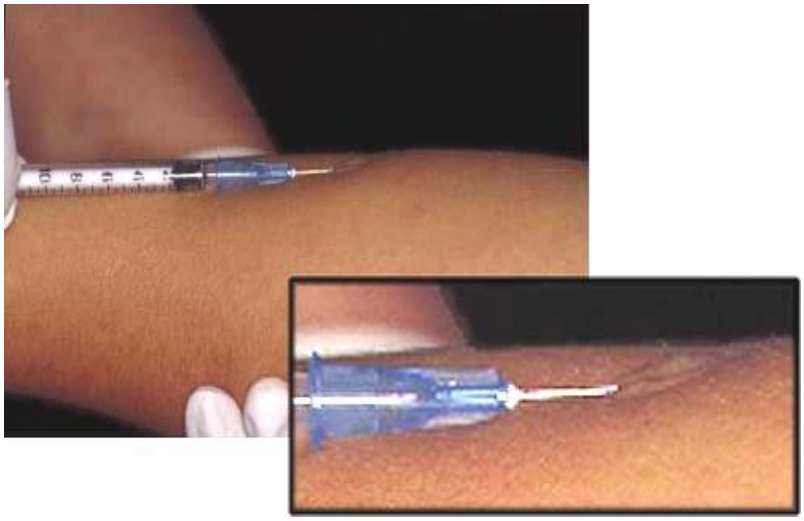
Medical Microbiology

A Brief Introduction

FOR

4TH YEAR STUDENTS

FACULTY OF SCIENCE



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Microorganisms are friend and foe

Humans are born into an environment laden with microorganisms, and colonization of the human body begins at the time of birth. Colonization simply implies the establishment of microorganisms on the body surface which, by extension, continues internally (oral cavity, gastrointestinal tract, ear canals, etc.). Throughout life, the skin and mucous membranes exposed to the outside world harbor a variety of indigenous bacteria, the **normal flora (**see, **H**ost **M**icrobe **I**nteraction for more details**)**.

A few hours after birth, the establishment of the normal flora on the surfaces of the body is well under way. Organisms acquired by an infant during passage through the birth canal are replaced by organisms derived from persons who attend the infant and from ingested foods. During the first day of life, many organisms find their niche for the life of the individual. Others take months or years to reach populations found in normal adults.

The number of different species of microorganisms living on the surfaces of the body is very large and even includes species that have not been fully characterized and classified. Some examples of the principal resident bacteria include *Staphylococcus*, *Micrococcus* and *Propionibacterium* found on the skin; *Lactobacillus*, *Streptococcus*, *Neisseria*, *Corynebacterium* and *Bacteroides* in the mouth; and *Bacteroides*, *Clostridium*, *Lactobacillus*, *Streptococcus* and many species of the Family *Enterobacteriaceae* in the

intestinal tract. Usually the normal flora causes no disturbances in the health of their host. In fact, they often benefit the host by out competing pathogenic bacteria, yeasts and protozoa which are encountered occasionally.

In the healthy individual, it is **not** normal for microorganisms to grow in areas that are inside the body; the lungs, heart, brain, spleen and muscles are typically free of microbes. Penetration of these areas by the normal flora or pathogens will incur the wrath of the immune system and result in a diseased state. Your body has a vast array of defenses to keep microbes out of the internal regions.

In the diagnosis of bacterial diseases of the human body, a general knowledge of the normal flora is essential. Exogenous pathogens must be distinguished from the indigenous species for the correct interpretation of bacteriological findings. Also, an increasing number of clinically-diagnosed bacterial diseases involves bacteria that are indigenous yet potentially pathogenic. Given an opportunity to infect an individual whose resistance is depressed, **or**, more specifically, to colonize an uncommon site (such as *E. coli* in the eye or urinary tract); certain of the normal flora may produce an endogenous bacterial disease. Examples of factors which lower the resistance of a host include radiation damage, prolonged use of antibiotics or steroids, and the debilitating effects of AIDS or other diseases. As the lives of more patients with major illnesses are prolonged by improved methods

of treatment, and as more infections caused by virulent exogenous bacteria are controlled by effective antimicrobial drugs, endogenous bacterial diseases have become more common.

Principles of Diagnosis

Some infectious diseases are distinctive enough to be identified clinically. Most pathogens, however, can cause a wide spectrum of clinical syndromes in humans. Conversely, a single clinical syndrome may result from infection with any one of many pathogens. Influenza virus infection, for example, causes a wide variety of respiratory syndromes that cannot be distinguished clinically from those caused by streptococci, mycoplasma, or more than 100 other viruses.

Most often, therefore, it is necessary to use **microbiologic laboratory methods** to identify a specific etiologic agent. Diagnostic medical microbiology is the discipline that identifies etiologic agents of disease. The job of the clinical microbiology laboratory is to **test specimens from patients for microorganisms that are, or may be, a cause of the illness and to provide information (when appropriate) about the in vitro activity of antimicrobial drugs against the microorganisms identified** (Fig. 1).

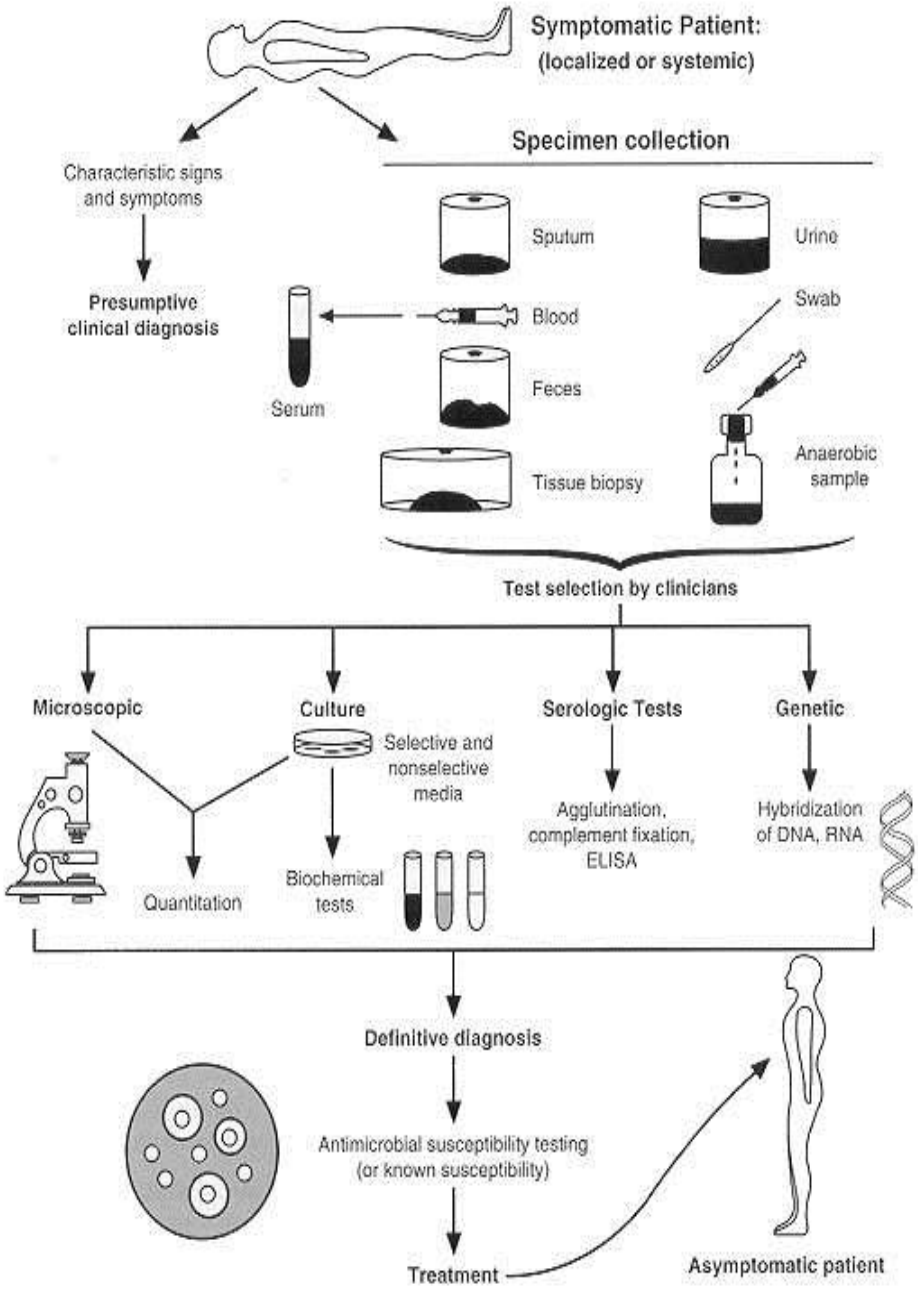


FIGURE (1): Laboratory procedures used in confirming a clinical diagnosis of infectious disease with a bacterial etiology.

The staff of a clinical microbiology laboratory should be qualified to advise the physician as well as process specimens. The physician should supply salient information about the patient, such as age and sex, tentative diagnosis or details of the clinical syndrome, date of onset, significant exposures, prior antibiotic therapy, immunologic status, and underlying conditions. The clinical microbiologist participates in decisions regarding the microbiologic diagnostic studies to be performed, the type and timing of specimens to be collected, and the conditions for their transportation and storage. Above all, the clinical microbiology laboratory, whenever appropriate, should provide an interpretation of laboratory results.

Manifestations of Infection

It is the successful entry of an organism into the human body with subsequent growth, multiplication and invasion of body tissue.

--Microorganisms may be:

1- **Pathogenic**.

2- **Non-pathogenic** including: -

a- Saprophytes from the cell.

b- Commensals from skin and mucous membranes of the host, which constitute the normal flora of the body which plays a role in

the body defense against pathogenic organisms, examples of these are:-

-- Salivary *streptococci*, produce H_2O_2 which is lethal to diphtheria and meningococci.

-- *Lactobacillus acidophilus*, ferment glycogen derived from the vaginal epithelium and produce a highly acidic vaginal secretion inhibitory to many kinds of bacteria as streptococci and staphylococci.

-- Some Commensals microorganisms may cause infection if they leave their normal places so they called opportunistic, e.g. *E. coli* which is Commensal in the gut, but it may cause urinary tract infection. Also, *Streptococcus viridans* Commensal in the mouth but it may cause subacute bacterial endocarditis if they reach the blood stream.

Another example is *Clostridium welchii* which is Commensal in the intestine, but they may cause gas gangrene in locally damaged tissue.

** **Pathogenic bacteria:**

Those bacteria which can cause diseases by invasion of the tissue by itself or by its toxins.

Pathogens should have the following characteristics:

1- **Transmissibility**:

It is the ability to grow profusely or to be shed in large number in body fluids.

2- **Infectivity**:

It is the ability to initiate infection related to (or depend on) dosage of the pathogen & phase of growth, virulence.

This occurs by overcome of the first line of defense which is the skin & mucous membrane.

For example, the infectivity dosage of *Salmonella typhi* is very small where large number of food- poisoning Salmonella must be ingested to produce acute vomiting and diarrhea.

3- **Virulence**.

It is the ability of organism to invade tissue, multiply & produce toxic effect substances.

The degree of virulence is measured by inoculation of the organism into a laboratory animal and we record LD_{50} (Lethal dose fifty) = the dose which kill 50% of laboratory animals.

Virulence of bacteria depends on:

A) Production of toxins. B) Invasiveness or aggressiveness.

Source of infection

A) **Exogenous**. B) **Endogenous**.

** **Exogenous infection through**:

1- Patients

This occurs by transmission of microorganisms from patient to healthy one e.g., T.B., syphilis, wheeping-cough, gonorrhoea Small-pox, Measles, & Influenza.

2- Healthy carriers.

Many pathogenic bacteria can affect certain persons and cause a limited sub- clinical infection, so it is unable to produce signs & symptoms of illness and they considered as source of infection to others.

3- Infected animals.

Here the disease is transmitted from animal to human by direct contamination. e.g., Anthrax, Rabies, Brucellosis & Bubonic- plug.

4- Soil.

Some saprophytic bacteria from the soil can infect human.

e.g., *Clostridium tetani* ---------- Tetanus.

Cl. Welchii ----------Gas gangrene.

** **Endogenous infection**:

Here the persons carry the bacteria, but they are harmless in their natural sites & pathogenic in other sites.

Examples of endogenous infection are: -

1- *E. coli*

They are harmless in the intestine, but they case urinary tract infection in urinary tract.

2- *Staph. aureus*

They are harmless in throat but harmful in wounds.

3- Pneumenococci

They are harmless in the throat & harmful in lung causing bronchopneumonia.

Spread of infection

Infections may be spread from the following:

1- **Respiratory**: By direct contact, by sneezing, coughing, speaking or indirectly transmitted.
2- **Skin**: In wounds & burns by contaminated hands and clothes.

3- **Venereal infection**: It is transmitted directly by sexual intercourse.
4- **Alimentary infection**: by contaminated hands, water or food through faecal- oral route transmission.
5- **Laboratory infection**: By culture & laboratory animals as in Brucellosis and Rikettsiosis.
6- **Blood sucking arthropods**: such as

Mosquito ---------- Malaria.

Flea ---------- Plug.

Louse ---------- Epidemic typhus.

The manifestations of an infection depend on many factors, including the site of acquisition or entry of the microorganism; organ or system tropisms of the microorganism; microbial virulence; the age, sex, and immunologic status of the patient; underlying diseases or conditions; and the presence of implanted prosthetic devices or materials. The signs and symptoms of infection may be localized, or they may be systemic, with fever, chills, and hypotension. In some instances, the manifestations of an infection are sufficiently characteristic to suggest the diagnosis; however, they are often nonspecific.

The clinical presentation of an infectious disease reflects the interaction between the host and the microorganism. This interaction is affected by the host immune status and microbial

virulence factors. Signs and symptoms vary according to the site and severity of infection. Diagnosis requires a composite of information, including history, physical examination, radiographic findings, and laboratory data.

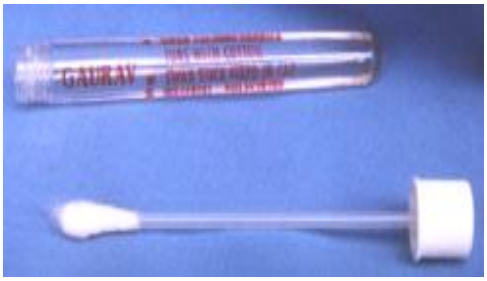
Microbial Causes of Infection

Infections may be caused by bacteria (including mycobacteria, chlamydiae, mycoplasmas, and rickettsiae), viruses, fungi, or parasites. Infection may be endogenous or exogenous. In endogenous infections, the microorganism (usually a bacterium) is a component of the patient's indigenous flora. Endogenous infections can occur when the microorganism is aspirated from the upper to the lower respiratory tract or when it penetrates the skin or mucosal barrier as a result of trauma or surgery. In contrast, in exogenous infections, the microorganism is acquired from the environment (e.g., from soil or water) or from another person or an animal. Although it is important to establish the cause of an infection, the differential diagnosis is based on a careful history, physical examination, appropriate radiographic and laboratory studies, including the selection of appropriate specimens for microbiologic examination. All of these allow the physician to request tests for the microorganisms most likely to be the cause of the infection.

Specimen Selection, Collection and Processing

Specimens selected for microbiologic examination should reflect the disease process and be collected in sufficient quantity to allow complete microbiologic examination.

The number of microorganisms per milliliter of a body fluid or per gram of tissue is highly variable, ranging from less than 1 to 10^8 or 10^{10} colony-forming units (CFU). Swabs (Fig. 2), although popular for specimen collection, frequently yield too small a specimen for accurate microbiologic examination and should be used only to collect material from the skin and mucous membranes.



(Fig. 2) SWAB CULTURE TUBE With cotton screw cap (Disposable).

Because skin and mucous membranes have a large and diverse indigenous flora, every effort must be made to minimize specimen contamination during collection. Contamination may be avoided by various means. The skin can be disinfected before aspirating or incising a lesion. Alternatively, the contaminated area may be bypassed altogether. Examples of such approaches are

transtracheal puncture with aspiration of lower respiratory secretions or suprapubic bladder puncture with aspiration of urine. It is often impossible to collect an uncontaminated specimen, and decontamination procedures, cultures on selective media, or quantitative cultures must be used. Specimens collected by invasive techniques, particularly those obtained intraoperatively, require special attention. Enough tissue must be obtained for both histopathologic and microbiologic examination. Histopathologic examination is used to distinguish neoplastic from inflammatory lesions and acute from chronic inflammations. The type of inflammation present can guide the type of microbiologic examination performed. If, for example, a caseous granuloma is observed histopathologically, microbiologic examination should include cultures for mycobacteria and fungi. The surgeon should obtain several samples for examination from a single large lesion or from each of several smaller lesions. If an abscess is found, the surgeon should collect several milliliters of pus, as well as a portion of the wall of the abscess, for microbiologic examination. Swabs should be kept out of the operating room. If possible, specimens should be collected before the administration of antibiotics. Above all, close communication between the clinician and the microbiologist is essential to ensure that appropriate specimens are selected and collected and that they are appropriately examined.

Microbiologic Examination

Direct Examination

Direct examination of specimens frequently provides the most rapid indication of microbial infection. A variety of microscopic, immunologic, and hybridization techniques have been developed for rapid diagnosis (Table 1). Direct examination of specimens reveals gross pathology. Microscopy may identify microorganisms. Immunofluorescence, immuno-peroxidase staining, and other immunoassays may detect specific microbial antigens. Genetic probes identify genus- or species-specific DNA or RNA sequences.

Table (1) Rapid tests commonly used to detect microorganisms in specimen.

Specimen	Test	Application
Blood	Giemsa	Plasmodia, microfilarie
	EIA	Hepatitis A and B virus, human immunodeficiency virus
Cerebrospinal fluid	Gram stain	Bacteria
	LA; COA	Haemophilus influenzae, Neisseria meningitidis
	India ink wet mount or LA	Streptococcus pneumoniae, Cryptococcus neoformans
Wound exudates, pus	Gram stain	Bacteria
Respiratory secretions	Gram stain	Bacteria
	Acid-fast stain	Mycobacteria, nocardiae
	IFA or genetic probe	*Legionella* species, *Streptococcus pyogenes*
	KOH wet mount	Fungi
	Gomori methenamine silver stain	Fungi, *Pneumocystis carinii*
	FA, EIA	Respiratory syncytial virus
Urine	Gram stain	Bacteria
Urethral or cervical scrapings or exudates	Gram stain, EIA, IFA, EIA, or genetic probe	Neisseria gonorrhoeae *Chlamydia trachomatis*, papillomaviruses
Genital ulcer	FA, EIA, or genetic probe	Herpes simplex virus
Feces	Methylene blue stain	Leukocytes
	Eosin wet mount, trichrome stain	Parasites
	EM, LA, EIA	Rotaviruses
	EIA	Adenoviruses, *Clostridium difficile*

Abbreviations: COA, coagglutination; EIA, enzyme immunoassay; IFA, immunofluorescent antibody; LA, latex agglutination.

Sensitivity and Specificity

The sensitivity of a technique usually depends on the number of microorganisms in the specimen. Its specificity depends on how morphologically unique a specific microorganism appears microscopically or how specific the antibody or genetic probe is for that genus or species. For example, the sensitivity of Cram stains is such that the observation of two bacteria per oil immersion field (X 1,000) of a Gram-stained smear of uncentrifuged urine is equivalent to the presence of $\geq 10^5$ CFU/ml of urine. The sensitivity of the Gram-stained smear for detecting Gram-negative coccobacilli in cerebrospinal fluid from children with *Haemophilus influenzae* meningitis is approximately 75 percent because in some patients the number of colony-forming units per milliliter of cerebrospinal fluid is less than 10^4. At least 10^4 CFU of tubercle bacilli per milliliter of sputum must be present to be detected by an acid-fast smear of decontaminated and concentrated sputum.

An increase in the sensitivity of a test is often accompanied by a decrease in specificity. For example, examination of a Gram-stained smear of sputum from a patient with pneumococcal pneumonia is highly sensitive but also highly nonspecific if the criterion for defining a positive test is the presence of any Gram-positive cocci. If, however, a positive test is defined as the presence of a preponderance of Gram-positive, lancet-shaped diplococci, the test becomes highly specific but has a sensitivity of only about 50 percent. Similar problems related to the number of

microorganisms present affect the sensitivity of immunoassays and genetic probes for bacteria, chlamydiae, fungi and viruses. In some instances, the sensitivity of direct examination tests can be improved by collecting a better specimen. For example, the sensitivity of fluorescent antibody stain for Chlamydia trachomatis is higher when endocervical cells are obtained with a cytobrush than with a swab. The sensitivity may also be affected by the stage of the disease at which the specimen is collected. For example, the detection of herpes simplex virus by immunofluorescence, immunoassay, or culture is highest when specimens from lesions in the vesicular stage of infection are examined. Finally, sensitivity may be improved through the use of an enrichment or enhancement step in which microbial or genetic replication occurs to the point at which a detection method can be applied.

Techniques

For microscopic examination, it is sufficient to have a compound binocular microscope equipped with low-power (1OX), high-power (40X), and oil immersion (1OOX) achromatic objectives, 10X wide-field oculars, a mechanical stage, a substage condenser, and a good light source. For examination of wet-mount preparations, a darkfield condenser or condenser and objectives for phase contrast increases image contrast. An exciter barrier filter, darkfield condenser, and ultraviolet light source are required for fluorescence microscopy.

For immunologic detection of microbial antigens, latex particle agglutination, coagglutination, and enzyme-linked immuno sorbent assay (ELISA) are the most frequently used techniques in the clinical laboratory. Antibody to a specific antigen is bound to latex particles or to a heat-killed and treated protein A-rich strain of *Staphylococcus aureus* to produce agglutination (**Fig. 3**). There are several approaches to ELISA; the one most frequently used for the detection of microbial antigens uses an antigen-specific antibody that is fixed to a solid phase, which may be a latex or metal bead or the inside surface of a well in a plastic tray. Antigen present in the specimen binds to the antibody as in (**Fig. 3**). The test is then completed by adding a second antigen-specific antibody bound to an enzyme that can react with a substrate to produce a colored product. The initial antigen antibody complex forms in a manner similar to that shown in (**Fig. 3**). When the enzyme-conjugated antibody is added, it binds to previously unbound antigenic sites, and the antigen is, in effect, sandwiched between the solid phase and the enzyme-conjugated antibody. The reaction is completed by adding the enzyme substrate.

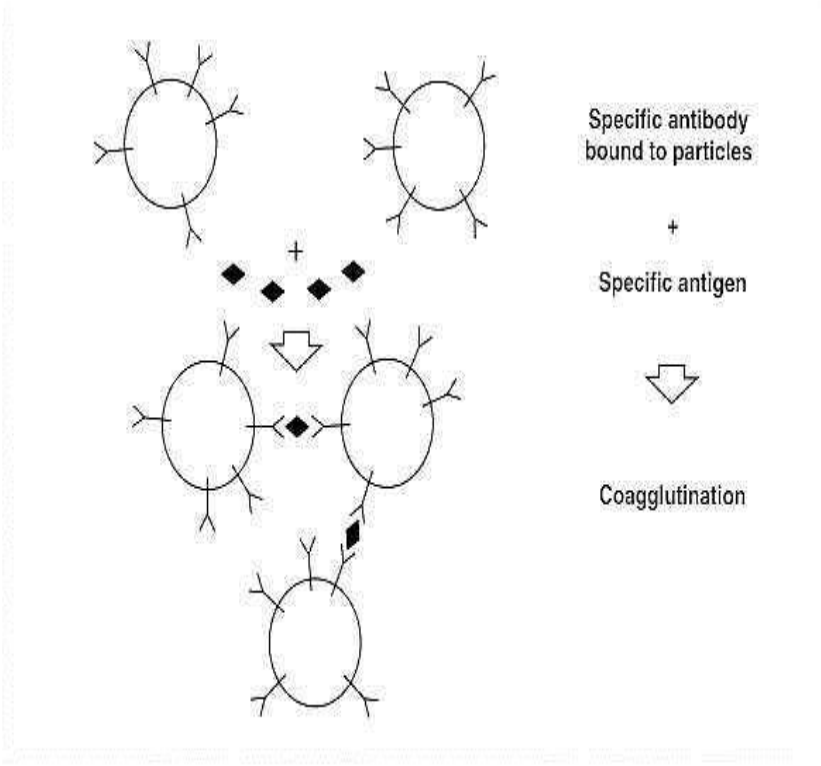
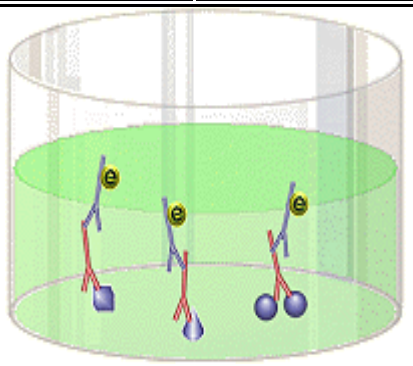
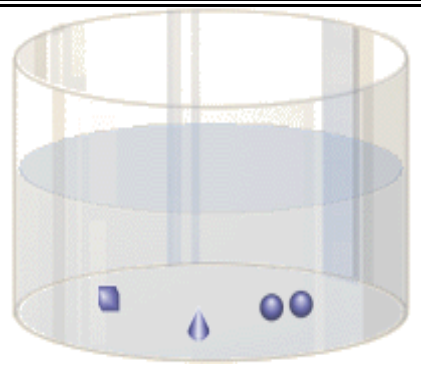


FIGURE (3): Agglutination test in which inert particles (latex beads or heat-killed *S. aureus* Cowan 1 strain with protein A) are coated with antibody to any of a variety of antigens and then used to detect the antigen in specimens or in isolated bacteria.

The ELISA Method

	Partially purified, inactivated HIV antigens pre-coated onto an ELISA plate
	Patient serum which contains antibodies. If the patient is HIV+, then this serum will contain antibodies to HIV, and those antibodies will bind to the HIV antigens on the plate.
	Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies.
	Chromogen or substrate which changes color when cleaved by the enzyme attached to the second antibody.

	
Positive ELISA Test	**Negative ELISA Test**

Genetic probes are based on the detection of unique nucleotide sequences with the DNA or RNA of a microorganism. Once such a unique nucleotide sequence, which may represent a portion of a virulence gene or of chromosomal DNA, is found, it is isolated and inserted into a cloning vector (plasmid), which is then transformed into Escherichia coli to produce multiple copies of the probe. The

sequence is then re-isolated from plasmids and labeled with an isotope or substrate for diagnostic use. Hybridization of the sequence with a complementary sequence of DNA or RNA follows cleavage of the double-stranded DNA of the microorganism in the specimen.

The use of molecular technology in the diagnoses of infectious diseases has been further enhanced by the introduction of gene amplication techniques, such as the polymerase chain reaction (PCR) in which DNA polymerase is able to copy a strand of DNA by elongating complementary strands of DNA that have been initiated from a pair of closely spaced oligonucleotide primers. This approach has had major applications in the detection of infections due to microorganisms that are difficult to culture (e.g. the human immunodeficiency virus) or that have not yet been successfully cultured (e.g. the Whipple's disease bacillus).

Culture

In many instances, the cause of an infection is confirmed by isolating and culturing microorganism either in artificial media or in a living host. Bacteria (including mycobacteria and mycoplasmas) and fungi are cultured in either liquid (broth) or on solid (agar) artificial media. Liquid media provide greater sensitivity for the isolation of small numbers of microorganisms; however, identification of mixed cultures growing in liquid media requires subculture onto solid media so that isolated colonies can be

processed separately for identification. Growth in liquid media also cannot ordinarily be quantitated. Solid media, although somewhat less sensitive than liquid media, provide isolated colonies that can be quantified if necessary and identified. Some genera and species can be recognized on the basis of their colony morphologies.

In some instances, one can take advantage of differential carbohydrate fermentation capabilities of microorganisms by incorporating one or more carbohydrates in the medium along with a suitable pH indicator. Such media are called differential media (e.g., eosin methylene blue or MacConkey agar) and are commonly used to isolate enteric bacilli. Different genera of the Enterobacteriaceae can then be presumptively identified by the color as well as the morphology of colonies.

Culture media can also be made selective by incorporating compounds such as antimicrobial agents that inhibit the indigenous flora while permitting growth of specific microorganisms resistant to these inhibitors. One such example is Thayer-Martin medium, which is used to isolate *Neisseria gonorrhoeae*. This medium contains vancomycin to inhibit Gram-positive bacteria, colistin to inhibit most Gram-negative bacilli, trimethoprim-sulfamethoxazole to inhibit *Proteus* species and other species that are not inhibited by colistin and anisomycin to inhibit fungi. The pathogenic *Neisseria* species, *N gonorrhoeae* and *N meningitidis*, are ordinarily resistant to the concentrations of these antimicrobial agents in the medium.

The number of bacteria in specimens may be used to define the presence of infection. For example, there may be small numbers (< 10^3 CFU/ml) of bacteria in clean-catch, midstream urine specimens from normal, healthy women; with a few exceptions, these represent bacteria that are indigenous to the urethra and periurethral region. Infection of the bladder (cystitis) or kidney (pyelone-phritis) is usually accompanied by bacteriuria of about > 10^4 CFU/ml. For this reason, quantitative cultures **(Fig. 4)** of urine must always be performed. For most other specimens a semiquantitative streak method **(Fig. 4)** over the agar surface is sufficient. For quantitative cultures, a specific volume of specimen is spread over the agar surface and the number of colonies per milliliter is estimated. For semiquantitative cultures, an unquantitated amount of specimen is applied to the agar and diluted by being streaked out from the inoculation site with a sterile bacteriologic loop **(Fig. 4)**. The amount of growth on the agar is then reported semiquantitatively as many, moderate, or few (or 3+, 2+, or 1+), depending on how far out from the inoculum site colonies appear. An organism that grows in all streaked areas would be reported as 3+.

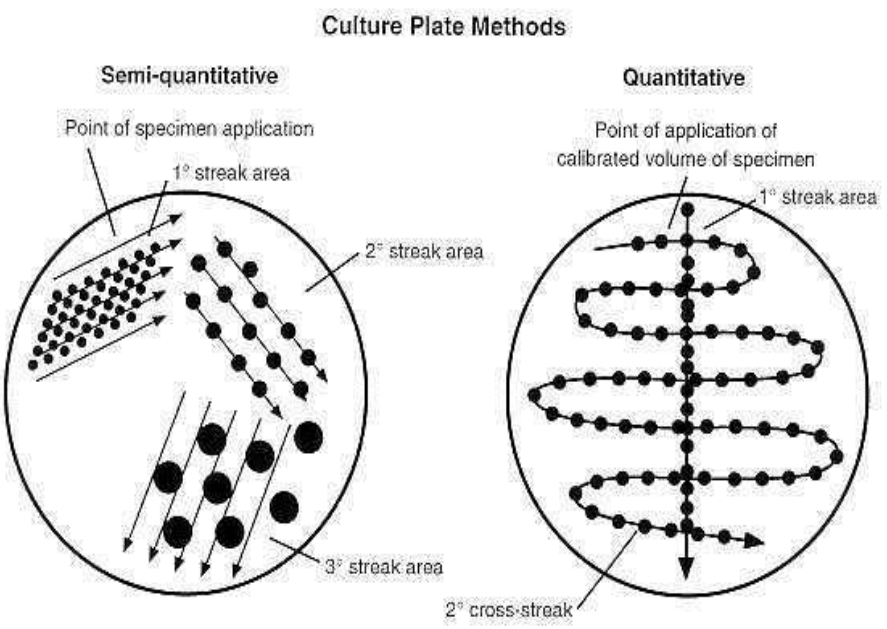


FIGURE (4): Quantitative versus semi quantitative culture, revealing the number of bacteria in specimens.

Chlamydiae and viruses are cultured in cell culture systems, but virus isolation occasionally requires inoculation into animals, such as suckling mice, rabbits, guinea pigs, hamsters, or primates. Rickettsiae may be isolated with some difficulty and at some hazard to laboratory workers in animals or embryonated eggs. For this reason, rickettsial infection is usually diagnosed serologically. Some viruses, such as the hepatitis viruses, cannot be isolated in cell culture systems, so that diagnosis of hepatitis virus infection is based on the detection of hepatitis virus antigens or antibodies.

Cultures are generally incubated at 35 to 37°C in an atmosphere consisting of air, air supplemented with carbon dioxide (3 to 10 percent), reduced oxygen (microaerophilic conditions), or no oxygen (anaerobic conditions), depending upon requirements of the microorganism. Since clinical specimens from bacterial infections often contain aerobic, facultative anaerobic, and anaerobic bacteria, such specimens are usually inoculated into a variety of general purpose, differential, and selective media, which are then incubated under aerobic and anaerobic conditions (**Fig. 5**).

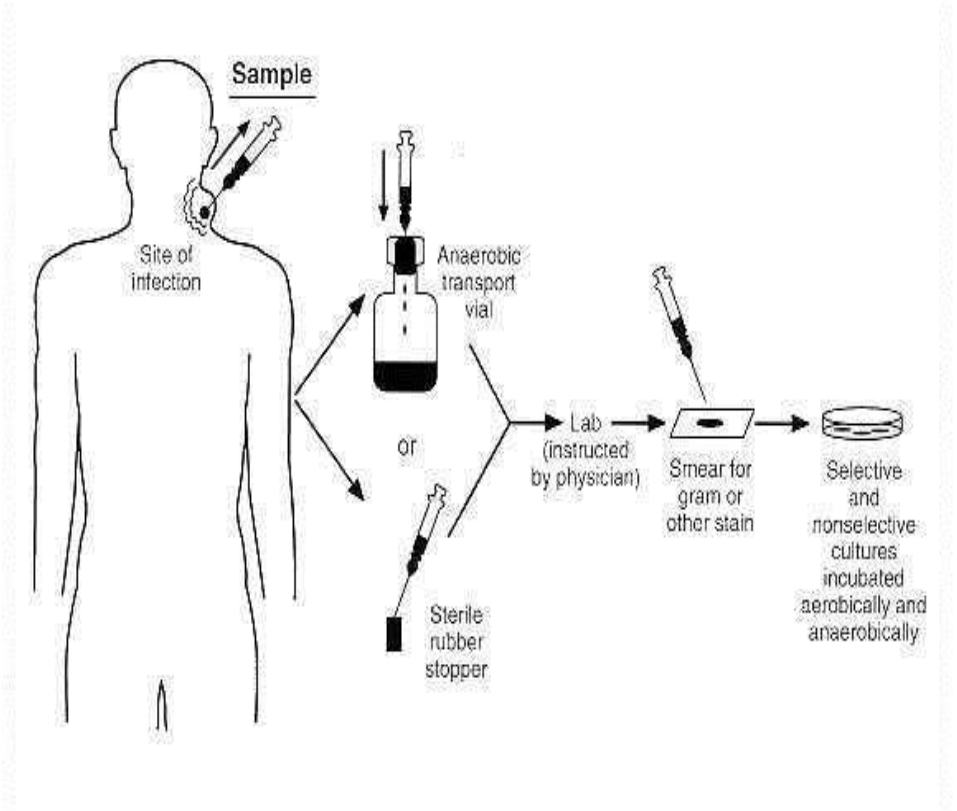


FIGURE 5: General procedure for collecting and processing specimens for aerobic and/or anaerobic bacterial culture.

The duration of incubation of cultures also varies with the growth characteristics of the microorganism. Most aerobic and anaerobic bacteria will grow overnight, whereas some mycobacteria require as many as 6 to 8 weeks.

Microbial Identification

Microbial growth in cultures is demonstrated by the appearance of turbidity, gas formation, or discrete colonies in broth; colonies on agar; cytopathic effects or inclusions in cell cultures; or detection of genus- or species-specific antigens or nucleotide sequences in the specimen, culture medium, or cell culture system.

Identification of bacteria (including mycobacteria) is based on growth characteristics (such as the time required for growth to appear or the atmosphere in which growth occurs), colony and microscopic morphology, and biochemical, physiologic, and, in some instances, antigenic or nucleotide sequence characteristics. The selection and number of tests for bacterial identification depend upon the category of bacteria present (aerobic versus anaerobic, Gram-positive versus Gram-negative, cocci versus bacilli) and the expertise of the microbiologist examining the culture. Gram-positive cocci that grow in air with or without added CO_2 may be identified by a relatively small number of tests. The identification of most Gram-negative bacilli is far more complex

and often requires panels of 20 tests for determining biochemical and physiologic characteristics. The identification of filamentous fungi is based almost entirely on growth characteristics and colony and microscopic morphology. Identification of viruses is usually based on characteristic cytopathic effects in different cell cultures or on the detection of virus- or species-specific antigens or nucleotide sequences.

Interpretation of Culture Results

Some microorganisms, such as *Shigella dysenteriae*, *Mycobacterium tuberculosis, Coccidioides immitis*, and influenza virus, are always considered clinically significant. Others that ordinarily are harmless components of the indigenous flora of the skin and mucous membranes or that are common in the environment may or may not be clinically significant, depending on the specimen source from which they are isolated. For example, coagulase-negative staphylococci are normal inhabitants of the skin, gastrointestinal tract, vagina, urethra, and the upper respiratory tract (i.e., of the nares, oral cavity, and pharynx). Therefore, their isolation from superficial ulcers, wounds, and sputum cannot usually be interpreted as clinically significant. They do, however, commonly because infections associated with intravascular devices and implanted prosthetic materials. However, because intravascular devices penetrate the skin and since cultures of an implanted prosthetic device can be made only after incision, the role of coagulase-negative staphylococci in causing infection

can usually be surmised only when the microorganism is isolated in large numbers from the surface of an intravascular device, from each of several sites surrounding an implanted prosthetic device, or, in the case of prosthetic valve endocarditis, from several separately collected blood samples. Another example, *Aspergillus fumigatus,* is widely distributed in nature, the hospital environment, and upper respiratory tract of healthy people but may cause fatal pulmonary infections in leukemia patients or in those who have undergone bone marrow transplantation. The isolation of *A. fumigatus* from respiratory secretions is a nonspecific finding, and a definitive diagnosis of invasive aspergillosis requires histologic evidence of tissue invasion.

Physicians must also consider that the composition of microbial species on the skin and mucous membranes may be altered by disease, administration of antibiotics, endotracheal or gastric intubation, and the hospital environment. For example, potentially pathogenic bacteria can often be cultured from the pharynx of seriously ill, debilitated patients in the intensive care unit, but may not cause infection.

Serodiagnosis

Infection may be diagnosed by an antibody response to the infecting microorganism. This approach is especially useful when the suspected microbial agent either cannot be isolated in culture by any known method or can be isolated in culture only with great

difficulty. The diagnosis of hepatitis virus infections can be made only serologically, since neither can be isolated in any known cell culture system. Although human immunodeficiency virus type 1 (HIV-1) can be isolated in cell cultures, the technique is demanding and requires special containment facilities. HIV-1 infection is usually diagnosed by detection of antibodies to the virus.

The disadvantage of serology as a diagnostic tool is that there is usually a lag between the onset of infection and the development of antibodies to the infecting microorganism. Although IgM antibodies may appear relatively rapidly, it is usually necessary to obtain acute- and convalescent-phase serum samples to look for a rising titer of IgG antibodies to the suspected pathogen. In some instances the presence of a high antibody titer when the patient is initially seen is diagnostic; often, however, the high titer may reflect a past infection, and the current infection may have an entirely different cause. Another limitation on the use of serology as a diagnostic tool is that immunosuppressed patients may be unable to mount an antibody response.

Antimicrobial Susceptibility

The responsibility of the microbiology laboratory includes not only microbial detection and isolation but also the determination of microbial susceptibility to antimicrobial agents. Many bacteria, in particular, have unpredictable susceptibilities to antimicrobial

agents, and their susceptibilities can be measured in vitro to help guide the selection of the most appropriate antimicrobial agent.

Antimicrobial susceptibility tests are performed by either disk diffusion or a dilution method. In the former, a standardized suspension of a particular microorganism is inoculated onto an agar surface to which paper disks containing various antimicrobial agents are applied. Following overnight incubation, any zone diameters of inhibition about the disks are measured and the results are reported as indicating susceptibility or resistance of the microorganism to each antimicrobial agent tested. An alternative method is to dilute on a log2 scale each antimicrobial agent in broth to provide a range of concentrations and to inoculate each tube or, if a microplate is used, each well containing the antimicrobial agent in broth with a standardized suspension of the microorganism to be tested. **The lowest concentration of antimicrobial agent that inhibits the growth of the microorganism is the minimal inhibitory concentration** (**MIC**). The MIC and the zone diameter of inhibition are inversely correlated (**Fig. 6**). In other words, the more susceptible the microorganism is to the antimicrobial agent, the lower the MIC and the larger the zone of inhibition. Conversely, the more resistant the microorganism, the higher the MIC and the smaller the zone of inhibition.

The term **susceptible** means that the microorganism is inhibited by a concentration of antimicrobial agent that can be attained in

blood with the normally recommended dose of the antimicrobial agent and implies that an infection caused by this microorganism may be appropriately treated with the antimicrobial agent. The term **resistant** indicates that the microorganism is resistant to concentrations of the antimicrobial agent that can be attained with normal doses and implies that an infection caused by this microorganism could not be successfully treated with this antimicrobial agent.

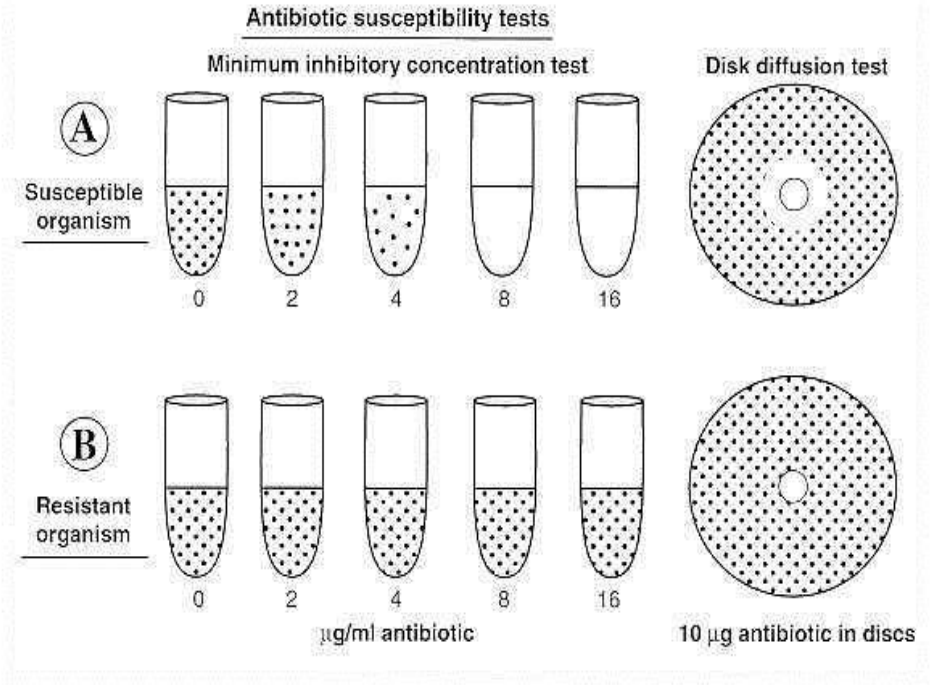


FIGURE 6: Two methods for performing antibiotic susceptibility tests.

(A) Disk diffusion method. (B) Minimum inhibitory concentration (MIC) method. In the example shown, two different

microorganisms are tested by both methods against the same antibiotic. The MIC of the antibiotic for the susceptible microorganism is 8 µg/ml. The corresponding disk diffusion test shows a zone of inhibition surrounding the disk. In the second sample, a resistant microorganism is not inhibited by the highest antibiotic concentration tested (MIC > 16 µg/ml) and there is no zone of inhibition surrounding the disk. The diameter of the zone of inhibition is inversely related to the MIC.

Microbiological culture

A culture of *Bacillus anthracis*

A **microbiological culture**, or **microbial culture**, is a method of growing a microbial organism to determine what it is, its abundance in the sample being tested, or both. It is one of the primary diagnostic methods of microbiology. A tool is often used to determine the cause of infectious disease by letting the agent multiply (reproduce) in predetermined media in laboratory.

The most common method of microbiological culture uses Petri dishes with a layer of agar-based growth medium in them to grow bacterial cultures. This is generally done inside of an incubator. Another method is liquid culture, where the bacteria are grown suspended in a liquid nutrient medium. Bottles of liquid culture are often placed in shakers in order to introduce oxygen to the liquid and maintaining the uniformity of the culture. The term culture can also, though infrequently and informally, be used as a synonym for tissue culture, which involves the growth of cells or tissues explanted from a multi-cellular organism.

Types of culture

- Blood culture
- Sputum culture
- Stool culture
- Culture of various fluids such as pleural fluid and peritoneal fluid
- Urine culture

BLOOD CULTURE

WHAT IS A BLOOD CULTURE

If you've been a patient in the hospital, you've probably had your blood drawn. Although it can be painful, it's done for an important reason.

Sometimes your blood is collected for a blood culture. The hospital lab tests your blood to look for microbes (germs) that may be causing infection. This information can help physicians prescribe the most effective medicine to get you better.

Why is a blood culture performed?

If you're hospitalized for pneumonia or a bladder infection, it can be difficult to choose the correct medicine to treat the infection. Most infections are caused by many different microbes, so it's important to figure out which of them is causing your infection.

The results of a blood culture can help physicians choose the correct medicine for your infection. For example, you may have a bladder infection. You go to the hospital to get treated, and the physicians give you some medicine. After three or four days, you're still not better. Could the medicine be targeting the wrong microbe? It's certainly possible. Your blood culture results can help indicate which microbe is the cause and your medicine can be changed. Blood culture results can give precise information about your infection.

Also, blood cultures that come back positive for back pain indicate a more severe type of infection, what is commonly referred to as

blood poisoning, which is associated with complications like shock, respiratory failure and death. Blood cultures that come back positive alert your physician that your infection is serious and requires aggressive treatment with intravenous antibiotics.

Finally, a blood culture is done to:

- Detect a bacterial infection, such as meningitis, osteomyelitis, or sepsis, in the bloodstream and identify the type of bacteria causing the infection.
- Detect a fungal infection, such as yeast, in the bloodstream.
- Detect endocarditis.
- Identify the best antibiotics to kill bacteria. This is called sensitivity testing.
- Evaluate unexplained fever or shock.

Test Overview

A blood culture is a test to detect infection in the blood. The blood does not normally contain any bacteria or fungi. A blood culture can detect and identify bacteria or fungi in the blood.

A bacterial infection in the blood, called bacteremia, is usually serious because the blood can spread the bacteria to any part of the body. A blood infection most commonly occurs with other serious infections (such as those affecting the kidneys, bowel, gallbladder, or heart valves).

A blood infection may also develop when the immune system is weakened. This can occur in infants and older adults from disease (such as cancer or AIDS) or from medications (such as corticosteroids or chemotherapy).

To test for infection in the blood, a sample of blood is collected and placed in a container with substances that promote the growth of bacteria or fungus. The type of bacteria or fungus that grows is identified by chemical tests and by examining the culture under a microscope. To increase the chances of identifying bacteria or fungi in the blood, two or three blood samples from different veins are usually taken. If no bacteria or fungus grow, the blood culture is called negative.

How To Prepare

No special preparation is required before having this test. Tell your health professional if you have recently taken antibiotics.

How It Is Done

The health professional drawing blood will:

- Wrap an elastic band around your upper arm to stop the flow of blood. This makes the veins below the band larger, so it is easier to put a needle into the vein.
- Clean the needle site with alcohol or iodine.
- Put the needle into the vein. More than one needle stick may be needed.

- Attach a tube to the needle to fill it with blood.
- Remove the band from your arm when enough blood is collected.
- Apply a gauze pad or cotton ball over the needle site as the needle is removed.
- Apply pressure to the site and then a bandage.

Blood is usually collected from at least two different locations, or it may be collected at two different times a few hours apart.

In some situations, people may have long-term catheters placed in a major vein because they are receiving chemotherapy or nutrition supplements for weeks or months at a time. For these people, blood will be collected from their catheters for this test.

Method used

The most important determinant of the sensitivity of blood cultures is the amount of blood inoculated into the blood. Not only does this increase the number of organisms introduced into the bottle (because the density of bacteria in the blood is usually very low, sometimes less than 1 organism per milliliter) but it also increases the nutrient content of the medium (since blood itself is extremely nutrient-rich). Some bottles can accommodate up to 20 ml of added blood. True, 20 ml, for two sets of culture bottles, is a lot of blood, but if it permits an accurate diagnosis to be made earlier, may save on total blood drawn subsequently, and for all but young children, is a very small fraction of total blood volume.

Subculturing positive blood culture bottles

Blood culture bottles (Trypticase Soy Broth, for aerobic culture) were inoculated with bacteria. Note: no blood was added, this is only for laboratory demonstration purposes. In an actual clinical setting, the bottle would be blood red.

In a feat of engineering, this double-sided needle is designed so that the first cover that comes off is the one that goes into the bottle, and only then will the second side cover come off.

		
Capped culture bottle, and capped needle	First cap off, inserted into bottle	Second cap off, ready for plating

Drops are then inoculated onto Blood agar and MacConkey agar, to streak for single colony isolation.

Blood Agar

MacConkey Agar

How It Feels

You may feel nothing at all from the needle puncture, or you may feel a brief sting or pinch as the needle goes through the skin. Some people feel a stinging pain while the needle is in the vein. However, many people do not feel any pain (or have only minor discomfort) once the needle is positioned in the vein. The amount of pain you feel depends on the skill of the health professional drawing the blood, the condition of your veins, and your sensitivity to pain.

Risks

There is very little risk of complications from having blood drawn from a vein.

- You may develop a small bruise at the puncture site. You can reduce the risk of bruising by keeping pressure on the site for several minutes after the needle is withdrawn.
- Rarely, the vein may become inflamed after the blood sample is taken. This condition is called phlebitis and is usually treated with a warm compress applied several times daily.
- Continued bleeding can be a problem for people with bleeding disorders. Aspirin, warfarin (Coumadin), and other blood-thinning medications can also make bleeding more likely. If you have bleeding or clotting problems, or if you take blood-thinning medication, tell your health professional before your blood is drawn.

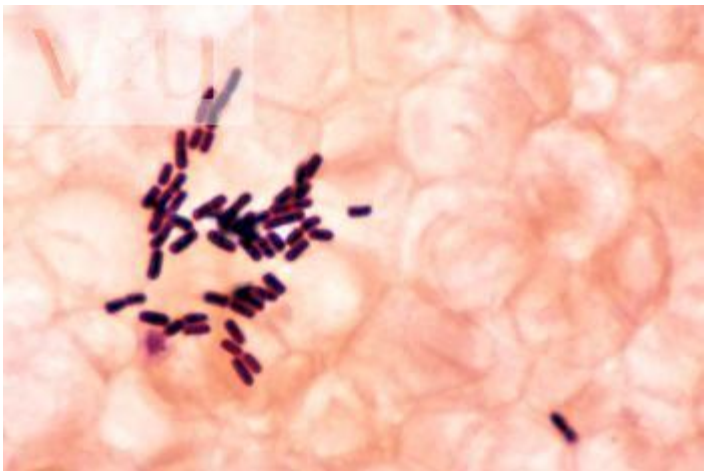
Results

A blood culture is a test to detect infection in the blood. Most bacteria can be seen in the culture within 2 to 3 days, but some types can take 10 days or longer to show up.

Fungal cultures can take up to 30 days for final results to be reported.

Blood culture	
Normal:	No bacteria or fungus are found. Normal culture results are called negative.
Abnormal:	Bacteria or fungus grow in the culture. Abnormal culture results are called positive.

If bacteria are found in the culture, another test is usually done to find the antibiotic that is most effective at killing the bacteria. This is called sensitivity or susceptibility testing. Sensitivity testing is important to treat a blood infection effectively and prevent the development of bacteria that are resistant to antibiotics.



Escherichia coli Bacteria from human blood culture. LM X1000.

What Affects the Test

Factors that can interfere with your test and the accuracy of the results include:

- Recent antibiotic treatment.
- Contamination of the blood sample by bacteria or fungus on the skin.

What To Think About

- Some types of bacteria can temporarily infect the blood when an infection of the kidneys, throat, lungs, or another part of the body is present. This may not indicate a serious infection of the blood; usually bacteria persist in the blood when a serious blood infection is present.
- About 5% of blood cultures are contaminated with normal skin bacteria (usually a type of staph bacteria). Therefore, it is sometimes difficult to determine whether the bacteria that grow in the culture are actually causing a blood infection or have contaminated the blood sample. When the same bacteria grow in several blood cultures, it is likely that those bacteria are in the blood and are not a contaminant. When staph bacteria grow in the culture in less than 48 hours, it is likely that the staph bacteria are in the blood and are not a contaminant.
- A culture that fails to grow any bacteria does not always rule out a blood infection. Factors such as the amount of blood taken, the timing of the blood sample, the type of culture done, and previous use of antibiotics can affect the growth of bacteria in the culture.

Sputum culture

Definition

Sputum is material coughed up from the lungs and expectorated (spit out) through the mouth. A sputum culture is done to find and identify the microorganism causing an infection of the lower respiratory tract such as pneumonia (an infection of the lung). If a microorganism is found, more testing is done to determine which antibiotics will be effective in treating the infection.

Purpose

A person with a fever and a continuing cough that produces pus-like material and/or blood may have an infection of the lower respiratory tract. Infections of the lungs and bronchial tubes are caused by several types of microorganisms, including bacteria, fungi (molds and yeast), and viruses. A chest x ray provides visual evidence of an infection; a culture can grow the microorganism causing the infection. The microorganism is grown in the laboratory so it can be identified, and tested for its response to medications, such as antifungal and antibiotics.

Description

Based on the clinical condition of the patient, the physician determines what group of microorganism is likely to be causing the infection, and then orders one or more specific types of cultures:

bacterial, viral, or fungal (for yeast and molds). For all culture types, the sputum must be collected into a sterile container. The sputum specimen must be collected carefully, so that bacteria that normally live in the mouth and saliva don't contaminate the sputum and complicate the process of identifying the cause of the infectious agent. Once in the laboratory, each culture type is handled differently.

Bacterial culture

A portion of the sputum is smeared on a microscope slide for a Gram stain. Another portion is spread over the surface of several different types of culture plates, and placed in an incubator at body temperature for one to two days.

A Gram stain is done by staining the slide with purple and red stains, then examining it under a microscope. Gram staining checks that the specimen does not contain saliva or material from the mouth. If many epithelial (skin) cells and few white blood cells are seen, the specimen is not pure sputum and is not adequate for culture. Depending on laboratory policy, the specimen may be rejected and a new specimen requested. If many white blood cells and bacteria of one type are seen, this is an early confirmation of infection. The color of stain picked up by the bacteria (purple or red), their shape (such as round or rectangular), and their size provide valuable clues as to their identity and helps the physician predict what antibiotics might work best before the entire test is

completed. Bacteria that stain purple are called gram-positive; those that stain red are called gram-negative.

During incubation, bacteria present in the sputum sample multiply and will appear on the plates as visible colonies. The bacteria are identified by the appearance of their colonies, by the results of biochemical tests, and through a Gram stain of part of a colony.

A sensitivity test, also called antibiotic susceptibility test, is also done. The bacteria are tested against different antibiotics to determine which will treat the infection by killing the bacteria.

The initial result of the Gram stain is available the same day, or in less than an hour if requested by the physician. An early report, known as a preliminary report, is usually available after one day. This report will tell if any bacteria have been found yet, and if so, their Gram stain appearance--for example, a gram-negative rod, or a gram-positive cocci. The final report, usually available in one to three days, includes complete identification and an estimate of the quantity of the bacteria and a list of the antibiotics to which they are sensitive.

Fungal culture

To look for mold or yeast, a fungal culture is done. The sputum sample is spread on special culture plates that will encourage the growth of mold and yeast. Different biochemical tests and stains

are used to identify molds and yeast. Cultures for fungi may take several weeks.

Viral culture

Viruses are a common cause of pneumonia. For a viral culture, sputum is mixed with commercially-prepared animal cells in a test tube. Characteristic changes to the cells caused by the growing virus help identify the virus. The time to complete a viral culture varies with the type of virus. It may take from several days to several weeks.

Special procedures

Tuberculosis is caused by a slow-growing bacterium called *Mycobacterium tuberculosis*. Because it does not easily grow using routine culture methods, special procedures are used to grow and identify this bacterium. When a sputum sample for tuberculosis first comes into the laboratory, a small portion of the sputum is smeared on a microscope slide and stained with a special stain, called an acid-fast stain. The stained sputum is examined under a microscope for tuberculosis organisms, which pick-up the stain, making them visible. This smear is a rapid screen for the organism, and allows the physician to receive a preliminary report within 24 hours.

To culture for tuberculosis, portions of the sputum are spread on and placed into special culture plates and tubes of broth that

promote the growth of the organism. Growth in broth is faster than growth on culture plates. Instruments are available that can detect growth in broth, speeding the process even further. Growth and identification may take two to four weeks.

Other microorganisms that cause various types of lower respiratory tract infections also require special culture procedures to grow and identify. *Mycoplasma pneumonia* causes a mild to moderate form of pneumonia, commonly called walking pneumonia; *Bordetella pertussis* causes whooping cough; *Legionella pneumophila*, Legionnaire's disease; *Chlamydia pneumoniae*, an atypical pneumonia; and *Chlamydia psittaci*, parrot fever.

Pneumocystis carinii causes pneumonia in people with weakened immune systems, such as people with AIDS. This organism does not grow in culture. Special stains are done on sputum when pneumonia caused by this organism is suspected. The diagnosis is based on the results of these stains, the patient's symptoms, and medical history.

Sputum culture is also called sputum culture and sensitivity.

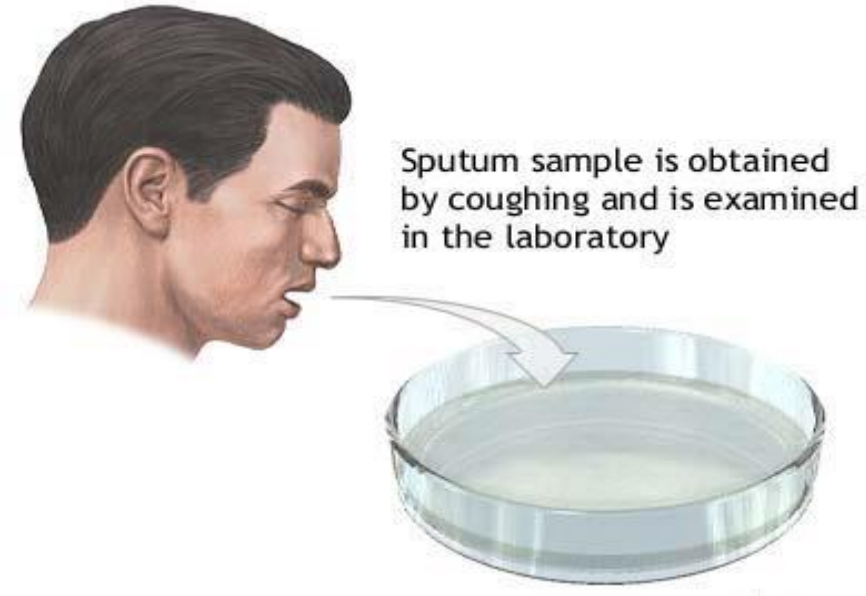
It is possible that sputum cultures will eventually be replaced in the diagnosis of tuberculosis by newer molecular techniques. These advanced methods speed the diagnostic process as well as improve its accuracy. As of late 2002, four molecular techniques are increasingly used in laboratories around the world to diagnose

TB. They include polymerase chain reaction to detect mycobacterial DNA in patient specimens; nucleic acid probes to identify mycobacteria in culture; restriction fragment length polymorphism analysis to compare different strains of TB for epidemiological studies; and genetic-based susceptibility testing to identify drug-resistant strains of mycobacteria.

Preparation

The specimen for culture should be collected before antibiotics are begun. Antibiotics in the person's system may prevent microorganisms present in the sputum from growing in culture.

The best time to collect a sputum sample is early in the morning, before having anything to eat or drink. The patient should first rinse his or her mouth with water to decrease mouth bacteria and dilute saliva. Through a deep cough, the patient must cough up sputum from within the chest. Taking deep breaths and lowering the head helps bring up the sputum. Sputum must not be held in the mouth but immediately spat into a sterile container. For tuberculosis, the physician may want the patient to collect sputum samples on three consecutive mornings.



A sputum sample is obtained by coughing deeply and expelling the material that comes from the lungs into a sterile cup. The sample is taken to a laboratory and placed in a medium under conditions that allow the organisms to grow. A positive culture may identify disease-producing organisms that may help diagnose bronchitis, tuberculosis, a lung abscess, or pneumonia.

If tuberculosis is suspected, collection of sputum should be carried out in an isolation room, with all attending healthcare workers wearing masks.

In addition to special precautions in collecting sputum when tuberculosis is suspected, workers in hospital laboratories must take extra care to inactivate unstained smear preparations that may contain *M. tuberculosis*. As of 2002, the most effective

deactivation technique is the use of a solution of 5% phenol in ethanol.

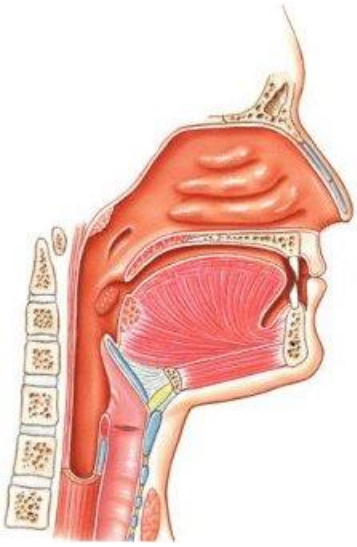
Normal results

Sputum from a healthy person would have no growth on culture. A mixture of microorganisms, however, normally found in a person's mouth and saliva often contaminates the culture. If these microorganisms grow in the culture, they may be reported as normal flora contamination.

Abnormal results

The presence of bacteria and white blood cells on the Gram stain and the isolation of a microorganism from culture, other than normal flora contamination, is evidence of a lower respiratory tract infection.

Microorganisms commonly isolated from sputum include: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Legionella pneumophila*, *Mycoplasma pneumonia*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bordetella pertussis*, and *Escherichia coli*.



ISOLATION OF BACTERIA FROM THE EAR, NOSE, & THROAT

Blood agar (BAP) is a common medium used to culture bacteria because 1) it is a great enrichment medium for **fastidious** bacteria, and 2) hemolysis of blood cells can be very useful as an identification test. Blood agar is made with 5% sheep blood. This You have actually used a variation of blood agar before, in **CNA** agar – (The CNA stands for "Colistin Naladixic Acid agar". CNA is used to select for and grow bacteria of potential medical importance, such as *Streptococcus pyogenes* (causes "Strep" throat) and *Staphylococcus aureus* (a cause of food poisoning and skin infections): the only difference is that CNA has an antibiotic that inhibits gram - bacteria.

Hemolysis is the breakdown of red blood cells: hemolysins are enzymes produced by some bacteria and are released into the medium around the bacterial colony. It can be a complete breakdown of the cells, with the release of hemoglobin and a clearing of the red from the surrounding medium around the colony. Or the hemolysis can be a partial breakdown, resulting in a greenish or green-yellow zone around the colony.

You will find a variety of bacteria in the throat: it is normal. This is true also of the ear and nose, but there is less diversity. In addition to your identification of different hemolytic reactions, you are also going to isolate a ***Staphylococcus*** species to be used in **the Kirby-Bauer test for antibiotic sensitivity**.

THE TEST OBJECTIVES:

Differentiate among various species from a clinical specimen.
Isolate a species of Staphylococcus for antibiotic susceptibility testing.
Identify the 3 hemolytic types on blood agar.

THE PROCEDURE:

1. **THROAT CULTURE:** Take a sterile swab and place it in the orifice of your patient. If performing a throat swab, be sure to hold the tongue down with the tongue depressor while going to the BACK of the throat with the swab.
2. **THROAT, NOSE, EAR CULTURES:** Prepare a streak plate (see earlier procedure if you need a reminder). However, you will use the swab for the first section of the plate, rolling it around to get as many bacteria off as possible). Switch over to your inoculating loop and continue on as you would a regular streak plate.
3. **KISS PLATE:** The agar plate is inoculated by kissing it---*gently*, because the agar medium is not very strong. You

also want to slightly touch the nose while kissing, so you can see the various populations in different areas.

4. Place the plates upside down in the candle jar. When all plates are in the jars, the candle will be lit, and the jars incubated at 37 ° C.

INTERPRETATION:

After incubation you will check the plates for:

- **different hemolytic reactions**
  - complete hemolysis = beta hemolysins produce a clear, yellow zone
  - partial helomysis = alpha hemolysins produce an opaque green/green-yellow zone
  - no hemolysis = gamma , no hemolysins, no zone

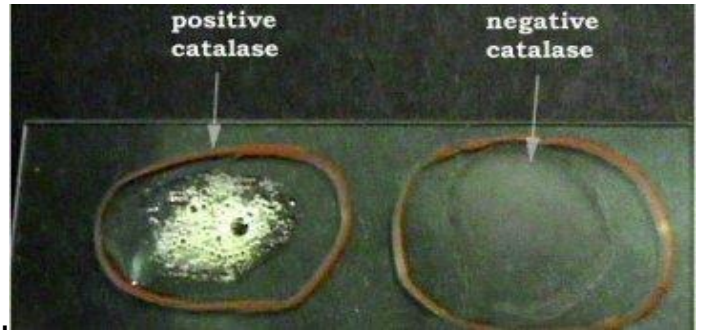
- **Different gram reactions** Gram stain a couple of different species. In particular, you are looking for a G+ coccus in clusters (potentially ***Staph***).
- **a *Staph* isolate**

1. Find a colony that is white, off white, or cream pigmented on the BAP. Gram stain. You will confirm that it is a ***Staph*** not only with a gram stain, but with a catalase test.
2. Once the colony is confirmed as a ***Staph*** species, subculture the colony onto a TSA plate, streak for isolation.
3. Incubate at 37^{o} C and then check for purity of the culture.
4. You will run the antibiotic susceptibility test on your own species of ***Staph*** in the next week or after.
5. **<u>One period</u>** before the day we are to perform the antibiotic sensitivity test, you need to subculture your ***Staph*** isolate into an TSB broth.

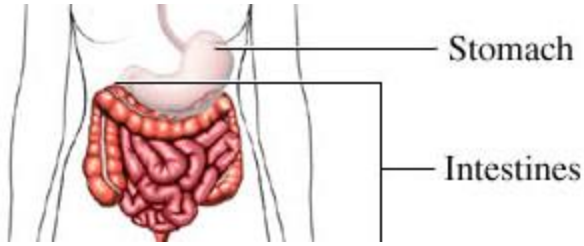
CATALASE TEST:

Place the inoculum from the BAP onto a microscope slide (this is not a smear, no water!).

Add one drop of H_2O_2 and watch for immediate bubbles (O_2 released from the hydrogen peroxide).



Stool Culture



Test Overview

A stool culture is done to identify bacteria or viruses that may be causing an infection **using MacConkey Agar**. More than 50 different kinds of bacteria normally live in the **intestines**. However, disease can result if large numbers of abnormal organisms (bacteria, viruses, fungi, or parasites) grow in the intestines. Certain types of viruses, fungi, or parasites can be identified with a stool culture. A stool culture may be done if you have persistent diarrhea.

For a stool culture, a stool sample is collected in a clean container and placed under conditions that allow bacteria or other organisms to grow. The type of infection is identified by noting the appearance of the growth, by performing chemical tests on the stool sample, and by looking at the sample under a microscope. If an infection is found, the test is positive. If there is no organism growth, the test is negative.

Usually several stool samples are collected over a period of days for accurate test results.

Why It Is Done

A stool culture is done to:

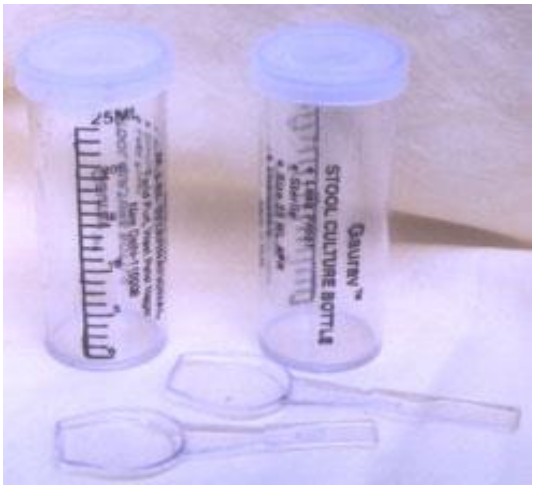
- Detect and identify certain types of bacteria, viruses, fungi, or parasites that can cause disease. Symptoms of an intestinal disease may include prolonged diarrhea, bloody diarrhea, an increased amount of gas, nausea, vomiting, loss of appetite, bloating, abdominal pain and cramping, and fever.
- Identify a person who may not have any symptoms of disease but who carries bacteria that can spread to others. This person is called a carrier. A person who is a carrier and who handles food is likely to infect others.

How To Prepare

No special preparation is required before having this test. Do not collect a stool sample if you have bleeding hemorrhoids. Women should not collect a stool sample during their menstrual period; wait until 3 days after your period has stopped. If you have recently taken antibiotics, traveled out of your native country, or had a recent test with contrast material, tell your health professional when you receive the stool sample collection container.

How It Is Done

The stool sample for this test may be collected at home or hospital.



STOOL CULTURE BOTTLE WITH SPOON 25ML (DISPOSABLE)

To collect the sample, you need to:

- Wear gloves before collecting your specimen.
- Pass stool (but not urine) into a dry container. You may be given a container that can be placed under the toilet seat. Either solid or liquid stools can be collected. Avoid mixing toilet paper, water, or soap with the sample.
- Seal the container and label it with your name, your health professional's name, and the date the sample was collected.
- Wash your hands well after collecting the sample to avoid spreading an infection.

- Deliver the sealed container as soon as possible to your health professional's office or directly to the lab.

Your health professional may collect a stool sample by gently inserting a cotton swab into the rectum of the patient if he/ she was unable to pass a stool sample.

How It Feels

Collecting a stool sample does not normally cause any discomfort.

Risks

There are no risks associated with collecting a stool sample. It is important to wear gloves before and wash your hands well after collecting the sample so that you do not spread an infection.

Results

A stool culture is done to identify bacteria, viruses, fungi, or parasites that may be causing an infection. Stool culture test results usually take 2 to 3 days.

Stool culture	
Normal:	No disease-causing (pathogenic) bacteria, viruses, fungi, or parasites are present or grow in the culture.
Abnormal:	Pathogenic bacteria (such as salmonella, shigella, campylobacter, certain types of *Escherichia coli*, or *Yersinia enterocolitica*) grow in the culture. Some of the more common diseases found using a stool culture include food poisoning and pseudomembranous enterocolitis. Fungi or parasites such as *Giardia lamblia* are found. Cholera and typhoid fever are less common diseases detected by stool culture.

If bacteria are found in the culture, sensitivity testing may be done to determine the best antibiotic to kill the bacteria.

Clostridium difficile **Organism**

Clostridium difficile is a bacterium commonly found in the intestinal tract but which, under the right circumstances such as after or during antibiotics therapy, can be the cause of enterocolitis.

What Affects the Test

Factors that can interfere with your test and the accuracy of the results include:

- Recent use of antibiotics, medication (such as bismuth) to control diarrhea, enemas, or laxatives.
- Recent X-ray tests using a contrast material containing barium.
- A stool sample contaminated with urine or blood.
- Delay in getting the stool sample to the lab for testing.

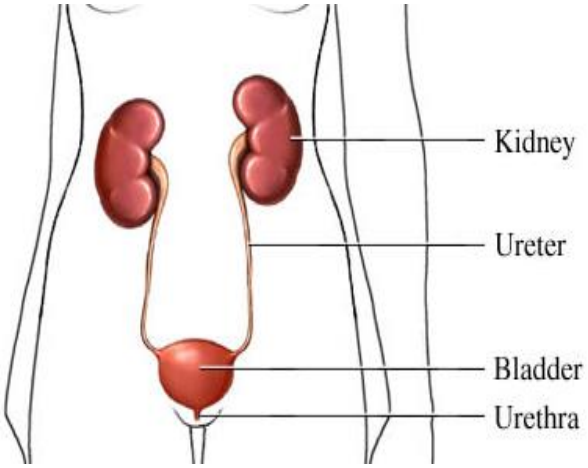
What To Think About

- A stool culture that does not grow any disease-causing (pathogenic) organism may not rule out an infection. Factors such as the amount of stool collected, the type of culture done, and previous use of antibiotics can prevent the growth of an organism in the culture.
- Sensitivity testing helps the health professional choose the best medication to kill the specific types of bacteria or fungi infecting a person.
- A test for parasites may be done using a sample of stool. Parasites are neither bacteria nor viruses and include organisms such as worms (pinworms, roundworms, tapeworms) and the protozoan *Giardia* that causes giardiasis. These parasites commonly infect the intestines. The parasites or their eggs can

often be seen during an examination of the stool sample under a microscope.

- A stool sample can also be checked for the presence of:
  - White blood cells, which may indicate an infection. If many white blood cells are present but no harmful bacteria grow in the stool culture, other diseases (such as inflammatory bowel disease) may be present.
  - Blood, which may indicate sores inside the intestines or stomach.
  - Poisonous substances (toxins) produced by some types of bacteria.

Urine Culture



Test Overview

A urine culture is a test to detect and identify organisms (usually bacteria) that may be causing a urinary tract infection (UTI). Urine in the bladder normally is sterile—it does not contain any bacteria or other organisms (such as fungi)—but a urine sample can pick up bacteria in the urethra during urination. A urine sample is kept under conditions that allow bacteria and other organisms to grow. If few organisms grow, the test is negative. If organisms grow in numbers large enough to indicate an infection, the culture is positive. The type of organisms causing the infection are identified with a microscope or by chemical tests.

Urinary tract infections are more common in women and girls than in men. This may be partly because the female urethra is shorter and closer to the anus, which allows bacteria from the intestines to

come into contact more easily with the urethra. Men also have an antibacterial substance in their prostate gland that reduces their risk.

If bacteria that can cause infection grow in the culture, another test may be done to determine which antibiotic will be most effective in treating the infection. This is called susceptibility or sensitivity testing.

Why It Is Done

A urine culture may be done to:

- Diagnose a urinary tract infection (UTI).
- Identify the best antibiotic to treat a urinary tract infection. This is called sensitivity testing.
- Determine whether a urinary tract infection has been cured.

How To Prepare

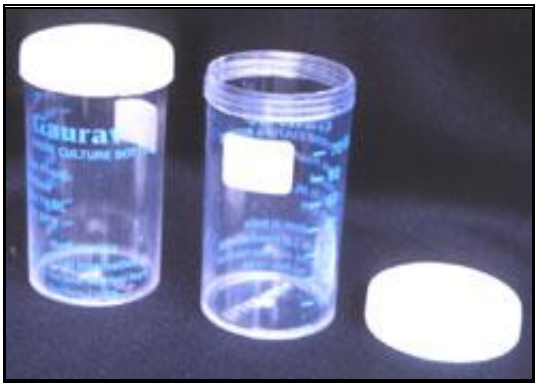
No special preparation is required before having this test. If you are currently taking or have recently taken antibiotics, tell your health professional.

How It Is Done

You will be asked to collect a clean-catch midstream urine sample for testing. The first urine of the day is preferred because bacterial levels will be higher.

Clean-catch midstream urine collection

This collection method prevents contamination of the sample.



URINE CULTURE BOTTLE
70ml with screw cap (Disposable)

- Wash your hands to make sure they are clean before collecting the urine.
- If the collection container has a lid, remove it carefully and set it down with the inner surface up.
- Clean the area around your genitals.
- After the urine has flowed for several seconds, place the collection container in the stream and collect of this "midstream" urine without interrupting the flow.
- Avoid touching the rim of the container to your genital area, and avoid getting toilet paper, hair, feces, or menstrual blood in the urine sample.

- Carefully replace the lid on the container. Wash your hands. Return the urine sample to the lab. If you are collecting the urine at home and cannot get it to the lab within an hour, refrigerate the sample. It can be refrigerated for up to 24 hours. Follow the instructions from your lab.

How It Feels

Collecting a urine sample does not normally cause any discomfort.

Risks

There are no risks associated with collecting a urine sample.

Results

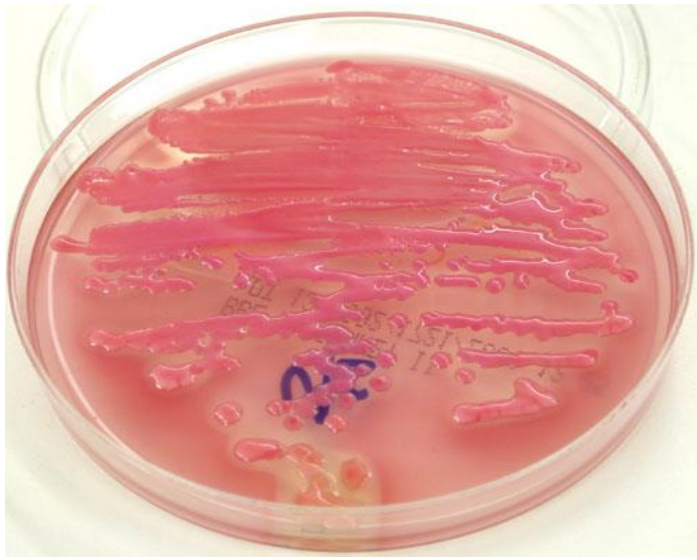
A urine culture is a test to detect and identify organisms (usually bacteria) that may be causing a urinary tract infection (UTI). Urine culture results are usually ready in 1 to 3 days. Some organisms take longer to grow in the culture; for this reason, results may not be available for several days.

Urine culture	
Normal:	No bacteria or other organisms (such as fungi) grow in the culture. The culture result is negative. Normal results may vary from lab to lab.

Abnormal:	Organisms (usually bacteria) grow in the culture. The culture result is positive. Urinary tract infections are usually caused by bacteria that normally live in the intestines, such as *E. coli*. The number of bacteria in a given quantity of urine is usually estimated. A count of 100,000 or more bacteria per milliliter (mL) of urine may indicate an infection. A count ranging from 100 to 100,000 could be due either to infection or to contamination of the sample (you may need a repeat urine culture). If the count is 100 or less, infection is unlikely; however, a count of 100 or less may also be seen if you are taking antibiotics for a UTI.

If test results indicate an infection, sensitivity testing may be done to determine the best antibiotic to kill the bacteria.

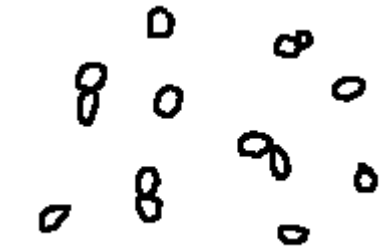
Lactose-fermenting, Gram negative rods.
MacConkey agar plate of *E. coli* from urine (>10^6 CFU/mL). Note pink, non-shiny, lactose$^+$ colonies that are characteristic for *E. coli*.



Urine culture on MacConkey agar. Lactose+, mucoid colonies identified as *E. coli* by API testing (not to be confused with mucoid nature of *Klebsiella pneumoniae*).

Yeast

Yeast cells may be contaminants or represent a true yeast infection. They are often difficult to distinguish from red cells and amorphous crystals but are distinguished by their tendency to bud. Most often they are Candida, which may colonize bladder, urethra, or vagina.



What Affects the Test

Factors that can interfere with your test and the accuracy of the results include:

- Using diuretics or drinking a large amount of liquid. This may dilute your urine and lower the concentration of bacteria, causing inaccurate test results.
- Recent use of antibiotics or taking high doses of vitamin C.
- Some organisms take several days to grow in the culture.

What To Think About

- A urine culture done in the early stage of a urinary tract infection (UTI) may be less accurate than one that is done after the infection becomes established.
- A urine culture may be done when an abnormal result from a urinalysis (such as an increased number of white blood cells) indicates an infection.
- A urine culture may be repeated after the UTI has been treated to make sure the infection is cured.
- To reduce the chance of contaminating the urine sample with bacteria (other than the bacteria causing the infection), a health professional may collect a urine sample by inserting a urinary catheter through the urethra into the bladder. Catheterization is sometimes done to collect urine from a person in the hospital who is very ill or unable to provide a clean-catch sample. This method reduces the risk that the sample will be contaminated but may occasionally cause a UTI.
- People who have a urinary catheter in place for a long time are at high risk of developing a UTI.
- Collecting a urine sample from a small child or baby may be done by using a special plastic bag with tape around its opening (a U bag). The bag is attached around the child's genitals until he or she urinates (usually within an hour). Then the bag is carefully removed. To collect a urine sample from a very sick baby, a

health professional may insert a needle through the baby's abdomen directly into the bladder.

- To diagnose tuberculosis that has spread to the urinary tract, a special test will be done using all of the first morning urine on three separate days.
- Sensitivity testing helps a health professional choose the best antibiotic for the specific type of bacteria infecting a specific person. Differences in the genetic material (DNA) in some types of bacteria make them resistant to certain antibiotics. In such cases, those antibiotics cannot kill all the bacteria. When an effective antibiotic is chosen, its entire course must be completed to prevent the bacteria from developing resistance to the antibiotic. Stopping antibiotic treatment early kills only the most sensitive bacteria, while bacteria that are more resistant can multiply and prolong the infection.

MACROSCOPIC URINALYSIS

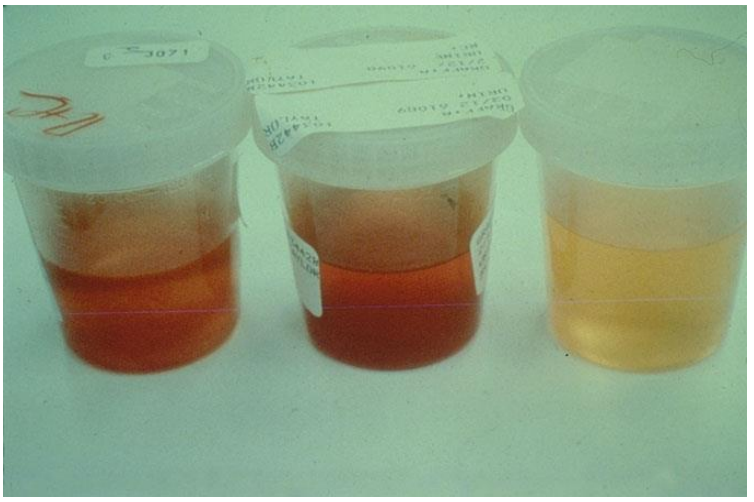
The first part of a urinalysis is direct visual observation. Normal, fresh urine is pale to dark yellow or amber in color and clear. Normal urine volume is 750 to 2000 ml/24hr.

Turbidity or cloudiness may be caused by excessive cellular material or protein in the urine or may develop from crystallization or precipitation of salts upon standing at room temperature or in the refrigerator. Clearing of the specimen after addition of a small

amount of acid indicates that precipitation of salts is the probable cause of tubidity.

A red or red-brown (abnormal) color could be from a food dye, eating fresh beets, a drug, or the presence of either hemoglobin or myoglobin. If the sample contained many red blood cells, it would be cloudy as well as red.

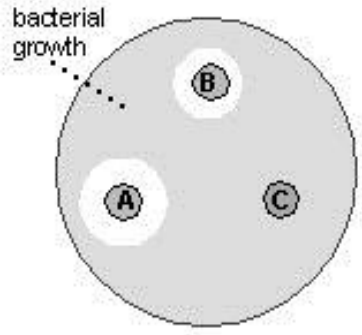
Examples of appearances of urine



Three urine samples are shown. The one at the left shows a red, cloudy appearance. The one in the center is red but clear. The one on the right is yellow, but cloudy.

KIRBY-BAUER TEST for Antibiotic Susceptibility

The Kirby-Bauer test for antibiotic susceptibility, called the disc diffusion test, is a standard that has been used for years. It has been super ceded in clinical labs by automated tests. But the K-B is still used in some labs, or used with certain bacteria.



The basics are easy: The bacterium is swabbed on the agar and the antibiotic discs are placed on top. The antibiotic diffuses from the disc into the agar in decreasing amounts the further it is away from the disc. If the organism is killed or inhibited by the concentration of the antibiotic, there will be **NO growth** in the immediate area around the disc: This is called the **zone of inhibition**.The zone sizes are looked up on a standardized chart to give a result of sensititive, resistant, or intermediate. Many charts have a corresponding column that also gives the **MIC** (minimal inhibitory concentration) for that drug.

You will need to subculture the isolated bacterium **(from ear, nose, or throat)** into a fresh TSB ***PRIOR to this lab*** in order to have a young culture to run the antibiotic sensitivity test. Have you made sure that the culture is a G+ coccus, catalase +? Is it growing well in the TSB broth?

However, you will see that there can be a lot of variation between species, for example **species** of ***Staph***, and even strains within one species. The Mueller-Hinton medium being used for the K-B is very high in protein, in particular.

Swabbed correctly

Not swabbed correctly

THE PROCEDURES:

1. Swab a Mueller-Hinton plate with each of the bacteria. Dip a sterile swab into the broth and express any excess moisture by pressing the swab against the side of the tube.
2. Swab the surface of the agar completely (**you do not want to leave any unswabbed agar areas at all**). In the pictures above and below, you can see what happens when the plate is not swabbed correctly with even coverage of the bacterium over the entire agar.

3. After completely swabbing the plate, turn it 90 degrees and repeat the swabbing process. (It is not necessary to re-moisten the swab.) Run the swab around the circumference of the plate before discarding it in the discard bag.
4. Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar.
5. **THE ANTIBIOTIC DISKS:**
    - The antibiotic dispensers have 8 antibiotic cartridges in them. If you do not see 8 disks come out onto your agar plate, you will have to manually remove the antibiotic from a free cartridge (see line below).
    - Each free antibiotic cartridge should have a little metal arm that allows you to dispense the disc right onto the agar. Even so, sometimes the discs pop out and fall in a place on the agar that you do not want it to be. Just quickly pick up the disc and move it to the appropriate place with the sterile forceps.
    - Lightly touch each disc with your sterile inoculating loop to make sure that it is in good contact with the agar surface. Incubate upside down and incubate at 37^{o} C

INTERPRETATION:

1. Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge. HOLDING THE PLATE UP TO THE LIGHT MIGHT HELP.
2. The disc diameter will actually be part of that number. If there is NO zone at all, report it as 0---even though the disc itself is around 7 mm.
3. Zone diameter is reported in millimeters, looked up on the **chart,** and result reported as S (sensitive), R (resistant), or I (intermediate).

4. Record the results for your sample in the table below.

Antibiotics	**isolate 1**		**isolate 2**	
	zone diameter	**S, R, or I**	**zone diameter**	**S, R, or I**

ZONE SIZE INTERPETIVE CHART FOR THE KIRBY-BAUER TEST					
ANTIMICROBIAL AGENT	**DISC CODE**	**R = mm or less**	**I = mm range**	**MS =**	**S = mm or more**
amoxicillin (Staph)	AMC	19			20
amoxicillin (other bacteria)	AMC	13	14-17		18
ampicillin (Staph)	AM	28			29
ampicillin (other bacteria)	AM	11	12-13		14
carbenicillin (Pseudomonas)	CB	13	14-16		17
carbenicillin (other bacteria)	CB	17	18-22		23
cefoxatime	CTX	14		15-22	23
cephalothin	CF	14	15-17		18
chloramphenicol	C	12	13-17		18
erythromycin	E	13	14-22		23
gentamycin	GM	12	13-14		15
methicillin (used for Staph only)	M (or DP)	9	10-13		14
penicillin	P	28			29
streptomycin	S	11	12-14		15
sulfamethoxazole-trimethoprim	SXT-TMP	10	11-15		16
tetracycline	TE	14	15-18		19

R = resistant I = intermediate S = sensitive
MS = moderate sensitive.

Antimicrobial Chemotherapy

The term chemotherapy was just used by **Enrlich (1904)** to describe the use of synthetic chemicals to destroy infective agents. The modern use of antimicrobial chemotherapy started with the clinical use of **sulfonamide in 1936**- but "the golden age" of antibiotics began with the production of **penicillin in 1941**, when this compound was mass produced and first made available for limited clinical trail. Nowadays, at least 30% of all hospitalized patients receive one or more courses of antibacterial chemotherapy.

The ideal antimicrobial agent is that agent which shows "selective toxicity" this means that the antimicrobial agent must produce toxic effect only on microbial cells but not on host cells. The feasibility of such selective toxicity depends primarily on the existence of biochemical and structural differences between the microbial cell and the host cell. For example, antibacterial agents that act via inhabiting the bacterial cell wall synthesis will show this selective toxicity where this cell wall absent in mammalian cells.

The term antimicrobial chemotherapy includes not only antibiotics (which substances produced by the various species of living microorganisms such as bacteria, fungi, and actinomycetes e.g. penicillins, cephalosporins, tetracyclins, etc.) but also applied to the use of synthetic chemicals (such as saulfonamides, trimethoprim, and quinolones).

The antibacterial agents are either bactericidal agent (destroy the bacterial cell) and/ or bacteriostatic (they only inhibit the growth of bacterial cells).

Why antimicrobial chemotherapy?

Antimicrobial agents exhibit selective toxicity it interferes at a concentration tolerated by the host, with some metabolic or synthetic process that exist only in the infectious organism not in the cells of the host it acts.

1- Inhibition of cell wall synthesis.
2- Alteration of cell membrane permeability or inhibit active transport across cell membrane.
3- Inhibition of protein synthesis, inhibit translation, transcription of genetic material.
4- Inhibit nucleic acid synthesis.
5- Competitive inhibition with PABA in sulfa.

Classification of Antimicrobial agents

1- According to the spectrum against bacteria:

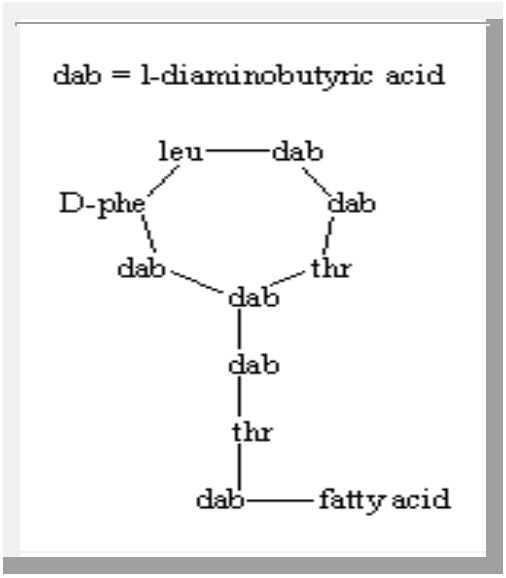
a) Agents acting mostly against Grame positive bacteria e.g., penicillin G, erythromycin, vancomycin.
b) Agents acting mostly against Grame negative bacteria e.g., aminoglycosides and polymyxins antibiotics.

c) Broad spectrum antibacterial agents e.g., chloramphenicl and tetracyclines. They act against both Grame positive and Grame negative bacteria.

2- According to the mechanism of action:

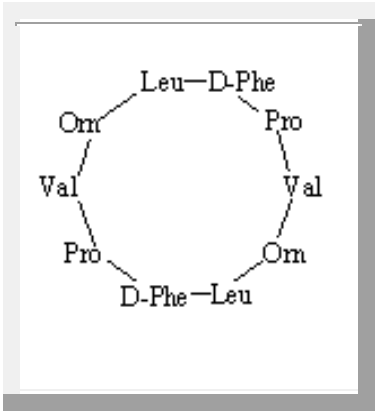
Which antibacterial agents affect bacterial cell membrane function?

Antibiotics including the **polymixins** and **gramicidin** act by interfering with the functioning of the bacterial cell membrane by increasing its permeability. **Gramicidin** is one of a family of cyclic decapeptides active against Gram-positive bacteria. **Polymixins** have a smaller peptide ring attached to a peptide chain ending with a branched fatty acid. They act specifically against Gram-negative bacteria, although chemically modified derivatives do have a broader spectrum of activity. These antibiotics are toxic to humans and are now rarely used in clinical practice.



Chemical structure of a polymixin

(for polymixin B, the fatty acid is 6-methyloctanoic acid)



Chemical structure of gramicidin

What inhibits bacterial cell wall synthesis?

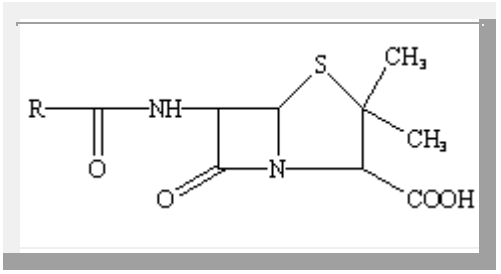
Peptidoglycan is an exclusively bacterial polymer and so potentially should provide an excellent target for selective chemotherapy.

Peptidoglycan is unique among biological polymers because it contains both L- and D- isomers of its constituent amino acids. Antibiotics may act at several stages during peptidoglycan synthesis. Some are valuable chemotherapeutic agents; others are too toxic for human use.

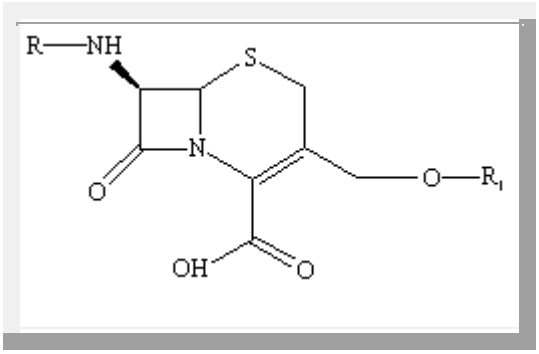
β- lactams

The β-lactam group of antibiotics includes an enormous diversity of natural and semi-synthetic compounds that inhibit several enzymes associated with the final step of peptidoglycan synthesis. All of this enormous family is derived from a β-lactam structure:

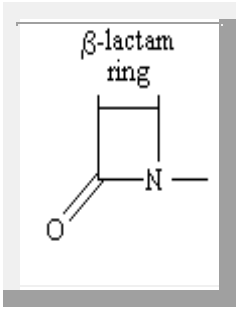
Clinically useful families of **β**- lactam compounds include the penicillins, cephalosporins, monobactams and carbapenems.



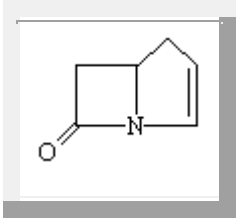
Penicillin nucleus



Cephalosporin nucleus



Monobactam nucleus



Carbapenem nucleus

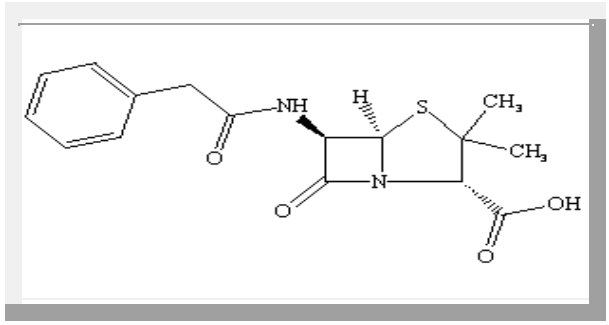
The targets for β-lactam drugs are the **penicillin binding proteins (**PBP's**)**, so called because they bind radioactive penicillin and can be detected by autoradiography of gels on which bacterial proteins have been separated electrophoretically. The penicillin

binding proteins have transpeptidase or carboxypeptidase activity and they act to regulate cell size and shape. They are also involved in septum formation and cell division. Bacteria have several individual penicillin binding proteins, each with a separate function. Conventionally these are numbered according to size, with PBP 1 as the largest protein. The PBP 1 of one bacterium will not necessarily have the same function as the PBP 1 of a different organism.

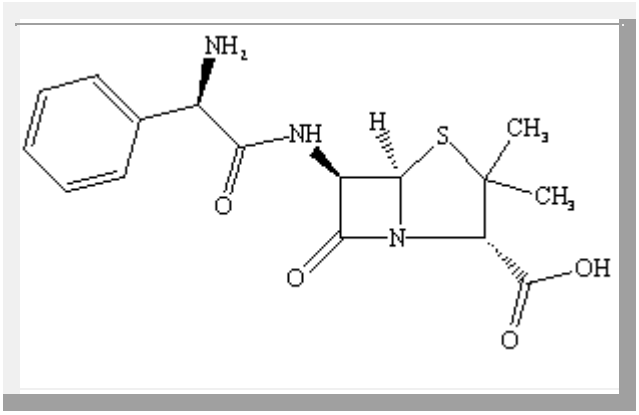
The β-lactam antibiotics may bind preferentially to different penicillin binding proteins and at sub-lethal concentrations may cause alterations in cell morphology. For example, mecillinam binds preferentially to *Escherichia coli* PBP 2 and causes spherical cells to form, whereas cephalexin causes *Escherichia coli* to grow as filaments as a result of its preferential binding to PBP 3. This indicates that PBP 2 in *Escherichia coli* is involved in cell elongation whereas its PBP 3 is has a role in the cell division of this bacterium.

The β-lactam antibiotics also stimulate the activity of **autolysins**. These are enzymes that are responsible for the natural turnover of cell wall polymers to permit growth of the cells. Under normal conditions, these enzymes produce controlled weak points within the peptidoglycan structure to allow for expansion of the cell wall structure. This activity is stimulated by β-lactams, causing a breakdown of peptidoglycan and leading to osmotic fragility of the cell and ultimately to cell lysis.

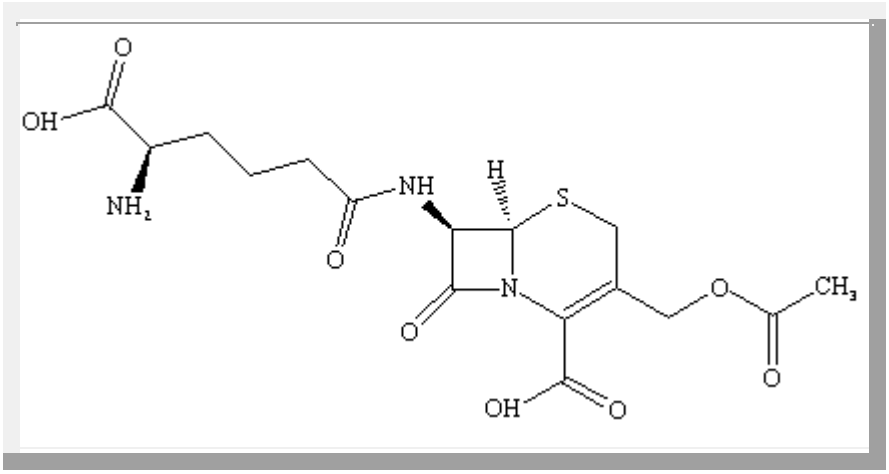
Some β-lactam antibiotics



Benzyl penicillin



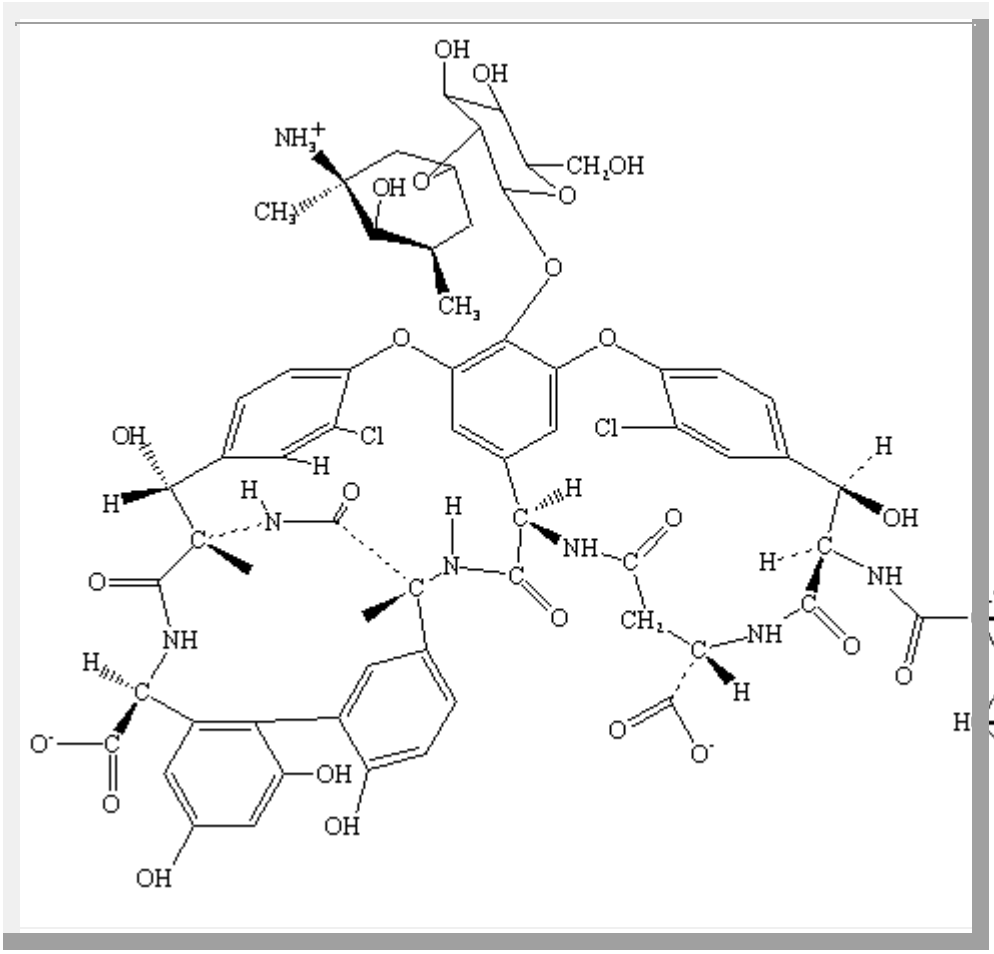
Ampicillin



Cephalosporin C

Vancomycin

The molecule of **vancomycin** is relatively large. The drug acts to prevent peptidoglycan subunits from being added to the growing cell wall polymer. This is accomplished by vancomycin binding to the D-alanyl D-alanine residue of the lipid-bound precursor. Its primary activity is against Gram-positive bacteria. It is particularly useful in the treatment of serious staphylococcal infections. In these cases, it is given either intramuscularly or intravenously since it is not absorbed from the gut. It is also used for the treatment of pseudomembranous colitis caused by *Clostridium difficile* when it is administered orally.

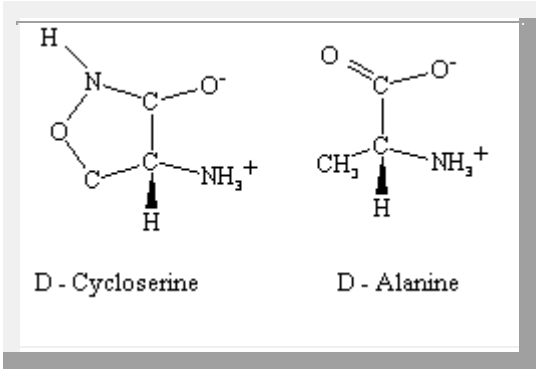


Chemical structure of vancomycin

Cycloserine

The simple, cyclic molecule **cycloserine** is an analogue of alanine that interferes with two steps in peptidoglycan synthesis. It is a competitive inhibitor of the racemase that converts L-alanine to D-alanine and it also prevents the action of the D-alanyl D-alanine synthetase. The stable ring structure of cycloserine holds the molecule in a sterically favourable position, permitting preferential

binding of this compound both to the racemase and to the synthetase, rather than their natural substrates. This results in competitive inhibition of these enzymes. Cycloserine is a neurotoxin and is not used clinically except for the treatment of drug-resistant *Mycobacterium tuberculosis*, or in other life-threatening infections where alternative therapies have failed.

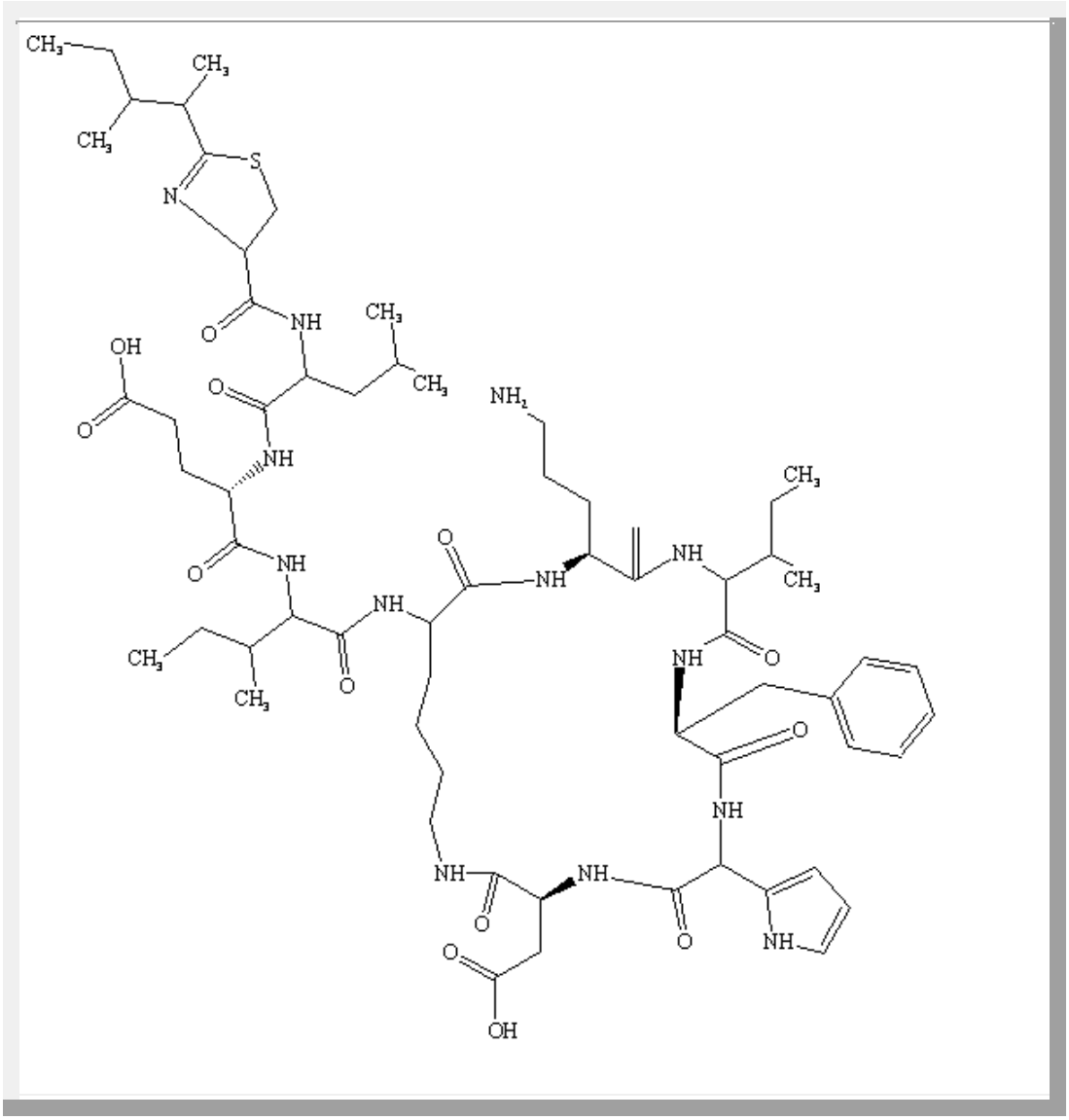


Chemical structure of cycloserine and its analogue, D-alanine

Bacitracin

The polypeptide antibiotic **bacitracin** is too toxic for human clinical use. It is, however, widely used in diagnostic laboratories to distinguish bactracin-sensitive *Streptococcus pyogenes* from other β- haemolytic streptococci. Its activity depends upon its ability to bind to the undecaprenyl pyrophosphate lipid carrier that transports the peptidoglycan monomers across the bacterial membrane. This impedes the dephosphorylation of the carrier, which, in turn, obstructs regeneration of the undecaprenyl phosphate and thus prevents recycling of the transport

mechanism. Bacitracin also interferes with sterol synthesis in mammalian cells by binding to pyrophosphate intermediates, accounting for its human toxicity.

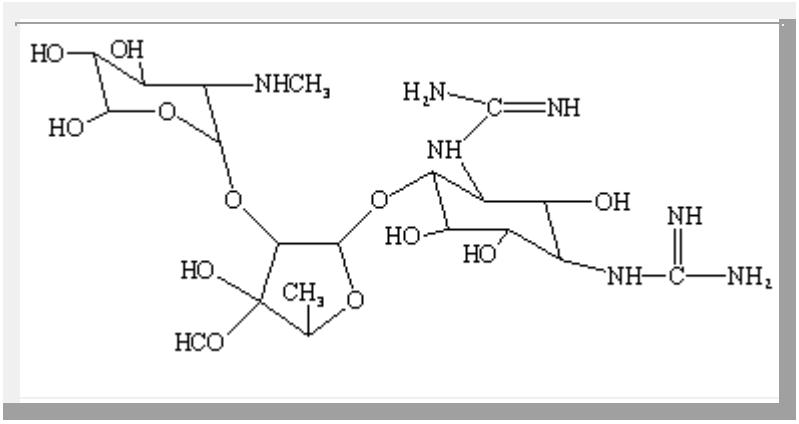


Chemical structure of bacitracin

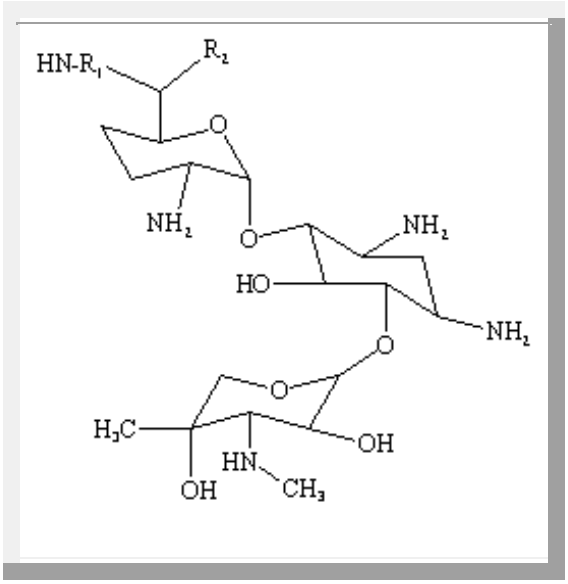
Which antibacterial agents are inhibitors of protein synthesis?

Aminoglycosides

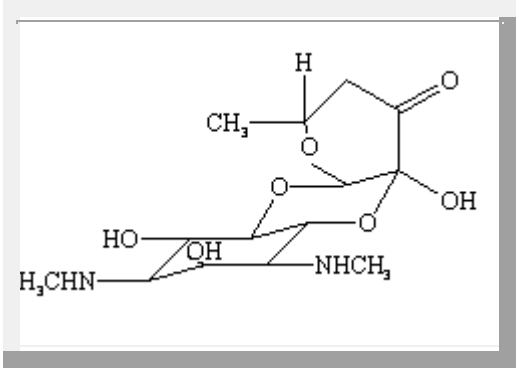
The aminoglycosides are a clinically important group of antibiotics that have a broad-spectrum of activity and that are bactericidal in action. The family includes **streptomycin**, **gentamicin**, **tobramycin**, **kanamycin**, **amikacin** and **netilmicin**. The aminocyclitols such as **spectinomycin** are closely related and have a similar mode of action. Aminoglycosides have a variety of effects within the bacterial cell but principally they inhibit protein synthesis by binding to the 30S ribosomal subunit to prevent the formation of an initiation complex with messenger RNA. They also cause misreading of the messenger RNA message, leading to the production of nonsense peptides. Another important function of the aminoglycosides is that they increase membrane leakage. Antibiotics such as **gentamicin** and **kanamycin** exist as mixtures of several closely related structural compounds; those like **netilmicin** and **amikacin** have a single molecular structure.



Chemical structure of streptomycin



Chemical structure of gentamicin

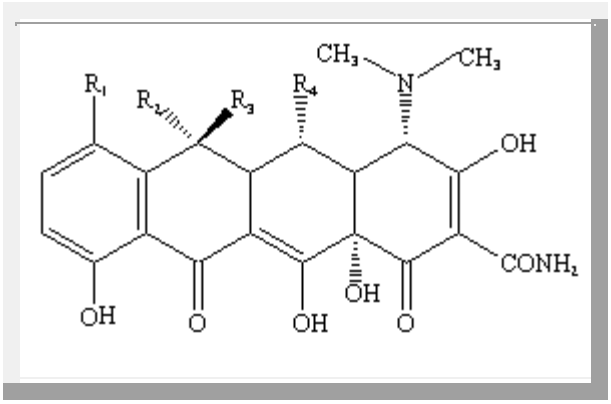


Chemical structure of spectinomycin

Aminoglycosides are toxic to humans, causing problems with kidney function and damage to the eighth cranial nerve. This leads to hearing loss and balance difficulties. The therapeutic use of the aminoglycosides requires careful monitoring to ensure adequate therapeutic levels are maintained, without the accumulation of the drug to toxic levels.

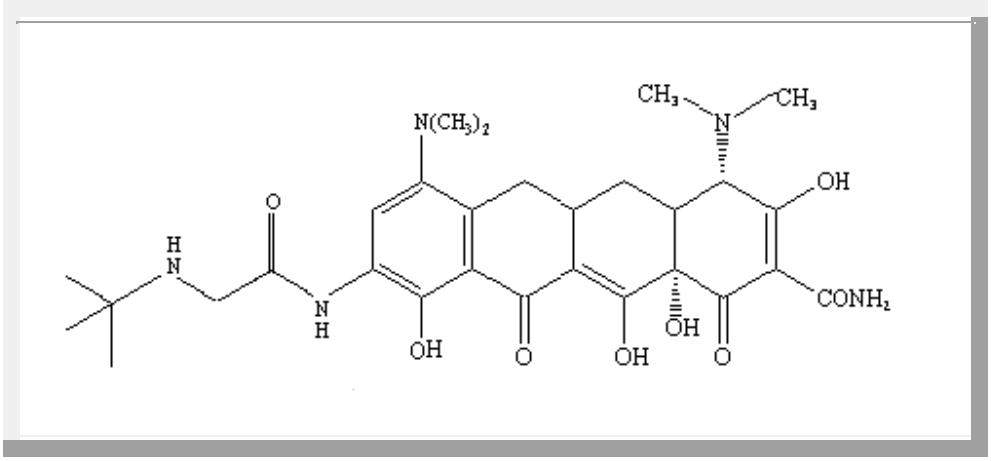
Tetracyclines

The **tetracyclines** are a family of antibiotics that have a four-ring structure. They are broad-spectrum agents that inhibit binding of the aminoacyl tRNA to the 30S ribosomal subunit in bacteria. The action is bacteriostatic and can therefore be reversed upon removal of the drug. The clinical use of tetracyclines is generally confined to adults. This is because tetracyclines affect bone development and can cause staining of teeth in children.



Chemical structure of a tetracycline

Tigecycline is a new tetracycline that is active against resistant *Staphylococcus aureus*.

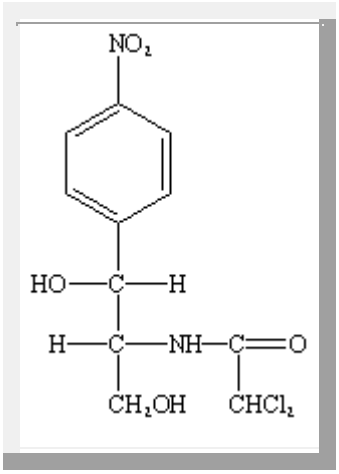


Chemical structure of tigecycline

Chloramphenicol

The broad-spectrum bacteriostatic agent **chloramphenicol** is toxic to humans. It has been recognised as a cause of aplastic

anaemia and so its use is confined to life-threatening infections where no alternative therapy is available. It acts by binding to the 50S ribosomal subunit and blocking the formation of the peptide bond by inhibiting peptidyl transferase activity. It is a potent inhibitor of mitochondrial protein synthesis in eukaryotic cells.

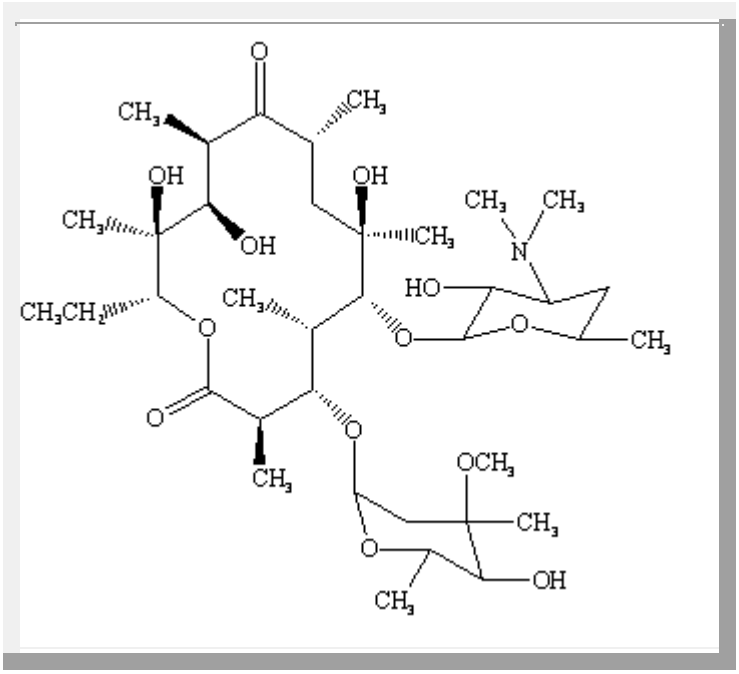


Chemical structure of chloramphenicol

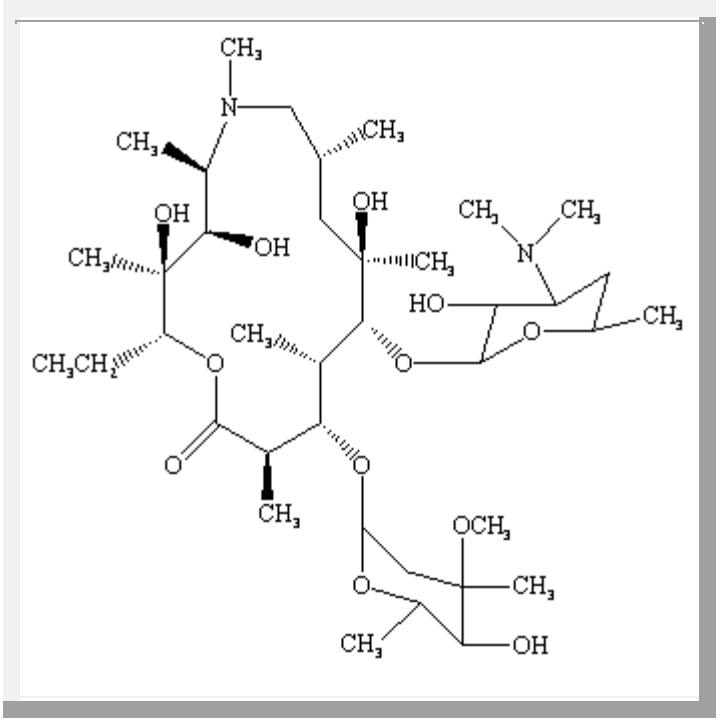
Macrolides and lincosamides

The macrolides are a group of antibiotics that have a large, lactone ring structure. These may be 14- or 16-membered rings. The most widely used macrolides are erythromycin and clarithromycin. These are relatively non-toxic antibiotics, most active against Gram-positive bacteria. Erythromycin is, however, the treatment of choice for Legionnaire's disease caused by the Gram-negative bacillus *Legionella pneumophila* and it is also active against *Haemophilus influenzae*, another Gram-negative bacillus.

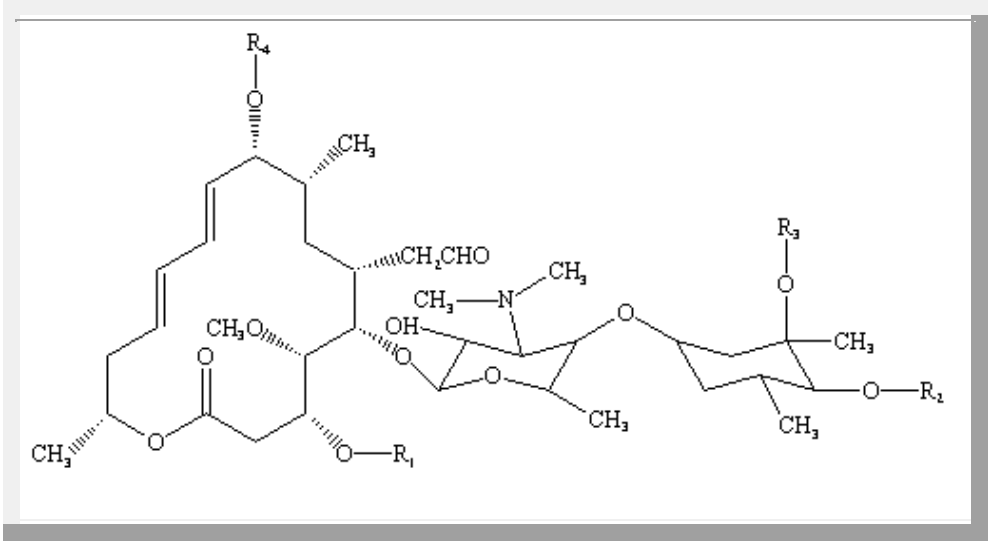
Erythromycin binds to the 50S ribosomal subunit and inhibits either peptidyl transferase activity or translocation of the growing peptide. Newer macrolides include azithromycin and clarithromycin. These have the same activity as erythromycin but they have better pharmacological properties.



Chemical structure of erythromycin

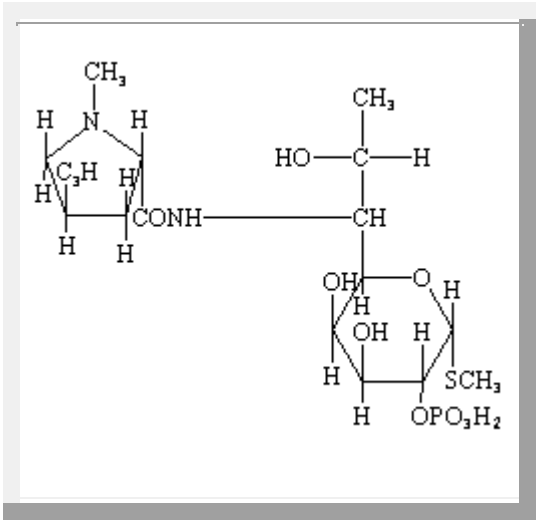


Chemical structure of azithromycin

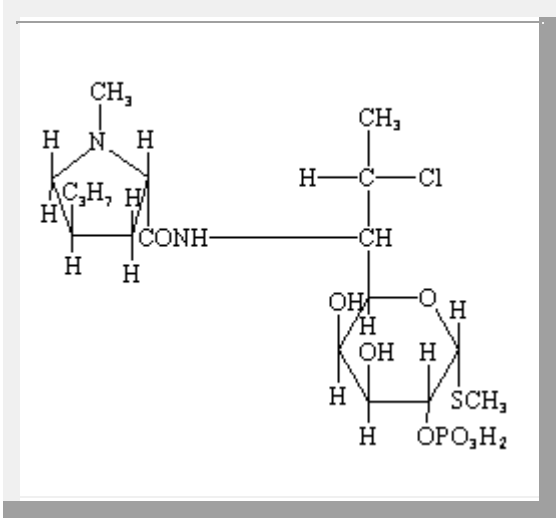


Chemical structure of a macrolide with a 16-membered ring

The lincosamide antibiotic **lincomycin** and its semi-synthetic derivative **clindamycin** have a similar mode of action.



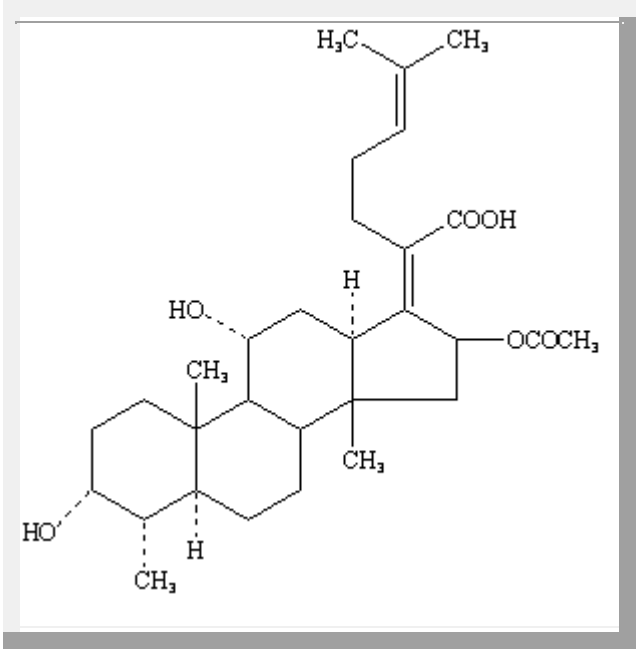
Chemical structure of lincomycin



Chemical structure of clindamycin

Fusidic acid

The steroid antibiotic **fusidic acid** is used to treat Gram-positive infections. It acts by preventing translocation of peptidyl tRNA. Resistant mutants may easily be selected, even during therapy and therefore fusidic acid is usually administered in combination with another antibiotic.

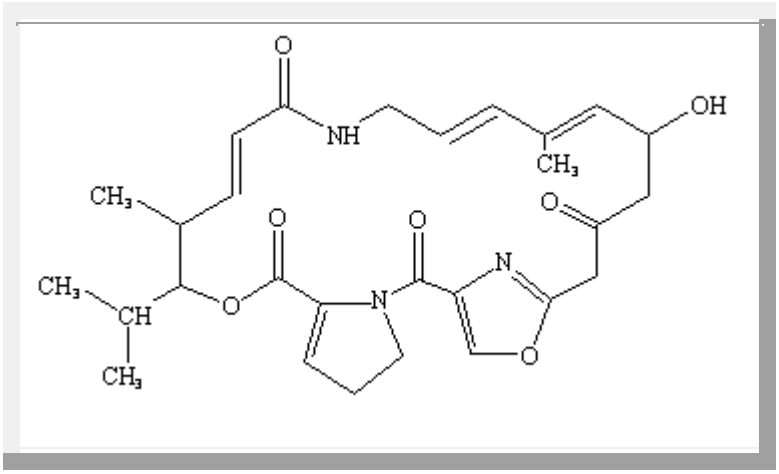


Chemical structure of fusidic acid

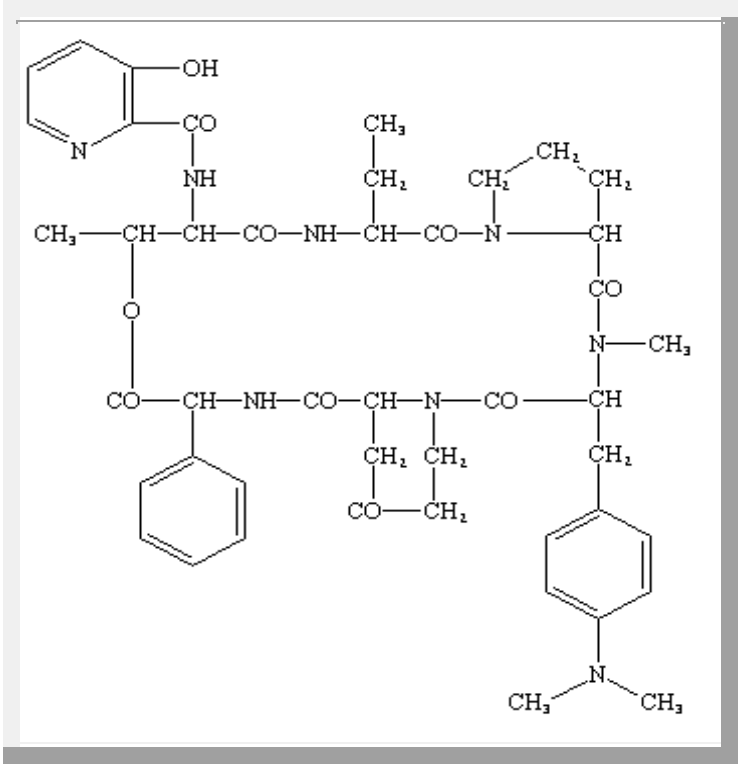
Streptogramins

The streptogramins fall into two groups, A and B. Streptogramins belonging to Group A have a large non-peptide ring, which is polyunsaturated. Streptogramins related to streptogramin B are

cyclic peptides. They differ in their modes of action although both inhibit bacterial protein synthesis. Group A streptogramins distort the ribosome to prevent binding of the t-RNA; Group B streptogramins are thought to block translocation of the growing peptide.



Chemical structure of streptogramin A



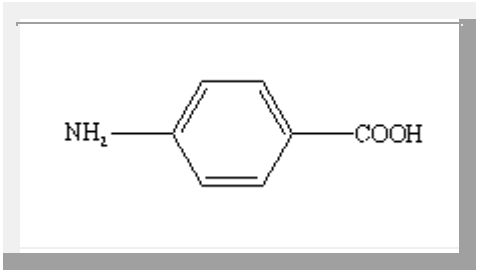
Chemical structure of streptogramin B

Which antibacterial agents are inhibitors of nucleic acid metabolism?

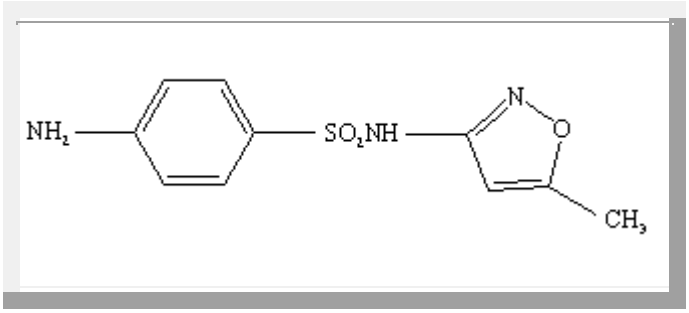
Nucleic acid metabolism may be interrupted at many steps. Antibacterial agents show selective toxicity either because humans lack the metabolic processes that act as targets, or because the bacterial targets are much more susceptible to particular chemicals than their eukaryotic counterparts.

Sulphonamides and trimethoprim

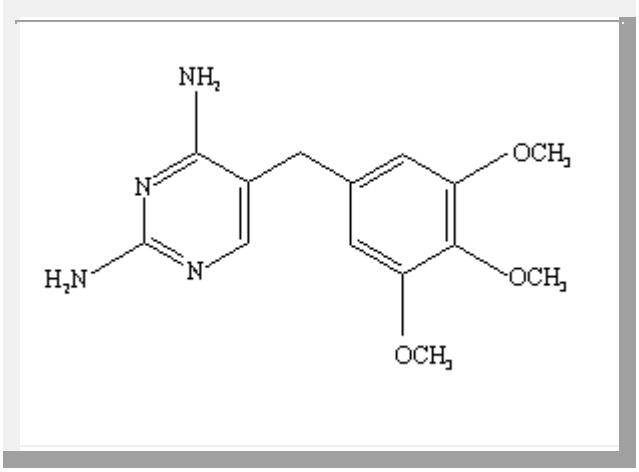
Humans are unable to make folic acid, a precursor of purine synthesis. We require an exogenous supply of this metabolite obtained from our diet. Many bacteria are, however, able to generate folic acid from *para*-amino benzoic acid (PABA) and this pathway provides a target for synthetic antimicrobial agents like the **sulphonamides** and **trimethoprim**. **Sulphonamides** act by inhibition of dihydropteroate synthetase because it acts as a structural analogue of the normal substrate, PABA. Trimethoprim inhibits dihydrofolate reductase, the next step in the folic acid biosynthetic pathway. **Trimethoprim** was first introduced to be used in combination with sulphonamides to potentiate their activity. Studies of the combination *in vitro* show that the combination is synergistic. This means that the combined activity of the drugs is more effective than the additive action of the individual components. The synergism observed *in vitro*, however depends upon maintaining a critical ratio of the two antimicrobials. Because of pharmacological constraints, this cannot be achieved in the body, raising doubts about the synergism *in vivo*. Furthermore, using two agents for chemotherapy significantly increases the risk of the patient developing an adverse reaction to the treatment. Such arguments led to the introduction and successful use of **trimethoprim** as a **single agent**.



Chemical structure of *para*-aminobenzoic acid



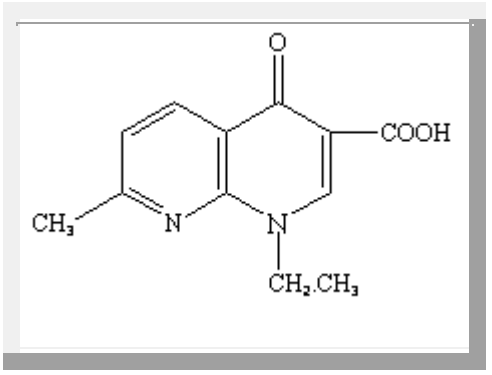
Chemical structure of sulphamethoxazole, a sulphonamide



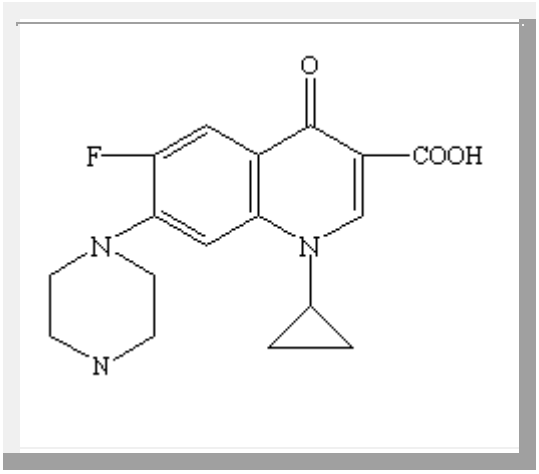
Chemical structure of trimethoprim

Quinolones

Bacterial DNA exists in a supercoiled form and the enzyme DNA gyrase, a topoisomerase, is responsible for introducing negative supercoils into the structure. Quinolone antibacterial drugs such as **nalidixic acid**, **norfloxacin**, **ofloxacin** and **ciprofloxacin** act by inhibiting the activity of the bacterial DNA gyrase, preventing the normal functioning of DNA. Humans do possess DNA gyrase but it is structurally distinct from the bacterial enzyme and remains unaffected by the activity of quinolones. These are broad-spectrum agents that rapidly kill bacteria and are well absorbed after oral administration. Overuse of these drugs in certain situations is selecting quinolone resistant mutants and these may threaten the long term use of such compounds.



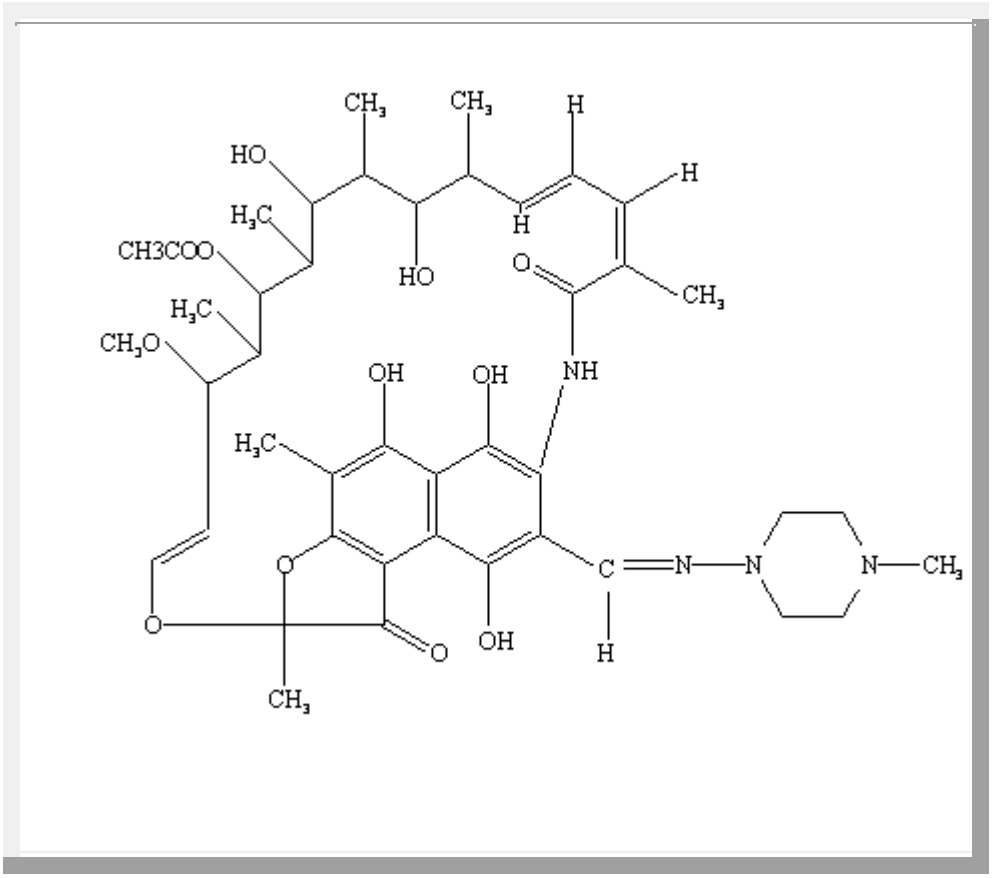
Chemical structure of nalidixic acid



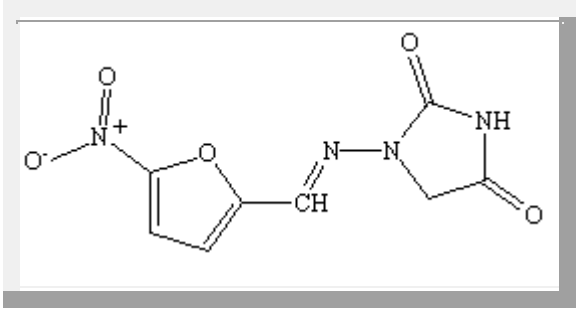
Chemical structure of ciprofloxacin

Which antibacterial agents are inhibitors of RNA metabolism?

The bacterial DNA-dependent RNA polymerase is inhibited by **rifampicin** but this drug has little effect on eukaryotic cells. It is active against the mitochondrial RNA polymerase but its penetration into mitochondria is so poor that it displays very little activity in intact eukaryotic cells. The action of rifampicin prevents production of messenger RNA and thus ultimately stops protein synthesis. Clinically, rifampicin is used in treating tuberculosis and for prophylaxis against meningococcal meningitis. In such cases, it is offered to close contacts of people with the disease. The synthetic antibacterial **nitrofuran compounds** also act by preventing messenger RNA production.



Chemical structure of rifampicin



Chemical structure of nitrofurantoin

رؤية الكلية

التميز في تعليم العلوم الأساسية والبحث العلمي للمساهمة فى التنمية المستدامة.

رسالة الكلية

تقديم تعليم مميز في مجالات العلوم الأساسية وإنتاج بحوث علمية تطبيقية للمساهمة فى التنمية المستدامة من خلال إعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية، وتطوير مهارات وقدرات الموارد البشرية، وتوفير خدمات مجتمعية وبيئية تلبي طموحات مجتمع جنوب الوادي، وبناء الشراكات المجتمعية الفاعلة.

ALL MY BEST WISHES

Prof. Dr. Wesam Salem

2023-2024

Microbial Toxins
2023/2024

Classified into three major groups :

1) Bacterial toxins: produced by bacteria
2) phycotoxin : produced by algae
3) mycotoxin : produced by fungi

Class 1 : Bacterial toxins

What is Bacterial Toxins?

Bacterial toxins are a virulence factors or by-products produced by pathogenic microbes that have taken up and entrance to the body fluid. Bacterium can enter a host by various means, such as consuming contaminated food or water. Bacteria can also be introduced through mucous membranes, either by direct contact with the source or as a consequence of breathing in air-borne bacteria. The type of bacterial toxins released depends on the species of invading bacteria.

The cellular structure of bacterium-cell influences what kinds of bacterial toxins are produced.

Microbial toxins: are soluble substances that alter normal metabolism of host cells with deleterious effects on the host are defined as toxins produced by micro-organisms, including bacteria and fungi. Microbial toxins promote infection and disease by directly damaging host tissues and by disabling the immune system. Some bacterial toxins, such as *Botulinum* neurotoxins, are the most potent natural toxins known. However, microbial toxins also have important uses in medical science and research. Potential applications of toxin research include combating microbial virulence, the development of novel anticancer

drugs and other medicines, and the use of toxins as tools in *neurobiology* and *cellular biology*.

Toxinosis is the effect of bacterial toxin alone, not necessarily involving *bacterial infection* (e.g. when the bacteria have died, but have already produced toxin, which are ingested) .

Pathogenicity is defined as the ability of bacterium to cause infection, the process of pathogensis is complex and represented as good sequences of events.

The major types of virulence factors

The ability of bacterium to cause diseases is called as virulence factors, and can be classified into three major groups:

(1) Factors that promote or aid bacterial colonization to the host cell surface:

(A) by fimbriae or pili-adherance to mucosal surfaces on the host cell

(B) non fimbrial adhesion-binding to host cell

(C) through the motility movement to mucosal surfaces

(D) involve (Bacteriocin , antibiotic) resistance

(E) secretion of IgA protease which prevent trapping of bacteria in mucin

(2) Virulence factors that facilate penetration and invasion to host Surfaces(tissues) and cells:

(A) Extracellular enzymes(Lipase-neuraminidase)

(B) Induction of actin rearrangement in host cells forced

The alteration the phagocytosis by producing non phagocytic host cells

(C) movement of bacteria within and between host cells

(D) Induce for more production non phagocytic cells

(3)virulence factors for avoiding or disrupting host defense mechanism

(A)resistance and degradation of antibodies

(B) Resistance to the lytic effect by immune system

(C) Inactivation of phagocytic cell

(D) Resistance to the effect of host- lysosome enzymes which damage bacterial-pathogen.

Classification of toxins

Toxin can be classified into many broad classes according to :

(A) Whether they are secreted (endo or exotoxin)

(B) Their bio-chemical structure

(C) Their cellular or tissue target of action

(D) Their mechanism of action and Their major biological effect

---

A-Classification according to where they are secreted

1-Exotoxin (secretion outside bacterial cell)

The general characters of exotoxins

1- are produced mainly by Gram-positive bacteria

2-they are protein molecules manufactured during the metabolism of bacteria during the end of stationary phase **as secondary metabolite**.

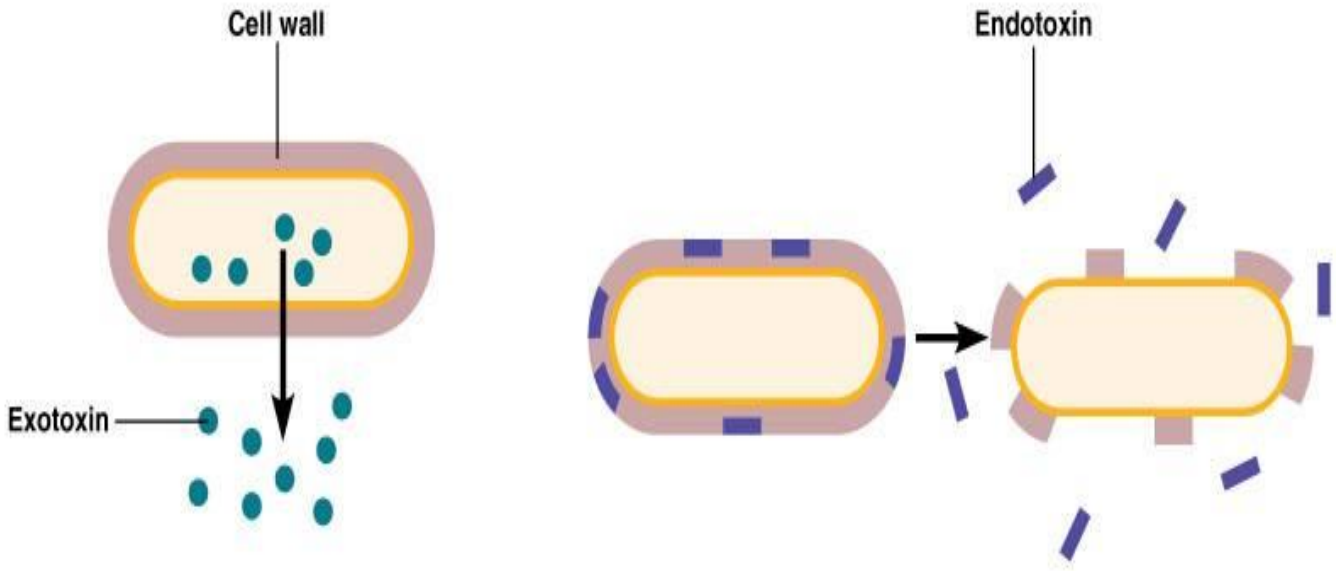
3-They are released into the surrounding environment and acting at sites distant from the synthezing bacteria, they dissolved in the blood fluid and circulate to their site of action, then the symptoms of diseases soon develop

2- Endotoxin (is an outer-membrane)

The general characters of endotoxins:

1-produced mainly by Gram –negative bacteria, are outer membrane layer and cell-wall associated, active only when the bacterial cells lysis or degraded

2- the Chemical structure are composed of lipid-polysaccharide complex, their presence indicated by certain signs and symptoms ,an increase in body temperature , body weakness , aches and general malaise



(a) **Exotoxins** are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then secreted or released following lysis into the surrounding medium.

(b) **Endotoxins** are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

B-Classiefied according to their mechanism of action

Membrane-damaging Toxin :

These cytolytic toxins causing lysis to the host cell by destroying the integrity of the cell plasma membranes , they are active against a wide range of host-tissue and the most famous group are:

Pore forming enzyme :

a- insert a transmembranous pore into a host cell membrane, These toxins form pores in the host -membranes resulting in leakage of the nutrients and the essential ions from the host- cell ,which leading to cell lysis,the best example for these enzymes such as phospholipases and

sphingomylinase which break down the lipid components out of the membrane, and causing disturbance of the plasma membrane-integrity,

b- This leading to the disrupting of the selective influx and efflux of ions across the membrane and disrupting the osmolic pressure. Generally, these toxins are produced as subunits that self-assemble as a pore on the eukaryotic host- membrane produced by a large number of different Gram-positive bacteria

c- Toxin acts as bacterial invasion;(spreading factors):

This is the ability of bacterium to invade tissues multiply and spread rapidly causing the inflammatory process .The most sings of toxins that affect the physical properties of host tissue-matrices

1-Hyaluronidase enzyme

It is original spreading factors produced by streptococci ,staphylococci, and Clostridia ,the enzyme attacks the interstitial cement (ground substance) of the connective tissue by depolymerizing hyaluronic acid .

2-Collagenase enzyme

Which degrade collagen and allowing bacteria to spread through subcutaneous tissue they are specially important in cellulitis caused by streptoccoci pyogenes , these toxins produced by Cl.histoliticum and Cl.perfringens ,which breakdown the collagen which are the framework of muscle leading to Gas gangarene

3-Neuroaminidase enzymes

is produced by intestinal pathogen such as *V.cholera* and shigella dysentaria, which degrade neuroaminic acids which is intercellullar cement of the epithelial cells of the intestinal mucosa.

4-Enzymes that cause hemolysis and leucolysis, these usually act in the animal cell membrane by insertion into the membrane (forming a pore that leading to cell lysis)

.

C-Toxins that interfere with cellular metabolism

There are several toxins that enter cells and alter their metabolism in some way. *Corynebacterium diphtheriae* secretes a toxin that is one of the best example of this topic.

1-The toxin firstly binds to a receptor on the host cell membrane and then enters to the cell by an endocytic process.

2- The toxin blocks protein synthesis by inactivating elongation factor 2(EF-2), which an enzyme necessary for the growth of the polypeptide chain in translation process.

3- The cell dies because it can no longer synthesize proteins.

Increase heartbeat, coma and death.

Cholera toxin

1-the causative agent of cholera is ***V. cholera***, characterized by comma shape grow only in alkaline enviroment , secreted an exotoxin that alters the regulatory control in a cell, rather than directly damaging it.

2- Cholera toxin binds specifically to intestinal epithelial cells. It then enters the cell and causes increased and uncontrolled production of cyclic AMP, which is the mediator of a number of regulatory systems in cells.

3-The increased levels of cyclic AMP cause unregulated secretion of chloride and bicarbonate ions from the epithelial cells lining the intestine. This is due to ionic imbalance results in a massive outflow of water from the cells into the lumen of the intestine,

4- Manifested as a profuse diarrhea odor watery –stool (more than 7 times daily). The loss of water can be so great that cholera victims die from extreme dehydration if their fluids and electrolytes are not replaced..

Toxin interfer with nerve cell function

The neurotoxins of the anaerobic bacteria ***clostridium tetani*** and ***clostridium botulinum*** are two of the most potent toxins known.

Clostridium tetani usually grows in deep, necrotic puncture wounds where the anaerobic condition are exist. The bacterium itself dose not spread far

from the initial site of infection, but the exotoxin which secretes go travels to the central nervous system causing severe neuromuscular dysfunction. *Cl. Tetani* is a good example of an organism that produces only a single toxin. The toxin binds to the surface of nerve cells is internalized, and is then transported to the spinal cord, where it becomes fixed to nerve synapses. It interferes specifically with synaptic transmission in inhibitory interneuron's, creating an imbalance in the excitatory and inhibitory transmissions to motor neuron's. leading to excessive stimulation of muscles, resulting in an uncontrolled, rigid muscle contraction, or spastic paralysis. Different muscles may be affected , such as the powerful massive muscles of the jaw, resulting in the classic condition of tetanus known as lockjaw. Often, back muscles are affected, causing arching of the back and sometimes even crushing of spinal processes in severe cases.

Cl. Botulism : Botulism occurs when the preformed toxin is ingested in contaminated food, often inadequately prepared home-canned foods. The organism itself normally causes no problem if ingested because it is unable to complete successfully with the normal gut.

.The Chemical properties of toxins

There are common characters for all toxins

A) soluble in water , they responded to reactions characteristic to the protein – substances.

B) Toxins are precipitated by the action of ammonium sulphate, zinc chloride , alcohol.

C) toxins are destroyed at 60˚C whereas (95%) of Toxin molecules are destroyed at (56˚C) also destroyed by acid reaction of PH 5.5

A very small dose of toxins will stimulate body cells to produce specific antibodies or antitoxins, which neutralize the toxins in definite proportions.

Animals are immunized against infections pathogenic bacteria by the injection of toxins in non-lethal doses.

Toxin have two main-part :

Toxin molecule → Toxophore (activety action of toxin)

Toxin molecule → Haptophore (combined with the tissue)

Toxins specificity

The most important characters of toxins is its specificity for a certain tissue.

Cl.tetani affect → Nerve tissue by toxin called neurotoxins

Cl.tetani affect → Erythrocytes by toxin called hemotoxins

Diphteriae toxin ⟶ affects the nerve center of the vascular system and produce degenerative changes in heart muscles.

BotulismToxin ⟶ causes paralysis of the muscles supplied by the cranial nerves .

Determination of the potency of toxins;(bacterial toxin assay)

Many method has been descriped for bacterial toxin assay based on in vivo(animal challenge test) or in vitro e.g. (tissue cultre techniques)

1-Biological assay methods (bioassay):

The bioassay descriping the whole animal test (e.g. mice lethality ,monkey,and guinea-pig skin test ,the doses supplied as follow

(M.L.D): The smallest definite dose of toxin which kill an animal (definite weight) in a definite time which varies from different toxins to others.

$L.D_{50\%}$:Lethal dose of toxin which required to kill 50% of experimental animals (mice) population within certain time.

(ITU): international toxin unit defined as amount of toxin required to kill mouse (20 gm. wt. /6 min).

Subunit arrangement in toxin (A+B)

Native structure

Protein toxin molecules
Consists of two component

Toxophore (subunit A):
(responsible for enzymatic activety)

Haptophore (subunit B): Responsible for binding to specific receptor of the tissue host

Subunit B

Concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme cross the membrane.

The enzymatic component is not active until it is released from the native (A+B) toxin.

Isolated A subunit are enzymatically active but lack binding and cell entry capability.

Isolated B subunit may bind to the target cells but they are nontoxic.

Toxin subunit (A+B) are synthesis separately and associated by non-covalent bonds during secretion interact and binding at the target cell surface

Bacterial toxins with similar enzymatic mechanisms may enter to target cells by different mechanisms.

e.g. the diphtheria toxin and pseudomonas exotoxin, which have identical mechanisms of enzymatic activity enter their host cells in different ways. The specific receptors for the B subunit of the toxin on target cells are usually glycoprotein called G-protein on the cell membrane .

The cholera toxin utilize GM1 as receptor

Tetanus toxin⟶ GT as receptor

Toxoid is define as an immunizing agent produced from exotoxins that elicits antitoxins production by the host- body. This was first discovered by Ehrlich who coined the term "toxoid" for this product ,which are detoxified toxins which retain their antigenic-property and their immunizing capacity. The

formation of toxoids can be accelerated by treating toxins with a variety of reagents including formalin, iodine, pepsin, ascorbic acid, ketones, etc. The mixture is maintained at 37C degrees at pH range 6 to 9 for several weeks. The resulting toxoids can be used for artificial immunization against diseases caused by pathogens. Toxoids are effective immunizing agents for example against diphtheria and tetanus that are part of the DPT vaccine.

The body responds to exotoxines by producing special antibodies called antitoxins , when toxins and antitoxins molecules combine with each other, the toxin is neutralize

Invasions Factors:

Is the ability of pathogenic bacteria to invade tissue they act locally to promote bacterial invasion multiply and spread to other tissue. Examples are extracellular enzymes that degrade tissue matrices or fibrin, allowing the bacteria to spread. This includes collagenase, hyaluronidase and streptokinase. some other toxins, also considered invasions, which able degrade membrane components, such as phospholipases and lecithinases.

.

Bacterial Toxigenesis

Toxigenesis: is the ability to produce toxins, is an underlying mechanism by which many bacterial pathogens produce disease. According to a chemical level, there are two main types of bacterial toxins, **lipopolysaccharides**, which are associated with the cell wall of Gram-negative bacteria, referred to as **endotoxins**. And the **proteins**, which referred to as **exotoxins**. are released from bacterial cells and may act as tissue sites a distance from the site of bacterial interlace. The **extracellular** is diffusible toxins .

BACTERIAL TOXINS

Endotoxins are cell wall-associated substances that are structural components of bacteria. Most endotoxins are located in the cell envelope. ,endotoxin refers specifically to the lipopolysaccharide (LPS) or lipooligosaccharide located in the outer membrane of Gram-negative bacteria. Although structural components of cells, soluble endotoxins may be released from growing bacteria or from cells that are lysed as a result of effective host defense mechanisms or by the activities of certain antibiotics.

Exotoxins are usually secreted by living bacteria during exponential growth. The production of the toxin is generally specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while non-virulent strains not produced, and the toxin is the major determinant of virulence it was thought that exotoxin production was limited mainly to Gram-positive bacteria,

A comparisons of Exotoxin and Endotoxins

Charcters	Exotoxins	Endotoxins
Usual source	Mainly gram – positive excreted by bacteria in the medium they are grown	Gram negative bacteria Intercellular substances liberated by bacterial distingration
Specificity	Highly specific for certain tissues and cells such as nerve muscle , producing typical symptoms	Not specific for tissue but produce general symptoms like fever
Toxicity	Very toxic , lethal with small amount	Weakly toxic in general

Stability	Labile at 60 'c , quickly losing toxicity when treated with various chemicals , or storing in room temperature Converted to toxoid	Stable , even at 100 'c or by storage or chemical-treated not converted
Chemical composition	Protein Highly antigenic stimulatin the formation of neutralizing antitoxin	Lipid –polysaccharide – peptide Not highly antigenic but do stimulate to formation antibodies
Coding gene	On plasmid	Original chromosomal DNA

TABLE 1. LETHALITY OF BACTERIAL PROTEIN TOXINS

Toxin	Toxic Dose (mg)	Host	Lethal toxicity	compared with:	
			Strychnine	Endotoxin (LPS)	Snake Venom
Botulinum toxin	$0.8x10^{-8}$	Mouse	$3x10^{6}$	$3x10^{7}$	$3x10^{5}$
Tetanus toxin	$4x10^{-8}$	Mouse	$1x10^{6}$	$1x10^{7}$	$1x10^{5}$
Shiga toxin	$2.3x10^{-6}$	Rabbit	$1x10^{6}$	$1x10^{7}$	$1x10^{5}$
Diphtheria toxin	$6x10^{-5}$	Guinea pig	$2x10^{3}$	$2x10^{4}$	$2x10^{2}$

A plus B Subunit Arrangement

Many protein toxins, notably those that act intracellularly (with regard to host cells), consist of two components: one component (**subunit A**) is responsible for the **enzymatic activity** of the toxin; the other component (**subunit B**) is concerned with **binding** to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (**A+B**) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are a variety of ways that toxin subunits may be synthesized and arranged: **A + B** indicates that the toxin is synthesized and secreted as two separate protein subunits that interact at the target cell surface; **A-B** or **A-5B** indicates that the A and B subunits are synthesized separately, but associated by noncovalent bonds during secretion and binding to their target; **5B** indicates that the binding domain of the protein is composed of 5 identical subunits. **A/B** denotes a toxin synthesized as a single polypeptide, divided into A and B domains that may be separated by proteolytic cleavage.

Attachment and Entry of Toxins

There are at least two **mechanisms of toxin entry into target cells.** In one mechanism called **direct entry**, the B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

receptor-mediated endocytosis (RME):an alternative mechanism, the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of **receptor-mediated endocytosis (RME).** The toxin is

internalized in the cell in a membrane-enclosed vesicle called an **endosome**. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

In both cases above, a large protein molecule must insert into and cross a membrane lipid bilayer, This activity is reflected in the ability of most A/B toxins, or their B components to insert into artificial lipid bilayers, creating ion permeable pathways.

A few bacterial toxins (e.g. diphtheria) are known to utilize both direct entry and RME to enter into the host cells, Bacterial toxins with similar enzymatic mechanisms may enter their target cells by different mechanisms. Thus, the diphtheria toxin and Pseudomonas exotoxin A, which have identical mechanisms of enzymatic activity, but enter their host cells in slightly different ways. The adenylate cyclase toxin of produced by Bacillus anthraces, act similarly to catalyze the production of cAMP from host cell intracellular ATP reserves. However, the anthrax toxin enters cells by receptor mediated endocytosis, whereas the pertussis adenylate cyclase traverses the cell membrane directly.

The **specific receptors** for the B subunit of toxins on target cells or tissues are usually gangliosides (glycoproteins) called **G-proteins** on the cell membrane. For example, the cholera toxin utilizes the ganglioside GM1, and tetanus toxin utilizes ganglioside GT1 and GD1b as receptors on host cells.

TABLE 2. BIOLOGICAL EFFECTS OF SOME BACTERIAL EXOTOXINS WITH ENZYMATIC ACTIVITY

TOXIN (subunit arr)*	ENZYMATIC ACTIVITY	BIOLOGICAL EFFECTS
Cholera toxin (A-5B)	ADP ribosylates eucaryotic adenylate cyclase Gs regulatory protein	Activates adenylate cyclase; increased level of intracellular cAMP promote secretion of fluid and electrolytes in intestinal epithelium leading to diarrhea
Diphtheria toxin (A/B)	ADP ribosylates elongation factor 2	Inhibits protein synthesis in animal cells resulting in death of the cells
Pertussis toxin(A-5B)	ADP ribosylates adenylate cyclase Gi regulatory protein	Blocks inhibition of adenylate cyclase; increased levels of cAMP affect hormone activity and reduce phagocytic activity
E. coli heat-labile toxin LT (A-5B)	ADP ribosylates adenylate cyclase Gs regulatory protein	Similar or identical to cholera toxin
Shiga toxin (A/5B	Glycosidase cleavage of ribosomal RNA (cleaves a single Adenine base from the 28S rRNA)	Inactivates the mammalian 60S ribosomal subunit and leads to inhibition of protein synthesis and death of the susceptible cells; pathology is diarrhea, hemorrhagic colitis (HC) and/or hemolytic uremic syndrome (HUS)
Pseudomonas Exotoxin A (A/B)	ADP ribosylates elongation factor-2 analogous to diphtheria toxin	Inhibits protein synthesis in susceptible cells, resulting in death of the cells
Botulinum toxin (A/B)	Zn^{++} dependent protease acts on	Inhibits presynaptic

	synaptobrevin at motor neuron ganglioside	acetylycholine release from peripheral cholinergic neurons resulting in flaccid paralysis
Tetanus toxin (A/B)	Zn^{++} dependent protease acts on synaptobrevin in central nervous system	Inhibits neurotransmitter release from inhibitory neurons in the CNS resulting in spastic paralysis
Anthrax toxin LF (A2+B)	Metallo protease that cleaves MAPKK (mitogen-activated protein kinase kinase) enzymes	Combined with the B subunit (PA), LF induces cytokine release and death of target cells or experimental animals
Bordetella pertussis AC toxin (A/B) and Bacillus anthracis EF (A1+B)	Calmodulin-regulated adenylate cyclases that catalyze the formation of cyclic AMP from ATP in susceptible cells, as well as the formation of ion-permeable pores in cell membranes	Increases cAMP in phagocytes leading to inhibition of phagocytosis by neutrophils and macrophages; also causes hemolysis and leukolysis
Staphylococcus aureus Exfoliatin B	Cleaves desmoglein 1, a cadherin found in desmosomes in the epidermis (also a superantigen)	Separation of the stratum granulosum of the epidermis, between the living layers and the superficial dead layers.

Toxins that affect nerve cell function

- *Clostridium botulinum*
- *Clostridium tetani*
- *Clostridium perferenges*

This microbial group are the most potent toxins known .

- ***Clostridium tetani***

Cl.tetani usually grows in deep, necrotic puncture wounds where anaerobic conditions exist. The bacterium itself does not spread far from the initial site of infection, but the exotoxin that secreting are travels to the centeral nervous system to cause severe neuromuscular dysfunction . The toxin binds to the surface of nerve cells is internalized and then transported to the spinal cord, and fixed to nerve synapsis. Creating an imbalance in the excitatory and inhibitory transmissions to motor neurons, resulting an uncontrolled, rigid muscle contraction or spastic paralysis resulting in the classic condition of tetanus known as **lock jaw**

- It is interesting some disease, such as tetanus , very small amount of toxin is required to produce the symptoms of disease, that the person immune system not be sufficient the toxin to produce antibodies. (Actively immunized) .

TABLE 3. SOME PORE-FORMING BACTERIAL TOXINS

Toxin	Bacterial source	Target	Disease
perfringiolysin O	Clostridium perfringens	Cholesterol	gas gangrene
Hemolysin	Escherichia coli	cell membrane	UTI
Listeriolysin	Listeria monocytogenes	Cholesterol	systemic; meningitis
anthrax EF	Bacillus anthracis	cell membrane	anthrax (edema)
alpha toxin	Staphylococcus aureus	cell membrane	abcesses
pneumolysin	Streptococcus pneumoniae	Cholesterol	pneumonia; otitis media
streptolysin O	Streptococcus pyogenes	Cholesterol	strep throat
Leukocidin	Staphylococcus aureus	phagocyte membrane	pyogenic infections

Superantigens: Toxins that Stimulate the Immune System

Several bacterial toxins can act directly on the T cells and antigen-presenting cells of the immune system. Impairment of the immunologic functions of these cells by toxin can lead to human disease. One large family of toxins in this category are the so-called **pyrogenic exotoxins** produced by staphylococci and streptococci, whose biological a
ctivities include potent stimulation of the immune system, pyrogenicity, and enhancement of endotoxin shock.

Pyrogenic exotoxins are secreted toxins of 22 kDa to 30 kDa, and include staphylococcal enterotoxins serotypes A-E, G, and H; group A streptococcal pyrogenic exotoxins A-C; staphylococcal exfoliatin toxin; and staphylococcal TSST-1.

These cytokines serve as mediators of the hypotension, high fever and diffuse erytheromatous rash that are characteristic of toxic-shock syndrome. The staphylococcal enterotoxins are superantigens, but it is not known if this activity contributes to vomiting or diarrhea characteristic of staphylococcal food poisoning.

Control of Synthesis and the Release of Protein Toxins

The regulation of synthesis and secretion of many bacterial toxins is tightly controlled by regulatory elements that are sensitive to environmental signals. For example, the production of diphtheria toxin is totally repressed by the availability of adequate amounts of iron in the medium for bacterial growth. Only under conditions of limiting amounts of iron in the growth medium does toxin production become derepressed. The expression of cholera toxin and related virulence factors (adhesins) is controlled by environmental osmolarity and temperature. In B. pertussis, induction of different virulence components is staggered, such that attachment factors are produced initially to establish

the infection, and toxins are synthesized and released later to counter the host defenses and promote bacterial survival.

The processes by which protein toxins are assembled and secreted by bacterial cells are also variable. Many of the classic exotoxins are synthesized with an NH terminal leader (signal) sequence consisting of a few (1-3) charged amino acids and a stretch of (14-20) hydrophobic amino acids. The signal sequence may bind and insert into the cytoplasmic membrane during translation such that the polypeptide is secreted while being synthesized. The signal peptide is cleaved as the toxin (protein) is released into the periplasm. Alternatively, the toxin may be synthesized intracytoplasmically, then bound to a leader sequence for passage across the membrane. Frequently, chaperone proteins are required to guide this process. Some multicomponent toxins, such as the cholera toxin, have their subunits synthesized and secreted separately into the periplasm where they are assembled. In Gram-negative bacteria, the outer membrane poses an additional permeability barrier that a protein toxin usually has to mediate if it is to be released in a soluble form. It has been proposed that some Gram-negative exotoxins (e.g. E. coli ST enterotoxin) might be released in membrane vesicles composed of outer membrane components. Since these vesicles possibly possess outer membrane-associated attachment factors, they could act as "smart bombs" capable of specifically interacting with and possibly entering target cells to release their contents of toxin.

Most toxigenic bacteria are free-living in nature and in associations with humans in a form which is phenotypically identical to the toxigenic strain but lacking the ability to produce the toxin.

A summary of bacterial protein toxins and their activities is given in Tables 4. Details of the mechanisms of action of these toxins and their involvement in

the pathogenesis of disease is discussed in chapters with the specific bacterial pathogens.

TABLE 4. SUMMARY: ACTIVITIES OF EXTRACELLULAR BACTERIAL TOXINS

NAME OF TOXIN	BACTERIA INVOLVED	ACTIVITY
Anthrax toxin (EF)	Bacillus anthracis	An adenylate cyclase enzyme that increases levels in intracellular cyclic AMP in phagocytes and formation of ion-permeable pores in cell membrane. Leads to edema and decreased phagocytic responses
Adenylate cyclase toxin (pertussis AC)	Bordetella pertussis	Acts locally to increase levels of cyclic AMP in phagocytes and formation of ion-permeable pores in cell membranes
Alpha toxin	Staphylococcus aureus	Protein subunits assemble into an oligomeric structure that forms an ion channel (pore) in the cell plasma membrane
Cholera enterotoxin (Ctx)	Vibrio cholerae	ADP ribosylation of G proteins stimulates adenlyate cyclase and increases cAMP in cells of the GI tract, causing secretion of water and electrolytes leading to diarrhea
E. coli LT toxin	Escherichia coli	Similar to cholera toxin
E. coli ST toxins	Escherichia coli	Binding of the heat-stable enterotoxins (ST) to a guanylate cyclase receptor results in an increase in cyclic GMP (cGMP) that adversely effects electrolyte flux. Promotes

		secretion of water and electrolytes from intestinal epithelium leading to diarrhea.
Shiga toxin	Shigella dysenteriae E. coli O157:H7	Enzymatically cleaves eucaryotic 28S rRNA resulting in inhibition of protein synthesis in susceptible cells. Results in diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS)
Perfringens enterotoxin	Clostridium perfringens	Stimulates adenylate cyclase leading to increased cAMP in epithelial cells. Result is diarrhea
ToxinA/ToxinB	Clostridium difficile	Modifies Rho, a subfamily of small GTP-binding proteins that are regulators of the actin cytoskeleton. Deamidation of the glutamine residue at position 63 of Rho to a glutamic acid produces a dominant active Rho protein unable to hydrolyze bound GTP. Pathological result is cell necrosis and bloody diarrhea associated with colitis
Botulinum toxin	Clostridium botulinum	Zn++ dependent protease that inhibits neurotransmission at neuromuscular synapses resulting in flaccid paralysis
Tetanus toxin	Clostridium tetani	Zn++ dependent protease that Inhibits neurotransmission at inhibitory synapses resulting in spastic paralysis
Diphtheria toxin (Dtx)		

	Corynebacterium diphtheriae	ADP ribosylation of elongation factor 2 leads to inhibition of protein synthesis in target cells
Exotoxin A	Pseudomonas aeruginosa	Inhibits protein synthesis; similar to diphtheria toxin
Anthrax toxin (LF)	Bacillus anthracis	Lethal Factor (LF) is a Zn++ dependent protease that induces cytokine release and is cytotoxic to cells by an unknown mechanism
Pertussis toxin (Ptx)	Bordetella pertussis	ADP ribosylation of G proteins blocks inhibition of adenylate cyclase in susceptible cells
Exfoliatin toxin*	Staphylococcus aureus	Cleavage within epidermal cells (intraepidermal separation); also acts as a superantigen
Staphylococcus enterotoxins*	Staphylococcus aureus	Superantigen causes massive activation of the immune system, including lymphocytes and macrophages; exact role in in emesis not not known
Toxic shock syndrome toxin (TSST-1)*	Staphylococcus aureus	Superantigen acts on the vascular system causing inflammation, fever and shock
Erythrogenic toxin [streptococcal pyrogenic exotoxin (SPE)]*	Streptococcus pyogenes	Super antigen same as TSST - inflammation, fever and shock; can cause localized erythematous reactions (scarlet fever)

Streptococcus pyogenes have been designated as superantigens. They represent a family of molecules with the ability to elicit massive activation of the immune system. These proteins share the ability to stimulate T cell proliferation by interaction with Class II MHC molecules on APCs and specific V beta chains of the T-cell receptor. The important feature of this interaction

is the resultant production of IL-1, TNF, and other lymphokines which appear to be the principal mediators of disease processes associated with these toxins.
Endotoxins are released by Gram-negative bacteria. At first, they are not as aggressively toxic as exotoxins due to the fact they remain largely contained in the cellular walls of bacteria. However, as these cells complete their life cycle and die, the circulating volume of this toxin increases. In addition, they cannot be used to make vaccines.

Normally, the body attempts to eliminate bacterial toxins before they can cause harm. The **immune system** is the first line of defense, but it may become over by the rate of bacterial replication. In fact, **inflammation** is an indication that bacterial overgrowth is occurring. In this case, the immune system will do the next best thing — move the bacteria out of the way. Usually, fat cells are the selected storage sites, which can lead to the formation of cysts and tumors.

Without intervention, bacterial toxins may eventually accumulate to the point where they move out of fat cells into other tissues of the body. This process may take years to unfold, but a degenerative disease is often the end result. In fact, many age-related conditions and metabolic disorders are associated with the long-term buildup of these toxins, including **heart disease**, cancer, **arthritis**, and diabetes.

An exotoxin is a poison secreted by bacterium, Exotoxins are vierulance , A very small amount can be fatal to a host organism and even though the **immune system** can often identify and attack the toxin, the toxin spreads so quickly that the host does not have an opportunity to mount a defense. Some governments historically have attempted to harness microorganisms that produce toxins in warfare, and the development of weaponized

microorganisms led to the creation of a treaty banning biological warfare out of concern that such organisms could get out of control.

Some organisms secrete exotoxins steadily, while others produce them as needed, and in some cases they are only released during cell- lysis, when a cell breaks up as an organism dies. An exotoxin can be classified by the types of tissues it targets, like neurotoxins that target neurons and enterotoxins that are designed to assault the digestive tract.

Using an exotoxin, a microorganism can attack remote areas, rather than needing to be in direct contact with the targeted tissue. The exotoxin can enter the bloodstream and travel, using the body's own circulatory system as a delivery method. Some are designed to assist with bacterial invasion, as with exotoxins that break down tissues to allow organisms to penetrate more deeply, while others do not have a known function.

Toxins which alter the mechanism of action into the host cells

Diphtheriae Toxin

The best known and studied bacterial toxin is the diphtheria toxin. Diphtheria toxin is highly potent; one molecule is thought to be sufficient to kill the host cell. The pathogenicity of ***C. diphtheriae*** is entirely due to the presence of this toxin Although ***C. diphtheriae*** remains localized in the throat during the infection, the secreted toxin is absorbed and enters to the bloodstream. The action of the toxin in the throat cause considerable damage to mucosal cells, leading to formation of a membrane (called a pseudo membrane) over the mucosal surface tissues , **consisting of fibrin, bacteria and inflammatory cells**. When the larynx is involved, a life- threatening obstruction of the airway can occur . the action of diphtheria toxin on other organs, especially the heart, can result in irregular, produced by ***Corynebacterium diphtheriae***.
Diphtheria toxin is a bacterial exotoxin of the A/B prototype. It is produced as

single polypeptide chain with a molecular weight of 60,000 daltons. The function of the protein is distinguishable into two parts: **subunit A,** with a Mol.wt. 21,000 daltons, contains the enzymatic activity for inhibition of elongation factor-2 involved in host protein synthesis; **subunit B,** with a Mol.wt. of 39,000 daltons, which is responsible for binding to the membrane of a susceptible host cell. The B subunit possesses a region T (translocation) domain which inserts into the endosome membrane thus curing the release of the enzymatic component into the cytoplasm.

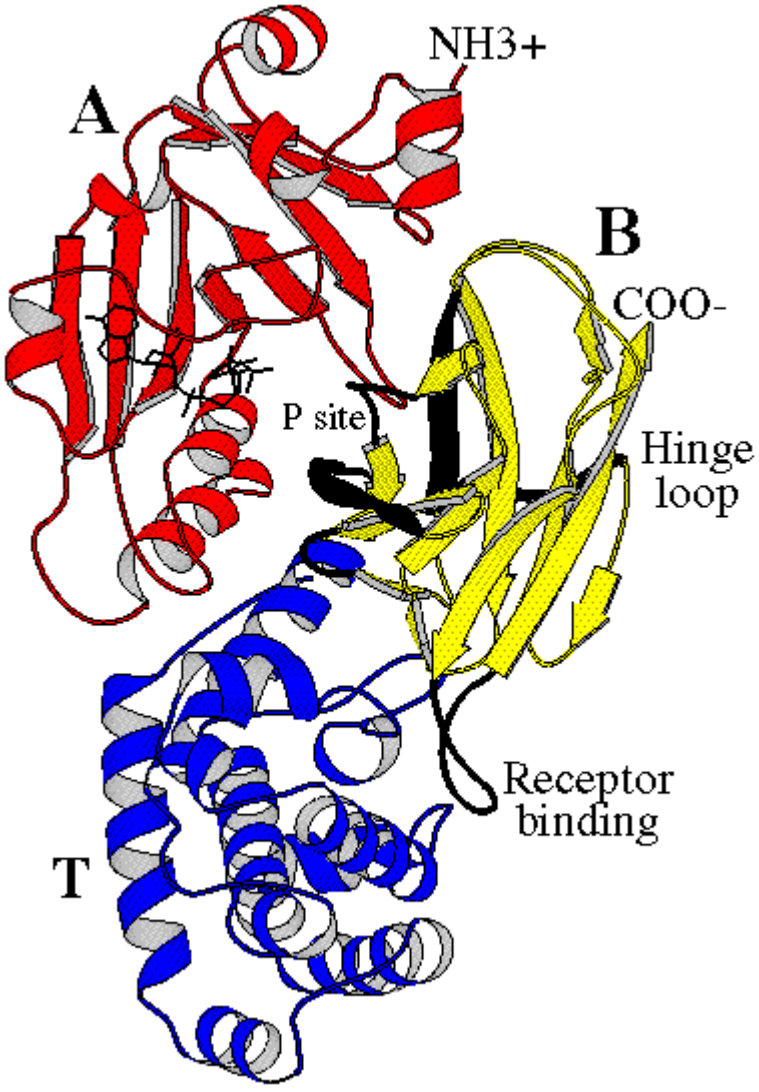


Figure 1. Diphtheria Toxin (Dtx). A (red) is the catalytic domain; B (yellow) is the binding domain which displays the receptor for cell attachment; T (blue) is the hydrophobic domain responsible for insertion into the endosome

membrane to secure the release of A. The protein is illustrated in its "closed" configuration.

In vitro, the native toxin is produced in an inactive form which can be activated by the proteolytic enzyme trypsin in the presence of thiol (reducing agent).

The enzymatic activity of fragment A is masked in the intact toxin. Fragment B is required to enable fragment A to reach the cytoplasm of susceptible cells. The C terminal end of Fragment B is hydrophilic and contains determinants that interact with specific membrane receptors on sensitive cell membranes and the N-terminal end of Fragment B (called the T domain) is strongly hydrophobic. The specific membrane receptor for the B fragment has been shown to be a transmembranous heparin-binding protein on the susceptible cell's surface. The diphtheria toxin enters its target cells by either direct entry or receptor mediated endocytosis. The first step is the irreversible binding of the C-terminal hydrophilic portion of Fragment B to the receptor. During RME, the whole toxin is then taken up in an endocytic vesicle. In the vesicle, the pH drops to about 5 which allows unfolding of the A and B chains. This exposes hydrophobic regions of both the A and B chains that can insert into the vesicle membrane. The result is exposure of the A chain to the cytoplasmic side of the membrane. There, reduction and proteolytic cleavage releases the A chain in the cytoplasm. The A fragment is released as an extended chain but retains its activey (enzymatic) globular conformation in the cytoplasm. The A chain catalyzes the ADP ribosylation of elongation factor-2 (EF-2) as shown in Figure 2.

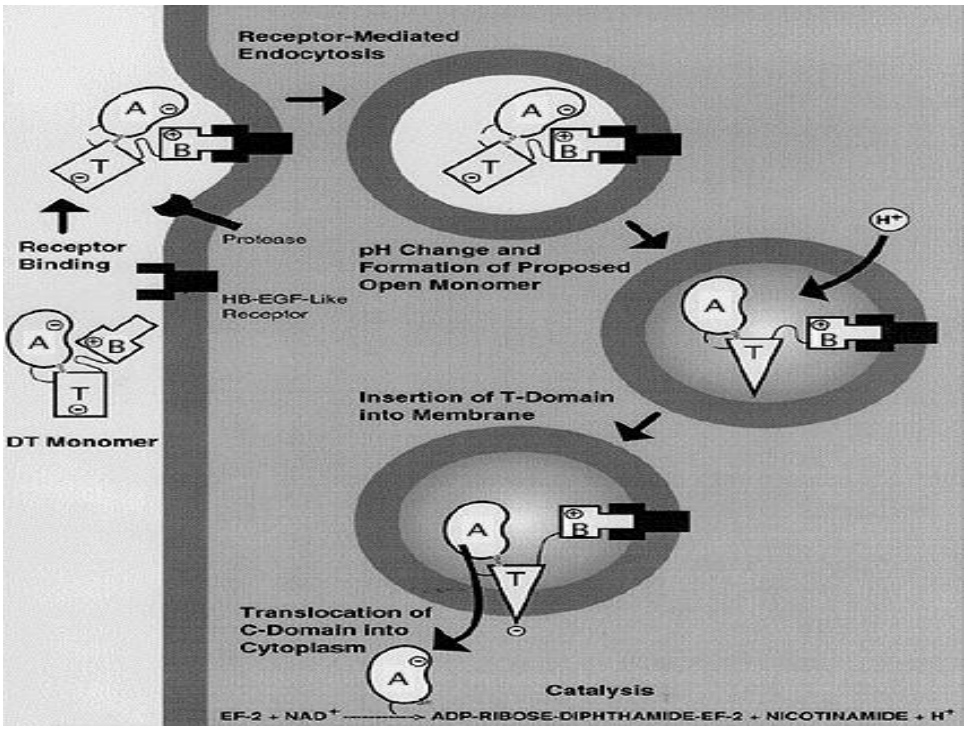


Figure 2. Entry and activity of diphtheria toxin (Dtx) in host- cells. The B domain of the toxin binds to receptor on a susceptible cell. The toxin is taken up in an endosome by receptor mediated encocytosis. Acidification of the endocytic vesicle allows unfolding of the A and B chains exposing the hydrophobic T domain of the toxin. The T domain inserts into the endosome membrane leading to translocation the A fragment into the cytoplasm where it regains its enzymatic configuration. The enzymatic A component utilizes NAD as a substrate. It catalyzes the attachment of the ADP-ribose portion of NAD

to elongation factor (EF-2) ,this due to inactivates its function in protein synthesis.

Infection agent :

- Diphtheria toxin is a bacterial exotoxin of the A,B protype .
- Produced as single polypeptide chain with a molecular weight of 60,000 , have two parts

Subunit A. ⟶ Mol.wt. 21,000 D.

Responsible for the enzymatic activity for inhibition of elongation factor -2 involved in host protein synthesis.

Subunit B ⟶ with Mol.wt. of 39.000 daltons is responsible for binding to the membrane of a susceptible host cell.

In vitro , the native toxin is produced in an inactive form which can be activated by the proteolytic enzyme trypsin the B.sub -unit is required to enable fragment A to reach the susceptible cells .

The C- terminal end of the fragement B is hydrophilic and contains determinants that interact with specific membrane receptors on sensitive cell membrane.

Entry to the host cells

The diphtheria toxin enters its target cells by either direct entry or by RME way (receptor mediated endocyst).

the first step is irreversible binding of the C- terminal hydrophilic portion of fragment B to the receptor .

During RME the whole toxine is then take up in an endocytic vesicle whereas the PH drops to about 5 which allows unfolding of the A and B . this exposes hydrophobic regions of both the A and B chains that can insert into the vesicle membrane the result is expoure of A chain to the cytoplasmic side of the membrane .there reduction and proteolytic cleavage releases the A chain in the cytoplasm .

Diphteria toxins is highly potent; one molecule is thought to be sufficient to kill the cell. The pathogenicity of ***C. diphtheriae*** is due to the excreation of this toxin ; the strains does not have the gene for toxin production are non-pathogenic. Although ***C. diphtheriae*** remains localized in the throat during the infection, the secreted toxin is absorbed and enters to the bloodstream. The action of the toxin in the throat cause considerable damage to mucosal cells, resulting in a membrane(called a pseudomembrane) over the mucosal surface , consisting of fibrin, bacteria and inflammatory cells. When the larynx is involved, a life- threatening obstruction of the airway can occur . the action of diphtheria toxin on other organs, especially the heart, can result in irregular heartbeat, coma and death.

in the action : a single molecule of subunit A within a host- cell is lethal and a single diphtheria bacillui is able to produce 5,000 mol /hours.

subunit A utilize NAD as a substrate : it catalyzes the attachment of the ADP-ribose portion of NAD to the elongation factor which inactivates its function in protein synthesis .

Diphtheria diseases

Infection agent : toxigenic strain of *corynebacterium diphtheria* reservoir :

Man , Age: > 15years

Incubation period: 1-7 days

Mode of transmission: contact with (direct droplet infection)

Period of communicability : until virulent bacilli have disappeared from discharges & lesions .

Usually recovery 2 weeks) or less & seldom more than 4 weeks effective antibiotic therapy terminate shedding .

The rare chronic carrier may shed the organism for 6 monthes .

Clinical picture depend on

(A) – the site of infection

(B) – immunization state of the host

(C) – whether or not toxin has escaped into the systemic circulation

Clinical classification by the location of diphtheria membrane (tonsiller , pharyngeal , conjunctiva , skin) .

The bacilli remain in the lesions where they are grow & multiply , producing soluble protein poison,

The symptoms of the disease as follow:

(1) Tonsillar & pharyngeal diphteria : begins Anorexia , malaise , low grade fever (38.5 ^{0}C).

(2) With in 1-2 days a membrane appears that may very in extent according to immune state of the host .

(3) The membrane initially is thin & gray resembling spider web that gradually extent or progress into larynx and trachea attempting to remove it , is followed by bleeding .

The virulance nature of diphtheria is due to :

1 – Intense local inflammation act the site of infection characterized by Coagulation necrosis, fibrinous exudate & formation of grash membrane due to irriating effect of dipheteriae bacilli as certain cytotoxin .

2 – the virulance effect of toxin : the toxaemia produce acute degenerative changes in the cells and various organs of the body .

3 – paralysis may occur for the

A – muscles of soft palate

B – extra ocular muscles

C – pharynx

D – diaphragm

Death resulted from : toxaemia -laryngeal stenosis bronchopheumonia , late myocardial failure .

Corynebacterium Dipheteria

General character: they are gram –positive, non-acid fast and non-motile bacilli occurring in palisade.

They show club shaped swelling at the ends and irregular shapes

Morphology

It is thin , slender , rod 3 to 6 u x 0.6 to 0.8 u showing clubbing at one or both ends . it is non-spore forming
non-capsulated and non-motile . it is gram-positive and shows pleomorphic .

The presence of metachromatic granules (babes-ernst granules) serve to distinguish it from diphtheoid forms(non bathogenic strains). The granules are colored dark purple with methylene blue .

The bacilli are arranges in characteristic fashion . it is seen in paris or groups . bacilli form various angles with each other like V or L . this is called Chinese letter arrangement .

Cultural character :

Enrichment of media with blood , serum or egg is necessary for good growth . The optimum temperature is 37'c and PH 7.2 . it is aerobic and facultative anaerobic .

(1) serum broth : turbidity pellicle formation , amount and nature of deposit is useful in identification of types of Corynebacterium diphtheriae .

(2) loeffler's slope culture shows abundant growth after 6 to 8 hours incubation . the colony is small , granular , moist , creamy and glistening with irregular edges .

(3) Blood tellurite medium (0.04%) it is useful in differentiation of corylebecterium diphteriae into gravis , mitis and intermedius types it acts as selective media inhibiting the growth of other organisms . Diphtheria bacilli reduce tellurite to metallic tellurite to metallic tellurium giving gray to black color to colonies .

Toxin consists of 2 factors A and B. A is a lethal factor and B is a spreading factor. Fragment A inhibits polypeptide chain elongation in the presence of nicotinamide adenine dinucleotide , by inactivating the elongation of EF-2. So toxin fragment –A inactivates EF-2 by catalyzing a reactions that yields free

nicotinamide plus an inactive adenosine diphosphate ribose EF-2 complex , it is presumed that abrupt arrest of protein synthesis is responsible for necrotizing and neurotoxic effects of diphtheria toxin .

Diphtheria toxin is protein in nature (mol wt. 62,000) it is extremely potent , lethal dose for 250 gm guinea pig is 0.0001 mg. it is labile .

It can be converted into toxoid (toxin that has lost toxicity but not antigenecity) by :

1 – prolonged storage at 37˚C .

2 – incubation at 37˚C for 4 to 6 weeks .

3 – 0.2 to 4% formalin.

The toxigenecity of diphtheria bacilli depends upon the presence of abeta phage which acts as genetic determinant controlling toxin production . Toxigenecity remains as long as the bacillus is lysogenic .when bacillus is cured of phage it looses toxigenecity .

Toxin production is also influenced by the concentration of iron in the medium . 0.1 mg of iron per ml is the optimum concentration in the medium for toxin production 0.5mg per ml of irn in the medium inhibits toxin production .

Other factors influencing the toxin production are osmotic pressure , PH and availability of suitable carbon and nitrogen .

The mechanism of action of toxin is not well understood. It inhibits protein synthesis and rapidly kill susceptible cell. It has affinity for myocardium, adrenal tissue and never endings.

Clinical symptoms for the diseases toxin

The bacilli remain confined to site of entry where they multiply and form toxin. This toxin produces area of necrosis. The resulting fibrinous exudate together with epithelial cells, leukocytes, erythrocytes and bacteria form pseudomembrane.

Diphtheria does not occur naturally in animal but infection can be produced experimentally, e.g. cat, dogs, chicks, pigeons, guinea pig, rabbits, etc. Subcutaneous injection in guinea pig, with culture of virulent diphtheria bacilli will cause death of animal in 1 to 4 days. At autopsy of guinea pig, we may find :

1. Gelatinous, hemorrhagic edema and necrosis at the site of inoculation .
2. swollen and congested lymph nodes .
3. peritoneal exudate which may be hemorrhagic .
4. congested abdominal viscera.
5. enlarged and hemorrhagic adrenals.
6. blood stained pleural exudate.
7. pericardial effusion.

Pathogenicity

Incubation period is 3 to 4 days. Pathogenicity is because of toxin production . corynebacterium diphtheriae doe not actively in vade deep tissues and never enters the bloodstearm . the site of infection may be :

1. Faucial
2. laryngeal
3. nasal
4. diaphram
5. genital, vulval, vaginal, etc.
6. conjunctival
7. cutaneous around mouth and nose, etc. Any how, fucial diphtheria is the most common .

Spore –forming bacilli

Family:Bacillaccea

Genus Bacillus anthraces

Gram +ve bacilli

a)aerobic spore forming

b)anaerobic spore forming resistant dry heat 100 C (1hr) and autoclave 120 C 20M

-the only pathogenic species B.anthracis

*mainly pathogen species :

Gram +ve, aerobic or facultative , catalase_positive , liquefied gelation , occur in long thread like forms , motile

-large 1.0:1.2 Mm

-causatatine organism of anthrax (sheep & cattle)

-culture characters:-

-aerobic grow on simple agar media

-colouies are rounded, hare "cut glass" appearance

-growth in gelatin stab resemble an "inverted fir tree"

-capsulated in vivo: when stain with polychrome methelene blue , the capsule appear as pink rim around blue bacilli {Mcfadyean reaction}

-the spore are central ,ovoid ,not stain with gram . stained with acid fast stain , spors can survive for many years

-culture character ;aerobes, grow on N.A at 37~c have medusa head appearance

VIRULANCE FACTOR :

1) poly-D-glutamic acid (protein) capsule poly peptide is antiphagocytic PLZ-mediated

2) anthrax-toxin :made of 3 types of protein

a) lethal factor (LF) protease

b) edema factor (EF) "adenylate cyclase "

c) protective antigen (PA)

LF & PA are lethal toxin : major causative agent of infected animal death

PA : binds to specific receptors and form pores in the human cell membrane through which EF & LF enter to the cell and do their action

Only one disease affect sheep, cattle ,goats by 3 ways

1-Cutaneous anthrax (malignant pustule) : microbe enter through skin abrasion , papule is formed and rapidly changes to vesicle then pustule and necrotic ulcer covered by a black eschai surrounded by manked edema

2-Pulmonary (inhalation) : transmitted by inhalation of spores mainly affect people handling wool ,characterized by haemorrhagic , bloody pleural effusion , septic shock and death

Respiratory anthrax : fever, malaise, difficulty breathing in 2-5 days

3-Gastrointestinal anthrax : fever ,abdominal pain ,vomiting with blood watery bowel

Transmitted by ingestion of infected under cooked meat

The organism cause invasion and leading to ulceration of gastrointestinal mucosa

anthraseis conseder themajor agent of "bioterrorism "

In U.S.A :22 case and 5 dead by sending spores via mail

Preventation

active immunization ., burning disposals , autoclaving the used material

Clostridium
Anerobic spore-forming bacilli

General characters

The genus clostridium consists of Gram-positive, anaerobic, spore forming bacteria ,.

Spindle shaped and highly pleomorhic bacilli.

Spores are wider than bacillary bodies , the genus contains bacteria causing three major diseases for human (tetanus , gas gangrene and food poisoning botulism .

Some pathogens, e.g.*Clostridium welchii* and *Clostridium tetani* are found normally in human and animal intestine as commensal microflora.

Clostridia are motile with peritricheate flagella, except *Clostridium perfringens* and Clostridium type VI . *Clostridium welchii* and Clostridium botulinum are capsulated while others are not , pathogenic clostridia forms powerful exotoxins .

Clostridium botulinum is non-invasive while Clostridium tetani has slight invasive properties .

Tetanus results from the action of powerful exotoxin it produces. The gas gangrene clostridia are toxigenic and invasive causing septicemia.

CLOSTRIDIUM TETANI

It is widely distributed in soil and in intestine of man and animals.

Morphology : It is slender, long, slightly, curved, Gram-positive 4.8 µ × 0.5 µ and occurring singly or in chain .It shows considerable variation in length. Spores are spherical, terminal and bulging, giving the bacilli drum-stick appearance It is non-capsulated and motile .

Cultural character

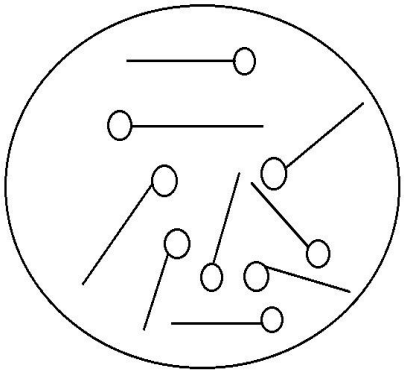
It is an obligatory anaerobe that grows only in absence of oxygen .

The characteristic of anaerobic bacilli is their inability to utilize oxygen as the final hydrogen acceptor .

It lacks cytochrome and cytochrome oxidase and so unable to break down hydrogen peroxide because it lacks catalase and peroxidase .

Therefore , hydrogen peroxide tends to accumulate to toxic concentration in presence of oxygen .

It also lacks superoxide dismutase and consequently permit the accumulation of toxic free radical superoxide.



Clostridium tetani

The optimum temperature is 37ºC and PH 7.4. It grows fairly well in ordinary media. Cultures have burnt organic smell .

Cooked meat medium

It grows well on this medium with turbidity and gas formation. The meat is not digested but is turned black after prolonged incubation.

Nutrient agar medium

It produces swarming growth forming fine film over the medium. By increasing the concentration of agar in the medium after 2 to 4 days incubation, colonies are irregularly round, 2 to 5 mm in diameter, translucent, grayish yellow with granular surface and ill-defined edges.

Blood agar medium

A zone of a hemolysis is produced. It later on develops into beta hemolysis, due to production of hemolysin (tetanolysin).

Lactose egg yolk milk medium

There is no opalescence, pearly layer, proteolysis or lactose fermentation.

Biochemical reactions

It does not ferment any sugar and is slightly proteolytic. It forms indole. Gelatin liquefaction occurs slowly. Coagulated serum is softened. Milk is not coagulated .

The spores of clostridium tetani withstand boiling for 15 to 90 minutes. Autoclaving at 121ºC for 20 minutes kills spores. Spores otherwise can survive in soil for years. Iodine (1% aqueous solution) and H_2O_2 (10 volumes) kill spores within a few minutes.

Antigen structure

The flagellar antigen differentiate clostridium tetani into 10 types but the toxin (neurotoxin) produced is pharmacologically and antigenically identical.

Type of toxin

clostridium tetani produced three type of toxin:

1. Hemolysin (tetanolysin)
2. Neurotoxin (tetanospasmin)
3. Non-spasmogenic peripherally active neurotoxin.

1 – Tetanolysin

It is heat labile and oxygen labile and is active against RBC of many animals (rabbit, horse, etc.). Its pathogenic role is not known. May be it acts as a leukotoxin.

2 - Tetanospasmin

It is oxygen stable and gets inactivated at 66ºC in 5 minutes (heat labile). Toxin has been crystallized. Horse is most susceptible. Birds and reptiles are highly resistant. It gets toxoided in presence of low concentration of formaldehyde. It is a good antigen and specifically neutralized by antitoxin. It is protein with 67,000 molecular weight. It acts like strychnine by inhibiting the synthesis and liberation of acetylcholine, thus, interfering with neuromuscular transmission. It can be fixed to cerebral gangliosides. It can

cause inhibition of postsynaptic spinal neuron by blocking the release of inhibitory mediator. This results in generalized muscular spasms, hyper-reflexia and seizures.

3 - Non-spasmogenic peripherally active neurotoxin Pathogenesis

Spores implantd in wound multiply only if conditions are favorable. Toxin so produced is absorbed by motor never ending. Toxin travels along the axis cylinders of peripheral never and reach central nervous system. It is fixed specifically by ganglioside of gray matter of nervous tissues. Its exact mode of action is not known but it may act at synaptic junctions between anterior horn cells and related internuncial neurons leading to abolition of spinal inhibition. As a result, muscle rigidity and spasm occurs .

If given orally it is destroyed by digestive enzyme and so is not effective. Subcutaneous, intramuscularly and intravenous route is equally effective. Intraneural route is more lethal.

If toxin is injected intramuscularly in one of the hind limbs of guinea pig or mice, spasm of inoculated limb appears. This is due to toxin acting on the segment of spinal cord. Subsequently spread of toxin up to the spinal cord causes ascending tetanus and likewise opposite hind limb is involved. If toxin is injected intravenously spasticity develops in the muscles of head and neck first and then spreads downwards (descending tetanus) .

Tetanus

Tetanus results from contamination of wound by clostridium tetani. The spores are found in soil. Germination and multiplication occur if certain factor like necrotic tissue, ionisable calcium salts and lactic acid are present. Infection of wound with pyogenic organisms increases the risk of tetanus. Toxin is probably absorbed from the area of infection and through motor never endings reach anterior horn cell. Other views are that toxin is absorbed through bloodstream and perineural lymphatics.

The incubation period is to days to several weeks and it depends upon site, nature of wound, doses, toxigenecity of organism and immune status of patient. In rural India tetanus is estimated to be the most common cause of death.

The clinical types of tetanus:

1 – Tetanus neonatorum : It occurs from contamination of cut surface of umbilical cord in infants. It has high rate of fatality.

2 – Potabortal and puerperal tetanus : It results from infection of genital tract with unsterile instrument and dressing. Puerperal tetanus is rare but most dangerous .

3 – splanchnic tetanus : There is involvement of muscle of deglutition and respiration with dysphagia.

4 – cephalic tetanus : It occurs from the wounds of head. There is unilateral and bilateral contraction of muscles of face.

Laboratory Diagnosis

The diagnosis is always clinical and bacteriological findings confirm the diagnosis.

1 – Microscopic examination : smears from wound material after Gram's staining show Gram-positive bacilli with typical drumstick appearance.

2 – Culture : Diagnosis by culture is more dependable. Excised bits of tissue from necrotic depth of wound is inoculated into cooked meat broth, blood agar and lactose egg yolk medium. The addition of polymyxin B to which clostridia resist, make the medium more selective .

If the material is grossly contaminated with other organisms, heating at 80 ° C for 10 minutes may be useful for destroying non-sporing organisms.

Animal inoculation : Mouse is a suitable laboratory animal for demonstration of toxigenecity 2 to 4 days' old cooked meat culture(0.2 ml) is inoculated into the root of tail of a mouse. A second mouse which has received tetanus antitoxin (1000 units) an hour earlier serves as control.

Symptoms appear in test animal in 12 to 24 hours with stiffness of tail. Rigidity develops to the inoculated side of the leg, opposite leg, trunk, fore limb in this order. The animal dies within 2 days. However appearance of ascending tetanus in animal is diagnostic.

Active immunization: Usually two injection 1 ml each of tetanus toxoid is given intramuscularly at the interval of 6 weeks. Third injection is given after 6 to 12 months. A full course of immunization confers immunity for 10 years. Toxin is given either alone or along with diphtheria toxoid and pertusis vaccine (triple vaccine) in which pertussis vaccine acts as adjuvant.

Passive immunization : It is an emergency procedure to be used only once. It is done by giving anti-tetanus serum (ATS). The recommended dose is 1800 IU subcutaneously or intramuscularly as early as possible after wounding. Unfortunately, it carries the risk of hypersensitivity and immune elimination (half life is 2 days).

Tetanus prophylaxis in wound

Nature of wound	Immune status		
	Immune	Partial immune	Non-immune
Clean (wound toilet performed within 6 hours)	Toxoid × 1	Toxoid × 1	Toxoid × 3
contaminated (soil, necrotic material present)	Toxoid × 1	Toxoid × 1 ATS Antibiotics	Toxoid × 3 ATS Antibiotics
Infected	Toxoid × 1 Antibiotics	Toxoid × 1 ATS Antibiotics	Toxoid × 3 ATS Antibiotics

NB: Immune patients having full course of 3 injections of toxoid. Partial immune patient has had 2 injections of toxoid. Non-immune patient has had no injection of toxoid or his immune status is not known.

Passive immunity without risk of hypersensitivity be obtained by use of human antitetanus immunoglobulin (ATG). This is effective in smaller dose (280 units) and has longer half life (3 to 5 weeks).

CLOSTRIDIUM PERFRINGENS (Clostridium welchii)

It is a normal inhabitant of the large intestine of man and animals. It is found in faces and contaminates the skin of perineum, buttocks and thigh. It also produces food poisoning and necrotic enteritis in man .

Morphology

It is a plump, Gram-positive bacillus with straight, parallel sides, rounded or truncated ends about 4 to 6μ × 1μ. It may occur singly or in chains. It is pleomorphic. Filaments and involution forms are common. It is capsulated and non-motile. Spores are central or subterminal.

Cultural characters

It is an anaerobe, growing rapidly at 37˚C .

(a) Cooked meat medium

Fairly good growth occurs at37˚C. The medium becomes turbid within 24 hours with production of gas. The meat is turned pink without digestion. The culture has sour odor.

(b) Nutrient agar

Two types of colonies appear after 24 hours of incubation ; (i) 2 to 4 mm round, smooth, butyrous emulsifiable colonies, (ii) Umbonate colonies with brownish opaque center and lighter radially striated periphery having crenated edges.

Biochemical reactions

Glucose, maltose, lactose and sucrose are fermented with production of acid and gas. In litmus milk it produces acid with gas. Milk is disrupted due to vigorous production of gas. This is called stormy clot. Indole is negative and H_2S is formed abundantly .

Resistance

Autoclaving at 121˚C for 18 minutes destroys the spores. Spores are resistant to antiseptics and disinfectants in common use.

Antigenic structures

Clostridium perfringens are differentiated into 6 types (A,B ,C,D,E,F) on the basis of toxin produced by the strains. Toxins are antigenic and antitoxic sera are used in routine typing of strain.

Toxin

Clostridium perfringens produces at least 12 distinct toxins, besides many other enzymes and biological active soluble substances. According to kind and quantity of toxins produced, different strains of *clostridium perfringens* are divided into 6 types, i.e. A to F. The 4 major toxins, alpha, beta, epsilon and iota are responsible for pathogenecity. Alpha toxin is more important and is produced by all strains of *clostridium perfringens*. Type A strains produce it more abundantly. It is responsible for toxemia of gas gangrene. It is lethal, derm-necrotic and hemolytic. It also shows lecithinase activity and gives positive Nagler's reaction.

NAGLER'S REACTION

Clostridium perfringens are cultured on plates containing 20 percent of human serum or egg yolk. The organism produces opalescence in media containing human serum and egg yolk. The opalescence is due to lecithinase activity of alpha toxin. Alpha toxin splits lipoproteins and liberates lipid. The

lipid deposits around the colony to give opalescence (fig. 29.2). the reaction is specific and is inhibited by alpha toxin antitoxin sera. It is a useful test for the rapid detection of clostridium perfringens in clinical specimen.

Beta, epsilon and iota toxins have lethal and necrotizing properties.

Besides toxins, *clostridium perfringens* also produces soluble substances with enzymatic properties, e.g. neuraminidase , hemagglutinin, fibrinolysin, hemolysin, histamine, etc.

Pathogenecity

Only type A and F are pathogenic for man. Type A is responsible for gas gangrene and food poisoning .

Gas gangrene

clostridium perfringens type A is the predominant agent causing gas gangrene. Other organisms associated with gas gangrene are *clostridium perfringens* , *clostridium edematiciens* and anaerobic streptococci. Organisms enter the wound usually along with foreign particles, e.g. soil, dust, etc. clostridia may be present on normal skin. Infection may be endogenous pathogen , Apart from this *clostridium perfringens* may cause gangrenous appendicitis, biliary tract infection, brain abscess, meningitis, pan-ophthalmitis, urogenital infection, etc. Rarely septicemia and endocarditis may occur

the presence of clostridium in wound does not constitute gas gangrene.

There are 3 types of anaerobic wound infection:

(1) Simple wound contamination with no invasion of underlying tissue. There is usually delay in wound healing.

(2) *Anaerobic cellulitis* : In which clostridia invade fascial plane with minimal toxin production and no invasion of muscle tissue. The disease is gradually in onset. It may be limited to gas abscess or extensive involvement of a limb occurs.

Infection clostridia is of low invasive power and poor toxigenecity. Toxemia is absent and prognosis is good.

(3) *Anaerobic myositis* : It is most serious and is associated with abundant formation of exotoxin. The clostridia multiply and elaborate toxin which causes further damage. **The lecithinase** (toxin) damages cell membranes, muscle fibers and increases capillary permeability. Resulting edema may cause increased tension and anoxia in affected muscle. Hemolytic anemia and hemoglobinuria are due to lysis of RBC by a toxin.

The collagenase destroy collagen barriers in tissue. Hyaluronidases break down intercellular substances. Abundant production of gas reduces blood supply by pressure effect extending the area of anoxic damage. Thus, there is spread of infection and lesion is progressive one.

The incubation period is 7 hours to 6 weeks. The disease develops with increasing pain, tenderness, edema of affected part with systemic signs of toxemia. Profound toxemia and prostration develops and death occurs due to circulatory failure.

. food poisoning Closteredia

Some strains of ***Closteredium botulinium*** type A produce food poisoning. They are characterized by marked heat resistance of spores and production of alpha and beta toxin. They are non-hemolytic strains. Incubation period is 10 to 12 hours. It starts with pain in abdomen , vomiting and diarrhea. Recovery takes place in 24 to 48 hours.

C. Enteritis necrotican

A severe and fatal enteritis due to F type strain may occur. The pathogenesis of this disease is suggested to be a low protein diet that predisposes to decreased levels of digestive proteases with subsequent

mobility to degrade the clostridia beta toxin. The proteases are further blocked by ingestion of trypsin inhibitors found in sweet potatoes .

Laboratory Diagnosis

A. *Hematological investigation*

a. Total leukocyte count usually shows no change. Increased count in secondary infection.
b. Differential leukocyte count shows no change .
c. Anemia, increased serum, bilirubin and hemoglobinuria may occur due to excessive RBC destruction .

B. *Bacteriological investigation* :

Specimens : They are collected from

1. Muscles at the edge of affected area.
2. Exudate from area where infection appears more active .
3. Necrotic tissue and muscle fragment.

Microscopic Examination

Gram- stained smear shows Gram-positive, long and thick bacilli. Gram-positive bacilli without spore are suggestive of *clostridium perfringens*.
Culture: material is inoculated on fresh blood agar and cooked meat media. Surface culture is incubated aerobically and anaerobically. Anaerobic culture is studies after 48 to 72 hours of incubation. Further identification is done by :

1. Nagler's reaction .
2. Biochemical reaction.
3. Animal pathogenecity.

Blood collected during bacteremia is cultured in cooked meat medium and glucose broth. It is identified in usual way.

Animal pathogenicity

One the hind limb of guinea pig 0.1 ml of 24 hour cooked meat broth is injected intramuscularly. Death of animal occurs in 24 to 48 hour. Autopsy shows swelling of injected limb with crepitation due to gas formation. The muscle becomes pink. Organism can be recovered from heart and spleen.

Bacteriological diagnosis of *clostridium perfringens* food poisoning

From the feces of patient and suspected food, isolation of non-hemolytic, non-motile anaerobic and Gram-positive bacilli is suggestive of clostridium perfringens infection.

Treatment : Antibiotics and surgery in gas gangrene.

Clostridium Botulinum

Clostridium Botulinum spores are widely distributed in soil, animal manure, sea mud, vegetables, etc. It causes botulism, a server form of food poisoning.

Morphology: It is Gram-positive about 5µ × 1µ, non-capsulated motile by peritrichate flagella producing subterminal, oval and bulging spores. It shows pleomorphism and occurs either singly or in pairs or chains.

Culture characters : It is strict anaerobic. There are 6 different types (A to F). They differ from one another in their culture characters. These types are identified on the base of immunological difference in toxin production. It grows at 20 to 35˚C in neutral or slightly alkaline medium.

(a) *Cooked meat medium*: After 2 to 4 days´ incubation there is abundant growth. There is blackening of meat particles and gas is also produced.

(b) *Nutrient agar medium* : Single colony is difficult to get because of tendency to spread. Colonies develops after 48 hours. Colony is irregular, 3 to 8 mm, glistening and with granular surface. Consistency of colony is butyrous and is emulsified easily.

(c) *Blood agar*: Colonies on blood agar medium are hemolytic.

(d) *Lacoste, egg yolk, milk agar medium* : All types of this organism produces opalescence and pearly layer. All are lactose negative.

Resistance : Spore is highly resistant, surviving several hours at 100˚C. It withstands 120˚C for 20 minutes. However, heat resistance is diminished at acid PH or high salt concentration.

Biochemical reactions : All types ferment glucose, maltose with acid and gas. There are two biochemical types of clostridium botulinum:

a. proteolytic (types A, B and F) .
b. Saccarolytic and non-proteolytic (type C, D and E). H2S is produced by all types.

Antigenic structure : Six types are distinguished by their toxin production. The toxins are antigenically distinct.

Toxin: It produces powerful exotoxin responsible for pathogenecity. Toxin has been isolated as crystalline protein which is most toxic substance known, i.e. 0,0003 mg is the lethal dose of mice. It is a neurotoxin which acts slowly by inhibiting release of acetylcholine at synapses and neuromuscular junctions. Flaccid paralysis results.

Toxin is stable. It resists digestion in the intestine and is absorbed through intestinal mucosa in an active form. It converted to toxoid. The most important and potent exotoxin is that of type A. The other types of toxin are less toxic.

Pathogenesis : clostridium botulinum is non-invasive and its pathogenecity is due to toxin produced in contaminated food. Botulism is performed by ingested of contaminated food. Human botulism is caused by type A, B and E. Source of this toxin is in preserved contaminated food, meat, canned vegetable and fishes, etc.

Incubation period is 12 to 36 hours. Vomiting thirst, constipation, difficulty in swallowing, speaking and breathing are the manifestations which may be followed by coma, delirium and death in 1 to 7 days .

Laboratory Diagnosis

Bacteriological Investigation

Specimen : Diagnosis is based on demonstration of bacillus or toxin in food or feces. In early stages toxin may be detected from patient's blood.

Culture: Isolation of organism (toxigenic strain) from vomit, food or feces

Demonstration of *clostridium botulinum* toxin: Specimen like food, vomit, etc. are grounded up and soaked overnight in equal volume of isotonic saline solution. It is centrifuged, and supernatant is divided into 3 parts. One portion is heated at 100˚C for 10 minutes. Penicillin is added (concentration being 100 units / ml).

One of the guinea pig is protected with polyvalent botulinum antitoxin and 2 ml of unheated material (2 ml) is injected into second guinea pig. The third animal is injected with 3 ml of heated material.

Second guinea pig develops toxin symptoms like dysphgea, flaccid paralysis and dies within 24 hours. First and third animals show no toxic symptoms.

Typing is done by passive protection with type specific antitoxin

Treatment : Active immunization with toxoid is effective. However, antitoxin is of no use in treatment as toxin already get fixed up to nervous tissue by the time disease becomes apparent.

clostridium tetani **Toxins that block nerve function**

Toxins that affect nerve cell function

- **clostridium botulinum clostridium tetani**

– Most lethal toxins known are tetanus and botulinum toxins

• Tetanus toxin produces irreversible muscle contraction

- Botulinum toxin blocks muscle contraction

Mechanism of Action

- General mechanism of both
  - Consist of single polypeptide chains with A and B regions
  - Binding to ganglioside receptors specific for nerve tissue
  - Activated by proteolysis and disulfide reduction, and they function intracellularly

Tetanus Toxin

- Acts at distance from central nervous system
  - Once bound to cell membranes, toxin is internalized probably by receptor-mediated endocytosis
  - Transported through axonal processes to the spinal cord
    - Toxin interferes with synaptic transmission by preferentially inhibiting release of neurotransmitter, such as glycine from inhibitory interneurons
    - Excitory and inhibitory effects of motor neurons become increasingly unbalanced, causing rigid muscle contractions
  - Cause of inhibitory synapse action

Botulinum Toxin

- General aspects
  - Intoxication, not infection; organism not needed after toxin produced
  - Toxin not destroyed by proteases of digestive tract; probably complexed with other proteins
- Mechanism
  - Affects peripheral nerve endings
  - Once across the gut, it is carried in the blood to neuromuscular junctions

- Bind to gangliosides at motor nerve endpoints and is taken up by cell
- Subsequent events unknown
  - Result in presynaptic block of release of acetylcholine
- ***Interruptions in nerve stimulation causes irreversible relaxation of muscles—leads to respiratory arrest***

the two most potent toxins known . ***Cl.tetani*** usually grows in deep, necrotic puncture wounds where anaerobic conditions exist. The bacterium itself does not spread far from the initial site of infection, but the exotoxine that secret travels to the centeral nervous system to cause severe neuromuscular dysfunction . The toxin binds to the surface of nerve cells is internalized and then transported to the spinal cord, and fixed to nerve synapsis. Creating an imbalance in the excitatory and inhibitory transmissions to motor neurons, resulting.

An uncontrolled, rigid muscle contraction or spastic paralysis resulting in the classic condition of tetanus known as lock jaw

- It is interesting some disease, such as tetanus , very small amount of toxin is required to produce the symptoms of disease, that the person immune system not be sufficient the toxin to produce antibodies. (actively immunized) .

The treatment involve the use of antitoxins

Antitoxins :

Are antibodies preparations derived from the serum of immune humans or animals to neutralize the toxin present already in the body. Injection of specific antitoxin .

specific immune antitoxin, aimed to immediate neutralization of toxin before able to bind the target cell. (no sufficient time)

Tetanus clostridia

Definition : tetanus is acute paralytic illness induced by toxins of the tetanus bacillus

Morphology : Cl.tetani (causative agent of tetanus) , it is a thin motile rod (4-8 M , 0.4 – 0.6 wid) , has periflagella, produce round terminal spores, give drumstick appears .

Gmthe , are obligate anaerobic on sugar and blood agar, PH 7.0 – 7.9 temp 37˚C.

Vegetative cells killed ⟶ O2 / spore survive long time expowre.

Resistance : Veg. cells of tetanus withstand of a temperat 60˚- 70˚C (30 min) . spores are very resistance survive in soil over long period of time , withstand boiling ⟶ 90 min.

Occurance : World wide affect all ages , more common in agricultural region & in under develop area.

Incubation periods: 10 day according to extent , charcters location of the wounds .

Clinical pictures : Is characterized by painful musculan contract (lock jaw) sardonic smile , Acommon 1st sign of tetanus is abdominal rigidity.

Toxines production : Cl.tetani produces an extremely potent toxin consists of two fraction

a) tetanus pasmin⟶ cause musle contraction
b) tetanolysin⟶ cause haemolyses erythrocytes

Pathogenesis and diseases

Sick people and animal, who discharge the organisms in their faces into the soil are sources of the injection . Spores of Cl.tetani demonstrated in 50% examined specimens .

Cl. Tetani gain entrance into the body of a newborn infant through the umbilical cord and into a woman during childbirth. It produce the exotoxines in the site of entry .

Microbes and spores (washed – off) from toxin , no produce dis. And rapidly destroyed by phagocytes.

Tetanus toxin reaches the motor center of the spinal cord via the peripheral nerves (more along the axial nerve cylinder).

Superficial Infections:

Even though staphylococci are able to infect all tissues of the body, the most common infections are of the superficial tissues. S. aureus is a coomon cause of boils, carbuncles, impetigo, and infections of surgical or accidental wounds and burns. Characteristically, these infections form an abscess a localized lesion with a cavity of destroyed (necrotic) tissue filled with pus (suppuration). Scar tissue forms on healing. These infections are usually treated with topical antibiotics, or if abscesses are present, by surgical drainage of the pus. In otherwise healthy individuals, these infections tend to resolve without serious consequences.

Toxic shock syndrome and scalded skin syndrome.

Causative agent Staphylococci group :Toxic shock syndrome usually begins suddenly with high fever, vomiting and diarrhea. In some cases, sore throat, headache, muscle aches, shock, kidney failure, and a red skin rash may be seen. Patients frequently shed the skin from their hands and feet after recovery. Without prompt supportive treatment, death may result. This syndrome was first recognized when a sharp increase in the number of cases was reported in the late 1970s and 1980. Intensive epidemiologic studies demonstrated the most cases occurred in females during or immediately after their menstrual period, and that most cases (99%) were associated with the use of tampons. Staphylococci that produced toxic shock syndrome toxin were found in high concentrations in affected females. It is now theorized that the introduction of tampons in the late 1970s created a condition in a small percentage of users that permitted the excessive growth of toxin producing staphylococci. Once this association was recognized, the use of such

tampons was discouraged and the number of cases of toxic shock syndrome greatly decreased.

Infection of infants or young children with exfoliatin producing strains of S. aureus may cause a condition termed scalded skin syndrome. Wide areas of skin have the appearance of scalded skin. This syndrome may be fetal, but is usually benign. Most patients are only moderately ill .

Food poisoning and colitis

Food poisoning is not an infection but an intoxication resulting from the ingestion of food containing performed staphylococcal enterotoxins. During the preparation of foods, it is very easy for the food handler to contaminate the food with staphylococci from the nose or from a skin lesion. If this food is not properly cooled and refrigerated at 4˚C , the staphylococci may multiply and release enterotoxins. Enough enterotoxin may be produced in 2 to 6 hours to cause severe symptoms. Foods that are not cooked after preparation for example, potato salads and cream pies are common sources of this type of food poisoning. Staphylococci also reproduce rapidly in cooked meats. Their ability to grow at relatively high (9%) salt concentrations makes cured and salted meats such as ham special concerns. Enterotoxins are fairly stable to heat and are not destroyed in foods that are cooked at moderate temperatures after the toxins have been formed. Such symptoms as severe nausea, vomiting, abdominal pain, diarrhea, and prostration may begin to occur as early as 2 hours after ingesting the toxin. There is an absence of fever and complete recovery generally occurs within a day or two. A number of enterotoxins are produced by S. aureus. These are designated A through E and produce similar symptoms.

In persons treated with oral broad- spectrum antibiotics, the normal microflora of the bowel can be greatly reduced. This may allow a selective overgrowth by S. aureus that is antibiotic resistant and also produced an enterotoxin B. Enterotoxin B causes direct damage to the intestinal mucosa and results in a

condition called staphylococcal colitis, which consists of diarrhea, abdominal cramps, and fever.

Botulism symptoms: Characteristic Triad

- Symmetric, descending (cranial nerves first, then upper extremities, then respiratory muscles, and lower extremities) flaccid paralysis with prominent bulbar palsies, particularly:
  - **Diplopia – double vision**
  - **Dysarthria – difficulty in speech articulation**
  - **Dysphonia – difficulty in voice production**
  - **Dysphagia – difficulty in swallowing**

Signs of Food-borne and Wound Botulism

- **Ventilatory (respiratory) problems**
- **Eye muscle paralysis (extraocular, eyelid)**
- **Dry mucous membranes in mouth/throat**
- **Dilated, fixed pupils**
- **Ataxia and Hypotension**
- **Nystagmus**
- **Decreased to absent deep tendon reflexes**

Clinical Features of Infant Botulism

- **Ventilatory difficulty**
- **Weakness/hypotonia**
- **Poor oral feeding/weak sucking**
- **Weak cry**
- **Poor head control**
- **Lethargy/somnulence**
- **Ocular abnormalities (mydriasis, ptosis)**
- **Cardiovascular abnormalities (hypotension, tachycardia)**

Mycotoxin

Mycotoxin

General characters

Definition : mycotoxin combines the greek word for fungi "mykes" and the latin word " toxicum" meaning poison . there are numerous varieties of fungi ranging from those growing in woods to microscopic species .

Moulds can be of great benefit as nutrients to man or as a source of antibiotic.

Toxic metabolic bi-products of fungi, known as mycotoxins, have received considerable attention during the past several years. Mycotoxins are known to cause serious health problems in animals including equine leuko-encephalo-malacia in horses and porcine edema in swine. Reduced weight gain, capillary fragility, reduced fertility, suppressed disease resistance, and even death have been attributed to mycotoxins. No animal is known to be resistant, but in general, older animals are more tolerant than younger animals. Some mycotoxins, fumonisin, aflatoxin, and ochratoxin in particular; have also been associated with human health problems. Certain mycotoxins are suspected carcinogens

- ***Aflatoxins are bis-furanocoumarin metabolites produced primarily by some strains of Aspergillus flovus group .***
- ***There are four main aflatoxins (B_1 ،B_2 ،G_1 ،G_2) plus two additional that are of significance and these are M1 and M2 .***
- ***Aflatoxins are potent liver toxins and also potent carcinogens.***
- ***Alfatoxin B1 is the most toxic of the group .***

- ***Alfatoxins are international mycotoxins intensively investigated in different parts of the world especially in warm temperature & humidity .***
- ***Aflatoxin has been found in number of products particularly oil seeds " peanuts , sunflower , cotton , seeds and corn "yellow corn ".***
- ***In Egypt , aflatoxins are widely detected in foods where the percentage of contaminated samples ranged between 7-44.5% ,oil seeds including peanuts & sun flower are the most contaminated seeds with aflatoxins 27.5% & 44.5% , respectively.***
- ***There are several factors affecting aflatoxin production on foods , these are moisture content of the foods , temperature , incubation period , darkness ,PH value, trace elements , and sodium chloride Nacl .***

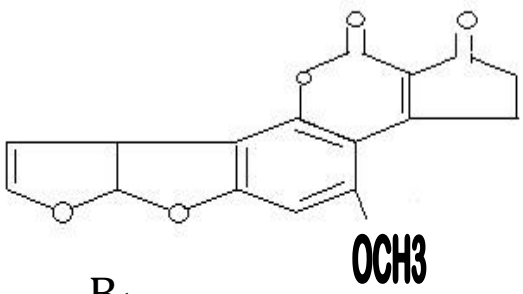
Alfatoxins – producing fungi

Aflatoxins are known to be produced by closely species of muld , Aspergillus flovus & A. porasiticus , both of which are especially A. fumigtusius , has been recognized an aflatoxigenic .

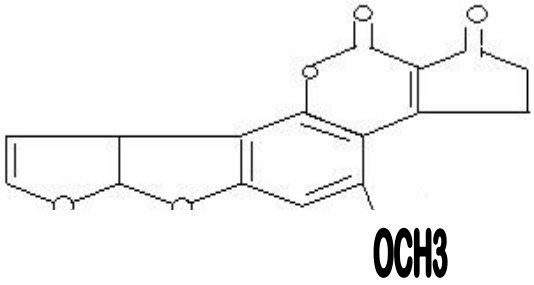
Aflatoxin Derivatives

- ***The aflatoxins are agroup of bis-furonoco.***
- ***Four derivatives are mostly common with A. flovus & A.parositicus , and these are B_1 , B_2 , G_1 , G_2***
- ***flatoxin B1 is the most toxic***

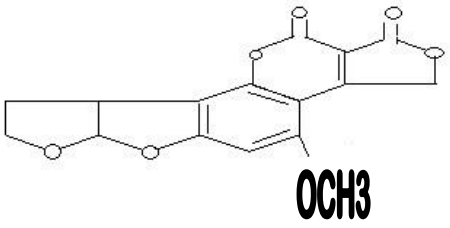
There are metabolites of aflatoxin described as M_1 & M_2, were first discovered in cow's milk ; these metabolites are also found in urine of animals including humans .



B_1



B_2



G1 G2

<u>Ecological factors affecting aflatoxin production</u>

<u>Effect of moisture content</u>

The moisture content of food & feed stuffs , including seeds & grains , is one of the factors affecting aflatoxin production .

A. flavus & A. parasiticus are cabable for producing aflotoxin on pea-nut seeds if the moisture content ranged from 14% to 25% . The production of aflatoxins was regularly increased by increasing the moisture content from 14% to 40% and strongly decreased with increasing the M.C from 50% to 90% , followed by sharply decreased at 100% M.C , there for14% M.C was the lower limit for production aflatoxins and 40% M.C was the best moisture content for the production of aflatoxin B1 , B2 , G1 , G2 .

There are 2-cotegories of grain –borne fungi .

A – field fungi: such as *Alternaria* , *fusarium* , *cladosporium* and *pulluoria* which invade grains during development of plants in the field or after they have been maturated , but before they are harvested , these fungi require a moisture content in equilibrium with a relative humidity of more than 90% in starchy grains cereals this is equivalent to 24-25% moisture content on wet-weight basis .

B – storage fungi : comprise mainly several species of Asperigllus which is particularly , A. glaucus group , in addition to A. candidus , A. ochroceus & A. flavus .

Storage fungi don't invade grain and seed to any degree before harvest , it require M.C. in equivalent to 13-18% M.C. on dry weight basis .

So, it is possible to said that the aflatoxin production on grains or seeds is completely associated with grains and seeds invasion .

Mycotoxin	Fungi associated	Symptoms/toxicology
Aflatoxin	*Aspergillus flavus, A. parasiticus*	liver necrosis, liver tumors, reduced growth, depressed immune response, carcinogen
Fumonisin	*Fusarium moniliforme, F. proliferatum*	equine leukoencephalomalacia, porcine pulmonary edema
Deoxynivalenol (DON)	*F. graminearum*	feed refusal, reduced weight gain, diarrhea, vomiting
Trichothecenes	*F. graminearum, F. culmorum, F. poae*	alimentary toxic aleukia, necrosis, hemorrhages, oral lesion in broiler chickens
Ochratoxins	*Penicillium verrucosum, Aspergillus ochraceus*	porcine nephropathy; various symptoms in poultry
Citrinin	*Penicillium sp., Aspergillus sp.*	kidney damage
Cyclopiazonic acid	*Penicillium sp., Aspergillus sp.*	Neurotoxin

Sterigmatocystin	*Aspergillus sp.*, and others	carcinogen, mutagen

- condition for aflatoxin production

1- **Aspergillus flavus produces the mycotoxin known as aflatoxin on a number of crops including corn, peanut, and cotton.**

2-Typically, the fungus has a yellow green appearance when it is growing on corn kernels.

3-The fungus is quite common in nature, but its population increases during hot dry weather.

4- Aflatoxin contamination is greater in corn that has been produced under stress conditions. Thus, drought, heat, insect, nematode, and fertilizer stress are all conductive to high levels of aflatoxins.

5-Seed companies are in the process of developing corn hybrids with some level of resistance to the fungus or that have less tendency to accumulate the toxin.

6- **Although these hybrids will tend to have lower levels of aflatoxin than others grown under the same conditions, the complete resistance is unlikely. Management practices such as irrigation, good insect control and timely fertilization may reduce stress to the corn plant and thus lower aflatoxin levels.**

7-Aflatoxin levels are regulated by the Food and Drug Administration (FDA) at 20 ppb (parts per billion) in food. Aflatoxin can also appear in milk of lactating animals fed aflatoxin-contaminated feed. The allowable limit level in milk is 0.5 ppb.

Aflatoxins

The cause of the disease was shown due toxins in peameal infected with Aspergillus Flavus

Types of aflatoxin :- B_1 , B_2 and, G_1 , G_2

A.flavus produce B_1,B_2,A. <u>parasiticus</u> produce G_1,G_2 as wellas B_1,B_2

Naturally occurring aflatoxins

Aflatoxins B_1,B_2 , G_1 and G_2 are found in foods and are produced by *A.Flavus* . *A.Parasiticus* , *A.oryzae* and *A.wentii*

Food products contaminated . (maize , sorghum , wheat) oil seeds : (ground nut , saybean , cotton) spices and milk

Physical and chemical properties

They are stable in U.V radiation . at temperature dgree > 100° C

They are refers to the group of difurano coumarine AF B_1, AF B_2 produce blue fluorescence

Toxicity and importance

According to FAO estimates, 25% of the world food crops are affected by mycotoxins each year

Aflatoxins are very potent toxins , carcinogenic , mutagenic and immune suppressive agents

AFB is hepatic – carcinogens and it is mutagen causing chromosomal aberrations

Acute Aflatoxin toxicity has been demonstrated in a wide rang of mammals , fish , birds

The liver is the principal target organ aflatoxins can accumulate in the brain

Aflatoxins have been implicated in sub- acute and chronic effects in humans these effects include primary liver cancer and also affect immunes

Some methods which lead to decrease aflatoxins :-

1 - Roasting of food may cause 40 – 60 % reduction

2 - Alkaline treatment of food destroy aflatoxins.

3 - Fermentation of contaminated seeds with aflatoxin cause of a loss of 60% of added aflatoxins.

General charcters for Fumonisins

1-Fumonisins are a common group of mycotoxins produced by fungi related to the genus Fusarium. The fungus Fusarium moniliforme (Fusarium verticillioides) is a common pathogen of corn, so common in fact that it is found wherever corn is grown.

2- **Fusarium moniliforme usually appears white to salmon colored, although it may not be visible on the corn kernel. This fungus often produces a symptom on the corn kernels referred to as "starburst," or a white streaking of the kernel .**

-Fumonisins have been implicated as a possible cause of human esophageal cancer, equine leukoencephalomalacia (ELEM), a serious disease in horses, and porcine edema – a disease in swine.

3- **<u>Poultry and cattle are not especially susceptible to fumonisins.</u> However, caution should be used in feeding moldy corn to these animals as other mycotoxins may also be present in rotted or moldy corn.**

4- **Fumonisins were found at levels above 5 ppm in 31.0% of suspect grain samples in North Carolina in 1992 and 1993 respectively. Contamination with fumonisins was more severe in 1998, and corn with fumonisin levels in excess of 15 ppm was rejected by some buyers.**

5- **no levels for fumonisins have been set by the FDA, but they will likely be less than 5 ppm for human consumption and horses. Buyers of corn used in feed may accept grain with higher levels of fumonisins than those recommended, depending on what portion of the feed ration is corn.is affected**

6- **Also, corn and corn products shipped to Europe may be regulated at a lower level in the future.**

8-Currently there are no corn hybrids resistant to the fungus F. moniliforme, which is the principle producer of fumonisins. Some

hybrids may be more susceptible to Fusarium than other hybrids, but no hybrid is known to be completely resistant.

Mangement for diseases:

Dry weather early in the season, followed by wet weather during silking of the corn plant, and insect infestation increase the amount of fungal infection of corn kernels. Typically, infection by F. moniliforme will not greatly affect the yield of corn. However, if conditions favorable for fungal growth continue up to harvest, fumonisin levels in harvested corn may exceed recommended levels for certain animals. Factors that influence fumonisin production in corn are not well understood at this time. Certainly, insects provide an avenue of infection for both Aspergillus and Fusarium. Hybrids genetically engineered to resist insects may have lower levels of fumonisins. Also, research is under way in the corn industry to engineer plants with an enzyme to degrade fumonisins.

General properties for Deoxynivalenol

1-Deoxynivalenol (DON or vomitoxin) is a mycotoxin produced by certain species of Fusarium, the most important of which is F. graminearum (Gibberella zeae).

2-This fungus causes Gibberella ear (also known as red ear rot) or stalk rot on corn and head scab in wheat. The fungus itself appears reddish to pinkish. The fungus may cause a reddish dicoloration of the cob and kernels.

3-The mycotoxin deoxynivalenol causes reduced weight gain and suppresses animal feeding, especially in swine. At high concentrations (greater than 10 ppm) vomiting and total feed refusal may occur.

4- FDA has recomended that total feed levels of DON not exceed 5 ppm for cattle and chicken, and 1 ppm for swine. DON levels for human food should be less than 1 ppm.

5-Red ear rot caused by F. graminearum is favored by warm wet weather after silking. Disease tends to be worse when corn is grown without rotation or after wheat as this pathogen also infects wheat. It may be worse when corn is grown in reduced tillage situations.

Products affected and Natural occurance

In cereals like wheat , triticale , oats , sorghum . and maize

Toxicity and importance :-

effects, difficulty in breathing , diarrhea and dry gangrene

Reproductive effects : reduced lactation , reduced feed intake and weight gain

Sterigmatocystin

is produced *by A.versicalor , A.nidulans , A.sydowil* and *P.luteum*

Natural occurance

Green coffee beens and cheese

A.versicolor **has been isolated from cereals grain products . fruits , dried meat products and grabe-fruit juice.**

Toxicity and importance

Are the same such as AFB,

Chronic symptoms include induction of hepatic in rats , pulmonary tumors in mice .

Laboratory animals has affected with kidney and liver damaged and diarrhea.

Ochratoxin A

Fugal producers

Ochratoxin has been obtained from *A.ochraceus* and *P.veridicatum*

Natural occurance

Ochratoxin B can occur naturally but is mush less toxic

The och . A,B have been detected in com beans , peanuts , wheat and rice.

Management for toxin production

Both A. flavus and F. moniliforme are widely distributed in nature and are favored by high temperature. Temperatures ranging from 80 to 100 degrees F and a relative humidity of 85 percent (18 to 20 percent moisture in the grain) are optimum for fungal growth and toxin production. Growth of these fungi does not occur below 12 to 13 percent moisture in the grain. In order to minimize the level of mycotoxins in corn, the following practices should be followed:

- **Use recommended crop production practices.**
- **Planted early.**
- **Irrigate to reduce drought stress.**
- **Minimize insect damage.**
- **Harvest crops early.**
- **Avoid kernel damage during harvest.**
- **Dry and store corn properly - 13 % or less moisture.**
- **Keep storage facilities clean.**
- **Dispose of corn screenings - do not feed to animals.**

Chronic mycotoxicoses

This disease caused by aflatoxin , ochratoxin A

at LD so o.l – 8 mg / kg and it cause :-

1-Reduction in weight gains.

2-Reductin in milk yield and eggs.

Biological effects of mycotoxins

1- **Carinogensis :- Aflatoxins cause cancer at dose of 30 mg / kg. citrinin, penicillic acid and ochratoxins are carcinogenic but trichothenes are not**
2- **Mutagenicity: Aflatoxin cause chromosomal aberration and DNA breakage.**
3- **Oestrogenism: enlargement of uterus in female enlargement of gland and reduction of testes in male of pig.**
4- **Hepatotoxins : they caused by aflatoxins(AFBD) the syndrome is liver injury as fatty and pale liver.**
5- **Nephrotoxicity : cause kidney damage.**
6- **Neurotoxicity: tremorgens caused by ergot**
7- **Dermal toxicosis : caused by trichothecencs and appeared as local imitation and inflammation**

Factors affecting an mycotoxin production

1 – Environmental factors

A) moisture content (MC)

when MC is high. The substrate is more susceptible to fungal growth and toxin production.

B) relative humidity (RH)

when it increase the fungal spores germination stimulates which leads to more fungal growth . Dry condition is more suitable for storage .

C) water activity (aw)

it is the free form of water and available for fungal growth.

D) temprature

toxigenic fungi (A. flavus and A.parasiticus) are widely distribrted at temp. rang.

- ***A. flavus*** can grow in temp-rang. 5.46˚C
- ***B. parasitions*** at low temp until 4˚C

E) light

fungi don't require light. The amount of aflatoxin formed under light condition equals to 50% of those formed under dark condition at the same condition F, O_2 , CO_2

High conc. Of o_2 stimulates growth and toxin formation and high conc of co_2 inhibite it .

2) chemical factors

3)Seed variety

Certain varities of seeds and grains support fungal growth but not support aflatoxin formation this due to chemical composition of seed the resist toxin formation some of these compounds are phenols , phenolic compounds.

Composition of seed substrate

a) carbohydrate content .
   high content of it stimulate toxin formation.
b) protein content
   high content is less susceptible to toxin .
c) mineral content
   Mg^{+2} stimulate toxin formation because it is cofactor for some enzymes.
   Zn^{+2} plays a key role in aflatoxin formation 20 enzymes that necessary for aflatoxin formation are zn^{+} dependant.
   Cd+2 makes inhibition as it is heavy metal.

Strategies for prevention and control of mycotoxin

Toxin producing fungi may invade at pre-harvesting, harvesting- time, during post-harvest and in storage.

The prevention strategies of mycotoxin .

1 – using of resistant varieties of seeds

2 – soil or plant treatment with safe pesticides chemicals.

3 – draying the crops.

4 – removing the broken seeds.

5 – using covered containers for crop storage.

- the pre-harvest control

1) Development of resistant plants

   Antifungal enzymes chitinase and B(1-3) glucanase found in of plant seeds may act as defense against pathogenic fungi so these enzymes may hydrolyze polysaccharides in fungal cell wall.

2) pesticides application

   using of fungicides can lead to increased mycotoxins contamination. This may happen if the application of fungicides isn't (sufficiently) to quickly eliminate the infection.

3) microbiological application

   field treatment with non-toxigenic strains of ***A. flavus and A. parasiticus*** haven been shown to reduce aflatoxin.

- Post-harvest control

1) Draying and moisture control during storage in many regions sundrying is the most used method.
2) Removing the broken seeds

   In case of fruits and fruit juices. Mycotoxin may remain in fruit even when the fungal rot has been removed.

3) control of atmosphere o_2 , co_2 using of covered containers for crop storage to decrease o_2 and increase co_2 and adjust storage temp Belarus.
4) Chemical agent

   Organic acids , essention oils fungicids and gamma radiation.

Algal toxin

ALGAE TOXINS

An introduction:

ALGAE : are more widely distributed groups of microorganisms.

They are natural in habitants of fresh and marine bodies of water through out the world.

Some algae produce toxins which cause infection to human or animal directly or by eating fish which are feed into toxic algae , indirectly and by drinking water that contaminated by toxic algae.

Can be classified into two main groups

Blue green toxigenic algae

It found in running and stagnant water, which appears at the surface of water due to the presence of gas vacuoles.

Toxins of blue-green algae are represented in three fresh water genera .

1- microcystis aeruginosa toxins

***include two toxins* :-**

A - **Fast Death Factor "FDF"**

Characterized as acyclic poly peptide is an endotoxin , obtained from lab grown cells during the early stages of growth , which can kill mice within 30-60 min

B - **Slow Death Factor "SDF"**

Which kill mice within 24-48 hr. which is associated with bacteria grown with blue-green algae .

2- Anabaena flos aquae

Type of toxic blue green algae more that of "FDF"

Is an alkaloid which can kill mice with in 2-10 min after Ip injection of the purified toxin , that called very fast death factor "VFDF"

3- Aphanizomenon :-

- Is more toxic than two are described and also is afast- acting toxin
- Is responsible for many deaths of iivestock and fish
- Reports found that one fraction of that toxin appears to be similar to the "saxitoxin"of gonyaulox cotenella toxins .

Eucaryotic toxigenic algae

Toxigenic sp. Are associated with human cases of shell fish poisoning . they represent types of eucaryatic algae that grow in marine water .

1- Gonyaulax Catenella

Is the causal agent of paralytic shell fish poisoning and infection of human occurs after eat shell fish "mussels and clams" that have been feeding on poisonous algae.

symptoms

1- numbness of lips, tongue and fingers after eating shell fish
2- leg, arms and neck become numb.
3- Feeling of dizziness , drowsiness and loss of mancular coordination.
4- Death due to respiratory failure within 2-12hr of eating poisonous shell fish .

Mechcanical of infection

under suitable condition ,groulth occurs rapidly "1-2 days" and that cause the water to turn to red or brown . clams and musses which feed on the poisonous algae and filter the water though their bodies . so the toxin is conc . is shell fish which people consmume as feed , that is indirectly method.

Chemically

The toxins from the type , is a heat – stable nonpratrin- substance called "soxitoxin" the amount of this toxin which can kill human is unknown but its potency is measured in terms of mouse units depending on extraction methods, purity of extraction.

2- Gymnedinium breve

Is adinoflagellate found in coastal water which is responsible for – shell fish poisnous to fish and tend to concentrate in their tissues and in instine and muscles .

3- Prymnesium parvum

Chemically ,thier toxins in characterized as proteolipids and all are toxic for fish in different degrees and are called " Inchthyo toxins "

some of it cause respiratory parlay sis

its an exo toxin secerated by algae into its surrounding these toxins is the causal agent for fish kills and demoging to other gill.breathing animals and lysis of red blood cells .

Toxicity of sea algal toxins to humans and animals

Marine algal toxins are responsible of more than 60000 intoxication / year with an overall mortality of about 1.5%. Human intoxications are due to consumption of seafood and respiratory exposure to aerosolized toxins.

Algal toxins are also responsible for extensive die-offs of fish and shellfish, as well as mortality in seabirds, marine mammals and other animals depending on marine food web. Lots of information's are available concerning acute intoxications , while little is known about environmental health effects of chronic exposure to low levels of algal toxins.

Toxins are produced by two algal groups, dinoflagellates and diatoms, representing about 2% of known phytoplankton species (60-80 species out of 3400-4000) and can reach humans directly (via consumption of shellfish) or through food web transfer to higher trophic levels .

Finally

Most toxins are neurotoxins and all are temperature stable , so cooking does not ameliorate toxicity in contaminated seafoods ; five seafood poisoning syndromes exists: paralytic shellfish poisoning, diarrhetic shellfish poisoning, and amnesic shellfish poisoning.

1 - Paralytic Shellfish Poisoning (PSP)

Is caused by the consumption of molluscan shellfish contaminated with a suite of heterocyclic guanidines collectively called saxitoxins (STXs) Causing cases of human Poisonings per year, with a 15% mortality rate.

In addition to human intoxications , PSP has been implicated in deaths of birds and humpback whales .

STX elicits its effects by inhibiting sodium channel conductance and there-by causing blockade of neuronal activity, mainly at the peripheral nervous system level, where its binding results in rapid onset of symptoms (less than 1 hr) that are classic for PSP: tingling and numbness of the perioral area and extremities, loss of motor control, drowsiness, incoherence, and in the case of high doses, respiratory paralysis.

2 - Neurotoxic Shellfish Poisoning (NSP)

Generally results from consumption of molluscan shellfish contaminated with brevetoxins (PbTx), a suite of nine structurally related ladderlike polycyclic ether toxins. Brevetoxins bind with high affinity sodium channel altering the voltage sensitivity of the channel, resulting in inappropriate opening of the channel under conditions in which it is normally closed, and inhibiting channel inactivation, resulting in persistent activation or prolonged channel opening. Symptoms of NSP include nausea, tingling and numbness of the perioral area, loss of motor control, and severe muscular ache. NSP has not been documented as a fatal intoxication in humans .

Gymnodinium breve red tides are also frequently associated with massive fish kills. The extreme sensivity of fish may result from lysis of cells passing through the gills, with direct transfer of toxin across the gill epithelium. *G. breve* was also responsible of a manatees dieoff in Florida concurrent with a persistent red tide. The demonstration of brevetoxin immunoreactivity in lymphoid tissue of the manatees raises the possibility of immunosuppression as a second mode by which brevetoxin exposure may affect human health, particularly in individuals with chronic exposure to aerosolized toxin during prolonged red tide incidents.

3- Ciguatera Fish Poisoning (CFP)

Is another seafood intoxication caused by ladderlike polyether toxins, primarily attributed to the dinoflagellate ‹Gambierdiscus toxicus, which produces a precursors to ciguatoxin which is biotransformed to ciguatoxins and bioaccumulated in the highest trophic levels carnivorous fishes associated with coral reefs are a frequent source of ciguatera. Baracuda, snapper, grouper, and jacks are particularly notorious for their potential to carry high toxin loads; however, smaller herbivorous fishes may also be ciguatoxic, particularly when viscera are consumed.

CFP is estimated to affect over 50,000 people annually and is no longer a disease limited to the tropics because of travel to the tropics and shipping of tropical fish species to markets elsewhere in the world. outbreaks are sporadic and unpredictable at others. The symptoms of ciguatera vary somewhat geographically as well as between individuals and incidents and may also vary temporally within an area, but they generally include early onset (2-6 hr) gastrointestinal disturbance-nausea, vomiting, and diarrhea-and may be followed by a variety of later onset (18 hr) neurologic sequelae such as numbness of the perioral area and extremities, reversal of temperature sensation, muscle and joint aches, headache, itching, tachycardia ‹hypertension, blurred vision, and paralysis. Ciguatera on rare occasions can be fatal. A chronic phase may follow acute intoxication and can persist for weeks, months, or even years.

4 - Diarrhetic Shellfish Poisoning (DSP)

Is a comparatively milder seafood intoxication that consists of rapid onset (3 hr) gastrointestinal symptoms such as vomiting and diarrhea that generally resolve within 2-3 days. The diarrhetic shellfish toxins (DTX) are a class of acidic polyether toxins consisting of at least eight congeners including the parent compound ‹okadaic acid. Okadaic acid, DTX-1, and DTX-2 are the primary congeners involved in shellfish poisoning, with the

other congeners believed to be either precursors or shellfish metabolites of the active toxins. The DTXs are inhibitors of ser/thr protein phosphatases. Ser/thr protein phosphatases are critical components of signaling cascades in eukaryotic cells that regulate a diverse array of cellular processes involved in metabolism, ion balance, neurotransmission, and cell cycle regulation. Diarrhea associated with DSP is most likely due to the hyperphosphorylation of proteins, including ion channels, in the intestinal epithelia, resulting in impaired water balance and loss of fluids. Okadaic acidlike polyether toxins have been identified as tumor promotors, thus raising the question of what effect low levels of chronic exposure to DSP toxins may have on humans as well as wildlife such as marine turtles .

5 -Amnesic Shellfish Poisoning (ASP)

Is the only shellfish intoxication caused by a diatom (Pseudo-nitzschia spp.). The first recorded occurrence of ASP was in Prince Edward Island, Canada in 1987 when approximately 100 people became ill and several died after consuming contaminated mussels. The toxic agent involved in the outbreak was identified as domoic acid.

Domoic acid is a water-soluble tricarboxylic amino acid that acts as an analog of the neurotransmitter glutamate and is a potent glutamate receptor agonist. The symptoms of ASP include gastrointestinal effects (e.g. nausea ، vomiting, diarrhea) and neurologic effects such as dizziness, disorientation ، lethargy, seizures, and permanent loss of short-term memory. Persistent activation of the kainate glutamate receptor results in greatly elevated intracellular Ca^{+2} through cooperative interactions with *N-methyl-d-aspartate and non-N-methyl-d-aspartate glutamate receptor subtypes followed by activation of voltage dependent calcium channels. Neurotoxicity due to domoic acid results from toxic levels of intracellular calcium, which leads to neuronal cell death and lesions in areas of the brain where glutaminergic pathways are heavily concentrated.*

The CA1 and CA3 regions of the hippocampus, an area responsible for learning and memory processing, are particularly susceptible. However, memory deficits occur at doses below those causing structural damage. Domoic acid has been identified as the causative agent in the mass mortality of pelicans and cormorants in Mon -terey Bay, California, in 1991 and in the extensive die-off of California sea lions in the same region in 1998. In both instances the vector for toxin transfer was anchovy.

General Characteristics of Producing Organisms

Marine algal toxins are produced by phytoplankton, phytobenthos and bacteria ، and are also called phycotoxins.

Phycotoxins are secondary metabolites produced by dinoflagellates and diatoms, which present pharmacologically active compounds which can be harmful to aquatic flora and fauna. Their role is both important for normal physiology of the cell and for the defense against external environmental insults, namely predators

Marine toxins are not dangerous per se, but they became an hazard when dinoflagellates and diatoms proliferate, under particular environmental conditions, i.e. eutrophication, and toxins can accumulate along different steps of trophic chains, particularly mollusks and fish. In these case, the so called HARMFUL ALGAL BLOOMS (HABs) occur, causing a great increase in cells and toxins concentrations .

Phycotoxins have an great and important toxicological role as they produce a huge number of human illness linked to seafood consumption and contaminated aerosol inhalation. They are also responsible for massive die - off of fish, shellfish and marine vertebrates

Generally speaking they are responsible for acute intoxications, which are well known from the toxicological, chemical and etiological point of view, while little is known concerning chronic exposure to low levels of toxins

More than 3000 dinoflagellates and diatoms species are known at present, but only 2% of then (about 60-80 species) have proved to be toxic or harmful. This little group of species, anyway, is responsible for about 60000 human intoxication/year, 1.5% of them fatal. Fatalities are generally linked to ingestion of saxitoxins, tetrodotoxin and, in rare cases, ciguatera and domoic acid .

Incidence of HABs has increased in recent years, both in frequency and in geographical distribution . Causes of this expansion are various ، and include on one side the increased awareness concerning the issue and the establishment of monitoring, surveillance and research programs on toxins. This lead to a faster and more detailed identification of blooms and toxic episodes .

On the other side, human activities can directly and indirectly contribute to this expansion. Ballast waters transport or shellfish transplantation can directly act by easing the transfer of toxic, non indigenous species from side to the other of the world. Local and regional environmental changes, i.e .eutrophication and pollution, and/or climate variations at the local or global scale can indirectly act by inducing algae proliferation, thus increasing toxins concentrations.

Algal blooms can be classified following various criteria: 1) the kind of bloom formed; 2) the chemical structure of the toxin; 3) the solubility in solvents; 4) the syndrome they induce.

There is no general rule to define harmful concentrations of cells in an algal bloom, the concentration in a HAB is species specific .
Some algae cause harm at low concentrations, with no discoloration in the water, e.g. Alexandrium tumarense where PSP toxins are detected in shellfish at concentrations below 10^3 cells/L, whereas other algae cause harmful effects when they occur in higher in higher concentrations, with discoloration of the water as a result, a "red tide". For

example Gyrodinium aureolum kills fish and benthic animals at concentrations higher than 10^7 cells/L

Five main classes of toxins have been identified starting from their ***chemical structure :***

1 - Amino acid-like compounds (domoic acid and derivatives)

2 - Purine derivatives (saxitoxins and derivatives)

3 - Cyclic imines (spirolides, gymnodines and pinnatoxin A)

4 - Linear and macrocyclic non-azotated polyethers (okadaic acid, pectentoxins, azaspiracid, primnesines)

5 - Trasfused polyehters (brevetoxins, yessotoxins, ciguatoxins).

All the toxins can be classified starting from their solubility in water and organic solvents :

1 - Hydrophilic compounds (saxitoxins, domoic acid, tetrodotoxin)

2 - Lipophilic compounds (okadaic acid, brevetoxins, ciguatoxins)

most known classification is that starting from the syndrome they induce. Starting from this principle 5 different syndromes can be identified.

(1) **Diarrhetic Shellfish Poisoning (DSP)**

Is caused by a group of toxins, represented by okadaic acid, and is characterized by gas -trointestinal symptoms (nausea, diarrhea, vomiting, abdominal pain) which following chronic exposure can evolve in digestive system tumors .

(2) **Paralytic Shellfish Poisoning (PSP)**

Is caused by saxitoxins and is characterized by gastrointestinal and neurological symptoms, with nausea ،vomiting, diarrhea, tingling or numbness around lips, gradual and more and more severe paralysis, respiratory difficulty, death through respiratory paralysis. It can cause death in humans .

(3) **Amnesic Shellfish Poisoning (ASP)**

Toxin is domoic acid, and main sign of this syndrome is loss of short term memory, accompanied by gastrointestinal and neurological symptoms.

(4) **Neurotoxic Shellfish Poisoning (NSP)**

Toxin is brevetoxin, and typical signs of toxicity are tingling and numbness of perioral area, loss of motor control and severe muscular ache. It is also responsible for some irritative episodes following exposure through contaminated aerosol.

(5) **One last syndrome is named ciguatera**

this is due to ciguatoxin. Together with tetrodotoxin, this is the only toxin transmitted by fish and not by shellfish. Typical symptoms are diarrhea, abdominal pain, nausea, vomiting, and lots of neurological signs. It can rarely cause death in humans.

When describing a syndrome, different toxins can be included as etiolo - gical agent, and that's the criteria followed in present chapter; it should be noted anyway that in some cases these toxins have only chemical similarity to the main toxin, causing the syndrome, and nay have a different action on humans and animals. So in the group of diarrheic toxins yessotoxins is included, even if its main action is at the neurological level .

Many other toxins have been studied and new ones are discovered in recent years, ut they are not included in a precise syndrome: tetrodotoxin ، palitoxin, Pfiesteria toxins among the others. These toxins will be described in next sections.

Lots of studies have been conducted to define ideal conditions for algal growth and toxin production, but no clear scenario has been identified

One of main question concerning phycotoxins is if they are produced by algae themselves or by symbiotic bacteria. In some species the production of toxins seems to be independent of bacteria presence, i.e. in Prorocentrum lima, producing okadaic acid. Studies conducted on saxitoxins production lead to no conclusive result, as these toxins have been found in autotrophous dinoflagellates, fresh water cyanobacteria, macrophytes and some bacteria .

Finally it has been proved that tetrodotoxin is produced by symbiotic bacteria, which can be found in various aquatic and terrestrial organisms

Phycotoxins have proved to have some antibacterial and antifungal activity; these activities are thought to allow dinoflagellates to inhibit growth of competitors like bacteria and fungi, as well as other algal species development.

. MECHANISM OF ACTION

Okadaic acid

Okadaic acid is an inhibitor of protein phosphatases (PP), which induce dephosphorylation of proteins by protein kinases (PK). The accumulation of phosphorilated proteins lead to tumor promotion and contraction of smooth muscles. This last effect is responsible for diarrhea and abdominal pain which are among principal symptoms.

Okadaic acid can also alter cell morphology, induce apoptosis and cell death and modify cell physiology: alteration of ions current across membrane ، of glucose balance, of resorption of glucocorticoids receptors, increase in T3 secretion.

Diarrhea, one of the main symptoms of DSP, is due to hyperphosphorilation of intestinal epithelia, with the loss of intestinal structure and of villi; this expose superior part of intestinal crypt cells and produce an important loss of water.

Paralytic Shellfish Poisoning (PSP)

This syndrome is caused by saxitoxins (STX), a group of toxins including about 20 different molecules .

STX was one of the first marine toxins recognized as responsible for human intoxications, the first report dating up to 1798, even if PSP symptoms were attributed to saxitoxins only
after 1920 .

Saxitoxins are responsible for about 2000 human cases/year, with a mortality rate ranging from 15 to 50% (van Dolah, 2000; Marcaillou-Le Baut et al., 2001).

The name of the toxin comes from the mollusk in which it was firstly identified, Saxidomus giganteus. It is produced by both temperate and tropical dinoflagellates of the genera Alexandrium, Gymnodium and
Pyrodinium

SYMPTOMS AND TREATMENT IN HUMANS

PSP, is the oldest known intoxication and one of the most dangerous for humans, with a high rate of mortality .

Native populations of Canada perfectly knew the existence of the toxin and prohibited consumption of mussels coming from contaminated areas ،which were considered as a food taboo (MarcaillouLe Baut et al., 2001).

It is a worldwide distributed poisoning, with cases reported for North and South America, Europe, Africa and Asia. It is commonly thought that it is indeed more probable that the toxin or the dinoflagellates have not been detected or searched for .

Symptoms observed during PSP poisoning are characteristic, easy to recognized and impossible to be confused with allergy and viral or bacterial pathologies .

First symptoms appear 5 to 30 minutes after ingestion of mussels which is called very fast death factor (VFDF) and develop following a precise sequence in few hours. The severity of signs depends on the dose ingested and on individual sensitivity .

Usually, recovery is complete in few days, even if in more severe intoxication death can occur following respiratory paralysis. Symptoms have been classified following the severity of intoxication, and are resumed in Table 2 .

Even if it is a well known toxin, no antidote has been found for its treatment .

Most efficient treatment is a symptomatic one, including gastric lavage and active charcoal or alkaline dinks administration, which favor the inactivation of the toxins and their elimination with urine. Indeed, their clearance via kidney is rapid, close to 24 hours

Forced ventilation is useful in more severe intoxications, when respiratory paralysis occurs, as it can counteract paralysis.

TOXICITY OF FRESH WATER ALGAL TOXINS TO HUMANS AND ANIMALS

Abstract: Algae and cyanobacteria are responsible of the presence of toxins in fresh waters. Algae are considered less dangerous than cyanobacteria, because even if they can proliferate quite intensively in eutrophic fresh waters, they rarely accumulate to form dense surface blooms like blue-green algae do. Thus the toxins they produce do no accumulate to levels high enough to become hazardous to human and animals health.

Cyanobacteria both planktonic and benthic species, can instead form huge agglomeration close to the shore, which can become very dense and concentrated. This material can take a long time to disperse and so become a risk for human health and mainly for animals, which can easely enter in contact with poisoned water. Lots of blue-green algae species have been found to produce toxins, and some authors assume that it could be prudent to assume that any cyanobacterial population can have a toxic potential. At present known toxins are classified as neurotoxins (anatoxin-a, anatoxin- a(s) and saxitoxin), cytotoxin or cylindrospermopsin and microcystins or nodularins. Anyway, starting from existing studies, it seems likely that other unidentified toxins exists.

Neurotoxins act by blocking neuronal signal transmission with two main mechanisms: anatoxins act as acetylcholine mimic (anatoxin-a) or as cholinesterase inhibitor (anatoxin-a (s)), causing an organophosphate like syndrome, while saxitoxin acts by blocking the sodium channel, thus disrupting sodium balance into nerve cells. Despite their high toxicity, their only occasionally are responsible of human intoxication, while neurotoxicity may be experienced by livestock and pets, that can drink polluted water or ingest scum material.

Cytotoxin is an alkaloid that blocks protein synthesis by binding to DNA or RNA. It's responsible for cytogenetic damages via DNA strand breaks and loss of whole chromosomes (aneuploidy) and has proved to be potentially carcinogenetic. Finally, it was found that cylindrospermopsin toxicity is associated with significant losses of glutathione and depletion of glutathione results in cell death. The fall in glutathione levels is due to an inhibition of the final common pathway of glutathione synthesis, and this in turn contributes to cylindrospermopsin cytotoxicity, as lower cell glutathione levels predispose to cylindrospermopsin toxicity. Numerous reports involving the poisoning of farm and wild animals following drinking water from lakes and ponds containing surface scum from cyanobacterial blooms exist, most have been documented in Australia. Australia was also interested by human poisoning episodes via drinking water, with patients escaping death only through skilled and intensive hospital care.

Microcystins are the most frequently occurring and widespread of the cyanotoxins; they act by blocking protein phosphatases 1 and 2a, causing toxicity at the hepatic level, as they use bile carrier to pass through cell membranes.

Microcystins toxicity is greater after intraperitoneal injection, but also intranasal exposure showed high toxicity, being this uptake route relevant for water sports activities (i.e. waterskiing). Nodularins toxicity has shown to be cumulative, as a single oral dose resulted in no hepatic damage, while the same dose applied daily over several days caused hepatic injury. Microcystins have also shown to be tumor-promoting agents, as they can increase the incidence of hepatic tumors in human too.

Fresh Water Algae, their toxicity is considerably lower than that of cyanobacteria, because algae do not have effective mechanism of accumulation and the toxic potency of their toxins is several order of magnitude lower than that of cyanotoxins. Available data reports about dinoflagellates within or related to the Peridinium genus as potential producers of toxins (ichtyotoxins). Ichtyotoxins caused fish kills and have shown to have an algicidal effect on the cyanobacteria Microcystis aeruginosa. It is possible that toxic blooms of dinoflagellates in freshwater occur more frequently than reported and that they affect the biota in those habitats.

Toxins production is considered as a defensive strategy for dinoflagellates from fish larvae preying. In human some allergic reaction has been reported after Uroglena spp. and Gonyostomum semen exposure, but clear toxic episode was reported.

General Characteristics of Fresh Water Toxins Producing Organisms

Freshwater algal toxins are produced by both algae and cyanobacteria (also called blue-green algae). Toxins are secondary metabolites of normal metabolism of the algae and of cyanobacteria, which present a different degree of toxicity: the less toxic induce dermatitis, the most dangerous are hepatotoxic. Generally speaking, algae produce less potent toxins and are rarely responsible of toxic episodes .

The algae that produce toxins are small unicellular autotroph organisms, which perform photosynthesis and organication. Cyanobacteria are organisms which have intermediate characteristics between algae and bacteria.

So they are photosynthetic, but their cellular structure is similar to that of bacteria, i.e. they lack a cellulose outer wall, do not reproduce sexually and do not have membrane-bound nuclei or specialized organelles. They posses an accessory pigment, phycocyanin, unique to cyanobacteria, which has a bluish color, thus cyanobacteria are also called blue-green algae

Bloom formation is eased by a stable water column, warm waters, high nutrient concentrations, high pH, low CO_2 and low grazing rate by zooplankton

Usually algae do not produce blooms, while blue-green algae produce important blooms. This differential behavior is partly responsible of the different toxicity and dangerousness of the two groups. Indeed, by not accumulating in the environment to form blooms, algae do not produce toxins amounts high enough to threaten human, livestock or wildlife health. On the contrary, blooms formed by cyanobacteria produce not only cells accumulation, but also an increase in toxins concentrations to levels hazardous to humans or livestock .

Main exposure way is through recreational and drinking waters, and absorption ways are ingestion, contact and inhalation. Human activities, causing , are among causes responsible for the increase in proliferation of both algae and cyanobacteria, by increasing the concentration of nutrients necessary for their development and growth. Cells proliferation highly impacts the quality of waters even when no evident bloom is formed.

At present little knowledge is available on toxins of algal origin, even if some evidence exists of toxicity of algae to humans and fish, due to exposure to Peridium polonicum.

Much more information exist on cyanobacteria toxins, which have been chemically characterized and for which producing organisms have been identified. At present 46 species of toxins producing blue-green algae have been identified, and 60% of studied strains have proved to contain toxins.

First evidence of cyanobacterial intoxications dates up to 1878, when a livestock poisoning in Australia was attributed to blue-green algae presence and to toxin production. "…Symptoms-stupor and unconsciousness, falling and remaining quiet, as if asleep, unless touched, when convulsions come on, with head and neck drawn back by rigid spasm, which subsides before death. Time- sheep, from one to six or eight hours; horses, eight to twenty four hours; dogs, four to five hours; pigs, three or four hours" .

All this considering, present chapter will focus on cyanobacteria toxins.

Cyanobacteria include both pelagic and benthic species, which form two different kind of aggregates.

Pelagic species can float in water column, vertically migrating depending on temperature, light and nutrient availability. These species contain intracellular gas vesicles which help the cells in buoyancy and in maintaining their position at desired depth in water column. In order to move through water column, blue-green algae change the dimensions and the number of vesicles. It has been observed that positive buoyancy is obtained by forming proteinaceous gas containing vesicle. Conversely, reduction of

buoyancy result from polysaccharide accumulation and increased cellular turgor pressure; polysaccharide act as ballast molecules, while increased pressure induces collapse of vesicles.

Vesicles dimensions are also adapted to atmospheric conditions, in particular to wind and waves, and are slowly adapted to weather changes. This can lead to an increase of surface concentration of cells and to the formation of floating scums when weather condition turn to less windy .

These scums can be redispersed by wind and waves action, which can also lead to persistence of scums themselves, by accumulating them to the shore. Wind and waves can also produce a slow dispersal by shore washing or cell disintegration, causing the release of toxins in the environment and potentially increasing toxicity of water .

Benthic species growth on different substrates (as mud or rocks) and form mats on it, which can then be washed by waves to the shore. When reaching the shore, they can be scavenged by dogs, livestock and wildlife ، thus causing toxicity. These cyanobacteria species have a smaller impact on human health with respect to pelagic species, because they have a smaller chance to be ingested, as they can be easily seen and avoided by man.

A major hazard for humans occurs when mats do not reach the surface . Mats form when water is extremely clear, as light should reach the bottom in order for blue-green algae to replicate, and thus water is perceived by users as safe because it does not present "floating pollution"

Three main classes of cyanotoxins exist :

-Microcystins or nodularins

-Neurotoxins

-Cytotoxin or cylindrospermopsin

FRESH WATER TOXINS

Laboratory studies have underlined that probably other unidentified toxins exist, starting from the toxic effects observed in cells cultures and fish eggs exposure trials, which could not be ascribed to know toxins .

Most species have been found to produce both microcystins and neuro - toxins; wild populations have been proved to be a mixture of toxic and non-toxic strains, so that not always the presence of a potentially toxin - producing species coincides with real presence of the toxin(s .(

These toxins have different mechanisms of action, which will be de - scribed in following sections. Briefly, they can be resumed as follow :

- Microcystins block protein phosphatases thus acting as cytotoxic com - pound, being liver their main target

-Neurotoxins act by blocking neuronal signalling ؛

- Cytotoxin blocks protein and glutathione synthesis.