Foodborne infections

B. Cereus
Large gram +ve(motile, lacks the capsule)
Resemble B anthracisin culture forming large, grey, irregular colonies
Clinical Significance
a. Food poisoning –food contaminated with organism or toxins formed by organism
Diarrheal type –abdominal pain and watery diarrhea caused by enterotoxin
o Associated with poultry, meats, soups, vegetables and desserts, symptoms usually 8-16 hours after ingestion, recover 12-24 hours from onset
Emetic type –vomiting caused by emetic toxin
o Associated with fried or boiled rice, symptoms usually 1-5 hours after ingestion, recover 6-24 hours after onset
Both diarrheal and emetic forms are usually mild and self-limiting
b. Serious infections in immunocompromised host

Laboratory Identification
a. B. cereus is normal stool flora, to diagnose food poisoning must culture suspected food NOT stool
b. Gram stain –large, gram-positive rods with spores, can stain gram-variable or gram-negative
c. Colony morphology –beta-hemolytic; large, feathery, spreading on Agar
d. Preliminary Identification
Beta-hemolytic
Motile
Penicillin –Resistant
Clostridium spp.
Most Clostridium species decompose proteins or form toxins and some do both. Their natural habitat is the soil or intestinal tract as saprophytes. The important pathogenic species are:
Clostridium botulinum: Causes botulism
Clostridium tetani: Causes tetanus
Clostridium perfringens: Causes gas gangrene
Clostridium difficile; causes pseudomembranouscolitis and antibiotic-associated diarrhoea.
Note: the toxins produced by the organisms of tetanus and botulism attack nervous pathways (neurotoxins), the organisms associated with gas gangrenattack soft tissues by producing toxins (histotoxic). C difficileand some strains of c. perfringensproduce an enterotoxin

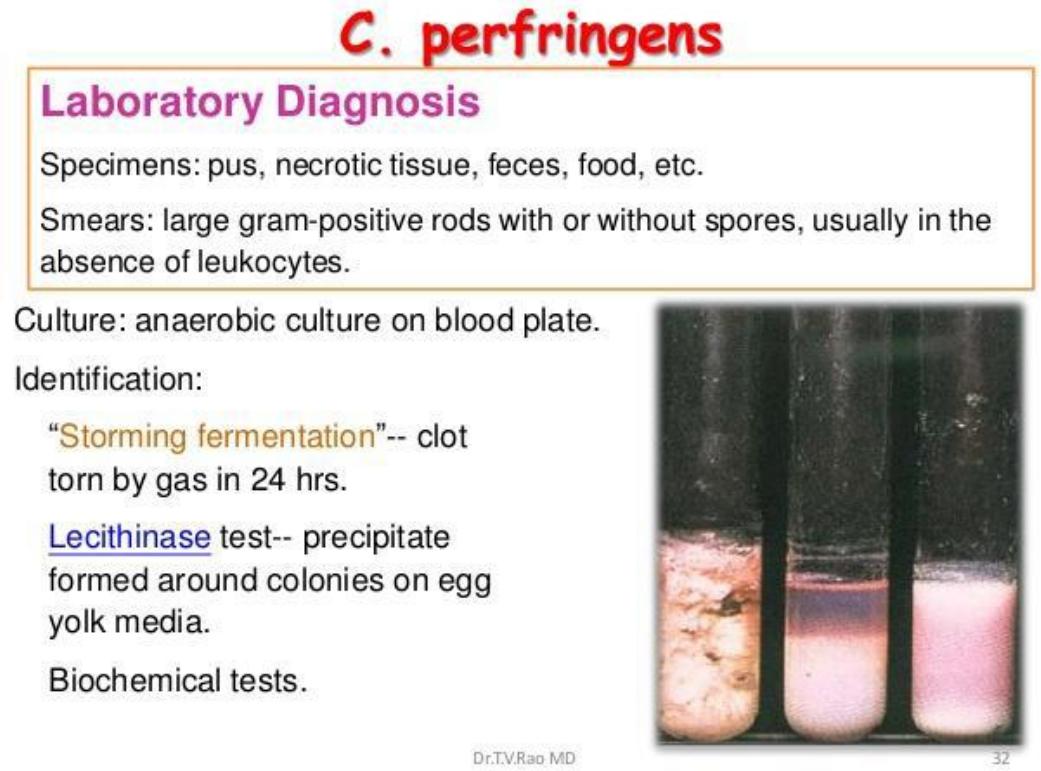
Morphology
- Large anaerobic gram positive motile rods.
- The spore is usually wider than the rods.
- Spores are placed centrally, terminally, or subterminallyaccording to the genus.

Unable to utilize O2 as the final oxygen acceptor.
Oxidase, catalase-ve
Types of Clostridium botulinum: They are from A –H according to the type of toxin produced.
- Types A, B, and E are the most commonly associated with illness
- Toxins of types A, B, and E have the following characteristics:

1. They are among the most highly toxic substances known.
2. They are neurotoxicproteins (MW = 150 000)
3. Lethal dose for human is 1 –2 mg
4. They are destroyed by heating for 20 minutes at 100 oC
5. Toxin production is under the control of a viral gene (Bacteriophageyielded from toxigenicstrain and it may infect non-toxigenicstrain and convert it to toxigenic).

Action of botulism toxin …
It is a neurotoxicprotein. All of its types (A, B, and E) are made of heavy and light chains linked by disulfide bonds. The heavy chain is thought to bind the toxin to the motor nerve end plate. The light chain blocks the calcium-mediated release of acetyl choline. The toxin acts by blocking the release of acetyl cholineat synapses and neuromuscular junctions causing flaccid paralysis.
…
Pathogenesis …
Botulism is intoxication. It results from ingestion of food in which Clostridium botulinumspores germinate and produce toxins under anaerobic conditions. These foods are spiced, smoked, vacuum-packed, or canned alkaline foods. The toxin acts by blocking the release of acetyl cholineat synapses and neuromuscular junctions causing flaccid paralysis.
Infant botulism …
It may result from honey feeding and cause signs of paralysis or sudden death.
Clostridium perfringens
This species implicated in gas gangrene and certain types of food poisoning.
Capsulated and non-motile
Subdivided into 5 types based on the four major lethal toxins they produce. Type A causes most of the human infections.
Identified by Nagler reaction, which exploits the action of its phospholipase on egg yolk medium; colonies are surrounded by zones of

turbidity and the effect is specifically inhibited if C. perfringens antiserum containing α-antitoxin is present on the medium.
Typical food poisoning strains produce heat resistant spores that can survive boiling for several hours, whereas the spores of strains that cause gas gangrene are inactivated within a few minutes by boiling.
Gas Gangrene: characterized by rapidly spreading oedema, myositis, necrosis of tissues, gas production and profound toxaemiaoccurring as complication of wound infection.
The main source of organism is animal and human excreta, and spores are distributed widely (soil and even air). The skin often bears spores, especially, in areas of the body that may be contaminated with intestinal organisms.



Treatment:

Clinical (surgical)

Antibiotic (immediately in high doses)—mixed infection, co-existence of coliform, gram positive cocci, and fecal anaerobes. Thus Penicillin, metronidazole and an aminoglycoside given in combination. Food poisoning *C. perfringens*can cause food poisoning if large numbers of an enterotoxin-producingstrain are ingested. The spores of some C.

perfringens strains are often particularly heat resistant and can withstand temperatures of 100°C for an hour or more. Thus, spores that survive initial cooking can convert to the vegetative form and multiply if food is not refrigerated or is rewarmed. After ingestion, the enterotoxin is released into the upper gastrointestinal tract, causing a fluid outpouring in which the ileum is most severely involved. *C. perfringens* is the 4thmost common cause of food poisoning after *campylobacter, salmonella, and staphylococcus.*

Lab:4

Environmental Factors Affecting Growth of Microorganisms

The growth of microorganisms is greatly affected by the chemical and physical nature of their environment. An understanding of the environmental factors that promote microbial growth aids in understanding the ecological distribution of microorganisms.

I. Temperature

Each microbial species requires a temperature growth range that is determined by the heat sensitivity of its particular enzymes, membranes, ribosomes, and other components.

Minimum growth temperature is the lowest temperature at which growth will occur; **maximum growth temperature** is the highest temperature at which growth will occur; and **optimum growth temperature** is the temperature at which the rate of cellular reproduction is most rapid. The optimum temperature for the growth of a given microorganism is correlated with the temperature of the normal habitat of the microorganism. For example, the optimum temperature for the growth of bacteria pathogenic to humans is near that of the temperature of human blood (35° to 37°C).

Most bacteria can be classified into one of three major groups based on their temperature requirements. **Psychrophiles** can grow at 0°C and have an optimum growth temperature of 15°C or lower; the maximum is around 20°C. **Mesophiles** have growth optima between 20° and 45°C. The majority of bacteria fall into this category. **Thermophiles** can grow at temperatures of 55°C or higher.

Boiling is probably one of the easiest methods of ridding materials of harmful bacteria. However, not all bacteria are equally sensitive to this high temperature. Some bacteria may be able to survive boiling even though they are unable to grow. These bacteria are termed **thermoduric.** Many of the spore formers (such as *B. subtilis*) can withstand boiling for 15 minutes because of their resistant endospores. Thus, both temperature and the species of bacteria will affect the disinfection of certain specimens. This is important to know when trying to kill pathogenic bacteria with heat.

II. pH

It is not surprising that pH dramatically affects bacterial growth. The pH affects the activity of enzymes—especially those that are involved in biosynthesis and growth.

Each microbial species possesses a definite pH growth range and a distinct pH growth optimum. Acidophiles have a growth optimum between pH 0.0 and 5.5; neutrophiles between 5.5 and 8.0; and alkalophiles 8.5 to 11.5. In general, different microbial groups have characteristic pH optima. The majority of bacteria and protozoa are neutrophiles.

Many bacteria produce metabolic acids that may lower the pH and inhibit their growth. To prevent this, buffers that produce a pH equilibrium are added to culture media to neutralize these acids. For example, the peptones in complex media act as buffers. Phosphate salts are often added as buffers in chemically defined media.

III. Osmotic pressure

Since bacteria are separated from their environment by a selectively permeable plasma membrane, they can be affected by changes in the osmotic pressure or water availability of their surroundings. Osmotic pressure is the force developed when two solutions of different solute concentrations are separated by a membrane that is permeable only to the solvent. The solvent is the liquid, usually water, that dissolves a substance (the solute). Water availability is expressed quantitatively in terms of water activity (aw). Pure water has an aw of 1.00, whereas cereals and other dried foods may have aw values of 0.60 or lower. If a bacterium is placed in a hypotonic solution (low solute, high-water content), water will enter the cell and cause it to burst unless something is done to prevent the influx. Most bacteria have rigid cell walls that maintain the shape and integrity of the cell; thus, hypotonic solutions are not harmful to these bacteria. When bacteria are placed in a hypertonic solution (high solute, lower water

content), water leaves, and the plasma membrane shrinks away from the wall, a process known as plasmolysis. This dehydrates the cell, and it ceases to grow. A few bacteria, called halophiles, are able to tolerate high (hypertonic) salt concentrations. Bacteria that can live in very salty environments are called extreme halophiles to distinguish them from the moderate halophiles that live in the sea. In an isotonic solution, the concentration of solutes is the same (iso means equal) outside and inside the bacterium. The bacterium is in osmotic equilibrium with its environment and does not change volume.

The Effects of Chemical Agents on Bacteria I: Disinfectants

Many factors influence the effectiveness of chemical disinfectants and antiseptics. The microbicidal (to kill) or microbiostatic (to inhibit) efficiency of a chemical is often determined with respect to its ability to deter microbial growth.

The Effects of Chemical Agents on Bacteria II: Antimicrobial Agents (Kirby-Bauer Method)

One method that is used to determine antibiotic susceptibility is the sensitivity disk method of Kirby- Bauer (named after W. Kirby and A. W. Bauer in 1966). In this method, antibiotics are impregnated onto paper disks and then placed on a seeded Mueller-Hinton agar plate using a mechanical dispenser or sterile forceps. The plate is then incubated for 16 to 18 hours, and the diameter of the zone of inhibition around the disk is measured to the nearest millimeter. The inhibition zone diameter that is produced will indicate the susceptibility or resistance of a bacterium to the antibiotic. Antibiotic susceptibility patterns are called antibiograms. Antibiograms can be determined by comparing the zone diameter obtained with the known zone diameter size for susceptibility. For example, a zone of a certain size indicates susceptibility, zones of a smaller diameter or no zone at all show that the bacterium is resistant to the antibiotic. Frequently one will see colonies within the zone of inhibition when the strain is antibiotic resistant. Many factors are involved in sensitivity disk testing and must be carefully controlled. These include size of the inoculum, distribution of the inoculum, incubation period, depth of the agar, diffusion rate of the antibiotic, concentration of antibiotic in the disk, and growth rate of the bacterium. If all of these factors are carefully controlled, this type of testing is highly satisfactory for determining the degree of susceptibility of a bacterium to a certain antibiotic.