PAVENDAR BHARATHIDASAN COLLEGE OF ARTS AND SCIENCE

APPLIED MICROBIOLOGY

I B.Sc., BIOTECHNOLOGY

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Unit IV

Gram-positive bacteria (Staphylococcus aureus, Mycoplasma)

Gram-positive bacteria are bacteria that give a positive result in the Gram stain test, which is traditionally used to quickly classify bacteria into two broad categories according to their cell wall.

Gram-positive bacteria take up the crystal violet stain used in the test, and then appear to be purple-coloured when seen through an optical microscope. This is because the thick peptidoglycan layer in the bacterial cell wall retains the stain after it is washed away from the rest of the sample, in the decolorization stage of the test.

Gram-negative bacteria cannot retain the violet stain after the decolorization step; alcohol used in this stage degrades the outer membrane of gram-negative cells, making the cell wall more porous and incapable of retaining the crystal violet stain. Their peptidoglycan layer is much thinner and sandwiched between an inner cell membrane and a bacterial outer membrane, causing them to take up the counterstain (safranin or fuchsine) and appear red or pink.

Despite their thicker peptidoglycan layer, gram-positive bacteria are more receptive to certain cell wall targeting antibiotics than gram-negative bacteria, due to the absence of the outer membrane.

Characteristics

- Cytoplasmic lipid membrane
- Thick peptidoglycan layer

- Teichoic acids and lipoids are present, forming lipoteichoic acids, which serve as chelating agents, and also for certain types of adherence.
- Peptidoglycan chains are cross-linked to form rigid cell walls by a bacterial enzyme DD-transpeptidase.
- A much smaller volume of periplasm than that in gram-negative bacteria.

Only some species have a capsule, usually consisting of polysaccharides. Also, only some species are flagellates, and when they do have flagella, have only two basal body rings to support them, whereas gram-negative have four. Both gram-positive and gram-negative bacteria commonly have a surface layer called an S-layer. In gram-positive bacteria, the S-layer is attached to the peptidoglycan layer. Gram-negative bacteria's S-layer is attached directly to the outer membrane. Specific to gram-positive bacteria is the presence of teichoic acids in the cell wall. Some of these are lipoteichoic acids, which have a lipid component in the cell membrane that can assist in anchoring the peptidoglycan.

Staphylococcus

The genus Staphylococcus also belongs to the class Bacilli, even though its shape is coccus rather than a bacillus. The name Staphylococcus comes from a Greek word for bunches of grapes, which describes their microscopic appearance in culture (Figure 5). Staphylococcus spp. are facultative anaerobic, halophilic, and nonmotile. The two best-studied species of this genus are S. epidermidis and S. aureus.

Strains of S. aureus cause a wide variety of infections in humans, including skin infections that produce boils, carbuncles, cellulitis, or impetigo. Certain strains of S. aureus produce a substance called enterotoxin, which can cause severe enteritis, often called staph food poisoning. Some strains of S. aureus produce the toxin responsible for toxic shock syndrome, which can result in cardiovascular collapse and death.

Mycoplasmas

Although Mycoplasma spp. do not possess a cell wall and, therefore, are not stained by Gram-stain reagents, this genus is still included with the low G+C gram-positive bacteria. The genus Mycoplasma includes more than 100 species, which share several unique characteristics.

They are very small cells, some with a diameter of about 0.2 μm, which is smaller than some large viruses. They have no cell walls and, therefore, are pleomorphic, meaning that they may take on a variety of shapes and can even resemble very small animal cells. Because they lack a characteristic shape, they can be difficult to identify. One species, M. pneumoniae, causes the mild form of pneumonia known as “walking pneumonia” or “atypical pneumonia.” This form of pneumonia is typically less severe than forms caused by other bacteria or viruses.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although S. aureus usually acts as a commensal of the human microbiota it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of S. aureus such as methicillin-resistant S. aureus (MRSA) is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for S. aureus has been approved.

An estimated 20% to 30% of the human population are long-term carriers S. aureus which can be found as part of the normal skin flora, in the nostrils, and as a normal inhabitant of the lower reproductive tract of women. S. aureus can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery. Each year, around 500,000 patients in hospitals of the United States contract a staphylococcal infection, chiefly by S. aureus. Up to 50,000 deaths each year in the USA are linked with S. aureus infections.

Role in health

In humans, S. aureus is not part of the normal microbiota present in the upper respiratory tract or gut mucosa or on the skin; rather, when it is prevalent here, it is a colonization.

S. aureus, along with similar species, can colonize and act symbiotically but can cause disease if they begin to take over the tissues they have colonized or invade other tissues, and as such they have been called "pathobionts"

Role in disease

While S. aureus usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population, it can sometimes cause disease. In particular, S. aureus is one of the most common causes of bacteremia and infective endocarditis. Additionally, it can cause various skin and soft-tissue infections, particularly when skin or mucosal barriers have been breached.

S. aureus infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person, and contact with objects used by an infected person such as towels, sheets, clothing, or athletic equipment. Joint replacements put a person at particular risk of septic arthritis, staphylococcal endocarditis (infection of the heart valves), and pneumonia.

Preventive measures include washing hands often with soap and making sure to bathe or shower daily.

S. aureus is a significant cause of chronic biofilm infections on medical implants, and the repressor of toxins is part of the infection pathway.

S. aureus can lay dormant in the body for years undetected. Once symptoms begin to show, the host is contagious for another two weeks, and the overall illness lasts a few weeks. If untreated, though, the disease can be deadly. Deeply penetrating S. aureus infections can be severe.

Skin infections

Skin infections are the most common form of S. aureus infection. This can manifest in various ways, including small benign boils, folliculitis, impetigo, cellulitis, and more severe, invasive soft-tissue infections.

S. aureus is extremely prevalent in persons with atopic dermatitis, more commonly known as eczema. It is mostly found in fertile, active places, including the armpits, hair, and scalp. Large pimples that appear in those areas may exacerbate the infection if lacerated. This can lead to staphylococcal scalded skin syndrome, a severe form of which can be seen in newborns.

The presence of S. aureus in persons with atopic dermatitis is not an indication to treat with oral antibiotics, as evidence has not shown this to give benefit to the patient. However, topical antibiotics combined with corticosteroids have been found to improve the condition. Colonization of S. aureus drives inflammation of atopic dermatitis; S. aureus is believed to exploit defects in the skin barrier of persons with atopic dermatitis, triggering cytokine expression and therefore exacerbating symptoms.

Food poisoning

S. aureus is also responsible for food poisoning. It is capable of generating toxins that produce food poisoning in the human body. Its incubation period lasts one to six hours, with the illness itself lasting from 30 minutes to 3 days. Preventive measures one can take to help prevent the spread of the disease include washing hands thoroughly with soap and water before preparing food. Stay away from any food if ill, and wear gloves if any open wounds occur on hands or wrists while preparing food. If storing food for longer than 2 hours, keep the food below 5 or above 63 °C.

Bone and joint infections

S. aureus is the bacterium commonly responsible for all major bone and joint infections. This manifests in one of three forms: osteomyelitis, septic arthritis, and infection from a replacement joint surgery.

Bacteremia

S. aureus is a leading cause of bloodstream infections throughout much of the industrialized world. Infection is generally associated with breaks in the skin or mucosal membranes due to surgery, injury, or use of intravascular devices such as catheters, hemodialysis machines, or

injected drugs. Once the bacteria have entered the bloodstream, they can infect various organs, causing infective endocarditis, septic arthritis, and osteomyelitis. This disease is particularly prevalent and severe in the very young and very old.

Without antibiotic treatment, S. aureus bacteremia has a case fatality rate around 80%. With antibiotic treatment, case fatality rates range from 15% to 50% depending on the age and health of the patient, as well as the antibiotic resistance of the S. aureus strain.

Mycoplasma

Mycoplasma is a genus of bacteria that lack a cell wall around their cell membranes. This characteristic makes them naturally resistant to antibiotics that target cell wall synthesis (like the beta-lactam antibiotics). They can be parasitic or saprotrophic. Several species are pathogenic in humans, including *M. pneumoniae*, which is an important cause of "walking" pneumonia and other respiratory disorders, and *M. genitalium*, which is believed to be involved in pelvic inflammatory diseases. *Mycoplasma* species are the smallest bacterial cells yet discovered, can survive without oxygen, and come in various shapes. For example, *M. genitalium* is flask-shaped (about 300 x 600 nm), while *M. pneumoniae* is more elongated (about 100 x 1000 nm). Hundreds of mycoplasma species infect animals.

Characteristics

- Over 100 species have been included in the genus Mycoplasma. Microbes of the class Mollicutes, to which Mycoplasma belongs, are parasites or commensals of humans, animals, and plants. The genus Mycoplasma uses vertebrate and arthropod hosts. Dietary nitrogen availability has been shown to alter codon bias and genome evolution in Mycoplasma and Phytoplasma.
- Mycoplasmal bacteria are also known as mollicutes. They are the simplest and the smallest free-living prokaryotes.
- Mycoplasmal bacteria have been found in the pleural cavities of cattle suffering from pleuropneumonia. These organisms are often called MLO (mycoplasma-like organisms) or PPLO (pleuropneumonia-like organisms).

Important characteristics of mycoplasmal bacteria

1. Cell wall is absent and plasma membrane forms the outer boundary of the cell.
2. Due to the absence of cell wall these organisms can change their shape and are pleomorphic.
3. Lack of nucleus and other membrane-bound organelles.
4. Genetic material is a single DNA duplex and is naked.
5. Ribosomes are 70S type.
6. Possess a replicating disc at one end which assist replication process and also the separation of the genetic materials.
7. Heterotrophic nutrition. Some live as saprophytes but the majority are parasites of plants and animals. The parasitic nature is due to the inability of mycoplasmal bacteria to synthesise the required growth factor.

Cell morphology

Due to the lack of a rigid cell wall, Mycoplasmataceae can contort into a broad range of shapes, from round to oblong. They therefore cannot be classified as rods, cocci or spirochetes.

Pathogenicity

The P1 antigen is the primary virulence factor of mycoplasma. P1 is a membrane associated protein that allows adhesion to epithelial cells. The P1 receptor is also expressed on erythrocytes which can lead to autoantibody agglutination from mycobacteria infection. Several Mycoplasma species can cause disease, including M. pneumoniae, which is an important cause of atypical pneumonia (formerly known as "walking pneumonia"), and M. genitalium, which has been associated with pelvic inflammatory diseases. Mycoplasma infections in humans are associated with skin eruptions in 17% of cases.

Sexually transmitted infections

Mycoplasma and Ureaplasma species are not part of the normal vaginal flora. Some Mycoplasma species are spread through sexual contact.

Infertility

Some mycoplasmae have a negative effect on fertility. M. hominis causes male sterility/Genitals inflammation in humans.

Infant mortality

Low birth-weight, preterm infants are susceptible to Mycoplasma infections

Parasitic infections

Entamoeba histolytica

- Entamoeba histolytica is an anaerobic parasitic amoebozoan, part of the genus Entamoeba. Predominantly infecting humans and other primates causing amoebiasis, E. histolytica is estimated to infect about 50 million people worldwide. E. histolytica infection is estimated to kill more than 55,000 people each year. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, E. dispar. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission.
- The word histolysis literally means disintegration and dissolution of organic tissues.

Transmission

The active (trophozoite) stage exists only in the host and in fresh loose feces; cysts survive outside the host in water, in soils, and on foods, especially under moist conditions on the latter. The infection can occur when a person puts anything into their mouth that has touched the feces of a person who is infected with E. histolytica, swallows something, such as water or food, that is contaminated with E. histolytica, or swallows E. histolytica cysts (eggs) picked up from contaminated surfaces or fingers. The cysts are readily killed by heat and by freezing temperatures, and survive for only a few months outside of the host

Pathology

In the vast majority of cases, infection is asymptomatic and the carrier is unaware they are infected. However, in an estimated 10% of cases E. histolytica causes disease. Once the trophozoites are excysted they colonize the large bowel, remaining on the surface of the mucus layer and feeding on bacteria and food particles. Occasionally, and in response to unknown stimuli, trophozoites move through the mucus layer where they come in contact with the epithelial cell layer and start the pathological process. E. histolytica has a lectin that binds to galactose and N-acetylgalactosamine sugars on the surface of the epithelial cells, The lectin normally is used to bind bacteria for ingestion. The parasite has several enzymes such as pore forming proteins, lipases, and cysteine proteases, which are normally used to digest bacteria in food vacuoles but which can cause lysis of the epithelial cells by inducing cellular necrosis and apoptosis when the trophozoite comes in contact with them and binds via the lectin. Enzymes released allow penetration into intestinal wall and blood vessels, sometimes on to liver and other organs. The trophozoites will then ingest these dead cells. This damage to the epithelial cell layer attracts human immune cells and these in turn can be lysed by the trophozoite, which releases the immune cell's own lytic enzymes into the surrounding tissue, creating a type of chain reaction and leading to tissue destruction. This destruction manifests itself in the form of an 'ulcer' in the tissue, typically described as flask-shaped because of its appearance in transverse section. This tissue destruction can also involve blood vessels leading to bloody diarrhea, amebic dysentery. Occasionally, trophozoites enter the bloodstream where they are transported typically to the liver via the portal system. In the liver a similar pathological sequence ensues, leading to amebic liver abscesses. The trophozoites can also end up in other organs, sometimes via the bloodstream, sometimes via liver abscess rupture or fistulas. In all locations, similar pathology can occur.

Transcriptomic study of E. histolytica for promoter analysis of variable expression class of all the genes reveals that the highly transcribed genes of E. histolytica belongs to virulence factor genes. The study also have reported about the presence of novel downstream regulatory motifs in E. histolytica

Pathogen interaction

E. histolytica may modulate the virulence of certain human viruses and is itself a host for its own viruses.

For example, AIDS accentuates the damage and pathogenicity of E. histolytica. On the other hand, cells infected with HIV are often consumed by E. histolytica. Infective HIV remains viable within the amoeba, although there has been no proof of human reinfection from amoeba carrying this virus.

Pathogenesis

E. histolytica causes tissue destruction which leads to clinical disease. E. histolytica–induced tissue damage by three main events: direct host cell death, inflammation, and parasite invasion.

Diagnosis

Diagnosis is confirmed by microscopic examination for trophozoites or cysts in fresh or suitably preserved faecal specimens, smears of aspirates or scrapings obtained by proctoscopy, and aspirates of abscesses or other tissue specimen. A blood test is also available but is only recommended when a healthcare provider believes the infection may have spread beyond the intestine (gut) to some other organ of the body, such as the liver. However, this blood test may not be helpful in diagnosing current illness because the test can be positive if the patient has had amebiasis in the past, even if they are not infected at present. Stool antigen detection and PCR are available for diagnosis, and are more sensitive and specific than microscopy.

Plasmodium vivax

Plasmodium vivax is a protozoal parasite and a human pathogen. This parasite is the most frequent and widely distributed cause of recurring malaria. Although it is less virulent than Plasmodium falciparum, the deadliest of the five human malaria parasites, P. vivax malaria infections can lead to severe disease and death, often due to splenomegaly (a pathologically enlarged spleen). P. vivax is carried by the female Anopheles mosquito; the males do not bite.

Clinical presentation

- Pathogenesis results from rupture of infected red blood cells, leading to fever. Infected red blood cells may also stick to each other and to walls of capillaries. Vessels plug up and deprive tissues of oxygen. Infection may also cause the spleen to enlarge.

- Unlike P. falciparum, P. vivax can populate the bloodstream with sexual-stage parasites—the form picked up by mosquitoes on their way to the next victim—even before a patient shows symptoms. Consequently, prompt treatment of symptomatic patients doesn't necessarily help stop an outbreak, as it does with falciparum malaria, in which fevers occur as sexual stages develop. Even when symptoms appear, because they are usually not immediately fatal, the parasite continues to multiply.
- Plasmodium vivax can cause a more unusual form of malaria with atypical symptoms. It has been known to debut with hiccups, loss of taste, lack of fever, pain while swallowing, cough and urinary discomfort.
- The parasite can go dormant in the liver for days to years, causing no symptoms and remaining undetectable in blood tests. They form what are called hypnozoites, a small stage that nestles inside an individual liver cell. This name derives from “sleeping organisms”. The hypnozoites allow the parasite to survive in more temperate zones, where mosquitoes bite only part of the year.
- A single infectious bite can trigger six or more relapses a year, leaving sufferers more vulnerable to other diseases. Other infectious diseases, including falciparum malaria, appear to trigger relapses.

Serious complications

Serious complications for malaria are dormant liver stage parasites, organ failures such as acute kidney failure. More complications of malaria can also be impairment of consciousness, neurological abnormalities, hypoglycemia and low blood pressures caused by cardiovascular collapse, clinical jaundice and or other vital organ dysfunctions and coagulation defects. The most serious complication ultimately being death.

Diagnosis

P. vivax and P. ovale that has been sitting in EDTA for more than 30 minutes before the blood film is made will look very similar in appearance to P. malariae, which is an important reason to warn the laboratory immediately when the blood sample is drawn so they can process the sample as soon as it arrives. Blood films are preferably made within 30 minutes of the blood draw and

must certainly be made within an hour of the blood being drawn. Diagnosis can be done with the strip fast test of antibodies.

Treatment

Chloroquine remains the treatment of choice for vivax malaria, except in Indonesia's Irian Jaya (Western New Guinea) region and the geographically contiguous Papua New Guinea, where chloroquine resistance is common (up to 20% resistance). Chloroquine resistance is an increasing problem in other parts of the world, such as Korea and India.

When chloroquine resistance is common or when chloroquine is contraindicated, then artesunate is the drug of choice, except in the U.S., where it is not approved for use. Where an artemisinin-based combination therapy has been adopted as the first-line treatment for P. falciparum malaria, it may also be used for P. vivax malaria in combination with primaquine for radical cure. An exception is artesunate plus sulfadoxine-pyrimethamine (AS+SP), which is not effective against P. vivax in many places. Mefloquine is a good alternative and in some countries is more readily available. Atovaquone-proguanil is an effective alternative in patients unable to tolerate chloroquine. Quinine may be used to treat vivax malaria but is associated with inferior outcomes.

32–100% of patients will relapse following successful treatment of P. vivax infection if a radical cure (eradication of liver stages) is not given.

Unit V

Environmental and Agricultural Microbiology

Waste management - waste water treatment

What is Wastewater?

• Wastewater is a term that is used to describe waste material that includes industrial liquid waste and sewage waste that is collected in towns and urban areas and treated at urban wastewater treatment plants.

Waste water treatment

• A process to convert wastewater - which is water no longer needed or suitable for its most recent use – into an effluent that can be either returned to the water cycle with minimal environmental issues or reused.

Wastewater Contaminants

• Suspended solids

• Biodegradable organics (e.g., BOD)

• Pathogenic bacteria

• Nutrients (N & P)

Where does wastewater come from?

• Residences

human and animal excreta and waters used for washing, bathing, and cooking.

• Commercial institution

• Dairy and industrial establishment

slaughterhouse waste, dairy waste, tannery wastewater, etc

Why treat wastewater?

• Causes a demand for dissolved oxygen (lower DO levels of streams)

• Adds nutrients (nitrate and phosphate) to cause excessive growth

• Increases suspended solids or sediments in streams (turbidity increase)

Objectives of wastewater treatment

- Reduce organic content i.e., BOD
- Removal/reduction of nutrients i.e., N,P

- Removal/inactivation of pathogenic microbes

Treatment stages – Primary treatment

- typical materials that are removed during primary treatment include
- fats, oils, and greases
- sand, gravels and rocks
- larger settle-able solids including human waste, and
- floating materials

Methods used in primary treatment

- Bar screens
- Grinding
- Grit Chamber
- Sedimentation Tank- primary Settling tank
- Chlorination of effluent

Sedimentation Tank primary Settling tank

- Remove grease, oil
- Fecal solid settle, floating material rise to the surface
- Produce a homologous liquid for later biological treatment
- Fecal sludge are pumped to sludge treatment plant

Secondary treatment

- Biological treatment
- activated sludge
- trickling filter
- oxidation ponds

Activated sludge process

- Primary wastewater mixed with bacteria-rich (activated) sludge and air or oxygen is pumped into the mixture
- Both aerobic and anaerobic bacteria may exist
- Promotes bacterial growth and decomposition of organic matter
- BOD removal is approximately 85%
- Microbial removal by activated sludge
- 80-99% removal of bacteria
- 90-99% removal of viruses

5 physical components

- **Aeration tank**
  oxygen is introduced into the system

- **Aeration source**
  ensure that adequate oxygen is fed into the tank
  provided pure oxygen or compressed air

- **Secondary clarifiers**
  activated-sludge solids separate from the surrounding wastewater

- **Activated sludge outflow line**
  Pump activated sludge back to the aeration tank

- **Effluent outflow line**
  discharged effluent into bay or tertiary treatment plant

Trickling filters

Trickling filters are beds made of coke (carbonized coal), limestone chips or specially fabricated plastic media

• Optimize their thickness by insect or worm grazing

• The primary wastewater is sprayed over the filter and microbes decompose organic material aerobically.

• Low pathogen removal

- Bacteria, 20-90%
- Viruses, 50-90%
- Giardia cysts, 70-90%

Stabilization or oxidation ponds

• Oxidation ponds are a few meters deep, and up to a hectare in size.

• They are low cost with retention times of 1 to 4 weeks.

• Odor and mosquitoes can be a problem

• Pathogen removal:

- Bacteria, 90-99%
- Virus, 90-99%
- Protozoa, 67-99%

• Mechanisms include the long detention time, high pH (10-10.5) generated by photosynthesis, predation, sunlight, temperature

Stabilization ponds are the preferred wastewater treatment process in developing countries due to low cost, low maintenance.

This is balanced by larger land requirement.

When the treatment is done

• Effluent back to stream after

– a final carbon filtration and

– chlorination/de-chlorination

• Sludge – very nutrient rich

– applied directly to land as fertilizer

– incinerated (good fuel after drying)

– composted

organic compost

What is Organic?

- Organic matter is matter that has come from a once-living organism.
- Organism: Animals, plants and microorganisms
- Once-living: Either live ones or dead bodies

What is Compost?

- Compost is the process that organic matter been decomposed and recycled as a fertilizer and soil amendment.Composting requires simply piling up waste outdoors and waiting for months.

biogas production,

Biogas Production From Sewage

What is biogas?

- Biogas is a methane rich flammable gas that results from the decomposition of organic waste material

- Biogas is produced by anaerobic digestion or fermentation of biodegradable materials such as biomass, manure, sewage, municipal waste, green waste, plant material and energy crops.
- Biogas also called as ‘Marsh gas’
- Biogas is a type of biofuel.
- This type of biogas comprises primarily methane and carbon dioxide