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Editorial

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We would like to thank all our esteem readers for their continuous support & encouragement in making this Journal a successful effort. Here's another issue of JHS with loads of valuable information, kindly flip a few pages to believe us…….

Hygiene is an old concept related to cleanliness, health and medicine, as well as to personal and professional care practices related to most aspects of living. Hygiene mostly means practices that prevent spread of disease-causing organisms. Since cleaning processes (e.g., hand washing) remove infectious microbes as well as dirt and soil, they are often the means to achieve hygiene.

Pesticides reaching to the soil are acted upon by several physical, chemical, and biological forces. However, physical and chemical forces are acting upon/degrading the pesticides to some extent, microorganism's plays major role in the degradation of pesticides. Many soil microorganisms have the ability to act upon pesticides and convert them into simpler non-toxic compounds. This process of degradation of pesticides and conversion into non-toxic compounds by microorganisms is known as "biodegradation". Not all pesticides reaching to the soil are biodegradable and such chemicals that show complete resistance to biodegradation are called "recalcitrant".

Our In Profile scientist of the month is "Alexis Carrel", an innovative surgeon whose experiments with the transplantation and repair of body organs led to advances in the field of surgery and the art of tissue culture. An original and creative thinker, Carrel was the first to develop a successful technique for suturing blood vessels together. For his work with blood-vessel suturing and the transplantation of organs in animals, he received the 1912 Nobel Prize in medicine and physiology. Carrel's work with tissue culture also contributed significantly to the understanding of viruses and the preparation of vaccines.

B. pertussis is a very small Gram-negative aerobic coccobacillus that appears singly or in pairs. Its metabolism is respiratory, never fermentative, and taxonomically, *Bordetella* is placed among the "Gram-negative Aerobic Rods and Cocci" in Bergey's Manual. *Bordetella* is not assigned to any family. The bacteria are nutritionally fastidious and are usually cultivated on rich media supplemented with blood. They can be grown in synthetic medium, however, which contains buffer, salts, an amino acid energy source, and growth factors such as nicotinamide (for which there is a strict requirement). Even on blood agar the organism grows slowly and requires 3-6 days to form pinpoint colonies.

Penicillin was the first naturally occurring antibiotic discovered. It is obtained in a number of forms from Penicillium moulds. Penicillin is not a single compound but a group of closely related compounds, all with the same basic ring-like structure (a β-lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate.

A microbiology laboratory is a unique environment that requires special practices and containment facilities in order to properly protect persons working with microorganisms. Safety in the laboratory is the primary concern.

Looking forward for your feedback & suggestions.

Body and Overall Hygiene

Hygiene (which comes from the name of the Greek goddess of health, Hygieia), is a set of practices performed for the preservation of health. Whereas in popular culture and parlance it can often mean mere 'cleanliness', hygiene in its fullest and original meaning goes much beyond that to include all circumstances and practices, lifestyle issues, premises and commodities that engender a safe and healthy environment. While in modern medical sciences there is a set of standards of hygiene recommended for different situations, what is considered hygienic or not can vary between different cultures, genders and groups.

Concept of hygiene

Hygiene is an old concept related to cleanliness, health and medicine, as well as to personal and professional care practices related to most aspects of living. In medicine and in home (domestic) and everyday life settings, hygiene practices are employed as preventative measures to reduce the incidence and spreading of disease. In the manufacture of food, pharmaceutical, cosmetic and other products, good hygiene is a key part of quality assurance i.e. ensuring that the product complies with microbial specifications appropriate to its use. The terms cleanliness (or cleaning) and hygiene are often used interchangeably, which can cause confusion. In general, hygiene mostly means practices that prevent spread of disease-causing organisms. Since cleaning processes (e.g., hand washing) remove infectious microbes as well as dirt and soil, they are often the means to achieve hygiene. Other uses of the term appear in phrases including: *body hygiene, personal hygiene, sleep hygiene, mental hygiene, dental hygiene,* and *occupational hygiene*, used in connection with public health. *Hygiene* is also the name of a branch of science that deals with the promotion and preservation of health, also called hygienic. Hygiene practices vary widely, and what is considered acceptable in one culture might not be acceptable in another.

Home and everyday life hygiene

Home hygiene pertains to the hygiene practices that prevent or minimize disease and the spreading of disease in home (domestic) and in everyday life settings such as social settings, public transport, the work place, public places etc.

Hygiene in home and everyday life settings plays an important part in preventing spread of infectious diseases. It includes procedures used in a variety of domestic situations such as hand hygiene, respiratory hygiene, food and water hygiene, general home hygiene (hygiene of environmental sites and surfaces), care of domestic animals, and home healthcare (the care of those who are at greater risk of infection).

At present, these components of hygiene tend to be regarded as separate issues, although all are based on the same underlying microbiological principles. Preventing the spread of infectious diseases means breaking the chain of infection transmission. The simple principle is that, if the chain of infection is broken, infection cannot spread. In response to the need for effective codes of hygiene in home and everyday life settings the International Scientific Forum on Home Hygiene has developed a risk-based approach (based on Hazard Analysis Critical Control Point (HACCP), which has come to be known as "targeted hygiene". Targeted hygiene is based on identifying the routes of spread of pathogens in the home, and applying hygiene procedures at critical points at appropriate times to break the chain of infection.

The main sources of infection in the home are people (who are carriers or are infected), foods (particularly raw foods) and water, and domestic animals. Additionally, sites that accumulate stagnant water—such as sinks, toilets, waste pipes, cleaning tools, face cloths—readily support microbial growth, and can become secondary reservoirs of infection, though species are mostly those that threaten "at risk" groups. Germs (potentially infectious bacteria, viruses etc.) are constantly shed from these sources via mucous membranes, faeces, vomit, skin scales, etc. Thus, when circumstances combine, people become exposed, either directly or via food or water, and can develop an infection. The main "highways" for spread of germs in the home are the hands, hand and food contact surfaces, and cleaning cloths and utensils. Germs can also spread via clothing and household linens, such as towels. Utilities such as toilets and wash basins, for example, were invented for dealing safely with human waste, but still have risks associated with them, which may become critical at certain times, e.g., when someone has sickness or diarrhea. Safe disposal of human waste is a fundamental need; poor sanitation is a primary cause of diarrhea disease in low income communities. Respiratory viruses and fungal spores are also spread via the air.

Good home hygiene means targeting hygiene procedures at critical points, at appropriate times, to break the chain of infection i.e. to eliminate germs before they can spread further. Because the "infectious dose" for some pathogens can be very small (10-100 viable units, or even less for some viruses), and infection can result from direct transfer from surfaces via hands or food to the mouth, nasal mucosa or the eye, 'hygienic cleaning' procedures should be sufficient to eliminate pathogens from critical surfaces. Hygienic cleaning can be done by:

- Mechanical removal (i.e. cleaning) using a soap or detergent. To be effective as a hygiene measure, this

process must be followed by thorough rinsing under running water to remove germs from the surface.

- Using a process or product that inactivates the pathogens in situ. Germ kill is achieved using a "microbiocidal" product i.e. a disinfectant or antibacterial product or waterless hand sanitizer, or by application of heat.
- In some cases combined germ removal with kill is used, e.g. laundering of clothing and household linens such as towels and bedlinen.

Hand hygiene

Hand hygiene is defined as hand washing or washing hands and nails with soap and water or using a waterless hand sanitizer.

Hand hygiene is central to preventing spread of infectious diseases in home and everyday life settings.

In situations where hand washing with soap is not an option (e.g. when in a public place with no access to wash facilities), a waterless hand sanitizer such as an alcohol hand gel can be used. They can also be used in addition to hand washing, to minimize risks when caring for "at risk" groups. To be effective, alcohol hand gels should contain not less than 60%v/v alcohol. Hand sanitizers are not an option in most developing countries. In situations with limited water supply, there are water-conserving solutions, such as tippy-taps. (A tippy-tap is a simple technology using a jug suspended by a rope, and a foot-operated lever to pour a small amount of water over the hands and a bar of soap.) In low-income communities, mud or ash is sometimes used as an alternative to soap.

The World Health Organization recommends hand washing with ash if soap is not available in emergencies, schools without access to soap and other difficult situations like post-emergencies where use of (clean) sand is recommended too. Use of ash is common and has in experiments been shown at least as effective as soap for removing bacteria.

Respiratory hygiene

Correct respiratory and hand hygiene when coughing and sneezing reduces the spread of germs particularly during the cold and flu season.

- Carry tissues and use them to catch coughs and sneezes
- Dispose of tissues as soon as possible
- Clean your hands by hand washing or using an alcohol hand sanitizer.

Laundry hygiene

Laundry hygiene pertains to the practices that prevent or minimize disease and the spreading of disease via soiled clothing and household linens such as towels. Items most likely to be contaminated with pathogens are those that come into direct contact with the body, e.g., underwear, personal towels, facecloths, nappies. Cloths or other fabric items used during food preparation or for cleaning the toilet or cleaning up material such as faeces or vomit are a particular risk.

Microbiological and epidemiological data indicates that clothing and household linens etc. are a risk factor for infection transmission in home and everyday life settings as well as institutional settings, although the lack of quantitative data directly linking contaminated clothing to infection in the domestic setting makes it difficult to assess the extent of the risk. Although microbiological data indicates that risks from clothing and household linens are somewhat less than those associated with hands, hand contact and food contact surfaces, and cleaning cloths, nevertheless these risks needs to be appropriately managed through effective laundering practices. In the home, this routine should be carried out as part of a multibarrier approach to hygiene which also includes hand, food, respiratory and other hygiene practices.

Infection risks from contaminated clothing etc. can increase significantly under certain conditions. e.g. in healthcare situations in hospitals, care homes and the domestic setting where someone has diarrhoea, vomiting, or a skin or wound infection. It also increases in circumstances where someone has reduced immunity to infection.

Hygiene measures, including laundry hygiene, are an important part of reducing spread of antibiotic resistant strains. In the community, otherwise healthy people can become persistent skin carriers of MRSA, or faecal carriers of enterobacteria strains which can carry multi-antibiotic resistance factors (e.g. NDM-1 or ESBL-producing strains). The risks are not apparent until, for example, they are admitted to hospital, when they can become "self infected" with their own resistant organisms following a surgical procedure. As persistent nasal, skin or bowel carriage in the healthy population spreads "silently" across the world, the risks from resistant strains in both hospitals and the community increases. In particular the data indicates that clothing and household linens are a risk factor

for spread of S. aureus (including MRSA and PVL-producing MRSA strains), and that effectiveness of laundry processes may be an important factor in defining the rate of community spread of these strains. Experience in the USA suggests that these strains are transmissible within families, but also in community settings such as prisons, schools and sport teams. Skin-to-skin contact (including unabraded skin) and indirect contact with contaminated objects such as towels, sheets and sports equipment seem to represent the mode of transmission.

During laundering, temperature, together with the action of water and detergent work together to reduce microbial contamination levels on fabrics. During the wash cycle soil and microbes are detached from fabrics and suspended into the wash water. These are then "washed away" during the rinse and spin cycles. In addition to physical removal, micro-organisms can be killed by thermal inactivation which increases as the temperature is increased. Chemical inactivation of microbes by the surfactants and activated oxygen-based bleach used in detergents also contributes to the hygiene effectiveness of laundering. Adding hypochlorite bleach in the washing process also achieves inactivation of microbes. A number of other factors can also contribute including drying and ironing.

Laundry detergents contain a mix of ingredients including surfactants, builders, optical brighteners, etc. Cleaning action arises primarily from the action of the surfactants and other ingredients, which are designed to maximise release and suspension of dirt and microbes into the wash liquid, together with enzymes and/or activated oxygen-based bleach which digest and remove stains. Although activated oxygen bleach is included in many powder detergents to digest and remove stains, it also produces some chemical inactivation of bacteria, fungi and viruses. As a rule of thumb, powders and tablets normally contain activated oxygen bleach, but liquids, and all products (liquid or powder) used for "coloureds" do not. Surfactants also exert some chemical inactivation action against certain species although the extent of their action is not known.

In 2013 the International Scientific Forum on Home Hygiene (IFH) reviewed some 30 studies of the hygiene effectiveness of laundering at various temperatures ranging from room temperature to 70 °C, under varying conditions. A key finding was the lack of standardisation and control within studies, and the variability in test conditions between studies such as wash cycle time, number of rinses etc. The consequent variability in the data (i.e. the reduction in contamination on fabrics) obtained, in turn makes it extremely difficult to propose guidelines for laundering with any confidence, based on currently available data. As a result, there is significant variability in the recommendations for hygienic laundering of clothing etc. given by different agencies.

Of concern is recent data suggesting that, in reality, modern domestic washing machines do not reach the temperature specified on the machine controls.

Medical hygiene at home

Medical hygiene pertains to the hygiene practices that prevents or minimizes disease and the spreading of disease in relation to administering medical care to those who are infected or who are more "at risk" of infection in the home. Across the world, governments are increasingly under pressure to fund the level of healthcare that people expect. Care of increasing numbers of patients in the community, including at home is one answer, but can be fatally undermined by inadequate infection control in the home. Increasingly, all of these "at-risk" groups are cared for at home by a carer who may be a household member who thus requires a good knowledge of hygiene. People with reduced immunity to infection, who are looked after at home, make up an increasing proportion of the population (currently up to 20%). The largest proportion are the elderly who have co-morbidities, which reduce their immunity to infection. It also includes the very young, patients discharged from hospital, taking immuno-suppressive drugs or using invasive systems, etc. For patients discharged from hospital, or being treated at home special "medical hygiene" procedures may need to be performed for them e.g. catheter or dressing replacement, which puts them at higher risk of infection.

Antiseptics may be applied to cuts, wounds abrasions of the skin to prevent the entry of harmful bacteria that can cause sepsis. Day-to-day hygiene practices, other than special medical hygiene procedures are no different for those at increased risk of infection than for other family members. The difference is that, if hygiene practices are not correctly carried out, the risk of infection is much greater.

Personal hygiene

Personal hygiene involves those practices performed by an individual to care for one's bodily health and well being, through cleanliness. Motivations for personal hygiene practice include reduction of personal illness, healing from personal illness, optimal health and sense of well being, social acceptance and prevention of spread of illness to others. What is considered proper personal hygiene can be cultural-specific and may change over time. In some cultures removal of body hair is considered proper hygiene. Other practices that are generally considered proper hygiene include bathing regularly, washing hands regularly and especially before handling food, washing scalp hair, keeping hair short or removing hair, wearing clean clothing, brushing one's teeth, cutting finger nails, besides other practices. Some practices are gender-specific, such as by a woman during her menstrual cycle. People tend to develop a routine for attending to their personal hygiene needs. Other personal hygienic practices would include covering one's mouth when coughing, disposal of soiled tissues appropriately, making sure toilets are clean, and

making sure food handling areas are clean, besides other practices. Some cultures do not kiss or shake hands to reduce transmission of bacteria by contact.

Personal grooming extends personal hygiene as it pertains to the maintenance of a good personal and public appearance, which need not necessarily be hygienic. It may involve, for example, using deodorants or perfume, shaving, or combing, besides other practices.

Excessive body hygiene and allergies

The hygiene hypothesis was first formulated in 1989 by Strachan who observed that there was an inverse relationship between family size and development of atopic allergic disorders – the more children in a family, the less likely they were to develop these allergies. From this, he hypothesised that lack of exposure to "infections" in early childhood transmitted by contact with older siblings could be a cause of the rapid rise in atopic disorders over the last thirty to forty years. Strachan further proposed that the reason why this exposure no longer occurs is, not only because of the trend towards smaller families, but also "improved household amenities and higher standards of personal cleanliness".

Although there is substantial evidence that some microbial exposures in early childhood can in some way protect against allergies, there is no evidence that we need exposure to harmful microbes (infection) or that we need to suffer a clinical infection. Nor is there evidence that hygiene measures such as hand washing, food hygiene etc. are linked to increased susceptibility to atopic disease. If this is the case, there is no conflict between the goals of preventing infection and minimising allergies. A consensus is now developing among experts that the answer lies in more fundamental changes in lifestyle etc. that have led to decreased exposure to certain microbial or other species, such as helminths, that are important for development of immuno-regulatory mechanisms. There is still much uncertainty as to which lifestyle factors are involved.

Although media coverage of the hygiene hypothesis has declined, a strong 'collective mindset' has become established that dirt is 'healthy' and hygiene somehow 'unnatural'. This has caused concern among health professionals that everyday life hygiene behaviours, which are the foundation of public health, are being undermined. In response to the need for effective hygiene in home and everyday life settings, the International Scientific Forum on Home Hygiene has developed a "risk-based" or targeted approach to home hygiene that seeks to ensure that hygiene measures are focussed on the places, and at the times most critical for infection transmission. Whilst targeted hygiene was originally developed as an effective approach to hygiene practice, it also seeks, as far as possible, to sustain "normal" levels of exposure to the microbial flora of our environment to the extent that is important to build a balanced immune system.

Excessive body hygiene of internal ear canals

Excessive body hygiene of the ear canals can result in infection or irritation. The ear canals require less body hygiene care than other parts of the body, because they are sensitive, and the body system adequately cares for these parts. Most of the time the ear canals are self-cleaning; that is, there is a slow and orderly migration of the skin lining the ear canal from the eardrum to the outer opening of the ear. Old earwax is constantly being transported from the deeper areas of the ear canal out to the opening where it usually dries, flakes, and falls out. Attempts to clean the ear canals through the removal of earwax can actually reduce ear canal cleanliness by pushing debris and foreign material into the ear that the natural movement of ear wax out of the ear would have removed.

Excessive body hygiene of skin

Excessive body hygiene of the skin can result in skin irritation. The skin has a natural layer of oil, which promotes elasticity, and protects the skin from drying. When washing, unless using aqueous creams with compensatory mechanisms, this layer is removed leaving the skin unprotected.

Excessive application of soaps, creams, and ointments can also adversely affect certain of the natural processes of the skin. For examples, soaps and ointments can deplete the skin of natural protective oils and fat-soluble content such as cholecalciferol (vitamin D3), and external substances can be absorbed, to disturb natural hormonal balances.

Medical hygiene

Medical hygiene pertains to the hygiene practices related to the administration of medicine, and medical care, that prevents or minimizes disease and the spreading of disease.

Medical hygiene practices include:

- Isolation or quarantine of infectious persons or materials to prevent spread of infection.
- Sterilization of instruments used in surgical procedures.
- Use of protective clothing and barriers, such as masks, gowns, caps, eyewear and gloves.
- Proper bandaging and dressing of injuries.
- Safe disposal of medical waste.
- Disinfection of reusables (i.e. linen, pads, uniforms)
- Scrubbing up, hand-washing, especially in an operating room, but in more general health-care settings as well, where diseases can be transmitted.

Most of these practices were developed in the 19th century and were well established by the mid-20th century. Some procedures (such as disposal of medical waste) were refined in response to late-20th century disease outbreaks, notably AIDS and Ebola.

Soil Microorganisms in Biodegradation of Pesticides and Herbicides

Pesticides are the chemical substances that kill pests and herbicides are the chemicals that kill weeds. In the context of soil, pests are fungi, bacteria insects, worms, and nematodes etc. that cause damage to field crops. Thus, in broad sense pesticides are insecticides, fungicides, bactericides, herbicides and nematicides that are used to control or inhibit plant diseases and insect pests. Although wide-scale application of pesticides and herbicides is an essential part of augmenting crop yields; excessive use of these chemicals leads to the microbial imbalance, environmental pollution and health hazards. An ideal pesticide should have the ability to destroy target pest quickly and should be able to degrade non-toxic substances as quickly as possible.

The ultimate "sink" of the pesticides applied in agriculture and public health care is soil. Soil being the storehouse of multitudes of microbes, in quantity and quality, receives the chemicals in various forms and acts as a scavenger of harmful substances. The efficiency and the competence to handle the chemicals vary with the soil and its physical, chemical and biological characteristics.

1. **Effects of pesticides:** Pesticides reaching the soil in significant quantities have direct effect on soil microbiological aspects, which in turn influence plant growth. Some of the most important effects caused by pesticides are : (1) alterations in ecological balance of the soil microflora, (2) continued application of large quantities of pesticides may cause ever lasting changes in the soil microflora, (3) adverse effect on soil fertility and crop productivity, (4) inhibition of N2 fixing soil microorganisms such as *Rhizobium, Azotobacter, Azospirillum* etc. and cellulolytic and phosphate solubilizing microorganisms, (5) suppression of nitrifying bacteria, *Nitrosomonas* and *Nitrobacter* by soil fumigants ethylene bromide, Telone, and vapam have also been reported, (6) alterations in nitrogen balance of the soil, (7) interference with ammonification in soil, (8) adverse effect on mycorrhizal symbioses in plants and nodulation in legumes, and (9) alterations in the rhizosphere microflora, both quantitatively and qualitatively.

2. **Persistence of pesticides in soil:** How long an insecticide, fungicide, or herbicide persists in soil is of great importance in relation to pest management and environmental pollution. Persistence of pesticides in soil for longer period is undesirable because of the reasons: a) accumulation of the chemicals in soil to highly toxic levels, b) may be assimilated by the plants and get accumulated in edible plant products, c) accumulation in the edible portions of the root crops, d) to be get eroded with soil particles and may enter into the water streams, and finally leading to the soil, water and air pollutions. The effective persistence of pesticides in soil varies from a week to several years depending upon structure and properties of the constituents in the pesticide and availability of moisture in soil. For instance, the highly toxic phosphates do not persist for more than three months while chlorinated hydrocarbon insecticides (eg. DOT, aldrin, chlordane etc) are known to persist at least for 4-5 years and some times more than 15 years.

   From the agricultural point of view, longer persistence of pesticides leading to accumulation of residues in soil may result into the increased absorption of such toxic chemicals by plants to the level at which the consumption of plant products may prove deleterious / hazardous to human beings as well as livestock's. There is a chronic problem of agricultural chemicals, having entered in food chain at highly inadmissible levels in India, Pakistan, Bangladesh and several other developing countries in the world. For example, intensive use of DDT to control insect pests and mercurial fungicides to control diseases in agriculture had been known to persist for longer period and thereby got accumulated in the food chain leading to food contamination and health hazards. Therefore, DDT and mercurial fungicides has been, banned to use in agriculture as well as in public health department.

3. **Biodegradation of Pesticides in Soil:** Pesticides reaching to the soil are acted upon by several physical, chemical, and biological forces. However, physical and chemical forces are acting upon/degrading the pesticides to some extent, microorganism's plays major role in the degradation of pesticides. Many soil microorganisms have the ability to act upon pesticides and convert them into simpler non-toxic compounds. This process of degradation of pesticides and conversion into non-toxic compounds by microorganisms is known as "biodegradation". Not all pesticides reaching to the soil are biodegradable and such chemicals that show complete resistance to biodegradation are called "recalcitrant".

   The chemical reactions leading to biodegradation of pesticides fall into several broad categories which are discussed in brief in the following paragraphs.

a) **Detoxification:** Conversion of the pesticide molecule to a non-toxic compound. Detoxification is not synonymous with degradation. Since a single chance in the side chain of a complex molecule may render the chemical non-toxic.

b) **Degradation:** The breaking down / transformation of a complex substrate into simpler products leading finally to mineralization. Degradation is often considered to be synonymous with mineralization, e.g. Thirum (fungicide) is degraded by a strain of *Pseudomonas* and the degradation products are dimethlamine, proteins, sulpholipaids, etc.

c) **Conjugation (complex formation or addition reaction):** In which an organism make the substrate more complex or combines the pesticide with cell metabolites. Conjugation or the formation of addition product is accomplished by those organisms catalyzing the reaction of addition of an amino acid, organic acid or methyl crown to the substrate, for e.g., in the microbial metabolism of sodium dimethly dithiocarbamate, the organism combines the fungicide with an amino acid molecule normally present in the cell and thereby inactivate the pesticides/chemical.

d) **Activation:** It is the conversion of non-toxic substrate into a toxic molecule, for eg. Herbicide, 4-butyric acid (2, 4-D B) and the insecticide Phorate are transformed and activated microbiologically in soil to give metabolites that are toxic to weeds and insects.

e) **Changing the spectrum of toxicity:** Some fungicides/ pesticides are designed to control one particular group of organisms / pests, but they are metabolized to yield products inhibitory to entirely dissimilar groups of organisms, for e.g. the fungicide PCNB fungicide is converted in soil to chlorinated benzoic acids that kill plants.

Biodegradation of pesticides / herbicides is greatly influenced by the soil factors like moisture, temperature, PH and organic matter content, in addition to microbial population and pesticide solubility. Optimum temperature, moisture and organic matter in soil provide congenial environment for the break down or retention of any pesticide added in the soil. Most of the organic pesticides degrade within a short period (3-6 months) under tropical conditions. Metabolic activities of bacteria, fungi and actinomycetes have the significant role in the degradation of pesticides.

4. **Criteria for Bioremediation / Biodegradation:** For successful biodegradation of pesticide in soil, following aspects must be taken into consideration. i) Organisms must have necessary catabolic activity required for degradation of contaminant at fast rate to bring down the concentration of contaminant, ii) the target contaminant must be bioavailability, iii) soil conditions must be congenial for microbial /plant growth and enzymatic activity and iv) cost of bioremediation must be less than other technologies of removal of contaminants.

According to Gales (1952) principal of microbial infallibility, for every naturally occurring organic compound there is a microbe / enzyme system capable its degradation.

5. **Strategies for Bioremediation:** For the successful biodegradation / bioremediation of a given contaminant following strategies are needed.

a) **Passive/ intrinsic Bioremediation:** It is the natural bioremediation of contaminant by tile indigenous microorganisms and the rate of degradation is very slow.

b) **Biostimulation:** Practice of addition of nitrogen and phosphorus to stimulate indigenous microorganisms in soil.

c) **Bioventing:** Process/way of Biostimulation by which gases stimulants like oxygen and methane are added or forced into soil to stimulate microbial activity.

d) **Bioaugmentation:** It is the inoculation/introduction of microorganisms in the contaminated site/soil to facilitate biodegradation.

e) **Composting:** Piles of contaminated soils are constructed and treated with aerobic thermophilic microorganisms to degrade contaminants. Periodic physical mixing and moistening of piles are done to promote microbial activity.

f) **Phytoremediation:** Can be achieved directly by planting plants which hyperaccumulate heavy metals or indirectly by plants stimulating microorganisms in the rhizosphere.

g) **Bioremediation:** Process of detoxification of toxic/ unwanted chemicals / contaminants in the soil and other environment by using microorganisms.

h) **Mineralization:** Complete conversion of an organic contaminant to its inorganic constituent by a species or group of microorganisms.

Alexis Carrel

Born 28 June 1873
Died 5 November 1944 (aged 71) Paris, France
Known for
New techniques in vascular sutures and pioneering work in transplantology and thoracic surgery
Profession
Surgeon, biologist
Institutions
University of Chicago
Rockefeller Institute for Medical Research.
Specialism transplantology, thoracic surgery
Notable prizes Nobel Prize in Physiology or Medicine (1912)

Alexis Carrel was an innovative surgeon whose experiments with the transplantation and repair of body organs led to advances in the field of surgery and the art of tissue culture. An original and creative thinker, Carrel was the first to develop a successful technique for suturing blood vessels together. For his work with blood-vessel suturing and the transplantation of organs in animals, he received the 1912 Nobel Prize in medicine and physiology. Carrel's work with tissue culture also contributed significantly to the understanding of viruses and the preparation of vaccines. A member of the Rockefeller Institute for Medical Research for thirty-three years, Carrel was the first scientist working in the United States to receive the Nobel Prize in medicine and physiology.

Carrel was born on June 28, 1873, in Sainte-Foy-les-Lyon, a suburb of Lyons, France. He was the oldest of three children, two boys and a girl, in a Roman Catholic family. His mother, Anne-Marie Ricard, was the daughter of a linen merchant. His father, Alexis Carrel Billiard, was a textile manufacturer. Carrel dropped his baptismal names, Marie Joseph Auguste, and became known as Alexis Carrel upon his father's death when the boy was five years old. As a child, Carrel attended Jesuit schools. Before studying medicine, he earned two baccalaureate degrees, one in letters (1889) and one in science (1890). In 1891, Carrel began medical studies at the University of Lyons. For the next nine years, Carrel gained both academic knowledge and practical experience working in local hospitals. He served one year as an army surgeon with the Alpine Chasseurs, France's mountain troops. He also studied under Leo Testut, a famous anatomist. As an apprentice in Testut's laboratory, Carrel showed great talent at dissection and surgery. In 1900, he received his medical degree but continued on at the University of Lyons teaching medicine and conducting experiments in the hope of eventually receiving a permanent faculty position there.

Early Success with Blood Vessel Sutures

In 1894, the president of France bled to death after being fatally wounded by an assassin in Lyons. If doctors had known how to repair his damaged artery, his life may have been saved, but such surgical repair of blood vessels had never been done successfully. It is said that this tragic event captured Carrel's attention and prompted him to try and find a way to sew severed blood vessels back together. Carrel first taught himself how to sew with a small needle and very fine silk thread. He practiced on paper until he was satisfied with his expertise, then developed steps to reduce the risk of infection and maintain the flow of blood through the repaired vessels. Through his careful choice of materials and long practice at various techniques, Carrel found a way to suture blood vessels. He first published a description of his success in a French medical journal in 1902.

Despite Carrel's growing reputation as a surgeon, he failed to acquire a faculty position at the university. His colleagues seemed indifferent to his research, and Carrel, in turn, was critical of the French medical establishment. The final split between Carrel and his peers came when Carrel wrote a positive account of a miracle he apparently witnessed at Lourdes, a small town famous since 1858 for its Roman Catholic shrine and often visited by religious pilgrims. In his article, Carrel suggested that there may be medical cures that cannot be explained by science alone, and that further investigation into supernatural phenomena such as miracles was required. This conclusion pleased neither the scientists nor the churchmen of the day.

In June, 1904, Carrel left France for the French-speaking city of Montreal, Canada; an encounter with French missionaries who had worked in Canada had sparked Carrel's interest in that country several years earlier. Shortly after his arrival, Carrel accepted an assistantship in physiology from the Hull Physiology Laboratory of the University of Chicago, where he remained from 1904 to 1906. The university provided him with an opportunity to continue the experiments he had begun in France.

Blood transfusion and organ transplantation seemed within reach to Carrel, now that he had mastered the ability to suture blood vessels. In experiments with dogs, he performed successful kidney transplants. His bold investigations began to attract attention not only from other medical scientists but from the public as well. His work was reviewed in both medical journals and popular newspapers such as the NEW YORK HERALD. In the era of Ford, Edison, and the Wright Brothers, the public was easily able to imagine how work in a scientific laboratory could lead to major changes in daily life. Human organ transplantation and other revolutions in surgery did not seem far off.

Begins Lifetime Career at Rockefeller Institute

In 1906, the opportunity to work in a world-class laboratory came to Carrel. The new Rockefeller Institute for Medical

Research (now named Rockefeller University) in New York City offered him a position. Devoted entirely to medical research, rather than teaching or patient care, the Rockefeller Institute was the first institution of its kind in the United States. Carrel would remain at the institute until 1939. At the Rockefeller Institute, Carrel continued to improve his methods of blood-vessel surgery. He knew that mastering those techniques would allow for great advances in the treatment of disorders of the circulatory system and wounds. It also made direct blood transfusions possible at a time when scientists did not know how to prevent blood from clotting. Without this knowledge, blood could not be stored or transported. In the JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION in 1910, Carrel described connecting an artery from the arm of a father to the leg of an infant in order to treat the infant's intestinal bleeding. Although the experiment was a success, the discovery of anticoagulants soon made such direct transfer unnecessary. For his pioneering efforts, Carrel won the Nobel Prize in 1912.

Carrel's success with tissue cultures through animal experiments led him to wonder whether human tissues and even whole organs, might be kept alive artificially in the laboratory. If so, lab-raised organs might eventually be used as substitutes for diseased parts of the body. The art of keeping cells and tissue alive and even growing, outside of the body is known as tissue culture. Successfully culturing tissue requires great technical skill. Carrel was particularly interested in perfusion -- a procedure of artificially pumping blood through an organ to keep it viable. Carrel's work with tissue culture contributed greatly to the understanding of normal and abnormal cell life. His techniques helped lay the groundwork for the study of viruses and the preparation of vaccines for polio, measles, and other diseases. Carrel's discoveries, in turn, built upon the successes of, among others, Ross G. Harrison, a contemporary anatomist at Yale who worked with frog tissue cultures and transplants.

One of Carrel's experiments in tissue culture became the subject of a sensationalized news story and was viewed as a monstrosity by the public. In 1912, Carrel took tissue from the heart of a chicken embryo to demonstrate that warm-blooded cells could be kept alive in the lab. This tissue, which was inaccurately depicted as a growing, throbbing chicken heart by some newspapers, was kept alive for thirty-four years -- outliving Carrel himself -- before it was deliberately terminated. The WORLD TELEGRAM, a New York newspaper, annually marked the so-called chicken heart's "birthday" each January.

Though working in the United States, Carrel had not bought a house there, and did not become a U.S. citizen. Rather, he spent each summer in France, and on December 26, 1913, Carrel married Anne-Marie Laure (Gourlez de la Motte) de Meyrie, a widow with one son, in a ceremony in Brittany. They had met at Lourdes, where Carrel made an annual pilgrimage each August. Eventually, the couple bought some property on the island of Saint Gildas off the coast of Brittany, and lived in a stone house there. They had no children together.

When World War I began, Carrel was in France. The French government called him to service with the army, assigning him to run a special hospital near the front lines for the study and prompt treatment of severely infected wounds. There, Madame Carrel, his wife of less than one year and a trained surgical nurse, assisted him. In collaboration with biochemist Henry D. Dakin, Carrel developed an elaborate method of cleansing deep wounds to prevent infection. The method was especially effective in preventing gangrene, and was credited with saving thousands of lives and limbs. The Carrel-Dakin method, however, was too complicated for widespread use, and has since been replaced by the use of antibiotic drugs.

After an honorable discharge in 1919, Carrel returned to the Rockefeller Institute in New York City. He resumed his work in tissue culture, and began an investigation into the causes of cancer. In one experiment, he built a huge mouse colony to test his theories about the relationship between nutrition and cancer. But the experiment produced inconclusive results, and the Institute ceased funding it after 1933. Nevertheless, Carrel's tissue culture research was successful enough to earn him the Nordhoff-Jung Cancer Prize in 1931 for his contribution to the study of malignant tumors.

Artificial Heart Collaboration with Charles A. Lindbergh

In the early 1930s, Carrel returned again to the challenge of keeping organs alive outside the body. With the engineering expertise of aviator Charles A. Lindbergh, Carrel designed a special sterilizing glass pump that could be used to circulate nutrient fluid around large organs kept in the lab. This perfusion pump, a so-called artificial heart, was germ-free and was successful in keeping animal organs alive for several days or weeks, but this was not considered long enough for practical application in surgery. Still, the experiment laid the groundwork for future developments in heart-lung machines and other devices. To describe the use of the perfusion pump, Carrel and Lindbergh jointly published THE CULTURE OF ORGANS in 1938. Lindbergh was a frequent sight at the Rockefeller Institute for several years, and the Lindberghs and the Carrels became close friends socially. They appear together on the July 1, 1935, cover of TIME magazine with their "mechanical heart."

Carrel's mystical bent, publicly revealed after his visit to Lourdes as a young man, was displayed again in 1935. That year Carrel published MAN, THE UNKNOWN, a work written upon the recommendation of a loose-knit group of intellectuals that he often dined with at the Century Club. In MAN, THE UNKNOWN, Carrel posed highly philosophical questions about mankind, and theorized that

mankind could reach perfection through selective reproduction and the leadership of an intellectual aristocracy. The book, a worldwide best-seller and translated into nineteen languages, brought Carrel international attention. Carrel's speculations about the need for a council of superior individuals to guide the future of mankind was seen by many as anti-democratic. Others thought that it was inappropriate for a renowned scientist to lecture on fields outside his own.

Unfortunately, one of those who disliked Carrel's habit of discussing issues outside the realm of medicine was the new director of the Rockefeller Institute. Herbert S. Gasser had replaced Carrel's friend and mentor, Simon Flexner, in 1935. Suddenly Carrel found himself approaching the mandatory age of retirement with a director who had no desire to bend the rules and keep him aboard. On July 1, 1939, Carrel retired. His laboratories and the Division of Experimental Surgery were closed.

Carrel's retirement coincided with the beginning of World War II in September, 1939. Carrel and his wife were in France at the time and Carrel immediately approached the French Ministry of Public Health and offered to organize a field laboratory, much like the one he had run during World War I. When the government was slow to respond, Carrel grew frustrated. In May, 1940, he returned to New York alone. As his steamship was crossing the Atlantic, Hitler invaded France.

Creates New Scientific Institute in Occupied Paris

Carrel made the difficult return to war-torn Europe as soon as he was able, arriving in France via Spain in February, 1941. Paris was under the control of the Vichy government, a puppet administration installed by the German military command. Although Carrel declined to serve as director of public health in the Vichy government, he stayed in Paris to direct the Foundation for the Study of Human Problems. The Foundation, supported by the Vichy government and the German military command, brought young scientists, physicians, lawyers, and engineers together to study economics, political science, and nutrition. When the Allied forces reoccupied France in August, 1944, the newly restored French government immediately suspended Carrel from his directorship of the Foundation and accused him of collaborating with the Germans. Mercifully, perhaps, a serious heart attack forestalled any further prosecution. Attended by French and American physicians, and nursed by his wife, Carrel died of heart failure in Paris on November 5, 1944. After his death, his body was buried in St. Yves chapel near his home on the island of Saint Gildas, Cotes-du-Nord.

Carrel's reputation remains that of a brilliant, yet temperamental man. His motivations for his involvement with the Nazi-dominated Vichy government remain the subject of debate. Yet there is no question that his achievements ushered in a new era in medical science. His pioneering techniques paved the way for successful organ transplants and modern heart surgery, including grafting procedures and bypasses.

JOKES

Wife : Ye aap ki shirt per lipstick ka nishan kahan se aaya?
Husband : Mein khud paresaan hu nishan dekhkar. Maine tu uss wakt shirt utari hui thi.

New way of writting answers in exams.
If you don't know the answer, then put lines like this :
||||||||||
and write below :
"Scratch here for ANSWERS"

1 boy on his way 2 home with his mom after school, saw a couple kissing on the road…
He suddenly shouted and said look mom they are fighting for CHEWING GUM.

Husband and Wife had a Fight.
Wife called Mom : He fought with me again, I am coming to you.
Mom : No beta, he must pay for his mistake, I am comming to stay with U!

Daughter: Mom aaj Ek ladke ne mere gal pe kiss kiya.
Mom: Tune usko chata mara ya nahi. . . . . .
Daughter: Mujhe achanak Gandhiji yaad agaye aur maine dusra gal agge kar diya.....

Boy: i love u...
Girl: sorry but I love sum1 else...
Boy: ok your happiness matters me more than ur love... .
Moral: moral voral kuch nahi jaha apni bezzati ho rahi ho waha acha dialogue maar

Dog was Chasing Sardar.. Sardar runs, but Laughing.. A Man asked y r u Laughing? Sardar replied I have put Airtel Sim, but the Hutch network is Following...

Student in a Hostel(dost se): Yaar dhokha ho gaya.
Dost: Kyon kya hua?
Student: Maine papa se books ke liye paise mangawaye, papa ne books hi bhej di.

1. Which of the following articles can be sterilized in an autoclave?
   A. Gloves B. Culture media
   C. Dressing material D. All of these

2. Which of the following disinfectants act by disrupting microbial membranes?
   A. Cationic detergents B. Halogens
   C. Heavy metals D. Aldehydes

3. Which of the following is best to sterilize heat labile solutions?
   A. Dry heat B. Autoclave
   C. Membrane filtration D. Pasteurization

4. The time required to kill 90% of the microorganisms in a sample at a specific temperature is the
   A. decimal reduction time B. thermal death point
   C. F value D. D value

5. Which of the following is best used for long term storage of microbial samples when carried out properly?
   A. Storage in a freezer at -10°C
   B. Storage in a freezer at ultra low temperatures (-70°C)
   C. Storage in a refrigerator on an agar slant
   D. Storage on a petri plate at room temperature

6. Which of the following is not a disinfectant containing a heavy metal?
   A. Silver nitrate B. Mercurochrome
   C. Copper sulfate D. Chlorine

7. Which is the most important surface active disinfectants?
   A. Amphoteric compounds B. Cationic compounds
   C. Non-ionic compounds D. Anionic compounds

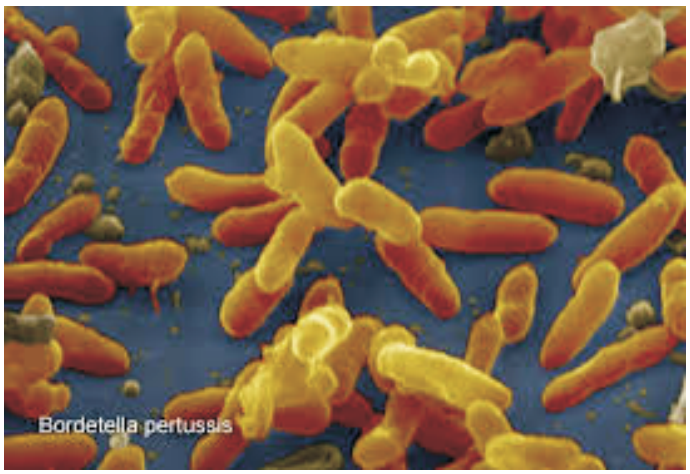
8. Which disinfectant would be most effective against *Staphylococcus* found in a blood spill?
   A. Phenol B. Cetylpyridinium chloride
   C. Hexachlorophene D. None of these

9. Milk is pasteurized in batch method by keeping it at
   A. 63°C for 30 minutes B. 72°C for 60 seconds
   C. 73°C for 30 minutes D. 72°C for 6 minutes

10. Preservation of foods by using salts and sugars works by
    A. raising pH
    B. lowering osmotic pressure
    C. creating a hypertonic environment
    D. creating a hypotonic environment

Answers: 1.D, 2.A, 3.C, 4.D, 5.B, 6.D, 7.B, 8.D, 9.A, 10.C.

Bordetella pertussis



Whooping cough (**pertussis**) is caused by the bacterium ***Bordetella pertussis***. *B. pertussis* is a very small Gram-negative aerobic coccobacillus that appears singly or in pairs. Its metabolism is respiratory, never fermentative, and taxonomically, *Bordetella* is placed among the "Gram-negative Aerobic Rods and Cocci" in Bergey's Manual. *Bordetella* is not assigned to any family. The bacteria are nutritionally fastidious and are usually cultivated on rich media supplemented with blood. They can be grown in synthetic medium, however, which contains buffer, salts, an amino acid energy source, and growth factors such as nicotinamide (for which there is a strict requirement). Even on blood agar the organism grows slowly and requires 3-6 days to form pinpoint colonies.

Bordetella pertussis colonizes the cilia of the mammalian respiratory epithelium (Figure 1). Generally, it is thought that *B. pertussis* does not invade the tissues, but some recent work has shown the bacterium in alveolar macrophages. The bacterium is a pathogen for humans and possibly for higher primates, and no other reservoir is known. Whooping cough is a relatively mild disease in adults but has a significant mortality rate in infants. Until immunization was introduced in the 1930s, whooping cough was one of the most frequent and severe diseases of infants in the United States.

Pathogenesis

The disease pertussis has two stages. The first stage, colonization, is an upper respiratory disease with fever, malaise and coughing, which increases in intensity over about a 10-day period. During this stage the organism can be recovered in large numbers from pharyngeal cultures, and the severity and duration of the disease can be reduced by antimicrobial treatment. Adherence mechanisms of *B. pertussis* involve a "**filamentous hemagglutinin**" (FHA), which is a fimbrial-like structure on the bacterial surface, and **cell-bound pertussis toxin** (**PTx**). Short range effects of soluble toxins play a role as well in invasion during the colonization stage.

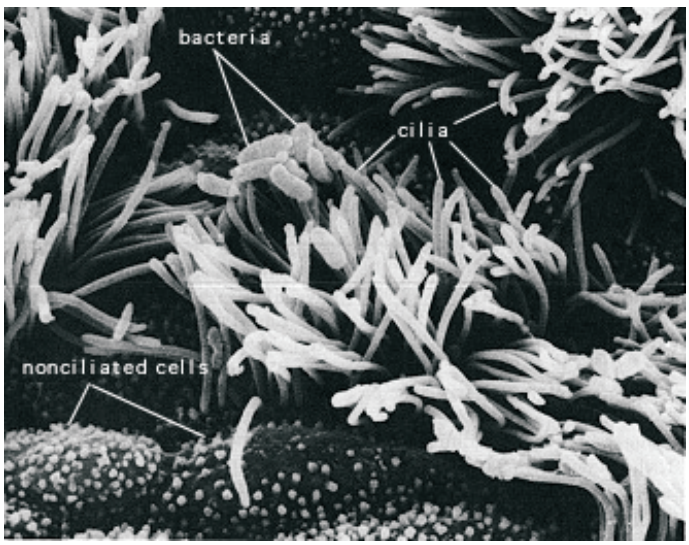


Figure 1. Colonization of tracheal epithelial cells by *Bordetella pertussis*

The second or toxemic stage of pertussis follows relatively nonspecific symptoms of the colonizaton stage. It begins gradually with prolonged and paroxysmal coughing that often ends in a characteristic inspiratory gasp (whoop). During the second stage, *B. pertussis* can rarely be recovered, and antimicrobial agents have no effect on the progress of the disease. As described below, this stage is mediated by a variety of soluble toxins.

Colonization

Studies of *B. pertussis* and its adhesins have focused on cultured mammalian cells that lack most of the features of ciliated epithelial cells. However, some generalities have been drawn. The two most important colonization factors are the filamentous hemagglutinin (FHA) and the pertussis toxin (PTx). Filamentous hemagglutinin is a large (220 kDa) protein that forms filamentous structures on the cell surface. FHA binds to galactose residues on a sulfated glycolipid called sulfatide which is very common on the surface of ciliated cells. Mutations in the FHA structural gene reduce the ability of the organism to colonize, and antibodies against FHA provide protection against infection. However, it is unlikely that FHA is the only adhesin involved in colonization. The structural gene for FHA has been cloned and expressed in *E. coli*, raising the possibility of its production for use in a component vaccine.

One of the toxins of *B. pertussis*, the pertussis toxin (PTx), is also involved in adherence to the tracheal epithelium.

Pertussis toxin is a 105 kDa protein composed of six subunits: S1, S2, S3, (2)S4, and S5. The toxin is both secreted into the extracellular fluid and cell bound. Some components of the cell-bound toxin (S2 and S3) function as adhesins, and appear to bind the bacteria to host cells. S2 and S3 utilize different receptors on host cells. S2 binds specifically to a glycolipid called lactosylceramide, which is found primarily on the ciliated epithelial cells. S3 binds to a glycoprotein found mainly on phagocytic cells.

The S1 subunit of pertussis toxin is the A component with ADP ribosylating activity, and the function of S2 and S3 is presumed to be involved in binding the intact (extracellular) toxin to its target cell surface. Antibodies against PTx components prevent colonization of ciliated cells by the bacteria and provide effective protection against infection. Thus, pertussis toxin is clearly an important virulence factor in the initial colonization stage of the infection.

Since the S3 subunit of pertussis toxin is able to bind to the surface of phagocytes, and since FHA will attach to integrin CR3 on phagocyte surfaces (the receptor for complement C3b), it has been speculated that the bacterium might bind preferentially to phagocytes in order to facilitate its own engulfment. The role of such self-initiated phagocytosis is not clear. Bacteria taken up by this abnormal route may avoid stimulating the oxidative burst that normally accompanies phagocytic uptake of bacterial cells which are opsonized by antibodies or complement C3b. Once inside of cells the bacteria might utilize other toxins (i.e. adenylate cyclase toxin) to compromise the bactericidal activities of phagocytes. In any case, there is some evidence that *Bordetella pertussis* can use this mechanism to get into and to persist in phagocytes as an intracellular parasite. If *B. pertussis* is an intracellular parasite it would explain why immunity to pertussis correlates better with the presence of specific cytotoxic T cells than it does with the presence of antibodies to bacterial products.

B. pertussis produces at least two other types of adhesins, two types of fimbriae and a nonfimbrial surface protein called pertactin, but their role in adherence and pathogenesis is not well established.

Toxins Produced by *B. pertussis*

B. pertussis produces a variety of substances with toxic activity in the class of exotoxins and endotoxins.

It secretes its own **invasive adenylate cyclase** which enters mammalian cells (*Bacillus anthracis* produces a similar enzyme, EF). This toxin acts locally to reduce phagocytic activity and probably helps the organism initiate infection. This toxin is a 45 kDa protein that may be cell-associated or released into the environment. Mutants of *B. pertussis* in the adenylate cyclase gene have reduced virulence in mouse models. The organisms can still colonize but cannot produce the lethal disease. The adenylate cyclase toxin is a single polypeptide with an enzymatic domain (i.e., adenylate cyclase activity) and a binding domain that will attach to host cell surfaces. The adenylate cyclase was originally identified as a hemolysin because it will lyse red blood cells. In fact, it is responsible for hemolytic zones around colonies of *Bordetella pertussis* growing on blood agar. Probably it inserts into the erythrocyte membrane which causes hemolysis. An interesting feature of the adenylate cyclase toxin is that it is active only in the presence of a eukaryotic regulatory molecule called calmodulin, which up-regulates the activity of the eukaryotic adenylate cyclase. The adenylate cyclase toxin is only active in the eukaryotic cell since no similar regulatory molecule exists in procaryotes. Thus, the molecule seems to have evolved specifically to parasitize eukaryotic cells. Anthrax EF (edema factor) is also a calmodulin-dependent adenylate cyclase.

It produces a highly **lethal toxin** (formerly called dermonecrotic toxin) which causes inflammation and local necrosis adjacent to sites where *B. pertussis* is located. The lethal toxin is a 102 kDa protein composed of four subunits, two with a mw of 24kDa and two with mw of 30 kDa. It causes necrotic skin lesions when low doses are injected subcutaneosly in mice and is lethal in high doses. The role of the toxin in whooping cough is not known.

It produces a substance called the **tracheal cytotoxin** which is toxic for ciliated respiratory epithelium and which will stop the ciliated cells from beating. This substance is not a classic bacterial exotoxin since it is not composed of protein. The tracheal cytotoxin is a peptidoglycan fragment, which appears in the extracellular fluid where the bacteria are actively growing. The toxin kills ciliated cells and causes their extrusion from the mucosa. It also stimulates release of cytokine IL-1, and so causes fever.

It produces the **pertussis toxin**, **PTx**, a protein that mediates both the colonization and toxemic stages of the disease. PTx is a two component, A+B bacterial exotoxin. The A subunit (S1) is an ADP ribosyl transferase. The B component, composed of five polypeptide subunits (S2 through S5), binds to specific carbohydrates on cell surfaces. The role of PTx in invasion has already been discussed. PTx is transported from the site of growth of the Bordetella to various susceptible cells and tissues of the host. Following binding of the B component to host cells, the A subunit is inserted through the membrane and released into the cytoplasm in a mechanism of direct entry. The A subunit gains enzymatic activity and transfers the ADP ribosyl moiety of NAD to the membrane-bound regulatory protein Gi that normally inhibits the eukaryotic

adenylate cyclase. The Gi protein is inactivated and cannot perform its normal function to inhibit adenylate cyclase. The conversion of ATP to cyclic AMP cannot be stopped and intracellular levels of cAMP increase. This has the effect to disrupt cellular function, and in the case of phagocytes, to decrease their phagocytic activities such as chemotaxis, engulfment, the oxidative burst, and bacteridcidal killing. Systemic effects of the toxin include lymphocytosis and alteration of hormonal activities that are regulated by cAMP, such as increased insulin production (resulting in hypoglycemia) and increased sensitivity to histamine (resulting in increased capillary permeability, hypotension and shock). PTx also affects the immune system in experimental animals. B cells and T cells that leave the lymphatics show an inability to return. This alters both AMI and CMI responses and may explain the high freqency of secondary infections that accompany pertussis (the most frequent secondary infections during whooping cough are pneumomia and otitis media).

Although the effects of the pertussis toxin are dependent on ADP ribosylation, it has been shown that mere binding of the B oligomer can elicit a response on the cell surface such as lymphocyte mitogenicity, platelet activation, and production of insulin effects.

The pertussis toxin gene has been cloned and sequenced and the subunits expressed in *E. coli*. The toxin can be inactivated and converted to toxoid for use in component vaccines.

Comparison between cholera toxin and pertussis toxin (ptx) in their ability to interfere with the regulation of the eucaryotic adenylate cyclase complex.

Normal regulation of adenylate cyclase activity in mammalian cells. Adenylate cyclase (AC) is activated normally by a stimulatory regulatory protein (Gs) and guanosine triphosphate (GTP); however the activation is normally brief because an inhibitory regulatory protein (Gi) hydrolyzes the GTP.

Adenylate cyclase activated by cholera toxin The cholera toxin A1 fragment catalyzes the attachment of ADP-Ribose (ADPR to the regulatory protein Gs, forming Gs-ADPR from which GTP cannot be hydrolyzed. Since GTP hydrolysis is the event that inactivates adenylate cyclase (AC), the enzyme remains continually activated.

Adenylate cyclase activated by pertussis toxin (The pertussis A subunit transfers the ADP ribosyl moiety of NAD to the membrane-bound regulatory protein Gi that normally inhibits the eukaryotic adenylate cyclase. The Gi protein is inactivated and cannot perform its normal function to inhibit adenylate cyclase. The conversion of ATP to cyclic AMP cannot be stopped.

Lipopolysaccharide. As a Gram-negative bacterium *Bordetella pertussis* possesses lipopolysaccharide (endotoxin) in its outer membrane, but its LPS is unusual. It is heterogeneous, with two major forms differing in the phosphate content of the lipid moiety. The alternative form of Lipid A is designated Lipid X. The unfractionated material elicits the usual effects of LPS (i.e., induction of IL-1, activation of complement, fever, hypotension, etc.), but the distribution of those activities is different in the two forms of LPS. For example, Lipid X, but not Lipid A, is pyrogenic, and its O-side chain is a very powerful immune adjuvant. Furthermore, Bordetella LPS is more potent in the limulus assay than LPS from other Gram-negative bacteria, so it is not reliable to apply knowledge of the biological activity of LPS in the *Enterobacteriaceae* to the LPS of *Bordetella*. The role of this unusual LPS in the pathogenesis of whooping cough has not been investigated.

Regulation of Virulence Factors in *B. pertussis*

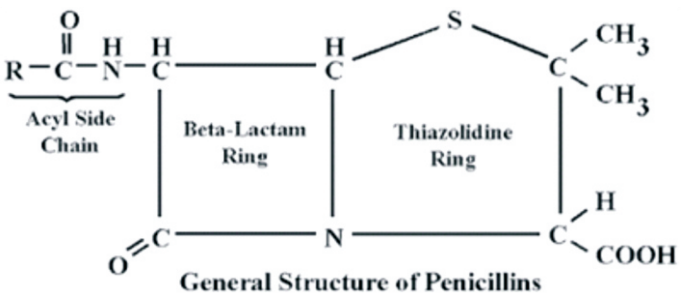
The production of virulence factors in *B. pertussis* is regulated in several different ways. Expression of virulence factors is regulated by the bvg operon.

First, the organisms undergo an event called phase variation resulting in the loss of most virulence factors and some undefined outer membrane proteins. Phase variation has been shown to occur at a genetic frequency of 10-4 - 10-6 generations and results from a specific DNA frame shift that comes about after the insertion of a single nucleotide into the bvg (also known as vir) operon.

A similar process called phenotypic modulation, occurs in response to environmental signals such as temperature or chemical content, and is reversible. This is an adaptive process mediated by the products of the bvg operon, and is an example of a two-component environmental-sensing (regulatory) system used by other bacteria. The expression of these regulatory proteins is itself regulated by environmental signals, such that entry into a host might induce components required for survival and production of disease.

Penicillin Production

Penicillin was the first naturally occurring antibiotic discovered. It is obtained in a number of forms from Penicillium moulds. Penicillin is not a single compound but a group of closely related compounds, all with the same basic ring-like structure (a β-lactam) derived from two amino acids (valine and cysteine) via a tripeptide intermediate. The third amino acid of this tripeptide is replaced by an acyl group (R) and the nature of this acyl group produces specific properties on different types of penicillin.



General Structure of Penicillins

There are two different types of penicillin.

Biosynthetic penicillin is natural penicillin that is harvested from the mould itself through fermentation.

Semi-synthetic penicillin includes semi synthetic derivatives of penicillin - like Ampicillin, Penicillin V, Carbenicillin, Oxacillin, Methicillin, etc. These compounds consist of the basic Penicillin structure, but have been purposefully modified chemically by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that produce new properties.

These modern semi-synthetic penicillins have various specific properties such as resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (or β-lactamase) (a penicillin-destroying enzyme produced by some bacteria) and an extended range of activity against some Gram-negative bacteria. Penicillin G is the most widely used form and the same one we get in a hypodermic form.

PENICILLIN G

Penicillin G is not stable in the presence of acid (acid-labile). Since our stomach has a lot of hydrochloric acid in it (pH2.0), if we were to ingest penicillin G, the compound would be destroyed in our stomach before it could be absorbed into the bloodstream, and would therefore not be any good to us as a treatment for infection somewhere in our body. It is for this reason that penicillin G must be taken by intramuscular injection - to get the compound in our bloodstream, which is not acidic at all. Many of the semi-synthetic penicillins can be taken orally.

Penicillium chrysogenum that produce antibiotics, enzymes or other **secondary metabolites** frequently require precursors like purine/pyrimidine bases or organic acids to produce said metabolites. Primary metabolism is the metabolism of energy production for the cell and for its own biosynthesis. Typically, in aerobic organisms (*Penicillium chrysogenum*) it involves the conversion of sugars such as glucose to pyruvic acid2 and the production of energy via the TCA cycle. Secondary metabolism regards the production of metabolites that are not used in energy production for example penicillin from *Penicillium chrysogenum*. In this case the metabolite is being utilized as a defence mechanism against other microorganisms in the environment. In essence *Penicillium chrysogenum* can kill off the competition to allow itself to propagate efficiently. It should be noted that these secondary metabolites are only produced in times of stress when resources are low and the organism must produce these compounds to kill off its competitors to allow it to survive.

MEDIA FORMULATION:

Lactose	: 1%
Calcium Carbonate	: 1%
Cornsteep Liquor	: 8.5%
Glucose	: 1%
Phenyl acetic acid	: 0.5g
Sodium hydrogen phosphate	: 0.4%
Antifoaming Agent	: Vegetable oil

FERMENTATION

To begin the fermentation process, a number of these spores will be introduced into a small (normally 250-500ml) conical flask where it will be incubated for several days. At this stage, explosive growth is the most desired parameter and as such the medium in the flask will contain high amounts of easily utilisable carbon and nitrogen sources, such as starch and corn-steep liquor. At this stage, the spores will begin to revive and form vegetative cells. Temperature is normally maintained at 23-28^{0}C and pH at ~6.5, although there may be some changes made to facilitate optimum growth. The flask will often have baffles in it and be on a shaking apparatus to improve oxygen diffusion in the flask.

Once the overall conditions for growth have been established and there is a viable vegetative culture active inside the flask, it will be transferred to a 1 or 2 litre *bench-top reactor*. This reactor will be fitted with a number of instruments to allow the culture to be better observed than it was in the shake flask. Typical parameters observed include

pH, temperature, and stirrer speed and dissolved oxygen concentration. This allows tweaking of the process to occur and difficulties to be examined. For example, there may not be enough oxygen getting to the culture and hence it will be oxygen starved. At this point, the cells should be showing filamentous morphology, as this is preferred for penicillin production. As before, cell growth is priority at this stage. At this stage, growth will continue as before, however, there are often sudden changes or loss in performance. This can be due to changes in the morphology of the culture (*Penicillium chrysogenum* is a filamentous fungi and hence pseudoplastic) that may or may not be correctable.

At this stage the medium being added to the reactor will change. Carbon and nitrogen will be added sparingly alongside precursor molecules for penicillin fed-batch style. Another note is that the presence of penicillin in the reactor is itself inhibitory to the production of penicillin. Therefore, we must have an efficient method for the removal of this product and to maintain constant volume in the reactor. Other systems, such as cooling water supply, must also be considered. If all goes well we should have penicillin ready for downstream processing. From here it can be refined and packaged for marketing and distribution to a global market.

References:

1. Hare, T; White, L / penicillin production
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Microbiology lab: Health & Safety

A microbiology laboratory is a unique environment that requires special practices and containment facilities in order to properly protect persons working with microorganisms. Safety in the laboratory is the primary concern. The three main elements of safe containment of microorganisms are (1) good laboratory practices and technique, (2) safety equipment, and (3) facility design.

There is a certain element of risk in anything you do, but the potential risks in a microbiology course are greater. Persons who work in a microbiology lab may handle infectious agents in additional to other hazards such as chemicals and radioactive materials. There have been many documented cases of lab personnel acquiring diseases due to their work. About 20% of these cases have been attributed to a specific incident, while the rest have been attributed to work practices in the lab. It is possible that you can be exposed to potentially harmful microbes when you isolate bacteria from environmental materials. So, you should consider environmental samples potentially hazardous and use BSL2 containment practices (see below). If you are immunocompromised or immunosuppressed, then you may be at greater risk of acquiring infections in this class than most students and should carefully consider whether you should enroll in this course.

Microbiology Lab Practices and Safety Rules

1. Wash your hands with disinfectant soap when you arrive at the lab and again before you leave.
2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers. Do not store food in areas where microorganisms are stored.
3. Purchase a lab coat and safety glasses, bring them to class, and use them. Alternatively, a long sleeved shirt that buttons or snaps closed is acceptable protective clothing. This garment must cover your arms and be able to be removed without pulling it over your head. Leave protective clothing in the lab and do not wear it to other non-lab areas.
4. Avoid loose fitting items of clothing. Wear appropriate shoes (sandals are not allowed) in the laboratory.
5. Keep your workspace free of all unnecessary materials. Backpacks, purses, and coats should be placed in the cubbyholes by the front door of the lab. Place needed items on the floor near your feet, but not in the aisle.
6. Disinfect work areas before and after use with 70% ethanol or fresh 10% bleach. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination.
7. Label everything clearly.
8. Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes in the lab unless absolutely necessary.
9. Inoculating loops and needles should be flame sterilized in a Bunsen burner before you lay them down.
10. Turn off Bunsen burners when not is use. Long hair must be restrained if Bunsen burners are in use.
11. When you flame sterilize with alcohol, be sure that you do not have any papers under you.
12. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.
13. Wear disposable gloves when working with potentially infectious microbes or samples (e.g., sewage). If you are working with a sample that may contain a pathogen, then be extremely careful to use good bacteriological technique.
14. Sterilize equipment and materials.
15. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors. [In the distant past, some lab personnel were taught to mouth pipette. This practice has been known to result in many laboratory-acquired infections. With the availability of mechanical pipetting devices, mouth pipetting is strictly prohibited.]
16. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.

17. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.
18. Familiarize yourself with the location of safety equipment in the lab (e.g., eye-wash station, shower, sinks, fire extinguisher, biological safety cabinet, first aid kit, emergency gas valve).
19. Dispose of broken glass in the broken glass container.
20. Dispose of razor blades, syringe needles, and sharp metal objects in the "sharps" container.
21. Report spills and accidents immediately to your instructor. Clean small spills with care (see instructions below). Seek help for large spills.
22. Report all injuries or accidents immediately to the instructor, no matter how small they seem.

Laboratory Safety Equipment

Biological Safety Cabinet

A biological safety cabinet (BSC) is used as a primary barrier against exposure to infectious biological agents. A BSC has High Efficiency Particulate Air (HEPA) filters. The airflow in a BSC is laminar, i.e. the air moves with uniform velocity in one direction along parallel flow lines.

Depending on the design, a BSC may be vented to the outside or the air may be exhausted into the room. BSCs are not chemical fume hoods. A percentage of the air is recirculated in most types of BSCs. HEPA filters only trap particulates, allowing any contaminant in non-particulate form to pass through the filter.

Proper Use of BSCs:

1. Operate the cabinet for five minutes before and after performing any work in it in order to purge airborne contaminants.
2. Before and after use, wipe the surface of the BSC with a suitable disinfectant, e.g., 70% alcohol or a recommended disinfection solution.
3. Place everything you will need inside the cabinet before beginning work, including a waste container. You should not have to penetrate the air barrier of the cabinet once work has begun.
4. Do not place anything on the air intake grills, as this will block the air supply.
5. You should prevent unnecessary opening and closing of door because this will disrupt the airflow of the cabinet.
6. Always wear a lab coat while using the cabinet and conduct your work at least four inches inside the cabinet.
7. Place burners to the rear of the cabinet to reduce air turbulence.
8. Do not work in the BSC while the ultraviolet light is on. Ultraviolet light can quickly injure the eye.
9. When finished with your work procedure, decontaminate the surfaces of any equipment.
10. Remove the equipment from the cabinet and decontaminate the work surface.
11. Thoroughly wash your hands and arms.

Eyewash and shower

Fire Extinguisher

First Aid Kit

Emergency Gas Valve

Cleaning Small Spills

First, contact your instructor or the Biology Department Safety Officer. If it is a small spill of a low hazard microorganism or sample, then you should clean the spill yourself.

The proper procedures for cleaning small spills of microorganisms or samples (BSL1 and BSL2 levels):

1. Wear a lab coat, disposable gloves, safety glasses or a face shield, and if needed, approved respiratory equipment.
2. Soak a paper towel(s) in an appropriate disinfectant (70% ethanol or a recommended disinfection solution) and place around the spill area.
3. Working from the outer edges into the centre, clean the spill area with fresh towels soaked in the disinfectant. Be sure to decontaminate any areas or surfaces that you suspect may have been affected by the spill. Allow 10 minutes contact time.
4. Place the paper towels and gloves into a biohazard bag and autoclave these materials to sterilize them.
5. Dispose of any contaminated clothing properly.
6. Wash your hands with a disinfectant soap.

Each lab is equipped with a spill response kit.

Biosafety Levels and Practices

Biosafety levels are selected to provide the end-user with a description of the minimum containment required for handling different microorganisms safely in a laboratory setting and reduce or eliminate exposure to potentially hazardous agents. Containment refers to safe methods for managing infectious material in the laboratory environment. These biosafety levels are applicable to facilities such as diagnostic, research, clinical, teaching, and production facilities that are working at a laboratory scale. The four biosafety levels are described as:

Biosafety Level 1 (BSL1)

Examples of BSL1 Agents: *Bacillus subtilus*, *Naegleria*

gruberi, many *Escherichia coli*, *Infectious Canine Hepatitis Virus*

BSL1 containment is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.

A BSL1 lab requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSCs) are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

Biosafety Level 2 (BSL2)

Examples of BSL2 Agents: *Bacillus anthracis*, *Bordetella pertussis, Brucella* spp., *Cryptococcus*

neoformans, *Clostridium botulinum, Clostridium tetani, Helicobacter pylori,* most *Salmonella* spp.,

Yersinia pestis, Mycobacterium leprae, *Shigella* spp., *Human Immunodeficiency Virus*, *Human blood*

The primary exposure hazards associated with organisms requiring BSL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring BSL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes. Primary containment devices such as BSCs and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of hand washing sinks and decontamination facilities (autoclaves).

Biosafety Level 3 (BSL3)

Examples of BSL3 Agents: *Myobacterium tuberculosis, Salmonella typhi, Vesicular Stomatitis Virus*, *Yellow Fever Virus*, *Francisella tularensis*, *Coxiella burnetti*

Laboratory personnel have specific training in handling these pathogenic and potentially lethal agents and are supervised by scientists who are experienced in working with these agents. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life-threatening disease. BSL3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. Additional features to prevent transmission of BSL3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

Biosafety Level 4 (BSL4)

Examples of BSL4 Agents: smallpox virus, Ebola virus, hemorrhagic fever viruses

This is the maximum containment available and is suitable for facilities manipulating agents that are dangerous/exotic agents, which post a risk of life threatening disease. These agents have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment represents an isolated unit, functionally and, when necessary, structurally independent of other areas.

BSL4 emphasizes maximum containment of the infectious agent by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.

TO BE CONTINUED...

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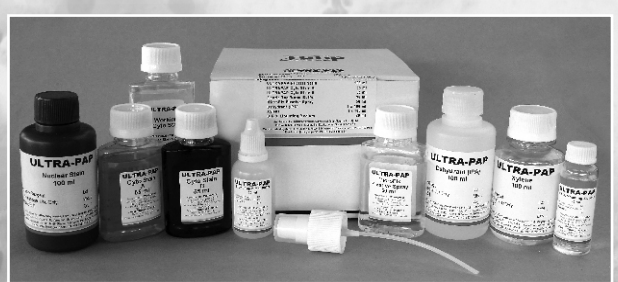

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