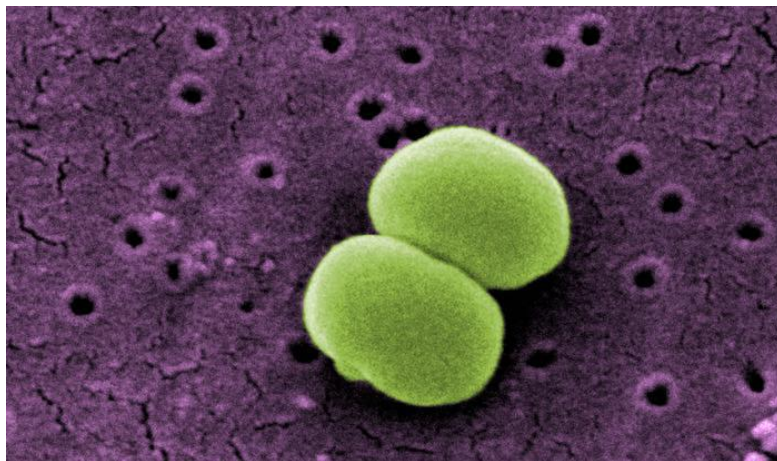

Figure 10: *Two Staphylococcus epidermidis bacteria observed by scanning electron microscopic (SEM) and digitally colored.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Janice Haney Carr.

2. **Phenotypic and Biochemical Properties:** *S. epidermidis* is a oxidase-negative and catalase-positive Gram-positive bacterium. It shows negative results for the Methyl Red test and Indole test. In contrast, it is positive for hydrogen sulfide (H_2S) production,

urease, nitrate reductase, Voges-Proskauer test and β-galactosidase activity test (Paul et al., 2021). These biochemical properties help distinguish *S. epidermidis* from other *Staphylococcus* species and other genera.

3. **Culture Conditions:** Staphylococci are generally cultured in blood, tryptic soy, or heart infusion agar. The culture can also be introduced onto mannitol salt agar containing 7.5% sodium chloride, as *S. epidermidis* can tolerate high salt concentrations.

4. **Identification Methods:** The identification of *S. epidermidis* typically employs a multifaceted approach, combining the analysis of colony morphology on selective media with confirmatory biochemical tests. Light microscopy offers an initial visual assessment, while catalase and slide coagulase testing provide key biochemical markers for differentiation. Zobell agar serves as a valuable tool for its selective isolation of *S. epidermidis* from marine environments (Paul et al., 2021). Conversely, on Baird-Parker agar supplemented with egg yolk, *S. epidermidis* colonies exhibit a characteristically small and black appearance. Real-time polymerase chain reaction (RT-PCR) is also being used to rapidly detect *S. epidermidis* (Kim et al., 2021). Besides, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) is applied to distinguish different CoNS including *S. epidermidis*. The final diagnosis relies on the combination of clinical symptoms and paraclinical studies. The selected tests depend not only on the suspected infection type but also on local guidelines.

5. **Virulence Factors and Antibiotic Resistance Genes/Proteins:** The main component contributing to the pathogenicity of *S. epidermidis* is biofilms. This occurs when the bacteria generate a substance called polysaccharide intercellular adhesin (PIA), which enables the pathogen to attach to the pre-existing biofilm and form many layers. These biofilms serve as a protective shield for the bacteria within, as they exhibit a decreasing metabolic rate, making them less susceptible to antibiotics, and consequently complicating infection clearance (Otto, 2009). Strains of *S. epidermidis* commonly exhibit resistance to many antibiotics, including gentamicin, clindamycin, fluoroquinolones, tetracycline, and sulfonamides, and rifamycin. Methicillin resistance presents a significant challenge in healthcare settings, with a concerning prevalence of 75-90% observed among hospital-acquired *S. epidermidis* isolates (Otto, 2009). Strains of Methicillin-Resistant *S. epidermidis* (MRSE) possess the *mecA* gene, which grants them resistance to methicillin and other beta-lactam antibiotics. This resistance complicates treatment options and enhances the persistence of infections.

6. **Diseases and Treatment:** *S. epidermidis* is a prevalent symbiotic organism found on the skin as well as mucous membranes of mammals including human. Typically, this bacterium poses no threat to individuals in good health. It is a disease with low virulence that can infect patients with weakened immune systems or those with implants. Moreover, *S. epidermidis* can form biofilm on any abiotic surface.

7. **Prevalence and Prevention:** *S. epidermidis* is responsible for 30 to 43% of infections in artificial joints. It also causes about 22% of bloodstream infections in intensive care units, almost 13% of infections in prosthetic valves, and most cases of sepsis in neonates. These infections increase mortality, prolonged hospitalization, and additional surgeries (Otto, 2009). Vaccination and decolonization are not suitable for *S. epidermidis*. It is widely accepted that prevention is the most effective approach to managing *S. epidermidis*

infections (Skovdal et al., 2022). This involves sterilizing medical equipment and the implementation of hand hygiene protocols for both patients and healthcare personnel who have contact with indwelling medical devices during surgery.

IX. STREPTOCOCCUS PNEUMONIAE

1. **Morphological Characteristics:** *Streptococcus pneumoniae (S. pneumoniae)* is a lancet-shaped diplococci, typically occurring in pairs of two cocci under microscopic examination. This Gram- positive pathogen is encapsulated in polysaccharides, which protects against phagocytosis, facilitating colonization and invasion of host tissues. Additionally, *S. pneumoniae* expresses surface proteins like pneumococcal surface protein A (PspA) and pneumolysin, pivotal in colonization and pathogenesis. Furthermore, it utilizes various adhesins to adhere to host cells and mucosal surfaces, facilitating colonization and the establishment of infections. These features collectively contribute to the pathogenicity and clinical significance of *S. pneumoniae* as a primary cause of respiratory tract and invasive infections.
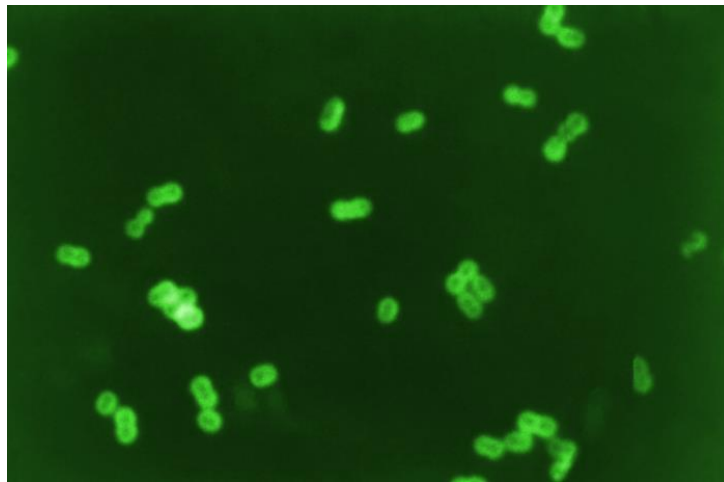

Figure 11*: Streptococcus pneumoniae are visible in this digitally colorized photomicrograph of the fluorescent processed sample, illustrating the diplococcus appearance of two lance-shaped cocci.*

Source: Public Health Image Library, Center for Disease Control and Prevention, Dr. M.S. Mitchell, 1964.

2. **Phenotypic and Biochemical Properties:** *S. pneumoniae* is catalase-negative, which distinguishes it from catalase-positive ones like *Staphylococcus aureus*. It demonstrates alpha-hemolysis on blood agar, characterized by a greenish halo encircling the colonies because of partial lysis of erythrocytes. Another distinctive trait of *S. pneumoniae* is the susceptibility to Optochin. The inhibition in the presence of optochin helps to differentiate this bacterium from other alpha-hemolytic streptococci such as *Streptococcus viridans* and *Streptococcus mitis*. Furthermore, *S. pneumoniae* is bile soluble, a unique feature that allows it to lyse in the presence of bile salts, facilitating its identification in laboratory settings. Lastly, *S. pneumoniae* is facultative anaerobic, capable of thriving in both aerobic and anaerobic conditions, reflecting its adaptability to various environments within the host.

3. **Culture Conditions and Media:** Luria-Bertani (LB) Agar in solid and liquid form is the most common media for culturing *S. pneumoniae*. Other well-known liquid media for this pathogen are Tryptic soy broth (TSB) and Todd Hewitt broth (THB). These media facilitate the isolation and growth of the desired bacterium, enabling the subsequent purification of its serotype-specific capsular polysaccharides, which serve as crucial antigens in vaccine formulations (Suárez & Texeira, 2019; Kim et al., 1996). Defibrinated blood of sheep, horse, and even humans can be supplemented into the agar as well due to the high metabolic needs of the bacteria and supports hemolysis observation (Suárez & Texeira, 2019).

4. **Molecular Identification:** *lytA* (major autolysin gene) and *piaB* (permease gene of the ABC transporter) were reported to be the target genes with markedly high specificity of 99.5% for the identification of *S. pneumoniae*. Another novel putative transcriptional regulator gene *sp2020* was recorded to possess even higher specificity (99.8%) (Tavares et al., 2019). Other genes with less specificity include *plyA* (pneumolysin), and *psaA* (lipoprotein component) (Sanz et al., 2018). Notably, different types of detection methods result in different specificity. One study found that identification of *lytA* and *blpA* using conventional Polymerase chain reaction (PCR) resulted in specificity of 87.71% and 89.47% respectively, while Real-time PCR gave significantly higher percentage with both 96.49% for *lytA* and *blpA* (Mosadegh et al., 2020).

5. **Virulence Factors:** *S. pneumoniae* possesses several key virulence factors that contribute to its pathogenicity. Firstly, the polysaccharide capsule is antiphagocytic, inhibiting complement deposition on bacterial surface and can protect itself from being trapped in neutrophil extracellular traps. There are also surface proteins PspA, PspC, and LytA which act as adhesins to host cell surfaces, inhibit complement deposition (PspA), involve in biofilm formation and haemolytic effects of *S. pneumoniae* on human red blood cells (LytA). Furthermore, metal-ion-binding proteins PsaA, PiaA, and PiuA are involved in iron uptake and play a role in promoting opsonophagocytosis of *S. pneumoniae*. Other enzymes include autolysin, pneumolysin, IgA protease are crucial pneumococcal-protein virulence factors as well (Aryal, 2022; Kadioglu et al., 2008).

6. **Diseases and Treatments:** *S. pneumoniae* is mostly known as the cause of community-acquired pneumonia, especially in children, the elderly, and immunocompromised patients. *S. pneumoniae* holds the ability to cause a broad array of illnesses, from moderate infections like otitis media and sinusitis to potentially fatal infections of the central nervous system like meningitis. Aside from mucosal infections, it also has the ability to infect the bone, resulting in osteomyelitis, the joint system, causing septic arthritis, and the blood, causing bacteremia. Interestingly, *S. pneumoniae* is detected frequently in patients with Overwhelming post-splenectomy infection (OPSI) (42%) (Theilacker et al., 2015). Primary antibiotics treatments for *S. pneumoniae* are β-lactam antibiotics including penicillins and cephalosporins, macrolides, and fluoroquinolones. However, due to the increasing resistance to β-lactam and macrolides, fluoroquinolones which possess the lowest rate of resistance are highly recommended. Another option can be vancomycin, although it may become resistant in the near future (Zahari et al., 2023).

7. **Prevalence:** The prevalence of young children infected with *S. pneumoniae* is observably higher than adults. A study in Central Vietnam found that *S. pneumoniae* exists in 31.8% of children, and 23.9% of those had clinical pneumonia. Furthermore, the detection of *S.*

pneumoniae was greater in the age groups of 0–1 and 2-3 years, and lowest in children older than five years old. (Wambugu et al., 2023). In a study with children under 5 years old in Ethiopia, the general prevalence of *S. pneumoniae* in is 18% (CI 95%) (Mekuria et al., 2023), while another paper reported to identify 40.7% cases infected with this pathogen among community-acquired pneumonia patients in the UK (Lansbury et al., 2023). Some of the most identified serotypes in these cases are 6A/B, 19F, and 23F (Wambugu et al., 2023; Mekuria et al., 2023).

X. STREPTOCOCCUS PYOGENES

Streptococcus pyogenes belongs to the Lancefield serogroup A and is often referred to as Group A Streptococcus (GAS).

1. **Morphological Characteristics:** *Streptococcus pyogenes (S. pyogenes)* presents a distinct morphological profile characterized by its round shape and tendency to form chain-like structures. This Gram- positive pathogen lacks flagella resulting in its non-motility, and it does not form spores. Notably, this pathogen possesses a protective capsule primarily composed of hyaluronic acid, differentiating it from other encapsulated bacteria. This capsule provides protection against macrophage engulfment. Moreover, *S. pyogenes* has fimbriae, which are hair-like protein structures on its surface, aiding in adherence to host cells and biofilm formation.

Figure 12: *A digitally regenerated image of Streptococcus pyogenes was created with scanning electron microscopy (SEM).*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC)/ Antibiotic Resistance Coordination and Strategy Unit, 2013.

2. **Phenotypic and Biochemical Properties:** *S. pyogenes* lacks the catalase enzyme, a characteristic feature that sets it apart from catalase-positive organisms. Notably, *S. pyogenes* displays beta-hemolysis on blood agar, indicative of its ability to completely lyse red blood cells. And the reason for the lysis of red blood cells is because of an enzyme called streptolysin O (SLO) which interacts with the cholesterol membrane on

red blood cells and completely lyses them (Duncan & Schlegel, 1975). Its sensitivity to bacitracin further aids in its identification, as it cannot survive in the presence of this antibiotic. Moreover, *S. pyogenes* is Pyrrolidonyl Aryl Sulfatase (PYR) positive, a trait that differentiates it from other beta-hemolytic enterococci, serving as a crucial diagnostic marker in clinical microbiology.
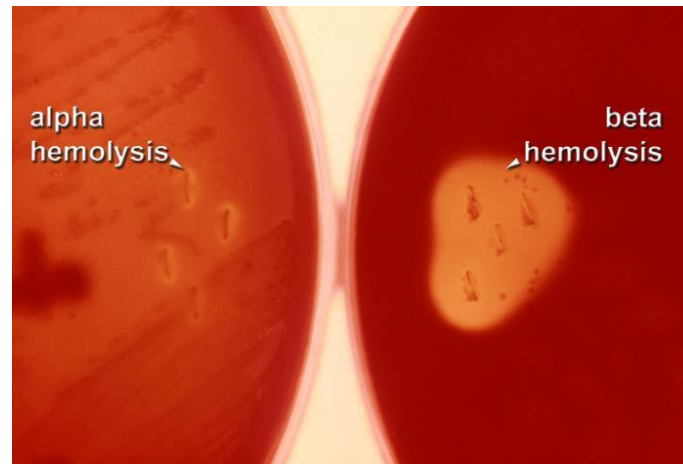

Figure 13*: The right plate is Group A Streptococcus pyogenes (GAS), a typical beta-hemolytic bacteria with clear zone of red blood cell lysis, while the left plate is Streptococcus mitis, an alpha-hemolytic bacteria. Two petri dishes of trypticase soy agar medium both contain 5% defibrinated sheep blood.*

Source: Public Health Image Library, Center for Disease Control and Prevention (CDC), Dr. Richard R. Facklam, 1977.

3. **Culture Conditions and Media:** Selective culture media commonly used for the growth of Streptococcus pyogenes include Columbia CNA agar with colistin and nalidixic acid, Phenylethyl Alcohol (PEA) agar, and Tryptic soy agar (TSA) with 5% defibrinated sheep blood (Spellerberg & Brandt, 2016). Columbia CNA agar has two antibiotics—nalidixic acid and colistin—that prevent gram-negative bacteria like *Enterobacteriaceae* and *Pseudomonas* species from growing, and favor gram-positive bacteria like *Staphylococci, Streptococci*, and *Enterococci*. Likewise, PEA agar is selective for *S. pyogenes* due to the inhibitory effect of PEA on gram-negative bacteria and fungi, while allowing the growth of gram-positive organisms.

4. **Molecular Identification:** *spy1258* is highly specific and is the most commonly used gene for the detection of *S. pyogenes*. Studies have shown that the *spy1258* PCR assay has the specificity of 93.85% to 100% for detecting the pathogen, as it only amplifies DNA from *S. pyogenes* and not from other *Streptococcus* species or common bacteria. Besides *spy1258*, genes encoded for virulence factors are also utilized as target genes such as *dnaseB, speB, and sof*. However, the specificity of these genes is relatively poorer than *spy1258* (Abraham & Sistla, 2016). Molecular identification method employed is usually PCR.

5. **Virulence Factors:** *S. pyogenes* possesses a wide range of virulence factors from the unique capsule and surface-associated proteins to secreted enzymes and toxins. A

hyaluronic capsule of *S. pyogenes* protects the pathogen from phagocytosis by the host immune system's neutrophils, while M protein embedded on the cell wall facilitates adherence to host cells and disrupts opsonization via the alternative complement pathway. Other cell wall components such as lipoteichoic acid and protein F (Sfbl), which is fibronectin-binding protein, further promotes epithelial host cell attachment. Moreover, some of the secreted virulence factors include Streptolysin O (SLO) and streptolysin S (SLS), which are hemolysins that lyse red blood cells, streptokinase which activates plasminogen to plasmin, aiding in tissue penetration, as well as hyaluronidase that breaks down hyaluronic acid in host tissues (Barnett et al., 2022). There are also Streptococcal pyrogenic exotoxins SpeA and SpeC which cause rash of scarlet fever and many other symptoms of Streptococcal Toxic Shock Syndrome (STSS) (Earhart et al., 2000).

6. **Diseases and Treatments:** *S. pyogenes* can cause a wide range of infections, from non-invasive ones such as pharyngitis (sore throat) and impetigo (mild skin infection), to severely fatal invasive ones such as necrotizing fasciitis, pneumonia, bacteremia, and Streptococcal Toxic Shock Syndrome (STSS). Necrotizing fasciitis is a deadly skin and soft tissue infection famously known as "flesh-eating disease", infected patients have a high fatality rate of 20-80% (Wallace & Perera, 2023). Streptococcal Toxic Shock Syndrome (STSS) is a rare disease defined by a series of acute symptoms including sudden shock, low blood pressure, organ failure, and in some worst cases, death. For pharyngitis, the recommended drug is penicillin. For fatal illnesses, it is advised to treat with vancomycin or clindamycin, along with removal of necrotic tissue in necrotizing fasciitis patients (Kanwal & Vaitla, 2023).

7. **Prevalence:** The prevalence of *S. pyogenes* among acute pharyngitis patients in Northwest Ethiopia is 9.1% which is comparatively lower than other regions such as Indonesia 13.5%, Jimma, Ethiopia 11.3%, Nepal 9.2%, Japan 5.8%, India 5.5%. However, it was more significant than studies from Romania (4%), Brazil (3.9%), Iran (2.5%), Saudi Arabia (1.5%), and Mexico (0.04–0.42%). Nonetheless, studies have recognized the prevalence of this bacteria is 16-45% in African, 28.6-37% in USA, 30% in Iran and 69.5% in Israel (Kebede et al., 2021). This comes to show that the detection of *S. pyogenes* depends on sample sizes, geographical features, climate conditions at the sample collection period, as well as the detection method. Notably, pharyngeal infection in children older than three years old is higher than other age groups due to the exposure with the pathogen in schools, playgrounds, etc., and naturally weaker immune system in children (Efstratiou & Lamagni, 2022).

ACKNOWLEDGMENT

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