JOURNAL OF
HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
VOLUME - VIII ISSUE - VI FEB-MAR 2016

# Editorial

## Contents



Here's another issue of JHS loaded with lot of valuable information, kindly flip a few pages to believe us……

**Mini Review Section** - Pure culture in microbiology, a laboratory culture containing a single species of organism. Pure culture involves not only isolation of individual microorganisms from a mixed population, but also the maintenance of such individuals and their progenies in artificial media, where no other microorganisms find way to grow.

**Current Trends Section -** The air catering business is very different from normal hotel operations. It lays a lot of emphasis on quality and hygiene, besides the taste of the food. Providers who produce meals for third parties bear a great deal of responsibility. Meals must not pose a risk to health and must be of flawless quality. To achieve this goal, it is important that the entire kitchen team pays attention to cleanliness and hygiene during their day-to-day work in the kitchen. This applies to personal and hand hygiene, correct handling of food and cleanliness in the kitchen and in the entire company.

**In Profile - Karl Landsteiner**, was an Austrian and American biologist and physician. He is noted for having first distinguished the main blood groups in 1900 (however, Jan Janský described and distinguished main blood groups before him), having developed the modern system of classification of blood groups from his identification of the presence ofagglutinins in the blood, and having identified, with Alexander S. Wiener, the Rhesus factor, in 1937, thus enabling physicians to transfuse blood without endangering the patient′s life. With Constantin Levaditi and Erwin Popper, he discovered the polio virus in 1909. He received the Aronson Prize in 1926. In 1930 he received the Nobel Prize in Physiology or Medicine. He was awarded a Lasker Award in 1946 posthumously and is recognized as the father of transfusion medicine.

**Bug of the Month -** *Klebsiella oxytoca* is a gram-negative bacterium with a cylindrical rod shape measuring 2 µm by 5µm. In the 1950's the strain M5aI was isolated with a notable characteristic of lacking a polysaccharide capsule. It was first named *Aerobacter aerogenes* and was later identified as *K. pneumoniae*, a strong pathogen that causes a form of Pneumoniae. It has recently been classified as *K. oxytoca* because it differs from *K. pneumoniae* in that it is indole-positive and able to grow on melezitose, but not 3-hydroxybutyrate.

**Did You Know? -** There are several antimicrobial susceptibility testing methods available today, and each one has their respective advantages and disadvantages. They all have one and the same goal, which is to provide a reliable prediction of whether an infection caused by a bacterial isolate will respond therapeutically to a particular antibiotict reatment. This data may be utilized as guidelines for chemotherapy, or at the population level as indicators of emergence and spread of resistance based on passive or active surveillance.

**Best Practices** - A surgical site infection is an infection that occurs after surgery in the part of the body where the surgery took place. Surgical patients commonly developed postoperative "irritative fever," followed by purulent drainage from their incisions, overwhelming sepsis, and often death. It was in late 1860s, Joseph Lister introduced the principles of antisepsis, after which postoperative infectious morbidity decreased substantially. Lister's work radically changed surgery from an activity associated with infection and death to a discipline that could eliminate suffering and prolong life.

Ease your mind with light humour in our Relaxed Mood section…….

So go on, enjoy reading & don't forget to give us your valuable inputs & feedback.

# Microbial Pure Culture

Microorganisms are generally found in nature (air, soil and water) as mixed populations. Even the diseased parts of plants and animals contain a great number of microorganisms, which differ markedly from the microorganisms of other environments. To study the specific role played by a specific microorganism in its environment, one must isolate the same in pure culture. Pure culture, in microbiology, a laboratory culture containing a single species of organism. A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample manyfold before inoculating the new medium. Both methods separate the individual cells so that, when they multiply, each will form a discrete colony, which may then be used to inoculate more medium, with the assurance that only one type of organism will be present. Isolation of a pure culture may be enhanced by providing a mixed inoculum with a medium favouring the growth of one organism to the exclusion of others.

Pure culture involves not only isolation of individual microorganisms from a mixed population, but also the maintenance of such individuals and their progenies in artificial media, where no other microorganisms find way to grow.

However, it is not easy to isolate the individual microorganisms from natural habitats and grow them under imposed laboratory conditions. For this, great deal of laboratory manipulation is required. If inoculums from any natural habitat is taken and allowed to grow in a culture medium, a large number of diverse colonies may develop that, due to crowdedness, may run together and, thereby, may lose individuality. Therefore, it is necessary to make the colonies well-isolated from each other so that each appears distinct, large and shows characteristic growth forms. Such colonies may be picked up easily and grown separately for detailed study.

There are a number of procedures available for the isolation of pure cultures from mixed populations. A pure culture may be isolated by the use of special media with specific chemical or physical agents that allow the enrichment or selection of one organism over another. Simpler methods for isolation of a pure culture include: (i) spread plating on solid agar medium with a glass spreader and (ii) streak plating with a loop. The purpose of spread plating and streak plating is to isolate individual bacterial cells (colony-forming units) on a nutrient medium. Both procedures (spread plating and streak plating) require understanding of the aseptic technique. Asepsis can be defined as the absence of infectious microorganisms. However, the term is usually applied to any technique designed to keep unwanted microorganisms from contaminating sterile materials.

**Common Methods of isolation of pure culture**

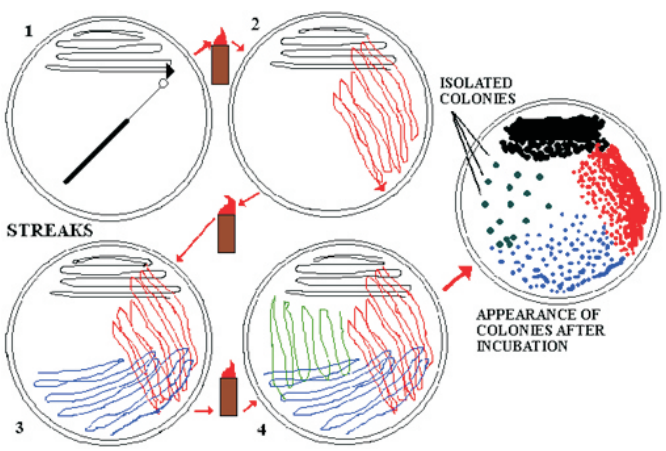
Pure culture of microorganisms that form discrete colonies on solid media, e.g., yeasts, most bacteria, many other microfungi, and unicellular microalgae, may be most commonly obtained by plating methods such as streak plate method, pour plate method and spread plate method.

But, the microbes that have not yet been successfully cultivated on solid media and are cultivable only in liquid media are generally isolated by serial dilution method.

**Streak Plate Method**

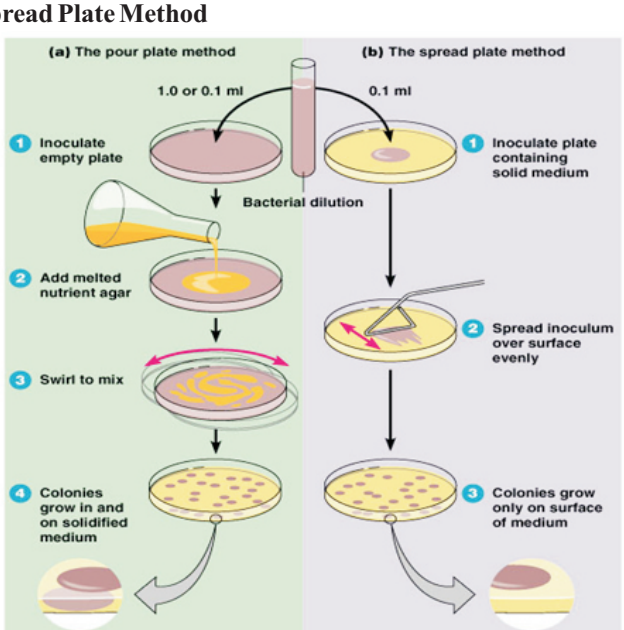
Streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.

Streaking is rapid and ideally a simple process of isolation dilution. The technique is done by diluting a comparatively large concentration of bacteria to a smaller concentration. The decrease of bacteria should show that colonies are sufficiently spread apart to effect the separation of the different types of microbes. Streaking is done using a sterile tool, such as a cotton swab or commonly an inoculation loop. Aseptic techniques are used to maintain microbiological cultures and to prevent contamination of the growth medium.There are many different types of methods used to streak a plate. Picking a technique is a matter of individual preference and can also depend on how large the number of microbes the sample contains.




The plate should show the heaviest growth in the first section. The second section will have less growth and a few isolated colonies, while the final section will have the least amount of growth and many isolated colonies.

**Spread Plate Method**



In this method the mixed culture of microorganisms is not diluted in the melted agar medium (unlike the pour plate method); it is rather diluted in a series of tubes containing sterile liquid, usually, water or physiological saline. The aim of this dilution is to inoculate a series of tubes with a microbial suspension so dilute that there are some tubes showing growth of only one individual microbeA drop of so diluted liquid from each tube is placed on the centre of an agar plate and spread evenly over the surface by means of a sterilized bent-glass-rod.

The medium is now incubated. When the colonies develop on the agar medium plates, it is found that there are some plates in which well-isolated colonies grow. This happens as a result of separation of individual microorganisms by spreading over the drop of diluted liquid on the medium of the plate.

**Pour Plate Method**

This method involves plating of diluted samples mixed with melted agar medium. The main principle is to dilute the inoculum in successive tubes containing liquefied agar medium so as to permit a thorough distribution of bacterial cells within the medium. Here, the mixed culture of bacteria is diluted directly in tubes containing melted agar medium maintained in the liquid state at a temperature of 42-45°C (agar solidifies below 42°C). The bacteria and the melted medium are mixed well. The contents of each tube are poured into separate Petri plates, allowed to solidify, and then incubated. When bacterial colonies develop, one finds that isolated colonies develop both within the agar medium (subsurface colonies) and on the medium (surface colonies). These isolated colonies are then picked up by inoculation loop and streaked onto another Petri plate to insure purity.

**Maintenance and Preservation of Pure Cultures**

Once a pure culture is obtained then methods are to be devised for their maintenance and preservation so that all the characteristics can be conserved. Some of the simple methods of culture maintenance and their preservation are described below.

**Refrigeration**

Live pure cultures can be successfully stored in their respective culture media in refrigerators or such cooling conditions at about 4°C. Generally, the metabolic activity of the organisms slows down and they become nearly inert at this temperature. However, the metabolism does not completely cease and hence, the organisms cannot be maintained for an indefinite period of time. At regular intervals, say 2-4 weeks, the culture may be taken out from the refrigerator and inoculated to fresh media, a process known as sub- culturing.

**Transfer to fresh media**

Periodic transfer to fresh, sterile media tubes can maintain microbial cultures. The frequency of transfer, however, varies with the organism. A culture the bacterium, Escherichia coli, for example, needs transfer at monthly intervals. After growth for 24 hours at 37°C, the slants can be stored at low temperature for 20-30 days to keep the culture viable. It is necessary to use the appropriate growth medium and proper storage temperature

**Overlying with mineral oil or liquid paraffin**

Covering the fresh growth in agar slants with sterile mineral oil or liquid paraffin can preserve many bacteria and fungi. The oil must be above the tip of the slanted surface. The cell viability in this method is very high as compared to frequent transfer and storage at low temperature.

**Freeze drying or lyophilization**

Freeze drying (lyophilization) is a rapid dehydration of organisms while they are in frozen state. In this process, the cell suspension is placed in small vials, which are, frozen by immersing in a mixture of dry ice and acetone liquid nitrogen. The vials are evacuated and dried under vacuum, sealed and stored at low temperature. Under such conditions, microbes can be stored for very long durations without upsetting their characteristics.

**Storage at sub-zero temperature**

In this method, the cultures are frozen in the presence of a protective agent such as glycerol or dimethylsulphoxide in liquid nitrogen (-196° C). This method is successful in many organisms particularly those which cannot be preserved under lyophilization.

**Checking purity of cultures**

It is sensible to check purity on suspicion of contamination of the working stock, and of the permanent stock when preparing new stock cultures. Evidence of purity is given by the uniformity of colony form on a dilution streak plate, cell form on stained microscope slides, and consistency with the appearance of the original culture.

If a culture becomes contaminated, go back to the working stock or permanent stock cultures, or buy in fresh supplies.

**References:**


1. BBL. 1973. BBL manual of products and laboratory procedures. Becton Dickson Microbiology Systems, Cockeysville, MD
2. Buchanan, E. D., and R. E. Buchanan. 1938. Bacteriology for students in general and household science, 4th ed. Macmillan Company, New York, NY.
3. Cappuccino, J. G., and N. Sherman. 2008. Microbiology a laboratory manual, 8th ed. Pearson/Benjamin Cummings, San Francisco, CA.
4. Lammert, J. M. 2007. Techniques in microbiology. A student handbook. Pearson/Prentice Hall, Upper Saddle River, NJ.
5. Levine, M. 1939. An introduction to laboratory technique in bacteriology, revised ed. The Macmillan Company, New York, NY.
6. Pelczar, M. J., Jr., and R. D. Reid. 1958. Laboratory exercises in microbiology, p. 45–47. McGraw-Hill Book Company, Inc., New York, NY.
7. Birkhaug KE. PRESERVATION OF BACTERIAL CULTURES UNDER LIQUID PARAFFIN. Science. 1932 Sep 9;76(1967):236–237. [PubMed]
8. Brown JH. VACUUM TUBES FOR THE STORAGE AND SHIPMENT OF BACTERIA. Science. 1926 Oct 29;64(1661):429–430. [PubMed]
9. Swift HF. A Simple Method for Preserving Bacterial Cultures by Freezing and Drying. J Bacteriol. 1937 Apr;33(4):411–421. [PMC free article] [PubMed]
10. Winslow CE, Brooke OR. THE VIABILITY OF VARIOUS SPECIES OF BACTERIA IN AQUEOUS SUSPENSIONS. J Bacteriol. 1927 Apr; 13(4): 235–243. [PMC free article] [PubMed]
11. PRESSCOTT, L.M., J.P. HARLEY and D.A. KLEIN. 1993. Microbiology. 2nd ed. Wm. C. Brown. Melbourne, Australia. 912 pp.
12. RAYMUNDO, A.K., A.F. ZAMORA, and I.F. DALMACIO. 1991. Manual on Microbiological Techniques. TLRC-UPLB. 112 PP.
13. STAINER, R.Y.,J.L. INGRAHAM, M.L. WHEELIS and G.R. PAINTER. 1986. The Microbial World. 5th ed. Prentice-hall, N.J. Englewood Cliffs, N.J. 689 pp.

# Hygiene in Airline Catering

**Introduction**

The air catering business is very different from normal hotel operations. It lays a lot of emphasis on quality and hygiene, besides the taste of the food.

Many cases of illness are reported every year in that may have been caused by microorganisms in food, in particular bacteria, viruses or parasites, and experts believe that the number of unreported cases is far higher. Providers who produce meals for third parties bear a great deal of responsibility. Meals must not pose a risk to health and must be of flawless quality. To achieve this goal, it is important that the entire kitchen team pays attention to cleanliness and hygiene during their day-to-day work in the kitchen. This applies to personal and hand hygiene, correct handling of food and cleanliness in the kitchen and in the entire company.

**Business**

The huge surge in passenger numbers will create significant growth opportunities in the travel catering sector and has already resulted in catering facilities being transformed, expanded and upgraded across the region.

For example, **Emirates** Flight Catering (EKFC), the region's largest caterer, providing in-flight catering for airlines flying into or out of Dubai International Airport, has already considerably enhanced the scope of its business, currently handling a throughput of 70 million plus passengers.

**Standards of hygiene and cleanliness** are now critical to maintaining a competitive advantage.

**Hygiene**

With the correct application of Disinfectants, Cleaners & sanitizers food poisoning bacteria can be reduced to safe levels following methodical and regular cleaning practices.

For cross-contamination prevention it is recommended that a two stage process as best practice with a stage 1 cleaning process followed by a stage 2 disinfection process bearing in mind that surfaces can only be disinfected if they are visibly clean.

**HACCP Concept** -The Hazard Analysis Critical Control Point (HACCP) concept is a systematic approach to the identification and assessment of food safety hazards and of defining means of their control.

The system focuses on prevention at every step of the production process rather than detection of unsafe food products at the end of production.

**Personal Hygiene**

Hand-washing top priority for flight caterer

No expenses spared by company when it come to hygiene

Many microorganisms live on your skin and particularly on your scalp but frequent washing helps to prevent them from breeding. This is why personal hygiene is so important. Washing your hair regularly frees the scalp of dandruff, which microorganisms feed on. As dirt cannot be seen under nail varnish and as the varnish can flake off, use fresh workwear and dish towels every day Microorganisms that can contaminate food accumulate on workwear and dish towels. Change your workwear and the towels used in the kitchen every day. The fabrics should be light-coloured and boil-washable. Sweat collects under rings, bracelets, wristwatches and the like, and this moisture is the ideal environment for bacteria to breed. Jewellery also prevents you from cleaning your hands and forearms properly. Always remove these objects before starting work. Always wash your hands in the designated washbasins and never in sinks in which food or crockery and cutlery are cleaned. Wash your hands thoroughly before starting work and wash them again regularly between individual work steps. A standard air catering has a bell system which is rung by the hygiene officer after every 3-4 hours which reminds the staff it is a hand washing time. After washing your hands, you should also disinfect them, at least after handling raw foods, in particular meat, poultry and eggs as well as after using the toilet. Do not cough or sneeze on food Even healthy people have bacteria in their nose and throat that can result in food poisoning. To ensure that these bacteria and any viruses they contain are not transferred to food via tiny droplets, always turn away from food if you have to cough or sneeze. Cough in your elbow and use a paper tissue to clean your noise. Then throw the tissue away, thoroughly wash your hands and disinfect them.

A procedure must be in place to ensure personal hygiene standards exist and are maintained in areas where open food or clean equipment is handled. The purpose is to prevent microbial and physical food and equipment contamination. Suitable, clean protective clothing shall be worn by all employees and visitors. · The company is to ensure that the protective clothing worn by staff handling open food is cleaned to commercial laundry standards (e.g. including mechanical washing and rinse with a disinfectant). Provision should be made for the storage of clean protective clothing to prevent contamination. Adequate provision should be made for the complete segregation between clean and soiled protective clothing. A designated area for the returned soiled clothing is to be provided. Employee Change Facilities & Lockers are to be provided to secure personal possessions away from production areas. Clean or soiled protective clothing should not be stored inside lockers. Periodic checks should be carried out by management to ensure compliance. Protective Hair Covering - Disposable protective hair covering should be worn by all persons working in or entering areas for handling of open food or clean equipment. · Suitable head covering should be provided and worn correctly to ensure complete enclosure of hair. Beards and moustaches should be covered with snoods.

Hand Hygiene - Gloves, if worn, should be suitable, disposable and changed frequently. Their disposal should be controlled to avoid product contamination. Cuts and grazes on exposed skin should be covered with a company-issued blue or appropriate colored waterproof dressing and covered by a disposable glove.

Jewellery - All employees and visitors shall follow the company rules on jewellery when working in or entering food production/handling areas. · The company rules must be based on risk assessment in regard to physical and microbial

contamination. Employees and visitors should be advised that eating, drinking and smoking are strictly restricted to designated areas e.g. staff canteen. Audit Verify that adequate protection and control measures are in place and adhered to by all employees and visitors.

Health monitoring procedures must be in place to prevent contamination of food through contact with infected people. Purpose is to prevent transmission of pathogenic microorganisms to food from infected employees and visitors.

Control of Food Handlers - Pre-employment Control Prior to employment, new food handlers shall be screened for current health status (e.g. questionnaires) and briefed on infection reporting requirements. · Annual Screening- All food handlers are required to report to management whenever suffering from the specified symptoms. Confirm continued compliance by annual screening (e.g. questionnaire) · Corrective Action- If a food handler confirms suffering from any of the symptoms specified, the person shall not be employed or assigned for food handling, until examined and subsequently cleared by a medical professional. Provided confirmation by medical professional, the person may be employed or assigned for a non-food handling task while awaiting clearance by the medical professional. Immediately report illness or symptoms like repeated diarrhoea and vomiting to the kitchen management People suffering from a disease that can be passed on via food or who exhibit symptoms that indicate communicable diseases may not handle food and must not enter the kitchen area if there is a risk that pathogens might be transferred. Especially with diarrhoea illnesses. Control of Visitors -Visitors must not touch open food or equipment at any time in food handling areas. They may however touch and/or eat food provided at a menu presentation held outside areas where open food is being prepared or handled.

Visitor Screening -Harmful contamination of food from visitors entering food handling areas shall be prevented by one of the following measures:

1. Visitor completes a Health Questionnaire for Visitors before entry 2. Visitor is presented with written instruction before entry
3. Visitor is presented with verbal instruction before entry.

Corrective Action- Visitor who confirms suffering from intestinal infection or flu-like symptoms should not be allowed to enter food production areas. Visitor who confirms suffering from other symptoms specified in the questionnaire may be allowed to enter food production areas, provided that the person agrees to put on proper protective gear, e.g. bandage, gloves, etc.

**Training & Hygiene checks**

a) Proper training of all the relevant staff covering basic food microbiology. b) Hand Swabs of the staff at random on daily basis. c) Equipment Random swabs to check the effectiveness of cleaning on a daily basis.

**Requirements in Air catering**


The equipment used in various areas should meet Food Safety criteria. Especially there should be Equipment Washing machine, Blast Chillers and Ice Cubes. It must have a cleaning and fumigation schedule well documented with implementation records. . It should have Microbiological lab manned by qualified microbiologists with facility to test water, ice, raw and finished food samples, equipments and hand swabs

Basic practices-When tasting food, take care to ensure that your own saliva does not come into contact with the food being prepared, as we naturally all have microorganisms in our mouth. Use a clean spoon to take a small sample of the food and then tip the food into a small bowl or directly onto another spoon you intend to use for tasting.

**Example**

Dubai: Emirates Flight Catering is more than just about delivering meals to airline passengers but also about delivering the highest standard of hygiene in the world.

Emirates Flight Catering (EKFC) used 11,000 litres of soap, 450 litres of hand sanitizer and 10 million square metres of tissue as it served 130,000 meals a day to passengers.

We have training programmes throughout the year and as we employ about 45 different nationalities, all newcomers have to attend basic food hygiene courses as well as refresher courses later on.

a) As part of their policy, employees with flu symptoms are not allowed to work and they have to undergo follow ups with an in-house doctor to ensure that they are free of symptoms and healthy enough to handle food.
b) Food handlers are required to wash their hands for no more than 20 seconds at critical moments during the day, including when handling raw materials, touching waste and after using the washroom.
c) We carry out random sampling by using swab tests on employees' hands and with the assistance of five microbiologists and 39 food safety assistants, we can determine within 48 hours if there are any bacteria present," noting that such tests include checking for E-Coli, salmonella and staphylococcus aureus.
d) The most common places that left out during handwashing are the finger tips and between the fingers," explaining that the number of washbasins around the premises has increased from 20 in 1990 to 153 in 2011.
e) A municipality official further stressed that hand sanitizers should be used following hand washing and should not be relied as the sole way to rid of bacteria.
f) At the same time, it is important to be environmentally friendly and not get into the habit of wasting water and paper. Handwashing is a habit that should extend to people's homes so that their children can also pick up and learn the importance of using a combination of soap and hand sanitizers.

**Onboard Flight Attendant/ Cabin Crew Training**

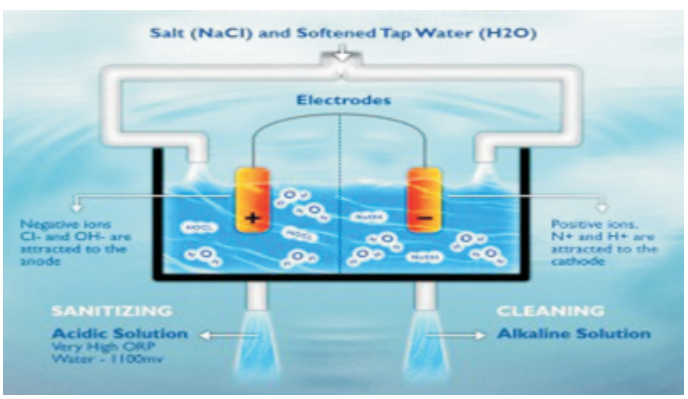
Personal Hygiene including proper hand washing prior to beverage service and meal service, reporting illness. · Maintaining method of cooling on board the aircraft until all food service(s) is complete. · Cooking, re-heating and/or maintaining hot food temperatures.

**Receiving area**

Food hygiene Only accept incoming goods that are of flawless quality Harmful microorganisms or


parasites can enter the storeroom via food with soiled or damaged packaging, resulting in contamination of the products already stored there. Always check incoming goods for flawless packaging and quality. Ensure uninterrupted cooling of products Food that has not been adequately cooled may be spoiled. Check incoming goods to determine whether delivered food is properly cooled. This is particularly important in the case of meat, poultry, fish and dairy products. Separate clean and unclean work tasks Microorganisms can be transferred from unclean to clean food during both processing and storage. Products like raw and cooked food must therefore be stored in separate fully covered containers. Used crockery and cutlery may also be contaminated, and food service and crockery return must therefore take place at different times or in different areas. Never use the same kitchen equipment to prepare raw and cooked foods without cleaning it extremely thoroughly in-between. Always keep highly perishable foods in cold storage and use them without delay.

**Sanitation**
**Introduction**




Electrolyzed Water using the ElectroCide System to provide highly effective cleaning, disinfecting and sanitizing solutions with low environmental impact Benefiting you and the environment:

- Potent but benign environmental sanitizer
- Suitable for a wide range of applications
- All work surfaces, floors, kitchens and bathrooms and more
- Effectively cleans, disinfects and sanitizes

Testing proves it kills pathogens faster, more effectively, and more safely than the most popular sanitizers.

- no need to mix or dilute the solutions
- no need for protective gloves or eyewear
- no special disposal procedures required
- no impact as an environmentally safe solution

Independent lab tests conducted in the United States during 2003 verified that Electrolyzed Water meets or exceeds regulatory (FDA, EPA and USDA) requirements for use in food service sanitation and disinfecting.

The treated water is available to use throughout the home or business to clean and sanitize. Electrolyzed Water is safe enough to drink but tough enough to provide thorough sanitizing properties without harming the environment.

**Sodium Hydroxide and Hypochlorous Acid**

In the electrolyzing process, sodium hydroxide and hypochlorous acid are produced. Both sodium hydroxide and hypochlorous acid are efficient disinfecting agents. The process decreases water's pH, creating more acidity within a solution that's still water. This acidic form of Electrolyzed Water is so safe it can be used on food-preparation surfaces and even on the food itself! Electrolyzed Water can be used to clean fruits and vegetables.

**What is Electrolyzed Water?**

EOW or EO, also known as electrolyzed oxidizing water, electro-activated water or electro-chemically activated water solution is produced by the electrolysis of ordinary tap water containing dissolved sodium chloride. The electrolysis of such salt solutions yielding a solution of sodium hypochlorite NaOCl which is the major component of ordinary household laundry bleach.

**How does it work?**

Both sodium hydroxide and hypochlorous acid are efficient disinfecting and sanitizing agents; the effectiveness of EOW is based on the increase at low pH creating an acidic form of EOW that functions as environmentally safe cleaning solution.

**How do I know it is working?**

The high oxidation of the water first damages bacteria cell walls, allowing infiltration by water. The microbe reaches capacity, causing an osmotic, or hydration, overload. The acidic fluid and water floods the cell faster than the cell can expel it, literally causing the cell to burst.

**What are the key ingredients in EOW?**

The key components are water, salt and an electrical charge producing: Hypochlorous Acid (.0088%), Hydrochloric Acid (.0096%), Dissolved Chorine. (.0001%) and Inert Ingredients (99.9815%)

**Safety and Efficacy**

| Safety Test Results | |
|---|---|
| Oral Toxicity | Negative |
| Skin Sensitivity | Negative |
| Eye Irritation | Negative |
| Skin Irritation | Negative |
| Odor | Negative |
| Taste | Negative |

| Type of Bacteria or Virus | Time to Eliminate | Prevents |
|---|---|---|
| Enterovirus | 5 Seconds | Gastroenteritis & others |
| Escherichia coli | 5 Seconds | Gastroenteritis cystitis & others |
| Haemophilus influenza | 5 Seconds | Respiratory infections |
| Kiebsiella pneumonia | 5 Seconds | Pneumonia |
| Proteus mirabilis | 5 Seconds | Gastroenteritis Urinary tract |
| Pseudomonas aeruginosa | 5 Seconds | Infections |
| Pseudomonas cepacia | 5 Seconds | Infections |
| Salmonella typhi | 5 Seconds | Gastroenteritis Typhoid fever |
| Staphylococcus aureus | 5 Seconds | Infections Endocarditis |
| Staphylococcus epidermidis | 5 Seconds | Hospital acquired infections |
| Streptococcus pneumoniae | 5 Seconds | Lobar pneumonia, meningitis, sinusitis |
| Streptococcus pyogenes | 5 Seconds | Pus, septicemia |
| E. Coli O-157 | 10 seconds | Colitis, anemia, kidney failure |
| Listeria Monocitogenes | 10 seconds | Meningitis, encephalitis |
| HIV | 60 seconds | AIDS |
| Candida albicans | 15 seconds | Infections, endocarditis |
| Bacillus cereus | 5 minutes | Food poisoning |

**Chlorine sanitation** is the commonly used sanitation process used in the catering for vegetable, fruits, and Chopping boards.

**Microbiological Laboratories**

Following tests must be carried out routinely for different types of food/ice and water:

A) Microbiological Test a) Total Plate Count b) Coliform Group of Bacteria c) Yeast & Molds d) E. coli e) Salmonella f) Staphylococcus aureus g) Bacillus cereus B) Chemical Analysis pH TDS (Total dissolved solids) Total Hardness Calcium Hardness Alkalinity and Acidity Fat contents in dairy products Gluten contents in Atta and Maida.

Use of microbiological testing is recommended as verification for the HACCP plan. Scope of analysis includes: 1. In-house produced ready-to-eat foods 2. Purchased ready-to-eat foods 3. Potable water and ice from caterer or aircraft 4. Cleaning effectiveness a) In-house produced ready-to-eat foods Random sampling and analysis of a limited number of potentially hazardous ready to eat foods may provide valuable information regarding effectiveness and reliability of in-house control procedures. If internal audit, including records verifies safe procedures and results of analysis of in-house produced foods are satisfactory. The auditor has verified that control system works according to expectations at time of audit. If internal audit, including records verifies safe procedures and results of analysis of in-house produced foods identify unsafe food properties, the auditor has detected weaknesses in the control system, which must be located and identified. Testing frequency depends on the performance of the control system as assessed by internal audit, as well as other factors such as number and frequency of alleged or confirmed food safety related customer complaints. Food safety related customer complaints may indicate the need for additional microbial testing even when other records confirm satisfactory control. Purchased ready-to-eat foods Microbiological testing of purchased ready-to-eat foods is often the most appropriate option for verification of safety of product, as alternatives may be costly audits of remotely located manufacturers. Frequency of testing should be determined primarily by performance history, such as alleged or confirmed problems with the purchased product in question, expressed through customer complaints or in-house observations. Potable Water & ice from caterer or aircraft Microbiological analysis is the most appropriate method of verification of safety of potable water and ice. Ice may be made in-house, at catering units or may fall into the "purchased ready-to-eat foods" category and therefore maybe sourced with certificates of analysis from the supplier. Analysis of water and ice may be performed by health authorities or by the caterer or the airline.

Cleaning effectiveness-Various testing methods are available to verify the effectiveness of the cleaning procedures for direct food contact surfaces and hands. Examples are swab tests, impression tests.

**Tray set up & Dishing out**

Section of hygienic impotance where the complete menu is aligned according to the load given by the aircraft. The belts & tables are cleaned & disinfected after every use

**Loading &off loading into Hi-loaders**

The loading vehicle is cleaned after every delivery & fumigated as per the timetable

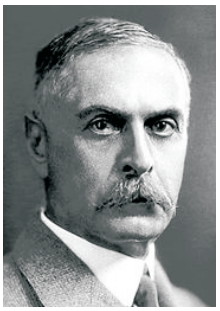
Hygiene is applied at every point from entrance till exit to ensure safe & healthy air travel.

Various Hygiene audits are conducted besides the various airline audits & ISO to ensure the hygiene standards

**References:**

[1] ACMSF (1995) Report on Verocytotoxin-Producing Escherichia coli. London: HMSO. AIFST (NSW Branch) Food Microbiology Group (1997), Food-borne Microorganisms of Public Health Significance, 5th Ed. [2] Boulton, D. J. & Maunsell, B (2004) Guidelines for Food Safety Control in European Restaurants. Teagasc – The National Food Centre. Republic of Ireland. [3] FAO/WHO (2003) Joint FAO/WHO Food Standards Program. Codex Committee on Food Hygiene. 35th Session. Risk profile for Enterohemorrhagic E.coli including the identification of the commodities of concern, including sprouts, ground beef and pork. [4] Food Safety Authority of Ireland (FSAI) (1999) The prevention of E. coli O157:H7 infection: a shared responsibility. Food Safety of Ireland. Dublin. [5] Food Standards Agency Scotland (2005) Cook Safe Food Safety Assurance System. Scottish HACCP Working Group. [6] USDA FSIS (2001) Draft Compliance Guidelines for Ready-to-eat Meat and Poultry Products.

## Karl Landsteiner



| | |
|---|---|
| Born: | June 14, 1868 Baden bei Wien (near Vienna), Austria-Hungary |
| Died: | June 26, 1943 (aged 75) New York City, New York, U.S. |
| Residence: | Austria<br>United States of America |
| Citizenship: | Austrian - American |
| Nationality: | Austrian |
| Fields: | Medicine, virology |
| Institutions: | University of Vienna<br>Rockefeller Institute for Medical Research, New York |
| Alma mater: | University of Vienna |
| Known for: | Development of blood group system, discovery of Rh factor, discovery of poliovirus |
| Notable awards: | Nobel Prize in Physiology or Medicine (1930)<br>Lasker-DeBakey Clinical Medical Research Award (1946 posthumously)<br>ForMemRS (1941) |

Karl Landsteiner was born in Vienna on June 14, 1868. His father, Leopold Landsteiner, a doctor of law, was a well-known journalist and newspaper publisher, who died when Karl was six years old. Karl was brought up by his mother, Fanny Hess, to whom he was so devoted that a death mask of her hung on his wall until he died. After leaving school, Landsteiner studied medicine at the University of Vienna, graduating in 1891. Even while he was a student he had begun to do biochemical research and in 1891 he published a paper on the influence of diet on the composition of blood ash. To gain further knowledge of chemistry he spent the next five years in the laboratories of Hantzsch at Zurich, Emil Fischer at Wurzburg, and E. Bamberger at Munich.

Returning to Vienna, Landsteiner resumed his medical studies at the Vienna General Hospital. In 1896 he became an assistant under Max von Gruber in the Hygiene Institute at Vienna. Even at this time he was interested in the mechanisms of immunity and in the nature of antibodies. From 1898 till 1908 he held the post of assistant in the University Department of Pathological Anatomy in Vienna, the Head of which was Professor A. Weichselbaum, who had discovered the bacterial cause of meningitis, and with Fraenckel had discovered the pneumococcus. Here Landsteiner worked on morbid physiology rather than on morbid anatomy. In this he was encouraged by Weichselbaum, in spite of the criticism of others in this Institute. In 1908 Weichselbaum secured his appointment as Prosector in the Wilhelminaspital in Vienna, where he remained until 1919. In 1911 he became Professor of Pathological Anatomy in the University of Vienna, but without the corresponding salary.

Up to the year 1919, after twenty years of work on pathological anatomy, Landsteiner with a number of collaborators had published many papers on his findings in morbid anatomy and on immunology. He discovered new facts about the immunology of syphilis, added to the knowledge of the Wassermann reaction, and discovered the immunological factors which he named haptens (it then became clear that the active substances in the extracts of normal organs used in this reaction were, in fact, haptens). He made fundamental contributions to our knowledge of paroxysmal haemoglobinuria.

He also showed that the cause of poliomyelitis could be transmitted to monkeys by injecting into them material prepared by grinding up the spinal cords of children who had died from this disease, and, lacking in Vienna monkeys for further experiments, he went to the Pasteur Institute in Paris, where monkeys were available. His work there, together with that independently done by Flexner and Lewis, laid the foundations of our knowledge of the cause and immunology of poliomyelitis.

Landsteiner made numerous contributions to both pathological anatomy, histology and immunology, all of which showed, not only his meticulous care in observation and description, but also his biological understanding. But his name will no doubt always be honoured for his discovery in 1901 of, and outstanding work on, the blood groups, for which he was given the Nobel Prize for Physiology or Medicine in 1930.

In 1875 Landois had reported that, when man is given transfusions of the blood of other animals, these foreign blood corpuscles are clumped and broken up in the blood vessels of man with the liberation of haemoglobin. In 1901-1903 Landsteiner pointed out that a similar reaction may occur when the blood of one human individual is transfused, not with the blood of another animal, but with that of another human being, and that this might be the cause of shock, jaundice, and haemoglobinuria that had followed some earlier attempts at blood transfusions.

His suggestions, however, received little attention until, in 1909, he classified the bloods of human beings into the now well-known A, B, AB, and O groups and showed that transfusions between individuals of groups A or B do not result in the destruction of new blood cells and that this catastrophe occurs only when a person is transfused with the blood of a person belonging to a different group. Earlier, in 1901-1903, Landsteiner had suggested that, because the characteristics which determine the blood groups are inherited, the blood groups may be used to decide instances of doubtful paternity. Much of the subsequent work that Landsteiner and his pupils did on blood groups and the immunological uses they made of them was done, not in Vienna, but in New York. For in 1919 conditions in Vienna were such that laboratory work was very difficult and, seeing no future for Austria, Landsteiner obtained the appointment of Prosector to a small Roman Catholic Hospital at The Hague. Here he published, from 1919-1922, twelve papers on new haptens that he had discovered, on conjugates with proteins which were capable of inducing anaphylaxis and on related problems, and also on the serological specificity of the haemoglobins of different species of animals. His work in Holland came to an end when he was offered a post in the Rockefeller Institute for Medical Research in New York and he moved there together with his family. It was here that he did, in collaboration with Levine and Wiener, the further work on the blood groups which greatly extended the number of these groups, and here in collaboration with Wiener studied bleeding in the new-born, leading to the discovery of the Rh-factor in blood, which relates the human blood to the blood of the rhesus monkey.

To the end of his life, Landsteiner continued to investigate blood groups and the chemistry of antigens, antibodies and other immunological factors that occur in the blood. It was one of his great merits that he introduced chemistry into the service of serology.

Rigorously exacting in the demands he made upon himself, Landsteiner possessed untiring energy. Throughout his life he was always making observations in many fields other than those in which his main work was done (he was, for instance, responsible for having introduced dark-field illumination in the study of spirochaetes). By nature somewhat pessimistic, he preferred to live away from people.

Landsteiner married Helen Wlasto in 1916. Dr. E. Landsteiner is a son by this marriage.

In 1939 he became Emeritus Professor at the Rockefeller Institute, but continued to work as energetically as before, keeping eagerly in touch with the progress of science. It is characteristic of him that he died pipette in hand. On June 24, 1943, he had a heart attack in his laboratory and died two days later in the hospital of the Institute in which he had done such distinguished work.

# JOKES

Maine Kaha- "I love you"
Girl-Mera Boyfriend hai
Maine Kaha- purana jayega tabhi to naya aayega OLX par bech de.

In maths exam, Santa got 1question
Prove Sin x= 6n
Santa cancelled 'n' from both the sides
Then six=6
& wrote "Dont mess with Santa d great"

bhikari: Bahen Bhoka Hu bhagwan KE Naam PE Khana De Do
Behan: Khana Abi Ni Paka
bhikari: FaceBook Pe BABA Pappu K Nam Se H khana Pak Jae To Wall Pe Update Kar Dena

A man saw a board at d centre Of a River,
He tried 2 read
but he can't read it.
So,he swim into d River &read
"CROCODILE INSIDE-DONT SWIM"

Banta: What's Marriage?
Santa: Marriage is the 7th sense of humans that destroys all the six senses and makes the person Non-sense.

Height of Job satisfaction-
A boy got a job in girls hostel...
After 2 months owner asked: Y u don't come 2 take ur salary?
Boy: KYA..?
salary bhi milegi..!

Girl- Main jb bhi phon krti hu tum Shaving kr rhe hote ho, Din me kitni baar shaving krte ho?
Boy- 30-40 baar.
Girl- pagal ho kya?
Boy- pagal nahi NAAI hu.

Boy- Ro Q rhi ho?
Girl- Mere marks bahut kam aaye hai.
Boy-Bata kitne aye hai?
Girl- Sirf 88 %
Boy- Khuda ka khof kar zalim itne me to 2 ladke pass ho jate

Boy to Diana Penty - wil u marry me???
Diana- wat will u do for me??
.
.
.
.
Boy- I will give u a better surname.!

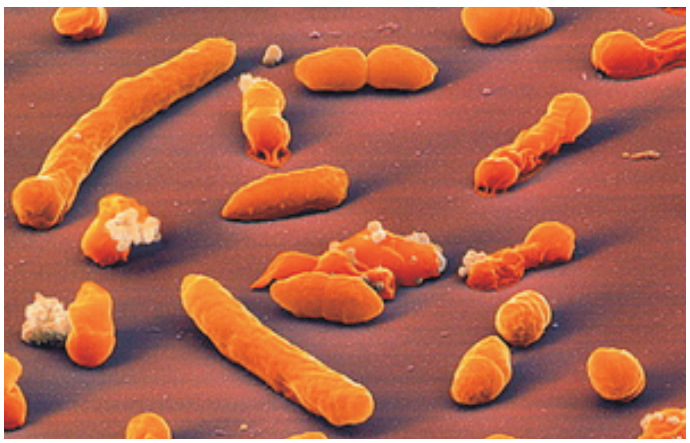
MBA student hugs a girl
Girl: what is dis ?
Boy: direct marketing
Girl: slaps a boy
Boy: what is dis ?? .
Girl: customer's feedback

# Microbiology Quiz

1. **Which of the following structures contains genes for1. The Tsetse fly is a transmission factor for which of the following organisms?**
   A. *Trichomonas vaginalis*
   B. *Trypanosoma gambiense*
   C. *Entamoeba histolytica*
   D. *Toxoplasma*
2. **The Ixodes tick is a transmission factor for which of the following organisms?**
   A. *Trichomonas vaginalis*
   B. *Leishmania donovani*
   C. *Babesia*
   D. *Giardia lamblia*
3. **Chagas' disease is commonly treated with Nifurtimox and is linked to the _____ microorganism.**
   A. *Naegleria*
   B. *Schistosoma*
   C. *Wucheria bancrofti*
   D. *Trypanosoma cruzi*
4. **Which of the following is not fungal related?**
   A. *Cryptococcus neoformans*
   B. *Candida albicans*
   C. *Tinea nigra*
   D. *Chlamydiae*
5. **Which of the following is not a DNA virus?**
   A. Adenovirus
   B. Calicivirus
   C. Papovirus
   D. Poxvirus
6. **Which of the following is not a RNA virus?**
   A. Reovirus
   B. Orthomyxovirus
   C. Deltavirus
   D. Herpesvirus
7. **Which of the following viruses is not a double strand linear DNA virus?**
   A. Poxvirus
   B. Papovavirus
   C. Adenovirus
   D. Herpesvirus
8. **Which of the following viruses is not a single strand linear RNA virus?**
   A. Togavirus
   B. Retrovirus
   C. Bunyavirus
   D. Picornavirus
9. **The Tzanck test is not used on which of the following viruses?**
   A. VZV
   B. HSV-2
   C. HHV-8
   D. HSV-1
10. **Which of the following microorganisms has not been linked to UTI's?**
    A. *E. coli*
    B. *Pseudomonas*
    C. *Klebsiella*
    D. *Haemophilus*

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

# *Klebsiella oxytoca*



**Description and Significance**

*Klebsiella oxytoca* is a gram-negative bacterium with a cylindrical rod shape measuring 2 µm by 5µm. In the 1950's the strain M5aI was isolated with a notable characteristic of lacking a polysaccharide capsule. It was first named *Aerobacter aerogenes* and was later identified as *K. pneumoniae*, a strong pathogen that causes a form of Pneumoniae. It has recently been classified as *K. oxytoca* because it differs from *K. pneumoniae* in that it is indole-positive and able to grow on melezitose, but not 3-hydroxybutyrate.

**Genome Structure**

The genome for *K. oxytoca* has been sequenced in several strains. Virginia Bioinformatics Institute sequenced the genome for *Klebsiella oxytoca*KOX105 and determined the plasma length to be 0.0546Mbp with 0 chromosomes. The Genome Sequencing Center at Washington University in St. Louis sample sequenced *Klebsiella oxytoca* M5aI strain VJSK009 to -5.5X WGS coverage in plasmids and determined total genome length to be 5.28Mbp with 56% total GC content . The strain *Klebsiella oxytoca* KCTC1686 has been sequenced showing genome length of 5.97Mbp. Currently, 10 strains of *Klebsiella oxytoca* are being sequenced as part of the Human Microbiome Project.

**Cell Structure and Metabolism**

*K. oxytoca* are Gram negative bacteria with a bi-membrane structure. In addition to the plasma membrane, they have an outer membrane composed of lipopolysaccharides (LPS) that have O antigens. Between these two membranes lies a thin layer of peptidoglycan. The LPS layer has a polysaccharide chain known as Lipid A, which produces endotoxins that contribute to their pathogenicity. *K. oxytoca* is an anaerobic specie that can fix nitrogen and hydrolyze cellulose. It is both catalase positive and indole positive. *K. oxytoca* produce β-lactamase, making them highly resistant to penicillin and ampicillin.

**Ecology**

*K. oxytoca* like other *Klebsiella spp.* can be found in a wide range of environments and are commonly referred to as ubiquitous and opportunistic in nature. *K. oxytoca* have been found in mammals and insects. In humans, this specie tends to colonize along the mucosa membranes of the colon and nasopharynx, and skin; however, they can be found colonizing on all parts of the body. Most infections of *K. oxytoca* are nosocomial, spreading via the hands of medical staff. Outbreaks occur in patients with immunodeficient diseases and patients whom are being treated with antibiotics. Outbreaks have also been reported occurring in both prenatal and neonatal infants.

**Pathology**

Species from the genus *Klebsiella* are frequently accountable for nosocomial infections in humans. They have the ability to colonize many different areas of the human body such as the skin, GI tract, sterile wounds, urine, and skin. While *Klebsiella oxytoca* does not cause as many of these infections as the more commonly known *Klebsiella pneumonia*, the genus *Klebsiella* was deemed responsible for 8% of all nonsocomial bacterial infections in the United States and Europe. Due to this incidence rate, *Klebsiella spp.* are considered one of the most significant infectious pathogens in the United States. The most common infections caused by *Klebsiella spp.* found in hospitals are as follows: urinary tract infections, pneumonia, wound infections, septicemia, neonatal septicemia, and nosocomial infections in ICU patients . *Klebsiella oxytoca* has increasingly been present in the blood samples of infants suffering from neonatal septicemia . Symptoms of neonatal septicemia include seizures, slow heart rate, temperature changes, jaundice, vomiting, diarrhea, low blood sugar, breaking difficulties, reduction in movements and sucking, and swollen abdomen.

Middle-aged men suffering with diseases such as alcoholism or diabetes are susceptible to *Klebsiella spp.* infections in the lungs. Such infections can lead to necrosis, hemorrhage, and inflammation of lung tissue resulting in bloody mucoid sputum, which is a common symptom . *K. oxytoca* is an opportunistic pathogen that can spread extremely quickly leading to nosocomial outbreaks. The gastrointestinal tract in hospital patients and hands of hospital staff are the two most common locations for the *Klebsiella spp.* to be found. Antibiotics, urinary catheters, invasive devices and contaminated respiratory equipment all increase the susceptibility of a person to a *Klebsiella spp.* infection.

**Current Research**

Raikher et al. (2010) analyzed the product obtained from the biomineralization of iron salt solutions by *Klebsiella oxytoca*. The resulting products, ferrihydrite nanoparticles, were determined to have magnetic properties that may be useful in the field of bioengineering and nanomedicine. *K. oxytoca* was previously known in geochemistry and industrial microbiology for mineralizing large amounts of iron in anaerobic conditions . In this study, *K. oxytoca* from a Russian lake was isolated from a sample of sapropel via a magnetic separator. After isolation, *K. oxytoca*was grown under microaerophilic and aerophilic conditions with varying light conditions in the presence of a yeast extract and ferric citrate. The resulting ferrihydrite nanoparticles magnetization curves were measured using a vibrating-coil magnetometer with a superconducting solenoid. These measurements resulted in a confirmation of the presences of two fractions of biogenic ferrihydrite. From the data gathered, it seemed the difference between the two fractions was magnetic susceptibility with the Fe12 particle having a higher

susceptibility than the Fe34. The magnetic susceptibility of these products results in a possibility of magnetic control being exerted over them.

*Klebsiella oxytoca* is known to breakdown cyanide, a chemical that is extremely toxic to humans. Wastewater that contains cyanide must be treated before it is released into the environment and the current methods of treating the water (e.g. alkaline chlorination and wet-air oxidation) are not only extremely expensive, but also, the reagents used in the process are hazardous. Tang et al. (2010) had previously shown that *Klebisialla oxytoca* was successful in degrading 1 mM cyanide into ammonia and recently they have looked into the molecular aspect of this . Electrophoresis and computer analysis were used to identify proteins. Some of the proteins were excised and further testing was perfromed to extract them and identify them using the NCBI database. The researchers identified 27 proteins that are known to be involved with carbohydrate, nucleotide, amino acid, and nitrogen metabolism, and oxidation-reduction reactions.

**Cool Factor**

*Klebsiella oxytoca* was determined to be the cause of spontaneous arthritis in the knee of a 30-month old child with no prior history of any bacterial infections . The child had not been hospitalized recently, was up to date on all vaccinations, and did not present with a urinary tract infection. Normally, cases of spontaneous arthritis in children are associated with *Staphylococcus aureus*, *Kingella kingae*, and *Streptococcus pneumonia* . The patient was treated with antibiotics specific to each of those bacteria, respectively, which ultimately proved to be ineffective. During this time no changes occurred in the patient's symptoms. The bacteria present in the synovial fluid of the knee was finally identified as *K. oxytaca*, and the antibiotic regimen was adjusted accordingly. The antibiotics, ceftriaxone and amikacin, were used for two days, then switched solely to ceftriaxone due to the results from antimicrobial susceptibility testing . X-rays taken post-antibiotic treatment showed a complete recovery in the child′s knee.

**References**


(1) Berkowitz, L.B., and Umeh, O. (2011). *Klebsiella* Infections. Medscape Reference.

(2) Charles River International Inc. *Klebsiella* species (*K. oxytoca*, *K. pneumoniae*). 2009.

(3) Doran, J.B., and Ingram, L.O. (1993). Fermentation of Crystalline Cellulose to Ethanol by *Klebsiella oxytoca* containing Chromosomally Integrated *Zymomonas mobilis* Genes. Department of Microbiology and cell Science at the Univeristy of Florida, Gainsville, Florida. pp. 533-538.

(4) Haartmann. (2012). Bode Science Center: Klebsiella oxytoca (bacterium incl. ESBL); [accessed 2012 Feb 11. Available from: http://www.bode-science-center.com/center/relevant-pathogens-from-a-z/klebsiella-oxytoca.html]

(5) Medscape Reference (2011). Drugs, Diseases & Procedures: Klebsiella Infections; [accessed 2012 Feb 15. Available from: http://emedicine.medscape.com/article/219907-overview#a0104]

(6) Mendard, A., et al. "First report of septic arthritis caused by *Klebsiella oxytoca*". *Journal of Clinical Microbiology*. 2010. Volume 48. p. 3012-3023.

(7) Morozkina, E.V., and Zvyagilskaya, R.A. (2007). Nitrate Reductases: Structure, Functions, and Effect of Stress Factors.

(8) Podschun R., and Ullmann, U. "*Klebsiella spp.* as a nonsocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors". *Clinical Microbial Revue*. 1998. Volume 11. p. 489-603.

(9) Raikher, Y. et al. "Magnetic properties of biomineral particles produced by bacteria Klebsiella oxytoca. Physics of the Solid State. 2010. Volume 52. p. 298-305.

(10) The Retroscope. (2011). Klebsiella oxytoca Colony Retroscope; [modified 2011 March 16; accessed 2012 Feb 14. Available from: http://www.retroscope.eu/wordpress/klebsiella-oxytoca/kleboxcolony/]

(11) Sahly, H., and Podschun, R. "Clinical, bacteriological, and serological aspects of *Klebsiella* infections and their spondylarthropathic sequelae". *Clinical and Diagnostic Laboratory Immunology*. 1997. Volume 4. p. 393-399.

(12) Science Photo Library Ltd. (2012). Science Photo Library: Klebsiella oxytoca bacteria; [accessed 2012 Feb 14. Available from: http://www.sciencephoto.com/media/11579/enlarge#]

(13) Tang, P. et al. "Proteomic analysis of the effect of cyanide on Klebsiella oxytoca." Current Microbiology. 2010. Volume 60. p. 224-228.

(14) U.S. National Library of Medicine (2011). Medline Plus: Neonatal sepsis; [accessed 2012 Feb 15. Available from: http://www.nlm.nih.gov/medlineplus/ency/article/007303.htm]

(15) Virginia Bioinformatics Institute (2012). Pathosystems Resource Integration Center: Klebsiella oxytoca KOX105; [accessed 2012 Feb 1. Available from:http://patricbrc.vbi.vt.edu/portal/portal/patric/Genome?cType=genome&cId=163830]

(16) Washington University in St. Louis. (2011). The Genome Institute at Washington University: Klebsiella oxytoca M5aI; [modified 2005 Dec 6; accessed 2012 Feb 1. Available from:http://genome.wustl.edu/genomes/view/klebsiella_oxytoca_m5al]

# Antibiotic Sensitivity Testing Methods

There are several antimicrobial susceptibility testing methods available today, and each one has their respective advantages and disadvantages. They all have one and the same goal, which is to provide a reliable prediction of whether an infection caused by a bacterial isolate will respond therapeutically to a particular antibiotict reatment. This data may be utilized as guidelines for chemotherapy, or at the population level as indicators of emergence and spread of resistance based on passive or active surveillance. Some examples of antibiotic sensitivity tesing methods are:

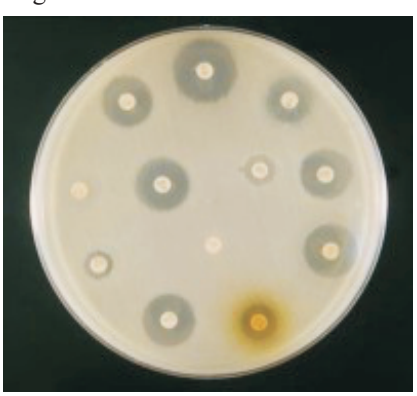
- Dilution method (broth and agar dilution method)
- Disk-diffusion method
- E-test
- Automated methods
- Mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin test
- Genotypic methods such as PCR and DNA hybridization methods

Selection of the appropriate method will depend on the intended degree of accuracy, convenience, urgency, availability of resources, availability of technical expertise and cost. Interpretation should be based on veterinary standards whenever possible, rather than on human medical standards, which may not always be applicable. Among these available tests, the two most commonly used methods in veterinary laboratories are the agar disk-diffusion method and the broth microdilution method.

## 1. DILUTION METHODS

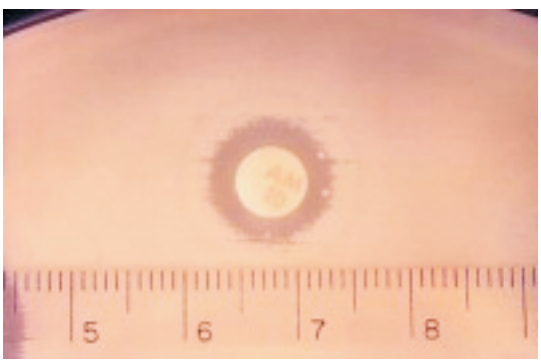
The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Microdilution testing uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. Macrodilution testing uses broth volumes at about 1.0 ml in standard test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC. The MIC is thus the minumum concentration of the antibiotic that will inhibit this particular isolate. The test is only valid if the positive control shows growth and the negative control shows no growth.

A procedure similar to broth dilution is agar dilution. Agar dilution method follows the principle of establishing the lowest concentration of the serially diluted antibiotic concentration at which bacterial growth is still inhibited.



On this agar plate, a bacterial isolate is tested for resistance to each of twelve different antibiotics. The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic. (A)The disk shows a large zone of inhibition; whereas (B) shows no zone of inhibition, indicating resistance of the isolate to the test antibiotic.

Presence of zone of inhibition is not automatically interpreted as susceptibility to the antibiotic; the zone width has to be measured and compared against a reference standard which contains measurement ranges and their equivalent qualitative categories of susceptible, intermediately susceptible or resistant.




For example, this E.coli isolate on the right has a zone of inhibition of 10.1mm around ampicillin (AM); since the zone diameter interpretation chart is as follows:

Resistant: 13mm or less
Intermediate: 14-16 mm
Susceptible: 17 mm or more

This particular E.coli isolate is read as resistant to ampicillin.

## 2. DISK DIFFUSION METHOD

Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance in private veterinary clinics.

A growth medium, usually Mueller-Hinton agar, is first evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 108colony forming units per ml). Commercially prepared disks, each of which are pre-impregnated with a standard concentration of a particular antibiotic, are then evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the agar such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" will be observed around that particular disk. The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. MIC measurement cannot be determined from this qualitative test,

which simply classifies the isolate as susceptible, intermediate or resistant.

**3. E-TEST**

E-test (AB Biodisk, Solna, Sweden) is a commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic. The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein. This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate. However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.

**4. AUTOMATED ANTIMICROBIAL SUSCEPTIBILITY TESTING SYSTEMS**

Several commercial systems have been developed that provide conveniently prepared and formatted microdilution panels as well as instrumentation and automated reading of plates. These methods are intended to reduce technical errors and lengthy preparation times.

Most automated antimicrobial susceptibility testing systems provide automated inoculation, reading and interpretation. These systems have the advantage of being rapid (some results can be generated within hours) and convenient, but one major limitation for most laboratories is the cost entailed in initial purchase, operation and maintenance of the machinery. Some examples of these include: Vitek System (bioMerieux, France), Walk-Away System (Dade International, Sacramento, Calif.), Sensititre ARIS (Trek Diagnostic Systems, East Grinstead, UK), Avantage Test System (Abbott Laboratories, Irving, Texas), Micronaut (Merlin, Bornheim-Hesel, Germany), Phoenix (BD Biosciences, Maryland) and many more.

**5. MECHANISM-SPECIFIC TESTS**

Resistance may also be established through tests that directly detect the presence of a particular resistance mechanism. For example, beta lactamase detection can be accomplished using an assay such as the chromogenic cephalosporinase test (Cefinase disk by BD Microbiology Systems, Cockeysville, MD and BBL DrySlide Nitrocefin, Becton Dickinson, Sparks, MD) and detection for chloramphenicol modifying enzyme chloramphenicol acetyltransferase (CAT) may utilize commercial colorimetric assays such as a CAT reagent kit (Remel, Lenexa, Kansas).

**6. GENOTYPIC METHODS**

Since resistance traits are genetically encoded, we can sometimes test for the specific genes that confer antibiotic resistance. However, although nucleic acid-based detections systems are generally rapid and sensitive, it is important to remember that the presence of a resistance gene does not necessarily equate to treatment failure, because resistance is also dependent on the mode and level of expression of these genes11.

Some of the most common molecular techniques utilized for antimicrobial resistance detection are as follows

Polymerase chain reaction (PCR) is one of the most commonly used molecular techniques for detecting certain DNA sequences of interest. This involves several cycles of denaturation of sample DNA, annealing of specific primers to the target sequence (if present), and the extension of this sequence as facilitated by a thermostable polymerase leading to replication of a duplicate DNA sequence, in an exponential manner, to a point which will be visibly detectable by gel electrophoresis with the aid of a DNA-intercalating chemical which fluoresces under UV light.

DNA hybridization. This is based on the fact that the DNA pyrimidines (cytosine and thymidine) specifically pair up with purines (guanine and adenine; or uracil for RNA). Therefore, a labeled probe with a known specific sequence can pair up with opened or denatured DNA from the test sample, as long as their sequences complement each other. If this "hybridization" occurs, the probe labels this with a detectable radioactive isotope, antigenic substrate, enzyme or chemiluminescent compound. Whereas if no target sequence is present or the isolate does not have the specific gene of interest, no attachment of probes will occur, and therefore no signals will be detected.

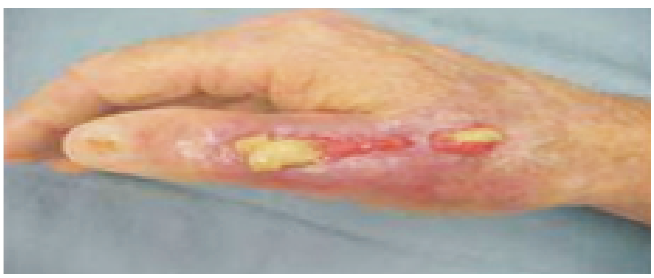
Modifications of PCR and DNA hybridization. With these basic principles, several modifications have been introduced which further improve the sensitivity and specificity of these standard procedures. Examples of such development were the use of 5'-fluorescence-labeled oligonucleotides, the development of molecular beacons, development of DNA arrays and DNA chips, among many others.

# To prevent SSI (Surgical site infections)

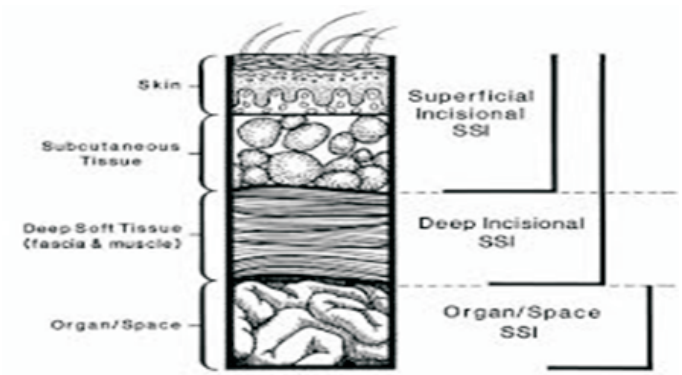
## INTRODUCTION

Surgical patients commonly developed postoperative "irritative fever," followed by purulent drainage from their incisions, overwhelming sepsis, and often death. It was in late 1860s, Joseph Lister introduced the principles of antisepsis, after which postoperative infectious morbidity decreased substantially. Lister's work radically changed surgery from an activity associated with infection and death to a discipline that could eliminate suffering and prolong life.

Definition-A surgical site infection is an infection that occurs after surgery in the part of the body where the surgery took place.



Surgical site infections can sometimes be superficial infections involving the skin only. Other surgical site infections are more serious and can involve tissues under the skin, organs, or implanted material.




*Cross-section of abdominal wall depicting CDC classifications of surgical site infection.*

Prevalence-SSIs are the third most frequently reported nosocomial infection, accounting for 14% to 16% of all nosocomial infections among hospitalized patients. SSIs were the most common nosocomial infection, accounting for 38% of all such infections.

When surgical patients with nosocomial SSI died, 77% of the deaths were reported to be related to the infection, and the majority (93%) were serious infections involving organs

Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following symptoms:

1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat.

Operating Room- A room in an operating suite where operations are performed

Surgical Personnel- Any healthcare worker who provides care to surgical patients during the pre-, intra-, or postoperative periods.

Surgical Team Member -Any healthcare worker in an operating room during the operation who has a surgical care role.

## MICROBIOLOGY

The distribution of pathogens isolated from SSIs has not changed markedly during the last decade Staphylococcus aureus, coagulase-negative staphylococci, Enterococcus spp., and Escherichia coli are mainly the most frequently isolated pathogens. An increasing proportion of SSIs are caused by antimicrobial-resistant pathogens, such as methicillin-resistant S. aureus (MRSA) or by Candida albicans.

### Pathogenesis

Most SSIs are believed to be acquired during surgery.Reports demonstrating matching strains of pathogens from the surgeon's fingers and postoperative infection.

### Best practices in Preventing SSI

### 1. Surgical Hand Preparation


"Wait, this one's a lawyer. We'd better wash our hands."

Surgical hand preparation is probably the most important SSI prevention strategy. Its importance is supported by expert opinion experimental studies and success stories of SSI reduction via mere hand hygiene promotion campaigns. It was recognized that the incidence of puerperal fever was high in obstetric clinic. After the compulsory introduction of hand antisepsis for obstetricians using chlorinated lime, he succeeded in lowering the incidence of this life-threatening, postpartum maternal infection. Either alcohol-based hand rubs or aqueous antiseptic scrubs can then be subsequently used between patients, provided hands are not visibly soiled. However, the rapid antimicrobial action, wider spectrum of activity, lower side effects and the absence of the risk of hand contamination by rinsing water in resource-poor areas might favour alcohol-based solutions. Perform a preoperative surgical scrub for at least 2 to 5 minutes using an appropriate antiseptic .Scrub the hands and forearms up to the elbows. After performing the surgical scrub, keep hands up and away from the body (elbows in flexed position) so that water runs from the tips of the fingers toward the elbows. Dry hands with a sterile towel and don a sterile gown and gloves. Clean underneath each fingernail. Do not wear hand or arm jewellery.

2. **Postponing Elective Surgery in the Case of Symptomatic Remote Infection** -Preparation of Surgical Patients - Eradicate or control all infections remote to the surgical site before elective surgery whenever possible .Screen patients for presence of hyperglycaemia and implement protocol to adequately control the serum blood glucose level (less than 11.1mmol/L) preoperatively and during the first 48 hours postoperatively (10-12). There is evidence for such measures to be applied in patients undergoing cardiothoracic operations, most notably coronary artery bypass graft (CABG).
Minimize the preoperative length of stay of the patients in the hospital, such as completing presurgical assessments and correcting underlying conditions before admission to hospital for operation and performing elective surgery, where possible, in ambulatory day centres
Educate the patients about the increased risk of smoking on postoperative surgical site infection and encourage patients to stop smoking or taking any tobacco consumption at least 30 days before the operation

3. **Expertise of the Surgeon** The surgeon's expertise and surgical technique is probably very important, although subjective and difficult to analyze. Furthermore, it is almost impossible to perform a randomized trial on this subject. An excellent surgical technique is believed to reduce SSI by: maintaining effective haemostasis while preserving adequate blood supply; gentle handling of tissue; removal of devitalized tissue; eradication of dead space; and appropriate management of the postoperative incision. These techniques can be learned and it has been suggested since the mid-1980s that surgical simulation has a beneficial impact on surgeons' experience and performance.
Antimicrobial prophylaxis. Administer a prophylactic antimicrobial agent only when indicated, and select it based on its efficacy against the most common pathogens causing SSI for a specific operation
For high-risk caesarean section, administer the prophylactic antimicrobial agent immediately after the umbilical cord is clamped

4. **Screening for MRSA Carriage on Admission** The rationale behind this approach is to detect MRSA skin carriage before incision, identify carriers and administer glycopeptides prophylaxis in the case of known carriage. .
As an example, Dutch investigators reported that nasal- and or pharynx decontamination with chlorhexidine before cardiac surgery significantly lowered deep SSIs, bacteraemia and lower respiratory tract infections.

5. **Preoperative Bathing or Showering** There is currently no evidence that preoperative showering with an antiseptic agent reduces SSI rates, despite the fact that it has been shown to reduce skin colonization. The CDC recommends that patients shower or bathe with an antiseptic agent prior to surgery

6. **Hair Removal**-In one study, SSI rates were 5.6% in patients who had hair removed by razor shave compared to a 0.6% rate among those who had hair removed by depilatory or who had no hair removed. The increased SSI risk associated with shaving has been attributed to microscopic cuts in the skin that later serve as sites for bacterial multiplication. Clipping hair immediately before an operation also has been associated with a lower risk of SSI than shaving or clipping the night before an operation

7. **Preoperative Skin Preparation** Skin preparation in the operating theatre immediately before surgery is routinely implemented in daily clinical practice worldwide, based on expert opinion. However, even with optimal preparation, true sterilization of the skin is impossible. To the best of our knowledge, there is no consensus on the best antiseptic agent to be used, although, very recently, a prospective randomized no blinded study revealed a superiority of 2% chlorhexidine combined with 70% isopropyl alcohol versus 10% povidone-iodine for preventing SSI after clean-contaminated surgery. For several decades, povidone-iodine or chlorhexidine have been generally used for skin antisepsis
In some comparisons of the two antiseptics when used as preoperative hand scrubs, chlorhexidine gluconate achieved greater reductions in skin microflora than did povidone-iodine and also had greater residual activity after a single application. Further, chlorhexidine gluconate is not inactivated by blood or serum proteins.

8. **Gloves & Adhesive Drapes** Sterile gloves and adhesive drapes are almost always used in the operating theatre. They contribute to prevent site contamination, but also reduce blood-borne pathogen transmission from patients to surgeons. However, many gloves reveal tiny punctures after use that mostly go unnoticed by the operating team and may double the SSI risk. Hence, the use of sterile gloves does not render surgical hand preparation unnecessary.[1] Double-gloving or glove-changing might reduce the risk of punctures but does not guarantee their absence

9. **Laminar Airflow in the Operating Theatre** several drawbacks of laminar air flow in the operating theatre were identified. Many hospitals in resource-rich countries are equipped with relatively expensive vertical or horizontal laminar airflow systems that reduce the bacterial burden in the air.

10. **Other Practices** use of staples versus sutures or the use of drains; both are reported to be similar in terms of SSI risk. Some studies concluded that primary closure in dirty abdominal surgery leads to less SSIs than delayed primary closure, whereas others report the opposite. Microbial sealing (with mechanical blockage of pathogen migration to the surgical wound) may be a new approach to reduce wound contamination, but this has yet to prove its effectiveness in reducing SSI rates.

11. **Postsurgical Wound Care** - hands must be cleaned before and after wound care.

12. **Public (mandatory) Reporting of HAI & SSI Rates** Public reporting of HAIs or SSIs is mandatory such a reporting method might influence healthcare workers' motivation or how to handle possible unexpected consequences

13. **Management of infected or colonized surgical personnel** work exclusion policies should be enforceable and include a

statement of authority to exclude ill personnel, they should also be designed to encourage personnel to report their illnesses and exposures

**14. Antimicrobial prophylaxis**

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent initiated just before an operation begins. AMP is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intraoperative contamination to a level that cannot overwhelm host defences. AMP does not pertain to prevention of SSI caused by postoperative contamination. Intravenous infusion is the mode of AMP delivery used most often in modern surgical practice. Essentially all confirmed AMP indications pertain to elective operations in which skin incisions are closed in the operating room.

**15. Operative characteristics:**

**Intraoperative issues**

**a. Operating room environment**

(1) Ventilation

Laminar airflow and use of UV radiation have been suggested as additional measures to reduce SSI risk for certain operations.. Filter all air, recirculated and fresh, through the appropriate filters per the American Institute of Architects' recommendations. Introduce all air at the ceiling, and exhaust near the floor

(2) Environmental surfaces Environmental surfaces in U.S. operating rooms (e.g., tables, floors, walls, ceilings, lights) are rarely implicated as the sources of pathogens important in the development of SSIs. Nevertheless, it is important to perform routine cleaning of these surfaces to reestablish a clean environment after each operation.

(3) Environmental Protection Agency (EPA)-approved hospital disinfectant should be used to decontaminate the affected areas before the next operation.

(4) Conventional sterilization of surgical instruments - Inadequate sterilization of surgical instruments has resulted in SSI outbreaks.

Flash sterilization of surgical instruments should only be used for emergency or unplanned cases. Flash sterilization of implant devices should be avoided .Standard procedures and staff proficiency of flash sterilization should be monitored. Flash sterilization record should be maintained and updated.

Scrub suits-Surgical team members often wear a uniform called a "scrub suit" that consists of pants and a shirt

Masks-The wearing of surgical masks during operations to prevent potential microbial contamination of incisions is a longstanding surgical traditionin combination with protective eyewear, such as goggles or glasses with solid shields, or chinlength face shields be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious

Surgical caps/hoods and shoe covers Surgical caps/hoods are inexpensive and reduce contamination of the surgical field by organisms shed from the hair and scalp.

Sterile gloves Sterile gloves are put on after donning sterile gowns Sterile gloves are worn to minimize transmission of microorganisms from the hands of team members to patients and to prevent contamination of team members' hands with patients' blood and body fluids. If the integrity of a glove is compromised (e.g., punctured), it should be changed as promptly as safety permits .Wearing two pairs of gloves (double-gloving) has been shown to reduce hand contact with patients' blood and body fluids when compared to wearing only a single pair.

Gowns and drapes Sterile surgical gowns and drapes are used to create a barrier between the surgical field and potential sources of bacteria. Gowns are worn by all scrubbed surgical team members and drapes are placed over the patient

Asepsis and surgical technique Rigorous adherence to the principles of asepsis by all scrubbed personnel is the foundation of surgical site infection prevention

Do not use tacky mats at the entrance to the operating room suite or individual operating rooms for infection control.

**Postoperative Issues**

a. Incision care -the incision is usually covered with a sterile dressing for 24 to 48 hours. Which has to be changed as directed by the doctor

b. Discharge planning-Unnecessary prolong stay has to be avoided. Educate the patient and family regarding proper incision care, symptoms of SSI, and the need to report such symptoms.

## REFERENCES


1. Garner JS. CDC guideline for prevention of surgical wound infections, 1985. Supercedes guideline for prevention of surgical wound infections published in 1982. (Originally published in 1995). Revised. Infect Control 1986;7(3):193-200.
2. Simmons BP. Guideline for prevention of surgical wound infections. Infect Control 1982;3:185-196.
3. Garner JS. The CDC Hospital Infection Control Practices Advisory Committee. Am J Infect Control 1993;21:160-2.
4. Hecht AD. Creating greater efficiency in ambulatory surgery. J ClinAnesth 1995;7:581-4.
5. Horwitz JR, Chwals WJ, Doski JJ, Suescun EA, Cheu HW, Lally KP. Pediatric wound infections: a prospective multicenter study. Ann Surg 1998;227:553-8.
6. INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY
   Guideline for Prevention of Surgical Site Infection, 1999
   Alicia J. Mangram, MD; Teresa C. Horan, MPH, CIC; Michele L. Pearson, MD; Leah Christine Silver, BS; William R. Jarvis, MD; The Hospital Infection Control Practices Advisory Committee
7. Preventing Surgical Site Infections
   IlkerUçkay; Stephan Harbarth; Robin Peter; Daniel Lew; Pierre Hoffmeyer; Didier Pittet

## Highlights of the coming issue


JOURNAL OF HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
Contents

**Mini Review**
Cervical Cancer – Symptoms, Diagnosis & Treatment.

**Current Trends**
UV light disinfection.

**In Profile**
Yellapragada Subbarow.

**Bug of the Month**
*Campylobacter jejuni*.

**Did You Know**
Endoclens.

**Best Practices**
To prevent Vancomycin-resistant enterococci (VRE).

Printed and published by D.G. Tripathi, Edited by Akshaya Tendulkar for and on behalf of Tulip Diagnostics (P) Ltd., Tulip House, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex, Post Office Goa - 403 202, India. Fax: 0832 2458544, **Website: www.tulipgroup.com.**