Chapter 8

Orientation to Hazards: Biological II

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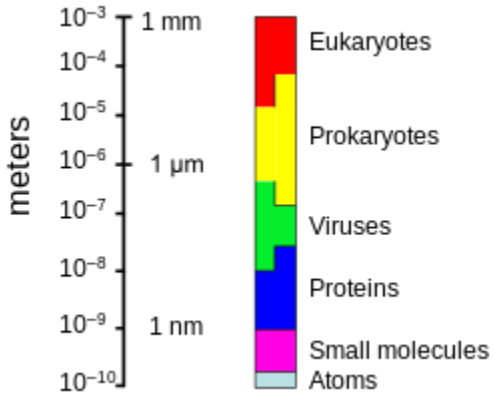
A microorganism, or microbe, is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells. Technically a microorganism or microbe is an organism that is microscopic. The scientific study of microorganisms began with their observation under the microscope in the 1670s by Anton van Leeuwenhoek. The microorganisms are classified into Bacteria, Fungi, Archaea, Protists, Microscopic plants (green algae), Microscopic animals (plankton) and Virus.Microorganisms can be found almost anywhere on Earth. Bacteria and archaea are almost always microscopic, while a number of eukaryotes are also microscopic, including most protists, some fungi, as well as some micro-animals and plants.Bacteria like archaea are prokaryotic - unicellular, and having no cell nucleus or other membrane-bound organelle.

Bacteria function and reproduce as individual cells, but they can often aggregate in multicellular colonies. Some species such as myxobacteria can aggregate into complex swarming structures, operating as multicellular groups as part of their life cycle, or form clusters in bacterial colonies such as *E. coli*. Their genome is usually a circular bacterial chromosome – a single loop of DNA, although they can also harbor small pieces of DNA called plasmids. These plasmids can be transferred between cells through bacterial conjugation. Bacteria have an enclosing cell wall, which provides strength and rigidity to their cells. In general, bacteria are between 0.2 and 2.0 um - the average size of most bacteria.Research studies have shown their size to play an important role in survival over time. Due to their small size, bacteria are able to exploit and thrive in various microenvironments. The small size of bacteria is also beneficial for parasitism and oligotrophy.



The following are the major categories of bacteria based on their shapes:

a) Cocci: Cocci bacteria appear spherical or oval in shape. For the most part, the shape is determined by the cell wall of the organism and therefore varies from one type of cocci bacteria to another. Cocci bacteria may exist as single cells or remain attached to each other. Attached Cocci bacteria include: **Diplococci** bacteria - Diplococci bacteria are the type of cocci bacteria that occur as a pair (two joined cells). Some examples of Diplococci bacteria include: *Streptococcus pneumonia*, *Moraxella catarrhalis*,

*Enterococcus*spp, *Neisseria gonorrhoea*. While some of these cells may be truly round shaped, others may appear elongated (ovoid) or bean-shaped/kidney shaped. For instance, some Neisseria cells may appear round while others are bean-shaped when viewed under the microscope. **Tetrad bacteria** - Tetrad bacteria are arranged in groups of four cells. Following division, the cells remain attached and grow in this attachment.Common examples of Tetrad bacteria include: *Pediococcus*, *Tetragenococcus*. **Sarcinaesarcina/Bacteria** - Sarcina bacteria occur in groups of 8 cells. Unlike tetrads that divide into two planes, Sarcinae is produced through the perpendicular plane division. Some of the characteristics associated with these bacteria include being strict anaerobes, Gram-positive bacteria and that measure between 1.5 and 3.0 um.Examples of Sarcinae bacteria include: *Sarcinaaurantiaca*, *Sarcina lutea*, *Sarcina ventriculi*. **Streptococci Bacteria**- Streptococci bacteria are a type of bacteria that arrange in a chain form (resembling chains). A majority of these bacterial cells are also ovoid in shape and may form paired chains. As members of the family Streptococcaceae, this group of bacteria is characterized by being non-motile, Gram-positive organisms. Examples of Streptococcus bacteria include: *Streptococcus pyogenes*, *Streptococcus pneumonia, S. mutans*. **Staphylococci Bacteria**- Staphylococci Bacteria are a type of bacteria that form grape-like clusters. This type of arrangement is the result of division that occurs in two planes. Two of the main characteristics of these organisms are that they are immobile, Gram-positive bacteria. Examples of Staphylococci bacteria include: *Staphylococcusepidermidis*, *Staphylococcushaemolyticus*, *Staphylococcusaureus* *Staphylococcuscapitis*.

b)Bacillus Bacteria (Rod-Shaped): Bacillus bacteria have the following traits: Are all rod-shaped, form endospores and are facultative anaerobes. bacillus bacteria are also arranged differently. While some exist as single, unattached cells (e.g. *Salmonellaentericas*ubsp, *Bacilluscereus*, and *Salmonellacholeraesuis*), others are attached. The following are the different types of bacillus arrangements: *Diplobacilli bacteria* - Like Diplococci bacteria, Diplobacilli occur in pairs. Following cell division, the two cells do not separate and continue existing as a pair. Examples of Diplobacilli bacteria include: *Coxiellaburnetii*, *Klebsiellarhinoscleromatis*, *Moraxellabovis*. **Coccibacilli bacteria** - Compared to other bacilli, Coccibacilli bacteria are shorter in length and thus appear stumpy. Examples of Coccibacilli include: *Chlamydia trachomatis*, *Haemophilus influenza*. Unlike cocci and bacilli bacteria, some types of bacteria appear curved when viewed under the microscope. However, they vary in shape making it possible to differentiate them from each other. These include: Vibrio bacteria - Generally, vibrio bacteria are comma-shaped and thus not fully twisted (curved rods). Examples of Vibrio bacteria include: *Vibrio mytili*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*. **Spirochete** - Spirochetes are characterized by a helical shape. Spirochetes are also flexible and have been shown to produce mycelium. The movement involves the use of axial filaments, which is one of the distinguishing features between the bacteria and other types of bacteria. Examples of Spirochetes include: Leptospira, Spirochaeta,
Treponema. **Spirilla** bacteria - Like Spirochetes, Spirilla bacteria possess a helical shape. However, they are more rigid and have the typical flagella found in other types of bacteria. Some examples of Spirilla bacteria include: *Aquaspirillum*, *Campylobacterjejuni*, *Spirillumwinogradskyi*.

In microbiology and bacteriology, Gram stain or Gram staining, also called Gram's method, is a method of staining used to classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who

developed the technique in 1884. Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol. They are stained pink or red by the counterstain, commonly safranin or fuchsine. Lugol's iodine solution is always added after addition of crystal violet to strengthen the bonds of the stain with the cell membrane. Gram staining is almost always the first step in the preliminary identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. Acid-fast staining is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called Ziehl-Neelsen staining techniques. Neelsen in 1883 used Ziehl'scarbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques was developed. The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups. This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus Mycobacterium, are resistant and can only be visualized by acid-fast staining.

Growth Curve

In a closed system with enough nutrients, a bacteria shows a predictable growth pattern that is the bacterial growth curve. It consists of four different phases. Read on to learn about the phases in detail. Phases of the Bacterial Growth Curve: Upon inoculation into a new nutrient medium, the bacteria shows four distinct phases of growth. Let us dive into each of the phases in detail.

Lag Phase: The bacteria upon introduction into the nutrient medium take some time to adapt to the new environment. In this phase, the bacteria does not reproduce but prepares itself for reproduction. The cells are active metabolically and keep increasing in size. The cells synthesise RNA, growth factors and other molecules required for cell division.

Log Phase: Soon after the lag phase, i.e., the preparation phase, the bacterial cells enter the log phase. The log phase is also known as the exponential phase. This phase is marked by the doubling of the bacterial cells. The cell number increases in a logarithmic fashion such that the cell constituent is maintained. The log phase continues until there is depletion of nutrients in the setup. The stage also comes to a stop if toxic substances start to accumulate, resulting in a slower growth rate. The cells are the healthiest at this stage and researchers prefer to use bacteria from this stage for their experimental processes. Plotting this phase on the bacterial growth curve gives a straight line. Upon calculation of the slope of this line, the specific growth rate of the organism is obtained. It is the measure of divisions per cell per unit of time.

Stationary Phase: In the stationary phase, the rate of growth of the cells becomes equal to its rate of death. The rate of growth of the bacterial cells is limited by the accumulation of toxic compounds and also depletion of nutrients in the media. The cell population remains constant at this stage. Plotting this phase on the graph gives a smooth horizontal linear line.

Death Phase: This is the last phase of the bacterial growth. At this stage, the rate of death is greater than the rate of formation of new cells. Lack of nutrients, physical conditions or other injuries to the cell leads to death of the cells.

Physical factors that affect microbial growth

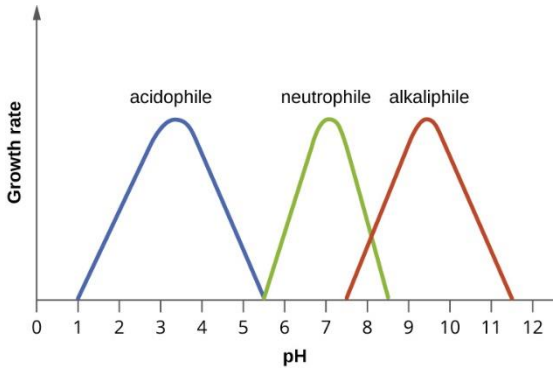
a) Temperature: Generally,an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will diminish and the protein (the enzyme) will denature. On the other hand, lowering temperature will decrease enzyme activity. At freezing temperatures enzyme activity can stop. Repeated cycles of freezing and thawing can denature proteins. In addition, freezing causes water to expand and also forms ice crystals, hence cells begin to rupture. Every bacterial species has specific growth temperature requirements which is largely determined by the temperature requirements of its enzymes. PSYCHROPHILES grow best between -5^{o}C and 20^{o}C, MESOPHILES grow best between 20^{o}C and 45^{o}C and
THERMOPHILES grow best at temperatures above 45^{o}C. THERMODURIC organisms can survive high temperatures but don't grow well at such temperatures. Organisms which form endospores would be considered thermoduric. Some organisms have exotic temperature requirements. *Thermus aquaticus* is a bright orange gram negative rod isolated from hot water and steam vents at Yellowstone Park. This organism grows best at temperatures between 70-75^{o}C (158-167^{o}F). Some of its unique enzymes are in demand for molecular biological and industrial applications.
b) **Oxygen**: Microbes display a great diversity in their ability to use and to tolerate oxygen. In part this is because of the paradoxical nature of oxygen which can be both toxic and essential to life. OBLIGATE AEROBES rely on aerobic respiration for ATP and they therefore use oxygen as the terminal electron acceptor in the electron transport chain. Pseudomonas is an example of this group of organisms. MICROAEROPHILES require O_2 for growth but they are damaged by normal atmospheric levels of oxygen and they don't have efficient ways to neutralize the toxic forms of oxygen such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The Streptococci are examples of this group. OBLIGATE ANAEROBES will die in the presence of oxygen because they lack enzymes like superoxide dismutase and catalase. Organisms like Clostridium, metabolize through fermentation and / or anaerobic respiration.
AEROTOLERANT organisms like Lactobacillus ferment and therefore do not use oxygen, however they do tolerate it. FACULTATIVE ANAEROBES are the most adaptable. They are capable of both fermentation and aerobic respiration. *Escherichiacoli* is an example of this class of organisms. ANAEROBIC PATHOGENS: *Clostridium tetani* - agent of tetanus, puncture wounds, produces a toxin which enters the spinal column and blocks the inhibitory spinal motor neurons. This produces generalized muscle spasms or spastic paralysis. *Clostridium botulinum* - this soil organism is the causative agent of botulism which typically occurs after eating home canned alkaline vegetables which were not heated enough during canning. The neurotoxin blocks transmission across neuromuscular junctions and this results in flaccid paralysis. *Clostridium perfringes* and *Clostridium sporogenes* - these organisms are associated with invasive infections known as GAS GANGRENE. *Clostridium difficile* - the causative agent of pseudomembranous colitis, a side effect of antibiotic treatment which eliminates the normal flora. MICROAEROPHILES: These organisms are all catalase negative, therefore the catalase test is useful in identification. They also have distinctive colonial morphology on blood agar which is differential for them. It is important to note if the colonies are alpha, beta, or gamma hemolytic. Group A Streptococcus - *Streptococcus pyogenes,* This beta hemolytic organism is also bacitracin sensitive. It is the cause of strep throat, rheumatic fever, glomerulonephritis and scarlet fever. Group D Streptococcus - Enterococcus - *Streptococcus faecalis*, This organism is a normal inhabitant of the large intestine. It is also a frequent cause of bladder infections. *Streptococcus pneumonia*, This

organism is a normal inhabitant of the respiratory tract. It is a frequent cause of pneumonia in people who have been compromised by other illness.

Based on the nutritional requirements, bacteria are classified as follows:

Energy source:	light:	phototrophic
	chemical:	chemotrophic
Electron source:	inorganic compounds:	lithotrophic
	organic compounds:	organotrophic
Carbon source:	CO_2:	autotrophic
	organic:	heterotrophic

Based on pH



bacterial requirements are classified as follows:

Most bacteria are neutrophiles, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7. Most familiar bacteria, like *Escherichiacoli*, *Staphylococci*, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0-6.0.Microorganisms that grow optimally at pH less than 5.55 are called acidophiles. Eg.*Lactobacillus* bacteria. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H^+ ions out of the cells. At the other end of the spectrum are alkaliphiles, microorganisms that grow best at pH between 8.0 and 10.5. *Vibrio cholerae*, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0.

Foodborne bacterial pathogens

Foodborne pathogens are mainly bacteria, viruses, or even parasites that are present in the food and are the cause of major diseases such as food poisoning. Foodborne pathogens are categorized according to the specific foods that are consumed. Foodborne illness occurs when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when a toxigenic pathogens establishes itself in a food product and produces a toxin, which is then ingested by the human host. Thus, foodborne illness is generally classified into: (a) foodborne infection and (b) foodborne intoxication. In foodborne infections, since an incubation period is usually involved, the time from ingestion until symptoms occur is much longer than that of foodborne intoxications. More than 200 different food-borne diseases have been identified. Aomng them, the common pathogenic bacteria associated with the fish and fishery products includes: *Aeromonas hydrophilia*, *Bacillus anthracis, Bacillus cereus/subtilis/lichniformis, Brucella/abortus/melitensis/suis, Campylobacter jejuni/coli, Clostridium botulinum/perfringens, Escherichia coli, Enterobacter sakazakii, Listeria monocytogenes, Mycobacterium paratuberculosis, Salmonella enterica, Shigella spp., Staphylococcus aureus, Vibrio cholera, V. cholerae* non-01*, V. parahemolyticus, V. vulnificus, V. fluvialis and Yersinia enterocolitica*. *Campylobacter* sp. (mostly associated with raw or undercooked poultry) is the major foodborne pathogen, causing more than two million infections per year, while *Salmonella*, mostly found in meat, poultry, and eggs, is responsible for more than one million cases of food poisoning. *Shigella*,

Escherichiacoli (mostly found in meat and unpasteurized milk), *Clostridiumbotulinum* (often found in improperly home-canned foods), *Clostridiumperfringens*, *Yersinia*, *Vibrio cholerae*, *V. vulnificus*, *V. parahaemolyticus*, *Staphylococcus aureus*, *Bacillus* spp., and *Listeria* (in uncooked meats, vegetables, unpasteurized milk, and soft cheese) also cause foodborne disease.

The specific bacterial pathogens, isolation and identification protocols are mentioned below:

a) *Clostridium botulinum*

- **Bacteria:** Anaerobic, spore-forming, motile GPR
- **Source:** Soils, sediments, intestinal tracts of fish/mammals, gills and viscera of crabs and other shellfish
- **Illness:** Intoxication (heat-labile neurotoxin)
- **Symptoms:** Weakness, vertigo, double vision, difficulty in speaking, swallowing and breathing, respiratory paralysis
- **Foods:** Semi-preserved seafood, improperly canned foods
- **Transmission:** Spores present in raw foods
- **Control:** Proper canning, a_w<0.93, pH <4.7
- **Isolation:** Inoculate the sample into cooked meat medium and incubate for 48-72 h. Streak onto blood agar medium supplemented with gentamycin and metronidazole ans incubate the plates under anaerobic conditions in anerobic jar for 48 h at 37^{o}C. After incubation observe for the growth.
- **Toxin testing:** The toxins produced by Clostridium botulinum is tested using mouse bio assay and also by other methods such as PCR, ELISA, endopeptidase assay, lateral flow tets

b) *Clostridium perfringens*

- **Bacteria:** Anaerobic, spore-forming, nonmotile GPR
- **Source:** Soil, dust, intestinal tract of animals and humans
- **Illness:** Infection (toxin released on sporulation)
- **Symptoms:** Intense abdominal cramps and diarrhea
- **Foods:** Temperature abuse of prepared foods such as meats, meat products, and gravy
- **Transmission:** Spores present in raw foods
- **Control:** Proper time/temperature control; preventing cross-contamination of cooked foods
- **Identification:** The bacterium is mainly identified by performing biochemical tests such as Grams staining, Litmus milk test, haemolysis (double zone), CAMP test
- **Toxin testing:**Nagler test

c) *Bacillus cereus*

- **Bacteria:** Facultatively aerobic, spore-forming, motile GPR
- **Source:** Soil, dust, raw foods
- **Illness:** 1) diarrheal type (infection, heat-labile toxin); 2) emetic type (intoxication, heat-stable toxin)
- **Symptoms:** 1) profuse watery diarrhea, abdominal pain; 2) vomiting, nausea
- **Foods:** 1) vegetables, salads, meats, casseroles; 2) rice **Transmission:** Spores present in raw foods
- **Control:** time/temperature; reheat cooked foods to >165^{o} F
- **Isolation:** The bacterium is isolated on commonly used microbiological media such as nutrient agar.

C) *Campylobacter jejuni*

- **Bacteria:** Microaerophilic, motile GNR
- **Source:** Intestines of poultry, livestock, domestic animals; streams and ponds
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, abdominal pain, headache, weakness
- **Foods:** undercooked chicken & hamburger, raw milk & clams
- **Transmission:** Contaminated foods & water; cross-contamination; person to person
- **Control:** Proper cooking, proper hand and equipment washing, sanitary food handling practices
- **Isolation:** The bacterium is isolated from the samples by using Bolton broth incubated at 42^{o}C for 24 h followed by streaking on chromogenic media incubated under micro-aerophilic conditions. The intense red colored colonies on a translucent agar facilitates the reading compared to charcoal based agar.
- **Identification:** PCR

d) Pathogenic *Escherichia coli* O157:H7

- **Bacteria:** Facultative anaerobic, motile or nonmotile GNR
- **Source:** Intestines of animals and poultry
- **Illness:** Hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP)
- **Symptoms:** HC) diarrhea & vomiting, HUS) diarrhea & acute renal failure, TTP) diarrhea, GI hemorrhage, Brain blood clots

- **Foods:** Meat, poultry, potatoes, raw milk
- **Transmission:** Cross-contamination, sewage pollution
- **Control:** Proper cooking, temp. control, preventing cross-contamination, proper personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using *E. coli* broth incubated initially at 25 ^{o}C for 2 h and at 42^{o}C for 8 h followed by streaking on chromogenic media incubated under aerophilic conditions (37 ^{o}C for 18-24 h). *E. coli* produces blue colour colonies.
- **Identification:** Biochemical tests and PCR

e) *Listeria monocytogenes*

- **Bacteria:** Microaerophilic, motile, GPR
- **Source:** Widespread in the environment
- **Illness:** Infection
- **Symptoms:** Mild flu-like symptoms to meningitis, abortions, septicemia, and death
- **Foods:** Coleslaw, raw milk, Mexican style soft cheese, smoked mussels
- **Transmission:** Cross-contamination, from raw to cooked food, contaminated raw foods
- **Control:** Proper cooking, preventing, cross-contamination, pasteurizing milk
- **Isolation:** The bacterium is isolated from the samples by using half-Fraser broth incubated at 30 ^{o}C for 24 h and later 0.1 ml of enriched broth (0.1 ml) was transferred to Fraser broth incubated at 37 ^{o}C for 24 h followed by streaking on selective media (Ottoviani and Agosti) or secondary selective media (PALCOM, OXFORD) and incubate under aerophilic conditions (37 ^{o}C for 18-24 h). β-D-glucosidase activity, common to the Listeria genus, is detected using a chromogenic substrate (X-glucoside). Its hydrolysis induces the formation of a blue to blue-green color in all Listeria colonies. PI-PLC is an enzyme only detected in pathogenic Listeria species: *L. monocytogenes* and *L. ivanovii*. AL medium contains phosphatidylinositol which, when it breaks down, produces an opaque halo around the colonies of these two bacterial species. The halo is visible after 24 hr for *L. monocytogenes* and 48 hr for *L. ivanovii*.
- **Identification:** Biochemical tests and PCR

f) ***Salmonella spp.***

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles

- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy products, egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination, personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using Buffered peptone water incubated at 37^{o}C for 24 h followed by enrichment in Rappaport and Vassiliadis broth (incubation at 41.5 ^{o}C for 24 h), Muller-Kauffman Tetrathionate Novobiocin broth (incubation at 37 ^{o}C for 24 h) and later streaking on XLD agar incubated at 37^{o}C for 24 h under aerophilic conditions. On XLD agar it produces red colour colonies with black centre.
- **Identification:** Biochemical, serological and PCR

g) *Shigella spp.*

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy & egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination, personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using *Shigella* broth supplemented with Novobiocin incubated initially at 44 ^{o}C for 24 h under anerobic conditions followed by streaking on MacConkey agar incubated under aerophilic conditions (35 ^{o}C for 20 h). Colonies are non-lactose fermenting (except S. sonnei) large, circular, convex, smooth, and translucent.
- **Identification:** Biochemical tests and Serological

h) *Pathogenic Staphylococcus aureus*

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever

- **Foods:** Poultry, poultry salads, meats, dairy products, egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using Baird parker agar supplemented with egg yolk and potassium telurite incubated initially at 35 ^{o}C for 24 h under anerobic conditions. *Staphylococcus aureus* is characterized by the formation of black, shiny, convex colonies surrounded by a lightening halo of the egg yolk. Coagulase negative staphylococci are almost completely inhibited and if, however, a culture does appear, areas of thinning would be absent.
- **Identification:** Mannitol fermentation, genotypic characterisation (pvl, spa typing, SCCmec typing) and phenotypic characterization (growth on ORSAB agar)

i) ***Vibrio cholerae***

- **Bacteria:** Facultative aerobic, motile, curved GNR
- **Source:** Naturally occurring in estuaries, bays and coastal water
- **Illness:** Infection (cholera or gastroenteritis)
- **Symptoms:** 01: watery diarrhea, vomiting, abdominal cramps; non-01: Diarrhea, abdominal cramps, fever
- **Foods:** Molluscan shellfish
- **Transmission:** Contaminated water, cross-contamination from raw to cooked seafood, contaminated raw seafood
- **Control:** Proper cooking, preventing cross-contamination, harvesting from approved waters
- **Isolation:** The bacterium is isolated from the samples by using alkaline peptone water incubated initially at 37 ^{o}C for 6-18 h under anerobic conditions followed by streaking on TCBS agar incubated under aerophilic conditions (37 ^{o}C for 18-20 h). *Vibrio cholera* produces flat yellow colonies with 2-3 mm in diameter
- **Identification:** Biochemical tests, Serological and PCR

j) ***Vibrio parahaemolyticus***

- **Bacteria:** Facultative aerobic, motile, curved GNR
- **Source:** Naturally occurring in estuaries and other coastal areas throughout the world
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, abdominal cramps, nausea, vomiting, headache
- **Foods:** Raw, improperly cooked, or cooked and contaminated fish and shellfish

- **Transmission:** Cross-contamination from raw to cooked seafood, consumption of raw seafood
- **Control:** Proper cooking, preventing cross-contamination
- **Isolation:** The bacterium is isolated from the samples by using alkaline salt peptone water incubated initially at 37 ^{o}C for 6-18 h under anerobic conditions followed by streaking on TCBS agar incubated under aerophilic conditions (37 ^{o}C for 18-20 h). *Vibrio parahaemolyticus* produces colorless colonies with a green center.
- **Identification:** Biochemical tests, pathotyping and PCR

k) *Yersinia enterocolitica*

- **Bacteria:** Facultative aerobic, motile, GNR
- **Source:** Soil, water, domesticated and wild animals
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, vomiting, abdominal pain, fever
- **Foods:** Meats, oysters, fish, raw milk
- **Transmission:** Cross-contamination from raw to cooked food, poor sanitation, time/temperature abuse
- **Control:** Preventing cross-contamination, proper sanitation and food handling practices
- **Isolation:** The bacterium is isolated from the samples by using buffered peptone water incubated initially at 4 ^{o}C for 1-3 weeks under anerobic conditions or treat the samples with alkali and later streaking on CIN or mVYE agar incubated under aerophilic conditions (30 ^{o}C for 24 h). *Vibrio parahaemolyticus* produces red (red bulls eye) colonies.
- **Identification:** Biochemical tests (Urea, TSI, LIM), PYZ and AA tests, Biotyping and Serotyping, Real time PCR