COMMENSAL BACTERIA

Many bacteria live within our gastrointestinal tract, on our bodies or in the environment with which we come into daily contact without there being any resulting disease. In these situations, the bacteria are non-pathogenic and are called commensal bacteria, which means “eating at the same table.” It is recognized, however, that many of these organisms can cause infections such as wound infections or septicaemia if they are introduced into body tissues, particularly if the person is immunocompromised.

Although many genera and species of heterotrophic bacteria have been isolated from water and have been found to colonize distribution systems, no outbreaks of associated human disease have been conclusively reported. Suspicions have been raised about several organisms, such as *Klebsiella* spp. and *Citrobacter* spp., but their frequent isolation and lack of involvement in human gastrointestinal disease make them very unlikely candidates. There are concerns about the potential for *Aeromonas* spp. and *Yersinia enterocolitica* to cause diarrhoeal disease.

Aeromonas

Species of *Aeromonas* are ubiquitous in the environment and commonly occur in soil, marine and freshwater habitats. Marine recreational waters pose a potential source of human infection. In a study in southern Italy, many of the isolated strains produced several virulence factors, and all isolates produced cytotoxin and haemolysin. Three isolates produced enterotoxin, and all isolates bound to human intestinal cells in varying Degrees.

Yersinia

Yersinia is a genus of heterotrophic bacteria with 11 recognized species, some of which cause disease in humans, and both pathogenic and non-pathogenic strains of *Yersinia* have been found in surface water and unchlorinated drinking water. The source of the organism is the environment or non-human hosts, such as wild animals and birds. However, only certain serotypes of *Yersinia enterocolitica* that occur in the environment is considered to be pathogenic for humans. This depends on the possession of virulence factors associated with pathogenesis of infection.

Klebsiella

Fifty-three per cent of surface water samples were positive for *Klebsiella pneumoniae*. The surface water isolates resembled the clinical isolates in the expression of virulence factors. *Klebsiella* is ubiquitous in nature and is a commensal organism of the gastrointestinal tract, where it does not cause disease. It may be involved in urinary tract infection, particularly in females, where it is transferred across the perineum to the urethra, and it may be involved in wound infections, particularly following bowel

Pseudomonas

There are many species of *Pseudomonas* that are widespread in the environment and commonly occurring in soil and water. They are capable of growth in low nutrient situations and can grow in water in distribution systems if they gain access and on materials used in domestic plumbing situations. They may colonize taps and grow on surfaces, such as plastic pipes in drink vending machines. *Pseudomonas aeruginosa* is the most important species for public health considerations, although it does not cause any effects if it is ingested. It is resistant to many antibiotics and can produce serious nosocomial infections if it gains access to the body through wounds or intravenous lines. Hospital control of infection procedures that limit the use of tap water is an effective measure to prevent disease. In the community, *P. aeruginosa* may readily colonize pools and lead to wound infections if persons with open wounds or sores use them. Care must also be taken in the care of contact lenses and contact lens solutions to prevent contamination by *P. aeruginosa* on taps, leading to eye infections from water contact.

Virulence factors

Pathogenic bacteria produce a variety of virulence factors — e.g., adherence factors, so that the organisms can attach to intestinal cells; enzymes, including haemolysin, that facilitate cell invasion; exotoxins; and several other factors that produce immunomodulation. The successful pathogen will possess a whole range of these factors, but some are critical; an example is *Vibrio cholerae* with and without cholera toxin gene, the former producing cholera and the latter being avirulent. It is important to appreciate that the possession of a single virulence factor by an organism not normally considered to be pathogenic may not be significant. The assessment of virulence should therefore include detection systems for a whole range of virulence factors.

Lab 2

Basic lab technique: bacterial morphology and staining

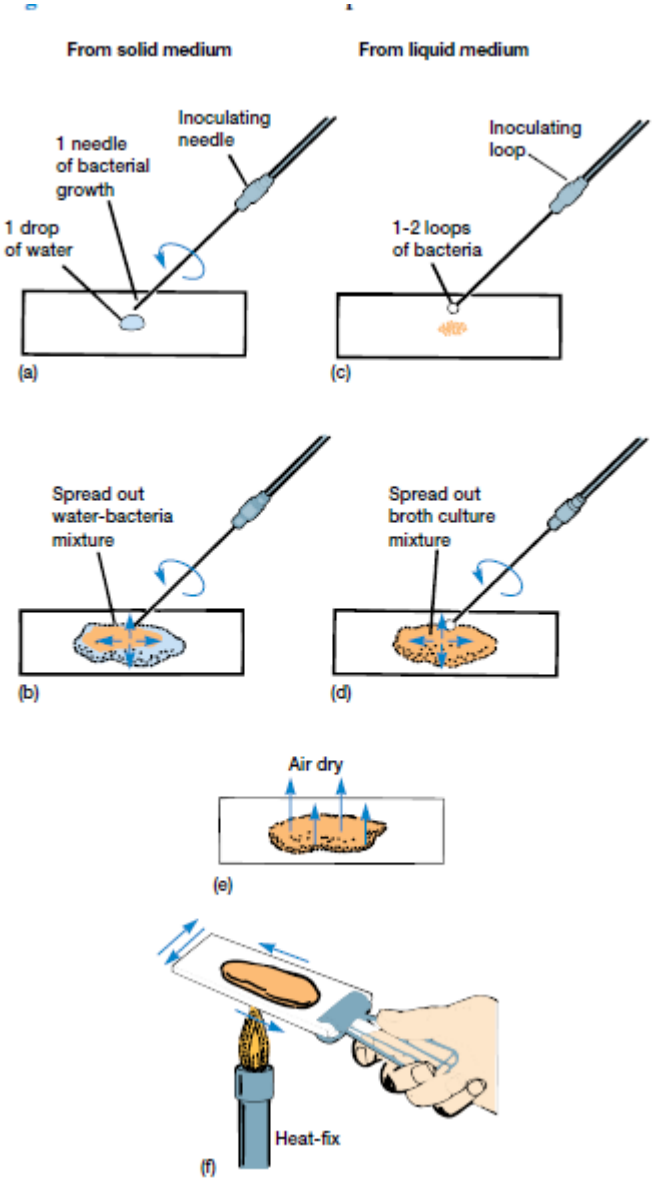
Negative, indirect, or **background staining** is achieved by mixing bacteria with an acidic stain such as nigrosin, India ink, or eosin, and then spreading out the mixture on a slide to form a film. The above stains will not penetrate and stain the bacterial cells due to repulsion between the negative charge of the stains and the negatively charged bacterial wall. Instead, these stains either produce a deposit around the bacteria or produce a dark background so that the bacteria appear as unstained cells with a clear area around them.

Smear Preparation and Simple Staining

A **bacterial smear** is a dried preparation of bacterial cells on a glass slide. In a bacterial smear that has been properly processed, (1) the bacteria are evenly spread out on the slide in such a concentration that they are adequately separated from one another, (2) the bacteria are not washed off the slide during staining, and (3) bacterial form is not distorted.

In making a smear, bacteria from either a broth culture or an agar slant or plate may be used.

The use of a single stain or dye to create contrast between the bacteria and the background is referred to as **simple staining.** Its chief value lies in its simplicity and ease of use. Simple staining is often employed when information about cell shape, size, and arrangement
is desired. In this procedure, one places the heat fixed slide on a staining rack, covers the smear with a small amount of the desired stain for the proper amount of time, washes the stain off with water for a few seconds, and, finally, blots it dry. Basic dyes such as **crystal violet** (20 to 30 seconds staining time), **carbolfuchsin** (5 to 10 seconds staining time), or **methylene blue** (1 minute staining time) are often used.


From solid medium
From liquid medium
Inoculating needle
1 needle of bacterial growth
1 drop of water
(a)
Inoculating loop
1-2 loops of bacteria
(c)
Spread out water-bacteria mixture
(b)
Spread out broth culture mixture
(d)
Air dry
(e)
Heat-fix
(f)

Shape		Arrangement	
Spherical	coccus (pl., cocci)	diplococcus (pairs)	
		streptococcus (chains)	
		staphylococcus (random or grapelike clusters)	
		micrococcus (square groups of four cells)	
Rod-shaped	bacillus (pl., bacilli)	streptobacillus (chains)	
Spiral	spirillum (pl., spirilla)	sarcina (cubical packets of eight cells)	
Incomplete spiral	vibrio (pl., vibrios)		
Irregular or variable shape	pleomorphic		

Lab 3

Gram stain

The **Gram stain** (named after Christian Gram, Danish scientist and physician, 1853–1938) is the most useful and widely employed differential stain in bacteriology. It divides bacteria into two groups—**gram negative** and **gram positive.**

The first step in the procedure involves staining with the basic dye crystal violet. This is the **primary stain.** It is followed by treatment with an iodine solution, which functions as a **mordant;** that is, it increases the interaction between the bacterial cell and the dye so that the dye is more tightly bound or the cell is more strongly stained. The smear is then decolorized
by washing with an agent such as 95% ethanol or isopropanol-acetone. Gram-positive bacteria retain the crystal violet-iodine complex when washed with the decolorizer, whereas gram-negative bacteria lose their crystal violet-iodine complex and become colorless. Finally, the smear is **counterstained** with a basic dye, different in color than crystal violet. This counterstain is usually safranin. The safranin will stain the colorless, gram-negative bacteria pink but does not alter the dark purple color of the gram-positive bacteria. The end result is that gram-positive bacteria are deep purple in color and gram-negative bacteria are pinkish to red in color.

Microbiological Culture Media

The survival and growth of microorganisms depend on available nutrients and a favorable growth environment. In the laboratory, the nutrient preparations that are used for culturing microorganisms are called **media** (singular, **medium**). Three physical forms are used: **liquid,** or **broth, media; semisolid media;** and **solid media.** The major difference among these media is that solid and semisolid media contain a solidifying agent (usually **agar**), whereas a liquid medium does not. Liquid media, such as nutrient broth, tryptic soy broth, or brain-heart infusion broth, can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests. Semisolid media can also be used in fermentation studies, in determining bacterial motility, and in promoting anaerobic growth. Solid media, such as nutrient agar or blood agar, are used (1) for the surface growth of microorganisms in order to observe colony appearance, (2) for pure culture isolations, (3) for storage of cultures, and (4) to observe specific biochemical reactions.